DISSERTATION

EFFECTS OF ENDOCRINE MODIFIERS ON GROWTH, CARCASS CHARACTERISTICS, AND BLOOD AND TISSUE METABOLITES OF FINISHING BEEF CATTLE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY ANTHONY CLAY BRYANT ENTITLED "EFFECTS OF ENDOCRINE MODIFIERS ON GROWTH, CARCASS CHARACTERISTICS, AND BLOOD AND TISSUE METABOLITES OF FINISHING BEEF CATTLE" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EFFECTS OF ENDOCRINE MODIFIERS ON GROWTH, CARCASS CHARACTERISTICS, AND BLOOD AND TISSUE METABOLITES OF FINISHING BEEF CATTLE

The effects of vitamin A and the interaction of steroids and beta-adrenergic agonists on growth and carcass performance, tissue metabolite profiles, and lipogenic enzyme activity were evaluated in beef cattle. In 1 experiment steers were fed 1 of 5 supplemental levels of vitamin A (0, 1,103, 2,205, 4,410, or 8,820 IU/kg DM). Final BW, G:F, ADG, HCW, LM area, marbling, and quality grade distribution did not differ among treatments. Except for d 56, no correlations between marbling score and tissue retinol concentrations or vitamin A intake were found. A negative correlation between liver retinol and α-tocopherol was observed, which may have potential health implications. Results suggest that vitamin A supplementation up to twice the NRC-suggested concentration has little effect on performance, marbling, or lipogenic enzyme activity in yearling steers and further suggest that 2,205 IU supplemental vitamin A/kg of DM is adequate for growing/finishing beef steers.

In 2 separate experiments, factorial arrangements of implant and beta-adrenergic agonist dosages were evaluated for effects on performance, carcass traits, blood metabolites, and lipogenic enzyme activity. In Exp. 1, steers were assigned to ractopamine (RAC; 0, 100, or 200 mg·steer^{-1}·d^{-1}) and implant/reimplant (IMP; None/None, Revalor-S/None, or Revalor-IS/Revalor-S) regimens, whereas in Exp. 2, heifers were assigned to ractopamine (0 or 250 mg·heifer^{-1}·d^{-1}) and implant (none,

Finaplix, or Revalor-200) treatments. No RAC x IMP interactions were noted for most carcass and performance traits. Cattle implanted or fed supplemental ractopamine had significantly greater final BW, HCW, ADG, and G:F than the respective controls. Despite no detectable difference in empty body fat (EBF), both marbling and quality grade were decreased significantly by IMP and numerically by RAC. Implanted cattle had decreased cortisol and increased GH, IGF-1, and NEFA. Although an IMP × RAC interaction was detected, BUN was decreased by IMP and RAC. No clear trends in lipogenesis were found. These data show that quality grade and marbling can differ significantly at equal EBF in finishing beef steers. Altogether, these data suggest that the modes of action of IMP and RAC are independent. Further research is needed to elucidate the exact mode of actions of these growth-enhancing products.

Key words: beta-adrenergic agonists, body composition, growth, hormone, implants, metabolite profiles, ractopamine, vitamin A

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Fear not, for I have redeemed you; I have summoned you by name; you are mine. When you pass through the waters, I will be with you; and when you pass through the rivers, they will not sweep over you. When you walk through the fire, you will not be burned; the flames will not set you ablaze. For I am the Lord, your God, the Holy One of Israel, your Savior. Isaiah 43:1-3.

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CHAPTER I

INTRODUCTION

The beef cattle industry in the United States has long focused on growth productivity and efficiencies as well as quality of the ultimate beef product being produced. These simultaneous goals are often in conflict because factors that positively affect 1 of these areas typically negatively affect the other. This paradox has led to segmentation and differentiation of the cattle market into a full spectrum of focuses for cattle operations that include maximizing productivity at one end of the spectrum and maximizing quality at the other end. These diverse focal objectives are partly driven by a wide breadth of consumer demands for product that is either affordable or that provides some unique quality. Moreover, consumers today expect all beef products to be safe, of high quality, and affordable. Growth promotants such as steroidal implants and the more recent approvals of beta-adrenergic agonists primarily focus on productivity gains to maximize gain, efficiencies, and yield of product; however, negative quality attributes such as decreased tenderness and decreased quality grade have been associated with their use. In contrast, some have recently observed potential quality grade improvements with the removal of part or all of the supplemental vitamin A from beef cattle diets. Research pertaining to these growth and nutritionally related areas will be the focus of the current review and research.

CHAPTER II

REVIEW OF LITERAURE

Metabolic Modifiers

Overview. Hormones are chemical messengers released by a tissue that can act on distant tissues, neighboring cells, or on the same cells that released the signaling molecule and that bind to receptors within the cell or on the cell surface and elicit a specific response (Roman, 2003). Hormones can be classified in different ways, but the most common method of categorizing hormones is by their structural origin. Hormones can be divided into the following categories: bioactive amines, fatty acid derivatives, peptides, and steroids. Bioactive amines are amino acid derivatives and include naturally occurring hormones such as the thyroid hormones, melatonin, serotonin, and the beta-adrenergic agonist catecholamines, epinephrine and norepinephrine, and also include the synthetic beta-adrenergic agonists like ractopamine, zilpaterol, and clenbuterol, which will be discussed in a subsequent section. Fatty acid-derived hormones include vitamin A and eicosanoids. Eicosanoids are derived from polyunsaturated fatty acids resulting from the transformation of arachidonic acid to form prostaglandins, thromboxanes, leukotrienes, and prostacyclins. Peptide hormones are very diverse and are the result of numerous processes involved in protein synthesis including signal transduction, gene transcription, post-transcriptional processing, translation, post-translational processing, and secretion. Peptide hormones vary in size ranging from 3 to 560 amino acids, and can consist of a single peptide chain or multiple linked peptide chains that may be glycosylated with different linkage types. Peptide and protein hormones include a long

list of molecules including insulin, glucagon, growth hormone (somatotropin), insulin-like growth factors, calcitonin, follicle stimulating hormone, parathyroid hormone, and numerous others. Steroid hormones are derivatives of cholesterol and include estrogen, progesterone, testosterone, cortisol, vitamin D, trenbolone acetate (a synthetic testosterone), zeranol (a fungal metabolite with estrogenic properties), and melengestrol acetate (a synthetic progestin).

Hormones can also be classified according to how they interact with the receptors on the cell surface or within the cell. Although not a completely accurate classification, these hormones are categorized as lipid soluble or water soluble. Lipid-soluble hormones freely diffuse across the lipid bilayer and interact with intracellular receptors in the cytosol or nucleus and include steroids, vitamin A, and the thyroid hormones. Water-soluble hormones are lipophobic and require interaction with a cell-surface receptor to affect intracellular signaling; the water-soluble hormones include the bioactive amines, peptide hormones, and the eicosanoids such as prostaglandin. Although eicosanoids are lipid soluble, they act in a similar manner as water-soluble proteins and bind to cell-surface receptors to initiate signal transduction. In the current research, the effects of vitamin A and the interaction of steroid hormones and beta-adrenergic agonists were studied, and thus will be the focus of this literature review.

Vitamin A

Overview. Vitamins are defined as a group of complex organic compounds present in minute amounts in natural foodstuffs that are essential to normal metabolism and lack of which in the diet causes deficiency diseases (McDowell, 2000). Vitamins include a mixed group of organic compounds that cannot be classified as easily into a category and,

unlike proteins, carbohydrates, and fats, are not related to each other. Vitamins are grouped according to whether they are water-soluble (e.g. B-vitamins and vitamin C) or fat-soluble (vitamins A, D, E, K). Most water-soluble vitamins are components of coenzymes, meaning that they are essential to the activity of the enzymes and act as a co-substrate, but do not form a permanent part of the enzyme's structure. Most water-soluble vitamins are not stored very well in the body, and, if in excess, are excreted rapidly via the urine; water-soluble vitamins are typically non-toxic. In contrast to water-soluble vitamins, fat-soluble vitamins are usually stored in some quantity in the body, are excreted via the bile into the feces, and can be toxic at excessive concentrations. Fat-soluble vitamins are involved in diverse processes in the body with vitamin A likely having the most breadth of functions.

Structure and Sources. Vitamin A plays a significant role in many essential biological processes (Blomhoff, 1994), and there is immense scientific interest in vitamin A and retinoids (Blomhoff and Blomhoff, 2006). In fact, as a result of its role in vision, cell division and differentiation, specification of positional information of cells and tissues, development of embryonic tissue and structures, reproduction, bone development, immunocompetency, hematopoiesis, skin health, antioxidant, and regulation of genes, vitamin A may be considered the most important vitamin from a practical standpoint (McDowell, 2000). Vitamin A is a term that includes any compound that possesses the biological activity of retinol, whereas retinoids are structural derivatives of vitamin A and include both naturally occurring forms of vitamin A and the numerous synthetic analogs of retinol that may or may not have biological activity and that may or may not be closely related to retinol, but they elicit biological vitamin A

activity (Blomhoff, 1994; Blomhoff and Blomhoff, 2006). Vitamin A compounds have 4 isoprenoid units joined in a head-to-tail manner (Blomhoff and Blomhoff, 2006). Most forms of vitamin A have a β-ionine ring at the head, followed by multiple isoprenoid chains with alternating double bonds. The structural forms of some naturally occurring retinoids are shown in Figure 2.1. In mammals, vitamin A is found primarily in the form of an alcohol (retinol), an aldehyde (retinal), or a carboxylic acid (retinoic acid). These vitamers exist in various isomeric forms and are typically complexed with proteins or organic acids such as fatty acids (Bonet et al, 2003). In most animal tissues, the parent retinoid, all-*trans* retinol, is typically found in the form of retinyl palmitate, but other esterified forms such as retinyl oleate or retinyl stearate are also found (Blomhoff and Blomhoff, 2006). Because of the double bonds, vitamin A can exist in different isomeric forms. The most active form of vitamin A found in mammalian tissue is the all-*trans* configuration, but 11-*cis* retinal is present in the retina of the eye, and several retinoic acids in both the *cis* and *trans* forms are found in other tissues (Blomhoff and Blomhoff, 2006).

Animal species are not capable of *de novo* synthesis of vitamin A; therefore, they rely on intake of plants, which can synthesize the precursors of vitamin A, carotenoids, to meet their nutritional needs. The carotenoids are comprised of a large group of pigments that are ubiquitous in nature and that are responsible for the orange, yellow, red, and purple colors of vegetation (Blomhoff and Blomhoff, 2006). More than 500 carotenoids have been isolated in nature, but to date, less than 60 have been shown to possess biological activity (Blomhoff, 1994; McDowell, 2000). Of these carotenoids, α-carotene, β-carotene, γ-carotene, and cryptoxanthine have been shown to have the greatest

provitamin A activity, with β-carotene having significantly more vitamin A activity than the other 3 (McDowell, 2000). Lycopene, another carotenoid, is not a precursor of vitamin A because it does not contain the β-ionine ring, but it is important for its antioxidant function (McDowell, 2000).

Vitamin A and carotenoids are rapidly destroyed by oxygen, heat, light, and acids (Frye et al., 1991; McDowell, 2000), and it has been reported that much of the carotene content is destroyed by field curing and ensiling of feedstuffs (Puls, 1994; McDowell, 2000). Consequently, most preserved or processed feedstuffs contain little vitamin A. Despite the fact that β-carotene is essentially 2 retinal molecules and that 1 mol of β-carotene should yield 2 mol of retinal, biological tests have consistently shown that only 1 molecule of vitamin A is formed from 1 molecule of β-carotene (McDowell, 2006). Once ingested, provitamin A carotenoids can be absorbed into the body and converted into retinal for later use and storage in the animal.

Absorption, Metabolism, and Storage. Vitamin A can be supplied to the body via naturally occurring carotenoids present in feedstuffs and in the form of supplemental vitamin A, which generally consists of esterified forms such as retinyl acetate. In ruminants, several reports have noted that significant amounts of both carotene and vitamin A may be degraded in the rumen (McDowell, 2000). In fact, Rode et al. (1990) reported that the ruminal degradation of biologically active vitamin A fed in the form of retinyl acetate was 80% for cattle fed high-concentrate diets and approximately 20% for high-roughage diets. Consequently, most companies have developed technologies to protect and stabilize vitamin A to minimize ruminal destruction (Alosilla et al., 2007).

Figure 2.2 illustrates the absorption and storage processes in the body. Carotenoids are absorbed by the enterocytes of the small intestine by passive diffusion; and it seems that the efficiency of carotenoid absorption may decrease with increasing intake of carotenoids (Blomhoff and Blomhoff, 2006). Moreover, the ability to absorb carotenoids is species-specific so that the carotene in some animals such as pigs, sheep, goats, and dogs must be cleaved before being absorbed; that humans, cattle, and horses can absorb carotenes intact. Carotenoids seem to be converted to retinoids in the small intestine by at least 2 different mechanisms. In 1 mechanism, carotenes such as β-carotene are cleaved symmetrically at the central double bond to form 2 molecules of retinal (McDowell, 2000). Carotenes may also be cleaved asymmetrically, resulting in products with different chain lengths; the longer of these chains can be shortened further enzymatically to form retinoic acid or retinal (Blomhoff and Blomhoff, 2006). Retinal formed by either method is then reduced to retinol. Vitamin A supplied to the animal in the form of retinyl esters, the usual form of supplemental vitamin A, are converted to retinol in the lumen of the small intestine before uptake at the brush border by the enterocytes via pancreatic triglyceride lipase and phospholipase B (Blomhoff and Blomhoff, 2006). The newly formed non-esterified retinol is then taken up into the enterocytes by a saturable facilitated diffusion.

Once in the enterocyte, and in the case of β-carotene, once in the form of retinol, most retinol binds to cellular retinol-binding protein II (CRBP-II), which then facilitates the reesterification of retinol with long-chain fatty acids, primarily palmitate (McDowell, 2000; Blomhoff and Blomhoff, 2006). Retinol esterification is facilitated by lecithin retinol acyl transferase (LRAT). Next, the nascent retinyl esters are incorporated into

chylomicrons and then secreted into the lymph system. It has also been reported that a significant amount of unesterified retinol is secreted directly into the portal circulation (Harrison, 2005). The lipoproteins move through the lymph system into general circulation where the triglycerides are hydrolyzed from the chylomicrons so that chylomicron remnants are formed. Then, the chylomicron remnants containing the intact retinyl esters are primarily cleared by the parenchymal cells of the liver. Other tissues such as bone marrow, peripheral blood cells, spleen, adipose tissue, skeletal muscle, and kidney may also uptake the chylomicron remnants for the their functions (Paik et al., 2004).

Within the hepatocytes (parenchymal cells), the retinyl esters are hydrolyzed so that the newly formed retinol may then bind to retinol-binding protein (RBP) in the endoplasmic reticulum (Blomhoff and Blomhoff, 2006) and then translocates to the Golgi apparatus. The complex then can take 1 of 2 routes: if retinol is needed for some function elsewhere in the body, the RBP-retinol complex may be secreted into the plasma; when there is sufficient vitamin A, the complex can be transferred to the stellate cells of the liver (Blomhoff, 1994) for reesterification with the aid of CRBP-I and LRAT and storage. This storage function allows the body to maintain a stable blood concentration of retinol, and only in severe vitamin A deficiency is the plasma concentration of retinol decreased (Blomhoff and Blomhoff, 2006).

In the blood, retinol is bound to RBP, which has a hydrophobic pocket to bind and protect the fat-soluble retinol (Zanotti and Berni, 2004). It also seems that RBP is necessary for mobilization of the hepatic stores of retinol into the blood and for subsequent cellular uptake in most tissues. In addition, 95% of the plasma RBP is also

bound to transthyretin. It is thought that the purpose of binding to transthyretin is to decrease the filtration of retinol in the kidney. The other 5% of plasma retinol is bound to albumin. The uptake of retinol into the cell from plasma seems to be a receptor-mediated process (Desvergne, 2007a; Blomhoff, 1994), and various forms of retinoid binding proteins seem to be important both in the blood and within tissues (cellular retinol binding protein, CRBP; cellular retinoic acid binding protein; CRABP) that either store or use vitamin A.

Cellular Action. Representations of the cellular actions of vitamin A are shown in Figures 2.3, 2.4, and 2.5. The major source of the synthesis of the active retinoid metabolites in cells is from the uptake of RBP-bound all-*trans* retinol from the plasma. Active retinoid metabolites may also be synthesized from retinyl esters, retinol, and carotenoids that are taken up from lipoproteins (Blomhoff and Blomhoff, 2006). All-*trans* retinoic acid, the major active cellular retinoid metabolite, is synthesized from all-*trans* retinol in a two-step process in which retinol is first oxidized to retinal, which is then oxidized to retinoic acid. Retinoic acid may also be synthesized from β-carotene's conversion to retinal and then to retinoic acid in the liver, intestine, kidney, and lung. Within the cell, the primary role of retinoic acid is to act as an activator of transcription factors (Blomhoff and Blomhoff, 2006). It must be noted that other retinoids, such as 11-*cis* retinal, which is isomerized to all-*trans* retinal when exposed to photons and starts a signal transduction cascade critical for proper photosensitivity and vision, are also biologically active and are also important for cellular functions.

The 2 biologically-active isomers of retinoic acid, all-*trans* retinoic acid and 9-*cis* retinoic acid, can bind to retinoid receptors, which belong to the nuclear-hormone

receptor superfamily along with steroids, vitamin D receptors (VDRs), thyroid hormone receptors (TRs), peroxisome proliferator-activated receptors (PPARs), and other orphan receptors (Villaroya et al., 1999). Retinoid receptors, which act as ligand-dependent transcription factors, exist as 2 separate subfamilies of nuclear hormone receptors: retinoic acid receptors (RARs) and retinoic-X receptors (RXRs; also known as rexinoid receptors). The RARs respond to both all-*trans* retinoic acid and 9-*cis* retinoic acid, and RXRs are specifically responsive to 9-*cis* retinoic acid. To date, there have been 3 subtypes (α, β, and γ) of both RARs and RXRs identified in mammalian tissues, and each is encoded by different genes (Villaroya et al., 1999; Bonet et al., 2003; Blomhoff and Blomhoff, 2006). To act as transcription factors, the RARs form heterodimers with RXRs, and RXRs may either form homodimers with themselves or act as obligatory heterodimer partners with other members of the nuclear receptor superfamily such as PPARs, VDRs, and TRs (Villaroya et al., 1999; Desvergne, 2007b, Ziouzenkova et al., 2007). The heterodimers and homodimers bind to specific DNA target sequences known as retinoic acid response elements (RAREs) or retinoid-X response elements (RXREs) in the promoter/enhancer regions of retinoic acid-responsive genes to promote or repress transcription and ultimately result in biological effects (Bonet et al., 2003). It seems that all-*trans* retinoic acid is more important than the 9-*cis* retinoic acid for activation of genes through the RAR-RXR heterodimer (Blomhoff and Blomhoff, 2006). The importance of 9-*cis* retinoic acid is currently under investigation, but numerous studies have demonstrated the potent effects of this molecule. On the other hand, few studies have identified 9-*cis* retinoic acid as an endogenous compound (Blomhoff and Blomhoff, 2006), and it is debated whether 9-*cis* retinoic acid is formed in the cell. It is currently

theorized that some RXR-containing heterodimers such as TR:RXR, VDR:RXR, and PPAR:RXR can be activated by ligands of 1 or both partner ligands (Bonet et al., 1999).

To add to the complexity and potential breadth of roles vitamin A can play, RARs can affect gene expression through both activation and repression of genomic actions, such as RARE or RXRE binding sites or transactivation of other transcription factors, including activating protein 1 (fos- and jun-heterodimer; AP1) and nuclear factor kappa-β (Bonet et al., 2003) . Retinoic acid can also modulate non-genomic actions that can be both either ligand-dependent or ligand-independent (Blomhoff and Blomhoff, 2006). For example, Ochoa et al. (2003) proposed that independent of its receptor, all-*trans* retinoic acid can modulate the activity of protein kinase C, an enzyme that regulates fundamental cellular functions such as proliferation, differentiation, and apoptosis, by competing with acidic phospholipids for the binding sites that ultimately affect signal transduction.

Cellular Actions of Vitamin A in Adipose Tissue. Mammals possess 2 kinds of adipose tissue, brown and white, each with different physiological roles. White adipose tissue stores energy in the form of triglycerides and can release free fatty acids based on the energy needs of the animal. Brown adipose tissue uses its fat stores for thermogenesis. In addition to energy regulation, adipose tissue controls other physiological functions via secretion of signaling proteins such as leptin, tumor necrosis factor, adiponectin, and thyroid hormone (Villaroya et al., 1999; Bonet et al., 2003). Although the liver is the primary storage organ for retinoids, adipose tissue can also take up circulating retinoids for storage. In addition, both tissues highly express RBP and CRBP (Bonet et al., 2003), which are important for solublizing retinol and for translocating the retinol for esterification or oxidation. Moreover, both RARs and RXRs

are expressed in white and brown adipose tissue, with the 3 subtypes expressed at different levels in each tissue.

Brown adipose tissue is the primary site of non-shivering thermogenesis, which is controlled by uncoupling protein-1 (UCP1), an inner mitochondrial membrane protein. In brown adipocytes, UCP1 uncouples fuel oxidation from phosphorylation of ATP, resulting in heat generation. Retinoic acid and β-carotene are transcriptional activators of UCP1, and there are 2 response elements that can bind either RAR:RXR or PPAR:RXR heterodimers (Bonet et al., 2003). Although UCP1 is not prevalent in adult humans, other uncoupling proteins such as UCP3 have been discovered in tissues other than brown adipose tissue. There is research showing that retinoids can upregulate UCP3 expression in skeletal muscle, and it is believed that UCP3 functions in energy metabolism and in controlling whether glucose or fat is oxidized (Bonet et al., 2003).

There is evidence that retinoids also play a role in adipogenesis. The formation of white adipose tissue from mesenchymal tissue begins before birth, and expansion takes place rapidly after birth as a result of increased fat cell size (i.e., hypertrophy) and proliferation of preadipocytes cells into adipocytes. The potential to generate new fat cells, hyperplasia, persists in the adult animal, and both adipocyte hypertrophy and hyperplasia occur during normal growth (Gregoire et al., 1998; Novakofski, 2004; Avram et al., 2007). Recent studies have suggested that the adipocyte lineage is derived from an embryonic stem cell precursor with the capacity to differentiate into adipocytes, chondrocytes, osteoblasts, and myocytes (Gregoire et al., 1998). Adipogenesis, the complex process by which new adipocytes are formed from precursor cells (preadipocytes), is generally thought of in 2 stages as displayed in Figures 2.5 and 2.6.

First, preadipocytes are recruited and proliferate; then, the preadipocytes differentiate into mature fat cells (Avram et al., 2007). Proliferation is the process by which preadipocytes replicate and increase in number; differentiation is the process by which undifferentiated, proliferating fibroblast-like preadipocytes become permanently cell cycle-arrested, spherical, lipid-filled, and functionally mature fat cells (Avram et al., 2007). In reality, both stages are complex processes that are affected by hormonal, nutritional, paracrine, and neuronal signals such as CAAT/enhancer binding protein (C/EBP) and PPARγ (Gregoire et al., 1998; Avram et al., 2007; Desvergne, 2007a). During the terminal phase of differentiation, *de novo* lipogenesis increases. Consequently, the sensitivity to insulin increases and the activity of enzymes involved in triacylglycerol metabolism such as acetyl-CoA carboxylase, stearoyl-CoA desaturase, glycerol-3-phosphate dehydrogenase, fatty acid synthase, and glyceraldehyde-3-phosphate dehydrogenase increase (Gregoire et al., 1998). Of course, there are many adipose depots in the body, and in cattle, these are typically grouped into perirenal, subcutaneous, intermuscular, and intramuscular. Allen et al. (1976) reported that adipocytes are developed in an overlapping sequence, beginning with perirenal and subcutaneous and ending with intermuscular fat and intramuscular marbling.

Retinoic acid was shown to be a potent inhibitor to adipocyte differentiation many years ago when researchers found that retinoic acid suppressed lipid accumulation and molecular markers of adipocyte differentiation such as stearoyl-CoA desaturase mRNA (Murray and Russell, 1980; Kuri-Harcuch, 1982; Stone and Bernlohr, 1990). It also seems that retinoic acid is only effective at inhibiting adipogenesis in the early stages of differentiation (Stone and Bernlohr, 1990; Xue et al., 1996). Many transcription factors

are cooperatively involved in adipocyte differentiation, including c-jun and c-fos, C/EBP, PPARγ, sterol regulatory element binding protein (SREBP), and adipocyte differentiation and determination factor-1 (ADD1; Xue et al., 1996; Villaroya et al., 1999; Ribot et al., 2001; Bonet et al., 2003). Adipocyte differentiation is initiated by the induction of C/EBPβ and C/EBPδ, after which PPARγ is induced followed by the induction of C/EBPα, which together synergistically induce adipogenesis. The addition of retinoic acid prevents the differentiation of adipocytes through multiple mechanisms. First, retinoic acid can interfere with the transcriptional activity of C/EBP proteins (Schwarz et al., 1997), and it is thought that retinoic acid acts through RARs to block the C/EBPβ-dependent activation of PPARγ and C/EBPα. This mechanism does not depend on binding of a liganded RAR to a response element (Schwarz et al., 1997). Other researchers have demonstrated that retinoic acid upregulates RAR and downregulates RXR and PPARγ expression (Kamei et al., 1994; Kawada et al., 1996, 2000; Brandebourg and Hu, 2005; Mercader et al., 2007). As a result, the formation of the RAR:RXR heterodimer may be favored over the PPARγ:RXR heterodimer (Bonet et al., 2003; Ziouzenkova et al., 2007). Altogether, the effects of retinoids on adipogenesis are complex and seem to depend on retinoid metabolism of both retinoic acid and retinaldehyde, carrier proteins, and both genomic and non-genomic factors (Villaroya et al., 1999; Schug et al., 2007; Ziouzenkova et al., 2007). Models illustrating these complex processes are shown in Figures 2.7, 2.8, and 2.9.

Vitamin A and Adiposity. Adipose depots in adult animals are comprised of mature adipocytes but also contain a finite number of preadipocytes that can proliferate and differentiate when the conditions are appropriate (Bonet et al., 2003). Adipose tissue

mass includes both the number and volume of adipocytes, and adipocyte volume is determined by the balance between lipogenesis and lipolysis. Adipocyte volume is determined by the relative rates of preadipocyte replication and differentiation, apoptosis, and cell acquisition (Bonet et al., 2003). In reviewing research that studied the effects of vitamin A on adiposity, several researchers noted a decrease in adiposity by animals given some form of vitamin A. Ribot et al. (2001) reported a 60% increase in the adiposity index (weight of fat expressed as percentage of body weight) of mice fed a vitamin A-deficient diet and showed a 50% decrease in adiposity index of mice fed a standard diet and also injected with retinoic acid.

Glycerol-3-phosphate dehydrogenase (GPDH) is used as a marker of adipocyte differentiation, and Torii et al. (1996) found a positive correlation ($r = 0.62$; $P < 0.01$) between GPDH activity and marbling scores. Moreover, there was a strong negative correlation between adipogenic activity and serum retinol concentration (Torii et al., 1996). In this study, however, Torri et al. (1996) cultured murine 3T3-L1 preadipocytes and then added the serum of Wagyu cattle to the culture, so this experiment was conducted across species, which could affect the inference level. Similarly and also using cultured cells, Suryawan and Hu (1997) and Brandebourg and Hu (2005) reported that GPDH was decreased significantly in cultured pig preadipocytes when treated with increasing and pharmacological levels of retinoic acid; however, no effect was noted when retinoic acid was applied at physiological concentrations. Ohyama et al. (1998) cultured stromal vascular cells containing preadipocytes prepared from Japanese Black cattle and studied thiazolidinedione, a known ligand of PPARγ, with and without the addition of retinoic acid in bovine adipose cells. The researchers observed that

thiazolidinedione by itself increased adipocyte differentiation as measured by increased GPDH and an increased number of lipid-laden cells. In contrast, the addition of all-*trans* retinoic acid completely blocked the effects of thiazolidinedione and decreased GPDH activity and the number of cells containing lipid droplets. Although retinoic acid decreased adipocyte differentiation in a dose-dependent manner, it is not known whether Ohyama et al. (1998) applied the retinoic acid at physiological or pharmacological concentrations.

Relationship between Vitamin A and Marbling in Hanwoo and Japanese Black Cattle. To this point, the discussion has focused on the effects of vitamin A on cellular activities including signal transduction that affect adipocyte differentiation, but now the focus will be on research that has evaluated the relationship between vitamin A and in vivo intramuscular fat deposition. Researchers in Japan and Korea were first to look at this relationship. Typically, the Japanese and Korean breeds of cattle are fed high-concentrate diets in confinement from a young age. Additionally, Asian consumers typically value beef based on the degree of marbling, and there can be a wide range in value based on marbling scores. Consequently, cattle reared in Korea and Japan are fed to an average age of over 30 mo (Nade et al., 2003), and considerable effort has been expended on ways to increase marbling scores in cattle. Many researchers have focused on the relationship between serum retinol and marbling scores in Hanwoo cattle and in Tajima and Wagyu strains of Japanese Black cattle (Adachi et al., 1999; Oka et al., 1998; Chae et al., 2003). It must be noted that these researchers did not fully describe their methods in detail, so extrapolation beyond these trials is difficult. Oka et al. (1998) collected blood immediately before slaughter from 57 Japanese Black cattle across 27

farms and found a strong correlation (r = -0.38; $P < 0.05$) between serum vitamin A concentrations and marbling scores. Similarly, Adachi et al. (1999) simply sampled 13 steers periodically during their lifetime and after the cattle were slaughtered and graded, marbling scores were ranked and categorized in top- and bottom-halves as either high or low marbling scores. The authors noted that the high marbling score cattle had lower serum retinol concentrations at each stage of fattening except for the initial stage. Similarly, Chae et al. (2003) found a correlation of r = -0.24 ($P < 0.01$) between the serum concentrations of vitamin A and marbling degree in 328 Hanwoo steers.

Two researchers examining the relationships between vitamin A and intramuscular fat deposition evaluated the effects of supplemental injectable or oral vitamin A on marbling score (Oka et al., 1998; Nade et al., 2003). In a series of 3 experiments in the Tajima strain of Japanese Black cattle, Oka et al. (1998) injected steers with 303 mg of vitamin A alcohol every 2 mo until slaughter (an equivalent of approximately 16,833 IU/d if prorated equally over the feeding period). The results of 1 experiment demonstrated that cattle administered injectable vitamin A periodically during the finishing period had decreased ($P < 0.05$) marbling scores compared with those that did not receive supplemental vitamin A. In contrast, in the other 2 experiments, marbling scores were not affected ($P > 0.05$) by injected supplemental vitamin A. The authors surmised that the difference in outcomes was a result of the age of the animals, and concluded that supplemental vitamin A had no effect in cattle after 23 mo of age because of the maturing of the adipocytes in the intramuscular adipose tissue. Nade et al. (2003) fed 5 sets of twins from the Wagyu strain of Japanese Black cattle 2 different levels of orally-fed vitamin A. There was no reference to the actual levels of vitamin A fed, and the only

notation was that the control cattle were fed according to the Japanese Feeding Standard for Beef Cattle (1995) and that the treatment group was fed at half that level. Nade et al. (2003) concluded that cattle fed at half the standard dose had higher marbling scores ($P < 0.05$) than those fed the standard dose.

Relationship between Vitamin A and Marbling and Growth in Cattle Breeds of the U S. In recent years, researchers from U.S. universities have focused on the effects of supplemental vitamin A or restriction of dietary vitamin A on fat deposition and adiposity in typical U.S. cattle breeds (Pyatt et al., 2005; Gorocica-Buenfil et al., 2007a, b, c; 2008). Pyatt et al. (2005) conducted 3 separate experiments in which Angus × Simmental cattle were fed either 2,300 or 7,250 IU of dietary vitamin A/kg. No differences between treatments in marbling scores or fat thickness were observed (Pyatt et al., 2005). In addition, Pyatt et al. (2005) did not find any correlation ($P > 0.10$) between marbling score and serum retinol concentration. In terms of growth performance, Pyatt et al. (2005) noted that ADG was increased in cattle fed 2,300 IU/kg compared with those that received 7,250 IU/kg; however, in the other 2 studies, no differences were detected. No differences in DMI were observed in any of the studies, but in 1 study, feed efficiency was improved in cattle fed 2,300 IU/kg compared with those that received 7,250 IU/kg (Pyatt et al., 2005).

Gorocica-Buenfil et al. (2007a, b, c; 2008) conducted research examining various vitamin A supplementation and restriction strategies and reported mixed results. For example, Gorocica-Buenfil et al. (2007a) reported that Angus-cross cattle (BW = 295 kg) that were fed 2,700 IU vitamin A/kg in a diet in which high-moisture corn was used as the sole grain source tended ($P = 0.11$) to have decreased marbling scores without any

effect on 12th rib fat thickness compared with those that received no supplemental vitamin A. Gorocica-Buenfil et al. (2007a) also reported that the vitamin A-supplemented cattle had decreased intramuscular adipose cell density and increased mean diameter of adipose cells; however, no effect on adipose cellularity was noted in the subcutaneous fat depot. In this same study, a slight increase in ADG by the cattle supplemented with vitamin A ($P = 0.08$) was observed compared with those fed no supplemental vitamin A.

In contrast to the results of Gorocica-Buenfil et al. (2007a), in lightweight Holstein steers (BW = 218 kg) that were fed 2,200 IU/kg of supplemental vitamin A before being restricted to no supplemental vitamin A for 131 or 243 d, Gorocica-Buenfil et al. (2007b) observed no differences in marbling scores, 12th rib fat thickness, or adipose cellularity in either the intramuscular or subcutaneous adipose tissue. In addition, no differences in ADG between treatments were noted, but feed efficiencies were slightly worsened in cattle that received no supplemental vitamin A during the finishing period compared with those fed 2,200 IU of supplemental vitamin A/kg.

In a third study (Gorocica-Buenfil et al., 2007c) with Angus-crossbred steers (BW = 295 kg), marbling in cattle supplemented with 2,200 IU vitamin A/kg was significantly lower than those receiving no supplemental vitamin A; however, the percentage of carcasses grading Choice or greater did not differ between treatments. Moreover, no differences in adipose cellularity were found for cattle fed either 0 or 2,200 IU/kg supplemental vitamin A, and the researchers did not detect any treatment differences ($P > 0.10$) in growth performance.

In a fourth study (Gorocica-Buenfil et al., 2008), researchers did not detect any differences in marbling or quality grade between Angus-based steers (BW = 224 kg) fed either 0 or 2,200 IU supplemental vitamin A/kg. The authors noted slightly worsened feed efficiencies in cattle that received no supplemental vitamin A during the finishing period compared to those fed 2,200 IU of supplemental vitamin A/kg, but no other differences in growth performance were observed.

Altogether, the effects of supplemental or restrictive use of vitamin A have been inconsistent. It is likely that many factors including breed, age, nutritional background, seasonal variation in vitamin A content of feedstuffs, utilization of vitamin A storage capacity in the body, duration of feeding, and stage of adipogenesis in relation to vitamin A supplementation or restriction have a large effect on the results of these trials and the benefit of decreasing vitamin A supplementation. It is also possible that the supplementation ranges that have been evaluated and that are used by nutritionists and feedyards are too small to yield consistent responses across all cattle breeds, nutritional backgrounds, and seasons.

Relationship between Vitamin A and Growth, Health, and Nutrient Requirements of Beef Cattle. Although the focus of the current review and research is on the relationship between vitamin A and fat deposition, one would be remiss to not review the literature as it pertains to the vitamin A requirements of beef cattle for growth and health functions. Numerous studies were conducted in the 1930s and then again in the 1960s and 1970s to evaluate the vitamin A requirements of finishing beef cattle (Guilbert and Hart, 1935; Guilbert et al., 1937; Jones et al., 1938; Hale et al., 1962; Perry et al., 1967, 1968; Kohlmeier and Burroughs, 1970; Kirk et al., 1971). These data were used by the

National Research Committee (NRC) to derive suggested requirements of vitamin A for cattle. The NRC (1996) lists the requirement for vitamin A in finishing beef cattle at 2,200 IU/kg of feed (DM basis). The requirements of other beef cattle (NRC, 1996) are 2,800 IU/kg for pregnant beef heifers and cows and 3,900 IU/kg for lactating cows and breeding bulls. Until the 1990s, additional research on vitamin A supplementation in U.S. beef cattle does not seem to have been conducted. Although not extremely pertinent to the focus of this dissertation, it should be mentioned that the nutrient requirements of dairy cattle as set by NRC (2001) are expressed per unit of BW rather than as a dietary concentration, being 80 IU/kg BW for growing dairy animals and 110 IU/kg BW for all adult dairy cattle (dry and lactating cows).

Hill et al. (1995) reported that feedlot steers receiving 2,134 IU of supplemental vitamin A/kg DM had greater gains and feed efficiencies than those receiving approximately 6,274 IU supplemental vitamin A/kg DM. In a receiving trial, Zinn et al. (1996) also observed greater ADG and feed efficiency in crossbred calves consuming 2,200 IU supplemental vitamin A/kg DM than in those consuming 11,000 IU supplemental vitamin A/kg DM; however, these differences were only noted during the first 28 d of the 56-d study. No carcass data were presented for either of these trials.

As was described previously, in 1 of 3 studies Pyatt et al. (2005) reported that ADG and feed efficiency were improved in cattle fed 2,300 IU/kg compared with those that received 7,250 IU/kg. Similarly, Gorocica-Buenfil et al. (2007a, b, c; 2008) conducted 4 trials (3 of which were with beef cattle) comparing 2,200 or 2,700 IU supplemental vitamin A/kg vs. no supplemental vitamin A in the finishing period of cattle. Compared with the non-supplemented cattle, vitamin A-supplemented cattle had greater ADG in 1

trial (Gorocica-Buenfil et al., 2007a) and improved feed efficiencies in 2 of the trials (Gorocica-Buenfil et al., 2007b, 2008). Taken together, these results seem to suggest that performance is lost by either supplementing too little or too much vitamin A. In addition, these data seem to support the validity of the NRC (1996) recommendations for vitamin A requirements.

In a survey of 42 consulting feedyard nutritionists, Vasconcelos and Galyean (2007) reported that the average recommendation for total dietary vitamin A was 5,215 IU vitamin A/kg DM, and the range was 2,205 to 11,023 IU vitamin A/kg. Thus, it seems that the industry is overfeeding vitamin A relative to requirements, and there might be some benefit in terms of performance or marbling or both associated with decreasing these formulation levels to NRC recommendations.

Vitamin A Requirements of Cattle and Assessment of Status. Both deficiencies and excesses of vitamin A can create serious health issues. Typically, the gold standard for assessing vitamin A status of animals is the concentration of retinol in the liver (Anderson et al., 1962; Herdt and Stowe, 1991; Puls, 1994; McDowell, 2000; Alosilla et al., 2007). Serum retinol concentrations are less sensitive to changes in vitamin A status because they are homeostatically regulated and will typically be in the "normal" range unless the animal is extremely deficient and until liver stores have become exhausted (Herdt and Stowe, 1991; Puls, 1994; McDowell, 2000; Alosilla et al., 2007). As a result, serum or plasma retinol concentrations are not useful indicators of the animal's vitamin A status (McDowell, 2000). Although there is some variation as to what retinol concentrations clinicians define as adequate, marginally deficient, and deficient, Puls (1994) defined that mature cattle (greater than 6 mo of age) are marginally

deficient in vitamin A if their liver retinol concentration is less than 100 μg/g (dry basis) and completely deficient when it falls below 30 μg/g. Although there is a wide range in published plasma and serum thresholds, likely because of the aforementioned reasons, Herdt and Stowe (1991) and Puls (1994) stated that adult cattle were deficient when the serum concentrations fell below 150 ng/mL, in which case it is likely that the animal has been deficient for some time. Other indicators of vitamin A deficiency include elevated cerebrospinal fluid pressure, night blindness, watery eyes, keratination of the eyes, cloudiness of the cornea, rough hair coat, edema of the joints and brisket, decreased feed intake and growth, abortions, stillbirths, low conception rates, diarrhea, urinary calculi, altered bone development, and susceptibility to respiratory infections (Frye et al., 1991; Puls, 1994; McDowell, 2000).

Sources and Factors Affecting Bioavailability. In general, fish oils, liver, milk fat, and egg yolks are significant sources of vitamin A. For cattle, vitamin A can either come from supplemental vitamin A or from provitamin A carotenoids. Supplemental vitamin A is typically esterified to fatty acids such as acetate or palmitate. Although the rumen degradation of supplemental vitamin A fed in the form of an esterified retinol can be high, especially in high-concentrate diets, most companies now coat the vitamin with carbohydrates, gelatin, and antioxidants to protect and stabilize the vitamin A thereby minimizing ruminal destruction (Frye et al., 1991; McDowell, 2000; Alosilla et al., 2007). The principal source of vitamin A for grazing livestock is provitamin A carotenoids such as β-carotene; the green parts of growing forage are high in carotene (Frye et al., 1991). Fresh forages can have vitamin A contents of 100,000 to more than 300,000 IU/kg (1 mg β-carotene = approximately 400 IU vitamin A; Frye et al., 1991;

Puls, 1994; NRC, 1996), whereas most grains have low content of vitamin A. Both carotene and vitamin A are destroyed by oxidation, heat, light, and storage. Forage slaughtering processes such as field curing and ensiling have been shown to rapidly degrade the vitamin A activity of feeds (Martin et. al., 1968; Puls, 1994; NRC, 1996; McDowell, 2000; NRC, 2001). As a result, numerous researchers (Jordan et al., 1963; Smith et al., 1964; Miller et al., 1970; Martin et al., 1971; McDowell, 2000) found that the liver stores of cattle grazing corn silages became depleted in vitamin A. The carotene content of feedstuffs decreases as the plants mature and in relation to the growing season; consequently, the body's storage and utilization of vitamin A and the supplementation needs of beef cattle will vary seasonally (Ralston and Dyer, 1959; Jordan et al., 1963; McDowell, 2000). Kohlmeier and Burroughs (1970) stated that yearling cattle entering the feedlot with medium vitamin A reserves (~100 μg/g, dry basis) need little or no supplemental vitamin A for 90 to 120 d. Mineral status and supplementation can also affect the availability of vitamin A. Minerals, especially Cu, are prooxidative in nature and can decrease the stability of vitamin A. In addition, a deficiency of Zn, which is needed for the production of RBP and hence mobilization of vitamin A from the liver, can affect vitamin A utilization (Puls, 1994; McDowell, 2000).

Although vitamin A deficiency can be common, vitamin A toxicity is not a practical problem. Ruminants seem to have a high tolerance for vitamin A, and it is presumed that vitamin A is safe up to 30 times the requirements for ruminants (McDowell, 2000). In dairy cattle, NRC (2001) stated that the presumed upper safe-limit is 66,000 IU/kg of diet for adult dairy cattle. Although not toxic, excess vitamin A can affect the absorption and transport, likely via competition, of other fat-soluble vitamins, and has been shown to

decrease vitamin E utilization (Dicks et al., 1959; Schelling et al., 1995; Franklin et al., 1998; McDowell, 2000). Given the role of vitamin E in immune function, it is not known whether excess vitamin A can negatively affect the health of animals.

Steroidal Implants

Although "metabolic modifiers" do not have a strict definition, they are typically considered to be a group of compounds that modify animal metabolism and affect how animals use absorbed nutrients, resulting in improved efficiency of production (NRC, 1994). The primary metabolic modifiers used in livestock are somatotropin (growth hormone), steroid hormones, and beta-adrenergic agonists. These products are known to increase skeletal muscle protein deposition and lean mass and decrease or dilute intramuscular fat deposition.

Structure and Mechanisms of Action. Steroid hormones such as estrogen and testosterone are synthesized from cholesterol in a sequence of enzymatic steps in the mitochondria and endoplasmic reticulum (Figure 2.10). Except for the formation of vitamin D_3, the conversion of cholesterol into pregnenolone via the cleavage of the side chain of cholesterol within the inner mitochondrial space is the rate-limiting step in the biosynthesis of steroid hormones. After release into circulation, steroid hormones complex with binding globulins for transport to the target cells, and then dissociate on arrival (Barrett, 2003a). The steroids enter the target cell primarily by diffusion across the plasma membrane and then attach to receptor proteins in the cytosol or nucleus. The binding of the hormone changes the conformation of the receptor so that the active site on the receptor can bind to the DNA-binding domain, the steroid hormone response element, and initiate transcription (Figure 2.11). Most steroids bind to the response element as

homodimers and bind to the DNA via Zn-fingers, which are loops of protein that are held together by a Zn ion that covalently binds 4 cysteine residues tetrahedrally (Igarashi, 2003). In contrast, the steroid hormone receptors for vitamin A, vitamin D, and thyroid hormones can bind as either homodimers or heterodimers of each other. Although steroid hormone receptors usually function as transcriptional activators, they can also behave as transcriptional repressors. For example, 1 theory is that a subtype of the retinoic acid receptors may bind with a thyroid hormone receptor to interfere with the binding of estrogen receptors (Igarashi, 2003). It must be noted that in recent years, plasma membrane-associated estrogen binding sites have been discovered, and evidence has been found that estrogen can trigger rapid signaling responses independent of nuclear localization and transcriptional effects (Moriarty et al., 2006). Because gene transcription and protein synthesis occur slowly over a period of time, evidence of membrane-associated receptors help explain why estrogen is able to evoke such quick responses (Moriarty et al., 2006). Moreover, estrogen has been shown to elicit or alter numerous signaling cascades involving G-proteins, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), ion channels, and growth factors (Moriarty et al., 2006).

Muscle Growth. Muscle growth and repair is a complex process. It integrates signals from numerous origins and involves continual synthesis and breakdown of proteins and the proliferation and differentiation of muscle precursor cells that are called myoblasts during the prenatal phase and satellite cells during their postnatal phase (Quinn et al., 2007). Skeletal muscle is comprised of multinucleated muscle fibers that are established embryonically via fusion of myoblasts (Yablonka-Reuveni et al., 2008). Before birth, the

terminal step in muscle differentiation is fusion of mononucleated myoblasts to form multinucleated myotubes, which then mature into myofibers (Wray-Cahen et al., 1998). The myofiber number is fixed at birth, and myofibers are terminally differentiated and do not have the ability to proliferate after birth (Hawke and Garry, 2001; Chung and Johnson, 2008). During postnatal growth, which will be the focus of this review, the addition of new myofiber nuclei and hence DNA needed to support skeletal muscle hypertrophy and repair originates from satellite cells. Satellite cells are muscle precursor cells that are located between the external basal lamina and the sarcolemma of muscle fibers (Mauro, 1961; Solomon and Bouloux, 2006; Chung and Johnson, 2008). Until maturity, satellite cells are proliferative, but after growth plateaus, satellite cells become quiescent until they are activated in response to muscle damage, mechanical loads, or other signals such as growth factors and steroids (Quinn et al., 2007; Yablonka-Reuveni et al., 2008). Satellite cells are classified as stem cells and once activated can reenter the cell cycle, and undergo proliferation and differentiation and fusion to contribute to hypertrophy of existing muscle fibers (Quinn et al., 2007). Technically, after satellite cells are activated and start proliferating, they are once again referred to as myoblasts (Chen et al., 2005); these myoblasts further differentiate into post-mitotic myotubes. Satellite cells lose their proliferative capacity to form myoblasts once they fuse with existing myofibers or fuse together to form new myofibers (Hawke and Garry, 2001). The abundance of satellite cells decreases from approximately 30% in young animals to around 5% in adult animals (Hawke and Garry, 2001; Chung and Johnson, 2008). It must be noted that there is some confusion and overlapping terminology used in the literature to describe muscle hyperplasia and hypertrophy. For example, nuclear

hyperplasia is the increase in muscle nuclei or an increase in nucleus-to-cytoplasm ratio; fiber hyperplasia is the increase in the number of muscle fibers (Quinn et al., 2007). Muscle hypertrophy can refer to the gross increase in muscle weight, cross-sectional area, and total protein content (Quinn et al., 2007). In addition, muscle hypertrophy can result from transcription and translation from existing myofiber DNA or from satellite cell fusion, and it seems that myonuclear incorporation from satellite cells lags behind the rate of hypertrophy (Petrella et al., 2008).

Numerous hormonal and cellular controls are used to stimulate, sustain, and regulate satellite cell activity (Hawke and Garry, 2001; Chung and Johnson, 2008; Yablonka-Reuveni et al., 2008). As will be discussed subsequently in more detail, myogenic transcription factors such as the myogenic regulatory factors (MRFs), and growth factors such as insulin-like growth factors (IGFs), fibroblast growth factor, and transforming growth factor are instrumental in regulating satellite cell activity (Wray-Cahen et al., 1998; Chung and Johnson, 2008; Yablonka-Reuveni et al., 2008). It seems that insulin-like growth factor-1 (IGF-1) increases both proliferation and differentiation of satellite cells, and that synthesis of IGF-1 can be stimulated by mechanical loads and exercise (Coolican et al., 1997; Hawke and Garry, 2001; Chung and Johnson, 2008). Fibroblast growth factor increases proliferation but inhibits differentiation (Hawke and Garry, 2001; Chung and Johnson, 2008). Moreover, myostatin, a member of the transforming growth factor-β family, inhibits both proliferation and differentiation by inhibiting myogenic transcription factors (Hawke and Garry, 2001; Chung and Johnson, 2008). It is likely that these proteins act in synchrony and as regulators of each other. Even more interesting are reports that thiazolidinediones (TZD), anti-diabetic compounds, in cell

culture can activate transdifferentiation of myoblasts into adipocytes (summarized by Chung and Johnson, 2008). Recent data in porcine muscle satellite cells indicated that TZDs blocked muscle differentiation and transdifferentiated satellite cells into adipoblasts. This transdifferentiation in culture was accompanied by increased expression of C/EBPα and PPARγ (Poulos and Hausman, 2006). Consequently, it is possible that satellite cells can adopt alternative lineages.

It is believed that consumers want lean beef but do not want to sacrifice the flavor that is associated with intramuscular marbling. In addition, producers do not want to sacrifice performance efficiencies offered from rapid weight gain. Perhaps this paradox can be solved by targeting and changing the fate of fat from different adipose depots. Although this is likely not practically feasible at the present time, it is an area that merits further research.

Implant Use in the Cattle Industry. The most common steroids used alone or in combination in beef cattle production are estradiol, testosterone, progesterone, zeranol, melengestrol acetate, and trenbolone acetate (Figure 2.12). These compounds are typically categorized as estrogens, androgens, and progestins based on an overlapping combination of their actions and structures. Estradiol 17-β, estradiol benzoate, and zeranol are classified as estrogens. Testosterone propionate and trenbolone acetate are synthetic androgens, and progesterone and melengestrol acetate are progestins. In the U.S., the first research with steroids and their effects on animal performance was conducted in poultry in the 1930s, utilizing estradiol benzoate and diethylstilbesterol (DES), an orally active estrogen (Hancock et al., 1991). Researchers began studying the effects of DES in cattle in the late 1940s and early 1950s. The history of steroidal

product approvals for use in cattle is shown in Table 2.1. In 1954, oral DES was approved for use in cattle, and Eli Lilly marketed it under the name of Stilbosol (Hancock et al., 1991). Implants with DES and implants with combinations of estradiol benzoate and progesterone and testosterone were approved in the late 1950s. Zeranol, a metabolite that has estrogenic activity of *Fusarium* fungus derived from corn, was approved in 1969 under the trade name of Ralgro (Raun and Preston, 1997). In 1987, the last steroidal compound to be approved for use in beef cattle was trenbolone acetate, a synthetic androgen (Raun and Preston, 1997). In the 1990s and 2000s, various combinations and doses of these separate and previously approved androgen and estrogen implants were approved by the FDA. Table 2.2 shows the steroidal products that are currently approved and used in beef cattle in the U.S. With the exception of Revalor-XS, most implants are targeted to have an expected duration and payout period of 80 to 140 d (Galyean, 1997; Preston, 1999). Revalor-XS is currently being intensively researched, but it is approved to support weight gain and feed efficiency for up to 200 d.

Although variation exists from among trials and with various combinations and doses of anabolic steroids, in general implants are used because they have been shown to improve growth rate by 10 to 30% and feed efficiency by 5 to 15% (Duckett et al., 1997; Preston, 1999; Montgomery et al., 2001; Nichols et al., 2002). Implants have also been shown to increase longissimus muscle (LM) area and to improve carcass yield and carcass leanness 5 to 8% (Johnson et al., 1996b; Dolezal, 1997; Duckett et al., 1997; Preston, 1999; Pritchard, 2000; Roeber et al, 2000; Schneider et al., 2007). As a result of heavier carcass weights and increased LM area at similar 12^{th} ribfat thickness and yield grades, carcasses from implanted steers yield more pounds of closely trimmed

boxed beef (Loy et al., 1988; Dolezal, 1997; Duckett et al., 1997; Paisley et al., 1999; Roeber et al, 2000; Pritchard, 2000; Platter et al., 2003; Schneider et al., 2007). However, numerous studies have also shown a decrease in tenderness as measured by Warner-Bratzler shear force and consumer sensory panels, (Kerth et al., 1996; Samber et al., 1996; Foutz et al., 1997; Roeber et al., 2000; Barham et al., 2003; Platter et al., 2003; Reiling and Johnson, 2003; Schneider et al., 2007; Smith et al., 2007), but this effect depends on the aging period of the meat. Similarly, Morgan (1997) and Scanga et al. (1998) concluded that implants increase the occurrence of dark-cutting beef. Implanting also increases or accelerates skeletal maturity as measured by the degree of ossification for the sacral, lumbar, and thoracic vertebra (Foutz et al., 1997; Morgan, 1997; Paisley et al., 1999; Pritchard, 2000; Duckett and Andrae, 2001; Roeber et al., 2000; Reiling and Johnson, 2003; Platter et al., 2003; Schneider et al., 2007).

Implants have also been shown to decrease quality grade and marbling score in beef cattle (Loy et al., 1988; Samber et al., 1996; Morgan, 1997; Pritchard, 2000; Duckett and Andrae, 2001; Montgomery et al., 2001; Reiling and Johnson, 2003; McPhee et al., 2006). Moreover, quality grade has been shown to decrease in a dose-dependent fashion in response to increased aggressiveness in implant regimens (Samber et al., 1996; Foutz et al., 1997; Morgan, 1997; Roeber et al., 2000; Platter et al., 2003). It must be noted that most studies were conducted so that cattle were slaughtered at time-constant endpoints. Nichols et al. (2002) theorized that some of the negative quality aspects associated with use of steroidal agents results from differences in physiological endpoints and suggested that part of the decrease in marbling might be compensated for by feeding implanted cattle longer and to heavier weights. Nichols et al. (2002) also stated that at

equal physiological maturity, carcass composition will be similar between implanted and non-implanted cattle. Owens et al. (1995) suggested that empty body fat (EBF) may be a good indicator of physiological maturity. Subsequently, Guiroy et al. (2001) developed a methodology to calculate EBF based on 12th rib fat thickness, HCW, USDA quality grade, and LM area. Subsequently, using these equations for EBF and 13 implant trials that involved 15 different implant strategies, Guiroy et al. (2002) calculated the adjusted final shrunk BW at 28% EBF, which is theoretically the standard body composition needed to achieve a small degree of marbling (i.e., low Choice) on the USDA scale (NRC, 1996). Accordingly, the BW at which animals reached the same EBF increased as the anabolic implant dose increased 14 to 42 kg and 30 to 39 kg in steers and heifers, respectively, depending on which implant regimen was used (Guiroy et al., 2002). Guiroy et al. (2001) reported that 28.61% EBF was needed to achieve a low Choice quality grade. Nichols et al. (2005) stated that the majority of cattle need to attain an EBF of 28.5 to 29.5% to grade to their genetic potential and suggested that if cattle are fed to the same EBF endpoint, they will grade similarly. Nonetheless, after analysis of the data summarized by Guiroy et al. (2002), it seems that the average EBF among implant-aggressiveness categories were similar; however, the percentages of carcasses grading Choice or greater decreased (Figure 2.13). In addition, even at a 30% EBF, many carcasses did not grade Choice or better. Schneider et al. (2007) conducted a study in heifers examining the response curves for growth and carcass measurements that were associated with increasing doses of estradiol and trenbolone acetate. Although the EBF among all 12 treatments did not differ statistically, marbling and quality grade trended down with increasing anabolic dose (Schneider et al., 2007). In comparing the 3

treatments of cattle that received identical doses of estrogen and trenbolone acetate (8 and 80 mg, respectively) on arrival and that were reimplanted with either 8:80, 14:140, or 20:200 mg of estradiol:trenbolone acetate, the percentage of Prime and Choice carcasses (Figure 2.14) and marbling score decreased dramatically with increasing anabolic dose (Schneider et al., 2007) without any differences in EBF. Similarly, in reviewing the trial data of Roeber et al. (2001) and Platter et al. (2003), J. D. Tatum (Colorado State University, unpublished data) showed that decreases in marbling score and quality grade that are associated with the use of steroidal implants were not mitigated by feeding implanted and non-implanted cattle to the same EBF endpoint.

More current research has focused on the doses and the timing of implant administration. Pritchard (2000) observed that cattle administered a lower-potency implant early in the finishing phase had greater marbling scores than those administered a higher anabolic dose, even though the cattle receiving the lower potency implant were administered a higher cumulative dose of anabolic steroids over the feeding period. In a follow-up study, Bruns et al. (2005) found that steers that receive an implant early in the finishing phase will have lower marbling scores than cattle that are either administered the same dose 57 d later or those cattle that did not receive any implant during the finishing phase. Moreover, no differences in EBF were detected among the 3 treatments. In contrast to these results, in cattle of similar weight and quality grade, Milton et al. (2000) observed no differences in quality grades or marbling when 20:200 mg of estradiol:trenbolone acetate (E:TBA) were administered either at arrival, d 35 or 70. Parr et al. (2006) studied the effects of giving a cumulative dose of 24:120 mg E:TBA either in 1 (24:120 mg E:TBA), 2 (12:60 mg E:TBA), or 3 (8:40 mg E:TBA) equally spaced

patterns and did not observe any differences in overall performance or carcass parameters. Schneider et al. (2007) also did not find any differences in marbling scores between cattle given a cumulative dose of 28:280 mg E:TBA as either 2 doses of 14:140 mg E:TBA or as 1 dose of 8:80 mg E:TBA and another dose of 20:200 mg E:TBA. However, Schneider et al. (2007) reported that heifers receiving a combination of 20:200 mg E:TBA had increased LM areas, lower marbling scores, and increased shear forces compared to those receiving 0:200 mg E:TBA implant. It is not known whether these differences were attributable to the estrogen component of the implant or to the difference in total anabolic dose. Bartle et al. (1992), Hutcheson et al. (1997), and Kreikemeier and Mader (2004) found that androgen and estrogen implants had additive effects to protein deposition and performance compared with using an estrogen or androgen implant alone. Herschler et al. (1995) also observed that estrogen plus androgen implant combinations were more negative to marbling and quality grade than estrogen- or trenbolone acetate-only implants. Some have hypothesized that the ratio of E:TBA (Herschler et al., 1995) and more specifically the estrogen portion (Gerken et al., 1995) is an important factor in the marbling effects noted in response to E:TBA implants. Gerken et al. (1995) stated that steers implanted with estrogenic steroids had significantly lower marbling scores than steers implanted with androgenic or combination implants. Herschler et al., (1995) surmised that 1:10 ratios of E:TBA had less of an effect on quality grade than 1:5 E:TBA ratios without any reduction in performance; however, the comparisons were made across different doses of both estrogen and trenbolone acetate so that few relative conclusions could be drawn. More research needs to be conducted to separate the effects cumulative anabolic dose from the effects that the estrogen and

trenbolone acetate components have separately on performance and quality grade when they are combined in an implant.

Cellular Actions of Growth Hormone and IGFs. From a physiological standpoint, it is believed that steroid hormones elicit increased protein deposition and muscle growth in cattle via stimulatory effects on growth hormone (GH) or IGF-1 or both (Hancock et al., 1991; Webb et al., 2002; Nichols et al., 2005). The complete mechanism by which implants work in the body is not fully understood, and the research related to mode of action of steroids specifically in cattle will be discussed in subsequent sections. Current research in human medicine is focusing on the cellular mechanisms by which GH and IGFs induce genomic and non-genomic actions. Growth hormone is a protein hormone that is primarily produced by the somatotrophs of the anterior pituitary gland and is controlled positively and negatively by numerous other protein hormones including growth hormone releasing hormone, somatostatin, and IGF-1. Growth hormone-releasing hormone is produced in the arcuate nucleus of hypothalamus and acts on the anterior pituitary to stimulate the release of GH (Barrett, 2003b). Somatostatin is synthesized in the periventricular region of the hypothalamus and is a potent inhibitor of GH release. Insulin-like growth factor-1 has a negative feedback loop to the somatotrophs to suppress release of GH, and its role and response to GH will be discussed further in a subsequent section. Because it is a protein and is water soluble, GH must bind to a cell-surface receptor to initiate cellular action. The GH receptor is monomeric and is classified as a tyrosine kinase-associated receptor; the receptor is related to several cytokine receptors such as interleukin-6, prolactin, and interferon. In the canonical signal transduction pathway (Figure 2.15), the GH receptor forms a

homodimer as GH binds to sites on 2 monomers of the GH receptor. After binding GH, the receptors are activated via docking of tyrosine kinases known as Janus kinase (JAK) to each monomer of the receptor. The JAKs then cross-phosphorylate each other and then the receptor, which allows transcription factors known as signal transducers and activators of transcription (STAT) to dock to the receptor and to be phosphorylated by JAK. Subsequently, the STATs disassociate from the receptor, dimerize, and translocate to the nucleus to bind to the response element and initiate gene transcription of proteins such as IGF-1.

In contrast, IGF-1, another protein hormone that has homology with insulin and which travels in circulation bound to IGF binding proteins (IGFBPs), binds to receptor-tyrosine kinases that have intrinsic kinase activity. The IGF-1 receptor is a tetramer of two α-chains and two β-subunits. The α-chains are entirely extracellular and are regulatory in nature; the β-chains span both the intracellular and extracellular regions of the cell and are the catalytic domains that contain the intrinsic tyrosine kinase activity. The α- and β-chains are connected to each other via disulfide bonds. With the binding of IGF-1 to the receptor (Figures 2.16 and 2.17), the receptor is autophosphorylated, which allows for docking of numerous endogenous substances including src homology (SHC) and insulin receptor substrate (IRS; LeRoith et al., 2001; Singleton and Feldman, 2001). The SHC can allow for binding of other proteins to initiate MAPK cascades via proteins such as son of sevenless (SOS), Ras, Raf, MEK, and ERK. The receptor-docked IRS can create binding sites for proteins such as PI3K, a heterodimer composed of a 110-kDa catalytic subunit and an 85-kDa or 55-kDa regulatory subunit, which can phosphorylate membrane-bound inositol phospholipids that allow for recruitment of Akt (also known as

protein kinase B; PKB/Akt). The PKB/Akt can then be phosphorylated by phosphoinositide-dependent kinases (PDKs) and subsequently activate or inactive other proteins such as mammalian target of rapamycin (mTOR) and myogenic transcription factors for further signaling to initiate synthesis or breakdown of various substrates (LeRoith et al., 2001; Rommel et al., 2001; Singleton and Feldman, 2001).

Insulin-like growth factors have been shown to be strong stimulators of proliferation and differentiation of myoblasts into post-mitotic myotubes and activators of hypertrophy of myotubes and myofibers (Singleton and Feldman, 2001; Quinn et al., 2007). It seems that these seemingly competing functions are partially reconciled by differences in signaling cascades. Moreover, IGF-1 seems to induce proliferation of myoblasts via MAPK cascades and stimulates differentiation of myoblasts primarily through PI3K pathways (Coolican et al., 1997; Xu and Wu, 2000; Hawke and Garry, 2001; Singleton and Feldman, 2001; Tureckova et al., 2001; Kim et al., 2005). However, as more research is conducted, the complexity of the GH, IGF, and muscle growth axes seems to increases. For example, recent research (Sun et al., 2007) suggests that the JAK-STAT pathway (via GH) is necessary for myoblast proliferation, and some evidence exits suggesting that IGF-1-induced activities of both MAPK and PI3K cascades are simultaneously necessary for muscle differentiation (Tiffin et al., 2004). In addition, some studies have shown that IGF-1-induced myoblast proliferation and differentiation, but not myotube hypertrophy, is dependent on a calcineurin/nuclear factor of activated T-cell (NFAT) signaling cascade (Delling et al., 2000). Calcineurin is a serine- and threonine-specific protein phosphatase that can sense Ca^{2+} through its activation of calmodulin (Aramburu et al., 2004). Calcineurin transduces signals to the nucleus by

dephosphorylating NFAT transcription factors (Delling et al., 2000). In addition, as summarized by Hameed et al. (2003), recent studies have even shown that human skeletal muscle produces 3 isoforms of IGF-1 via alternative splicing.

Stimulation of the PI3K-PKB/Akt pathway by IGF-1 has been shown to be the primary cascade for muscle hypertrophy, and it seems that PKB/Akt can activate numerous downstream pathways (Rommel et al., 2001; Bodine et al., 2001; Hoffman and Nader, 2004; Quinn et al., 2007). Specifically, the IGF-induced PI3K-PKB/Akt pathway has been shown to activate mTOR, a kinase that can activate transcription factors (Bodine et al., 2001; Rommel et al., 2001), and the 2 families of myogenic transcription factors, the MRFs and the myocyte enhancer factors (MEF2s; Xu and Wu, 2000; Sun et al., 2004; Tureckova et al., 2001; Tiffin et al., 2004). Of the 4 MRFs, myogenic determination factor 1 (MyoD) and myogenic factor 5 (Myf5) are important in proliferation and for determining the fate of myoblasts and satellite cells, and myogenin and myogenic regulatory factor 4 (MRF4) are involved in the actual execution of myoblast and satellite-cell differentiation (Xu and Wu, 2000; Hawke and Garry, 2001; Sun et al., 2004; Chen et al., 2005). The MRFs and MEFs physically interact with each other to increase the transcription of many muscle-specific genes (Xu and Wu, 2000; Sun et al., 2004); moreover, it has been shown that the IGF-PI3K-Akt pathways can target and stimulate the activities of myogenin, MyoD, and MEF2 (Xu and Wu, 2000; Tureckova et al., 2001; Sun et al., 2004).

The Somatomedin Hypothesis and Research in non-Bovine Tissues. According to the somatomedin hypothesis, which was proposed many years ago (Salmon and Daughaday, 1957; Daughaday et al., 1972), the effects of growth hormone on somatic growth are

mediated by the action of somatomedins. Although the activities of somatomedins such as increased glucose uptake were similar to insulin, they could not be blocked by insulin antibodies (Kaplan and Cohen, 2007). Furthermore, because it was discovered that the somatomedins had growth and mitogenic activities, they were called insulin-like growth factors. Currently, there are 2 insulin-like growth factors that have been characterized: IGF-1 and IGF-2. Of the 2, IGF-1 has received more attention; IGF-1 is mostly derived from the liver where its expression was found to be regulated by GH (Stewart and Rotwein, 1996). The GH-induced somatic growth also can be mediated by local production and autocrine/paracrine actions of IGF-1 (LeRoith et al., 2001).

Although several studies have shown that GH treatment increases IGF-1 mRNA in skeletal muscle cell lines, there has been much debate as to whether the GH effects on skeletal muscle are direct or indirect, and what the exact role of IGF-1 is (Kaplan and Cohen, 2007). Recently, Kim et al. (2005) observed GH administration in wild-type mice that contained functional IGF-1 receptors significantly increased serum and hepatic IGF-1, IGF-1 mRNA, increased muscle mass, stimulated proliferation of satellite cells, and caused hypertrophy of myofiber in skeletal muscle. In contrast, none of these effects was noted in mice that lacked a functional IGF-1 receptor. Kim et al. (2005) also noted that IGF-1 mRNA did not increase in the skeletal muscle of mice which overexpressed a dominant-negative IGF-1 receptor; however, there was a numerical tendency ($P = 0.07$) for the IGF-1 mRNA to be increased in the skeletal muscle of wild-type mice. Murphy et al. (1987) found that IGF-1 mRNA were present in numerous tissues, but highest in the liver, and others (Isaksson et al., 1982; Russell and Spencer, 1985; Schlechter et al., 1986) have found that direct injection of GH into specific limbs increased growth,

thereby indicating that GH has local effects that could be independent of circulating IGF-1. Similarly, other researchers (Klover and Hennighausen, 2007) have shown that selective deletion of STAT5, the transcription factor induced by GH that is involved in the expression of IGF-1, in skeletal muscle of mice significantly decreased postnatal muscle growth, skeletal size, lean mass, and skeletal muscle IGF-1 mRNA with only a slight decrease in circulating IGF-1 levels. Moreover, the selective deletion of STAT5 in the liver (Klover and Hennighausen, 2007) and IGF-1 production in the liver (Sjogren et al., 1999) did not affect growth. These data suggest that the autocrine/paracrine effects of IGF-1 in the muscle are more important than the endocrine effects (Klover and Hennighausen, 2007). LeRoith et al. (2001) proposed that endocrine and locally produced IGF-1 are responsible for the effects of GH and that GH may act in an IGF-independent manner in some tissues. Furthermore, it has been suggested that GH and IGF-1 promote growth in multiple ways, including collaborating with each other and also behaving in independent but overlapping manners (Frank, 2007). Regardless, research does support the necessary intermediate role of IGF-1 in skeletal muscle growth (Sjogren et al., 1999; Kim et al., 2005). Although it is known that GH increases gluconeogenesis and lipolysis, whereas IGF-1 behaves in a seemingly antagonistic way to increase glucose uptake and adipogenesis and inhibit gluconeogenesis, Kaplan and Cohen (2007) proposed that IGFs actually augment the role of GH by amplifying its anabolic actions such as increased protein synthesis and decreased proteolysis and by countering its catabolic effects such as lipolysis and gluconeogenesis.

