EFFECTS OF TRENBOLONE ACETATE AND 17 BETA-ESTRADIOL IMPLANTATION ON SKELETAL MUSCLE PROTEIN METABOLISM IN STEERS

By

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ABSTRACT

EFFECTS OF TRENBOLONE ACETATE AND 17 BETA-ESTRADIOL IMPLANTATION ON SKELETAL MUSCLE PROTEIN METABOLISM IN STEERS

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Four experimental treatment (trmt) groups were utilized to elucidate the mode of action of trenbolone acetate (300mg; TBA), 17β-estradiol (24 mg; E2) and combined treatments (TBA+E2) on skeletal muscle protein deposition in yearling steers (n=48). In conjunction with estimates of skleletal muscle protein (skl prot) accretion and breakdown rates, blood samples were collected (n=16) and analyzed for serum concentrations of growth hormone (GH), insulin (INS) and cortisol (CT). Deposition of skl prot was increased 84, 48 and 7% by TBA+E2, E2 and TBA, respectively. Skeletal muscle protein breakdown was unaffected by implant treatment and averaged 1.39%/d. Mean GH concentration was decreased by collective TBA trmt, which seems to occur through modulation of GH pulse amplitude. Serum INS concentration was not altered due to anabolic implantation of steers. Serum CT levels were dramatically depressed upon both TBA and TBA+E2 trmt. These results imply that enhanced skl prot gains are not a consequence of decreased degradation rates and that anabolic action is directly related to changes in the hormonal milieu affecting muscle protein metabolism.

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INTRODUCTION

Efficient production of lean, nutritious and palatable meat products is a continual concern of the livestock industry. Utilization of various anabolic compounds has proven effective towards improving feed conversion efficiency, and increasing both growth rate and lean tissue deposition in ruminants (Galbraith and Topps, 1981). Anabolics are generally defined as substances that increase nitrogen retention and protein deposition in animals (Heitzman et al., 1980), where tissue active agents presently utilized are sex-steroids or closely related compounds possessing androgenic, estrogenic and progestogenic activity. Although enhanced animal performance resulting from the use of anabolic implants has been well established in the beef industry, the actual mode of action of these compounds, with regard to skeletal muscle accretion, has not been fully elucidated in cattle. European researchers (Harris et al., 1983; Sinnett-Smith et al., 1983; Lobley, 1985) have suggested that implantation of castrated male ruminants with combined treatments of trenbolone acetate (a synthetic androgenic xenobiotic) and 17β-estradiol, enhance skeletal muscle protein accretion by decreasing rates of muscle protein degradation. This

response of decreased skeletal muscle protein degradation, however, has not always been elicited when other anabolic implant treatments have been tested in steers (Gopinath and Kitts, 1982). In addition, inconsistancies exist whether anabolic treatment decreases (Sinnett-Smith et al., 1983; Lobley, 1985) or increases (Gopinath and Kitts, 1982) rates of muscle protein sythesis in ruminants. Due to these discrepancies, this present study was designed to further investigate the action of estrogenic and androgenic implants on skeletal muscle protein metabolism in steers.

LITERATURE REVIEW

Muscle Protein Metabolism and Turnover Concepts

Traditional concepts viewed skeletal muscle as a protein reservoir whose constituent amino acids could be mobilized and expended during conditions of fast or stress (Goldberg and Chang, 1978). Although skeletal muscle represents some 50% of the total body protein pool (Reeds and Palmer, 1986), it also possesses the capability to actively synthesize and degrade proteins. Skeletal muscle, in non-ruminants, is the primary tissue which catabolizes branched-chain amino acids (Goldberg and Chang, 1978; Lindsay and Buttery, 1980). Additionally, this tissue can oxidize leucine to carbon dioxide and convert carbon skeletons of aspartate, asparagine, glutamate, isoleucine and valine into intermediates of the tricarboxylic acid cycle (Goldberg and Chan, 1978). Proteins of skeletal muscle, in both growing and mature animals, are subject to continual synthesis and degradation. This dynamic process of protein turnover is considered to be a homeostatic mechanism providing an organism with a system that continually redistributes amino acids (Waterlow et al. 1978). This process is important for the constant maintenance of the tissue free amino acid pool, which constitutes only 0.5% of the total amino acids in the body (Swick, 1982), besides providing substrate for protein synthesis in a variety of cells (Bergen, 1974; 1978).

Quantitatively, protein turnover is large and may amount to more than five times the daily dietary protein consumed, therefore, amino acids obtained from protein breakdown must be reutilized for continuing protein synthesis (Swick, 1982). Various muscle tissues differ greatly with respect to their protein turnover rates (Millward, 1980). Additionally, individual constituent muscle proteins of muscle tissue may also turnover at different rates (Swick and Song, 1974; Bates and Millward, 1983). Protein turnover is an energy expensive process, requiring approximately 4 ATP equivalents per each amino acid incorportated into protein (Millward et al., 1975). Lobley et al. (1980) reported that the energetic cost of protein synthesis, accounted for a maximum of 30% of the heat produced in cattle, whereas Van Es (1980) estimated some 40-60% of metabolizable energy is utilized for lean deposition in growing animals.

Skeletal muscle accretion in immature animals may account for as much as 65% of the total daily protein gain (Reeds and Palmer, 1986). In growing animals, approximately 30% of protein synthesized is retained, and is dependent on both rates of synthesis and degradation (Swick, 1982; Mulvaney et al., 1985). These rates may be represented as protein gained (accretion), which is equal to protein synthesized minus protein degraded (Millward et al., 1975). Obviously, for accretion to occur in skeletal muscle, protein synthesis must exceed degradation. However,

accretion may also prevail if synthesis is maintained while degradation is reduced, or both degradation and synthesis rates are reduced with degradation rates exceeding those of synthesis. Overall, rates of synthesis and degradation are considered as being in a non-steady state. Differential equations formulated by Schimke (1973) provide a method to quantify these non-steady rates and how they effect protein accretion. Fractional rates, numerically expressed as a percent of a total pool per unit time (Waterlow et al., 1978), for both synthesis and degradation, appear to parallel each other (McCarthy et al., 1983). Fractional rates, however, tend to decrease with the increase of age in a variety of domestic animals (McCarthy et al., 1983; Mulvaney et al., 1985; Jones et al., 1986), where overall growth seems to be modulated by alterations of fractional breakdown rates (Millward et al., 1975; Mulvaney et al., 1985).

Although the reduction of protein degradation seems to be an attractive alternative towards enhancing protein accretion, protein degradation is critical for supplying substrate for protein synthesis, and is essential for the destruction of abnormally transcribed proteins (Goldberg and Dice, 1974). Proteolysis of muscle proteins can be linked to hormonal changes during both normal and pathological states (Tischler, 1981), where altered proteolysis may be mediated through the prostaglandin system, after being induced by trauma (Reeds and Palmer,

1986).

Fractional breakdown rate has been postulated as a controlling factor for DNA-unit size (Millward and Waterlow, 1978). The DNA-unit, first proposed by Cheek et al., (1971) is a defined volume of cytoplasm of the multinucleated muscle cell which is "managed" by a single nucleus. Briefly, the size and number of DNA units are indicative of a protein:DNA ratio, where the ultimate size of the muscle is dependent on the number of DNA units (Millward and Waterlow, 1978). Reduced rates of synthesis throughout development are a consequence of increased DNA-unit size (Millward and Waterlow, 1978), and is associated with small changes in the RNA:DNA ratio during development (Young, 1974). Consequently, a decrease in both ribosome number and activity results in the decrease of fractional synthesis rates of skeletal protein with age.

Overall, a number of factors effect or control muscle growth. Primary influence of sex and genotype are important determinants (Reeds and Palmer, 1985). Individual muscle stimulus, involving passive stretch induced by the stretch reflex in innervated muscles, is an additional factor (Laurent and Millward, 1980; Baracos and Goldberg, 1986). Furthermore, maintenance and growth of skeletal muscle tissue are collectively dependent on nutritional status (Bergen, 1974; Trenkle, 1974; Millward and Waterlow, 1978) and various hormonal components (Young, 1980: Buttery, 1983).

The Effect of Glucocorticoids on Growth and Protein Metabolism

The biological action of glucocorticoids on skeletal muscle is both catabolic and antianabolic (Young, 1980; Goldberg et al., 1980; Sharpe et al., 1986a). In eviscerated rats, glucocorticoid administration increased the release of muscle amino acids into blood, while concommitantly decreasing the incorporation of amino acids into muscle (Baxter and Forsham, 1972). In contrast to the catabolic action on muscle tissue, glucocorticoid adiministration increases protein content of the liver (Munro, 1970). Subsequently, Odedra et al. (1983) reported that corticosterone treatment in rats, increased both hepatic protein mass and size. Additionally, these researchers (Odedra et al., 1983) reported that fractional synthesis rate of liver protein was maintained over a period of chronic corticosterone treatment, in comparison to a marked decrease in protein synthesis demonstrated by muscle tissue. In general, substrate provided by the catabolic action of glucocorticoids on muscle, lymph and adipose tissues, enhance gluconeogenesis in the liver, whereas general glucocorticoid action in the periphery is hyperglycemic due to glucose sparing (Sharpe et al., 1986a).

Several experiments involving cattle have demonstrated a negative relationship between circulating cortisol, the primary glucocorticoid in ruminants, and growth (Purchas et al., 1971; Trenkle and Topel, 1978; Purchas et al., 1980).

In contrast, Lange and Lindermann (1972) did not show this relationship in cattle, while Purchas (1973) reported that glucocorticoid administration increased growth rate of ruminants. Recently, Sillence (1985) demonstrated that administration of an adrenal blocker (trilostane), reduced plasma corticosterone concentrations and increased both growth rate and feed conversion efficiency in rats. Henricks and co-workers (1984) found that a significant difference in circulating cortisol concentrations exsisted between bulls and heifers. These reseachers (Henricks et al., 1984) further suggested that plasma cortisol concentration was of little utility towards predicting individual growth rates, and that a better approach of comparison would be to measure the effect of a hormonal challenge (adrenocorticotrophin) on resulting blood cortisol levels.

Glucocorticoid action is directly mediated by target cells. Receptors have been located in the cytosol of rat skeletal muscle (Snochowski et al., 1980) and liver (Carlstedt-Duke et al., 1984). Many researchers have hypothesized that unbound or "free" glucocorticoids are the active form which produce physiological responses. Barnett and Star (1981) tested this hypothesis in sheep and found a significant negative correlation between free, but not total cortisol, and growth rate. In contrast, Pardridge (1981) and Siiteri et al. (1982) proposed that cortisol binding globulin (transcortin) actively participates in the uptake

of cortisol by target cells.

Glucocorticoids are antagonists of insulin (Millward et al., 1983) and testosterone (Young and Pluskal, 1977). Cortisol-insulin antagonism may be due to a reduction in receptor numbers and/or an inhibition of post-receptor events (Grunfield et al., 1981). The ability of insulin to decrease the proteolytic effect of glucocorticoids, compelled Tischler (1981) to propose that an glucocorticoid:insulin ratio may be used as a physiological determinant of muscle protein turnover status. This hypothesis is supported by data published by Coward et al. (1977), where further calculation of correlation coefficients (Tischler, 1981; Sharpe et al., 1986a) showed a linear-reciprocal relationship between plasma corticosterone:insulin ratio and total protein content of gastrocnemius muscle in rats. Exogenous glucocorticoid administration has demonstrated an ability to reduced serum somatomedin A levels in rats (Asakawa et al., 1982). Since subsequent growth hormone treatment failed to alleviate this response, Asakawa and co-workers (1982), concluded that glucocorticoids reduced serum somatomedins by inhibiting the stimulatory effect of growth hormone on somatomedin production.

The role of glucocorticoids on reducing protein synthesis has been established in rats, in vivo (Rannels and Jefferson, 1980; Odedra et al., 1983), and in vitro (McGrath and Goldspink, 1982). This effect may be attributed by the

formation of a glucocorticoid-steroid receptor complex interacting with DNA, influencing the amount or activity of specific mRNA (Baxter et al., 1972). Rannels and Jefferson (1980) reported that glucocorticoids inhibited translation as a consequence of impaired peptide-chain initiation. These results were indicative of increased single 40S and 60S ribosomal subunits (unpairing of the polyribosomal complex), as determined by sucrose-density gradient technique.

Although glucocorticoid treatment has produced marked decreases in protein synthesis, changes in degradation, as measured in vivo, have not been as definitive. Protein degradation in perfused rat hemicorpus muscle and cathepsin D activity in gastroenemius muscle were unaffected by cortisone acetate treatment (Rannels and Jefferson, 1980). Santidrian et al. (1981) injected adult male rats subcutaneously with 10 mg of corticosterone/100 g live body weight/day for 8 days. Daily urinary excretion of 3-methylhistidine, utilized as a measure of skeletal muscle myofibrillar protein degradation, increased considerably during the first 4 days during treatment, however, constant corticosterone injections past day 4 resulted in levels of 3-methylhistidine which declined toward control concentrations (Santidrian et al., 1981). Odedra et al. (1983) reported a similar time-course effect of corticosterone on rat muscle protein degradation, where urinary 3-methylhistidine excretion from young growing rats

doubled on the third day of treatment and returned to normal rates by day 5. McGrath and Goldspink (1982) showed that isolated rat soleus muscles, treated with a variety of both synthetic and natural glucocorticoids, demonstrated decreased rates of protein degradation. In contrast to these findings, an increase in myofibrillar protein breakdown in L6 myoblasts was reported by Ballard and Francis (1983) after dexamethazone treatment.

In summary, glucocorticoids directly diminish rat muscle protein synthesis, in vivo (Rannels and Jefferson, 1980; Odedra et al., 1983). In contrast, physiological concentrations of glucocorticoids seem to affect protein degradation only temporarily (Sharpe et al., 1986a). Although enhanced protein degradation has been demonstrated, these results are paradoxical, and may be confounded by experimental conditions, method of measurement (usually indirect), type and doseage of glucocorticoid, as well as relative potency of synthetic or natural glucocorticoids used as treatments (McGrath and Goldspink, 1982).

Insulin Effect on Growth and Protein Metabolism

Insulin is a pancreatic polypeptide hormone which is essential for growth in mammals (Young, 1980; Martin et al., 1984; Weekes, 1986). This hormone has exhibited pronounced effects on protein and carbohydrate metabolism, stimulating uptake of both amino acids and glucose by extrahepatic tissues (Prior and Smith, 1982). The exact mode of insulin action on the cell has not been fully elucidated, however,

Goldfine (1978) discussed the ability of insulin to produce temporal cell effects, i.e. rapid stimulation of cell membrane transport, intermediate effects on protein synthesis and long-term effects on DNA synthesis and cell replication. Insulin may exert a direct effect on the cell for a tetrameric receptor, isolated on plasma cell membranes, has demonstrated high affinity for this peptide hormone (Czech, 1984). Several reseachers have proposed that insulin should be classified as a "secondary" hormone, or one which serves a supportive role in growth (Davis et al., 1984; Florini, 1985). This proposal is more compelling due to results presented by Ewton and Florini (1981), who demonstrated that the apparent anabolic action of insulin is through cross-reactivity with somatomedin receptors. Since insulin has not shown an ability to induce somatomedin production (Schalch et al., 1979), it may be argued that insulin action is not indirect. More typically, insulin probably acts synergistically with other growth factors promoting cell growth (Straus, 1981).

