MECHANISM OF ESTROGEN ACTION WHICH ELICITS THE ANABOLIC RESPONSE OBSERVED IN RUMINANTS

by

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CHAPTER I

INTRODUCTION

Consumer awareness of the diet/health issue has increased demand for lean meat. As reviewed by Cross (1988), results of the National Consumer Retail Beef Study indicate that consumers believe beef products are too fat and that the diet/health image improves with fat trimming, resulting in consumers purchasing more beef. Cross (1988) stated that "the U.S. Beef Industry has produced over 6 billion pounds of waste fat each of the past several years." To decrease the fat content of meat, beef packers and retailers are implementing close (quarter inch) or complete (no external fat) trimming procedures. Trimmed fat is a waste to the industry, both in terms of the feed cost of producing excess fat and additional processing costs. The beef industry is being challenged to produce lean beef efficiently.

Implants have had a significant impact upon the beef industry since their introduction nearly 35 years ago. Anabolic agents used today are compounds which contain estrogen {Compudose - 17-β estradiol; Synovex and Steeroid/Heiferoid - 17-β estradiol benzoate plus progesterone (steers) or testosterone propionate (heifers)} or are non-steroidal compounds which have estrogenic activity {Ralgro - zeranol, a resorcylic acid

lactone, originally isolated as a fungal product in moldy corn}. Recently, attention is being focused on the use of trenbolone acetate (a potent synthetic androgen), especially in combination with estrogenic anabolic agents.

Anabolic estrogens increase growth, efficiency and lean tissue deposition in ruminants. Research has been conducted on the growth response and physiological changes which result from estrogen administration and are reported in several reviews (Preston, 1975; Heitzman, 1981; Preston, 1987). Although the effects of anabolic estrogens are well documented, the mode of action which elicits these effects has not been clearly defined (Buttery and Sinnett-Smith, 1984; Gopinath and Kitts, 1986; Florini, 1987; Preston, 1987). It has been hypothesized that estrogens either act indirectly by increasing circulating concentrations of somatotropin which then gives rise to the observed anabolic response or act directly on the skeletal muscle cell, stimulating protein synthesis.

Research on the mechanism of estrogen action is of high priority. Such data would provide insight into factors which regulate growth at the cellular and molecular level, and might lead to manipulation of growth at this level. Once the mechanism of action is known, more efficacious anabolic agents might be discovered.

It was the intent of this research to identify the mechanism of anabolic estrogen action in ruminants. Specific objectives were to determine if the anabolic actions of 17-β estradiol and bovine somatotropin are additive; to determine if catechol estrogen formation is involved in the estrogenic anabolic response; to determine the effects of progesterone on the anabolic activity of estrogens; and to determine 17-β estradiol clearance rate in feedlot steers.

The following is a summary of the literature regarding anabolic effects of estrogens and possible mechanisms for regulating anabolism.

CHAPTER II

REVIEW OF LITERATURE

Effects of Anabolic Estrogens on Protein Accretion

Anabolic estrogens improve daily gain, feed efficiency, nitrogen retention and increase protein deposition in growing cattle and sheep (Preston, 1975; Heitzman, 1979; Muir et al., 1983; Basson et al., 1985; Preston, 1987). Fecal nitrogen is unaltered indicating that the anabolic effects of estrogens on nitrogen metabolism are post absorptive (VanderWal, 1976). Urinary nitrogen, however, is decreased by anabolic estrogens (Davis et al., 1970b; Grebing et al., 1970; VanderWal, 1976); therefore, increased nitrogen retention and lean tissue deposition is observed. Estrogens also decrease plasma urea nitrogen (PUN) and plasma amino acids (Oltjen and Lehmann, 1968; Preston, 1975; Gopinath and Kitts, 1986). Plasma urea nitrogen reduction: 1) occurs within 24 h and reaches maximal reduction within 12-19 d post administration with diethylstilbestrol (DES; Preston, 1968); and 2) occurs prior to, but parallels urinary nitrogen reduction (Grebing et al., 1970). Therefore, it has been suggested that PUN depression be used as an indicator of anabolic activity (Preston, 1986 unpublished data).

As reviewed by Galbraith (1980), endogenous urea production is primarily dependent on amino acid deamination (Sykes, 1978). Therefore, PUN reduction may result from an increase in amino acid requirements for protein deposition or from a decrease in amino acid levels produced in protein turnover (Millward et al., 1976; Gopinath and Kitts, 1986). Thus PUN reduction would indicate a protein sparing effect (Donaldson and Heitzman, 1983).

> Improvements in nitrogen retention based on changes in tissue metabolism rather than intake must be accompanied by a decrease in the catabolism of amino acids and an alteration in the rate of protein synthesis or protein degradation or both. (Lobely et al., 1985)

Body protein accretion is a function of both protein synthesis and protein degradation. When zeranol (Z; a non-steroidal compound with estrogenic activity) is administered alone or in combination with trenbolone acetate (TBA; a potent synthetic androgen), protein synthesis is unaltered or decreased and protein degradation is decreased, resulting in protein accretion (Buttery, 1983; Sinnett-Smith et al., 1983). Tucker and Merkel (1987) indicate that estrogens decrease protein synthesis and degradation. For accretion to occur, degradation must be depressed to a greater extent than synthesis. Hayden et al. (1987), however, observed that 17-β estradiol (E_2) had no effect on protein degradation, but did tend to increase protein synthesis. Roeder et al.

(1986) studied the <u>in vitro</u> effects of E_2 and Z on protein synthesis and degradation in muscle cell culture. Estradiol decreased protein synthesis but was without effect on protein degradation. No effects in either protein synthesis or degradation were observed with Z.

In addition to increased protein accretion, the combination of Z and TBA promotes an increase in muscle fiber size and oxidative muscle fiber content, a proposed mechanism whereby ATP production can be increased to support protein synthesis following administration of anabolic agents (Clancy et al., 1986).

While it is generally thought that estrogens are anabolic only in ruminants, DeWilde and Lauwers (1984) implanted boars, barrows and gilts with either E_2 plus progesterone, TBA or testosterone and observed decreased weight gain and feed intake, resulting in a longer time on feed compared to controls. There was a decrease in fat and increase in lean with implantation, however. Anderson et al. (1988) implanted boars with increasing dosages of E_2 (0, .6, 1.2 and 1.8 mg E_2/d) which improved daily gain, feed conversion, carcass protein and decreased carcass fat when compared to non-implanted barrows and improved daily gain and feed conversion when compared to control boars. In a second study, nitrogen retention was increased in barrows, but not boars, when implanted with 1.5 mg E_2 dipropionate/d.

Somatotropin

Somatotropin (ST) also increases growth and protein deposition (Trenkle and Topel, 1978; Goldberg et al., 1980; Buttery, 1983; Johnsson et al., 1985) and several reviews have been written on ST (Bauman et al., 1982; Davis et al., 1984; Spencer, 1985; Hart and Johnsson, 1986; Bauman and McCutcheon, 1986; Gluckman et al., 1987; Tucker and Merkel, 1987; Machlin, 1976; Young, 1976).

Administration of ST mimics many of the observed effects of estrogen. Somatotropin decreases plasma amino acid and urea nitrogen levels as well as urinary nitrogen; thus, ST also increases nitrogen retention (Davis et al., 1970a,b; Moseley et al., 1982; Eisemann et al., 1986). These factors suggest increased tissue uptake of amino acids and protein accretion (Trenkle, 1981). Somatotropin increases protein accretion by increasing protein synthesis (transcription and translation) with no effect on protein degradation (Bergen, 1974; Goldberg et al., 1980; Buttery, 1983).

Since many of the effects of estrogen and ST are similar, it has been postulated that the anabolic effect of estrogens is mediated through an increase in ST secretion (Preston, 1975; Trenkle, 1976; Heitzman, 1979; Heitzman, 1981; Gopinath and Kitts, 1984; Preston, 1987). In support of this hypothesis, anterior pituitary weight, cell size and number (especially acidophiles, which

produce and secrete ST) as well as ST secretion and concentration have been reported to increase following estrogen administration in ruminants (Trenkle, 1970; Preston, 1975; Trenkle, 1981; Gopinath and Kitts, 1984; Grigsby and Trenkle, 1986)) and Wien et al. (1983) reported an 189% increase in plasma ST. It has been concluded, however, that the anabolic effect of DES is not ST mediated because plasma ST did not increase during the first 28 d following DES administration (Wien et al., 1983) and a gain response was noted two weeks prior to increased plasma ST following DES administration, with plasma ST remaining elevated thereafter (Muir et al., 1983). Hutcheson and Preston (1971), however, observed a 31% increase in plasma ST within 14 d after DES administration and Gopinath and Kitts (1984) observed ST secretion rates approximately 100% higher than controls within 20 d following estrogen administration. In contrast, plasma ST is not always increased following Z treatment (Buttery and Sinnett-Smith, 1984).

Phelps et al. (1988) observed an immediate increase in ST release from cultured anterior pituitary cells from growing sheep when Z was introduced. However, ST release was not stimulated when adult rat or adult sheep pituitary cells were treated with Z. This indicated a maturational difference in the ability of the pituitary cells to respond to exogenous anabolic agents.

Cultured bovine pituitary cells incubated with E_2 did not alter the amount of bST mRNA or ST secretion into the culture medium compared to control cells (Silverman et al., 1988). Preincubation with E_2, however, increased the response to a challenge of ST releasing factor by 142%. These pituitaries were removed from "recently killed cattle at a local slaughter house" and it is possible that the cattle may have been previously estrogenized (via implantation) which could affect these results as in vivo secretion rates and plasma ST concentrations are elevated with E_2 implantation and ST release has been shown to increase following Z administration in vitro with lamb pituitary cells.

As previously cited, ST stimulates protein synthesis without altering protein degradation and it appears that estrogens do not stimulate protein synthesis. Therefore, if secretion of ST is responsible for the mechanism of estrogen action, then, as was stated by Buttery (1983), "surely muscle protein synthesis would be stimulated." Furthermore, if protein synthesis is not stimulated, what then accounts for the depression in plasma amino acids and why the shift to oxidative muscle fibers, hypothesized to provide more energy for increased muscle protein synthesis?

As recently reviewed by Preston (1987), estrogens increase both synthesis and release of ST from rat

pituitary cells *in vitro* (Simard et al., 1986) and increase plasma ST *in vivo* (Lloyd et al., 1971). Yet there is no observed anabolic response from estrogens in rats (Preston, 1975). Somatotropin administration, however, does promote anabolic growth and has been used to evaluate the biopotency of ST in rats (Groesbeck and Parlow, 1987). Therefore, if increased ST secretion is the mechanism of anabolic estrogen action, then "why do estrogens depress the growth of rats" and "what then is the mode of anabolic action of estrogens in ruminants?" (Preston, 1987). Wright (1961) has suggested that species differences in anabolic response to estrogen is due to differences in energy substrate utilization (monogastrics-glucose; ruminants-volatile fatty acids). Research has yet to be conducted to confirm this hypothesis.

Mechanism of Anabolic Estrogen Action

Additivity of Estrogen and Bovine Somatotropin

Due to the equivocal results regarding ST as the mediator of anabolic estrogen action, research is required to specifically answer this question. If estrogen and ST are administered simultaneously, both at their optimum dose for anabolic activity, non-additive results would indicate that the two mechanisms of anabolic activity are similar, or that ST mediates estrogen action. Additive results, on the other hand, would indicate two independent

mechanisms of anabolic activity, indicating that the mechanism of estrogen action must be through an alternate pathway.

Little research has been reported on the optimum dose of estrogen or ST required to elicit maximal improvements in growth and protein deposition. Cain et al. (1986) reported that feed efficiency and daily gain increased linearly with a 72 mg Z implant, indicating that 36 mg, the recommended dosage, is not optimal. Similar results were observed by Preston (1984). Preston (1987) found that the Synovex dosage (20 mg E_2 benzoate/200 mg progesterone) was optimal for steers, however, it may be insufficient for heifers. Wagner et al. (1979) found that 30-40 µg of E2 are required daily for optimal steer response. Potter and Wagner (1987) found different doses of E_2 were required to elicit maximal improvements in intake and daily gain (15 versus 30 µg E_2/d, respectively), which are below the dose (62 µg) delivered by the Compudose implant (Compudose technical manual, White, 1982). Basson et al. (1985) reported that the average daily release of E_2 from Compudose implants was 84-91 µg/d.

Research to date indicates that approximately 15 mg (42 µg/kg) of ST/d is required to elicit improvements in growth and protein deposition in steers (Ivy et al., 1986b). In swine, the dose relationship is dependent upon

the performance variable measured (Ivy et al., 1986a; Wolfrom et al., 1986).

As reviewed by Preston (1987), several studies have concluded that the relationship between estrogen and ST is additive; however, these studies were not obviously conducted at the optimum dosages for each hormone (Wolfrom and Ivy, 1985; Wolfrom et al., 1985; Ivy et al., 1986b; Roche and Quirke, 1986). Wolfrom et al. (1985) found a linear increase in gain and feed efficiency with increasing doses of ST. Roche and Quirke (1986) found that estrogen treatment increased carcass weight and conformation whereas ST did not, perhaps because ST was not given at the optimum dose. Ivy et al. (1986b) indicated an optimum ST dose of 15 mg/d but used a 36 mg Z implant, which is probably suboptimal (Preston, 1984; Cain et al., 1986; Preston, 1987).

It was our goal to perform independent E_2 and ST trials to determine the optimum dose of both hormones and then tested individually and in combination to determine if their effects are additive or not.

Recently, Wagner et al. (1988a,b and unpublished data) evaluated the effects of E_2 (Compudose) and bovine ST (bST; 960 mg released over a 14-d period; reinjected every 12 to 14 d), alone and in combination, on urinary nitrogen excretion, growth performance, carcass and plasma constituents. Urinary nitrogen excretion was 61.8, 52.2,

51.2 and 45.3 g/d and PUN concentrations were 17.8, 14.2, 14.4 and 13.2 mg/dl for control, E_2, bST and E_2 plus bST treatments, respectively. Significant E_2 and bST main effects were observed in both urinary nitrogen excretion and PUN depression. The interaction was nonsignificant for urinary nitrogen excretion, indicating an additive response. There was an additional 12% decrease in urinary nitrogen excretion when E_2 and bST were combined, compared to either hormone alone. Although there was an additional 8% reduction in PUN when E_2 and bST were combined compared to either hormone alone (20%), the interaction was significant ($P = .03$) indicating that the PUN depression was not additive. Plasma bST concentrations were 2.5, 6.3, 98.5 and 95.0 ng/ml for control, E_2, bST and E_2 plus bST, respectively. Administration of E_2 did not significantly elevate plasma bST concentrations even though plasma bST concentrations were increased 2.5 fold (152%) above controls. This increase may not have been detected in the statistical analysis due to the supra-physiological elevations in plasma bST resulting from injection of bST (39-fold increase above controls). In a second study by Wagner et al. (1988a), steers were placed on the above treatments for 140 d, after which time the steers were slaughtered and carcass composition was evaluated. Similar to trial 1, plasma bST concentrations were 7, 14, 71 and 70 ng/ml for control, E_2, bST and E_2

plus bST, respectively. Both hormones increased insulin-like growth factor I concentrations (E_2, 49% over controls, nonsignificant; bST, 154% over controls); however, this was non-additive (162% over controls when E_2 and bST were combined). Daily gain was 1.25, 1.41, 1.37 and 1.57 kg per d and gain/feed was .145, .163, .178 and .189 for controls, E_2, bST and E_2 plus bST, respectively. This represented increases above control of 13, 10 and 26% for E_2, bST and E_2 plus bST for daily gain and 12, 19 and 27%, respectively, for gain/feed. Effects of E_2 and bST were additive for daily gain, gain/feed, carcass protein, moisture and bone. Both hormones decreased carcass fat, but the reduction was not additive, nor was there an additive effect on increased ribeye area. It was concluded that both E_2 and bST are anabolic and additive in response based on growth, gain/feed, protein, bone and nitrogen excretion, and that the anabolic actions of E_2 are by some other mechanism or in addition to increased endogenous bST secretion.

Alternate Mechanisms of Anabolic Estrogen Action

Catechol Estrogen Formation

Catechol estrogens (CE) are a class of estrogen metabolites which are formed in many tissues such as the liver, pituitary and hypothalamus (MacLusky et al., 1981). Estrogens are metabolized to CE by hydroxylation at either

the 2 or 4 carbon of the aromatic ring. The CE resemble the catecholamines (CA; epinephrine, norepinephrine and dopamine) as they each have an ortho-dihydroxy substituted benzene ring. The CE are potent inhibitors of tyrosine hydroxylase, the enzyme which converts tyrosine to L-dopa (Lloyd and Weisz, 1978; Pfeiffer et al., 1986). As this is the rate limiting enzyme in CA synthesis (Granner, 1985), CE would decrease the synthesis of CA. This enzyme is usually regulated via negative feedback inhibition from the CA. Due to the structural similarity of the CE and CA, it is hypothesized that the CE are acting as competitive inhibitors of tyrosine hydroxylase via the pterin cofactor (Lloyd and Weisz, 1978). The CE also compete for dopamine receptors (Schaeffer and Hsueh, 1979; Paden et al., 1982) and inhibit catechol-o-methyl transferase (COMT; Ball et al., 1972), a cytosolic enzyme in many tissues, which catalyzes the methylation of the hydroxyl group on the benzene ring of CE and CA in their metabolism (Granner, 1985; Pfeiffer et al., 1986). The COMT enzyme converts 2-or 4-hydroxy E_2 to 2- or 4-methoxy E_2 or to 2-hydroxy E_2-3-methyl-ether, dependent upon tissue location (Pfeiffer et al., 1986). Suppression of this enzyme should increase the half-life of the circulating CA and CE. If the CE are completely inhibiting tyrosine hydroxylase, however, no new CA would be formed.

Reeds (1987) postulated that growth regulation may result from hormone-receptor mediated responses, namely enzyme phosphorylation and metabolic control. Regarding protein metabolism, he noted four areas where enzyme phosphorylation may be of importance: 1) initiation of polypeptide translation controlled by phosphyorlation of eukaryotic initiation factor 2; 2) phosphyorlation state of protein S-6 of the small ribosomal subunit; 3) phosphorylation of aminoacyl tRNA synthetase enzymes; and 4) amino acid catabolic enzymes.

If CE formation is required to elicit the anabolic response, involvement of the cAMP-second messenger system is implied if the CE have CA or β-agonist like properties (Buttery and Dawson, 1987; Sejrsen and Jensen, 1987). This would involve protein kinase activity and enzyme regulation of events (possibly the four areas mentioned in the preceding paragraph) which may lead to anabolic activity (Sejrsen and Jensen, 1987; Smith, 1987). More specifically, however, in muscle glycogen synthesis would be decreased due to decreased glycogen synthetase and glycogenolysis would increase due to increased phosphorylase, as well as gluconeogenesis in the liver, which would increase glucose. Lipolysis and free fatty acids would increase and uptake and utilization of glucose in skeletal muscle would decrease. As suggested by Wright (1961), could the difference in anabolic response between

species be due to energy substrate utilization? Ruminants continuously utilize glucogenic pathways because of microbial production of volatile fatty acids (VFA) in the rumen, which are absorbed and used as the major source of energy via the TCA cycle or converted to glucose (propionic acid) via gluconeogenesis. If uptake and utilization of glucose were decreased in skeletal muscle following conversion of estrogen to CE, it would seem that the ruminant might demonstrate an anabolic response since it uses other sources of energy (acetate and butyrate produced in the rumen, which would be similar to the products of lipolysis) and since the ruminant has a glucose conservation mechanism whereby 66% of produced glucose is recycled rather than oxidized, with glucose oxidation inhibition most likely regulated in the muscle tissue (Lindsay, 1981).

Therefore, it seems that the ruminant may be functionally able to utilize these alternate metabolic products whereas the monogastric is not presented with these metabolites and would not be similarly affected. In support of this, VanderWal (1976) reported that growth was not improved and slaughter weight was less than controls when calves were injected with DES at 5 weeks of age (immature ruminant), whereas with calves 11 or 14 weeks of age, growth was increased and maintained until slaughter. Increased growth, feed efficiency and carcass weight were

observed in rabbits following E_2 administration (Daley et al., 1987) and in guinea pigs following DES administration (Preston et al., 1956). Rabbits and guinea pigs have a large cecum with hind gut fermentation and VFA production.

Recently, Welsh et al. (1987) reported that clenbuterol (a β-adrenergic agonist), forskolin (an adenyl cylcase activator), 1-methyl-3-isobutyl xanthine (a phosphodiesterase inhibitor) as well as ST releasing hormone all stimulated ST secretion. This suggests that the cAMP-second messenger system may regulate ST secretion. Cyclic AMP is stimulated by the β-adrenergic agonists which are similar in structure and action to CA.

Prolactin has been shown to increase growth and nitrogen retention in some species (Bauman et al., 1982), however, effects of prolactin in ruminants are inconclusive (Bauman and McCutcheon, 1986). Prolactin has been reported to increase following DES administration (Davis et al., 1978; Wien et al., 1983). Dopamine inhibits prolactin secretion, however, estrogen can override this by decreasing dopamine receptors and stimulating prolactin transcription (Granner, 1985; Norman and Litwack, 1987).

Baile et al. (1986) reported that dopamine controls ST secretion in rats. Intraventricular injections of dopamine in rats inhibits ST secretion and blockage of dopamine receptors stimulates ST release. They indicated

that this effect is probably through increased somatostatin. Therefore, if CE decrease dopamine synthesis and compete for dopamine receptors, this could increase ST secretion by decreasing somatostatin, removing the ST release inhibition.

Norman and Litwack (1987) and Scanes and Lauterio (1984) have also indicated that the CA stimulate ST releasing hormone and ST secretion. Could CE be involved in this pathway?

With these factors in mind, it is hypothesized that the formation of CE might be required for the anabolic actions of estrogens in ruminants and that CE act as a neurohormone.

Skeletal Muscle Estrogen Receptors

It is also possible that estrogens exert their action directly on the muscle (Heitzman, 1979). In addition to estrogen receptors in the hypothalamus, pituitary, uterus, kidney and liver (Norman and Litwack, 1987), several studies have indicated the presence of estrogen receptors in ovine and bovine skeletal muscle (Buttery, 1983; Meyer and Rapp, 1985; Bechet et al., 1986; Frey et al., 1988). Although the number of estrogen receptors in muscle tissue is much less compared to uterine tissue (1000 fold), Meyer and Rapp (1985) reported that bovine skeletal muscle receptors are specific, with affinity similar to that of

classical estrogen target tissue receptors. It was suggested that the two tissue receptors are identical; thus, similar modes of action could be postulated (nuclear binding, increased RNA and protein synthesis).

Meyer and Rapp (1985) suggested that cytosolic estrogen receptor content was dependent upon hormone treatment as they observed an 81% decrease in cytosolic estrogen receptor content when calves had been treated with E_2 and trenbolone acetate (Revalor) prior to receptor measurement. This was believed to be due to transfer of the E_2-receptor complex to the nucleus. This observation was confirmed by the results of Bechet et al. (1986), indicating an increase of nuclear estrogen binding sites after implantation with Z. In contrast, following implantation, Frey et al. (1988) observed a decrease in cytosolic E_2 receptor binding capacity in semintendenosis muscle and a decrease in nuclear E_2 receptor binding capacity in triceps brachii. Meyer and Rapp (1985) concluded that due to the high affinity of the bovine skeletal muscle receptor, a slight elevation in plasma E_2 could stimulate receptor binding and protein synthesis.

In addition to estrogen binding, Bechet et al. (1986) reported binding of progesterone to the estrogen binding site in nuclei isolated from bovine diaphragm, which does not fulfill the criteria for a classical estrogen receptor. No progesterone binding was observed, however,

in cytosolic estrogen receptors isolated from bovine musculis brachialis and biceps brachii (Meyer and Rapp, 1985).

Problems associated with the muscle estrogen receptor hypothesis are: 1) estrogens are believed to decrease protein degradation rather than to stimulate protein synthesis and 2) estrogen receptors are also located in rat skeletal muscle (Dube et al., 1976; Dionne et al., 1979; Dahlberg, 1982) yet there is no anabolic effect in rats following estrogen administration.

Although some reports support a possible direct effect of estrogen on skeletal muscle, more research is needed to determine if estrogen directly stimulates muscle protein synthesis, resulting in the anabolic effect.

Bovine Somatotropin Receptors

Breier et al. (1988a) indicated that E_2 and nutritional status influenced hepatic ST receptors in steers. A 51% increase in ST binding was observed when steers were on a high plane compared to a low plane of nutrition. This was primarily due to a high affinity binding site. They also observed an increase in affinity (binding) of a low affinity binding site with improved nutritional status. Estradiol implantation increased ST binding at both levels of nutrition, with greater binding observed on the high nutritional plane. Implantation

increased the capacity of the high affinity receptor in the steers on the high nutritional plane from 1.9 to 6.6 pmol/100mg (251% increase). Similar effects were observed in the capacity of the low affinity ST receptors, however, the magnitude of difference was less (50% increase). Affinity of either the high or low affinity binding site was not affected by E2 implantation. Weight gain was increased with implantation, and weight gain was correlated with capacity of the high affinity ST binding site, plasma IGF-I levels (Breier et al., 1988b). Thus it was concluded that E_2 stimulated IGF-I and increased growth rate which may be mediated by an increase in ST receptors. It was suggested that in ruminants, ST receptor modulation is an important factor in growth regulation.

Insulin-Like Growth Factors

It has been proposed that the ST affect on protein synthesis may be mediated via a direct effect of somatomedin C (SmC; insulin-like growth factor I or IGF-I) in muscle which is stimulated by ST (Spencer, 1981; Buttery, 1983; Davis et al., 1984; Spencer, 1985; Florini, 1987). Insulin-like growth factor I indirectly mediated ST effects on long bone growth in an autocrine or paracrine manner in rats (Schlechter et al., 1986; Isgaard et al., 1988).

Correlations between growth rate and circulating IGF-I concentrations are not always observed (Orlowski and Chernausek, 1988). These researchers indicated that hypopsectomized rats treated with rST had increased growth rates and tibial epiphyseal plate widths with no increase in IGF-I. Tissue levels of IGF-I were doubled (liver) and tripled (kidney), however. It was also concluded that IGF-I may be mediating ST actions via autocrine or paracrine mechanisms. At low concentrations, IGF-I and IGF-II are lipolytic in ovine adipose tissue and may therefore mediate this action of ST (Lewis et al., 1988). Since mammary tissue lacks ST binding, it has been suggested that IGF mediate ST actions on lactation. Dehoff et al. (1988) concluded that both IGF-I and IGF-II receptors are in bovine mammary tissue with type I receptors predominating. Lactation was associated with increased concentrations of both type I and II receptors, especially type I. Shamay et al. (1988) reported that IGF-I is mitogenic in undifferentiated bovine mammary epithelial cells, however, galactopoiesis was not effected by IGF-I.

Sex steroid induced changes in ST metabolism may mediate increased growth during puberty (Mansfield et al., 1988). Davis et al. (1984) reported that pubertal increases in IGF concentrations have been observed.

Insulin-like growth factor I is usually increased following ST administration (Wien et al., 1983; Cohick et al., 1987). Contradictory results have been reported on IGF-I response following estrogen administration. Wien et al. (1983) reported a 119% increase in IGF-I following DES administration, whereas, Moffit (1980) and Wangsness et al. (1981) have reported no effect of compounds containing estrogen activity on plasma IGF-I. Although not significant, Wagner et al. (1988a) observed a 49% increase in IGF-I after E_2 implantation and a 100% increase in plasma ST. Injection with bST, however, increased IGF-I by 154% and bST by 914%. No additive effects were observed when E_2 and bST were administered simultaneously. The plasma elevations in bST and IGF-I resulting from bST are supra-physiologic and may mask E_2 effects, however.

Breier et al. (1988b) found that steers on a low plane of nutrition had evelvated mean concentration, peak height and intergrated area of plasma ST and decreased IGF-I concentrations. Estradiol implantation increased baseline ST concentrations in steers on a low nutritional status and increased mean concentration, baseline, peak height and intergrated area of plasma ST and increased IGF-I concentrations on both levels of nutrition. Following an intravenous injection of bST, IGF-I concentrations were increased in steers on the high nutritional plane. Plane of nutrition did not alter IGF-

II concentrations. Implantation with E_2 increased IGF-II concentrations from 1100 to 1850 µg/l. It was concluded that E_2 implantation increased both ST secretion and ST receptors (Breier et al., 1988a), IGF-I and IGF-II concentrations and growth rate in ruminants and it was suggested that modulation of ST receptors are thus an important regulatory mechanism of the somatotropic axis.

Murphy et al. (1987) reported a 14 fold increased uterine expression of IGF-I mRNA 6 h following E_2 administration in ovariectomized prepubertal rats. There was no change, however, in serum IGF-I concentration or hepatic or renal IGF-I mRNA. It was suggested that IGF-I may be important for E_2 action in the uterus in addition to its role in mediating ST action; IGF-I may have a generalized role in growth rather than a specific mediator of ST. To further evaluate this hypothesis, IGF-I expression was measured in hypophysectomized, ovariectomized rats after they were administered the following treatments: Control, E_2, ST or E_2 plus ST. IGF-I expression was increased 20, 8 and 10 fold above controls for E_2, ST and the combination, respectively. Estradiol was more potent in stimulating IGF-I expression than ST or the combination, which was actually antagonistic, indicating that E_2 may indeed have a generalized role in growth.

Insulin-like growth factor I (Haynes et al., 1987; Isgaard et al., 1988) and IGF-II (Haynes et al., 1987) mRNA are dependent on ST. However, IGF-II mRNA is less dependent on ST, but is tissue specific. Valentino et al. (1988) indicated that the central nervous system, brain, pituitary, liver, lung, kidney and skeletal muscle of rats contained immunoreactive IGF-II receptors. Since IGF-II is less dependent on ST but E_2 stimulates IGF-II, perhaps this is another mechanism which could lead to additive responses when E_2 and ST are administered simultaneously.

Maiter et al. (1988) suggested that IGF-I production and growth may be regulated differently than ST receptor concentration. Pulsatile ST administration was required for maximal stimulation of IGF, while continuous ST exposure was required for up-regulation of rat liver ST receptors. It was also reported that insulin regulates ST receptors and thyroid hormones regulate IGF receptors (Spencer, 1985).

Effects of Progesterone on Anabolic Estrogen Action

Two commercially available implants, Synovex-S and Steeroid, contain both E_2 benzoate (20 mg) and progesterone (200 mg). As reviewed by Galbraith and Topps (1981), melengesterol acetate (a synthetic progestogen) and progesterone were ineffective as growth promotants in steers and lambs, respectively. Melengesterol acetate,

however, increased the lean to bone ratio in heifer carcasses and a combination of progesterone and E_2 resulted in up to 27% improvements in growth in steers, with varying responses in carcass data reported. This latter effect, however, is likely due primarily to E_2 rather than progesterone.

For behavioral estrus to be expressed, progesterone levels must be low when estrogen levels are elevated, however, estrus behavior may be regulated by the level of progesterone prior to the increase in estrogen (Carrick and Shelton, 1969; Levasseur and Thibault, 1980; Davidge et al., 1987). Davidge et al. (1987) observed linear decreases in estrous behavior with increasing progesterone dosages from 0 to 500 mg/d. Progesterone was injected twice daily for 5 d in ovariectomized cows. Estrus was induced 72 h post progesterone treatment by the injection of 2 mg of E_2. It was suggested that progestrone treatment may have blocked E_2 action by down regulating brain E_2 receptors (Kato, 1977). Melampy et al. (1957) observed similar results, indicating that 30 to 60 mg of progesterone (12 h before or 12 h after estrogen administration) was required for suppression of estrus behavior. At lower dosages, however, estrus behavior was observed and progesterone was synergistic with E_2 when injected 12 h prior to, with or 12 h post E_2.

With these factors in mind, it was our interest to determine if "priming" with progesterone prior to estrogen administration alters the anabolic response to estrogens in ruminants.

17-β Estradiol Metabolism and Clearance

Over 25 different estrogens have been identified in the tissues and body fluids of humans, the most important consisting of the classical estrogens, namely E_2, estrone and estriol (Bidlingmaier and Knorr, 1978). The most biologically active estrogen is E_2, followed by estrone and then estriol, a weak estrogen.

As reviewed by Bidlingmaier and Knorr (1978) and Reed and Murray, (1979), only a small percentage (1-3%) of circulating estrogen is in the biologically active free form, the remainder is bound to plasma proteins (primarily β-globulin, referred to as the sex hormone binding globulin (SHBG), and albumin). Concentrations of SHBG are increased with increased plasma concentrations of estrogens. The SHBG are believed to have the following functions: 1) transport of E_2 and testosterone to effector sites; 2) storage, as free hormones are available upon steroid dissociation from the protein; 3) protection from rapid metabolism and excretion; 4) buffer protection against steroid hormone inundation of extravascular spaces; and 5) regulation of the free

androgen to free estrogen ratio (Bidlingmaier and Knorr, 1978; Reed and Murray, 1979).

Human females have a metabolic clearance rate for E_2 of approximately 1000 liters/d (Reed and Murray, 1979). As cited by (Bidlingmaier and Knorr, 1978), Sandberg and Slaunwhite (1957) using radio-labelled E_2 calculated a 22 min half-life for circulating 17-β estradiol in humans. Likewise, Breuer and Breuer (1973) observed a rapid initial disappearance of radio-labelled E_2 following injection. They observed a second pool which had a slower half-life of 120 min. The fast clearance pool was thought to be a reflection of injected E_2 distribution within the body, while the slow clearance pool was largely determined by metabolism and excretion of E_2. Estradiol and estrone are metabolized in the liver via hydroxylation primarily on the 2 (catechol estrogen formation) and 16 carbons (Figure 2.1; Bidlingmaier and Knorr, 1978). In addition, in bovine blood, estrone is metabolized to 17-α estradiol via 17-α-A-hydroxy steroid dehydrogenase (Dorfman and Ungar, 1965; Gorski and Erb, 1959). The liver is also an important site of steroid conjugation and it was stated that

> the decisive metabolic step for estrogen elimination is esterification with glucuronic or sulfuric acid to water-soluble conjugates. (Bidlingmaier and Knorr, 1978)

These conjugates are primarily excreted through the kidneys as glucuronides and are eliminated via the urine.

