

/EFFECTS OF ESTRADIOL-17 $\beta$  IMPLANTS FROM BIRTH TO SLAUGHTER ON  
PERFORMANCE, CARCASS, SENSORY TRAITS AND ENDOCRINE ASPECTS OF  
YOUNG BULLS AND STEERS/  
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by

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This thesis is dedicated to Fred and Dolores Hopkins, the authors parents. Their continuous encouragement, support, caring and love throughout my life has enabled me to attain my goals.

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## CHAPTER 1

### GENERAL INTRODUCTION

Increased concern about fat in the American diet has prompted consumer preference for leaner beef. Consequently, the beef industry has renewed its interest in feeding non-castrated males in an effort to produce meat more efficiently and meet consumer's desire for a leaner product. Field (1971) and Seideman et al. (1982) reviewed research comparing intact males with castrates and concluded that intact males grow more rapidly, utilize feed more efficiently and produce a higher-yielding carcass with less fat and more meat than castrates. However, bull carcasses generally tend to have less marbling, lower quality grades, darker-colored lean and lower tenderness ratings than castrates of the same age or weight (Cross, 1982; Seideman et al., 1982). In addition, production from intact males has encountered strong resistance by the producer and feeder due to management and handling problems (aggressive behavior, equipment destruction, etc.) and by the packer due to price differential (which is attributed to darker, coarser lean and lower quality grades). In order to realize the benefits of using young bulls for meat production, methods to improve behavior, management, packer acceptance, carcass quality and consumer acceptance merit consideration.

The use of exogenous hormone treatments for steers is common in the U.S. Some researchers have thought that exogenous hormone treatments might moderate the negative characteristics associated with feeding young bulls without detrimental effects on growth, feed efficiency or carcass yield (Johnson et al., 1984). Baker and Arthaud (1972) reviewed the use of estrogenic agents in young bulls and were unable to identify any consistent trends for improving the negative attributes of bulls. However, some recent studies using hormone treatment of young bulls indicate



possible advantages in behavior, masculinity, carcass characteristics and meat palatability traits (Gray et al., 1986; Unruh et al., 1986; Greathouse et al., 1983).

## CHAPTER II

### GENERAL REVIEW OF LITERATURE

#### Bulls versus Steers

Live Performance. Prewaning growth and performance differences of bulls versus steers has been less significant than differences during the postweaning growing and finishing phases. Bailey et al. (1966a) reported growth rates up to 7 mo were not significantly different between bulls and steers. Although steers tended to have greater gains from birth to 120 d of age, Brown et al. (1962) found no differences in weaning weights of bulls and steers. In contrast, work done by Marlowe and Gaines (1958), using performance-test records of 2007 creep-fed and 4166 non-creep-fed calves, concluded that bull calves gained 5% faster than steer calves. After adjusting to a 210 d weaning age, non-creep-fed bulls were 7.3 kg heavier than steers.

Many researchers are in general agreement that bulls are superior in performance over steers during the feedlot phase (Champagne, 1969; Hedrick et al., 1969; Warwick et al., 1970; Field, 1971; Arthaud et al., 1977; Brethour, 1982). Seideman et al. (1982) reviewed several studies quantifying differences between bulls and steers in feedlot performance. These studies generally show that bulls gain more rapidly and more efficiently than steer mates and produce leaner carcasses. Field (1971) and Seideman et al. (1982) reported a 17% advantage in feed efficiency (FE) for bulls over steers when fed to the same weight. Arthaud et al. (1977) reported that at all ages ( 12, 15, 18 and 24 mo of age), bulls gained faster, required less feed per unit of gain and produced carcasses with lower fat percentages than steers. Champagne et al. (1969) found bulls to have superior ADG and FE over three

castrate groups (castrated at 2, 7, and 9 mo of age). Bulls gained 24% faster and consumed 22% less feed/unit gain than males castrated at 13 mo of age (Ford and Gregory, 1983). Field (1971) reviewed 15 research papers and reported that ADG and FE averaged 1.16 kg/d and 6.8 kg, respectively, for bulls and .96 kg/d and 7.8 kg, respectively for steers. Bidart et al. (1970) found that bulls consumed 6.0 Mcal of digestible energy per kilogram of edible product compared with 12.8 Mcal for steers. Berg and Butterfield (1968) suggested that a high plane of nutrition more strongly expresses the detrimental effects of castration when considering growth rate and feed efficiency. Performances of bulls, steers and heifers fed high concentrate diets were evaluated in two studies conducted by Hedrick et al. (1969). Bulls had significantly faster gains and heavier slaughter weights than steers and heifers when slaughtered at either constant-weight or time-on-feed endpoints. In addition, bulls were more efficient in feed utilization than steers and heifers at either endpoint.

Crouse et al. (1985) indicated that most of the feed efficiency and much of the carcass leanness advantage of bulls over steers is lost if bulls are fed to attain the same quality grade and (or) composition endpoint as steers. Final weight and carcass composition affect relative performance because steers fatten sooner. Galbraith et al. (1978) suggested that superior growth performance of bulls is due, at least partially, to hormonally mediated differences in their nitrogen metabolism resulting in a greater deposition of lean tissue and thus a more rapid weight gain.

Handling, Slaughter-Dressing. A significant problem in feeding bulls for slaughter is their management before slaughter. Bulls can be raised without fighting if they are never mixed with strange bulls after weaning (Seideman et al., 1982). Oltjen (1982) suggested that bulls be carefully managed to avoid fighting and stress prior to slaughter. Furthermore, keeping familiar bulls together and slaughtering them immediately upon arrival at the slaughter plant may reduce the incidence of dark,

firm and dry (DFD) or "dark-cutting" carcasses. Oltjen (1982) also reported on a Canadian study which indicated a 73% incidence of "dark-cutters" when bulls were mixed compared with 2% of those not mixed. Price and Tennesen (1981) indicated similar findings of 71% DFD when bulls were mixed prior to slaughter. In addition, it is not uncommon to have a 15-20% incidence of DFD carcasses when bulls are held overnight at the slaughter plant prior to slaughter.

Many difficulties are encountered during the slaughter process of bulls. Smith (1982) outlined these problem as follows: a) bullocks are less manageable in holding pens, b) bullocks are more difficult to stun, c) bullock heads often must be manually skinned because hide-pullers will not perform the task, d) bullock hides are very difficult to pull resulting in tearing of the carcass and broken hock tendons (causing the carcass to drop onto the floor), e) bullock bone structure is more dense, causing problems with saws and hock cutters, f) standard rail switches cannot support weights of heavy bullock carcasses, g) bullock hides are wide and thick and cause breakdowns of hide-fleshing equipment, h) bullock hides may need to be passed through the fleshing machine two or three times, i) bullock hides are extra heavy, j) bullock hides may not cure completely in conventional periods of curing-time, and k) bullock hides must be separated from those of other classes and are worth less money. In reviewing several studies, Seideman et al. (1982) concluded that the difficulties of hide removal were considered a serious disadvantage.

Heavy carcasses are an additional problem with bulls. In an attempt to characterize the ideal beef carcass for the packer, Bowling (1982) suggested that in beef carcasses with an adequate degree of fatness, weight is not a problem until it reaches 408.2 kg; with inadequately finished carcasses, problems occur at 385.5 kg. If large-framed cattle are fed as bullocks, it is quite likely that they will not be adequately finished at conventional live or carcass weights (Bowling, 1982). On the other hand, if small-framed cattle are fed as bullocks, they can be fed to

considerably heavier weights than would be normal for them (as steers) without becoming excessively fat.

With boxed beef being the major marketing system for most large packing plants in the U.S.A., it is important to produce a product which will fit fabrication and boxing standards. Price constraints are often placed on carcasses of atypical weights or fatness. This is important as bulls are often too heavy when they reach an acceptable fatness (at least .51 cm) for the boxed beef trade (Gray et al., 1986; Cross and Allen, 1982). Other factors that determine whether or not bullocks are selected for boxed beef fabrication include size of pizzle eye, fullness and thickness of neck, and size of jump muscles (carcass masculinity indicators), round weight and ribeye size (Binger, 1982). In addition, color and texture of lean are considered when determining the use of bullock for boxed beef fabrication (Binger, 1982). Johnson et al. (1984) and Gray et al. (1983) found that carcass masculinity was reduced by using estrogenic or zeranol implants from birth to slaughter.

Carcass Characteristics. Allen (1982) summarized a composite of research trials comparing bulls and steer mates and reported bulls to have heavier carcasses (281.6 kg vs. 267.6 kg) that were trimmer and yielded a higher percentage of salable product (66.9% vs. 61.8%). He also indicated that of the 1.4 yield grade advantage revealed by bulls, approximately, .6 units of this was attributable to trimness with another .6 units attributed to their larger longissimus areas. In addition, Allen (1982) speculated that the remaining difference in yield grade may be the result of bulls having less kidney, heart and pelvic fat. Bailey et al. (1964), Champagne et al (1969) and Jacobs et al. (1977) also reported greater carcass yields and larger rib eyes for bulls. Jacobs et al. (1977) also reported that bull carcasses yielded 5.5% more boxed beef and cutting losses were 17% less than for steers. Ntunde et al. (1977) reported that bulls required longer to feed to .76 cm fat thickness but yielded heavier, leaner

carcasses that contained a greater percentage of trimmed chuck and forequarter cuts with less trimmable fat than steers.

Cross and Allen (1982) summarized several trials and found the average in boneless round, loin, rib and chuck for bulls exceeded that of steers by 2.65% according to the estimating equation of Murphey et al. (1960). However, differences of 4.8 to over 9.0% using actual cutout data were found by Jacobs et al. (1977) and Champagne et al. (1969). Differences in percentage of bone are small, but bull carcasses have much higher muscle to bone ratios than steer carcasses (Berg and Butterfield, 1968).

Field (1971) reviewed 15 research articles and reported similar dressing percentages for bulls and steers (59.7 and 59.6%, respectively) but fat thickness was less (9.3 mm) for bulls than for steers (14.3 mm). Smith et al. (1983) also found young bulls to have less subcutaneous fat (.97 cm for 1,245 bullocks and 1.40 cm) than for 1,044 steers fed for comparable periods.

There is a great deal of data which support the distinct advantage that bulls have over steers in yields of lean meat. An important factor in the strong resistance to bulls from both processors and retailers, resulting in a major price discount, is lower USDA quality grades for bullocks. Binger (1982) concluded that the difficulty of selling young bulls on a live basis is because a relatively low proportion of bull carcasses will grade USDA Choice. Several reports (Stiffler et al., 1983; Brethour, 1982; Cross and Allen, 1982; Smith et al., 1982; Field, 1971) indicate that bullocks deposit less marbling and have lower quality grades than steers when fed for comparable periods on the same diet. Seideman et al. (1982) concluded that carcasses from young bulls generally had lower USDA quality grades, less marbling, darker lean color and coarser-textured lean. Data from 16 research studies reveal that bullocks had a mean marbling score of "slight-typical" and a mean USDA quality grade of average Good while steers had "modest minus" marbling and low Choice grade (Cross

and Allen, 1982). Smith and Merkel (1982) reviewed 21 studies and reported mean marbling scores of "slight-typical" for bullocks and "small plus" for steers. The data accumulated by Cross and Allen (1982), and Smith and Merkel (1982) suggest that bullocks and steers fed for comparable periods on the same diet will differ in marbling by approximately one to one and two-thirds marbling scores and in USDA quality grade by about two-thirds to one full grade.

Maturity is also an important factor in determining quality grade, and researchers have noted differences between bulls and steers. Glimp et al. (1971) found bull carcasses to be physiologically more mature based on bone ossification and lean color than steers of the same chronological age, but by current grade standards none would be discounted on quality grade. Negligible differences in carcass maturity were found between bulls and steers when animals were slaughtered at 12 months of age (Arthaud et al., 1977). However, carcasses from bulls slaughtered at 15, 18 and 24 months of age tended to be physiologically more mature (skeletal and lean maturity) than carcasses from steers at the same chronological age. Field (1971), Price and Tennesen (1981), and Riley et al. (1983) indicated that meat from bulls is darker in color and coarser in texture than meat from steers.