The effect of insulin on growth has been demonstrated by Romsos et al. (1971), where these researchers reversed chronic tissue wasting and enhanced weight gain in diabetic pigs, compared to non-diabetic controls after insulin treatment. In growing cattle, Trenkle and Topel (1978) reported that plasma insulin concentration was positively correlated to fattening and negatively correlated to percent lean. In addition, these researchers (Trenkle and Topel,

1978) also showed that insulin concentration increased as both body weight and feed intake increased. Similarly, Eversole et al. (1981) reported that serum insulin concentration was positively correlated to average daily gain and fat deposition in cattle. An increase in insulin response, after administration of a hypoglycemic agent (tolbutamide), was demonstrated in lean Fresian steers, compared to Herefords having a greater percentage of empty body fat (Gregory et al., 1980). This response was consistent through two sampling periods at 12 and 20 months of age, however, insulin response was not correlated to percent body fat within breed (Gregory et al., 1980). Since overall plasma insulin concentration increased over time in both treatment groups, Gregory et al., (1980) concluded that high insulin secretion in cattle is associated with age-related adiposity, rather than differences in fatness between animals of the same age. Etherton and Kensinger (1984) stated that a positive correlation between growth and insulin could not be attained due to the variation of insulin concentration throughout the day. Since this variation may be amplified, especially with respect to feeding (Bassett, 1974; Godden and Weekes, 1981), Etherton and Kensinger (1984) further proposed that measurements of insulin receptor sensitivity, secretion and metabolic clearance rate may provide for a better prediction of the physiological action of insulin on growth.

Insulin is an essential hormone for protein anabolism.

Fuller et al. (1977) showed that continuous infusion of insulin and glucose in growing female pigs, decreased urinary nitrogen by 25%, versus glucose infused alone. This response was similar to those reported by Manchester and Young (1958), where insulin appeared to lower plasma amino acid levels and increase direct uptake of glucose by muscle tissue. Call et al. (1972) reported an 83.1% and 66.3% reduction in both plasma non-essential and essential amino acids, 40 minutes after an intravenous insulin injection in sheep. The stimulatory effect of insulin on amino acid uptake, seems to be limited to peripheral tissues, since insulin does not seem to elicit this effect in hepatic tissues (Brockman et al., 1975).

Cattle hind-limb studies have been utilized to study the effect of insulin to increase uptake of amino acids in peripheral tissues (consisting predominantly of skeletal muscle). Ahmed et al. (1983) reported that insulin injection in steers fed adequate protein and in a post-absorptive state, markedly increased both branched-chain and total amino acid uptake in the hind-quarter. Similarly, Prior and Smith (1983) reported that insulin treatment of diabetic-induced steers, transiently reversed a 2-3 fold increase in plasma branched-chain amino acid levels.

In an attempt to control problems of secondary hormonal and metabolic response due to hypoglycemia, DeFronzo et al. (1981) proposed utilization of an euglycemic clamp technique. This method was employed in sheep, by Weekes

(1986) who additionally demonstrated that insulin infusion lowered plasma alpha-amino nitrogen and alanine concentrations. Weekes (1986) further concluded that insulin action increased amino acid uptake in skeletal muscle, while concomittantly diminishing release of alanine from extraheptic tissue.

Insulin has been reported to stimulate protein synthesis in muscle (Young, 1970; Jefferson, 1980). King and Kahn (1981), demonstrated that insulin promoted [^{3}H]-thymidine incorporation into DNA. Wool (1972) suggested that insulin effect on protein synthesis was related to an alteration of muscle ribosome structure, whereas Flaim et al. (1983) proposed that insulin accelerated protein synthesis in rat cardiac muscle by increasing peptide-chain initiation. Pain et al. (1983) reported that protein synthesis decreased in a variety of muscle tissues excised from young streptozotocin-diabetic rats. Upon insulin withdrawl, fast twitch gastrocnemius muscle exhibited a decrease in both protein synthesis:RNA and total RNA concentration, whereas soleus and heart muscle, which are more resistent to insulin inhibition, demonstrated a slight decrease in ribosomal concentration (Pain et al., 1983).

Besides enhancing protein synthesis, insulin has shown an ability to retard protein degradation. Goldberg (1979) reported that insulin reduced overall protein breakdown through regulation of lysosomal function. In perfused heart muscle, Long et al. (1984) found that decreasing insulin

concentration in the perfusate was associated with both increased protein degradation and dense lysosome enzyme activity. In contrast to these findings, McElligott and Bird (1981) found no significant changes in muscle cathepsin- or serine- protease activities in diabetic rats when compared to control animals.

In summary, the primary role of insulin is to maintain homeostasis in the post-absorptive animal. In the ruminant, disposal of exogenous glucose loads is not prevalent, however, insulin is capable of inhibiting hepatic glucose output (Brockman, 1983) and stimulating glucose uptake in the periphery (Prior et al., 1984). Insulin's effect on protein synthesis is stimulatory, where it can promote uptake of branched-chain amino acids into skeletal muscle (Ahmed et al., 1983; Prior and Smith, 1983). A direct mode of action of insulin on muscle protein degradation has been suggested; however, this effect remains to be fully elucidated (Weekes, 1986). Insulin plays a predominate role in stimulating lipogenesis in bovine adipocytes, both in vitro (Etherton and Evock, 1986), and in vivo (Emery, 1979). Although direct action of insulin on cellular growth currently remains inconclusive, it is plausible to assume that this polypeptide effects growth through synergism with other growth factors (Straus, 1981).

Growth Hormone Effect on Growth and Protein Metabolism

Growth hormone (GH; somatotrophin) is a single chain polypeptide, which is stored and secreted by acidophile cells in the anterior pituitary. Basically GH is a product of gene transcription and translation in the pituitary, which is regulated by hypothalamic hormones (Bennett and Whitehead, 1983), nutrient availability (Davis et al. 1984) and hormones exogenous to the hypothalmus (Davis et al., 1984). The primary regulators of GH secretion are two hypothalamic neurohormones, growth hormone releasing factor (GRF) and somatostatin, whose actions are stimulatory and inhibititory on GH secretion, respectively.

Growth hormone is known to effect a wide range of metabolic and growth promoting effects in a variety of tissues, and is vital with respect to the metabolism of protein, carbohydrate and fat. Furthermore, GH is a primary hormone affecting the partitioning of nutrients for support of physiological and developmental processess (Bauman et al. 1982).

The effect of chronic administration of exogenous GH eliciting a somatotrophic response on growth of rats, has been known since the work of Evans and Simpson (1931). Presently, abounding evidence has been reported showing that exogenous GH treatment similarly effects growth of farm animals (Hart and Johnsson, 1986). In sheep, Muir et al., (1983) demonstrated that 7 mg of ovine GH (oGH) injected daily in an olive oil base, to ensure slow release, improved

feed conversion efficiency 7.4% without an effect on average daily gain or feed intake. Johnsson et al. (1985) reported that daily bGH injections in lambs significantly increased average daily gain, feed conversion efficiency and weight of lean tissue and bone. Butler-Hogg et al. (1987) reported similar findings in both lamb gain performance and carcass composition as Johnsson et al. (1985), in addition that GH also significantly decreased subcutaneous fat deposition. In identical twin heifer calves, daily GH injections produced marked increases in growth rate (Brumby, 1959). More recently, it has been reported that bGH administration increases nitrogen retention (Mosely et al., 1982; Eisemann et al., 1986) and protein content of the 9-10-11 ribs (Peters, 1986) in steers.

Although GH produces an anabolic affect in meat producing animals, attempts to relate endogenous circulating GH concentration with growth, have not been successful. Negative correlations between GH and growth were reported in beef steers (Trenkle, 1970; Eversole et al., 1981) and in Holstein heifers (Purchas et al., 1971) In contrast, young bulls showed a positive correlation of both body weight at slaughter and specific growth rate to plasma GH concentration (Purchas et al. 1970). A likely reason for the discrepancy found between these studies may be due to utilization of single or infrequent blood collections. Growth hormone secretion is quite dynamic in rats, cattle and sheep (McAtee and Trenkle, 1971; Davis and Borger, 1974;

Tannenbaum and Martin, 1976). Since GH secretion is episodic, Davis et al. (1984) proposed utilizing patterns of secretion, i.e. plasma GH pulse, frequency and amplitude for assessing growth response to GH. This suggestion is supported by the studies of Jannson et al. (1982a,b), where these reseachers found that multiple injections of GH gave a greater growth response in rats. In contrast to these results, however, Mosely et al. (1982; 1987) reported that both multiple injections and/or continuous infusion of either bGH or GRF gave similar increases in nitrogen retention in cattle.

Influence of gonadal steroids on GH have been reviewed by Davis et al. (1984). Basically, estrogens and testosterone increased blood GH concentrations, where estrogens seem to enhance GH secretory activity from the anterior pituitary (Davis et al., 1984). The effect of sex differences on GH have been reported in cattle (Reynert et al., 1976), where plasma GH baseline concentration and pulse amplitude were higher in bulls than in heifers. Rapidly growing breeds have demonstrated a positive relationship between growth and increased GH secretion, which lead Davis et al. (1979) to propose that GH secretion may be a valuable predictor of genetic growth potential. In agreement with Davis et al. (1979), O'Sullivan et al. (1986) reported that genetically small, versus large rats, had lower plasma GH peak amplitudes, baseline and mean plasma concentrations.

The primary effect of GH on muscle is anabolic (Young,

1970; Young and Pluskal, 1977). Early studies reported GH administration enhanced whole-body nitrogen retention and promoted positive nitrogen balance in animals (Wheatly et al., 1966; Davis et al., 1970). Administration of GH has stimulated amino acid uptake in rat muscle (Noall et al., 1957; Riggs and Walker, 1960). Manchester et al. (1959) demonstrated that GH treatment in both hypophysectomized and intact rats, increased incorporation of radioactive glycine in isolated diaphragm muscle. Similarly, Kostyo and Knobil (1959) showed that GH administeration increased [^{14}C]-leucine incorporation in excised rat diaphragm muscle.

In a series of experiments Kostyo and colleagues (Rillema and Kostyo, 1971; Kostyo and Nutting, 1973; Dreskin and Kostyo, 1980) reported that bGH increased both uptake of amino acids and protein synthesis in rat muscle. Each experiment reported a similar time trend, in which protein synthesis occuring 15-30 minutes after GH administration. Dreskin and Kostyo (1980) summarized these experiments, stating that GH has a direct effect on rat skeletal muscle and that the lag-period demonstrated is needed for an increase of ribosomal activity to occur. This hypothesis is supported by Dreskin and Kostyo (1980), who by using sucrose density gradients, showed that GH increased isolated muscle polyribosome fractions. Additional effects of GH on protein synthesis may be attributed to alteration of RNA metabolism (Manchester, 1970). In support of this proposal, Florini and Breuer (1966) demonstrated that GH treatment increased

both protein synthesis and total RNA concentration in hypophysectomized rat skeletal muscle.

Growth hormone does not seem to affect protein degradation (Goldberg, 1968). Flaim et al. (1978) reported that GH did not elicit an effect on rat gastrocnemius muscle degradation or cathepsin D activity. Additionally, Harper et al. (1987) demonstrated that bGH had no effect on protein turnover in cultured primary ovine muscle tissue.

Presently, data has been reported showing that GH action on muscle is mediated by somatomedins (Clemmons et al., 1981). Ewton and Florini (1980; 1981) showed that proliferation and amino acid uptake by myoblasts were stimulated by somatomedins and not GH. In myotube preparations, however, GH did promote amino acid uptake (Ewton and Florini, 1980), suggesting that this peptide hormone may induce somatomedin mediated growth in muscle cells. In arguement of the original theory proposed by Salmon and Daughaday (1957), recent data provide information that GH directly binds and increases proliferation of both rabbit (Eden et al., 1983; Madsen et al., 1983) and rat (Isaksson et al.,1982) chondrocytes.

In summary, GH administration in domestic animals increases lean tissue accretion, while concomittantly decreasing overall fat deposition (Hart and Johnsson, 1986). Presently, it is believed that GH indirectly stimulates skeletal muscle growth by enhancing somatomedin production (Clemmons et al., 1981; Florini, 1985). However, the direct

mode of action of GH on growth and cellular proliferation should not be completely discredited, for under certain cell culture conditions this hormone has demonstrated high-affinity for GH receptors, and has elicited post-receptor binding events (Eden et al., 1983; Madsen et al., 1983). Growth hormone seems to enhance protein accretion strictly by increasing protein synthesis, while having no apparent effect on protein degradation (Hart and Johnsson, 1986).

Anabolics-General Concepts

Anabolic treatment usually increases ruminant growth rate, feed efficiency and total carcass protein deposition (Galbraith and Topps, 1981; Roche and Quirke, 1986). Utilization of anabolic compounds have proven as attractive stimulators of animal performance, especially with regard to castrated male cattle (Roche and Quirke, 1986). Usually the consequence of castration is a reduction of androgen secretion, which subsequently affects metabolism related to growth (Galbraith et al., 1978). General effects of castration most noted are decreased musculature, primarily in the forequarter, and increased distribution of carcass fat (Berg and Butterfield, 1976).

Anabolic compounds presently utilized are closely related to gonadal steroids, e.g. androgens, estrogens and progestins. Heitzman (1975) proposed that "correct" anabolic agents should be administered to the different sex types of cattle in order to attain maximum performance. Since intact females normally produce estrogens, better

growth rates should be obtained after administration of an androgen (Roche and Quirke, 1986). Androgen and estrogen combinations have demonstrated additive effects on growth rates in steers (Heitzmen et al., 1977b; Buttery and Sinnett-Smith, 1984), whereas exogenous steroid adminsteration usually does not elicit a substantial growth response in bulls (Roche and Quirke, 1986).

The Effect of Estrogens on Growth and Performance

Estrogenic compounds have demonstrated stimulatory effects on growth in ruminants since the 1940's (Galbraith and Topps, 1981). After comparing estrogenic implants, containing 30 mg of stilbene, estriol, estrine, α-estradiol, β-estradiol and diethylstilbesterol (DES) in steer calves, Preston et al., (1978) reported that gain and feed efficiency were only stimulated by β-estradiol (24 and 13%, respectively) and DES (12 and 19%, respectively). In addition to the estrogenic compounds listed above, hexestrol has also shown to improve growth rates of steers (Perry et al., 1955; Aitken and Crichten, 1956). More recently, Rumsey et al., (1981) reported that DES treated steers, produced greater proportions of separable lean and bone, in addition to further increases of live weight gain and feed efficiency of 17 and 12%, when compared to non-implanted steers. Furthermore, Rumsey et al., (1981) demonstrated that DES increased both gain/unit protein and energy intake by approximately 20% within steer total empty body. Although

DES has been proven as an attractive stimulator of protein gain in steers, this product has been banned in most countries due to residue safety concerns. A natural occuring estrogen (17β-estradiol), however, seems to be an excellent alternative to DES for providing accellerated gains in cattle (Turner et al, 1981; Wellington, 1985). Experimentation to provide an implant that would release efficacious doses of estrogen over periods of 200 days was attempted by Lilly Research Laborotories (Greenfield, ID). After a series of studies, Lilly Research Laborotories began marketing a cylindrical silicone rubber matrix implant peripherally encoated with 24 or 45 mg of crystalline 17β-estradiol (Compudose 200 or 365). It was reported by Utley and co-workers (1981), and Wagner (1983) that a 17β-estradiol implant length consisting of 2.54 cm elicited a maximum growth response in steers. Additionally, these implants demonstrated an ability to release efficacious amounts of estradiol for up to 400 days in steers (Wagner, 1983). This prolonged release of estradiol, however, is in contrast to data presented by Sawyer (1987), who reported that silastic implants containing 24 mg of estradiol gave an anabolic response in steer calves for only 66 days. Wagner (1983) further reported that 2.54 cm estradiol implants provided release of approximately 5.4 mg of 17β-estradiol per day, resulting in plasma 17β-estradiol concentrations of 54 pg/ml, as compared to control levels equalling 0.77 pg/ml. In agreement with Wagner (1983), similar increases in

blood 17β-estradiol concentrations have been reported by Chudleigh et al. (1982) in steers.