Lesser amounts are excreted via the liver through biliary excretions into the intestine. Furthermore, the intestinal conjugates are hydrolyzed and free estrogens are reabsorbed with small amounts of fecal excretion occurring due to this enterohepatic circulation of estrogens. In ruminants, however, Preston (1975) indicated that 45-95% and 4-30% of DES was eliminated via the feces and urine, respectively.

Little work has been conducted on E_2 clearance rate in beef cattle. Kinetic parameters of estrogen in the circulatory system need to be evaluated in order to understand estrogen clearance and metabolism. This information will provide insight on the length of effectiveness of circulating E_2 and aid in interpreting plasma E_2 concentrations in implanted cattle.

Following implantation of calves with Synovex C (10 mg E_2 plus 100 mg progesterone), Castree et al. (1988) reported elevations in plasma E_2 28 and 108 d post-implantation (7.04 versus 14.06 and 6.29 versus 8.26 pg/ml in control and implanted calves, respectively). Rumsey and Beaudry (1979) reported that plasma E_2 concentrations in non-implanted steers ranged from less than 1 to 32 pg/ml with an overall mean of 3.7 over a three trial experiment. Sixty d following Synovex implantation, plasma E_2 concentrations ranged from 6.9 to 181 with a mean of 31 pg/ml in one experiment and ranged from less

than 1 to 310 with a mean of 65.1 in another. Plasma E_2 concentrations had returned to baseline concentrations by 120 d post implantation. Large variations were observed in plasma E_2, especially in the implanted steers.

Implantation of E_2 with either TBA, testosterone or progesterone increases the time plasma E_2 levels are elevated due to a slower, more sustained release of E_2 (Heitzman et al., 1981). Their research indicated that non-implanted steers had plasma E_2 concentrations less than 10 pg/ml. Implantation with E_2 alone elevated plasma E_2 to approximately 100 pg/ml, which decreased to approximately 30 pg/ml by 30 d and to baseline by approximately 60 d post implantation. Implantation of E_2 plus TBA, however, elevated plasma E_2 concentrations to 30-40 pg/ml and sustained concentrations at this level for 98 d. Steers implanted with TBA plus E_2 had improved daily gains for 98 d compared to controls, whereas, implantation with E_2 alone improved daily gain for only 35 d, when plasma E_2 concentrations were approaching baseline concentrations. Similar plasma E_2 results were reported by Riis and Suresh (1976) using radio-labelled E_2 implants with and without TBA. In addition, 95% of the radioactivity was excreted within 20 d after implantation with E_2 alone, whereas, 107 d were required to account for all the radioactivity when E_2 and TBA were combined.

Glascock and Hoekstra (1959) observed that radio-labelled hexestrol was concentrated in organs known to respond to estrogens (uterus, vagina, mammary gland and pituitary) and the excretory organs (kidney, liver and intestines). However, 24 h following the radio-labelled hexestrol injection, they observed that less than two% of the labelled hormone remained in the tissues of sheep and goats. Therefore, 98% of the label not only was cleared from the circulatory system, but from the tissues as well.

Calculating E_2 clearance rate in E_2 implanted cattle by measuring the amount of E_2 in the plasma ipsilateral versus contralateral to the implant, half-lives of 1.8 to 6.8 min have been reported (Harrison, 1981, as cited by Heitzman et al., 1984). This half-life is faster than that previously reported in humans. Due to the lack of information on E_2 clearance rate in cattle, the objective of this research was to determine E_2 clearance and to determine the effect of implantation on E_2 clearance rate in feedlot steers. This information will provide insight on the length of effectiveness of circulating E_2 in implanted cattle and will allow us to determine if implantation alters the kinetic parameters of estrogen in the circulatory system, which may effect the clearance, metabolism, utilization and/or mode-of-action of estrogen.

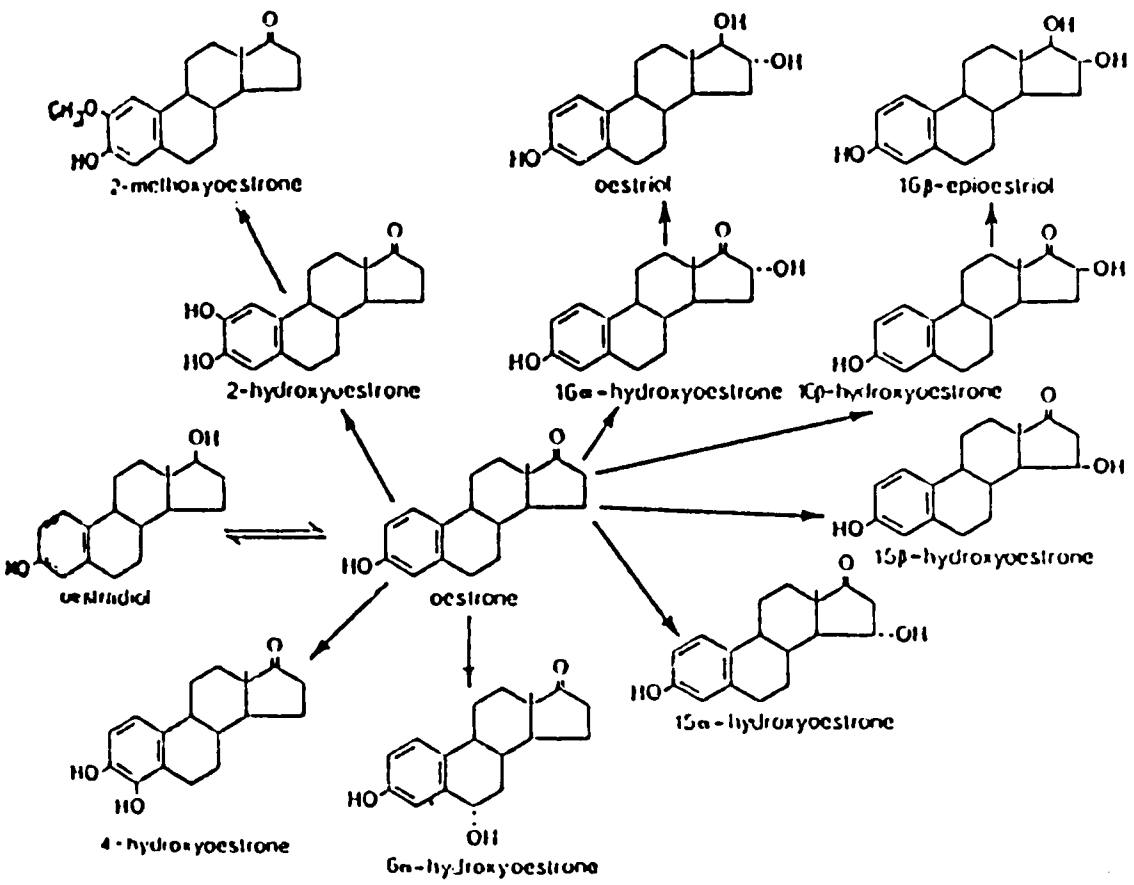

Figure 2.1. Metabolism of estrogens (Bidlingmaier and Knorr, 1978).

CHAPTER III

TITRATION OF THE 17-β ESTRADIOL DOSAGE WHICH MAXIMIZES THE ANABOLIC RESPONSE IN FEEDLOT STEERS

Abstract

The objective of this study was to determine the optimum dosage of 17-β estradiol (E_2) required to elicit maximum depression in plasma urea nitrogen (PUN), an indicator of anabolic activity. Forty-two steers (343 kg) were blocked by weight into six pens. Pellets containing E_2 were implanted subcutaneously in the ear on d 0. Six steers were placed on each of the following E_2 treatments (TRT): 0, .1, .5, 1, 2.5, 5, 10 mg E_2/implant; two .5 mg E_2 implants were used to achieve the 1 mg dosage. The trial was conducted for 21 d. Steers were weighed and blood samples taken via jugular vein puncture on d 0, 1, 4, 7, 14 and 21 at 1400 h, approximately 4 h post feeding. No TRT ($P = .28$), but, time ($P < .0001$) and a trend for TRT by time ($P = .08$) differences were observed in PUN depression. Mean PUN levels were 13.6, 11.6, 9.46, 10.2 and 11.7 on d 1, 4, 7, 14 and 21, respectively; maximal reduction occurred on d 7 ($P < .05$). Mean PUN levels on d 7 were 11.6, 11.3, 8.64, 7.92, 8.83, 10.1 and 7.86 for 0, .1, .5, 1, 2.5, 5 and 10 mg E_2 dosages, respectively.

Opposite, but similar, responses were observed in plasma E_2. Linear ($P < .005$), quadratic ($P < .05$) and cubic ($P < .01$) orthogonal and broken line regression on d 7 PUN indicated that PUN depression increased with E_2 TRT of .5 mg and above, with maximal reduction calculated to occur at a .7 mg E_2 implant dose (33 µg E_2/d). PUN reduction was similar ($P > .05$), however, for the .5, 1, 2.5 and 10 mg E_2 TRT. There were no main effect differences in daily gain ($P > .05$); however, when TRT effects were orthogonally partitioned, a linear ($P < .01$) increase in daily gain was observed with increasing levels of E_2. Results of this study indicate E_2 TRT reduces PUN in a dose-dependent manner.

(Key Words: 17-β Estradiol, Plasma Urea Nitrogen Depression, Steers, Anabolic Activity.)

Introduction

Since many of the effects of estrogen and somatotropin (growth hormone) are similar (improved daily gain and feed conversion as well as decreased urinary nitrogen, PUN and amino acid nitrogen, and resulting increased nitrogen retention and lean tissue deposition (Preston, 1968; Davis et al., 1970a,b; Grebing et al., 1970; Preston, 1975; Trenkle and Topel, 1978; Heitzman, 1979; Goldberg, et al., 1980; Buttery, 1983; Muir et al., 1983; Basson et al., 1985; Johnsson et al., 1985; Eisemann

et al., 1986; Gopinath and Kitts, 1986; Preston, 1987)), it has been postulated that the anabolic effect of estrogen is mediated through an increase in somatotropin secretion (Preston, 1975; Trenkle, 1976; Heitzman, 1979; Heitzman, 1981; Gopinath and Kitts, 1984; Preston, 1987).

Several studies have concluded, however, that the relationship between estrogen and somatotropin is additive; however, these studies were not obviously conducted at the optimum dosages for each hormone (Wolfrom and Ivy, 1985; Wolfrom et al., 1985; Ivy et al., 1986b; Roche and Quirke, 1986; Wagner et al., 1988a,b). Furthermore, little research has been reported on the optimum dosage of estrogen or somatotropin required to elicit maximal improvements in growth and lean tissue deposition in feedlot steers. Therefore, the objective of this experiment was to determine the optimum dosage of E_2 required to maximize PUN depression; plasma E_2 levels and gain were also evaluated.

Materials and Methods

Forty-two steers (343 kg) were blocked by weight into six pens with seven head per pen. Steers were fed ad libitum on a diet balanced to meet or exceed NRC (1984) requirements (Table 3.1). Pellets containing 17-β

estradiol (E_2; zero order release[1]) were implanted subcutaneously in the ear on d 0. Six steers were randomly allotted to each of the following E_2 treatments (one steer.pen^{-1}. treatment^{-1}): 0, .1, .5, 1, 2.5, 5 and 10 mg E_2/implant; two .5 mg E_2 implants were used to achieve the 1 mg E_2 dosage. These dosages were chosen so the .5, 1.0 and 2.5 mg E_2 dosages would be in the middle of the titration curve, giving reported daily payout concentrations of E_2 in commercially available implants (White, 1982; Basson et al., 1985) and the optimum dosage of E_2 indicated by Wagner et al. (1979) and Potter and Wagner (1987) for maximal improvements in daily gain. The trial was conducted for 21 d, the theoretical duration of E_2 release from the implants. Steers were weighed and blood samples taken via jugular vein puncture ipsilateral to the implant on d 0, 1, 4, 7, 14 and 21 at 1400 h, approximately 4 h post feeding. Samples were collected in heparinized tubes, transported on ice, centrifuged and stored refrigerated or at -20°C until analyzed for PUN and E_2, respectively. Hematocrits were determined using an Autocrit II[2] centrifuge. Samples were analyzed for PUN

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[1]Innovative Research of America, 3361 Executive Parkway, Toledo, OH 43606.

[2]Clay Adams, Division of Becton, Dickinson and Company, Parsippany, NJ 07054.

using a spectrophotometric assay (Chaney and Marbach,1962; Searle, 1984) and E_2 via a double antibody radioimmunoassay kit[3] modified for bovine plasma (Hancock, 1989).

Data were analyzed by analysis of variance as a split plot in time design for PUN and E_2 concentrations and hematocrit determinations using the General Linear Model Procedure (SAS, 1985). Effects included in the main plot analysis were treatment (E_2 dosage), pen and the treatment by pen interaction. The latter term was used as the error term to test main plot effects. Subplot effects included time, treatment by time interaction and residual effects which were used as the error term to test subplot effects. Treatment, time and treatment by time differences were tested by protected least significant difference. Linear, quadratic and cubic contrasts[4] were made among treatments to detect differences in E_2 dosage response over time. This was tested using a one-degree of freedom F-test.

Multiple regression analysis was performed to orthogonally partition the treatment, time and treatment by time effects to determine the optimum dosage of E_2 required for maximal depression in PUN. The optimum

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[3]Diagnostic Products Corporation, 5700 W. 96th St. Los Angeles, CA 90045.

[4]University of Missouri, Agricultural Experiment Station Statistician Department, Columbia, MO 65211.

dosage of E_2 was calculated by solving for the first derivative of the resulting regression equations and then solving for zero. Broken line regression analysis was also performed to determine optimum dosage of E_2. In this analysis, d 7 treatment effects on PUN were analyzed by linear regression. Dosages were serially deleted from the model starting at the upper end of the titration curve (i.e., 10, 5, 2.5 and 1, respectively). The following F test was performed to determine significant reductions in the residual sums of squares (RSS) resulting from dosage deletion:

$$F_{calc} = \frac{RSS1 - RSS2 \;/\; (df1 - df2)}{RSS2 \;/\; df2}$$

where RSS1 and RSS2 are the residual sums of squares and df1 and df2 are the associated degrees of freedom for the equation with n dosages included and for the equation with n - 1 dosages included, respectively. This F value was tested against $F_{df1 - df2,\ df2}$ at α=.10. The optimum E_2 dosage was determined to be the point of intersection of the resulting best fit linear regression line and the mean PUN concentration of all dosages demonstrating PUN reduction from analysis of variance.

Daily gain was determined for the overall 21-d period; therefore, the model for daily gain included treatment, pen and treatment by pen, with the latter term used as the error term. Treatment effects were orthogonally partitioned. A similar model was used for d 0 PUN concentrations.

Results and Discussion

Plasma E_2 concentrations were determined in order to evaluate release of E_2 from the implants. As d on trial increased, plasma E_2 concentrations also increased with increasing dosage of E_2, with maximum elevation observed ($P < .01$) on d 7 (Figure 3.1). Plasma E_2 concentrations declined towards baseline by d 21, the theoretical duration of E_2 release from the implants. Similar elevations have been reported in steers implanted with Synovex-S (Rumsey and Beaudry, 1979) and E_2 (Heitzman et al., 1981) and in calves implanted with Synovex-C (Castree, et al., 1988).

Plasma E_2 concentration increased as E_2 dosage increased, with .5 mg and above being higher ($P < .05$) than controls. The 5 mg implant did not ($P > .05$) elevate plasma E_2 over controls, nor did it differ from controls in the linear, quadratic or cubic responses over time ($P > .05$), possibly due to an improper E_2 level in this implant. In addition, the 1 mg implant dose, which was composed of two .5 mg implants, appeared to have a different E_2 release pattern in that plasma E_2 was elevated earlier and decreased sooner, as indicated by different ($P < .05$) linear and cubic responses over time compared to all other treatments. Therefore, the 1 and 5 mg implant dosages may have had problems associated with release and dosage, respectively.

On d 7, mean plasma E_2 concentrations were 5.32, 15.1, 40.9, 61.6, 37.3, 16.1 and 65.2 pg/ml for 0, .1, .5, 1, 2.5, 5 and 10 mg E_2 implant dosages, respectively. Heitzman et al. (1981) reported maximal improvements in daily gain when steers were implanted with E_2 plus trenbolone acetate (Revalor). This combination resulted in a slower, more sustained release of E_2 from the implant, which elevated plasma E_2 to 30 to 40 pg/ml and sustained plasma concentrations of E_2 at this level for 98 d. Implantation with E_2 alone only improved daily gain for 35 d, at which time plasma E concentrations were returning to baseline. In our study, implants elevated plasma E_2 concentrations to levels similar to or slightly above those reported by Heitzman et al. (1981). In addition, baseline (d 0) and plasma E_2 concentrations in steers receiving 0 mg E_2/implant were similar to values reported by Rumsey and Beaudry (1979) in non-implanted steers.

There were no treatment ($P = .08$) or treatment by time ($P = .77$) differences observed in hematocrit values. The mean hematocrit for the experiment was 40.3%.

As there were no treatment differences in PUN concentration on d 0 (avg = 12.6 mg/dl), d 0 was not included in the split plot in time analysis of variance for PUN depression. There were no treatment differences in PUN response ($P = .28$); however, time (Table 3.2;

P < .0001) and a trend for treatment by time (Figure 3.2 and Table 3.3; P = .08) differences were observed in PUN. Plasma urea nitrogen response over time was similar, but opposite, to the plasma E_2 response (Figure 3.2). The 1 and 5 mg implants gave a PUN response that reflected plasma E_2 concentrations in that PUN depression occurred faster and returned towards baseline earlier for the 1 mg implant, and the 5 mg implant gave a smaller PUN depression than several of the other implant dosages.

Estradiol treatment maximized the reduction in PUN on d 7 (P < .05) after which time PUN concentrations returned towards baseline values (Table 3.2). Preston (1968) reported similar observations when diethylstilbestrol (DES) was either fed or implanted in lambs. The PUN reduction was very dynamic, occurring within 24 h following DES administration, with maximal reduction occurring between 12 and 19 d, and then returning to baseline. It was suggested that the "rebound" observed following maximal PUN reduction may in part result from decreasing dosage. Implants used in our study were designed to give zero-order release. Therefore, release and plasma levels of E_2 should have been relatively constant for the 21-d period; however, plasma E_2 concentrations generally decreased after d 7. When DES was fed, a partial "rebound" was observed in PUN following maximal reduction.

On d 7, as E_2 implant dosage increased, PUN depression increased ($P < .05$) with an E_2 dosage of .5 mg and above (Table 3.3 and Figure 3.2). There was a negative correlation ($r = -.50$; $P < .0007$) between d 7 PUN and d 7 E_2 concentration.

The optimum dosage of E_2 required for maximal PUN reduction was calculated on d 7 PUN, the time of maximal reduction. Linear ($P < .01$), cubic ($P < .005$) and quartic ($P < .05$) orthogonal partition of the treatment effects indicated that as E_2 dosage increased, PUN depression increased, decreased and then increased, due primarily to the smaller response to the 5 mg implant in comparison to the other E_2 dosages (Figure 3.3).

Since there appeared to be problems associated with the 1 and 5 mg implant, they were deleted from the model for optimum dosage determination. This resulted in linear ($P < .005$), quadratic ($P < .05$) and cubic ($P < .01$) effects when treatment effects were partitioned (Figure 3.4). The cubic regression equation ($PUN = -.2533\ (D^3) + 3.2728\ (D^2) - 7.7883\ (D) + 11.8037$; $r^2 = .57$) was used to calculate the optimum dosage of E_2 required for maximal PUN reduction, by solving for the first derivative of the regression equation and then solving for zero. This gave optimum implant dosages of 1.4 and 7.2 mg E_2. Therefore, the optimum dosage was between .5 (from analysis of variance) and 7.2 mg E_2/implant. Since, the first

derivative was attempting to solve a polynomial equation to points including 0.5 mg E_2/implant and above which were not significantly different from each other, a broken line regression analysis was also used to determine the optimum dosage of E_2 for maximum PUN depression (Figure 3.5). The optimum E_2 implant dosage was determined to be .7 mg E_2 (33 µg of E_2/d). This dosage is similar to that reported by Wagner et al. (1979) and Potter and Wagner (1987) for maximal improvements in daily gain.

Although main effects indicated no difference ($P = .15$) in daily gain with increasing E_2 dosage, when the treatment effect was partitioned, a linear ($P < .01$) increase in daily gain was observed with increasing E_2 dosage (Figure 3.6). Wagner et al. (1979) and Potter and Wagner (1987) observed a plateau in daily gain with increasing E_2 dosages, however, their trials were conducted for a much longer period of time (i.e., 190 d) and are probably more realistic measures of a growth response than can be observed in a 21-d period.

In conclusion, PUN depression increased with an E_2 implant dosage of .5 mg and above, with maximal reduction in PUN calculated to occur with a .7 mg E_2 implant (33 µg E_2/d).

TABLE 3.1. COMPOSITION OF DIET[a]

Ingredient	%[b]
Steam flaked sorghum grain	30.00
Corn silage	42.00
Cottonseed hulls	10.00
Cottonseed meal	11.48
Cane molasses	4.00
Fat	.50
Urea	.49
Calcium carbonate	.84
Sodium chloride	.12
Vitamin A premix	.12
Trace mineral premix	.12
Tylosin premix	.33
Total	100.00

[a]Formulated to contain 16.3% CP; 3.21 Mcal DE/kg; 3.12% crude fat; 36% NDF; 30% roughage equivalent; .65% Ca; .37% P; .10% Na; .92% K; .17% S; 43 ppm Zn; 5221 IU/kg Vitamin A on a dry matter basis.

[b]As fed basis.

TABLE 3.2. EFFECTS OF TIME ON LEAST-SQUARES MEAN PLASMA UREA NITROGEN (PUN)[a]

Days on trial	PUN, mg/dl
1	13.6[b]
4	11.6[c]
7	9.5[d]
14	10.2[e]
21	11.7[c]

[a]Time effects ($P < .0001$; SE = .24).

[b,c,d,e]Means with different superscripts differ ($P < .05$).

TABLE 3.3. LEAST-SQUARES MEANS FOR PLASMA UREA NITROGEN (MG/DL) RESPONSE OVER TIME AS INFLUENCED BY E_2 IMPLANT DOSAGE[a]

E_2 implant	Days on trial				
	1	4	7	14	21
0 mg	12.7^{b}	11.5^{b}	11.5^{b}	11.3^{b}	12.0^{bc}
.1	14.2^{b}	12.7^{b}	11.3^{b}	11.8^{b}	13.1^{b}
.5	12.9^{b}	11.3^{b}	8.6^{c}	9.8^{bc}	11.0^{bc}
1.0	14.2^{b}	10.8^{b}	7.9^{c}	9.7^{bc}	12.6^{b}
2.5	14.2^{b}	11.5^{b}	8.8^{c}	9.6^{bc}	12.1^{bc}
5.0	13.9^{b}	12.1^{b}	10.1^{bc}	10.4^{bc}	11.1^{bc}
10.0	13.1^{b}	11.0^{b}	7.9^{c}	8.9^{c}	10.1^{c}

[a]Treatment by time interaction (P = .08; SE = .63).

[b,c]Means with different superscripts within E_2 dosage differ (P < .05).

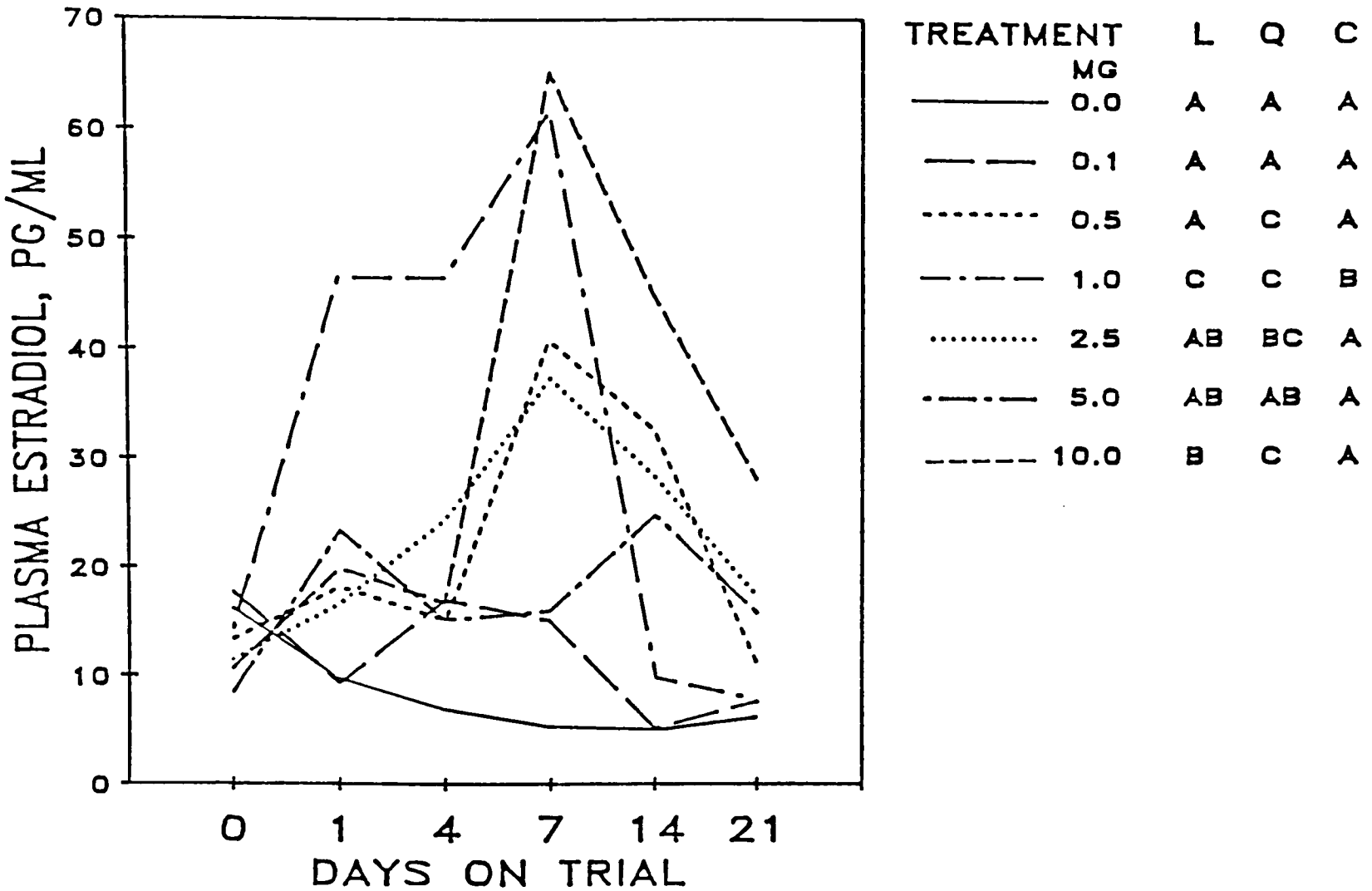

Figure 3.1. Least-squares means for plasma E_2 response over time in feedlot steers following E_2 implant administration. Treatment by time interaction ($P < .0001$; SE = 5.50). Significant linear (L), quadratic (Q) and cubic (C) contrasts ($P < .005$) among treatments as days on trial increased. Treatments having at least one letter in common are similar ($P > .05$) for contrast comparison.

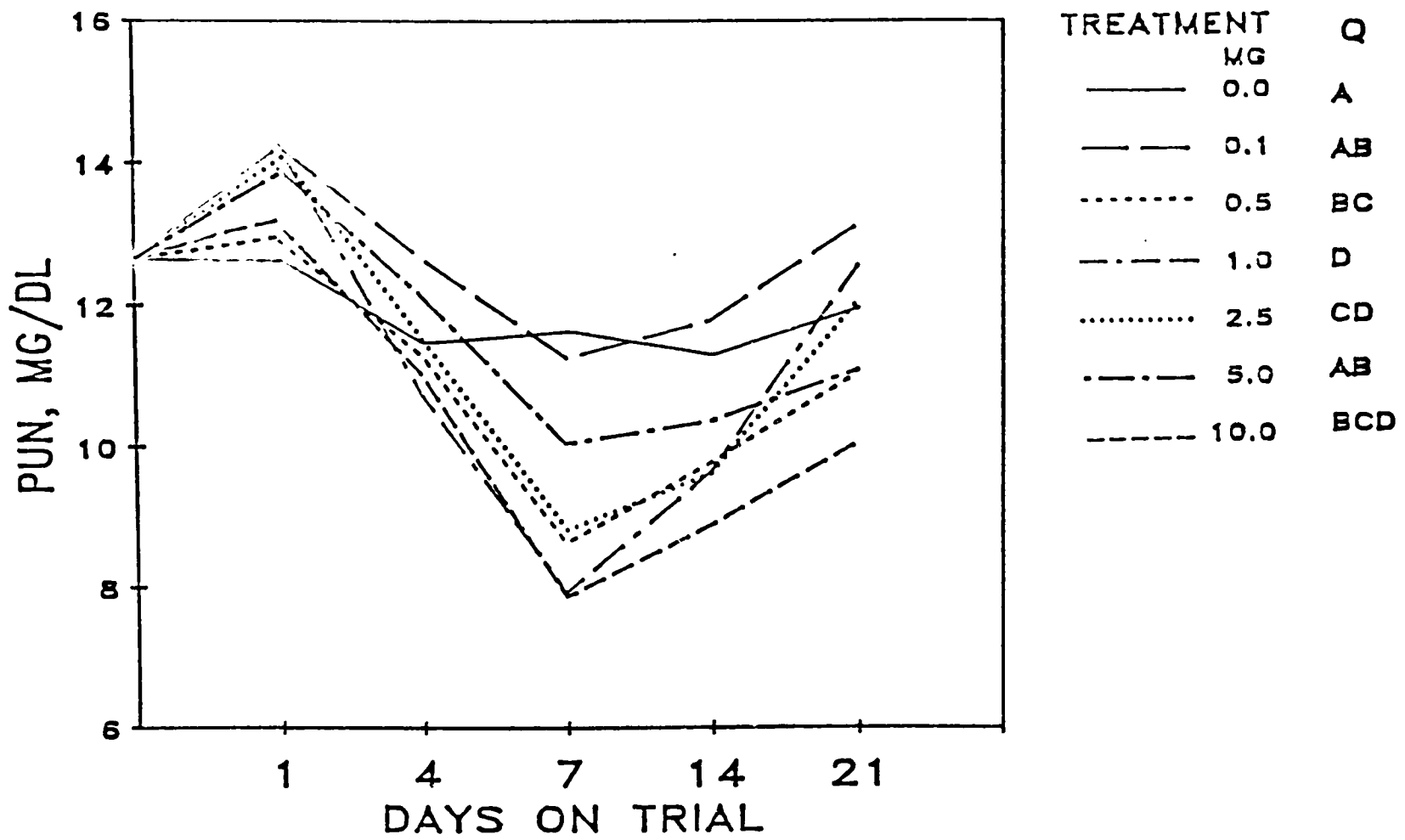

Figure 3.2. Least-squares means for plasma urea nitrogen (PUN) response over time in feedlot steers following E_2 implant administration. Treatment by time interaction ($P = .08$; $SE = .63$; $treatment_{linear}$ by $time_{linear}$ ($P < .05$); $treatment_{cubic}$ by $time_{quadratic}$ ($P < .005$). Significant quadratic (Q) contrasts ($P < .005$) among treatments as days on trial increased. Treatments having at least one letter in common are similar ($P > .05$) for contrast comparison.