Marbling disadvantages in the form of price discounts due to these factors (quality grade, marbling, lean color and lean-texture) need to be considered in determining the feasibility of feeding young bulls for slaughter.

Palatability and Retail Acceptance. The palatability of bull meat is lower and more variable at any age between 6 and 24 mo than meat from steers of the same age (Cross, 1982). Smith and Merkel (1982) were in agreement and stated that the bulk of scientific data suggest that young bull beef is usually less juicy, less tender and less desirable than beef from steers.

In a study by Arthaud et al. (1977), taste panel tenderness scores showed the

longissimus muscles to be more tender for steers than for bulls. However, the average value for bulls were slightly to moderately tender, which is still acceptable. In addition, Arthaud et al. (1977) found only small differences between bulls and steers in juiciness, flavor and odor evaluations. Breidenstein and Carpenter (1983) considered tenderness to be the most important characteristic related to palatability. Field (1971), Cross (1982) and Seideman et al. (1982) summarized numerous studies and concluded that steers were almost always superior to bulls in tenderness. Reagen et al. (1971) found steaks from bull carcasses were considerably more variable in palatability attributes (especially tenderness) than those of steer carcasses. Cross (1982) reported that consumer tests have generally concurred with results of trained taste-panel tests. In addition, Arthaud et al. (1977) and Jacobs et al. (1977) reported that Warner-Bratzler shear values tended to be higher for bulls than for steers. Although steaks from bulls are generally less tender when compared with steaks from castrate males, tenderness is acceptable in intact males slaughtered at young ages (Jacobs et al., 1977; Gregory et al., 1983). Seideman et al. (1982) concluded that the greater variability in tenderness of bullock beef is a significant problem.

Cross (1982) indicated the difference in tenderness of bull beef may be linked to connective tissue during sexual development or a cold-shortening effect related to the rate of postmortem chill. The decreased subcutaneous fat of young bulls may be allowing cold shortening to occur. Electrical stimulation may reduce the incidence of cold shortening and, in addition may reduce the variability of tenderness in bull beef (Riley et al., 1983). These reductions may be effective in assuring "acceptable" tenderness in marginally tender beef from young bulls.

Jacobs et al. (1977) stated that consumers will ultimately determine the type of beef produced in this country. When comparing bulls and steers of equal fatness, Baron (1979) found in a consumer test that households preferred the appearance, flavor, juiciness, tenderness and overall eating quality of steers compared with bulls.



However, over 85% of the consumers surveyed by Jacobs et al. (1977) indicated that retail cuts from bulls were "as good" or "better" than beef they normally purchased. Over 44% of these consumers felt that "leanness" was most important in visual selection of retail beef when color, leanness and marbling were considered. Interestingly, 47% felt that "marbling" was least important. This is contrary to the emphasis placed on marbling in the marketing and grading system. Smith (1982) stated that although bullock beef was less desirable in lean color and texture, it sold more rapidly because of the superior lean-to-fat ratio. Although consumers can usually detect differences in palatability between bull and steer beef, this does not imply that beef from young bulls is "unacceptable" (Cross, 1982).

Production of beef from young bulls would be very beneficial to the producer because of the superior growth rate, feed efficiency and carcass cutability of bulls over steers. If bulls are properly managed on a high level of nutrition and slaughtered at a young age, they yield an acceptable product. Although steers and heifers have the quality advantages of marbling, finer lean texture and lighter lean color, bulls are leaner. Perhaps production should be aimed towards the increasingly diet-health conscious consumer demanding leaner beef.

#### Hormones Related to Postnatal Growth

Hormone regulation of growth involves multiple hormones acting in concert (Davis et al., 1984). The complex integrated system includes hormones such as growth hormone, somatomedins, insulin, glucocorticoids, gonadal steroid hormones and perhaps others. Although most of these hormones have been identified and some of their interactions defined, specifics of hormonal mechanisms of action are poorly understood at best and difficult to discuss.

Growth Hormone, Somatomedins and Insulin. Growth hormone (GH) is known to be a distinct pituitary hormone while insulin is a pancreatic hormone. Somatomedins

are formed in the liver and are GH dependent. These hormones are responsible for a wide range of metabolic and growth-promoting effects in vivo (Davis et al., 1984 and Guyton, 1981). Metabolism of carbohydrates, lipids and proteins are affected by GH, somatomedins (SM) and insulin. However, no single hormone has control of muscle growth. Rather, several hormones act in concert by regulating protein metabolism of muscle within limits of the animals genetic potential. This review deals primarily with protein metabolism and muscle hypertrophy.

Growth hormone and insulin are both necessary and have a profound effect on muscle hypertrophy (Gray et al., 1986). Insulin has an apparent indirect effect on growth by enhancing SM secretion (Schalch et al., 1979). There appears to be a synergistic effect between GH and insulin, each performing its specific function that is separate from the other. Each hormone promotes cellular uptake of a different selection of amino acids, all of which are required for growth (Guyton, 1981). Furthermore, both hormones are thought to inhibit the catabolism of protein.

Riggs and Walker (1960) found that the administration of GH stimulated amino acid uptake and their incorporation into skeletal muscle proteins (Manchester et al., 1959) of hypophsectomized rats. In addition, Russell (1957) and Davis et al. (1970) reported that GH-treated animals exhibited reduced nitrogen excretion and an overall positive nitrogen balance. A positive relationship between GH and total muscle mass and a negative relationship between GH and fat content was found by Trenkle and Topel (1978) and Wagner and VeenHuizen (1978) in studies done with cattle and sheep. Machlin (1975) reported that GH decreased fat synthesis and increased protein deposition in GH-treated rats. In addition, Mosely et al. (1982) infused steers with exogenous GH and found increased serum concentrations of GH and nitrogen accretion.

Trenkle (1974) indicated that postnatal muscle growth occurs solely through cellular hypertrophy and that the number of nuclei and the number of myofibrils

maintain a constant ratio. Therefore, hypertrophy can be attributed to increased protein accretion occurring either through increased protein synthesis or decreased protein catabolism (Trenkle, 1974).

Thyroid Hormones. Thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) are the two thyroid hormones that play a dual role in stimulating oxidative metabolism and anabolic functions of cells (Mosier, 1981). It seems that thyroid hormones increase the metabolic activities of most tissues in mammals as well as affecting their growth and development (Guyton, 1981). Thyroid hormones regulate tissue oxygen consumption, mineral balance and the synthesis and metabolism of proteins, carbohydrates and lipids (Eberhardt et al., 1980).

Thyroid hormones are essential for normal growth and development. A deficiency of these hormones during the neonatal period results in severe retardation of many organ systems, in the growth and development of body weight and length, and the central nervous system (Eberhardt, 1980 and Florini, 1985). However, too high levels promote protein catabolism.

It is suggested that thyroid hormones play significant roles in several mechanisms involved in growth. Thyroid hormones may exert direct stimulatory effects on growth such as increasing overall hepatic and muscle protein synthesis, or it may have permissive effects via other hormones such as GH (Davis et al., 1984). Chernousek et al. (1982) indicated that growth failure and reduced serum SM concentration associated with hypothyroidism are probably not due to decreased binding of GH to its cellular receptor. Rather, the growth failure may be caused by decreased GH secretion and decreased SM production. Thyroid hormones may play a role in restoring "normal" tissue response to growth promoting agents.

All mechanisms of thyroid hormones are not fully understood, but those presented above seem to be significant.

Glucocorticoids. The protein catabolic effects of glucocorticoids (GLC) in skeletal muscle are well established (Beitz, 1985; and Florini, 1985). The catabolic effect seems to be due to the muscle protein synthesis rate (Rannels et al., 1978) being reduced to less than the degradative rate so that a) a net efflux of amino acids from muscle, b) an increased rate of amino acid catabolism in muscle cells and c) an increased rate of amino acid conversion to glucose in liver occurs (Beitz, 1985). Glucocorticoids have an indirect effect on muscle protein synthesis because they increase hepatic uptake of amino acids and thus reduce the amino acid supply available for muscle (Guyton, 1981 and Beitz, 1985). Furthermore, GLC (cortisol) diverts the action of GH away from muscle to promote protein synthesis and, thus lipolysis in adipose tissue (Beitz, 1985). Additionally, Beitz (1985) theorized that, because of opposing actions and interactions with GH, the balance of insulin and GLC seems highly important to the growth-promoting effects of GH in muscle.

Androgens. Testosterone (T), the most abundant and most potent male steroid, produced primarily in the testes (also in adrenal cortex), is responsible for development of secondary sex characteristics, maintenance of secondary sexual organs, stimulation of spermatogenesis (through control of LH), and aggressive behavior (Frandsen, 1974; Amann and Schanbacher, 1983).

Androgens, especially T, are associated with increased musculature as well as skeletal growth in both male and female animals. Androgens have definite protein anabolic activity (Trenkle and Marple, 1983). Florini (1985) summarized a wide range of studies on effects of the administration of T and its derivatives on castrated animals and various preparations from muscle. Administration of T and its derivatives increased ribosome activity and RNA polymerase, elevated ribosome content and activated several specific enzymes involved in protein synthesis.

Mayer and Rosen (1977) suggested that the anabolic actions of T might result from its displacement of cortisol from the GLC receptor. This would indicate an anticatabolic effect rather than a direct anabolic effect for T in muscle. However, Florini (1985) reviewed several studies and concluded from a preponderance of evidence that T acts as an anabolic hormone through a direct interaction with cytoplasmic receptors, and this leads to the typical migration of the hormone-receptor complex to the nucleus, activation of RNA synthesis and accumulation of muscle protein.

Davis et al. (1984) cited several studies which indicated plasma GH concentrations are higher in intact than castrate males. Conversely, treatment of castrate males with androgens increased plasma GH concentrations (Davis, et al. 1984). This finding suggests that GH may be playing a significant role in the androgen mechanism. The effects of T and other androgens have been investigated to a large extent in animal production. Gortsema et al. (1974) and Galbraith et al. (1978) reported that castrates have lower rates of gain compared with intact bulls, as a result of lower concentrations of endogenous steroid hormones. Galbraith et al. (1978) suggested that the hormone differences between castrates and bulls affect the nitrogen metabolism of bulls resulting in a greater deposition of lean tissue. Schanbacher et al. (1980) found growth rate, feed efficiency, and carcass yields of rams and T-implanted castrates were superior to non-implanted castrates. They also indicated decreased fat associated with T and concluded T to be the primary testicular hormone responsible for superior performance and preferred carcass traits of young market rams.

Manipulation of serum LH can effect T but will not be discussed. Although the mechanisms of action are not totally understood, it is evident that androgens play a significant role in growth and development.

Estrogens. The widespread use of estrogens and estrogen-like compounds to stimulate growth in meat animals has received much research attention. However, there has been surprisingly little study of endogenous effects of estrogens on muscle growth and development (Florini, 1985).

Powers and Florini (1975) experimented with cultured muscle cells and found that estrogens were inactive in stimulating protein synthesis under conditions in which a small, but significant, effect of T could be demonstrated. Knudsen and Max (1980) also reported no increase in protein content of the levator ani muscle after injection of estradiol. However, they found estrogen receptors to be present in muscle suggesting that the action may be direct. In contrast, Trenkle (1975) investigated the role of exogenous estrogens and stated that their effects are caused by action on other hormones such as GH. Florini (1985) indicated with such little evidence available on the direct effects of endogenous estrogens, more research is necessary to establish the hypothesis.

#### Anabolic Growth Promotants

Efficient lean beef production is essential for the cattle producer to financially survive in the United States. Effective use of growth promoting implants are one of the most profitable management tools available to the beef cattle industry (Corah, 1984). Implants contain anabolic growth stimulants and are usually placed under the skin on the back of the ear for slow absorption into the blood.