To date, research conducted on the effect of 17β-estradiol on growth has been favorable. An average increase in rate of gain and feed efficiency of 15% and 18% has been demonstrated by numerous feedlot trials (Wagner, 1983). Wagner (1983) also reported that implantation of steers with 24 mg of 17β-estradiol increased total carcass weight. Similarly, Mathison and Stobbs (1983) showed that steers receiving 24 mg 17β-estradiol implants had heavier carcasses (+17 kg) and higher dressing percentages when compared to controls.

The Effect of Trenbolone Acetate on Growth and Performance

The anabolic steroid trenbolone acetate (TBA; 17β-acetoxy-4,9,11-androstatrien-3-one) was first synthesized by Velluz and co-workers (1967). It is a testosterone analog which exhibited anabolic activities 10 and 50 times greater than those demonstrated by testosterone propionate and testosterone in a variety of test animals (Neumann, 1975). Trenbolone acetate treatment has demonstrated pronounced increases in both live weight gain and feed efficiency in cattle, sheep, pigs (Gailbraith and Topps, 1981; Grandadam et al., 1975), rats (Vernon and Buttery, 1976, 1978a,b) and rabbits (Lobley et al., 1983). In cattle, TBA and TBA plus estrogen combinations have successfully increased nitrogen retention (Galbraith and Topps, 1981). Implantation of growing heifers with 300 mg of TBA produced marked increases

in both live weight gain and feed efficancy when compared to non-implanted control heifers (Heitzman and Chan, 1974). Best et al. (1972) reported similar findings with heifers on pasture, obtaining an average 38% increase in live weight gain over the entire experimental period. Additionally, treatment of cull cows with 300 mg of TBA resulted in improved feedlot gain performance (Galbraith, 1980a). In contrast to the aformentioned heifer studies, Benner (1983) showed that 200 and 300 mg TBA treatments did not produce a significant increase in feedlot heifer performance.

Trenbolone acetate has increased both gain and feed efficiency in steers, however, these results are not as great as those of single estrogens, or estrogen and TBA treatment combinations (Bouffault and Willemart, 1983). Heitzman et al. (1981) reported increases of steer live weight gain (11, 12, 24 and 45%), cold carcass weight (4, 6, 6.7 and 8.8%), and decreases in feed conversion ratios (5.5, 9, 12.5 and 25%) between treatments of TBA, 17β-estradiol, TBA plus 17β-estradiol, and combined TBA and 17β-estradiol (Revalor) implants, respectively. Similar findings have been reported in steers implanted with varied combinations of estrogens and TBA; TBA with 17β-estradiol (Heitzman et al., 1977a; Hicks et al., 1985), TBA with hexestrol (Stollard et al., 1977; Heitzmann et al., 1977b), and TBA with zeranol (Roche and Davis, 1978). Comparison of dual implant combinations and their effect on steer performance has been reported by Lowman and Scott (1983)

from data combined from 4 commercial beef farms. These reseachers (Lowman and Scott, 1983) showed that the largest response achieved throughtout the various studies was by TBA plus estradiol, followed by estradiol plus zeranol, and TBA plus zeranol. Additionally, Verbeke et al. (1975) and VanderWaal (1975b) reported that implantation with 140 mg of TBA plus 20 mg of estradiol appeared to be more effective than 20 mg of estradiol plus 200 mg of testosterone, towards improving carcass characteristics in veal calves.

Treatment of young bulls with TBA does not seem to cause a growth response, however, Galbraith (1982) showed that combined treatment with TBA plus hexestrol stimulated bull growth. Additionally, a recent report by Wood et al. (1986) demonstrated that TBA plus estradiol combinations increased both muscle and fat deposition in bull carcasses.

Upon review of the activity of TBA in ruminants, Bouffault and Willemart (1983) summarized that TBA enhances overall growth performance by increasing average daily gain, feed efficiency, carcass weight and carcass conformation, while decreasing carcass fat content. This is in partial contrast, however, to Wood et al. (1986) who reported that TBA plus estradiol implantation in both bulls and steers increased total carcass fat.

The Mode of Action of Estrogens and Androgens-Introduction

Administration of anabolic implants to ruminants usually stimulate protein deposition (Galbraith and Topps, 1981).

A possible mode of action of these steroids, besides altering endogenous hormone concentrations and/or the affinity of receptors to endogenous hormones, is the direct action of the anabolic agent, or its metabolites on target tissues.

Direct Action of Androgens on Muscle

Few studies have actually demonstrated anabolic effects on muscle tissue by direct action of testosterone, however those that have seem to suggest that this is accomplished by increasing net muscle protein synthesis (Buttery and Sinnett-Smith, 1984). Upon castration, growing guinea pigs showed a marked decrease in muscle RNA, which was returned to normal concentrations after testosterone administration (Kochakian et al., 1964). Furthermore, Grigsby et al. (1976) reported that testosterone treatment of rabbits, increased [^{3}H]-leucine incorporation into myofibrillar proteins.

Isolation of androgen receptors was first demonstrated by Jung and Balieu (1972) who reported that testosterone, but not dihydrotestosterone (DHT), bound to androgen receptors of the rat levator ani muscle. This result is in contrast to those of reproductive tissues, i.e. prostrate, where testosterone is a "pre-hormone", primarily reduced by 5α-reductase to DHT, which binds to the androgen receptor (Mainwaring, 1977). Skeletal muscle has limited 5α-reductase activity (Bardin et al. 1978). Recently, testosterone receptors have been isolated in the cytosol of skeletal muscle in rats, pigs and humans (Snochowski et al.,

1980, 1981a, 1981b). Testosterone has been reported to stimulate the labelling index of muscle cell DNA, as measured by [^{3}H]- thymidine incorporation into DNA of primary myoblasts and cultured muscle L6 cells (Powers and Florini, 1975). Testosterone and TBA treatment of cultured L6 myoblasts did not cause an effect on degradation rates (Ballard and Francis, 1983), however, in rat prostate TBA demonstrated a high-binding affinity for androgen receptors (Raynaud et al., 1981). Additionally, Raynaud and co-workers (1981) also reported that a low-binding affinity of TBA for glucocorticoid receptors exsisted. These results, in addition to research presented by Snochowski et al (1980), disproves the hypothesis of Mayer and Rosen (1975) who stated that androgens compete with glucocorticoids for binding at glucocorticoid receptors. Trenbolone acetate has been reported to reduce the apparent binding of testosterone to cytosolic muscle receptors (Buttery and Sinnett-Smith, 1984). Another mode of action of TBA on target cells may be the aromatization of testosterone to an estrogen, where the newly formed estrogen than mediates a growth response. (Longcope, et al., 1978). In support of this hypothesis, Pottier and Cousty (1981) demonstrated that certain metabolites derived from TBA have aromatized A-rings.

Direct Action of Estrogens on Muscle

Estrogens have a stimulatory effect on protein synthesis, as demonstrated by the increase of certain plasma proteins

(transcortin and sex binding globulin) after estrogen administration (Michel and Balieu, 1983). Estrogen receptors have been characterized in the cytosol of rat skeletal muscle (Dahlberg, 1982). Additionally, the presence of cytosolic estrogen receptors have been confirmed in sheep and bovine skeletal muscle (Buttery and Sinnett-Smith, 1984; Meyer and Rapp, 1985). Diethylestilbesterol and 17β-estradiol treatment have demonstrated increased activity of glucose 6-phosphate dehydrogenase in rat levator ani muscle, which may suggest that estrogens directly effect muscle tissue (Knudsen and Max, 1980). In constrast to these results, DES did not demonstrate any effect on rates of L6 muscle cell protein degradation (Ballard and Francis, 1983), which is in agreement with a proposal by Michel and Balieu (1983), stating that synthetic estrogens can act only indirectly on various target tissues.

Indirect Effects of Estrogens and Trenbolone Acetate on Thyroid Hormones

Treatment of steers, wethers, and heifers with TBA usually causes a reduction of plasma thyroid hormones (Table 1). Normal growth is dependent on an euthyroid condition in mammals, where varied blood thyroid concentrations, either low (Brown et al., 1981) or high (Goldberg et al., 1980), will decrease the occurance of muscle protein accretion. Buttery and Sinnett-Smith (1984) hypothesized that the decreased thyroid hormone concentration associated with TBA treatment may explain the reduction of protein synthesis

Table 1. Hormonal Changes Demonstrated in Ruminants After Treatment with Trenbolone Acetate and Estrogens.

Treatment	Animal	GH	PRL	INS	Other	References
TBA	beef cows	NS	NS			Galbraith (1980a)
TBA	wethers	NS	NS	NS	T4(-)	Donaldson and Hart (1981)
E2		(+)	NS	(+)		
TBA+E2		NS	NS	NS	E2(+),T4(-)	
TBA+HEX	bulls	NS	NS	NS		Galbraith (1979)
TBA	steers				T4(-)	Heitzman et al. (1980)
TBA+HEX					T4(-)	
TBA	heifers				T4(-)	
TBA+HEX	bulls	NS		NS		Galbraith and Coelho (1978)
TBA+HEX	steers	NS		NS		
TBA+HEX	steers	NS	NS	NS		Galbraith and Geraghty (1978)
TBA+HEX	steers	(+)	NS	NS		Peters and Read (1982)
TBA	heifers	NS	NS			Galbraith and Miller (1977)
TBA	female lambs				C(-)	Thomas and Rodway (1982a)
TBA+E2	wethers				C(-)	Singh et al. (1983)
TBA+E2	wethers				C(-)	Thomas and Rodway (1983a)
TBA					C(-)	
TBA	female lambs				C(-)	Sillence et al. (1987)
TBA+E2	female lambs				C(-)	
TBA	wethers			NS	C(NS)	
E2	wethers			NS	C(+)	
TBA+E2	wethers			NS	C(NS)	
TBA	female lambs			NS	C (+)	Sharpe et al. (1986b)
				NS	Cf(NS)	
TBA	steers	NS			T4(-)	Heitzman et al. (1977a)
TBA+E2		NS			T4(-)	

Table 1 Continued.

Treatment	Animal	GH	PRL	INS	Other	References
TBA+HEX	steers	(+)		NS		Galbraith and
TBA		NS		NS		Watson (1978)
HEX		(+)		NS		
T3A	heifers	NS	NS	NS		Galbraith (1980b)
TBA+HEX	steers	NS	NS	NS		Galbraith and Geraghty (1982)
TBA+HEX	bulls	NS	NS		T,DHT(-) C(NS)	Galbraith (1982)
TBA+HEX	wethers		NS	NS		Coelho et al. (1981)
TBA+HEX	steers				T3,T4(NS)	Stebbings (1983)
E2	steers	(+)		NS	T4(NS)	Grigsby and Trenkle (1986)
DES	steers	(+)		(+)		Trenkle (1970)
DES	wethers	(+)				Davis et al. (1977)

[a] adapted from Buttery and Sinnett-Smith (1984)
NS= no significant change
(+)= increased plasma concentration
(-)= decreased plasma concentration
E2= estradiol
T4= thyroxine
T3= triiodothyronine
T= testosterone
DHT= dihydrotesosterone
C= total cortisol
Cf= free cortisol
HEX= hexestrol
PRL= prolactin
TBA= trenbolone acetate

that they observed in sheep. Lower concentrations of thyroid hormone have not been observed in cattle implanted with estradiol (Trenkle, 1983), however, zeranol appears to depress thyroxine in lambs (Wiggins et al., 1979). Additionally, Donaldson and Hart (1981) reported that suppressed blood thyroxine concentrations were prominant in ewes treated with combined treatments of estradiol and TBA.

Indirect Effects of Estrogens and Trenbolone Acetate on Glucocorticoids

Trenbolone acetate treatment reduces plasma glucocorticoid concentration in rats (Thomas and Rodway, 1982a, 1983b), and sheep (table 1). Thomas and Rodway (1983a) found that both TBA, and combined TBA and estradiol treatments reduced circulating cortisol levels in lambs, while single estradiol administration increased plasma cortisol concentration. This response agrees with results presented by Seal and Doe (1963), who reported that pregnancy caused an increase in estradiol concentration, which subsequently causes an elevation in plasma cortisol concentrations in humans, rats, mice, guinea pigs and rabbits. Glucocorticoids enhance activity of hepatic deaminative enzymes, such as tyrosine amino transferase (TAT; Lin and Knox, 1957). Activity of TAT was reduced in both rats (Rodway and Galbraith, 1979; Thomas and Rodway, 1982b; 1983b) and lambs (Thomas and Rodway, 1983a) after administration of TBA. Adrenocorticotrophin challenge on

the adrenals has demonstrated to produced a smaller increase in corticosterone secretion in TBA treated rats and lambs (Thomas and Rodway, 1982b; 1983a; 1983b). In addition, Thomas and Rodway (1983b) also reported that exogenous corticosterone injection induced hepatic TAT activity in TBA treated rats. Together, these data provide evidence that TBA may be directly effecting adrenal output of glucocorticoids. Thomas and Rodway (1982b) attempted to investigate the direct action of TBA on cultured adrenal cells from female rats. Additions of 10^{-10} and 10^{-12}M ACTH, significantly increased cell production of corticosterone, whereas treatment with 10^{-6}M trenbolone reduced corticosterone release into the media by 44 and 81%, respectively. Sharpe et al. (1986b) examined the effect of TBA and zeranol on skeletal muscle glucocorticoid receptors, total plasma cortisol concentration, free cortisol concentration and transcortin capacity in sheep. Skeletal muscle glucocorticoid receptors showed no affinity for TBA, however, TBA treatment reduced receptor binding capacity. This response was suggested to be due to a reduction of receptor numbers (Sharpe et al., 1986b). Additionally, transcortin capacity was decreased with TBA, as compared to an increase in binding capacity demonstrated by zeranol treatment. Sharpe et al., (1986) further reported that free cortisol concentration increased with a concomittant decrease in transcortin numbers after TBA treatment, however, these workers did not present any further evidence

that this increase in plasma free cortisol concentration was correlated to lamb growth rate.

Indirect Effects of Estrogens and Trenbolone Acetate on Growth Hormone

A common occurance often noted after estrogen treatment in ruminants, is an increase in mean growth hormone concentration in plasma (Trenkle, 1969; Preston, 1975). Single TBA treatment does not seem to produce this response (table 1), although there is evidence that combined hexestrol and TBA increases blood GH concentrations in steers (table 1). A consistent change demonstrated in cattle and sheep upon estrogen treatment, is an increase of pituitary gland weight (Trenkle, 1983), whereas this response has not been found to be associated with TBA treatment (Donaldson and Hart, 1981). Early studies revealed that concentration of GH in the pituitary is not increased after estradiol treatment, but the glands are actually heavier and contain more total growth hormone (Struempler and Burroughs, 1959). Estradiol implanted cattle have demonstrated increases in both plasma mean and baseline GH concentrations, where the overall increase in mean GH concentration is attributed to enhanced GH pulse frequency (Grigsby and Trenkle, 1986). Similar findings have been reported in lambs (Davis et al., 1977), although GH pulse amplitude tended to be increased, in contrast to pulse frequency. Davis et al. (1977) reported that estradiol stimulated GH secretion with no apparent effect on

GH clearance rates. Some evidence suggests that estradiol can bind directly to cells of the anterior pituitary (Carlsson et al., 1987), which further suggests that estradiol may effect sensitivity of the anterior pituitary to GRF (Trenkle, 1983).