Tissue Responses to Steroidal Implants in Bovines. As previously mentioned, it is believed that the growth responses associated with steroid hormones in cattle is likely

through the stimulatory effects on growth hormone (GH) and IGF-1 (Hancock et al., 1991; Trenkle, 1997; Webb et al., 2002; Nichols et al., 2005). In a review, Trenkle (1983) stated that 1 of the most consistent changes observed in cattle treated with estradiol is the increase in weight of the anterior pituitary gland. Through magnetic resonance imaging, Carroll et al. (2007) also observed that the pituitary size of a zeranol-treated sheep was 3 times that of its control sibling. Furthermore, the concentration of GH in the pituitary is not increased, but the total amount is increased as a result of the increase in size (Trenkle, 1983). Clegg and Cole (1954) reported an increased number of acidophils in DES-treated cattle. In agreement, Thomson et al. (1996a) observed an increase in percentage of somatotrophs compared with mammosomatotrophs in steers implanted with an E:TBA combination implant 24 d before slaughter. In contrast, the pituitary size from ruminants administered only trenbolone acetate is not altered (Donaldson et al. 1981). Numerous studies have shown an increase in circulating concentrations of GH associated with use of estrogen-containing implants, in beef cattle (Borger et al., 1973; Preston, 1975, 1999; Trenkle, 1970, 1983, 1997; Grigsby and Trenkle, 1986; Hongerholt et al., 1992). Moreover, Trenkle (1983) reported that circulating GH is increased following administration of estrogens and testosterone propionate but not trenbolone acetate. Hayden et al. (1992) observed an increase in serum GH of cattle administered estrogen-only implants but not estrogen-trenbolone acetate combinations relative to negative controls. Similarly, Hunt et al. (1991) did not observe any difference in GH in either trenbolone acetate-only implants or estrogen-trenbolone acetate implants; however, Hongerholt et al. (1992) showed an increase in GH associated with estrogen-plus-trenbolone acetate implants.

Growth hormone is released in discrete and episodic intervals within each day (Trenkle, 1997). Steroid hormones have been shown to increase the baseline GH and the frequency of release in steers administered an estradiol implant without any change in amplitude (Grigsby and Trenkle, 1986). In contrast, Hayden et al. (1992) reported trends for increased amplitude, duration, and frequency without any changes in baseline concentrations when steers were administered estrogen implants. However, the amplitude, duration, and frequency of GH release tended to decrease with exposure to trenbolone acetate-only or trenbolone acetate-plus-estrogen implants (Hayden et al., 1992).

Trenkle (1983) postulated that androgens act directly on the muscle and that estrogens act primarily on the hypothalamus or anterior pituitary to increase secretion of growth hormone. Some have reported that the relationship between estrogen and GH seems to be additive, which suggests that the estrogens do not solely exert actions via GH (Enright et al., 1990; Hancock et al., 1991; Preston et al., 1995; Ono et al., 1996; Rumsey et al., 1996; Elasser et al., 1998). Furthermore, Trenkle (1983, 1997) concluded that anabolic agents seem to work through more than a single mechanism of action and that the anabolic responses observed in cattle to exogenous steroids cannot be solely a result of an increase in GH secretion.

In a recent study with the objective to further elucidate the mechanisms of action for androgens and estrogens, Hassan et al. (2001) perifused estradiol-17β, testosterone and its metabolites into bovine hypothalamic and anterior pituitary slices and found that neither estrogen nor testosterone affected GH release by direct action on the anterior pituitary cells. In contrast, dihydrotestosterone (DHT) and 3α-diol, which are produced from the

reduction of testosterone via 5α-reductase, increased GH release directly from the somatotrophs (Hassan et al., 2001). When the hypothalamus and anterior pituitary slices were placed in series, perifusion of estrogen into the hypothalamus cells increased growth hormone-releasing hormone (GHRH) and GH and decreased somatostatin (SS) concentrations (Hassan et al., 2001). Shirasu et al. (1990) and Painson et al. (1992) also showed that estrogen acted directly on the hypothalamus of rats to induce secretion to GHRH and GH. When testosterone was infused at a constant rate in-series, GHRH and SS increased at the same rate so that GH release was not affected. Argente et al. (1990) also observed that SS mRNA was increased by testosterone and decreased by estradiol. Hassan et al. (2001) reported that infusion of testosterone and its metabolites in-series each increased GH, GHRH, and SS when administered at a pulsatile rate. The researchers noted that the increase in GH release from pulsatile infusion of testosterone into the hypothalamus was nullified, and that SS release was increased by the addition of aromatase inhibitor. The authors hypothesized that the aromatization of testosterone and into estrogen in the hypothalamus is partially responsible for the testosterone-induced GH release in the hypothalamus (Hassan et al., 2001); however, they concluded that the differences in release patterns of GHRH and SS in response to estrogen and androgens may be responsible for gender-specific GH patterns.

Research results have demonstrated that implants increase circulating IGF-1 (Lee et al., 1990; Preston et al., 1995; Johnson et al, 1996a,1998a; Dunn et al., 2003; Pampusch et al., 2003). Hunt et al. (1991) reported a statistically significant increase in serum IGF-1 in steers that were given estrogen (24 mg) and trenbolone acetate (120 mg) in combination and observed a non-significant increase in trenbolone acetate-only

implants (120 mg). Similarly, Mader and Kreikemeier (2006) administered estrogen-only, trenbolone acetate-only, and estrogen-and-trenbolone acetate implants (separately) and only reported statistical increases in circulating IGF-1 with the combination use of both estrogen and trenbolone acetate implants; however, all implanted cattle had numerically greater serum concentrations of IGF-1 than negative controls. It is not known whether these differences in degree of significance were a result of the estrogen component or the differences in cumulative anabolic dose.

The IGF binding protein-3 (Johnson et al, 1996a) and IGF-1 mRNA levels in longissimus (Johnson et al, 1996a, 1998b; Dunn et al., 2003; Pampusch et al., 2003) or semimembranosus muscles (White et al., 2003) and liver (White et al., 2003) were increased in steers that were implanted with a combined estrogen and trenbolone acetate implant relative to those that were not implanted. These results suggest that perhaps both liver (endocrine) and local (autocrine/paracrine) production of IGF are necessary for anabolic responses associated with steroid implants in cattle. Johnson et al. (1996a) also observed an increase in satellite-cell proliferation when exposed to sera of steers implanted with an E:TBA of 24:120 mg. Similarly, satellite-cell cultures from implanted steers had greater fusion percent, myotube nuclei, and thymidine incorporation rates than those from non-implanted steers (Johnson et al., 1998a). Kamanga-Sollo et al. (2004) observed that estradiol and trenbolone acetate can independently and in a dose-dependent fashion increase the IGF-1 mRNA and increase the rate of satellite cell proliferation in bovine satellite cell cultures in vitro. No differences in myostatin mRNA levels have been observed between implanted and nonimplanted cattle (Pampusch et al., 2003; White et al., 2003; Kamanga-Sollo et al., 2004). Both estrogen and androgen receptors

have been discovered in muscle fibers and satellite cells (Sauerwein and Meyer, 1989; Kamanga-Sollo et al., 2004; Sinha-Hikim et al., 2004; Dayton and White, 2008); moreover, an androgen response element has been found on the upstream promoter of the human IGF-1 gene (Wu et al., 2007). These findings support the assertion by Trenkle (1997) that steroids, both estrogens and androgens, can possibly have a direct effect on muscle cells.

Thomson et al. (1996b) observed increased protein synthesis with only numerical increases in protein degradation when bovine fetal muscle cells were incubated with serum from steers that had been treated with bovine somatotropin with or without steroidal implants. Similarly, Hayden et al. (1992) detected no differences in myofibrillar protein degradation as measured by the ratio of urinary N-methylhistidine:creatinine between implanted and nonimplanted cattle. Kerth et al. (2003) reported increased amino acid uptake and protein synthesis and decreased protein degradation in primary bovine muscle cells treated with muscle extract from heifers that were implanted compared with those that were not implanted. Lobley et al. (1985) showed a decrease in N-methylhistidine elimination and hence decreased muscle protein degradation after steers were implanted with an E:TBA dosage of 20:140 mg. Some have also noted decreases in blood urea N in implanted cattle suggesting increased use of N for protein deposition (Hongerholt et al., 1992; Mader and Kreikemeier, 2006).

Other than the typical carcass measures of fat such as marbling and 12^{th} rib fat thickness, few trials have been conducted examining the effects of steroidal hormones on cellularity and adipogenesis in beef cattle. Smith et al. (2007) did not detect any differences in mRNA production of the lipogenic enzymes acetyl CoA carboxylase,

lipoprotein lipase, and stearoyl-CoA desaturase between cattle that either did not receive an anabolic implant and those that received 2 separate doses of 28 mg of estradiol benzoate and 200 mg of trenbolone acetate during the feeding period. The authors reported, however, that the number of intramuscular adipocytes per gram of tissue was greater for the implanted cattle than for those that were not implanted (Smith et al., 2007), but no differences in cellularity were noted in subcutaneous adipose tissue. More recently, Parr et al. (2008) reported that after 28 d of exposure, finishing steers implanted with 24:120 mg E:TBA had decreased abundance of PPARγ and stearoyl CoA desaturase mRNA and a tendency for reduced C/EBPβ mRNA expression; these transcription factors and enzymes are involved in adipogenesis. Singh et al. (2003) also reported that both testosterone and DHT downregulated C/EBPα and PPARγ mRNA expression in pluripotent mesenchymal cells.

With respect to other blood constituents numerous studies have reported a decrease in plasma concentrations of glucocorticoids such as cortisol associated with estradiol or trenbolone acetate use in cattle (Grigsby and Trenkle, 1986; Lee et al., 1990; Jones et al., 1991; Hayden et al., 1992). Corticosteroids are catabolic to muscle tissue, and corticosteroid administration has resulted in decreased protein synthesis and increased protein degradation (Hancock et al., 1991). In human medicine, glucocorticoids inhibit the physiological secretion of GH (Solomon and Bouloux; 2006) and decrease IGF-1 production at target organ (Schakman et al., 2008). Moreover, Ma et al. (2001) concluded that glucocorticoids upregulate the expression of myostatin, which is a negative regulator of skeletal muscle mass, and Yang et al. (2005) suggested that glucocorticoids increase activity of the C/EBP cascade. In addition, several authors have

reported that anabolic steroids can preferentially bind and displace corticosteroids from their receptors via competitive inhibition in the muscle (Mayer and Rosen, 1975; Hancock et al., 1991; Trenkle, 1997; Eason et al., 2003). As a result, some have postulated that decreased secretion of glucocorticoids resulting from exposure to steroids could result in anabolic effects in muscle protein (Trenkle, 1983; Preston, 1999).

Trenkle (1997) reported that estrogenic implants increased the activity of thyroid glands and listed several older studies that have shown decreases in the plasma concentration of thyroxine in response to estrogen implants. Grigsby and Trenkle (1986) did not observe any difference in thyroid hormone concentrations in the blood of steers administered an estrogen-only implant. In more recent experiments, Kahl et al. (1992) observed that plasma thyroxine levels for each 2-wk collection over an 8-wk period were lower in steers receiving 20 mg estradiol benzoate:200 mg progesterone than in those that were not implanted. In contrast, in heifers Mader and Kreikemeier (2006) only observed decreases in thyroxine concentrations with trenbolone acetate-only implants and only in winter compared with negative control steers. Thyroxin concentrations increased with estrogen-trenbolone acetate combination implants, and no changes were detected in estrogen-only implants (Mader and Kreikemeier, 2006). There seems to be no clear change in thyroxine in implanted vs. nonimplanted cattle.

Some have reported an increase in plasma insulin concentrations in implanted cattle (Trenkle, 1970), whereas others have not (Grigsby and Trenkle, 1986; Hayden et al., 1992). Trenkle (1997) postulated that in those studies in which an increase in plasma insulin was found, the pancreas was likely responding to the increased feed intake, which is often observed in implanted cattle.

Interestingly, Hancock et al. (1991) suggested that catecholestrogens, a class of estrogen metabolites that resemble catecholamines in that the C2 or C4 positions are hydroxylated, might be an alternative mechanism for estrogenic effects. In her dissertation, Hancock (1989) reported "similar anabolic activity" as measured by blood urea N with estrogen or catecholestrogen. Little other data can be found to support or refute this observation or elucidate the mechanisms of action of catecholestrogens.

In summary, the effects of androgens and estrogens seem to exert both independent and interdependent actions and potentially via direct and indirect actions on muscle. It is quite remarkable that after 50 yr of use, the exact mechanism by which steroidal implant elicit action is not known.

Beta-adrenergic Agonists

Structure and Mechanisms of Action. Naturally occurring bioactive amines include the catecholamines epinephrine, norepinephrine, and dopamine. Catecholamines are synthesized primarily in the adrenal medulla and are derivatives of the amino acid tyrosine produced from a series of steps involving hydroxylase, decarboxylase, and methyltransferase enzymes (Figure 2.18). To exert action, catecholamines interact with cell-surface adrenergic receptors that are typically classified as α- or β-adrenoceptors Consequently, catecholamines are categorized with other compounds having similar structure that are together called phenethanolamines, which are chemicals with an ethanolamine group, which is attached to a phenyl ring group that can have various substituents attached (Moody et al., 2000). These compounds can have a variety of configurations and can bind selectively to specific α- or β-adrenoceptor subtypes. The adrenoceptors are G-protein coupled receptors (guanine nucleotide binding proteins),

which are a super-family of 7-transmembrane domain receptors in which the binding of the ligand (e.g., catecholamines) and subsequent activation of the receptor is coupled to a membrane-bound heterotrimeric G-protein complex (α, β, γ; Nelson and Cox, 2000; Roman, 2003; Figure 2.19). The α-subunit binds and hydrolyzes GTP (guanosine triphosphate), a purine nucleotide that acts as a source of energy similar to ATP. In the inactivated state, the α-subunit has GDP (guanosine diphosphate) bound to it. After the ligand binds, the trimeric G-protein complex associates with the receptor, and GDP is exchanged for GTP (Nelson and Cox, 2000; Roman, 2003). Next, the trimeric αβγ-GTP complex separates from the receptor and then, the β and γ dimeric complex disassociates from the α-GTP complex, each of which can subsequently bind to their effectors. G-proteins can be further categorized based on what effectors they act on and whether they stimulate or repress the activity of the effectors. The most classical effectors in a G-protein cascade are the transmembrane enzymes adenylate cyclase and phospholipase C (Figure 2.20). Those G-proteins that stimulate adenylate cyclase are called Gαs proteins, whereas those that inhibit adenylate cyclase are known as Gαi proteins; the proteins that stimulate phospholipase C are known as Gαq proteins (Barrett, 2003a). Adenylate cyclase catalyzes the conversion of ATP to cyclic AMP, which then can activate certain intracellular enzymes such as protein kinase A via disassociation of the catalytic and regulatory units of the enzyme. Protein kinase A aids in the transfer of a phosphate group from ATP to serine or threonine residues in various proteins and can thereby further activate or repress enzymes. Phosphorylation has been shown to activate hormone-sensitive lipase, the rate-limiting enzyme for adipocyte triacylglycerol degradation, and inactivate acetyl CoA carboxylase, the rate-limiting enzyme for long-chain fatty acid

biosynthesis (Mersmann, 1998). In 1 of the most classical Gs signaling cascades, protein kinase A, activated by epinephrine, causes the inactivation of glycogen synthase and the activation of glycogen phosphorylase, resulting in glycogenolysis (Roman, 2003). Protein kinase A (PKA) has also been shown to increase the activity of transcription factors such as cyclic AMP response element binding protein (CREB), activating protein 1 (AP1), and SP1. The PKA signaling pathways can interact with both MAPK and PI3K signaling cascades as well (Lynch and Ryall, 2008).

The activation of phospholipase C from the ligand binding of Gq receptor causes the cleavage of phosphatidyl-inositol bisphosphate to the products diacylglycerol and inositol trisphosphate (Roman, 2003). Diacylglycerol has been shown to active protein kinase C, another serine/threonine kinase; and inositol trisphosphate induces the release of Ca from the endoplasmic reticulum, which in turn can activate other signaling cascades and enzymes such as protein kinase C.

Attenuation of signal can occur through a few different mechanisms. First, G-protein may return to the basal inactivated state from the hydrolysis of GTP to GDP via the slow intrinsic GTPase activity of the Gα protein; subsequent to this action, the trimeric subunits then reassociate (Nelson and Cox, 2000). The phosphorylation of serine and threonine residues on the ligand-receptor complex by G-protein receptor kinases (GRKs) such as β-adrenergic receptor kinase (βARK) may also attenuate the signal. These GRKs are mobilized to the cell surface by association with the Gβγ dimer, and the phosphorylation sites allow for the binding of arrestin proteins to the receptor, thereby preventing interaction with the receptor and the G-protein. The binding of arrestin also facilitates the uncoupling from the G-protein (desensitization) and removal of the

receptor from the cell surface via endocytosis (downregulation), thereby decreasing the availability of adrenoceptors at the cell surface (Nelson and Cox, 2000; Roman, 2003). This process of downregulation and desensitization as cells have chronic exposure to ligands decreases signal transduction and thereby cellular response. Cells may be "resensitized" as the receptors in the endocytotic vesicles are dephosphorylated and returned to the cell surface (Nelson and Cox, 2000).

As mentioned previously, adrenoceptors are typically classified as α- or β-adrenoceptors, and norepinephrine and epinephrine can bind to each type with different affinities. All adrenoceptors are classical G-protein coupled receptors but can act through Gαs, Gαi, or Gαq proteins (Barrett, 2003a). Currently, there have been 2 types of the α-receptors (α_1 and α_2) and 3 types of the β-receptors (β_1, β_2, and β_3) identified, with subtypes (A, B, C) within each of the α receptors (Barrett, 2003a). The primary structure of the β_1-adrenoceptor contains more than 400 amino acid residues with approximately 75 to 80% homology between livestock species and 30 to 50% between the 3 subtypes (Mersmann, 1998; Moody et al., 2000; Figure 2.19). The adrenoceptors are present on most mammalian cells, but the distribution of the subtypes varies among tissues and among species (Mersmann, 1998). Ligands are categorized into receptor subtypes based on their affinity for binding to the adrenoceptors; however, ligands that are classified as 1 subtype (e.g. β_1 vs. β_2) also can bind to other receptor subtypes. Minneman et al. (1979) stated that most tissues contain a mixture of the β-subtypes. Sillence and Matthews (1994) and Van Liefde et al. (1994) reported that in competitive-ligand binding studies, there are predominantly β_2 receptors with a small percentage of β_1 and essentially no β_3 receptors in skeletal muscle and adipose tissue of cattle. Despite the observation of

McNeel and Mersmann (1999) that β1 adrenoceptors comprise the majority of mRNA transcripts in porcine tissue, Sillence et al. (2005) reported that there was an absence of a significant number of β_1 adrenoceptors in skeletal muscle, so that β_2 adrenoceptors predominate. The investigators also concluded that porcine adipose tissue was comprised of both β_1 and β_2 receptors; cardiac tissue was predominantly β_1 adrenoceptors but also contained some β_2 receptors (Sillence et al., 2005). It must be noted, however, that these conclusions were derived based on tests with agonists classified as β_1 and β_2 in prototypical tissues, so that the results could represent the affinity of these βAA instead of the abundance of receptor types (Mersmann, 1998). Moreover, Mersmann (1998) stated that when several βAA are tested, there are no clear conclusions regarding which adrenoceptor is being detected and the proportion of each; this author also stated that the lack of antibodies for the receptor subtypes preclude any conclusions as to adrenoceptor subtypes on muscle and adipose tissue of livestock. Liang and Mills (2001) and Mersmann (2002) stressed the importance of using species-specific adrenoceptors because of the observation that βAA had different binding affinities for β_2 adrenoceptors in pigs compared with other species. Nonetheless, it is thought that the variation in subtype and tissue distribution of each subtype provides for specificity and variation in response within and between species and cell types.

All the α_1-adrenoceptors act through Gαq and hence activate phospholipase C, whereas α_2-adrenoceptors are coupled to Gαi proteins, which inhibit adenylate cyclase activity (Mersmann, 1998). In contrast, β-adrenoceptors are associated with Gαs proteins and activate adenylate cyclase to increase production of cyclic AMP (Barrett, 2003a). Because norepinephrine and epinephrine can bind to both α- and β-adrenoceptors,

specificity of action is determined by tissue abundance of each type of receptor, as well as binding affinity of the ligand for each type of receptor. For example, epinephrine binds β-receptors with a higher affinity, and norepinephrine primarily acts through α-receptors (Barrett, 2003a). Although the functions and actions vary by tissue, α-adrenoceptors are involved in vasoconstriction of arteries and vein. In contrast, β_1-adrenoceptors increase contractility of the heart and lipolysis in adipose tissue, β_2-adrenoceptors cause smooth muscle relaxation, vasodilation of arteries to skeletal muscle, glycogenolysis, and gluconeogenesis (Barrett, 2003a; Boulpaep, 2003). Consequently, in human medicine, the interest in β-adrenergic agonists (βAA) has centered on their effects on respiratory and cardiac functions to relieve ailments such as asthma and high-blood pressure. Many βAA that were filtered from the parent human-medicine product streams were given to their animal-health subsidiaries to be tested, and as a result, some βAA have been approved for improvement of performance of livestock species.

Synthetic β-adrenergic agonists (βAA) that are currently approved or have been researched in beef cattle include clenbuterol, cimaterol, ractopamine, L-644,969, and zilpaterol (Figure 2.21). Mersmann (1998) and Moody et al. (2000) provide excellent reviews of the topic area. These phenethanolamines are often called repartitioning agents because of their ability to shift nutrient utilization away from adipose and toward muscle protein (Moody et al., 2000). Clenbuterol, 1 of the first and most well known βAA to be studied in livestock, was reported to increase muscle mass and decrease fat mass (Mersmann, 1998); other βAA were subsequently investigated with similar results. In livestock, these βAA have been shown to increase weight gain, improve feed efficiency, and increase carcass yield and leanness. The efficacy of βAA varies by species, with

cattle and sheep having the largest responses, followed by swine and then poultry (Mersmann, 1998; Moody et al., 2000). Moreover, the efficacy among specific βAA compounds can vary within species and may be a function of adrenoceptor specificity and binding affinity for specific βAA. Moody et al. (2000) reported that of the synthetic βAA, ractopamine is selective for β_1 adrenoceptors, whereas clenbuterol, cimaterol, salbutamol, L-644,969, and zilpaterol preferentially bind β_2 adrenoceptors. However, as with the catecholamines, it is likely that these βAA can elicit action via binding to both adrenoceptors subtypes. Ryall et al. (2006) proposed that βAA predominantly bind to 1 receptor subtype, but that there is a selectivity ratio, meaning that they can bind to other receptor types. In support of this premise, Colbert et al. (1991) stated that ractopamine possessed significant β_1AA and β_2AA properties. Moreover, Mills et al. (2003a, b) revealed that ractopamine stereoisomers seemed to be more effective at eliciting cAMP responses from β_2 adrenoceptors than β_1 adrenoceptors. Verhoeckx et al. (2005) demonstrated that although zilpaterol primarily exerts action via β_2 adrenoceptors, it also can bind and increase intracellular cyclic AMP concentrations via β_1 receptors.

Beta-Adrenergic Agonist Responses in Skeletal Muscle. The tissue and growth response associated with βAA varies by species, compound, dose, and duration; however, the 2 predominant tissue effects resulting from administration of βAA are increased muscle mass and decreased adipose mass (Mersmann, 1998). Both Elanco and Intervet/Schering-Plough, the manufacturers of ractopamine and zilpaterol, respectively, have attempted to differentiate their products based on the receptor-subtype classification of their βAA. Elanco has used this reasoning to suggest that ractopamine has less effect on tenderness because it has less effect on protein degradation; Intervet/Schering-Plough

states that because zilpaterol is a β_2AA it will have a greater beneficial effect on growth than ractopamine. As previously stated, this differentiation is likely not as black-and-white as the 2 companies have implied in that it seems that skeletal muscle is primarily made of β_2 adrenoceptors (Sillence et al., 2005) and because ractopamine and zilpaterol have been shown to elicit some actions via both β_1 and β_2 adrenoceptors (Mills et al., 2003a, b; Verhoeckx et al., 2005). In truth, the effects of βAA on protein synthesis vs. protein degradation seem to be inconsistent, in that researchers have observed various combinations of increased protein synthesis and decreased protein degradation among experiments (Mersmann, 1998). Protein degradation is typically measured by protease activity or mRNA expression. Goll et al. (2008) described 4 classes of proteolytic enzymes in skeletal muscle. The lysosomal system is composed of cathepsins, and the caspase system is composed of cysteine proteases. The other 2 are the calpain system and the proteasome. Goll et al. (2008) stated that the latter 2 have the major role in metabolic turnover of myofibrillar proteins such as actin and myosin. The proteasome is an ATP-dependent proteolytic system that involves the attachment of ubiquitin proteins to a polypeptide that is to be degraded. Yimlamai et al. (2005) found that clenbuterol attenuated the ubiquitin-proteasome pathway to induce muscle hypertrophy. Calpains are non-lysosomal, Ca-dependent cysteine proteases that are present as μ- and m-calpain based on the molar concentrations of Ca needed for enzyme activity (Goll et al., 2003). Calpastatin, another protein, is also a member of the calpain family, and it acts to inhibit the proteolytic activity of the μ- and m-calpain (Goll et al., 2003). Numerous studies have shown that calpain activity is decreased in response to epinephrine (Sensky et al., 1996), cimaterol (Wang and Beermann, 1988), and L-644,969 (Koohmaraie and

Shackelford, 1991; Pringle et al., 1993). However, others have reported an increase in m-calpain without any change or a decrease in μ-calpain in response to L-644,969 (Kretchmar et al., 1989; Kretchmar et al., 1990; Koohmaraie et al., 1991), clenbuterol (Higgins et al., 1988; Luño et al., 1999), and cimaterol (Parr et al., 1992). Bergen et al. (1989) did not detect any difference in calpain activity in pigs fed ractopamine. Some have also reported increased calpastatin activities in response to clenbuterol (Higgins et al., 1988; Luño et al., 1999), L-644,969 (Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991; Killefer and Koohmaraie, 1992; Wheeler and Koohmaraie, 1992; Pringle et al., 1993), and cimaterol (Parr et al., 1992). Calpastatin is an inhibitor of calpains; moreover, recent data revealed that calpains can block the PI3K cascade by decreasing PKB/Akt and mTOR phosphorylation (Smith and Dodd, 2007). In addition, in rats, clenbuterol has been shown to increase the phosphorylation of PKB/Akt and other proteins in the PI3K cascade (Kline et al., 2007). It should be noted that the PI3K signaling cascade is 1 mechanism by which IGF-1 exerts its actions, which provides for potential overlap between modes of action for steroids and βAA.

In terms of tenderness, several have reported increased Warner-Bratzler shear forces (WBSF) in response to L-644,969 in lambs (Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991; Pringle et al., 1993) and steers (Wheeler and Koohmaraie, 1992). Similarly, several reports indicate that cattle (Luño et al., 1999) and (Schiavetta et al., 1990) and sheep (Hamby et al., 1986) fed clenbuterol have significantly greater WBSF than controls. With ractopamine, the effects on WBSF have been similar, although to a lesser degree. Most researchers have reported decreased tenderness values in pigs (Uttaro et al., 1993; Stoller et al., 2003; Carr et al., 2005) and cattle (Gruber et al., 2008;

Avedaño-Reyes et al., 2006) fed ractopamine. Gruber et al. (2008) reported that cattle supplemented with 200 mg/steer daily of ractopamine had 9.0, 7.7, and 9.6% greater WBSF, sliced-shear force, and trained sensory panel values, respectively. In a compilation of in-house trials examining the effects of ractopamine on tenderness, Platter and Choat (2008) reported that feeding 200 mg/steer daily of ractopamine resulted in a 4.3% increase ($P = 0.09$) in WBSF in strip loins that were aged 14 d. Avedaño-Reyes et al. (2006) showed that 5- to 14-d aged beef from cattle supplemented with ractopamine (300 mg·steer^{-1}·d^{-1}) or zilpaterol (60 mg·steer^{-1}·d^{-1}) for the last 33 d of the feeding period had 10.0% and 16.4% greater WBSF values than that of control cattle. Similarly, Platter et al. (2008) summarized that after 14 d of aging, steaks from cattle that were fed 200 mg·steer^{-1}·d^{-1} of ractopamine or 7.5 mg/kg zilpaterol had 6.5 and 48.7% greater WBSF values. Research with zilpaterol in U.S. cattle is just beginning, and more tenderness data should follow.

Wheeler and Koohmaraie (1992) measured urinary creatinine and N-methylhistidine in steers treated with L-644,969 and reported decreased fractional degradation rate of protein and an increased fractional accretion rate. In terms of protein synthesis, Bergen et al. (1989) observed an increase in fractional synthesis rate in pigs fed ractopamine compared with controls. Adeola et al. (1992a) reported that the effect of ractopamine in fractional synthesis and degradation rates of protein depended on protein supplementation. At higher dietary protein levels, rates of synthesis and breakdown were increased with ractopamine supplementation, resulting in an overall increase in protein accretion. Byrem et al. (1998) infused cimaterol into hindlimbs of steers and found that cimaterol increased blood flow and the net uptake of amino acids for up to 14 d from

initiation; overall protein content was also increased. Eisemann et al. (1988) and Eisemann and Bristol (1998) also reported increased blood flow to the hindlimbs, as well as increased heart rate. Hoey et al. (1995) similarly observed reduced diastolic blood pressure with a subsequent increased heart rate and contractility in response to clenbuterol. In agreement with increased protein deposition and hence N retention, many authors have shown decreased plasma urea N concentrations in response to cimaterol (Chickou et al., 1991), clenbuterol (Ricks et al., 1984), and ractopamine (Eisemann and Bristol, 1998; See et al., 2004)

Increases in total RNA or α-actin mRNA abundance have been reported in response to ractopamine (Grant et al., 1993) and L-644,969 (Koohmaraie et al., 1991; Pringle et al., 1993). Others have demonstrated an increase in myosin light chain mRNA in response to ractopamine (Smith et al., 1989) and clenbuterol (Smith et al., 1995). Bergen et al. (1989) reported that the protein concentration and total protein per semitendinosus muscle was increased in pigs fed ractopamine up to 42 d. In addition, the DNA concentration in the muscle decreased without any change in total DNA content of the muscle (Bergen et al., 1989). In the muscle of lambs fed L-644,969 for 6 wk, Koohmaraie et al. (1991) noted increased protein content and concentration, decreased DNA concentration, a numerical increase in total DNA content of the muscle, and increased protein:DNA ratios. Pringle et al. (1993) showed increased total muscle content of DNA, RNA, and protein, and increased protein:DNA ratios in lambs fed L-644,969 after 2 to 6 wk on treatment. In lambs fed cimaterol for 8 wk, Kim et al. (1987) observed decreased DNA concentrations and increased protein:DNA ratios. O'Connor et al. (1991a) fed cimaterol to lambs for 3 or 6 wk. At both time periods the protein:DNA

ratio was increased, and the DNA concentration was decreased, whereas total DNA content remained the same between treatments. Beermann et al. (1987) fed lambs cimaterol for 7 or 12 wk, and after 7 wk, the authors noted increased protein content, no change in DNA content, decreased DNA concentration, increased RNA content, and increased protein:DNA ratios compared with the controls. After 12 wk, the investigators observed increased protein content, decreased DNA concentration, increased RNA content, and increased protein:DNA ratios, and a numerical increase in DNA content (Beermann et al., 1987). Grant et al. (1990) reported that ractopamine or isoproternol, another βAA, could enhance the proliferative activity of satellites cells in embryonic chicken breast muscle. Altogether, these data suggest that the increase in muscle mass and size could be from combined effects of hypertrophy and hyperplasia. It may be a time response with initial growth occurring through hypertrophy; after longer feeding periods, satellite cells could be recruited to supply additional DNA for maintenance and growth of muscle. Nonetheless, given that the 2 currently-approved βAA, ractopamine and zilpaterol, are only fed for the last 28 to 42 d and 20 to 40 d of the feeding period, respectively, the majority of muscle growth is likely via hypertrophy. Chung and Johnson (2008) proposed that fiber hypertrophication cannot be sustained very long without additional DNA and that, in addition to internalization of receptors, this may be another contributor to decreased responsiveness to βAA over time. It is also evident that the protein accretion is likely a result of a combination of increased protein synthesis and decreased protein degradation.

Beta-Adrenergic Agonist Responses in Adipose Tissue. Most of the focus of βAA has been placed on muscle growth; however, βAA can also affect carcass fat. Mersmann

(1998) stated emphatically that βAA stimulate adipocyte triacylglycerol degradation and inhibit fatty acid and triacylglycerol synthesis in vitro. In addition, several authors have reported increased levels of plasma nonesterified fatty acids in response to exposure to βAA such as cimaterol (Beermann et al., 1987; Kim et al., 1987; Chickou et al., 1991; O'Connor et al., 1991b; Byrem et al., 1998), clenbuterol (Blum and Flueckiger, 1988; Eisemann et al., 1988), and ractopamine (Adeola et al., 1992b). Some have also observed a decrease in plasma insulin concentrations in response to cimaterol (Beermann et al., 1987; O'Connor et al., 1991b) and clenbuterol (Eisemann and Huntington, 1988) and decreased insulin binding to adipocytes (Liu and Mills, 1990). In contrast, studies by Eisemann and Bristol (1998) did not reveal any changes in plasma concentration of insulin when sheep were fed ractopamine, but there was a tendency toward increased tissue sensitivity and responsiveness to insulin. Corresponding to decreased insulin concentrations, some investigators have shown increases in plasma glucose concentrations in response to cimaterol (Chickou et al., 1991; O'Connor et al., 1991b), clenbuterol (Blum and Flueckiger, 1988; Eisemann et al., 1988), and ractopamine (Adeola et al., 1992b), but others have not detected differences in plasma glucose in response to cimaterol (Beermann et al., 1987; Byrem et al., 1998) or ractopamine (Eisemann and Bristol, 1998). Similarly, the effects of βAA on other endocrine functions such as GH, IGF-1, and thyroid hormones have been inconsistent (Moody et al., 2000). Both Mersmann (1998) and Moody et al. (2000) concluded that given this lack of consistent effect on circulating GH and that some studies have shown additive effects of βAA and GH, βAA likely elicit actions independently of the somatotropic axis. In addition, GH typically stimulates increases in intake, whereas βAA typically do not. In

rat muscle, however, clenbuterol has been shown to induce local IGF-1 production (Awede et al., 2002). As has been described previously, the steroidal and βAA pathways can interact intracellularly; both have been shown to utilize the MAPK and PI3K signaling cascades (Lynch and Ryall, 2008). The cross-talk and the overlapping of signaling cascades are becoming more evident as scientists dive deeper into cellular mechanisms. In fact, Pearen et al. (2006) stated that there is selective and specific cross-talk between βAA and nuclear hormone signaling. In addition, it is likely that these potentially interdependent signals are highly dependent on duration, and hypertrophy and hyperplasia require different mechanisms. At this time the effects of βAA on insulin sensitivity, glucose metabolism, and endocrine actions are unclear.

Schiavetta et al. (1990) fed clenbuterol to steers for 50 d and then slaughtered half the negative controls and half the treatment cattle; they subsequently fed the remaining cattle for an additional 78 d without any supplemental clenbuterol. Subcutaneous adipocytes of the clenbuterol-fed cattle were smaller and had less volume after both 50 and 128 d, but no differences in adipocyte numbers were detected (Schiavetta et al., 1990). Conversely, after the 78-d withdrawal, the total number of subcutaneous adipocytes in the clenbuterol cattle was greater than the control cattle. Although not statistically different, the authors noted a 23% decrease in the volume of intramuscular adipocytes in the clenbuterol-fed cattle after the 78-d withdrawal, which correlated to the 25% decrease in marbling score associated with the clenbuterol cattle. In heifers, Miller et al. (1988) also fed clenbuterol for 50 d and demonstrated a decrease in subcutaneous and intramuscular adipocyte size and volume; however, among the 2 trial groups, the response of the number of adipocytes varied with an increase in adipocyte number in 1 group and no difference in

the other. The activity of several lipogenic enzymes, such as FAS, NADP-malic dehydrogenase, 6-phosphogluconate dehydrogenase, and glucose-6-phosphate dehydrogenase, and the activity of fatty acid binding protein were all decreased with clenbuterol supplementation (Miller at al., 1988). Although somewhat mixed, these responses suggest that βAA increase lipolysis and decrease lipogenesis (Mersmann, 1998; Moody et al., 2000; Mills et al., 2003b).

Excretion of Beta-Adrenergic Agonists. Thus far, the focus of this review has primarily been on how the body utilizes βAA for growth and how βAA elicit their responses; however, absorption and excretion of βAA are also important. Although the percent varies slightly by species, the absorption of βAA in livestock is "rapid and extensive" and seems to be well over 70% for most livestock species (Smith, 1998). In addition, the primary route of excretion seems to be urinary as glucuronide- and sulfate-conjugates (Smith and Shelver, 2002), with a significantly smaller portion excreted via the bile and feces. The plasma half-lives vary among βAA, with the halogenated βAA such as clenbuterol having much longer half-lives than the βAA that contain hydroxyl groups in the aromatic rings such as ractopamine (Smith, 1998). Similarly, the bioavailability, which is assessed based on how much of the parent compound reaches the target tissue in and unchanged state, differs by what substituents are on the aromatic ring (Smith, 1998); however, there are few available studies to assess this in livestock. Because of the presence of chloride on its aromatic rings, clenbuterol has a long half-life and high bioavailability to tissues. Clenbuterol has also been implicated in numerous cases of food poisoning in Europeans who ingested liver from animals that were illegally fed clenbuterol. Symptoms included tachycardia, nervousness, muscle pain, tremor,

headache, and dizziness (Martínez-Navarro, 1990; Salleras et al., 1995; Moody et al., 2000). The primary tissues in which residues have been found are liver, kidney, lungs, and the pigmented ocular and hair tissues, with less residing in muscle and adipose (Smith, 1998, 2000). Smith and Shelver (2002) recently studied the residues in liver and kidney after 0-, 3-, or 7-d withdrawal from animals fed ractopamine for 7 d. The investigators revealed that residues decreased rapidly, with no residues being detectable 7 d after withdrawal. Additionally, residues were greater in kidney than in liver. The authors also demonstrated that ractopamine was detectable in the urine of sheep and cattle for 5 and 7 d after withdrawal, respectively. Similarly, Shelver and Smith (2006) tested for residues in the liver, kidney, muscle, and urine of sheep fed zilpaterol for 10 d and found residues to be highest in liver and kidney. In agreement with Stachel et al. (2003), residues declined dramatically between d 0 and 2 of withdrawal. Urinary zilpaterol was excreted rapidly and reached a steady state 7 to 10 d after withdrawal (Stachel et al., 2003; Shelver and Smith, 2006). The U.S. Food and Drug Administration has set a 3-d withdrawal for zilpaterol.

Beta-Adrenergic Agonist Use and Current Research with Ractopamine in the Cattle Industry. A significant amount of research was conducted in the 1980s and 1990s to evaluate the effects of some βAA on cattle performance. Johnson (2004) summarized the results of these early trials with cimaterol, L-644,969, clenbuterol, ractopamine, and zilpaterol (Table 2.3). The ranges in responses were highly variable; relative to controls the improvement in ADG for steers in response to βAA ranged from -9% to 30%, and the improvement in G:F ranged from -1% to 33% (Johnson, 2004). In terms of carcass response to βAA, dressing percent improved from 1 to 8% in steers, and the increase in

muscle mass ranged from 2 to 41% (Johnson, 2004). The decrease in fat percent ranged from 2 to 26%. In the older research, few trials examined the effects of βAA on performance and carcass merit; however, the ranges in responses to βAA in heifers were similar (Johnson, 2004). It must be noted that the older research summarized by Johnson (2004) was across many βAA and across many doses and durations; as a result, the production responses noted in this summary were variable.

Because the focus of the current research is ractopamine, this section will center on βAA that are currently approved and used in beef cattle in the U.S. Ractopamine and zilpaterol were approved for use in finishing beef cattle in the U. S. in 2003 and 2006, respectively. Since that time, a significant amount of research has been initiated to examine the production response in commercial settings. Ractopamine hydrochloride (Optaflexx-45; Elanco Animal Health, Greenfield, IN) is approved to feed to finishing beef cattle for the last 28 to 42 d of the feeding period, with a recommended dosage of 70 to 430 mg·animal^{-1}·d^{-1} (8.2 to 24.6 g/ton; 90% DM basis). The label approval for ractopamine is for increased rate of gain, improved feed efficiency, and increased carcass leanness. Zilpaterol hydrochloride (4.8%; Zilmax; Intervet/Schering-Plough Animal Health, Millsboro, DE) is approved to feed the last 20 to 40 d of the finishing period and has a 3-d withdrawal before slaughter. Zilpaterol is approved to feed at 6.8 g/ton (7.5 mg/kg; 90% DM basis) to provide 60 to 90 mg·animal^{-1}·d^{-1}, and the label claims for zilpaterol include increased rate of gain, improved feed efficiency, and increased carcass leanness.

Steers. In a summary of 6 studies evaluating the effects of ractopamine fed for the last 28 to 32 d of the finishing period at 0, 100, or 200 mg·steer^{-1}·d^{-1}, Laudert et al.

(2005a,b) reported a 3.7 and 6.7 kg increase in final live weight for the cattle fed the 100 and 200 mg/d relative to the negative controls. For the final 28 to 32 d, the same cattle had a 9.4 and 17.4% increase in ADG and a 9.2 and 15.9% improvement in feed efficiency for the 100 and 200 mg·steer^{-1}·d^{-1} treatments, respectively. It should be noted that these improvements were described just for the final 28- to 32-d period in which the ractopamine was fed. These improvements would be diluted by 5- to 6-fold if calculated from d 0 because typical finishing cattle are fed 150 to 180 d, which is how most producers would evaluate the use of products economically. On a carcass basis for the same trials (Laudert et al., 2005b) reported a 2.4 and 5.6 kg increase in HCW for the cattle fed the 100 and 200 mg/d, respectively, relative to the negative controls. The 200 mg·steer^{-1}·d^{-1} cattle also had a 0.23 percentage unit increase in dressing percent and a 2.0 cm^2 increase in LM area relative to the negative controls. No changes in 12th rib fat or yield grade were detected among treatments. Neither marbling score nor quality grade distribution differed among treatments; the percentage of carcasses grading Choice or greater for the 200 mg·steer^{-1}·d^{-1} treatment was 2.0 percentage units greater numerically than the negative controls.

In a compilation of 5 dose-titration studies, Schroeder et al., (2005a,b,c) evaluated the effects of 0, 10, 20, and 30 mg/kg of supplemental ractopamine fed to steers the last 28 to 42 d of the finishing period. The steers consumed approximately 0, 100, 200, and 300 mg·steer^{-1}·d^{-1} for the 0, 10, 20, and 30 mg/kg treatments, respectively, and for comparison purposes will be summarized as such. Most responses were similar to those reported by Laudert et al. (2005 a,b). A 6.8-, 7.2-, and 9.9-kg increase in final live weights were noted for the 100, 200, and 300 mg·steer^{-1}·d^{-1} diets, respectively, relative to the negative

control (Schroeder et al., 2005a). Additionally, compared with the control cattle, ADG was increased 17.3, 19.7, and 26.0% for the 100, 200, and 300 mg·steer^{-1}·d^{-1} treatments, respectively, during the period in which ractopamine was fed. Feed efficiency was also improved by 13.6, 15.9, and 20.5% for the 100, 200, and 300 mg·steer^{-1}·d^{-1} treatments, respectively. As observed by Laudert et al. (2005a), no differences in DMI were evident among treatments (Schroeder et al., 2005a). From a carcass viewpoint, HCW was increased by 3.0, 6.1, and 8.3 kg for the steers receiving 100, 200, and 300 mg/d, respectively, compared with the controls. Dressing percent was 0.3 and 0.4 percentage units greater for the 200 and 300 mg/d cattle, respectively, relative to the controls, and LM area was increased by 1.9, 2.6, and 3.2 cm^2 for the 100, 200, and 300 mg·steer^{-1}·d^{-1} treatments, respectively (Schroeder et al., 2005a, b). No differences in 12th rib fat thickness were observed; however, a decrease in overall yield grade was noted for cattle that consumed 300 mg/d (Schroeder et al., 2005b). In examination of the composition of the carcasses, (Schroeder et al., 2005b) found that the cattle fed 200 and 300 mg/d had 3.5% greater carcass protein (15.35 vs. 14.82%), and carcasses from the 200 mg/d cattle had 5.4% less fat (29.5 vs. 31.2%) than controls. Consequently, the carcass protein gain per day for the 200 and 300 mg/d cattle was 101 and 114% greater than the controls, respectively (101.2 and 108.0 vs. 50.4 g/d).

Across 4 studies, Van Koevering et al. (2006a,b) examined the effects of both dose and duration on growth performance and carcass traits of cattle fed ractopamine in which steers were fed 0, 100, or 200 mg/d of ractopamine for the final 28, 35, or 42 d of the finishing period. The ADG of the cattle fed 100 and 200 mg/d was 6.9 and 11.1% greater than the negative controls, respectively, and feed efficiency was improved by 8.0 and

11.4% for the 100 and 200 mg/d treatments, respectively relative to the controls (Van Koevering et al., 2006a). Accordingly, HCW was increased by 2.9 and 5.0 kg for cattle fed the 100 and 200 mg/d diets, respectively, and LM area was increased by 1.5 and 2.8 cm^2 for the 100 and 200 mg/d treatments, respectively, compared with the controls (Van Koevering et al., 2006b). Although 12^{th} rib fat thickness was not affected, yield grade decreased in a dose-dependent manner, but marbling score did not differ among treatments (Van Koevering et al., 2006b). Overall, these responses were smaller than the results of Laudert et al. (2005) and Schroeder et al. (2005), but the trends were similar.

In another study, Van Koevering et al. (2006c) fed 0 or 200 $mg \cdot steer^{-1} \cdot d^{-1}$, and cattle were weighed every 7 d from d 0 to 42. Final live weight and HCW were increased by 8.0 and 4.8 kg, respectively, and ADG and G:F were improved by 14.4 and 14.2%, respectively, for cattle fed ractopamine for an average of 35 d. In a serial slaughter study, Winterhollar et al. (2007) examined the effects of feeding ractopamine at 200 $mg \cdot steer^{-1} \cdot d^{-1}$ for the final 28 d of the finishing period; in addition, cattle were slaughtered in 3 groups after 150, 171, or 192 d. The investigators did not detect an interaction between ractopamine × days on feed and reported an increase of 11 and 8 kg in live weight and HCW, respectively, in the ractopamine steers. Over the entire finishing period, ADG and G:F was increased by 4.5 and 4.0%, respectively, which equates to 27.4 and 24.3% for the final 28 d in which ractopamine was fed. Dressing percent was 0.29 percentage units greater numerically for the ractopamine heifers, and LM area was 1.74 cm^2 larger for the heifers supplemented with ractopamine. No differences in 12^{th} rib fat thickness, yield grade or marbling score were detected.

In a large-pen study involving 164 pen comparisons of cattle assigned to control or ractopamine (200 mg·steer^{-1}·d^{-1}) for the final 28 to 35 d of the finishing period, T. C. Bryant (Colorado State University, unpublished data), reported an 1.5% improvement in ADG and feed efficiency in the ractopamine-fed cattle over the entire feeding period. In addition, the ractopamine cattle had 3.6-kg heavier HCW and yielded 0.26 percentage units more than the negative control. The cattle fed ractopamine had 3.24 percentage units fewer carcasses grading Prime and Choice and 1.20 percentage units more carcasses grading sub-Select. However, there was a 3.80 percentage unit increase in the percentage of Yield Grade 1 and 2 and a 1.41 percentage unit decrease in the percentage of Yield Grade 4 and 5. Additionally, the mortality of the cattle fed ractopamine was 2-fold greater than that of the negative controls during the period in which ractopamine was fed. Bryant hypothesized that the increase in mortality was associated with either a compromised cardiac-respiratory function or a decrease in gut motility.

The smaller growth responses noted in trial of T. C. Bryant (unpublished data) compared with those of Laudert et al. (2005a) are attributed to the difference between a true biological response and what is actually observed in the field. In a true commercial setting, all cattle cannot be fed a set dose of ractopamine because feedlot diets are formulated for cattle of varying weights, breeds, and backgrounds. Consequently, intake varies significantly within a feedlot; for example, a 0.9 kg SD in intake in a feedyard, which is not uncommon, would result in a range in ractopamine dose of 120 mg·animal^{-1}·d^{-1} on a pen-level basis. This range in ractopamine dose would likely be even greater on an individual-animal basis. Additionally, relative to controls, some studies have shown smaller marginal increases in growth response at doses both less than and greater than

200 mg·animal^{-1}·d^{-1}. As a result, the cattle that eat less than 200 mg·animal^{-1}·d^{-1} will have less response compared to the controls, and those that eat more than 200 mg·animal^{-1}·d^{-1} will benefit marginally less. This variation in intake may also contribute to the increase in incidence in mortality that was observed in the study (T. C. Bryant, unpublished data).

In addition, in a commercial setting, other factors including milling and logistics have a large effect, and there can also be a difference in how line graders evaluate carcass merit vs. how others such as university students or even USDA supervisors evaluate carcass merit in a research-trial setting. As a result, in evaluating the economic returns of various products such as βAA in a commercial setting, the true treatment effect must include these factors and not just the isolated biological response. In addition, although many studies claim there are no differences in various measurements such as quality grade or mortality, in many cases the studies do not have sufficient replication to detect statistical differences. Consequently, the analyses of many trials are susceptible to Type-II errors, in which the investigators claim there are no differences when in reality they could not detect the differences because of lack of power. The remaining studies evaluated in this literature review will focus on the biological response, such that as many outside variables and biases that affect the ability to assess the biological response are removed. Both types of research are needed, but from a production standpoint other variables in the commercial application of products must be kept in mind.

In evaluating the interactions of ractopamine and breed type, Gruber et al. (2007) fed 0 or 200 mg·steer^{-1}·d^{-1} of ractopamine to British, Continental crossbred, and Brahman crossbred calf-fed steers the final 28 d of the finishing period. No interactions between breed type and ractopamine were detected. The final live BW of the ractopamine cattle

was 7.3 kg greater than the controls, and the ractopamine steers had 15.3% greater ADG and 17.2% greater G:F the final 28 d. The HCW was 5.5 kg greater, and LM area was 2.3 cm^2 larger for the ractopamine-fed steers than for the controls. Marbling score was 2.1% lower for the ractopamine steers, and the percentage of carcasses grading Choice or greater was 3.8 percentage units less for the ractopamine treatment, but differences were not statistically different. No other differences were noted. As previously mentioned these investigators also examined the effects of ractopamine on WBSF and found that strip loins from the ractopamine-supplemented cattle were less tender than strip loins from controls.

Although much of the focus with beta-agonists has been on typical non-dairy, *Bos indicus* and *Bos taurus* breeds of cattle, a significant number of Holsteins are also fed for beef production in the U.S. In a series of 4 studies, Vogel et al. (2005 a,b) evaluated the effect of feeding 0, 200, or 300 mg·steer^{-1}·d^{-}1 of ractopamine for 28 to 38 d before slaughter in calf-fed Holstein steers. Other than slightly lower 12th-rib fat thickness (0.58 vs. 0.64 cm) and corresponding yield grade (2.63 vs. 2.71), no differences in growth or carcass characteristics were noted between the 200 and 300 mg/d treatments. Comparing the control to the 200 mg/d treatments, final live BW and HCW were increased by 8.0 and 4.7 kg, respectively (Vogel et al., 2005a). Similarly, ADG and feed efficiency were improved by 17.5 and 14.4%, respectively, during the period in which ractopamine was fed. The LM area increased by 1.8 cm^2, and marbling score was decreased by 17 units (3.3%). No other differences were noted among the 3 treatments. Thus, all-in-all, the response in calf-fed Holsteins was similar to native steers. In addition, because most

grids for Holsteins have a minimum threshold for LM area, ractopamine may have more value in Holstein cattle raised for beef compared with native breeds.

Heifers. In a compilation of 5 dose-titration studies, Schroeder et al., (2005d,e,f) evaluated the effects of 0, 10, 20, and 30 mg/kg of supplemental ractopamine fed to heifers the last 28 to 42 d of the finishing period. On average, the heifers consumed 0, 94, 189, and 283 mg·heifer^{-1}·d^{-1} for the 0, 10, 20, and 30 mg/kg treatments, respectively, and for comparison purposes will be summarized by dose instead of dietary concentration. Relative to the negative controls, the final live BW for the heifers that consumed 94, 189, and 283 mg/d increased by 3.0, 6.6, and 9.3 kg, respectively (Schroeder et al., 2005d). Additionally, compared with the control heifers, ADG during the period in which ractopamine was fed was increased 8.1, 9.7, and 21.0% for the 94, 189, and 283 mg·heifer^{-1}·d^{-1} treatments, respectively. Feed efficiency was also improved by 6.9, 14.0, and 17.1% for the 94, 189, and 283 mg·heifer^{-1}·d^{-1} treatments, respectively. In contrast to the findings of Laudert et al. (2007), no differences in DMI were noted among treatments (Schroeder et al., 2005d). With respect to carcass characteristics, HCW was increased by 2.9 and 5.1 kg for the heifers receiving 189 and 283 mg/d, respectively, compared with the controls; however, unlike steers, dressing percent was not affected by treatment (Schroeder et al., 2005d). The LM area increased by 3.2 cm^2 for the 283 mg·heifer^{-1}·d^{-1} diets (Schroeder et al., 2005e), but no treatment differences in 12th rib fat, yield grade, or marbling were detected. Relative to the composition of protein and fat in the carcass, (Schroeder et al., 2005f) demonstrated that the heifers fed 283 mg/d had 3.7% greater carcass protein concentration (15.33 vs. 14.78%) and 5.6% less carcass fat (30.4 vs. 32.2%) than controls. The carcass protein gain per day for the

189 and 283 mg/d cattle was 30.6 and 70.3% greater than the controls, respectively (58.1 and 75.8 vs. 44.5 g/d).

Compared with results in steers, heifers seemed less responsive to supplemental ractopamine; however, heifers consume less feed than steers, and the resulting doses were less than those evaluated for steers. Consequently, in 2 trials, Laudert et al. (2007) evaluated the effects of ractopamine fed at levels equal or greater than that in steers. Diets consisting of 0 or 200 mg·heifer^{-1}·d^{-1} or 30.3 mg/kg ractopamine were fed for the last 28 to 32 d of the finishing period (Laudert et al., 2007). The heifers on the 30.3 mg/kg treatment consumed 7.76 kg/d, so that their daily consumption of ractopamine averaged 235 mg. With the addition of 200 or 235 mg·heifer^{-1}·d^{-1}, the final live BW increased by 3.4 and 6.0 kg, respectively (Laudert et al., 2007). The cattle supplemented with ractopamine consumed 2.9% less feed than the control cattle. The feed efficiencies of the heifers fed 200 and 235 mg·heifer^{-1}·d^{-1} improved by 12.1 and 17.8%, respectively. Relative to the control treatment, the cattle fed 200 and 235 mg/d had 11.2 and 18.7% greater ADG, respectively; moreover, the 235 mg/d cattle had 5.0% greater ADG than the 200 mg/d heifers (Laudert et al., 2007). No other differences between the 200 and 235 mg·heifer^{-1}·d^{-1} treatments were noted. The HCW was increased by 4.6 and 6.1 kg for the 200 and 235 mg/d heifers, respectively, compared with the control heifers, and LM area were 1.4 and 1.9 cm^2 larger than the controls (Laudert et al., 2007). Dressing percent was increased by 0.46 and 0.36 percentage units for the 200 and 235 mg/d heifers, respectively, compared with the controls, but no other differences were noted among treatments. Although the response to ractopamine in heifers was still slightly less

than steers, the difference in response to ractopamine for steers and heifers decreased when heifers were compared across equal doses.

In 2 studies designed to evaluate the response associated with ractopamine (0 or 200 mg·heifer^{-1}·d^{-1}) for feeding durations of 14, 28, or 42 d, Homm et al. (2008) reported that BW gain was 5.9, 9.6, and 11.2 kg greater than the controls for 14, 28, and 42 d, respectively. The HCW did not differ among treatments at d 14; however, HCW was 5.5 add 8.3 kg heavier than controls after 28 and 42 d, respectively. Other than a larger LM area after 42 d, dressing percent, marbling score, and yield grade did not differ among treatments for any of the feeding durations (Homm et al., 2008).

In 1 of 2 experiments, Quinn et al. (2008) fed heifers 200 mg·heifer^{-1}·d^{-1} for the last 28 d of the feeding period observed a 9.6% improvement in G:F, but did not detect any other differences in performance or carcass merit. In a second experiment, these investigators evaluated dose and duration with heifers being fed 1 of 5 treatments: 1) negative control; 2) ractopamine fed for the last 28 d at 200 mg/d; 3) ractopamine fed for the last 28 d at 300 mg/d; 4) ractopamine fed for the last 42 d at 200 mg/d; and 5) ractopamine fed in an increasing step-up fashion every 2 wk of 100, 200, and 300 mg/d. Other than a decrease in DMI for the 300 mg/d treatment, no differences in growth or carcass merit were noted among the heifers supplemented with ractopamine; however, on a carcass basis, weight, feed efficiency, and gain all were increased in the ractopamine treatment groups relative to the controls. Interestingly, the only other difference noted was that the ractopamine-fed cattle had greater fat thickness over the 12th rib (Quinn et al., 2008). The response to ractopamine in this trial was drastically less than those noted in other heifer trials with ractopamine.

In 1 of 2 experiments, Sissom et al. (2007a) fed heifers 0 or 200 mg/d for the last 28 d of the finishing period and with 3 slaughter times of 129, 150, and 170 d. No interactions of ractopamine with days-on-feed were detected, and the only statistically different outcomes were a 2.4% increase in G:F (12.9% over the 28-d ractopamine-feeding period) and a 0.97 cm^2 increase in LM area. The response to ractopamine in this experiment seems to be the least of any trial published to date.

Dietary and Ruminal Effects of Feeding Ractopamine. In swine, the addition of ractopamine to the diets has been shown to increase the protein and lysine requirements (Dunshea et al., 1993; Apple et al., 2004). Most feedyards typically feed 1 diet after cattle are transitioned to a finishing diet, and diets are typically formulated to meet the protein requirements of the cattle during the period of most rapid growth. Because cattle growth decreases during the feeding period as cattle mature, it is likely that cattle are fed in excess of their protein requirements at the end of the finishing period. However, because βAA elicit growth protein deposition responses, the protein requirements are likely greater in cattle fed βAA than those that are not. In addition, in recent reports researchers have suggested that optimal cattle performance will be realized as long as cattle are fed adequate amounts of degradable intake protein (DIP; Gleghorn et al., 2004). Walker et al. (2006) evaluated the effects of ractopamine (0 or 200 $mg \cdot heifer^{-1} \cdot d^{-1}$) and 3 different protein sources on performance and carcass characteristics. The 3 protein sources, urea, solvent soybean meal, and expeller soybean meal, are known to provide different amounts of DIP, with urea providing the most and expeller soybean meal providing the least (NRC, 1996). The diets with urea, solvent soybean meal, and expeller soybean meal provided 688, 761, and 808 g of metabolizable protein/d (MP),

respectively; MP originates from the undegradable intake protein (UIP) supplied and from microbial synthesis, which is based on energy intake (NRC, 1996). Interactions between protein source and ractopamine were noted for final BW, ADG, and LM area; however, when the data were analyzed on a carcass weight basis, the interaction for ADG was not evident. In general, the performance of the control heifers, which were not fed ractopamine, was improved by increasing the supply of UIP and hence MP supply. In contrast, the growth response in ractopamine-fed heifers was greater in the diets that supplied more DIP and less overall MP. The authors concluded that the supplying adequate dietary DIP in the form of urea was sufficient to meet the MP requirements of heifers fed ractopamine in a typical finishing diet (Walker et al., 2006).

Walker and Drouillard (2008) evaluated the effects of ractopamine on ruminal protein metabolism in cannulated Holstein steers. In the 2 × 2 × 2 factorial arrangement, these investigators also examined the protein degradation associated with grain processing method (dry-rolled vs. steam-flaked) and the addition of dried distillers grains. The ruminal ammonia concentrations were less when ractopamine was fed with dry-rolled corn; however, no differences in ruminal ammonia were detected between ractopamine treatments when fed with steam-flaked corn. To add to the complexity, ruminal amino acid concentrations were less in the ractopamine diets when dried distiller's grains were not included, but were not affected when ractopamine was fed in combination with dried distiller's grains (Walker and Drouillard, 2008). Despite these interactions, the authors commented that the addition of ractopamine, steam-flaked corn, and dried distiller's grains decreased ammonia concentrations in the rumen, and concluded that ractopamine may influence ruminal degradation of dietary protein (Walker and Drouillard, 2008). If

true, this finding may explain why cattle seem to be more responsive to ractopamine when ruminally available N is added as was reported by (Walker et al., 2006). Despite potential changes in ruminal fermentation, Abney et al. (2007) did not detect any differences in urine pH or acid-base balance in individually fed steers fed 0 or 200 mg/d of ractopamine for 30 d.

Implants and Ractopamine. Although the canonical mechanisms by which steroids and βAA exert action seem to be independent, significant intracellular cross-talk may exist to provide overlap in the modes of action for the 2 categories of growth promotants. Sissom et al. (2007a) conducted a study examining at the interactions of implants and ractopamine in finishing heifers. In a 2 × 2 factorial arrangement of treatments, Sissom et al. (2007a) fed ractopamine at 0 or 200 mg·heifer^{-1}·d^{-1} for the final 28 d before slaughter. Heifers were implanted with either a single 20:200 mg E:TBA dose at arrival or a dose of 8:80 mg E:TBA at arrival followed by a 20:200 mg E:TBA at d 58 of the feeding period. No interactions between implant regimen and ractopamine were noted. Relative to main effects, ADG and feed efficiency were improved by 2.2 and 3.9%, respectively, for the ractopamine-fed cattle when analyzed over the entire 182-d finishing period; f or comparison purposes this would equate to 14.0 and 25.4% increases in ADG and G:F for the 28-d ractopamine feeding period. The HCW was increased by 5 kg in the ractopamine cattle, LM area was increased by 2.55 cm^2, and 12th rib fat thickness was decreased by 0.09 cm in the ractopamine heifers relative to controls (Sissom et al., 2007a). The percentage of cattle grading Choice or greater was numerically 6.2 percentage units less in the ractopamine-fed heifers. Although no statistical interactions were detected, the authors stated that a possible interaction between steroid hormone

implants and ractopamine existed (Sissom et al., 2007a). However, depending on whether implant aggressiveness is defined on the basis of total dose over the feeding period or on the basis of initial dose, it could be argued which implant regimen was more aggressive. Consequently, it is difficult to draw conclusions about the interaction of ractopamine and steroid implants from the results of this trial. In this same trial, the investigators examined the semimembranosus muscle for abundance of β-adrenoceptor subtype mRNA and found no difference in the expression of β_1- adrenoceptor mRNA, although they noted a tendency for an increase in expression of β_2- adrenoceptor mRNA (Sissom et al., 2007a). Winterhollar et al. (2007; summarized previously) observed no changes in β_1- and β_3-adrenoceptor mRNA abundance in response to ractopamine, but these researchers reported an increase in β_2-adrenoceptor mRNA abundance, which is similar to the findings of Sissom et al. (2007a). Spurlock et al. (1994) found that the receptor density in porcine adipose was decreased with exposure to ractopamine, whereas the number of receptors in skeletal muscle, thought to be β_2-adreneoceptors, was not changed. In contrast, Rothwell et al. (1987) reported a decrease in the density of β_2-adrenoceptor density in the skeletal muscle of rats administered clenbuterol. Gunawan et al. (2007) reported that the abundance of β_1-adrenoceptor mRNA was not changed, but the expression of β_2-adrenoceptor was decreased in pigs fed diets with 20 mg/kg of ractopamine. Based on the results of their experiments and summaries by others, Gunawan et al. (2007) hypothesized that ractopamine likely functions through β_2-adrenoceptors, and that the loss of response noted with βAA over time may be partially attributable to a decrease in receptor abundance. Finally, Sissom et al. (2007a) observed an interaction between implant and ractopamine treatments with a decrease in IGF-

mRNA associated with the ractopamine heifers that were reimplanted; however, the non-implanted heifers fed ractopamine tended to have greater IGF-1 mRNA than those not fed ractopamine, but no explanation was offered for this difference in response by implant regimen.

Walker et al. (2007) conducted a split-plot design in which 6 Holstein steers were implanted with either a 24:120 mg E:TBA dose or nothing, and steers were fed no ractopamine during the first 28 d of the trial and 200 mg/d for d 29 to 56. The authors reported that circulating IGF-1 and longissimus IGF-1 mRNA was increased in the implanted steers and decreased in the ractopamine-fed steers compared with controls. In addition, the abundance of mRNA for the β_1- and β_2-adrenoceptors in longissimus was decreased in the ractopamine-treated steers; however, because there was not a true negative control for the ractopamine treatment and because the observations are confounded with time, caution should be used when interpreting these results.

Although the effects of ractopamine and other βAA on the density and abundance of β_2-adrenoceptors seem inconsistent, a majority of data supports that their abundance decreased on exposure to ractopamine. More research is needed in this area.

Current Research with Zilpaterol in the Cattle Industry. Because ractopamine and zilpaterol were approved in 2003 and 2006, respectively, the majority of βAA research in U.S. beef cattle to date has focused on ractopamine; however, a few studies have been published with zilpaterol. Because zilpaterol has been approved for use in feedlot cattle in Mexico for a few years, Plascencia et al. (1999) evaluated its effects before U.S. approval. Zilpaterol was fed at 0 or 6 mg/kg (60 mg/d; 90% DM basis) to crossbred steers. The current approval in the U.S. is for feeding at 7.5 mg/kg (90% DM basis), so

the concentration fed in this trial was slightly less than is fed in U.S. feedlots. In addition, the cattle were fed zilpaterol for the final 42 d of the finishing period, whereas the approval in the U.S. is the final 20 to 40 d. Plascencia et al. (1999) observed a 20-kg increase in final live BW and a 13-kg increase in HCW in the zilpaterol cattle compared with controls. The ADG and feed efficiency improved by 36.6 and 28.1%, respectively, in the zilpaterol-fed cattle, which if standardized over a 140-d feeding period would equate to 11.0 and 8.4% improvements in ADG and feed efficiency, respectively. Dressing percent was increased by 2.2 percentage units, and LM area was 2.2 cm^2 larger in the carcasses of cattle fed zilpaterol. Marbling score was decreased by 1.7% in the zilpaterol cattle. Although the duration is slightly longer, zilpaterol seems to elicit much larger responses in growth at a much lower dose than ractopamine.

For approval of zilpaterol, 3 trials were conducted and submitted to FDA (2006), examining its effects on growth and carcass measurements in steers and heifers when fed at 7.5 mg/kg (90% DM basis) for a 20- or 40-d feeding period, followed by a 5-d withdrawal period. The data (FDA, 2006) were not separated by both sex and days on the product. Because there have been slightly smaller responses to βAA in heifers, the focus of this review will first be placed on gender differences. Steers fed zilpaterol (75 mg/d) over an average of 30 d had 13-kg heavier final BW and 16-kg heavier HCW. Accordingly, dressing percent was 1.6 percentage units greater in zilpaterol-fed steers (FDA, 2006). Over the final 30-d period, zilpaterol steers had 35.9 and 29.4% improvements in ADG and feed efficiency, respectively. If calculated to a standardized 168-d feeding period, this would equate to 6.4 and 5.2% improvements in ADG and feed efficiency, respectively. The LM area was 8.2 cm^2 larger in the zilpaterol carcasses;

yield grade was 0.34 units less (11.6%) for the carcasses of cattle fed zilpaterol, and carcass protein was 4.3% greater in the zilpaterol carcasses (FDA, 2006). Although it was not reported or analyzed, based on the ADG and feed efficiency data, DMI decreased by 4.0% in the zilpaterol-fed steers.