Implants currently used in the U.S. include: 1) Ralgro®, active ingredient 36 mg zeranol, for all classes of growing and finishing cattle (except breeding replacements) - Ralgro® is also approved for use in lambs with a 12 mg zeranol dose; 2) Compudose®, active ingredient estradiol-17 $\beta$  (natural), for growing and finishing steers; 3) Synovex-S®; and 4) Steeroid®, both contain 20 mg estradiol benzoate and

200 mg progesterone, for use in growing and finishing steers; 5) Synovex-H®; 6) Heiferoid®, both contain 20 mg estradiol benzoate and 200 mg testosterone propionate for use in growing and finishing heifers; and 7) Synovex-C®, a half dose of Synovex-S® for use in suckling calves (Corah, 1984; Gray et al., 1986; Muir, 1985).

Anabolic growth promotants or agents are commonly divided into two classes; estrogenic or androgenic based on their overall effects on metabolism (Buttery et al., 1978). The implants mentioned above all contain estrogen in some form as the active ingredient with the exception of Ralgro® (zeranol). Zeranol is a resorcylic-acid lactone and is not classed as an estrogen. However, it has a structure and configuration similar to some synthetic estrogens (i.e. stilbene, figure 1) and is known to have estrogenic properties (Beverly, 1984). Estrogens are the major class of anabolic growth promotants used for ruminants (Muir, 1985) and have been shown to increase ADG and FE from 10 to 20% in growing and finishing animals (Preston, 1975; Heitzam, 1980; Muir, 1985). However, estrogens are not anabolic for swine and cause increased fattening in poultry (Trenkle, 1969).

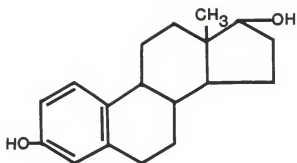
Diethylstilbestrol (DES), a synthetic estrogen, was the first estrogen shown to increase gain of implanted heifers (Dinussou et al., 1948). In 1954 DES was found to be active in cattle and sheep (Burrough et al., 1954). Although DES was removed from the market on November 1, 1979 in accordance with the Delaney Amendment of the U.S. Constitution, it has provided a better understanding of the use and actions of estrogenic compounds.

Modes of Action of Estrogenic Agents. Anabolic agents have consistently been shown to enhance ADG and FE in ruminants. However, studies on their modes of action have been far less numerous and consistent and highly speculative at best. Trenkle and Burroughs (1978) proposed four possible avenues by which growth may be enhanced with the use of estrogenic anabolic agents: 1) increased production of androgens from

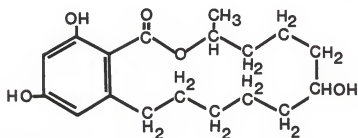
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Figure 1. Structural characteristics of estradiol-17 $\beta$ , zeranol, diethylstilbestrol and estradiol benzoate.

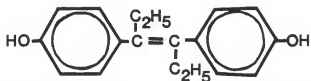




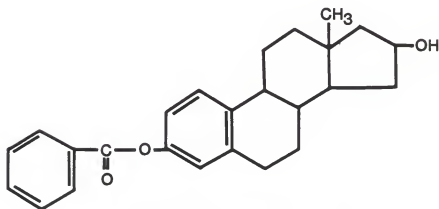
Estradiol 17 $\beta$  (the major estrogen)



6-(6,10-dihydroxyundecyl)- $\beta$ -resorcylic acid- $\mu$ -lactone  
(Zeranol)



$\alpha,\alpha'$ -diethyl-4-4' dihydroxystilbene  
(Diethylstilbestrol)



Estradiol Benzoate

the adrenal cortex, 2) increased thyroid activity, 3) increased GH secretion and 4) a direct effect at the tissue level.

Zeranol and various estrogens have increased both adrenal weight and adrenocorticotrophic hormone (ACTH) secretory cell number (Wiggins et al., 1976). Hutcheson and Preston (1971) reported increased growth of androgen-sensitive secondary-sex glands of castrate males, which provides evidence of adrenal androgen secretion. Wiggins et al. (1976) proposed that stimulation of adrenal androgen production may be directly responsible for the increased seminal vesicle weights observed in castrates treated with DES. It is not certain whether the adrenal cortico steroid production is increased directly by the estrogenic compound or is a result of increased ACTH production (Beverly, 1984). These proposed modes of action involving increased androgen production appear to be worthy of further study.

Zeranol and other estrogenic compounds have increased pituitary and thyroid weights and secretory activity in lambs and steers (Borger et al., 1973a; Wiggins et al., 1976). However, Rothenbacker et al. (1975) and Wiggins et al. (1979) reported depressed thyroid secretory activity and decreased  $T_4$  concentrations in lambs. There is an optimal level of thyroid secretion for growth, and metabolism which lies within a rather narrow range (Beverley, 1984). With such a critical balance necessary for anabolic effect, he also suggests that stimulation of thyroid activity might exceed anabolic secretory limits and give a decrease in growth. Further evidence of increased  $T_4$  concentrations (outside narrow range) being associated with decreased weight gains (growth) or weight loss has been reported by Falconer and Draper (1968) and Muir and Wien (1983). In addition, these elevated levels may be protein catabolic (Goldberg et al., 1980).

It has long been recognized that GH is a critical and important factor in normal growth. Growth hormone is known to increase amino acid uptake by muscle, increase protein synthesis and nitrogen retention, in addition to increasing bone growth and

body weight gain (Beverly, 1984). Therefore, it is not surprising that the most widely accepted theory for exogenous estrogenic compound activity is through increased GH secretion. Beverly (1984) proposed three possible modes by which zeranol enhances GH levels and thus growth rate. These modes include: 1) directly stimulating release of GH from the pituitary, 2) stimulating hypothalamic release of GH releasing factors or inhibiting somatostatin, thus allowing secretion of GH and insulin, and 3) stimulating GH release and thus enhancing somatomedin status in the body.

Improvement in nitrogen balance has generally been attributed to increased plasma GH concentrations or pituitary weights of estrogen or zeranol-treated ruminants (Wiggins et al., 1976; Olsen et al., 1977; Trenkle and Burroughs, 1978; Beverly, 1984). However, SM release is thought to mediate GH action as indicated in an earlier section. Wangsness et al. (1981) found no change in serum SM concentrations in lambs treated with zeranol, suggesting that increased SM concentrations may not be involved in the growth response of zeranol. Most experimental evidence regarding an increased growth response from zeranol treatment is attributed to the increased levels of plasma GH (Beverly, 1984). However, Buttery et al. (1978) suggested that the increase in growth is achieved by a different mechanism than that of GH. Their work indicated that, while both GH and zeranol increased protein accretion, zeranol actually decreased the rate of protein synthesis and degradation. Since protein degradation is decreased to a greater extent than synthesis, the net effect of zeranol is increased protein accretion. However, the majority of earlier evidence suggests that increased growth in zeranol-treated animals is a response to elevated GH levels. This must be interpreted with some caution as Trenkle and Burroughs (1978) suggested that it might also indicate a decreased metabolic clearance rather than an increase secretory rate of GH.

Increased plasma insulin concentrations have been reported in conjunction with increased GH levels as a response to estrogens (Preston, 1975; Wiggins et al., 1976)

and zeranol treatment (Olsen et al., 1977; Wangsness et al., 1981). However, Borger et al. (1973a) reported unchanged levels of insulin in steers in response to estrogen treatment. Considering the interrelationships among GH, insulin, SM, and growth, Preston (1975) suggested that increased insulin might be responsible for the anabolic actions by stimulating protein synthesis. However, this hypothesis needs further investigation.

Since estrogen receptors have been found in rat skeletal muscle (Knudsen and Max, 1980), the possibility of estrogens eliciting their actions directly at the tissue level cannot be ruled out. Buttery et al. (1978) suggested that the direct tissue action may be the result of estrogens competing with GLC receptors, thereby blocking the GLC protein-catabolic activity.

The endocrine system is very complex and integrated and not fully understood. The picture becomes even more complicated when the endocrine system is manipulated with hormone implants, feed additives, etc. Researchers are increasing their understanding about the endocrine system; however, more research is required to understand the modes of action of anabolic agents.

Effects of Estrogenic Anabolic Agents on Female and Castrate Male Performance, Carcass and Palatability Traits. Anabolic agents have been widely accepted and used in cattle to increase ADG and FE in lambs (Jackson et al., 1974; Wiggins et al., 1979), heifers (Sharp and Dyer, 1971; Preston, 1975; Price and Makarechian, 1982), steer calves (Ward et al., 1978; Donovan et al., 1983; Simms et al., 1983; Simms, 1984) and growing and finishing steers (Klosterman et al., 1955; Laflamme and Burgess, 1973; Casey et al., 1974; Fink et al., 1979; Lomas, 1982; Laudert and Davis, 1983; Cain et al., 1984; Preston, 1984). Most of this work has used either DES or zeranol as the anabolic agent. Conversely, Lamm et al. (1980) reported no effect on ADG pre-weaning for heifers and steers implanted with 12, 24 or 36 mg of zeranol at birth and subsequent reimplanting at 100-d intervals.

Marchello et al. (1970) reported no differences in cutability between carcasses from steers implanted with DES and nonimplanted steers. However, Hedrick et al. (1969) indicated that steers implanted with 24 mg of DES tended to have a higher yield of retail cuts than nonimplanted steers. Klosterman et al. (1955) and Cahill et al. (1956) also found an increase in the amount of edible meat in steers which had been implanted with 84 mg DES.

Sharp and Dyer (1971) found that steers implanted with zeranol had increased percentages of carcass protein and moisture but reduced percentages of fat. They also reported increased nitrogen retention in lambs. Borger et al. (1973a) were in agreement and reported more carcass moisture, less carcass fat and similar amounts of protein on a fat and moisture-free basis when comparing zeranol-implanted steers with nonimplanted steers.

Skeletal maturity, measured by the degree of ossification in the cartilagenous tip of the spinous process of the first thoracic vertebra, may be influenced by growth promotants. Ralston et al. (1975) conducted one trial in which implanting yearling steers with 36 mg of zeranol and 15 mg of DES, at 330 kg initial weight, had no effect on percentage of cartilage ash. However, in a second trial implanting steer calves at a lighter weight (192 kg initial weight) and reimplanting resulted in a reduced amount of cartilage ash compared with nonimplanted steers. These data are supported by Sharp and Dyer's (1971) study which indicated zeranol delayed physiological maturity of the growing ruminant.

Longissimus roasts from steers implanted with 24 mg of DES tended to have lower taste-panel flavor and tenderness ratings than nonimplanted steers (Bailey et al., 1966b). Cahill et al. (1956) reported that longissimus steaks from steers implanted with 84 mg of DES had lower taste-panel tenderness ratings than nonimplanted steers.

Effects of Estrogenic Anabolic Agents on Bulls. The influence of estrogenic anabolic agents on young bulls intended for slaughter remains unresolved because much of the research is inconsistent. In a review of 38 studies using estrogenic implants (DES, Synovex®, or zeranol), Baker and Arthaud (1972) reported no consistent effect on performance of feedlot bulls. Gray et al. (1983) suggested the growth response of bulls to estrogen or zeranol implants appears to be dependent on age at initial implantation.

Ralston (1978) found gains from birth to weaning to be similar between implanted (at birth) and nonimplanted bull calves. However, in another study he indicated that bull calves implanted with 36 mg of zeranol at birth and again at 90 d of age were slightly heavier at weaning than nonimplanted bulls. Corah et al. (1979), Lamm et al. (1980) and Simms (1984) found that implanting early in life (near birth) and continuing through slaughter produced similar gains when compared with controls. Conversely, Cooper and Kirk (1982) and Greathouse et al. (1983) reported an increase in ADG and(or) FE compared with nonimplanted controls. Implanting later in life (postweaning or later) has also produced conflicting results. Implanting postweaning has resulted in unchanged (Martin et al., 1979b; Price et al., 1983; Ford and Gregory, 1983; Patterson et al., 1983; Gray et al., 1983; Johnson et al., 1984) or increased performance (Brethour, 1983; Brethour and Schanbacher, 1983; Gregory and Ford, 1983; Vanderwert et al., 1984).