Indirect Effects of Estrogens and Trenbolone Acetate on Insulin

Estrogen administration causes an increase in plasma insulin concentration in both sheep (Donaldson and Hart, 1981) and cattle (Trenkle, 1970). In contrast to estradiol, TBA or TBA plus estradiol treatment combinations do not significantly effect plasma insulin concentration (table 1). Trenbolone acetate has actually been reported to antagonize the effect of estradiol on increasing plasma insulin concentration in sheep implanted with dual treatments of TBA and estradiol (Donaldson and Hart, 1981). The estrogenic effect on insulin secretion may be indirectly related to the diabetogenic effect elicited by elevated blood GH concentrations (Trenkle, 1983). There is evidence that GH directly acts on rat pancreas cells (Whittaker and Taylor, 1977). Additionally, mediatation of insulin secretion may be a direct effect caused by estrogen binding to receptors on pancreatic beta-islet cells (Sandberg et al., 1973).

Indirect Effects of Estrogens and Trenbolone Acetate on Prolactin

Circulating concentration of prolactin, in ruminants, is not effected by either TBA or estradiol treatment (table 1).

The Effect of Trenbolone Acetate and Estrogen Treatment on Blood Metabolites

A common trend noted in the literature (table 2), is that anabolic implantation does not effect plasma glucose, free fatty acids, or total protein concentration. However, Galbraith (1980a) showed a significant decrease of plasma free fatty acids in beef cows after TBA treatment (table 2). Trenbolone acetate and TBA plus an estrogen, usually promotes a decrease in serum albumin and plasma urea nitrogen concentration in ruminants (table 2). Lower plasma urea nitrogen levels have also been reported in cattle and lambs upon treatment with synthetic estrogens (Preston, 1975). In addition, Heitzman et al. (1977a) reported that TBA, or combined TBA estradiol treatments, did not significantly change serum calcium, magnesium, inorganic phosphorus, copper, sodium and potassium. However, in contrast to TBA treatment, synthetic estrogens have shown an ability to increase calcium and phosphorous retention in lambs (Whitehair et al., 1953; Braithwaite et al., 1972).

Table 2. Changes of Blood Proteins and Metabolites in Cattle After Trenbolone Acetate and/or Estrogen Treatment

Treatment	Animal	Serum Albumin	Serum Protein	Plasma Urea	Plasma Glucose	Plasma FFA	Reference
TBA+HEX	steers	(-)	(-)	(-)	NS	NS	Galbraith
	bulls	NS	NS	NS	NS	NS	and Coelho (1978)
TBA	cows	(-)		(-)		(-)	Galbraith (1980a)
TBA+HEX	bulls	NS	NS		NS	NS	Galbraith (1979)
TBA+HEX	steers			(-)	NS		Peters and Read (1982)
TBA	heifers	(-)	NS	(-)			Galbraith and Miller (1977)
TBA	steers	(-)	NS	(-)	NS		Heitzman et al.
TBA+E2		(-)	NS	(-)	NS		(1977a)
TBA	steers				NS	NS	Galbraith
HEX					NS	NS	and Watson
TBA+HEX					NS	NS	(1978)
TBA	heifers	(-)	NS	(-)	NS	NS	Galbraith (1980b)
TBA+HEX	bulls	(-)	NS	(-)	NS	NS	Galbraith (1982)

TBA= trenbolone acetate
HEX= hexestrol
E2= estradiol
FFA= free fatty acids
NS= non-significant
(-)= decrease concentration

The Effects of Estrogens and Trenbolone Acetate on Protein Metabolism

To date, limited research has been reported on the effect of estrogens on skeletal muscle protein metabolism in large domestic animals, in vivo. Early studies utilizing nitrogen-balance techniques showed that synthetic estrogens decreased urinary nitrogen output in ruminants and improved overall nitrogen retention (Trenkle, 1975). VanderWaal et al. (1975a) demonstrated that treatment of bull calves with DES, estradiol, and combinations of estradiol plus testosterone and estradiol plus TBA increased nitrogen retention 214, 171, 188 and 551 g respectively, over a 30 day treatment period. These researchers (Vanderwaal et al., 1975a), however, did not show a significant decrease in nitrogen retention after single testosterone, TBA or zeranol administration. In contrast to young bull calves, Chan et al. (1975) reported that young growing heifers treated with 300 mg of TBA, retained significantly more nitrogen. More recently, two experiments using steers treated with a combination of estradiol and zeranol, showed a significant increase of 50% in average nitrogen retention when compared to non-implanted animals (Griffiths, 1982). In an attempt to pinpoint the mechanism in which TBA enhances muscle protein accretion, Vernon and Buttery (1976) by utilization of [^{14}C]-tyrosine infusion (Garlick and Marshall, 1972) and $NaH^{14}CO_3$ (Millward, 1970) techniques, reported that this xenobiotic collectively decreased both fractional synthesis

and breakdown rates of rat skeletal muscle. In contrast to to these findings, Martinez et al., (1984) showed that testosterone treatment stimulated protein synthesis in rat gastrocnemius muscle. Together, these data indicate that TBA and testosterone are effective through different modes of action. In a series of experiments with female rats, Vernon and Buttery (1978 a,b) reported that TBA treated animals had greater carcass nitrogen retention, which was a result of conjoined decreases in both muscle protein synthesis and degradation rates. In an additional study to further elucidate the response of skeletal muscle protein degradation to TBA administeration, Vernon and Buttery (1981) reported that both free and total activity of proteolytic cathepsin D decreased in rat skeletal muscle. With further regard to the effect of TBA on reducing muscle protein synthesis rates, Vernon and Buttery (1978a) reported that treated rat muscle demonstrated increased RNA content, however, this increase was coupled with significantly decreased RNA activity.

Comparable to the rat studies (Vernon and Buttery, 1978a,b; 1981), similar findings of decreased muscle protein degradation and synthesis rates have been reported in ruminants implanted with combined TBA and estrogen treatments. Bohorov et al. (1987) found that TBA plus estradiol implants significantly decreased rate of lamb muscle protein degradation. Lobley et al. (1982) reported superior nitrogen retentions in steers treated with Revalor

(43.7 vs. 24.7 g nitrogen/day), which was strictly an outcome of decreased rates of protein degradation. In castrated male sheep, Sinnett-Smith et al. (1983) showed that TBA plus estradiol treatment increased growth rate, as a result of decreased muscle protein degradation, which was measured by muscle cathepsin D activity. The same researchers (Sinnett-Smith et al., 1983) found similar findings in female lambs, in addition that TBA plus estradiol treatment also decreased both DNA concentration and mixed-muscle protein synthesis rates. Similarly, Lobley et al. (1985) showed that estimates of whole body protein synthesis and protein oxidation in beef steers declined significantly by weeks 5 and 2 respectively, after TBA and estradiol implantation. Additionally, both Harris et al., (1983) and Lobely et al., (1985), reported that a slight decrease in urinary 3-methylhistidine concentration occurred after implantation with TBA and estradiol, suggesting that a reduction of myofibrillar protein breakdown is induced by this anabolic treatment in cattle.

In summary, administration of both TBA and estrogens in the ruminant causes enhanced muscle protein accretion. The exact mode of estrogen action on skeletal muscle has not yet been elucidated. However, TBA seems to cause a decrease in both protein synthesis and degradation rates, where the depression in degradation exceeds that of synthesis, allowing for the occurance of net protein deposition (Sinnett-Smith et al., 1983; Lobely et al., 1985).

GENERAL EXPERIMENTAL PLAN

The growth promoting effect of 17β-estradiol (E2), trenbolone acetate (TBA), and a combination of these single implants (TBA+E2) on weight gains, dry matter intake (DMI) and feed efficiency (F/G) of steers fed a 1.14 Mcal/kg NEg diet were measured. Estimates of implant effectiveness, to increase individual skeletal muscle protein fractional accretion rates (FAR), were calculated by using a non-invasive urea dilution technique (Kock and Preston, 1979; Hammond et al., 1984). Urinary 3-methylhistidine (3MH) excretion (Harris and Milne, 1981; McCarthy et al., 1983) was measured to evaluate the effect of the implant treatment on fractional breakdown rates (FBR) of skeletal muscle proteins. Muscle protein fractional synthesis rates (FSR) were calculated by adding FAR plus FBR. Excreted urinary creatinine concentration was used as an additional indicator of muscle mass size (Waterlow, 1969). Measurement of serum 17β-trenbolone (TB) and E2 concentrations were obtained to ensure implant delivery. Finally, serum concentrations of growth hormone (GH), insulin (INS), cortisol (CT) and plasma glucose were quantified to determine indirect implant treatment response on skeletal protein metabolism.

MATERIALS AND METHODS

Feedlot Study

Experimental Animals, Treatments and Design

Forty-eight crossbred steers were blocked by breed, frame size and body weight, (initial average live weight= 295 kg) into four groups of twelve. Each group was assigned to one of four implant treatments; control (C), 300 mg TBA (Finaplix, Distrivet, Paris), 24 mg E2 (Compudose 200, Elanco, Indianapolis, IN) and 300 mg TBA plus 24 mg E2.

Cattle within each treatment group were randomly assigned to two pens. Pens were randomized within the facility to decrease the possibility of treatment bias, contributed from the environment. Animals were housed in pens, which were concrete-based and approximately 40% sheltered. The trial was initiated on February 2 and continued for 130 days.

Experimental Diet

Prior to the experimental period the steers were maintained on a corn silage-based diet supplemented with soybean meal, vitamins and minerals. Two weeks were allotted to adjust the animals to the experimental diet (tables 3, 4, and 5). All steers were fed daily during the experiment at approximately 0900 h. Both feed and water were offered free choice.

Table 3. Composition of the Experimental Diet

	% of Diet DM
Corn, silage, well eared	70.8
Corn, grain, high moisture	18.7
Protein supplement	10.5

Table 4. Composition of the Protein Supplement

	% of Supplement DM
Soybean, seeds, meal solvent extracted 44% protein	89.10
Limestone, grnd.	5.90
Calcium phosphate dibasic, from deflourinated phosphoric acid	1.90
Trace mineral salt [a]	1.90
Vitamins A,D,E [b]	0.24
Selenium 200 [c]	0.77

[a] Zn= 0.35%, Mn= 0.20%, Fe= 0.20%, Cu= 0.030%, Co= 0.005%, I= 0.007%, NaCl= 97.0%

[b] A= 220 IU/kg, D= 264 IU/kg, E= 44 IU/kg

[c] Se= 0.15 mg/kg

Table 5. Nutrients in the Experimental Diet [a]

DM, % [b]	= 41.7
NEm, Mcal/kg	= 1.76
NEg, Mcal/kg	= 1.14
Crude protein, % [b]	= 11.00
ADF, % [b]	= 19.07
Ca, %	= 0.55
P, %	= 0.35
K, %	= 0.99
Salt, %	= 0.25

[a] Values are on a dry matter basis.

[b] Values are pooled averages from analyses of feed samples collected biweekly over 130 days.

Data Collection

Individual live weights were obtained prior to the morning feeding for each collection period (d 40 and 80 post-implantation). Two-day consecutive weights were taken at the beginning and end of the trial. Feed refusals were measured at the end of each collection period. Feed samples consisting of both total mixed ration and individual feed ingredients were collected every two weeks. All feeds were composited for each period and frozen at -20°C until analyzed for DM, crude protein (CP) and acid-detergent fiber (ADF) content (AOAC, 1965). Dry matter intakes were determined on an individual pen basis.

Statistical Analyses

The ADG data representative of periods 1 through 2 (d 40-80 post-implantation) and periods 2 through 3 (d 40-80 post-implantation) were analyzed by split-plot analysis of variance (Gill and Hafs, 1971), consisting of a 2^2 factorial main-plot, and a 2-sample period sub-plot (appendix A). The ADG data presented for the total implant period (period 1 through 3, d 0-80), and DMI and F/G for the entire experimental period, where analyzed by 2^2 factorial analysis of variance (appendix A), as described by Gill (1978). Collectively, all the feedlot data generated are least square means together with standard error of means (GLM procedure, SAS Institute Inc., 1985). Additionally, the effects of TBA, E2, and TBA+E2 were specifically compared to C, by Dunnetts-t analysis (Gill, 1978). For statistical

models with split-plot structure, error terms and critical values were modified as recommended by Gill (1986) in order to increase comparison sensitivity of treatments with few animals.

Metabolism Study

Animals, Design, Management

The same animals and diet (tables 3, 4 and 5) from the feedlot trial were used for this aspect of the study. The steers were fed ad libitum daily at 0900 h. Individual feed intakes and refusals were recorded daily, and water was available at all times.

Steers from each pen (feedlot trial) were randomly assigned to one of three metabolism groups (table 6). Each of these groups consisted of a total of 16 animals, with 4 steers representing one of four implant treatments, as mentioned previously. During collection periods, the steers were housed inside a metabolism room in 86 x 198 cm slotted-floor metabolism stalls. Prior to each collection the steers were adapted to the metabolism room for 5 days.

Collection Schedule

Presented in table 6 is a three period sampling schedule for each of the 48 steers. Period 1 is denoted as d 0 and represents the sampling time prior to implantation. Periods 2 and 3 are representative of collections obtained during d 30-39 and 71-79 post-implantation, respectively. Within

Table 6. Sampling Scheme for Metabolism Data Collection

Metabolism Group #	Action	Period 1	Period 2	Period 3
		-days relative to implantation-		
1	urea infusion	0	30	71
	blood collection		31	72
	urine collection		34-39	73-79
2	urea infusion	0	32	71
	urine collection		34-39	73-79
3	urea infusion	0	31	71
	urine collection		34-39	73-79

each period, body mass and urine excretion were monitored. Exact times for each experimental activity are listed in table 6. Only group 1 (table 6) was utilized to obtain blood samples during a 12 h collection for hormone analyses.

Implantation

After each metabolism group was initially sampled (per 1), the animals were confined, haltered and implanted. Before blocking, all animals were checked to ensure that they were not implanted prior to the experimental period. Implants (TBA and E2) were inserted in the middle one-third of the right and left ears, respectively. Implant retention was determined on d 14 and 80 for all treatments.

