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Application of Growth Enhancing Compounds in Modern Beef Production

Executive Summary

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For more than 50 years, beef cattle producers have safely used growth promotants to enhance muscle leanness, increase average daily gain, stimulate feed intake moderately, and subsequently enhance the rate of gain compared to the amount of food needed to achieve that gain, referred to as feed efficiency. These improvements generally are characterized by an increase in average daily gain ranging from 8% to 28% and improvements in feed efficiency from 5% to 20% in treated cattle compared to non-treated cattle. Factors such as cattle type and growth promotant type, ingredients, and strength of dose affect cattle response to treatment. Many different formulations of growth promotants are approved for use in raising cattle, but the most common are anabolic steroid implants administered primarily through small pellets being placed under the skin on the back of the animal's ear. The active ingredients in these growth promotants generally belong to one of three major categories of hormones: androgens, estrogens, and progestins. These are the same hormones that are found endogenously (naturally occurring) in all animals.

Growth promotants are required to go through a comprehensive, multi-step scientific review by the Food and Drug Administration (FDA) to ensure animal health and human food safety. Approved products are regularly evaluated and must be continually proven safe to remain on the market. Steroidal implants, specifically, have been proven safe over multiple years of study and have a zero-day withdrawal prior to harvest, because research shows that by harvest time, no residue remains that would be concerning to human health. In many applications, the final growth promoting implant is given greater than 100 days before harvest.

Once an implant is administered in the back of cattle's ear, the growth promoting compound is slowly released from the carrier matrix. The common term used to describe this is "payout." The compounds enter the bloodstream and act on a specific receptor to elicit a biological response very quickly since they have a very short half-life in the bloodstream. In most instances, the half-life of these compounds is around 20 minutes once released from the implant. After binding to a specific receptor on skeletal muscle, for example, the compound is metabolized rapidly by the liver and cleared from the body. Compounds are excreted from cattle both in the feces and urine, predominantly as biologically inactive steroid metabolites.

Certain cattle types benefit from more potent growth promoting combinations to optimize gain and muscle deposition. The majority of the increase in body weight obtained in cattle administered implants is due to increased lean tissue (muscle) mass. Therefore, use of growth-promoting implants increases the production of lean, edible, wholesome beef.

More recently, another growth-enhancing technology called β-adrenergic agonists has been approved by FDA. These compounds have been marketed in the beef industry during the last decade, so they are a relatively new production tool. β-adrenergic agonists are orally active. They are administered to cattle during the last 20 to 42 days on feed and are absorbed through the digestive system into the bloodstream. These compounds bind with high affinity to β-adrenergic receptors present on cells of tissues in cattle. Three subtypes of β-adrenergic receptors are on the cell surface: β_1, β_2, and β_3. In cattle, economically important tissues such as skeletal muscle (lean tissue) and adipose tissue (fat) each have abundant numbers of β_2-adrenergic receptors on their cell surfaces. Once a β-adrenergic agonist binds to its receptor on the surface of a cell, a cascade of events is set in motion that ultimately changes cellular activity. We refer to this as a direct, receptor-mediated effect. In skeletal muscle, β-adrenergic agonist binding to its receptor results in signals that cause the muscle cell to incorporate a greater amount of protein and water. This is accomplished through increasing the rate of new protein being made (synthesis) and/or decreasing the speed by which the muscle cell breaks down existing protein (degradation). Research suggests that β-adrenergic agonists may impact both of these processes in skeletal muscle of cattle. The net effect is the muscle cell undergoes "hypertrophy" (increase in size of existing cells).

Several types of adipose tissue exist that are economically important in cattle (e.g., subcutaneous or backfat, intramuscular or marbling, and mesenteric or omental). β-adrenergic agonists appear to have variable effects on these different types of adipose tissue based on factors such as receptor number and affinity. Generally, β-adrenergic agonists binding to a receptor on a fat cell will trigger increased fat breakdown (lipolysis) and impair

synthesis of new fat (lipogenesis). The net effect is reduction in some adipose tissue deposits. Nutrients that would have been stored in adipose tissue can be shuttled to other tissues such as skeletal muscle to support increased growth.

These biological effects occur very rapidly after β-adrenergic agonists bind to their receptor. Following the initiation of the signal, β-adrenergic agonists lose affinity for the receptor, metabolize to an inactive form, and are cleared by the body very rapidly. As with growth-promoting implants, β-adrenergic agonists have been thoroughly tested by the FDA for efficacy and food safety.

In closing, growth promotants have been safely used in beef cattle production for more than 50 years. Growth enhancing compounds, including steroidal implants and beta-adrenergic agonists, increase production and improve feed efficiency of beef cattle. These changes in performance result in an economic benefit to beef cattle producers and affect the relative price competitiveness of beef as compared to other protein sources. The safe use of growth-enhancing compounds benefit the consumer in two ways. First, consumers benefit from the reduced production cost associated with the use of this technology in beef production. Second, consumers benefit from the increase in lean protein options provided through beef from cattle raised with growth-enhancing technologies.

Introduction

Anabolic steroids have been widely used in the beef cattle industry for over 50 years as safe and effective growth-promoting agents, and today, more than 90% of all feedlot cattle in the United States receive some type of steroidal implant during their lifetime (NAHMS, USDA 2000). Generally, implants have been shown to increase growth rate 8% to 28%, improve feed efficiency 5% to 20%, and enhance lean tissue mass of the carcass 3% to 10% (Duckett and Owens, 1997). These improvements in growth rate and feed efficiency create tremendous production benefits throughout the beef industry, particularly in productivity (i.e., pounds of beef produced per animal). This is illustrated in Figures 1 and 2, demonstrating that while beef cow numbers have declined significantly during the past 30 years, beef production increased approximately 23%.

This improvement in production efficiency has multiple benefits: 1) it clearly and dramatically reduces production costs by reducing the amount of feed required per unit of gain (Avery and Avery, 2007); 2) it reduces the amount of land necessary to produce equivalent amounts of food for consumers; 3) it limits the production of greenhouse gases by reducing the number of animals required to produce equivalent amounts of beef (Avery and Avery, 2007); and 4) it extends cost savings to consumers by providing a year-round, affordable supply of beef at reduced prices (Lawrence and Ibarburu, 2009).

Feed efficiency due to steroidal implants often reduces the cost of production by $20 to $80 per head above and beyond the actual cost of the implant (Preston, 1999). This equates to a $10 to $17 return-on-investment for producers, who can then pass along cost savings to consumers. With record feed and input costs seen since 2008, these growth-enhancing technologies are more important to cattle industry profitability and food supply availability and affordability than ever before. Additionally, enhanced carcass lean tissue (protein) accretion appears to be another major benefit to steroidal implants (Johnson et al., 1996a). Specifically, the value of the retail product can be increased more than $100 per head due to the improvements in lean tissue deposition (Hancock et al., 1991). Since animal products contribute significantly to the total caloric and nutrient intake in the human population, altering the composition of growth toward more lean tissue and less adipose tissue results in a healthier product with fewer calories that still is rich in beneficial nutrients.