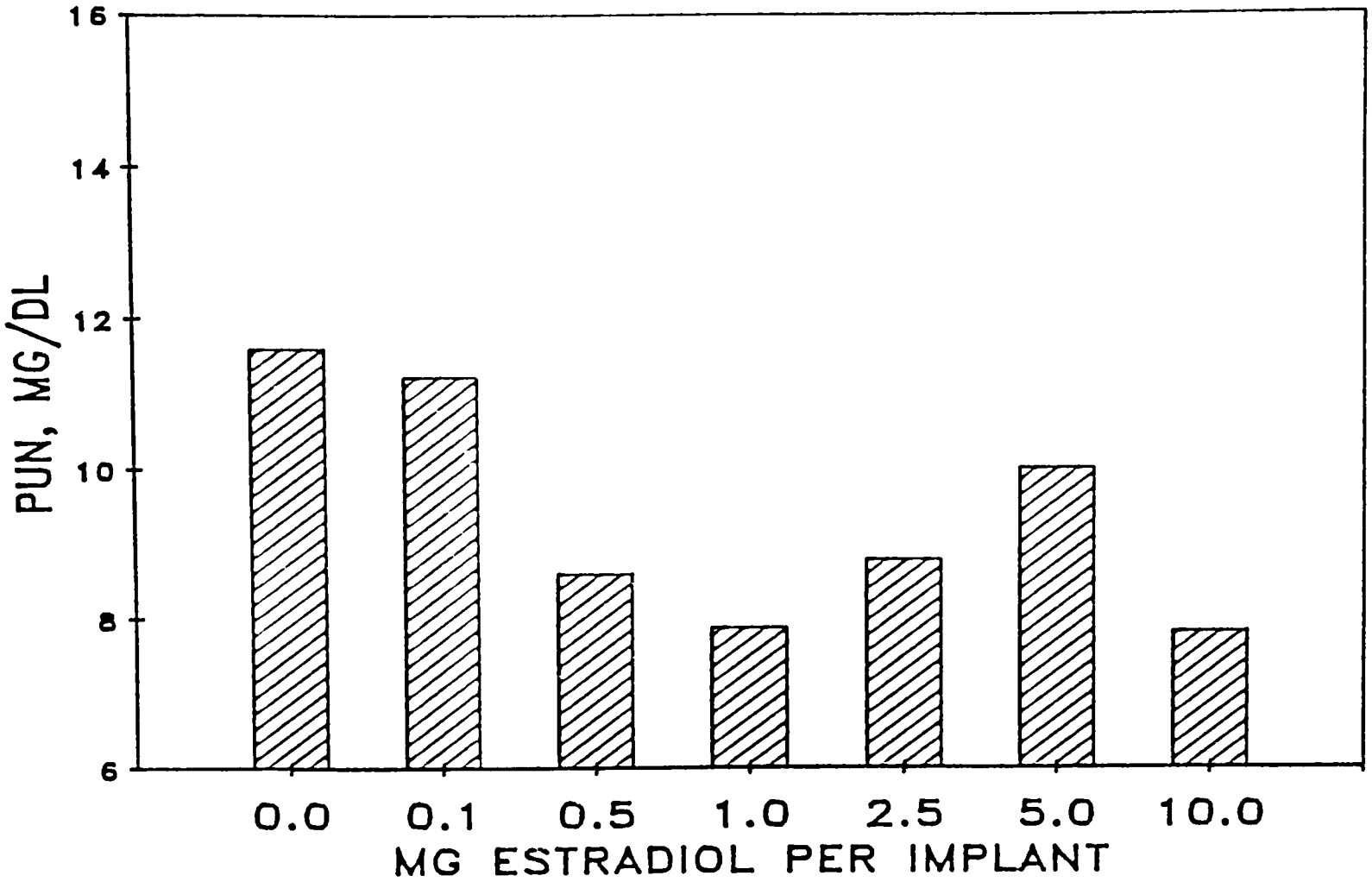

Figure 3.3. Least-squares means for day seven plasma urea nitrogen (PUN) response in feedlot steers following E_2 implant administration. Main effect of E_2 implantation ($P < .0006$; SE = .65). Linear ($P < .01$), cubic ($P < .005$) and quartic ($P < .05$) effects with increasing E_2 dosage.

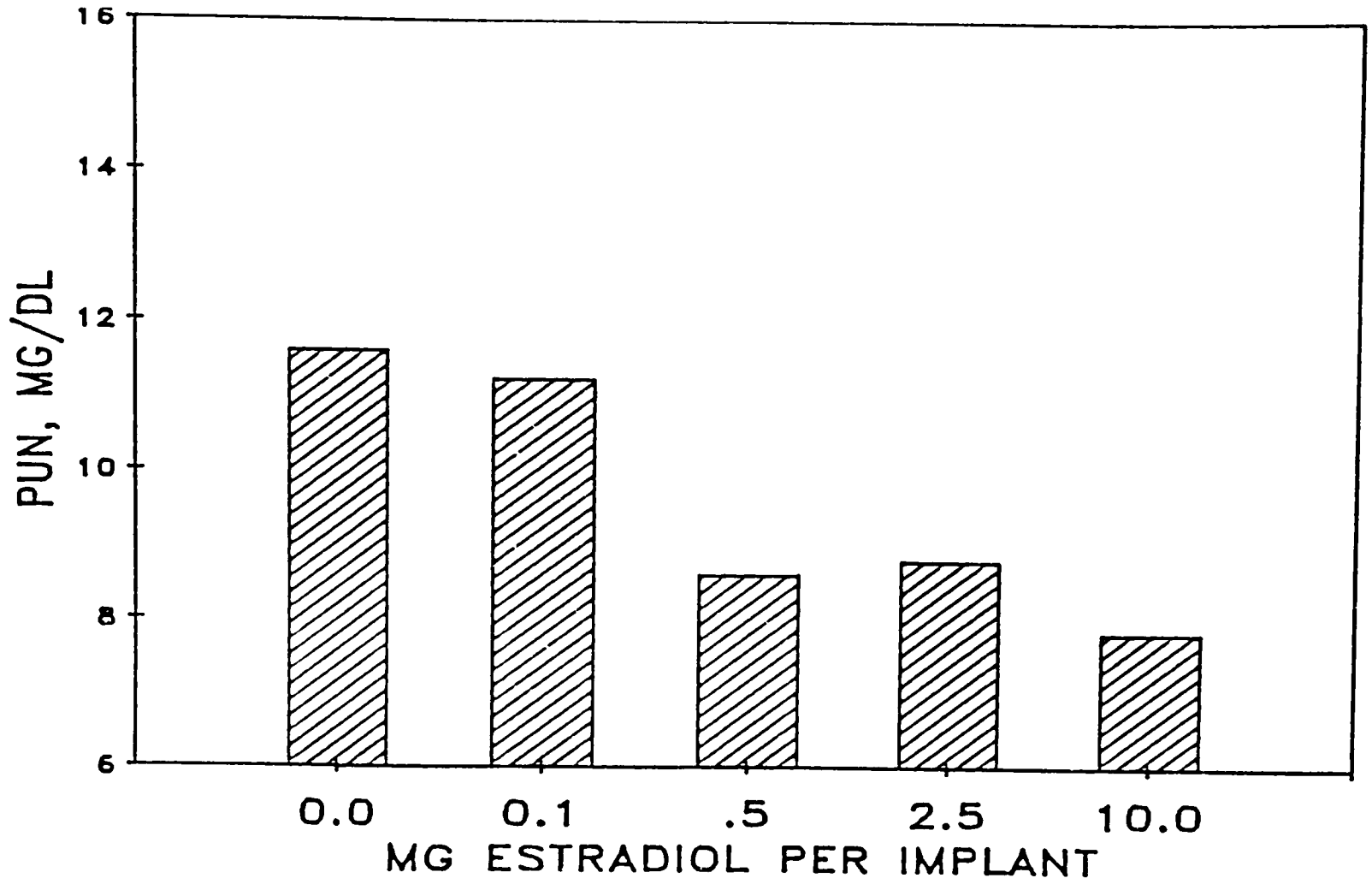

Figure 3.4. Least-squares means for day seven plasma urea nitrogen (PUN) response in feedlot steers following E_2 implant administration; deleting the 1 and 5 mg treatments (see text). Main effect of E_2 implantation ($P < .0012$; SE = .65). Linear ($P < .005$), quadratic ($P < .05$) and cubic ($P < .01$) effects with increasing E_2 dosage.

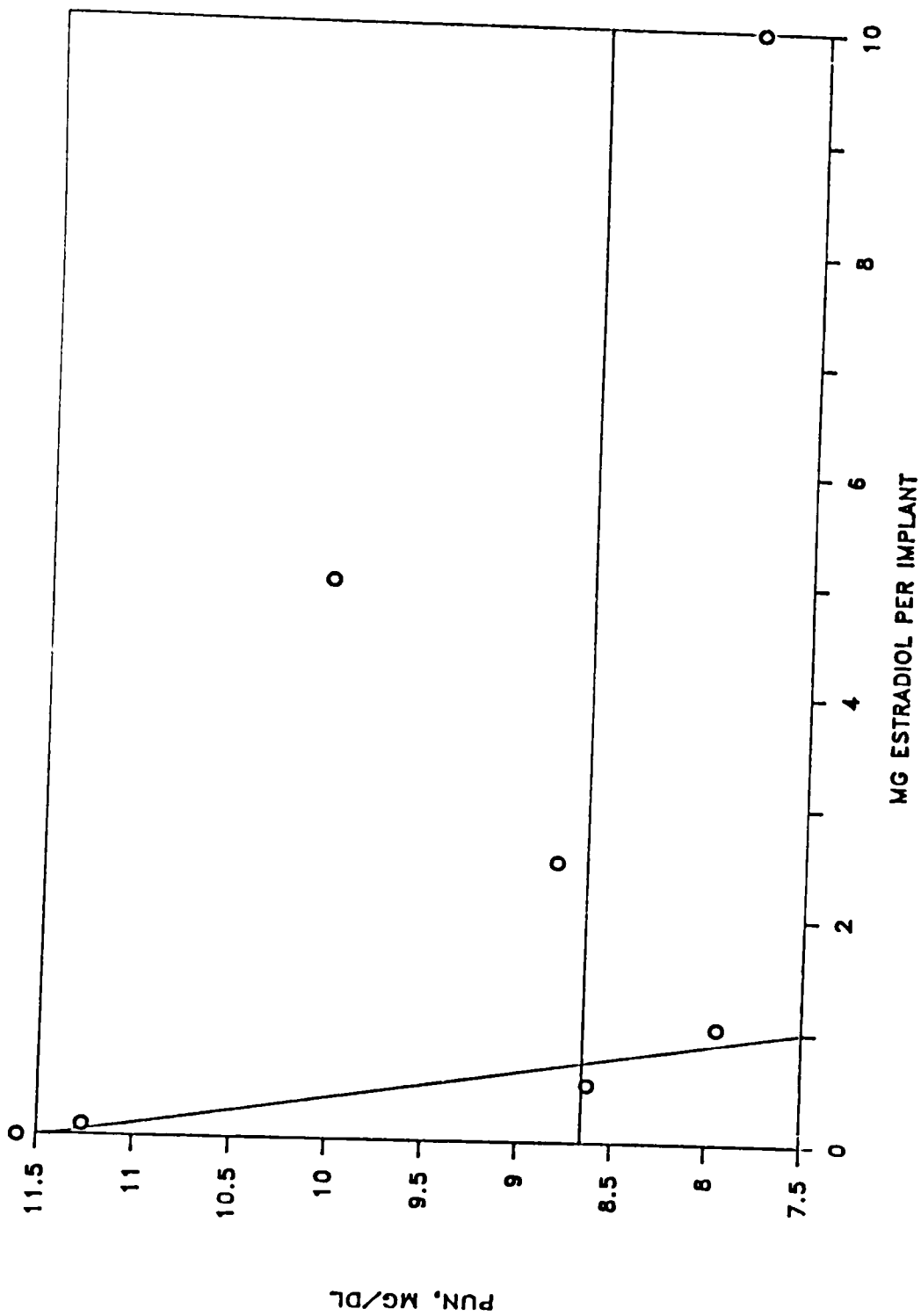

Figure 3.5. Broken line regression analysis for day seven plasma urea nitrogen (PUN) response in steers following E_2 implant administration. Optimum dosage = .7 mg E_2/implant.

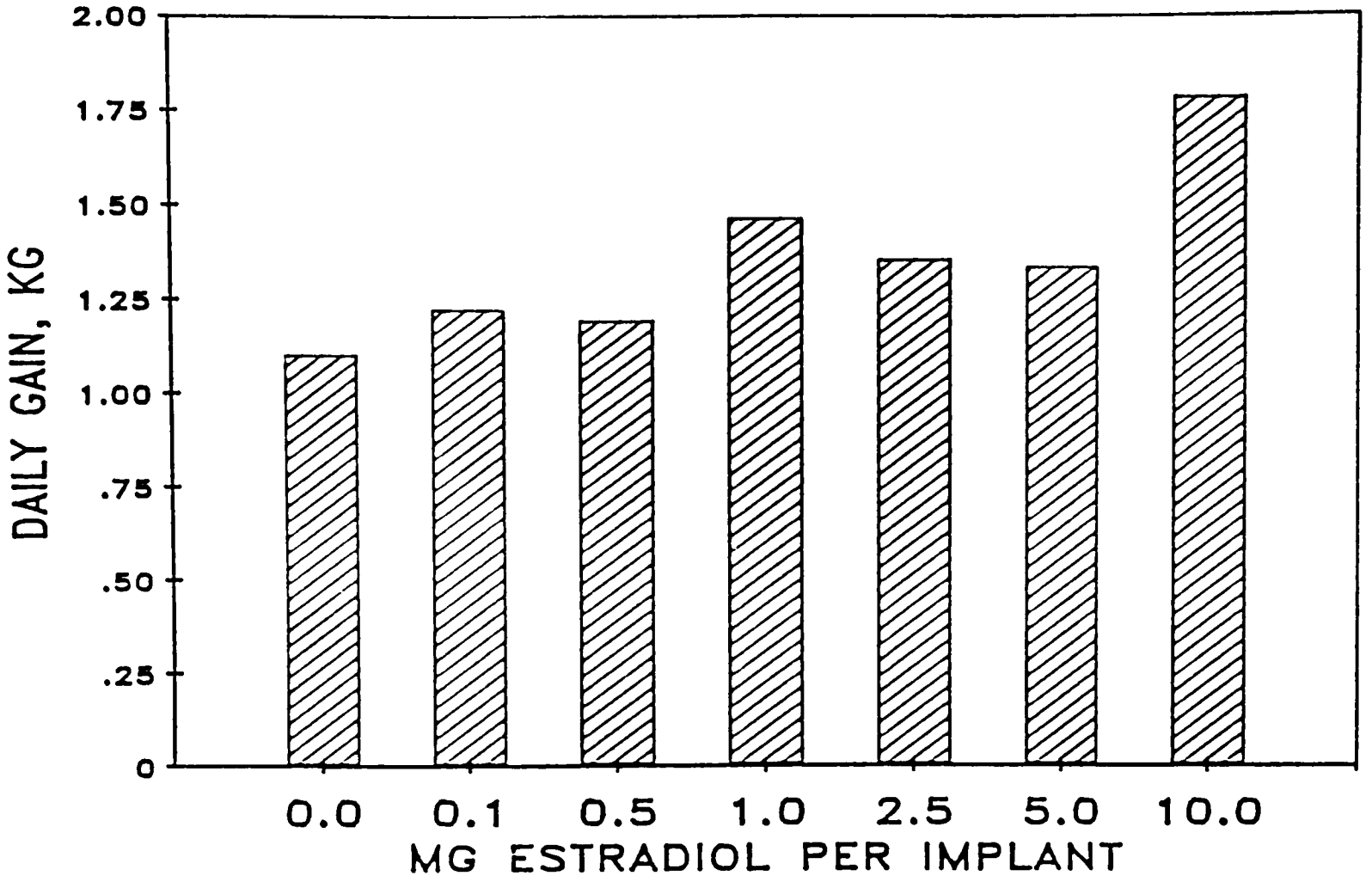

Figure 3.6. Least-squares means for daily gain response in feedlot steers following E_2 implant administration. Main effect of E_2 implantation (P = .1482; SE = .17). Linear (P < .01) increase in daily gain with increasing E_2 dosage.

CHAPTER IV

TITRATION OF THE RECOMBINANT BOVINE SOMATOTROPIN DOSAGE WHICH MAXIMIZES THE ANABOLIC RESPONSE IN FEEDLOT STEERS

Abstract

The objective of this study was to determine the optimum dosage of recombinant bovine somatotropin (bST) required to elicit maximum depression in plasma urea nitrogen (PUN), an indicator of anabolic activity. Twenty-four steers (389 kg) were blocked by weight into six pens. The bST vehicle was a .03 M sodium bicarbonate/.15 M sodium chloride buffer (pH = 9.4); injection solutions were prepared every 5 to 6 d. Six steers were placed on each of the following treatments (TRT): 0, 8, 16 and 32 mg bST/. Treatments were administered once daily via subcutaneous injections for 21 d. Steers were weighed and blood samples taken via jugular puncture on d 0, 1, 4, 7, 10, 13, 16 and 21 at 1400 h, approximately 4 h post feeding. Delta PUN (DPUN) was calculated as PUN - d 0 PUN. There were no TRT by time interactions (P = .80) in DPUN. Mean DPUN levels were -2.01, -3.50, -4.23, -3.39, -3.87, -4.23 and -3.88 on d 1, 4, 7, 10, 13, 16 and 21, respectively; bST TRT maximized the reduction in DPUN on d 7 (P < .05). Mean

DPUN levels were -2.13, -3.56, -4.37 and -4.29 for 0, 8, 16 and 32 mg bST/d, respectively. Linear ($P < .01$) and quadratic ($P < .05$) orthogonal contrasts indicated that DPUN depression increased with bST administration, with maximal reduction calculated to occur with 23 mg (59 µg/kg) bST/d. The equation which best described DPUN depression was: $DPUN = .00455 (TRT^2) - .213 (TRT) - 2.134$ ($r^2 = .51$). As bST dosage increased, daily gain increased and then plateaued between 16 and 32 mg bST/d, with maximal increases in daily gain calculated to occur with 24.5 mg bST. Results of this study indicate that bST reduces PUN in a dosage dependent manner.
(Key Words: Bovine Somatotropin, Plasma Urea Nitrogen Depression, Anabolic Activity.)

Introduction

Since many of the anabolic effects of estrogen and somatotropin (growth hormone) are similar (improved daily gain and feed conversion as well as decreased urinary nitrogen, PUN and amino acid nitrogen, and resulting increased nitrogen retention and lean tissue deposition (Preston, 1968; Davis et al., 1970a,b; Grebing et al., 1970; Preston, 1975; Trenkle and Topel, 1978; Heitzman, 1979; Goldberg et al., 1980; Buttery, 1983; Muir et al., 1983; Basson et al., 1985; Johnsson et al., 1985; Eisemann et al., 1986; Gopinath and Kitts, 1986; Preston, 1987)),

it has been postulated that the anabolic effect of estrogen is mediated through an increase in somatotropin secretion (Preston, 1975; Trenkle, 1976; Heitzman, 1979; Heitzman, 1981; Gopinath and Kitts, 1984; Preston, 1987).

Several studies have concluded that the relationship between estrogen and somatotropin is additive; however, these studies were not obviously conducted at the optimum dosage for each hormone (Wolfrom and Ivy, 1985; Wolfrom et al., 1985; Ivy et al., 1986b; Roche and Quirke, 1986; Wagner et al., 1988a,b). Furthermore, little research has been reported on the optimum dosage of estrogen or somatotropin required to elicit maximal improvements in growth and lean tissue deposition in feedlot steers. Therefore, the objective of this experiment was to determine the optimum dosage of bST required to maximize PUN depression; plasma bST and gain were also evaluated.

Materials and Methods

Twenty-four steers (389 kg) were blocked by weight into six pens with four head per pen. Steers were fed ad libitum on a diet balanced to meet or exceed NRC (1984) requirements (Table 4.1). Six steers were randomly allotted to each treatment (one steer.pen^{-1}.treatment^{-1}). The treatments were 0, 8, 16 and 32 mg recombinant bovine

somatotropin[1] (bST).head^{-1}.d^{-1}. The bST injection vehicle was a .03 M sodium bicarbonate/.15 M sodium chloride buffer (pH = 9.4). Injection solutions were prepared every 5 or 6 d. Treatments were administered once daily via subcutaneous injections in the front shoulder, alternating injection site every other d. The trial was conducted for 21 d, similar in duration to our previous 17-β estradiol dose titration study (Hancock and Preston, 1989a). Steers were weighed and blood samples taken via jugular vein puncture on d 0, 1, 4, 7, 10, 13, 16 and 21 at 1400 h, approximately 4 h post feeding. Samples were collected in tubes containing disodium EDTA, transported on ice, centrifuged and the plasma stored refrigerated or at -20°C until analyzed for PUN and bST, respectively. Hematocrits were determined using an Autocrit II[2] centrifuge. Samples were analyzed for PUN using a spectrophotometric assay (Chaney and Marbach, 1962; Searle, 1984) and bST via a double antibody radioimmunoassay (Hancock, 1989). Delta PUN (DPUN) was calculated as PUN concentration on the d collected minus PUN concentration on d 0 and was used to evaluate PUN depression.

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Data were analyzed by analysis of variance as a split plot in time design for DPUN and hematocrit determinations using the General Linear Model Procedure (SAS, 1985). Effects included in the main plot analysis were treatment (bST dosage), pen and the treatment by pen interaction. The latter term was used as the error term to test main plot effects. Subplot effects included time, treatment by time interaction and the residual effects which were used as the error term to test subplot effects. Treatment and time differences were tested by protected least significant difference.

Multiple regression analysis was performed to orthogonally partition the treatment and time effects to determine the optimum dosage of bST required for maximal depression in PUN. The optimum dosage of bST was calculated by solving for the first derivative of the resulting regression equation and then solving for zero.

Daily gain was determined for the overall 21-d period and the model for daily gain included treatment, pen and treatment by pen, with the latter term used as the error term. Treatment effects were tested by LSD and orthogonally partitioned. Day 0 PUN concentrations were also evaluated for initial differences using the above model.

Results and Discussion

There were initial differences observed (P = .04) in PUN concentrations (12.5, 11.1, 11.2 and 10.0 mg/dl on d 0 for steers on the 0, 8, 16 and 32 mg bST TRT, respectively). Not only were the initial PUN concentrations different, they also decreased with increasing bST dosage to be applied. Therefore, delta PUN, the difference in concentration on the d collected minus the concentration on d 0, was utilized to evaluate PUN depression.

No treatment (P = .70) or treatment by time (P = .14) effects were observed in hematocrit percentages. The mean hematocrit for the experiment was 44.1%.

There were no (P = .94) treatment by time interactions in the DPUN response (Figure 4.1). Partition of the time effects (P < .0001), indicated linear, quadratic, cubic and quartic (P < .005) effects in DPUN over time (Figure 4.1). As days on trial increased, PUN depression increased with maximal reduction in DPUN occurring on d 7 (P < .05; Table 4.2), after which time DPUN concentrations increased, decreased and then increased again, accounting for the linear, quadratic, cubic and quartic effects.

As cited by Bauman and McCutcheon (1986), daily ST dosages of 50 to 400 µg/kg have been used in cattle and sheep, resulting in improvements in growth rate and nitrogen retention, however, there has been only one

reported dose response study (Ivy et al., 1986b). In our bST dose response study, linear ($P < .01$) and quadratic ($P < .05$) orthogonal contrasts indicated that DPUN depression increased with increasing doses of bST administration, then plateaued between 16 and 32 mg bST/d (Figure 4.2). The optimum dosage (D) of bST required for maximal PUN reduction was calculated by solving for the first derivative of the quadratic regression equation ($DPUN = .00455\ (D^2) - .21304\ (D) - 2.13385$; $r^2 = .51$) and then solving for zero. Maximal PUN reduction was calculated to occur with 23 mg of bST/d, which corresponds to 59 $\mu g.kg^{-1}.d^{-1}$.

Daily gain for the 21-d period is reported in Figure 4.3. Daily gains for all steers were somewhat low (.2 to .8 kg/d). These steers were brought from feedlot pens to a working area every d for bST injections and, therefore, handling was greater than under normal feedlot circumstances. There was a significant linear effect ($P < .05$) and a trend for a quadratic effect ($P = .19$) in daily gain with increasing bST dose. Similar to the DPUN response, as bST dose increased, daily gain increased and then tended to plateau between 16 and 32 mg bST/d; these gains were higher ($P < .05$) than gains observed in the control steers. Maximal increases in daily gain were calculated to occur with 24.5 mg bST/d, which corresponds to 63 $\mu g.kg^{-1}.d^{-1}$ (daily gain = $-.001075\ (D^2) + .052773$

(D) + .161514; r^2 = .35). These results support the optimum dose obtained with the PUN depression response. There was a trend for a negative correlation between daily gain and DPUN on d 7 (r = -.32; P < .13).

Results of our study are in accordance with those of Ivy et al., (1986b) who titrated pituitary derived bST dosages of 0, 6, 12 and 24 mg in steers (356 kg) for a duration of 42 d and found an optimum dosage of 15 mg (42 µg/kg) bST/d for maximal improvements in gain and feed conversion. Unlike our study, however, they did not observe a quadratic decrease in blood urea nitrogen with increasing levels of bST, rather they observed a linear decrease.

Plasma bST concentrations are reported in Figure 4.4. The 0 mg bST treatment represents the mean plasma bST concentration of all steers on d 0 and for the 0 mg bST treatment over time. A 95% confidence interval around this mean was determined to have lower and upper limits of 0 and 45 ng bST/ml, respectively. In looking at these data, it is important to bear in mind that blood samples were collected once daily, 24 h post bST injection, that both biological and analytical variations were large (SE = 9.97 ng/ml) and plasma bST concentrations in the 0 mg bST treated steers (controls) were several fold greater than reported concentrations of circulating bST in nontreated steers (Moseley et al., 1982; Grigsby and Trenkle, 1986;

Breier et al., 1988b; Wagner et al., 1988a,b). The episodic bST release and plasma bST evaluation which may relate to the handling required for daily injections of bST, limits the practicality of bST use in the feedlot industry. Despite these problems, it is interesting to note that higher plasma concentrations of bST, but still within the 95% confidence interval, were observed for steers receiving 32 mg bST/d. As the optimum dose of bST required for maximal PUN depression, or anabolic activity, was determined to be between the 16 and 32 mg doses, the elevation in plasma bST on d 7 for the 32 mg treatment may reflect an excess of bST above that required for the anabolic response.

In conclusion, results indicated that decreased PUN and daily gain increased with increasing bST administration, and that maximal reduction in PUN and increased daily gain were calculated to occur with 23 and 24.5 mg of bST/d, respectively, or about 60 $\mu g.kg^{-1}.d^{-1}$.

TABLE 4.1. COMPOSITION OF DIET[a]

Ingredient	%[b]
Steam flaked sorghum grain	35.10
Corn silage	42.00
Cottonseed hulls	10.00
Cottonseed meal	8.52
Cane molasses	2.00
Fat	.40
Urea	.45
Calcium carbonate	.84
Sodium chloride	.12
Vitamin A premix	.12
Trace mineral premix	.12
Tylosin premix	.33
Total	100.00

[a]Formulated to contain 14.8% CP; 3.24 Mcal DE/kg; 3.10% crude fat; 36% NDF; 30% roughage equivalent; .62% Ca; .34% P; .10% Na; .81% K; .15% S; 40 ppm Zn; 5214 IU/kg Vitamin A on a dry matter basis.

[b]As fed basis.

TABLE 4.2. EFFECTS OF TIME ON LEAST-SQUARES MEANS FOR DELTA PLASMA UREA NITROGEN (DPUN)[a]

Days on trial	DPUN, mg/dl
1	-2.01[b]
4	-3.50[cd]
7	-4.23[e]
10	-3.39[c]
13	-3.87[de]
16	-4.23[e]
21	-3.88[de]

[a]Time effects ($P < .0001$; SE = .17).

[b,c,d,e]Means with different superscripts differ ($P < .05$).

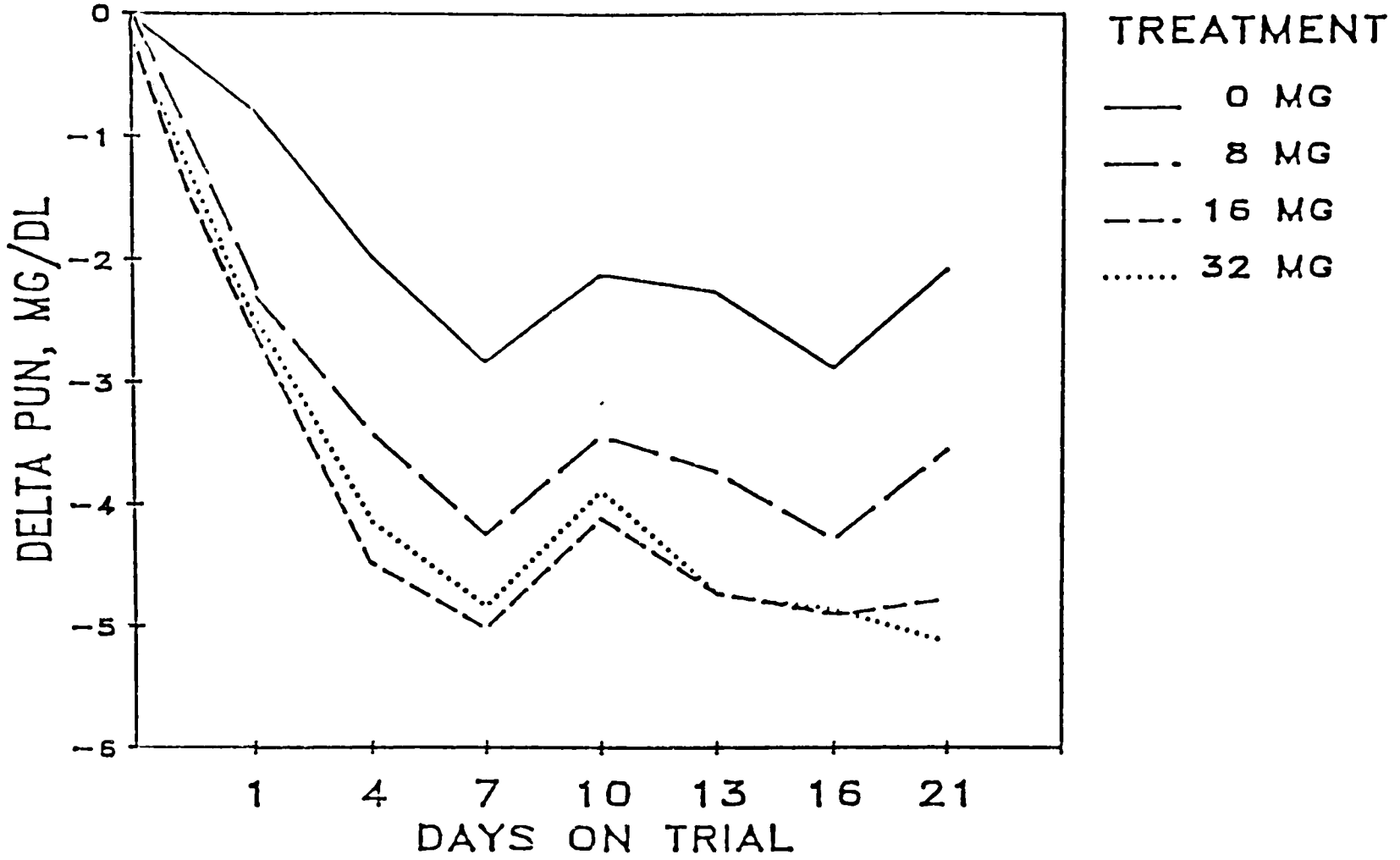

Figure 4.1. Least-squares means for delta plasma urea nitrogen (DPUN) response over time in feedlot steers following somatotropin administration. Time effects ($P < .0001$; SE = .17). Partition of time effects indicated linear, quadratic, cubic and quartic effects in DPUN ($P < .005$). Treatment by time interaction ($P = .94$; SE = .33).

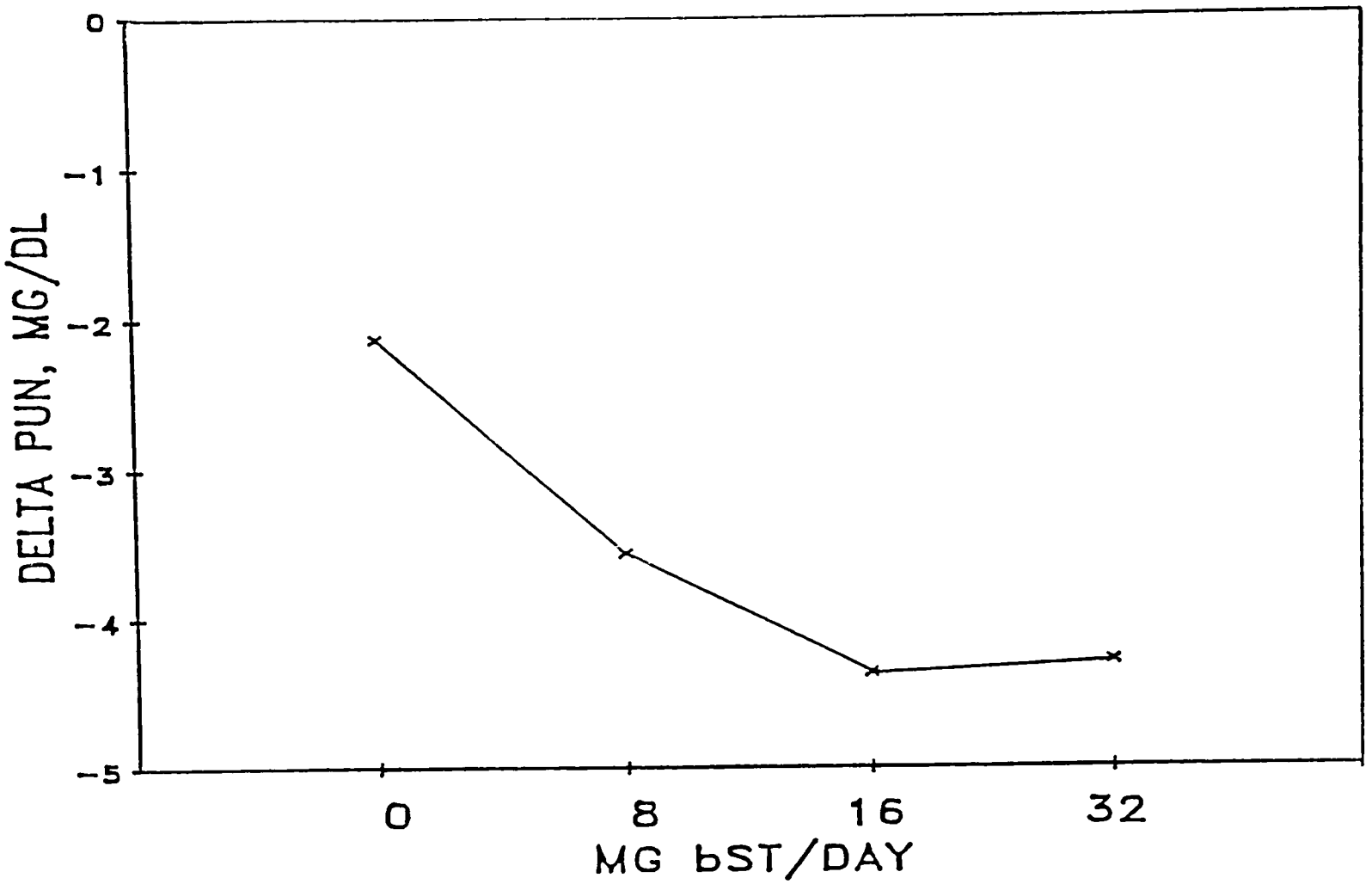

Figure 4.2. Least-squares means for main effects for delta plasma urea nitrogen (DPUN) response in feedlot steers following somatotropin administration. Treatment effects ($P < .01$; SE = .46). Linear ($P < .01$) and quadratic ($P < .05$) increase in DPUN with increasing somatotropin dosage.