Urea Dilution Technique

Steers were weighed prior to feeding on two consecutive days before urea infusion (table 6). Live weights (LW) were averaged and the volume of urea infusate needed (20% urea w/v in 0.9% saline) to provide 130 mg urea/kg LW was calculated. Before the infusion the steers were restrained in a squeeze chute, haltered and fitted with a 30 cm length of 18 gauge polyvinyl tubing (SLV 105, ICO Rally, Palo Alto, CA). Duplicate blood samples were taken intially (T0) and 12 min (T12) after the midpoint of the infusion and were dispensed in 15 ml tubes treated with sodium heparin (Becton-Dickinson, Rutherford, NJ). At T0, each catheter was flushed with 10 ml of 3.5% sodium citrate and capped after urea infusion. Subsequently, approximately 8-10 ml of blood

were removed and discarded before obtaining a T12 sample. Blood samples were chilled immediately in ice and stored at 4°C until centrifuged at 2,000 x g for 30 min. Plasma was decanted and frozen at -20°C until analyzed. Plasma urea nitrogen concentrations were assayed (Crocker, 1967) by using Sigma procedure no. 535 (Sigma Diagnostics, St. Louis, MO). Urea space (US) was calculated by dividing the quantity of urea nitrogen infused by the difference in plasma urea nitrogen concentration before and after infusion.

Both empty body water (EBH2O) and protein (EBPROT) were calculated by using multiple regression equations, obtained from mixed-beef breeds, which including LW as an independant variable (Hammond et al., 1984, 1985).

$$EBH2O\ (kg) = 53.4 + 0.54\ US + 0.16\ LW$$

$$EBPROT\ (kg) = 5.0 + 0.14\ US + 0.072\ LW$$

Urine Collection

Urine was collected by placing steers in individual 85 x 142 cm collection crates. Total urine was collected for 3 consecutive days by employing a weak vacuum (2-3 cm Hg) on the bottom of a 66 x 66 cm plexiglass collector under the crate (modified, McCarthy, 1981). Approximately 100 ml of 50% H_2SO_4 was added in a 5 l collection container to maintain the urine at pH 2-3. The containers were emptied once daily and total volumes were recored. Ten percent aliquots were obtained each day and passed through 3 layers

of cheese-cloth, composited and frozen at -20°C. Approximately 100 ml of urine was filtered for specific analyses.

Urinary 3-Methylhistidine Analysis

One milliliter of urine was deproteinized in a 12x60 mm test tube containing 1 ml of 0.1 N hydrochloric acid. The HCl was previously spiked with a 0.5 mM/ml norleucine internal standard (Nle; Sigma Chemical Co., St. Louis Mo.). The solution was briefly vortexed and approximately 1 ml was transferred into an ultrafiltration device (Amicon Corp., Danvers, MA.), which was fitted with a Millipore membrane (10,000 NMWL) and centrifuged at 1500 x g for 20 min. Two external standards, (Waters Assoc., Milford MA; Pierce Chemical Co., Rockford IL) were prepared with an addition of a stock Nle solution (1.25 mM/ml). Twenty-five microliters of the filtered urine solution were transferred into 6x50 mm glass culture tubes (Thomas Scientific, Swedesboro, NJ), which were placed together with the external standards in a Millipore reaction vessel (Waters Assoc.). The samples were then subjected to vacuum on the Pico Tag work station (Waters Assoc.) until dry (< 65 millitorr). This procedure was repeated after rehydration of the sample with additions of a sodium acetate prederivatization solution, a phenylisothiocyanate reagent (precolumn PITC derivatization) and seqential washes of methanol and water. Final dilutions of the phenylthiocarbamyl amino acids were prepared by the addition of a Waters diluent (refer to appendix B for

detailed sample preparation). Samples were analyzed by reverse phase HPLC, utilizing a binary gradient system. The mobile phase (Pico Tag eluent #1 and #2, Waters Assoc.) flow rate was set at 1 ml/min and column (Pico Tag, Waters Assoc.) temperature was 45°C. A total of 10 ul of sample (refrigerated, 5°C) was injected by the Waters Intelligent Sample Processor (WISP). The absorbance detector (Waters Model 720) was set at both 245 nm and 0.1 absorbance units full scale (AUFS). 3-methylhistidine was eluted off the column at a retention time of 30 min post-injection (figure 1). Calculation of the unknown 3MH (pmol/1.25 ul sample) was accomplished by the Waters Data Module (Model 730). Basic computation of daily excreted 3MH (mM/d) is presented in appendix B.

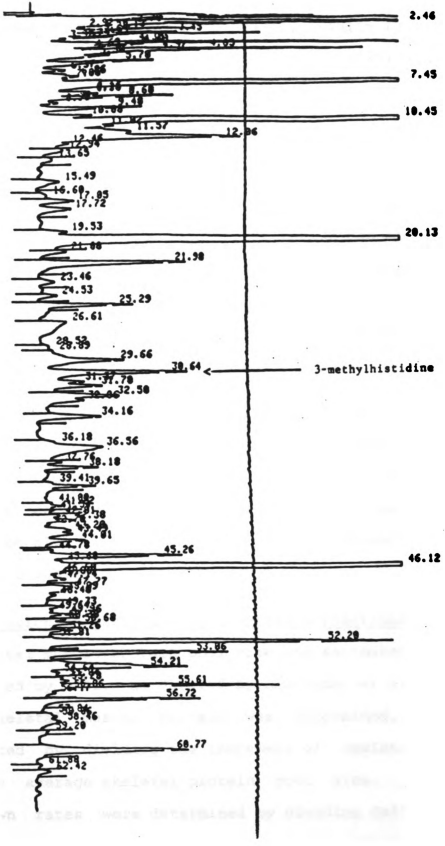
Creatinine Analysis

Creatinine concentrations were determined in urine samples (diluted 1:40) using Sigma Diagnostic procedure no. 555-A (Heinegard and Tiderstrom, 1973).

Growth Hormone, Insulin, and Cortisol Analyses

During periods 1, 2 and 3 steers from metabolism group 1 (n=16) were catheterized 2 days prior to sampling. Starting at 0700 h, blood samples were taken every 30 min for 12 h. Blood was stored at 20°C for approximately 6 h, and at 4°C overnight. The following day sera was obtained by centrifugation at 2000 x g for 30 min. Sera was decanted and stored at -20°C until analyzed for concentrations of

Figure 1. A reverse phase HPLC chromatograph of a steer urine sample. Presented are peaks of individual unknown amino acids and their respective retention times. 3MH is eluted from a C18 column by utilization of a binary gradient system at approximately 30 min after sample injection.


2.46
7.45
10.45
11.57
12.96
20.13
21.98
25.29
29.66
38.64
3-methylhistidine
32.58
34.16
36.56
39.65
45.26
46.12
52.28
54.21
55.61
56.72
60.77

growth hormone (Purchas et al., 1971), insulin (Villa-Godoy et al., 1988) and cortisol (Purchas et al., 1985).

Glucose

During the 12 h bleed for periods 1, 2 and 3, hourly blood samples were aliquoted into 5 ml potassium oxalate treated tubes (Becton-Dickinson) and chilled on ice. Chilled samples were immediately centrifuged for 30 min at 2000 x g. Plasma was then pipetted into plastic tubes and stored at -20^{o}C. Samples at 0700, 1100, 1300 and 1700 h were then assayed for glucose concentrations utilizing Sigma Diagnostic procedure no. 315 (Trinder, 1969).

17β-Trenbolone and 17β-Estradiol

Three sera samples at 1200, 1400 and 1600 h, during periods 2 and 3, were assayed for both 17β-trenbolone (Henricks et al., 1982) and 17β-estradiol concentrations (Breuel et al., 1988), courtesy of Henricks and co-workers (Clemson University, Clemson, SC).

Calculation of Skeletal Muscle Protein Fractional Rates

Skeletal muscle protein pool size was estimated as EBPROT multiplied by 0.52, as derived by Schroeder et al.,(1987a). Once skeletal muscle protein was determined, FAR was calculated by dividing the increment of skeletal protein gain by average skeletal protein pool size. Fractional breakdown rates were determined by dividing daily urinary 3MH excretion (adjusted for non-skeletal muscle sources,

Afting et al., 1981) by the concentration of the mixed skeletal muscle 3MH pool (Nishizawa et al., 1979). Fractional synthesis rates were then estimated by adding FAR plus FBR.

Statistical Analyses

All metabolism data presented on a whole period basis, (i.e. per 1-3), were analyzed by 2^2 factorial analysis of variance (appendix A), as suggested by Gill (1978). Whereas, data represented as multiple periods were analyzed by split-plot analysis of variance (Gill and Hafs, 1971), with a 2-factor main plot and treatment periods as subplots (appendix A). Effects of specific interactions between C, TBA, E2, and TBA+E2 on all the metabolism data were determined by contrasting treatment combinations with a Bonferroni-t test. This additional group of comparisons were included to further test for additive effects that may have been elicited by the dual implant treatment. The specific Bonferroni contrasts performed were C vs E2 and TBA, and TBA+E2 vs E2 and TBA. For models with split-plot structure, both the error terms and critical values utilized for Bonferroni-t analysis were modified to increase comparison sensitivity of treatments with few animal numbers (Gill, 1986). Empty body water, EBPROT and skeletal protein that had a significant treatment x period interaction, were compared by Bonferroni-t analysis, utilizing comparisons of treatment trends (Gill, 1986) between periods 1 and 3. All data presented in tables and graphs are least square means

with standard errors of the mean, as determined by GLM procedure (SAS Institute, 1985). Additionally, serum mean concentration, baseline concentration, pulse frequency, pulse amplitude and duration of the amplitude of both GH and INS were indentified by the Pulsar program, which uses alogarithms for pattern recognition (Merriam and Wachter, 1982).

RESULTS

Feedlot Study

Steer Live-Weight Gain. Prior to implantation, the average initial live body weight among all steers was 319 kg. Subsequently, steer weights increased to 355, 362, 377 and 387 kg, and 404, 414, 428 and 452 kg, 40 and 80 d after implantation for C, TBA, E2, and TBA+E2 treatments, respectively. Corresponding ADG (table 7) demonstrated that E2 and TBA+E2 administration increased the rate of steer gain 34 and 55% ($P<.01$) during the first 40 d, as compared to C. However, only TBA+E2 treatment maintained an increased weight gain response ($P<.01$) during the remainder of the experimental period (40-80 d). Overall, the ADG for C, TBA, E2 and TBA+E2 treated animals, for the entire implant period, averaged 1.09, 1.19, 1.32 and 1.56 kg/d, respectively. During this time interim only E2 ($P<.05$) and TBA+E2 ($P<.01$) significantly stimulating growth rate, when compared to C steers. Additionally, further factorial analysis revealed that both E2 ($P<.0001$) and TBA ($P<.01$) implantation, dependently stimulated the rate of steer gain, during the entire 80 d implant period.

Feed Intake and Efficiency. Both steer DMI and F/G values (table 8) are presented on a pen basis, allowing the pen to represent the experimental unit. Due to pen limitations, F/G and DMI were only obtained for the complete 130 d trial,

Table 7. Effect of TBA, E2 and Combined Treatments on Steer Average Daily Gain.[a]

Trt	n	Days 0-40 [b]	Days 40-80 [c]	Days 0-80 [d]
		kg/d	kg/d	kg/d
C	10	0.97 [e]	1.22 [e]	1.09 [e]
TBA	11	1.10 [f]	1.28	1.19 [g]
E2	11	1.30 [f]	1.35 [f]	1.32 [f]
TBA+E2	11	1.50	1.60	1.56
SEM		0.06	0.08	0.06

[a] Statistical comparisons presented were generated from Dunnett's-t analysis:
Values with e vs f superscripts within columns differ (P<.01).
Values with e vs g superscripts within columns differ (P<.05).

[b] E2 (P<.0001) and TBA (P<.01) treatment increased steer ADG during days 0-40.

[c] E2 treatment increased steer ADG (P<.01) during days 40-80.

[d] E2 (P<.0001) and TBA (P<.01) administration increased steer ADG over the entire treatment period.

Table 8. Effect of TBA, E2 and Combined Treatments on Steer Dry Matter Intake and Feed Efficiency [a]

Trt	n [b]	DMI(kg) [c]	F/G [d]
C	2	7.49	7.47 [e]
TBA	2	7.33	7.02
E2	2	7.55	6.79
TBA+E2	2	7.95	6.14 [f]

[a] Statistical comparisons presented were generated from Dunnett's-t analysis:
Values with e vs f superscripts within columns differ (P<.01).

[b] Pen (n=2) is representative of the experimental unit.

[c] E2 treatment increased steer DMI (P<.03).

[d] E2 treatment decreased steer F/G (P<.10).

which includes both combined pre-implant and implant administered periods. Overall, the average daily steer DMI for the total experimental period was 7.6 kg. Although specific treatment comparisons to C did not demonstrate any differences (P>.10) among implant treatments, additional factorial analysis of variance showed that E2 treatments collectively tended to increase feed intake (7.4 vs 7.8 kg/d; P<.03).

Feed efficiency, expressed as kg of DMI/LBW gain are presented in table 8. Although numerical trends are evident in the sequential order of C, TBA, E2 and TBA+E2 respectively, only the TBA+E2 treatment differed (P<.01) when specifically compared to C steers. Furthermore, E2 (P<.10) treatment did tend to increase feed efficiency, as compared to steers treated with TBA (P>.10).

Metabolism Study

Empty Body Water. Steer EBH2O values (table 9) were not found to be different (P>.05) among treatments during the pretreatment period (179.2 $\pm$ 4.6 kg). Empty body water did increase over time, which was indicative by a general period effect (P<.001). Also, a period x treatment interaction exsisted between E2 (P<.0002), whereas, only a period x TBA trend, over the experimental period, was evident (P>.10). Furthermore, both E2 and TBA administration increased overall steer EBH2O content during both periods 2 (P<.01 and P<.04) and 3 (P<.0008 and P<.04). Upon further examination

of the data, specific Bonferroni comparisons of treatment time trends (between periods 1 through 3) showed that E2 increased EBH2O, as compared to C ($P<.05$), whereas, TBA did not exhibit any effect ($P>.05$) on EBH2O content. Additionally, single E2 ($P<.05$) and TBA ($P<.01$) treatments from periods 1 through 3, did not successfully, increase EBH2O content, as compared to TBA+E2 (43.8 kg and 32.6 kg vs 56.5 kg, respectively).

Empty Body Protein and Skeletal Muscle Protein. These results (tables 10 and 11) are discussed together because skeletal muscle protein (skl prot) was calculated from EBPROT values by utilization of a constant conversion factor, thus any statistical relevance within any one set of results should pertain to those of the other. Empty body protein and skl prot values between treatments, within period 1 were not different ($P>.10$), and averaged 51.6 $\pm$ 1.4 kg and 26.8 $\pm$ .76 kg. Both groups demonstrated a time x period effect ($P<.0001$), a period x E2 interaction ($P<.0001$), and period x TBA trend ($P<.10$). Treatments containing E2 and TBA significantly increased both EBPROT and skl prot during periods 2 ($P<.03$ and $P<.09$) and 3 ($P<.0008$ and $P<.05$). A change in treatment differences from periods 1 through 3 were found between E2 and C ($P<.05$). Additionally, TBA ($P<.01$) and E2 ($P<.05$) treatments did not increase either EBPROT or skl prot, as compared to TBA+E2.

Table 9. Effect of TBA, E2 and Combined Treatments on Empty Body Water in Steers.[a,b]

Trt	n	Period 1	Period 2 [c]	Period 3 [c]
		kg		
C	10	177.87	196.84	209.84
TBA	11	180.33	200.96	212.96
E2	11	177.12	203.94	220.94 [d]
TBA+E2	11	181.64	223.18	238.18 [e]
SEM		4.65	5.75	4.97

[a] Statistical contrasts presented are Bonferroni time trends between periods 1 vs 3:

[d] E2 treatment increased steer EBH2O from periods 1 to 3, as compared to C ($P<.05$).