The beef cattle industry started using anabolic growth promotants following the published observations (Dinusson et al., 1948), which found both rate and efficiency of gain increased in heifers that were implanted with diethylstilbestrol (DES) compared to non-treated controls. Following this initial discovery, Burroughs et al. (1954 a, b) reported feeding DES to steers also improved rate and efficiency of gain. FDA approved DES for use in beef cattle production in 1954. Following DES approval and 18 years of successful use by the cattle-feeding industry, both as

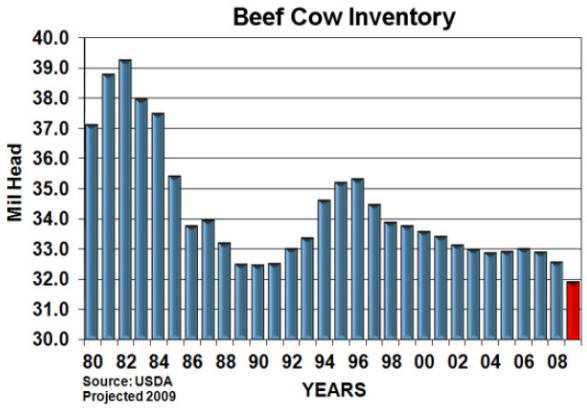


Figure 1. USDA beef cow inventory numbers have been declining since the high in 1982. Beef cow inventory in 2009 is projected to be more than 18% smaller than in 1982.

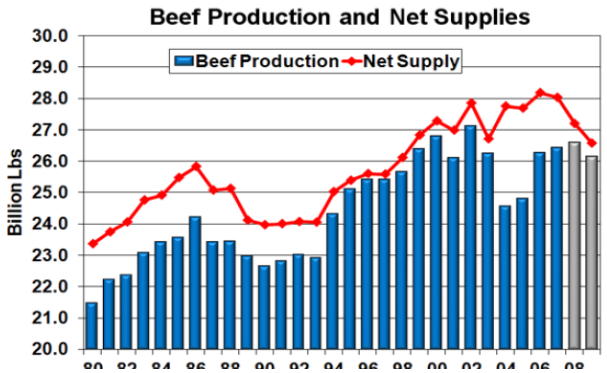


Figure 2. According to USDA, beef production has increased more than 14% during the same period

an implant or as an oral feed additive, FDA was forced in the early 1970s to re-evaluate its approval of DES. Research at the time indicated an increased incidence of adenocarcinoma in the female offspring of women who were prescribed exceptionally high doses of DES during pregnancy by their medical doctors to prevent miscarriages (Herbst et al., 1971). In addition, Cole et al. (1975) reported DES caused carcinoma in rats that were genetically predisposed to cancer. Thus, after years of deliberation, DES was banned for use in beef cattle as a precautionary measure in 1979. This action was mandated through the Delaney Clause, a 1958 amendment to the Federal Food, Drug and Cosmetic Act, that prevents FDA from approving products that could result in cancer-causing residues of chemicals in food. Therefore, growth promotants containing compounds with any potential for carcinogenicity – even if only in extreme circumstances – are subject to additional, stringent safety testing requirements before they can be approved for use in food animal production.

Other steroids with anabolic effects were examined for their potential as growth-enhancing compounds for livestock production, and after thorough evaluation for any potential human health consequences, have received FDA approval for use in both feedlot cattle and cattle on pasture. Since 1956, 14 growth promoting compounds have received FDA approval (Appendix 1). Approved products are regularly evaluated and must be continually proven safe to remain on the market. As of April 2009, 30 individual growth promoting products have been through the rigorous FDA approval process and are available for use in beef cattle production (Appendix 2).

With the controversy surrounding the withdrawal of DES' approved use in beef cattle, subsequent product approvals have been subject to greater scrutiny. All growth-promoting compounds are tested under the New Animal Drug Application (NADA) process before approval as mandated by FDA. This process is a very thorough, science-based regulatory review overseen by scientists at the FDA's Office of New Animal Drug Evaluation within the Center for Veterinary Medicine (CVM). Approval of a new growth-enhancing compound requires, on average, 75 independent studies that document human food safety, target animal safety, efficacy, environmental safety, and user safety.

The risk of detectable residue levels from implants, which are administered in the middle third on the back of the ear, is negligible. The ear provides adequate blood flow for consistent steroid release from the implant, and the ears do not enter the human food supply because they are removed and disposed of at harvest. The lack of detectable residues in beef from implanted cattle is due to very rapid metabolism of the anabolic steroid once it is released from the implant. Once metabolized by the liver, the anabolic steroids are excreted rapidly in the feces and urine of beef cattle. Lange et al. (2001) illustrated the effects of correct and off label usage of hormonal implants on the residue levels of the muscle, fat, liver and kidney tissue (Appendix 3). By using the manufacturer's recommended dosage no apparent differences in estrogen, zeranol or testosterone content can be detected in muscle tissue between implantation and control animals. The liver and kidneys of an animal act as filters to remove toxins and waste. Because of the function of the liver and kidneys, we can expect some accumulation of steroid hormones in these tissues. As expected implantation of cattle results in an apparent increase in the accumulation of trenbolon, zeranol, and estrogen. Additionally, some testosterone, zeranol, and trenbolone residue of can be seen in the perirenal fat of animals treated with steroid hormones compared to controls. This is likely due to the fact that steroid hormones are cholesterol based lipids. However, the daily intake of steroid hormones is negligible compared to the average daily production in humans (Table 1).

Based on these papers, if a prepubescent girl was to eat 453.6 g (1 lb) of meat a day and that meat was obtained from cattle implanted at 10 times the manufacturer's recommendation then she would consume approximately 0.031 μg of testosterone from that meat. That 0.031 μg of testosterone would be approximately 1/1000th of her daily production. Because the amount of steroidal hormones that are ingested daily is minute in comparison to the amount produced naturally the effect of residue increases due to implantation is insignificant.

In addition to steroidal implants, a newer class of orally active growth promotants, known as β-adrenergic agonists (ractopamine-HCl; 2003 and zilpaterol-HCl; 2006), has been approved for use in finishing beef cattle in the last decade. The products provide similar production benefits as steroidal implants, but differ in application and mode of action. β-adrenergic agonists are fed during the last 20 to 42 days of the cattle finishing period, depending on the specific product. These products preferentially increase carcass lean tissue at the end of the feeding period. This alteration in carcass lean-to-fat ratio has very positive effects on feed efficiency during the last 20 to 42 days of the feeding period. In addition, β-adrenergic agonists have tremendous impacts on retail yield of red meat from the carcass, further supporting producers' ability to meet consumer demand for beef with fewer animals.

Table 1. Daily production of steroid hormones in humans compared to total daily intake.

	Testosterone	Estrogens (17β-estradiol+estrone)		
	Daily production (μg/d)	Daily intake (μg/d)	Daily production (μg/d)	Daily intake (μg/d)
Men	6480	0.07	140	0.10
Women	240	0.05	630	0.08
Boys (prepubertal)	65	0.05	100	0.08
Girls (prepubertal)	32	0.04	54	0.07

Adapted from Hartmann et al. (1998)

Classification of compounds

Anabolic steroids. Steroidal compounds have been used effectively and safely for growth promotion in the beef cattle industry for over 50 years. Commonly used steroid compounds include estrogens, androgens, and progestins. These steroids can be classified as naturally occurring or synthetic as described in Table 2:

The natural hormones listed in Table 2 are found in all mammals, regardless of gender. The three synthetic compounds used in beef cattle production to enhance growth rate and feed efficiency are zeranol, trenbolone acetate, and melegestrol acetate. Zeranol is classified as a nonsteroidal macrolide and is in a class of naturally occurring (found in nature; not produced in a laboratory) products referred to as β-resorcyclic acid lactones. Zeranol was originally isolated from corn mold, and although it is a nonsteroidal compound, it has been shown to have estrogen-like biological activity in cattle. Trenbolone acetate (TBA) was synthesized in 1967. Trenbolone acetate is a testosterone analogue that has 10 to 50 times the anabolic activity compared to testosterone (Bouffault and Willemart, 1983). This androgen growth promotant often is used in combination with an estrogen (most commonly E_2) to maximize growth rate and efficiency in cattle, especially steers. In 2008, it was estimated that nearly two-thirds of all implants marketed in the U.S. were single implants of various concentrations of TBA and E_2 (TBA/E_2) (P. Parker, personal communications). Growth-promoting implants containing various combinations of TBA and E_2 are the prominent type of implants used in the industry today. Melengestrol acetate (MGA) is approved for use in feedlot heifers to suppress estrus and enhance efficiency of growth. Even though this is an exogenous, synthetic progestin, unique characteristics of this compound allow it to be active when fed to heifers at 0.40 mg/head/day, thus it is not necessary to administer this compound as an implant. With rapid metabolism in the animal's body and no detectable residues in edible tissues, no withdrawal period is required for MGA in heifers so cattle producers can feed the product safely up until harvest. Currently, MGA is approved only for feedlot heifers and cannot be fed to steers.