Implanting young bulls from near birth to slaughter resulted in decreased live (Unruh et al., 1983; 1986) and carcass masculinity scores (Ralston, 1978; Unruh et al., 1983; 1986). However, when implanting was delayed until postweaning, masculinity was not affected (Ford and Gregory, 1983; Price et al., 1983; Gray et al., 1983). Scrotal circumference and testicular weights were reduced in bulls implanted prepubertally with an estrogenic compound (Ralston, 1978; Corah et al., 1979; Deschamps et al., 1982; Greathouse et al., 1983; Unruh et al., 1983; Juniewicz et al.,

1985; Unruh et al., 1986). In addition, prepubertal estrogen treatment reduced serum testosterone concentrations, spermatozoa number and seminiferous tubular diameter (Juniewicz et al., 1985). However, implantation later in life had minimal effects upon scrotal circumference, testicle weight (Ford and Gregory, 1983; Gregory and Ford, 1983; Unruh et al., 1983) or testicular function (Juniewicz et al., 1985).

Similar dressing percentages have been reported for bulls implanted with either 36 mg or 72 mg of DES (Garrigus et al., 1969), which is in agreement with results of Laflamme and Burgess (1973).

Several researchers (Williams et al., 1975; Martin et al., 1979b; Calkins and Clanton, 1984) have found that implantation of prepubertal bull calves with exogenous estrogen treatments resulted in increased kidney, heart and pelvic and(or) subcutaneous carcass fat. However, others have indicated that implanting with zeranol beginning later in life has had little effect on carcass composition (Ford and Gregory, 1983; Gregory and Ford, 1983). These findings are in agreement with the earlier conclusion of Baker and Arthaud (1972).

Hedrick et al. (1969) and Hunsley et al. (1967) found that DES implanted bulls tended to have a lower yield of retail cuts when compared with nonimplanted bulls. This may have been due to increased levels of fat in the DES implanted bulls. However, Koger et al. (1960) reported no significant differences in yield of trimmed lean cuts for implanted and nonimplanted bulls.

Steen et al. (1978) and Johnson et al. (1984) reported that bulls implanted with estrogenic compounds had higher yield grades than nonimplanted bulls. However, Corah et al. (1979) found no effect of zeranol treatment on yield grade. In addition, Steen et al. (1978) indicated no effect on loin eye area or quality grades. Greathouse et al. (1983) and Johnson et al. (1984) were in agreement, indicating that implantation had no effect on marbling scores or quality grades. Conversely, Hunsley et al. (1967), Corah et al. (1979) and Calkins and Clanton (1984) reported

improvement in carcass quality grade due to implant treatment. While Schanbacher and Prior (1982) reported reduced quality grade and longissimus areas.

Implanting from birth to slaughter resulted in similar trained taste-panel evaluations for connective tissue amount and myofibrillar and overall tenderness in implanted bulls and steers (Gray et al., 1984). In addition, implanted bulls tended to have lower shear values than nonimplanted bulls. Greathouse et al. (1983) found that bulls implanted from birth to slaughter had increased taste-panel flavor intensity and decreased taste-panel detectable connective tissue amount than nonimplanted bulls. However, Johnson et al. (1984) and Gregory et al. (1983) reported no effect on palatability or shear force values of bulls implanted during the feeding phase only. Pelton (1984) conducted a take-home consumer taste-panel study and found that bulls and steers implanted from birth to slaughter were more tender than either bulls implanted from weaning to slaughter or nonimplanted bulls.

The use of estrogenic anabolic agents in bulls has not been investigated to the extent that it has in heifers and especially steers. More research is needed in the area of exogenous effects on carcass and palatability traits because current research results are inconsistent.



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## CHAPTER III

EFFECTS OF ESTRADIOL-17 $\beta$  IMPLANTS FROM BIRTH TO SLAUGHTER ON PERFORMANCE, CARCASS, SENSORY TRAITS AND ENDOCRINE ASPECTS OF BULLS AND STEERS

## Summary

Twenty-eight Simmental bull calves were randomly allotted to one of three treatments at birth. Ten calves remained as intact bulls and were implanted (IB) with estradiol-17 $\beta$  (E<sub>2</sub>)(Compudose®); nine calves were castrated within 3 d of birth and implanted (IS) with Compudose®; and the remaining calves were intact, non-implanted control bulls (CB). Implanted calves were implanted within 3 d of birth and at 200 d intervals until slaughter. Live weights were monitored at 28 d intervals from 8.5 to 14.8 mo while masculinity scores (MS), scrotal circumferences (SC) and hip heights were measured at 8.5 and 14.8 mo. Blood samples were taken every 14 d from 9.8 through 14.8 mo and serum was analyzed for estradiol-17 $\beta$  (E<sub>2</sub>) and testosterone (T) levels. Testicle weights (TW) were obtained after slaughter, while carcass quality, yield traits and MS were obtained at 24 h postmortem. Modified oven-broiled longissimus (LD) steaks were evaluated by a trained sensory panel and a Warner-Bratzler shear (WBS) device. Implanted steers were less (P<.05) masculine than IB, and IB were less (P<.05) masculine than CB at both 9.1 and 14.8 mo. Further, SC were smaller (P<.05) for IB than for CB at both 8.5 and 14.8 mo. Control bulls had heavier weaning weights (P<.05) than IB and higher (P<.05) feedlot average daily gains (ADG) than IS; whereas IB were intermediate in ADG. Implanted steers required more (P<.05) feed/unit of gain than CB and IB. Implanting young bulls with E<sub>2</sub> increased (P<.05) serum E<sub>2</sub> levels and tended (P<.10) to reduce T levels compared with CB. Levels of serum E<sub>2</sub> in IS and IB were drastically increased (P<.05) for 4 to 6 wk, following re-implantation during the feedlot phase then declined sharply. Serum T levels were reduced (P<.05) in IB from 9.8 through 12.1 mo of age and tended

( $P < .10$ ) to be lower than CB through 14.8 mo. Hot carcass weights and dressing percentages were not different ( $P < .05$ ) among treatments. Implanted steer carcasses were more youthful than CB carcasses, and IB tended ( $P = .07$ ) to be more youthful than CB. The incidence of heat ring (dark, coarse band formation) was higher ( $P < .05$ ) for IB than for IS, likely because fat thickness was less ( $P < .05$ ) for IB than for IS. Ribeye areas were smaller ( $P < .05$ ) and yield grades were higher for IS than for CB. Testicle weights were not significantly different between IB and CB, although variability was much greater for IB. Spermatozoa concentrations were lower ( $P < .05$ ) for IB than for CB. Carcass masculinity was lowest ( $P < .05$ ) for IS, and IB were less ( $P < .05$ ) masculine than CB. The only differences in longissimus sensory traits was that IB steaks were juicier than CB steaks. However, WBS values were lower ( $P < .05$ ) for IS than for CB. These findings indicate that implanting young bulls with  $E_2$  from birth to slaughter decreases masculinity development, which makes them more acceptable to meat packers.

(Key Words: Bulls, Steers, Implant, Estradiol-17 $\beta$ , Performance, Carcass)

## Introduction

The use of young bulls for meat production is a consideration for some producers because bulls grow more rapidly, utilize feed more efficiently and produce a higher-yielding carcass with less fat and more meat than castrates (Field, 1971; Seidman et al., 1982). However, bull carcasses generally have less marbling, lower quality grades, darker-colored lean and lower tenderness ratings than castrates of the same age or weight (Cross, 1982; Seidman et al., 1982), although other researchers have found meat from bulls and steers to be comparable in palatability (Field et al., 1966; Hedrick et al., 1969). In addition to lower quality grades, bull carcasses are usually discounted by the packer due to coarser textured lean, excessive fullness and thickness of neck and larger sizes of pizzle eye, jump muscle, round and ribeye, thus making the carcass less desirable for boxed beef fabrication.

Some researchers have indicated that exogenous hormone treatment might moderate the negative characteristics associated with feeding young bulls without detrimental effects on growth, feed efficiency or carcass yield (Greathouse et al., 1983; Johnson et al., 1984; Gray et al., 1986; Unruh et al., 1986). Baker and Arthaud (1972) reviewed several experiments on the use of estrogen or estrogen-like exogenous anabolic agents in bulls and reported varied responses. On the other hand, the use of estrogen-like anabolic agents have consistently increased growth rate, improved feed efficiency and increased carcass leanness in steers. The inconsistent results between bulls and steers may be due to different modes of action in the intact male versus the castrate.

Recent concern about the use of exogenous substances which are not naturally occurring has prompted an increased interest in the use of a natural anabolic agent such as estradiol-17 $\beta$ . The purpose of our study was to determine the effects of estradiol-17 $\beta$  (Compudose®) implantation of bulls and steers beginning near birth and

reimplanting at 200-d intervals until slaughter on their growth performance, masculine development, carcass and meat sensory traits, and serum concentrations of estradiol-17 $\beta$  and testosterone.

## Materials and Methods

Management and Live Animal Measurements. Twenty-eight Simmental fall-born (September 18 to December 7, 1983) male calves were randomly assigned at birth to one of three treatments. Ten calves remained as bulls and were implanted (IB) near birth with 24 mg estradiol-17 $\beta$  (Compudose®). Nine calves were castrated and implanted (IS) within 3 d with Compudose®. The remaining nine calves were nonimplanted control bulls (CB). The implants were administered within 3 d after birth and at approximately 200 d intervals thereafter until slaughter. Implants were inserted between the skin and cartilage in the middle one third of the ear as recommended. Calves were maintained with their dams on native bluestem pasture (IFN 2-00-892), supplemented with prairie hay (IFN-1-03-191) and range cubes to meet NRC (1976) requirements. Calves were weaned in mid May at an average age of 7.5 mo, then pre-conditioned for 2 wk prior to shipment to the Kansas State University Beef Cattle Research Unit.

Calves were received on June 4, 1984 and allowed one week for acclimation. Feed and water were withheld for 16 h prior to obtaining initial feedlot weights. At that time hip height and scrotal circumference measurements were obtained. Masculinity was scored 2 wk after the onset of the feedlot phase and again just prior to slaughter by a four member panel. The scoring scale ranged from 1 to 5 with 1=steer and 5=very masculine. Scoring was based on physical appearance of the head, neck, forearm, jump muscle and overall proportion (not to include scrotal development) relative to these characteristics of average young bulls at that age (Appendix 1).

Calves were started on a 53% concentrate diet which was adjusted up for the next 4 wk until a final ad libitum full-feed diet consisted of 85% concentrate (Table

TABLE 1. DIET COMPOSITION<sup>a</sup> OF CONTROL BULLS AND ESTRADIOL 17- $\beta$  IMPLANTED BULLS AND STEERS

Ingredient	Starting %	Finishing %
	%	
Grain sorghum (IFN 4-20-893)	46.22	78.22
Corn silage (IFN 3-28-250)	47.00	15.00
Soybean meal (IFN 5-20-637)	4.15	4.15
Urea (IFN 5-05-070)	.58	.58
Ground limestone (IFN 6-02-632)	.45	.45
Sodium chloride (IFN 6-04-152)	.40	.40
Potassium chloride (IFN 6-03-755)	.20	.20
Dehydrated molasses (IFN 4-04-695)	.15	.15
Fat (IFN 4-00-376)	.08	.08
Rumensin 60	.02	.02
Trace minerals	.02	.02
Vitamin A (30,000 IU/gm)	.01	.01

<sup>a</sup>dry matter basis.



1). All animals remained on this diet until slaughter.

Cattle within a treatment were assigned to one of three pens so average pen weights at the start of the feeding period were approximately equal. Each treatment had three replicates (pens) with either three or four head/pen.

Individual weights were obtained at 28 d intervals with calves held off feed and water for 16 h before weighing. Feed consumption for each pen for the 28 d periods were also obtained for feed efficiency (FE) calculations and Average daily gains were (ADG) calculated over the feedlot phase.