[e] TBA+E2 treatment increased steer EBH2O from periods 1 to 3, as compared to TBA ($P<.01$) and E2 ($P<.05$).

[b] Split-plot analysis demonstrated an E2, and TBA x period interaction ($P<.0002$ and $P<.10$, respectively).

[c] E2 and TBA treatments increased EBH2O during both periods 2 ($P<.01$ and $P<.04$) and 3 ($P<.0008$ and $P<.04$), respectively.

Table 10. Effect of TBA, E2 and Combined Treatments on Empty Body Protein in Steers.[a,b]

Trt	n	Period 1	Period 2 [c]	Period 3 [c]
		kg	kg	kg
C	10	51.14	58.23	62.21
TBA	11	51.94	58.94	63.52
E2	11	51.18	59.90	65.99 [d]
TBA+E2	11	52.42	65.39	70.90 [e]
SEM		1.43	1.82	1.53

[a] Statistical contrasts presented are Bonferroni time trends between periods 1 vs 3:

[d] E2 treatment increased steer EBPROT from periods 1 to 3, as compared to C ($P<.05$).

[e] TBA+E2 treatment increased steer EBPROT from periods 1 to 3, as compared to TBA ($P<.01$) and E2 ($P<.05$).

[b] Split-plot analysis demonstrated an E2 and TBA x period interaction ($P<..0001$ and $P<.10$, respectively).

[c] Main effects of E2 and TBA on EBPROT were demonstrated during periods 2 ($P<.03$ and $P<.09$) and 3 ($P<.0008$ and $P<.05$), respectively.

Table 11. Effect of TBA, E2 and Combined Treatments on Skeletal Muscle Protein in Steers.[a,b]

Trt	n	Period 1	Period 2 [c]	Period 3 [c]
		kg	kg	kg
C	10	26.59	30.28	32.34
TBA	11	27.00	30.64	33.02
E2	11	26.61	31.14	34.31 [d]
TBA+E2	11	27.26	33.99	36.86 [e]
SEM		0.76	0.95	0.80

[a] Statistical contrasts presented are Bonferroni time trends between periods 1 and 3:

[d] E2 treatment increased steer skeletal muscle protein from periods 1 to 3, as compared to C ($P<.05$).

[e] TBA+E2 treatment increased steer skeletal muscle protein from periods 1 to 3, as compared to TBA ($P<.01$) and E2 ($P<.05$).

[b] Split-plot analysis demonstrated an E2, and TBA x period effect ($P<.0001$ and $P<.10$, respectively).

[c] Main effects of E2 and TBA on muscle protein were demonstrated during periods 2 ($P<.03$ and $P<.09$) and 3 ($P<.0008$ and $P<.05$), respectively.

Daily Skeletal Muscle Protein Accretion. As presented in table 12, daily skeletal muscle protein accretion increased during both period intervals 1-2 ($P<.008$ and $P<.08$) and 1-3 ($P<.0001$ and $P<.06$), as a result of implanting steers with both E2 and TBA. In contrast to these periods, only an E2 treatment trend ($P<.10$) towards increasing skl prot deposition was observed during period interval 2-3. Overall, the average daily protein deposited for the entire treatment period was 60, 70, 93 and 120 g for the C, TBA, E2 and TBA+E2 groups, respectively. Futhermore, specific Bonferroni contrasts revealed that TBA+E2 enhanced steer muscle protein depostion by 28% ($P<.05$) and 72% ($P<.01$), as compared to E2 and TBA treated animals during the total 80 d implant period. Similarly, during period interval 1-2, TBA+E2 improved muscle deposition over both E2 ($P<.05$) and TBA ($P<.01$) treatments, however, this response was not maintained through period interval 2-3.

Skeletal Muscle Protein Fractional Accretion Rates Similarly to the muscle protein deposition values presented in table 12, steer skeletal muscle FAR (table 13), during both period intervals 1-2 and 1-3, were greater after treatment with TBA+E2 vs E2 ($P<.05$) or TBA ($P<.01$). These differences, however, were not observed amongst the treatment groups ($P>.10$) through the next treatment interval (2-3). Average rates calculated for periods 1-3 indicate that TBA+E2 increased FAR approximately 60% ($P>.01$), as compared to the single TBA treatment, whereas, dual

Table 12. Effect of TBA, E2 and Combined Treatments on Average Daily Skeletal Muscle Protein Deposited in Steers.[a]

Trt	n	Period Intervals 1-2[b]	2-3[c]	1-3[d]
		g/d		
C	10	82.29	51.77	65.09
TBA	11	83.00	59.57	69.80
E2	11	111.48	79.23	93.31
TBA+E2	11	182.01[e]	71.65	119.84[f]
SEM		20.05	12.03	8.04

[a] Statistical comparisons presented were generated from Bonferroni-t analysis:

[e] TBA+E2 treatment increased skeletal muscle protein deposition, as compared to both E2 ($P<.05$) and TBA ($P<.01$) during period interval 1-2.

[f] TBA+E2 treatment increased skeletal muscle protein deposition, as compared to both E2 ($P<.05$) and TBA ($P<.01$) during the entire treatment period.

[b] Main effects of E2 ($P<.002$) and TBA ($P<.08$) were demonstrated on muscle protein deposition during time interval 1-2.

[c] Main effect of E2 ($P<.10$) was demonstrated on muscle protein deposition during time interval 2-3.

[d] E2 ($P<.0001$) and TBA ($P<.06$) increased total muscle protein deposited over the entire treatment period.

Table 13. Effect of TBA, E2 and Combined Treatments on Skeletal Muscle Protein Fractional Accretion Rates in Steers.[a,b]

Trt	n	Period Intervals 1-2 [c]	2-3 [c]	1-3 [d]
		%/d		
C	10	0.386	0.170	0.218
TBA	11	0.397	0.194	0.231
E2	11	0.499	0.239	0.301
TBA+E2	11	0.689 [e]	0.206	0.367 [f]
SEM		0.060	0.037	0.026

[a] Statistical comparisons presented were generated from Bonferroni-t analysis:

[e] TBA+E2 treatment increased steer FAR, as compared to both E2 ($P<.05$) and TBA ($P<.01$) during period interval 1-2.

[f] TBA+E2 treatment increased steer FAR, as compare to TBA ($P<.01$) during the entire treatment period.

[b] Split-plot analysis demonstrated a period x E2 interaction ($P<.05$).

[c] E2 increased FAR during both period intervals 1-2 ($P<.001$) and 2-3 ($P<.10$).

[d] E2 treatment increased FAR over the entire treatment period ($P<.0001$).

implantation (TBA+E2) caused a numerical increased in steer FAR by 21.9%, as compared to E2 treatment.

Skeletal Muscle Fractional Breakdown Rates. As observed in table 14 all of the experimental treatments seemed to cause a transient decline in skeletal muscle FBR during the initial implantation period (per.2). Skeletal muscle FBR among all treatment groups during the entire experimental period, however, did not differ (P>.10) and averaged 1.39 %/d.

Skeletal Muscle Protein Synthesis Rates. Table 15 demonstrates a treatment trend, which suggests that TBA, E2 and TBA+E2 treatments sequentially increase skeletal muscle FSR. Additionally, this group of data also provides information that TBA+E2 numerically increases FSR approximately, 10 and 5% beyond those rates exhibited from both single TBA and E2 implant treatments. Collectively, E2 treatment tended to increase FSR during period interval 1-2 (P<.09), however, this response was not maintained through period 2-3. In contrast to period interval 2-3, E2 did successfully increase total muscle protein FSR (P<.06) through the entire treatment period.

Urinary 3-Methylhistidine Excretion. Absolute and relative steer urinary 3MH excretion concentrations are represented in tables 16 and 17. Treatment values for 3MH (m moles/d), increased between all treatments over the three consecutive sampling periods (P<.001). Additionally, an E2 x period trend exsisted (P<.10) during the entire treatment period.

Table 14. Effect of TBA, E2 and Combined Treatments on Skeletal Muscle Protein Fractional Breakdown Rates in Steers.

		Period			
Trt	n	1	2	3	Avg 2&3 [a]
		%/d			
C	10	1.34	1.36	1.45	1.40
TBA	11	1.37	1.33	1.42	1.38
E2	11	1.37	1.31	1.46	1.39
TBA+E2	11	1.37	1.28	1.53	1.40
SEM		0.10	0.07	0.06	0.05

[a] Values averaged over periods 2-3 are representative of the entire implant treatment period.

Table 15. Effect of TBA, E2 and Combined Treatments on Skeletal Muscle Protein Fractional Synthesis Rates in Steers.

		-------- Period Interval -------		
Trt	n	1-2 [a]	2-3	1-3 [b]
		---------- %/d ----------		
C	10	1.75	1.62	1.62
TBA	11	1.73	1.62	1.61
E2	11	1.82	1.70	1.69
TBA+E2	11	1.98	1.73	1.77
SEM		0.09	0.07	0.06

[a] E2 treatment increased FSR during period interval 1-2 ($P<.09$).

[b] E2 treatment increased FSR over the entire treatment period ($P<.06$).

Table 16. Effect of Implant Treatments on Steer[a] Absolute Urinary 3-Methylhistidine Excretion.

		Period		
Trt	n	1	2	3
		m moles/d		
C	10	1.44	1.67	1.91
TBA	11	1.51	1.64	1.91
E2	11	1.40	1.66	2.03
TBA+E2	11	1.51	1.76	2.28[b]
SEM		0.12	0.09	0.09

[a] Statistical comparisons were generated from Bonferroni-t analysis:

[b] TBA+E2 treatment increased urinary 3MH excretion, as compared to TBA ($P<.10$) during period 3.

Table 17. Effect of Implant Treatments on Steer Relative Urinary 3-Methylhistidine Excretion.

		Period		
Trt	n	1	2	3
		m moles/kg LBW		
C	10	4.81	4.74	4.84
TBA	11	4.87	4.55	4.72
E2	11	4.52	4.51	4.87
TBA+E2	11	4.87	4.62	5.23
SEM		0.35	0.25	0.09

After performing interperiod contrasts, no differences (P>.10) were found among any of the the treatment combinations, however, analysis within period 3 demonstrated that the TBA+E2 treated animals excreted more 3MH/d compared to TBA treated animals (P<.10). In contrast to the absolute 3MH excretion data, values presented on a live weight basis (table 17) did not exhibit any treatment or period effects.

Urinary Creatinine Excretion. Presented in tables 18 and 19 are absolute and relative urinary creatinine concentrations. Relative creatinine concentrations (table 19) show that E2 treatment increases (P<.03) overall steer urinary creatinine concentration during experimental period 3. Absolute creatinine values (table 18) demonstrate both a general period effect, and a period x E2 interaction (P<.004). Furthermore, initial values of absolute creatinine concentration (table 18) during period 1, were not different (P>.10) and averaged 8.40 g/d among treatments. A definite treatment trend during both periods 2 and 3, suggests that the concentration of urinary creatinine increases in the treatment order of C, TBA, E2 and TBA+E2, respectively. Within period 2 (P<.10) and 3 (P<.001) collective E2 treatment increased urinary creatinine excretion. Furthermore, during period 3, TBA+E2, as compared to TBA showed to increase urinary creatinine concentration by 21% (P<.01).

3-Methylhistidine:Creatinine Ratios. These ratios are presented in table 20. Overall, no relative differences

Table 18. Effect of Implant Treatments on Steer Absolute Urinary Creatinine Excretion.[a,b]

Trt	n	Period 1	Period 2[c]	Period 3[c]
		g/d		
C	10	8.42	8.29	9.70
TBA	11	8.26	8.77	9.41
E2	11	8.48	8.98	11.12
TBA+E2	11	8.47	9.58	11.66[d]
SEM		0.47	0.47	0.51

[a] Statistical comparisons presented were generated from Bonferroni-t analysis:

[d] TBA+E2 treatment increased urinary creatinine excretion, as compared to TBA ($P<.01$) during period 3.

[b] Split-plot analysis demonstrated an E2 x period interaction ($P<.004$).

[c] E2 increased urinary creatinine concentration during both periods 2 ($P<.10$) and 3 ($P<.001$).

Table 19. Effect of Implant Treatments on Steer Relative Urinary Creatinine Concentration.[a]

Trt	n	Period 1	Period 2	Period 3[b]
		mg/kg LBW		
C	10	28.11	23.41	24.67
TBA	11	27.32	24.21	23.33
E2	11	27.39	24.37	26.45
TBA+E2	11	27.20	25.06	26.66
SEM		1.63	0.99	1.13

[a] Split-plot analysis demonstrated an E2 x period interaction (P<.10).

[b] E2 treatment increased relative urinary creatinine excretion during period 3 (P<.03).

Table 20. Effect of Implant Treatments on Steer 3-Methylhistidine-to-Creatinine Ratios.

		Period		
Trt	n	1	2	3
C	10	.0289	.0343	.0337
TBA	11	.0333	.0321	.0349
E2	11	.0297	.0317	.0314
TBA+E2	11	.0309	.0317	.0333
SEM		.0040	.0020	.0020

between treatment combinations were identified upon statistical analyses.

Serum 17 beta- Estradiol and Trenbolone Concentrations. Serum E2 and TBA concentrations during periods 2 and 3 are shown in table 21. During period 2, E2 treatment in steers caused an increase in circulating E2 concentration of approximately 5 pg/ml ($P<.04$), whereas, TBA implantation increased sera TB concentrations to 652 and 1672 pg/ml in both the TBA and TBA+E2 implanted steers ($P<.03$). Similarly, within period 3, TBA maintained serum TB levels ($P<.0001$), which averaged approximately 908 pg/ml between the two TBA treatments. In contrast to TBA during period 3, E2 concentrations declined, averaging 8.2 vs 6.9 pg/ml ($P<.02$) between the E2 and non-E2 implanted steers.

Growth Hormone. Treatment effects on steer GH concentration over the entire experimental period are graphically represented in figure 2. Within period 1, GH concentration among treatments did not differ and averaged 4.6 $\pm$ 0.47 ng/ml. Upon implantation, TBA treatments, as compared to the controls, had similar GH concentrations of 3.54 vs 3.99 ng/ml, respectively. In contrast to the TBA treatments, E2 during both periods 2 and 3, showed to increase circulating GH concentration. Within period 2, E2 increased GH 46% ($P<.10$), as compared to TBA+E2. In addition, E2 produced a 29% and 44% increase in serum GH, as compared to both C and TBA. Overall, during period 2 TBA treatment decreased steer serum GH concentration (3.54 vs

Table 21. Steer Serum Concentrations (pg/ml) of both 17 beta-Estradiol and Trenbolone (TB) after Implant Administration.[a]

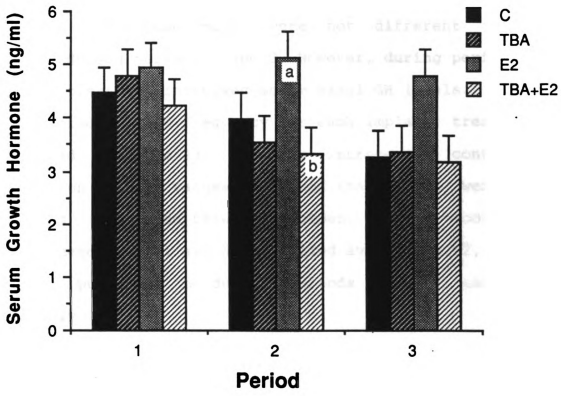
		- Period 2 -		- Period 3 -	
Trt	n	E2 [b]	TB [c]	E2 [b]	TB [c]
C	4	8.9	0	6.5	7
TBA	4	10.2	652	7.0	709
E2	4	14.2	0	7.7	6
TBA+E2	4	15.4	1672	8.8	1108
SEM		2.3	461	0.5	125

[a] Values are pooled means determined from samples obtained at 1200, 1400 and 1600 h during a total 12 h blood collection.