In heifers fed zilpaterol at 7.5 mg/kg (69 mg/d; 90% DM basis) for an average of 30 d, final BW and HCW were 12 and 7 kg heavier, respectively. Dressing percent and LM area increased by 1.4 percentage units and 6.4 cm^2, respectively (FDA, 2006). The ADG and feed efficiency of zilpaterol-fed heifers improved 18.9 and 20.5%, respectively, over the final 30-d feeding period, or 3.4 and 3.7% if standardized over an entire 168-d feeding period. Marbling score in heifers only decreased by 18 points (3.9%). The decrease in numerical yield grade was 9.3%, and the carcass protein increased by 6.8% when zilpaterol was fed. Although it was not reported or analyzed, based on the ADG and feed efficiency data, DMI decreased by 5.6% in the zilpaterol-fed heifers.

Since approval and because the manufacturer has recommended feeding zilpaterol for the last 20 d of the finishing period, Intervet/Schering-Plough (2007) has also conducted and summarized 4 post-registration studies examining the effects of feeding zilpaterol (7.5 mg/kg; 90% DM basis; 77 $mg \cdot steer^{-1} \cdot d^{-1}$) to steers for 20 d followed by a 3-d withdrawal period. Unlike the FDA trials (2006), in these studies, cattle were weighed at arrival and at slaughter. The ADG and feed efficiencies improved by 3.5 and 2.7%, respectively, over the entire feeding period, which equates to 29.2 and 22.8% improvements over the entire 168-d (average) feeding period. Final live and carcass weights were increased by 9.1 and 14.1 kg, respectively, and dressing percent increased by 1.4 percentage units. The percentage of steers grading Choice or greater decreased by

7.4 percentage units by zilpaterol, and the percentage of Yield Grade 1 and 2 carcasses increased 6.4 percentage units, whereas the percentage of Yield Grade 4 and 5 carcasses decreased 7.9 percentage units (Intervet/Schering-Plough, 2007).

Vasconcelos et al. (2008) evaluated the growth and carcass responses of steers to feeding zilpaterol (7.5 mg/kg; 90% DM basis; 72 mg/d) for the final 0, 20, 30, or 40 d, following a 3-d withdrawal, of the finishing period for cattle slaughtered after 136, 157, 177, or 198 d (4 × 4 factorial arrangement). With the exception of KPH, no duration of zilpaterol feeding × slaughter group interactions were detected. In terms of growth performance over the average of the 30-d zilpaterol feeding period, cattle fed zilpaterol had 8.2- and 17.2-kg heavier final BW and HCW, respectively. Over the entire feeding period, ADG and G:F of steers fed zilpaterol were improved by 3.7 and 4.6%, respectively, with improvements during the last 43 d of 12.5 and 15.3% in ADG and G:F, respectively. In addition, DMI decreased linearly with increasing zilpaterol-feeding duration (8.7 vs. 8.9 kg/d for controls and zilpaterol, respectively, for the final 43-d period). As a result, G:F increased linearly with duration of feeding. Dressing percent increased 2.0 percentage units and increased linearly with increasing feeding duration of zilpaterol. Longissimus muscle area increased 10.6% (9.6 cm^2) for the zilpaterol-fed cattle, and fat thickness over the 12^{th} rib decreased linearly with increasing feeding duration of zilpaterol and averaged 1.5 vs. 1.3 cm for controls and zilpaterol, respectively. Accordingly, calculated yield grade was 17.5% less for the zilpaterol-fed cattle vs. controls (3.1 vs. 2.6). Marbling score decreased linearly with increasing duration of zilpaterol-feeding and was 9.7% lower for the zilpaterol cattle (401 vs. 384 units). Consequently, the percentage of carcasses grading Choice or greater decreased by

18.8 percentage units (59.5 vs. 40.7%) in the zilpaterol-treated cattle compared with the controls (Vasconcelos et al., 2008).

Zilpaterol and Ractopamine Comparisons. Few studies have directly compared the effects of ractopamine and zilpaterol on growth and carcass responses. Because of variation in duration of feeding, comparison across trials is difficult. Avendaño-Reyes et al. (2006) fed steers ractopamine (300 mg·steer^{-1}·d^{-1}) or zilpaterol (60 mg·steer^{-1}·d^{-1}) for the last 33 d of the feeding period and reported 10.6 and 19.5 kg greater finishing live BW, respectively, than for negative control cattle. Similarly, HCW were 13.6 and 21.9 kg greater for the ractopamine- and zilpaterol-fed cattle. Over the 33-d feeding period, ADG was 31.7 and 35.4% greater, and G:F was 34.1 and 36.8% greater for the ractopamine and zilpaterol steers, respectively. Calculated across the entire 138-d feeding period, the ADG was increased by 7.6 and 8.5%, and G:F was increased by 8.1 and 8.8%, respectively. Dressing percent was increased by 1.5 and 2.0 percentage units for the ractopamine and zilpaterol cattle, respectively. Longissimus muscle area tended to be increased by 5.42 cm^2 (8.12%) and 8.5 cm^2 (12.7%) in the carcasses of the cattle fed ractopamine and zilpaterol, respectively. Fat thickness over the 12th rib was decreased 17.6% in the carcasses of cattle fed zilpaterol but only a 5.5% non-significant difference was noted in the carcasses of steers fed ractopamine; the investigators did not contrast the zilpaterol and ractopamine treatments statistically.

In another direct comparison of ractopamine and zilpaterol and a comparison more representative of doses fed in the U.S., Platter et al. (2008) fed 200 mg·steer^{-1}·d^{-1} of ractopamine or 7.5 mg/kg (as-fed basis; ~75 mg·steer^{-1}·d^{-1}) for the final 33-d of the finishing period (i.e., ractopamine fed for 33 d; zilpaterol fed for 30 d plus a 3-d

withdrawal). Final BW was increased by 8 and 3 kg for the ractopamine and zilpaterol cattle, respectively, and HCW in the ractopamine and zilpaterol-fed cattle were 5 and 13 kg heavier, respectively. Accordingly, dressing percent was increased 1.93 percentage units in the zilpaterol cattle, with no change observed in the ractopamine-fed cattle. Improvements of 24.2 and 10.5% were observed in ADG for the ractopamine and zilpaterol cattle, respectively, and feed efficiency was improved by 18.8 and 16.7% by the ractopamine and zilpaterol treatments, respectively. When standardized to a 168-d cumulative feeding period, ADG was increased by 5.8 and 2.5%, and feed efficiency was improved by 4.5 and 4.0%, respectively, for steers fed ractopamine and zilpaterol, respectively. The DMI was decreased by 8.6% for the zilpaterol cattle during the last 33 d of the trial. Although not statistically different, marbling scores for the carcasses of cattle fed ractopamine and zilpaterol were 12 and 21% less, respectively, than for the controls.

Zilpaterol and Gene Expression in Tissue. The exact mode of action of βAA is still ambiguous, and studies with ractopamine (Sissom et al., 2007a; Walker et al., 2007) have revealed inconsistent responses in β-adrenoceptor and IGF-1 mRNA abundance in the skeletal muscle. In cultured bovine satellite cells, Sissom et al. (2007b) evaluated the effects of the addition of several different molar concentrations (0, 100 p*M*, 1 n*M*, 10 n*M*,, 100 n*M*, 1 μ*M*, and 10 μ*M*) of zilpaterol and reported that zilpaterol did not have any effect on [^{3}H]-incorporation into proliferating myoblasts. Overall, the abundance of β_1-, β_2-, and β_3-adrenoceptor mRNA decreased as the concentration of zilpaterol increased in myoblasts, and the expression of IGF-1 mRNA trended upwards with increasing concentration of zilpaterol. The authors also observed that myosin heavy

chain mRNA increased for the 1 n*M* zilpaterol treatment and decreased for the 10 n*M* and 1 μ*M* treatments. Interestingly, Western blots revealed that the protein content of β_2-adrenoceptor increased with increasing concentrations of zilpaterol in myoblasts but decreased with increasing concentrations of zilpaterol in myotubes. Because of numerous potential differences that may not mimic what is truly occurring in the body, it is difficult to extrapolate in vitro research to in vivo settings.

In a study examining the interactions between 24:120 mg E:TBA and feeding of zilpaterol (8.3 mg/kg DM basis) for the last 30 d, followed by a 3-d withdrawal, Baxa et al. (2008) observed no effects of zilpaterol feeding on β_1- or β_2-mRNA abundance in the semimembranosus muscle. The expression of IGF-1 in muscle was not affected by the feeding of zilpaterol. The authors also concluded that the expression of myosin heavy chain isoforms (1, 2a, and 2x) tended to decrease in the skeletal muscle of steers fed zilpaterol. It is not known whether these changes in mRNA correlate to decreased protein translation.

In the study by Vasconcelos et al. (2008), which was previously described, Rathmann et al. (2008) reported that the feeding of zilpaterol did not alter the mRNA abundance of calpastatin, IGF-1, or β_1- or β_2-adrenoceptors in the semimembranosus muscle of steers. The investigators also commented that the expression of myosin heavy chain isoform 2a was decreased with the feeding of zilpaterol in 2 of the 4 slaughter groups. Luqué et al. (2008) did not detect any differences in the mRNA expression of myosin heavy chain 1, 2a, or 2x between Holstein steers fed 200 mg/d of ractopamine or 75 mg/d of zilpaterol. Expression of calpastatin and β_1- and β_2-adrenoceptors did not differ among zilpaterol, ractopamine, or control treatments; however, numerically, the abundance of mRNA for

the β_2-adrenoceptors decreased with increasing potency of βAA (i.e., zilpaterol > ractopamine > control). Additionally, in a separate experiment evaluating the effects of feeding 75 mg·steer^{-1}·d^{-1} of zilpaterol in calf-fed Holsteins for 0, 20, 25 or 30d, Luqué et al. (2008) observed a numerical increase in calpastatin expression with zilpaterol. Further research using multiple assay procedures and methodologies needs to be conducted to elucidate the apparent discrepancies in the cellular and tissue responses to βAA.

Summary of Growth and Carcass Responses to Ractopamine and Zilpaterol. A summary of the responses relative to a control are presented in Table 2.4. In steers (non-Holstein), research has shown that during the feeding period in which ractopamine or zilpaterol is fed (i.e., the final 28 to 42 d or final 20 to 40 d, respectively), ractopamine and zilpaterol improve ADG by 11 to 27% and 11 to 37%, respectively. Only 1 study (FDA, 2006) could be found evaluating the effects of zilpaterol in heifers; consequently, their response to zilpaterol will not be discussed. In heifers fed ractopamine, the increases in ADG have been slightly less at 5 to 18% than has been typical for steers. Feed efficiency has been improved by 12 to 23% and 13 to 29% for ractopamine and zilpaterol, respectively in steers. Similar to ADG in heifers, the response in feed efficiency in ractopamine-fed heifers is slightly less than in steers at 9 to 24%. There is a slight trend for a decrease in DMI associated with both ractopamine and zilpaterol, but most trials have not detected significant differences. Dressing percent is increased 1 to 2% in ractopamine-fed cattle and 1 to 5% in zilpaterol-fed steers. Similarly, HCW is increased 1 to 2% in ractopamine-fed steers and 4 to 8% in zilpaterol-fed steers. Although the live BW gains are similar between cattle fed zilpaterol and ractopamine, the

carcass gains are significantly different, and it seems that the transfer of live weight to carcass weight approaches 100% or more. No definitive theory has been proposed as to what is occurring physiologically to support this observation.

As expected, because of the increased protein gain, marbling scores are decreased with feeding βAA. Although most trials do not detect significant differences, the trend is clearly downward, and most of these trials likely do not have sufficient power to detect differences. As mentioned previously, Bryant (unpublished data) observed ($P < 0.01$) a 3.24 percentage unit decrease in the number of carcasses grading Prime and Choice in cattle that were fed ractopamine. This trial was well-replicated with 164 blocks. Moreover, as previously alluded to, in a commercial setting, 100% of the biological responses will not be attained because of barriers and costs in feedyard systems, such as diet formulation for the average of the yard to optimize milling and feeding logistics.

Summary of Research Needs with Vitamin A, Steroids, and Beta-Adrenergic Agonists in Cattle. Clearly, vitamin A, steroid implants, and βAA have the potential to affect protein or fat deposition or both and growth in beef cattle. The objective of the current research is to further elucidate their effects in vivo and to further define their metabolic actions.

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Figure 2.1. Structures of common retinoids and carotenoids.

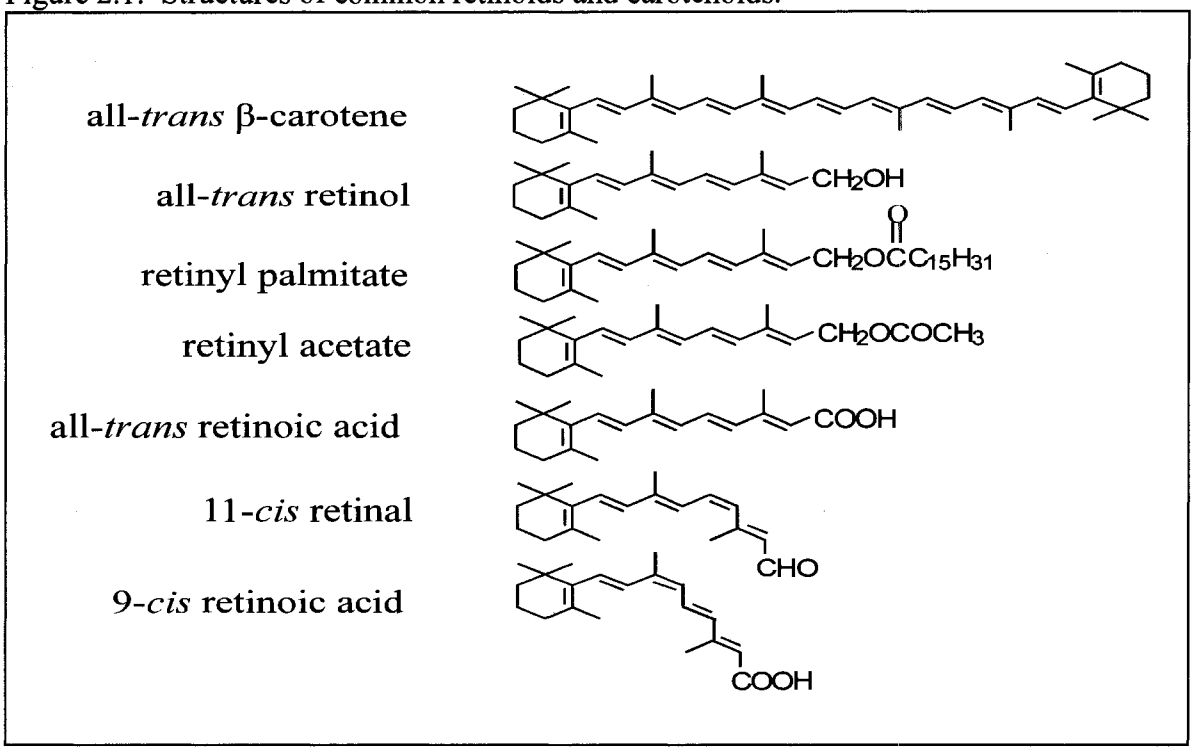

Figure 2.2. Major pathways for retinoid transport in the body (Blomhoff and Blomhoff, 2006).

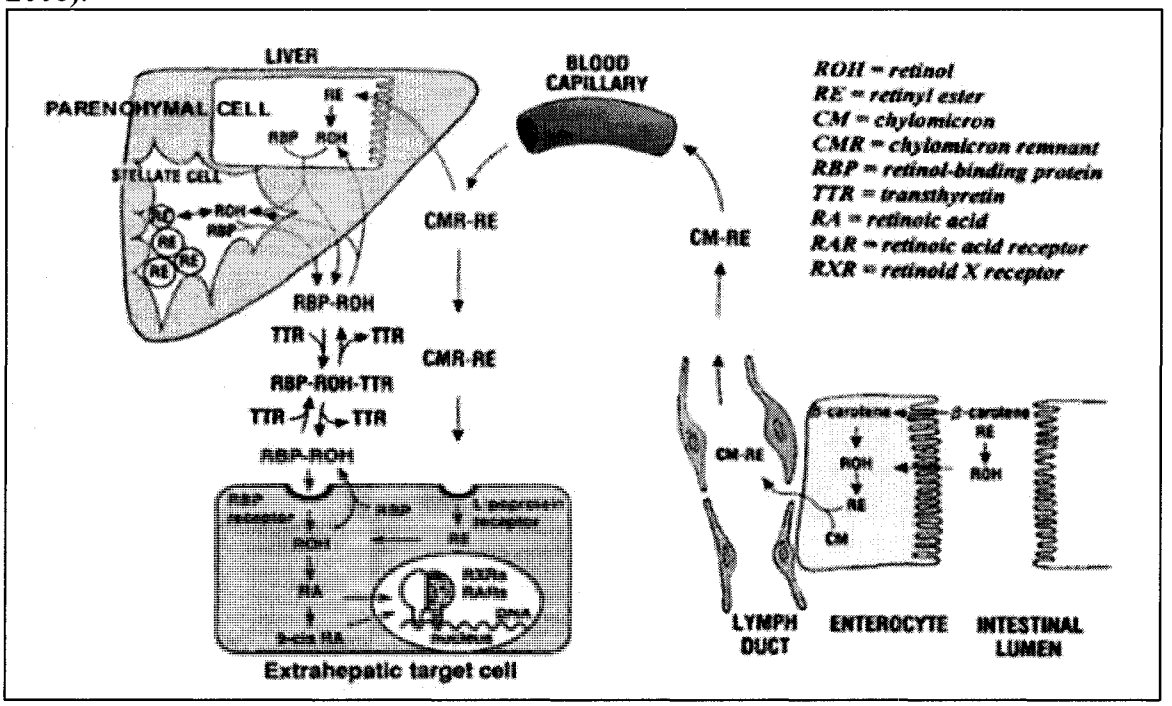

Figure 2.3. Cellular actions of vitamin A in the cell (Villaroya et al., 1999).

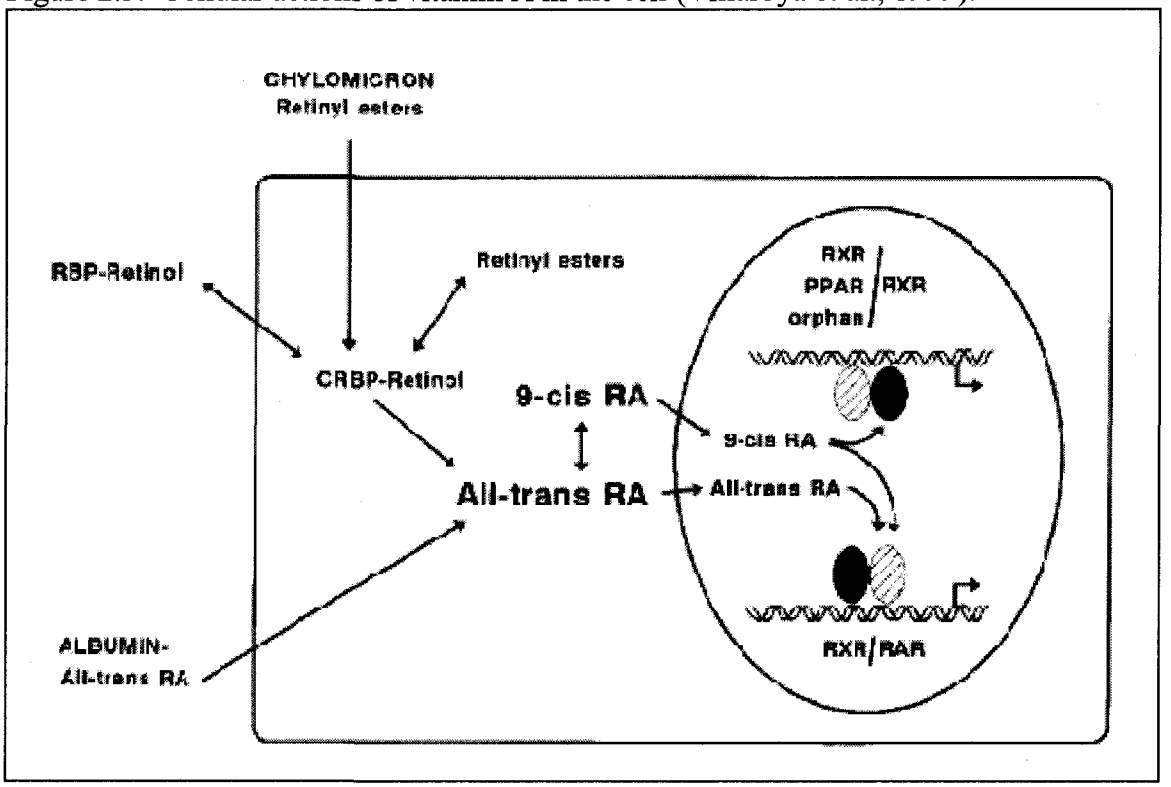

Figure 2.4. The metabolism of vitamin A into retinoic receptors (Ziouzenkova, 2007).

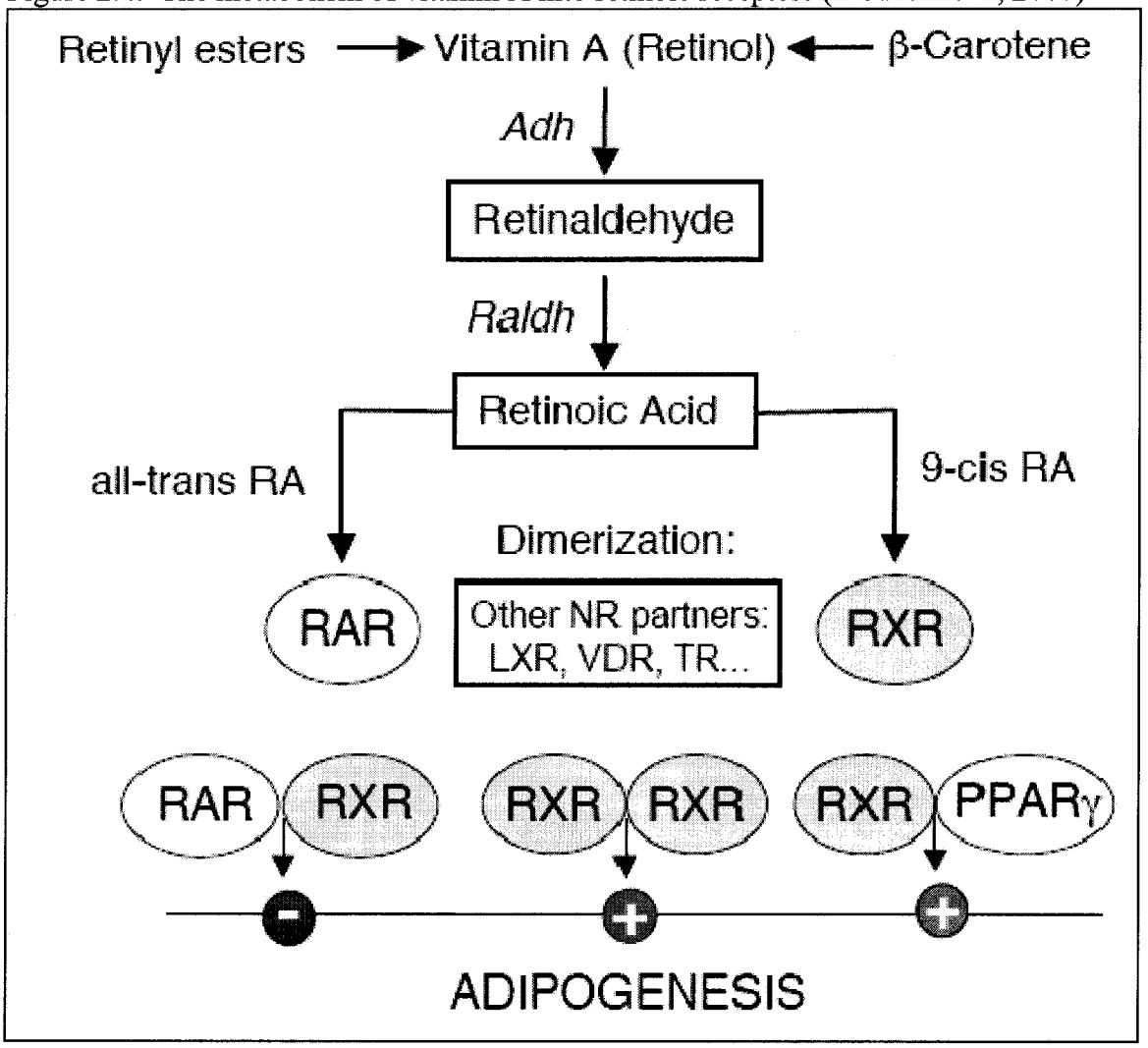

Figure 2.5. An overview of adipogenesis (Avram et al., 2007).

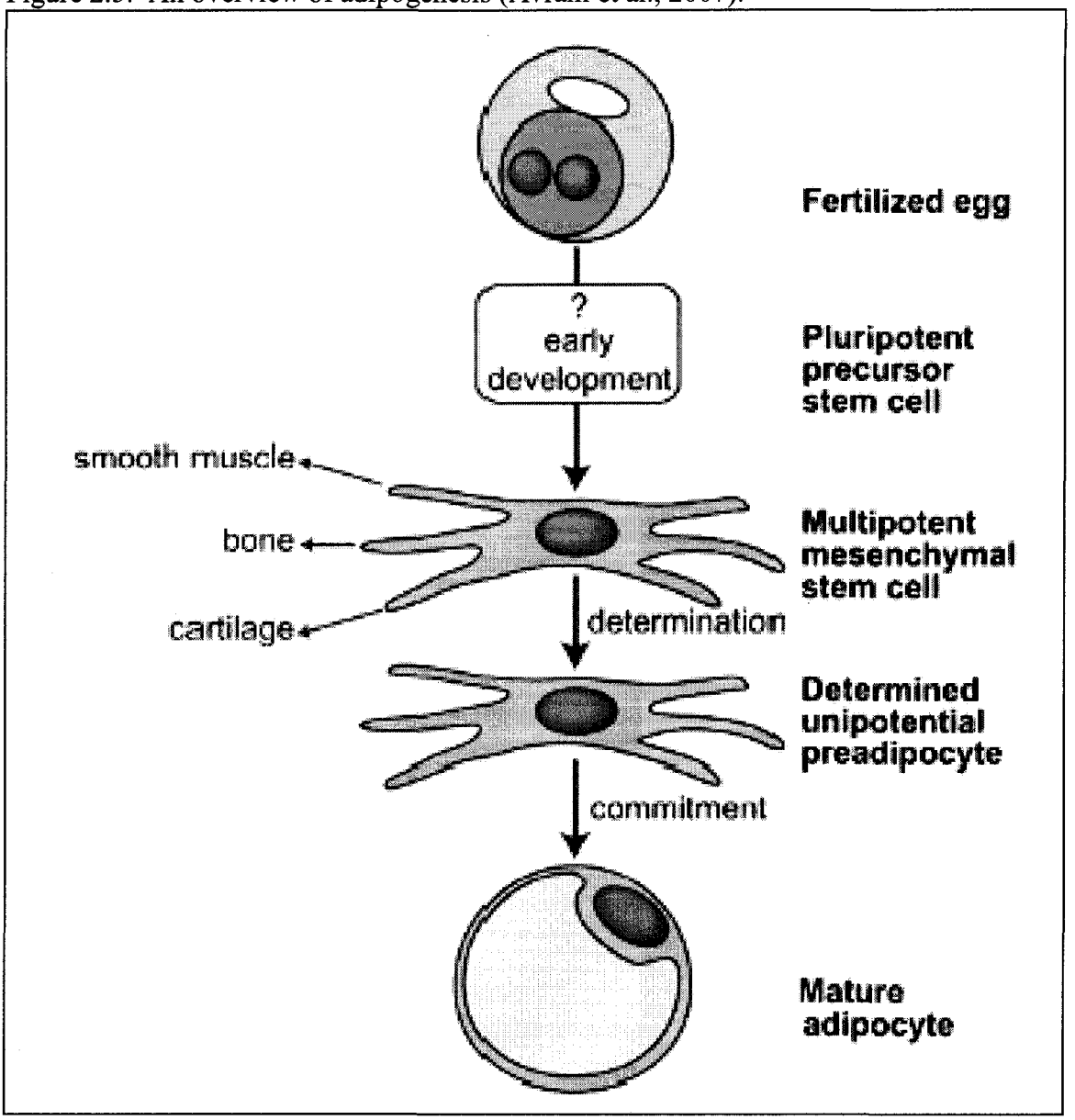

Figure 2.6. Proliferation and differentiation in adipogenesis (Avram et al., 2007).

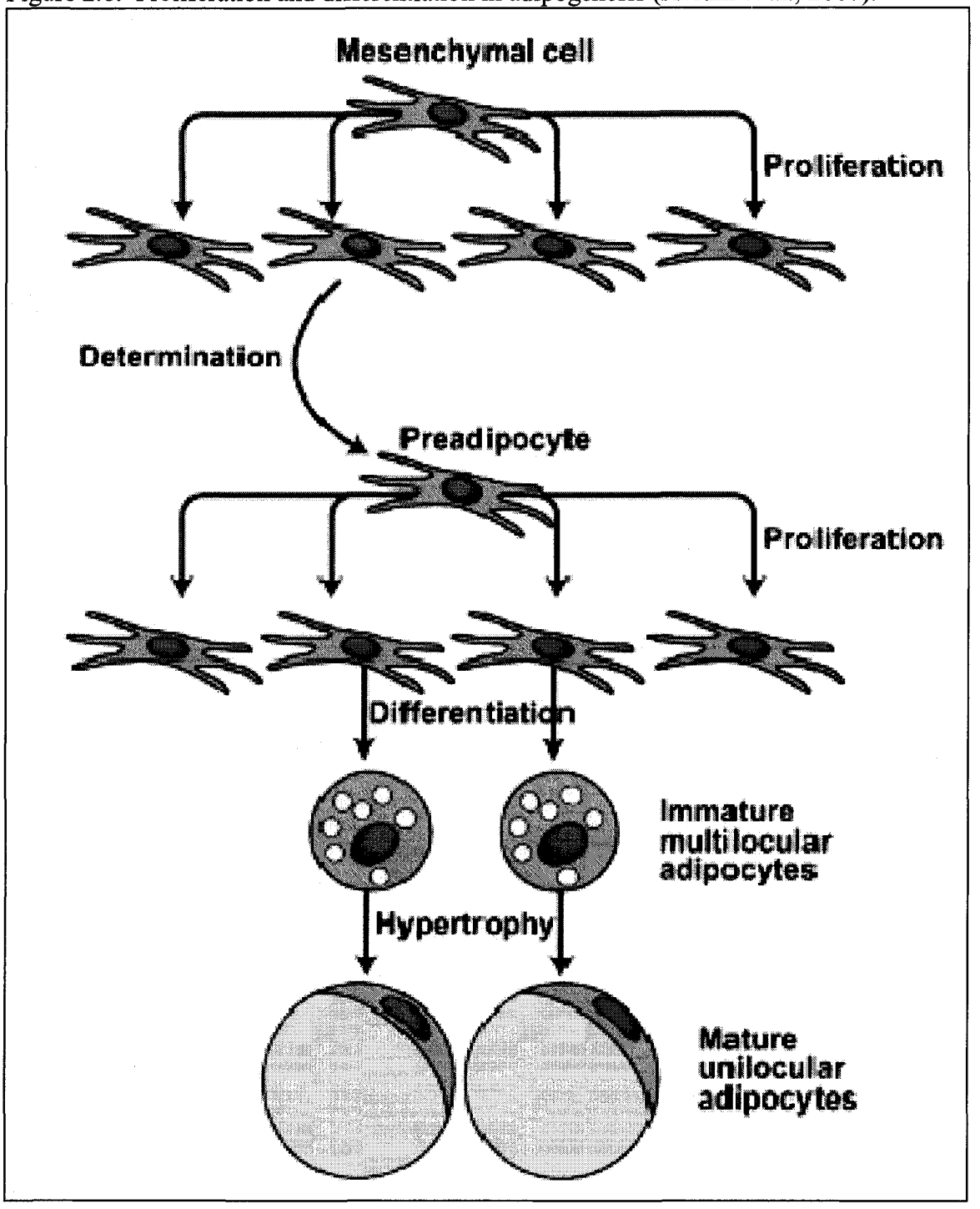

Figure 2.7. Actions of vitamin A in the cell (Desvergne, 2007a).

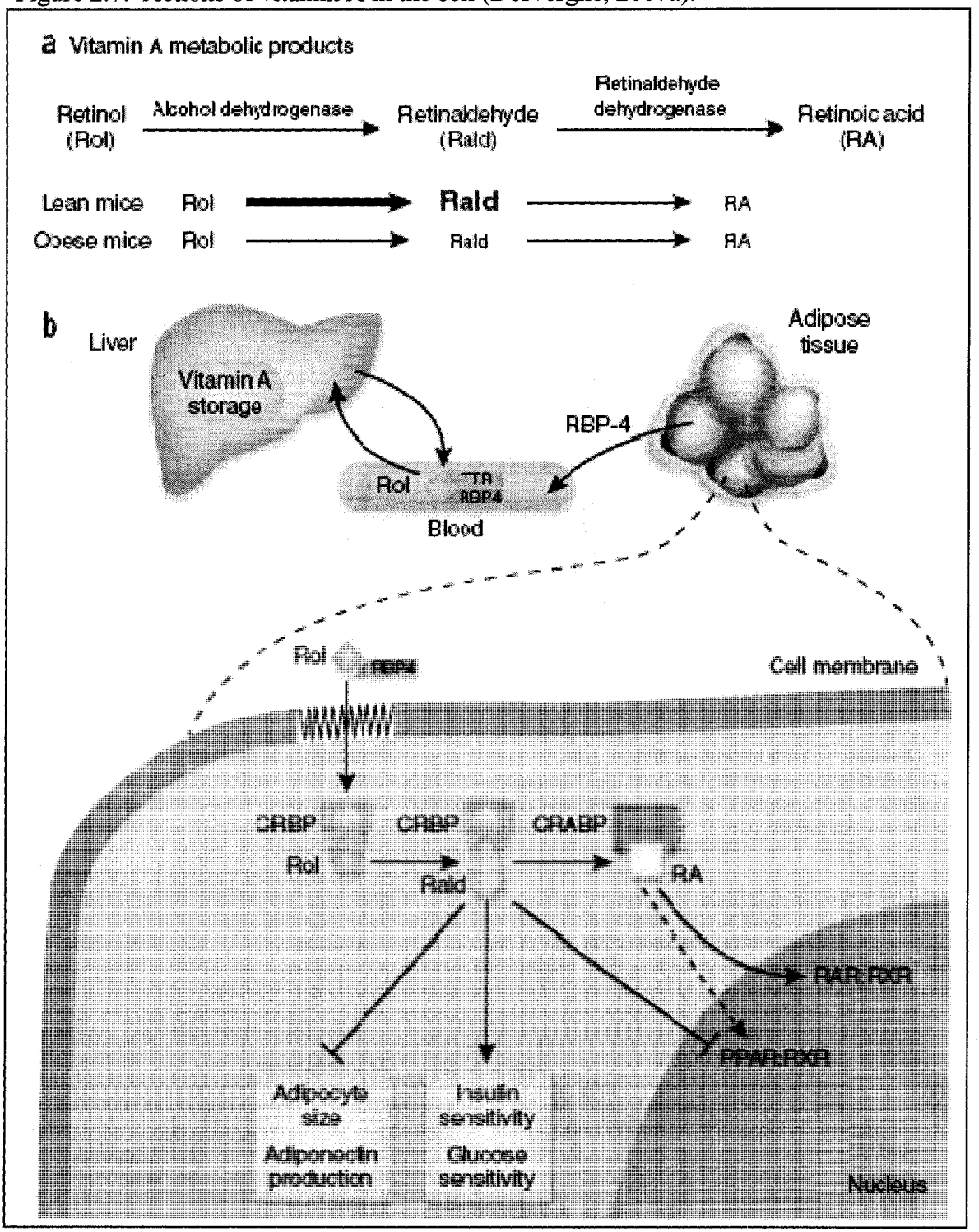

Figure 2.8. Proposed genomic mechanisms of vitamin A on adipocyte differentiation (Kawada et al., 2000).

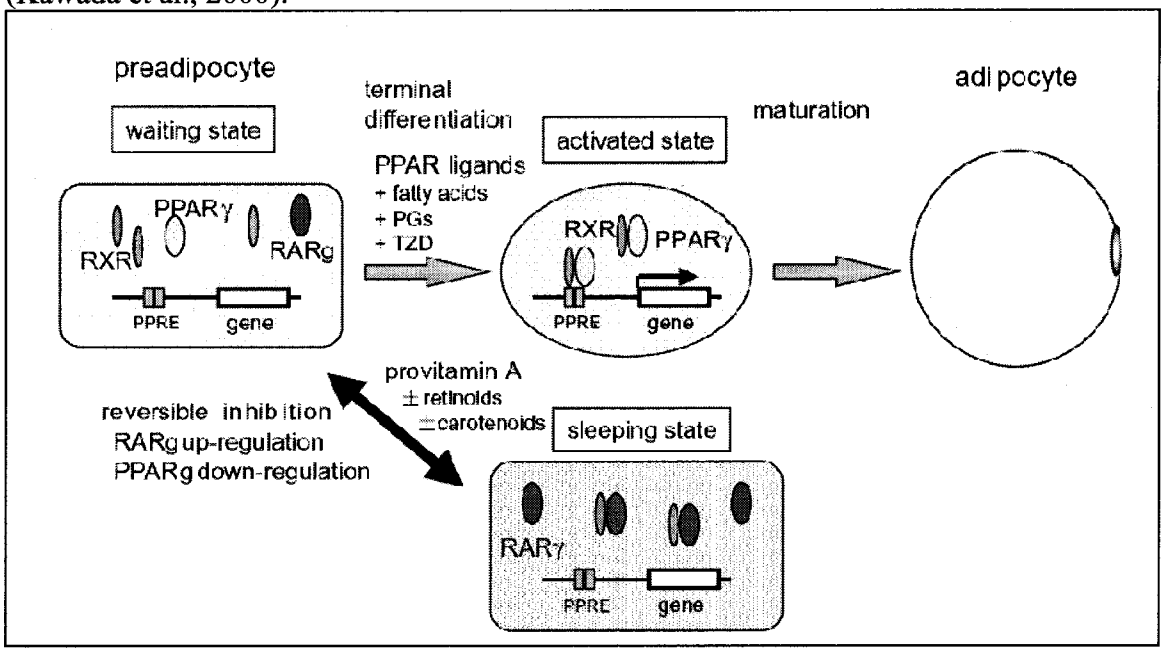

Figure 2.9. Alternative genomic mechanisms of vitamin A on adipocyte differentiation (Villaroya et al., 1999).

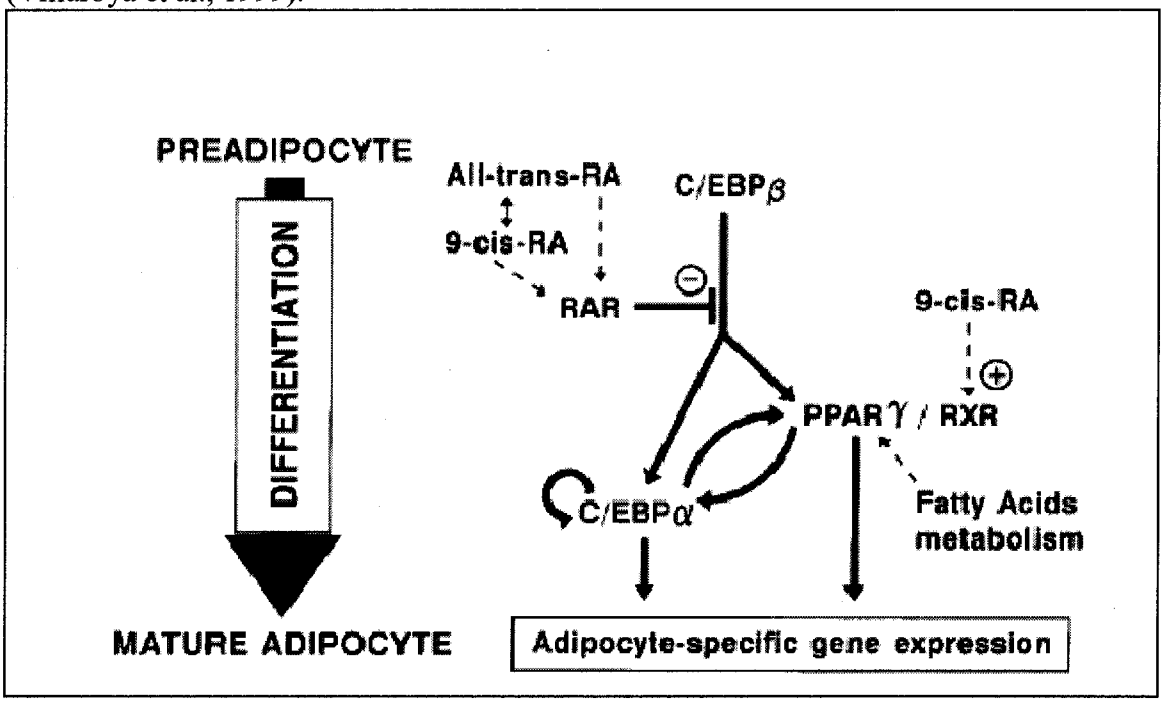

Figure 2.10. Steroid biosynthesis (R. A. Bowen, 2006).

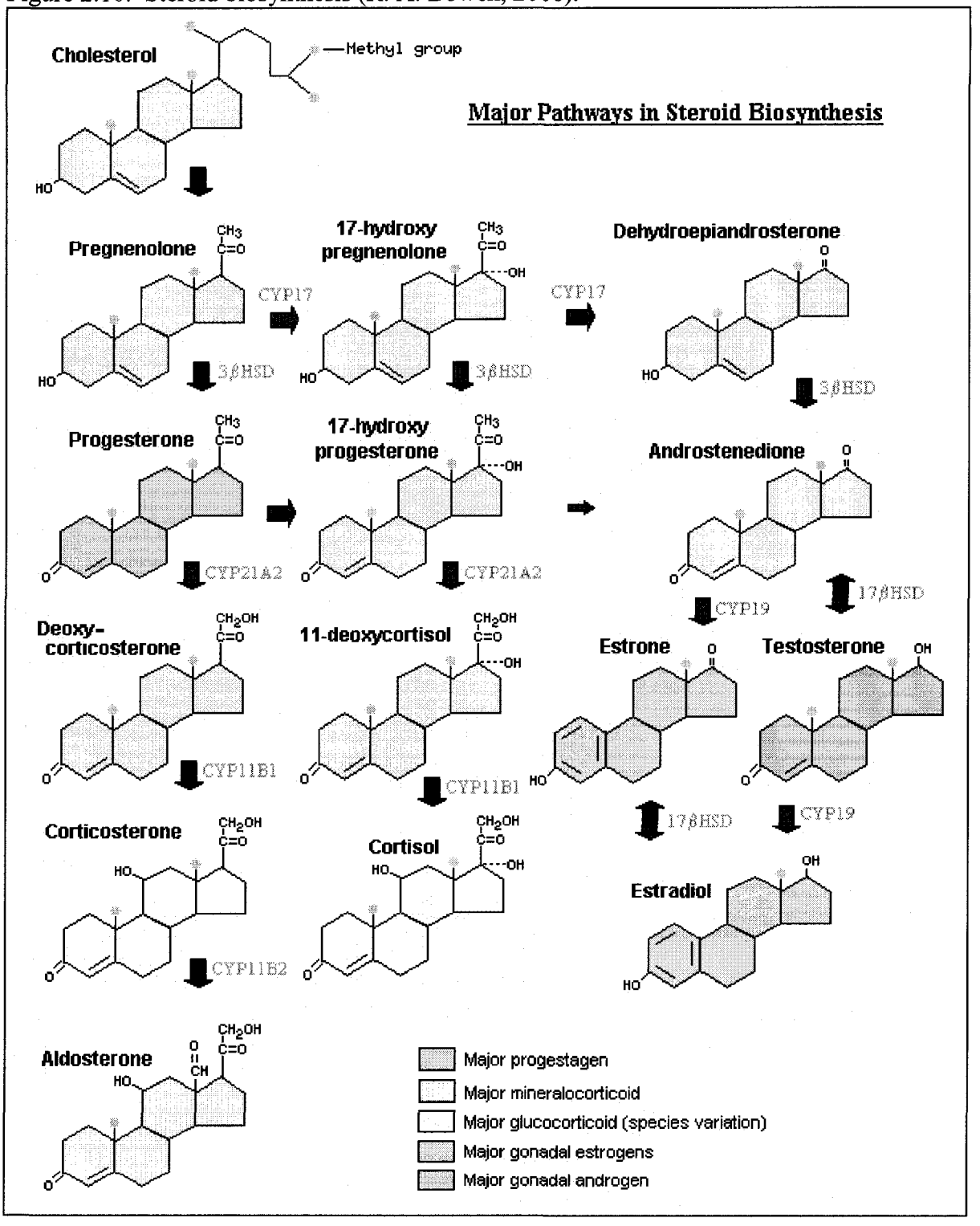

Figure 2.11. Action of steroid hormones (Barrett, 2003).

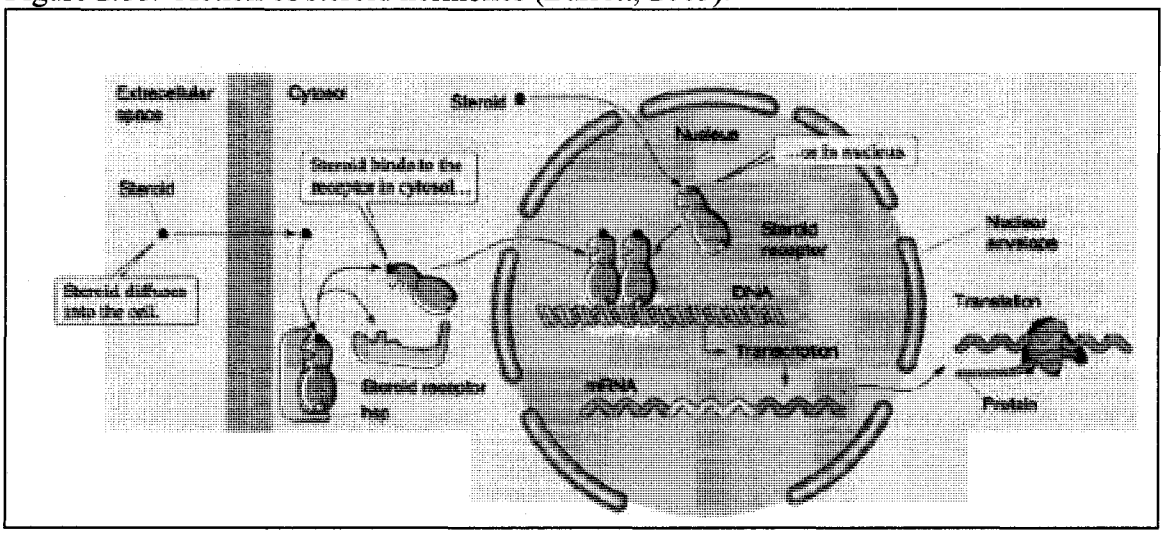

Figure 2.12. Chemical structures of common steroids used in beef cattle production (NRC, 1994).

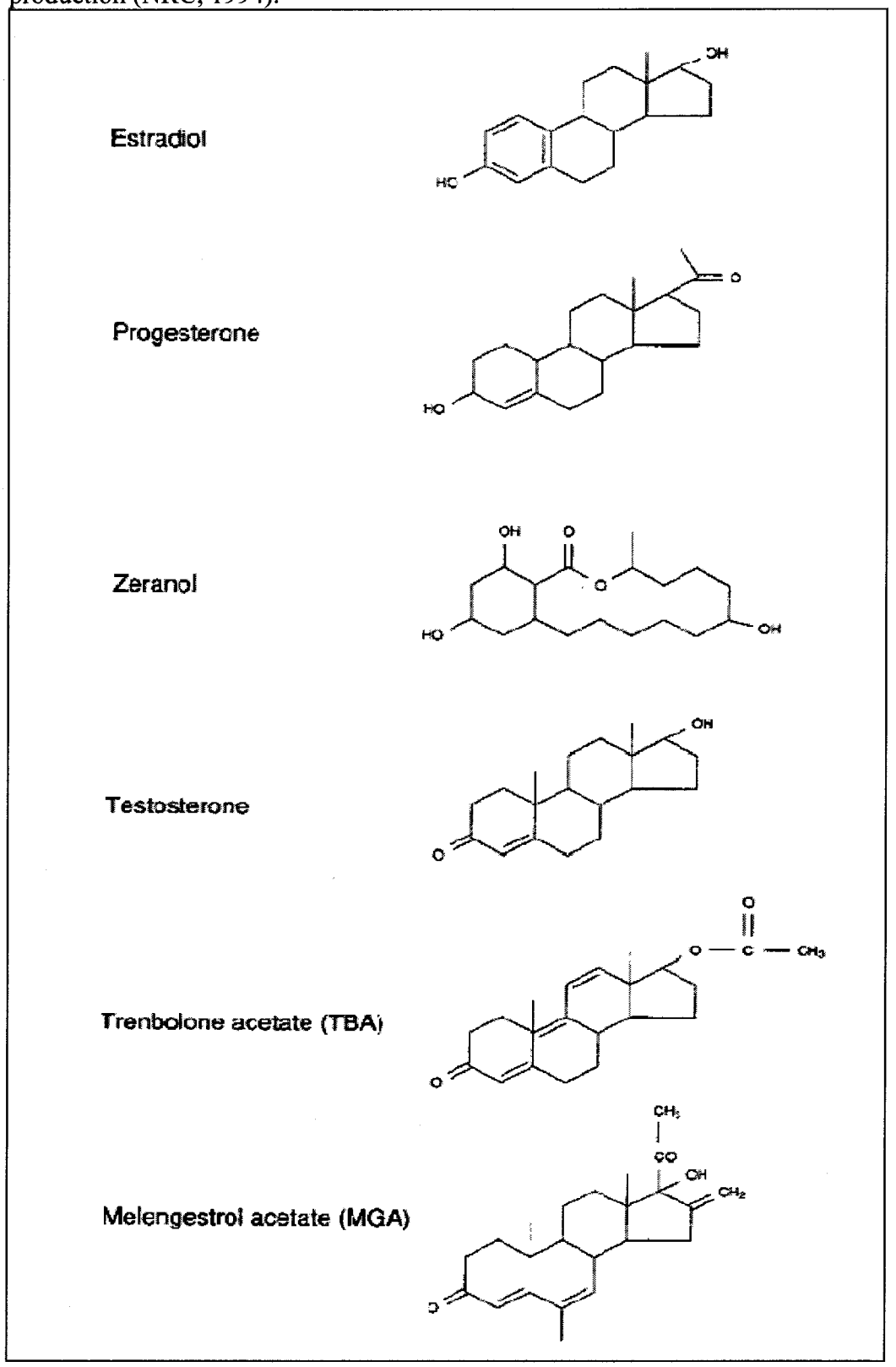

Table 2.1. Chronology of approval of steroidal and beta-adrenergic agonist agents for use in cattle in the United States (Raun and Preston, 1997; Preston, 1999).

Year	Event
1954	Oral diethylstilbesterol (DES) approved for cattle
1956	Estradiol benzoate/progesterone implants approved for steers
1956	DES implants approved for cattle
1958	Estradiol benzoate/testosterone propionate implants approved for heifers
1968	Oral melengestrol acetate approved for heifers
1969	Zeranol implants (36 mg) approved for cattle
1970	Oral DES dose range increase approved in cattle
1979	All use of DES banned in cattle
1982	Silastic (silicone/rubber) estradiol implant approved for cattle
1984	Estradiol benzoate/progesterone implants approved for calves
1987	Trenbolone actetate (TBA) implants approved for cattle
1991	Estradiol/TBA (1:5 ratio) implants approved for steers
1993	Bovine somatotropin approved for lactating dairy cows
1994	Estradiol/TBA (1:10 ratio) implants approved for heifers
1995	Zeranol implant-dose increase (72 mg) approved for cattle
1996	Estradiol/TBA (1:5 ratio) implants approved for stocker cattle
1996	Estradiol/TBA (1:10 ratio) implants approved for steers
2003	Ractopamine approved for use in finishing beef cattle
2006	Zilpaterol approved for use in finishing beef cattle

Table 2.2. List of steroidal implants that are FDA-approved and used for beef cattle.

Compounds	Dose, mg	Brand Names	Manufacturer[b]	Carrier[c]	Approval Group
Single-Ingredient Implants					
Zeranol	36	Ralgro	ISP	L	All Steers, Feedlot Heifers
	72	Ralgro Magnum	ISP	L	Feedlot Steers
Estradiol	25.7	Compudose	VL	SR	All Steers, Heifers
	43.9	Encore	VL	SR	All Steers, Heifers
Trenbolone Acetate	200	Finaplix-H Component T-H	ISP VL	L	Feedlot Heifers
Combination-Ingredient Implants					
Estradiol benzoate[a] Progesterone	10 100	Synovex-C Component E-C	FD VL	PEG	Suckling calves
Estradiol benzoate[a] Progesterone	20 200	Synovex-S Component E-S	FD VL	PEG	Stocker, Feedlot Steers
Estradiol benzoate[a] Testosterone propionate	20 200	Synovex-H Component E-H	FD VL	PEG	Stocker, Feedlot Heifers
Estradiol Trenbolone Acetate	8 40	Revalor-G Component TE-G	ISP VL	C	Stocker Steers, Heifers
Estradiol Trenbolone Acetate	16 80	Revalor-IS Component TE-IS	ISP VL	C	Feedlot Steers
Estradiol Trenbolone Acetate	8 80	Revalor-IH Component TE-IH	ISP VL	C	Feedlot Heifers
Estradiol Benzoate[a] Trenbolone Acetate	14 100	Synovex Choice	FD	PEG	Feedlot Steers
Estradiol Trenbolone Acetate	24 120	Revalor-S Component TE-S	ISP VL	C	Feedlot Steers
Estradiol Trenbolone Acetate	14 140	Revalor-H Component TE-H	ISP VL	C	Feedlot Heifers
Estradiol Benzoate[a] Trenbolone Acetate	28 200	Synovex Plus	FD	PEG	Feedlot Steers, Heifers
Estradiol Trenbolone Acetate	20 200	Revalor-200 Component TE-200	ISP VL	C	Feedlot Steers, Heifers
Estradiol Trenbolone Acetate	40 200	Revalor-XS	ISP	C, X7[d]	Feedlot Steers

[a]Estradiol benzoate contains 72.4% estradiol when calculated on a molecular-weight basis (Herschler et al., 1995).

[b]Manufacturer: FD = Fort Dodge Animal Health; ISP = Intervet Schering-Plough; VL = VetLife

[c]Carrier: C= cholesterol; L = lactose; PEG = polyethylene glycol; SL = silastic rubber.

[d]All implant pellets use a cholesterol carrier, but 6 of the 10 pellets are coated with X7 polymer, a proprietary delayed-release formula.

Figure 2.13. Relationship between empty body fat and percentage of USDA Prime and Choice carcasses with increasing aggressiveness of implant regimen (adapted from Guiroy et al., 2001).

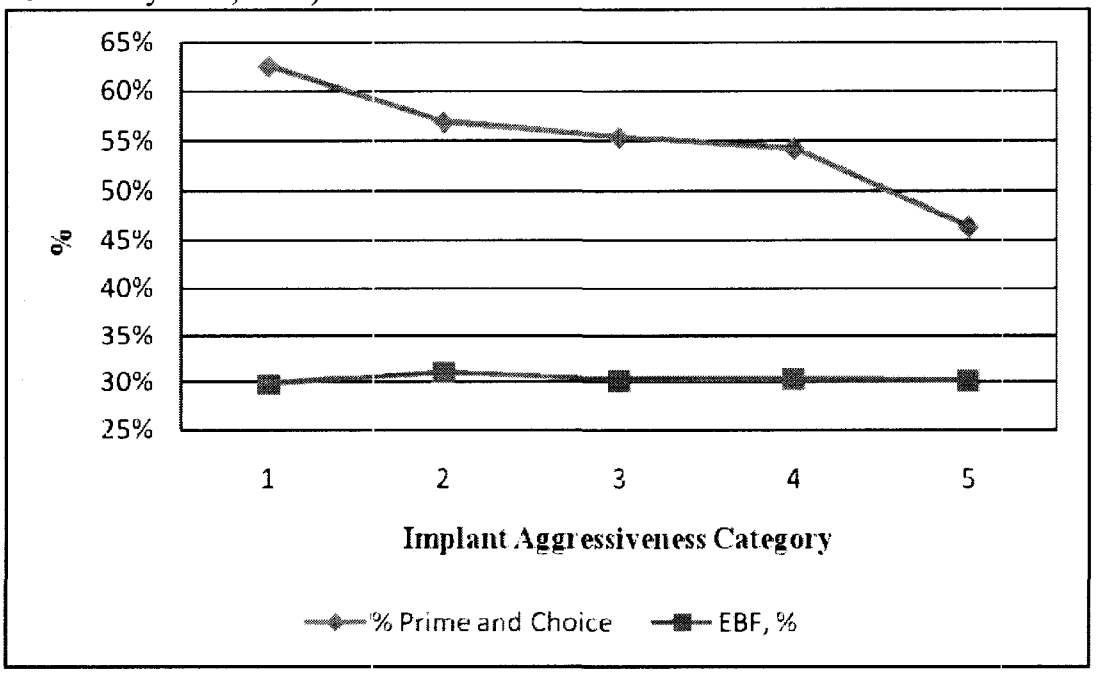

Figure 2.14. Relationship between empty body fat and percentage of USDA Prime and Choice carcasses of heifers given 8:80 mg of estradiol:trenbolone acetate at arrival and given increasing anabolic doses at reimplant (adapted from Schneider et al., 2007).

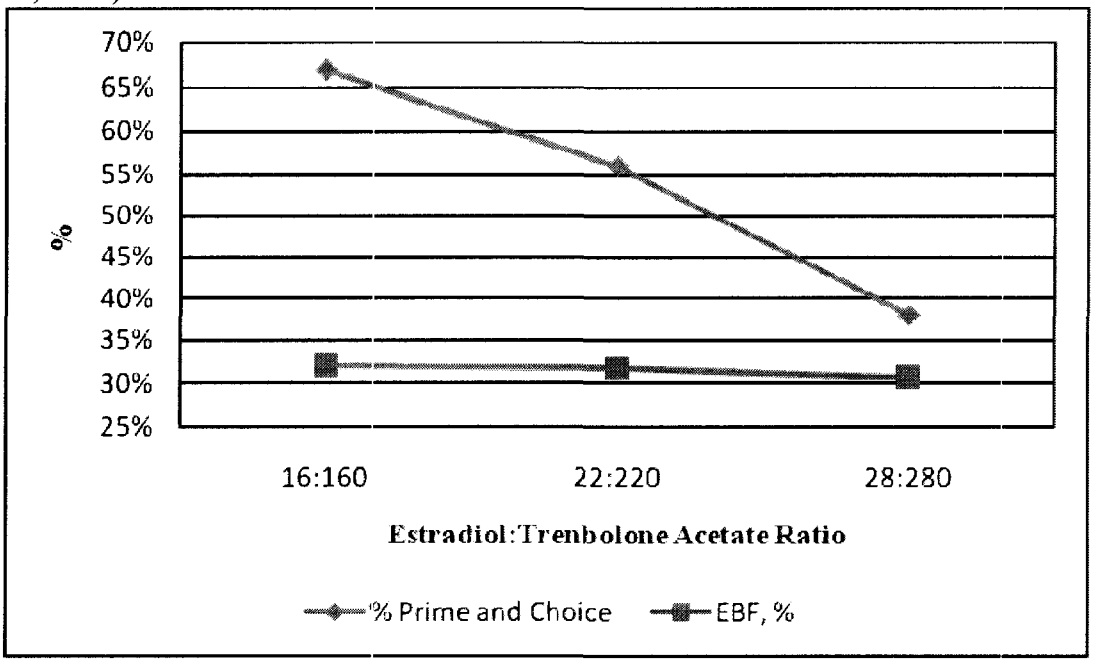

Figure 2.15. Signaling pathways used by GH (LeRoith et al., 2001).

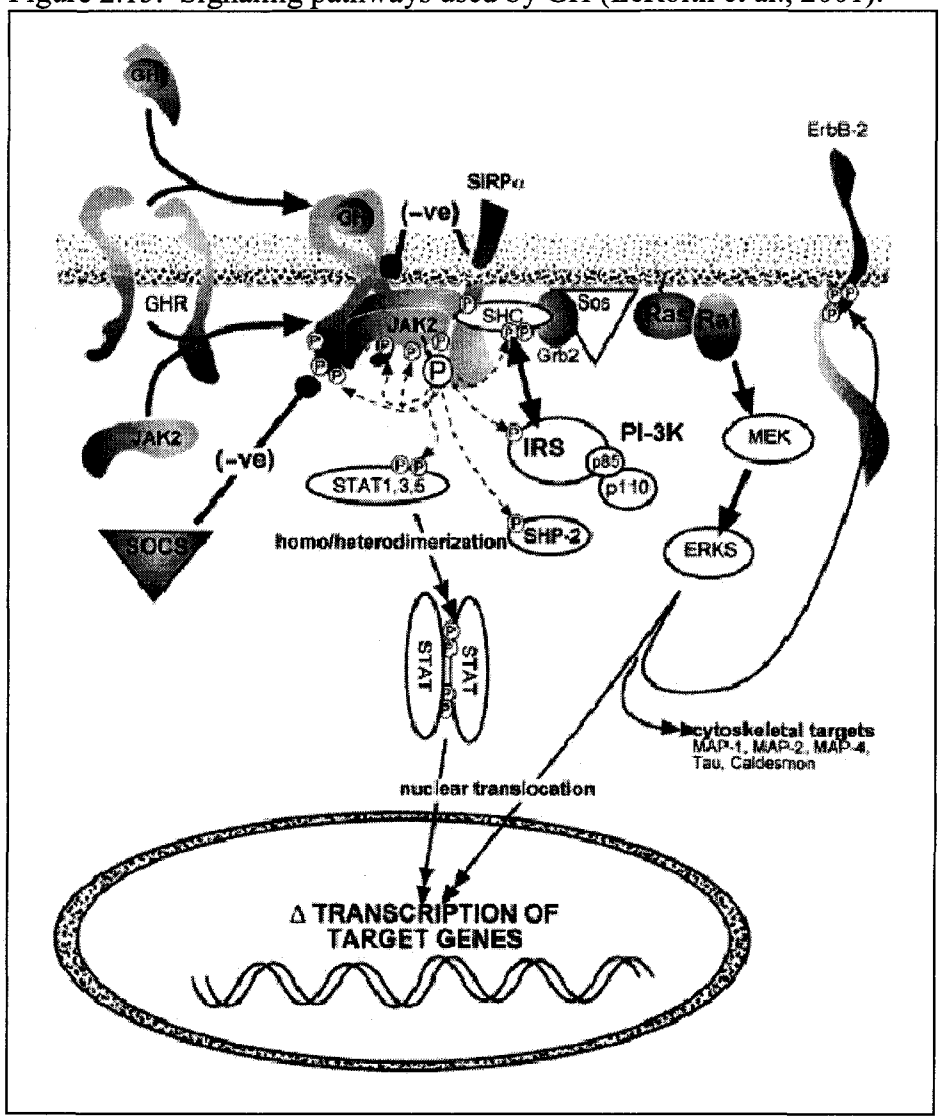

Figure 2.16. Signaling pathways used by IGF-1 (LeRoith et al., 2001).

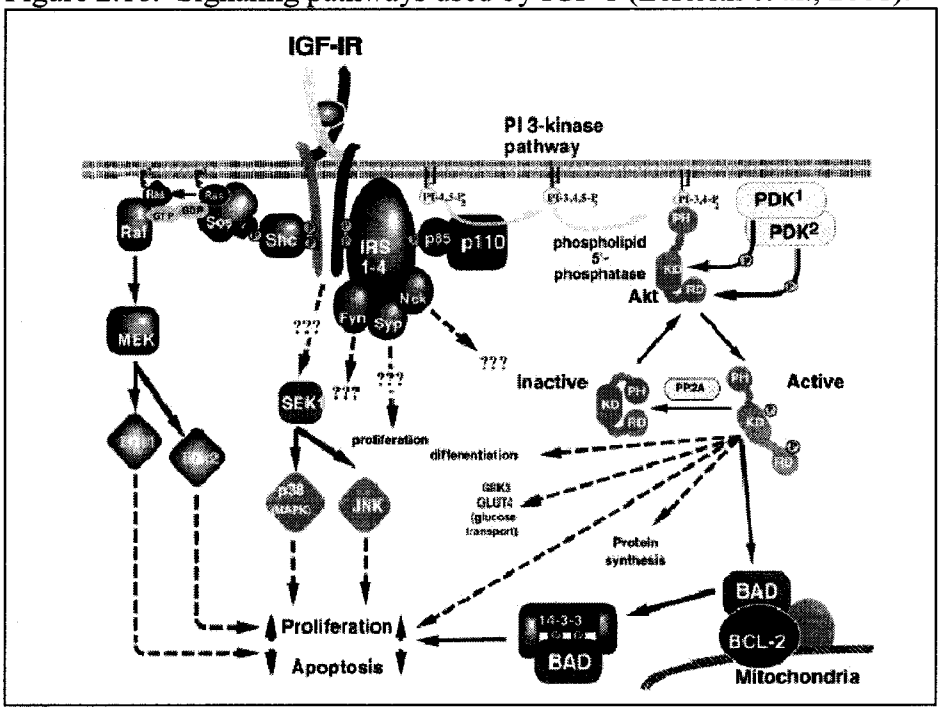

Figure 2.17. Alternative view of signaling pathways used by IGF-1 (Rommel et al., 2001).

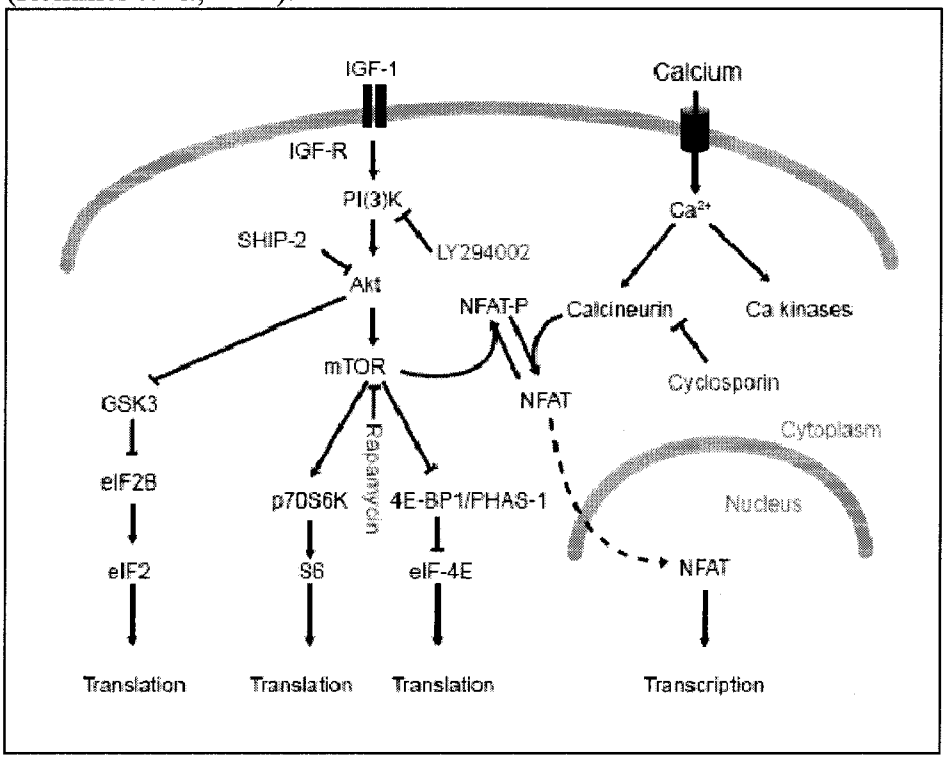

Figure 2.18. Synthesis of catecholamines.