Blood Collection and Analyses. Blood samples were obtained every 2 wk, beginning 6 wk after calves were started on feed. Collection started approximately 1 hr after dawn to avoid any possible photoperiod effects. Blood was collected via jugular-venipuncture immediately after the animal was restrained. Blood was allowed to coagulate at 5C for 23 to 26 h prior to centrifugation and sera harvest. Sera samples were stored at -20C for subsequent analysis for concentrations of testosterone (T) and estradiol-17 $\beta$  (E<sub>2</sub>) by radioimmunoassay techniques. Testosterone concentrations were determined in duplicate 200  $\mu$ l aliquots of serum by the procedure (Appendix II) previously described by Pruitt (1983) using Niswender rabbit anti-testosterone sera #250 (Gay and Kerlan, 1978). Estradiol-17 $\beta$  concentrations were determined in 5 ml aliquots of sera by the procedure (Appendix III) previously described by Skaggs et al. (1986).

Slaughter and Carcass Traits. Animals were withheld from feed and water 16 h prior to final weighing, then returned to normal feeding regimen for 72 h. The heavy half of each treatment was removed from feed and water for 12 h prior to slaughter at the Kansas State University Meats Laboratory. Animals were slaughtered upon arrival at the laboratory to minimize preslaughter stress. The second half of the animals

were fed for 2 additional weeks and treated the same as the first group prior to slaughter. This was to allow time for product to be processed in the small slaughter facility.

Paired testicle weights were obtained during the slaughter process. At 24 h postmortem, carcass masculinity and USDA (1976) quality and yield grade data were obtained. Carcass masculinity was evaluated based on pizzle eye size and jump muscle (gluteus) and crest development (Appendix IV) with an overall score determined by giving equal weight to all factors.

A section of the longissimus muscle at the 11th-12th rib region was removed at 48 h postmortem, then vacuum packaged and aged at 2-4C for 10 d. A 2.54 cm thick steak was removed from each longissimus muscle for sensory panel evaluation and an adjacent 2.54 cm thick steak was removed for Warner-Bratzler shear (WBS) force determination. Steaks were double wrapped in freezer paper, frozen and stored at -20C until evaluation.

Steaks used for either sensory-panel evaluation or WBS force were thawed overnight at 5C before cooking. Two steaks representing each treatment were evaluated at each sensory session. The steaks were modified oven-broiled at 166C in a rotary oven to an internal temperature of 70C (monitored with thermocouples) for both sensory and WBS evaluation. A mechanical coring device was used to remove 1.27 cm diameter cores which were served warm to a six member trained sensory panel (AMSA, 1978). The panel evaluated each steak for flavor intensity, juiciness, myofibrillar tenderness, overall tenderness and connective tissue amount (Appendix V). Steaks used for WBS force were allowed to cool to room temperature for 2 h prior to shear force determination.

Determination of Spermatozoa Concentration. Content of spermatozoa in testes and epididymides was determined in homogenates of these tissues as previously described

by Amann and Lambiase (1969). Briefly, 10g of testicular peranchyma and the entire epididymis were homogenized, separately for 2 min in blenders containing 200 ml of saline-Triton-Thimerosol solution (.85% saline to which had been added 1mg/liter Triton X-100 and 1 mg/liter Thimersol (Sigma Chemical Co., St. Louis, Mo.)). Homogenates were filtered through cheese cloth, diluted and then sperm cells enumerated in hemocytometer counting chambers. Spermatozoa concentrations were expressed on a total testicle basis.

Statistical Analysis Data were analyzed by analysis of variance and means were separated using multiple t-tests calculated by the General Linear Models (GLM) Procedure of the Statistical Analysis System (SAS, 1982). Live animal measures and carcass data were analyzed by one-way analysis of variance with implant being the main effect. Age was tested as a covariate, but had no effect on any of the traits so it was not used.

Serum hormone concentrations were analyzed by a split-plot, repeated measures analysis. Implant treatment was treated as the whole plot factor while time was the subplot factor. In addition, hormone concentrations lacked homogeneity so logarithmic transformations were used to stabilize variance for statistical analysis.

## Results and Discussion

Animal Performance. Performance data are presented in Table 2. Adjusted 205-d weaning weights were greater ( $P < .05$ ) for CB than for IB. However, average daily gains during the feedlot phase were greater ( $P < .05$ ) for CB than for IS; whereas ADG for IB were intermediate to CB and IS. Feed per kilogram of gain was similar for CB and IB, but IS were less ( $P < .05$ ) efficient than either CB or IB. This is attributed to the steers depositing fat earlier and having lower ADG than their bull counterparts. Although slaughter and carcass weights were not significantly different, CB tended ( $P < .10$ ) to be heavier than IS, and IB were intermediate in weight.

Ralston (1978) and Corah et al. (1979) reported that bulls implanted with zeranol starting early in life had similar ADG to CB. In addition, implanting during the postpubertal feeding period resulted in similar ADG as CB (Baker and Arthaud, 1972; Ford and Gregory, 1983; Johnson et al., 1984). Ford and Gregory (1983) also reported no effect of implantation on FE of IB compared to CB. In addition, Corah et al. (1979) reported similar slaughter weights between CB and IB (birth to slaughter). However, Greathouse et al. (1983) reported that zeranol implantation from birth through slaughter of small-framed bulls increased both pre- and postweaning gains over CB. Further, Fabry et al. (1983) reported a positive gain response from zeranol implantation of double muscled Belgian White-Blue bulls.

Rate of gain during the feedlot phase was relatively constant for all treatment groups except that CB tended ( $P < .10$ ) to gain faster from 12.1 to 13.9 mo (figure 2). Gray et al. (1986) found similar feedlot gains between bulls implanted at birth and CB, but noted the patterns of those gains appeared to be altered. Non-implanted bulls increased in weight faster from 7.8 through 9.5 mo and from 11.3 to 12.2 mo of age; whereas implanted bulls increased in weight faster from 12.2 through 14.1 mo of age. This was in contrast to Martin et al. (1979) who reported faster ADG during the

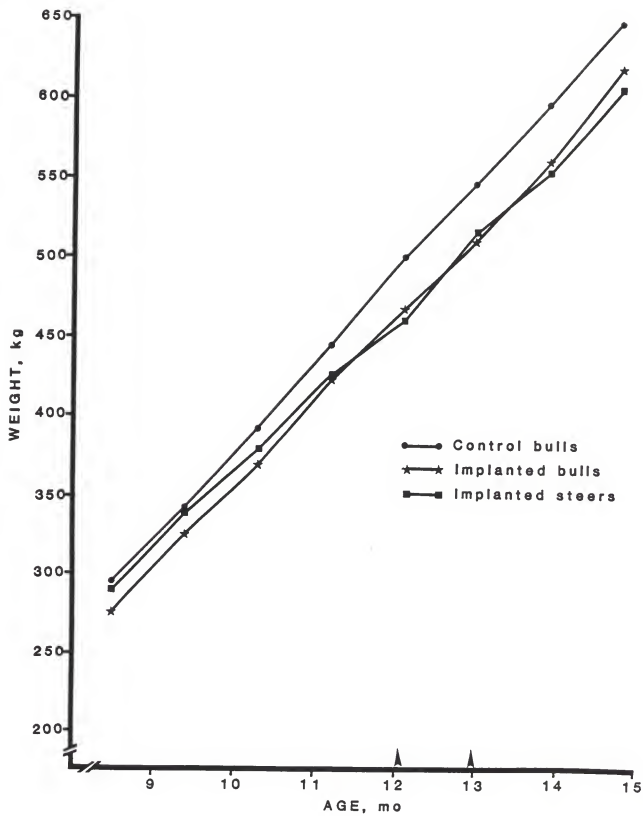
TABLE 2. WEIGHTS AND PERFORMANCE CHARACTERISTICS OF CONTROL BULLS AND ESTRADIOL-17 $\beta$ -IMPLANTED BULLS AND STEERS

Trait	Treatments			
	Implanted		Control Bulls	SE <sup>a</sup>
	Steers	Bulls		
No. of animals	9	10	9	
Weaning wt., kg	284 <sup>bc</sup>	270 <sup>b</sup>	292 <sup>c</sup>	8.02
Slaughter wt., kg	606	617	649	18.94
Hot-carcass wt., kg	380	388	414	12.58
Average daily gain, kg	1.54 <sup>b</sup>	1.65 <sup>bc</sup>	1.72 <sup>c</sup>	.05
Feed/gain (DM basis)	6.80 <sup>b</sup>	5.96 <sup>c</sup>	6.12 <sup>c</sup>	.06

<sup>a</sup>Standard error of the mean.

<sup>bc</sup>Means in the same row with different superscript letters differ ( $P < .05$ ).

Figure 2. Live weights of control bulls and estradiol-17 $\beta$  implanted bulls and steers. Arrows indicate time of reimplantation with half of the IS and IB being implanted at each time.



first 56 d on feed for bulls fed diethylstilbestrol (DES). These contrasting results of estrogenic anabolic agents on the performance of bulls may be associated with breed, age at initial implantation, dosage level, re-implantation schedule or time of slaughter as related to altered growth of implanted bulls.

Animal Measurements. Hip heights and masculinity evaluations are presented in Table 3. Hip heights were similar ( $P>.10$ ) for all treatments at 8.5 and 14.8 months of age. Live masculinity scores were lowest ( $P<.05$ ) for IS, and IB were less ( $P<.05$ ) masculine than CB. Unruh et al. (1983, 1986) reported similar results for these traits for bulls implanted from near birth until slaughter compared to CB. However, bulls implanted with zeranol postweaning were not different in masculinity than control bulls (Ford and Gregory, 1983; Price et al., 1983; Unruh et al., 1983). Therefore, time of implantation appears to be important in affecting masculinity.

Scrotal circumferences were smaller ( $P<.05$ ) for IB at 8.5 mo and just prior to slaughter (14.8 mo) than for CB. However, testicle weights were not different ( $P>.10$ ) between IB and CB. There tended to be more variation in testicle weights of IB than for CB. Estrogenic treatment decreased scrotal circumference and testicle size in rams (Wiggins et al., 1976; Riesen et al., 1977) and prepubertally treated bulls (Corah et al., 1979; Deschamps et al., 1982; Greathouse et al., 1983; Unruh et al., 1983, 1986; Juniewicz et al., 1985). In contrast, no affect was observed on scrotal circumference of bulls implanted postweaning (Price et al., 1983; Unruh et al., 1983).

Epididymal weights were similar ( $P>.10$ ) for IB and CB. However,  $E_2$  treatment decreased ( $P<.05$ ) spermatozoa concentration in IB. Juniewicz et al. (1985) reported lower testicle and epididymal weights in bulls implanted prepubertally with zeranol. They also found that spermatozoa were absent in testicular homogenates from IB. In addition, seminiferous tubular diameter was smaller and only spermatogonia and sertoli cells were present for zeranol-implanted bulls; whereas control bulls had all



TABLE 3. HIP HEIGHTS AND MASCULINITY CHARACTERISTICS OF CONTROL BULLS AND ESTRADIOL-17 $\beta$ -IMPLANTED BULLS AND STEERS

Trait	Treatments			
	Implanted		Control Bulls	SE <sup>b</sup>
	Steers	Bulls		
Hip height at 8.5 mo, cm	116.2	114.5	116	1.62
Hip height at slaughter, cm	136.5	134.2	134.8	1.51
Masculinity score at 9.1 mo <sup>a</sup>	1.2 <sup>c</sup>	2.2 <sup>d</sup>	3.9 <sup>e</sup>	.24
Masculinity score at slaughter <sup>a</sup>	1.4 <sup>c</sup>	3.6 <sup>d</sup>	4.6 <sup>e</sup>	.17
Scrotal circumference at 8.5 mo., cm		19.5 <sup>c</sup>	27.0 <sup>d</sup>	1.17
Scrotal circumference at slaughter, cm		38.7 <sup>c</sup>	42.4 <sup>d</sup>	1.11
Paired testicle wt., gm		623	693	56.00
Spermatozoa concentration, total testis basis		21 x 10 <sup>6c</sup>	75 x 10 <sup>6d</sup>	4.9 x 10 <sup>6</sup>
Spermatozoa concentration, total epididymis basis		4 x 10 <sup>7c</sup>	41 x 10 <sup>7d</sup>	4.5 x 10 <sup>7</sup>
Epididymal wt., gm		23.9	28.1	.87

<sup>a</sup>Scores of 1 to 5: 2=slightly masculine, 3=moderately masculine, 4=masculine.