[b] E2 implantation increased serum E2 concentration during periods 2 ($P<.04$) and 3 ($P<.02$).

[c] TBA implantation increased serum TB concentration during periods 2 ($P<.03$) and 3 ($P<.0001$).

Figure 2. Steer Serum Growth Hormone Concentrations. Data are least-squares means $\pm$ SEM; n=4 for values representative of each treatment. Statistical comparisons presented were generated from Bonferroni-t analysis: During period 2 TBA+E2 treated steers tended to demonstrated lower mean serum GH concentrations ($P<.10$). Overall, TBA treatment decreased serum GH concentration during period 2 (3.54 vs 4.56 ng/ml; $P<.06$).



a,b superscripts within period differ (P<.10)

4.56 ng/ml; P<.06). Furthermore, the E2 effect on GH concentration was maintained throughout period 3, exhibiting numerical increases of 50, 42 and 46% over TBA+E2, TBA and C treatments, respectively.

Serum GH basal means are presented in table 22. Comparisons of these means were not different among treatments during periods 1 and 3. However, during period 2, overall E2 treatment increased serum basal GH levels.

Growth hormone pulse frequency for each implant treatment is reported in table 23. Specific intraperiod contrasts performed on these values indicated that there were no differences associated between treatment groups. Pooled GH pulse frequencies that are demonstrated averaged 2.12, 2.06 and 1.87 pulses/12 h during periods 1, 2 and 3, respectively.

Growth hormone pulse amplitudes are shown in table 24. Amplitudes measured during period 1 were not different among treatments (P>.10) and averaged 8.6 ng/ml. After implantation, however, TBA treatment depressed overall GH pulse amplitude during both periods 2 (P<.03) and 3 (P<.02). Additionally, during periods 2 and 3, TBA+E2 treatment depressed steer GH pulse amplitude (P<.05), as compared to E2 implant treatment.

Growth hormone pulse duration is presented in table 25. Both intraperiod and interperiod comparisons did not indicate any treatment differences (P>.10), except for during period 3, where collective TBA treatment decreased GH

Table 22. Effect of Implant Treatments on Serum Growth Hormone Basal Mean Concentration in Steers.

		Period		
Trt	n	1	2 [a]	3
		ng/ml		
C	4	2.43	1.95	2.00
TBA	4	2.69	1.89	2.68
E2	4	2.86	2.54	1.88
TBA+E2	4	2.74	2.55	2.52
SEM		0.38	0.31	0.40

[a] E2 treatment increased serum GH basal mean concentration during period 2 ($P<.06$).

Table 23. Effect of Implant Treatments on Growth Hormone Pulse Frequency in Steers .

		Period		
Trt	n	1	2	3
		pulses/12 h		
C	4	2.25	2.50	1.75
TBA	4	2.25	1.75	1.25
E2	4	2.00	2.00	3.00
TBA+E2	4	2.00	2.00	1.50
SEM		0.44	0.53	0.62

Table 24. Effect of Implant Treatments on Growth Hormone Pulse Amplitude in Steers.[a]

Trt	n	Period 1	Period 2[b]	Period 3[b]
		ng/ml		
C	4	9.61	10.13	6.18
TBA	4	8.62	6.91	2.79
E2	4	9.70	12.66	10.38
TBA+E2	4	6.57	3.71[c]	3.11[c]
SEM		1.84	2.43	1.97

[a] Statistical comparisons presented were generated from Bonferroni-t analysis:

[c] TBA+E2 treatment decreased serum GH pulse amplitude, as compared to E2 ($P<.05$) during both periods 2 and 3.

[b] TBA decreased GH pulse amplitude during periods 2 ($P<.03$) and 3 ($P<.02$).

Table 25. Effect of Implant Treatments on Growth Hormone Pulse Duration in Steers.

		Period		
Trt	n	1	2	3 [a]
		min/pulse		
C	4	142.50	141.25	118.75
TBA	4	156.25	138.75	61.25
E2	4	143.75	250.00	156.25
TBA+E2	4	105.00	116.25	78.75
SEM		30.98	54.32	34.01

[a] TBA treatment decreased GH pulse duration during period 3 ($P<.07$).

pulse duration (P<.07), as compared to non-TBA treatments. Similarly to the pulse amplitude data (table 24), GH duration seems to be enhanced by E2, as compared to C, TBA, and TBA+E2 during both periods 2 and 3. This particular trend is also found in table 25, where E2 treatment enhanced GH duration 115% and 100% over the dual implant group (TBA+E2), during periods 2 and 3, respectively.

Insulin. Mean INS concentrations during the pre-implant and implant administered treatment periods are shown in figure 3. Although actual statistical analyses did not reveal any treatment differences, some treatment trends are prevalent (figure 3) and should be noted. After the initial implantation of the steers, E2 seemed to enhance serum INS concentration, whereas, both TBA and TBA+E2 actually depressed serum INS concentration. Overall, C serum INS levels rose consistantly throughout all 3 experimental periods. Additionally, all treatment INS values converged together during period 3 and averaged approximately .831 $\pm$ .115 ng/ml among all treatments.

Data presented in table 26 are pooled INS parameters. Overall, values consisting of INS pulse frequency, pulse amplitude, and duration of pulse amplitude, increased consistently over time.

Cortisol Concentration. Cortisol data are graphically depicted in figure 4. Prior to implantation, treatment values did not differ (P>.10) and averaged approximately 3.5 $\pm$ .42 ng/ml. Within period 2, E2 increased CT

Figure 3. Steer Serum Insulin Concentrations. Data are least-squares means ± SEM; n=4 for values representative of each treatment.

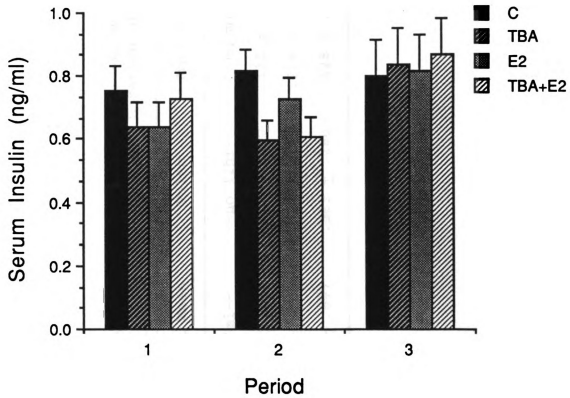
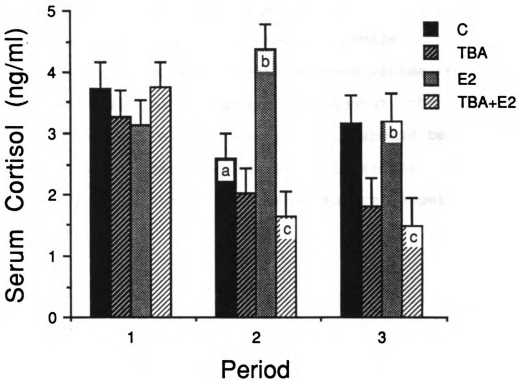

Serum Insulin (ng/ml)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
Period
C
TBA
E2
TBA+E2

Table 26. Pooled Treatment Means and Standard Errors of the Means for Serum Insulin Means, Basal Means, Pulse, Amplitude and Duration of Amplitude Over 3 Periods

Period	Mean	Basal Mean	Pulse	Amplitude	Duration
	--ng/ml--	--ng/ml--	--no/12hr--	--ng/ml--	--min/pulse--
1	.689 ± .079	.677 ± .077	.250 ± .250	.116 ± .117	31.87 ± 32.48
2	.687 ± .067	.652 ± .059	.500 ± .338	.224 ± .148	33.75 ± 23.52
3	.832 ± .115	.784 ± .097	.563 ± .260	.428 ± .292	45.00 ± 26.52

Figure 4. Steer Serum Cortisol Concentrations. Data are least-squares means $\pm$ SEM; n=4 for values representative of each treatment. Statistical comparisons presented were generated from Bonferroni-t analysis: E2 increased serum cortisol concentration, compared to C ($P<.05$) and TBA+E2 ($P<.01$) during period 2. Similarly, TBA+E2 treatment demonstrated lower circulating cortisol levels ($P<.01$) than E2 during period 3. Overall, collective TBA treatments depressed steer serum cortisol concentration during both periods 2 ($P<.001$) and 3 ($P<.005$).



a,b superscripts within period differ (P<.05)
b,c superscripts within period differ (P<.01)

concentration, as compared to C ($P<.05$) and TBA+E2 ($P<.01$). During period 3, however, E2 concentrations returned back towards C levels, but still remained greater than those demonstrated by steers implanted with TBA+E2 ($P<.01$). A common occurance exhibited by steers treated with TBA was a consistant decline in circulating CT concentrations. This effect was elicited during both periods 2 ($P<.001$) and 3 ($P<.005$), as compared to non-TBA treated animals.

Plasma Glucose. Implant effects on mean plasma glucose concentrations were not observed throughout the varied treatment periods (table 27). However, it should be noted that a prevalent decline in all treatment glucose concentrations, was shown over the entire experimental period ($P<.001$).

Table 27. Effect of Implant Treatments on Plasma Glucose Concentrations in Steers.[a,b]

		Period		
Trt	n	1	2	3
		mg/dl		
C	4	85.52	86.84	81.83
TBA	4	89.22	86.73	81.85
E2	4	90.67	88.56	85.79
TBA+E2	4	90.65	87.67	83.59
SEM		2.34	2.19	2.88

[a] Values presented are pooled means from blood samples obtained at 0700, 1100, 1300 and 1700h during a 12 h blood collection.

[b] Glucose values declined with respect to time ($P<.001$).

DISCUSSION

This present study has demonstrated that both estrogenic, and estrogenic plus androgenic implant treatments, stimulated steer growth. Associated with enhanced rate of gain was an increase in skeletal muscle protein accretion. This particular response seems to be mediated by increasing skeletal muscle FSR, since skeletal muscle FBR was not changed after either E2 or TBA administration. In general, this response, demonstrated in skeletal muscle protein turnover, seems to be attributed to alterations in various endogenous blood hormone concentrations, which are changed dependently on the type of anabolic implant treatment that is administered to steers.

As noted by the unbalanced treatment replication in the data presented, four animals were removed from the data-base for being significant aberrant observations (Gill, 1978). Data presented on a pen basis, (DMI and F/G, table 8) were adjusted by employing correction factors formulated from individual feed intakes (obtained during the metabolism phase of the study) from animals that were removed from the experiment. Final animal numbers per treatment were 10, 11, 11, and 11 for C, TBA, E2 and TBA+E2 treatments, respectively.

Gain Performance. Only steers treated with E2 and TBA+E2 demonstrated a significant increase in growth performance, as compared to C. The overall effect of TBA, E2 and TBA+E2

implants during the entire treatment period towards increasing rate of gain was 9, 21 and 43% (table 7) while improving F/G by 6, 9 and 18%, respectively (table 8). Comparable results in steers have been reported by a number of different researchers. Bouffault and Willemart (1983) indicated that TBA alone gave significant improvements in both ADG and F/G, however, these results were less than those obtained from single E2 administration, which were still markedly less than the increases produced by TBA+E2. Heitzman et al. (1977b) showed an increased gain response of 14 and 42% in steers treated with TBA and TBA+E2. Similar results were presented by Roche and co-workers (1978) where Finaplix (TBA) implantation of steers increased their growth rates 14%, as compared to C. Subsequently, Roche and Quirke (1986) reported a predominant increase in ADG by 20% in yearling steers given E2 once, in conjunction with 3 consecutive treatments of TBA. Wagner (1983), after summarized numerous feeding trials conducted with E2 in both the United Kingdom and Latin America, concluded that Compudose (E2) yielded a 15% increase in cattle rate of gain together with an additional improvement of approximately 8% in F/G. Additionally, Wellington (1985) reported a 21% increase in steer gain, while being maintained over a 200 d pasture trial, after 24 mg E2 implantation. A trial which most closely resembled the feedlot aspect of this present study was performed by Heitzman et al. (1981) who compared sham-implanted steers to 20 mg E2, 140 mg TBA, 140 mg TBA

plus 20 mg E2 (TBA+E2) and combined 140 mg TBA and 20 mg E2 (TBA*E2) implanted steers. Weight gain and feed intake were measured individually over two consecutive sampling periods (0-35 and 0-98 d). During the first 35 d, implant gains increased for E2, TBA, TBA+E2 and TBA*E2 by 44, 26, 87 and 62%, respectively, however, over the entire 90 d steer performance declined to 11, 12, 24 and 45%, over C, for the respective treatments mentioned above. Heitzman et al. (1981) reported that TBA, E2 and TBA+E2 decreased F/G 9, 6 and 13%, respectively. Collectively, these results are in agreement with this present experiment where TBA, E2, and TBA+E2 decreased F/G by 6, 9, and 18%, respectively.

Although results previously presented are repesentative of different breed types, diets, feeding regimes and enviromental conditions, they are quite comparable to the results obtained from this present experiment, especially with regard to the sequential order of the gain performance previously reported by Bouffault and Willemart (1983).

Empty Body Composition. Lack of methodology allowing accurate and precise measurement of the composition of gain in live animals has effectively limited collection of information pertinent to the understanding of the efficiency of growth. Terminal composition measurements following slaughter by grinding and chemical analyses of the entire animal is considered the most accurate measure of body composition (Jesse et al., 1976). This technique, however, inhibits further measure of individual growth. In large

domestic animals, indirect methods utilizing serial determination of body composition, have proven more suitable towards adequately assessing growth processes.

Because the percentages of ash, water and protein approach consistency in the fat-free body, composition of the entire animals can be estimated if either fat or water content can be determined (Reid et al., 1955). Preston and Kock (1973) reported that urea could be infused in cattle, and provide as a suitable marker for estimating bovine EBH2O. Results presented by these researchers were highly correlated to estimates obtained from carcass specific gravity (Preston and Kock, 1973; Kock and Preston, 1979) and coefficients of rib soft tissue (Kock and Preston, 1979). Hammond et al. (1984) compared US with direct measurements of EBH2O in steers and found that a significant relationship existed between both these parameters ($r=0.96$; $P<.001$). Recently Bartle et al. (1987) validated urea dilution in diverse types of crossbred cattle of different ages and frame sizes by using carcass specific gravity, compared to US estimates of empty body composition ($r^2 = .67$, $P<.001$).

An important concern in estimating body water in ruminants is the ability to differentiate gut or reticulo-ruminal water (RRW) from EBH2O. Since RRW is not known to be related to any empty body components it may introduce a large and variable error into estimation of body composition. Since the animals in this experiment were not water restricted prior to infusion, an interest arose to see

if this component may pose a problem towards overestimating EBH2O. Represented in table 28, are the daily excreted urine volumes from steers utilized in this present experiment. These data are given as indirect indicators of water consumption among animals in the different implant groups during the respective collection periods. Overall, there were no statistical differences in steer urine volume among any treatment within each period (table 28). Recently Bartle and Preston (1986) reported that a ^{15}N-urea infusate did not equilibrate in the RRW pool within the 12 min time interval required to estimate US, however, this isotope did equilibrate within the plasma and urine pools during this particular time interim. Therefore, Bartle and Preston (1986) concluded that US estimated at 12 min will overestimate EBH2O only by the volume of urine produced during this time, and that RRW influences urea dilution estimation of body composition only as a component of live weight.