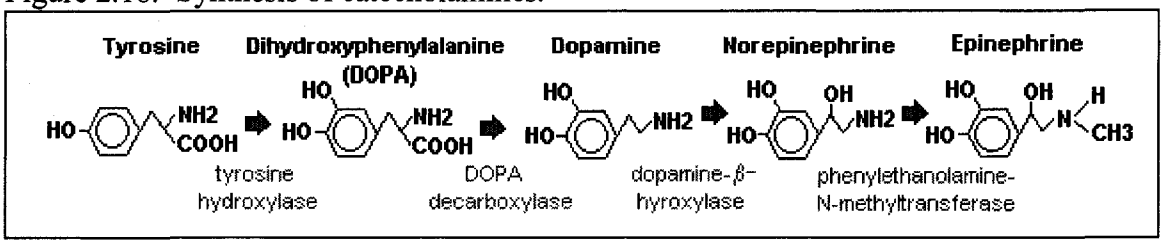

Figure 2.19. Primary structure of the β_1-adrenergic receptor. Amino acids in circles are conserved across the the β_1 adrenoceptor of humans, pigs, and cattle (80%). Shaded amino acids are conserved across the human β_1, β_2, and β_3 adrenoceptors (31%; Moody et al., 2000).

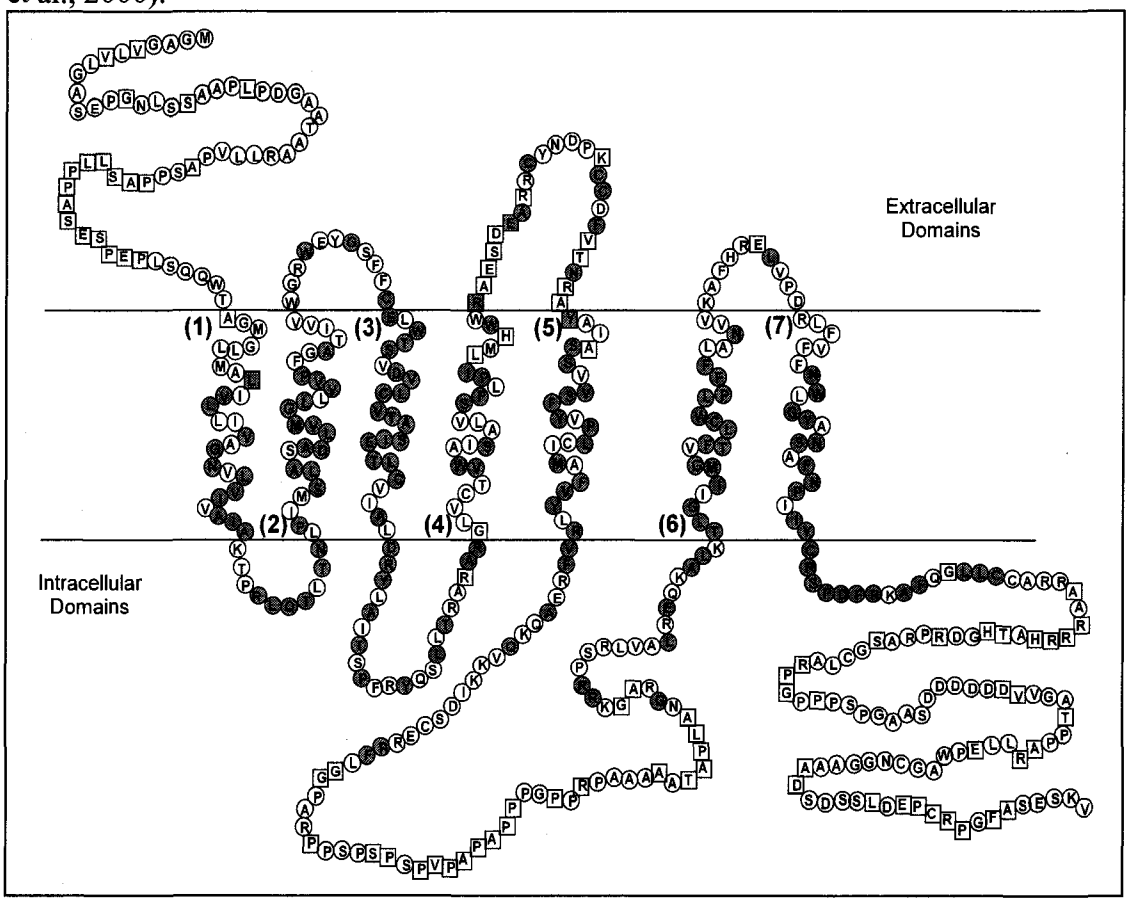

Figure 2.20. Actions of beta-adrenergic agonists (Barrett, 2003).

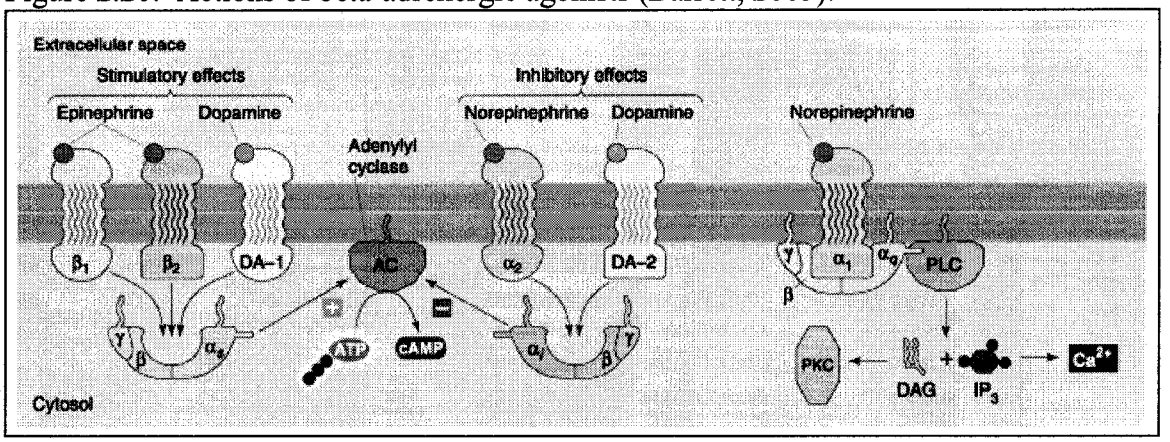

Figure 2.21. Structures of beta-adrenergic agonists that have been studied in livestock.

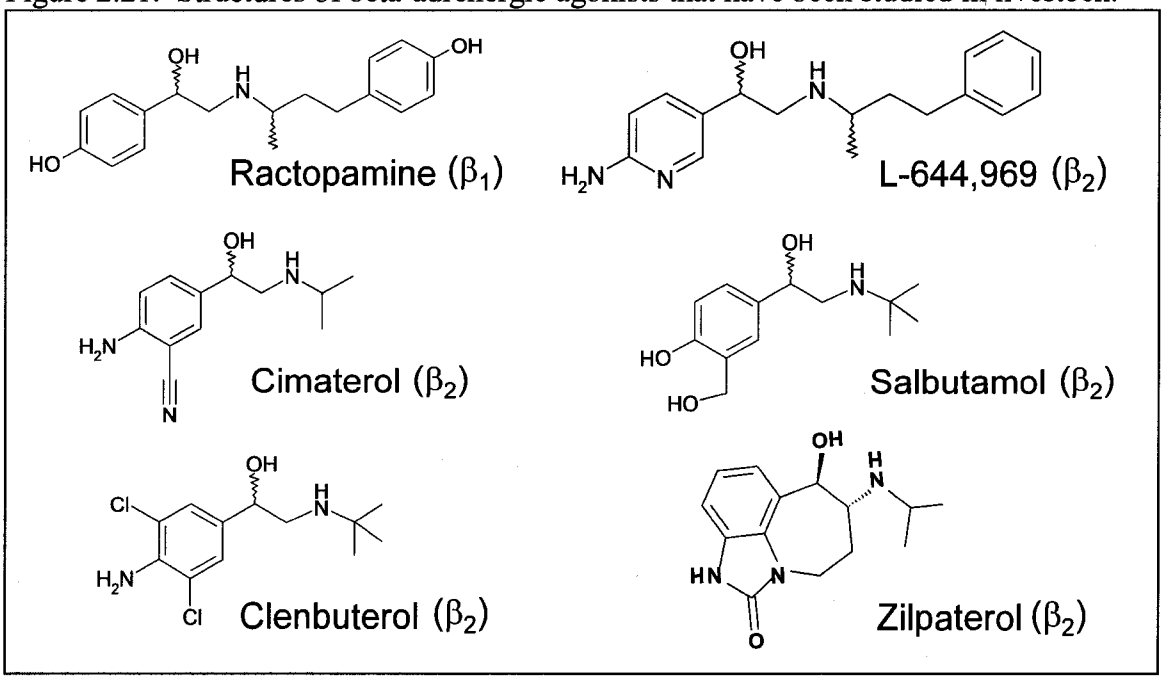

Table 2.3. Older research showing comparative responses of finishing steers and heifers to various βAA (Johnson, 2004).

Compound[a]	Dose	Days	ADG	Feed efficiency	DMI	Yield	Muscle	Fat
			Control = 100					
Steers								
Cimaterol	49	91	130	70	100	108	141	74
Clenbuterol	10	98	91	101	93	101	111	65
Clenbuterol	7	50	134	67	101	101	128	91
L-644,969	7.5	84	117	80	94	107	113	71
Ractopamine	200	42	111	90	100	101	102	98
Ractopamine	200	28-42	120	84	100	101	104	95
Zilpaterol	50	52	113	85	98	104	112	82
Heifers								
Clenbuterol	10	50	97	86	NA	103	118	60
Ractopamine	200	28-42	118	86	102	100	101	99
Zilpaterol	53	52	114	88	100	104	NA	NA

[a]See Johnson (2004) for references.

Table 2.4. Current research showing percentage improvement of various growth and carcass measures in response to ractopamine or zilpaterol.[a]

Compound[b]	Dose[c]	Days	ADG	Feed eff.[d]	DMI	Yield	Live BW	HCW	Marb.[e]
Beef steers									
Ractopamine[f]	200	28-32	117.4	84.1	99.6	100.4	101.1	101.5	99.3
Ractopamine[g]	200	28-42	119.7	84.1	100.0	100.5	101.3	101.9	99.5
Ractopamine[h]	200	28-42	111.1	88.6	98.6	100.4	101.0	101.3	98.8
Ractopamine[i]	200	28-42	114.4	87.6	100.2	99.9	101.3	101.3	NA
Ractopamine[j]	200	28	127.4	76.6	100.7	100.5	101.9	102.2	100.8
Ractopamine[k]	200	28	115.3	85.3	99.0	100.3	101.3	101.5	98.0
Ractopamine[l]	200	33	124.2	81.3	101.0	100.1	101.5	101.4	97.3
Zilpaterol[m]	60	42	136.6	71.9	98.8	103.6	104.6	104.8	98.3
Zilpaterol[n]	75	20-40	135.9	70.7	96.0	102.7	101.9	104.6	90.7
Zilpaterol[o]	77	20	129.2	77.2	100.7	102.2	101.5	103.6	NA
Zilpaterol[p]	72	20-40	112.5	86.7	99.6	103.1	103.4	104.5	90.3
Zilpaterol[q]	60	30	135.4	73.1	99.4	103.3	104.1	107.5	NA
Zilpaterol[l]	75	30	110.5	83.3	91.4	103.0	100.6	103.6	95.3
Holstein steers									
Ractopamine[r]	200	28-38	114.6	85.7	98.1	100.3	101.2	101.4	98.5
Beef Heifers									
Ractopamine[s]	200	28-32	111.2	87.9	97.1	100.7	100.7	101.4	99.8
Ractopamine[t]	200	28-42	117.7	86.0	101.6	99.7	101.3	100.9	100.9
Ractopamine[u]	200	28	106.5	91.3	97.6	NA	NA	100.3	98.2
Ractopamine[v]	200	28	114.0	75.6	98.7	NA	NA	101.5	97.1
Ractopamine[w]	200	28	104.6	87.4	99.4	NA	NA	100.7	99.6
Zilpaterol[n]	~69	20-40	118.9	79.5	94.4	102.3	101.5	103.8	96.1

[a]Expressed relative to control; control = 100; calculated only for period in which βAA was fed.
[b]Ractopamine = ractopaming hydrochloride; zilpaterol = zilpaterol hydrochloride
[c]Dose, mg·animal^{-1}·d^{-1}.
[d]Feed efficiency; based on change in feed:gain ratio.
[e]Marbling score.
[f]Laudert et al. (2005a,b).
[g]Schroeder et al. (2005a,b,c).
[h]Van Koevering et al. (2006a,b).
[i]Van Koevering et al. (2006c).
[j]Winterhollar et al. (2007).
[k]Gruber et al. (2007).
[l]Platter et al. (2008).
[m]Plascencia et al. (1999).
[n]FDA (2006).
[o]Intervet (2007).
[p]Vasconcelos et al. (2008).
[q]Avendaño-Reyes et al. (2006).
[r]Vogel et al. (2005a,b).
[s]Laudert et al. (2007).
[t]Schroeder et al. (2005d,e,f).
[u]Quinn et al. (2008).
[v]Sissom et al. (2007; Exp 1.)
[w]Sissom et al. (2007; Exp 2).

CHAPTER III

EVALUATION OF THE EFFECT OF DIETARY SUPPLEMENTAL VITAMIN A CONCENTRATION ON FEEDYARD PERFORMANCE, CARCASS MERIT, SERUM METABOLITES, AND LIPOGENIC ENZYME ACTIVITY IN YEARLING STEERS

ABSTRACT

Three hundred sixty, single-source, black, yearling steers (average BW = 316.1 ± 9.1 kg) fed a 91% concentrate (steam-flaked corn base) diet were used to evaluate the effects of supplemental vitamin A concentration on performance, DMI, and carcass traits. Steers were blocked into 8 weight replicates and assigned randomly to pens (n = 9/pen) and to diets containing 0, 1,103, 2,205, 4,410, or 8,820 IU of supplemental vitamin A/kg of dietary DM. Final BW (586, 580, 590, 585, and 584 kg for 0, 1,103, 2,205, 4,410, and 8,820 IU vitamin A/kg DM, respectively) did not differ ($P = 0.392$) among treatments. Feed efficiency, ADG, and daily DMI also did not differ ($P > 0.10$) among treatments within each 28-d period or for the overall trial. Although not significant, the 2,205 IU supplemental vitamin A treatment had the greatest final BW and overall ADG and DMI. From d 57 to slaughter, average DMI (10.33, 10.28, 10.57, 9.75, and 10.22 kg/steer daily for 0, 1,103, 2,205, 4,410, and 8,820 IU vitamin A/kg of DM, respectively) was less ($P < 0.02$) for steers receiving 4,410 IU vitamin A/kg of DM than for steers in the other treatments, and DMI was greater ($P = 0.06$) for the 2,205 IU vitamin A/kg of DM treatment than for the 8,820 IU/kg of DM treatment. Marbling score, HCW, LM area,

and 12th rib fat thickness did not differ ($P > 0.10$) among treatments. Similarly, the percentage of carcasses grading ≥ USDA Choice (62.6, 52.8, 64.0, 58.4, and 58.4% for 0, 1,103, 2,205, 4,410, and 8,820 IU vitamin A/kg of DM, respectively), Select, or ≤ Standard did not differ ($P > 0.10$) among treatments. Except for d 56 ($P = 0.0498$; r = 0.18), no correlations ($P > 0.10$) between marbling score and any plasma or liver tissue retinol or α-tocopherol levels or vitamin A intake were found, and no differences ($P > 0.10$) in lipogenic enzyme activity were detected among treatments.

Results of this trial suggest that vitamin A supplementation at a concentration up to twice the NRC recommendation has little effect on performance or marbling in typical yearling feedlot steers. Results of this trial and previous research suggest that 2,205 IU supplemental vitamin A/kg of DM (20,000 IU/steer daily) is adequate to meet the vitamin A requirements, to prevent the capacity of the gastrointestinal tract to absorb other fat-soluble nutrients such as vitamin E from being exceeded in typical yearling feedlot steers, and to optimize supplementation costs.

Key words: carcass quality, lipogenesis, marbling, vitamin A

INTRODUCTION

Quality grade distributions vary seasonally in cattle slaughtered throughout the year with relatively more Prime and Choice carcasses being slaughtered in the late autumn and early spring than during other times of the year. Berger and Faulkner (2003) postulated that this seasonal variation in carcass quality may result, in part, from the effects of seasonal variation in vitamin A intake. Most cattle slaughtered in the late Autumn and early spring, enter feedlots in the summer directly from lush green pastures, which have high concentrations of carotenoids, precursors of vitamin A. The liver is known to be the

primary storage depot for vitamin A; therefore, cattle placed into feedlots from lush pastures likely have elevated concentrations of vitamin A stored in the liver (Perry et al., 1966; Kohlmeier et al., 1970).

Several researchers have reported that that retinoic acid, a form of vitamin A, may inhibit adipocyte differentiation (Kamei et al., 1994; Kawada et al., 1996; Torri et al., 1996; Schwarz et al., 1997; Suryawan and Hu, 1997; Hida et al., 1998; Ohyama et al., 1998; Kawada et al., 2000). In addition, other research has shown a negative correlation between marbling and serum retinol concentration in Japanese Black cattle (Oka et al., 1992; Adachi et al., 1999; Chae et al., 2003).

Typical finishing diets recommended by 42 consulting nutritionists contained an average of 5,215 IU vitamin A/kg of DM (Vasconcelos and Galyean, 2007). In contrast, NRC (1996) suggested that the vitamin A requirement of feedlot cattle is 2,200 IU/kg of DM. Excess vitamin A intake by feedlot cattle may delay the depletion of vitamin A reserves in the livers of cattle placed in the feedlot directly from lush pastures, thereby delaying or limiting adipocyte differentiation. Feedlot diets that are low in vitamin A might hasten the depletion of vitamin A reserves and thereby diminish the inhibitory effect on adipocyte differentiation.

The objectives of this research were to study the effects of dietary vitamin A concentration on plasma and liver vitamin A and E concentrations, lipogenic enzyme activity, marbling score, and feedyard performance in yearling steers.

MATERIALS AND METHODS

Before the initiation of this experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Animals. Four hundred twenty-five single-source black, yearling steers (BW = 316.1 ± 9.1 kg) were purchased from the Flint Hills region of Southeastern Kansas via Eastern Livestock Company. All cattle had been managed similarly by a single producer. Since August 2002, the steers had grazed native bluestem varieties, which typically have peak nutritive value from May through July. One hundred twenty-five steers arrived at Colorado Beef (Lamar, CO) on July 15, 2003, and 300 steers arrived on July 18, 2003. All steers had access to long-stemmed grass hay and water on arrival, and all cattle were processed within 24 h of arrival. Processing included treatment for parasites and flies with Dectomax (doramectin; Pfizer Animal Health, Exton, PA) and Durasect Pour-On (permethrin and pyrethrin; Pfizer Animal Health), respectively, vaccination with Bovishield IBR-BVD (bovine rhinotracheitis and viral diarrhea; Pfizer Animal Health) and Ultrabac CD (*Clostridium perfringens* Types B, C, & D Bacterin-Toxoid; Pfizer Animal Health), and application of Component-ES implants (200 mg progesterone and 20 mg estradiol benzoate; Ivy Animal Health, Inc., Overland Park, KS). After processing, cattle were trailed to Southeastern Colorado Research Center (previously Continental Beef Research; CBR) in Lamar, CO and maintained on a starter diet (Table 3.1) until the start of the trial.

Allotment. On July 22, 2003 steers were weighed individually and tagged with an electronic ID tag. Steers were ranked by BW, and those weighing greater than ± 2 SD

deviations from the mean BW were excluded from the study. The remaining steers were stratified by weight into 8 blocks, and within each block, cattle were assigned randomly to 1 of 5 treatments. By following this procedure, eight pens with 9 steers/pen were available for each treatment. On July 23, 2003 steers were returned through the chute, individually weighed, and tagged with ear tags identifying treatment (1 to 5), replicate (1 to 8), and animal number (1 to 9) in each pen. Cattle were then sorted into their respective treatment pens, and the trial was started.

Treatments. Five dietary supplemental concentrations of vitamin A providing 0, 1,103, 2,205, 4,410, or 8,820 IU vitamin A/kg of DM were used. Vitamin A was supplied in the form of retinyl acetate. According to NRC (1996) the only source of naturally occurring vitamin A in the finishing diet was corn silage; however, β-carotenes and vitamin A are destroyed and become biologically unavailable as a result of ensiling, oxidation, and heat (Martin et. al., 1968; Puls, 1994; NRC, 1996; McDowell, 2000), which is likely why some researchers (Jordan et al., 1963; Smith et al., 1964; Miller et al., 1970; Martin et al., 1971; McDowell, 2000) have found that the liver stores of cattle consuming corn silages became depleted of vitamin A. Moreover, the vitamin A concentration of the basal diet (with no added vitamin A) was below the detection limits of the assay conducted by the commercial laboratory. (SDK Laboratories, Hutchinson, KS).

Diets. All cattle received the same basal diets for the entire experiment (Table 3.1). Starting, Step 1, and Step 2 diets containing 16.5 mg monensin/kg DM (trade name Rumensin, Elanco Animal Health, Greenfield, IN) were used to acclimatize the steers to a high-concentrate diet. Tylosin (trade name Tylan, Elanco Animal Health) was included

in the Step 1 and Step 2 diets at 2.2 and 6.6 mg/kg of DM, respectively. The finishing diets contained monensin and tylosin at 33 and 11 mg/kg DM, respectively. The Finish 1 diet contained corn silage as a roughage source, whereas sorghum silage was used in the Finish 2 diet because corn silage was no longer available at CBR. All diets contained a minimum of 13.5% CP and 5 IU/ kg of DM of vitamin E (supplied in the form of DL-α-tocopherol).

All diets were fed twice daily. Diet transitions were simultaneous for all treatments between d 3 and 5 on each diet. All diets used for each treatment were manufactured immediately before feeding using the stationary mixer in the feed mill at CBR. Finishing diet and feed commodities were sampled every 2 wk during the trial. All diet and feed commodity samples were shipped via UPS to a commercial laboratory (SDK Laboratories) for routine DM, NDF, CP, non-protein N, ether extract, and mineral analyses. In addition, finishing diet and supplement samples were sampled every 4 wk, shipped, and assayed for vitamin A and E concentrations.

Dry Matter Intake Determination. Feed refusals were weighed and sampled for DM determination whenever feed became spoiled, either as a result of adverse weather conditions or because of feed being left in the bunk for 3 d consecutively, on weigh days, and at the conclusion of the trial. Feed refusal samples were evaluated for DM content at CBR by drying the samples for 48 h in a 60°C convection oven. Dry matter consumption for each pen was calculated by subtracting the amount of DM refusals from the amount of DM delivered and dividing the result by animal-days for the pen.

Supplements. Supplements were manufactured at the beginning and throughout the duration of the trial as needed (typically weekly) at CBR according to Standard Operating

Procedures (Table 3.2). Supplements contained minerals, urea, vitamins A (except for the 0 IU vitamin A/kg of DM) and E, monensin, and tylosin. Vitamin A was mixed into the supplement from a premix containing 110,250,000 IU/kg of retinyl acetate.

Weighing Conditions. The initial BW used for the analysis was the average of the 2 individual BW measurements (scale readability ± 1 lb; scale calibrated with 1,000 lb of certified weights before use) obtained at the beginning of the trial. Individual interim weights were obtained on d 56 as the cattle were reimplanted with Revalor-S (120 mg trenbolone acetate and 24 mg estradiol-17β; Intervet/Schering-Plough Animal Health, Millsboro, DE), d 112, and d 141. Interim pen BW (scale readability ± 5 lb; scale calibrated with 1,000 lb of certified weights before use) were obtained on d 28 and d 84. Final BW was the average of 2 individual BW measurements obtained on d 141 and on 1 of the 2 d during the final sampling period (d 142 and d 143; December 12 and 13, 2003) before slaughter. A 4% pencil shrink was applied to all weights before data analyses.

Blood, Liver, and Adipose Sampling. Blood and liver tissue samples were collected from 3 animals per replicate on d 0, 56, 112, and 142 or 143. The 3 sampled animals from each replicate (n = 120) were selected randomly on d 0, and the same 3 animals were used on the remaining sampling days. Blood was collected via jugular venipuncture into heparinized vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) to determine plasma retinol and α-tocopherol concentrations. Samples were placed in an aluminum foil-covered rack and stored on ice in for approximately 2 to 3 h before being centrifuged at 2,500 × *g* for 20 min at room temperature. Plasma was decanted and stored in acid-washed polyethylene tubes. The tube racks were then wrapped in aluminum foil, and the samples were refrigerated overnight until the following day, at which time the samples

were transported on ice to Colorado State Univ. (Fort Collins, CO) where the samples were stored at -80°C until analyzed. At all times from sample collection through analysis, every effort was made to minimize light exposure to prevent the breakdown of the retinol.

Liver tissue biopsy samples were obtained from the same animals that were used for the blood collection using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000). In short, on the right side of each animal between the 11^{th} and 12^{th} ribs, a 10 cm × 10 cm area was clipped, scrubbed 3 times with Betadine (Purdue Products, L.P., Stamford, CT), then scrubbed with 70% (vol/vol) ethyl alcohol, and the area was locally anesthetized with 5 mL of lidocaine hydrochloride. A 1-cm incision was made with a #11 scalpel blade between the 11^{th} and 12^{th} ribs on a line from the tubercoxae to the point of the shoulder. A core sample of liver weighing approximately 50 mg was taken using the true-cut technique as described by Pearson and Craig (1980) using a modified Jamshidi bone marrow biopsy punch (0.7cm in diameter x 14 cm in length). Briefly, the biopsy probe was inserted into the liver, and negative pressure was applied with a 20-cc syringe to aspirate the sample into the biopsy probe. All biopsy instruments were cold-sterilized in 50% Nolvasan (Fort Dodge Animal Health, Overland Park, KS): 50% deionized water in an enclosed stainless-steel instrument container before use on each animal, and a new pair of sterile gloves was used for each biopsy. Following collection, each sample was rinsed immediately with phosphate buffered saline (PBS; pH 7.4) and placed into an acid-washed polyethylene tube, capped, and stored on ice. The plasma and liver samples were refrigerated overnight until the following day, at which time the samples were transported on ice to Colorado State Univ.

where the samples were stored at -80°C until analyzed. As with the plasma samples, every effort was made to minimize light exposure to prevent the breakdown of the retinol.

Because of the increased sampling time required for adipose tissue biopsies, the final collection was conducted over a 2-d period. Biopsies were sampled from the adipose tissue on d 142 or 143 from 1 randomly chosen steer, which was selected from 1 of the 3 designated sampling steers in each replicate. Adipose tissue biopsies were obtained from (1 animal per pen; n = 40) the right side of the tail-head a few days (d 142 or 143) before slaughter (d 147). At the time of sampling, the injection site and incision site were clipped of hair, scrubbed 3 times with (Purdue Products) and then 70% (vol/vol) ethyl alcohol. Scrubbing procedures were repeated 3 times. The incision site was anesthetized with 5 mL of lidocaine hydrochloride, which was injected into the epidural space between the last lumbar and first coccygeal vertebra. An incision 2.5 to 3.5 cm in length was made between the tail head and the tuber ischii, and approximately 5 g of adipose tissue was removed and washed with PBS. Incisions were then sutured with sterile #2 cat gut suture material. All biopsy instruments were cold-sterilized in 50% Nolvasan (Fort Dodge Animal Health, Overland Park, KS): 50% deionized water in an enclosed stainless-steel instrument container before use on each animal and a new pair of sterile gloves was used for each biopsy. After the initial biopsy, animals were monitored twice daily in the morning and evening for 1 wk. No post-surgical complications were observed for any of the animals.

Immediately after collection and rinsing, the subcutaneous adipose tissue samples were weighed, wrapped in aluminum foil, labeled, snap-frozen in liquid N and stored at -

80°C until activity of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and lipoprotein lipase (LPL) activities were determined.

Liver and Plasma Retinol and α-Tocopherol. Plasma and liver retinol and α-tocopherol concentrations were determined by high-performance liquid chromatography (HPLC). Liver and plasma retinol was analyzed according to the procedure of Alosilla et al. (2007). The α-tocopherol was determined following the procedure of Njeru et al. (1992) for plasma and of Njero et al. (1995) for liver.

Fatty Acid Synthase Enzyme Activity. Fatty acid synthase activity was determined as described by Moibi et al. (2000). At the time of assay, frozen adipose tissue samples were pulverized in liquid N and homogenized (30s at 4°C) in 3 volumes of phosphate bicarbonate buffer (70 m*M* $KHCO_3$, 85 m*M* K_2HPO_4, 9 m*M* KH_2PO_4, 1 m*M* DTT; pH 8). The homogenate was centrifuged at 10,000 x *g* for 10 min, and the resulting supernatant fluid was centrifuged at 105,000 x *g* for 60 min at 4°C to obtain adipose tissue cytosol. The supernatant fraction was brought to saturation with an ammonium sulfate solution (3 m*M* EDTA and 1 m*M* β-mercaptoethanol) and stirred for 60 min on ice. The precipitate was collected by centrifugation at 105,000 x *g* for 60 min. Protein content was assayed according to the method of Bradford (1976).

Fatty acid synthase activity was determined in duplicate according to the method of Nepokroeff et al. (1975) by measuring the malonyl-CoA-and acetyl-CoA-dependent oxidation of NADPH using a UV-visible automated spectrophotometer equipped with a temperature controller set at 30°C. For each assay, reference (blank) and sample cuvettes were measured simultaneously, and the decrease in absorbance at 340 nm was monitored. The change in concentration of NADPH during oxidation was calculated as described by

Moibi et al. (2000). Fatty acid synthase activity was expressed as nmol NADPH oxidized·min^{-1}·mg protein^{-1}.

Acetyl-CoA Carboxylase Enzyme Activity. Acetyl-CoA carboxylase activity was determined as described by Moibi et al. (2000). Frozen subcutaneous tissue samples were pulverized under liquid N and homogenized with a buffer containing 50 m*M* Tris-HCl (pH 7.5 at 4°C), 50 m*M* NaF, 0.25 *M* mannitol, 1 m*M* EDTA, 1 m*M* ethylene glycol-bis, 1 m*M* dithiotheritol, 5 m*M* sodium pyrophosphate, 1 mM PMSF, 1 mM benzamidine, and 4 μg/mL soybean trypsin inhibitor. Homogenates were centrifuged at 14,000 x *g* for 20 min at 4°C. The supernatant fraction of the samples was made to a final concentration of 2% (wt/vol) PEG, stirred for 10 min at 4°C, and then centrifuged at 10,000 x *g* for 10 min at 4°C. Acetyl-CoA carboxylase protein was precipitated from the supernatant fraction in a 10% (wt/vol) PEG solution, stirred on ice for 10 min, and centrifuged at 10,000 x *g* as described previously. Precipitate was collected and washed with 10% PEG (wt/vol)/homogenizing buffer. After centrifugation (10,000 x *g*, 10 min), the pellet was re-suspended in a buffer containing 100 m*M* Tris-HCl (pH 7.5 at 4°C); 1 m*M* EDTA; 1 m*M* EGTA; 1 m*M* DTT; 50 m*M* NaF; 5 m*M* sodium pyrophosphate; 10% (vol/vol) glycerol; 0.02% (wt/vol) sodium azide; 4 μg/mL each of aprotinin, leupeptin, pepstatin-A, and soybean trypsin inhibitor; and 1 m*M* benzamidine. Protein content was determined using the Bradford method (Bradford, 1976).

Acetyl-CoA carboxylase activity was determined as described by (Lopaschuck et al., 1994), except that the HPLC procedure was replaced by measuring the rate of incorporation of [^{14}C] bicarbonate into an acid-stable compound (malonyl-CoA; Thampy and Wakil, 1985). Briefly, 3 parts of enzyme extract were pre-incubated for 5 min at

37°C in 1 part of buffer containing 0.24 *M* Tris-acetate, 3 mg/mL BSA, 20 m*M* mg-acetate, 40 m*M* citrate, and 5.2 m*M* β-mercaptoethanol. The reaction was initiated by adding 10 μL of pre-incubated enzyme in a final assay mixture of 165 μL containing 60.6 m*M* Tris-acetate, 2.12 m*M* ATP, 1.32 μ*M* β-mercaptoethanol, 5.0 m*M* Mg-acetate, 10 m*M* potassium citrate, 1.06 mM acetyl-CoA, 18.18 m*M* $NaHCO_3$, 0.33 μCi/μmol $Na^{14}CO_3$, and 1 mg/mL fatty acid free-BSA (pH 7.5). After a 4-min incubation at 37°C in a shaking water bath, the reaction was stopped by adding 25 μL of 10% (vol/vol) perchloric acid. Reactions tubes were placed in a desiccator under vacuum, and tubes were centrifuged at 2,900 x *g* for 20 min. The supernatant fraction (160 μL) was transferred into glass scintillation vials and evaporated to dryness at 80°C under vacuum. The residue was dissolved in 100 μL of H_2O and mixed with 4 mL of scintillation fluid. Acetyl-CoA carboxylase activity was expressed as nanomoles of ^{14}C-bicarbonate incorporated into malonyl-CoA·min^{-1}·mg $protein^{-1}$.

Lipoprotein Lipase Enzyme Activity. Activity of lipoprotein lipase (LPL) in subcutaneous adipose tissue was determined by modifications of the methods of Krauss et al. (1973) and Rao and Hawkins (1976). The modifications were as follows: a weighed amount of frozen tissue was diced into small pieces, placed in 5 volumes of 0.15 *M* KCL and homogenized at high speed for 1 min. The substrate was then prepared by sonification of 100 mg of triolein in 12 mL of 0.194 *M* Tris-HCl plus 0.15 *M* NaCl buffer, pH 8.6, containing 0.05% (vol/vol) Triton X-100 and 200 mg of bovine serum albumin, and activated by incubation for 30 min at 37°C with 0.1 part of calf serum. The assay mixture was incubated at 37°C for 90 min in a metabolic shaker, and the free fatty acids released were determined (Smith, 1975).

Energy Recovery. Net energy values for each diet were calculated from estimates of energy expended for maintenance (EM, Mcal/d) and energy retained (EG, Mcal/d) derived from BW, actual growth performance data, and DMI using the following equations for medium-framed yearling steers (NRC, 1996):

EM = 0.077 × mean shrunk $BW^{0.75}$ (kg) where shrunk BW (SBW) = full BW × 0.96;

EG = (0.0635 × (equivalent empty $BW^{0.75}$) × (empty body $gain^{1.097}$)), where equivalent empty BW (kg) = (mean SBW × ((reference BW at a Choice endpoint) /final SBW) × 0.891), and empty body gain (kg/d) is the shrunk daily weight gain × 0.956

The NEm and NEg values of the diets were then calculated using the quadratic equation:

NEm (Mcal/kg DM) = $((-b \pm \sqrt{b^2-4ac}) / 2a)$, where

$a = 0.877 \times DMI$,

$b = (-0.877 \times EM) - (0.41 * DMI) - EG$, and

$c = 0.41 \times EM$

NEg (Mcal/kg DM) = 0.877 x NEm - 0.41.

Slaughter Procedures and Carcass Data. Steers were allowed to recover from blood-sampling and liver and adipose biopsies for 72 h, after which all animals were transported on d 147 (December 17, 2003) to the commercial abattoir (Swift; Greeley, CO) for slaughter using conventional, humane procedures. Dressing percent was determined using the HCW and the average of the 2 final live BW. All carcass data were jointly collected by the author and Colorado State Univ. Meat Science students. On the day of slaughter, the sequential order of steer slaughter was recorded, and carcass identification

numbers were matched with ear tag numbers. Following a 36-h chill, routine carcass measurements were obtained. Preliminary yield grade and KPH were assessed by 2 individuals, and the results were averaged for statistical analysis. Longissimus muscle area (LMA) and marbling percent of the lean surface at the $12^{th}/13^{th}$ rib interface and fat thickness at the ¾-measure opposite the lean surface between the $12^{th}/13^{th}$ rib interface were measured via the Computer Vision System Ribeye Camera (RMS Research Management Systems, USA Inc., Fort Collins, CO), which used colorimetric video-imaging. Yield grades for each animal were calculated from the adjusted 12^{th} rib fat thickness, LMA, HCW, and KPH. Marbling score was also assessed by 2 individuals, and data were averaged before analysis. The incidence of dark cutting beef, HCW, and USDA quality and yield grades were obtained from the carcass kill sheets supplied by the packing plant. A separate quality grade was also determined based on the marbling scores that were assessed by representatives of Colorado State Univ.

Pen Observations. Pens were checked daily shortly after the morning feeding to monitor cattle for health problems. Cattle exhibiting symptoms of injury or disease were removed from the pen, treated according to diagnosis and the corresponding standard CBR antibiotic-treatment regime, and immediately returned to the pen.

Data Analysis. Data for feedyard performance, HCW, marbling score, marbling percent, 12^{th} rib fat, LMA, KPH, calculated yield grade, dressing percent, and lipogenic enzyme activities were analyzed on a pen-mean basis using the Mixed procedure of SAS (Release 8.0; SAS Inst., Inc., Cary, NC) for a randomized block design. Treatment was included in the model as a fixed effect, and weight block was included in the model as a random effect. All independent variables included in the model were considered class

variables. When the overall F-value for treatment was significant ($P < 0.05$), least squares means were separated using Fisher's LSD test generated by the PDIFF statement in SAS. Orthogonal polynomials were used to evaluate linear and quadratic responses among supplemental concentrations of vitamin A. Orthogonal coefficients for unequally spaced treatments were generated using the IML procedure of SAS.

USDA yield and quality grade data were evaluated as categorical data with a binomial distribution using the GLIMMIX procedure of SAS. Treatment was included in the model as a fixed effect, and block was included as a random effect. All independent variables included in the model were considered class variables.

Plasma and liver retinol and α-tocopherol concentrations were analyzed on a pen-mean basis using the Mixed procedure of SAS for a randomized block design with repeated measures as described by Littell et al. (1998). Fixed effects included in the model were treatment, period, and the interaction between treatment and period. Weight block was included in the model as a random effect. The subject of the repeated statement was the interaction between treatment and replicate. First-order autoregressive (AR1) covariance structure was used. Class variables included treatment, replicate, and period. When the overall F-value for treatment was significant ($P < 0.10$), least squares means were separated using Fisher's LSD test generated by the PDIFF statement in SAS. Orthogonal polynomials were used to evaluate linear and quadratic responses among supplemental concentrations of vitamin A. As before, orthogonal coefficients for unequally spaced treatments were generated using the IML procedure of SAS. Finally, Pearson correlation coefficients, with animal as the experimental unit, were determined

for the correlation among plasma and liver tissue retinol and α-tocopherol concentrations and marbling score using the CORR procedure of SAS.

RESULTS AND DISCUSSION

Health. Because of the low incidence of morbidity and mortality of the trial cattle, statistical analysis of the health data was not performed. During the experiment, 20 steers (0 for 0 IU vitamin A/kg DM; 7 for 1,103 IU vitamin A/kg DM; 6 for 2,205 IU vitamin A/kg DM; 3 for 4,410 IU vitamin A/kg DM; and 4 for 8,820 IU vitamin A/kg DM) were pulled and treated. Five steers died during the trial period. One steer mortality occurred on each of the following dates: August 11 (4,410 IU vitamin A/kg of DM), August 21 (4,410 IU vitamin A/kg DM), October 14 (8,820 IU vitamin A/kg of DM), November 8 (2,205 IU vitamin A/kg of DM), and November 28 (2,205 IU vitamin A/kg of DM).

Supplemental Vitamin A Intake. Cumulative average daily supplemental vitamin A intake is presented in Table 3.4. Based on actual average daily DMI and formulated dietary concentrations, steers fed the 0, 1,103, 2,205, 4,410, and 8,820 IU vitamin A/kg of DM treatments consumed 0, 10,367, 21,260, 40,997, and 85,578 IU of supplemental vitamin A/(steer•d), respectively, from d 0 to slaughter. The average daily supplemental vitamin A intake increased linearly ($P < 0.001$) with increasing supplemental concentration of vitamin A. In contrast to the expected quadratic response, this observed linear response is likely a function of the actual DMI response noted.

Least squares means for feedyard growth performance, intake, feed efficiency, and energy recovery are presented in Tables 3.3 and 3.4. Live weights were not affected ($P > 0.171$) by supplemental vitamin A concentration for any period. In addition, slaughter weights did not differ ($P = 0.392$) and averaged 586, 580, 590, 585, and 584 kg for 0,

1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. Previous researchers (Perry et al., 1968; Kohlmeier et al., 1970) observed that feedlot cattle consuming 1,433 to 3,087 IU supplemental vitamin A/kg of DM had greater ADG and BW than those not receiving any supplemental vitamin A. Kohlmeier et al. (1970) conducted 3 feedlot trials with yearling steers and diets containing 0 to 3,087 IU supplemental vitamin A/kg of DM and found that no supplemental vitamin A was required as long as plasma and liver vitamin A levels remained above 25 μg/dL and 2 μg/g, respectively. Chapman et al. (1964) and Perry et al. (1966) found no advantage of supplementing vitamin A to cattle grazing summer pasture. Furthermore, Kohlmeier et al. (1970) concluded that most cattle entering the feedlot from grazing conditions have sufficient vitamin A reserves to last for 90 to 120 d, but that 1,433 IU supplemental vitamin A/kg of DM was needed to ensure that incoming cattle did not become deficient in vitamin A during the feeding period. Similarly, Embry et al. (1962) suggested that 1,268 IU/kg of DM was sufficient to meet the vitamin A requirements of feedlot steers.

Average Daily Gain. Daily gain did not differ among treatments from d 0 to d 56 ($P = 0.324$), d 57 to slaughter ($P = 0.116$), or for the entire feeding period ($P = 0.367$); ADG for the entire experiment averaged 1.90, 1.86, 1.93, 1.89, and 1.88 kg/d for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. As previously mentioned, Perry et al. (1968) found that cattle consuming 12,500 to 20,000 IU of supplemental vitamin A daily had greater ADG than those not receiving any supplemental vitamin A. Hill et al. (1995) reported that feedlot steers receiving 24,000 IU supplemental vitamin A/(steer•d), which equated to approximately 2,134 IU supplemental vitamin A/kg of DM, had greater ADG than those receiving 70,000 IU of

supplemental vitamin A/d (approximately 6,274 IU supplemental vitamin A/kg of DM). In a receiving trial, Zinn et al. (1996) also observed greater ADG by crossbred calves consuming 2,200 IU supplemental vitamin A/kg of DM than by those consuming 11,000 IU supplemental vitamin A/kg of DM; however, this difference was only noted for the first 28-d of the 56-d trial period.

In more recent research, in 1 of 3 studies, Pyatt et al. (2005) reported that ADG was increased in cattle fed 2,300 IU/kg compared with those that received 7,250 IU/kg; however, in the other 2 studies, no differences in ADG were detected with different vitamin A concentrations. Gorocica-Buenfil et al. (2007a; 2008) observed a slight increase in ADG in cattle supplemented with 2,700 IU/kg ($P = 0.08$) or 2,200 IU/kg ($P = 0.14$), respectively, compared with those that were fed no supplemental vitamin A. Conversely, Gorocica-Buenfil et al. (2007b,c) noted no differences in ADG among Holstein or Angus-cross cattle that were had different cumulative vitamin A intakes.

Daily Dry Matter Intake. From d 57 to slaughter, average DMI (10.33, 10.28, 10.57, 9.75, and 10.22 kg/(steer•d) for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively) was lower ($P < 0.02$) for steers receiving 4,410 IU supplemental vitamin A/kg DM than for steers in other treatments, and DMI was greater ($P = 0.06$) for the 2,205 IU supplemental vitamin A/kg of DM treatment than for the 8,820 IU supplemental vitamin A/kg of DM treatment. As a result, from d 57 to slaughter intake increased quadratically ($P = 0.062$) with increasing level of supplemental vitamin A. From d 0 to slaughter, daily DMI did not differ among treatments ($P = 0.306$) and averaged 9.55, 9.40, 9.64, 9.30, and 9.48 kg/(steer•d) for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. The numerically increased

DMI associated with the 2,205 IU supplemental vitamin A/kg of DM treatment could partly explain the numerically greater slaughter weight and ADG associated with this treatment. Hill et al. (1995) reported numerically greater DMI by steers consuming an 83% concentrate diet containing 2,134 IU supplemental vitamin A/kg of DM than in those consuming 6,274 IU supplemental vitamin A/kg of DM. Similarly, Zinn et al. (1996) reported numerically greater DMI in crossbred calves fed a receiving diet with 2,200 IU supplemental vitamin A/kg DM than in those receiving 11,000 IU supplemental vitamin A/kg of DM. Other studies (Pyatt et al., 2005; Gorocica-Buenfil et al., 2007a,b,c; 2008) have not detected differences in DMI among cattle fed varying levels of supplemental vitamin A.

Feed Efficiency. Feed efficiency (G:F) ratio did not differ among treatments from d 0 to d 56 ($P = 0.996$), d 57 to slaughter ($P = 0.890$), and for the entire feeding period ($P = 0.841$), which averaged 0.235, 0.231, 0.235, 0.233, 0.233 for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. As expected DMI:ADG ratios followed similar patterns as G:F ratios. Hill et al. (1995) observed statistically improved feed efficiency in steers consuming 2,134 IU supplemental vitamin A/kg of DM than in those consuming 6,274 IU supplemental vitamin A/kg of DM. Likewise, Zinn et al. (1996) observed statistically improved feed efficiency in crossbred calves consuming 2,200 IU supplemental vitamin A/kg of DM than in those consuming 11,000 IU supplemental vitamin A/kg of DM, but this difference was only evident for the first 28-d of the 56-d trial period.

In 1 of 3 studies, Pyatt et al. (2005) reported that feed efficiency was improved in cattle fed 2,300 IU/kg compared with those that received 7,250 IU/kg; however, in the

other 2 studies, no differences were detected. Gorocica-Buenfil et al. (2007a,b; 2008) noted slightly worsened feed efficiencies in cattle that received no supplemental vitamin A during the finishing period when compared to those fed 2,700 or 2,200 IU of supplemental vitamin A/kg. In contrast, Gorocica-Buenfil et al. (2007c) observed no differences in feed efficiency between steers fed either 0 or 2,200 IU supplemental vitamin A/kg.

Cost of Gain and Cost of Vitamin A. No differences for cost of gain were detected between treatments from d 0 to 56 ($P = 0.964$), d 57 to slaughter ($P = 0.870$), or for the overall trial ($P = 0.695$). Overall cost of gain, not including the cost of the vitamin A supplementation, averaged \$77.05, \$77.84, \$76.60, \$75.64, and \$77.22/cwt for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. At the current industry average concentration of 5,215 IU supplemental vitamin A/kg of DM (Vasconcelos and Galyean, 2007) and at an average assumed consumption of 9 kg/(animal•d), the cost of vitamin A is approximately \$0.28/animal over an average feeding period of 170 d. These trial data and previous research suggest that 2,205 IU supplemental vitamin A/kg of DM is adequate to meet the vitamin A requirements of typical feedlot steers. By decreasing current concentrations of supplemental vitamin A to 2,205 IU/kg of DM, cattle feeders could cut supplementation costs to approximately \$0.12/animal resulting in tremendous annual savings.

Calculated Net Energy. Dietary NEm concentrations from d 0 to slaughter were not affected ($P = 0.776$) by dietary supplemental vitamin A concentrations, and averaged 2.24, 2.23, 2.24, 2.27, and 2.24 Mcal/kg for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. Similarly, no differences ($P = 0.776$) in

dietary NEg concentrations from d 0 to slaughter were detected among dietary supplemental vitamin A treatments. For the overall trial, NEg averaged 1.55, 1.54, 1.55, 1.58, and 1.55 Mcal/kg for 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively.

Carcass Merit. Least squares means and frequency distributions showing the effect of supplemental vitamin A concentration on carcass quality and cutability traits are presented in Table 3.5. No measured carcass parameters were affected ($P > 0.10$) by supplemental concentration of vitamin A. Except for d 56 ($P = 0.050$; $r = 0.18$), no correlations ($P > 0.10$) between marbling score and any plasma or liver tissue retinol or α-tocopherol concentrations or vitamin A intake were found. Thus, it seems that the effect of supplemental vitamin A on marbling is still uncertain. Previous studies have shown a negative correlation between serum retinol concentration and marbling in cattle (Oka et al., 1992; Adachi et al., 1999; Chae et al., 2003). Nonetheless, these previous studies were conducted primarily in a retrospective fashion in that supplemental dietary vitamin A treatments were not applied to the cattle. Instead, cattle used in these studies were chosen in a random fashion, and biochemical assay results from these cattle were correlated to their individual marbling scores.

With supplemental vitamin A and in a series of 3 experiments in the Tajima strain of Japanese Black cattle (Oka et al., 1998) in which cattle were injected with 303 mg of vitamin A alcohol every 2 mo until slaughter (an equivalent of approximately 16,833 IU/d prorated equally over the feeding period), 1 experiment showed that cattle administered injectable vitamin A periodically during the finishing period had decreased ($P < 0.05$) marbling scores compared with those that did not receive supplemental

vitamin A; however, in the other 2 experiments, marbling scores were not affected ($P > 0.05$) by injected supplemental vitamin A. The authors surmised that the difference in outcomes was a result of the age of the animals, and concluded that supplemental vitamin A had no effect in cattle after 23 mo of age as a result of the maturing of the adipocytes in the intramuscular adipose tissue. In the Wagyu strain of Japanese Black cattle, Nade et al. (2003) fed cattle 2 different levels of oral vitamin A. In this study, there was no reference to the actual levels of vitamin A fed, and the only notation was that the control cattle were fed according to the Japanese Feeding Standard for Beef Cattle (1995) and that the treatment group was fed at half that level. Nade et al. (2003) reported that cattle fed at half the standard dose had increased marbling scores ($P < 0.05$).

In reference to the studies conducted with Japanese Black cattle (Oka et al., 1992; Oka et al., 1998; Adachi et al., 1999; Nade et al., 2003), it must be noted that these cattle are very unique and are known to have a significant genetic potential for marbling, whereas the cattle in the present experiment were typical U.S. cattle and graded 49 to 60 percent Prime and Choice. In addition, the Japanese cattle are typically over 30 mo of age when slaughtered, whereas the cattle in the present study and most cattle in the U.S. are less than 30 mo of age when slaughtered. In Japan, most cattle are raised in confinement from a very young age, whereas in the U.S., most cattle graze native forage for the majority of their life before entry to the feedlot.

More recent research with supplemental vitamin A in beef cattle of the U.S. has yielded inconsistent results. In 3 separate experiments in which Angus × Simmental cattle were fed either 2,300 or 7,250 IU of dietary vitamin A/kg, Pyatt et al. (2005) did not observe differences in marbling scores or 12^{th} rib fat. In addition, Pyatt et al. (2005)

did not find any correlation ($P > 0.10$) between marbling score and serum retinol concentration. Moreover, Arnett et al. (2007) showed that marbling score increased in lambs fed 6,600 vs. 0 IU/ of supplemental vitamin A/kg and found a positive correlation ($r = 0.30$; $P < 0.10$) between marbling score and serum retinol. Similarly, research conducted to evaluate various vitamin A supplementation and restriction strategies (Gorocica-Buenfil et al., 2007a,b,c; 2008) showed mixed results. For instance, Gorocica-Buenfil et al. (2007a) reported that Angus-cross cattle (BW = 295 kg) that were fed 2,700 IU vitamin A/kg in a diet in which high-moisture corn was used as the sole grain source tended ($P = 0.11$) to have decreased marbling scores without any effect on 12^{th} rib fat thickness compared with those that received no supplemental vitamin A. In contrast, in lightweight Holstein steers (BW = 218 kg) that were fed 2,200 IU/kg of supplemental vitamin A before being restricted to no supplemental vitamin A for 131 or 243 d, Gorocica-Buenfil et al. (2007b) observed no differences in marbling scores or 12^{th} rib fat thickness. In a third study (Gorocica-Buenfil et al., 2007c) with Angus-cross steers (BW = 295 kg), marbling in cattle supplemented with 2,200 IU vitamin A/kg was significantly lower than those receiving no supplemental vitamin A; however, the percentage of carcasses grading Choice or greater was not affected by vitamin A level. In a fourth study (Gorocica-Buenfil et al., 2008), no differences in marbling or quality grade were detected between Angus-based steers (BW = 224 kg) fed either 0 or 2,200 IU supplemental vitamin A/kg. It seems that the effect of supplemental vitamin A on marbling and quality grade is highly variable, likely being affected by the flux of liver stores of vitamin A throughout the animal's life and in relation to the maturing of the intramuscular adipose depot.

Lipogenic Enzyme Activity. As previously noted, no differences in marbling score or 12th rib fat thickness were detected ($P > 0.10$) among supplemental vitamin A treatments. The activities of the ACC, FAS, and LPL did not differ among treatments ($P > 0.10$), and orthogonal contrasts did not reveal any trends ($P > 0.10$). Acetyl-CoA carboxylase regulates the first controlling step of fatty acid synthesis in adipose tissue, and it aids in the conversion of acetyl CoA to malonyl-CoA via addition of CO_2. Fatty acid synthase is a multi-enzyme complex, which first catalyzes the combination of a primer acetyl-CoA and malonyl-CoA and which uses NADPH as a donor of reducing equivalents during numerous cycles to form long-chain fatty acids. Both ACC and FAS work in the cytosol of the adipose tissue. In contrast, LPL is anchored to the endothelial walls of the blood capillaries and acts to hydrolyze lipoproteins so that the non-esterified fatty acids can enter the cytosol for re-esterifcation and storage. No other trials evaluating the response of these enzymes to supplemental vitamin A could be found in the literature; however, the results agree with the lack of differences in subcutaneous and intramuscular fat among vitamin A treatments. Gorocica-Buenfil et al. (2007a) reported that, compared with a diet without supplemental vitamin A, Angus-cross cattle (BW = 295 kg) supplemented with 2,700 IU vitamin A/kg in a typical high-moisture corn diet had decreased intramuscular adipose cell density and increased mean diameter of adipose cells; however, no effect on adipose cellularity was noted in the subcutaneous fat depot. In contrast, in lightweight Holstein steers (BW = 218 kg) that were fed 2,200 IU supplemental vitamin A/kg before being restricted to no supplemental vitamin A for 131 or 243 d, Gorocica-Buenfil et al. (2007b) observed no differences in adipose cellularity in either the intramuscular or subcutaneous adipose tissue. In a third study (Gorocica-

Buenfil et al.; 2007c) with Angus-cross steers (BW = 295 kg), no differences in adipose cellularity were found for cattle fed either 0 or 2,200 IU/kg supplemental vitamin A.

Plasma and Liver Retinol Profiles. Plasma and liver tissue retinol concentrations are shown in Table 3.6, and graphical observations of plasma and liver retinol are shown in Figures 3.1 and 3.3, respectively. Plasma retinol did not differ among treatments (P = 0.342), and the period × treatment interaction was not significant (P = 0.328). Additionally, no significant (P > 0.10) orthogonal trends in plasma retinol were detected for any periods. Although caution should be used in comparing assay results among laboratories and studies because of differences in samples, procedures, conditions, etc., for the sake of comparison and discussion, initial plasma retinol concentrations averaged 146.7 ng/mL. Pyatt et al. (2005) reported initial serum concentrations of 114.5 ng/mL. Serum retinol concentrations for Gorocica-Buenfil et al. (2007a,b,c; 2008) averaged 350 ng/mL, 279 ng/mL, 323 ng/mL, and 295 ng/mL, respectively.

Liver retinol has been reported to be a better indicator of vitamin A status than plasma (Ralston and Dyer, 1959; Smith et al., 1964; Miller et al., 1970; Westendorf et al., 1990; Puls, 1994; Alosilla et al., 2007) because plasma retinol is maintained at a relatively constant level in the body via hepatic stores so that bodily processes are maintained (Goodman and Blaner, 1984; Alosilla et al., 2007). Analysis of liver tissue revealed interesting trends (Figure 3.3). The period × treatment interaction was significant (P < 0.001) for liver retinol. Within period, liver retinol differed (P = 0.006) among treatments on d 0, with the 8,820 IU/kg treatment having lower liver retinol than the 2,205 IU/kg treatment, whereas all other treatments did not differ (P > 0.10) from each other. On d 56, liver retinol increased linearly (P < 0.001) with increasing level of

supplemental vitamin A. Moreover, the 0 IU/kg treatment had lower ($P < 0.10$) liver retinol than all other treatments, and the 8,820 IU/kg treatment had higher ($P < 0.10$) liver retinol concentrations than all other treatments. On d 112, liver retinol concentrations increased linearly and quadratically ($P < 0.009$) with increasing levels of supplemental vitamin A. In addition, all individual treatment means differed from each other ($P < 0.10$), with the 0 IU/kg treatment having the lowest liver retinol, and the 8,820 IU/kg treatment having the highest liver retinol concentration. On the last sampling day, liver retinol increased linearly and quadratically ($P < 0.011$) with increasing dietary vitamin A concentrations. Following the previous patterns, the 0 IU/kg treatment had lower liver retinol ($P < 0.10$) than all other treatments, and the 8,820 IU/kg treatment had higher ($P < 0.10$) liver retinol than all other treatments. In addition, the 1,103 IU/kg treatment had higher ($P < 0.001$) liver retinol than the 0 IU/kg treatment, but it was lower than all other treatments ($P < 0.10$).

Despite the fact that the liver retinol has been reported to be a better indicator of vitamin A status (Puls, 1994; McDowell, 2000; Alosilla et al., 2007), except for 1 sentinel animal (Gorocica-Buenfil et al., 2007b), recent studies (Pyatt et al., 2005; Gorocica-Buenfil et al., 2007a,b,c; 2008) have not taken liver tissue samples at the beginning of the trial. In the current trial, liver retinol concentrations averaged 163.9 μg/g (dry basis). At slaughter, the liver retinol concentrations averaged 92.5, 342.7, 457.8, 467.8, and 841.5 μg/g (dry basis) for the 0, 1,103, 2,205, 4,410, and 8,820 IU supplemental vitamin A/kg of DM, respectively. It is interesting to note that the liver retinol in the 0 IU/kg treatment was less than 100 μg/g on d 142 and 143. Adult cattle are typically thought to be marginally deficient in vitamin A when their liver retinol falls

below 100 μg/g and fully deficient when liver retinol decreases below 30 μg/g (Puls, 1994). Kohlmeier and Burroughs (1970) stated that cattle that entered the feedlot with 20 to 40 μg/g (wet basis; 67 to 133 μg/g dry basis) will have sufficient reserves for 90 to 120 d. In the present study, liver retinol concentrations were observed to be below the 100 μg/g (dry basis) threshold between d 112 and d 142/143. On d 142/143, the plasma retinol for the 0 IU/kg treatment was the only 1 that decreased from the previous period, which might have been the signal that the liver stores of retinol had been depleted sufficiently to decrease plasma retinol. It is known that the liver contains about 90% of the body's vitamin A (Puls, 1994), and present data seem to substantiate that liver retinol is a better indicator of vitamin A status than plasma retinol when cattle are not in a deficient state.

Gorocica-Buenfil et al. (2007a,b,c; 2008) reported values for hepatic retinol concentrations at slaughter. Assuming a 30% DM liver content and reporting on a DM basis, at slaughter the liver concentration of retinol for the cattle receiving no supplemental vitamin A was 22 μg/g, whereas the liver retinol of those receiving 2,700 IU supplemental vitamin A/kg was 259 μg/g (Gorocica-Buenfil et al., 2007a). Similarly, Gorocica-Buenfil et al. (2007b) observed that liver retinol concentrations for the cattle receiving no supplemental vitamin A for the last 112 or 243 d were 21 μg/g compared with 149 μg/g for the cattle supplemented with 2,200 IU/kg. Gorocica-Buenfil et al. (2007c) reported liver retinol concentrations of 129 μg/g at slaughter for cattle receiving no supplemental vitamin A and 343 μg/g for those receiving 2,200 IU of supplemental vitamin A/kg. Gorocica-Buenfil et al. (2008) observed liver retinol concentrations of 21 and 127 μ/g for cattle fed 0 or 2,200 IU supplemental vitamin A/kg. In 3 of these 4

studies (Gorocica-Buenfil et al., 2007a,b; 2008), the liver retinol reported in the cattle receiving no supplemental vitamin A was below both the marginally deficient and deficient thresholds as defined by Puls (1994). Moreover, Gorocica-Buenfil et al. (2007b, 2008) both reported lower efficiency during the finishing period, and Gorocica-Buenfil et al. (2008) reported a trend for lower ADG for the finishing period for cattle that were not fed supplemental vitamin A. Neither of these studies, however, showed any differences in marbling between the cattle as a result of supplementation with vitamin A. There does not seem to be a clear pattern among these trials with respect to liver retinol concentrations at slaughter and marbling score. As has been noted, because blood concentrations of retinol tend to be maintained until hepatic stores are depleted, retinol concentrations in the blood are only good indicators when an animal is extremely deficient in vitamin A (Puls, 1994; McDowell, 2000; Alosilla et al., 2007). In addition, because marbling deposition occurs over the life of the animal and over the entire feeding period, it seems that the liver retinol concentration at the beginning of the feeding period would be more pertinent and applicable than serum or plasma retinol. Hepatic stores of retinol may have to be below a certain critical threshold before the preadipocytes differentiate into mature fat cells, and this point-in-time likely differs according to the environment and genetics of the animal. In addition, to truly ascertain these "critical thresholds" to make recommendations for feeding of vitamin A, it is necessary to develop dose-response curves over time and to examine both the initial liver retinol concentrations and also its rate of decline until slaughter.

Plasma retinol did not show any significant trends ($P > 0.10$) with increasing level of dietary vitamin A; however, liver retinol increased linearly and quadratically with

increasing level of supplemental vitamin A. Moreover, the correlation between liver retinol and plasma retinol (Table 3.7) was only significant ($P = 0.07$) on d 56, and the correlation coefficient was small (r = 0.17). Ralston and Dyer (1959), Smith et al. (1964), and Alosilla et al. (2007) also reported no correlation between liver and plasma vitamin A and found little value in plasma retinol concentrations. Few other studies have evaluated both liver and plasma retinol concentrations over the same time periods; however, some studies (Gorocica-Buenfil et al., 2007a,b; 2008) have shown a numerically positive relationship between serum retinol and either increased supplemental vitamin or restricted supplemental vitamin A. Conversely, Gorocica-Buenfil et al. (2007c) observed that the serum retinol in the cattle not given any supplemental vitamin A stayed constant from the initiation of treatments until slaughter.

Altogether, data from the present trial and from previous research suggest that the results from studies conducted in a similar fashion to this one will continue to provide variable results and conclusions. First, the backgrounds and previous nutritional history of the animals in these studies is variable and in large part not known. In addition, only the current study assayed for initial liver concentration of retinol, which is considered the gold standard for assessing vitamin A status. It is known that carotene and hence vitamin A content of feed sources changes seasonally (Puls, 1994; McDowell, 2000). Consequently, based on the previous nutritional history and seasonal time of the year, cattle will have tremendous variation in hepatic stores of vitamin A. Moreover, the duration in the finishing phase of most beef cattle is less than 1/3 of their life-span, and it has been shown that marbling and thereby quality grade can be significantly influenced by practices and nutritional backgrounds (e.g., genetics, age at weaning and feedlot entry,

implants, energy intake, castration, etc.) before feedlot entry (Myers et al., 1999; Schoonmaker et al., 2002, 2004, Platter et al., 2003; Bruns et al., 2004). In addition, it is likely that there are interactions among these various factors that influence marbling. Consequently, much more data are needed before extrapolating results and making recommendations for all beef cattle across all nutritional backgrounds and seasons, because the answer likely depends on numerous factors. Adding to the complexity is that feedyards only have the capability, both physically and economically, to feed a finite number of diets; however, perhaps dietary vitamin A requirements and hence formulations should vary seasonally. A more comprehensive approach to examine the relationship of total vitamin A intake and liver and blood retinol concentrations from birth to slaughter and their effects on carcass quality seems warranted.

Plasma and Liver α-Tocopherol Profiles. Plasma and liver tissue α-tocopherol concentrations are shown in Table 3.6, and graphical observations of plasma and liver α-tocopherol are shown in Figures 3.2 and 3.4, respectively. Plasma α-tocopherol concentrations did not differ among treatments ($P = 0.503$), the period × treatment interaction was not significant ($P = 0.127$), and no significant orthogonal responses were detected among the dietary vitamin A concentrations ($P > 0.10$). Liver and adipose α-tocopherol concentrations have been the preferred indicators of vitamin E status (McDowell, 1989; Puls, 1994; Njeru et al., 1995), in that plasma typically only reflects the dietary vitamin E that has been consumed recently. In addition, Njeru et al. (1995) showed that the liver and adrenal tissues have a much higher concentration of α-tocopherol than does the adipose tissue of yearling beef heifers.

For liver α-tocopherol, the period × treatment interaction was significant ($P = 0.007$). Although there was a linear trend ($P = 0.041$) for decreasing liver α-tocopherol with increasing dietary vitamin A, no differences in liver α-tocopherol were observed on d 0. It is interesting to note the period effect ($P < 0.001$) for liver α-tocopherol (Figure 3.4). In particular, it seems that there was a significant decrease in liver α-tocopherol from d 0 to d 56. The first 60 d of the feeding period are typically when the highest morbidity and stress occur. Carter et al. (2005) showed that plasma vitamin E concentrations decreased over time from d 0 to 28 and 42 of the receiving period. In addition, Nockels et al. (1996) observed that liver and plasma α-tocopherol decreased when cattle that had diets adequate in vitamin E were stressed via injections of epinephrine and ACTH. Moreover, Rivera et al., (2002) found that serum IgG titers increased linearly with supplemental levels of vitamin E. Consequently, it is likely that the decrease in liver α-tocopherol was related to the normal health-related issues that are associated with the first 2 mo of the feeding period.

On d 112, liver α-tocopherol decreased linearly ($P = 0.005$) with increasing supplemental vitamin A. On d 142 and 143, the liver α-tocopherol increased both linearly and quadratically ($P < 0.065$) with increasing level of dietary vitamin A. Moreover, the 0 and 1,103 IU/kg treatments had significantly ($P < 0.10$) higher concentrations of liver α-tocopherol than the 3 other treatments. With each increasing level of supplemental vitamin A, the numerical values of liver α-tocopherol decreased in ranking. In other words, in the order that the dietary concentration of vitamin A was increased, the liver α-tocopherol concentrations decreased in sequential order. There was a moderately strong negative correlation between liver retinol and liver α-tocopherol for

d 56 ($P = 0.0157$; r = -0.22), d 112 ($P = 0.011$; r = -0.24), d 142/143 ($P < 0.001$; r = -0.38), and overall ($P < 0.001$; r = -0.43), and the correlation seemed to get stronger and more negative over time. Puls (1994) noted that excessive vitamin A can destroy vitamin E. Research (Dicks et al., 1959; Hill et al., 1995; Schelling et al., 1995; Zinn et al., 1996; Eicher et al., 1997; Franklin et al., 1998) has shown interactions in performance with supplemental levels vitamin A and E and has indicated that supplementing beef or Holstein cattle with vitamin A in excess of NRC suggestions can decrease plasma concentrations of vitamin E. Although not studied in the previously mentioned research, this fact along with the correlation between vitamin E supplementation and immune response (Nockels et al., 1996; Rivera et al., 2002) may suggest that feeding high levels of vitamin A could adversely affect health and performance of feedlot cattle. These observations could be the result of fat-soluble vitamins and their carriers exceeding the capacity for absorption in the lower gastrointestinal tract. A receiving trial evaluating effects of supplemental vitamins A and E concentrations and their interactions on the performance, health, and carcass merit of incoming cattle seems warranted.

IMPLICATIONS

Data from the present experiment did not show any relationship between supplemental vitamin A and marbling score, and the results of recently published research are inconsistent. The dietary backgrounds of cattle and their resulting hepatic stores of retinol likely explain the variability in response to vitamin A supplementation or restriction in beef cattle. Typical finishing diets in High Plains feedlots contained an average of 5,215 IU vitamin A/kg DM. The performance and tissue measurements from the present study and the data reviewed by NRC (1996) suggest that the vitamin A

requirement by feedlot cattle is approximately 2,205 IU/kg of DM. In addition, recent research provides evidence that feeding supplemental vitamin A in excess of 2,205 IU/kg of DM could overwhelm the capacity of the lower gastrointestinal tract to absorb other nutrients, including fat-soluble vitamins such as vitamin E, which could adversely affect the health and performance of feedlot cattle. Decreasing the current supplemental vitamin A concentrations from the industry average of 5,215 to 2,205 IU/kg of DM could result in $0.16/animal savings over the feeding period. For a 30,000-animal feedyard and assuming 2.2 turns per yr, this could result in savings of more than $10,000/yr. Further research to evaluate the performance, health, and carcass effects of various concentrations of supplemental vitamins A and E in receiving diets should be conducted. Moreover, a more comprehensive approach to examine the relationship of total vitamin A intake and liver and blood retinol concentrations from birth to slaughter and their effects on carcass quality seems warranted.