<sup>b</sup>Standard error of the mean.

<sup>c,d,e</sup>Means in the same row with different superscript letters differ (P<.05).

stages of the spermatogenic cycle present. Corah et al. (1979) reported that bulls implanted at 28 d of age through slaughter were classified as not being fertile based on semen evaluation. However, Juniewicz et al. (1985) reported that postpubertal implantation had an insignificant effect on testicular function.

Hormonal Concentrations. Serum  $E_2$  levels are illustrated in figure 3. Implanted bulls had higher ( $P<.05$ )  $E_2$  levels than CB from 12.5 through 13.9 mo. The IB tended ( $P>.10$ ) to have higher  $E_2$  levels than either IS or CB at all sampling times, except at 10.3 or 14.8 mo. This overall trend was likely due to the additive effect of the implant and endogenous  $E_2$  production. Estradiol levels for IB increased ( $P<.05$ ) sharply at 12.5 mo while  $E_2$  levels for IS increased ( $P<.05$ ) at 12.5 and 13.5 mo. We attribute these increases to re-implantation at 12.1 and 13.0 mo (the older one half of the animals in each treatment were re-implanted at 12.1 mo; the younger one half at 13 mo). The  $E_2$  levels remained higher ( $P<.05$ ) for 6 wk in IB and for 4 wk in IS, then decreased ( $P<.10$ ) rapidly for both treatments at 14.4 mo. Control bulls tended to have a gradual increase in  $E_2$  from 9.8 mo to slaughter. Fabry et al. (1983) and Schanbacher (1984) reported that estrogen-implanted bulls had a suppressed release of pulsatile LH. Although,  $E_2$  was not quantified in either study, the suppression of LH would suggest reduced testicular  $E_2$  secretion.

Serum T levels are illustrated in figure 4. Because steers had very low levels of T their values are not included in figure 4. Implantation of bulls with  $E_2$  reduced ( $P<.05$ ) serum T levels from 9.8 through 12.1 mo of age, and tended ( $P>.10$ ) to reduce T secretion through 14.8 mo. The more variable differences seen in T levels from 12.5 through 14.8 mo may be due to re-implantation confounded with pubertal development. Testosterone levels increased ( $P<.05$ ) rapidly from 9.8 mo to a peak at 10.8 mo in CB. However, T levels for IB increased ( $P<.05$ ) from 9.8 to 10.3 mo, then remained similar ( $P>.10$ ) until slaughter. Fabry et al. (1983) also reported decreased T levels in zeranol-treated, double-muscled bulls. In addition, Gray et al. (1986)

Figure 3. Serum estradiol-17 $\beta$  concentrations of control bulls and estradiol-17 $\beta$  implanted bulls and steers. Values within the graph represent standard error of the mean for each point. Arrows indicate time of reimplantation, with half of the IS and IB being implanted at each time.

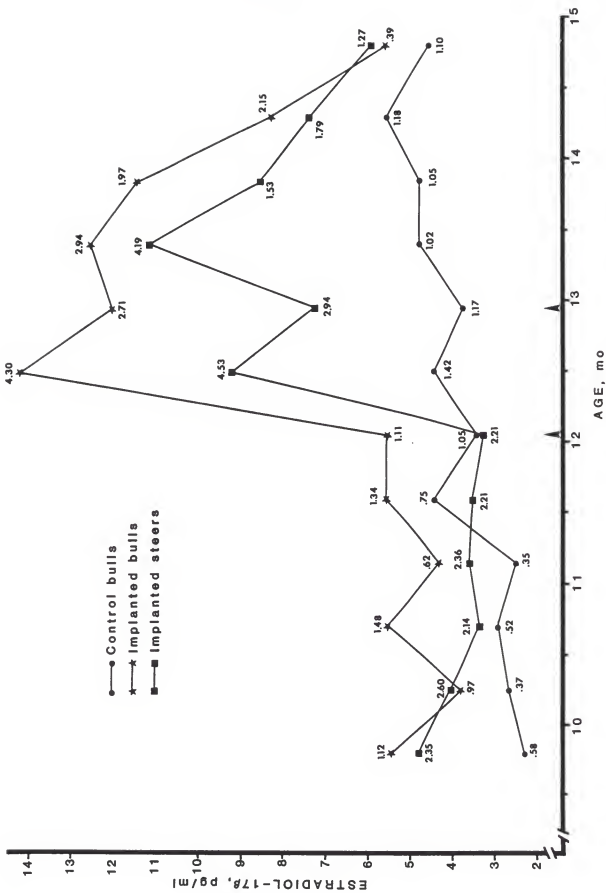
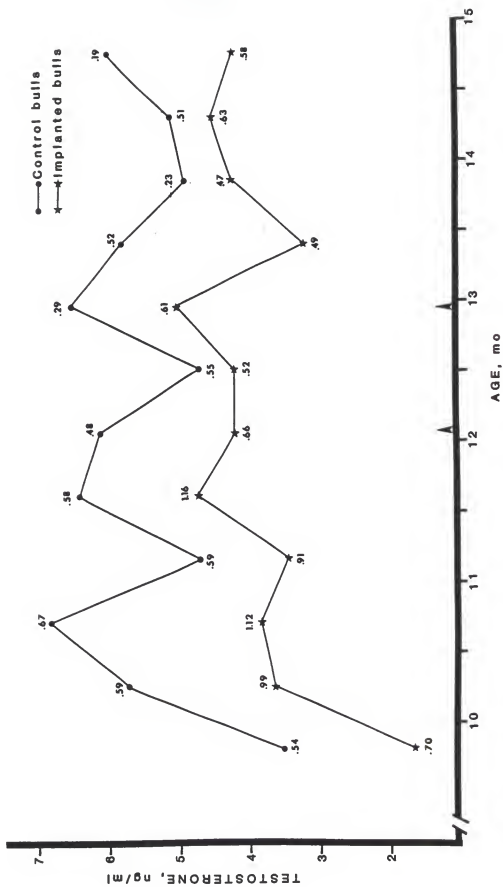


Figure 4. Serum testosterone concentrations of control bulls and estradiol-17 $\beta$  implanted bulls. Values within the graph represent standards errors of the mean for each point. Arrows indicate time of reimplantation, with half of the IS and IB being implanted each time.



observed that serum T levels were reduced for 8.3 through 13.0 mo of age for bulls implanted with zeranol from birth. However, they reported no difference from 13.9 to 14.8 mo and also noted that CB had peak T levels around 11 mo of age.

Carcass Characteristics. Dressing percentages were not different among the three treatment groups (Table 4). This is not surprising considering the similarity in slaughter and carcass weights. Ford and Gregory (1983) Greathouse et al. (1983) and Gray et al. (1986) reported similar results with zeranol implanted and CB.

Implanted-steer carcasses were more ( $P<.05$ ) youthful than CB carcasses, and IB carcasses tended ( $P=.07$ ) to be more youthful than CB carcasses. Greathouse et al. (1983) and Gray et al. (1986) reported older skeletal maturity scores in zeranol implanted bulls from birth to slaughter. However, Gray et al. (1983) observed no differences in skeletal or overall maturity scores in zeranol-IB either pre- or postweaning. Johnson et al. (1984) implanted bulls at approximately 1 y of age and reported similar skeletal and overall maturity scores to CB.

Marbling scores were not different among treatments, although CB tended to be the lowest. Similar marbling scores for IS and IB were unexpected because IB had significantly less ( $P<.05$ ) 12th rib adjusted fat thickness than IS (.50 cm and .80 cm, respectively). Greathouse et al. (1983) and Johnson et al. (1984) were in agreement, indicating that implantation had no effect on marbling scores or quality grades. Conversely, Hunsley et al. (1967), Corah et al. (1979) and Calkins and Clanton (1984) reported improvement in carcass quality grade due to implant treatment. Implanting bulls with zeranol from early in life until slaughter increased carcass fatness in several studies (Greathouse et al., 1983; McKenzie, 1983; Calkins and Clanton, 1984; Gray et al., 1986). However, implanting beginning later in life had minimal effects on fat thicknesses of young bulls (Gray et al., 1983; Gregory and Ford, 1983; Johnson et al., 1984).

Kidney, pelvic and heart (KPH) fat percentages were similar among treatment

TABLE 4. CARCASS CHARACTERISTICS OF CONTROL BULLS AND ESTRADIOL-17 $\beta$ -IMPLANTED BULLS AND STEERS

Trait	Treatments			SE <sup>C</sup>
	Implanted		Control Bulls	
	Steers	Bulls		
Dressing percentage	62.6	62.9	63.8	.53
Carcass maturity	A <sup>62</sup> d	A <sup>66</sup> de	A <sup>76</sup> e	3.80
Marbling score	Slight <sup>90</sup>	Slight <sup>94</sup>	Slight <sup>62</sup>	15.20
Adjusted 12th rib fat thickness, cm	.80 <sup>d</sup>	.50 <sup>e</sup>	.70 <sup>de</sup>	.06
Kidney, pelvic and heart fat, %	2.00	1.75	1.94	.15
Longissimus area, cm <sup>2</sup>	88.1 <sup>d</sup>	93.1 <sup>d</sup>	100.6 <sup>e</sup>	1.12
Yield grade	2.4 <sup>d</sup>	1.9 <sup>e</sup>	1.9 <sup>e</sup>	.14
Jump muscle and crest <sup>a</sup>	1.4 <sup>d</sup>	3.4 <sup>e</sup>	5.0 <sup>f</sup>	.28
Pizzle eye size <sup>b</sup>	1.8 <sup>d</sup>	3.0 <sup>e</sup>	4.2 <sup>f</sup>	.32

<sup>a</sup>Scores of 1 to 6: 2=barely evident, 3=slightly prominent and 4=moderately prominent.

<sup>b</sup>Scores of 1 to 7: 2=moderately small, 3=slightly small and 4=slightly large.

<sup>c</sup>Standard error of the mean.

<sup>d,e,f</sup>Means in the same row with different superscript letters differ ( $P < .05$ ).



groups. However, longissimus areas (REA) were smaller and yield grades were higher ( $P < .05$ ) for IS than for CB. Yield grades (YG) for IB and CB were equal, although CB had larger ( $P < .05$ ) REA. Preston (1975) indicated that although estrogen treatment usually leads to decreased fat deposition in steers, bull carcasses generally exhibited increased fat deposition. Greathouse et al (1983) reported that implanted bulls tended to have increased KPH fat over CB. However, Gray et al. (1986) reported no effect on KPH fat of bulls implanted from birth to slaughter. Our results contrast those of Greathouse et al. (1983) who reported larger REA and higher YG in bulls implanted with zeranol from birth to slaughter. However, others have reported no difference in REA of bulls implanted pre- or postweaning with estrogenic compounds (Steen et al., 1978; Gray et al., 1983; Gregory and Ford, 1983; Johnson et al., 1984).

Carcass masculinity (as measured by jump muscle, crest and pizzle eye development) was lowest ( $P < .05$ ) for IS, and IB were less ( $P < .05$ ) masculine than CB. Consequently, implanting young bulls with  $E_2$  effectively retarded masculinity and made bull carcasses more acceptable. These results agree with those of Unruh et al. (1986) who used zeranol implants.

Lean color and firmness were not different among treatments, but CB tended ( $P < .10$ ) to be darker than IS and IB (Table 5). Implanted steers had finer ( $P < .05$ ) textured RE than IB and CB. This agrees with the conclusion of Field (1971) that meat from bulls is darker in color and coarser in texture than meat from steers. Greathouse et al. (1983) found that bulls implanted from near birth to slaughter had a finer lean texture. Unruh et al. (1986) found similar lean color, texture and firmness scores for CB and IB. The incidence of heat ring (dark coarse band) was higher ( $P < .05$ ) for IB than IS, whereas CB were intermediate. The higher incidence of heat ring in IB was likely due to less insulation (less fat cover) over the RE muscle during chilling. However, Unruh et al. (1986) reported less heat ring in zeranol IB than CB.