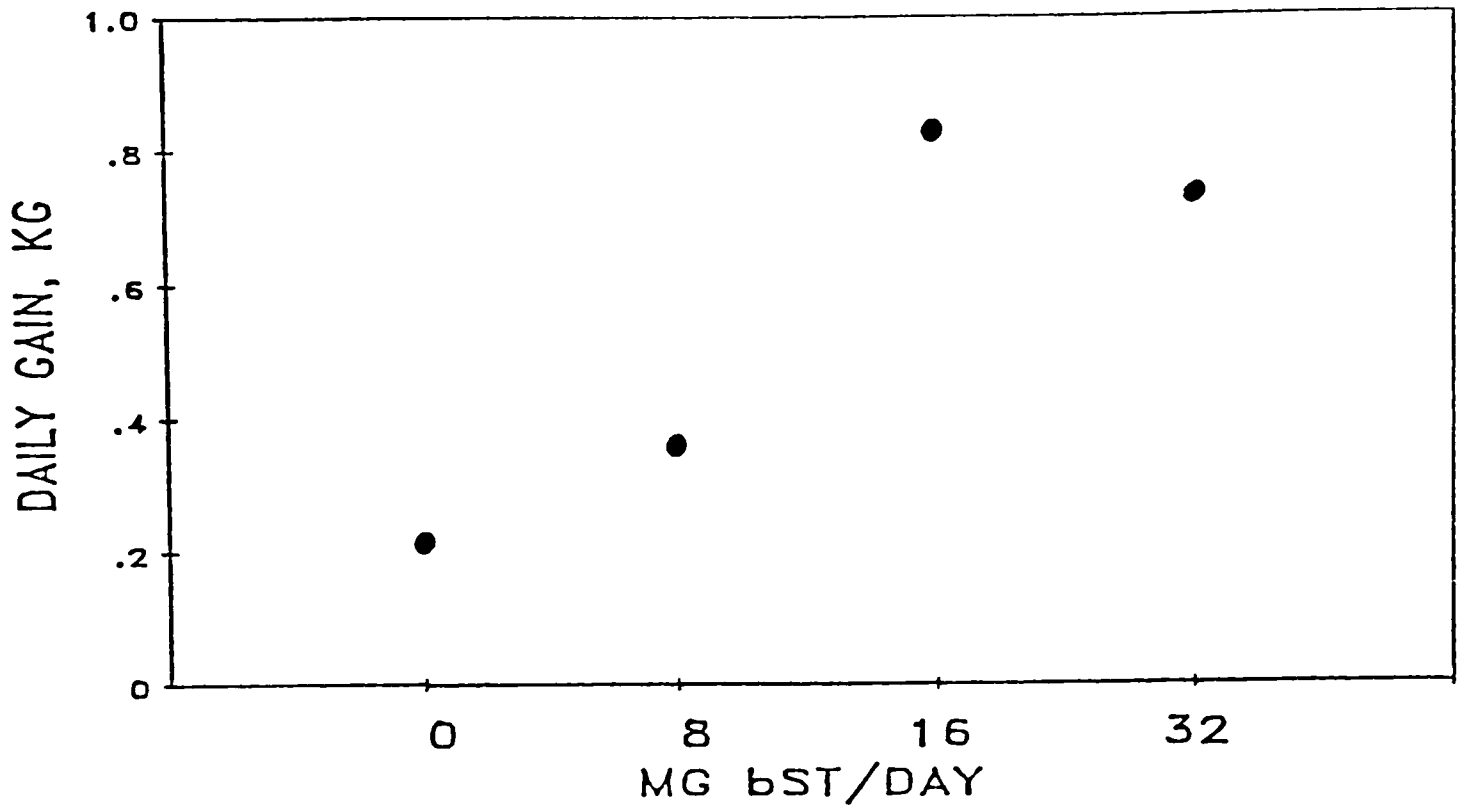

Figure 4.3. Least-squares means for main effects for daily gain in feedlot steers following somatotropin administration. Treatment effects ($P < .05$; SE = .19). Linear ($P < .05$) and quadratic ($P = .19$) increase in daily gain with increasing somatotropin dosage.

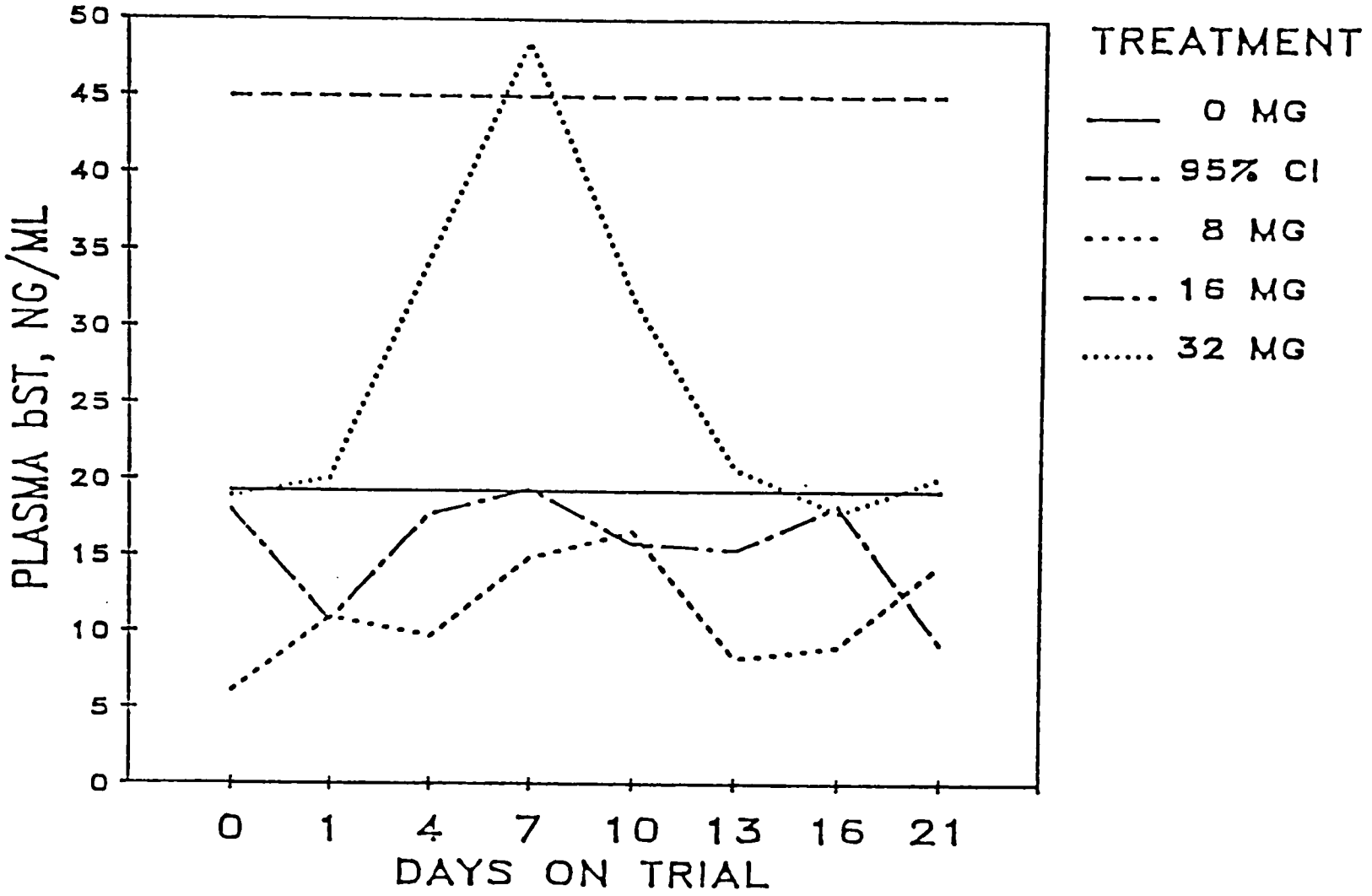

Figure 4.4. Means for plasma somatotropin response over time in feedlot steers following somatotropin administration (SE = 9.97 ng/ml).

CHAPTER V

INTERACTION BETWEEN 17-β ESTRADIOL AND RECOMBINANT BOVINE SOMATOTROPIN IN THE ANABOLIC RESPONSE OF FEEDLOT STEERS

Abstract

To evaluate the mechanism of anabolic estrogen action in ruminants, three trials were conducted to determine if the anabolic actions of 17-β estradiol (E_2) and recombinant bovine somatotropin (bST) are additive. In trials 1 and 2, 54 British type steers were randomly allotted to treatments in a 3 X 3 factorial design (0, .5 and 1 mg E_2/implant and 0, 41 and 82 μg bST.kg^{-1}.d^{-1} in trial 1; 0, 1 and 2 mg E_2/implant and 0, 41 and 82 μg bST.kg^{-1}.d^{-1} in trial 2). In trial 3, 32 British type steers were randomly allotted to treatments in a 2 X 2 factorial design (0 and 2 mg E_2/d; 0 and 82 μg bST.kg^{-1}.d^{-1}, both via daily injections). In trial 1, as E_2 dosage increased, there was a linear ($P < .001$) increase in plasma E_2 and quadratic ($P < .05$) increases in plasma bST and daily gain. In trial 2, although there was a 20% increase in plasma E_2 following E_2 implantation this was not significant ($P > .10$), however, on d 7, there was a linear increase in plasma E_2 collected ipsilateral ($P < .10$) and contralateral ($P < .05$) to the implant. These increases were approximately 20 and 60% for steers

implanted with 1 and 2 mg E_2, respectively, which were similar to the percent improvements observed over all d on trial in trial 1. In trials 1 and 2, implantation with E_2 failed ($P > .10$) to reduce PUN. Despite this lack of E_2 effect, in general, when E_2 was administered in combination with bST, there was an additional (9-10%) depression in PUN, compared to the reduction in PUN with bST alone, suggesting possible additivity with these combinations. Plasma E_2 concentrations in non-implanted steers were several fold higher than expected and may explain the lack of PUN response to E_2 implantation. As bST dosage increased, linear increases were observed in PUN depression in trials 1 ($P < .005$) and 2 ($P < .001$). In trial 3, administration of E_2 elevated plasma E_2 (39% increase; $P < .003$). Plasma E_2 concentrations in non-treated steers, however, were elevated as in trials 1 and 2. On d 7, administration of bST elevated ($P < .03$) plasma bST. Administration of both E_2 and bST decreased PUN ($P < .0002$). There was no interaction between E_2 and bST administration ($P > .10$), indicating additivity between these two anabolic agents. Plasma urea nitrogen was reduced by 24 and 29% with E_2 and bST administration, respectively. When E_2 and bST were administered together, PUN was reduced by 44%. Results indicate an additive effect with combined administration of the two anabolic agents. Therefore, the anabolic actions of E_2 appear to

be by some other mechanism or in addition to increased endogenous bST secretion.

(Keywords: 17-β Estradiol, Bovine Somatotropin, Anabolic, Additive, Plasma Urea Nitrogen Depression, Steers.)

Introduction

Due to the similarities in the anabolic effects of estrogen and somatotropin (ST) it has been postulated that the anabolic effect of estrogen is mediated through an increase in ST secretion (Preston, 1975; Trenkle, 1976; Heitzman, 1979; Heitzman, 1981; Gopinath and Kitts, 1984; Preston, 1987; Hancock and Preston, 1989a,b). However, ST stimulates protein synthesis without altering protein degradation (Bergen, 1974; Goldberg et al., 1980; Buttery, 1983) and it appears that estrogens do not stimulate muscle protein synthesis (Roeder et al., 1986; Tucker and Merkel, 1987), but rather decrease protein degradation.

Furthermore, as recently reviewed by Preston (1987), estrogens increase both synthesis and release of ST from rat pituitary cells in vitro (Simard et al., 1986) and increase plasma ST in vivo in rats (Lloyd et al., 1971). Yet there is no observed anabolic response from estrogens in rats (Preston, 1975). Administration of ST, however, does promote anabolic growth in rats and has been used to evaluate the biopotency of ST (Groesbeck and Parlow, 1987).

Due to the equivocal results regarding ST as the mediator of anabolic estrogen action, research is required to specifically answer this question. Several studies have concluded that the relationship between estrogen and ST is additive; these studies were not obviously conducted, however, at the optimum dosages for each hormone (Wolfrom and Ivy, 1985; Wolfrom et al., 1985; Ivy et al., 1986b; Roche and Quirke, 1986; Wagner et al., 1988a,b).

The objective this research was to test the optimum dosage of E_2 and ST individually and in combination to determine if their anabolic effects are additive or not in feedlot steers, thus indicating whether or not ST mediates estrogen action.

Materials and Methods

Trial 1. Fifty-four British type steers (370 kg) were blocked by weight and breed type (27 red baldy and 27 black baldy steers, three pens each) into six pens with nine head per pen. Steers were fed ad libitum on a diet balanced to meet or exceed NRC (1984) requirements (Table 5.1). Treatments were arranged in a 3 X 3 factorial design. The two factors were 17-β estradiol[1] (E_2; 0, .5

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and 1 mg/implant) and recombinant bovine somatotropin[2] (bST; 0, 41 and 82 μg.kg^{-1}.d^{-1}). Six steers were randomly allotted to each of the above treatments or combination thereof (one steer.pen^{-1}.treatment combination^{-1}). The .5 mg E_2 and 41 μg bST dosages were previously determined to be the minimum dosage of each hormone required for maximal plasma urea nitrogen (PUN) reduction, an indicator of anabolic activity (Hancock and Preston, 1989a,b). Therefore, these dosages (and a dosage two-fold higher) were tested individually and in combination to determine if the anabolic effects of E_2 and bST are additive or not.

The trial was conducted for 7 d, the minimum time required for maximal PUN reduction for both E_2 and bST (Hancock and Preston, 1989a,b). Pellets containing E_2 were implanted subcutaneously in the right ear on d 0. The bST injection vehicle was a .03 M sodium bicarbonate buffer in .15 M sodium chloride (pH = 9.4). Injection solutions were prepared every 3 or 4 d and bST treatments were administered once daily via subcutaneous injections in the front shoulder, alternating injection site every other d. Steers were weighed and blood samples taken via jugular vein puncture ipsilateral to the implant on d 0, 1, 4 and 7 at 1400 h, approximately 5 h post feeding. On

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d 7, a blood sample was also collected contralateral to the implant to evaluate E_2 clearance. Samples were collected in heparinized tubes, transported on ice, centrifuged and the plasma stored at -20°C until analyzed for PUN, amino acid nitrogen (AAN), E_2, bST and glucose. Hematocrits were determined using an Autocrit II[3] centrifuge. Samples were analyzed for PUN (Chaney and Marbach, 1962; Searle, 1984), glucose[4] and AAN (Goodwin 1968; Gopinath and Kitts, 1986) using spectrophotometric assays and E_2[5] and bST via double antibody radioimmunoassays (Hancock, 1989).

Data were analyzed by analysis of variance as a split plot in time design using the General Linear Model Procedure (SAS, 1985). Effects included in the main plot analysis were breed type, pen within breed type, E_2, bST, and the interaction between E_2 and bST. The combined interactions (E_2 by bST by breed type and E_2 by bST by pen within breed type) were used as the error term to test main plot effects. Subplot effects included time (d on trial), and the interactions of time by E_2, time by bST,

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time by E_2 by bST and the residual effect, which was used as the error term to test subplot effects. Least-squares means are reported.

Multiple regression analysis were performed to orthogonally partition the effects of E_2, bST, E_2 by bST, time and interactions thereof.

Daily gain was determined for the overall 7-d period; therefore, the model for daily gain included only main plot effects indicated above. A similar model was used to evaluate d 0 PUN, d 7 ipsilateral plasma E_2, d 7 contralateral plasma E_2 and the difference between ipsilateral and contralateral samples.

Trial 2. Fifty-four British type steers (with some Brahman breeding; 369 kg) were blocked by weight and breed type {27 red baldy (three pens), 9 red solid (one pen), 9 black baldy (one pen) and 9 black solid (one pen)} into six pens with nine head per pen. This trial was identical to trial 1 except E_2 dosages were 0, 1 and 2 mg/implant. Dosage was increased in this trial due to a lack of PUN response in trial 1. The data were analyzed using a similar statistical model as reported in trial 1, except breed type was not included in the model as there was only one pen for three of the types used.

Trial 3. Thirty-two British type steers (361 kg) were blocked by weight and type (4 red baldy, 13 black solid, 15 black baldy) into eight pens with four head per

pen. Steers were fed ad libitum on a diet balanced to meet or exceed NRC (1984) requirements (Table 5.1). Treatments were arranged in a 2 X 2 factorial design. The two factors were F_2 (0 and 2 mg/d) and bST (0 and 82 $\mu g.kg^{-1}.d^{-1}$). Eight steers were randomly allotted to each of the above treatments or combination thereof (one steer.pen^{-1}.treatment $combination^{-1}$). The E_2 was dissolved in 100% ethanol and was administered via daily subcutaneous injections. This E_2 injection dosage (2 mg/d) was chosen based on preliminary PUN response studies (see Appendix A, Hancock, 1989). Other procedures were similar to those used in trials 1 and 2. Plasma samples were analyzed for PUN and E_2 on d 0, 1, 4 and 7 and for bST on d 7. The statistical model was similar to that used in trial 2.

Results and Discussion

Trial 1. Main plot effects over time for E_2 and bST on mean plasma E_2 concentrations are reported in Table 5.2. As E_2 dosage increased, there was a linear ($P < .001$) increase in plasma E_2 (28 and 62% increase with .5 and 1 mg E_2/implant, respectively), indicating that E_2 was released from the implants. Plasma E_2 concentrations on the 0 mg E_2/implant were several fold higher, however, than expected in non-implanted steers (less than 10 pg/ml; Heitzman et al., 1981; Hancock and Preston, 1989a).

Rumsey and Beaudry (1979) reported that plasma E_2 concentrations in non-implanted steers ranged from less than 1 to 32 pg/ml with an overall mean of 3.7 over a three-trial experiment; sixty d following Synovex implantation, plasma E_2 concentrations ranged from 6.9 to 181 with a mean of 31 pg/ml in one trial and ranged from less than 1 to 310 with a mean of 65.1 in another. Therefore, it appears that the steers used in this trial were still experiencing estrogen release from an implant (Synovex-C) administered approximately 11 months prior to the start of this trial.

Administration of bST did not affect plasma E_2 concentrations ($P > .50$), nor was there an E_2 by bST interaction ($P > .50$; Table 5.2).

There was an interaction ($P < .01$) between E_2 administration and d on trial (Table 5.3) and was due to an $E_{2linear}$ by $time_{cubic}$ interaction ($P < .001$). Plasma E_2 decreased as d on trial increased in steers receiving the 0 mg E_2 implant, whereas plasma E_2 increased on d 1, decreased on d 4 and increased on d 7 in steers receiving .5 and 1 mg E_2 implants. Linear ($P < .05$), quadratic ($P < .05$) and cubic ($P < .001$) effects were observed in plasma E_2 concentrations as d on trial increased (Table 5.4). Plasma E_2 concentrations increased on d 1, decreased on d 4 and were similar to baseline (d 0) concentrations on d 7.

On d 7, plasma samples were collected both ipsilateral (Table 5.5) and contralateral (Table 5.6) to the implant. The difference (delta) between the ipsilateral and contralateral plasma E_2 concentration was taken to represent E_2 clearance (Table 5.7). Similar to the previously reported effects of E_2 over d on trial (Table 5.2), there was a linear ($P < .005$) increase in ipsilateral, contralateral and delta plasma E_2 concentrations on d 7 as E_2 dosage increased. The lower contralateral plasma E_2 concentrations compared to ipsilateral plasma E_2 concentrations in non-E_2 implanted steers are indicative of metabolic clearance of E_2. Clearance of E_2 in the non-E_2 implanted steers substantiates the statement regarding the influence of prior implantation with Synovex-C, as this is possibly the source of E_2 being cleared. It appears that implantation did not affect E_2 clearance rate since delta plasma E_2 were similar over implant levels. This is in accordance with our work on E_2 clearance rate in implanted cattle using a single non-radiolabelled E_2 infusion technique (Hancock and Preston, 1989c).

A quadratic ($P < .05$) response in plasma bST concentrations was observed as E_2 dosage increased (Table 5.8). Plasma bST concentrations were elevated in steers receiving the .5 mg E_2 implant. Similar to our previous bST dosage titration study (Hancock and Preston, 1989b),

plasma bST concentrations in steers receiving no E_2 or bST were several fold greater than reported concentrations of circulating bST in non-treated steers (Moseley et al., 1982; Grigsby and Trenkle, 1986; Breier et al., 1988b; Wagner et al., 1988a,b). Unlike the prior titration study, however, as bST dosage increased, plasma bST concentrations increased linearly ($P < .001$) and were similar to the increases in plasma bST observed by Wagner et al. (1988a,b) following bST administration. There was a bST by d on trial interaction ($P < .0001$). This was due to a bST_{linear} by $time_{linear}$ interaction ($P < .001$; Table 5.9). As d on trial increased, bST concentrations in steers receiving no bST remained relatively constant, while bST concentrations in steers receiving 41 $\mu g.kg^{-1}.d^{-1}$ increased and then plateaued at approximately 65 ng bST/ml, while bST concentrations in steers receiving 82 $\mu g.kg^{-1}.d^{-1}$ continued to increase. As d on trial increased, plasma bST concentrations increased linearly ($P < .001$) and quadratically ($P < .05$; Table 5.4).

As there were no treatment differences in PUN concentration on d 0 (avg = 9.88 mg/dl), d 0 was not included in the split plot in time analysis of variance for PUN depression. As d on trial increased, there was a linear ($P < .005$) and quadratic ($P < .005$) decrease in PUN (Table 5.4); there were no interactions with time ($P > .10$). Therefore, main plot effects of E_2 and bST on

mean PUN response are reported in Table 5.10. As bST dosage increased from 0 to 82 $\mu g.kg^{-1}.d^{-1}$, there was a linear ($P < .005$) increase in PUN depression. This was unexpected as our previous bST dosage titration study indicated that 41 $\mu g.kg^{-1}.d^{-1}$ reduced PUN, with no further PUN reduction with 82 $\mu g.kg^{-1}.d^{-1}$ (Hancock and Preston, 1989b). Implantation with E_2 failed to decrease PUN ($P > .10$). This is also in contrast with our E_2 dosage titration study (Hancock and Preston, 1989a), where maximum PUN depression resulted with implantation of a minimum dosage of .5 mg E_2/implant. There was no interaction between E_2 and bST administration ($P > .10$). Despite the lack of overall E_2 effects, when .5 and 1 mg E_2 were administered in combination with 41 μg $bST.kg^{-1}.d^{-1}$, there was an additional 9% depression in PUN when compared to the reduction in PUN with bST alone, suggesting possible additivity with these combinations. In contrast, there was no indication of additivity when E_2 was administered with 82 μg $bST.kg^{-1}.d^{-1}$.

Lack of E_2 response on PUN depression may be explained by the elevated initial and control (0 mg E_2/implant) plasma E_2 concentrations. If the steers were already "estrogenized," as indicated by elevated plasma E_2 as well as lower contralateral compared to ipsilateral plasma E_2 concentrations previously discussed, then a lack of response to E_2 could be expected. In support of this,

when steers were double implanted with Synovex-S, there was no further improvement in performance over steers implanted with a single implant (Preston, 1987).

Neither implantation with E_2 nor injection of bST decreased AAN ($P > .50$; Table 5.11) nor were there any differences in AAN concentration as d on trial increased ($P > .50$; avg = 3.84 mg/dl; Table 5.4). This is in contrast to the results of Gopinath and Kitts (1986) who reported a decrease in AAN over a 56-d period after implantation with estrogenic anabolic agents. A lack of E_2 response in AAN depression may relate to the lack of PUN depression response previously discussed, however. Concentrations of AAN reported in our study are two-fold lower than those reported by Gopinath and Kitts (1986). This also might indicate possible prior estrogenization if estrogenic anabolic agents decrease amino acid nitrogen as indicated by Gopinath and Kitts (1986).

There were no effects ($P > .10$) of E_2, bST or the interaction between E_2 and bST on plasma glucose (avg = 90.9 mg/dl; Table 5.12) or packed cell volume (hematocrit; avg = 42.2%; Table 5.13). Plasma glucose concentrations were somewhat higher, however, than normal values (55 to 80 mg/dl; Reid, 1968; Hancock et al., 1988) and may be associated with the daily handling of the steers. Linear ($P < .001$), quadratic ($P < .005$) and cubic ($P < .001$) effects were observed in plasma glucose concentration as d

on trial increased (Table 5.4). There was a bST by d on trial interaction ($P < .008$) in plasma glucose concentrations, which was due to a $bST_{quadratic}$ by $time_{quadratic}$ interaction ($P < .05$; Table 5.14). Hematocrit determinations decreased linearly ($P < .001$) and quadratically ($P < .005$) as d on trial increased (Table 5.4).

Although main effects of E_2 implantation or bST administration indicated no significant difference in daily gain with increasing E_2 or bST dosage ($P < .09$ and $> .10$, respectively), when these effects were partitioned, a quadratic ($P < .05$) increase in daily gain was observed with increasing E_2 dosage and a linear ($P < .10$) increase in daily gain was observed with increasing bST dosage (Table 5.15). A 7-d period for evaluation of daily gain is tenuous, however.

Trial 2. This trial was conducted due to the lack of PUN response in trial 1 following E_2 implantation. The E_2 dosages were increased to 1 and 2 mg E_2/implant in order to assure that E_2 levels were optimal for PUN reduction. Main plot effects of E_2 and bST on mean plasma E_2 concentration are reported in Table 5.16. Similar to trial 1, plasma E_2 concentrations on the 0 mg E_2/implant were about two-fold higher than expected in non-implanted steers. Unlike trial 1, E_2 implantation did not ($P > .10$) elevate plasma E_2 concentrations over the 7-d trial.

Numerically, however, plasma E_2 concentrations in steers implanted with 1 and 2 mg E_2/implant were increased 20%, which was similar to the plasma E_2 elevation in steers on trial 1 receiving the .5 mg E_2/implant dosage. There was, however, a trend for a E_2 by time interaction ($P = .09$), which was due to an $E_{2quadratic}$ by $time_{cubic}$ interaction ($P < .10$; Table 5.17). Plasma E_2 concentrations in steers implanted with the 0 mg E_2 implant remained relatively constant over d on trial. However, plasma E_2 concentrations in steers implanted with the 1 mg E_2 implant were elevated on d 1, whereas all steers had similar plasma E_2 concentrations on d 4, and on d 7, steers implanted with 2 mg E_2 had elevated plasma E_2 concentrations giving a cubic response in plasma E_2 concentrations as d on trial increased ($P < .01$; Table 5.18). Prior implant history is not known on these steers.

As in trial 1, plasma samples were collected both ipsilateral (Table 5.19) and contralateral (Table 5.20) to the implant on d 7 and the difference (delta) represented E_2 clearance (Table 5.21). In contrast to the main plot effects of E_2 implantation over d on trial, on d 7 there was a linear increase in ipsilateral ($P < .10$) and contralateral ($P < .05$) plasma E_2 concentrations as E_2 dosage increased. The increases were approximately 20 and 60% for steers implanted with 1 and 2 mg E_2, respectively,

which were similar to the percentage increases observed over all d on trial in trial 1. Unlike trial 1, there were no differences (P >.50) in delta E_2 concentrations due to E_2 implantation, even though implantation resulted in approximately a 30% numeric increase in the difference between ipsilateral and contralateral plasma E_2 concentrations. In addition, delta E_2 concentrations in steers implanted with 0 mg E_2 were higher in trial 2 than trial 1, due to higher baseline concentrations. Similar to trial 1, it appears that the steers used in this study were experiencing estrogen release, however, we do not have prior implantation history on these steers. As in trial 1, implantation did not appear to alter the clearance rate of E_2.

A linear (P < .10) increase in plasma bST concentrations was observed as E_2 dosage increased (Table 5.22). Plasma bST concentrations in steers receiving no E_2 or bST were extremely elevated, even greater than in trial 1. A quadratic (P < .005) response in plasma bST concentrations was observed as bST dosage increased. Plasma bST concentrations were elevated in steers receiving 41 µg bST.kg^{-1}.d^{-1} and 82 µg bST.kg^{-1}.d^{-1}, however, but to a greater extent in steers receiving 41 µg bST.kg^{-1}.d^{-1}. As d on trial increased, plasma bST concentrations increased linearly (P < .001; Table 5.18). There were also E_2 by d on trial, bST by d on trial and E_2

by bST by d on trial interactions in plasma bST concentrations (Table 5.23). In general, all plasma bST concentrations were extremely elevated in this trial compared to previous studies (Hancock and Preston, 1989b; Wagner et al., 1988a,b) and the variation in plasma bST was large as indicated by large SE (Tables 22 and 23). In addition, it is important to bear in mind that blood samples were collected once daily, 24 h post bST injection.

As there were no treatment differences in PUN concentration on d 0 (avg = 12.4 mg/dl), d 0 was not included in the split plot in time analysis of variance for PUN depression. As d on trial increased, there was a linear ($P < .005$) and quadratic ($P < .001$) decrease in PUN (Table 5.18). There were no interactions with time ($P > .10$), however. Therefore, main plot effects of E_2 and bST on mean PUN response are reported in Table 5.24. As in trial 1, as bST dosage increased from 0 to 82 $\mu g.kg^{-1}.d^{-1}$, there was a linear ($P < .001$) increase in PUN depression, implantation with E_2 failed to decrease PUN ($P > .10$) and there was no interaction between E_2 and bST administration ($P > .50$). Likewise, despite the lack of E_2 effects, when 1 and 2 mg E_2 were administered in combination with 41 and 82 μg bST.$kg^{-1}.d^{-1}$, there was an additional 10% depression in PUN when compared to the reduction in PUN with bST alone, suggesting possible

additivity with these combinations. Further research is required, however, to answer this due to the lack of E_2 response on PUN depression. As in trial 1, this lack of response may be explained by elevated initial plasma E_2 concentrations.

Similar to trial 1, neither implantation with E_2 nor injection of bST influenced ($P > .10$) plasma AAN (avg = 3.73 mg/dl; Table 5.25), glucose (avg = 100.6 mg/dl; Table 5.26) or packed cell volume (avg = 41.2%; Table 5.27) and unlike trial 1, daily gain (avg = -.80 kg; Table 5.28) was not affected ($P > .10$) by either E_2 or bST administration. Again, plasma glucose concentrations were higher than normal and plasma AAN concentrations were lower than expected. Negative daily gains were observed in this 7-d trial and are probably a result of daily handling of the steers.

Trial 3. Due to the lack of PUN response following E_2 implantation in trials 1 and 2, a third study was conducted. In this study, E_2 was administered via daily injections at a dosage of 2 mg E_2, which is above the optimum dosage of E_2 required for maximal PUN reduction as well as performance (Wagner et al., 1979; Potter and Wagner, 1987; Hancock, 1989; Hancock and Preston, 1989a). Administration was via daily injection to allow more control over E_2 dosage delivery.

Administration of E_2 elevated ($P < .003$) plasma E_2 concentrations (39% increase; Table 5.29). As in trials 1 and 2, plasma E_2 concentrations in steers receiving no E_2 or bST were several fold higher than expected in non-implanted steers. As in trial 1, these steers had been previously implanted with Ralgro (zeranol) at branding time (over one year prior to this trial). Plasma E_2 concentrations did not change ($P > .10$) as d on trial increased (Table 5.30).

On d 7, plasma bST concentrations were increased with bST administration ($P < .03$; Table 5.31). E_2 administration did not statistically increase ($P > .10$) plasma bST concentrations, however, numerically mean concentrations were increased 110% (due to an increase in plasma bST when E_2 and bST were combined; interaction ($P > .10$)).

As there were no treatment differences in PUN concentration on d 0 (avg = 12.6 mg/dl), d 0 was not included in the split plot in time analysis of variance for PUN depression. Administration of both E_2 and bST decreased PUN ($P < .0002$; Table 5.32). There was no interaction between E_2 and bST administration ($P > .10$), indicating additivity between these two anabolic agents. Plasma urea nitrogen was reduced by 24 and 29% with E_2 and bST administration, respectively. When E_2 and bST were administered together, PUN was reduced by 44%. As d on

trial increased, there was a linear ($P < .05$) decrease in PUN (Table 5.30).

Recently, Wagner et al. (1988a, b and unpublished data) evaluated the effects of E_2 (Compudose) and bST (960 mg released over a 14-d period; reinjected every 12 to 14 d), alone and in combination, on urinary nitrogen excretion, growth performance, carcass and plasma constituents. Urinary nitrogen excretion was 51.8, 52.2, 51.2 and 45.3 g/d and PUN concentrations were 17.8, 14.2, 14.4 and 13.2 mg/dl for control, E_2, bST and E_2 plus bST treatments, respectively. Significant E_2 and bST main effects were observed in both urinary nitrogen excretion and PUN depression. The interaction was nonsignificant for urinary nitrogen excretion, indicating an additive response. There was an additional 12% decrease in urinary nitrogen excretion when E_2 and bST were combined, compared to either hormone alone. Although there was an additional 8% reduction in PUN when E_2 and bST were combined compared to either hormone alone, the interaction was significant ($P < .028$) indicating that PUN depression was not additive. Grebing et al. (1970) indicated that PUN reduction occurs prior to, but parallels urinary nitrogen reduction following estrogen administration. Wagner et al. (1988b) also noted additive effects for daily gain, gain/feed, carcass protein, moisture and bone when both E_2 and bST were administered together.