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Table 3.1. Formulated ingredient and chemical composition of the diets fed in the vitamin A trial.[a]

	Diet				
Item[a]	Starter	Step-one	Step-two	Finish1	Finish2
Steam-flaked corn	47.48	57.41	67.33	77.77	79.75
Alfalfa hay	30.00	20.00	10.00		
Corn silage	17.41	15.49	13.57	9.32	
Sorghum silage					7.84
CCDS[b]	3.00	3.00	3.00	3.00	3.00
Yellow grease		1.00	2.00	3.50	3.50
Soybean meal	1.16	1.31	1.45	2.80	2.31
Supplement	0.95	1.80	2.66	3.61	3.61
Theoretical nutrients					
DM, % as-fed	63.96	64.99	66.05	69.10	68.40
CP	13.50	13.50	13.50	13.50	13.50
Non-protein nitrogen[c]	1.00	2.00	3.00	3.50	3.50
ADF	18.10	14.33	10.55	6.20	5.47
NDF	24.28	20.12	15.96	10.94	11.19
Crude fiber	14.10	10.92	7.74	4.13	4.76
eNDF[d]	17.69	14.11	10.53	6.28	4.68
fNDF[e]	20.00	15.00	10.00	4.00	4.00
NEm, mcal/kg[f]	1.87	1.99	2.11	2.24	2.22
NEg, mcal/kg[g]	1.22	1.32	1.42	1.54	1.53
Ca	0.70	0.70	0.70	0.70	0.70
P	0.26	0.26	0.26	0.26	0.27
K	1.29	1.05	0.81	0.70	0.70
Mg	0.25	0.25	0.25	0.25	0.25

[a]Percentage of DM unless stated otherwise.
[b]Condensed corn distillers soluble (i.e. corn steep).
[c]CP equivalent.
[d]Effective NDF; calculated from NRC (1996).
[e]Forage NDF.
[f]Net energy for maintenance.
[g]Net energy for gain.

Table 3.2. Dry matter composition of the supplements fed for the vitamin A trial.

	Diet				
Ingredient[a]	Starter	Step-one	Step-two	Finish1	Finish2
Limestone	38.689	43.167	44.708	44.525	27.449
Urea	16.702	29.667	34.313	30.663	29.548
Salt	26.427	13.889	9.416	6.931	6.932
Magnesium oxide	11.205	8.611	7.721	7.042	
MIN-AD[b]					28.392
Potassium chloride				7.153	3.918
Mineral oil	2.008	2.056	2.034	2.024	2.035
Colorado Beef TM[c]	2.643	1.333	0.866	0.638	0.757
Rumensin 80[d]	0.951	0.500	0.339	0.527	0.494
Tylan 100[e]		0.056	0.113	0.139	0.130
Vitamin E premix[f]	0.529	0.278	0.188	0.139	0.147
Vitamin A premix[g]	0.846	0.444	0.301	0.222	0.200

[a]Percentage of DM.

[b]Dolomitic limestone, a significant source of magnesium carbonate and calcium carbonate; MIN-AD, Inc., Greeley, CO.

[c]Colorado Beef trace mineral premix: Co = 340 ppm; Cu = 7.7%; Mn = 6%; Zn = 22.4%; and Se = 300 ppm.

[d]Monensin, 176 g/kg.

[e]Tylosin, 221 g/kg.

[f]198,450 IU vitamin E/ kg; DL-α-tocopherol.

[g]Vitamin A premix (110,250,000 IU vitamin A/kg; retinyl acetate) blended with ground corn at ratios of 0:8, 1:7, 2:6, 4:4, and 8:0 for the 0, 1,103, 2,205, 4,410, and 8,820 IU per kg of DM treatments, respectively.

Table 3.3. Least squares means for the effect of supplemental vitamin A concentration on cumulative feedyard growth performance.

	Vitamin A, IU/kg of DM						Treatment	
Item	0	1,103	2,205	4,410	8,820	SEM[a]	*P*-value	Contrast[b]
Body weight, kg								
Initial	315.8	315.6	316.0	316.8	316.5	9.1	0.3789	NS
d 28	372.6	372.9	375.3	372.6	374.2	10.0	0.9256	NS
d 56	423.8	418.7	422.9	428.6	424.1	9.7	0.1711	NS
d 84	492.1	485.1	490.6	492.4	491.2	9.9	0.5847	NS
d 112	539.1	532.8	540.4	544.3	539.5	10.4	0.2766	NS
Slaughter	586.2	579.6	589.9	584.6	583.8	9.2	0.3920	NS
ADG, kg/d								
d 0-56	1.93	1.84	1.91	2.00	1.92	0.06	0.3241	NS
d 57-slaughter	1.89	1.87	1.94	1.81	1.86	0.04	0.1162	NS
d 0-slaughter	1.90	1.86	1.93	1.89	1.88	0.02	0.3669	NS
DMI, kg/d								
d 0-56	8.36	8.06	8.22	8.60	8.34	0.34	0.1595	NS
d 57-slaughter	10.33^{xy}	10.28^{xy}	10.57^{x}	9.75^{z}	10.22^{y}	0.29	0.0023	Q, 0.062
d 0-slaughter	9.55	9.40	9.64	9.30	9.48	0.30	0.3055	NS
Feed:gain								
d 0-56	4.34	4.41	4.33	4.32	4.34	0.20	0.9790	NS
d 57-slaughter	5.51	5.53	5.45	5.39	5.51	0.19	0.8476	NS
d 0-slaughter	5.02	5.07	5.00	4.93	5.03	0.17	0.6952	NS
G:F								
d 0-56	0.235	0.231	0.235	0.233	0.233	0.011	0.9955	NS
d 57-slaughter	0.184	0.183	0.185	0.187	0.183	0.007	0.8898	NS
d 0-slaughter	0.201	0.199	0.202	0.204	0.200	0.007	0.8412	NS

[a]Standard error of the mean, n = 8 pens/treatment.

[b]Orthogonal contrasts: L = linear; Q = quadratic; $P < 0.10$.

[x,y,z]Row means that do not have a common superscript letter differ, $P < 0.10$.

Table 3.4. Least squares means for the effect of supplemental vitamin A concentration on cumulative feedyard efficiency, energy recovery and vitamin A intake.

	Vitamin A, IU/kg of DM						Treatment	
Item	0	1,103	2,205	4,410	8,820	SEM[a]	*P*-value	Contrast[b]
Cost of gain[c], $/cwt								
d 0-56	69.26	70.67	69.29	68.95	69.26	3.06	0.9641	NS
d 57-slaughter	85.40	85.72	84.51	83.71	85.49	2.93	0.8702	NS
d 0-slaughter	77.05	77.84	76.60	75.64	77.22	2.51	0.6949	NS
Calculated NEm, Mcal/kg of DM								
d 0-56	2.57	2.57	2.59	2.55	2.57	0.089	0.9846	NS
d 57-slaughter	2.23	2.22	2.23	2.29	2.23	0.053	0.2293	NS
d 0-slaughter	2.24	2.23	2.24	2.27	2.24	0.055	0.7761	NS
Calculated NEg, Mcal/kg of DM								
d 0-56	1.85	1.84	1.86	1.82	1.84	0.078	0.9846	NS
d 57-slaughter	1.55	1.54	1.54	1.60	1.55	0.047	0.2293	NS
d 0-slaughter	1.55	1.54	1.55	1.58	1.55	0.048	0.7761	NS
Supplemental Vitamin A Intake, IU/d[d]								
d 0-56	0	8,882	18,116	37,929	73,545	1,498	<0.0001	L, <0.001
d 57-slaughter	0	11,333	23,307	42,995	90,111	1,307	<0.0001	L, <0.001
d 0-slaughter	0	10,367	21,260	40,997	85,578	1,360	<0.0001	L, <0.001

[a]Standard error of the mean, n = 8 pens/treatment.

[b]Orthogonal contrasts: L = linear; Q = quadratic; $P < 0.10$.

[c]Cost of gain is based on a $300/ton (DM) diet cost and does not include the cost of vitamin A.

[d]All treatment mean comparisons differed ($P < 0.001$).

[w,x,y,z]Row means that do not have a common superscript letter differ, $P < 0.10$.

Table 3.5. Least squares means and frequency distributions for the effect of supplemental vitamin A concentration on carcass quality and cutability.

	Vitamin A, IU/kg of DM							
Item	0	1,103	2,205	4,410	8,820	SEM[a]	Treatment *P*-value	Contrast[b]
HCW, kg	376.7	369.8	377.7	372.0	374.4	6.9	0.3165	NS
Dressing percent	64.24	63.80	64.00	63.64	64.11	0.26	0.3666	Q, 0.085
12th rib fat, cm	1.40	1.38	1.42	1.45	1.36	0.048	0.6001	NS
LM area, cm^2	87.3	86.9	88.0	86.1	87.5	1.27	0.8412	NS
KPH[c], %	2.05	2.00	2.08	2.07	2.05	0.03	0.3784	NS
Calculated YG	3.13	3.02	3.09	3.19	3.04	0.10	0.6859	NS
USDA yield grade								
1	6.94	5.56	5.56	1.39	6.94		0.6660	NS
2	25.00	33.33	26.39	25.00	30.56		0.7873	NS
1 and 2	31.94	38.89	31.94	26.39	37.50		0.5226	NS
3	61.26	48.77	59.88	58.54	54.31		0.6970	NS
4 and 5	6.86	12.38	8.24	15.15	8.24		0.5508	NS
Marbling score[d]	419	412	429	424	424	8	0.6338	NS
Marbling percent[e]	3.26	3.27	3.51	3.47	3.38	0.16	0.7286	NS
USDA quality grade								
Prime and Choice	57.00	48.59	62.61	48.59	59.81		0.3256	NS
Select	40.33	48.74	33.38	44.70	38.86		0.1901	NS
Sub-Select	2.67	2.67	4.01	6.71	1.33		0.4332	Q, 0.098
Quality grade-CSU[f]								
Prime and Choice	62.57	52.79	63.96	58.38	58.38		0.7359	NS
Select	35.97	45.78	33.17	38.77	40.17		0.6536	NS
Sub-Select	1.46	1.43	2.87	2.85	1.45		0.9258	NS

[a]Standard error of the mean, n = 8 pens/treatment.

[b]Orthogonal contrasts: L = linear; Q = quadratic; $P < 0.10$.

[c]Kidney, pelvic, and heart fat.

[d]Marbling score units: 300 = Slight[00]; 400 = Small[00]; 500 = Modest[00]; 600 = Moderate[00].

[e]Area of marbling within the LM divided by the total LM area; determined via colorimetric measures.

[f]Quality grade based on marbling scores as assessed by representatives of Colorado State Univ.

Table 3.6. Least squares means for the effect of supplemental vitamin A concentration on plasma and liver retinol and α-tocopherol concentrations and on lipogenic enzyme activities of adipose tissue.

	Vitamin A, IU/kg of DM							
Item	0	1,103	2,205	4,410	8,820	SEM[a]	*P*-value	Contrast[b]
Plasma retinol[c], ng/mL								
Overall	212.6	254.9	236.3	214.8	231.8	17.7	0.3418	NS
d 0	143.3	134.8	165.0	133.9	156.4			
d 56	190.6	195.5	251.3	231.7	227.5	N/A	N/A	N/A
d 112	263.7	285.4	188.3	202.3	265.7			
d 142/143	252.8	404.0	340.6	291.3	277.7			
Liver retinol[d], μg/g of dry weight								
d 0	174.9^{yz}	176.4^{yz}	184.1^{y}	145.0^{yz}	139.0^{z}	9.8	0.0062	L, 0.001
d 56	154.4^{x}	228.2^{y}	235.6^{y}	235.6^{y}	314.6^{z}	8.0	< 0.0001	L, < 0.001
d 112	153.4^{v}	291.8^{w}	352.9^{x}	404.0^{y}	606.6^{z}	14.9	< 0.0001	LQ, < 0.009
d 142/143	92.5^{w}	342.7^{x}	457.8^{y}	467.8^{y}	841.5^{z}	20.3	< 0.0001	LQ, < 0.011
Plasma α-tocopherol[e], μg/dL								
Overall	704.0	734.8	757.1	734.3	751.6	23.5	0.5027	NS
d 0	673.0	759.4	680.2	699.7	728.6			
d 56	817.7	735.1	761.4	791.6	717.2	N/A	N/A	N/A
d 112	597.1	654.5	819.6	760.2	790.7			
d 142/143	728.1	790.2	767.2	685.5	786.8			
Liver α-tocopherol[f], μg/g of dry weight								
d 0	33.14	33.53	31.78	28.53	28.36	2.07	0.2448	L, 0.041
d 56	23.38^{y}	15.74^{z}	17.29^{yz}	20.79^{yz}	19.88^{yz}	1.39	0.0040	NS
d 112	24.58^{yz}	25.62^{y}	22.09^{yz}	19.43^{yz}	18.39^{z}	1.91	0.0412	L, 0.005
d 142/143	35.74^{y}	34.15^{y}	22.63^{z}	21.24^{z}	18.85^{z}	3.64	0.0042	LQ, < 0.065
Fatty acid synthase activity, nmol of NADPH oxidized•minute^{-1}•mg of protein^{-1}								
d 142/143	3.89	4.12	3.90	4.37	4.29	0.26	0.6135	NS
Acetyl-CoA carboxylase Activity, nmol of $H^{14}CO_3$ incorporated•minute^{-1}•mg of protein^{-1}								
d 142/143	3.98	4.22	4.15	4.47	4.59	0.32	0.5338	NS
Lipoprotein lipase activity, μmol of NEFA hydrolyzed•h^{-1}•g of tissue^{-1}								
d 142/143	2.76	2.64	2.56	2.83	2.72	0.21	0.8961	NS

[a]Standard error of the mean, n = 8 pens/treatment.

[b]Orthogonal contrasts: L = linear; Q = quadratic; $P < 0.10$.

[c]Plasma retinol: treatment, $P = 0.342$; period, $P < 0.001$; period × treatment, $P = 0.328$.

[d]Liver retinol: treatment, $P < 0.001$; period, $P < 0.001$; period × treatment, $P < 0.001$.

[e]Plasma α-tocopherol: treatment, $P = 0.503$; period, $P = 0.325$; period × treatment, $P = 0.127$.

[f]Liver α-tocopherol: treatment, $P = 0.0006$; period, $P < 0.0001$; period × treatment, $P = 0.007$.

[v,w,x,y,z]Row means that do not have a common superscript letter differ, $P < 0.10$.

Table 3.7. Correlations between concentrations of retinol and α-tocopherol in both the plasma and liver.

	Treatment day				
Item[a]	0	56	112	142/143	Overall
Plasma retinol vs. plasma α-tocopherol					
r	0.11	0.16	-0.17	-0.07	0.04
P-value	0.227	0.089	0.064	0.461	0.658
Liver retinol vs. liver α-tocopherol					
r	0.05	-0.22	-0.24	-0.38	-0.43
P-value	0.543	0.016	0.011	< 0.001	< 0.001
Liver retinol vs. plasma retinol					
r	0.03	0.17	0.00	0.11	0.04
P-value	0.724	0.066	0.992	0.258	0.682
Liver α-tocopherol vs. plasma α-tocopherol					
r	0.01	0.11	0.04	0.08	0.07
P-value	0.925	0.279	0.699	0.427	0.467

[a]Calculated from individual animal data.

Table 3.8. Correlations between marbling score and concentrations of retinol and α-tocopherol in both the plasma and liver and vitamin A intake.

	Treatment day				
Item[a]	0	56	112	142/143	Overall
Plasma retinol					
r	-0.07	-0.07	0.13	0.04	0.05
P-value	0.471	0.436	0.150	0.701	0.619
Plasma α-tocopherol					
r	0.03	0.05	-0.06	-0.13	-0.06
P-value	0.769	0.571	0.494	0.166	0.520
Liver retinol					
r	-0.01	0.18	0.11	0.06	0.08
P-value	0.944	0.050	0.248	0.533	0.392
Liver α-tocopherol					
r	-0.12	0.04	0.05	-0.16	-0.10
P-value	0.189	0.665	0.621	0.093	0.262
Vitamin A intake[b]					
r					0.13
P-value					0.411

[a]Calculated from individual animal data unless otherwise stated.
[b]Calculated from pen data.

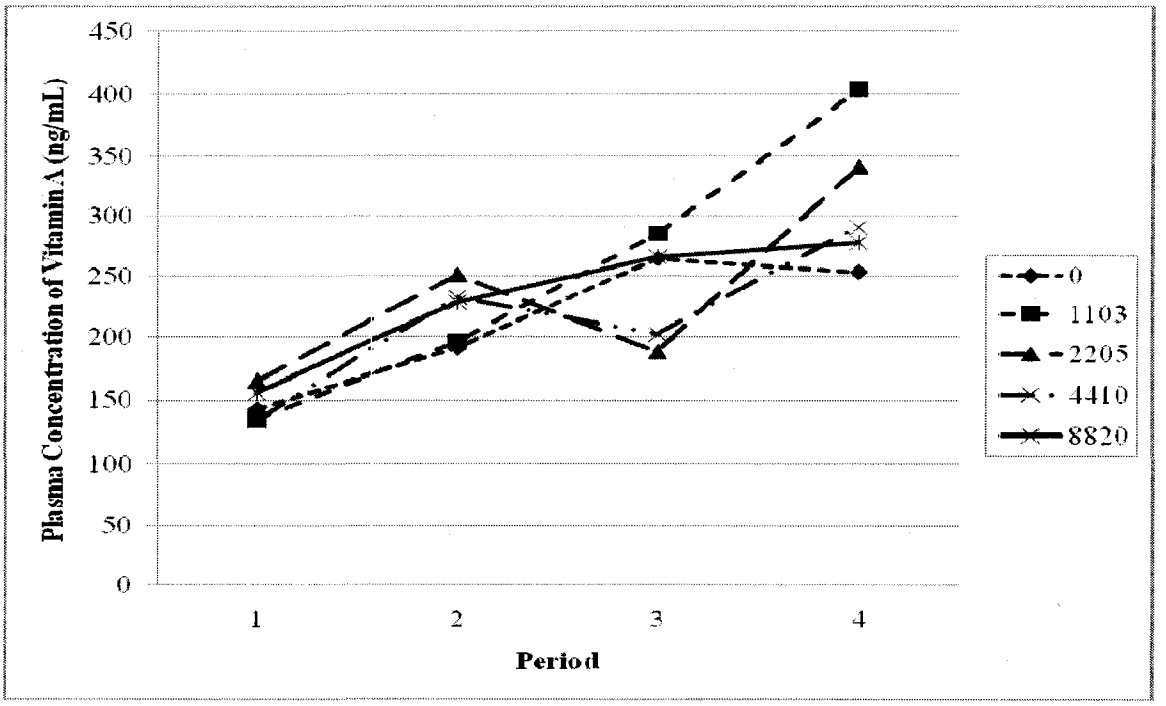

Figure 3.1. Plasma concentrations of retinol (ng/mL) for each period (d 0, 56, 112, and 142/143) and for each of the 5 dietary supplemental vitamin A treatments (0, 1,103, 2,205, 4,410, and 8,820 IU/kg of DM). Treatment, $P = 0.342$; period, $P < 0.001$; period × treatment, $P = 0.328$.

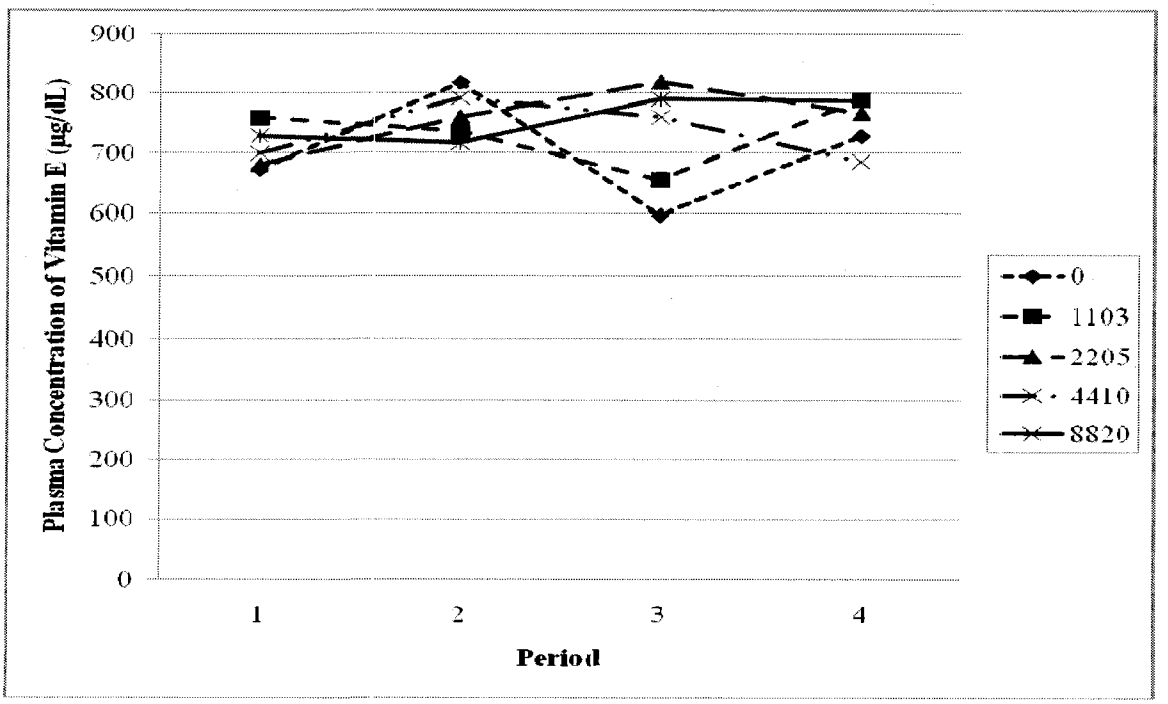

Figure 3.2. Plasma concentrations of α-tocopherol (µg/dL) for each period (d 0, 56, 112, and 142/143) and for each of the 5 dietary supplemental vitamin A treatments (0, 1,103, 2,205, 4,410, and 8,820 IU/kg of DM). Treatment, $P = 0.503$; period, $P = 0.325$; period × treatment, $P = 0.127$.

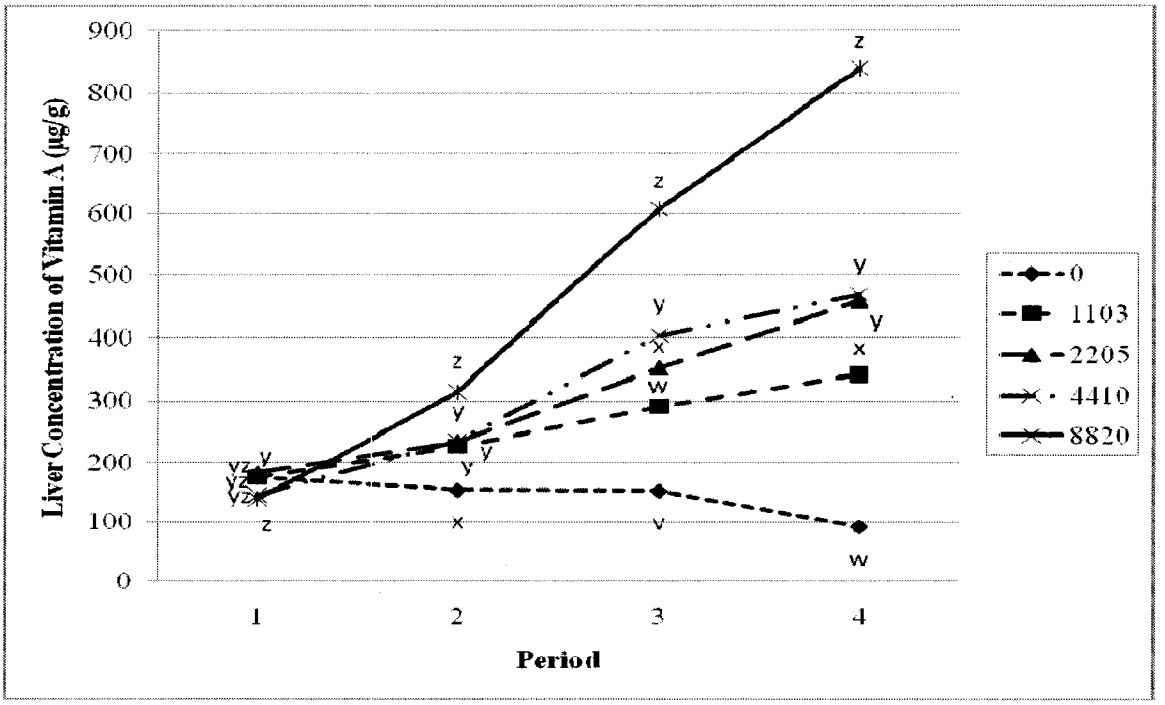

Figure 3.3. Liver concentrations of retinol (µg/g of DM) for each period (d 0, 56, 112, and 142/143) and for each of the 5 dietary supplemental vitamin A treatments (0, 1,103, 2,205, 4,410, and 8,820 IU/kg of DM). Treatment, $P < 0.001$; period, $P < 0.001$; period × treatment, $P < 0.001$.

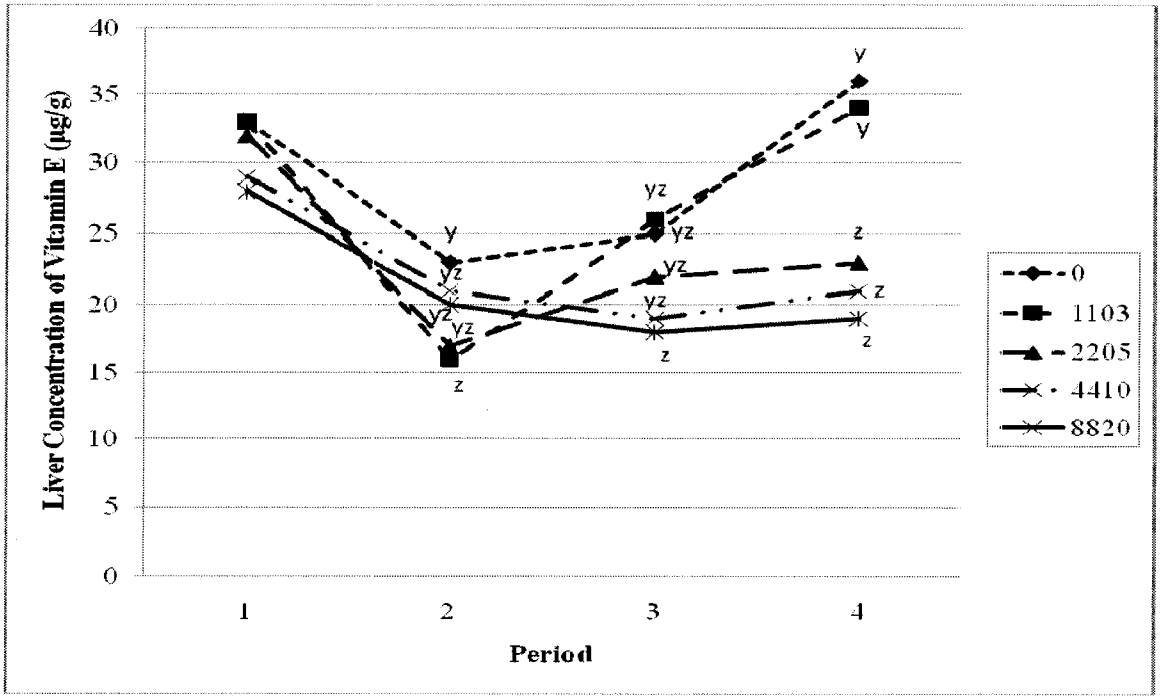

Figure 3.4. Liver concentrations of α-tocopherol (µg/g of DM) for each period (d 0, 56, 112, and 142/143) and for each of the 5 dietary supplemental vitamin A treatments (0, 1,103, 2,205, 4,410, and 8,820 IU/kg of DM). Treatment, $P = 0.001$; period, $P < 0.001$; period × treatment, $P = 0.007$.

CHAPTER IV

EFFECTS OF RACTOPAMINE AND COMBINED TRENBOLONE ACETATE AND ESTRADIOL IMPLANT REGIMENS ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS IN FEEDLOT STEERS

ABSTRACT

Yearling steers (n = 486; initial BW = 305 kg ± 10.4 kg) were used to evaluate the effects of ractopamine (Optaflexx; Elanco Animal Health; RAC) and implant/reimplant (IMP) regimen on performance and carcass traits. Steers were blocked by initial BW into 6 replicates and assigned randomly to treatments (9/pen). The 3 x 3 factorial arrangement included RAC doses of 0 (R0), 100 (R100), or 200 (R200) mg·steer^{-1}·d^{-1} fed for 28 d and IMP regimens of None/None (N/N), Revalor-S/None (S/N), or Revalor-IS/Revalor-S (I/S). Except for KPH and skeletal maturity, no RAC x IMP interactions were noted ($P > 0.10$). Compared with R0, steers fed R200 had 26% ($P < 0.001$) greater ADG and 27.6% ($P < 0.001$) greater G:F during the final 28-d supplementation period, resulting in 4.7% ($P = 0.009$) greater G:F from d 0 to slaughter. Carcasses from R200 were 6.3 kg ($P = 0.042$) heavier than those from R0. Marbling, empty body fat (EBF), and USDA quality grade did not differ among RAC treatments ($P > 0.10$). For IMP, I/S cattle had 6.0% ($P < 0.001$) and 23.5% ($P < 0.001$) greater ADG and 4.9% ($P = 0.007$) and 12.3% ($P < 0.001$) greater G:F over the 168-d feeding period than S/N and N/N, respectively. The I/S steers had 12.6 kg ($P = 0.001$) and 41.1 kg ($P < 0.001$) greater HCW than S/N and N/N, respectively. Despite no difference ($P > 0.10$) in EBF, marbling score was decreased for I/S ($P < 0.001$) and S/N ($P = 0.001$) relative to N/N,

resulting in 14.6 and 11.4 percentage unit fewer USDA Prime and Choice carcasses with I/S ($P = 0.008$) and S/N ($P = 0.039$) than with N/N. These data suggest that the effects of IMP and RAC on growth and carcass traits are independent, and that USDA quality grade and marbling can differ significantly despite carcasses having equal EBF. Further research is needed to elucidate the exact mode of actions of these growth-enhancing products.

Key words: beta-adrenergic agonists, body composition, growth, hormone, implants, ractopamine

INTRODUCTION

Steroidal implants and beta-adrenergic agonists are believed to elicit growth responses via separate modes of action. Steroidal implants primarily act by binding to cytosolic receptors, which then act as trans-activating factors in the nucleus to stimulate gene expression and hence translation of growth-enhancing protein hormones such as insulin-like growth factor and growth hormone. In contrast, ractopamine, a beta-adrenergic agonist, binds to cell-surface beta-adrenergic receptors to initiate signal transduction pathways and transcription of proteins such as myosin and actin, which are the primary proteins of skeletal muscle. Although steroidal implants and beta-agonists work through separate mechanisms, both act to increase protein deposition. Therefore, whether the growth responses to steroidal implants and beta-adrenergic agonists depend on each other or are additive in a production setting is not known. The purpose of this study was to determine the effects of ractopamine (Optaflexx; Elanco Animal Health; RAC) supplementation and various implant/reimplant regimens on growth performance and carcass characteristics of finishing steers.

MATERIALS AND METHODS

Before the initiation of this experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Animals and management. Four hundred eighty-six yearling steers from 6 different sources were used in this study. Steers arrived at the feedlot from May 27, 2004 through June 3, 2004. All steers had access to long-stemmed grass hay and water on arrival, and all cattle were processed within 24 h of arrival. Processing included treatment for parasites and flies with Dectomax (doramectin, Pfizer Animal Health, Exton, PA) and Durasect Pour-On (permethrin and pyrethrin, Pfizer Animal Health), respectively, and vaccination with Bovishield-4 (bovine rhinotracheitis - viral diarrhea - parainfluenza-3 - respiratory syncytial virus vaccine, Pfizer Animal Health) and for *Clostridial* species with Ultrabac CD (*Clostridium perfringens* Types B, C, & D Bacterin-Toxoid, Pfizer Animal Health).

All steers received the same basal diets for the entire experiment (Table 4.1). The finishing diets contained monensin (trade name Rumensin; Elanco Animal Health, Greenfield, IN) and tylosin (trade name Tylan; Elanco Animal Health) at 33 and 11 mg/kg DM, respectively. All diets were fed twice daily. Diet transitions were simultaneous for all treatments between d 3 and 5 on each diet. Seven days before the initiation of the feeding of RAC treatments (35 d before slaughter), cattle were transitioned to new sequences of feeding to minimize effects associated with changes in feeding times. Cattle were fed in the following order of RAC treatments: 0 $mg \cdot steer^{-1} \cdot d^{-1}$, 100 $mg \cdot steer^{-1} \cdot d^{-1}$, and 200 $mg \cdot steer^{-1} \cdot d^{-1}$. All diets used for each treatment were

manufactured immediately before feeding with the on-site stationary mixer at Continental Beef Research (CBR). Cattle were allowed to consume diets on an ad libitum basis. Before the RAC treatment period, diets were sampled weekly and analyzed for DM determination. Fresh weekly samples were composited into a monthly sample, which was shipped to SDK Laboratories (Hutchinson, KS) for routine DM, NDF, CP, non-protein N, ether extract, and mineral analyses (Table 4.2). During the RAC-supplementation period, each of the 3 RAC treatment diets was sampled 3 times/wk, and the 3 samples were also shipped to SDK Laboratories. The weekly samples were composited and analyzed for DM, CP, non-protein N, ADF, ether extract, Ca, and P (Table 4.3). In addition, before analysis of the RAC treatment diets, personnel at SDK Laboratories split each diet sample and shipped a subsample of each weekly composite sample to Eurofins Scientific (345 Adams Ave., Memphis, TN) to be assayed for ractopamine hydrochloride and monensin.

A Type-B supplement containing minerals, urea, vitamins, monensin, and tylosin was manufactured at the start and throughout the duration of the trial as needed (typically weekly) at CBR. During the RAC-supplementation period, 1 of 3 additional, ground corn-based Type-B supplements was added to the respective RAC treatment diets. According to treatment, the Type-B ractopamine supplements were formulated to contain 0, 441, and 882 mg/kg of RAC (DM basis). All RAC treatment Type-B supplements were manufactured by Akey (Lewisburg, OH) and were shipped to CBR 2 wk before the initiation of RAC treatments.

Feed refusals were weighed and sampled for DM determination whenever feed became off-conditioned, either as a result of adverse weather conditions or because of

feed remaining in the bunk for 3 d consecutively, on weigh days, and at slaughter. Feed refusal samples were evaluated for DM content on-site by drying the samples for 48 hours at 60°C in a convection oven. Dry matter consumption for each pen was calculated by subtracting the amount of DM refusals from the amount of DM delivered and dividing the result by animal-days for the pen.

Experimental Design and Treatments. On June 9, 2004 (d -1) steers were individually weighed, tagged with an electronic identification tag, and classified according to breed (British, Continental, Longhorn, dairy, less than 1/4 Brahman, and greater than 1/4 Brahman) and color (black, red, and yellow/white/gray). Steers that had been treated for any ailment and those that were classified as Longhorn, dairy, or greater than 1/4 Brahman were excluded from the study. Steers were then ranked by BW, and steers that were more than 2 SD from the mean BW were also excluded from the study. Steers were sorted by breed, color within breed, and weight within breed × color category; and then were assigned to 1 of 6 weight blocks within each breed × color category, so that an equal number of animals were assigned to each weight block. Steers were then sorted by weight block, breed within weight block, color within weight × breed block, and individual BW within weight × breed × color block (lightest to heaviest). In groups of 9 and in ascending order of individual BW within weight × breed × color block, 1 of 9 treatments was assigned randomly to the eligible 486 individual animals. Treatments were arranged in a 3 × 3 factorial layout of RAC level and implant/reimplant regimen (Table 4.4). Ractopamine supplementation levels included: 1) 0; 2) 100; and 3) 300 mg·steer^{-1}·d^{-1}. Implant/reimplant regimens included: 1) None/None; 2) Revalor-S (120 mg of trenbolone acetate and 24 mg of estradiol-17 Beta; Intervet/Schering-

Plough Animal Health, Millsboro, DE)/None; and 3) Revalor-IS (80 mg of trenbolone acetate and 16 mg of estradiol-17 Beta; Intervet/Schering-Plough Animal Health)/Revalor-S. By following this procedure, 6 weight blocks each with 9 treatments and 9 animals in each weight block × treatment combination were formed. Fifty-four CBR pens (6 x 18 m; soil surfaced pens; bunk spacing = 68 cm/animal) were grouped into six, 9-pen clusters, and 1 of the 6 steer weight blocks was assigned randomly to each pen cluster. Treatments were then assigned randomly to pens within cluster. As a result, six, 9-animal pens were available for each treatment, with each pen having equal representation of breeds and colors. The factorial layout used in this study resulted in 6 weight blocks, 18 pen replicates per each RAC treatment, 18 pen replicates per each implant/reimplant regimen, and 6 replicates for each RAC-supplementation × implant/reimplant treatment subclass.

On June 10, 2004, steers were weighed individually (scale readability ± 1 lb; scale calibrated with 1,000 lb of certified weights before use) tagged with ear tags identifying treatment (1 to 9), weight block (1 to 6), and animal number (1 to 9) in each pen, and implanted according to the implant/reimplant treatment regimen. Cattle were sorted into their respective treatment pens, and the trial was initiated. On d 56, all steers were weighed individually, and cattle on the Revalor-IS/Revalor-S treatment were reimplanted with Revalor-S. On d 112 cattle were individually weighed, and 12^{th} rib fat thickness was estimated by ultrasound technology. Weight and 12th rib fat measurements were used to assess marketing endpoints and to set a date for the initiation of feeding of RAC treatments (28 d before slaughter). The heavy weight blocks (blocks 5 and 6) began receiving RAC treatments on October 20, 2004 (d 132), whereas weight blocks 1 to 4

began receiving RAC treatments on November 3, 2004 (d 146). Steers in all blocks received their respective RAC treatment for 28 d consecutively. Cattle were weighed individually at the initiation of the RAC-feeding period and on the 2 d consecutively before slaughter.

Ten steers were excluded from the dataset before data analysis (Appendix Tables 4.17 and 4.18). Conditions that required an animal to be removed from the study, during the study period, were considered adverse drug experiences and were reported to Pharmacovigilance at Elanco Animal Health within 24 h of the incident.

Weather events involving substantial moisture accumulation occurred on several days coinciding with the RAC treatment period (Appendix Table 4.20). The moisture from these weather patterns created undesirable pen conditions. In addition, anomalies in intake patterns were anecdotally observed and could have affected ADG, feed efficiency, gut fill, and hence final BW. Despite these possible effects, all treatments should have been affected equally.

Slaughter and Carcass Data Collection. Steers were slaughtered in 2 groups (blocks 5 to 6 and blocks 1 to 4). On the day of slaughter, steers in each group were transported approximately 402 km to Swift and Company, Greeley, CO where they were slaughtered using conventional and humane procedures. Carcasses were chilled in a cooler with an air temperature of 2°C for 36 h, and sprayed intermittently (2 min on, 8 min off) with a fine mist of 2°C water for the first 8 h of the chill period. Following the carcass-chilling period, a USDA grading supervisor assigned marbling scores and lean maturity assessments to each carcass. Longissimus muscle area (LMA) measurements for each carcass were obtained by applying blotting paper (LS-601; Life Science

Products; Frederick, CO) to the LM of the left side of each carcass. The outline of the LM remaining on the blotting paper for each carcass was traced and subsequently scanned using software designed to measure area (MeatScan Image Analysis Software; AEW Consulting; Lincoln, NE). A panel of 2 evaluators (Colorado State Univ. personnel) independently evaluated each carcass and recorded measurements/assessments of adjusted fat thickness, adjusted preliminary yield grade, and skeletal maturity. Values for each trait from the 2 evaluators were averaged, resulting in a single value for each grade factor for each carcass. One evaluator from Colorado State Univ. assessed the percentage of kidney, pelvic, and heart fat (KPH). Skeletal and lean maturities were recorded on the following continuous scale: 100 = A-Maturity and 200 = B-Maturity. Marbling scores were assigned to each carcass using the following continuous scale: 300 = Traces; 400 = Slight; 500 = Small; 600 = Modest; 700 = Moderate; and 800 = Slightly Abundant. Yield grade for each carcass was calculated as: 2.50 + (2.50 × adjusted fat thickness, inches) + (0.20 × percent KPH) + (0.0038 x hot carcass weight, pounds) – (0.32 × LM area, square inches). As described by Guiroy et al. (2002), percent EBF was calculated as: 17.76207 + (4.68142 × adjusted fat thickness, cm) + (0.01945 × hot carcass weight, kg) + (0.8185 × marbling score/100) – (0.06754 × LM area, cm^2).

Statistical Analyses. Analyses of growth traits and non-categorical carcass characteristics were conducted using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The initial statistical model included RAC treatment, IMP treatment, and all the two-way interactions between RAC treatment, IMP treatment, and weight block as fixed effects. The two-way interactions of RAC treatment × weight block and IMP treatment × weight block were subsequently removed from the model if not significant ($P > 0.10$).

The final analysis of variance (ANOVA) model for all growth performance traits and non-categorical carcass characteristics included RAC treatment, IMP treatment, and their two-way interaction as fixed effects and weight block as a random effect. Least squares means were separated ($P < 0.10$) using a protected pairwise *t*-test (PDIFF option of SAS) when *F*-tests were significant at $\alpha = 0.10$.

The USDA yield and quality grade data were evaluated as categorical data (binomial proportion) using the GLIMMIX procedure of SAS. Ractopamine treatment, IMP treatment, and the two-way interaction between RAC and IMP treatment were included in the model as fixed effects, and weight block was included as a random effect. Simple arithmetic frequencies are presented. All independent variables included in the model were considered class variables.

RESULTS AND DISCUSSION

A primary objective of this study was to determine the effects of RAC supplementation in combination with various implant/reimplant regimens on feedlot performance and carcass traits of steers and to determine whether responses to RAC and implant/reimplant regimens were independent. Except for KPH and skeletal maturity, no interactions between RAC-supplementation level and implant/reimplant regimen were noted ($P < 0.10$), indicating that the responses associated with various implant/reimplant regimens and RAC-supplementation level are independent. Consequently, this discussion will be focused on the main effects of RAC supplementation and implant/reimplant regimen treatments on growth performance and carcass traits.

Ractopamine Supplementation. Performance data for RAC treatments are presented in Tables 4.5 and 4.6. Although there seemed to be a numeric trend for increased live

BW at slaughter associated with increasing RAC-supplementation level, no statistical differences were detected in BW on any weigh day ($P < 0.10$). Steers receiving 100 and 200 mg·steer^{-1}·d^{-1} RAC showed a 26% (0.25 kg/d) improvement ($P < 0.001$) in ADG during the final 28-d supplementation period compared with steers receiving the control diet (0 mg·steer^{-1}·d^{-1}). Others have shown an 11.1 to 27.4% increase in ADG in steers fed 200 mg of RAC the final 28 d of the feeding period (Laudert et al., 2005a,b; Schroeder et al., 2005a,b,c; Van Koevering et al., 2006a,b,c; Winterhollar et al., 2007; Gruber et al., 2007; Platter et al., 2008). Despite this increase, no statistical differences in ADG over the entire feeding period were noted among the RAC treatments ($P = 0.333$). The fact that statistical differences in ADG noted during the RAC treatment period were not observed for the entire feeding period is likely a result of a dilution effect. Ractopamine was only fed for 28 d of the 168 d average trial period and only accounted for 17% of the entire feeding period. The increase in ADG and live BW associated with the RAC treatments during the final 28 d of the feeding period was not sufficiently large to significantly shift the mean ADG of the entire feeding period. If more replicates had been used, perhaps these differences would have become significant.

Compared with the controls, steers receiving the 100 mg·steer^{-1}·d^{-1} RAC treatments showed significant improvements in G:F ratio during the periods from the initiation of RAC treatment feeding to slaughter (26.7%, 0.028 units; $P < 0.001$) and from d 0 to slaughter (3.0%, 0.005 units; $P = 0.077$). Moreover, greater improvements in G:F ratio were observed in cattle on the 200 mg·steer^{-1}·d^{-1} treatment during the periods from d 56 to slaughter (4.7%, 0.007 units; $P = 0.030$), from the initiation of the RAC treatment feeding to slaughter (27.6%, 0.029 units; $P < 0.0001$), and from d 0 to slaughter (4.7%,

0.008 units; $P = 0.0032$). Others have shown 12.4 to 23.4% improvements in feed efficiency in steers fed 200 mg/d for the final 28 d of the finishing period (Laudert et al., 2005a,b; Schroeder et al., 2005a,b,c; Van Koevering et al., 2006a,b,c; Winterhollar et al., 2007; Gruber et al., 2007; Platter et al., 2008). Feed efficiency did not differ ($P > 0.10$) between steers receiving the 100 and 200 mg·steer^{-1}·d^{-1} for any period. In addition, average daily feed intake, expressed on a DM basis, did not differ ($P > 0.10$) between RAC treatments for any period.

Carcass data for the 28-d RAC treatments are presented in Tables 4.7, 4.8, and 4.9. No differences were detected ($P > 0.10$) between RAC-supplementation treatments for adjusted fat thickness, lean maturity, or overall maturity. Steers receiving the 200 mg·steer^{-1}·d^{-1} RAC treatment produced carcasses that were 6.3 kg (1.7%; $P = 0.042$) and 5.3 kg (1.4%; $P = 0.086$) heavier than carcasses produced by steers receiving 0 and 100 mg·steer^{-1}·d^{-1} RAC, respectively. Others have observed 1.3 to 2.2% increases in the carcass weights of steers fed 200 mg RAC/d for the final 28 d before slaughter (Laudert et al., 2005a,b; Schroeder et al., 2005a,b,c; Van Koevering et al., 2006a,b,c; Winterhollar et al., 2007; Gruber et al., 2007; Platter et al., 2008). Steers supplemented with 200 mg·steer^{-1}·d^{-1} RAC had 2.3 cm^2 ($P = 0.018$) and 2.3 cm^2 ($P = 0.017$) larger LM area measurements than the steers receiving 0 and 100 mg·steer^{-1}·d^{-1} RAC, respectively. Steers receiving the 200 mg·steer^{-1}·d^{-1} RAC treatment had a 0.46 percentage unit (0.7%; $P = 0.014$) and 0.63 percentage unit greater (1.0%; $P = 0.001$) dressing percent compared with those from the 0 and 100 mg·steer^{-1}·d^{-1} RAC treatment steers, respectively. Previous research has indicated -0.1 to 0.5% increases in dressing percent with RAC at 200 mg/d (Laudert et al., 2005a,b; Schroeder et al., 2005a,b,c; Van

Koevering et al., 2006a,b,c; Winterhollar et al., 2007; Gruber et al., 2007; Platter et al., 2008). No statistical differences in carcass weight ($P = 0.733$), LM area ($P = 0.979$), or dressing percent ($P = 0.331$) between the 100 mg·steer^{-1}·d^{-1} RAC treatment and the controls were noted. Marbling and empty body fat (Table 4.7), as well as USDA quality grade distribution and incidence of dark-cutting beef (Table 4.8) did not differ ($P > 0.10$) between RAC treatments. Similarly, mean calculated yield grades (Table 4.7) and distribution of yield grades (Table 4.9) did not differ ($P > 0.10$) between RAC treatments. A RAC × implant/reimplant treatment interaction was detected for KPH ($P = 0.062$) and skeletal maturity ($P = 0.059$), but reasons for these interactions are not readily evident.

Implant/Reimplant Regimen. Performance data for implant/reimplant regimen treatments are presented in Tables 4.5 and 4.6. On d 56 (reimplant), live BW of the Revalor-S/None and Revalor-IS/Revalor-S steers did not differ from each other ($P = 0.784$) but were 12.5 kg ($P < 0.001$) and 11.9 kg ($P < 0.001$) heavier, respectively, than the None/None steers. At the beginning of the RAC-treatment period, steers administered Revalor-IS at arrival and Revalor-S at reimplant had 13.0 kg ($P = 0.002$) and 49.6 kg ($P < 0.001$) heavier live BW than steers on the Revalor-S/None and None/None implant/reimplant regimens, respectively. In addition, the live BW of the Revalor-S/None cattle at the start of RAC treatments were 36.6 kg heavier ($P < 0.001$) than those of the None/None cattle. At slaughter, these BW differences increased, and steers administered Revalor-IS at arrival and Revalor-S at reimplant had 16.6 kg ($P = 0.001$) and 56.1 kg ($P < 0.001$) heavier live BW than steers on the Revalor-S/None and None/None implant/reimplant regimens, respectively. In addition, the live BW at

slaughter of the Revalor-S/None cattle were 39.5 kg heavier ($P < 0.001$) than those of the None/None cattle.

From d 0 to 56 (reimplant), steers receiving Revalor-S and Revalor-IS on d 0 had similar ADG ($P = 0.885$) and G:F ratios ($P = 0.660$). However, during the same period and compared with cattle not receiving an initial implant, greater ADG was noted in the Revalor-S/None (12.6%, 0.22 kg/d; $P < 0.001$) and Revalor-IS/Revalor-S (12.3%, 0.22 kg/d; $P < 0.001$) cattle, and improved G:F was observed in the Revalor-S/None (11.2%, 0.023 units; $P < 0.001$) and Revalor-IS/Revalor-S (10.2%, 0.021 units; $P < 0.001$). For all other periods except for the final 28 d, statistical differences ($P < 0.10$) between every implant/reimplant regimen existed. For the entire feeding period, the Revalor-IS/Revalor-S cattle showed 6.0% (0.10 kg/d; $P = 0.001$) and 23.5% (0.34 kg/d; $P < 0.0001$) improvements in ADG compared with the Revalor-S/None and None/None treatments, respectively. The Revalor-S/None steers gained 16.5% (0.24 kg/d; $P < 0.001$) faster than the None/None treatment from d 0 to slaughter. Similarly, over the same period, 4.9% (0.008 units; $P = 0.001$) and 12.3% (0.020 units; $P < 0.001$) improvements in G:F were observed in the Revalor-IS/Revalor-S steers compared with the Revalor-S/None and None/None treatments, respectively. The Revalor-S/None steers converted feed to gain 7.4% (0.012 units; $P < 0.001$) more efficiently than the None/None treatment from d 0 to slaughter. These increases in growth and efficiency are similar to responses summarized and observed by others (Johnson et al., 1996b; Duckett et al., 1997; Preston, 1999; Montgomery et al., 2001; Nichols et al., 2002).

Dry matter intake did not differ ($P = 0.539$) among implant/reimplant treatments from d 0 to 56; however, differences ($P < 0.10$) in intake were noted in all other periods, and

intake tended to numerically increase with increasing number of implants administered. Over the entire feeding period, the Revalor-IS/Revalor-S cattle consumed 0.91 kg/d ($P < 0.001$) more feed than the None/None cattle, and the Revalor-S/None steers consumed 0.78 kg/d ($P < 0.001$) more feed than the None/None treatment. Although not significant ($P = 0.400$), the Revalor-IS/Revalor-S steers ate 0.13 kg/d more feed than the Revalor-S/None cattle, and this consumption pattern was consistent during each trial period. In agreement with these results, Duckett et al. (1997) and Johnson et al. (1996b) summarized that steroid implants typically elicit an increase in intake over negative controls. Although a significant interaction between RAC treatment and implant/reimplant regimen was detected for RAC dose, this is likely an artifact that resulted from the fact that intake increased with increasing implant/reimplant regimen aggressiveness and that the control RAC diets did not contain any RAC; therefore, dose did not increase with intake for the control RAC steers. Although no RAC treatment × implant/reimplant treatment interactions for performance variables were detected, it seemed that the non-implanted cattle benefited more from RAC supplementation in terms of live and carcass weight relative to the RAC dose than the other implant treatments (Appendix Tables 4.11 and 4.13).

Carcass data for implant/reimplant treatments are presented in Tables 4.7, 4.8, and 4.9. Carcass weights, dressing percent, and LM area increased with increasing number of implants. The Revalor-IS/Revalor-S steers had 12.6 kg ($P = 0.001$) and 41.1 kg ($P < 0.001$) heavier carcass weights than steers on the Revalor-S/None and None/None implant treatments, respectively. Similarly, the Revalor-S/None steers had 28.5 kg heavier carcass weights ($P < 0.001$) than the non-implanted controls. Dressing percent

responded in a similar fashion, with the Revalor-IS/Revalor-S steers having 0.34 percentage unit ($P = 0.064$) and 1.02 percentage unit ($P < 0.001$) greater yields than the Revalor-S/None and None/None cattle, respectively. The Revalor-S/None cattle had 0.68 percentage unit greater dressing percent ($P = 0.001$) than the None/None steers. Likewise, the Revalor-IS/Revalor-S steers had 3.36 cm^2 ($P = 0.001$) and 0.55 cm^2 ($P < 0.001$) bigger LM than the Revalor-S/None and None/None cattle, respectively. The Revalor-S/None cattle had 4.45 cm^2 larger LM ($P < 0.001$) than the non-implanted controls.

Lean maturity scores decreased with increasing number of implants, with the Revalor-IS/Revalor-S steers having lower lean maturity scores ($P = 0.018$) than the None/None steers. The lean maturity for the Revalor-S/None treatment did not differ from the Revalor-IS/Revalor-S ($P = 0.417$) or the None/None ($P = 0.101$) treatments. As previously noted, a RAC × implant/reimplant treatment interaction was noted for skeletal maturity ($P = 0.059$), and individual treatment responses are reported in Appendix Tables 4.10 and 4.13. Overall maturity was greater for the Revalor-S/None ($P = 0.001$) and Revalor-IS/Revalor-S ($P = 0.001$) treatments compared with the None/None treatment. A RAC × implant/reimplant treatment interaction was also detected for KPH ($P = 0.062$).

In contrast, no differences in adjusted fat thickness were detected ($P > 0.10$) among implant regimens. Similarly, no differences ($P > 0.10$) in yield grade distributions or incidence of dark cutters were associated with implant/reimplant treatments, nor did empty body fat differ ($P = 0.369$) among implant treatments. On the contrary, marbling score was decreased for the Revalor-IS/Revalor-S ($P < 0.001$) and Revalor-S/None ($P = 0.001$) treatments relative to the negative control treatment. As a result, the percentage of

Prime and Choice carcasses numerically decreased with increasing number of implants, whereas the percentage of Select and Standard carcasses increased with increasing number of implants. The Revalor-IS/Revalor-S treatment had 14.64 percentage units fewer carcasses grading Prime and Choice ($P = 0.008$) than the None/None treatment, and the Revalor-S/None treatment had 11.39 percentage units fewer Prime and Choice carcasses ($P = 0.039$) than the negative controls. The percentage of Prime and Choice carcasses did not differ ($P = 0.518$) between the Revalor-S/None and Revalor-IS/Revalor-S treatment.

The fact that marbling scores and quality grade distributions differed among implant/reimplant treatments, whereas empty body fat did not differ suggests that implants affect fat deposition. Numerous trials have shown that implants decrease quality grade and marbling score in beef cattle (Loy et al., 1988; Johnson et al., 1996b; Samber et al., 1996; Morgan, 1997; Pritchard, 2000; Duckett and Andrae, 2001; Montgomery et al., 2001; Reiling and Johnson, 2003; Bruns et al., 2005; McPhee et al., 2006). Moreover, quality grade has been shown to decrease in a dose-dependent fashion in response to increased aggressiveness in implant regimens (Samber et al., 1996; Foutz et al., 1997; Morgan, 1997; Roeber et al., 2000; Platter et al., 2003).

As will be discussed more thoroughly in the next chapter, previous researchers (Owens et al., 1995; Nichols et al., 2002; Guiroy et al., 2002) have suggested that marbling and thereby quality grade will not be affected by implant regimens as long as cattle are slaughtered at common empty body fat endpoints. In the present study, marbling and the percentage of carcasses grading Prime and Choice were decreased by increasing implant regimen aggressiveness, despite the fact that empty body fat did not

differ among implant treatments. In contrast to the results with implant regimen, marbling and quality grade distribution were not affected by RAC treatments. This finding may suggest that the potential of cattle to deposit marbling is determined early in the feeding phase, so that products such as implants, which are used during the early stages of marbling deposition and that partition the deposition of muscle and fat, may alter and even retard the development of intramuscular adipocytes. Ractopamine and steroidal implants are known to elicit responses via different signal transduction pathways. The data from the current trial may suggest that RAC does not affect the deposition of intramuscular fat itself but instead may simply increase net protein deposition. If implants were administered later in the feeding period, then perhaps marbling would be affected to a lesser degree; however, this practice could result in lesser net returns as a result of decreased saleable carcass weight associated with the potential for increased cattle handling, lower cumulative anabolic dosages, and the decreased efficiency of muscle vs. fat deposition later in the feeding period. Compared with steroidal implants, RAC seems to have been targeted during the optimal portion of the growth curve and finishing period to increase protein deposition and carcass weight with minimal effects on fat deposition.

IMPLICATIONS

In summary, RAC seems to increase protein accretion and improve animal performance beyond that observed when implants are administered in the absence of RAC. More research is needed to elucidate optimal implant regimens when RAC is supplemented in a commercial feedlot setting under varying economic conditions. To ensure maximum profitability, growth and carcass changes associated with use of

steroidal hormones and beta-adrenergic agonists must be considered when evaluating alternative production systems.

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Table 4.1. Specifications for diets fed during the experiment, DM basis.

Ingredient,%	Starting	Step-1	Step-2	Finishing: Before ractopamine period	Finishing: Ractopamine period
Alfalfa hay	33.02	23.88	13.47		
Steam-flaked corn	41.48	51.59	64.50	81.07	79.19
Sorghum silage	20.00	15.00	12.00	7.66	7.64
CCDS[a]	3.00	3.00	3.00	3.00	2.99
Yellow grease	0.00	1.00	2.00	3.50	3.49
Soybean meal	2.05	4.42	2.93	0.96	0.94
Supplement[bc]	0.44	1.11	2.11	3.81	3.70
Ractopamine supplement[de]					2.04
Calculated chemical composition[f]					
DM, %	59.6	65.1	67.2	70.6	71.2
CP, %	14.50	14.00	13.50	13.50	13.50
Non-protein N, %	0.75	1.00	2.00	4.00	4.00
Neutral detergent fiber, %	27.89	23.17	18.26	11.66	11.66
Forage NDF, %	24.00	18.00	12.00	4.00	4.00
Crude fiber, %	16.83	12.99	9.43	4.67	4.67
NEm Mcal•kg^{-1}	1.73	1.87	2.02	2.22	2.22
NEg, Mcal•kg^{-1}	1.11	1.23	1.37	1.54	1.54
Fat (ether extract), %	2.73	3.89	5.15	6.98	6.98
Ca, %	0.70	0.70	0.70	0.70	0.70
P, %	0.27	0.30	0.29	0.28	0.28
K, %	1.56	1.35	1.04	0.70	0.70
Mg, %	0.24	0.25	0.25	0.25	0.25
Zn, ppm	70	70	70	70	70
Vitamin A, IU/kg	3308	3308	3308	3308	3308
Vitamin E, IU/kg	33.08	33.08	33.08	33.08	33.08

[a]Condensed corn distillers soluble (i.e., corn steep).
[b]Formulated to provide monensin in the finishing diet at 33 mg/kg (DM basis).
[c]Formulated to provide tylosin in the finishing diet at 11 mg/kg (DM basis).
[d]Formulated to provide ractopamine at the following supplemental concentrations (as-fed basis): control at 0 mg/kg; 100 mg·steer^{-1}·d^{-1} treatment at 441 mg/kg; and 200 mg·steer^{-1}·d^{-1} treatment at 882 mg/kg. Samples of the 441 and 882 mg/kg ractopamine supplements tested at 97 and 93% of claim, respectively.
[e]Formulated to provide ractopamine at the following dietary concentrations (DM basis): control at 0 mg/kg; 100 mg·steer^{-1}·d^{-1} treatment at 10.4 mg/kg; and 200 mg·steer^{-1}·d^{-1} treatment at 20.7 mg/kg.
[f]Diets were formulated to meet or exceed all nutrient requirements for finishing steers (NRC, 1996).

Table 4.2. Chemical composition of diets based on assayed results, DM basis.

Item	Starting	Step-1	Step-2	Finishing: Before ractopamine period
Number of composited samples	4	2	2	4
DM, %	60.92±0.88	61.14±0.14	63.49±0.29	71.27±0.38
CP, %	13.78±0.29	13.15±0.41	13.56±0.26	13.08±0.39
Non-protein N, %	0.76±0.09	0.96±0.02	1.92±0.11	3.14±0.08
NDF, %	27.56±2.28	20.36±0.12	18.21±1.15	12.60±0.44
Fat (ether extract), %	2.98±0.31	4.07±0.09	5.50±0.07	6.66±0.23
Ca, %	0.81±0.05	0.70±0.00	0.73±0.01	0.81±0.06
P, %	0.27±0.00	0.29±0.00	0.31±0.00	0.30±0.01
K, %	1.72±0.12	1.26±0.02	0.95±0.01	0.82±0.09
Mg, %	0.31±0.01	0.29±0.00	0.27±0.00	0.23±0.01

Table 4.3. Chemical composition of ractopamine treatment diets fed during the last 28-d period based on assayed results, DM basis.

	Ractopamine Treatment Diet[a]		
Item	0 mg/d	100 mg/d	200 mg/d
Number of composited samples	6	6	6
DM[b], %	68.51±0.24	68.79±0.20	68.68±0.19
CP, %	13.38±0.11	13.36±0.14	13.45±0.07
Non-protein N, %	3.68±0.15	3.82±0.17	3.86±0.13
ADF, %	6.53±0.19	6.26±0.07	6.35±0.31
NEm, Mcal/kg	2.00±0.0046	2.01±0.0013	2.00±0.0068
NEg, Mcal/kg	1.27±0.0037	1.28±0.0013	1.28±0.0071
Total digestible nutrients, %	78.73±0.12	78.90±0.04	78.84±0.20
Fat (ether extract), %	6.99±0.05	7.06±0.16	7.23±0.18
Ca, %	0.80±0.05	0.84±0.03	0.85±0.03
P, %	0.31±0.01	0.32±0.02	0.33±0.01
Monensin[c], mg/kg	37.8±1.49	37.6±2.04	37.0±0.39
Monensin[c], % of claim	111±4.5	108±6.2	109±1.5
Ractopamine[c], mg/kg	<3.9±0.10	7.59±0.47	16.84±1.15
Ractopamine[c], % of claim	N/A	72.3±4.2	81.1±5.3

[a]Mean ± standard error.
[b]As-received at SDK Laboratories, 1000 Corey Road, Hutchinson, KS 67501.
[c]As analyzed by Eurofins Scientific, 345 Adams Ave., Memphis, TN 38103.

Table 4.4. Implant and ractopamine treatment combinations.

Treatment number	Treatment codes and ractopamine color codes	Ractopamine levels during ractopamine treatment phase (last 28 d)[a]	Implant/Reimplant regimen[b]
1	000282S00 Yellow	Control (0)	Negative control
2	000282S30 Yellow	Control (0)	Revalor-S/None
3	000282S43 Yellow	Control (0)	Revalor-IS/Revalor-S
4	100282S00 White	~100 mg/d	Negative control
5	100282S30 White	~100 mg/d	Revalor-S/None
6	100282S43 White	~100 mg/d	Revalor-IS/Revalor-S
7	200282S00 Red	~200 mg/d	Negative control
8	200282S30 Red	~200 mg/d	Revalor-S/None
9	200282S43 Red	~200 mg/d	Revalor-IS/Revalor-S

[a]Ractopamine was fed to target either 100 or 200 mg·steer^{-1}·d^{-1}.
[b]Revalor-S = 120 mg of trenbolone acetate and 24 mg of estradiol-17-Beta; Revalor-IS = 80 mg of trenbolone acetate and 16 mg of estradiol-17-Beta; Intervet/Schering-Plough Animal Health, Millsboro, DE.

Table 4.5. Least squares means of growth performance for main effects of ractopamine supplementation and implant/reimplant regimen.

	Ractopamine, mg·steer^{-1}·d^{-1}				Implant/Reimplant regimen					
Trait[a]	0	100	200	P_{RAC}	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S	$P_{Implant}$	SEM	$P_{R \times I}$ [c]
Number of pens	18	18	18	-	18	18	18	-	-	-
Initial BW, kg[b]	304.6	304.6	304.2	0.778	304.5	304.6	304.3	0.869	10.4	0.327
d 56 BW, kg[b]	409.5	412.3	412.7	0.346	403.4^{x}	415.9^{y}	415.3^{y}	<0.001	11.2	0.495
End-minus-28 BW, kg[b]	546.8	543.5	546.2	0.667	516.8^{x}	553.4^{y}	566.4^{z}	<0.001	8.8	0.623
End BW, kg[b]	573.5	577.1	579.5	0.427	544.9^{x}	584.4^{y}	601.0^{z}	<0.001	10.1	0.615
ADG; d 0 to 56	1.87	1.92	1.94	0.219	1.76^{x}	1.99^{y}	1.98^{y}	<0.001	0.031	0.581
ADG; d 0 to end-minus-28	1.71	1.69	1.71	0.656	1.51^{x}	1.76^{y}	1.85^{z}	<0.001	0.028	0.613
ADG; d 56 to end-minus-28	1.61	1.54	1.57	0.196	1.33^{x}	1.61^{y}	1.77^{z}	<0.001	0.034	0.831
ADG; d 56 to end	1.46	1.47	1.49	0.71	1.27^{x}	1.50^{y}	1.66^{z}	<0.001	0.040	0.890
ADG; End-minus-28 to end	0.99^{x}	1.25^{y}	1.24^{y}	0.001	1.04^{x}	1.15^{x}	1.28^{y}	0.005	0.091	0.870
ADG; d 0 to end	1.60	1.62	1.64	0.333	1.43^{x}	1.66^{y}	1.76^{z}	<0.001	0.034	0.629
F:G; d 0 to 56	4.70^{x}	4.54^{y}	4.46^{y}	0.032	4.88^{x}	4.39^{y}	4.43^{y}	<0.001	0.098	0.938
F:G; d 0 to end-minus-28	5.55	5.54	5.42	0.259	5.91^{x}	5.42^{y}	5.18^{z}	<0.001	0.081	0.785
F:G; d 56 to end-minus-28	6.22	6.39	6.23	0.388	6.84^{x}	6.27^{y}	5.73^{z}	<0.001	0.114	0.604
F:G; d 56 to end	6.75^{x}	6.60^{xy}	6.46^{y}	0.092	7.05^{x}	6.64^{y}	6.13^{z}	<0.001	0.126	0.900
F:G; End-minus-28 to end	10.06^{x}	7.70^{y}	7.62^{y}	<0.001	8.36	8.66	8.36	0.791	0.550	0.885
F:G; d 0 to end	5.94^{x}	5.78^{y}	5.66^{y}	0.012	6.15^{x}	5.74^{y}	5.49^{z}	<0.001	0.083	0.876

[a]ADG = average daily gain, kg; F:G = feed:gain ratio, DM basis.
[b]Weights decreased by 4% to represent a standard industry shrink.
[c]Ractopamine × implant/reimplant regimen interaction remained in the model regardless of significance value.
[x,y,z]Means in the same row within a main effect that do not have a common superscript letter differ, $P < 0.10$.

Table 4.6. Least squares means of intake and gain efficiency for main effects of ractopamine supplementation and implant/reimplant regimen.

	Ractopamine, mg·steer^{-1}·d^{-1}				Implant/Reimplant regimen					
Trait[a]	0	100	200	P_{RAC}	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S	$P_{Implant}$	SEM	$P_{R \times I}$[b]
Number of pens	18	18	18	-	18	18	18	-	-	-
DMI; d 0 to 56	8.75	8.73	8.62	0.669	8.59	8.73	8.77	0.539	0.231	0.337
DMI; d 0 to end-minus-28	9.47	9.33	9.24	0.321	8.88^{x}	9.56^{y}	9.61^{y}	<0.001	0.197	0.197
DMI; d 56 to end-minus-28	9.94	9.72	9.65	0.247	9.06^{x}	10.09^{y}	10.15^{y}	<0.001	0.201	0.242
DMI; d 56 to end	9.80	9.62	9.53	0.312	8.86^{x}	9.96^{y}	10.13^{y}	<0.001	0.190	0.153
DMI; End-minus-28 to end	9.37	9.32	9.20	0.78	8.25^{x}	9.58^{y}	10.06^{z}	<0.001	0.225	0.239
DMI; d 0 to end	9.45	9.33	9.23	0.341	8.78^{x}	9.56^{y}	9.68^{y}	<0.001	0.190	0.135
G:F; d 0 to 56	0.214^{x}	0.221^{xy}	0.226^{y}	0.033	0.206^{x}	0.229^{y}	0.227^{y}	<0.001	0.005	0.948
G:F; d 0 to end-minus-28	0.181	0.181	0.186	0.199	0.170^{x}	0.185^{y}	0.194^{z}	<0.001	0.003	0.757
G:F; d 56 to end-minus-28	0.162	0.158	0.162	0.388	0.147^{x}	0.160^{y}	0.175^{z}	<0.001	0.003	0.652
G:F; d 56 to end	0.149^{x}	0.153^{xy}	0.156^{y}	0.058	0.143^{x}	0.151^{y}	0.164^{z}	<0.001	0.003	0.789
G:F; End-minus-28 to end	0.105^{x}	0.133^{y}	0.134^{y}	<0.001	0.126	0.119	0.127	0.356	0.008	0.632
G:F; d 0 to end	0.169^{x}	0.174^{y}	0.177^{y}	0.009	0.163^{x}	0.175^{y}	0.183^{z}	<0.001	0.002	0.798
Dose[c], mg/d	0^{x}	96.5^{y}	190.6^{z}	<0.001	86.6^{x}	98.3^{y}	102.2^{y}	0.001	3.07	0.015

[a]DMI = dry matter intake, kg/d; G:F = gain:feed ratio, DM basis.