With the exception of MGA, which is orally active, the rest of the anabolic steroids are administered as compressed pellet-implants with various inert carrier compounds. These steroid-containing implants are administered in the back of the middle of the ear of cattle. Proper implanting of these compounds is important for both efficacy and safety. Once administered in the back of the ear, the active steroids dissolve slowly into the bloodstream in the ear. The compounds then are carried by special binding proteins in the bloodstream to all tissues of the body.

Table 2. Growth promoting steroid hormone classifications

Estrogens (female hormone)	
Natural	Estradiol-17beta
Estrogen-like activity	Zeranol
Androgens (male hormone)	
Natural	Testosterone
Synthetic	Trenbolone acetate
Progestins (hormone of pregnancy)	
Natural	Progesterone
Synthetic	melengestrol acetate (orally-active)

β-*adrenergic agonists*. β-adrenergic agonists (β-AA) are classified as phenethanolamine compounds and are approved for use in food-animal production in several countries, including the United States. These compounds are neither steroids nor peptide growth factors; rather, they are compounds similar to endogenous catecholamines, such as norepinephrine and epinephrine, are found in all animals, including humans.

In the United States, ractopamine hydrochloride and zilpaterol hydrochloride are both approved as growth promotants for beef cattle (Table 3). In both cases, these compounds (Figure 4) are orally active in the parts per million (ppm) concentration range. In addition, these compounds are fed at the very end of the feeding period, immediately prior to harvest (last 20 to 42 days). Ractopamine, marketed by Elanco Animal Health as Optaflexx®, was approved (June 2003) to be fed the last 28 to 42 days prior to slaughter with no pre-harvest withdrawal. Zilpaterol, marketed by Intervet/Schering-Plough Animal Health as Zilmax®, was approved (August 2006) to be fed to cattle the last 20 to 40 days prior to slaughter. Zilmax was approved by FDA with a 72-hour withdrawal time prior to harvest.

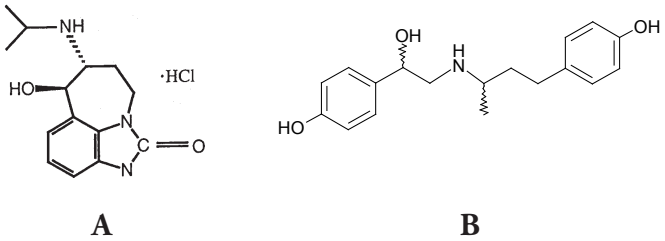
Strategic use of steroidal implants as growth promotants

Cattle producers have developed strategies to use the different combinations of implants based on breed and sex of cattle, marketing conditions, body condition and estimated days on feed. Because implants promote the deposition of muscle rather than fat, the resulting beef carcasses tend to be leaner, with less marbling when harvested at similar days-on-feed as animals that have not been implanted. Therefore, in order to achieve the same degree of marbling, implanted cattle must be fed for longer and to a heavier body weight.

Implant strategies can be tailored to each animal type and marketing opportunity by increasing or decreasing the dose of hormone administered. For example, in large-frame continental

Table 3. β-adrenergic agonists (phenethanolamines; orally active)

Natural	catecholamines (epinephrine, norepinephrine)
Synthetic	ractopamine HCl and zilpaterol HCl



A **B**

Figure 4. Chemical structures of two approved β-adrenergic agonists for beef cattle in the United States. Panel A) Zilpaterol HCl, Panel B) Ractopamine HCl

cattle that may be surpassing acceptable carcass weight, a lower dose TBA/E_2 combination implant may provide an adequate dose of anabolic steroids to achieve acceptable gains without impacting quality grade at a given carcass weight. However, smaller-framed British breeds may benefit from higher dose combination implants to increase gain and body size and improve feed conversion efficiency without substantially reducing quality grade due to their genetic propensity to deposit intramuscular fat (marbling).

While no withdrawal prior to harvest exists for implants, in order to gain the optimal benefit of the implant, it is necessary to leave the implant in the animal until the majority of the compound has paid out, ranging from 50 days to 200 days (Appendix 2). After implantation, the steroid hormones are released from the compressed pellet into the bloodstream through a process commonly referred to as "pay out." Upon entering the bloodstream, the hormones are converted into their biologically active form, meaning that estradiol benzoate will be converted into estradiol (E_2) and trenbolone acetate will be converted into trenbolone (TBOH). The insoluble steroid then reversibly binds to specific carrier proteins in the blood (steroid binding globulins and albumin) for delivery to target cells. While it is possible to "stack" implants — administer an implant while another implant is still paying out — producers tend to use a combination implant that achieves the same purpose but in a more controlled manner. For this reason, it is advised that producers inquire about the implant history of an animal so an appropriate implant strategy can be applied and the optimal economic return can be achieved.

Implant strategies typically are developed by estimating the days on feed for cattle to achieve the desired harvest weight, and working back. For example, in Figure 3, the implant strategy will change based on the weight of cattle arriving at the feedyard, even though they will all be harvested at similar harvest weights.

Typically, when cattle are implanted more than one time, strategies are developed in which the cattle are "stair-stepped" from mild to aggressive implants. Researchers at South Dakota State University (Bruns et al., 2005) found aggressive implanting during the early growth phases of the animals evaluated substantially reduced quality grade (decreased marbling/intramuscular fat deposition) at harvest. Therefore, utilizing mild implants during the rapid growth phases, followed by more aggressive implants when growth has slowed down, tends to provide adequate growth enhancement with minimal impact on capacity to deposit marbling.

Performance response to steroidal implants

Currently, 29 growth promoting products are approved for use and marketed in the U.S., 27 of which are steroidal implants (Appendix 2). These products vary in active ingredients, dosage and carrier compounds. The dose of active hormone is the primary determinant of performance response; however, if nutrients (primarily protein and/or energy) are limiting, cattle will not respond to an increased dose of hormone. Another determinant of the absolute response to implants is the inherent genetic potential for growth of each animal. As the growth rate of the non-implanted animals increases, so does the added benefit from the implant. However, the percentage response to the implant may not change dramatically. With respect to feedlot performance, Duckett and Owens (1997) reviewed 33 independent implant studies that compared performance of non-implanted cattle to those given a combination androgenic/estrogenic implant. Implanting increased average daily gain 21% and improved feed efficiency 11% in feedlot cattle. In addition, carcass weight was increased 7% due to implanting. The majority of feedlot implant studies have been conducted using a time-constant termination point for all treatments. Given this restriction, the aforementioned review also reported a 5% increase in ribeye size, a 7% reduction in fat cover, a 5% reduction in marbling score, and a 17% reduction in percent of carcasses grading Choice or better. This indicates that although implanted cattle gain faster than non-implanted cattle, they do not accumulate fat at a rate proportional to their increased growth. If cattle are harvested at different fat-content endpoints, we would normally expect lower marbling content.