TABLE 5. LONGISSIMUS QUALITY CHARACTERISTICS OF CONTROL BULLS AND ESTRADIOL-17 $\beta$ -IMPLANTED BULLS AND STEERS

Trait	Treatments			SE <sup>e</sup>
	Implanted		Control Bulls	
	Steers	Bulls		
Lean firmness <sup>a</sup>	6.2	6.3	6.4	.46
Lean texture <sup>b</sup>	5.4 <sup>f</sup>	4.1 <sup>g</sup>	4.0 <sup>g</sup>	.30
Lean color <sup>c</sup>	3.7	3.7	4.5	.33
Heat ring (Dark coarse band) <sup>d</sup>	1.0 <sup>f</sup>	1.8 <sup>g</sup>	1.3 <sup>fg</sup>	.19

<sup>a</sup>Scores of 1 to 8: 5=slightly firm, 6=moderately firm, 7=firm.

<sup>b</sup>Scores of 1 to 8: 4=slightly coarse, 5=slightly fine, 6=moderately fine.

<sup>c</sup>Scores of 1 to 9: 3=light cherry red, 4=cherry red, 5=slightly dark red.

<sup>d</sup>Scores of 1 to 5: 1=none, 2=slight.

<sup>e</sup>Standard error of the mean.

<sup>f,g</sup>Means in the same row with different superscript letters differ ( $P < .05$ ).

Longissimus Sensory Traits. Implanting young bulls and steers from birth to slaughter with  $E_2$  had no significant effect on sensory-panel flavor intensity, connective tissue amount, myofibrillar tenderness or overall tenderness evaluations of longissimus steaks (Table 6). Although not significant, IB tended to be more tender than CB; whereas IS were intermediate in tenderness. In addition, longissimus steaks from IB were more ( $P<.05$ ) juicy than CB. Greathouse et al. (1983) reported that longissimus steaks from implanted bulls had greater flavor intensity, but similar juiciness scores to steaks from CB. Unruh et al. (1986) found similar flavor intensity and juiciness scores for IB and CB. Gray et al. (1983) found that bulls implanted from birth to slaughter were similar to IS for sensory-panel traits of flavor intensity, connective tissue amount, myofibrillar and overall tenderness. Warner Bratzler Shear force values were lowest ( $P<.05$ ) for IS and tended ( $P=.07$ ) to be lower for IB than for CB. This is in agreement with Gray et al. (1983) who reported lower WBS values for bulls implanted from birth to slaughter compared to CB. From our findings and those of other researchers, life-long implanting of bulls with  $E_2$  zeranol has no negative effects on meat sensory traits and tends to improve some sensory traits.

Conclusions. The results of our study indicate that implanting young bulls from birth to slaughter with  $E_2$  improved some of the negative traits associated with the use of bulls for meat production. Implantation from birth to slaughter decreased weaning weight, but did not affect feedlot ADG. Implanting from birth to slaughter decreased masculinity development and scrotal circumference. In addition,  $E_2$  implantation of young bulls tended to increase marbling, decrease carcass maturity and resulted in a lighter-colored lean than CB.

Control bulls had heavier weaning weights, higher ADG and required less feed/unit gain than IS. However, IS were less masculine, had more youthful carcasses, had finer lean texture and lower WBS values than CB.

TABLE 6. TASTE PANEL EVALUATION AND WARNER-BRATZLER SHEAR VALUES OF THE LONGISSIMUS MUSCLE OF CONTROL BULLS AND ESTRADIOL-17 $\beta$ -IMPLANTED BULLS AND STEERS

Trait	Treatments			SE <sup>c</sup>
	Implanted		Control Bulls	
	Steers	Bulls		
Flavor intensity <sup>a</sup>	5.8	5.8	5.4	.15
Juiciness <sup>a</sup>	5.4 <sup>de</sup>	5.8 <sup>d</sup>	5.2 <sup>e</sup>	.21
Connective tissue amount <sup>a</sup>	6.5	6.4	6.0	.18
Myofibrillar tenderness <sup>b</sup>	5.4	5.8	5.2	.25
Overall tenderness <sup>b</sup>	5.6	5.8	5.4	.24
Warner-Bratzler shear, Kg.	4.0 <sup>d</sup>	4.4 <sup>de</sup>	5.2 <sup>e</sup>	.64

<sup>a</sup>Scores of 1 to 8: 6=slightly intense, slightly juicy or slight amount, 7=very intense, very juicy or practically none.

<sup>b</sup>Scores of 1 to 8: 5=slightly tender, 6=moderately tender and 7=very tender.

<sup>c</sup>Standard error of the mean.

<sup>d,e</sup>Means in the same row with different superscript letters differ ( $P < .05$ ).

Implantation of young bulls with  $E_2$  from birth to slaughter increased serum  $E_2$  levels and decreased serum T levels compared to CB. Re-implantation during the feedlot phase drastically increased  $E_2$  levels for 4 and 6 wk, then levels declined sharply in IS and IB, respectively. Further,  $E_2$  implantation decreased testicular function.

For large-framed cattle such as Simmentals, castration and implantation with  $E_2$  results in the most desirable combination of performance, carcass and meat quality traits. Even though IB had less fat cover and lower yield grade numbers than IS, carcasses from IS were very desirable in composition.

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## Appendix I

Live Masculinity Score Descriptions<sup>a</sup>

1. Steer: no prominent facial features over the eye brow and jaw; narrow and long head in relation to body size; refined head crest and neck; smooth, little muscular development of crest, shoulder, rib and hindquarter; and fine hair coat texture, especially over the head.
2. Slightly masculine: slight prominence of eye brow and facial features; somewhat narrow and long head in relation to body size; somewhat refined head, crest, and neck; somewhat smooth and moderate muscular development; and moderate fine hair coat.
3. Moderately masculine: somewhat prominent brow and facial features; moderate relationship of length and width of head to body size; slight fullness of head, crest and neck; moderate muscular development of crest, shoulder, rib and hindquarter; and slightly coarse hair coat.
4. Masculine: prominent brow and facial features; moderately wide head in relation to length of head; full head, crest and neck; muscular through crest, shoulder, rib and hindquarter; and coarse hair texture.
5. Very masculine: very prominent eye brow and facial features; relatively wide head in relation to length; very full head, crest and neck; high degree of muscular development through the crest, shoulder, rib and hindquarter; and very coarse hair texture.

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<sup>a</sup> Scores made relative to the normal development of young bulls at the observed age.

## Appendix II

## TESTOSTERONE RADIOIMMUNOASSAY

1. Thaw standards, serum samples, antiserum, and standard serum overnight in refrigerator (5°C).
2. To estimate recovery of testosterone during solvent extraction of serum, add 10  $\mu\text{l}$   $^3\text{H}$ -1, 2, 6, 7-testosterone ( $^3\text{H}$ -T) in 95 % EtOH (~2,500 cpm/10 $\mu\text{l}$ ) to six 16 x 100 mm disposable culture tubes (Tracer tubes). Add 10  $\mu\text{l}$   $^3\text{H}$ -T to four scintillation vials (7 ml minivials) and set aside to air dry (total count tubes).
3. Add appropriate dilution (50, 100, or 200  $\mu\text{l}$ ) of serum in duplicate to 16 x 100 mm tubes and aliquot random serum dilutions to Tracer tubes (T).
4. Extract serum with minimum of 10 volumes (10x serum dilution) ethyl acetate. Vortex for 5 min.
5. Freeze extracts (~30 min) and decant solvent phase into 12 x 75 mm disposable culture tubes. Dry down extract under air in 40°C water bath. Decant tracer tubes directly into scintillation vials and set aside to air dry.
6. Prepare standard curves of testosterone<sup>a</sup> by pipetting 0.1 ml of each standard concentration (1 ml total/tube) in duplicate 12 x 75 mm tubes which corresponds to 2000, 1500, 1000, 750, 500, 250, 100, 50, 25 and 10 pg/tube. NOTE: BE CERTAIN that standards in EtOH are at room temperature before pipeting volumes! Pipets are calibrated for accuracy when solutions are at room temperature (i.e., 22-26°C).
7. Include Hot-Buffer tubes (HBT, 0.2 ml assay tracer only), nonspecific binding tubes (NSB; 0.2 ml phosphate-buffer saline-gelatin (PBSG) in place of antiserum and 0.2 ml assay tracer), and zero tubes ( $\emptyset$ ; 0 standard-0.2 ml assay tracer and 0.2 ml antiserum)
8. Add 0.2 ml testosterone antiserum<sup>b</sup>  
     0.2 ml  $^3\text{H}$ -T<sup>c</sup>  
     0.1 ml PBSG<sup>d</sup>  
     to all samples  $\emptyset$  tubes, and standards.
9. Add 0.2 ml  $^3\text{H}$ -T and 0.8 ml PBSG to HBT tubes.  
     Add 0.2 ml  $^3\text{H}$ -T and 0.2 ml PBSG to NSB tubes.
10. Vortex all tubes for 2-3 sec, incubate overnight (14 to 18 h) at 5°C covered with foil or plastic wrap.

11. Pipet 0.5 ml ice-cold dextran-coated charcoal (DCC) solution <sup>e</sup> into all tubes except HBT tubes. Add DCC to approximately 144 tubes at a time, vortex (2-3 sec) in backwards order (i.e., first tubes to receive DCC are the last ones vortexed), and load into centrifuge to begin centrifugation within 10 min after beginning addition of DCC.
12. Centrifuge at 5°C for 10 min at 1,500 x g (2,400 rpm).
13. Pipet 0.5 ml supernatant into scintillation vials. Do not contaminate supernatant by pipetting some of the DCC pellet. Add 5 ml scintillation fluid<sup>f</sup> to each vial. Cap, label, and vortex for 1 min on shaker.
14. Count for 4 min in liquid scintillation (B)-counter.
15. Add scintillation fluid (5 ml) to tracer and total count tubes and handle as in 14.

<sup>a</sup>Testosterone (Sigma, T-1500) standards are made from a stock solution of 100 ng/ml 95% EtOH, dried down, and reconstituted in PBSG. They are stored frozen in 1 ml aliquots in concentrations of 10, 25, 50, 100, 250, 500, 750, 1,000, 1,500, and 2,000 pg/0.1 ml.

<sup>b</sup>Stock solution of testosterone antiserum is diluted to give 45% (40-50%) binding in B<sub>0</sub> or  $\phi$  tubes. Approximate dilution is 1:20,000.

<sup>c</sup>Stock solution of assay tracer is made by diluting <sup>3</sup>H-T in PBSG to achieve 16,000 cpm/0.2 ml assay buffer (PBSG).

<sup>d</sup>PBS is made by combining the following phosphate buffers to achieve pH 7.4.

- (1) 0.01 M phosphate-buffered saline, monobasic "A"

To a 1000 ml volumetric flask, add:

1.38 g Na<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O

8.7 g NaCl

0.1 g Thimerosal (antibacterial compound)

Add double distilled deionized water to 1000 ml mark, invert to mix or add stirring bar. Store indefinitely at 5°C.

- (2) 0.01 M phosphate-buffered saline, dibasic "B"

To a 1000 ml volumetric flask, add:

1.42 g Na<sub>2</sub>HPO<sub>4</sub>

8.7 g NaCl  
0.1 g Thimerosal

Add double distilled deionized water to 1000 ml mark, invert to mix or add stirring bar. Store indefinitely at 5°C.

PBSG is made by combining "A" and "B" buffers to pH 7.4 (approx. 6B: 1A, v/v). Add 0.1% gelatin (Sigma, G-2500), heat and stir to dissolve. Do not heat over 30°C. Store at 5°C.