[b]Ractopamine × implant/reimplant regimen interaction remained in the model regardless of significance value.

[c]Dose of ractopamine based on actual DMI.

[x,y,z]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

Table 4.7. Least squares means of carcass characteristics for main effects of ractopamine supplementation and implant/reimplant regimen.

	Ractopamine, mg·steer^{-1}·d^{-1}				Implant/Reimplant regimen					
Trait[a]	0	100	200	P_{RAC}	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S	$P_{Implant}$	SEM	$P_{R \times I}$ [d]
Number of pens	18	18	18	-	18	18	18	-	-	-
HCW, kg	362.1^{x}	363.1^{x}	368.4^{y}	0.092	341.3^{x}	369.8^{y}	382.4^{z}	<0.001	5.9	0.509
Dressing percent	63.09^{x}	62.92^{x}	63.55^{y}	0.003	62.62^{x}	63.30^{y}	63.64^{z}	<0.001	0.29	0.215
LM area, cm^2	84.0^{x}	84.0^{x}	86.3^{y}	0.024	80.7^{x}	85.2^{y}	88.5^{z}	<0.001	1.3	0.311
AFAT, cm	1.52	1.47	1.48	0.479	1.44	1.52	1.51	0.210	0.034	0.606
KPH, %	2.25	2.21	2.25	0.349	2.34^{x}	2.21^{y}	2.17^{y}	<0.001	0.028	0.062
YG	3.32	3.27	3.21	0.324	3.25	3.31	3.24	0.532	0.057	0.988
SMAT[b]	155.1	155.0	156.4	0.159	151.1^{x}	157.9^{y}	157.5^{y}	<0.001	0.57	0.059
LMAT[b]	168.8	168.6	168.9	0.981	171.2^{x}	168.3^{xy}	166.8^{y}	0.052	1.21	0.514
OMAT[b]	159.7	159.3	160.6	0.341	157.5^{x}	161.4^{y}	160.7^{y}	0.001	1.59	0.367
Marbling[c]	534.7	537.8	531.4	0.746	556.2^{x}	527.3^{xy}	520.5^{y}	0.001	8.24	0.505
Empty body fat[e], %	30.64	30.42	30.38	0.622	30.25	30.64	30.55	0.369	0.24	0.705

[a]HCW = hot carcass weight; LM = longissimus muscle; AFAT = adjusted fat thickness; KPH = kidney, pelvic, and heart fat percent; YG = calculated yield grade; SMAT = skeletal maturity; LMAT = lean maturity; OMAT = overall maturity.

[b]100 = A - maturity; 200 = B - maturity.

[c]300 = Traces^0, 400 = Slight^0, 500 = Small^0, 600 = Modest^0, 700 = Moderate^0, 800 = Slightly Abundant^0.

[d]Ractopamine × implant/reimplant regimen interaction remained in the model regardless of significance value.

[e]Empty body fat, %, (Guiroy et al., 2002) = 17.76207 + (4.68142 x AFAT, cm) + (0.01945 x HCW, kg) + (0.8185 x marbling/100) – (0.06754 x LM area, cm^2).

[x,y,z]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

Table 4.8. Quality grade distribution of carcasses within the main effects of ractopamine supplementation and implant/reimplant regimen.

Quality grade marketing category[a]	Ractopamine, mg·steer^{-1}·d^{-1}				Implant/Reimplant regimen				
	0	100	200	P_{RAC}	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S	$P_{Implant}$	$P_{R \times I}$[b]
USDA Prime	2.58	4.32	2.52	0.999	5.06	3.16	1.25	0.712	0.887
Upper ⅔ USDA Choice	19.35	20.99	18.87	0.809	23.42^{x}	13.92^{y}	21.88^{x}	0.079	0.527
Lower ⅓ USDA Choice	41.94	37.04	40.88	0.638	43.04	43.04	33.75	0.156	0.718
USDA Choice or greater	63.87	62.35	62.26	0.950	71.52^{x}	60.13^{y}	56.88^{y}	0.024	0.612
USDA Choice	61.29	58.02	59.75	0.819	66.46^{x}	56.96^{y}	55.63^{y}	0.034	0.598
USDA Select	35.48	37.65	37.74	0.990	27.85^{x}	39.87^{y}	43.13^{y}	0.106	0.653
No Roll	0.65	0.00	0.00	0.999	0.63	0.00	0.00	0.999	0.999
Dark Cutter[c]	1.94	4.94	1.89	0.999	4.43	0.63	3.75	0.986	0.954

[a]Quality grade marketing category determined by expert marbling score: USDA Prime = Slightly Abundant^{0} to Abundant^{99}; Upper ⅔ USDA Choice = Modest^{0} to Moderate^{99}; Lower ⅓ USDA Choice = Small^{0} to Small^{99}; USDA Choice or higher = Small^{0} to Abundant^{99}; USDA Choice = Small^{0} to Moderate^{99}; USDA Select = Slight^{0} to Slight^{99}; No Roll = Traces^{0} to Traces^{99}.

[b]Ractopamine × implant/reimplant regimen interaction remained in the model regardless of significance value.

[c]Incidence of dark cutter was determined independently of marbling score and USDA quality grade.

[x,y,z]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

Table 4.9. USDA yield grade distribution of carcasses within the main effects of ractopamine supplementation and implant/reimplant regimen.

	Ractopamine, mg·steer^{-1}·d^{-1}				Implant/Reimplant Regimen				
Calculated yield grade[a]	0	100	200	P_{RAC}	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S	$P_{Implant}$	$P_{R \times I}$ [b]
Yield grade 1	2.63	2.56	5.03	0.549	3.27	3.21	3.80	0.982	0.993
Yield grade 2	28.29	32.69	30.19	0.707	30.07	32.05	29.11	0.877	0.868
Yield grade 1 and 2	30.92	35.26	35.22	0.654	33.33	35.26	32.91	0.922	0.887
Yield grade 3	54.61	50.64	51.57	0.780	54.25	47.44	55.06	0.364	0.435
Yield grade 4	14.47	12.82	13.21	0.853	11.76	16.67	12.03	0.344	0.574
Yield grade 5	0.00	1.28	0.00	0.999	0.65	0.64	0.00	0.999	0.999
Yield grade 4 and 5	14.47	14.10	13.21	0.915	12.42	17.31	12.03	0.335	0.642

[a]Based on calculated yield grade from longissimus muscle area, adjusted fat thickness, hot carcass weight, and kidney, pelvic, and heart fat.

[b]Ractopamine × implant/reimplant regimen interaction remained in the model regardless of significance value.

APPENDIX A

Appendix Table 4.1. Laboratory proximate analysis of the Control diet during the ractopamine feeding period[a,b]

Diet	Dates	DM[c]	CP[c]	NPN[c]	ADF[c]	Fat	Ca	P
Control	Week 1	68.43	13.47	3.33	6.17	7.01	0.88	0.34
	Week 2	69.43	13.02	3.25	6.56	6.78	0.59	0.30
	Week 3	68.73	13.69	3.84	6.32	7.02	0.91	0.32
	Week 4	67.95	13.52	3.99	6.70	6.96	0.80	0.31
	Week 5	67.82	13.09	3.56	6.09	7.05	0.74	0.30
	Week 6	68.71	13.46	4.13	7.33	7.11	0.86	0.31
Average		68.51	13.38	3.68	6.53	6.99	0.80	0.31
Number of samples		6	6	6	6	6	6	6
Standard deviation		0.59	0.26	0.36	0.46	0.11	0.12	0.02
Coefficient of variation, %		0.86	1.96	9.76	6.98	1.62	14.82	4.80

[a]SDK Labs, 1000 Corey Road, Hutchinson, KS 67501

[b]Unless otherwise noted, values are percentages and are reported on a DM basis.

[c]DM = dry matter, CP = crude protein, NPN = non-protein N, ADF = acid detergent fiber.

Appendix Table 4.2. Laboratory proximate analysis of the 100 mg/d ractopamine diet during the ractopamine feeding period.[a,b]

Diet	Dates	DM[c]	CP[c]	NPN[c]	ADF[c]	Fat	Ca	P
100 mg ractopamine	Week 1	68.61	13.14	3.51	5.95	7.38	0.85	0.33
	Week 2	69.23	13.16	3.58	6.35	6.43	0.91	0.36
	Week 3	68.91	13.86	3.86	6.25	6.98	0.83	0.31
	Week 4	69.40	13.53	4.15	6.35	7.22	0.83	0.32
	Week 5	68.14	12.92	3.38	6.39	6.82	0.69	0.30
	Week 6	68.45	13.56	4.43	6.27	7.52	0.90	0.30
Average		68.79	13.36	3.82	6.26	7.06	0.84	0.32
Number of samples		6	6	6	6	6	6	6
Standard deviation		0.48	0.35	0.41	0.16	0.40	0.08	0.02
Coefficient of variation, %		0.70	2.59	10.67	2.57	5.67	9.45	7.13

[a]SDK Labs, 1000 Corey Road, Hutchinson, KS 67501

[b]Unless otherwise noted, values are percentages and are reported on a DM basis.

[c]DM = dry matter, CP = crude protein, NPN = non-protein N, ADF = acid detergent fiber.

Appendix Table 4.3. Laboratory proximate analysis of the 200 mg/d ractopamine diet during the ractopamine feeding period. [a,b]

Diet	Dates	DM[c]	CP[c]	NPN[c]	ADF[c]	Fat	Calcium	Phosphorus
200 mg ractopamine	Week 1	68.35	13.21	3.30	5.37	6.98	0.86	0.34
	Week 2	69.12	13.56	3.73	6.02	6.53	0.89	0.34
	Week 3	69.16	13.60	3.96	6.60	7.33	0.82	0.31
	Week 4	68.68	13.47	4.18	7.43	7.57	0.93	0.32
	Week 5	67.92	13.30	3.82	5.79	7.16	0.75	0.31
	Week 6	68.83	13.58	4.15	6.91	7.79	0.84	0.33
Average		68.68	13.45	3.86	6.35	7.23	0.85	0.33
Number of samples		6	6	6	6	6	6	6
Standard deviation		0.48	0.16	0.33	0.76	0.45	0.06	0.01
Coefficient of variation, %		0.69	1.21	8.43	12.04	6.18	7.28	4.24

[a]SDK Labs, 1000 Corey Road, Hutchinson, Kansas 67501

[b]Unless otherwise noted, values are percentages and are reported on a DM basis.

[c]DM = dry matter, CP = crude protein, NPN = non-protein N, ADF = acid detergent fiber.

Appendix Table 4.4. Ractopamine Type B Premix assay results for the 0, 100, and 200 mg/d treatments[a].

	Control, 0 mg ractopamine		100 mg ractopamine		200 mg ractopamine	
Type B assay date	Claim,[b] mg/kg	Results,[b] mg/kg	Claim,[b] mg/kg	Results,[b] mg/kg	Claim,[b] mg/kg	Results,[b] mg/kg
09/17/04	0	<2.5	441	-----	882	-----
09/29/04	-----	-----	441	433.9	882	831.0
10/05//04	0	<2.5	441	424.1	882	805.2
Type B average	0	<2.5	441	429.0	882	818.1
Number of samples		2		2		2
Mean theory[c], %				97.3		92.8

[a]Eurofins Scientific, 345 Adams Ave., Memphis TN.

[b]As-fed basis.

[c]Feed additive result divided by the feed additive claim x 100.

Appendix Table 4.5. Monensin and ractopamine assay results for the Control diet during ractopamine feeding.[a]

Dates	Diet	Monensin		Ractopamine	
		Claim,[b] mg/kg	Results,[b] mg/kg	Claim,[b] mg/kg	Results,[b] mg/kg
Week 1	Control	23.3	28.2	0.0	< 2.5
Week 2		23.3	25.2	0.0	< 2.5
Week 3		23.3	27.7	0.0	<2.5
Week 4		23.3	25.2	0.0	<2.5
Week 5		23.3	21.2	0.0	< 2.5
Week 6		23.3	26.7	0.0	< 2.5
Average of diet		23.3	25.7	0.0	< 2.5
Number of samples			6		6
Mean theory[c], %			110.3		---
Standard deviation			2.54		---
Coefficient of variation, %			9.9		---

[a]Eurofins Scientific, 345 Adams Ave., Memphis, TN 38103.

[b]As-fed basis.

[c]Feed additive result divided by the feed additive claim x 100.

Appendix Table 4.6. Monensin and ractopamine assay results for the 100 mg/d diet during ractopamine feeding.[a]

Dates	Diet	Monensin		Ractopamine	
		Claim,[c] mg/kg	Results,[c] mg/kg	Claim,[c] mg/kg	Results,[c] mg/kg
Week 1	100 mg ractopamine	23.3	21.8	7.2	5.3
Week 2		23.3	29.4	7.2	4.9
Week 3		23.3	28.2	7.2	4.2
Week 4		23.3	22.6	7.2	4.7
Week 5		23.3	21.4	7.2	5.6
Week 6		23.3	26.9	7.2	6.3
Average of diet		23.3	25.1	7.2	5.2
Number of samples			6		6
Mean theory[c], %			107.7		72.1
Standard deviation			3.54		0.74
Coefficient of variation, %			14.1		14.3

[a]Eurofins Scientific, 345 Adams Ave., Memphis, TN 38103.

[b]As-fed basis.

[c]Feed additive result divided by the feed additive claim x 100.

Appendix Table 4.7. Monensin and ractopamine assay results for the 200 mg/d diet during ractopamine feeding.[a]

Dates	Diet	Monensin		Ractopamine	
		Claim,[c] mg/kg	Results,[c] mg/kg	Claim,[c] mg/kg	Results,[c] mg/kg
Week 1	200 mg ractopamine	23.3	24.6	14.2	10.1
Week 2		23.3	26.8	14.2	9.7
Week 3		23.3	25.9	14.2	10.7
Week 4		23.3	25.8	14.2	12.1
Week 5		23.3	24.7	14.2	11.7
Week 6		23.3	24.9	14.2	14.8
Average of diet		23.3	25.5	14.2	11.5
Number of samples			6		6
Mean theory[c], %			109.4		81.0
Standard deviation			0.86		1.84
Coefficient of variation, %			3.4		15.9

[a]Eurofins Scientific, 345 Adams Ave., Memphis, TN 38103.

[b]As-fed basis.

[c]Feed additive result divided by the feed additive claim x 100.

Appendix Table 4.8. Least squares means of performance for implant/reimplant regimen within each ractopamine-supplementation treatment.

			0 mg/d			100 mg/d			200 mg/d		
Trait[a]	$P > F$[b]	SEM	None/ None	Rev-S/ None	Rev-IS/ Rev-S	None/ None	Rev-S/ None	Rev-IS/ Rev-S	None/ None	Rev-S/ None	Rev-IS/ Rev-S
Number of pens			6	6	6	6	6	6	6	6	6
Initial BW, kg[c]	0.327	10.4	304.1	304.5	305.3	304.5	305.6	303.8	305.0	303.8	303.8
d56 BW, kg[c]	0.495	11.5	398.2	416.0	414.4	404.6	416.6	415.8	407.5	415.1	415.6
End-minus-28 BW, kg[c]	0.623	9.6	513.3	558.1	569.1	518.0	548.3	564.2	519.0	553.7	565.9
End BW, kg[c]	0.615	11.1	535.6	585.2	599.7	548.4	582.4	600.7	550.6	585.6	602.5
ADG; d 0 to 56	0.581	0.05	1.68	1.99	1.95	1.79	1.98	2.00	1.83	1.99	2.00
ADG; d 0 to end-minus-28	0.613	0.04	1.48	1.80	1.87	1.51	1.72	1.85	1.51	1.77	1.86
ADG; d 56 to end-minus-28	0.831	0.05	1.35	1.66	1.82	1.33	1.55	1.74	1.32	1.63	1.77
ADG; d 56 to end	0.890	0.05	1.22	1.51	1.66	1.28	1.48	1.65	1.28	1.52	1.67
ADG; End-minus-28 to end	0.870	0.11	0.83	1.00	1.13	1.12	1.26	1.35	1.17	1.18	1.36
ADG; d 0 to end	0.629	0.04	1.38	1.67	1.75	1.45	1.65	1.77	1.46	1.68	1.78
F:G; d 0 to 56	0.938	0.13	5.01	4.49	4.60	4.84	4.36	4.43	4.80	4.32	4.26
F:G; d 0 to end-minus-28	0.785	0.12	5.89	5.47	5.29	5.96	5.46	5.20	5.90	5.33	5.03
F:G; d 56 to end-minus-28	0.604	0.18	6.62	6.26	5.78	6.96	6.41	5.79	6.93	6.14	5.61
F:G; d 56 to end	0.900	0.18	7.10	6.80	6.36	7.10	6.62	6.08	6.96	6.50	5.94
F:G; End-minus-28 to end	0.885	0.74	10.12	10.11	9.96	7.83	7.69	7.58	7.14	8.17	7.56
F:G; d 0 to end	0.876	0.12	6.24	5.88	5.71	6.17	5.71	5.46	6.04	5.64	5.31

[a]ADG = average daily gain, kg/d; F:G = feed:gain ratio, DM basis.

[b]Significance of interaction between ractopamine supplementation treatment and implant/reimplant regimen. Main-effect *P*-values are not presented here.

[c]Weights decreased by 4% to represent a standard industry shrink.

Appendix Table 4.9. Least squares means of intake and feed efficiency for implant/reimplant regimen within each ractopamine-supplementation treatment.

			0 mg/d			100 mg/d			200 mg/d		
Trait[a]	$P > F$[b]	SEM	None/ None	Rev-S/ None	Rev-IS/ Rev-S	None/ None	Rev-S/ None	Rev-IS/ Rev-S	None/ None	Rev-S/ None	Rev-IS/ Rev-S
Number of pens			6	6	6	6	6	6	6	6	6
DMI; d 0 to 56	0.337	0.28	8.40	8.92	8.93	8.65	8.66	8.86	8.73	8.60	8.51
DMI; d 0 to end-minus-28	0.197	0.25	8.71	9.81	9.88	9.02	9.40	9.59	8.91	9.45	9.35
DMI; d 56 to end-minus-28	0.242	0.27	8.90	10.41	10.51	9.25	9.86	10.05	9.03	10.00	9.90
DMI; d 56 to end	0.153	0.26	8.65	10.25	10.50	9.10	9.76	10.00	8.83	9.87	9.89
DMI; End-minus-28 to end	0.239	0.34	7.89	9.78	10.46	8.67	9.45	9.83	8.20	9.51	9.88
DMI; d 0 to end	0.135	0.24	8.58	9.81	9.98	8.96	9.40	9.63	8.80	9.46	9.44
G:F; d 0 to 56	0.948	0.006	0.200	0.224	0.219	0.207	0.230	0.226	0.210	0.233	0.235
G:F; d 0 to end-minus-28	0.757	0.004	0.170	0.184	0.190	0.168	0.184	0.192	0.170	0.188	0.199
G:F; d 56 to end-minus-28	0.652	0.004	0.152	0.161	0.173	0.144	0.157	0.173	0.145	0.163	0.179
G:F; d 56 to end	0.789	0.004	0.142	0.147	0.158	0.141	0.152	0.165	0.145	0.154	0.169
G:F; End-minus-28 to end	0.632	0.009	0.105	0.102	0.108	0.130	0.133	0.137	0.142	0.124	0.137
G:F; d 0 to end	0.798	0.004	0.161	0.171	0.176	0.162	0.175	0.184	0.166	0.178	0.188
Dose[c], mg/d	0.015	5.10	0^{v}	0^{v}	0^{v}	89.8^{w}	97.9^{wx}	101.9^{x}	170.0^{y}	197.1^{z}	204.8^{z}

[a]DMI = dry matter intake, kg/d; G:F = gain:feed ratio, DM basis.

[b]Significance of interaction between ractopamine supplementation treatment and implant/reimplant regimen. Main-effect *P*-values are not presented here.

[c]Dose of ractopamine based on actual DMI.

[w,x,y,z]Means in the same row that do not have a common superscript letter differ, $P < 0.10$. Main-effect *P*-values are not presented here.

Appendix Table 4.10. Least squares means of carcass characteristics for implant/reimplant regimen within each ractopamine-supplementation treatment.

			0 mg/d			100 mg/d			200 mg/d		
Trait[a]	$P > F^b$	SEM	None/ None	Rev-S/ None	Rev-IS/ Rev-S	None/ None	Rev-S/ None	Rev-IS/ Rev-S	None/ None	Rev-S/ None	Rev-IS/ Rev-S
Number of pens			6	6	6	6	6	6	6	6	6
HCW, kg	0.509	6.7	335.1	370.2	381.0	341.5	365.4	382.4	347.3	373.9	384.0
Dressing percentage	0.215	0.34	62.5	63.25	63.52	62.26	62.8	63.69	63.09	63.85	63.72
LM area, cm^2	0.311	1.55	79.04	84.39	88.72	79.62	84.07	88.39	83.42	87.10	88.46
AFAT, cm	0.606	0.058	1.45	1.55	1.60	1.42	1.47	1.50	1.45	1.55	1.45
KPH, %	0.062	0.045	2.37^x	2.19^y	2.21^y	2.23^y	2.23^y	2.16^y	2.42^x	2.21^y	2.13^y
YG	0.988	0.093	3.28	3.37	3.31	3.28	3.30	3.22	3.19	3.27	3.17
SMAT[bc]	0.059	0.98	149.3^w	157.3^{yz}	158.5^z	152.3^x	157.2^{yz}	155.5^y	151.5^{wx}	159.2^z	158.5^z
LMAT[bc]	0.514	2.10	172.3	168.8	165.3	171.5	168.5	165.8	169.8	167.5	169.5
OMAT[bc]	0.367	1.84	156.7	161.5	160.8	158.3	160.7	158.8	157.5	162.0	162.3
Marbling[d]	0.505	11.7	547.7	531.3	525.2	556.5	529.2	527.8	564.3	521.5	508.5
Empty body fat[e], %	0.705	0.37	30.14	30.82	30.95	30.28	30.46	30.53	30.32	30.63	30.18

[a]HCW = hot carcass weight; LM = longissimus muscle; AFAT = adjusted fat thickness; KPH = kidney, pelvic, and heart fat percent; YG = calculated yield grade; SMAT = skeletal maturity; LMAT = lean maturity; OMAT = overall maturity.

[b]Significance of interaction between ractopamine supplementation treatment and implant/reimplant regimen. Main-effect *P*-values are not presented here.

[c]100 = A maturity; 200 = B maturity.

[d]300 = Traces, 400 = Slight, 500 = Small, 600 = Modest, 700 = Moderate, 800 = Slightly Abundant.

[e]Empty body fat, % (Guiroy et al., 2002) = 17.76207 + (4.68142 x AFAT, cm) + (0.01945 x HCW, kg) + (0.8185 x marbling/100) – (0.06754 x LM area, cm^2).

[w,x,y,z]Means in the same row that do not have a common superscript letter differ, $P < 0.10$. Main-effect *P*-values are not presented here.

Appendix Table 4.11. Least squares means of performance for ractopamine-supplementation treatment within each implant/reimplant regimen.

			None/None			Revalor-S/None			Revalor-IS/Revalor-S		
Trait[a]	$P > F^b$	SEM	0 mg/d	100 mg/d	200 mg/d	0 mg/d	100 mg/d	200 mg/d	0 mg/d	100 mg/d	200 mg/d
Number of pens			6	6	6	6	6	6	6	6	6
Initial BW, kg[b]	0.327	10.4	304.1	304.5	305.0	304.5	305.6	303.8	305.3	303.8	10.4
d 56 BW, kg[b]	0.495	11.5	398.2	404.6	407.5	416.0	416.6	415.1	414.4	415.8	11.5
End-minus-28 d BW, kg[b]	0.623	9.6	513.3	518.0	519.0	558.1	548.3	553.7	569.1	564.2	9.6
End BW, kg[b]	0.615	11.1	535.6	548.4	550.6	585.2	582.4	585.6	599.7	600.7	11.1
ADG; d 0 to 56	0.581	0.05	1.68	1.79	1.83	1.99	1.98	1.99	1.95	2.00	0.05
ADG; d 0 to end-minus-28	0.613	0.04	1.48	1.51	1.51	1.80	1.72	1.77	1.87	1.85	0.04
ADG; d 56 to end-minus-28	0.831	0.05	1.35	1.33	1.32	1.66	1.55	1.63	1.82	1.74	0.05
ADG; d 56 to end	0.890	0.05	1.22	1.28	1.28	1.51	1.48	1.52	1.66	1.65	0.05
ADG; End-minus-28 to end	0.870	0.11	0.83	1.12	1.17	1.00	1.26	1.18	1.13	1.35	0.11
ADG; d 0 to end	0.629	0.04	1.38	1.45	1.46	1.67	1.65	1.68	1.75	1.77	0.04
F:G; d 0 to 56	0.938	0.13	5.01	4.84	4.80	4.49	4.36	4.32	4.60	4.43	4.26
F:G; d 0 to end-minus-28	0.785	0.12	5.89	5.96	5.90	5.47	5.46	5.33	5.29	5.20	5.03
F:G; d 56 to end-minus-28	0.604	0.18	6.62	6.96	6.93	6.26	6.41	6.14	5.78	5.79	5.61
F:G; d 56 to end	0.900	0.18	7.10	7.10	6.96	6.80	6.62	6.50	6.36	6.08	5.94
F:G; End-minus-28 to end	0.885	0.74	10.12	7.83	7.14	10.11	7.69	8.17	9.96	7.58	7.56
F:G; d 0 to end	0.876	0.12	6.24	6.17	6.04	5.88	5.71	5.64	5.71	5.46	5.31

[a]ADG = average daily gain, kg/d; F:G = feed:gain ratio, DM basis.

[b]Significance of interaction between ractopamine supplementation treatment and implant/reimplant regimen. Main-effect *P*-values are not presented here.

[c]Weights decreased by 4% to represent a standard industry shrink.

Appendix Table 4.12. Least squares means of intake and feed efficiency for ractopamine-supplementation treatment within each implant/reimplant regimen.

			None/None			Revalor-S/None			Revalor-IS/Revalor-S		
Trait[a]	$P > F$[b]	SEM	0 mg/d	100 mg/d	200 mg/d	0 mg/d	100 mg/d	200 mg/d	0 mg/d	100 mg/d	200 mg/d
Number of pens			6	6	6	6	6	6	6	6	6
DMI; d 0 to 56	0.337	0.28	8.40	8.65	8.73	8.92	8.66	8.60	8.93	8.86	8.51
DMI; d 0 to end-minus-28	0.197	0.25	8.71	9.02	8.91	9.81	9.40	9.45	9.88	9.59	9.35
DMI; d 56 to end-minus-28	0.242	0.27	8.90	9.25	9.03	10.41	9.86	10.00	10.51	10.05	9.90
DMI; d 56 to end	0.153	0.26	8.65	9.10	8.83	10.25	9.76	9.87	10.50	10.00	9.89
DMI; End-minus-28 to end	0.239	0.34	7.89	8.67	8.20	9.78	9.45	9.51	10.46	9.83	9.88
DMI; d 0 to end	0.135	0.24	8.58	8.96	8.80	9.81	9.40	9.46	9.98	9.63	9.44
G:F; d 0 to 56	0.948	0.006	0.200	0.207	0.210	0.224	0.230	0.233	0.219	0.226	0.235
G:F; d 0 to end-minus-28	0.757	0.004	0.170	0.168	0.170	0.184	0.184	0.188	0.190	0.192	0.199
G:F; d 56 to end-minus-28	0.652	0.004	0.152	0.144	0.145	0.161	0.157	0.163	0.173	0.173	0.179
G:F; d 56 to end	0.789	0.004	0.142	0.141	0.145	0.147	0.152	0.154	0.158	0.165	0.169
G:F; End-minus-28 to end	0.632	0.009	0.105	0.130	0.142	0.102	0.133	0.124	0.108	0.137	0.137
G:F; d 0 to end	0.798	0.004	0.161	0.162	0.166	0.171	0.175	0.178	0.176	0.184	0.188
Dose[c], mg/d	0.015	5.10	0^{v}	89.8^{w}	170.0^{y}	0^{v}	97.9^{wx}	197.1^{z}	0^{v}	101.9^{x}	204.8^{z}

[a]DMI = dry matter intake, kg/d; G:F = gain:feed ratio, DM basis.

[b]Significance of interaction between ractopamine supplementation treatment and implant/reimplant regimen. Main-effect *P*-values are not presented here.

[c]Dose of ractopamine based on actual DMI.

[w,x,y,z]Means in the same row that do not have a common superscript letter differ, $P < 0.10$. Main-effect *P*-values are not presented here.

Appendix Table 4.13. Least squares means of carcass characteristics for ractopamine-supplementation treatment within each implant/reimplant regimen.

			None/None			Revalor-S/None			Revalor-IS/Revalor-S		
Trait[a]	$P > F$[b]	SEM	0 mg/d	100 mg/d	200 mg/d	0 mg/d	100 mg/d	200 mg/d	0 mg/d	100 mg/d	200 mg/d
Number of pens			6	6	6	6	6	6	6	6	6
HCW, kg	0.509	6.7	335.1	341.5	347.3	370.2	365.4	373.9	381.0	382.4	384.0
Dressing percentage	0.215	0.34	62.5	62.26	63.09	63.25	62.8	63.85	63.52	63.69	63.72
LM area, cm^2	0.311	1.55	79.04	79.62	83.42	84.39	84.07	87.10	88.72	88.39	88.46
AFAT, cm	0.606	0.058	1.45	1.42	1.45	1.55	1.47	1.55	1.60	1.50	1.45
KPH, %	0.062	0.045	2.37^{x}	2.23^{y}	2.42^{x}	2.19^{y}	2.23^{y}	2.21^{y}	2.21^{y}	2.16^{y}	2.13^{y}
YG	0.988	0.093	3.28	3.28	3.19	3.37	3.30	3.27	3.31	3.22	3.17
SMAT[b]	0.059	0.98	149.3^{w}	152.3^{x}	151.5^{wx}	157.3^{yz}	157.2^{yz}	159.2^{z}	158.5^{z}	155.5^{y}	158.5^{z}
LMAT[b]	0.514	2.10	172.3	171.5	169.8	168.8	168.5	167.5	165.3	165.8	169.5
OMAT[b]	0.367	1.84	156.7	158.3	157.5	161.5	160.7	162.0	160.8	158.8	162.3
Marbling[c]	0.505	11.7	547.7	556.5	564.3	531.3	529.2	521.5	525.2	527.8	508.5
Empty body fat[e], %	0.705	0.37	30.14	30.28	30.32	30.82	30.46	30.63	30.95	30.53	30.18

[a]HCW = hot carcass weight; LM = longissimus muscle; AFAT = adjusted fat thickness; KPH = kidney, pelvic, and heart fat percent; YG = calculated yield grade; SMAT = skeletal maturity; LMAT = lean maturity; OMAT = overall maturity.

[b]Significance of interaction between ractopamine supplementation treatment and implant/reimplant regimen. Main-effect *P*-values are not presented here.

[c]100 = A maturity; 200 = B maturity.

[d]300 = Traces^0, 400 = Slight^0, 500 = Small^0, 600 = Modest^0, 700 = Moderate^0, 800 = Slightly Abundant^0.

[e]Empty body fat, % (Guiroy et al., 2002) = 17.76207 + (4.68142 x AFAT, cm) + (0.01945 x HCW, kg) + (0.8185 x marbling/100) – (0.06754 x LM area, cm^2).

[w,x,y,z]Means in the same row that do not have a common superscript letter differ, $P < 0.10$. Main-effect *P*-values are not presented here.

Appendix Table 4.14. Quality grade distribution of carcasses within the main effects of ractopamine supplementation and implant/reimplant regimen based on a line-grader's assessment.

	Ractopamine, mg·steer^{-1}·d^{-1}			Implant/Reimplant regimen		
Quality grade marketing category[a]	0	100	200	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S
USDA Prime	7.10	6.79	6.29	10.13	6.33	3.75
USDA Choice	63.23	58.02	57.86	63.29	59.96	58.75
USDA Choice or higher	70.32	64.81	64.15	73.42	63.29	62.50
USDA Select	28.39	32.10	35.22	22.78	36.71	36.25
No Roll	1.29	1.85	0.00	1.90	0.00	1.25
Dark Cutter	0.00	1.23	0.63	1.90	0.00	0.00

[a]Quality grade marketing category determined by on-line grader assessment into whole quality grades.

[xyz]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

Appendix Table 4.15. USDA Yield Grade distribution of carcasses within the main effects of ractopamine supplementation and implant/reimplant regimen based on a line-grader's assessment.

	Ractopamine, mg·steer^{-1}·d^{-1}			Implant/Reimplant regimen		
USDA Yield Grade[a]	0	100	200	None/ None	Revalor-S/ None	Revalor-IS/ Revalor-S
USDA Yield Grade 1	1.95	2.48	4.40	3.16	1.92	3.75
USDA Yield Grade 2	26.62	30.43	27.04	24.68	30.77	28.75
USDA Yield Grade 1 and 2	28.57	32.92	31.45	27.85	32.69	32.50
USDA Yield Grade 3	51.95	49.69	53.46	53.80	48.72	52.50
USDA Yield Grade 4	18.83	14.29	12.58	17.72	14.74	13.13
USDA Yield Grade 5	0.65	3.11	2.52	0.63	3.85	1.88
USDA Yield Grade 4 and 5	19.48	17.39	15.09	18.35	18.59	15.00

[a]Based on USDA Yield Grade as assessed by an on-line USDA grader into whole yield grades.

Appendix Table 4.16. Dark cutter incidence by ractopamine × implant/reimplant treatment subclass.

Ractopamine treatment	Implant/Reimplant treatment	Grading supervisor	Line grader
Control	None/None	2	0
Control	Revalor-S/None	0	0
Control	Revalor-IS/Revalor-S	1	0
100 mg/d	None/None	4	2
100 mg/d	Revalor-S/None	1	0
100 mg/d	Revalor-IS/Revalor-S	3	0
200 mg/d	None/None	1	1
200 mg/d	Revalor-S/None	0	0
200 mg/d	Revalor-IS/Revalor-S	2	0

Appendix Table 4.17. Number of steers per ractopamine × implant/reimplant treatment subclass.

Ractopamine treatment	Implant/Reimplant treatment	Initial count	Dead count	Removal count	Final count
Control	None/None	54	1	1	52
Control	Revalor-S/None	54	0	3	51
Control	Revalor-IS/Revalor-S	54	1	1	52
100 mg/d	None/None	54	0	0	54
100 mg/d	Revalor-S/None	54	0	0	54
100 mg/d	Revalor-IS/Revalor-S	54	0	0	54
200 mg/d	None/None	54	0	2	52
200 mg/d	Revalor-S/None	54	0	1	53
200 mg/d	Revalor-IS/Revalor-S	54	0	0	54

Appendix Table 4.18. Summary of steers that were removed during the study.

Ractopamine treatment	Implant/Reimplant treatment	Death or removal	Reason	Date	Tag	Pen
200 mg/d	Revalor-S/None	Removal	Bull	7/09/04	3835	226
Control	Revalor-S/None	Removal	Bull	7/09/04	3263	427
Control	Revalor-S/None	Removal	Cripple	8/17/04	3246	323
Control	None/None	Dead	AIP	8/23/04	3166	428
Control	Revalor-IS/Revalor-S	Removal	Poor performer	9/30/04	3346	317
200 mg/d	None/None	Removal	Poor performer	10/01/04	3717	214
Control	Revalor-IS/Revalor-S	Dead	Nervous system	10/19/04	3344	317
Control	None/None	Removal	Poor performer	10/22/04	3162	428
200 mg/d	None/None	Removal	Poor performer	11/03/04	3746	322
Control	Revalor-S/None	Removal	Respiratory	11/16/04	3256	117

Appendix Table 4.19. Important activity dates during the study.

Date	Days on feed	Activity
06/09/04	d -1	Initial individual BW and electronic identification
06/10/04	d 0	Treatment assignment, initial implant, and individual BW
07/08/04	d 28	Pen BW
08/05/04	d 56	Individual BW and reimplant for Revalor-IS/Revalor-S steers
09/02/04	d 84	Pen BW
09/30/04	d 112	Individual BW and ultrasound for 12th rib fat thickness
10/13/04	d 125	Begin transition of heavy blocks (5-6) to new feeding sequence
10/20/04	d 132	Begin ractopamine treatment feeding to blocks 5-6
10/27/04	d 139	Begin transition of light blocks (1-4) to new feeding sequence
11/03/04	d 146	Begin ractopamine treatment feeding to blocks 1-4
11/15/04	d 158	Individual BW for blocks 5-6
11/16/04	d 159	Individual BW for blocks 5-6
11/17/04	d 160	Slaughter of blocks 5-6 in Greeley, CO
11/19/04	d 162	Grading and carcass data collection for blocks 5-6
11/29/04	d 172	Individual BW for blocks 1-4
11/30/04	d 173	Individual BW for blocks 1-4
12/01/04		Slaughter of blocks 1-4 in Greeley, CO
12/03/04		Grading and carcass data collection for blocks 1-4

Appendix Table 4.20. Dates of moisture accumulation and activities during final 2 months of the study.

Date	Moisture	Activity
10/04/04	Rain	
10/11/04	Rain	
10/13/04	Rain	Begin transition of heavy blocks (5-6) to new feeding sequence
10/20/04		Begin ractopamine treatment feeding to blocks 5-6
10/21/04	Rain	
10/27/04		Begin transition of light blocks (1-4) to new feeding sequence
11/01/04	Snow	
11/03/04		Begin ractopamine treatment feeding to blocks 1-4
11/09/04	Rain	
11/10/04	Rain	
11/13/04	Snow	
11/14/04	Freezing rain	
11/15/04		Individual BW for blocks 5-6
11/16/04	Rain	Individual BW for blocks 5-6
11/17/04	Rain	Slaughter of blocks 5-6 in Greeley, CO
11/18/04	Rain	
11/19/04	Rain	Grading and carcass data collection for blocks 5-6
11/20/04	Snow	
11/21/04	Snow	
11/22/04	Rain	
11/26/04	Rain	
11/28/04	Snow	
11/29/04	Snow	Individual BW for blocks 1-4
11/30/04		Individual BW for blocks 1-4
12/01/04		Slaughter of blocks 1-4 in Greeley, CO

CHAPTER V

EFFECTS OF RACTOPAMINE AND TRENBOLONE ACETATE IMPLANTS WITH OR WITHOUT ESTRADIOL ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, BLOOD METABOLITES, AND ADIPOGENIC ENZYME ACTIVITY IN FEEDLOT HEIFERS

ABSTRACT

Finishing beef heifers (n = 63; initial BW = 305 kg ± 10.4 kg), all of which were sired by 1 of 3 Charolais bulls bred to Angus, Red Angus, and Hereford composite dams, were used to evaluate effects of ractopamine (Optaflexx; Elanco; RAC) and implant (IMP) treatment on performance, carcass traits, blood metabolites, and lipogenic enzyme activity. Heifers were blocked by initial BW into 21 replicates and assigned randomly to implant treatments and fed as a group until d 87. Heifers were ranked and blocked again by the d 70-BW within implant treatment, and 1 of 2 RAC treatments was assigned randomly to the 48 heifers of the 63 original heifers. The 3 x 2 factorial arrangement included RAC doses of 0 (R0) or 250 (R250) mg·heifer^{-1}·d^{-1} and IMP regimens of None (N), Finaplix-H (TO), or Revalor-200 (TE). Blood samples were collected from all heifers on d 0, 2, 7, 14, 28, 42, 56, 70, 91, 93, 98, 105, 112, and 119, and s.c. adipose samples were collected on d 119. For growth and carcass measurements, no RAC × IMP interactions ($P > 0.10$) were detected. The RAC-supplemented heifers had a 2.0% greater final BW and HCW and increased ADG and G:F ($P < 0.10$) with no significant differences in marbling score. For IMP, TE cattle had 4.6% ($P = 0.001$) and 2.7% ($P = 0.038$) greater final BW and 5.3% ($P = 0.001$) and 2.8% ($P = 0.037$) greater HCW than

the N and TO treatments, respectively. Despite no difference ($P > 0.10$) in empty body fat, marbling score tended to be decreased for TE ($P = 0.122$) relative to N, resulting in fewer Prime and Choice carcasses with TE ($P = 0.013$) and TO ($P = 0.060$) than with N. Heifers with implants had decreased cortisol and increased GH, IGF-1, and NEFA ($P < 0.10$) compared with non-implanted controls. Although an IMP × RAC interaction was detected ($P = 0.001$), TE and RAC-supplemented heifers had decreased BUN. No clear trends in lipogenic enzyme activity were found. Altogether, these data suggest that the modes of action of IMP and RAC are independent. Further research is needed to elucidate the exact mode of actions of these growth-enhancing products.

Key words: beta-adrenergic agonists, body composition, growth, hormone, implants, lipogenesis, metabolite profiles, ractopamine

INTRODUCTION

Steroidal implants and beta-adrenergic agonists are known to elicit growth responses via separate modes of action; however, both act to increase protein deposition. Therefore, whether the growth responses to steroidal implants and beta-adrenergic agonists depend on each other and are additive in a production setting is not known. In addition, it is not known whether the response to beta-agonists is similar in steers and heifers. The purpose of this study was to determine the effects of ractopamine supplementation and trenbolone-acetate-containing implant treatments with and without estrogen on growth performance and carcass characteristics of finishing beef heifers. A further objective of this trial is to further elucidate the mode(s) of action by which steroids and beta-adrenergic agonists elicit growth responses.

MATERIALS AND METHODS

Before the initiation of this experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State Univ. Animal Care and Use Committee.

Animals. Sixty-three Charolais-crossbred heifers born and reared at the Eastern Colorado Research Center (ECRC; Akron) of Colorado State Univ. were used for this experiment. Heifers were transported to the Colorado State Univ. Beef Research Feedlot at the Agriculture Research Development and Education Center (ARDEC; Fort Collins, CO) when the heifers were 6 to 8 mo of age. Before arrival at ARDEC, heifers were vaccinated for *Clostridial* organisms, bovine rhinotracheitis, and bovine viral diarrhea. As described in the next section, 48 of the 63 heifers were chosen to be included in the experiment based on BW and sire. All 48 of the selected heifers were approximately 9 to 11 mo of age at the beginning of the study, and all were sired by 1 of 3 Charolais bulls bred to Angus, Red Angus, and Hereford composite dams.

Experimental Design and Treatments. On January 19, 2004 (d -1), the 63 heifers were weighed individually. Heifers were ranked by BW, and within groups of 3 cattle, were assigned randomly to 1 of 3 implant (IMP) treatments: 1) None; 2) Finaplix-H (200 mg trenbolone acetate, Intervet/Schering-Plough Animal Health, Millsboro, DE); or Revalor-200 (200 mg trenbolone acetate and 20 mg estradiol-17 Beta, Intervet/Schering-Plough Animal Health). On January 20, 2004, heifers were weighed, blood samples were collected, and IMP treatments were applied. Heifers were fed in a group setting until d 87. Consequently, DMI and G:F data were not collected until the period of d 91 through slaughter.

During the study, BW were collected on d 0, 2, 7, 14, 28, 42, 56, 70, 91, 93, 98, 112, 119, and 120. Weights could not be collected on d 105. Heifers were ranked by the d 70 BW, and those that weighed more than ± 2 SD of the mean BW were excluded from the study. In addition, heifers that were not sired by 1 of the 3 Charolais bulls were excluded. In groups of 2 and in ascending order of individual BW within IMP treatment, 1 of 2 ractopamine (0 or 250 mg/heifer daily; RAC; trade name Optaflexx, Elanco Animal Health, Greenfield, IN) treatments was assigned randomly to the remaining 48 heifers. Treatments were arranged in a factorial arrangement of ractopamine and implants. In ascending order of BW, each pair within implant × ractopamine treatment was assigned a block number (1 to 8), resulting in 8 weight blocks of 6 treatments for a total of 48 pens of individual animals. Each block of 6 treatments was assigned randomly to a group of contiguous individual pens. Within the groups of 6 pens, IMP treatments were assigned randomly in groups of 2, and ractopamine treatments were assigned randomly within the 2-pen groups of IMP treatments within the 6-pen blocks. On d 87, cattle were sorted into their respective individual treatment pens, and ractopamine treatments began on d 91.

Diets. Before the start of the experiment, cattle were transitioned to the finishing diet (Table 5.1) through a 5-step feeding program that began with a receiving diet (1.07 Mcal/kg of NEg and 14% CP). Heifers were then transitioned from the receiving diet through a series of incremental increases in NEg (from 1.07 to 1.46 Mcal/kg NEg) and decreases in CP (from 14.0 to 12.8% CP) until the finishing diet was achieved (Table 5.1). Melengestrol acetate (MGA; Pfizer Animal Health, Exton, PA) was fed to provide 0.45 mg·heifer^{-1}·d^{-1} until d 70 of the trial, after which MGA was withdrawn because

there was not an approved combination clearance for MGA and RAC at the time the study was conducted. The finishing diet contained 30 mg monensin/kg of DM (trade name Rumensin, Elanco Animal Health) and 8.8 mg of tylosin/kg DM (trade name Tylan, Elanco Animal Health).

All diets were fed once daily to allow ad libitum access to feed throughout the day, and all diets used for each treatment were manufactured immediately before feeding in a mobile mixer at the ARDEC. Feed was dispensed from the feed truck into plastic containers and weighed to the nearest 0.045 kg before delivery of the daily ration into each feed bunk.

Beginning on d 91, heifers in the RAC treatment group were administered RAC via a Type-B, medicated, ground corn supplement formulated to contain 551 mg/kg of RAC and 1.5% mineral oil. The supplement was thoroughly hand-mixed into each animal's daily feed issue at a rate of 0.45 kg/heifer. A similar non-medicated premix (identical formulation to that of medicated supplement, except that ractopamine was excluded) was hand-mixed into each control animal's daily feed issue at a rate of 0.45 kg/heifer. Four batches of supplement were manufactured at the start of the RAC-feeding period, and RAC was fed for the final 29 d of the finishing period.

Finishing diet and feed commodities were sampled every 2 wk during the trial. All composited diet and feed commodity samples were shipped via UPS to a commercial laboratory (Servi-Tech Laboratories, Dodge City, KS) for routine chemical analyses. In addition, samples from each batch of supplement were shipped to Eurofins Scientific (345 Adams Ave., Memphis, TN) to be assayed for ractopamine hydrochloride

(Appendix Table 5.1). All DM assays were conducted at ARDEC by drying the samples for 48 h in a 60°C convection oven.

Feed refusals were weighed and sampled for DM determination whenever feed remained in the bunk for 3 d consecutively, on weigh days, and at the conclusion of the trial. Feed refusal samples were evaluated for DM content at ARDEC by drying the samples for 48 h in a 60°C convection oven. Dry matter consumption for each pen was calculated by subtracting the amount of DM refusals from the amount of DM delivered.

Blood and Adipose Sampling. Corresponding to weigh days, blood samples were collected from all heifers on d 0, 2, 7, 14, 28, 42, 56, 70, 91, 93, 98, 105, 112, and 119. Blood was collected via jugular venipuncture into both heparinized-plasma and uncoated-serum vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) to determine concentrations of non-esterified fatty acids (NEFA), IGF-1, insulin, glucose, estradiol-17β, cortisol, GH, blood urea N (BUN), epinephrine, and norepinephrine. After collection, samples were maintained at room temperature for 4 to 5 h before being centrifuged at 2,000 × *g* for 25 min at 4°C. Plasma and serum were decanted and stored in acid-washed polyethylene tubes at -80°C until analyzed.

Biopsies were sampled from the adipose tissue on d 119 from 24 of the 48 heifers. Four of the 8 blocks were chosen randomly to sample the adipose, such that all adipose samples from all animals within each of the 4 blocks were collected. Adipose tissue biopsies were obtained from the right side of the tail-head 2 d before slaughter. At the time of sampling, the injection site and incision site were clipped of hair, scrubbed 3 times with Betadine (Purdue Products, L.P., Stamford, CT) and then 70% ethyl alcohol. Scrubbing procedures were repeated 3 times. The incision site was anesthetized with 5

mL of lidocaine hydrochloride, which was injected into the epidural space between the last lumbar and first coccygeal vertebra. An incision 2.5 to 3.5 cm in length was made between the tail-head and the tuber ischii, and approximately 5 g (fresh-weight basis) of adipose tissue was removed and washed with PBS. Incisions were then sutured with sterile #2 cat gut suture material. All biopsy instruments were cold-sterilized in 50% Nolvasan (Fort Dodge Animal Health, Overland Park, KS): 50% deionized water in an enclosed stainless-steel instrument container before use on each animal, and a new pair of sterile gloves were used for each biopsy. After the initial biopsy, animals were monitored twice daily (morning and evening) for 1 wk. No post-surgical complications were observed for any of the animals biopsied.

Immediately after collection and rinsing, the subcutaneous adipose tissue samples were weighed, wrapped in aluminum foil, labeled, snap-frozen in liquid N and stored at -80°C until activity of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and lipoprotein lipase (LPL) were determined.

Blood Urea Nitrogen. Serum urea nitrogen (BUN; mg/dL) was analyzed with a commercial kit (Stanbio Procedure #2050; Stanbio Laboratory, Boerne, TX) using a spectrophotometer (ELx800; BioTek Instruments, Winooski, VT; $\lambda = 630$ nm). This procedure is a modification of the Berthelot reaction, in which urea in the sample is hydrolyzed by the enzyme urease to yield ammonia and carbon dioxide. The ammonium ions then react with a mixture of salicylate, sodium nitroprusside, and hypochlorite to yield a blue-green chromophore. The color intensity is proportional to the urea concentration in the sample. Standards with concentrations of 0, 7.5, 15, and 30 mg/dL urea N were prepared. Serum samples (1.5 μL) and standards were assayed undiluted in

duplicate in a 96-well microtiter plate. The CV limit for duplicate samples was set at 5% for precision. Inter- and intra-assay CV were 9.3 and 2.1%, respectively.

Epinephrine and Norepinephrine. Plasma concentrations (pg/mL) of epinephrine and norepinephrine were analyzed with a commercial kit (3 CAT EIA; Labor Diagnostika Nord, Nordhorn, Germany; distributed by Rocky Mountain Diagnostics, Colorado Springs, CO). This competitive ELISA test kit uses a 96-well plate; epinephrine, norepinephrine, and dopamine are bound to the solid phase of the microtiter plate. Acylated catecholamines from the sample and solid phase-bound catecholamines compete for a fixed number of antiserum binding sites. When equilibrium is reached, free antigen and free antigen antiserum complexes are removed by washing. The antibody bound to the solid phase catecholamine is detected by an anti-rabbit IgG-peroxidase conjugate using tetramethylbenzidine as a substrate. The amount of antibody bound to the solid phase catecholamine is inversely proportional to the catecholamine concentration of the sample. The absorbance was read at 450 nm with a reference wavelength of 630 nm. Standards with concentrations of 0, 1, 4, 16, 64, and 256 ng/mL of epinephrine, 0, 4, 16, 64, 256, and 1,024 ng/mL of norepinephrine, and 0, 10, 40, 160, 640, and 2,560 ng/mL of dopamine were prepared. Plasma samples (600 μL) and standards were assayed undiluted in duplicate in a 96-well microtiter plate, with a CV limit of 10% for precision. As per manufacturer's instructions, the read concentrations of the plasma samples were divided by 30; sample sizes required for plasma were 30 times that of urine. A significant percentage of the samples was below the detection limits for dopamine; consequently, those results were not summarized. Inter-assay CV were 22.4

and 17.4% for epinephrine and norepinephrine, respectively. Because of limited quantities of plasma, intra-assay comparisons were not made.

Steroid and Protein Hormones. Serum concentrations of GH, estradiol-17β, cortisol, and IGF-1 were quantified under the direction of Dr. Dennis Hallford (Dept. of Animal and Range Sciences, New Mexico State Univ.). Serum concentrations of GH (ng/mL) were determined using a double-antibody radioimmunoassay (RIA) in a single assay as described by Hoefler and Hallford (1987). Inter-assay CV was 7.6%. Serum estradiol-17β (pg/mL) was quantified by solid-phase RIA as described by Kane et al. (2004), with inter- and intra-assay CV of 8.8 and 3.4%, respectively. Serum cortisol (ng/mL) concentrations were determined using a commercial RIA kit as described by Kiyma et al. (2004), with inter- and intra-assay CVs of 3.2 and 4.3%, respectively. Serum IGF-1 (ng/mL) was quantified by RIA as described by Berrie et al. (1995), and inter- and intra-assay CV were 14.8 and 14.7%, respectively.

Non-Esterified Fatty Acids. Serum concentrations of non-esterified fatty acids (NEFA, μEq/L) were analyzed by a commercially available in vitro enzymatic colorimetric procedure (NEFA-C; Wako Chemicals USA, Richmond, VA) adapted for a 96-well plate. This enzymatic method relies on the addition of 2 color reagent solutions. The addition of Color Reagent A Solution, which contains acyl-CoA synthetase (ACS), catalyzes the acylation of coenzyme A (CoA) by the NEFA present in serum; the resulting product is acyl-CoA. The addition of Color Reagent B Solution, which contains acyl-CoA oxidase (ACOD), catalyzes the oxidation of acyl-CoA to produce hydrogen peroxide. Next, hydrogen peroxide in the presence of peroxidase (POD), which is also present in Color Reagent B Solution, permits the oxidative condensation of 3-methyl-N-

ethyl-N-(*B*-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple-colored product that can be measured colorimetrically at 550 nm.

Each sample was analyzed in duplicate. In the procedure, 5 μL of sample serum were pipetted into the appropriate wells of the microplate. Standards with concentrations of 0, 125, 250, 500, and 1,000 μEq/L were prepared by diluting a NEFA standard solution provided in the kit, and 5 μL of each standard were then pipetted into individual wells. A volume of 100 μL of Color Reagent A Solution was pipetted into all wells. All plates were mixed using a plate shaker and were placed in an oven at 37°C for 5 min. Microtiter plates were removed from the oven, and the absorbance was measured at 550 and 660 nm with a spectrophotometer (ELx800; BioTek Instruments). Next, 200 μL of Color Reagent B Solution were pipetted into all wells. All plates were again mixed and placed in the oven for an additional 5-min period; then, the racks were removed, and the optical density of the standards and samples was determined at 550 and 660 nm. The average of the first reading was subtracted from the second reading, and NEFA concentrations were determined from a regression of the standard curve. Inter- and intra-assay CV were 6.5 and 5.3%, respectively.

Glucose. Serum concentrations of glucose (mg/dL) were analyzed by a commercially available in vitro enzymatic colorimetric procedure (Autokit Glucose; Wako Chemicals USA, Richmond, VA) adapted for a 96-well plate. The color reagent in the test contains mutarotase, glucose oxidase, peroxidase, 4-aminoantipyrine, and ascorbate oxidase. The α-D-glucose in the samples is converted to β-D-glucose via mutarotase. The glucose oxidase then converts the β-D-glucose to hydrogen peroxide and gluconic acid. The resulting hydrogen peroxide induced the oxidative condensation between phenol and 4-

aminoantipyrine in the presence of peroxidase to a red pigment. The amount of glucose in the sample is determined by measuring the absorbance of the red color.

Each sample was analyzed in duplicate. In the procedure, 3 μL of sample serum were pipetted into the appropriate wells of the microplate. Standards with concentrations of 0, 40, 60, 80, 100 and 200 mg/dL were prepared by diluting a glucose standard solution provided in the kit, and 3 μL of each standard were then pipetted into individual wells. A volume of 350 μL of color reagent/buffer solution was pipetted into all wells. All plates were mixed using a plate shaker and were placed in an oven at 37°C for 5 min. Microtiter plates were removed from the oven, and the absorbance was measured at 515 and 660 nm with a spectrophotometer (ELx800, BioTek Instruments). Glucose concentrations were determined from a regression of the standard curve. Inter- and intra-assay CV were 3.3 and 3.5%, respectively.

Insulin. Serum concentrations of insulin (ng/mL) were analyzed with a commercial kit (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden; distributed by ALPCO Diagnostics, Windham, NH). This solid phase 2-site ELISA test kit uses a 96-well plate, and the method is based on the direct sandwich technique, in which 2 monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to the microtiter plate well. The unbound enzyme-labeled antibody is removed via washing, and the bound conjugate is detected by reaction with tetramethylbenzidine. Acid is added to stop the reaction, after which the samples are read in a spectrophotometer.

Each sample was analyzed in duplicate. In the procedure, 25 μL of sample serum were pipetted into the appropriate wells of the microplate. Stock standards with concentrations of 0, 0.25, 0.5, 1, 2, 4, and 6 ng/mL were supplied with the kit, and 25 μL of each standard were then pipetted into individual wells. Then 50 μL of an enzyme conjugate were added to the wells. After incubation on a shaker for 2 h at room temperature, the plates were washed 6 times, and 200 μL of substrate tetramethylbenzidine was added. After an additional incubation for 15 min, 50 μL of stop solution were added. Plates were placed on the shaker for another 5 sec, and absorbance was measured at 450 nm. Inter- and intra-assay CV were 6.6 and 3.4%, respectively.

Fatty Acid Synthase Enzyme Activity. Fatty acid synthase (FAS) activity was determined as described by Moibi et al. (2000). At the time of assay, frozen adipose tissue samples were pulverized in liquid N and homogenized (30s at 4°C) in 3 volumes of phosphate bicarbonate buffer (70 m*M* $KHCO_3$, 85 m*M* K_2HPO_4, 9 m*M* KH_2PO_4, 1 m*M* DTT; pH 8). The homogenate was centrifuged at 10,000 x *g* for 10 min, and the resulting supernatant fluid was centrifuged at 105,000 x *g* for 60 min at 4°C to obtain adipose tissue cytosol. The supernatant fraction was brought to saturation with an ammonium sulfate solution (3 m*M* EDTA and 1 m*M* β-mercaptoethanol) and stirred for 60 min on ice. The precipitate was collected by centrifugation at 105,000 x *g* for 60 min. Protein content was assayed according to Bradford (1976).

Fatty acid synthase activity was determined in duplicate according to the method of Nepokroeff et al. (1975) by measuring the malonyl-CoA-and acetyl-CoA-dependent oxidation of NADPH using a UV-visible automated spectrophotometer equipped with a

temperature controller set at 30°C. For each assay, reference (blank) and sample cuvettes were measured simultaneously, and the decrease in absorbance at 340 nm was monitored. The change in concentration of NADPH during oxidation was calculated as described by Moibi et al. (2000). Fatty acid synthase activity was expressed as nmol NADPH oxidized·min^{-1}·mg $protein^{-1}$.

Acetyl-CoA Carboxylase Enzyme Activity. Acetyl-CoA carboxylase (ACC) activity was determined as described by Moibi et al. (2000). Frozen subcutaneous tissue samples were pulverized under liquid N and homogenized with a buffer containing 50 m*M* Tris-HCl (pH 7.5 at 4°C), 50 m*M* NaF, 0.25 *M* mannitol, 1 m*M* EDTA, 1 m*M* ethylene glycol-bis, 1 m*M* dithiotheritol, 5 m*M* sodium pyrophosphate, 1 m*M* PMSF, 1 m*M* benzamidine, and 4 μg/mL soybean trypsin inhibitor. Homogenates were centrifuged at 14,000 x *g* for 20 min at 4°C. The supernatant samples were made to a final concentration of 2% (wt/vol) PEG, stirred for 10 min at 4°C, and then centrifuged at 10,000 x *g* for 10 min at 4°C. Acetyl-CoA carboxylase protein was precipitated from the supernatant fraction in a 10% (wt/vol) PEG solution, stirred on ice for 10 min, and centrifuged at 10,000 x *g* as described previously. Precipitate was collected and washed with 10% (wt/vol) PEG/homogenizing buffer. After centrifugation (10,000 x *g*, 10 min) the pellet was resuspended in a buffer containing 100 m*M* Tris-HCl (pH 7.5 at 4°C), 1 m*M* EDTA, 1 m*M* EGTA, 1 m*M* DTT, 50 m*M* NaF, 5 m*M* sodium pyrophosphate; 10% (vol/vol) glycerol; 0.02% (wt/vol) sodium azide, 4 μg/mL each of aprotinin, leupeptin, pepstatin-A, and soybean trypsin inhibitor, and 1 m*M* benzamidine. Protein content was determined using the Bradford method (Bradford, 1976).

Acetyl-CoA carboxylase activity was determined as described by (Lopaschuck et al., 1994), except that the HPLC procedure was replaced by measuring the rate of incorporation of [^{14}C] bicarbonate into an acid-stable compound (malonyl-CoA; Thampy and Wakil, 1985). Briefly, 3 parts of enzyme extract were pre-incubated for 5 min at 37°C in 1 part of buffer containing 0.24 *M* Tris-acetate, 3 mg/mL BSA, 20 m*M* mg-acetate, 40 m*M* citrate, and 5.2 m*M* β-mercaptoethanol. The reaction was initiated by adding 10 µL of pre-incubated enzyme in a final assay mixture of 165 µL containing 60.6 m*M* Tris-acetate, 2.12 m*M* ATP, 1.32 µ*M* β-mercaptoethanol, 5.0 m*M* Mg-acetate, 10 m*M* potassium citrate, 1.06 m*M* acetyl-CoA, 18.18 m*M* $NaHCO_3$, 0.33 µCi/µmol $Na^{14}CO_3$, and 1 mg/mL fatty acid free-BSA (pH 7.5). After a 4-min incubation at 37°C in a shaking water bath, the reaction was stopped by adding 25 µL of 10% (vol/vol) perchloric acid. Reactions tubes were placed in a desiccator under vacuum, and tubes were centrifuged at 2,900 x *g* for 20 min. The supernatant fraction (160 µL) was transferred into glass scintillation vials and evaporated to dryness at 80°C under vacuum. The residue was dissolved in 100 µL of H_2O and mixed with 4 mL of scintillation fluid. Acetyl-CoA carboxylase activity was expressed as nanomoles of ^{14}C-bicarbonate incorporated into malonyl-CoA·min^{-1}·mg protein^{-1}.

Lipoprotein Lipase Enzyme Activity. Activity of lipoprotein lipase (LPL) in subcutaneous adipose tissue was determined by modifications of the methods of Krauss et al. (1973) and Rao and Hawkins (1976). The modifications were as follows: a weighed amount of frozen tissue was diced into small pieces, placed in 5 volumes of 0.15 *M* KCL and homogenized at high speed for 1 min. The substrate was then prepared by sonification of 100 mg of triolein in 12 mL of 0.194 *M* Tris-HCl plus 0.15 *M* NaCl

buffer, pH 8.6, containing 0.05% (vol/vol) Triton X-100 and 200 mg of BSA, and activated by incubation for 30 min at 37°C with 0.1 part of calf serum. The assay mixture was incubated at 37°C for 90 min in a metabolic shaker, and the free fatty acids released were determined (Smith, 1975).

Slaughter and Carcass Data Collection. On the day of slaughter (May 19, 2004), heifers were transported approximately 60 km to Swift and Company, Greeley, CO where they were slaughtered using conventional and humane procedures. Carcasses were chilled in a cooler with an air temperature of 2°C for 36 h, and sprayed intermittently (2 min on, 8 min off) with a fine mist of 2°C water for the first 8 h of the chill period. Following the carcass-chilling period, a panel of 2 evaluators (Colorado State Univ.) independently evaluated each carcass and recorded measurements/assessments of adjusted fat thickness, adjusted preliminary yield grade, skeletal maturity, lean maturity, marbling score, and percentage of kidney, pelvic, and heart fat (KPH). Values for each trait from the 2 evaluators were averaged, resulting in a single value for each grade factor for each carcass. Skeletal and lean maturities were recorded on the following continuous scale: 100 = A-Maturity and 200 = B-Maturity. Marbling scores were assigned to each carcass using the following continuous scale: 300 = Traces; 400 = Slight; 500 = Small; 600 = Modest; 700 = Moderate; and 800 = Slightly Abundant. Yield grade for each carcass was calculated as: 2.50 + (2.50 × adjusted fat thickness, inches) + (0.20 × percent KPH) + (0.0038 x hot carcass weight, pounds) – (0.32 × LM area, square inches). Longissimus muscle area (LMA), fat area, grade percent fat, and total percent fat of the lean surface at the $12^{th}/13^{th}$ rib interface and fat thickness at the ¾-measure opposite the lean surface between the $12^{th}/13^{th}$ rib interface were measured via the Computer Vision

System Ribeye Camera (RMS Research Management Systems, USA Inc., Fort Collins, CO), which used colorimetric video-imaging. Fat area is defined as the cumulative 12th rib fat area as measured from 6 different sections. Grade percent fat and total percent fat are adjusted and unadjusted measurements, respectively, of the area of fat within the LM. As described by Guiroy et al. (2002), percent empty body fat (EBF) was calculated as: 17.76207 + (4.68142 × adjusted fat thickness, cm) + (0.01945 × hot carcass weight, kg) + (0.8185 × marbling score/100) – (0.06754 × LM area, cm^2).

Data Analyses. A 4% pencil shrink was applied to all BW before data analyses. Growth performance, HCW, marbling score, marbling percent, 12th rib fat thickness, LMA, KPH, calculated yield grade, dressing percent, and lipogenic enzyme activities were analyzed on an individual animal basis using the Mixed procedure of SAS Release 8.0 (SAS Inst., Inc., Cary, NC; 1999) for a randomized block design. The final analysis of variance (ANOVA) model for all growth performance traits and carcass characteristics included RAC treatment, IMP treatment, and their two-way interaction as fixed effects and weight block as a random effect. All independent variables included in the model were considered class variables. Least squares means were separated ($P < 0.10$) using a protected pairwise *t*-test (PDIFF option of SAS) when *F*-tests were significant at $\alpha = 0.10$.

The USDA yield and quality grade data were evaluated as categorical data (binomial proportion) using the GLIMMIX procedure of SAS. Ractopamine treatment and IMP treatment were included in the model as fixed effects, and weight block was included as a random effect. The two-way interactions of RAC treatment and IMP treatment were not statistically significant ($P > 0.10$) for any dependent variable and were not included in the

model. Simple arithmetic frequencies are presented. All independent variables included in the model were considered class variables.

Blood metabolite concentrations were analyzed on an individual animal basis using the Mixed procedure of SAS Release 8.0 for a randomized block design with repeated measures as described by Littell et al. (1998). Fixed effects included in the model were RAC treatment, IMP treatment, period, and all the corresponding two- and three-way interactions. In addition, the blood concentration of each metabolite on d 0 was used as a covariate in the analysis. Weight block and the three-way interaction of block, RAC treatment, and IMP treatment were included in the model as random effects. The subject effect of the repeated statement was period. A spatial power covariance structure was used with each sampling period defined in relative weeks. Class variables included RAC treatment, IMP treatment, block, animal, and period. When the overall *F*-value for treatment was significant ($P < 0.10$), least squares means were separated using Fisher's LSD test generated by the PDIFF statement in SAS.

RESULTS AND DISCUSSION

Growth Performance and Carcass Traits

Interactions. For growth and carcass variables, no interactions between RAC-supplementation and IMP treatment were observed ($P < 0.10$). Consequently, only the main-effect results will be discussed for growth and carcass measures.