A small number of studies have been conducted where cattle, having been treated with different dosages of implant, are harvested at multiple times and, hence, at fat-content endpoints.

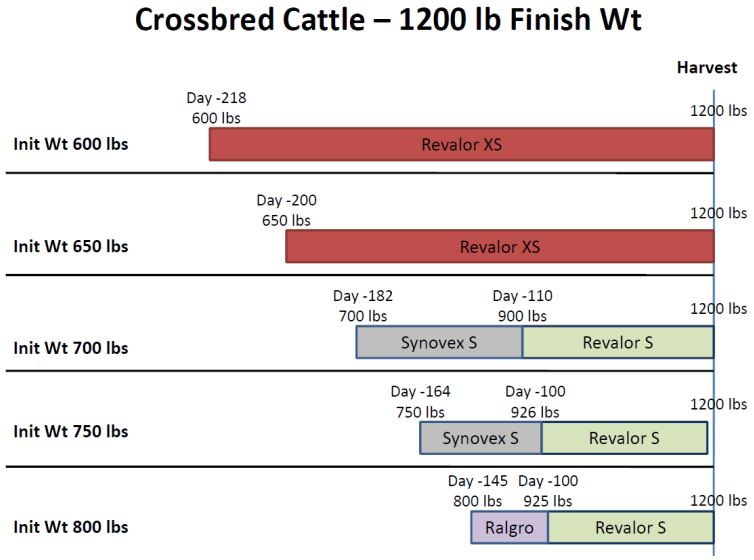


Figure 3. Implant protocol (Courtesy of Beckett Consulting). The diagram above illustrates an example of an implant protocol for cattle of a projected finish weight of 1,200 lbs, with varying intake weights ranging from 600 to 800 lbs. Feedyards project final weights and gain potential of cattle upon arrival, and implant protocols are assigned to each lot to achieve production targets (gain, efficiency and carcass characteristics) within the constraints of cattle biological type and weight.

Hutcheson et. al (1997) reported that when the dosage of a TBA/E_2 implant was increased by 50% (120/24 vs. 80/16 mg TBA/mg E_2), an additional 22 days-on-feed resulted in similar average daily gain (ADG), feed-to-gain ratio (F:G) and a similar percent of carcasses grading Choice and Prime for the higher dosage compared to the lower dosage at the earlier time endpoint. However, at the higher dosage and the later time point, hot carcass weight also increased by 55 lbs. Preston and coworkers reported that based on a review of 24 studies, steers and heifers implanted with combination TBA/E_2 implants required an additional 12 and 15 days on feed, respectively, to attain a similar degree of marbling compared to non-implanted animals. Cornell University researchers calculated that live empty body weight (the weight of a live animal with an empty digestive tract) of steers implanted twice in the feedyard with combination TBA/E_2 implants would be 97 lbs heavier at comparable body fatness compared to steers which receive no feedyard implant, and steers would have similar quality grade. Anderson (1991) reported the difference between implanted and non-implanted feedyard cattle would be 128 lbs.

Steroid metabolism in cattle

Most steroidal growth promotants are administered as subcutaneous implants, placed in the middle third of the back of the ear. Implant products are compressed pellets, with a high concentration of active steroid compound and a small portion of inert ingredients such as either lactose, cholesterol, or polyethylene glycol polymers. Estrogenic implants range in dosage from 10 to 72 mg, and TBA-containing products range from 40 to 200 mg. Many products contain both an androgen and an estrogen. Melengestrol acetate is an orally-active feed additive, which is absorbed from the digestive tract of cattle and enters the bloodstream.

After implantation, the steroid hormones are released from the compressed pellet into the bloodstream through a process commonly referred to as "pay out." Upon entering the bloodstream, the hormones are converted into their biologically active form, meaning that estradiol benzoate will be converted into estradiol (E_2) and trenbolone acetate will be converted into trenbolone (TBOH). The insoluble steroid then reversibly binds to specific carrier proteins in the blood (steroid binding globulins and albumin) for delivery to target cell types on tissues such as skeletal muscle, adipose tissue and bone.

Once the steroid hormone enters the bloodstream, the concentration of the hormone in circulation can easily be measured. The concentration of implanted steroid hormones in the bloodstream is a result of two independent events, release rate of the steroids from the implant and clearance and excretion of the steroid hormone from the body.

There appears to be two partitions or pools of excreted steroid from animals following administration. One that occurs very rapidly, called fast pool, and a second system that clears the steroids at a slower rate (slow pool). Hancock et al. (1987) reported that infused E_2 had a very short half-life of 7.7 minutes in the fast pool and a longer half-life of 41.5 minutes in the slow pool. Therefore, once E_2 was released and entered the circulation, it was cleared very rapidly from the animal. The increased circulating E_2 levels observed following implantation were most likely the result of release of new steroid from the implant rather than slow clearance rate from plasma (Hancock et al., 1987). These are key findings indicating the biology for low tissue residue of steroids following administration of steroidal implants to cattle. However, the animal must have a mechanism for compensating for increased E_2 concentrations. Moran et al. (1991) reported no significant differences in circulating E_2 (13.1 pg/mL vs. 16.8 pg/mL) in heifers implanted with either one or two E_2 implants.

Circulating TBOH levels follow similar patterns after implantation compared to E_2. Henricks et al. (1982) reported that on the day following implantation, plasma TBOH rose to more than 900 pg/mL in heifers implanted with 300 mg TBA. The circulating levels gradually decreased to 400 pg/mL on day 90 post-implantation. In bulls, Istasse et al. (1988) reported that TBOH increased to about 1000 pg/mL and was sustained at that level until week 8 and then began to decline until week 11 when the bulls were re-implanted and the circulating TBOH rose again. There tends to be an interaction in TBOH levels for TBA implanted and TBA/E_2 implanted steers. Hunt et al. (1991) observed that serum TBOH was more than 1,000 pg/mL in steers implanted with TBA alone. However in steers implanted with TBA/E_2, the serum TBOH was approximately 550 pg/mL, or almost half the concentration of steers receiving TBA alone. In contrast, Istasse et al. (1988) found that plasma concentrations of TBOH tended to be higher with higher doses of E_2. Bulls implanted with 200 mg TBA + 60 mg E_2 had 964 pg/mL compared to 844 pg/mL in bulls implanted with 200 mg TBA + 40 mg E_2. Similarly, Hayden et al. (1992) reported TBOH levels in TBA/E_2 implanted steers were twice as high as those in steers implanted with TBA alone (1,672 pg/mL vs. 652 pg/mL). The authors suggested that this may be due to E_2 competition with hepatic TBOH metabolism.

In addition, the half-life of one steroid often is influenced by simultaneous administration of another steroid (Harrison et al., 1983). Previous studies have shown that the combined administration of TBA/E_2 can have interactive effects on payout from the implant which, in turn, can result in different circulating levels of the steroid post-implantation. In addition, these changes in circulating steroid concentrations then, in turn, could impact clearance rate of the individual steroid and the major metabolites of these steroids. All these changes ultimately impact the amount of steroid that is sequestered by individual tissues that in turn can affect residue levels.

Excretion

The risk of detectable residue levels that could be harmful to human health from implanted growth promotants is negligible. Circulating steroid levels in implanted ruminants are influenced by the release rate of the steroid from the implant and the metabolic clearance rate of the steroid in the animal's body. Metabolism of both estrogens and androgens occurs in the liver through a series of hydroxylation and reduction steps. The liver also is the site of steroid conjugation. Once conjugated, the metabolites are water-soluble and can be excreted through the kidneys and eliminated from the body in the urine. In ruminants, it appears approximately 50% of steroids can be eliminated through the

feces. This excretion occurs following modifications of original steroid molecule in the liver to alter the original steroid molecule to a less active metabolite. These alternative metabolites go from the liver to through the bile back into small intestines and are excreted in the feces as significantly less active compounds compared to what was administered to the animal as an implant.