Similar to trials 1 and 2, there were no differences ($P > .50$) in hematocrit values (avg = 38.8%) due to E_2 or bST administration (Table 5.33). Hematocrit values remained constant as d on trial increased ($P > .50$; Table 5.30).

Daily gain was increased with bST administration ($P < .0006$), but not with E_2 administration ($P > .50$; Table 5.34). A 7-d period is a short time for evaluating a gain response, however.

In both trials 1 and 2, there was a lack of PUN response to E_2 implantation, which makes it difficult to evaluate additive effects of E_2 and bST administration. Prior research has indicated PUN depression with estrogen administration (Preston, 1968; Grebing et al., 1970; Preston, 1975; Gopinath and Kitts, 1986; Hancock and Preston, 1989a). The lack of E_2 response in these studies was unexpected, especially after having conducted a dose titration study with the E_2 implants used in this study and observing a dose related PUN reduction with E_2 administration. The lack of response in these studies was probably due to elevated E_2 concentrations prior to and during these studies. Following E_2 administration in trial 3, however, PUN depression was observed despite elevated plasma E_2 concentrations. It is also possible that the implants used in these studies were not releasing

the indicated amounts of E_2, however, elevations in plasma E_2 were observed.

In trial 3, where PUN was reduced following both E_2 and bST administration, there was an additive effect with the combined administration of the two anabolic agents. Similarly, additive results in performance, nitrogen excretion and carcass protein, moisture and bone have been previously reported (Wolfrom and Ivy, 1985; Wolfrom et al., 1985; Ivy et al., 1986b; Roche and Quirke, 1986; Wagner et al., 1988a,b). Therefore, the anabolic actions of E_2 appear to be by some other mechanism or in addition to increased endogenous bST secretion.

TABLE 5.1. COMPOSITION OF DIET[a]

Ingredient	%[b]
Steam flaked sorghum grain	39.80
Cottonseed hulls	27.10
Cottonseed meal	19.60
Cane molasses	3.30
Fat	1.50
Urea	.65
Ammonium sulfate	.09
Calcium carbonate	1.39
Sodium chloride	.16
Vitamin A premix	.52
Vitamin E premix	.30
Trace mineral premix	.15
Water	5.00
Tylosin premix	.44
Total	100.00

[a]Formulated to contain 18.0% CP; 3.13 Mcal DE/kg; 3.95% crude fat; 40% NDF; 29% roughage equivalent; .75% Ca; .40% P; .11% Na; .83% K; .19% S; 44 ppm Zn; 4068 IU/kg Vitamin A on a dry matter basis.

[b]As fed basis.

TABLE 5.2. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST ON IPSILATERAL PLASMA E_2 CONCENTRATIONS (PG/ML) FOR STEERS IN TRIAL 1[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	.5	1	
0	19.0	23.9	28.2	23.7
41	16.8	21.3	33.2	23.8
82	19.9	26.5	28.8	25.1
E_2 mean	18.6	23.9	30.1	

[a]Main effect of E_2 implantation ($P < .001$; SE = 2.03). Linear ($P < .001$) increase in plasma E_2 with increasing E_2 dosage.

[b]Main effect of bST administration ($P > .50$; SE = 2.03).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 3.51).

TABLE 5.3. LEAST-SQUARES MEANS FOR EFFECTS OF E_2 BY DAYS ON TRIAL INTERACTION ON IPSILATERAL PLASMA E_2 CONCENTRATIONS (PG/ML) FOR STEERS IN TRIAL 1[a]

mg E_2/ implant	Days on trial			
	0	1	4	7
0	25.0	20.6	13.1	15.5
.5	22.3	29.8	17.9	25.6
1	24.8	44.3	20.0	31.3

[a] E_2 by days on trial interaction ($P = .013$; $SE = 3.22$). $E_{2\,linear}$ by days on $trial_{cubic}$ interaction ($P < .001$).

TABLE 5.4. LEAST-SQUARES MEANS FOR EFFECTS OF DAYS ON TRIAL FOR PLASMA VARIABLES AND HEMATOCRIT DETERMINATIONS FOR STEERS IN TRIAL 1

	Days on trial			
Variable	0	1	4	7
E_2, pg/ml[a]	24.0	31.6	17.0	24.2
bST, ng/ml[b]	24.4	48.0	76.9	90.0
PUN, mg/dl[c]	9.88	9.16	7.20	7.08
AAN, mg/dl[d]	3.83			3.85
Glucose, mg/dl[e]	88.9	86.7	95.7	92.4
Hematocrit, %[f]	43.2	42.3	41.8	41.6

[a]Effects of days on trial ($P < .0001$; SE = 1.86). Linear ($P < .05$), quadratic ($P < .05$) and cubic ($P < .001$) response in plasma E_2 with increasing days on trial.

[b]Effects of days on trial ($P < .0001$; SE = 5.84). Linear ($P < .001$) and quadratic ($P < .05$) increase in plasma bST with increasing days on trial.

[c]Effects of days on trial ($P < .0001$; SE = .15). Linear ($P < .005$) and quadratic ($P < .005$) decrease in PUN with increasing days on trial.

[d]Effects of days on trial ($P > .50$; SE = .11).

[e]Effects of days on trial ($P < .0001$; SE = 1.06). Linear ($P < .001$), quadratic ($P < .005$) and cubic ($P < .001$) response in plasma glucose with increasing days on trial.

[f]Effects of days on trial ($P < .0001$; SE = .16). Linear ($P < .001$) and quadratic ($P < .005$) decrease in hematocrit value with increasing days on trial.

TABLE 5.5. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA E_2 CONCENTRATIONS (PG/ML) COLLECTED IPSILATERAL TO THE IMPLANT ON DAY 7 FOR STEERS IN TRIAL 1[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	.5	1	
0	16.9	30.1	33.0	26.6
41	14.5	24.4	28.0	22.3
82	15.3	22.3	33.1	23.6
E_2 mean	15.5	25.6	31.3	

[a]Main effect of E_2 implantation ($P < .0001$; SE = 2.21). Linear ($P < .005$) increase in plasma E_2 with increasing E_2 dosage.

[b]Main effect of bST administration ($P > .10$; SE = 2.21).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 3.83).

TABLE 5.6. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA E_2 CONCENTRATIONS (PG/ML) COLLECTED CONTRALATERAL TO THE IMPLANT ON DAY 7 FOR STEERS IN TRIAL 1[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	.5	1	
0	10.36	11.7	17.6	13.2
41	8.20	11.1	13.9	11.1
82	9.59	13.2	17.1	13.3
E_2 mean	9.38	12.0	16.2	

[a]Main effect of E_2 implantation ($P < .0001$; SE = .9). Linear ($P < .005$) increase in plasma E_2 with increasing E_2 dosage.

[b]Main effect of bST administration ($P > .10$; SE = .9).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 1.7).

TABLE 5.7. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR DELTA PLASMA E_2 CONCENTRATIONS (PG/ML) ON DAY 7 FOR STEERS IN TRIAL 1[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	.5	1	
0	6.51	14.16	15.4	12.0
41	6.26	13.33	14.0	11.2
82	5.67	9.16	16.0	10.3
E_2 mean	6.15	12.2	15.1	

[a]Main effect of E_2 implantation ($P < .004$; SE = 1.9). Linear ($P < .005$) increase in delta plasma E_2 with increasing E_2 dosage.

[b]Main effect of bST administration ($P > .50$; SE = 1.9).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 3.5).

TABLE 5.8. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA bST CONCENTRATIONS (NG/ML) FOR STEERS IN TRIAL 1[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	.5	1	
0	28.3	31.2	25.3	28.3
41	33.1	68.6	36.6	46.1
82	80.5	134.4	100.4	105.1
E_2 mean	47.3	78.1	54.1	

[a]Main effect of E_2 implantation ($P < .05$; SE = 8.45). Quadratic ($P < .05$) increase in plasma bST with increasing E_2 dosage.

[b]Main effect of bST administration ($P < .001$; SE = 8.45). Linear ($P < .001$) increase in plasma bST with increasing bST dosage.

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 14.8).

TABLE 5.9. LEAST-SQUARES MEANS FOR EFFECTS OF bST BY DAYS ON TRIAL INTERACTION FOR PLASMA bST CONCENTRATIONS (NG/ML) FOR STEERS IN TRIAL 1[a]

µg bST. kg-1.d-1	Days on trial			
	0	1	4	7
0	20.6	22.4	38.3	31.8
41	19.0	38.0	61.9	65.5
82	33.5	83.6	130.7	172.7

[a]bST by days on trial interaction ($P < .0001$; SE = 10.3). bST_{linear} by $days\ on\ trial_{linear}$ interaction ($P < .001$).

TABLE 5.10. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PUN CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 1[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	.5	1	bST mean
0	9.43	9.17	9.96	9.52
41	8.30	7.55	7.59	7.81
82	6.15	6.13	6.07	6.12
E_2 mean	7.96	7.62	7.87	

[a]Main effect of E_2 implantation ($P > .10$; SE = .30).

[b]Main effect of bST administration ($P < .005$; SE = .30). Linear ($P < .005$) decrease in PUN concentration with increasing bST dosage.

[c]Interaction of E_2 and bST administration ($P > .10$; SE = .52).

TABLE 5.11. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR AAN CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 1[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	.5	1	bST mean
0	3.67	3.84	3.83	3.78
41	4.07	3.79	3.56	3.81
82	3.94	3.93	3.89	3.92
E_2 mean	3.89	3.86	3.76	

[a]Main effect of E_2 implantation (P > .50; SE = .13).

[b]Main effect of bST administration (P > .50; SE = .13).

[c]Interaction of E_2 and bST administration (P > .50; SE = .22).

TABLE 5.12. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA GLUCOSE CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 1[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	.5	1	
0	97.0	81.0	86.5	88.2
41	92.9	88.2	92.9	91.3
82	93.3	92.3	94.2	93.3
E_2 mean	94.4	87.2	91.2	

[a]Main effect of E_2 implantation ($P > .10$; SE = 2.39).

[b]Main effect of bST administration ($P > .10$; SE = 2.39).

[c]Interaction of E_2 and bST administration ($P > .10$; SE = 4.14).

TABLE 5.13. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR HEMATOCRIT DETERMINATIONS (%) FOR STEERS IN TRIAL 1[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	.5	1	bST mean
0	44.1	41.7	43.4	43.1
41	41.5	42.3	42.1	42.0
82	42.3	40.7	41.8	41.6
E_2 mean	42.6	41.6	42.4	

[a]Main effect of E_2 implantation (P > .50; SE = .74).

[b]Main effect of bST administration (P > .10; SE = .74).

[c]Interaction of E_2 and bST administration (P > .50; SE = 1.28).

TABLE 5.14. LEAST-SQUARES MEANS FOR EFFECTS OF bST BY DAYS ON TRIAL INTERACTION FOR PLASMA GLUCOSE CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 1[a]

µg bST. kg-1.d-1	Days on trial			
	0	1	4	7
0	88.5	81.2	93.7	89.3
41	90.8	89.2	92.0	93.3
82	87.5	89.7	101.4	94.5

[a]bST by days on trial interaction ($P < .008$; SE = 1.84). $bST_{quadratic}$ by $days\ on\ trial_{quadratic}$ interaction ($P < .05$).

TABLE 5.15. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR DAILY GAIN (KG) FOR STEERS IN TRIAL 1[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	.5	1	bST mean
0	-.62	.79	-.20	-.01
41	.65	.80	.18	.55
82	.82	.65	.22	.56
E_2 mean	.29	.75	.07	

[a]Main effect of E_2 implantation (P = .089; SE = .22). Quadratic (P < .05) increase in daily gain with increasing E_2 dosage.

[b]Main effect of bST administration (P = .1187; SE = .22). Linear (P < .10) increase in daily gain with increasing bST dosage.

[c]Interaction of E_2 and bST administration (P > .10; SE = .37).

TABLE 5.16. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST ON IPSILATERAL PLASMA E_2 CONCENTRATIONS (PG/ML) FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	1	2	
0	23.1	25.9	27.8	25.6
41	16.2	27.4	23.2	22.3
82	26.2	25.0	27.5	26.2
E_2 mean	21.8	26.1	26.2	

[a]Main effect of E_2 implantation ($P > .10$; SE = 2.52).

[b]Main effect of bST administration ($P > .10$; SE = 2.52).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 4.36).

TABLE 5.17. LEAST-SQUARES MEANS FOR EFFECTS OF E_2 BY DAYS ON TRIAL INTERACTION FOR IPSILATERAL PLASMA E_2 CONCENTRATIONS (PG/ML) FOR STEERS IN TRIAL 2[a]

mg E_2/ implant	Days on trial			
	0	1	4	7
0	18.1	25.4	20.2	23.5
1	21.6	36.3	19.1	27.4
2	24.8	21.7	20.6	37.6

[a]E_2 by days on trial interaction (P = .0878; SE =4.13). $E_{2\text{quadratic}}$ by days on $\text{trial}_{\text{cubic}}$ interaction (P < .10).

TABLE 5.18. LEAST-SQUARES MEANS FOR EFFECTS OF DAYS ON TRIAL FOR PLASMA VARIABLES AND HEMATOCRIT DETERMINATIONS FOR STEERS IN TRIAL 2

	Days on trial			
Variable	0	1	4	7
E_2, pg/ml[a]	21.5	27.8	19.9	29.5
bST, ng/ml[b]	36.4	132.8	539.0	729.8
PUN, mg/dl[c]	12.4	11.7	10.1	10.8
AAN, mg/dl[d]	3.74			3.71
Glucose, mg/dl[e]	100.2	100.4	100.1	101.9
Hematocrit, %[f]	41.3	41.7	41.4	40.6

[a]Effects of days on trial ($P < .01$; SE = 2.39). Cubic ($P < .01$) response in plasma E_2 with increasing days on trial.

[b]Effects of days on trial ($P < .001$; SE = 138.0). Linear ($P < .001$) increase in plasma bST with increasing days on trial.

[c]Effects of days on trial ($P < .0001$; SE = .21). Linear ($P < .005$) and quadratic ($P < .001$) decrease in PUN with increasing days on trial.

[d]Effects of days on trial ($P > .50$; SE = .08).

[e]Effects of days on trial ($P > .50$; SE = 1.93).

[f]Effects of days on trial ($P < .0004$; SE = .18).

TABLE 5.19. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA E_2 CONCENTRATIONS (PG/ML) COLLECTED IPSILATERAL TO THE IMPLANT ON DAY 7 FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	1	2	
0	22.4	32.6	38.2	31.1
41	19.0	21.0	41.4	27.1
82	29.2	28.5	33.1	30.3
E_2 mean	23.5	27.4	37.6	

[a]Main effect of E_2 implantation (P = .1365; SE = 5.0). Linear (P < .10) increase in plasma E_2 with increasing E_2 dosage.

[b]Main effect of bST administration (P > .50; SE = 5.0).

[c]Interaction of E_2 and bST administration (P > .50; SE = 8.7).

TABLE 5.20. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA E_2 CONCENTRATIONS (PG/ML) COLLECTED CONTRALATERAL TO THE IMPLANT ON DAY 7 FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	1	2	
0	15.7	16.4	24.9	19.0
41	11.9	16.6	26.0	18.2
82	13.6	16.8	17.0	15.8
E_2 mean	13.7	16.6	22.6	

[a]Main effect of E_2 implantation ($P < .003$; SE = 1.8). Linear ($P < .05$) increase in plasma E_2 with increasing E_2 dosage.

[b]Main effect of bST administration ($P > .10$; SE = 1.7).

[c]Interaction of E_2 and bST administration ($P > .10$; SE = 3.1).

TABLE 5.21. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR DELTA PLASMA E_2 CONCENTRATIONS (PG/ML) ON DAY 7 FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	1	2	bST mean
0	6.7	16.2	13.7	12.2
41	7.1	4.4	16.4	9.3
82	15.6	17.7	10.1	14.4
E_2 mean	9.8	12.8	13.4	

[a]Main effect of E_2 implantation ($P > .50$; SE = 4.48).

[b]Main effect of bST administration ($P > .50$; SE = 4.36).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 7.80).

TABLE 5.22. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA bST CONCENTRATIONS (NG/ML) FOR STEERS IN TRIAL 2[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	1	2	
0	140.5	54.5	31.1	75.3
41	367.2	448.1	1297.8	704.4
82	246.6	306.4	343.3	298.8
E_2 mean	251.4	269.7	557.4	

[a]Main effect of E_2 implantation (P = .176; SE = 128.0). Linear ($P < .10$) increase in plasma bST with increasing E_2 dosage.

[b]Main effect of bST administration ($P < .004$; SE = 128.0). Quadratic ($P < .005$) increase in plasma bST with increasing bST dosage.

[c]Interaction of E_2 and bST administration (P = .1318; SE = 225.0).

TABLE 5.23. LEAST-SQUARES MEANS FOR E_2, bST AND E_2 BY bST INTERACTIONS WITH DAYS ON TRIAL FOR PLASMA bST CONCENTRATIONS (NG/ML) FOR STEERS ON TRIAL 2[abc]

Days on trial	µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	1	2	bST mean
	0	125.0	28.7	38.7	64.1
0	41	16.9	23.3	46.0	28.7
	82	19.6	12.7	16.8	16.3
	E_2 mean	53.8	21.6	33.8	
	0	201.5	149.8	17.7	123.0
1	41	203.7	122.7	250.3	192.2
	82	93.2	84.4	71.9	83.2
	E_2 mean	166.1	118.9	113.3	
	0	197.9	20.5	20.1	79.5
4	41	1027.5	1205.6	1011.0	1081.4
	82	358.4	235.7	774.3	456.1
	E_2 mean	527.9	487.2	601.8	
	0	37.5	19.0	47.9	34.8
7	41	220.8	440.8	3883.9	1515.1
	82	515.1	893.0	510.1	639.4
	E_2 mean	257.8	450.9	1480.6	

[a] E_2 by days on trial interaction ($P < .09$; SE = 243.7). $E_{2linear}$ by $time_{linear}$ interaction ($P < .01$).

[b] bST by days on trial interaction ($P < .03$; SE = 243.7). $bST_{quadratic}$ by $time_{linear}$ interaction ($P < .001$).

[c] E_2 by bST by days on trial interaction ($P < .01$; SE = 410.2). $E_{2quadratic}$ by $bST_{quadratic}$ by $time_{linear}$ ($P < .001$) and $E_{2quadratic}$ by $bST_{quadratic}$ by $time_{quadratic}$ ($P < .005$) interactions.

TABLE 5.24. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PUN CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 2[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	1	2	bST mean
0	12.8	12.2	12.7	12.6
41	11.9	10.3	11.0	11.1
82	9.6	8.9	8.4	9.0
E_2 mean	11.4	10.5	10.7	

[a]Main effect of E_2 implantation ($P > .10$; SE = .53).

[b]Main effect of bST administration ($P < .0001$; SE = .53). Linear ($P < .001$) decrease in PUN concentration with increasing bST dosage.

[c]Interaction of E_2 and bST administration ($P > .50$; SE = .93).

TABLE 5.25. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR AAN CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	1	2	bST mean
0	4.02	3.79	3.87	3.89
41	3.79	3.96	3.31	3.69
82	3.53	3.60	3.66	3.60
E_2 mean	3.78	3.78	3.61	

[a]Main effect of E_2 implantation ($P > .10$; SE = .11).

[b]Main effect of bST administration ($P > .10$; SE = .11).

[c]Interaction of E_2 and bST administration ($P > .10$; SE = .20).

TABLE 5.26. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA GLUCOSE CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 2[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/implant			bST mean
	0	1	2	
0	100.3	102.3	92.7	98.4
41	93.2	102.5	103.9	99.9
82	107.0	105.9	97.8	103.6
E_2 mean	100.2	103.6	98.1	

[a]Main effect of E_2 implantation ($P > .50$; SE = 4.92).

[b]Main effect of bST administration ($P > .50$; SE = 4.92).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 8.52).

TABLE 5.27. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR HEMATOCRIT DETERMINATIONS (%) FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	1	2	bST mean
0	42.5	41.1	41.9	41.8
41	39.6	40.1	41.3	40.3
82	42.3	40.7	41.8	41.6
E_2 mean	41.5	40.6	41.6	

[a]Main effect of E_2 implantation (P > .50; SE = .72).

[b]Main effect of bST administration (P > .10; SE = .71).

[c]Interaction of E_2 and bST administration (P > .50; SE = 1.27).

TABLE 5.28. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR DAILY GAIN (KG) FOR STEERS IN TRIAL 2[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/implant 0	1	2	bST mean
0	-1.53	- .82	- .71	-1.02
41	-1.00	-1.34	- .63	- .99
82	-1.11	- .45	.36	- .40
E_2 mean	-1.21	- .87	- .32	

[a]Main effect of E_2 implantation (P > .10; SE = .38).

[b]Main effect of bST administration (P > .10; SE = .38).

[c]Interaction of E_2 and bST administration (P > .50; SE = .70).

TABLE 5.29. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA E_2 CONCENTRATIONS (PG/ML) FOR STEERS IN TRIAL 3[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/d		bST mean
	0	2	
0	28.6	41.1	34.8
82	26.1	35.2	30.7
E_2 mean	27.4	38.1	

[a]Main effect of E_2 administration ($P < .003$; SE = 2.28).

[b]Main effect of bST administration ($P > .10$; SE = 2.28).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = 3.25).

TABLE 5.30. LEAST-SQUARES MEANS FOR EFFECTS OF DAYS ON TRIAL FOR PLASMA VARIABLES AND HEMATOCRIT DETERMINATIONS FOR STEERS IN TRIAL 3

	Days on trial			
Variable	0	1	4	7
E_2, pg/ml[a]	31.4	29.3	36.8	33.6
PUN, mg/dl[b]	12.6	10.6	9.4	9.6
Hematocrit, %[c]	38.6	38.8	38.8	39.0

[a]Effects of days on trial ($P > .10$; SE = 3.48).

[b]Effects of days on trial ($P < .002$; SE = .22). Linear ($P < .05$) decrease in PUN with increasing days on trial.

[c]Effects of days on trial ($P > .50$; SE = .26).

TABLE 5.31. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PLASMA bST CONCENTRATIONS (NG/ML) FOR STEERS IN TRIAL 3[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/d		bST mean
	0	2	
0	14.6	13.4	14.0
82	359.9	777.1	568.5
E_2 mean	187.3	395.2	

[a]Main effect of E_2 administration ($P > .10$; SE = 163.2).

[b]Main effect of bST administration ($P < .026$; SE = 163.2).

[c]Interaction of E_2 and bST administration ($P > .10$; SE = 230.8).

TABLE 5.32. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR PUN CONCENTRATIONS (MG/DL) FOR STEERS IN TRIAL 3[abc]

μg bST. $kg^{-1}.d^{-1}$	mg E_2/d		bST mean
	0	2	
0	13.02	9.95	11.49
82	9.25	7.26	8.26
E_2 mean	11.14	8.61	

[a]Main effect of E_2 administration ($P < .0002$; SE = .40).

[b]Main effect of bST administration ($P < .0001$; SE = .40).

[c]Interaction of E_2 and bST administration ($P > .10$; SE = .56).

TABLE 5.33. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR HEMATOCRIT DETERMINATIONS (%) FOR STEERS IN TRIAL 3[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/d		bST mean
	0	2	
0	39.6	39.0	39.3
82	38.7	37.9	38.3
E_2 mean	39.2	38.4	

[a]Main effect of E_2 administration ($P > .10$; SE = .67).

[b]Main effect of bST administration ($P > .10$; SE = .67).

[c]Interaction of E_2 and bST administration ($P > .50$; SE = .97).

TABLE 5.34. LEAST-SQUARES MEANS FOR MAIN EFFECTS OF E_2, bST AND THE INTERACTION OF E_2 AND bST FOR DAILY GAIN (KG) FOR STEERS IN TRIAL 3[abc]

µg bST. $kg^{-1}.d^{-1}$	mg E_2/d		bST mean
	0	2	
0	.69	.67	.68
82	1.86	2.25	2.05
E_2 mean	1.27	1.46	

[a]Main effect of E_2 administration (P > .50; SE = .54).

[b]Main effect of bST administration (P = .0006; SE = .54).

[c]Interaction of E_2 and bST administration (P > .50; SE = .79).

CHAPTER VI

ALTERNATE MECHANISMS OF ANABOLIC ESTROGEN ACTION: EFFECTS OF CATECHOL ESTROGEN AND PROGESTERONE ON ANABOLIC ESTROGEN ACTION IN FEEDLOT STEERS

Abstract

Forty British type steers (362 kg) were blocked by weight and breed type (5 red baldy, 1 red solid, 16 black solid, 18 black baldy) into eight pens with five head per pen. Eight steers were randomly allotted to each of the following five treatments (one steer.pen^{-1}.treatment^{-1}): 5 d pretreatment with 200 mg progesterone (P_4)/d followed by 7 d control (100% ethanol) or 17-β estradiol (E_2; 2 mg/d) administration; or no P_4 pretreatment, followed by 7 d control, E_2 (2 mg/d) or 4-hydroxy E_2 (catechol (C) E_2; 2.12 mg/d) administration. Steers were weighed and blood samples taken via jugular vein puncture on d 0, 1, 4 and 7 at 1400 h, approximately 5 h post feeding, following initiation of estrogen treatment. Samples were analyzed for PUN, E_2 and hematocrit on d 0, 1, 4 and 7, and bST on d 7. There were no treatment differences ($P > .80$) in daily gain (avg = .76 kg), d 7 bST concentration (avg = 14.4 ng/ml) or hematocrit value (avg = 38.8%). There was a significant cubic contrast ($P < .05$) in plasma E_2 between treatments as d on trial increased. Steers which

received E_2 and CE_2 had elevations in plasma E_2 concentrations as d on trial increased, compared to steers which did not receive E_2 ($P < .10$). Administration of E_2 and CE_2 reduced ($P < .05$) PUN by 24 and 21%, respectively, with no difference in PUN reduction between E_2 and CE_2 administration ($P > .05$). Pretreatment for 5 d with 200 mg P_4/d did not block E_2 induced PUN depression ($P < .05$). Results indicate that CE_2 formation may be involved in the anabolic action of estrogens and that pretreatment with P_4 did not block E_2 induced PUN depression.
(Key Words: 17-β Estradiol, Catechol Estradiol, Progesterone, Plasma Urea Nitrogen Depression, Steers, Anabolic Activity.)

Introduction

Due to the similarities in the anabolic effects of estrogen and somatotropin (ST), it has been postulated that the anabolic effect of estrogen is mediated through an increase in ST secretion (Preston, 1975; Trenkle, 1976; Heitzman, 1979; Heitzman, 1981; Gopinath and Kitts, 1984; Preston, 1987; Hancock and Preston, 1989a,b). However, additive results in performance, plasma urea nitrogen depression (an indicator of anabolic activity), nitrogen excretion and carcass protein, moisture and bone have been previously reported with combined administration of estrogen and ST (Wolfrom and Ivy, 1985; Wolfrom et al.,

1985; Ivy et al., 1986b; Roche and Quirke, 1986; Hancock and Preston, 1989d; Wagner et al., 1988a,b). Therefore, the anabolic actions of estrogen appear to be by some other mechanism or in addition to increased endogenous bST secretion.

Catechol estrogens (CE) are a class of estrogen metabolites which are formed in many tissues such as the liver, pituitary and hypothalamus (MacLusky et al., 1981) via hydroxylation at either the 2 or 4 carbon of the aromatic ring, resemble the catecholamines (CA; epinephrine, norepinephrine and dopamine), are potent inhibitors of tyrosine hydroxylase (Lloyd and Weisz, 1978; Pfeiffer et al., 1986), compete for dopamine receptors (Schaeffer and Hsueh, 1979; Paden et al., 1982), and inhibit catechol-o-methyl transferase (COMT; Ball et al., 1972). It is hypothesized that the formation of CE might be related to the anabolic action of estrogens in ruminants and that CE act as a neurohormone.

Progesterone "priming" has been reported to block E_2 induced estrus behavior in ovariectomized cows (Melampy et al., 1957; Davidge et al., 1987). It was suggested that P_4 treatment blocks E_2 action by down-regulating brain E_2 receptors (Kato, 1977). It was our interest to determine if "priming" with P_4 prior to estrogen administration alters the anabolic action of estrogens in ruminants.

Materials and Methods

Forty British type steers (362 kg) were blocked by weight and breed type (5 red baldy, 1 red solid, 16 black solid, 18 black baldy) into eight pens with five head per pen. Steers were fed ad libitum on a diet balanced to meet or exceed NRC (1984) requirements (Table 6.1). Eight steers were randomly allotted to each of the following treatments (one steer.pen^{-1}.treatment^{-1}): 1) 5 d pretreatment with 200 mg progesterone (P_4)/d followed by 7 d control (100% ethanol) administration; 2) 5 d pretreatment with 200 mg P_4/d followed by 7 d 17-β estradiol (E_2; 2 mg/d) administration; 3) no P_4 pretreatment, 7 d control administration; 4) no P_4 pretreatment, 7 d E_2 (2 mg/d) administration; and 5) no P_4 pretreatment, 7 d 4-hydroxy E_2 (catechol (C) E_2; 2.12 mg/d) administration. Subcutaneous injections of P_4, E_2 and CE_2 were administered daily in an injection vehicle of 100% ethanol. Concentrations of injection solutions were 50 mg/ml, 2 mg/ml and 2.12 mg/ml for P_4, E_2 and CE_2, respectively. This E_2 injection dosage (2 mg/d) was chosen based on preliminary plasma urea nitrogen (PUN) response studies (Hancock, 1989). The CE_2 dosage was a molar equivalent dosage of E_2. Ascorbic acid (73 mM) was added to the CE_2 injection vehicle, which was prepared daily, to prevent oxidation.

The P_4 pretreatment period was 5 d based on research by Davidge et al. (1987) who demonstrated that behavioral estrus was blocked with a 5 d P_4 pretreatment prior to E_2 administration to induce estrus. The E_2 treatment period of this trial was conducted for 7 d, the minimum time required for maximal PUN reduction for E_2 (Hancock and Preston, 1989a). Steers were weighed and blood samples taken via jugular vein puncture on d 0, 1, 4 and 7 at 1400 h, approximately 5 h post feeding, following initiation of estrogen treatment. Samples were collected in heparinized tubes, transported on ice, centrifuged and the plasma stored at -20°C until analyzed for PUN, E_2 and hematocrit on d 0, 1, 4 and 7, and bST on d 7. Hematocrits were determined using an Autocrit II[1] centrifuge. Samples were analyzed for PUN (Chaney and Marbach, 1962; Searle, 1984) using a spectrophotometric assay and E_2[2] and bST via double antibody radioimmunoassays (Hancock, 1989).

Data were analyzed by analysis of variance as a split plot in time design using the General Linear Model Procedure (SAS, 1985). Effects included in the main plot analysis were pen, treatment and the interaction of pen by treatment. The latter term was used as the error term to

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[1]Clay Adams, Division of Becton, Dickinson and Company, Parsippany, NJ 07054.

[2]Diagnostic Products Corporation, 5700 W. 96th St. Los Angeles, CA 90045.

test main plot effects. Subplot effects included time (d on trial), the interaction of treatment by time and residual effect which was used as the error term to test subplot effects. Treatment differences were tested by protected least significant difference. Regression analysis was performed to orthogonally partition the effects of time into linear, quadratic and cubic components. Linear, quadratic and cubic contrasts[3] were made among treatments to detect treatment differences over time. This was tested using a one-degree of freedom F-test.

Daily gain was determined for the overall 7-d period; therefore, the model for daily gain included only main plot effects indicated above. A similar model was used to evaluate d 0 PUN and d 7 bST concentrations.

Results and Discussion

There were no treatment differences ($P > .80$) in daily gain (avg = .76 kg), d 7 bST concentration (avg = 14.4 ng/ml) or hematocrit value (avg = 38.8%). There was a linear ($P < .05$) increase in hematocrit value, however, as d on trial increased (38.6, 38.9, 38.6 and 39.4% on d 0, 1, 4 and 7, respectively).

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[3]University of Missouri, Agricultural Experiment Station Statistician Department, Columbia, MO 65211.

Compared to steers receiving no E_2, administration of E_2 elevated plasma E_2 concentrations by 34% ($P > .05$) in steers which were primed with P_4 prior to E_2 administration and 44% ($P < .05$) in steers receiving only E_2 (no P_4 prime), respectively (Table 6.2). A linear ($P < .01$) increase in plasma E_2 was observed as d on trial increased. There was a significant cubic contrast ($P < .05$) between treatments as d on trial increased. Control steers and steers which were primed with P_4 and then received no E_2 had similar ($P > .10$) cubic responses in plasma E_2 concentrations as d on trial increased, both of which were different responses ($P < .10$) than in steers which received E_2 or CE_2. Steers which received E_2 and CE_2 had elevations in plasma E_2 concentrations as d on trial increased, compared to steers which did not receive E_2. Similar to our previous research (Hancock and Preston, 1989d), plasma E_2 concentrations on d 0 and on the 0 mg E_2 treatment were several fold higher, however, than expected in non-implanted steers.