Ractopamine Effects. Performance data for RAC treatments are presented in Table 5.2. Before the feeding of RAC, no differences in period BW were observed ($P > 0.10$); however, the final live BW was 10.1 kg heavier (2.0%; $P = 0.054$) for the heifers fed 250 mg or RAC/d. Other studies have reported a 0.7 to 1.3% increase in the final BW of

heifers fed 200 mg ractopamine/d for 28 d (Schroeder et al., 2005d; Laudert et al., 2007), but few studies have examined the effects of feeding more than 200 mg/d of RAC to heifers. However, Schroeder et al. (2005d) noted a 9.3 kg (1.8%) increase in the final live BW of heifers fed 283 mg RAC/d compared with negative control heifers. Laudert et al. (2007) reported a 6.0 kg (1.1%) increase in the final live BW of heifers fed 235 mg of RAC/d. Plots of the period BW for the RAC main effect are shown in Figures 5.3 and 5.4, and differences in period BW are charted in Figures 5.5 and 5.6. Similarly, average daily gain did not differ from d 0 to 56 ($P = 0.861$), d 0 to the beginning of the RAC-feeding period ($P = 0.827$), and from d 56 to the beginning of the RAC-feeding period ($P = 0.899$). However, ADG during the RAC-feeding period was 60.7% greater (0.98 vs. 0.61 kg/d; $P = 0.001$) for the heifers fed RAC compared with the controls. The percent improvement in ADG the final 28 d was extremely large compared with the 21.0 and 18.7% increases observed by Schroeder et al. (2005d) and Laudert et al. (2007), respectively, in heifers fed 283 and 235 mg of RAC/d and the 11.2 and 17.7% improvements in ADG observed in heifers fed approximately 200 mg of RAC/d for the final 28 d of the finishing period (Schroeder et al., 2005d; Laudert et al., 2007). As a result, ADG from d 56 to slaughter and from d 0 to slaughter were 16.8% ($P = 0.001$) and 6.2% ($P = 0.057$) greater, respectively, for the RAC heifers than for controls. Figure 5.7 shows the period ADG during the RAC-feeding period. From these charts, it seems that the increased ADG is large when the feeding of RAC is initiated, and it diminishes as RAC is fed for longer periods.

The DMI did not differ during the RAC-feeding period; however, G:F was improved by 60.3% (0.078 vs. 0.125 kg/d; $P = 0.001$). Similar to ADG, the percent improvement

in G:F the final 28 d of the current trial was much greater than the 19.5 and 21.8% increases observed by Schroeder et al. (2005d) and Laudert et al. (2007), respectively, in heifers fed 283 and 235 mg of RAC/d, and the 13.5 and 15.0% improvements in G:F observed in heifers fed approximately 200 mg of RAC/d for the final 28 d of the finishing period (Schroeder et al., 2005d; Laudert et al., 2007). A full explanation of why the performance response in these heifers was so much greater than those of previous trials cannot be given; however, it is likely partially attributable to the fact that these heifers were fed as individual animals and that each heifer consumed a more precise dose of ractopamine than would be expected in a pen setting with multiple animals.

Carcass data and quality and yield grade distributions for the RAC treatments are presented in Tables 5.3 and 5.4, respectively. As expected from the live growth performance, HCW was 6.5 kg greater (2.0%; $P = 0.068$) for the heifers receiving 250 mg of RAC/d. Most studies have noted a 0.3 to 1.5% increase in HCW in heifers fed 200 mg/d of RAC for 28 d (Schroeder et al., 2005d, Laudert et al., 2007; Sissom et al., 2007; Quinn et al., 2008). Schroeder et al. (2005d) reported a 5.1 kg (1.6%) increase in HCW of heifers fed 283 mg of RAC/d. Similarly, Laudert et al. (2007) noted a 6.1 kg (1.8%) increase in HCW in heifers fed 235 mg of RAC/d. Dressing percent did not differ between RAC treatments in the present experiment, and although LM area was numerically greater for the RAC heifers (82.4 vs. 79.9 cm^2), no statistical differences were detected ($P > 0.10$). In contrast, both Schroeder et al. (2005e) and Laudert et al. (2007) reported increases in dressing percent and LM area of heifers fed 283 and 235 mg of RAC/d, respectively, and Laudert et al. (2007) observed increase LM areas of in carcasses of heifers fed approximately 200 mg of RAC/d. In the present study, lean ($P =$

0.533), skeletal ($P = 0.809$), and overall maturities ($P = 0.855$) did not differ between treatments. The RAC heifers did have slightly more 12th rib fat (1.24 vs. 1.12 cm ; $P = 0.072$), but KPH and calculated yield grade did not differ between treatments ($P > 0.10$). Neither Schroeder et al. (2005e) or Laudert et al. (2007) observed any change in 12th rib fat thickness, yield grade, or KPH in heifers fed greater than 200 mg of RAC/d. In addition, the distribution of yield grades did not differ between treatments ($P > 0.10$). Empty body fat ($P = 0.643$), cumulative fat area in the LM ($P = 0.392$), grade percent fat ($P = 0.279$), and total percent fat ($P = 0.336$) did not differ between RAC and control treatments. Although mean marbling scores were 5.8% less for the RAC heifers, the differences were not significant ($P = 0.266$). In agreement with present findings, Schroeder et al. (2005e) and Laudert et al. (2007) noted numerical decreases in marbling score, but statistical differences were not detected. The corresponding percentage of carcasses grading Choice or greater in the present study was numerically less for the RAC heifers (58.3 vs. 75.0%; $P = 0.186$); however, with a small sample size (n = 48), caution should be taken in extrapolating these quality grade results to the general population of cattle.

Implant Effects. Performance and carcass data for IMP treatments are presented in Tables 5.2, 5.3, and 5.4, respectively. Plots of the period BW for the IMP main effect are shown in Figure 5.1, and differences in period BW are charted in Figure 5.2. Initial and d56 BW did not differ among IMP treatments ($P > 0.10$); however, at the start of the RAC-feeding period, the Revalor-200 heifers weighed 16.2 kg ($P = 0.007$) more than the non-implanted controls, and at slaughter, the Revalor-200 heifers weighed 22.9 (4.6%; $P = 0.001$) and 13.4 kg (2.7%; $P = 0.038$) more than the control and Finaplix heifers,

respectively. The ADG from d 0 to 56 was 13.5% greater ($P = 0.009$) for the Revalor-200 cattle than the controls. From d 56 to the beginning of the RAC-feeding period (d 91), the ADG of the Revalor-200 heifers was 13.6% ($P = 0.002$) and 6.7% ($P = 0.088$) greater than the control and Finaplix heifers, respectively. The ADG did not differ ($P > 0.10$) among treatments during the RAC-feeding period; however, as shown in Figure 5.2, the BW difference of the Revalor-200 treatment compared with the control continued to increase. For the entire feeding period, the ADG of the Revalor-200 cattle was 16.3 ($P = 0.001$) and 9.2% ($P = 0.022$) greater than the control and Finaplix heifers, respectively, which is similar to the response in the previous trial as well as the growth rates summarized and observed by others (Johnson et al., 1996b; Duckett et al., 1997; Preston, 1999; Montgomery et al., 2001; Nichols et al., 2002). Additionally, the Finaplix heifers tended ($P = 0.112$) to gain 6.5% greater than the controls from d 0 to slaughter. During the period in which intake data were collected (d 91 to slaughter), DMI was 5.8 ($P = 0.056$) and 10.3% ($P = 0.002$) greater for the Revalor-200 heifers than the control and Finaplix heifers, respectively; however, G:F was only numerically improved ($P > 0.10$) with the Finaplix (15.9%) and Revalor-200 (29.5%) implants during the final 28 d of the trial.

The HCW was 17.1 kg (5.3%; $P = 0.001$) and 9.2 kg (2.8%; $P = 0.037$) greater for the Revalor-200 –treated heifers than for the non-implanted controls and the Finaplix cattle, respectively. In addition, the carcasses of the Finaplix heifers were 7.9 kg heavier ($P = 0.067$) than the non-implanted heifers. Dressing percent for the controls (65.2%) were numerically less than for the Finaplix (65.5%) and Revalor-200 (65.6%) heifers, but the differences were not significant ($P > 0.10$). Similar to HCW, the LM areas of the

Revalor-200 carcasses were 7.7% ($P = 0.026$) and 7.0% ($P = 0.039$) greater than the control and Finaplix treatments, respectively. Skeletal maturity for the carcasses from the Revalor-200 treatment was greater ($P = 0.036$) than the non-implanted controls and tended ($P = 0.108$) to be greater than the Finaplix heifers. Skeletal maturity did not differ ($P = 0.604$) between the Finaplix and control carcasses. These results are in agreement with the previous trial and tend to support previous data showing that estrogen hastens ossification of cartilage to bone (Foutz et al., 1997; Morgan, 1997; Paisley et al., 1999; Pritchard, 2000; Duckett and Andrae, 2001; Roeber et al., 2000; Reiling and Johnson, 2003; Platter et al., 2003; Schneider et al., 2007). Following a similar trend to skeletal maturity, the lean maturity of the Revalor-200 carcasses was significantly greater than that from carcasses of non-implanted ($P = 0.001$) and Finaplix-implanted heifers ($P = 0.005$). As a result, overall maturity of the Revalor-200 carcasses was significantly greater than in carcasses from the non-implanted ($P = 0.001$) and Finaplix ($P = 0.005$) heifers, and the overall maturity of the controls and Finaplix carcasses did not differ ($P = 0.318$).

In agreement with the previous trial, empty body fat (EBF), a measure of compositional endpoint, did not differ among IMP treatments ($P = 0.585$) and averaged 28.7% for the implanted heifers, which is above the "critical" 28% (NRC, 1996) and 28.6% (Guiroy et al., 2001) EBF thresholds needed to attain a USDA low-Choice quality grade. Despite being fed to similar compositional endpoints and to endpoints beyond suggested thresholds, marbling scores tended ($P = 0.122$) to differ among treatments, with the marbling score of the Finaplix and Revalor-200 carcasses being 4.3 and 12.6% less than controls, respectively. In addition, the marbling score of the Revalor-200

carcasses was 8.7% less than the Finaplix carcasses. In agreement with marbling score changes, the grade percent fat ($P = 0.018$) and total percent fat ($P = 0.026$), which are 2 colorimetric measurements of marbling, were significantly lower for the Revalor-200 carcasses compared with the non-implanted controls ($P < 0.10$), and the total percent fat in the Revalor-200 carcasses was less than the Finaplix carcasses ($P = 0.064$). Moreover, the percentage of carcasses grading USDA Choice and greater differed among treatments ($P = 0.042$), even though the EBF did not differ among treatments. The Finaplix and Revalor-200 treatments had 31.3 ($P = 0.060$) and 50.0 ($P = 0.013$) percentage units fewer carcasses, respectively, grading USDA Prime or Choice than carcasses from the non-implanted controls.

Numerous trials have shown that implants decrease USDA quality grade and marbling score in beef cattle (Loy et al., 1988; Johnson et al., 1996b; Samber et al., 1996; Morgan, 1997; Pritchard, 2000; Duckett and Andrae, 2001; Montgomery et al., 2001; Reiling and Johnson, 2003; Bruns et al., 2005; McPhee et al., 2006). Moreover, quality grade has been shown to decrease in a dose-dependent fashion in response to increased aggressiveness in IMP regimens (Samber et al., 1996; Foutz et al., 1997; Morgan, 1997; Roeber et al., 2000; Platter et al., 2003).

It must be noted that most studies, including the current one, were conducted so that cattle were slaughtered at time-constant endpoints. Nichols et al. (2002) theorized that some of the negative quality aspects associated with use of steroidal agents is a result of differences in physiological endpoints and suggested that some of the decreased marbling may be negated by feeding implanted cattle longer and to heavier BW. Nichols et al. (2002) also stated that at equal physiological maturity, carcass composition will be

similar between implanted and non-implanted cattle. Owens et al. (1995) suggested that EBF might be a good indicator of physiological maturity. Subsequently, Guiroy et al. (2001) developed a methodology to calculate EBF based on 12th rib fat thickness, HCW, USDA quality grade, and LM area. Using these equations for EBF and 13 experiments that involved 15 different IMP strategies, Guiroy et al. (2002) calculated the adjusted final shrunk BW at 28% EBF, which is theoretically the standard body composition needed to achieve a small degree of marbling (i.e., low Choice) on the USDA scale (NRC, 1996). Accordingly, the BW at which animals reached the same EBF increased as the anabolic implant dose increased 14 to 42 kg and 30 to 39 kg in steers and heifers, respectively, depending on the IMP regimen that is used (Guiroy et al., 2002).

The data in the both the current IMP × RAC trials (Chapter IV and the present experiment) are in agreement with others such as Guiroy et al. (2002), who showed that the percentages of carcasses grading USDA Choice or greater decreased even though the average EBF among IMP-aggressiveness categories were similar. In addition, Guiroy et al. (2002) reported that even at a 30% EBF, many carcasses did not grade USDA Choice or greater. Schneider et al. (2007) conducted a study in heifers examining the response curves for growth and carcass measurements that were associated with increasing doses of estradiol and trenbolone acetate. Although the EBF among all 12 treatments were not statistically different, marbling and quality grade trended down with increasing anabolic dosage (Schneider et al., 2007). In comparing the 3 treatments of cattle that received the identical doses of estrogen and trenbolone acetate (8 and 80 mg, respectively) at the time of arrival processing and that were reimplanted with either 8:80, 14:140, or 20:200 mg of estradiol:trenbolone acetate, the percentage of USDA Prime and Choice carcasses and

marbling score decreased dramatically with increasing anabolic dose (Schneider et al., 2007) without any differences in EBF. Similarly, in reviewing the trial data of Roeber et al. (2001) and Platter et al. (2003), J. D. Tatum (unpublished data; Colorado State Univ., Fort Collins) showed that decreases in marbling score and quality grade that are associated with the use of steroidal implants were not mitigated by feeding implanted and non-implanted cattle to the same EBF endpoint. Collectively, these results do not agree with the hypothesis of Nichols et al. (2005), which suggested that if cattle are fed to the same EBF endpoint, they will grade the similarly.

It is not clear whether these marbling and grading differences between the Finaplix and Revalor-200 treatments are a result of differences in cumulative doses of steroid hormone or the difference in estrogen content. Parr et al. (2006) studied the effects of giving a cumulative dose of 24:120 mg of estradiol:trenbolone acetate (E:TBA) either in 1 (24:120 mg of E:TBA), 2 (12:60 mg of E:TBA), or 3 (8:40 mg of E:TBA) equally spaced patterns and did not observe any differences in overall performance or carcass measures among the treatments. Schneider et al. (2007) also did not detect differences in marbling scores between cattle given a cumulative dose of 28:280 mg E:TBA as either 2 doses of 14:140 mg E:TBA or as 1 dose of 8:80 mg E:TBA and another dose of 20:200 mg E:TBA. However, Schneider et al. (2007) reported that heifers receiving a combination of 20:200 mg E:TBA had increased LM areas, lower marbling scores, and increased shear forces compared with those receiving 0:200 mg E:TBA implant. It is not known whether these differences were attributable to the estrogen component of the implant or to the difference in total anabolic dose. Bartle et al. (1992), Hutcheson et al. (1997), and Kreikemeier and Mader (2004) found that androgen and estrogen implants

had additive effects on protein deposition and performance compared with using an estrogen or androgen implant alone. Herschler et al. (1995) also observed that estrogen plus androgen implant combinations were more negative to marbling and quality grade than estrogen- or trenbolone acetate-only implants. Some have hypothesized that the ratio of E:TBA (Herschler et al., 1995) and more specifically the estrogen portion (Gerken et al., 1995) is an important factor in the marbling effects noted in response to E:TBA implants. Gerken et al. (1995) stated that steers implanted with estrogenic steroids had significantly lower marbling scores than steers implanted with androgenic or combination implants. Herschler et al., (1995) surmised that 1:10 ratios of E:TBA had less effects on quality grade than 1:5 E:TBA ratios without any decreases in performance; however, the comparisons were made across different doses of both estrogen and trenbolone acetate, so that few relative conclusions could be drawn. More research needs to be conducted to separate the effects of cumulative anabolic dose from the effects that the estrogen and trenbolone acetate components have separately on performance and quality grade when they are combined in a single implant.

In agreement with the results presented in Chapter IV, the 12th rib fat thickness, mean calculated yield grade, and yield grade distribution did not differ among IMP treatments ($P > 0.10$). Many recent trials have noted decreased calculated yield grade, increased LM area without any differences in EBF (Roeber et al., 2000; Platter et al., 2003; Schneider at al., 2007) or fat thickness (Herschler et al., 1995; Johnson et al., 1996b; Foutz et al., 1997; Roeber et al., 2000; Platter et al., 2003; Bruns et al., 2005; Duckett and Andrae, 2007; Schneider at al., 2007) associated with IMP use. Although noted in Chapter IV but not the present experiment, several others have reported decreased KPH associated

with implanted cattle compared with non-implanted cattle (Herschler et al., 1995; Johnson et al., 1996b; Platter et al., 2003; Schneider et al., 2007), whereas others have not (Herschler et al., 1995; Foutz et al., 1997; Roeber et al., 2000; Bruns et al., 2005; Duckett and Andrae, 2007).

Blood Metabolites

Estradiol-17β. An IMP × RAC interaction was detected ($P = 0.015$) for serum estradiol-17β (Figure 5.8). As expected, the estradiol concentration was greater in heifers that were administered an estrogen-containing implant. The concentrations of estradiol-17β did not differ ($P > 0.10$) between non-implanted heifers and those administered Finaplix, regardless of whether they received supplemental RAC. Those heifers administered a Revalor-200 and either fed or not-fed RAC had greater ($P < 0.10$) estradiol-17β concentrations than either the control or Finaplix heifers. In agreement with others (Lee et al., 1990; Johnson et al.; 1996b; Henricks et al., 1997), with current uncoated implants, the release of estradiol is rapid and peaked within 7 d of administration (Figure 5.9). Newer coating technologies such as those used in the production of Revalor-XS (Intervet/Schering-Plough Animal Health, Millsboro, DE) may alter the release of estrogen and trenbolone acetate and hence alter the patterns of protein and fat deposition observed with uncoated implants. Interestingly, within the Revalor-200 treatment, the heifers fed RAC had greater ($P = 0.005$) estradiol concentrations than those that did not receive supplemental RAC. This observation cannot be explained, and no other data could be found to support or negate this finding.

Cortisol. No changes ($P = 0.499$) in cortisol were associated with feeding RAC. An IMP × period interaction was detected ($P = 0.007$) for serum cortisol (Figure 5.10). For

each collection period between and including d 7 and 112, serum cortisol concentrations were greater ($P < 0.10$) for the non-implanted controls than for the Revalor-200 heifers. Moreover, the cortisol concentrations of the Finaplix heifers were less than ($P < 0.10$) those of the controls on d 70, 93, 98, 105, and 112 and were typically intermediate between the control and Revalor-200 treatments for other periods from d 14 through slaughter. No changes ($P > 0.10$) in cortisol were associated with feeding RAC. Others have also observed a decrease in circulating concentrations of serum cortisol associated with the use of estrogen or trenbolone acetate implants (Grigsby and Trenkle, 1986; Lee et al., 1990; Jones et al., 1991; Hayden et al., 1992; Isaacson et al., 1993). In human medicine, glucocorticoids inhibit the physiological secretion of GH (Solomon and Bouloux; 2006) and decrease IGF-1 production at target organs (Schakman et al., 2008). Moreover, Ma et al. (2001) concluded that glucocorticoids upregulate the expression of myostatin, which is a negative regulator of skeletal muscle mass, and Yang et al. (2005) suggested that glucocorticoids increase activity of the C/EBP cascade. In addition, several have reported that anabolic steroids can preferentially bind and displace corticosteroids from their receptors via competitive inhibition in the muscle (Mayer and Rosen, 1975; Hancock et al., 1991; Trenkle, 1997; Eason et al., 2003). As a result, it is possible that decreased secretion of glucocorticoids resulting from exposure to steroids could result in indirect anabolic effects in muscle protein.

IGF-1. No changes ($P = 0.188$) in IGF-1 were associated with feeding RAC. A 2-way period × IMP interaction was observed ($P < 0.001$) for serum IGF-1; consequently, data are summarized by IMP treatment within each period (Figure 5.10). On d 56, the IGF-1 concentrations of the non-implanted heifers were less ($P = 0.045$) than those of the

Revalor-200, but the IGF-1 concentrations in the Finaplix heifers did not differ from the non-implanted or Revalor-200 heifers. For every collection period from d 70 to slaughter, the IGF-1 concentrations in the Revalor-200 heifers were greater ($P < 0.10$) than the Finaplix and non-implanted cattle. In addition, from d 70 to slaughter, the Finaplix heifers had greater concentrations than the non-implanted heifers. The present experiment design does not allow us to separate the effects of the estrogen component of the implant from the cumulative anabolic dose. Consequently, it is not known whether the apparently additive effect of estrogen and trenbolone acetate on circulating IGF-1 was caused by the additional anabolic dose or to the estrogen component itself.

Recent research results have demonstrated that implants increase circulating IGF-1 (Lee et al., 1990; Preston et al., 1995; Johnson et al, 1996a,1998a; Dunn et al., 2003; Pampusch et al., 2003). Hunt et al. (1991) reported a statistical increase in serum IGF-1 in steers that were given estrogen (24 mg) and trenbolone acetate (120 mg) in combination and observed a numerical increase with trenbolone acetate-only implants (120 mg). Similarly, Mader and Kreikemeier (2006) administered estrogen-only, trenbolone acetate-only, and estrogen-and-trenbolone acetate implants (separately) and only reported statistical increases in circulating IGF-1 with the combination use of both estrogen and trenbolone acetate implants; however, all implanted cattle had numerically greater serum concentrations of IGF-1 than the negative controls. It is not known whether these differences in degree of significance were a result of the estrogen component or the differences in cumulative anabolic dose.

The IGF binding protein-3 (Johnson et al, 1996a) and IGF-1 mRNA levels in longissimus (Johnson et al, 1996a, 1998b; Dunn et al., 2003; Pampusch et al., 2003) or

semimembranosus muscles (White et al., 2003) and liver (White et al., 2003) were increased in steers that were implanted with a combined estrogen and trenbolone acetate implant relative to those that were not implanted. These results suggest that perhaps both liver (endocrine) and local (autocrine/paracrine) production of IGF are necessary for anabolic responses associated with steroid implants in cattle. These data also suggest that RAC does not elicit a response via the GH-IGF-1 pathway.

Growth Hormone. Similar to IGF-1, an IMP × period interaction was detected ($P = 0.037$) for serum GH (Figure 5.12). Growth hormone is known to be released in a pulsatile manner, and more revealing information and conclusions may have been reached if samples had been collected serially within day, and if amplitude, frequency, and area under the curve of GH release had been measured. Although the GH patterns seemed somewhat erratic, for each collection from d 42 to 119, except for d 93 and 112, the Revalor-200 heifers had greater ($P < 0.10$) serum GH concentrations than the non-implanted control heifers. In addition, the Revalor-200 heifers had greater ($P < 0.10$) GH concentrations than the Finaplix heifers on d 56, 70, 98, and 105. Serum GH concentrations did not differ between the Finaplix and controls for any collection period. Unlike in IGF-1, these data seem to suggest that the estrogen component of the implant and not the anabolic dose is associated with increased circulating concentrations of GH in the Revalor-200 heifers.

In a review, Trenkle (1983) stated that 1 of the most consistent changes observed in cattle treated with estradiol is the increase in weight of the anterior pituitary gland. Through magnetic resonance imaging, Carroll et al. (2007) also observed that the pituitary size of a zeranol-treated sheep was 3 times that of its control sibling.

Furthermore, the concentration of GH in the pituitary is not increased, but the total amount is increased as a result of the increase in size (Trenkle, 1983). Clegg and Cole (1954) reported an increased number of acidophils in DES-treated cattle. In agreement, Thomson et al. (1996a) observed an increased in percentage of somatotrophs in comparison to mammosomatotrophs in steers implanted with an E:TBA combination implant 24 d before slaughter. In contrast, the pituitary size from ruminants administered only trenbolone acetate was not altered (Donaldson et al. 1981). Numerous studies have shown an increase in circulating concentrations of GH associated with use of estrogen-containing implants, in beef cattle (Borger et al., 1973; Preston, 1975, 1999; Trenkle, 1970, 1983, 1997; Grigsby and Trenkle, 1986; Hongerholt et al., 1992). Moreover, Trenkle (1983) reported that circulating GH is increased following administration of estrogens and testosterone propionate but not trenbolone acetate. Hayden et al. (1992) observed in increase in serum GH of cattle administered estrogen-only implants but not estrogen-trenbolone acetate combinations relative to negative controls. Similarly, Hunt et al. (1991) did not observe difference in GH in cattle given either trenbolone acetate-only implants or estrogen-trenbolone acetate implants. However, Hongerholt et al. (1992) showed an increase in GH associated with estrogen-plus-trenbolone acetate implants.

Growth hormone is released in discrete and episodic intervals within each day (Trenkle, 1997). Steroid hormones have been shown to increase the baseline GH and the frequency of release in steers administered an estradiol implant without any change in amplitude (Grigsby and Trenkle, 1986). In contrast, Hayden et al. (1992) reported trends for increased amplitude, duration, and frequency without any changes in baseline concentrations when steers were administered either estrogen implants. Nonetheless, the

amplitude, duration, and frequency of GH release tended to decrease with exposure to trenbolone acetate-only or trenbolone acetate-plus-estrogen implants (Hayden et al., 1992).

Trenkle (1983) postulated that androgens act directly on the muscle, and that estrogens primarily act on the hypothalamus or anterior pituitary to increase secretion of growth hormone. Some have reported that the relationship between estrogen and GH seems to be additive, which suggests that the estrogens do not solely exert actions via GH (Enright et al., 1990; Hancock et al., 1991; Preston et al., 1995; Ono et al., 1996; Rumsey et al., 1996; Elasser et al., 1998). Furthermore, Trenkle (1983, 1997) concluded that anabolic agents seem to work through more than 1 mechanism of action, and that the anabolic responses observed in cattle to exogenous steroids cannot be solely a result of an increase in GH secretion.

In a recent study with the objective to further elucidate the mechanisms of action for androgens and estrogens, Hassan et al. (2001) perifused estradiol-17β, testosterone, and its metabolites into bovine hypothalamic and anterior pituitary slices and found that neither estrogen nor testosterone affected GH release by direct action on the anterior pituitary cells. Conversely, dihydrotestosterone (DHT) and 3α-diol, which are produced from the reduction of testosterone via 5α-reductase, increased GH release directly from the somatotrophs (Hassan et al., 2001). When the hypothalamus and anterior pituitary slices were placed in series, perifusion of estrogen into the hypothalamus cells increased growth hormone-releasing hormone (GHRH) and GH and decreased somatostatin (SS) concentrations (Hassan et al., 2001). Shirasu et al. (1990) and Painson et al. (1992) also showed that estrogen acted directly on the hypothalamus of rats to induce secretion to

GHRH and GH. When testosterone was infused at a constant rate in-series, GHRH and SS increased at the same rate so that GH release was not affected. Argente et al. (1990) also observed that SS mRNA was increased by testosterone and decreased by estradiol. Hassan et al. (2001) reported that infusion of testosterone and its metabolites in-series each increased GH, GHRH, and SS when administered at a pulsatile rate. The researchers noted that the increase in GH release from pulsatile infusion of testosterone into the hypothalamus was nullified and that SS release was increased by the addition of aromatase inhibitor. Hassan et al. (2001) hypothesized that the aromatization of testosterone into estrogen in the hypothalamus is partially responsible for the testosterone-induced GH release in the hypothalamus. The authors concluded, however, that the differences in release patterns of GHRH and SS in response to estrogen and androgens may be responsible for gender-specific GH patterns.

No changes ($P = 0.625$) in GH were associated with feeding RAC. Together with the IGF-1 results, these data suggest that RAC does not elicit a response via the GH-IGF-1 pathway.

Insulin and Glucose. No changes ($P = 0.690$) in insulin were associated with administration of steroid hormones. A 2-way RAC × period interaction was observed ($P = 0.074$) for serum insulin concentrations (Figure 5.13). Likely because of random chance, the serum concentrations of insulin were different ($P < 0.10$) between the control and RAC-supplemented heifers on d 56 and 70, before the initiation of RAC feeding. Insulin concentrations did not differ on d 91, when RAC-supplementation began. The control heifers had lower ($P < 0.10$) insulin concentrations than the RAC heifers on d 93, 98, and 119. It is not known whether this was a result of random chance or to treatment;

however, with the exception of a period effect ($P < 0.001$) no differences were detected ($P > 0.10$) for the main effects or their interactions for serum glucose concentrations (mean = 85.8 mg/dL; Figure 5.14).

Others have reported no changes in circulating insulin (Trenkle, 1970; Borger et al., 1973; Grigsby and Trenkle, 1986; Hayden et al., 1992) and glucose concentrations (Borger et al., 1973; Hongerholt et al., 1992), whereas Enright et al. (1990) reported increased plasma glucose associated with steroid hormone administration. With use of beta-adrenergic agonists, some have observed a decrease in plasma insulin concentrations in response to cimaterol (Beermann et al., 1987; O'Connor et al., 1991b) and clenbuterol (Eisemann and Huntington, 1988) and decreased insulin binding to adipocytes (Liu and Mills, 1990). Studies by Eisemann and Bristol (1998), however, did not reveal any changes in plasma concentration of insulin when sheep were fed RAC, but there was a tendency toward increased tissue sensitivity and responsiveness to insulin. Corresponding to decreased insulin concentrations, some investigators have shown increases in plasma glucose concentrations in response to cimaterol (Chickou et al., 1991; O'Connor et al., 1991b), clenbuterol (Blum and Flueckiger, 1988; Eisemann et al., 1988), and RAC (Adeola et al., 1992b). Others, however, have not detected any differences in plasma glucose in response to cimaterol (Beermann et al., 1987; Byrem et al., 1998) or RAC (Eisemann and Bristol, 1998); moreover, Walker et al. (2006) reported greater decreases in plasma glucose concentrations of heifers after 13 d of RAC feeding compared with those that were not fed RAC.

Epinephrine and Norepinephrine. No differences in plasma epinephrine (mean = 251.3 pg/mL) were detected ($P > 0.10$) among treatments, periods, or their interaction. A

period × IMP interaction was noted ($P = 0.001$) for plasma norepinephrine (Figure 5.15). On d 2, 7, 14, and 28, the non-implanted heifers had lower ($P < 0.10$) concentrations of norepinephrine than the Finaplix heifers. In each of these collection periods, the Revalor-200 heifers had intermediate plasma concentrations of norepinephrine, with lower ($P < 0.10$) concentrations than the Finaplix heifers on d 2 and 14 and greater concentrations than the controls on d 14 and 28. After d 28, no differences in plasma norepinephrine were detected ($P > 0.10$) among treatments. It is not know whether the intermediate response observed in the Revalor-200 heifers was a result of chance or to possible mitigating effects of added estrogen. No other studies could be found assessing changes in circulating catecholamine concentrations associated with steroid implant or beta-adrenergic agonist use.

Blood Urea Nitrogen. A RAC × IMP interaction was detected ($P = 0.001$) for BUN concentrations (Figures 5.16, 5.17, and 5.18). Within the non-implanted and Finaplix heifers, the addition of supplemental RAC decreased ($P < 0.10$) BUN concentrations. Within the Revalor-200 treatment, no differences ($P > 0.10$) were observed between the heifer fed RAC and controls. Moreover, the serum BUN concentrations of the Finaplix/RAC did not differ ($P < 0.10$) from either RAC treatment of the Revalor-200 heifers. The administration of an implant and/or supplementation with RAC decreased BUN, suggesting that more circulating urea was mobilized to muscle tissue to support protein deposition.

Others have also reported a decrease in circulating BUN associated with the use of steroidal implants (Enright et al., 1990; Hongerholt et al., 1992; Cecava and Hancock, 1994; Preston et al., 1995; Mader and Kreikemeier, 2006). With use of beta-adrenergic

agonists, decreased concentrations of circulating BUN have been associated with the use of cimaterol (Chikhou et al., 1991) and RAC (Eisemann and Bristol, 1998). In addition, in feedlot heifers, Walker et al. (2006) reported greater numerical decreases in plasma BUN concentrations after 13 d of RAC feeding compared with heifers that were not fed RAC.

Non-Esterified Fatty Acids. No changes ($P = 0.991$) in serum concentrations of NEFA were associated with feeding RAC. Circulating NEFA were increased ($P < 0.10$) in implanted heifers (Figures 5.19, 5.20, and 5.21). Limited data are available examining the effects of implants on circulating NEFA. Enright et al. (1990) observed no differences in plasma NEFA of steers administered an estradiol-only implant. In sheep, Lough et al. (1993) reported decreased numerical concentrations of trenbolone-acetate implanted rams compared to non-implanted controls, but the differences were not statistically different. Typically, increased concentrations of serum NEFA indicate mobilization of fat stores to provide energy support for the physiological functions of other tissues. Present findings could indicate that protein deposition and muscle building may require increased energy.

In contrast to present results, several authors have reported increased concentrations of plasma NEFA in response to exposure to beta-adrenergic agonists such as cimaterol (Beermann et al., 1987; Kim et al., 1987; Chickou et al., 1991; O'Connor et al., 1991b; Byrem et al., 1998), clenbuterol (Blum and Flueckiger, 1988; Eisemann et al., 1988), and RAC (Adeola et al., 1992b)

Lipogenic Enzyme Activity. An implant × RAC interaction ($P = 0.045$) was observed for FAS activity (Figure 5.22). Within IMP treatment, the addition of RAC increased (*P*

= 0.001) the FAS activity in the Finaplix heifers, but no other differences were noted ($P >$ 0.10) within the other 2 IMP treatments. Across implant × RAC treatment combinations, the Finaplix-RAC heifers had greater ($P < 0.10$) FAS activity than the no-implant/control, no-implant/RAC, and the Finaplix/controls. For the activity of ACC, an implant × RAC interaction was detected ($P = 0.043$). Similar to FAS, the activity of ACC did not differ ($P > 0.10$) between RAC treatments within the control and Reavlor-200 heifers (Figure 5.23); however, ACC activity increased with RAC supplementation in the Finaplix heifers. Across treatments, the Finaplix/RAC heifers had greater ($P <$ 0.10) ACC activity than the Finaplix/controls and either non-implanted heifer group. No differences ($P > 0.10$) in implant, RAC, or their 2-way interaction were observed for LPL activity.

Limited data are available which examine the effects of implants or beta-agonists on lipogenic enzyme activity. In agreement with the current data, Smith et al. (2007) did not detect any differences in mRNA production of the lipogenic enzymes ACC, LPL, and stearoyl-CoA desaturase between cattle that either did not receive an anabolic implant and those that received 2 separate doses of 28 mg of estradiol benzoate and 200 mg of trenbolone acetate during the feeding period. However, the authors reported that the number of intramuscular adipocytes per gram of tissue were greater for the implanted heifers than for their non-implanted counterparts (Smith et al., 2007); no differences in cellularity were noted in subcutaneous adipose tissue. More recently, Parr et al. (2008) reported that after 28 d of exposure, finishing steers implanted with 24:120 mg E:TBA had decreased abundance of peroxisome proliferators-activated receptor γ (PPARγ) and stearoyl CoA desaturase mRNA and a tendency for decreased CCAAT/enhancer-binding

protein β (C/EBPβ) mRNA expression; these transcription factors and enzymes are involved in lipogenesis. Singh et al. (2003) also reported that both testosterone and dihydrotestosterone downregulated C/EBPα and PPARγ mRNA expression in pluripotent mesenchymal cells.

IMPLICATIONS

Results of the present experiment suggest that IMP and RAC can act synergistically to alter growth and carcass performance. Additionally, USDA quality grade and marbling score can differ despite cattle being slaughtered at equal EBF. To determine compositional endpoints at which quality grade will be equivalent, new equations with independent slopes and intercepts must be modeled from implant trial data instead of applying existing equations to trial results. Further research should be initiated to evaluate the dose-equivalencies of estradiol-17β and trenbolone acetate to determine whether these components individually have differential effects on growth and carcass quality. Combination implants seem to elicit action via GH and IGF-1 pathways and also seem to increase lipolysis without affecting lipogenesis. Furthermore, as measured by classical metabolite indicators of growth and protein and fat anabolism and catabolism, steroids and beta-adrenergic agonists seem to elicit dissimilar metabolic responses, suggesting different modes of action. Further research is needed to elucidate the mechanisms of action of both steroidal implants and beta-adrenergic agonists at both the cellular level and in terms of changes in organ mass.

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Table 5.1. Formulated ingredient and analyzed chemical composition of the finishing diet fed to heifers in the ractopamine × implant trial, DM basis.

Ingredient, %	
Alfalfa hay	14.17
Steam-flaked corn (85.6% DM)[a]	72.10
Sorghum-sudan silage	2.63
Protein supplement[b]	2.21
Soybean meal	1.92
Limestone	1.07
Dried distillers grain	5.90
Ground corn[c]	—
Chemical composition[d]	
DM, %	78.7
CP, %	12.8
Crude fiber, %	6.6
NDF, %	16.1
ADF, %	9.0
NEm, Mcal/kg	2.16
NEg, Mcal/kg	1.46
TDN, %	84.6
Fat (ether extract), %	2.6
Ca, %	0.25
P, %	0.21
K, %	0.64
S, %	0.14
Mg, %	0.09
Zn, ppm	33
Fe, ppm	75
Mn, ppm	27
Cu, ppm	13

[a]Steaming was maintained for approximately 55 min at 98.9°C. Bulk density = 0.36 kg/L. Flaked corn was air-dried before feeding.

[b]Protein supplement (55.9% CP) provided per kilogram of diet DM: 30 mg of monensin; 8.8 mg tylosin phosphate; 30 mg of Zn as $ZnSO_4$; 10 mg of Cu as $CuSO_4$; 20 mg of Mn as $MnSO_4$; 0.5 mg of I as $(Ca(IO_3)_2(H_2O)$; 0.1 mg of Co as $CoCO_3$; and 0.1 mg of Se as Na_2SeO_3.

[c]Ground corn supplement was used as a carrier to deliver the treatment ingredient as a rate of 0.45 kg·heifer^{-1}·d^{-1}.

[d]Diets were formulated to meet or exceed all nutrient requirements for finishing heifers (NRC, 1996).

Table 5.2. Least squares means of growth performance for main effects of ractopamine supplementation and implant treatment.

	Ractopamine, mg/d			Implant					
Trait[a]	0	250	P_{RAC}	None	Finaplix	Revalor-200	$P_{Implant}$	SEM	$P_{R \times I}$ [c]
Number of animals	24	24		16	16	16			
Initial BW, kg[b]	346.6	347.0	0.885	347.4	347.0	345.9	0.909	9.8	0.987
d56 BW, kg[b]	440.2	440.0	0.962	435.0	440.4	444.9	0.116	9.5	0.419
End-minus-28 BW, kg[b]	483.0	482.5	0.906	474.7^{x}	482.6^{xy}	490.9^{y}	0.024	10.3	0.955
End BW, kg[b]	500.8^{x}	510.9^{y}	0.054	495.1^{x}	504.6^{x}	518.0^{y}	0.003	10.2	0.777
ADG; d 0 to 56	1.67	1.66	0.861	1.56^{x}	1.67^{xy}	1.77^{y}	0.031	0.052	0.403
ADG; d 0 to end-minus-28	1.50	1.49	0.827	1.40^{x}	1.49^{x}	1.59^{y}	0.008	0.042	0.923
ADG; d 56 to end-minus-28	1.22	1.21	0.899	1.14	1.21	1.31	0.208	0.075	0.123
ADG; d 56 to end	0.95^{x}	1.11^{y}	0.0006	0.94^{x}	1.00^{x}	1.14^{y}	0.017	0.048	0.139
ADG; End-minus-28 to end	0.61^{x}	0.98^{y}	0.0006	0.70	0.76	0.93	0.156	0.086	0.733
ADG; d 0 to end	1.29^{x}	1.37^{y}	0.057	1.23^{x}	1.31^{x}	1.43^{y}	0.0012	0.036	0.736
DMI; End-minus-28 to end	7.71	7.78	0.691	7.70^{x}	7.39^{x}	8.15^{y}	0.0069	0.162	0.401
F:G; End-minus-28 to end	14.56^{x}	8.71^{y}	0.047	12.08	12.85	9.97	0.699	2.48	0.924
G:F; End-minus-28 to end	0.078^{x}	0.125^{y}	0.0004	0.088	0.102	0.114	0.246	0.011	0.777

[a]ADG = average daily gain, kg/d; F:G = feed:gain ratio, DM basis.

[b]Weights decreased by 4% to represent a standard industry shrink.

[c]Ractopamine × implant interaction remained in the model regardless of significance value.

[x,y,z]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

Table 5.3. Least squares means of carcass characteristics for main effects of ractopamine supplementation and implant treatment.

	Ractopamine, mg/d			Implant					
Trait[a]	0	250	P_{RAC}	None	Finaplix	Revalor-200	$P_{Implant}$	SEM	$P_{R \times I}$[d]
HCW, kg	327.6^{x}	334.1^{y}	0.068	322.5^{x}	330.4^{y}	339.6^{z}	0.0012	6.1	0.429
Dressing percent	65.4	65.4	0.992	65.2	65.5	65.6	0.618	0.3	0.233
LM area, cm^2	79.9	82.4	0.250	79.0^{x}	79.5^{x}	85.1^{y}	0.046	1.9	0.505
AFAT, cm	1.12^{x}	1.24^{y}	0.072	1.20	1.10	1.24	0.166	0.055	0.156
KPH, %	2.30	2.27	0.694	2.31	2.25	2.30	0.798	0.068	0.282
YG	2.84	2.88	0.803	2.93	2.86	2.81	0.802	0.129	0.922
SMAT[b]	166.7	165.8	0.809	162.5^{x}	164.7^{xy}	171.6^{y}	0.090	2.96	0.953
LMAT[b]	160.0	161.7	0.533	155.3^{x}	158.8^{x}	168.4^{y}	0.0006	2.29	0.168
OMAT[b]	163.3	163.8	0.855	158.9^{x}	161.7^{x}	170.0^{y}	0.0009	2.03	0.563
Marbling[c]	581.7	548.1	0.266	598.8	572.8	523.1	0.122	26.0	0.729
Empty body fat[e], %	28.7	29.0	0.643	29.2	28.6	28.7	0.585	0.45	0.343
Fat area[f], mm^2	3.13	2.89	0.392	3.24	3.05	2.74	0.328	0.252	0.368
Grade fat[g], %	1.96	1.76	0.279	2.11^{x}	1.90^{xy}	1.57^{y}	0.056	0.166	0.599
Total fat[g], %	2.89	2.59	0.336	3.08^{x}	2.92^{x}	2.21^{y}	0.059	0.266	0.736

[a]HCW = hot carcass weight; LM = longissimus muscle; AFAT = adjusted fat thickness; KPH = kidney, pelvic, and heart fat percent; YG = calculated yield grade; SMAT = skeletal maturity; LMAT = lean maturity; OMAT = overall maturity.

[b]100 = A-maturity; 200 = B-maturity.

[c]300 = Traces^0, 400 = Slight^0, 500 = Small^0, 600 = Modest^0, 700 = Moderate^0, 800 = Slightly Abundant^0.

[d]Ractopamine × implant interaction remained in the model regardless of significance value.

[e]Empty body fat, % (Guiroy et al., 2002) = 17.76207 + (4.68142 x AFAT, cm) + (0.01945 x HCW, kg) + (0.8185 x marbling/100) – (0.06754 x LM area, cm^2).

[f]Fat area is defined as the cumulative 12th rib fat area as measured from 6 different sections.

[g]Grade percent fat and total percent fat are adjusted and unadjusted measurements, respectively, of the area fat within the LM.

[x,y,z]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

Table 5.4. Quality and Yield grade distributions of carcasses within the main effects of ractopamine supplementation and implant treatment.

	Ractopamine, mg/d			Implant				
	0	250	P_{RAC}	None	Finaplix	Revalor-200	$P_{Implant}$	$P_{R \times I}$[a]
Quality grade marketing category[b]								
USDA Prime	12.50	0.00	0.976	6.25	12.50	0.00	0.821	
USDA Choice	62.50	58.33	0.750	87.50^{x}	50.00^{y}	43.75^{y}	0.055	
USDA Choice or greater	75.00	58.33	0.186	93.75^{x}	62.50^{y}	43.75^{y}	0.042	
USDA Select	25.00	41.67	0.186	6.25	37.50	56.25	0.042	
Calculated yield grade[c]								
Yield grade 1	0.00	4.17	0.985	0.00	6.25	0.00	1.000	
Yield grade 2	70.83	54.17	0.232	62.50	50.00	75.00	0.352	
Yield grade 1 and 2	70.83	58.34	0.367	62.50	56.25	75.00	0.535	
Yield grade 3	25.00	37.50	0.356	31.25	37.50	25.00	0.747	
Yield grade 4	4.17	4.17	1.000	6.25	6.25	0.00	1.000	

[a]The ractopamine × implant interaction was not significant ($P > 0.10$) and was removed from the model.

[b]Quality grade marketing category determined by expert marbling score: USDA Prime = Slightly Abundant^{0} to Abundant^{99}; USDA Choice or higher = Small^{0} to Abundant^{99}; USDA Choice = Small^{0} to Moderate^{99}; USDA Select = Slight^{0} to Slight^{99}.

[c]Based on calculated yield grade from LM area, adjusted fat thickness, hot carcass weight, and percentage of kidney, pelvic, and heart fat.

[x,y,z]Means in the same row within main effect that do not have a common superscript letter differ, $P < 0.10$.

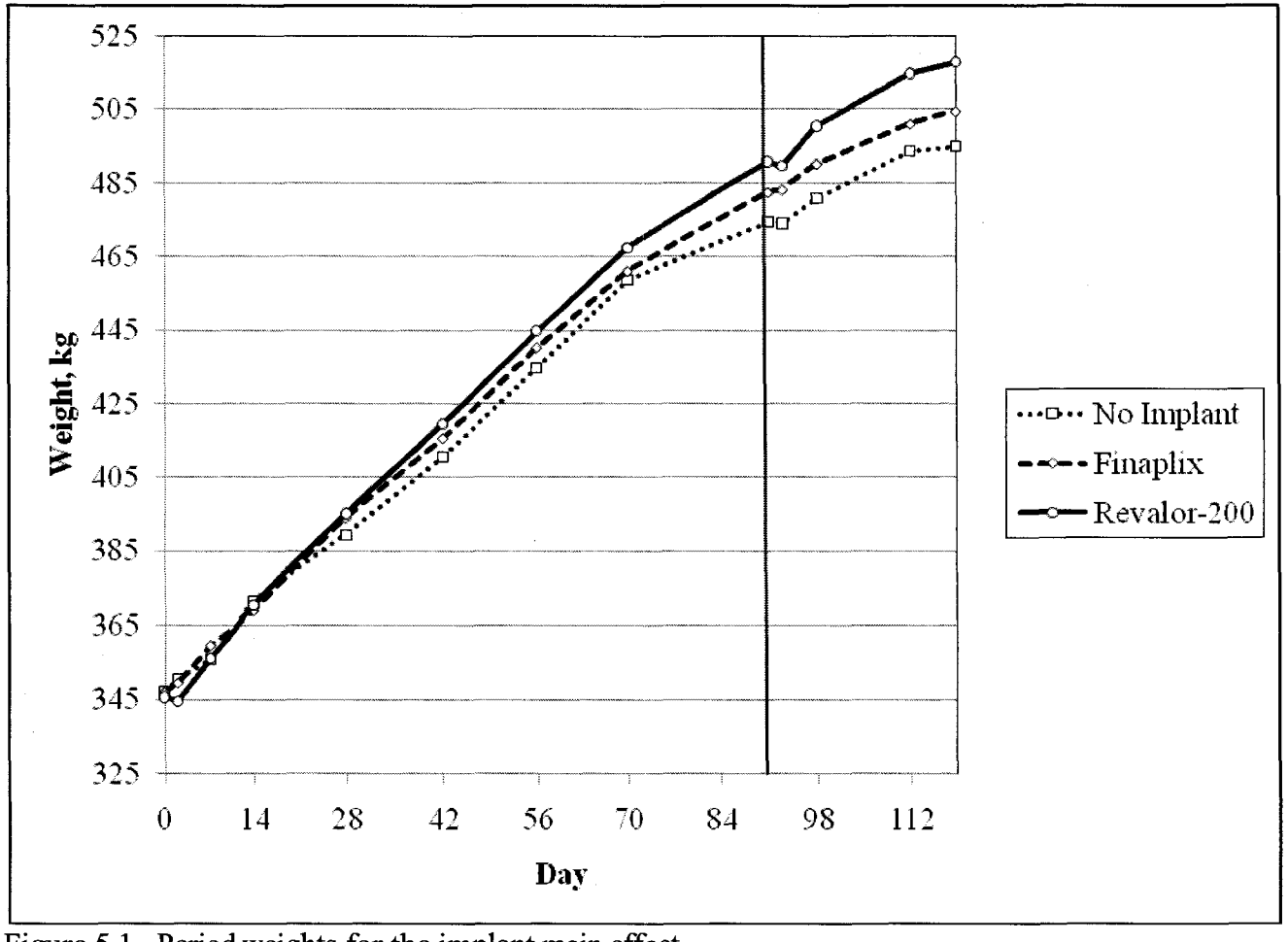

Figure 5.1. Period weights for the implant main effect.

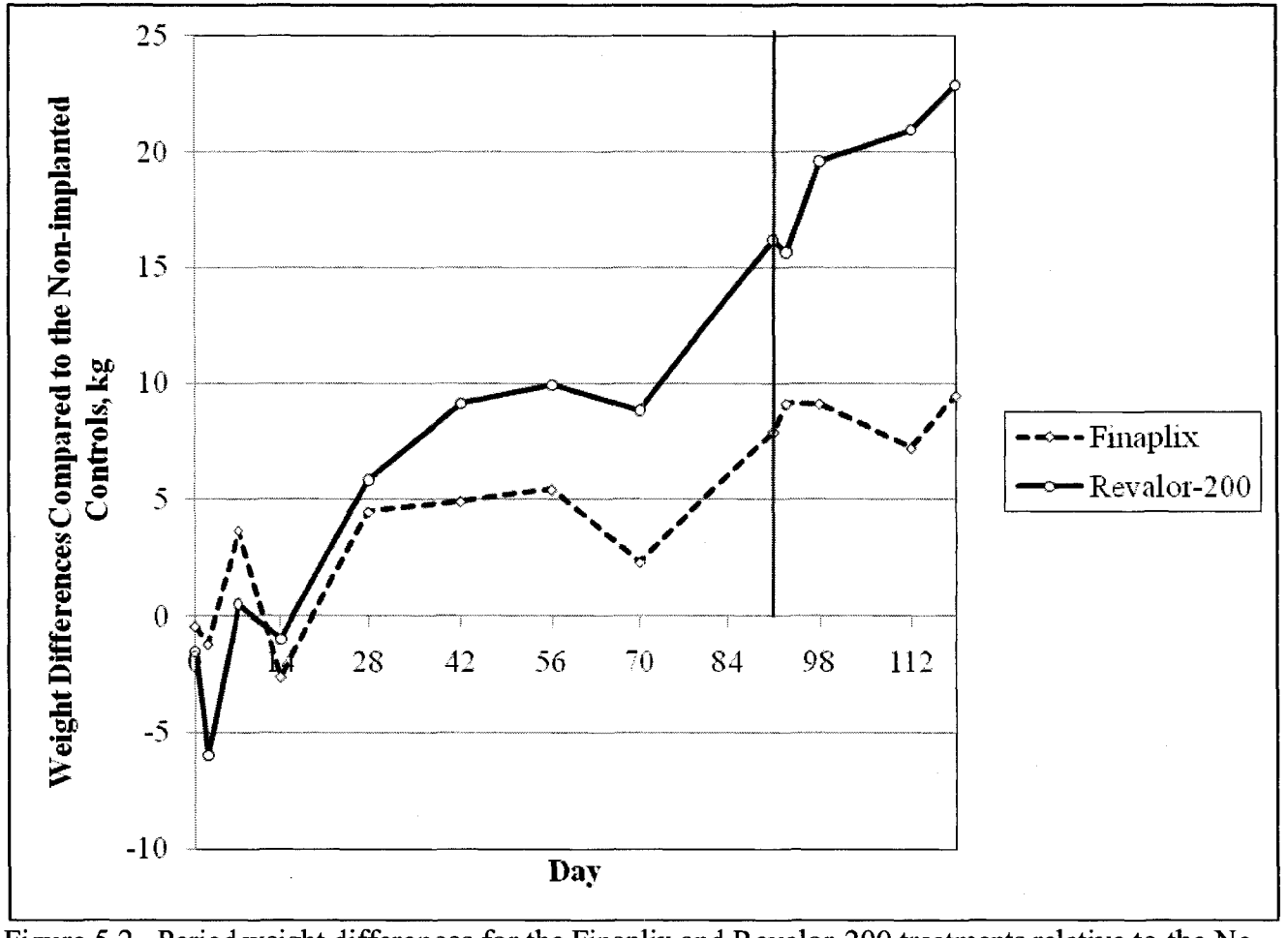

Figure 5.2. Period weight differences for the Finaplix and Revalor-200 treatments relative to the No Implant treatment.

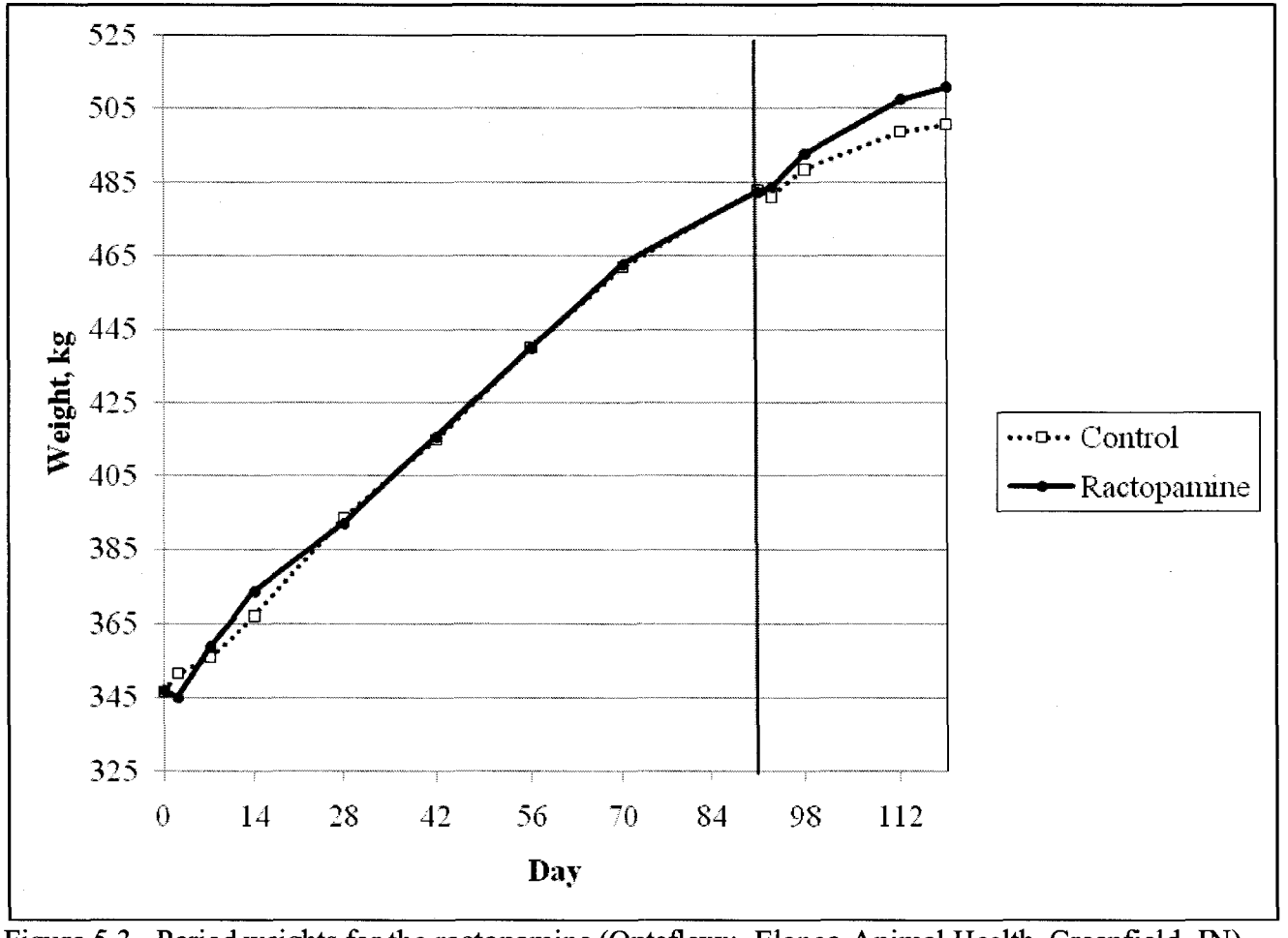

Figure 5.3. Period weights for the ractopamine (Optaflexx; Elanco Animal Health, Greenfield, IN) main effect.

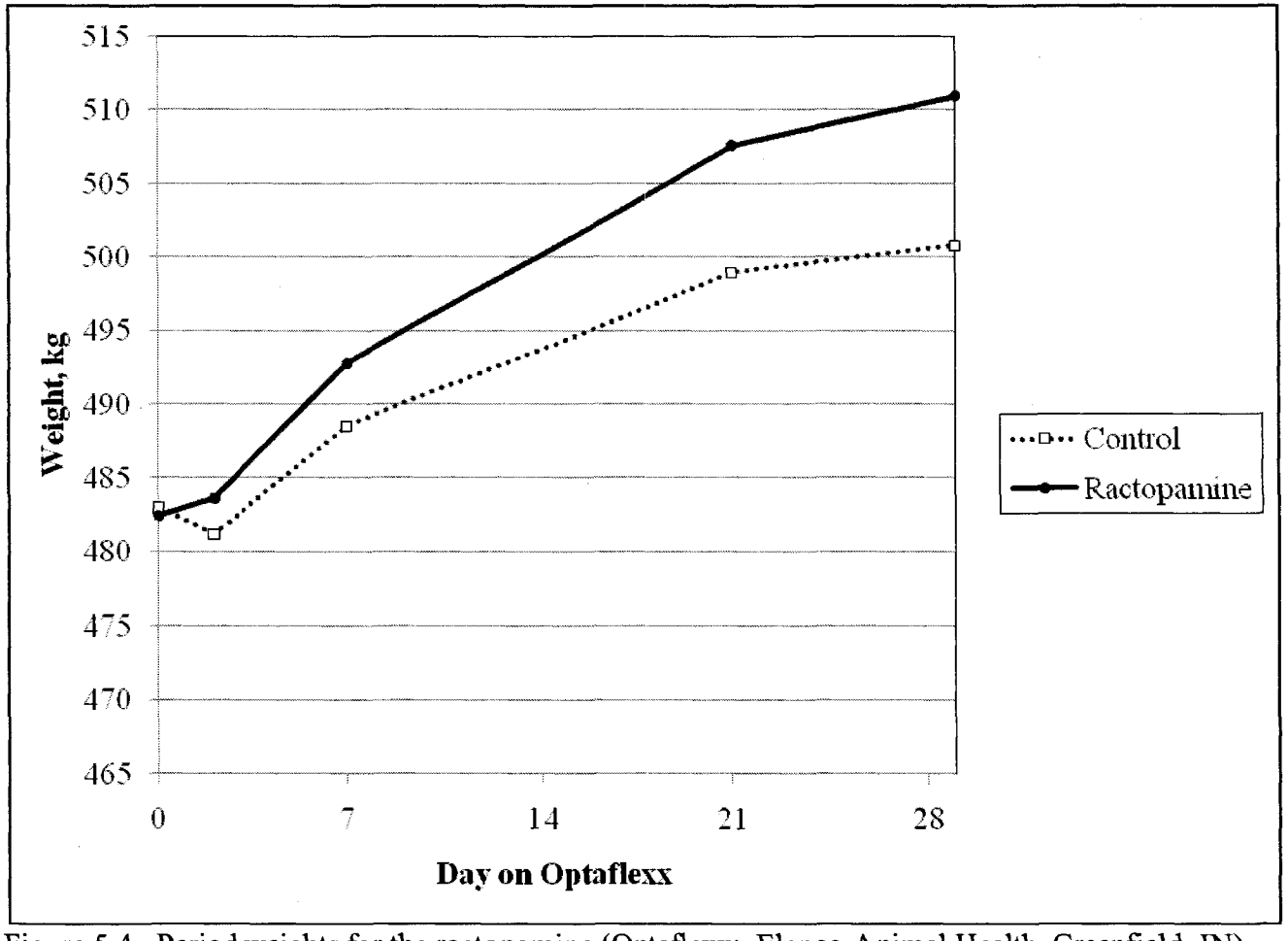

Figure 5.4. Period weights for the ractopamine (Optaflexx; Elanco Animal Health, Greenfield, IN) main effect during the ractopamine-feeding period.

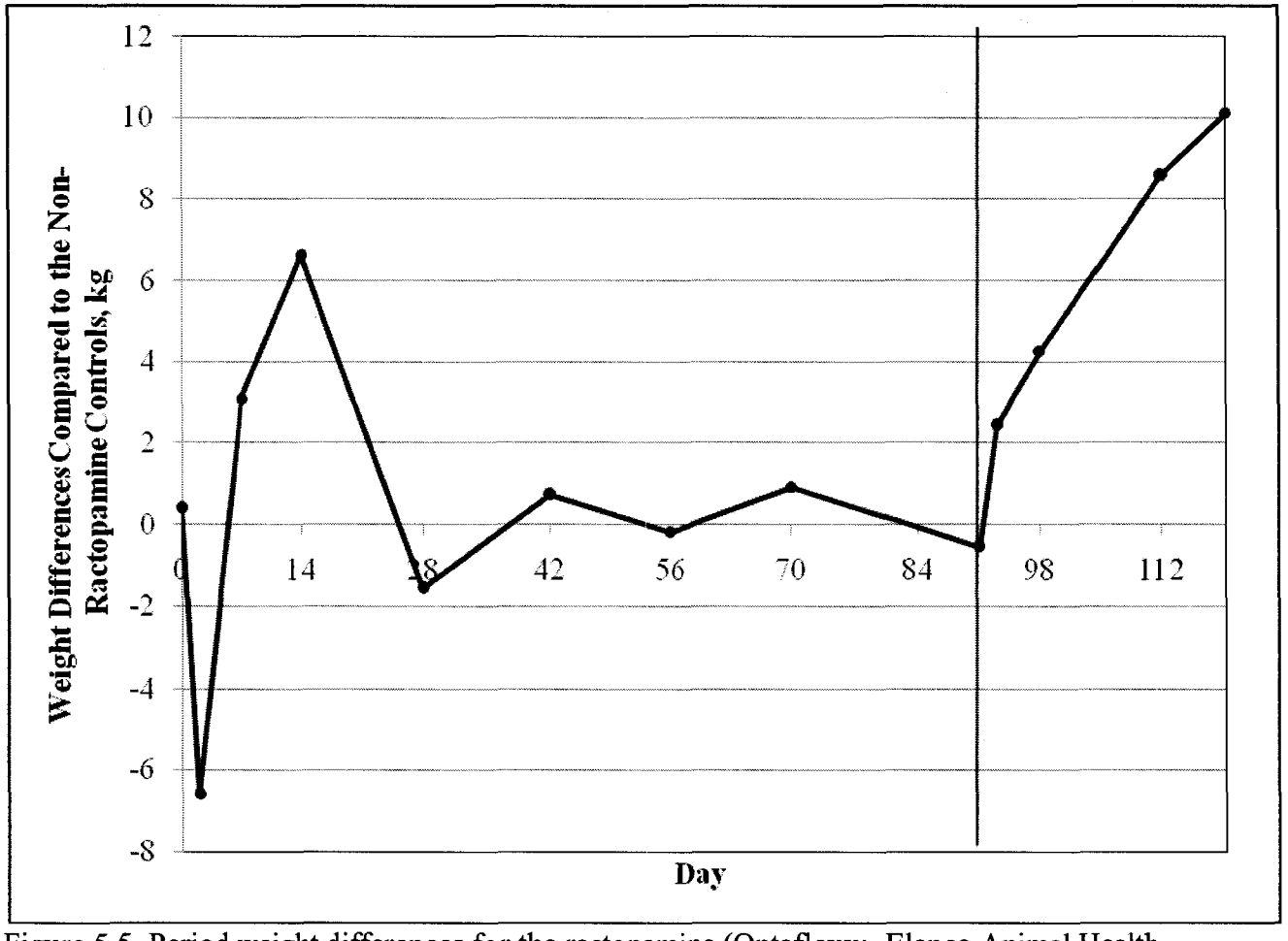

Figure 5.5 Period weight differences for the ractopamine (Optaflexx; Elanco Animal Health, Greenfield, IN) treatment relative to the no-ractopamine treatment.

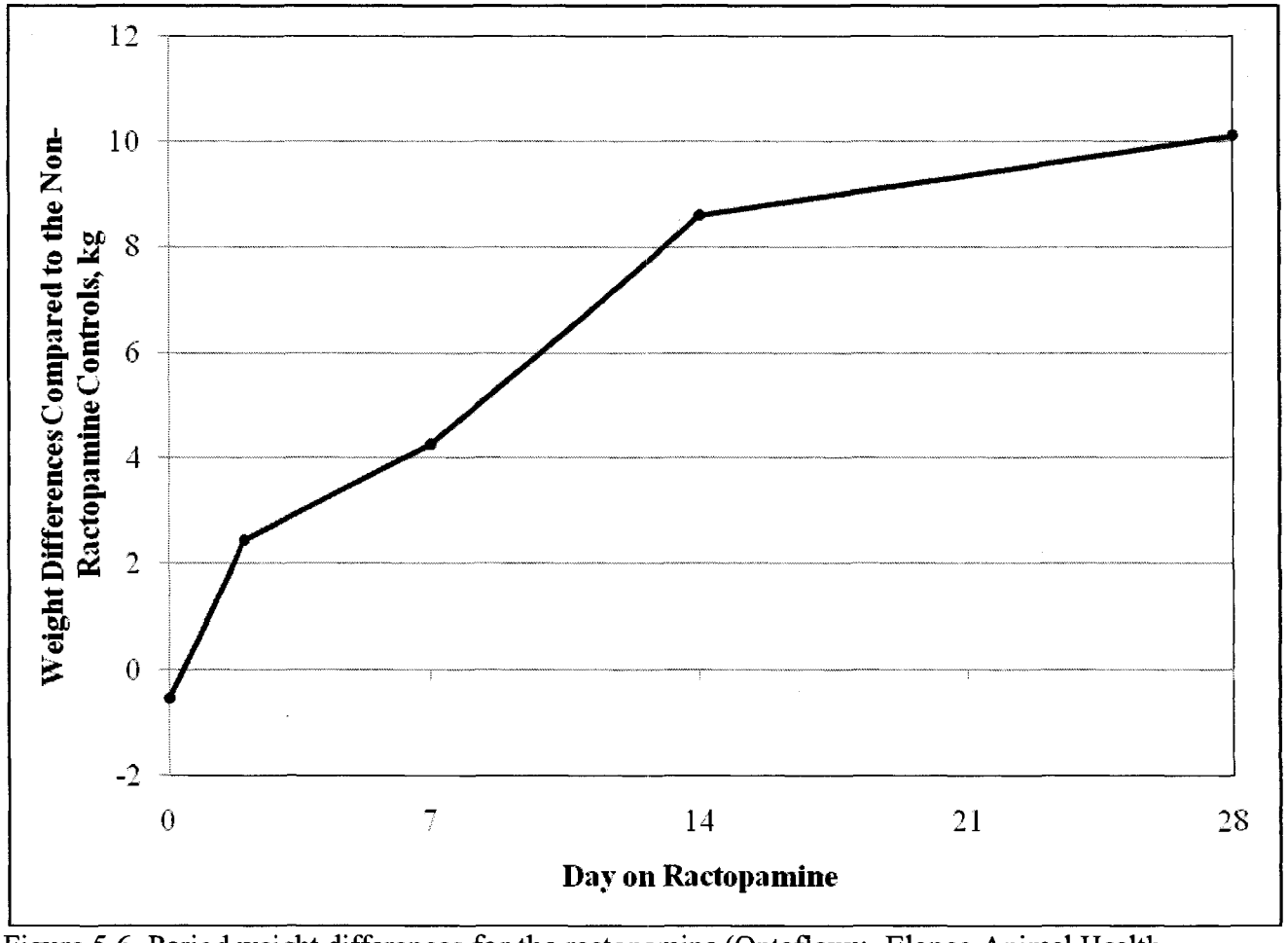

Figure 5.6 Period weight differences for the ractopamine (Optaflexx; Elanco Animal Health, Greenfield, IN) treatment during the ractopamine-feeding period.