The dynamics of steroid payout, clearance and excretion in cattle that receive growth-promoting implants allows for safe use of these steroid hormones to enhance growth and lean tissue deposition with nearly non-detectable hormone residues compared to untreated animals. Plasma kinetic studies have revealed that hydrolysis of a single IV injection of radiolabeled trenbolone acetate to the alcohol derivative, trenbolone, was extremely rapid (Pottier et al., 1975). Trenbolone was estimated to have a half-life of 1.5 hours following a single intravenous (IV) injection (Pottier et al., 1975). These authors followed up their initial study of a single dose of TBA with an experiment describing plasma kinetics following administration of an ear implant containing radiolabeled TBA. The half-life of radiolabeled TBA in the implant was estimated between 68 and 84 days (Pottier et al., 1975). The authors reported that the majority of modified steroid was excreted in the bile and urine. Total radioactivity recovered at slaughter indicated approximately 2/3 of the activity was in the bile fraction and 1/3 in urine component. The authors found that milk and other tissues were not tissues of excretion or storage for the radiolabeled steroid. This indicates that edible tissue harbor extremely low levels of these compounds, ensuring no detectable levels are present in the beef consumed.

The level of steroid present at any given time is related to the dose and release pattern from the implant that was administered due to a very short half-life once in circulation. The implication of this in beef production is that these steroid hormone implants are administered early in the feeding period (80 to 120 days prior to harvest). The active ingredients are allowed to be released from the implants over this time period, but once released, the active ingredients bind to their receptors (see next section) on target tissues and are metabolized and excreted very rapidly. This assures no risk of residues of steroid hormones in edible tissues like skeletal muscle and fat.

A comprehensive review on the topic of steroid hormone residues in beef was published by Doyle (2000) and serves has as an excellent reference to much of the residue research conducted in the last 30 years. Hancock et al., (1991) reported that estradiol-17β concentrations in lean skeletal muscle and liver were nearly identical and indistinguishable in samples collected from steers administered an estrogenic implant containing 24 mg estradiol-17β compared to untreated steers. Briefly, lean tissue from implanted steers contained 3.5 pg/g E_2, as compared to 5.8 pg/g in lean from non-implanted steers. Liver samples from the estrogen-implanted steers contained 10 pg/g E_2 compared to 4 pg/g E_2 in non-implanted liver samples. Likewise, Henricks et al. (1983) reported that muscle samples from steers administered an estrogenic implant contained similar E_2 concentrations compared to non-implanted samples (17 vs. 14 pg/g, respectively). In addition, E_2 concentrations of liver samples were greater in implanted steers (42 pg/g) as compared to non-implanted steers (14 pg/g). A study by Henricks et al. (1982) evaluated tissue concentrations of trenbolone following administration of a trenbolone acetate implant to heifers. There were no differences in concentrations of TBOH in subcutaneous fat samples between implanted and non-implanted heifers (45 pg/g vs. 25 pg/g). Trenbolone concentrations were greater in liver samples from implanted compared to non-implanted heifers (94 pg/g vs. 36 pg/g). Taken together, these data indicate that the metabolism of steroids in edible tissues like skeletal muscle and adipose tissue is very rapid with no marked change in concentration of these steroids in edible tissues. Tissues such as the liver and kidney are important in the metabolism and excretion of these compounds from the animal's body. Research indicates that these tissues may have slightly elevated levels of metabolites of steroid hormones following administration to the cattle due to the increased steroid metabolism.

It is important to note that analytical techniques have improved dramatically in the last decade, allowing for detection of traces of hormones in the part-per-trillion levels. These values must be put in context with published maximum safe levels that have been established for steroid hormones. As described above, the biology supports rapid metabolism of original steroid to less active metabolite; excretion of these altered compounds through the urine and feces; and nearly non-detectable levels of these metabolites in edible tissues such as skeletal muscle and adipose tissue. Federal regulatory approvals require the determination of the no hormonal effect level in the most sensitive species (Lone, 1997). Once this dose is established, it is divided by a safety factor of 100 to give an acceptable daily human intake (ADI) per unit of body weight. The potential daily intake (PDI) is estimated from expected daily intake of animal tissues multiplied by the residue of the compound in the tissue sample of animals receiving the growth promotant (Preston, 1999). A product will never be approved if the PDI exceeds the ADI. For synthetic growth promotants, like TBA, the ADI is more than 1,200 times the PDI, allowing for a very wide safety margin (Preston, 1999). Finally, the normal (endogenous) daily human production of natural sex steroid hormones like estrogen, progesterone, and testosterone greatly exceeds the PDI from beef produced with steroidal implants (Preston, 1999).

In addition, federal regulators have determined it is safe to consume beef from cattle administered naturally occurring steroids such as estradiol, testosterone, and progesterone. They monitor this through a process called allowable incremental increase (AII). It is mandated the AII cannot exceed 1% of the level of these naturally occurring hormones produced by humans. These numbers must be calculated from humans that would produce the least amount of each of these hormones.

Although FDA sets the acceptable levels of steroids allowable in edible tissues, USDA's Food Safety and Inspection Service (FSIS) is responsible for monitoring the presence of violative residues through its National Residue Program (FSIS, 2008). Data collected during 2007 noted no violative residue for compounds that would be considered growth enhancing compounds in beef cattle such as trenbolone, zeranol, MGA, and ractopamine. These data reaffirm that the U.S. cattle producer uses these compounds judiciously and they pose no risk to consumers when used according to label directions.

Mode of action: Anabolic steroids

Composition of growth

Each muscle fiber is a large, multinucleated cell primarily composed of two proteins, actin and myosin. The number of these fibers is fixed at birth in many species of mammals, including cattle. Therefore, to increase muscle mass, a process of hypertrophy must occur, which is the increase in size of existing cells through the accumulation of additional protein. The accumulation or accretion of protein is a function of two important processes that occur continuously in skeletal muscle: protein synthesis and protein degradation. Skeletal muscle is a dynamic tissue that is constantly "turning" itself over. For skeletal muscle growth (hypertrophy) to occur, the rate of protein synthesis must exceed the rate of protein degradation.

Receptors for estrogens, androgens, and progestins are located in most all cell types but in vastly different proportions. The concentration and binding affinity of these receptors affects the ability of the steroid to elicit a biological response in that cell type. These effects are often referred to as "direct" effects since the steroid is binding to a receptor present on the target tissue such as skeletal muscle. Muscle tissue contains both androgen and estrogen receptors, but the concentrations of these receptors in muscle are often 1,000 times less than in reproductive tissues. However, the relative binding affinity for the androgen receptor in skeletal muscle and prostate are identical. Androgen receptors in muscle tissue have been characterized in several species including rat, porcine, bovine, ovine, and human. Similarly, estrogen receptors in muscle tissue also have been characterized in rat and bovine.