<sup>e</sup>Dextran-coated charcoal (DCC). Add 1.0% dextran T-70 (Pharmacia) to double-distilled deionized water and stir until dissolved. Add 0.5% charcoal (Norit A, Fisher Scientific). Make-up fresh suspension for each assay. Store at 5°C until needed. When adding to assay tubes, stir continuously.

<sup>f</sup>Complete counting cocktail 3a70B (Research Products International Corp.)

## Appendix III

## ESTRADIOL RADIOIMMUNOASSAY

1. Remove antiserum, serum samples, and standard serum from freezer and thaw overnight in refrigerator at 5°C. Standards can be removed a few hours before needed.
2. Add 10 µl  $^3\text{H}$ -2, 4, 6, 7 estradiol (~ 2,500 cpm/10 µl) to six 20 x 150 mm tubes (random tracers) and to four scintillation vials to estimate recovery of estradiol from solvent extraction.
3. Add serum (5 ml) to 20 x 150 mm tubes in duplicate and to random tracer tubes.
4. Extract serum with 2 volumes ethyl acetate (2x serum volume). Vortex for 5 min on shaker.
5. Freeze extraction tubes to separate solvent from aqueous phases (~ 60 min).
6. Decant solvent into 16 x 100 mm tubes. BE CAREFUL to not allow the aqueous pellet to thaw (i.e., remove only a dozen or so tubes from the freezer at one time). If thawing occurs, simply refreeze.
7. Dry down solvent in 40°C water under air. (This will require approx 60 to 90 min depending on the volume). Thaw pellet. Re-extract with 2x vol. of solvent. Vortex 1 min. Freeze and decant into same 16 x 100 mm tube as in 6.
8. When dry, rinse down inside of tubes with an additional 1 ml ethyl acetate. Vortex 10 sec and dry down again.
9. Rinse down inside tubes again with 1 ml ethyl acetate and then carefully decant into 12 x 75 mm disposable culture tubes and air-dry in water bath.
10. Decant tracer tubes directly into scintillation vials and set aside to air dry.
11. Remove standard stock estradiol<sup>a</sup> (1 ng/ml) from freezer or if already done so, be certain it is room temperature (22-26°C) before pipetting dilution from standard concentrations. Pipet in duplicate the following volumes for standards: 5, 10, 15, 30, 60, 120, 250, and 500 µl which corresponds to 5, 10, 15, 30, 60, 120, 250, and 500 pg/tube. Dry down in water bath under air as described above.
12. Include Hot Buffer tubes (HBT; 0.2 ml assay tracer), nonspecific binding tubes (NSB; 0.2 ml PBSG<sup>c</sup> in place of antiserum, and 0.2 ml assay tracer and zero tubes (0; 0 standards, 0.2 ml antiserum, and 0.2 ml assay tracer).
13. Make-up estradiol antiserum<sup>b</sup> in PBSG.
14. Add 0.2 ml antiserum to all sample tubes, 0 tubes, and to all standard tubes. Add equivalent amount of buffer to NSB tubes. Vortex and then incubate for 30 min at room temperature.

15. Dilute  $^3\text{H}$ -estradiol<sup>d</sup> in PBSG and add 0.2 ml to all samples, 0, and standard tubes. Vortex and incubate overnight (14 to 18 h) at 5°C covered with aluminum foil or plastic wrap. (Add 0.2 ml  $^3\text{H}$ -E<sub>2</sub> and 0.8 ml PBSG to HBT tubes and 0.2 ml  $^3\text{H}$ -E<sub>2</sub> and 0.3 ml PBSG to NSB tubes).
16. Pipet 0.5 ml ice-cold dextran-coated charcoal<sup>e</sup> (DCC) solution into all tubes except HBT tubes. Add to tubes in one direction and vortex (2-3 sec) in backwards order. Within 10 min of adding DCC to tubes, centrifugation step should begin.
17. Centrifuge at 1,500 x g (2,400 rpm) at 52C for 10 min.
18. Pipet 0.5 ml supernatant fluid into 7-ml scintillation vials add 5 ml counting cocktail<sup>f</sup>, cap, label, shake for 1 min.
19. Add scintillation fluid<sup>f</sup> (5 ml) to tracer and total count tubes after they have dried down.
20. Count all vials for 4 min in liquid scintillation (B) counter.

<sup>a</sup> Estradiol-17 $\beta$  (Sigma, E-8875) was prepared by serially diluting 10 mg to a stock 1 ng/ml solution in 95% EtOH.

<sup>b</sup> Stock solution of estradiol antiserum (A gift from Norman R. Mason, Eli Lilly and Company, Indianapolis, IN) is diluted to give 50% (45-55%) binding in B<sub>0</sub> or 0 tubes. Approx. dilution 1:35,000.

<sup>c</sup> PBS is made by combining the following phosphate buffers to achieve pH 7.4.

1. 0.01 M phosphate-buffered saline, monobasic 'A'  
To a 1000 ml volumetric flask, add:  
1.38 g Na<sub>2</sub>PO<sub>4</sub>-H<sub>2</sub>O  
8.7 g NaCl  
0.1 g Thimerosal (antibacterial compound)

Add double-distilled deionized water to 1000 ml mark, invert to mix or add stirring bar. Store indefinitely at 5°C.

2. 0.01 M phosphate-buffered saline, dibasic 'B'  
To a 1000 ml volumetric flask, add:  
1.42 g Na<sub>2</sub>HPO<sub>4</sub>  
8.7 g NaCl  
0.1 g Thimerosal

Add double-distilled water to 1000 ml mark, invert or stir with stirring bar, and store indefinitely at 5°C.

PBSG is made by combining 'A' and 'B' buffers to pH 7.4 (approx 6B:1A, v/v). Add 0.1% gelatin (Sigma, G-2500), heat and stir to dissolve (do not heat above 30°C). Store at 5°C for no more than 2 weeks.

<sup>d</sup> Stock solution of assay tracer is made by diluting  $^3\text{H}$ -2, 4, 6, 7-estradiol (Amersham TRK.322) to PBSG to achieve 16 to 18,000 cpm/0.2 ml.

- <sup>e</sup> Dextran-coated charcoal (DCC). Add 1% dextran T-70 (Pharmacia) to double-distilled deionized water and stir until dissolved. Add 1.5% charcoal (Norit A, Fisher Scientific) and stir until dissolved. Make-up fresh suspension for each assay. Store at 5°C until needed. When adding to assay tubes, stir continuously.
- <sup>f</sup> Complete counting cocktail 3a70B (Research Products International Corp.).

## Appendix IV

## Carcass Evaluation Score Descriptions

MasculinityPizzle Eye

- 1=small
- 2=moderately small
- 3=slightly small
- 4=slightly large
- 5=moderately large
- 6=large
- 7=very large

Jump Muscle and Crest

- 1=none
- 2=barely evident
- 3=slightly prominent
- 4=moderately prominent
- 5=prominent
- 6=very prominent

Longissimus QualityLean Firmness

- 1=very soft
- 2=soft
- 3=moderately soft
- 4=slightly soft
- 5=slightly firm
- 6=moderately firm
- 7=firm
- 8=very firm

Lean Texture

- 1=very coarse
- 2=coarse
- 3=moderately coarse
- 4=slightly coarse
- 5=slightly fine
- 6=moderately fine
- 7=fine
- 8=very fine

Lean Color

- 1=pale red
- 2=very light cherry red
- 3=light cherry red
- 4=cherry red
- 5=slightly dark red
- 6=moderately dark red
- 7=dark red
- 8=very dark red
- 9=black

Heat Ring (Dark Coarse Band)

- 1=none
- 2=slight
- 3=moderate
- 4=severe
- 5=extremely severe



## Appendix V

## Sensory Panel Evaluation Score Descriptions

Flavor intensity

- 1=extremely bland
- 2=very bland
- 3=moderately bland
- 4=slightly bland
- 5=slightly intense
- 6=moderately intense
- 7=very intense
- 8=extremely intense

Juiciness

- 1=extremely dry
- 2=very dry
- 3=moderately dry
- 4=slightly dry
- 5=slightly juicy
- 6=moderately juicy
- 7=very juicy
- 8=extremely juicy

Connective tissue amount

- 1=abundant
- 2=moderately abundant
- 3=slightly abundant
- 4=moderate
- 5=slight
- 6=traces
- 7=practically none
- 8=none

Myofibrillar and overall tenderness

- 1=extremely tough
- 2=very tough
- 3=moderately tough
- 4=slightly tough
- 5=slightly tender
- 6=moderately tender
- 7=very tender
- 8=extremely tender

EFFECTS OF ESTRADIOL-17 $\beta$  IMPLANTS FROM BIRTH TO SLAUGHTER ON  
PERFORMANCE, CARCASS, SENSORY TRAITS AND ENDOCRINE ASPECTS OF  
YOUNG BULLS AND STEERS

by

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San Luis Obispo, 1983

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AN ABSTRACT OF A MASTER'S THESIS

Submitted in partial fulfillment of the  
requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

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### Abstract

Twenty-eight Simmental bull calves were randomly allotted to one of three treatments at birth. Ten calves remained as intact bulls and were implanted (IB) with estradiol-17 $\beta$  (E<sub>2</sub>)(Compudose®); nine calves were castrated within 3 d of birth and implanted (IS) with Compudose®; and the remaining calves were intact, non-implanted control bulls, (CB). Implanted calves were implanted within 3 d of birth and at 200 d intervals until slaughter. Live weights were monitored at 28 d intervals from 8.5 to 14.8 mo while masculinity scores (MS), scrotal circumferences (SC) and hip heights were measured at 8.5 and 14.8 mo. Blood samples were taken every 14 d from 9.8 through 14.8 mo and serum was analyzed for estradiol-17 $\beta$  (E<sub>2</sub>) and testosterone (T) levels. Testicle weights (TW) were obtained after slaughter, while carcass quality, yield traits and carcass masculinity scores were obtained at 24 h postmortem. Modified oven-broiled longissimus (LD) steaks were evaluated by a trained sensory panel and a Warner-Bratzler shear (WBS) device. Implanted steers were less (P<.05) masculine than IB, and IB were less (P<.05) masculine than CB at both 9.1 and 14.8 mo. Further, SC were smaller (P<.05) for IB than for CB at both 8.5 and 14.8 mo. Control bulls had heavier weaning weights (P<.05) than IB and higher (P<.05) feedlot average daily gains (ADG) than IS; whereas IB were intermediate in ADG. Implanted steers required more (P<.05) feed/unit of gain than CB and IB. Implanting young bulls with E<sub>2</sub> increased (P<.05) serum E<sub>2</sub> levels and tended (P<.10) to reduce T levels compared with CB. Levels of serum E<sub>2</sub> in IS and IB were drastically increased (P<.05) for 4 to 6 wk, following re-implantation during the feedlot phase then declined sharply. Serum T levels were reduced (P<.05) in IB from 9.8 through 12.1 mo of age and tended (P<.10) to be lower than CB through 14.8 mo. Hot carcass weights and dressing percentages were not different (P<.05) among treatments. Implanted steer carcasses were more youthful than CB carcasses, and IB tended (P=.07) to be more youthful than CB. The incidence of heat ring (dark, coarse band formation) was

higher ( $P < .05$ ) for IB than for IS, likely because fat thickness was less ( $P < .05$ ) for IB than for IS. Ribeye areas were smaller ( $P < .05$ ) and yield grades were higher for IS than for CB. Testicle weights were not significantly different between IB and CB, although variability was much greater for IB. Spermatozoa concentrations were lower ( $P < .05$ ) for IB than for CB. Carcass masculinity was lowest ( $P < .05$ ) for IS, and IB were less ( $P < .05$ ) masculine than CB. The only differences in longissimus sensory traits was that IB steaks were juicier than CB steaks. However, WBS values were lower ( $P < .05$ ) for IS than for CB. These findings indicate that implanting young bulls with  $E_2$  from birth to slaughter decreases masculinity development, which makes them more acceptable to meat packers.

(Key Words: Bulls, Steers, Implant, Estradiol-17 $\beta$ , Performance, Carcass)