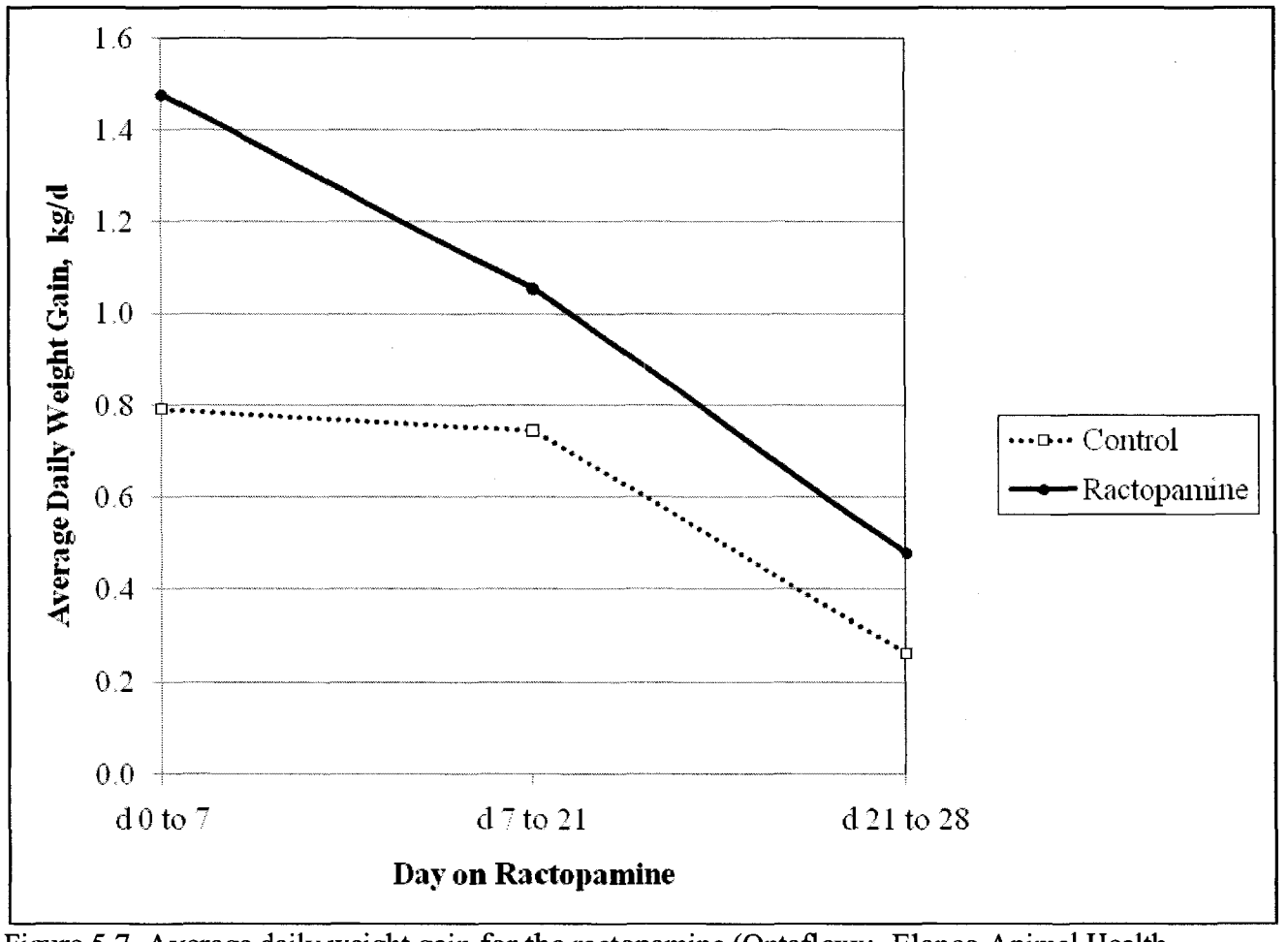

Figure 5.7 Average daily weight gain for the ractopamine (Optaflexx; Elanco Animal Health, Greenfield, IN) main effect during the ractopamine-feeding period.

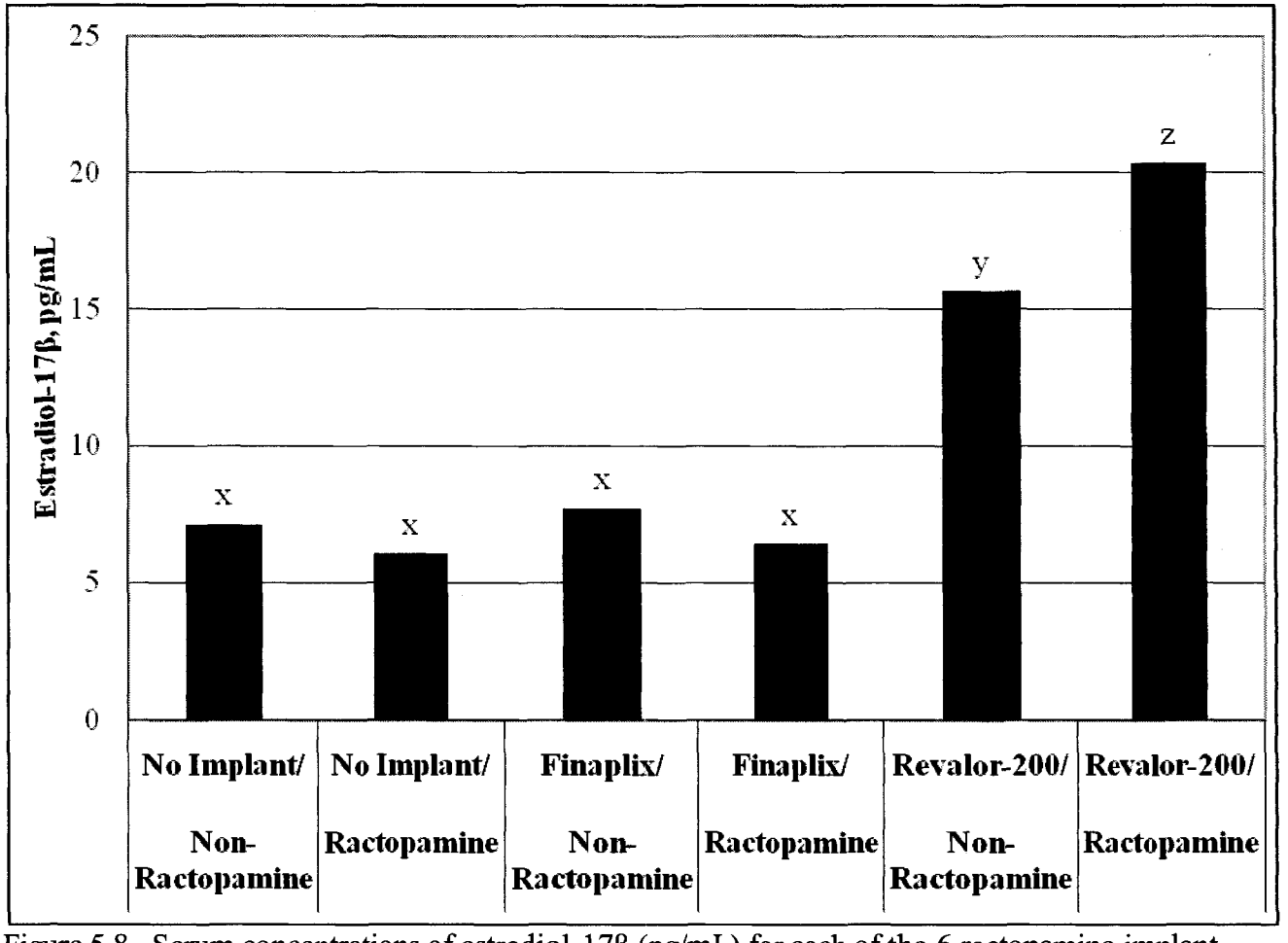

Figure 5.8. Serum concentrations of estradiol-17β (pg/mL) for each of the 6 ractopamine-implant treatment combinations across periods. Implant, $P < 0.001$; ractopamine, $P = 0.407$; implant × ractopamine, $P = 0.015$; period, $P < 0.001$; period × implant, $P = 0.642$; period × ractopamine, $P = 0.910$; implant × ractopamine × period, $P = 0.934$. ^{x,y,z}Treatment combinations that do not have a common letter differ, $P < 0.10$.

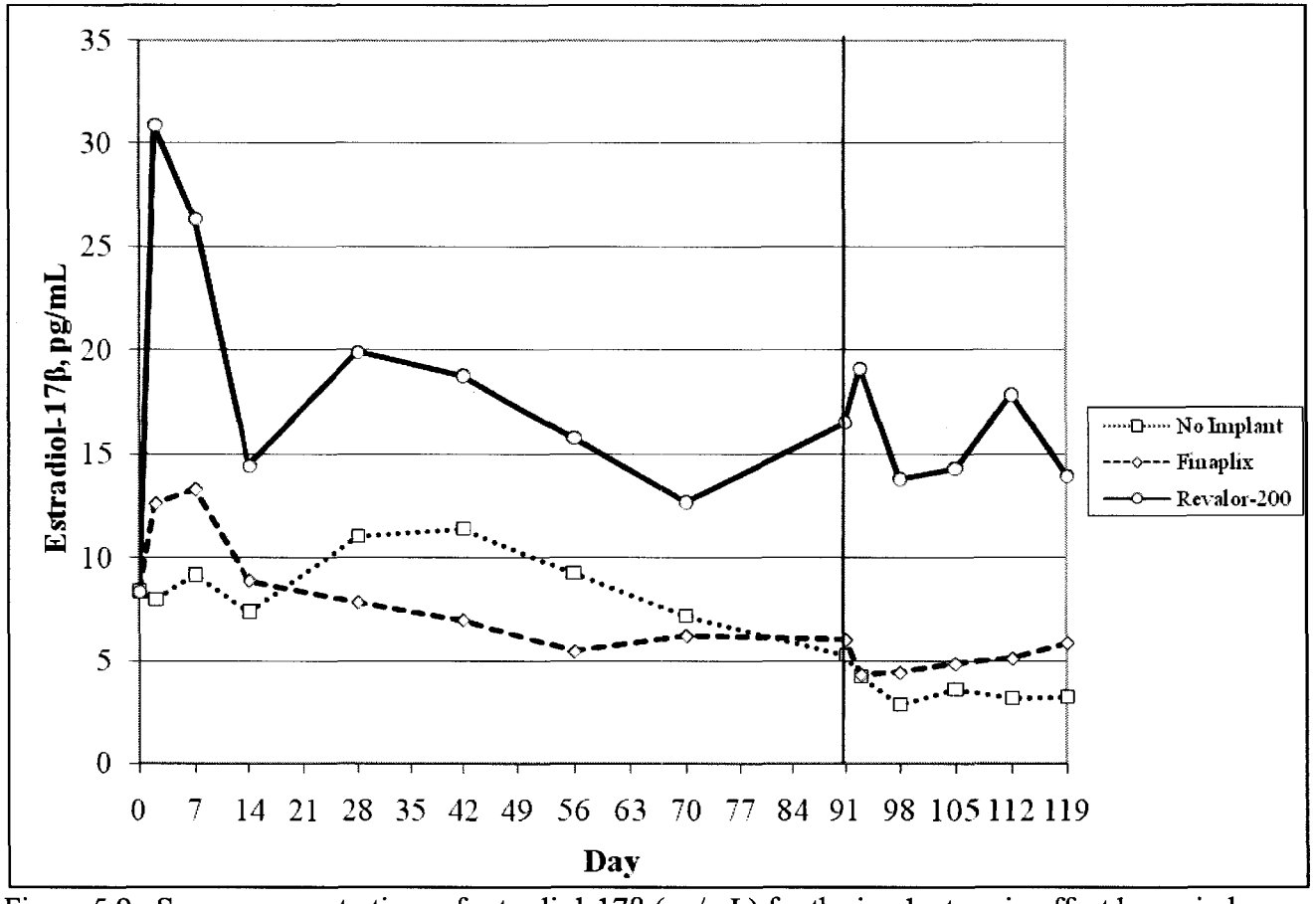

Figure 5.9. Serum concentrations of estradiol-17β (pg/mL) for the implant main effect by period. Treatment differences are not shown.

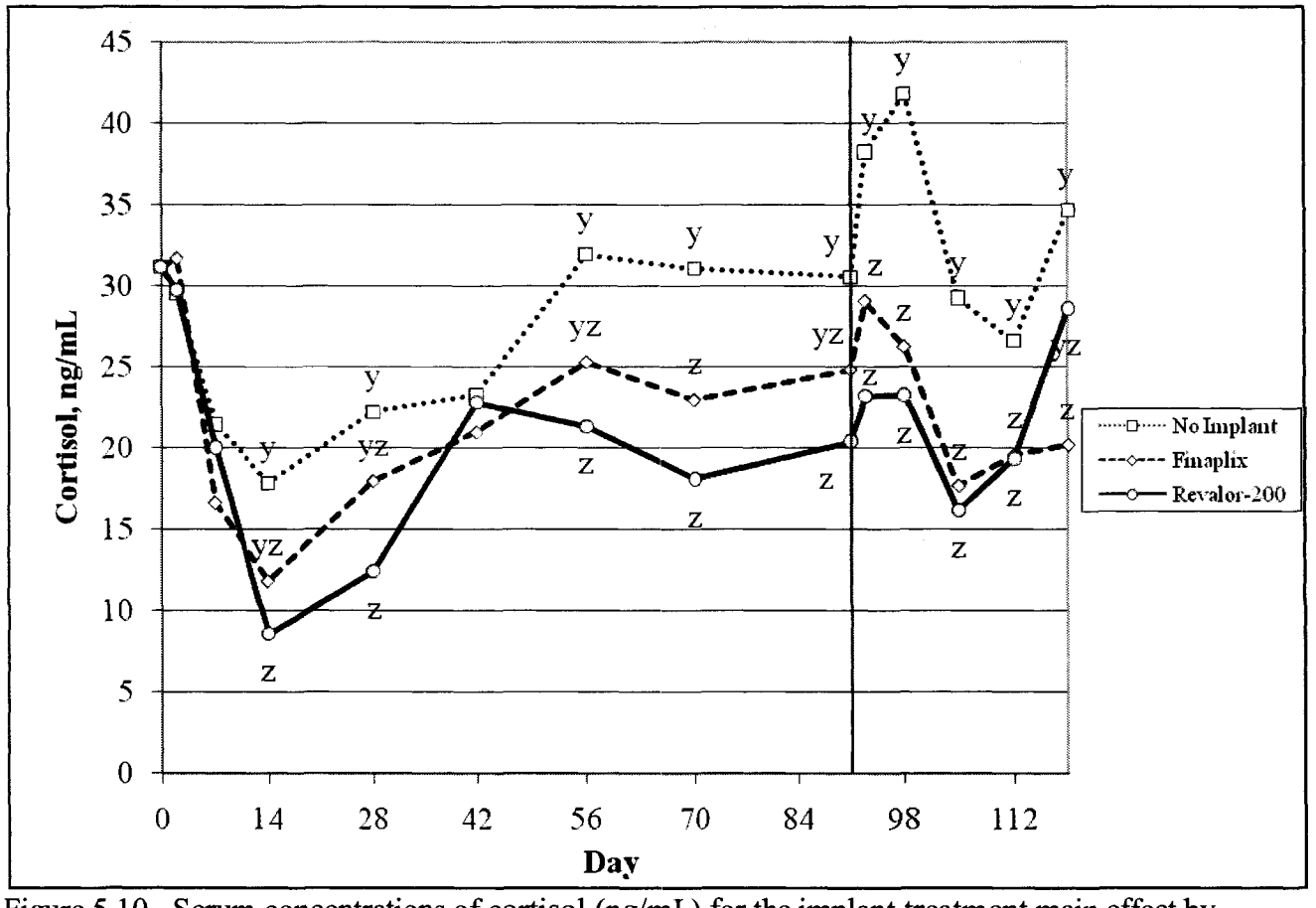

Figure 5.10. Serum concentrations of cortisol (ng/mL) for the implant treatment main effect by period. Implant, $P = 0.002$; ractopamine, $P = 0.499$; implant × ractopamine, $P = 0.569$; period, $P < 0.001$; period × implant, $P = 0.007$; period × ractopamine, $P = 0.696$; implant × ractopamine × period, $P = 0.812$. [y,z]Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

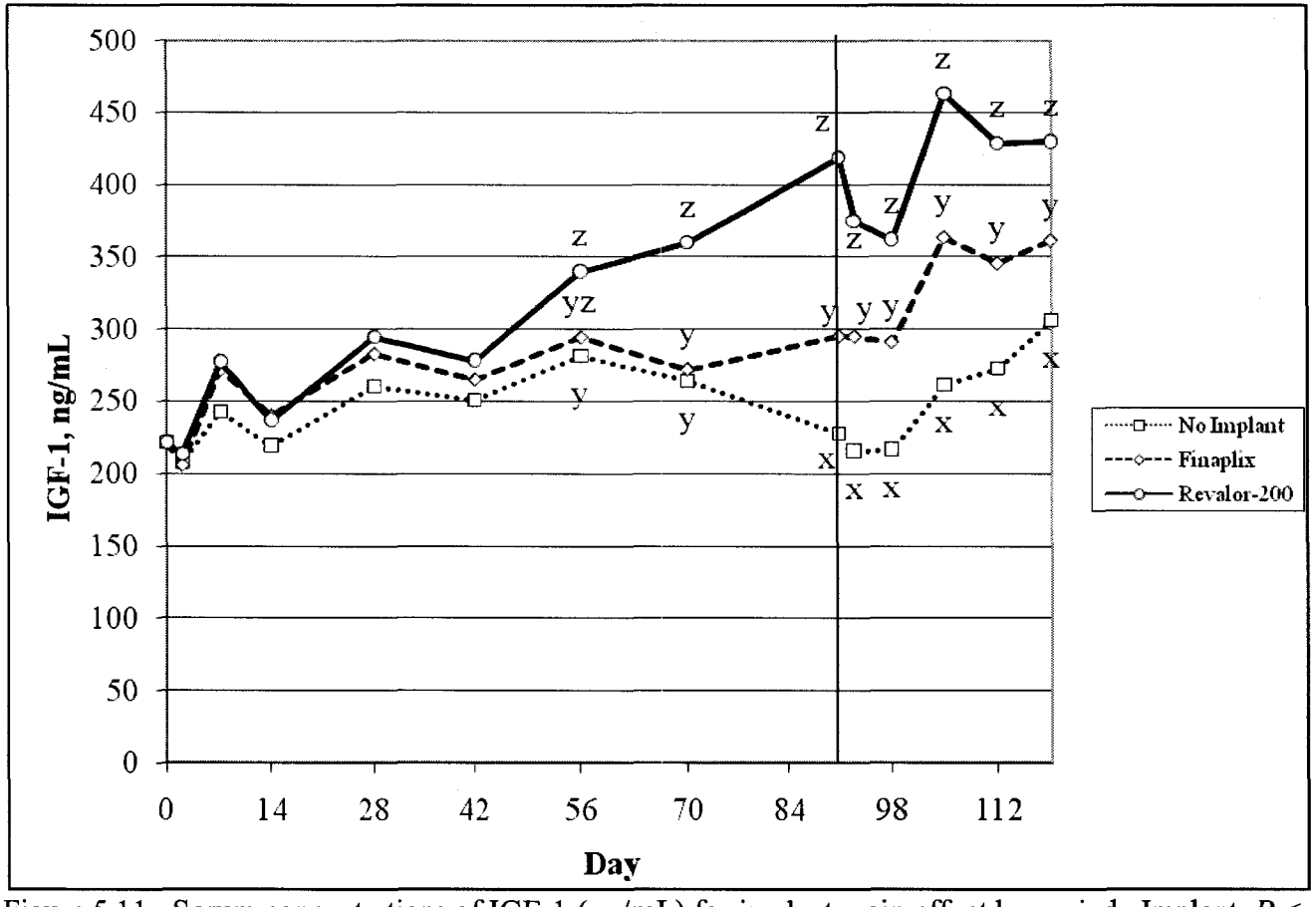

Figure 5.11. Serum concentrations of IGF-1 (ng/mL) for implant main effect by period. Implant, $P < 0.001$; ractopamine, $P = 0.188$; implant × ractopamine, $P = 0.715$; period, $P < 0.001$; period × implant, $P < 0.001$; period × ractopamine, $P = 0.310$; implant × ractopamine × period, $P = 0.173$. [x,y,z]Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

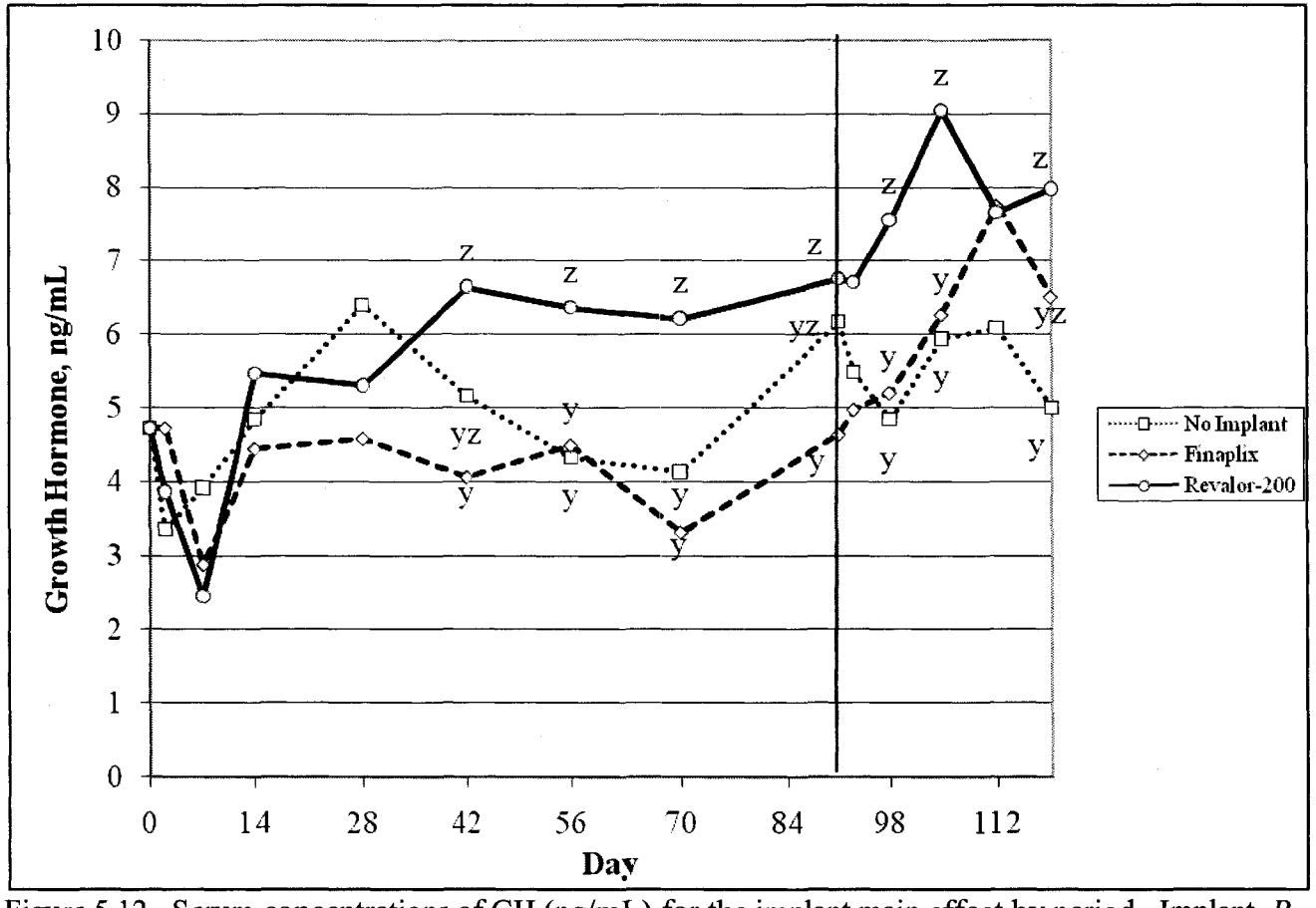

Figure 5.12. Serum concentrations of GH (ng/mL) for the implant main effect by period. Implant, $P = 0.061$; ractopamine, $P = 0.625$; implant × ractopamine, $P = 0.296$; period, $P < 0.001$; period × implant, $P = 0.037$; period × ractopamine, $P = 0.552$; implant × ractopamine × period, $P = 0.172$. [x,y,z]Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

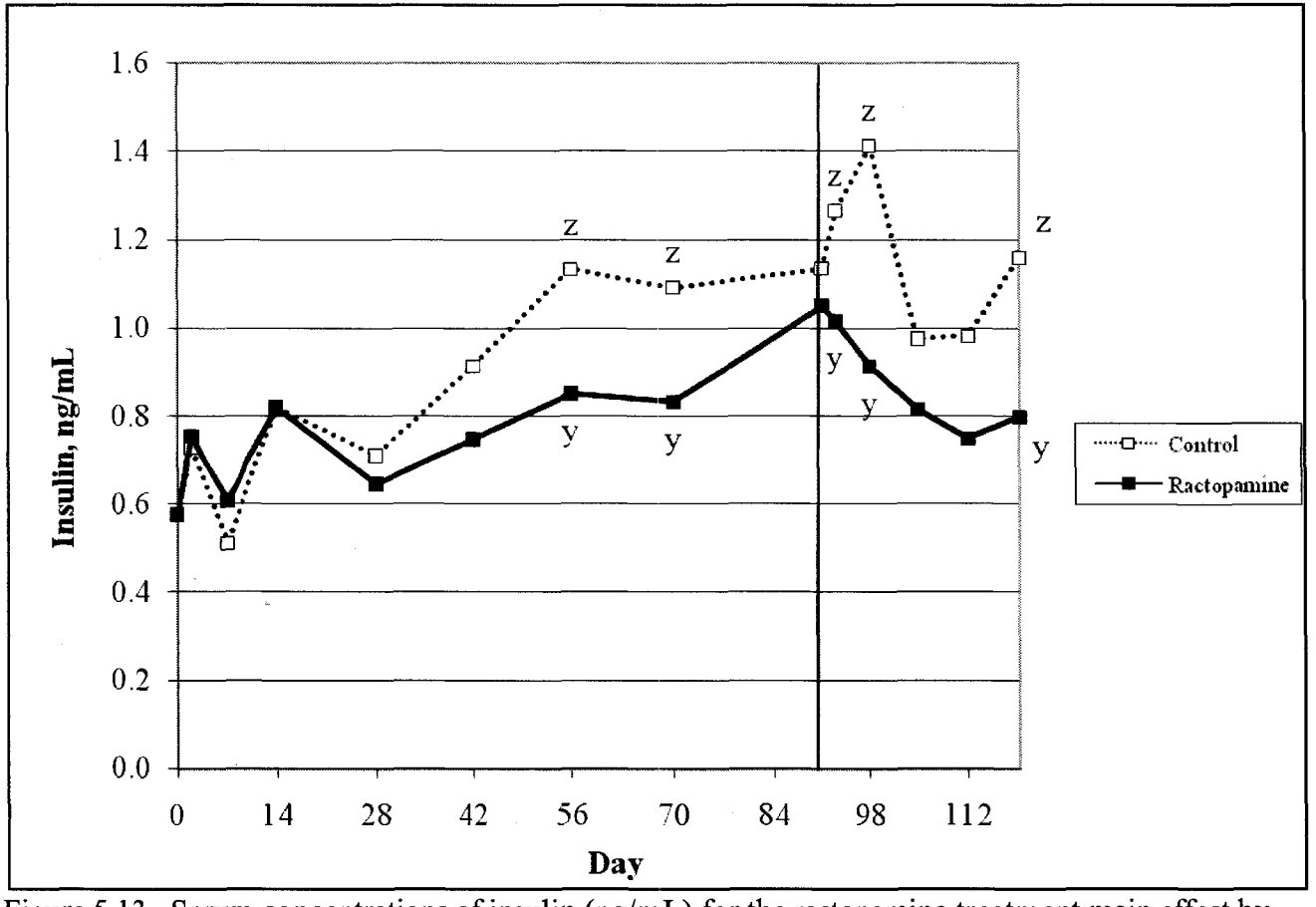

Figure 5.13. Serum concentrations of insulin (ng/mL) for the ractopamine treatment main effect by period. Implant, $P = 0.6904$; ractopamine, $P = 0.059$; implant × ractopamine, $P = 0.217$; period, $P < 0.001$; period × implant, $P = 0.145$; period × ractopamine, $P = 0.074$; implant × ractopamine × period, $P = 0.482$. ^{y,z}Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

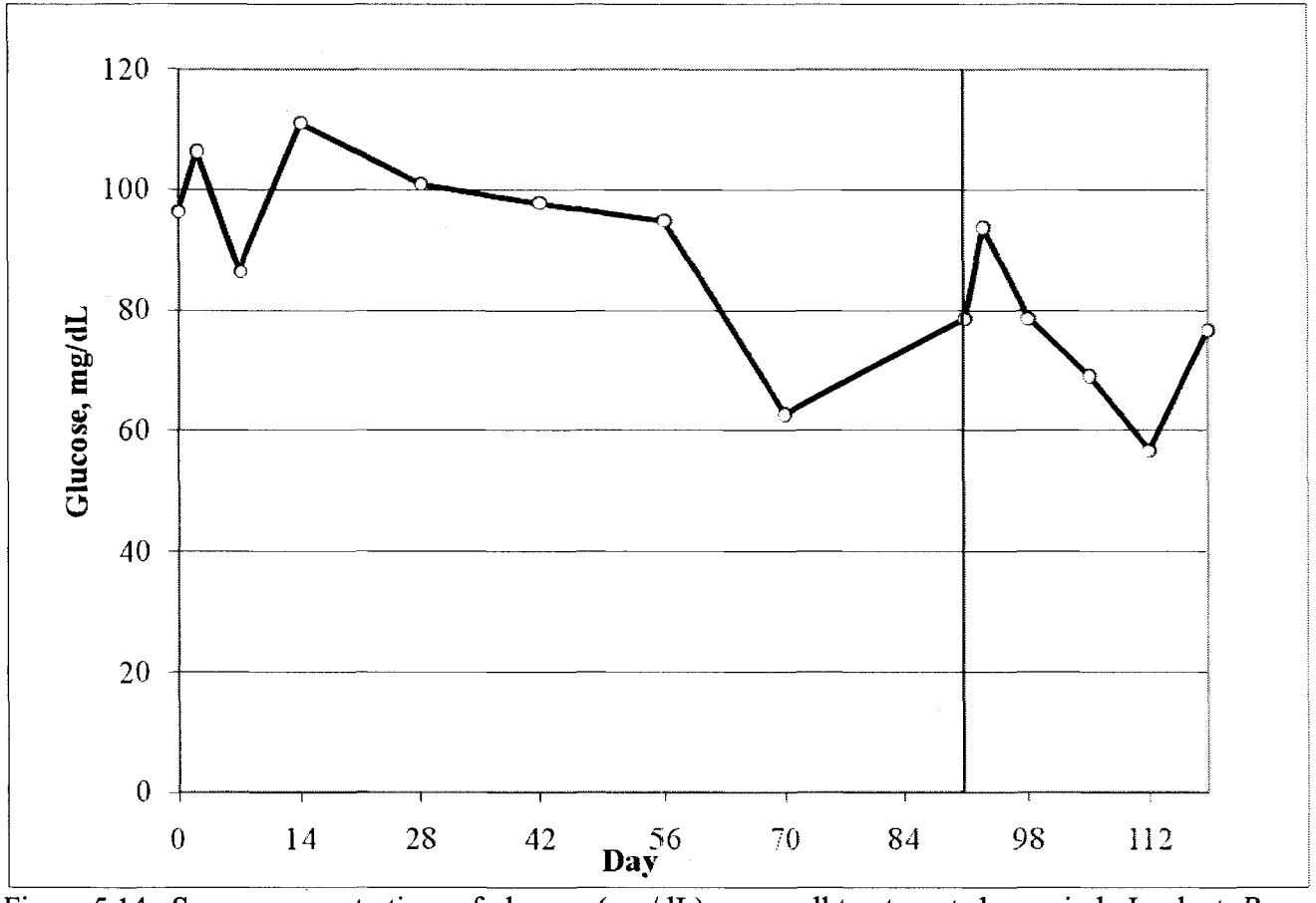

Figure 5.14. Serum concentrations of glucose (mg/dL) across all treatments by period. Implant, $P = 0.621$; ractopamine, $P = 0.650$; implant × ractopamine, $P = 0.471$; period, $P < 0.001$; period × implant, $P = 0.3635$; period × ractopamine, $P = 0.240$; implant × ractopamine × period, $P = 0.857$. [y,z]Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

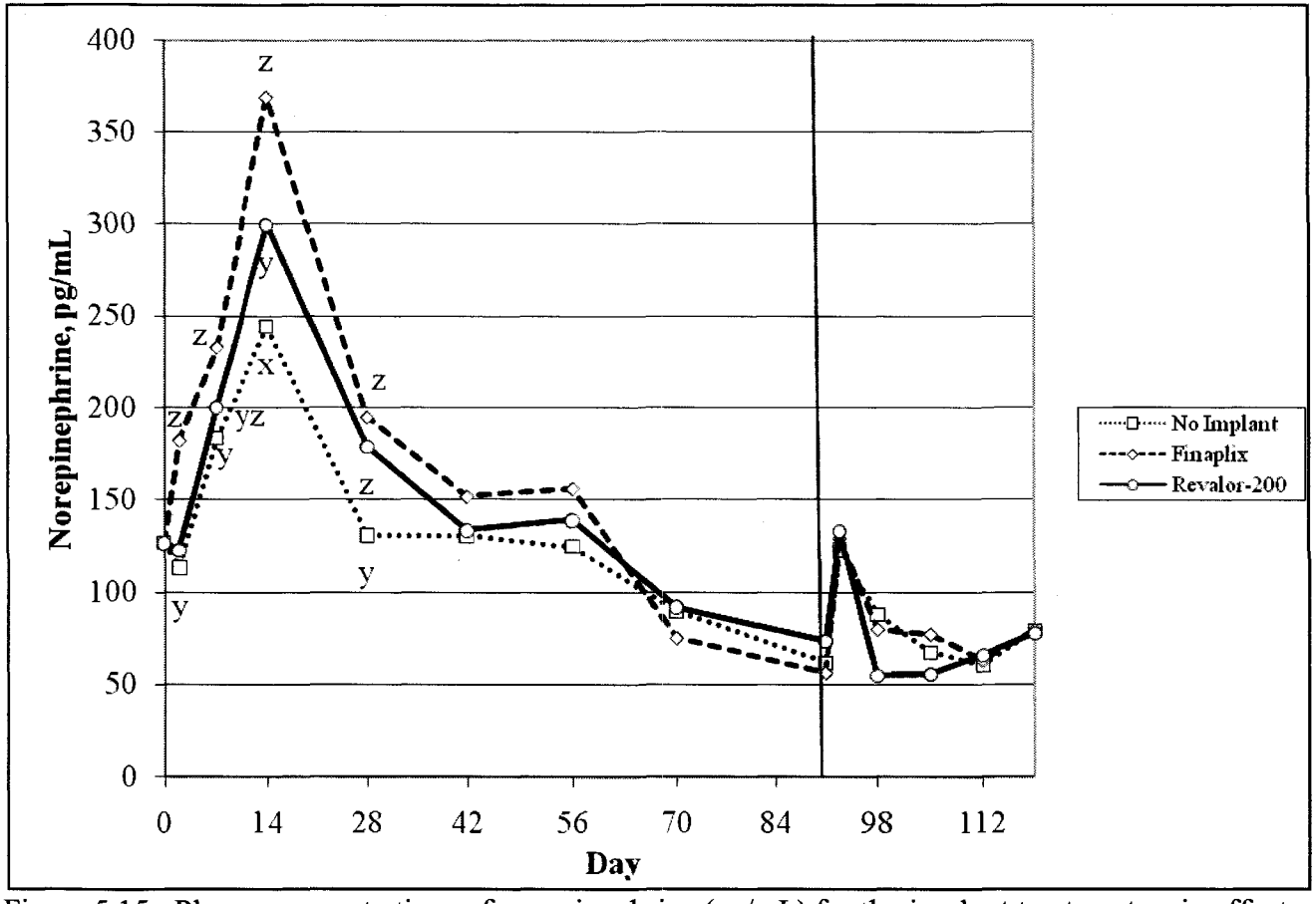

Figure 5.15. Plasma concentrations of norepinephrine (pg/mL) for the implant treatment main effect by period. Implant, $P = 0.017$; ractopamine, $P = 0.208$; implant × ractopamine, $P = 0.795$; period, $P < 0.001$; period × implant, $P = 0.001$; period × ractopamine, $P = 0.684$; implant × ractopamine × period, $P = 0.989$. ^{y,z}Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

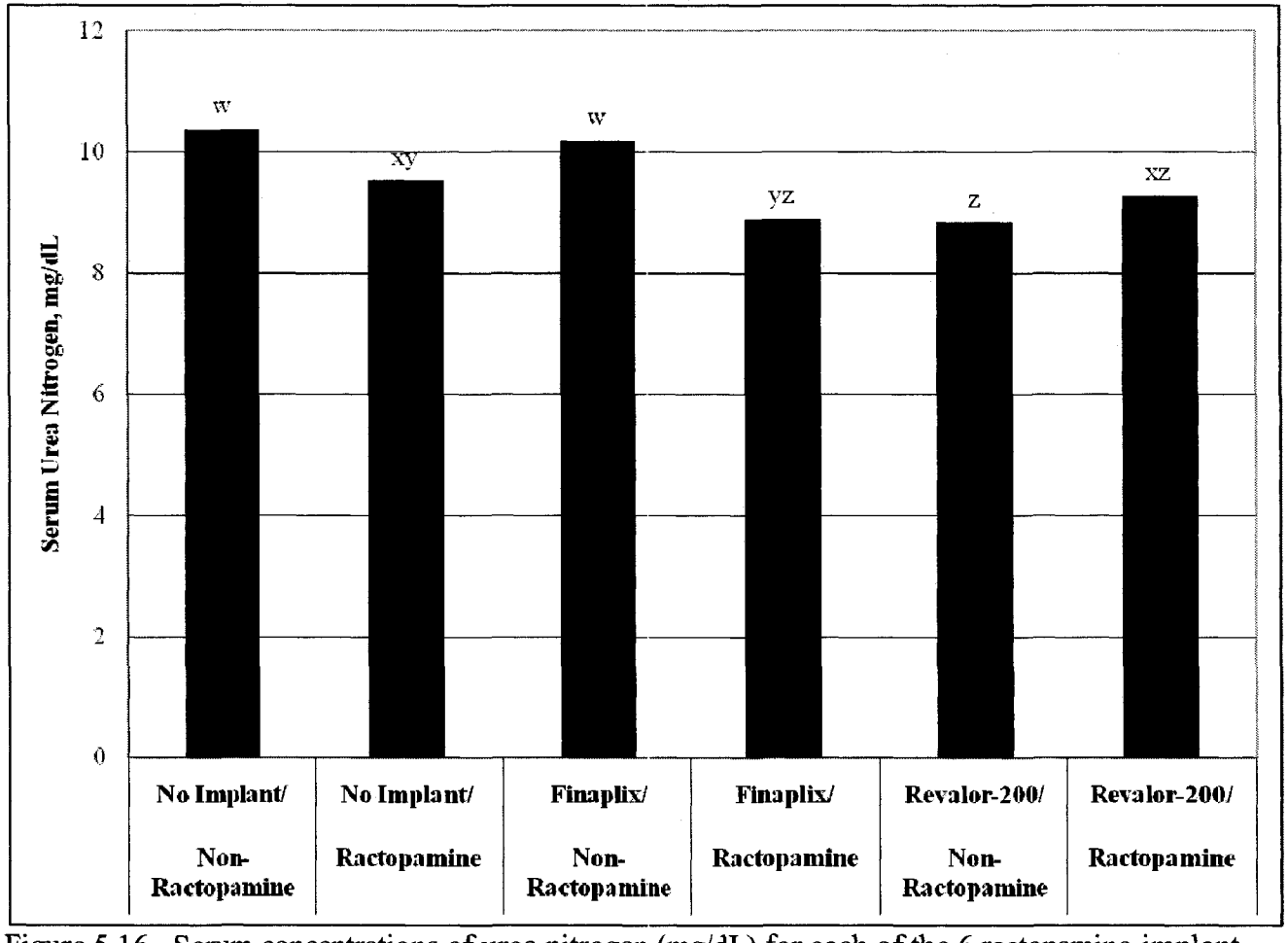

Figure 5.16. Serum concentrations of urea nitrogen (mg/dL) for each of the 6 ractopamine-implant treatment combinations across periods. Implant, $P = 0.001$; ractopamine, $P = 0.015$; implant × ractopamine, $P = 0.001$; period, $P < 0.001$; period × implant, $P = 0.798$; period × ractopamine, $P = 0.117$; implant × ractopamine × period, $P = 0.997$. ^{w,x,y,z}Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

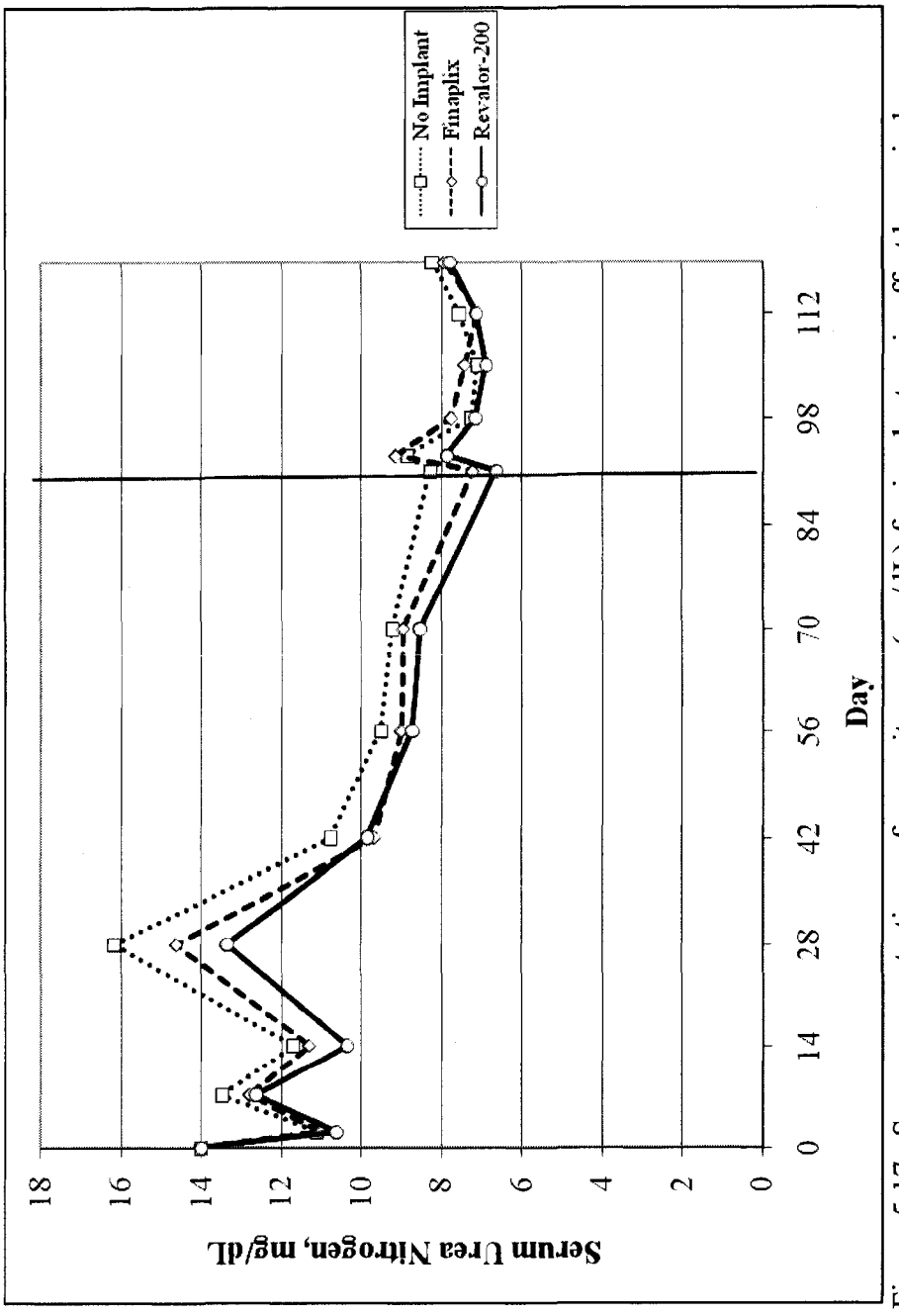

Figure 5.17. Serum concentrations of urea nitrogen (mg/dL) for implant main effect by period. Treatment differences are not shown.

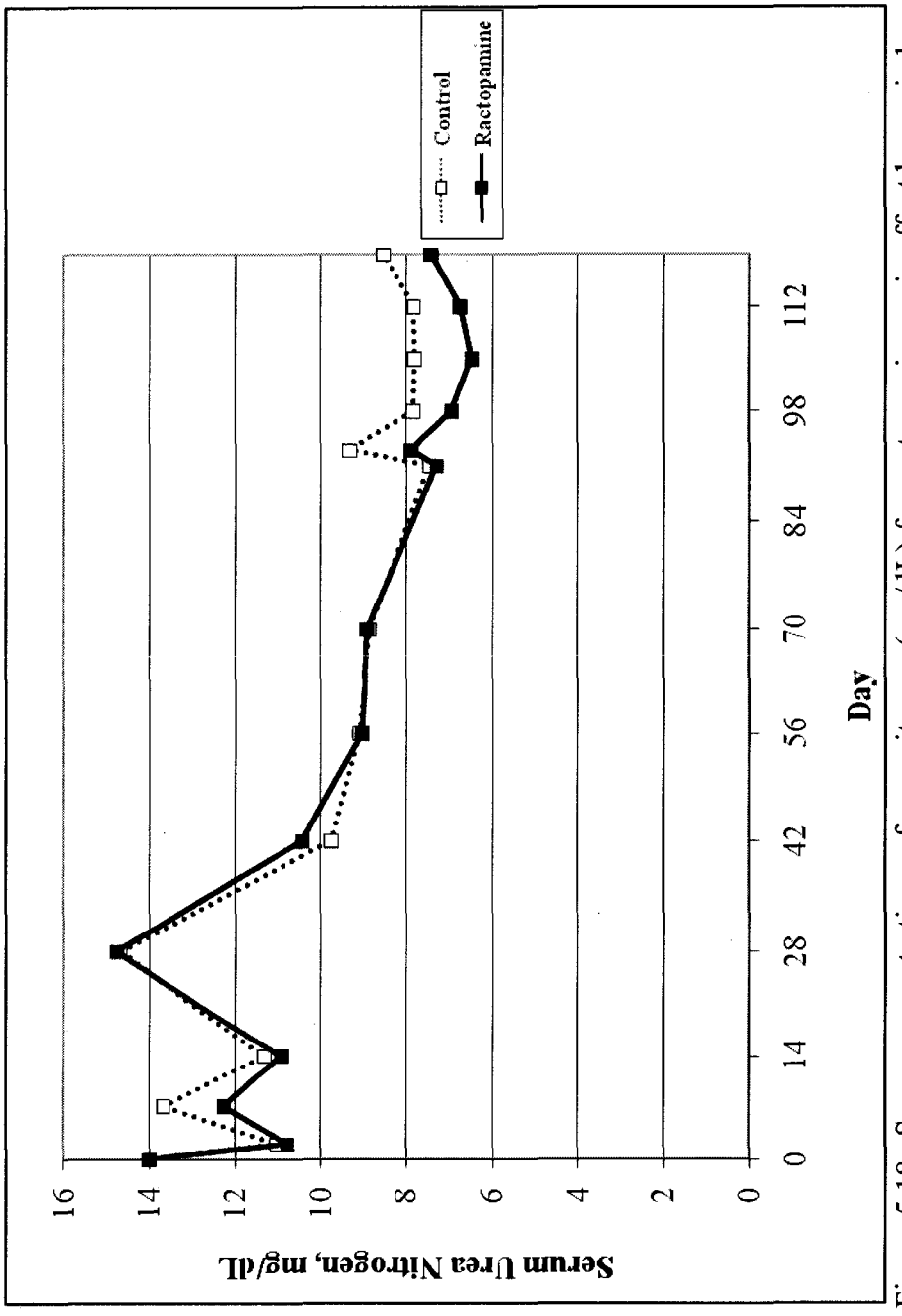

Figure 5.18. Serum concentrations of urea nitrogen (mg/dL) for ractopamine main effect by period. Treatment differences are not shown.

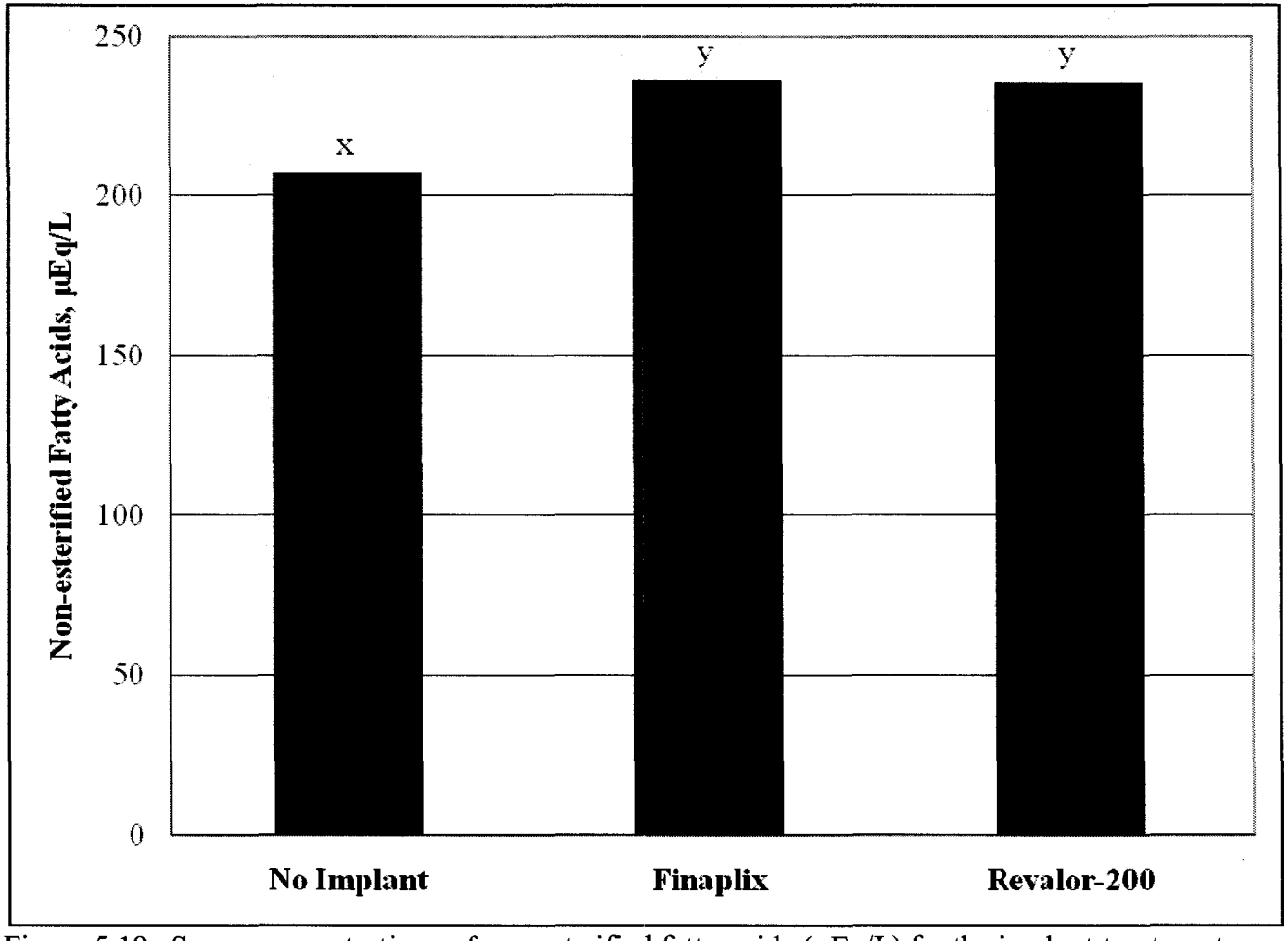

Figure 5.19. Serum concentrations of non-esterified fatty acids (µEq/L) for the implant treatment main effect across periods. Implant, $P = 0.043$; ractopamine, $P = 0.991$; implant × ractopamine, $P = 0.814$; period, $P < 0.001$; period × implant, $P = 0.798$; period × ractopamine, $P = 0.288$; implant × ractopamine × period, $P = 0.138$. ^{x,y}Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

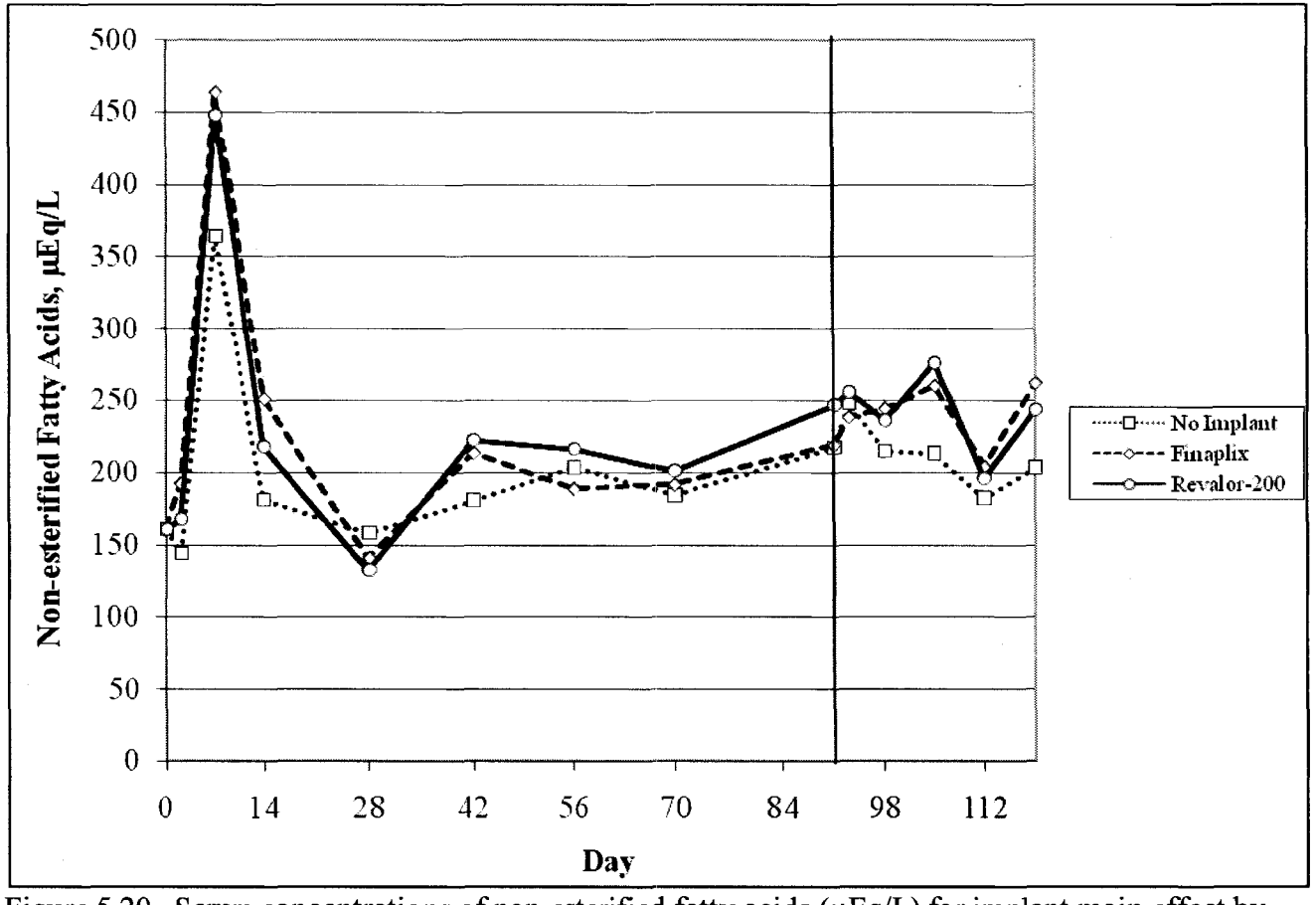

Figure 5.20. Serum concentrations of non-esterified fatty acids (µEq/L) for implant main effect by period. Treatment differences are not shown.

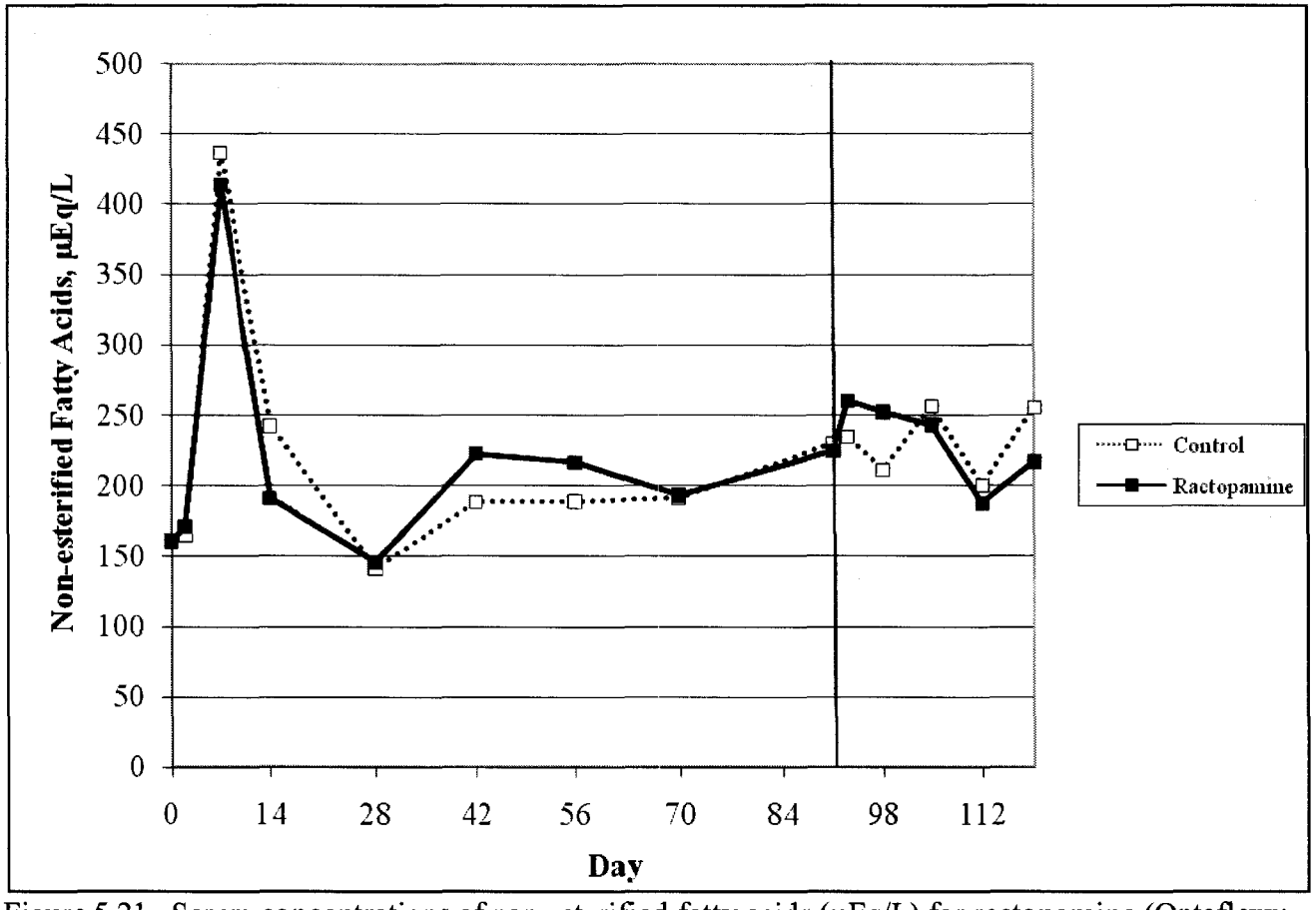

Figure 5.21. Serum concentrations of non-esterified fatty acids (µEq/L) for ractopamine (Optaflexx; Elanco Animal Health, Greenfield, IN) main effect by period. Treatment differences are not shown.

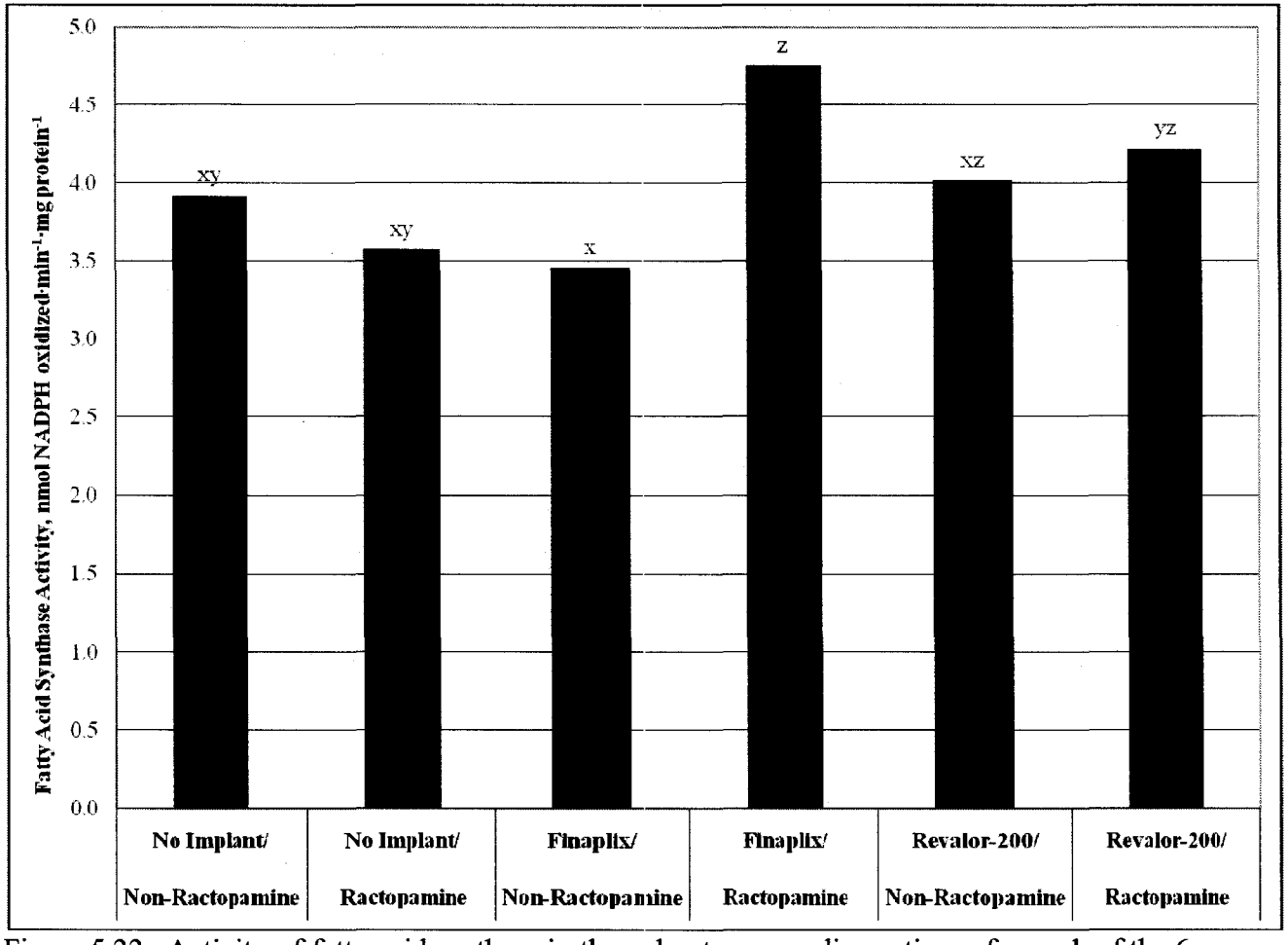

Figure 5.22. Activity of fatty acid synthase in the subcutaneous adipose tissue for each of the 6 ractopamine-implant treatment combinations. Implant, $P = 0.135$; ractopamine, $P = 0.379$; implant × ractopamine, $P = 0.045$. ^{x,y,z}Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

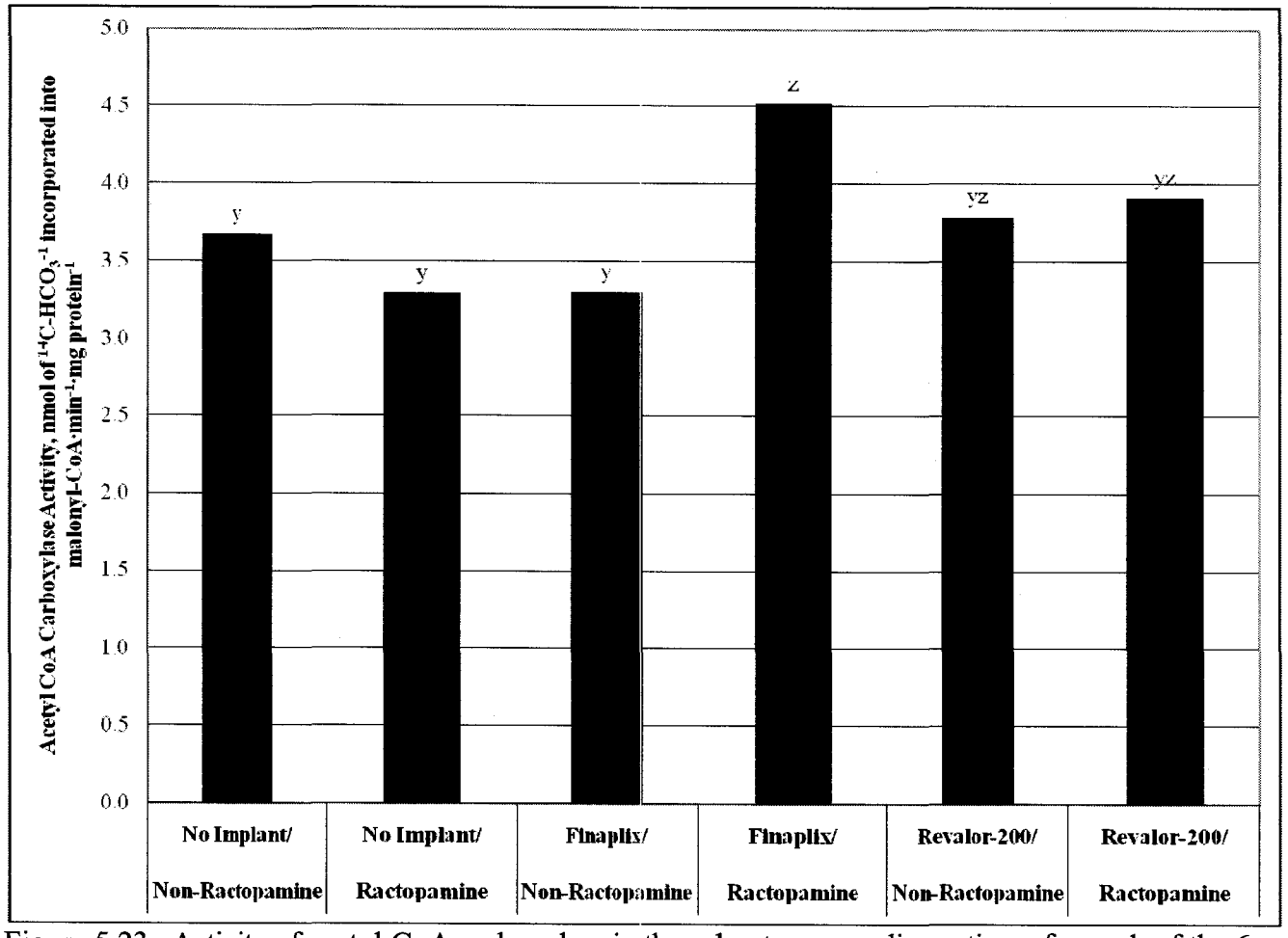

Figure 5.23. Activity of acetyl CoA carboxylase in the subcutaneous adipose tissue for each of the 6 ractopamine-implant treatment combinations. Implant, $P = 0.192$; ractopamine, $P = 0.296$; implant × ractopamine, $P = 0.043$. ^{y,z}Treatment combinations that do not have a common letter within period differ, $P < 0.10$.

APPENDIX B

Appendix Table 5.1. Ractopamine Type B Premix assay results for the 0 and 250 mg/d treatments.

	Control, 0 mg ractopamine		250 mg ractopamine	
Type B supplement batch	Claim[b] mg/kg	Results[b] mg/kg	Claim[b] mg/kg	Results[b] mg/kg
1	0	<2.5	551	546.5
2	0	<2.5	551	540.5
3	0	<2.5	551	532.9
4	0	<2.5	551	531.2
Type B average	0	<2.5	551	537.8
Number of samples		4		4
Mean theory[c], %				97.6

[a]Eurofins Scientific, 345 Adams Ave., Memphis TN 38103.
[b]As-fed basis.
[c]Feed additive result divided by the feed additive claim x 100.