Administration of a combined TBA/E_2 implant consistently increases growth rate and carcass protein accumulation in cattle. In yearling steers, implanting with a combined TBA/E_2 implant increased carcass protein mass approximately 10% on day 40 following implantation (Johnson et al., 1996a). The increased mass of carcass protein on day 40 was due to an 82% increase in estimated daily carcass protein gain in steers implanted with TBA/E_2 the first 40 days (Johnson et al., 1996a). Although carcasses from cattle implanted with TBA/E_2 had approximately 8% to 10% more protein after 115 days, the majority of this effect was brought about by rapid changes in protein accretion the first 40 days following implantation. Carcass water amounts followed the protein values closely, suggesting lean tissue deposition was enhanced with TBA/E_2 implants. Similarly, Hutcheson et al. (1997) reported steers implanted with TBA/E_2 implants had 8 kg (17.6 lbs) more protein in the live empty body. This effect was greater with combined implant (120 mg TBA and 24 mg E_2) compared to single active ingredient steroid implants, so the authors concluded that the combined TBA/E_2 implant had additive effects at increasing protein mass in steers. Daily protein accretion rates were 163 g/day for TBA/E_2 implanted steers compared to 101 g/day in non-implanted control steers. In addition, percentage water was increased similar to protein content. The water increase, coupled with the increase in protein mass, resulted in an overall increase in lean tissue mass of TBA/E_2 implanted steers in both of these data sets (Johnson et al., 1996a; Hutcheson et al., 1997). Although lean tissue accumulation was increased following administration of a combined TBA/E_2 implant, adipose tissue or fat accumulation was unaffected in the carcass (Johnson et al., 1996a) and live empty body (Hutcheson et al., 1997). Hutcheson et al. (1997) speculated that any observed changes in fat accumulation following implanting were simply a dilution effect due to increased lean tissue accumulation and not a direct reduction in lipogenesis.

Protein metabolism

Much of the early work with TBA and rodent models conducted in the 1970s focused on the effect of trenbolone on rate of both protein synthesis and degradation in the whole body or target tissues such as skeletal muscle. Classic papers by Vernon and Buttery (1976, 1978a, b) reported that trenbolone injection to female rats reduced both the rate of protein synthesis and degradation in skeletal muscle. The reduction in rate of protein degradation was greater than the drop in synthesis so protein accumulation was increased. When this work was conducted in larger mammals such as cattle, a different effect was noted. Hayden et al. (1992) reported implanting steers with 300 mg TBA and 24 mg E_2 as separate implants had no effect on the rate of skeletal muscle protein degradation. Consequently, the authors concluded the increased skeletal muscle protein mass was due to increased protein synthesis rates in the implanted steers. The magnitude of skeletal muscle protein accretion was very similar to those reported in Johnson et al. (1996a) and Hutcheson et al. (1997).

Hormonal changes

Steroids have both direct and indirect effects on muscle growth. In the case of estrogens, the direct effects are thought to be secondary to indirect effects, mediated by changes in other hormone profiles. The primary effect of estrogens is through an altered somatotrophic axis. Estrogens increase pituitary size and increase the proportion of somatotrophs in the pituitary. The pituitary is also more responsive to somatotrophin releasing factor (SRF). Insulin-like growth factor-I (IGF-I) production is increased and both somatotrophin (ST) and IGF-I binding characteristics are altered. These changes work together to produce higher circulating ST, a more efficacious release pattern, and a more responsive muscle, resulting in stimulus of muscle growth.

Increased ST does not explain all of the effects of estradiol. Exogenous ST has been shown to increase growth of estradiol-implanted cattle; estradiol is additive in affecting circulating metabolites and growth factors. Effects of estradiol and ST are nearly additive when calorie consumption is restricted to the level of cattle without estradiol implants. Other hormones such as insulin and thyroid hormones also are altered, supporting increased muscle growth.

Many research studies over the years have investigated the effects of anabolic steroids on changes in concentrations of important hormones that regulate growth. These effects often are referred to as "indirect" effects since the anabolic steroids are affecting a non-target tissue to synthesize and release an important growth-enhancing hormone that, in turn, impacts a target tissue such as skeletal muscle. From a postnatal growth perspective,

growth hormone (somatotropin) concentrations have been positively correlated with overall growth rate. Many of the growth-enhancing effects of growth hormone are mediated through another very important growth factor called insulin-like growth factor I, which is a potent anabolic growth factor found in the general circulation. It is generally recognized that the majority of IGF-I found in circulation is synthesized in and secreted by the liver (Florini et al., 1996). Insulin-like growth factor-I also is produced by skeletal muscle fibers as well as satellite cells and is known to act in both autocrine and paracrine manners (Jennische et al., 1987; Lewis et al., 2002). Jennische and Matejka (1992) have shown that muscle cells preferentially use locally produced IGF-I in an autocrine and paracrine mode. Thus, locally produced IGF-I is extremely important in skeletal muscle growth. This is further supported by the findings of Liu et al. (2000), who found that mice lacking the liver-specific IGF-I gene had much lower circulating IGF-I levels yet had normal postnatal growth. These data suggest that indeed locally produced IGF-I acted in an autocrine/paracrine mechanism to mediate postnatal skeletal muscle growth. IGF-I has been shown to be potent stimulator of protein synthesis in skeletal muscle and, at the same time, can reduce the rate of protein degradation. Previous research demonstrated that administration of a combined TBA/E_2 implant resulted in increased circulating IGF-I and IGF-I messenger RNA (mRNA) levels in the longissimus muscles of implanted steers as compared to non-implanted steers 30 to 40 days after implantation (Johnson et al., 1996b; 1998b; White et al., 2003). In addition, Pampusch et al. (2003) reported that IGF-I mRNA levels in longissimus muscle biopsy samples from implanted steers were greater than those of non-implanted steers as quickly as 12 days after implantation. These results suggest that the muscle of implanted steers may produce more IGF-I than that of non-implanted cattle. Additionally, circulating levels of IGF-I will be greater in sera from cattle implanted with TBA/E_2 compared to non-implanted cattle. Taken together, these effects of implanting will have positive effects on enhancing protein accretion in existing skeletal muscle fibers.

For the increase in protein mass to be sustained long-term in skeletal muscle, eventually the fiber will need more "machinery" or added DNA to aid in the process of protein synthesis. This is an important role of the muscle satellite cell. Satellite cells lie between the basal lamina and sarcolemma of individual muscle fibers. They are capable of proliferating/dividing and ultimately "fuse" into the adjoining fiber to donate their nuclei to support the ramped-up protein synthesis. Consequently, factors that can impact rate of satellite cell incorporation into existing fibers will have a positive impact on postnatal muscle hypertrophy. TBA/E_2 administration to steers resulted in an increase in the number of actively proliferating, satellite cells within 35 days of implantation (Johnson et al., 1998a). It is thought that the enhanced IGF-I production by the muscle fiber after administration of the steroid implants mediated the increased proliferative activity of these satellite cells. In addition, *in vitro* studies have revealed that trenbolone and estradiol can directly increase the rate of cell proliferation of cultured satellite cells isolated from bovine skeletal muscle (Kamanga-Sollo et al., 2004). Based on the discussion in the previous section, increased proliferative activity of satellite cells should enhance the rate of muscle growth in cattle. Taken together, these findings strongly support a mechanism for steroid implant-induced muscle growth in beef cattle that involves increases in the local production of muscle IGF that, in turn, enhances satellite cell activity and consequently increases skeletal muscle growth.