As there were no treatment differences in PUN concentration on d 0 (avg = 13.1 mg/dl), d 0 was not included in the split plot in time analysis of variance for PUN depression. As d on trial increased, there was a linear ($P < .05$) decrease in PUN (Table 6.3). Administration of E_2 reduced PUN by 24% ($P < .05$; Table 6.3). Similar reductions in PUN were observed with CE_2

administration (21%; $P < .05$). There was no difference in PUN reduction between E_2 and CE_2 administration ($P > .05$). This observation indicates that catechol estrogen formation may be involved in the anabolic action of estrogens. Further research is required, however, to determine if CE_2 is merely binding to the E_2 receptor, resulting in PUN reduction or if the catechol structure has catecholamine or β-agonist like properties. If CE_2 formation is required to elicit the anabolic response, involvement of the cAMP-second messenger system is implied if the CE have catecholamine or β-agonist like properties (Buttery and Dawson, 1987; Sejrsen and Jensen, 1987). This would involve protein kinase activity and enzyme regulation of events which may lead to anabolic activity (Sejrsen and Jensen, 1987; Smith, 1987).

Reeds (1987) postulated that growth regulation may result from hormone-receptor mediated responses, namely enzyme phosphorylation and metabolic control. Regarding protein metabolism, he noted four areas where enzyme phosphorylation may be of importance: 1) initiation of polypeptide translation controlled by phosphorylation of eukaryotic initiation factor 2; 2) phosphorylation state of protein S-6 of the small ribosomal subunit; 3) phosphorylation of aminoacyl tRNA synthetase enzymes; and 4) amino acid catabolic enzymes.

Other possible enzyme regulated processes are glycogen synthesis and breakdown, and lipolysis. More specifically, if CE_2 have catecholamine like actions, in muscle glycogen synthesis would be decreased due to decreased glycogen synthetase and glycogenolysis would increase due to increased phosphorylase, as well, gluconeogenesis would be increased in the liver, which would increase glucose. Lipolysis and free fatty acids would increase uptake and utilization of glucose in skeletal muscle would decrease. As suggested by Wright (1961), could the difference in anabolic response between species to estrogens be due to energy substrate utilization? Ruminants continuously utilize glucogenic pathways because of microbial production of volatile fatty acids (VFA) in the rumen, which are absorbed and used as the major source of energy via the TCA cycle or converted to glucose (propionic acid) via gluconeogenesis. If uptake and utilization of glucose were decreased in skeletal muscle following conversion of estrogen to CE_2, it would seem that the ruminant might demonstrate an anabolic response since it uses other sources of energy (acetate and butyrate produced in the rumen, which would be similar to the products of lipolysis) and since the ruminant has a glucose conservation mechanism whereby 66% of produced glucose is recycled rather than oxidized, with

glucose oxidation inhibition most likely regulated in the muscle tissue (Lindsay, 1981).

Therefore, it seems that the ruminant may be functionally able to utilize these alternate metabolic products whereas the monogastric is not presented with these metabolites and would not be similarly affected. In support of this, VanderWal (1976) reported that growth was not improved and slaughter weight was less than controls when calves were injected with diethylstilbestrol at 5 weeks of age (immature ruminant), whereas with calves 11 or 14 weeks of age, growth was increased and maintained until slaughter. Increased growth, feed efficiency and carcass weight were observed in rabbits following E_2 administration (Daley et al., 1987) and in guinea pigs following diethylstilbestrol administration (Preston et al., 1956). Rabbits and guinea pigs have a large cecum with hind gut fermentation and VFA production.

Another possible area for CE_2 involvement is in E_2 induced increased plasma bST concentrations following estrogen administration. Recently, Welsh et al. (1987) reported that clenbuterol (a β-adrenergic agonist), forskolin (an adenyl cyclase activator), 1-methyl-3-isobutyl xanthine (a phosphodiesterase inhibitor) as well as ST releasing hormone all stimulated ST secretion. This suggests that the cAMP-second messenger system may regulate ST secretion. Baile et al.

(1986) reported that dopamine controls ST secretion in rats. Intraventricular injections of dopamine in rats inhibits ST secretion and blockage of dopamine receptors stimulates ST release. They indicated that this effect is probably through increased somatostatin. Likewise, dopamine inhibits prolactin secretion; however, estrogen can override this by decreasing dopamine receptors and stimulating prolactin transcription (Granner, 1985; Norman and Litwack, 1987). Therefore, if CE decreases dopamine synthesis and competes for dopamine receptors, CE could be involved in the increased ST secretion observed following E_2 administration.

Significant linear and quadratic ($P < .05$) contrasts in PUN were observed between treatments as d on trial increased (Table 6.3). In general, steers which were pretreated with P_4 and then did not receive E_2 had a different response over d on trial compared to the other treatments. Plasma urea nitrogen concentrations increased from d 1 to 4, then decreased from d 4 to 7, whereas steers receiving no P_4 or E_2 had relatively constant PUN concentrations over d on trial and steers which received E_2 or CE_2 had decreasing PUN concentrations.

For behavioral estrus to be expressed, P_4 levels must be low when estrogen levels are elevated, however, estrus behavior may be regulated by the level of P_4 prior to the increase in estrogen (Carrick and Shelton, 1969; Levasseur

and Thibault, 1980; Davidge et al., 1987). Davidge et al. (1987) observed linear decreases in estrous behavior with increasing P_4 dosages from 0 to 500 mg/d. Progesterone was injected twice daily for 5 d in ovariectomized cows. Estrus was induced 72 h post P_4 treatment by the injection of 2 mg of E_2, depending on P_4 dosage. It was suggested that P_4 treatment may have blocked E_2 action by down-regulating brain E_2 receptors (Kato, 1977). Melampy et al. (1957) observed similar results, indicating that 30 to 60 mg of P_4 was required for suppression of estrus behavior. At lower dosages, however, estrus behavior was observed and P_4 was synergistic with E_2 when injected 12 h prior to, with or 12 h post E_2.

With these factors in mind, it was our interest to determine if "priming" with P_4 prior to estrogen administration alters the anabolic response of estrogens in ruminants. If P_4 treatment down-regulates E_2 receptors, a lack of anabolic response would also be expected if the mechanism of anabolic estrogen action is via E_2 receptor mediated effects. Pretreatment for 5 d with 200 mg P_4/d did not block E_2 induced PUN depression ($P < .05$; Table 6.3). If P_4 pretreatment down-regulated E_2 receptors not involved in the growth response, these results may indicate anabolic E_2 actions in addition to E_2 receptor mediated actions, such as the hypothesized CE_2 effects (involvement of the cAMP-second messenger system

and enzyme regulation of events which may lead to anabolic activity and growth regulation). We are currently evaluating P_4 pretreatment effects on E_2 receptor content in bovine skeletal muscle as well as CE_2 binding to E_2 receptors.

Two commercially available implants, Synovex-S and Steeroid, contain both E_2 benzoate (20 mg) and P_4 (200 mg). As reviewed by Galbraith and Topps (1981), melengesterol acetate (a synthetic progestogen) and P_4 were ineffective as growth promotants in steers and lambs, respectively. Melengesterol acetate, however, increased the lean to bone ratio in heifer carcasses and a combination of P_4 and E_2 resulted in up to 27% improvements in growth in steers, with varying responses in carcass data. This latter effect, however, is likely due primarily to E_2 rather than P_4. Heitzman et al. (1981) indicated that implantation of E_2 with either TBA, testosterone or P_4 increases the time plasma E_2 levels are elevated due to a slower, more sustained release of E_2.

In conclusion, results of this research indicate that CE_2 formation may be involved in the anabolic action of estrogens and that pretreatment with P_4 did not block E_2 induced PUN depression. Further research is required to evaluate the effects of CE_2 and P_4 on the anabolic E_2 response in ruminants. Other alternate mechanisms of estrogen action have recently been elucidated. Breier et

al. (1988a,b) concluded that since E_2 implantation increased both ST secretion and ST receptors as well as insulin-like growth factor I and II concentrations, and growth rate, modulation of ST receptors are an important regulatory mechanism of the somatotropic axis and growth regulation in ruminants.

TABLE 6.1. COMPOSITION OF DIET[a]

Ingredient	%[b]
Steam flaked sorghum grain	39.80
Cottonseed hulls	27.10
Cottonseed meal	19.60
Cane molasses	3.30
Fat	1.50
Urea	.65
Ammonium sulfate	.09
Calcium carbonate	1.39
Sodium chloride	.16
Vitamin A premix	.52
Vitamin E premix	.30
Trace mineral premix	.15
Water	5.00
Tylosin premix	.44
Total	100.00

[a]Formulated to contain 18.0% CP; 3.13 Mcal DE/kg; 3.95% crude fat; 40% NDF; 29% roughage equivalent; .75% Ca; .40% P; .11% Na; .83% K; .19% S; 44 ppm Zn; 4068 IU/kg Vitamin A on a dry matter basis.

[b]As fed basis.

TABLE 6.2. LEAST-SQUARES MEANS FOR EFFECTS OF TREATMENT, DAYS ON TRIAL AND TREATMENT BY DAYS ON TRIAL INTERACTION FOR PLASMA E_2 CONCENTRATIONS (PG/ML) FOR FEEDLOT STEERS[abc]

	Days on trial					
Treatment	0	1	4	7	Cubic contrast[c]	Treatment mean[a]
Control	39.4	17.8	32.8	24.4	D	28.6[de]
P_4	28.8	16.3	32.2	27.6	D	26.2[d]
P_4/E_2	15.5	34.9	48.9	53.5	E	38.2[ef]
E_2	27.7	48.7	43.6	44.2	E	41.1[f]
CE_2	18.1	37.1	37.9	39.1	E	33.0[def]
Days on trial mean[b]	25.9	31.0	39.1	37.8		

[a]Main effects of treatment ($P < .05$; SE = 3.85).

[b]Effects of days on trial ($P < .02$; SE = 3.35). Linear ($P < .01$) increase in plasma E_2 as days on trial increased.

[c]Treatment by days on trial interaction ($P < .04$; SE = 7.90). Significant cubic contrast ($P < .05$) between treatments as days on trial increased. Treatments having at least one letter in common are similar ($P > .10$) for contrast comparison.

[d,e,f]Means with different superscripts within treatment differ ($P < .05$).

TABLE 6.3. LEAST-SQUARES MEANS FOR EFFECTS OF TREATMENT, DAYS ON TRIAL AND TREATMENT BY DAYS ON TRIAL INTERACTION FOR PUN (MG/DL) FOR FEEDLOT STEERS[abc]

	Days on trial			Contrast[c]		Treatment
Treatment	1	4	7	L	Q	mean[a]
Control	13.1	12.8	13.2	DE	D	13.2[d]
P_4	11.8	13.7	12.5	E	E	12.7[d]
P_4/E_2	11.3	10.2	9.9	D	D	10.5[e]
E_2	11.2	9.1	9.5	D	D	10.0[e]
CE_2	11.5	9.8	9.9	D	D	10.4[e]
Days on trial mean[b]	11.8	11.1	11.0			

[a]Main effects of treatment ($P < .004$; SE = .64).

[b]Effects of days on trial ($P < .03$; SE = .21). Linear ($P < .05$) decrease in PUN as days on trial increased.

[c]Treatment by days on trial interaction ($P < .006$; SE = .48). Significant linear (L) and quadratic (Q; $P < .05$) contrasts between treatments as days on trial increased. Treatments having at least one letter in common are similar ($P > .05$) for contrast comparison.

[d,e]Means with different superscripts within treatment differ ($P < .05$).

CHAPTER VII

17-β ESTRADIOL CLEARANCE RATE IN FEEDLOT STEERS

Abstract

Three trials were conducted to determine the clearance rate of E_2 and to determine the effect of implantation on E_2 clearance rate in feedlot steers. In trial 1, six steers (402 kg) were randomly allotted to the following treatments (three steers/treatment): control (C), infused with 100% ethanol, or 17-β estradiol (E_2), infused with 1.24 μg E_2/kg. Steers were jugular catheterized, placed in outdoor stanchions and infused with their respective treatments. Following infusion, the catheters were flushed with 5 ml ethanol followed by 5 ml heparinized physiological saline. Serial blood samples were collected at the following times (T): 0 (initial), 15, 30, 45, 60, 90, 120, 240, 360 and 720 min post infusion. In trial 2, five steers (225 kg) were placed in metabolism stalls 1 week prior to the initiation of the study, fed a diet at 1.2X maintenance and randomly allotted to C, or E_2 infused at a dosage of 1.24 μg E_2/kg. Serial blood samples were collected at the following T: -4, -2, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 52.5, 60, 90, 120, 240, 360, 720 and 1440 min post infusion. One week later, treatments were reversed. In

trial 3, 12 Hereford steers (309 kg) were allotted to either a control (six steers) or implant treatment (Compudose; six steers). Steers were fed ad libitum for 40 d on a feedlot diet to meet NRC requirements. Steers were then weighed (control = 331 kg; implant = 342 kg), placed in metabolism stalls and fed at 1.5X maintenance. On d 50 and 51, six steers were infused with C and six steers were infused with 1.24 μg E_2/kg. One week later, infusion treatments were reversed. Sampling times were identical to those in trial 2 with the addition of a 1 min sample. In all three trials, plasma E_2 concentrations were elevated ($P < .05$) in E_2 infused steers for 30 to 35 min post infusion, after which time E_2 concentrations had reached baseline concentrations. The following two-pool biexponential equations best described the E_2 clearance:

Trial 1: $C = 4.644\ e^{-6.434\ (h)} + .441\ e^{-.983\ (h)}$

Trial 2: $C = 2.436\ e^{-30.876\ (h)} + .714\ e^{-2.346\ (h)}$

Trial 3: $C = 5.645\ e^{-58.505\ (h)} + .939\ e^{-2.380\ (h)}$

where C is the E_2 concentration at time h in hours, ng/ml; 6.434 and .983, 30.876 and 2.346, 58.505 and 2.380 (h^{-1}) are the slopes of the fast and slow components of the E_2 clearance curves in trials 1, 2 and 3, respectively; and 4.644 and .441, 2.436 and .714, 5.645 and .939 (ng/ml) are the Y-intercepts of the fast and slow components of the E_2 clearance curves in trials 1, 2 and 3, respectively. Based on these equations, infused E_2 had a short half-life

of 6.46, 1.34 and .93 min (fast pool) and 42.29, 17.72 and 19.3 min (slow pool) in trials 1, 2 and 3, respectively. In trial 3, implantation with E_2 elevated ($P < .0001$) plasma E_2 concentrations (8.23 versus 13.25 pg/ml for control and implanted steers, respectively). There was no influence of implantation ($P > .10$) on the slope, intercept or half-life components of the clearance curve. Results indicate that once E_2 enters the circulatory system, through infusion, it is cleared very rapidly from the plasma and that implantation did not alter the E_2 clearance rate, however, it did elevate plasma E_2 concentrations.
(Keywords: 17 -β Estradiol, Clearance Rate, Implantation, Steers.)

Introduction

Glascock and Hoekstra (1959) observed that radio-labelled hexestrol was concentrated in organs known to respond to estrogens (uterus, vagina, mammary gland and pituitary) and the excretory organs (kidney, liver and intestines). However, 24 h following the radio-labelled hexestrol injection, they observed that less than two% of the labelled hormone remained in the tissues of sheep and goats. Therefore, 98% of the label not only was cleared from the circulatory system, but from the tissues as well.

Calculating E_2 clearance rate in E_2 implanted cattle by measuring the amount of E_2 in the plasma ipsilateral versus contralateral to the implant gave half-lives of 1.8 to 6.8 min (Harrison, 1981, as cited by Heitzman et al., 1984).

Due to the lack of information on E_2 clearance rate in cattle, the objective of this research was to determine the clearance rate of E_2 and to determine the effect of implantation on E_2 clearance rate in feedlot steers. This information will provide insight into the length of effectiveness of circulating E_2 in implanted cattle and will allow us to determine if implantation alters the kinetic parameters of estrogen in the circulatory system, which may affect the clearance, metabolism, utilization and/or mode-of-action of estrogen.

Materials and Methods

Trial 1. Six steers (402 kg) were randomly allotted to the following treatments (three steers/treatment): 1) control (C), infused with 2 ml 100% ethanol; 2) 17-β estradiol (E_2), infused with 2 ml 100% ethanol containing .25 mg E_2/ml (.50 mg E_2/steer). Steers were jugular catheterized, placed in outdoor stanchions and infused with their respective treatments. Following infusion, the catheters were flushed with 5 ml ethanol followed by 5 ml heparinized physiological saline. Serial blood samples

were collected at the following times (T): 0 (initial), 15, 30, 45, 60, 90, 120, 240, 360 and 720 min post infusion. Samples were collected in heparinized tubes, centrifuged and the plasma stored at -20°C until analyzed for E_2[1] concentration via a double antibody radioimmunoassay (Hancock, 1989).

Data were analyzed by analysis of variance using the General Linear Model Procedure (SAS, 1985) to evaluate plasma E_2 concentrations over time in both control and E_2 infused steers. Effects included in the analysis were steer, time and residual effects which were used as the error term. Means were separated by protected least significant difference. Nonlinear regression was used to evaluate the E_2 clearance curve in E_2 infused steers using an exponential equation (SAS, 1985). Preliminary plasma E_2 concentrations (T 0) were excluded from this analysis. To determine whether increased goodness of fit (decreased residual sums of squares (RSS)) for the two-pool model was significant, the following F-test was performed (Munson and Rodbard, 1980; Motulsky and Ransnas, 1987):

$$F_{calc} = \frac{RSS1 - RSS2 \;/\; (df1 - df2)}{RSS2 \;/\; df2}$$

where RSS1 and RSS2 are the residual sums of squares and df1 and df2 are the associated degrees of freedom for the

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[1]Diagnostic Products Corporation, 5700 W. 96th St. Los Angeles, CA 90045.

one- and two-pool models, respectively. This F value was tested against $F_{df1 - df2, df2}$ at α=.05. Nonlinear regression was performed on individual animal clearance curves to determine individual slopes and intercepts for the fast and slow pools of the E_2 clearance curve.

Trial 2. Five steers (225 kg) were placed in metabolism stalls 1 week prior to the initiation of this study so that they would be adjusted to the environment prior to the study. Steers were fed a diet (Table 7.1) at 1.2X maintenance. Steers were randomly allotted to the following treatments: C, infused with 100% ethanol or E_2 infused at a dosage of 1.24 µg E_2/kg (equivalent to E_2 dosage in trial 1). The concentration of E_2 in the injection vehicle was .1 mg E_2/ml of 100% ethanol. Steers were jugular catheterized and infused with their respective treatments. Following infusion, the catheters were flushed with 5 ml ethanol followed by 5 ml heparinized physiological saline. Serial blood samples were collected at the following T: -4, -2, 2, 4, 6, 8, 10, 12, 15, 20, 25, 30, 35, 40, 45, 52.5, 60, 90, 120, 240, 360, 720 and 1440 min post infusion. One week later, treatments were reversed and the trial was repeated. Plasma samples were collected, stored and analyzed, and the data were statistically analyzed as previously described in trial 1. An analysis of variance was

performed to determine if there were differences between trials 1 and 2 in E_2 clearance curve parameters.

Trial 3. Twelve Hereford steers (309 kg) were allotted to either a control (six steers) or implant treatment (Compudose; six steers). Steers were fed ad libitum for 40 d on a feedlot diet to meet NRC requirements (Table 7.2). Steers were then weighed (control = 331 kg; implant = 342 kg), placed in metabolism stalls and fed at 1.5X maintenance. On d 50 and 51, six steers (3 control steers and 3 implanted steers) were infused, via a jugular catheter, with 100% ethanol and six steers (3 control steers and 3 implanted steers) were infused with 1.24 µg E_2/kg in 100% ethanol. One week later, infusion treatments were reversed. Infusion procedures, sampling times, sample collection, storage and analysis and statistical analysis were identical to procedures described in trial 2. In addition, a 1 min sample was collected. Effects included in the analysis of variance model for plasma E_2 evaluation were implant and time. An additional analysis of variance was conducted on the kinetic parameters determined from the nonlinear regression procedure to determine if prior implantation had an effect on the kinetic parameters of E_2 clearance.

Results and Discussion

Due to differences ($P < .05$) in fast pool intercepts and fast and slow pool slopes between trials 1 and 2, the two trials will be discussed individually.

Trial 1. Plasma E_2 concentrations in the E_2 infused steers were elevated several orders of magnitude greater than would be expected from E_2 released from an implant (Rumsey and Beaudry 1979; Heitzman et al., 1981; Castree et al., 1988; Hancock and Preston, 1989a,c). Infusion of E_2 had a significant effect ($P < .0001$; SE = .117 ng/ml) on plasma E_2 concentrations over time (Figure 7.1). Plasma E_2 concentrations were higher ($P < .05$) than initial E_2 concentrations at 15 and 30 min post E_2 infusion in the E_2 infused steers. Plasma E_2 concentrations were similar ($P > .05$) to initial E_2 concentrations, however, at all remaining sampling times, indicating a rapid clearance of E_2 from the circulatory system. By 90 min post infusion, plasma E_2 concentrations were below initial E_2 concentrations and continued to decrease throughout the collection period. Although the E_2 concentrations after 90 min were not significantly different ($P > .5$) from initial E_2 concentrations, the differences were numerically large (113 pg/ml at initial sampling compared with 80, 59, 19, 15 and 4 pg/ml at 90, 120, 240, 360 and 720 min samplings, respectively) and may have biological implications.

Plasma E_2 concentrations were similar ($P > .12$; SE = .045 ng/ml) over time in control steers (Figure 7.2). One steer, however, had an elevated plasma E_2 concentration at 15 min (.441 ng/ml), and then demonstrated a clearance response similar to the E_2 infused steers. Removing this steer from the analysis resulted in significant differences ($P < .0001$; SE = .009 ng/ml) in plasma E_2 concentrations over time in control steers (Figure 7.2). Plasma E_2 concentrations were higher ($P < .05$) than initial E_2 concentrations at 30, 45 and 90 min post infusion. There was a six fold increase in E_2 concentration from 60 to 90 min. One possible reason for this peak could be an endogenous E_2 release in response to handling and sampling the steers, especially since these steers were in outdoor stanchions and were not accustomed to the environment or handling.

Individual steer clearance curve components are reported in Table 7.3. The following two-pool biexponential equation best described the E_2 clearance:

$$C = 4.644\ e^{-6.434\ (h)} + .441\ e^{-.983\ (h)}$$

where C is the E_2 concentration at time h in hours, ng/ml; 6.434 and .983 (h^{-1}) are the slopes of the fast and slow components of the E_2 clearance curve, respectively; and 4.644 and .441 (ng/ml) are the Y-intercepts of the fast and slow components of the E_2 clearance curve, respectively. Based on this equation, infused E_2 has a

short half-life of 6.46 min (fast pool) and 42.29 min (slow pool). Volume of distribution was 24.6% of body weight, based on the Y-intercept.

Trial 2. Due to the rapid half-life of E_2 observed in trial 1, plasma samples were collected at very frequent intervals in trial 2. There were no differences over time in plasma E_2 concentrations in control infused steers ($P > .05$; avg = 6.32 pg/ml; SE = 1.50 pg/ml). Similar to trial 1, E_2 infusion had a significant effect ($P < .0001$; SE = 54.9 pg/ml) on plasma E_2 concentrations over time (Figure 7.1). Plasma E_2 concentrations were higher ($P < .05$) than initial E_2 concentrations at 2, 4, 6, 8, 10, 12, 15, 20, 25, 30 and 35 min post infusion in the E_2 treated steers. Plasma E_2 concentrations were similar ($P > .05$) to initial E_2 concentrations at all remaining sampling times. Unlike trial 1, with increasing time, plasma E_2 concentrations did not decrease below initial concentrations.

Individual steer clearance curve components are reported in Table 7.4. The following two-pool biexponential equation best described the E_2 clearance:

$$C = 2.436\ e^{-30.876\ (h)} + .714\ e^{-2.346\ (h)}$$

where C is the E_2 concentration at time h in hours, ng/ml; 30.876 and 2.346 (h^{-1}) are the slopes of the fast and slow components of the E_2 clearance curve, respectively; and 2.436 and .714 (ng/ml) are the Y-intercepts of the fast

and slow components of the E_2 clearance curve, respectively. Based on this equation, infused E_2 has a short half-life of 1.34 min (fast pool) and 17.72 min (slow pool). Volume of distribution was 39.4% of body weight, based on the Y-intercept.

Trial 3. Implantation with E_2 elevated ($P < .0001$) plasma E_2 concentrations (8.23 versus 13.25 pg/ml for control and implanted steers, respectively). Similar to trial 2, there was no influence ($P > .10$) of sampling time in the steers infused with ethanol and plasma E_2 concentrations were elevated ($P < .05$) in E_2 infused steers for 30 min post infusion, after which time E_2 concentrations had reached baseline concentrations (Figure 7.1). There was no influence of implantation ($P > .10$) on the slope, intercept or half-life (.93 and 19.3 min in fast and slow pools, respectively) components of the clearance curve (Table 7.5). Volume of distribution was 18.9% of body weight, based on the Y-intercept.

Individual steer clearance curve components are reported in Table 7.5. The following two-pool biexponential equation best described the E_2 clearance:

$$C = 5.645\ e^{-58.505\ (h)} + .939\ e^{-2.380\ (h)}$$

where C is the E_2 concentration at time h in hours, ng/ml; 58.505 and 2.380 (h^{-1}) are the slopes of the fast and slow components of the E_2 clearance curve, respectively; and 5.645 and .939 (ng/ml) are the Y-intercepts of the fast

and slow components of the E_2 clearance curve, respectively.

These results indicating a rapid clearance of E_2 from the circulatory system are in accordance with the results of Harrison (1981), cited by Heitzman et al. (1984), who reported E_2 half-lives of 1.8 to 6.8 min in implanted cattle. These half-lives are faster, however, than those reported for human E_2 clearance. Human females have a metabolic clearance rate for E_2 of approximately 1000 liters/d (Reed and Murray, 1979). As cited by (Bidlingmaier and Knorr, 1978), Sandberg and Slaunwhite (1957), using radio-labelled E_2, calculated a 22-min half-life for circulating E_2 in humans. Breuer and Breuer (1973) observed a rapid initial disappearance of radio-labelled E_2 following injection. They observed a second pool which had a slower half-life of 120 min. The fast clearance pool was thought to be a reflection of injected E_2 distribution within the body, while the slow clearance pool was largely determined by metabolism and excretion of E_2.

Estradiol and estrone are metabolized in the liver via hydroxylation primarily on the 2 (catechol estrogen formation) and 16 carbons. The liver is also an important site of steroid conjugation, and it was stated that "the decisive metabolic step for estrogen elimination is esterification with glucuronic or sulfuric acids to water-

soluble conjugates" (Bidlingmaier and Knorr, 1978). The glucuronic conjugates are primarily excreted through the kidneys as glucuronides and are eliminated via the urine. Lesser amounts are excreted via the liver through biliary excretion into the intestine. Furthermore, the intestinal conjugates are hydrolyzed and free estrogens are reabsorbed with small amounts of fecal excretion occurring due to this enterohepatic circulation of estrogens. In ruminants, however, Preston (1975) indicated that 45-95% and 4-30% of diethylstilbestrol was eliminated via the feces and urine, respectively.

In conclusion, once E_2 enters the circulatory system, through infusion, it is cleared very rapidly from the plasma. Implantation did not alter the E_2 clearance rate, however, it did elevate plasma E_2 concentrations. Therefore, elevated plasma E_2 concentrations observed in implanted cattle are most likely a result of the release rate from the implant rather than a slow clearance rate of E_2 from the plasma.

TABLE 7.1. COMPOSITION OF DIET IN TRIAL 2[a]

Ingredient	%[b]
Steam flaked sorghum grain	47.74
Corn silage	31.99
Cottonseed hulls	12.00
Cottonseed meal	3.00
Cane molasses	3.37
Calcium carbonate	.80
Sodium chloride	.17
Potassium chloride	.04
Urea	.49
Vitamin A premix	.24
Trace mineral premix	.16
Total	100.00

[a]Formulated to contain 12.4% CP; 3.26 Mcal DE/kg; 2.61% crude fat; 34% NDF; 25% roughage equivalent; .57% Ca; .29% P; .13% Na; .75% K; .15% S; 40 ppm Zn; 4587 IU/kg Vitamin A on a dry matter basis.

[b]As fed basis.

TABLE 7.2. COMPOSITION OF DIET IN TRIAL 3[a]

Ingredient	%[b]
Steam flaked sorghum grain	24.46
Corn silage	43.73
Cottonseed hulls	20.88
Cottonseed meal	7.92
Cane molasses	1.89
Calcium carbonate	.51
Sodium chloride	.12
Dicalcium phosphate	.24
Vitamin A premix	.10
Trace mineral premix	.15
Total	100.00

[a]Formulated to contain 11.7% CP; 2.98 Mcal DE/kg; 2.32% crude fat; 47% NDF; 46% roughage equivalent; .53% Ca; .37% P; .10% Na; .89% K; .15% S; 45 ppm Zn; 5236 IU/kg Vitamin A on a dry matter basis.

[b]As fed basis.

TABLE 7.3. INDIVIDUAL STEER CLEARANCE CURVE COMPONENTS DETERMINED FROM NONLINEAR REGRESSION ANALYSIS OF PLASMA E_2 CONCENTRATIONS POST E_2 INFUSION IN TRIAL 1

Steer	Fast pool intercept ng/ml	Slow pool intercept ng/ml	Fast pool slope h^{-1}	Slow pool slope h^{-1}	r^2	$S_{y.x}$ ng/ml
1	2.62	.53	8.41	1.50	.999	.006
2	5.89	.65	6.46	1.07	.999	.017
3	5.42	.15	4.43	.38	.999	.009
Lsmean	4.644	.441	6.434	.983		
SE	.715	.124	6.057	.207		

TABLE 7.4. INDIVIDUAL STEER CLEARANCE CURVE COMPONENTS DETERMINED FROM NONLINEAR REGRESSION ANALYSIS OF PLASMA E_2 CONCENTRATIONS POST E_2 INFUSION IN TRIAL 2

Steer	Fast pool intercept ng/ml	Slow pool intercept ng/ml	Fast pool slope h^{-1}	Slow pool slope h^{-1}	r^2	$S_{y.x}$ ng/ml
1	2.73	.86	19.94	2.37	.986	.092
2	3.38	.41	41.84	2.22	.996	.025
3	1.19	.82	25.31	2.66	.997	.028
4	2.89	.82	47.18	2.48	.997	.028
5	1.99	.67	20.11	2.23	.996	.037
Lsmean	2.436	.714	30.876	2.346		
SE	.554	.096	4.692	.160		

TABLE 7.5. INDIVIDUAL STEER CLEARANCE CURVE COMPONENTS DETERMINED FROM NONLINEAR REGRESSION ANALYSIS OF PLASMA E_2 CONCENTRATIONS POST E_2 INFUSION IN TRIAL 3[a]

Steer	Fast pool intercept ng/ml	Slow pool intercept ng/ml	Fast pool slope h^{-1}	Slow pool slope h^{-1}	Fast pool half-life min	Slow pool half-life min	r^2	$S_{y.x}$ ng/ml
Control								
1	5.75	1.39	52.41	2.70	.79	15.43	.997	.064
2	7.43	.57	39.64	2.06	1.05	20.18	.999	.017
3	6.12	.38	13.38	.98	3.11	42.30	.993	.160
4	7.82	.82	100.72	2.48	.41	16.77	.997	.047
5	4.42	.90	54.68	3.13	.76	13.28	.998	.037
6	3.12	.80	44.54	2.81	.93	14.81	.997	.032
Lsmean	5.78	.81	50.89	2.36	1.18	20.46		
Implanted								
7	2.78	1.14	42.54	2.06	.98	20.21	.994	.066
8	2.62	.59	44.56	2.00	.93	20.77	.995	.042
9	6.71	1.36	86.47	3.75	.48	11.10	.992	.080
10	8.57	.99	89.20	2.17	.47	19.19	.999	.033
11	9.25	1.29	61.62	2.34	.67	17.81	.999	.047
12	3.16	1.05	72.32	2.09	.57	19.87	.994	.056
Lsmean	5.51	1.07	66.12	2.40	.68	18.16		
Overall Lsmean	5.65	.94	58.51	2.380	.93	19.31		
SE	1.02	.13	10.08	.29	.29	3.33		

[a]No influence of implantation on slopes, intercepts or half-lives ($P > .10$).

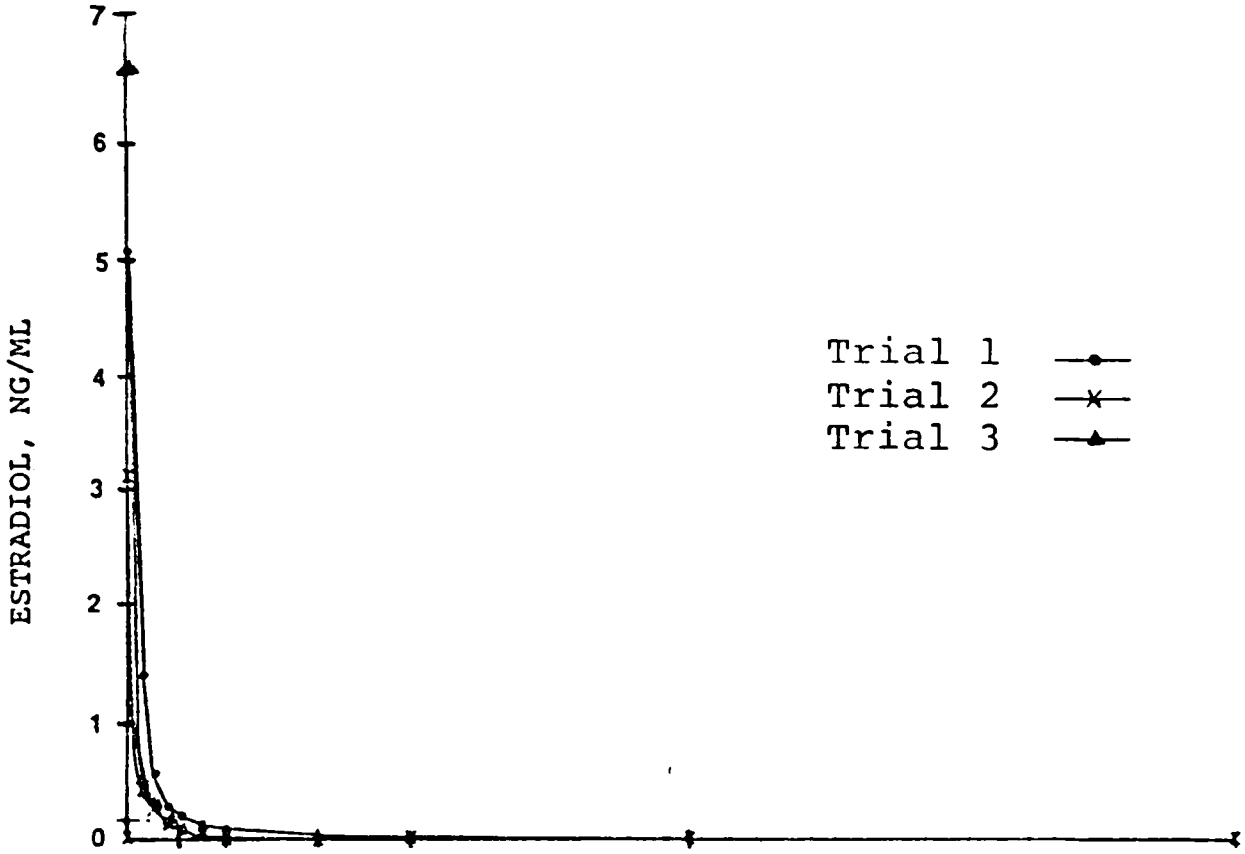

Figure 7.1. Clearance of E_2 for steers in trials 1, 2 and 3 post E_2 infusion.

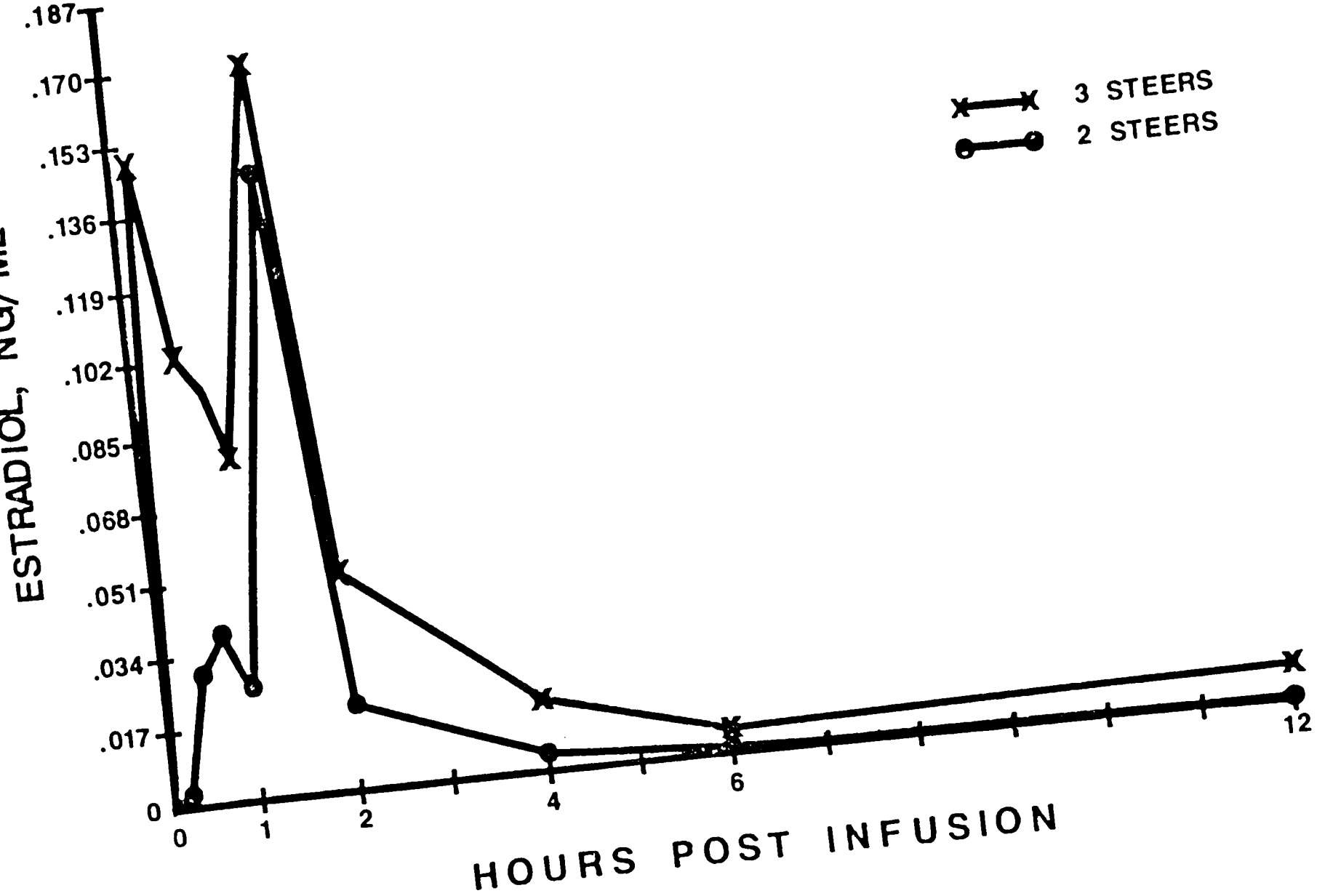

Figure 7.2. Least-squares mean E_2 concentration in control steers in trial 1.

CHAPTER VIII

INTEGRATED SUMMARY

Consumer awareness on the diet/health issues has increased demand for lean meat. Anabolic estrogens increase growth, efficiency and lean tissue deposition in ruminants. The mode of action which elicits these effects has not been clearly defined. Therefore, it was the intent of this research to identify the mechanism of anabolic estrogen action in ruminants. Specific objectives were to determine if the anabolic actions of estradiol (E_2) and somatotropin (ST) are additive; to determine if catechol estrogen formation is involved; to determine the effects of progesterone on the anabolic activity of estrogens; and to determine estradiol clearance rate.

Dose titration studies were conducted to determine the optimum dosage of E_2 and ST required for maximal plasma urea nitrogen (PUN) reduction, an indicator of anabolic activity. These optimum dosages were then tested individually and in combination to determine if their anabolic effects were additive. Non additive results would indicate that ST mediates estrogen action, a hypothesized mechanism of estrogen action. Additive results would indicate an alternate mechanism. Alternate mechanisms tested were the involvement of catechol

estrogens and progesterone. The effect of implantation on E_2 clearance rate was determined.

Depression in PUN increased with E_2 dosages of 24 mcg/d and with ST dosages of 41 $mcg.kg^{-1}.d^{-1}$ and above. Administration of E_2 increased plasma E_2 and ST. Administration of ST increased plasma ST and increased PUN depression. In two trials, E_2 implantation failed to reduce PUN. Plasma E_2 in non-treated steers was several fold higher than expected and may explain the lack of PUN response. When PUN was reduced following both E_2 and ST administration, there was an additive effect with combined administration. Plasma urea nitrogen was reduced by 24, 29 and 44% with E_2, ST and E_2 plus ST, respectively. Therefore, the anabolic actions of E_2 appear to be by some other mechanism or in addition to increased endogenous ST secretion. Similar PUN reductions were observed between catechol E_2 and E_2 administration, therefore, catechol E_2 may be involved in anabolic estrogen action. Pretreatment with progesterone did not block E_2 induced PUN depression and E_2 implantation did not alter the rapid E_2 clearance rate.

LITERATURE CITED

Anderson, D.B., W.P. Waitt, R.E. Paxton, J.F. Wagner, D.H. Mowrey and D.J. Jones. 1988. The effects of estrogen implantation on growth and carcass composition of intact male pigs. J. Anim. Sci. 66 (Suppl. 1):290 (Abstr.).

Baile, C.A., M.A. Della-Fera and F.C. Buonomo. 1986. The neurophysiological control of growth. In: P.J. Buttery, D.B Lindsay and N.B. Haynes (Ed.) Control and Manipulation of Animal Growth. pp 105. Butterworths, London.

Ball, P., R. Knuppen, M. Haupt and H. Breuer. 1972. Interactions between estrogens and catechol amines III. Studies on the methylation of catechol estrogens, catechol amines and other catechols by the catechol-o-methyltransferase of human liver. J. Clin. Endocr. 34:736.

Basson, R.P., W.E. Dinusson, L. Embry, D.L. Feller, P.E. Gorham, H.P. Grueter, D.D. Hinman, J. MacAkill, C. Parrott, J. Riley, T.L. Stanton, D.C. Young and J.F. Wagner. 1985. Comparison of the performance of estradiol silicone rubber implant-treated steers to that of zearalone or estradiol + progesterone. J. Anim. Sci. 61:1023.

Bauman, D.E., J.H. Eisemann and W.B. Currie. 1982. Hormonal effects on partitioning of nutrients for tissue growth: Role of growth hormone and prolactin. Fed. Proc. 41:2538.

Bauman, D.E. and S.N. McCutcheon. 1986. The effects of growth hormone and prolactin on metabolism. In: L.P. Milligan, W.L. Grovum and A. Dobson (Ed.) Control of Digestion and Metabolism in Ruminants. pp 436. Prentice-Hall, Englewood Cliffs, NJ.

Bechet, D.M., B.N. Perry, A. Toong and R.D. Lovell. 1986. Oestrogen specific binding sites in bovine muscle nuclei. J. Steroid Biochem. 24:1127.

Bergen, W.G. 1974. Protein synthesis in animal models. J. Anim. Sci. 38:1079.

Bidlingmaier, F. and D. Knorr. 1978. Oestrogens: physiological and clinical aspects. In: Z. Laron (Ed.) Pediatric and Adolescent Endocrinology, Vol. 4. pp 1. S. Karger AG, Basel (Switzerland).

Breier, B.H., P.D. Gluckman and J.J. Bass. 1988a. The somatotrophic axis in young steers: influence of nutritional status and oestradiol-17β on hepatic high-and-low-affinity somatotrophic binding sites. J. Endocr. 116:169.

Breier, B.H., P.D. Gluckman and J.J. Bass. 1988b. Influence of nutritional status and oestradiol-17β on plasma growth hormone, insulin-like growth factors-I and -II and the response to exogenous growth hormone in young steers. J. Endocr. 118:243.

Breuer, J. and Breuer, H. 1973. Half-life of [4-^{14}C] oestradiol-17β in vivo, and metabolism of [4-^{14}C] oestrone and [4-^{14}C] oestriol in vitro in normal subjects and in patients with liver cirrhosis. Z. Klin. Chem. Klin. Biochem. 11:263.

Buttery, P.J. 1983. Hormonal control of protein deposition in animals. Proc. Nutr. Soc. 42:137.

Buttery, P.J. and J.M. Dawson. 1987. The mode of action of beta-agonists as manipulators of carcass composition. In: J.P. Hanrahan (Ed.) Beta-Agonists and Their effects on Animal Growth and Carcass Quality. pp 29. Elsevier Applied Science, London.

Buttery, P.J. and P.A. Sinnett-Smith. 1984. The mode of action of anabolic agents with special reference to their effects on protein metabolism-some speculations. In: J.F. Roche and D. O'Callagan (Ed.) Manipulation of Growth in Farm Animals. pp 211. Marinus Nijhoff, Dordrecht, Netherlands.

Cain, M.F., J. Henson, R.D. Williams and P.G. Lemieux. 1986. Effect of 72 mg zeranol on gain and efficiency of feedlot steers. J. Anim. Sci. 63 (Suppl. 1):398 (Abstr.).

Carrick, M.J. and J.N. Shelton. 1969. Oestrogen-progesterone relationships in the induction of oestrus in spayed heifers. J. Endocrinol. 45:99.

Castree, J.W., R.P. Wettemann, K.S. Lusby, E.R. Cole, T.C. Fox, M.A. Kimbrough, K.W. Kugler and B.G. McDaniel. 1988. Plasma estradiol after implanting calves with estradiol benzoate. Oklahoma Agricultural Experiment Station. Animal Science Research Report. pp 38.

Chaney, A.L. and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130.

Clancy, M.J., J.M. Lester and J.F. Roche. 1986. The effects of anabolic agents and breed on the fibers of the longissimus muscle of male cattle. J. Anim. Sci. 63:83.

Cohick, W.S., R. Slepetis, K. Plaut and D.E. Bauman. 1987. Effect of exogenous somatotropin on serum somatomedin-C (SmC) and hepatic metabolism of lactating cows. J. Anim. Sci. 65 (Suppl. 1):248 (Abstr.).

Cross, H.R. 1988. Developing a better product. The use of biotechnology can help to meet carcass targets. Feedstuffs/Cattle. Special supplement. 2:1.

Dahlberg, E. 1982. Characterization of the cytosolic estrogen receptor in rat skeletal muscle. Biochimica et Biophysica Acta 717:65.

Daley, C.A., A.V. Rodiek, F.D. Schrieber and D.A. Daley. 1987. The synergistic effect of anabolic sex steroids on growth, body composition and reproductive tissue. J. Anim. Sci. 65 (Suppl. 1):289 (Abstr.).

Davidge, S.T., J.L. Wiebold, P.L. Senger and J.K. Hillers. 1987. Influence of varying levels of blood progesterone upon estrous behavior in cattle. J. Anim. Sci. 64:126.

Davis, S.L., U.S. Garrigus and F.C. Hinds. 1970a. Metabolic effects of growth hormone and diethylstilbestrol in lambs. II. Effects of daily ovine growth hormone injections on plasma metabolites and nitrogen - retention in fed lambs. J. Anim. Sci. 30:236.

Davis, S.L., U.S. Garrigus and F.C. Hinds. 1970b. Metabolic effects of growth hormone and diethylstilbestrol in lambs. III. Metabolic effects of DES. J. Anim. Sci. 30:241.

Davis, S.L., D.L. Ohlson, J. Klindt and M.S. Anfinson. 1978. Episodic patterns of prolactin and thyrotropin secretion in rams and wethers: Influence of testosterone and diethylstilbestrol. J. Anim. Sci. 46:1724.

Davis, S.L., K.L. Hossner and D.L. Ohlson. 1984. Endocrine regulation of growth in ruminants. In: J.F. Roche and D. O'Callagan (Ed.) Manipulation of Growth in Farm Animals. pp 151. Marinus Nijhoff, Dordrecht, Netherlands.

Dehoff, M.H., R.G. Elgin, R.J. Collier and D.R. Clemmons. 1988. Both type I and II insulin-like growth factor receptor binding increase during lactogenesis in bovine mammary tissue. Endocrinology 122:2412.

DeWilde, R.O. and H. Lauwers. 1984. The effect of parenteral use of estradiol, progesterone, testosterone and trenbolone on growth and carcass composition in pigs. J. Anim. Sci. 59:1501.

Dionne, F.T., R.L. Lesage, J.Y. Dube and R.R. Tremblay. 1979. Estrogen binding proteins in rat skeletal and perineal muscles: In vitro and in vivo studies. J. Steroid Biochem. 11:1073.

Donaldson, I.A. and R.J. Heitzman. 1983. Reduction of urea synthesis in growing heifers implanted with trenbolone acetate. Proc. Nutr. Soc. 42:59A (Abstr.).

Dorfman, R.I. and F. Ungar. 1965. Metabolism of Steroid Hormones. pp 402. Academic Press, New York.

Dube, J.Y., R. Lesage and R.R. Tremblay. 1976. Androgen and estrogen binding in rat skeletal and perineal muscles. Can. J. Biochem. 54:50.

Eisemann, J.H., H.F. Tyrrell, A.C. Hammond, P.J. Reynolds, D.E. Bauman, G.L. Haaland, J.P. McMurtry and G.A. Varga. 1986. Effect of bovine growth hormone administration on metabolism of growing hereford heifers: Dietary digestibility, energy and nitrogen balance. J. Nutr. 116:157.

Florini, J.R. 1987. Hormonal control of muscle growth. Muscle and Nerve 10:577.

Frey, R.S., F.D. McCarthy and D.J. Johnson. 1988. Characterization of estrogen and glucocorticoid receptors in skeletal muscle of lambs treated with estradiol-17 beta and trenbolone acetate. J. Anim. Sci. 66 (Suppl. 1):290 (Abstr.).

Galbraith, H. 1980. The effect of trenbolone on acetate growth, blood hormones and metabolites, and nitrogen balance of beef heifers. Anim. Prod. 30:389.

Galbraith, H. and J.H. Topps. 1981. Effect of hormones on growth and body composition of animals. Nutr. Abstr. Rev. Series B. 51:521.

Glascock, R.F. and W. G. Hoekstra. 1959. Selective accumulation of tritium-labelled hexoestrol by the reproductive organs of immature female goats and sheep. Biochem. J. 72:673.

Gluckman P.D., B.H. Breier and S.R. Davis. 1987. Physiology of the somatotropic axis with particular reference to the ruminant. J. Dairy Sci. 70:442.

Goldberg, A.L., M. Tischler, G. DeMartino and G. Griffin. 1980. Hormonal regulation of protein degradation and synthesis in skeletal muscle. Fed. Proc. 39:31.

Goodwin, J.F. 1968. The colorimetric estimation of plasma amino nitrogen with DNFB. Clin. Chem. 14:1080.

Gopinath, R. and W.D. Kitts. 1984. Growth hormone secretion and clearance rates in growing beef steers implanted with estrogenic anabolic compounds. Growth. 48:499.

Gopinath, R. and W.D. Kitts. 1986. Effect of estrogenic anabolic compounds on plasma nitrogenous compounds in growing beef steers. Nutr. Reports Intl. 33:505.

Gorski, J. and R.E. Erb. 1959. Characterization of estrogens in the bovine. Endocrinology 64:707.

Granner, D.K. 1985. Hormones of the adrenal gland. In: D.W. Martin, Jr., P.A. Mayes, V.W. Rodwell and D.K. Granner (Ed.) Harper's Review of Biochemistry. pp 548. Appleton-Century-Crofts, East Norwalk, CT.

Grebing, S.E., D.P. Hutcheson and R.L. Preston. 1970. Early reduction in urinary-N by diethylstilbestrol in lambs. J. Anim. Sci. 31:763.

Grigsby, M.E. and A. Trenkle. 1986. Plasma growth hormone, insulin, glucocorticoids and thyroid hormones in large, medium and small breed of steers with and without an estradiol implant. Domestic Anim. Endocrinology 3:261.

Groesbeck, M.D. and A.F. Parlow. 1987. Highly improved precision of the hypophysectomized female rat body weight gain bioassay for growth hormone by increased frequency of injections, avoidance of antibody formation, and other simple modifications. Endocrinology 120:2582.

Hancock, D.L. 1989. Mechanism of estrogen action which elicits the anabolic response observed in ruminants. Ph.D. Thesis, Texas Tech University, Lubbock.

Hancock, D.L. and R.L. Preston. 1989a. Titration of the 17-β estradiol dosage which maximizes the anabolic response in feedlot steers. J. Anim. Sci. (manuscript in preparation).

Hancock, D.L. and R.L. Preston. 1989b. Titration of the recombinant bovine somatotropin dosage which maximizes the anabolic response in feedlot steers. J. Anim. Sci. (manuscript in preparation).

Hancock, D.L. and R.L. Preston. 1989c. Estradiol clearance rate in feedlot steers. J. Anim. Sci. (manuscript in preparation).

Hancock, D.L. and R.L. Preston. 1989d. Interaction between 17-β estradiol and recombinant bovine somatotropin in the anabolic response of feedlot steers. J. Anim. Sci. (manuscript in preparation).

Hancock, D.L., J.E. Williams, H.B. Hedrick, E.E. Beaver, S.M. Hannah, S.J. Miller, M.R. Ellersieck, G.B. Garner, R.E. Morrow and J.R. Gerrish. 1988. Effects of previous forage systems on feedlot performance, body composition and plasma variables of finishing steers. J. Anim. Sci. 66:2272.

Harrison, L.P. 1981. An Investigation into the Effects of Anabolic Steroids on Ruminants. Ph.D. Thesis, University of Reading.

Hart, I.C. and I.D. Johnsson. 1986. Growth hormone and growth in meat producing animals. In: P.J. Buttery, D.B Lindsay and N.B. Haynes (Ed.) Control and Manipulation of Animal Growth. pp 135. Butterworths, London.

Hayden, J.M., W.G. Bergen, D.R. Hawkins and R.A. Merkel. 1987. The effect of 17-B estradiol (E) and trenbolone acetate (T) on muscle protein turnover in steers. J. Anim. Sci. 65 (Suppl. 1):265 (Abstr.).

Heitzman, R.J. 1979. Growth stimulation in ruminants. In: W. Haresign and D. Lewis (Ed.) Recent Advances in Animal Nutrition. pp 133. Butterworths, London.

Heitzman, R.J. 1981. Mode of action of anabolic agents. In: J.M. Forbes and M.A. Lomax (Ed.) Hormones and Metabolism in Ruminants. pp 129. Agricultural Research Council, London.

Heitzman, R.J., D.N. Gibbons, W. Little and L.P. Harrison. 1981. A note on the comparative performance of beef steers implanted with the anabolic steroids trenbolone acetate and oestradiol-17β, alone or in combination. Anim. Prod. 32:219.

Heitzman, R.J., A. Carter, S.N. Dixon, D.J. Harwood and M. Phillips. 1984. Recent studies on pharmacokinetics and residues of anabolic agents in beef cattle and other farm animals. In: J.F. Roche and D. O'Callagan (Ed.) Manipulation of Growth in Farm Animals. pp 1. Marinus Nijhoff, Dordrecht, Netherlands.

Hutcheson, D.P. and R.L. Preston. 1971. Stability of diethylstilbestrol and its effect on performance in lambs. J. Anim. Sci. 32:146.

Hynes, M.A., J.J. Van Wyk, P.J. Brooks, A.J. D'Ercole, M. Jansen and P.K. Lund. 1987. Growth hormone dependence of somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II messenger ribonucleic acids. Mol. Endocrinol. 1:233.

Isgaard, J., C. Moller, O.G. Isaksson, A. Nilsson, L.S. Mathews and G. Norstedt. 1988. Regulation on insulin-like growth factor messenger ribonucleic acid in rat growth plate by growth hormone. Endocrinology 122:1515.

Ivy, R.E., C.D. Baldwin, G.W. Wolfrom and D.E. Mouzin. 1986a. Effects of various levels of recombinant porcine growth hormone (rpGH) injected intramuscularly in barrows. J. Anim. Sci. 63 (Suppl. 1):218 (Abstr.).

Ivy, R.E., G.W. Wolfrom and C.K. Edwards, III. 1986b. Effects of growth hormone and RALGRO (zeranol) in finishing beef cattle. J. Anim. Sci. 63 (Suppl. 1):217 (Abstr.).

Johnsson, I.D., I.C. Hart and B.W. Butler-Hogg. 1985. The effects of exogenous bovine growth hormone and bromocriptine on growth, body development, fleece weight and plasma concentrations of growth hormone, insulin and prolactin in female lambs. Anim. Prod 41:207.

Kato, J. 1977. Steroid hormone receptors in brain hypothalamus and hypophysis. In: J.R. Pasqualini (Ed.) Receptors and Mechanisms of Action of Steroid Hormones. Part II. pp 603-671. Marcel Dekker, New York.

Levasseur, M. and C. Thibault. 1980. Reproductive life cycles. In: E.S.E. Hafez (Ed.) Reproduction in Farm Animals. pp 130-149. Lea and Febiger, Philadelphia.

Lewis, K.J., P.C. Molan, J.J. Bass and P.D. Gluckman. 1988. The lipolytic activity of low concentrations of insulin-like growth factors in ovine adipose tissue. Endocrinology 122:2554.

Lindsay, D.B. 1981. Some peculiarities in the metabolism of ruminants. In: J.M. Forbes and M.A. Lomax (Ed.) Hormones and Metabolism in Ruminants. pp 25. Agricultural Research Council, London.

Lloyd, H.M., J.D. Meares, J. Jacobi and F.J. Thomas. 1971. Effects of stilboestrol on growth hormone secretion and pituitary cell proliferation in the male rat. J. Endocrinol. 51:473.

Lloyd, T. and J. Weisz. 1978. Direct inhibition of tyrosine hydroxylase activity by catechol estrogens. J. Biol. Chem. 253:4841.

Lobely, G.E., A. Connell, G.S. Mollison, A. Brewer, C.I. Harris, V. Buchan and H. Galbraith. 1985. The effects of a combined implant of trenbolone acetate and oestradiol-17β on protein and metabolism in growing beef steers. Br. J. Nutr. 54:681.

Machlin, L.J. 1976. Role of growth hormone in improving animal production. In: F. Coulston and F. Korte (Ed.) Anabolic Agents in Animal Production. pp 43. Georg Thieme Publishers, Stuttgart.

MacLusky, N.J., F. Naftolin, L.C. Krey and S. Franks. 1981. The catechol estrogens. J. Steroid Biochem. 15:111.

Maiter, D., L.E. Underwood, M. Maes, M.L. Davenport and J.M. Ketelslegers. 1988. Different effects of intermittent and continuous growth hormone (GH) administration on serum somatomedin-C/insulin-like growth factor I and liver GH receptors in hypophysectomized rats. Endocrinology 123:1053.

Mansfield, M.J., C.R. Rudlin, J.F. Crigler, Jr., K.A. Karol, J.D. Crawford, P.A. Boepple and W.F. Crowley, Jr. 1988. Changes in growth and serum growth hormone and plasma somatomedin-C levels during suppression of gonadal sex steroid secretion in girls with central precocious puberty. J. Clin. Endocrinol. Metab. 66:3.

Melampy, R.M., M.A. Emmerson, J.M. Rakes, L.J. Hanka and P.G. Eness. 1957. The effect of progesterone on the estrous response of estrogen-conditioned ovariectomized cows. J. Anim. Sci. 16:967.

Meyer, H.H.D. and M. Rapp. 1985. Estrogen receptors in bovine skeletal muscle. J. Anim. Sci. 60:294.

Millward, D.J., P.J. Garlick, W.P.T. James, P.M. Sender and J.C. Waterlow. 1976. Protein turnover. In: D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan (Ed.) Protein Metabolism and Nutrition. pp 49. Butterworth, London.

Moffitt, P.E. 1980. The response of yearling steers to Synovex-S, its relationship to plasma somatomedin and the response of bulls and steers to various growth stimulants. Ph.D. Dissertation. The Ohio State Univ., Columbus.

Moseley, W.M., L.F. Krabill and R.F. Olsen. 1982. Effect of bovine growth hormone administered in various patterns on nitrogen metabolism in the holstein steer. J. Anim. Sci. 55:1062.

Motulsky, H.J. and L.A. Ransnas. 1987. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. Faseb. J. 1:365.

Muir, L.A., S. Wien, P.F. Duquette, E.L. Ricks and E.H. Cordes. 1983. Effects of exogenous growth hormone and diethylstilbestrol on growth and carcass composition of growing lambs. J. Anim. Sci. 56:1315.

Munson, P.J. and D. Rodbard. 1980. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107:220.

Murphy, L.J., L.C. Murphy and H.G. Friesen. 1987. Estrogen induces insulin-like growth factor-I expression in the rat uterus. Mol. Endocrinol. 1:445.

Norman, A.W. and G. Litwack. 1987. Hormones. Academic Press, Inc. Harcourt Brace Javanovich, New York.

NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed.). National Academy Press, Washington, DC.

Oltjen, R.R. and R.P. Lehmann. 1968. Effect of diethylstilbestrol on the blood plasma amino acid patterns of beef steers fed finishing diets. J. Nutr. 95:399.

Orlowski, C.C. and S.D. Chernausek. 1988. Discordance of serum and tissue somatomedin levels in growth hormone-stimulated growth in the rat. Endocrinology 123:44.

Paden, C.M., B.S. McEwen, J. Fishman, L. Snyder and V. DeGroff. 1982. Competition by estrogens for catecholamine receptor binding in vitro. J. Neurochem. 39:512.

Pfeiffer, D.G., N.J. MacLusky, E. Barnea, F. Naftolin, L.C. Krey, D.L. Loriaux and G.R. Merriam. 1986. Are catechol oestrogens obligatory mediators of oestrogen action in the central nervous system? I. Characterization of pharmacological probes with different receptor binding affinities and catechol oestrogen formation rates. J. Endocr. 110:489.

Phelps, C.J., J.P. Wiggins and P.J. Wangsness. 1988. Effects of zeranol on in vitro growth hormone release by lamb and rat pituitary cells. J. Anim. Sci. 66:2614.

Potter, E. and J.F. Wagner. 1987. Feed additive effects - ionophores, growth stimulants. Feed Intake by Beef Cattle: Symposium. Oklahoma State University.

Preston, R.L. 1968. Reduction of plasma urea-N by diethylstilbestrol in ruminants. Proc. Soc. Exp. Biol. Med. 129:250.

Preston, R.L. 1975. Biological responses to estrogen additives in meat producing cattle and lambs. J. Anim. Sci. 41:1414.

Preston, R.L. 1984. Zeranol dosage for feedlot steers. J. Anim. Sci. 59 (Suppl. 1):399 (Abstr).

Preston, R.L. 1987. Role of anabolic and repartitioning agents in the production of lean beef. Southwest Nutrition and Management Conference.

Preston, R.L., E. Cheng and W. Burroughs. 1956. Growth and other physiological responses to diethylstilbestrol in diet of rats and guinea pigs. Proc. Iowa Acad. Sci. 63:423.

Reed, M.J. and M.A.F. Murray. 1979. The Oestrogens. In: C.H. Gray and V.H.T James (Ed.) Third edition, Hormones in Blood, Volume 3. pp 263-353. Acedemic Press, New York.

Reeds, P.J. 1987. Metabolic control and future opportunities for growth regulation. Anim. Prod. 45:149.

Reid, R.L. 1968. The physiopathology of undernourishment in pregnant sheep, with particular reference to pregnancy toxemia. Adv. Vet. Sci. 12:163.

Riis, P.M. and T.P. Suresh. 1976. The effect of a synthetic steroid (trienbolone) on the rate of release and excretion of subcutaneously administered estradiol in calves. Steroids. 27:5.

Roche, J.F. and J.F. Quirke. 1986. The effects of steroid hormones and xenobiotics on growth of farm animals. In: P.J. Buttery, D.B Lindsay and N.B. Haynes (Ed.) Control and Manipulation of Animal Growth. pp 39. Butterworths, London.

Roeder, R.A., S.D. Thorpe, F.M. Byers, G.T. Schelling and J.M. Gunn. 1986. Influence of anabolic agents on protein synthesis and degradation in muscle cells grown in culture. Growth. 50:485.

Rumsey, T.S. and N. Beaudry. 1979. Plasma estrogen concentrations in non-implanted and synovex-S implanted feedlot steers. Bull. Environm. Contam. Toxicol. 23:405.

Sandberg, A.A. and Slaunwhite, W.R., Jr. 1957. Studies on phenolic steroids in human subjects. II. The metabolic fate and hepato-biliary-enteric circulation of ^{14}C-estrone and ^{14}C-estradiol in women. J. Clin. Invest. 36:1266.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

Scanes, C.G. and T.J. Lauterio. 1984. Growth hormone: Its physiology and control. J. Exp. Zool. 232:443.

Schaeffer, J.M. and A.J.W. Hsueh. 1979. 2-hydroxyestradiol interaction with dopamine receptor binding in rat anterior pituitary. J. Biol. Chem. 254:5606.

Schlechter, N.L., S.M. Russell, E. M. Spencer and C. S. Nicoll. 1986. Evidence suggesting that the direct growth-promoting effect of growth hormone on cartilage in vivo is mediated by the local production of somatomedin. Proc. Natl. Acad. Sci. USA. 83:7932.

Searle, P.L. 1984. The Berthelot or indophenol reaction and its use in the analytical chemistry of nitrogen: A review. Analyst 109:549.

Sejrsen, K. and M.V. Jensen. 1987. An overview of endocrine mechanisms important to regulation of metabolism and growth in farm animals. In: J.P. Hanrahan (Ed.) Beta-Agonists and Their effects on Animal Growth and Carcass Quality. pp 44. Elsevier Applied Science, London.

Shamay, A., N. Cohen, M. Niwa and A. Gertler. 1988. Effect of insulin-like growth factor I on deoxyribonucleic acid synthesis and galactopoiesis in bovine undifferentiated and lactating mammary tissue in vitro. Endocrinology 123:804.

Silverman, B.L., S.L. Kaplan, M.M. Grumbach and W.L. Miller. 1988. Hormonal regulation on growth hormone secretion and messenger ribonucleic acid accumulation in cultured bovine pituitary cells. Endocrinology 122:1236.

Simard, J., J.F. Hubert, T. Hosseinzadeh and F. Labrie. 1986. Stimulation of growth hormone release and synthesis by estrogens in rat anterior pituitary cells in culture. Endocrinology 119:2004.

Sinnett-Smith, P.A., N.W. Dumelow and P.J. Buttery. 1983. Effects of trenbolone acetate and zeranol on protein metabolism in male castrate and female lambs. Brit. J. Nutr. 50:225.

Smith, S.B. 1987. Effects of β-adrenergic agonists on cellular metabolism. Reciprocal Meat Conference Proceedings 40:65.

Spencer, G.S. 1981. Proceedings of a Colloquium on Somatomedins. Bristol: Somatomedin Club.

Spencer, G.S.C. 1985. Hormonal systems regulating growth. A review. Livest. Prod. Sci. 12:31.