Summary of mode of action

Combined TBA/E_2 implants increase carcass protein by approximately 10% when compared to non-implanted steers. Much of this increase occurs the first 40 days following implantation. Growth-promoting implants containing anabolic steroids have been shown to induce postnatal skeletal muscle hypertrophy, in part, through increased circulating and locally produced (skeletal muscle) concentrations of a very important growth factor for skeletal muscle, IGF-I. Insulin-like growth factor-I is a potent stimulator of skeletal muscle growth and differentiation. It is thought to stimulate skeletal muscle protein synthesis and reduce skeletal muscle protein degradation. Specifically, TBA and estradiol-17β (TBA/E_2) implant administration to cattle has been reported to increase circulating IGF-I and skeletal muscle IGF-I mRNA levels compared to non-implanted, control steers in several experiments. In addition, TBA/E_2 administration to steers resulted in an increase in the number of actively proliferating, satellite cells within 35 days of implantation. It is thought that the enhanced IGF-I production by the muscle fiber after administration of the steroid implants mediates the increased proliferative activity of these satellite cells. Based on the discussion in the previous section, increased proliferative activity of satellite cells should enhance the rate of muscle growth in cattle. Taken together, these findings strongly support a mechanism for steroid implant-induced muscle growth in beef cattle that involves increases in the local production of muscle IGF that, in turn, enhances satellite cell activity and consequently increases skeletal muscle growth.

The effects listed above can be observed within days after implant administration. Early muscle growth stimulus is primarily hypertrophic in nature, which has been shown by depressed DNA/protein ratios. Prolonged (weeks-long) exposure to combined estrogenic/androgenic implants produces hyperplasia (increase in satellite cell nuclei) as well. In this case, quantity of muscle protein is increased but normal DNA/protein ratios are observed, indicating that proliferation of satellite cells resulted in increased quantity of DNA in the muscle. Cell culture studies have shown that the mitogenic activity of sera from implanted steers is increased, providing support for the line of thinking that implants initially increase hypertrophy and ultimately increase hyperplasia to support increased muscle mass.

Proposed mechanism of action of β-adrenergic agonists in beef cattle

One of the most pronounced effects of feeding a β-adrenergic agonist to ruminants is the preferential dramatic increase in skeletal muscle mass and/or cross-sectional area of individual muscles. Due to the dramatic increase in skeletal muscle hypertrophy following β-adrenergic agonist administration to ruminants, one would expect satellite cell proliferation and subsequent fusion

of the satellite cells to provide a source of DNA to support the rapid changes in muscle mass, similar to action of steroid implants. However, the majority of previous work suggests during the three to five weeks of β-adrenergic agonist-stimulated muscle hypertrophy, no change in number of nuclei occurred. A constant DNA amount (nuclei number) coupled with rapid changes in muscle mass and, consequently, protein accumulation results lower DNA concentration of individual muscles in β-adrenergic agonist-fed animals compared to untreated controls. Since DNA accumulation during rapid periods of muscle hypertrophy does not occur due to feeding a β-adrenergic agonist, many researchers have focused on the direct binding of β-adrenergic agonists to their receptors (β-adrenergic receptors), affecting either rate of protein synthesis or protein degradation or both. Skeletal muscle in cattle has been shown to have abundant numbers of β-adrenergic receptors on the cell surface. Previous research has shown that many β-adrenergic agonists are capable of increasing protein synthesis and decreasing protein degradation. The net effect of these changes are dramatic changes in accretion of protein within skeletal muscle tissue. It appears that β-adrenergic agonists cause existing nuclei within the muscle fiber to become much more efficient at increasing muscle protein accumulation without the support of additional DNA from satellite cells. These compounds cause the existing muscle fibers to exhibit muscle hypertrophy very efficiently without the need for additional nuclei. This effect is brought about due to direct binding of β-adrenergic agonists to its receptor on skeletal muscle tissue. Following receptor activation, key pathways regulating protein accretion are regulated resulting in an increased protein accumulation in the muscle fiber.

Conclusions

In closing, growth promotants have been safely used in beef cattle production for over 50 years. Growth-enhancing compounds, including steroidal implants and β-adrenergic agonists, increase production and improve feed efficiency of beef cattle. The changes in performance result in an economic benefit to beef cattle producers and impacts the relative price competitiveness of beef as compared to other dietary protein sources. Long-term use of the growth enhancing technologies has proven that the compounds are a safe, effective way to enhance lean-tissue deposition in cattle. The compounds are rapidly metabolized and excreted from the animal, assuring no risk of potential residues in the edible tissues. The safe use of growth-enhancing compounds benefits the consumer. First, consumers benefit from the reduced production cost associated with the use of this technology in beef production. Second, consumers benefit from the improved lean protein options through beef from cattle reared with growth-enhancing technologies. Combined, growth promoting technologies are an important tool for production of lean, healthy beef in the United States.

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Appendix 1. Chronological sequence of FDA approval of growth promotants used in the U.S. beef cattle industry.

Growth Promotant	Year of FDA Approval
Oral diethylstilbestrol (DES)	1954
DES implant	1957
Estradiol benzoate / progesterone (steers)	1956
Estradiol benzoate / testosterone propionate (heifers)	1958
Oral melengestrol acetate (heifers)	1968
Zeranol (36 mg) implants (cattle)	1969
Oral DES removed from market	1972
DES implants removed from market	1973
Silastic estradiol implant (cattle)	1982
Estradiol benzoate / progesterone (calves)	1984
Trenbolone acetate (TBA) implants (cattle)	1987
Estradiol (17-β) / TBA implants (steers)	1991
Bovine somatotropin (lactating dairy cows)	1993
Estradiol (17-β) / TBA implants (heifers)	1994
Zeranol (72 mg) implants (cattle)	1995
Estradiol (17-β) / TBA implants (stocker cattle)	1996
Ractopamine (cattle)	2003
Zilpaterol (cattle)	2006

Appendix 2. Steroidal implant comparison chart

Implant	Company	Zeranol	Estradiol Benzoate	Estrogenic Effect	Progesterone	Testosterone Propionate	Trenbolone Acetate	Approximate Effective Days	Approved Animals
Ralgro	Merck Animal Health	36 mg		11-13 mg*				70-90	Suckling beef calves, growing beef cattle, feedlot steers and heifers
Compudose	Elanco Animal Health			25.7 mg				175	Steers, feedlot heifers
Encore	Elanco Animal Health			43.9 mg				350	Steers, feedlot heifers
Component E-C	Elanco Animal Health		10 mg	7 mg**	100 mg			Suckling Period	Suckling calves up to 400 lbs, 45 days of age
Component E-S	Elanco Animal Health		20 mg	14 mg**	200 mg			120	Steers >400 lbs
Component E-H	Elanco Animal Health		20 mg	14 mg**		200 mg		120	Heifers >400 lbs
Component T-S	Elanco Animal Health						140 mg	110-130	Feedlot steers
Component T-H	Elanco Animal Health						200 mg	120	Feedlot heifers
Component TE-S	Elanco Animal Health			24 mg			120 gm	110-130	Feedlot steers
Component TE-H	Elanco Animal Health			14 mg			140 mg	120	Feedlot heifers
Component TE-IS	Elanco Animal Health			16 mg			80 mg	110	Feedlot steers
Component TE-IH	Elanco Animal Health			8 mg			80 mg	100-120	Feedlot heifers
Component TE-200	Elanco Animal Health			20 mg			200 mg	110-130	Feedlot steers, heifers
Component TE-G	Elanco Animal Health			8 mg			40 mg	120-140	Stockers, steers, heifers
Synovex C	Pfizer Animal Health		10 mg	7 mg**	100 mg			Suckling Period	Steers, heifers[1]
Synovex S	Pfizer Animal Health		20 mg	14 mg**	200 mg			120	Steers >400 lbs
Synovex H	Pfizer Animal Health		20 mg	14 mg**		200 mg		120	Heifers >400 lbs
Synovex Choice	Pfizer Animal Health		14 mg	10 mg**			100 mg	120	Feedlot steers
Synovex Plus	Pfizer Animal Health		28 mg	20 mg**			200 mg	130	Feedlot steers, heifers
Revalor G	Merck Animal Health			8 mg			40 mg	120-140	Stocker cattle only
Revalor-IH	Merck Animal Health			8 mg			80 mg	100-120	Feedlot heifers only
Revalor-IS	Merck Animal Health			16 mg			80 mg	110	Feedlot steers only
Revalor-H	Merck Animal Health			14 mg			140 mg	120	Feedlot heifers
Revalor-S	Merck Animal Health			24 mg			120 mg	110-130	Feedlot steers
Revalor 200	Merck Animal Health			20 mg			200 mg	110-130	Feedlot steers, heifers
Revalor XS	Merck Animal Health			16 mg (uncoated), 24mg (coated)			80 mg (uncoated) 120 mg (coated)	200-230	Feedlot steers only
Finaplix-H	Merck Animal Health						200 mg	100-110	Feedlot heifers