Sykes, A.R. 1978. An assessment of the value of plasma urea nitrogen and albumin concentraions as monitors of the protein status of sheep. In: The Use of Blood Metabolites in Animal Production, Br. Soc. Anim. Prod., Occ. Publ. No. 1. pp 143.

Trenkle, A. 1970. Plasma levels of growth hormone, insulin and plasma protein-bound iodine in finishing cattle. J. Anim. Sci. 31:389.

Trenkle, A. 1976. The anabolic effect of estrogens on nitrogen metabolism of growing and finishing cattle and sheep. In: F. Coulston and F. Korte (Ed.) Anabolic Agents in Animal Production. pp 79. Georg Thieme Publishers, Stuttgart.

Trenkle, A. 1981. Endocrine regulation of energy metabolism in ruminants. Fed. Proc. 40:2536.

Trenkle, A. and D.G. Topel. 1978. Relationships of some endocrine measurements to growth and carcass composition of cattle. J. Anim. Sci. 46:1604.

Tucker, H.A. and R.A. Merkel. 1987. Applications of hormones in the metabolic regulation of growth and lactation in ruminants. Fed. Proc. 46:300.

Valentino, K.L., H. Pham, I. Ocrant and R.G. Rosenfeld. 1988. Distribution of insulin-like growth factor II receptor immunoreactivity in rat tissues. Endocrinology 122:2753.

VanderWal, P. 1976. General aspects of the effectiveness of anabolic agents in increasing protein production in farm animals, in particular bull calves. In: F. Coulston and F. Korte (Ed.) Anabolic Agents in Animal Production. pp 60. Georg Thieme Publishers, Stuttgart.

Wagner, J.F., R.P. Basson, L.H. Carroll, J.L. Hudson, J. McAskill, R.S. Nevin and A.P. Raun. 1979. Factors affecting payout of estradiol 17β ($E_2\beta$) from a silicone rubber implant and effect on performance in finishing steers. J. Anim. Sci. 49 (Suppl. 1):416 (Abstr.).

Wagner, J.F., T. Cain, D.B. Anderson, P. Johnson and D. Mowrey. 1988a. Effect of growth hormone (GH) and estradiol ($E_2\beta$) alone and in combination on beef steer growth performance, carcass and plasma constituents. J. Anim. Sci. 66 (Suppl. 1):283 (Abstr.).

Wagner, J.F., R.E. Paxton, D.B. Anderson, E.L. Potter and D. Mowery. 1988b. The effect of bovine growth hormone (bGH) and estradiol ($E_2\beta$) alone and in combination on urinary nitrogen excretion in beef steers. J. Anim. Sci. 66 (Suppl. 1):253 (Abstr.).

Wangsness, P.J., R.F. Olsen and R.J. Martin. 1981. Effects of breed and zeranol implantation on serum insulin, somatomedin-like activity and fibroblast proliferative activity. J. Anim. Sci. 52:57.

Welsh, T.H., Jr., S.B. Smith, M.R. Sutton and K.A. Wagner. 1987. Growth hormone releasing factor and clenbuterol regulation of bovine growth hormone secretion in vitro. J. Anim. Sci. 65 (Suppl. 1):279 (Abstr.).

White, D.R. 1982. Controlled release implant for beef cattle. Compudose Technical Manual. Elanco Prod. Co., a division of Eli Lilly and Co., Indianapolis, IN.

Wien, S., P.F. Duquette, E.L. Rickes and L.A. Muir. 1983. Concentrations of somatomedin C and anterior pituitary hormones in plasma of wether lambs injected daily with DES or ovine GH for 8 weeks. J. Anim. Sci. 57 (Suppl. 1):210 (Abstr.).

Wolfrom, G.W. and R.E. Ivy. 1985. Effects of exogenous growth hormone in growing beef cattle. J. Anim. Sci. 61 (Suppl. 1):249 (Abstr.).

Wolfrom, G.W., R.E. Ivy and C.D. Baldwin. 1985. Effects of growth hormone alone and in combination with RALGRO (Zeranol) in lambs. J. Anim. Sci. 61 (Suppl. 1):249 (Abstr.).

Wolfrom, G.W., R.E. Ivy and C.D. Baldwin. 1986. Effect of native porcine growth hormone (npGH) injected intramuscularly in barrows. J. Anim. Sci. 63 (Suppl. 1):219 (Abstr.).

Wright, A.A. 1961. Endocrine control of metabolism with reference to the effect of oestrogens on growth. In: D. Lewis (Ed.) Digestive Physiology and Nutrition of the Ruminant. pp 242. Butterworths, London.

Young, V.R. 1976. An overview of protein synthesis, degradation and the regulation of protein content in skeletal muscle. In: F. Coulston and F. Korte (Ed.) Anabolic Agents in Animal Production. pp 20. Georg Thieme Publishers, Stuttgart.

APPENDICES

APPENDIX A

PRELIMINARY 17-β ESTRADIOL

DOSAGE TRIALS

TRIAL 1.

1. Twelve heifers (avg = 286 kg) were placed in two pens (6/pen) and fed ad libitum on the following diet balanced to meet NRC requirements; ten steers (avg = 300 kg) were placed in two pens (4 steers in one pen and 6 steers in another pen), fed ad libitum on the following diet:

Ingredient[a]	%[b]
Steam flaked sorghum grain	23.11
Corn silage	21.15
Cottonseed hulls	28.50
Cottonseed meal	21.47
Cane molasses	2.93
Urea	.36
Calcium carbonate	1.75
Sodium chloride	.25
Vitamin A premix	.24
Trace mineral premix	.24
Total	100.00

[a]Formulated to contain 16.4% CP; 2.92 Mcal DE/kg; 2.15% crude fat; 47% NDF; 44% roughage equivalent; .84% Ca; .34% P; .13% Na; .99% K; .17% S; 53 ppm Zn; 5474 IU/kg Vitamin A.

[b]Dry matter basis.

2. Heifers were randomly allotted to the following treatments: 0, .3 or 3 mg 17-β estradiol (E_2) every other d (EOD). Steers were randomly allotted to the following treatments: 0 or 3 mg E_2 EOD. Subcutaneous injections of E_2 were administered EOD in an injection vehicle of 80% sesame oil and 20% of 100% ethanol. Cattle were weighed and blood samples taken via jugular vein puncture on d 0, 1, 4 and 7. Plasma urea nitrogen (PUN) concentrations were determined.

3. Data were analyzed by analysis of variance as a split plot in time. Main plot effects were pen, E_2 dosage and pen by E_2 dosage, with the latter term used as the

error term to test main plot effects. Subplot effects included time, E_2 dosage by time interaction and residual effects. The residual effects were used as the error term to test subplot effects. Multiple regression analysis was performed to partition E_2 dosage, time and E_2 dosage by time interactions into linear and quadratic components. Daily gain was calculated over the 7 d period, therefore, only main plot effects were included in the statistical model for daily gain.

4. Due to significant ($P < .05$) differences in d 0 PUN between heifers and steers and due to the lack of a .3 mg E_2 dosage for the steers, data were analyzed individually for heifers and for steers. There were no differences in daily gain due to E_2 administration in heifers ($P > .85$; avg = -.25 kg/d; SE = .77) or steers ($P > .64$; avg = .09 kg/d; SE = .27).

5. There were no differences in d 0 PUN concentrations between treatments in heifers ($P > .50$; avg = 11.8; SE = 1.59) or steers ($P > .30$; avg = 9.04; SE = .86). Therefore d 0 was not included in the split plot in time analysis of variance.

6. Least-squares means for PUN concentrations (mg/dl) in heifers are reported in the following table:

Days on trial	E_2 dosage, mg EOD[c] 0	.3	3	Days on trial mean[a]
1	12.7	9.18	8.88	10.2
4	12.8	9.41	7.95	10.1
7	12.9	9.15	6.79	9.61
E_2 dosage mean[b]	12.8	9.24	7.87	

[a]Main effects of E_2 dosage ($P < .10$; SE = 1.19). Quadratic ($P < .10$) increase in PUN depression with increasing E_2 dosage.

[b]Effects of days on trial ($P > .70$; SE = .56).

[c]E_2 dosage by days on trial interaction ($P > .70$; SE = .97).

7. In heifers, results indicate a quadratic ($P < .10$) increase in PUN depression with increasing E_2 dosage (28 and 38% increase with .3 and 3 mg EOD when compared to controls), with maximal reduction calculated to occur with a dosage of 1.7 mg E_2 EOD (in sesame oil/ethanol vehicle). However, an analysis of variance on d 4 and d 7 PUN concentrations indicated that the .3 and 3 mg E_2 treatments were similar ($P > .05$).

8. Least-squares means for PUN concentrations (mg/dl) in steers are reported in the following table:

Days on trial	E_2 dosage, mg EOD[c] 0	3	Days on trial mean[a]
1	7.60	8.01	7.81
4	8.90	7.36	8.13
7	9.22	6.66	7.94
E_2 dosage mean[b]	8.57	7.34	

[a]Main effects of E_2 dosage ($P > .10$; SE = .70).

[b]Effects of days on trial ($P > .70$; SE = .23).

[c]E_2 dosage by days on trial interaction ($P < .0008$; SE = .10). E_2 dosage$_{linear}$ by time$_{linear}$ interaction ($P < .001$).

9. There were no differences in PUN concentrations due to E_2 dosage ($P > .10$) or days on trial ($P > .70$). However, there was a treatment by time interaction ($P < .0008$), which was due to an E_2 dosage$_{linear}$ by time$_{linear}$ interaction ($P < .001$). On d 7, PUN was reduced by 28% for steers receiving E_2 compared to controls.

TRIAL 2.

1. The twelve heifers (avg = 336 kg) and nine (avg = 376 kg) of the steers in used in trial 1 were kept in the same pens, fed the same diet and reallotted to the following treatments: 0 mg E_2 EOD, .3 E_2 EOD or .6 mg E_2 every fourth d (E4D). Subcutaneous injections of E_2 were administered in an injection vehicle of 100% ethanol. Cattle were weighed and blood samples taken via jugular vein puncture on d 0, 1, 4, 6, 8 and 9. Plasma urea nitrogen (PUN) concentrations were determined.

3. Data were analyzed (by sex as in trial 1) by analysis of variance as a split plot in time. Main plot effects were pen, E_2 dosage and pen by E_2 dosage, with the latter term used as the error term to test main plot effects. Subplot effects included time, E_2 dosage by time interaction and residual effects. The residual effects were used as the error term to test subplot effects. Regression analysis was performed to partition time effects into linear, quadratic, cubic and quartic components. Daily gain was calculated over the 7 d period, therefore, only main plot effects were included in the statistical model for daily gain.

4. There were no differences in daily gain due to E_2 administration in heifers ($P = .056$; avg = -.38 kg/d; SE = .26). However, numerically daily gain increased with E_2 administration, and was higher when administered EOD compared to E4D (-.88, .13 and -.38 kg/d for heifers receiving 0 mg E_2, .3 mg E_2 EOD and .6 mg E_2 E4D, respectively). In the steers there was a significant increase in daily gain with E_2 administration ($P < .008$; -1.35, .90 and .54 kg/d for steers receiving 0 mg E_2, .3 mg E_2 EOD and .6 mg E_2 E4D, respectively; SE = .32). Daily gain was greater ($P < .05$) in steers injected with E_2, however, there was no difference ($P > .05$) in daily gain due to time of injection.

5. There were no differences in d 0 PUN concentrations between treatments in heifers ($P > .80$; avg = 16.3; SE = 1.06) or steers ($P > .70$; avg = 15.0; SE = 1.30). Therefore d 0 was not included in the split plot in time analysis of variance.

6. Least-squares means for PUN concentrations (mg/dl) in heifers are reported in the following table:

Days on trial	E_2 dosage[c] 0	.3 EOD	3 E4D	Days on trial mean[a]
1	17.2	15.7	14.1	15.6
4	17.0	14.0	14.4	15.1
6	15.6	15.2	14.2	15.0
8	14.6	14.8	14.5	14.6
9	12.6	12.8	12.0	12.5
E_2 dosage mean[b]	15.4	14.5	13.9	

[a]Main effects of E_2 dosage ($P > .50$; SE = 1.22).

[b]Effects of days on trial ($P < .0018$; SE = .53). Linear ($P < .001$), quadratic ($P < .05$) and cubic ($P < .05$) responses in PUN concentration as days on trial increased.

[c]E_2 dosage by days on trial interaction ($P > .50$; SE = .92).

7. Least-squares means for PUN concentrations (mg/dl) in steers are reported in the following table:

Days on trial	E_2 dosage[c] 0	.3 EOD	3 E4D	Days on trial mean[a]
1	15.2	15.7	14.7	15.2
4	14.5	12.6	13.2	13.4
6	15.2	15.5	15.1	15.2
8	14.0	14.1	15.1	14.4
9	14.2	13.9	14.7	14.2
E_2 dosage mean[b]	14.6	14.4	14.6	

[a]Main effects of E_2 dosage ($P > .50$; SE = 1.64).

[b]Effects of days on trial ($P = .0083$; SE = .36). Cubic ($P < .005$) and quartic ($P < .05$) responses in PUN concentration as days on trial increased.

[c]E_2 dosage by days on trial interaction ($P > .40$; SE = .62).

8. Although E_2 dosage nor E_2 dosage by time effects were significant, numerically on d 1 and d 4 PUN concentrations were reduced in both heifers and steers. However, there was a lack of PUN response thereafter, which may be attributed to prior estrogen treatment in trial 1.

Trial 3.

1. Twenty-one steers (411 kg) were placed in one pen and fed ad libitum on the following diet:

Ingredient[a]	%[b]
Steam flaked sorghum grain	46.10
Corn silage	22.83
Cottonseed hulls	13.59
Cottonseed meal	11.71
Cane molasses	2.29
Fat	.60
Urea	.67
Calcium carbonate	1.26
Sodium chloride	.18
Vitamin A premix	.16
Trace mineral premix	.16
Tylosin premix	.45
Total	100.00

[a]Formulated to contain 16.3% CP; 3.21 Mcal DE/kg; 3.12% crude fat; 36% NDF; 30% roughage equivalent; .65% Ca; .37% P; .10% Na; .92% K; .17% S; 43 ppm Zn; 5221 IU/kg Vitamin A.

[b]Dry matter basis.

2. Steers were randomly allotted to the following treatments (7 steers/treatment): 0, .2 or 2 mg E_2/d. Subcutaneous injections of E_2 were administered daily in an injection vehicle of 100% ethanol. Blood samples taken via jugular vein puncture on d 0, 1, 4 and 7. Plasma urea nitrogen (PUN) concentrations were determined.

3. Data were analyzed by analysis of variance as a split plot in time. Main plot effects were E_2 dosage and steer within E_2 dosage, with the latter term used as the error term to test main plot effects. Subplot effects included time, E_2 dosage by time interaction and residual effects. The residual effects were used as the error term to test subplot effects. Multiple regression analysis was performed to partition E_2 dosage, time and E_2 dosage by time interactions into linear and quadratic components.

4. There were no differences in d 0 PUN concentrations between treatments ($P > .50$; avg = 8.52; SE = .70). Therefore d 0 was not included in the split plot in time analysis of variance.

5. Least-squares means for PUN concentrations (mg/dl) in steers are reported in the following table:

Days on trial	E_2 dosage, mg/d[c] 0	.2	2	Days on trial mean[a]
1	9.22	7.56	7.91	8.23
4	10.00	8.36	6.81	8.39
7	9.47	7.40	5.38	7.42
E_2 dosage mean[b]	9.56	7.77	6.70	

[a]Main effects of E_2 dosage ($P < .01$; SE = .62). Linear ($P < .05$) and quadratic ($P < .10$) increase in PUN depression with increasing E_2 dosage.

[b]Effects of days on trial ($P < .003$; SE = .62). Linear ($P < .01$) and quadratic ($P < .05$) decrease in PUN concentration with increasing days on trial.

[c]E_2 dosage by days on trial interaction ($P < .002$; SE = .35). E_2 dosage$_{linear}$ by days on trial$_{linear}$ interaction ($P < .001$).

6. The E_2 dosage by days on trial interaction ($P < .002$) was due to an E_2 dosage$_{linear}$ by days on trial$_{linear}$ interaction ($P < .001$). Plasma urea nitrogen concentrations in steers receiving no E_2 were relatively constant as days on trial increased, however, PUN concentrations in steers receiving .2 mg E_2/d increased on d 4 and decreased on d 7 (all concentrations were lower than controls), whereas PUN concentrations in steers receiving 2 mg E_2/d continued to decrease as days on trial increased.

7. Results indicate that PUN depression is increased ($P < .01$) with E_2 administration, with maximal reduction calculated to occur with 1.2 mg E_2/injection (in 100% ethanol) per day.

APPENDIX B

17-β ESTRADIOL RADIOIMMUNOASSAY

A. Prepare gel phosphate buffered saline (gel PBS; pH=7.5) to be used in standard preparation. The formula for this buffer was obtained from Betty Pettigean at South Dakota University.

1. Weigh:
   - 2.84 g dibasic sodium phosphate (Na_2HPO_4; anhydrous)
   - .74 g disodium ethylenediamine tetraacetate (EDTA; our EDTA has two waters of hydration, therefore, use .819 g to account for this)
   - 2.00 g sodium azide (NaN_3; antimicrobial agent)
   - 16.20 g sodium chloride (NaCl)

2. Place above ingredients into a 2 liter volumetric flask containing approximately 1400 ml distilled water. Stir with a magnet.

3. Bring to volume with distilled water; however, check pH and adjust if necessary before bringing to final volume. The pH should be 7.5.

4. Weigh 4 g of Knox gelatin into a 250 ml beaker. Add approximately 100 ml of PBS. Heat, while stirring to dissolve the gelatin. Do not over heat. Add this back to the PBS, for .2% gel PBS.

B. Preparation of 17-β estradiol (E_2) standards. Standards in the E_2 RIA kit purchased from Diagnostic Products Corporation will not work for bovine samples.

1. Stock solution - 1 mg E_2/ml. Weigh .05 g of E_2 (purchased from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178). Place in 50 ml volumetric flask. Bring to volume with 100% ethanol (Texas Tech - Chemistry). Store in freezer.

2. Make the following standard concentrations with gel PBS: 5000, 1000, 500, 100, 50, 10 and 5 pg E_2/ml.

C. 17-β estradiol double antibody radioimmunoassay. Purchase antiserum, tracer, precipitating solution and

reference controls from Diagnostic products Corporation (DPC; 5700 W. 96th St., Los Angeles, CA 90045). Prior to ordering, you must contact Steve Abernathy, Radiation Safety Officer (742-3876) and radioactive materials need to be delivered to his attention. Use the DCP protocol with the above standards (in the range you are interested in).

Gamma counters available on campus:

Food and Nutrition	Dr. Julian Spallholz	(742-3124)
Biology	Dr. John Burns	(742-2715)
Medical Biochemistry	Dr. Gwynne Little	(743-2507)

D. Log/logit analysis to determine unknown concentrations. This transforms the data such that linear regression can be used for calculation of unknown concentrations. We have this analysis programmed on LOTUS. Assay sensitivity is approximately 1 pg/ml. The following curve parameters should be expected:

Slope	-2.0709
Intercept	3.3205

Standard pg/ml	% Bound
5	89.1
10	79.8
50	40.6
100	29.2
500	9.53
1000	5.46
5000	1.75

E. Avoid thawing and refreezing of samples.

APPENDIX C

BOVINE SOMATOTROPIN RADIOIMMUNOASSAY

Procedure for iodination used at North Carolina State University. Vickey Hedgepest (919-737-2504)

COLUMN PREPARATION:

1. Obtain a 10 ml disposable pipet, place a 6 mm glass bead inside the column, mark off 17 cm from the bead, then 2 cm followed by 1 cm. Place pipet on a ring stand.

2. Pre-swell P60, P10 and AG1X8 (Biorad, 1414 Harbor Way South, Richmond, CA 94804; catalog numbers 1501630, 1501040 and 1401451, respectively) in .05 M phosphate buffered saline (PBS; pH=7.5). P60 and P10 separate on the basis of molecular weight (gel filtration). AG1X8 is an anion exchange resin which traps free ^{125}I and acidic aggregates. Therefore, there should be no free ^{125}I peak in the fractions following iodination.

3. Place .05 M PBS (pH=7.5) into the column. Use this to remove any air bubbles trapped in the tip.

4. Gel suspension should be a fairly thick slurry (75% settled gel). Pack the column as follows (see handout entitled, Gel Filtration Theory and Practice from Pharmacia, Inc., 800 Centennial Ave., Piscataway, NJ 08854): approximately 17 cm of pre-swollen P60, 2 cm pre-swollen P10 and 1 cm of pre-swollen AG1X8. Use a glass pipet to transfer gel to the column. Never let the column run dry. Let the gel settle, then allow buffer to flow through the column (control flow rate with a clamp on the bottom of the column). Try to get the top of the gel line as level as possible.

5. After the column is packed, condition the column with approximately 20 ml of .05 M PBS-1% bovine serum albumin (BSA; pH=7.5) followed with .05 M PBS (pH=7.5). The .05 M PBS is the eluent.

IODINATION AND PURIFICATION:

1. The reaction should be conducted in a fume hood approved for radioactive iodinations. Make sure that thimersol or other preservatives are not in any of

the reagents to be used in the reaction as they will interfere with the reaction.

2. Prepare all reagents ahead of time except for the somatotropin, chloramine T, sodium metabisulfite and potassium iodide. Have these weighed out into volumetric flasks ahead of time and bring to volume just prior to iodination. The volumetric flasks containing chloramine T and sodium metabisulfite should be wrapped in aluminum foil to prevent light damage.

3. Place 40 µl of .5 M sodium phosphate buffer (pH=7.5) into a 1.5 ml plastic conical centrifuge tube. This is a strong buffer used to neutralize the iodine which is in a sodium hydroxide solution (to protect the hormone). Then add 10 µl (1 mCi) of sodium ^{125}I (purchased from Amersham, 2636 South Clearbrook Drive, Arlington Heights, IL 60005; catalog number IMS.30). Gently flick the reaction flask with your finger to mix. Add 3 µg (10 µl; keep the volume low to have a narrow peak and increased separation efficiency) of recombinant bovine somatotropin (in .05 M sodium phosphate buffer, pH=7.5). Gently flick the reaction flask with your finger to mix.

4. The following steps must be conducted as quickly as possible. Therefore, have all materials ready. Add 10 µl of the oxidizing agent chloramine T (1.25 mg/ml in .05 M sodium phosphate buffer, pH=7.5). Wait 60 seconds (gently flick the reaction flask during this time period), and then stop the reaction by adding 20 µl of sodium metabisulfite (2.5 mg/ml in .05 M sodium phosphate buffer, pH=7.5). Then add 150 µl of potassium iodide (10 mg/ml in .05 M sodium phosphate buffer, pH=7.5) and 100 µl of PBS-1%BSA. Flick with finger. The mixture is transferred to the conditioned column with a transfer pipet (make sure the meniscus of the buffer is at the top of the AG1X8 resin and try not to disturb the resin when loading the mixture). Allow the mixture to penetrate the resin (bring meniscus down to the top of the resin) and then add the .05 M PBS eluent (add several mls with a transfer pipet then start the drip). Never let the column go dry. One ml fractions are collected into plastic tubes containing .5 ml of .01 M PBS-1%BSA, pH=7.0 (with thimersol, an antimicrobial agent; this prevents the iodinated hormone from sticking to the tube). Mark a line on the tube to indicate where approximately 1.5 ml total volume should be to aid in fraction collection. Collect 20

fractions. Mix each fraction, then take 10 µl from each and count on a gamma counter. Labelled somatotropin (fractions 3-7) will come out after void volume (fractions 1-2).

NOTES:

1. Potassium phosphate buffer can be used instead of sodium phosphate buffer. This buffer will not precipitate after freezing and should be used if buffer will be stored frozen.

2. Use Eppendorf pipets and add the indicated volumes under the surface of the liquid in the reaction flask.

3. Use disposable transfer pipets and column pipet so that everything can be disposed of in radioactive waste.

4. Swell beads (1 tablespoon/250 mls) in .05 M sodium phosphate buffer overnight (24 hours). P60 and P10 may have film on top, if so remove with a pipet. These are "fines" and are damaged. Degas the gel with a vacuum until bubbles are no longer visible on sides and bottom of the flask. Also degas buffer.

5. When pouring the column, swirl to release bubbles and work at room temperature (cold temperatures are slow to give off gas).

6. Conditioning the column serves two purposes: first, it packs the column and second, it loads the column with protein (BSA) so that the iodinated protein of interest won't adhere to the walls of the column.

7. Need two people, one to do the iodination, the other to assist.

8. The KI is a scavenger which picks up free ^{125}I. PBS/BSA quenches the reaction.

9. Account for water in chloramine T and albumin.

10. .5 M Sodium Phosphate Buffer (pH=7.5)

    11.454 g NaH_2PO_4
    59.624 g Na_2HPO_4
    dilute to 1 liter with distilled water

11. .05 M Sodium Phosphate Buffer (pH=7.5)

1:10 dilution of the above buffer.

12. .05 M Phosphate Buffered Saline (PBS; pH=7.5)

Same as in number 10, but 8.19 g NaCl/liter is added and a 1:10 dilution is made.

13. PBS-1%BSA

Add 1% Bovine Serum Albumin to PBS.

14. .01 M PBS-1%BSA with Thimersol (pH=7.0)

A. 1.38 g NaH_2PO_4
8.19 g NaCl
dilute to 1 liter

B. 1.42 g Na_2HPO_4
8.19 g NaCl
dilute to 1 liter

Add A to B until pH=7.0. Add thimersol at .1 g/liter and BSA at 1%.

TALC/RESIN/TCA TEST:

Procedure received from V.S. Hedgepeth, North Carolina State University

1. Quality of labeled somatotropin should be tested using the talc/resin/TCA test (Tower et al., 1980, Methods in Enzymology 70:322).

2. <u>Reagents:</u>

A. 1% BSA (Bovine Serum Albumin) in 0.01M PBS pH 7.0, Sigma Chemical Company.

B. 10% TCA (Trichloracetic Acid), Sigma Chemical Company.

C. TALC Tablets, U. S. Products, Inc., 5341 Derry Ave., Unit Q, Agoura Hills, CA 91301, or TALC Powder, Sigma Chemical Company.

D. AG1x8 Resin, Bio-Rad Laboratories.

3. Procedure:

A. Add 900 µl of PBS-1%BSA to each tube.

B. Dilute Tracer (iodinated hormone) to concentration you plan to use in your assay with PBS-1%BSA.

C. Add 100 µl of diluted tracer to each tube.

D. Vortex all tubes.

E. a. To the tubes designated for TCA test, add 1.0 ml 10% TCA to each tube.

b. To the tubes designated for TALC test, add 1 talc tablet to each tube or 100 mg talc powder.

c. To the tubes designated for Resin Test, add 150 mg (.150 gm) of AG1x8 to each tube.

F. Vortex all tubes.

G. Rinse down the sides of TALC and Resin tubes with 1.0 ml PBS-1%BSA each.

H. Centrifuge 15 min at 3000 RPM.

I. Pipet off the supernate into a second tube labelled to correspond with the initial tube.

J. Count in gamma counter -- (Remember to cap liquid tubes before putting them into sleeves to facilitate removal and avoid contamination).

4. Calculations:

$$\frac{\text{cpm ppt}}{\text{cpm ppt + cpm liquid}} \times 100 = \% \text{ bound}$$

Following Results indicate valid hormone iodination:

>90% TCA Test)____These should agree within 3%.
>90% TALC Test)
<25% Resin Test

5. Tips & Technical Notes:

A. 0.1% PBS-gel will not work as a substitute for PBS-1.0%BSA.

B. Do at least duplicates for each test. Suggest 3 tubes/test.

C. If tracer is diluted to PBS-gel instead of BSA, test will work but be sure to change initial BSA addition to 1.0 ml instead of 900 µl.

D. Initial findings show differences in binding between AG11A8 and AG1x8 resins.

RADIOIMMUNOASSAY:

Procedure from T. Cain and Dr. J. Wagner at Lilly Research Labs.

1. Stock solution of somatotropin (1 mg/ml) is solubilized in .01 M PBS, pH=10.8. pH is then adjusted to 9.5 and solution is brought to volume with .01 M PBS, pH=9.5. Stock somatotropin solution is diluted with PBS-1%BSA (.01 M, pH=7.0, with thimersol). The following concentrations are used in the assay: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, .7812 and .3906 ng somatotropin/ml. We are currently using recombinant derived bovine somatotropin.

2. Pipet .1 ml of each standard in triplicate into disposable borosilicate glass test tubes.

3. Total tubes will contain only labeled somatotropin. Add .1 ml of tracer at step 5. Nothing else should be added to these tubes.

4. Place .1 ml of plasma in duplicate into disposable borosilicate glass test tubes.

5. Labeled somatotropin is diluted with .01 M PBS-1%BSA, pH=7.0, with thimersol such that .1 ml contains approximately 10,000 CPM. Pipet .1 ml of ^{125}I somatotropin into each tube. Vortex.

6. Dilute anti somatotropin serum with 2% normal rabbit serum (NRS; purchased from Sigma) in PBS/EDTA at a dilution which will obtain approximately 40% binding (1:40,000). Pipet .1 ml of this antiserum into all tubes except the NSB and TOTAL tubes. Vortex.

7. Pipet .1 ml of 2% NRS PBS/EDTA (antiserum diluent) into each NSB tube.

8. Incubate at room temperature approximately 20 hours.

9. Following incubation, add to each tube .1 ml of sheep anti rabbit gamma globulin (diluted 1:16 with PBS/EDTA; purchased from Antibodies Incorporated, P.O. Box 1560, Davis, CA 95617, 916-758-4400). Vortex. Refrigerate for approximately 24 hours.

10. Following incubation, termination steps are as follows:

    a) Add 2 ml of cold .01 M PBS, pH=7.0, with thimersol, to each tube just prior to centrifuging.

    b) Centrifuge for 30 minutes at 2800 rpm.

    c) Pour off supernatant, drain and wipe tubes dry.

    d) Count the precipitate for 1 minute in a gamma counter.

11. Calculate using log/logit analysis.

12. The following curve parameters should be expected:

Slope	-1.6332
Intercept	1.1322

Standard ng/ml	% Bound
.391	89.5
.781	82.2
1.56	66.1
3.13	50.3
6.25	36.9
12.5	29.8
25.0	23.6
50.0	18.7
100	13.6

Buffers:

1. .01 M PBS-1%BSA is described in the iodination protocol.

2. PBS/EDTA

1.42 g dibasic sodium phosphate
18.612 g disodium EDTA
8.190 g sodium chloride
2 ml of 1:1000 thimersol solution

The pH of this solution is about 5.5. Add NaOH pellets while stirring to adjust pH to 7.0. Use this to make 2% NRS and to dilute second antibody.

APPENDIX D

HYPOPHYSECTOMIZED FEMALE RAT WEIGHT GAIN BIOASSAY FOR BOVINE SOMATOTROPIN

1. The bioactivity of bovine somatotropin (bST; Lilly Research Laboratories, Greenfield IN 46140) was tested using the current standardized procedure by the modified hypophysectomized (hypox) female rat (Taconic Laboratory Animals and Services, 33 Hover Ave., Germantown NY 12526-9608) body weight gain method (Groesbeck and Parlow, 1987, Endocrinology 120:2582).

2. Proper care of the hypox rats, selection of hypox rats for bioassay and solubilization of bST were conducted as described by Groesbeck and Parlow (1987).

3. bST injections (0, 20 or 100 µg $bST.d^{-1}.rat^{-1}$; 8 rats/treatment) were administered subcutaneously twice daily at 0700 and 1700 h for seven consecutive d (d 0 to d 6) and rats were weighed once daily at 0730 h for 8 consecutive d (d 0 to d 7).

4. Data were analyzed by analysis of variance as a split plot in time for weight. Main plot effects included dose and rat within dose, with the latter term used as the error term to test main plot effects. Subplot effects included time, dose by time interaction and residual effects. The residual effects were used to test subplot effects. Multiple regression analysis was used to partition dose, time and dose by time effects into linear, quadratic and cubic components. Daily gain was calculated for the 7 d period, therefore, only main plot effects were used in the daily gain model for analysis of variance.

5. Least-squares means for daily gain were .29, 2.09 and 3.97 g/d for rats receiving 0, 20 and 100 µg bST/d treatments, respectively. Linear and quadratic ($P < .001$) increases in daily gain were observed as bST dosage increased. Daily gains observed in this study were similar to those reported by Groesbeck and Parlow (1987). Therefore, the estimated biopotency of our bST was 1.0 IU/mg.

6. Least-squares means for daily body weights are reported in the following table[abc]:

Days on trial	bST dosage, µg/d 0	20	100	Days on trial mean[a]
0	111	111	112	112
1	109	112	117	113
2	110	115	122	116
3	111	119	127	119
4	111	121	130	121
5	113	123	133	123
6	113	124	135	124
7	113	126	140	126
bST dosage mean[b]	111	119	127	

[a]Main effects of bST dosage ($P < .0001$; SE = 2.12). Linear ($P < .001$) increase in weight with increasing bST dosage.

[b]Effects of days on trial ($P < .0001$; SE = .34). Linear ($P < .001$) and quadratic ($P < .005$) increase in weight as days on trial increased.

[c]bST dosage by days on trial interaction ($P < .0001$; SE = .61). The following interactions were significant: $Dose_{linear}$ by $time_{linear}$ ($P < .001$), $dose_{quadratic}$ by $time_{linear}$ ($P < .001$), $dose_{linear}$ by $time_{quadratic}$ ($P < .01$), $dose_{quadratic}$ by $time_{quadratic}$ ($P < .05$) and $dose_{linear}$ by $time_{cubic}$ ($P < .005$).

7. Results indicate that rat weight increased as bST dosage and days on trial increased (primarily resulting from dosage linear and quadratic by time linear and quadratic responses).