* Zeranol is about 31% to 36% active estrogen

** Estradiol benzoate contains 72.34% estradiol (E_2)

[1] Synovex C is also recommended for improvement in rate of weight gain in steers weighing greater than 400 pounds and fed in confinement for slaughter when used as part of a re-implant program in which an initial Synovex C implant is followed at approximately 70 days by Synovex S

Appendix 3. Residue levels of steroidal implants in muscle, fat, liver and kidney tissue.

Estrogen Content (pg/g)		Loin Muscle						Perirenal Fat					
Dose	**n**	**E2-17β[d]**	**CV**	**E2-17α[e]**	**CV**	**E1[f]**	**CV**	**E2-17β[d]**	**CV**	**E2-17α[e]**	**CV**	**E1[f]**	**CV**
none	2	3.6	11.8	<3.0	69.5	<1.1	0.0	<4.2	0.0	<4.9	0.0	<3.4	0.0
200 mg testosterone propionate, 20 mg estradiol benzoate[a]	2	2.6	49.0	<1.5	0.0	<1.1	0.0	21.5	23.0	<4.9	0.0	6.9	29.9
600 mg testosterone propionate, 60 mg estradiol benzoate[b]	2	4.3	3.3	<1.5	0.0	<1.1	0.0	66.0	2.1	<4.9	0.0	13.6	46.4
2000 mg testosterone propionate, 200 mg estradiol benzoate[c]	2	18.0	15.7	<1.5	0.0	<1.1	0.0	197.5	9.7	<4.9	0.0	63.0	42.7

Trenbolone Content (pg/g)		Loin Muscle						Perirenal Fat					
Dose	**n**	**TbOH-17β[g]**	**CV**	**TbOH-17α[h]**	**CV**	**TbO[i]**	**CV**	**TbOH-17β[g]**	**CV**	**TbOH-17α[h]**	**CV**	**TbO[i]**	**CV**
none	2	<1.0	0.0	<1.6	0.0	<1.0	0.0	<1.7	0.0	<2.7	0.0	<1.6	0.0
200 mg trenbolone acetate[a]	2	94.0	27.1	<1.6	0.0	<2.4	82.5	278.0	56.0	22.0	38.6	115.0	2.5
600 mg trenbolone acetate[b]	2	158.5	35.2	7.7	42.2	7.8	22.8	822.5	79.2	46.5	71.5	134.0	0.0
2000 mg trenbolone acetate[c]	2	274.5	21.9	18.5	26.8	13.1	42.1	2388.5	6.8	202.5	3.1	961.5	9.8

[a] Manufacturer's recommended dosage
[b] 3 times manufacturer's recommended dosage
[c] 10 times manufacturer's recommended dosage
[d] estradiol-17β
[e] estradiol-17α
[f] estrone
[g] trenbolone-17β
[h] trenbolone-17α
[i] trendione

Appendix 3. Residue levels of steroidal implants in muscle, fat, liver and kidney tissue.

Estrogen Content (pg/g)		Liver						Kidney					
Dose	**n**	**E2-17β[d]**	**CV**	**E2-17α[e]**	**CV**	**E1[f]**	**CV**	**E2-17β[d]**	**CV**	**E2-17α[e]**	**CV**	**E1[f]**	**CV**
none	2	<10.2	25.8	62.0	77.6	<6.7	0.0	<18.1	78.0	52.0	40.8	<7.7	20.2
200 mg testosterone propionate, 20 mg estradiol benzoate[a]	2	30.0	33.0	249.5	57.5	23.0	24.6	72.0	80.5	247.0	11.5	50.1	115.5
600 mg testosterone propionate, 60 mg estradiol benzoate[b]	2	55.5	1.3	466.5	32.3	32.0	8.8	107.0	38.3	417.0	3.1	40.0	14.1
2000 mg testosterone propionate, 200 mg estradiol benzoate[c]	2	143.0	20.8	1568.5	57.5	119.5	58.6	163.5	53.2	1596.5	59.4	157.5	58.8

Trenbolone Content (pg/g)		Liver						Kidney					
Dose	**n**	**TbOH-17β[g]**	**CV**	**TbOH-17α[h]**	**CV**	**TbO[i]**	**CV**	**TbOH-17β[g]**	**CV**	**TbOH-17α[h]**	**CV**	**TbO[i]**	**CV**
none	2	<3.8	0.0	<5.9	0.0	<3.6	0.0	<3.9	0.0	<6.1	0.0	<3.7	0.0
200 mg trenbolone acetate[a]	2	382.0	15.2	4999.5	3.0	19.5	18.1	67.5	38.8	354.5	2.6	<3.7	0.0
600 mg trenbolone acetate[b]	2	649.5	89.2	8868.5	28.4	53.0	80.0	192.0	50.8	336.0	35.8	<10.4	90.9
2000 mg trenbolone acetate[c]	2	1912.0	34.0	30467.0	15.5	136.5	28.5	421.5	21.6	1070.5	44.1	62.5	35.1

[a] Manufacturer's recommended dosage
[b] 3 times manufacturer's recommended dosage
[c] 10 times manufacturer's recommended dosage
[d] estradiol-17β
[e] estradiol-17α
[f] estrone
[g] trenbolone-17β
[h] trenbolone-17α
[i] trendione

Testosterone Content (pg/g)									
Dose	**n**	**Loin Muscle**	**CV**	**Liver**	**CV**	**Kidney**	**CV**	**Perirenal Fat**	**CV**
none	2	9.2	73.8	32.5	6.5	225.0	14.5	<9.8	61.6
200 mg testosterone propionate, 20 mg estradiol benzoate[a]	2	10.5	88.6	24.5	8.7	387.5	35.6	84.5	7.5
600 mg testosterone propionate, 60 mg estradiol benzoate[b]	2	14.5	14.6	24.0	5.9	451.5	25.2	283.0	15.0
2000 mg testosterone propionate, 200 mg estradiol benzoate[c]	2	69.5	66.1	50.5	35.0	548.0	20.4	608.5	4.5

Zeranol Content (pg/g)									
Dose	**n**	**Loin Muscle**	**CV**	**Liver**	**CV**	**Kidney**	**CV**	**Perirenal Fat**	**CV**
none	2	<2.7	0.0	65.0	10.9	<11.0	12.9	<4.7	0.0
36 mg zeranol[a]	2	7.1	17.1	500.0	50.6	237.0	23.9	38.5	20.2
108 mg zeranol[b]	2	24.0	29.5	1432.0	24.3	555.5	16.4	51.0	49.9
360 mg zeranol[c]	2	41.0	55.2	5961.0	51.5	2427.5	32.2	179.0	88.5

[a] Manufacturer's recommended dosage
[b] 3 times manufacturer's recommended dosage
[c] 10 times manufacturer's recommended dosage