Validation of varied published US equations, with comparisons to actual chemical composition, were reported by Rule et al. (1986). These workers showed that the EBH2O equation utilized in this experiment (Hammond et al., 1984) overestimated EBH2O volume in 6 mo. old steers by 13.8% ($P<.05$). In contrast, EBH2O based on this same equation for 12 and 18 mo. old steers did not differ ($P>.05$) between actual and estimated values of EBH2O (Rule et al., 1986).

Since animals were not slaughtered at the end of the

Table 28. Steer Daily Urine Excretion for the Implant Treatments During the Preimplant and Treatment Periods.[a]

Trt	n	Period 1	Period 2	Period 3
		liters/d		
C	10	3.19	3.45	3.87
TBA	11	3.21	3.57	3.02
E2	11	3.13	2.26	3.15
TBA+E2	11	3.37	2.34	3.99
SEM		0.44	0.49	0.55

[a] Values were derived from a pooled 3 day total urine collection.

experimental period to obtain actual percentages of carcass water and protein, further attempts to examine the validity of these values (derived from US) were done by comparison to actual carcass data derived from another study (table 29). Although these data are from animals used from a different experiment, they are similar with respect that they were large-framed and predominantely exotic-cross steers.

Comparisons in table 29 show that EBPROT and calculated skl prot values are very similar between groups at similar live weights, however, values calculated from US consistently overestimated EBH2O by approximately 7%. This overestimation of EBH2O is also reflected in the EBH2O/ EBPROT ratios at each weight comparison. The ratios generated from this study decline over time, showing a similar time trend as those reported by Haecker (1920), relating the effect of increased adipose deposition, at the expense of H2O in animals of increasing maturity. In summary, although possibly slightly overestimated, the present values calculated from US were accepted as representative estimates for both EBPROT and skl prot in the experimental steers during the three sampling periods.

Empty Body and Skeletal Muscle Protein. Empty body protein values are presented in table 10. By utilization of the treatment means generated from this table, EBPROT gains, as calculated for the entire treatment period, averaged 125, 134, 179, and 230 g/d for treatments C, TBA, E2 and TBA+E2, respectively. These values are within the range reported by

Table 29. Mean Comparisons of Empty Body Water, Protein and Skeletal Muscle Protein of Structurally Similar Steers at Different Live Weights.[a]

LBW -kg-	EBH2O -kg-	EBPROT -kg-	SKL PROT -kg-	EBH2O/EBPROT -H-	-S-
319	179.2	51.6	26.8	3.47	
(298)	(167.0)	(52.0)	(27.0)		(3.20)
424	220.4	65.6	34.1	3.35	
(390)	(207.9)	(64.0)	(33.0)		(3.20)

[a] Values in parentheses were derived by Schroeder (S) (1987b), whereas values not in parentheses were calculated by utilizing US techniques in this present study (H).

McCarthy et al. (1983), who showed EBPROT gains of approximately 135 and 184 g/d in small-framed Hereford vs large-framed crossbred steers. Additional studies utilizing crossbred beef steers, which demonstrated similar EBPROT gains, were reported by Byers (1980) and Garret (1979).

Schroeder et al. (1987a) found that 52% of EBPROT is associated with skl prot (table 11). This constant value is comparable to those reported by Berg and Butterfield (1976), who demonstrated that approximately 52-57% of skeletal protein was derived from EBPROT in a variety of cattle types.

Fractional accretion rates of skeletal muscle (table 13) were determined by dividing the increment, or difference of skeletal protein gain, by the average skeletal muscle pool size per the number of days between sampling (table 6). Muscle protein FAR for the entire treatment period for this study were .22, .23, .30 and .37%/d for C, TBA, E2, and TBA+E2, respectively. These values are somewhat higher then those presented by McCarthy (1981), who reported muscle FAR to average .19%/d for both small- and large-framed cattle. Fractional growth rates (FGR) for EBPROT calculated from treatment means in table 10 represented above, averaged .27, .28, .35 and .42%/d for the respective treatments listed above. As would be expected FGR was consistently greater than FAR due to the contributing non-skeletal muscle protein sources. Comparisons between the differences of the low and high gain treatment groups (C vs TBA+E2) for FGR and FAR

showed a 55 and 68% increase in these respective rates upon combined implant treatment. An interesting point to note is that muscle protein FAR response to TBA+E2 was 13% greater than those of FGR, which demonstrates an ameliorated response of skl prot to implant treatment, as compared to total EBPROT.

Skeletal muscle protein gains (table 12) for C, TBA, E2, and TBA+E2 groups are 65, 78, 93 and 120 g/d, respectively. Overall, these rates were similar to those reported by McCarthy et al. (1983), which ranged from 77.5 to 101.0 g/d. Similarly to this study, Gopinath and Kitts (1982) reported that the effect of varied anabolic implants on steer muscle protein gain averaged 62, 82, 101, and 106 g/d for C, Zeranol, DES and estradiol benzoate plus progesterone (Synovex-S) treatment groups, respectively. After comparing the data from Gopinath and Kitts (1982) to those of this present study, C values were found to be similar, while E2, as compared to zeranol, increased deposition 43 vs 32% over respective C, while DES increased gains 62%, as compared to the response demonstrated by E2 (+43%). The implant treatments from both studies which produced the largest response were TBA+E2 and Synovex-S which both increased muscle protein deposition 84% and 71%, as compared to C.

Both skl prot deposited and skeletal muscle FAR in this experiment, showed similar decreases over time within all treatments. This is a typical response in maturing animals, as a result of declining muscle protein synthesis and

breakdown rates (figure 5). Although a normal occurrence, values listed (tables 12 an 13) decline quite rapidly after the 30 d treatment period. Especially noted is a 52 and 70% decrease in FAR after E2 and TBA+E2 treatment. Initial thought in relation to this event was that US measurements were overestimating EBPROT values within period 2. Although this condition may be a possibility, after viewing the skeletal muscle protein values in table 28 from Schroeder et al. (1987b), the results presented from this study seem to be within range for animals of comparable live weights. Since skeletal muscle FAR, in the C group, also dropped fairly dramatically (from .40 to .17%), the initial increase of accretion may be attributed a compensatory gain response. Prior to the experiment, problems with the urine collection system were encountered, so the animals were restricted fed to limit gains. Upon adaptation to the experimental diet (tables 3 and 4), additional nutrients may have caused an acceleration of protein deposition to occur in these steers. Clearly, an additional increase in muscle protein was seen during the period after initial implantation of both E2 and TBA+E2 (tables 12 and 13). In agreement with the above hypothesis, values obtained from beef steers, reported by Fox et al. (1972) indicated that compensating animals (from 364 to 454 kg), deposited relatively more protein and less fat than C during initial full feeding. Increased deposition of fat, however, was more prevalent in compensating steers during the end of the full feeding period, as related

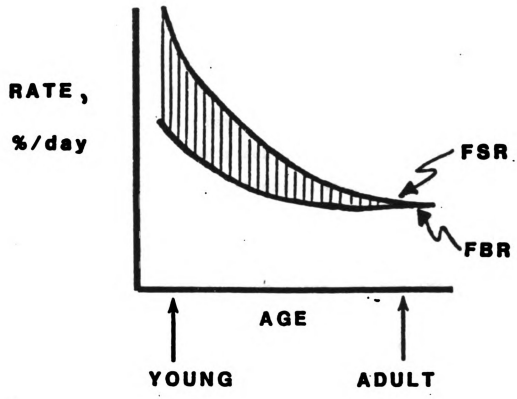


Figure 5. Effect of maturity on muscle protein fractional synthesis, breakdown and accretion rates (Mulvaney, 1984).

to non-compensating animals. These later results reported by Fox et al. (1972) may explain then that the decline in protein deposition during period interval 2- 3 from this present study, was dependent on an increase of fat rather than protein accretion.

Urinary 3-Methylhistidine Excretion. Measurement of urinary 3MH has been widely used as an index of myofibrillar protein degradation in rats and humans (Young and Munro, 1978). This, in vivo, approach of assessing protein breakdown has been validated in steers (McCarthy et al., 1983; Harris and Milne, 1979), however, it does not appear to be a reliable index in sheep (Harris and Milne, 1980) or pigs (Harris and Milne, 1981). The basis of the validation of this procedure entailed a rapid quantitative recovery of radio-labelled 3MH within 5-7 d post-injection, in the urine. McCarthy et al. (1983) reported that approximately 90% of the radioactivity injected into yearling Charolais-cross heifers was recovered within 120 h after administration. Similar results to those of McCarthy et al. (1983) were shown by Harris and Milne (1979). Additionally, Harris and Milne (1979) reported that recovered radioactive 3MH in urine was structurally unchanged. This result agrees with data presented by Young and Munro (1978) showing that 3MH, once released from muscle peptides, after muscle protein degradation, is not reutilized towards protein synthesis but quantitatively excreted in the urine.

Presently, controversy regarding 3MH measurement as a

index of myofibrillar breakdown exists due to the contribution of non-skeletal muscle sources of 3MH. This concept was initially proposed by Millward et al. (1980), after reporting that approximately 25% of the 3MH excreted in the urine is from skeletal muscle. Although the validity of the experimental procedures of Millward et al. (1980) were questioned by Harris (1981), the contribution of non-skeletal muscle still remains a problem towards approximating the relative amount of skeletal muscle 3MH towards total urinary 3MH excretion. Data presented (table 16) from this experiment were calculated with a 20% correction factor to adjust for the non-muscle sources of 3MH, as based on results by Afting et al. (1981).

Reports by Nishizawa et al. (1979) demonstrated that more than 93% of total 3MH, in cattle tissues, occur in skeletal muscle. Although the skeletal muscle pool has the largest store of 3MH, faster turnover rates of other contributing tissues need to be considered. Recently, Millward and Bates (1983) investigated the tissue origin of 3MH in female rats, based on estimates of both 3MH pool size and turnover rates of the respective tissues. These researchers found that skeletal and intestinal tissue contributed 52 and 22% of the total excreted 3MH, while other non-skeletal tissues represented the additional 26% of the total urinary 3MH.

Until recently, concern of another contributing source towards increasing urinary 3MH excretion in ruminants was from the dietary components being consumed by the animal

(Nishizawa et al., 1979; Wolht et al., 1982). Although this is a prevalent factor when feeding meat products (Huszar et al., 1983), diets commonly feed to ruminants do not contain enough 3MH to provide additive concentrations which would be excreted in the urine (Gur-Chiang and Bergen, 1986).

Urinary 3MH values in this study increased over time (table 16), indicating greater protein degradation in association with an increase in the skeletal muscle protein pool. These data are comparable to those presented by Gopinath and Kitts (1982, 1984) in growing beef steers. Similarly, Harris and Milne (1979) reported that there is a high correlation between 3MH excretion and body weight (r=.997) in male and female Fresian cattle. In contrast to these studies, McCarthy et al. (1983) did not demonstrate a consistent increase of 3MH excretion in both small- and large-framed steers, as associated with increasing skeletal muscle mass. Concentrations of steer 3MH excreted in the urine from this present experiment were 1.47, 1.68 and 2.03 mmoles for steers averaging 319, 369 and 424 kg, respectively. These values are quite comparable to values reported by McCarthy et al. (1983) and Gopinath and Kitts, (1984) from steers having similar body weights.

Similar to the present study, anabolic treatment did not depress 3MH excretion from steers implanted with TBA and Ralgro (Griffith, 1982), or with Synovex-S or DES (Gopinath and Kitts, 1982). In contrast to the aforementioned compounds, zeranol was reported to increase muscle protein

degradation in steers (Gopinath and Kitts, 1982). Lobley et al. (1985) further reported that a decline in 3MH excretion existed in 250-520 kg Hereford-Fresian cross steers upon implantation of 140 mg TBA plus 20 mg E2, however, this decrease was not numerically sufficient to account for the increase in body protein accretion.

3-methylhistidine values expressed on a liveweight basis (table 17) from this experiment did not change over time because 3MH values rose in concert with live body weights. This result is in contrast to work presented by McCarthy et al. (1983), who demonstrated that a decline in 3MH concentrations/kg LW existed during increasing age of steers. Similarly, Harris et al. (1983), reported that TBA+E2 administration, decreased relative 3MH concentration in steers at both 4 and 7 wks after implantation.

Creatinine. This urinary metabolite is a product of muscle creatine phosphate metabolism, and was measured in this experiment as a global index of muscle mass (Waterlow, 1969).

Urinary creatinine concentrations, as similar to skeletal muscle protein values, increased with age of the steers in this study (table 18). Comparable results have been demonstrated in steers (McCarthy et al., 1983; Gopinath and Kitts, 1984) and also in heifers (Benner, 1983). Upon E2 and TBA+E2 treatment urinary creatinine concentrations rose in comparison to both the C and TBA treated groups. Furthermore, these results parallel those presented for skl

prot (table 11). The average concentration of creatinine in this experiment ranged from 8.40 to 10.47 g/d across all periods. These values are comparable to large-framed crossbred steers (McCarthy et al., 1983), but are slightly lower than concentrations reported by Gopinath and Kitts (1983) from similar weight steers.

3-Methylhistidine-to-Creatinine Ratios. These values were determined as an index of muscle protein degradation per unit of muscle mass. In this study there were no changes in these values (table 20) among treatments over time. These results agree with the FBR results (table 14), showing that TBA and TBA+E2 do not affect protein degradation. Similar results were found by Gopinath and Kitts (1982) after steers were implanted with DES, Synovex and Ralgro. In comparison, McCarthy et al. (1983) reported that these ratios decreased with increasing animal maturity, indicating that the rate of muscle degradation may decline with age. In contrast, Gopinath and Kitts (1984) reported that 3MH/creatinine ratios increased over time in growing steers.

Skeletal Muscle Protein Fractional Breakdown Rates. The total skeletal muscle 3MH pool was calculated by multipling skeletal muscle protein pool by 594 ug/kg, which represents the bound 3MH contribution in bovine mixed-muscle protein (Nishizawa et al., 1979). Actual FBR of the muscle protein pool were calculated by dividing the amount of daily urinary 3MH excreted by the bound 3MH in the skeletal muscle pool.

Fractional breakdown rates determined from C in this study (table 14) did not decline over time, as reported by McCarthy et al. (1983) and Nishizawa et al. (1979), in steers. However, FBR tended to increase slightly from periods 1 to 3 (table 14), which is a similar trend as those reported by Gopinath and Kitts (1984). The overall FBR values presented from this experiment may be representative of animals at a stage of maturity where FBR has already plateaued (figure 5), compared to the curvilinear decrease demonstrated by young growing animals, increasing in age. Average FBR calculated from this study was 1.39%/d for all combined treatments (table 14). This value is slightly lower than those reported by McCarthy et al. (1983) and Gopinath and Kitts (1984) from steers with similar live weights. In contrast, higher FBR values were presented by Benner (1983) for heifers treated with TBA. The specific skeletal muscle FBR during the entire implant period averaged 1.40, 1.38, 1.39 and 1.40%/d for C, TBA, E2, and TBA+E2, respectively. Results from Gopinath and Kitts (1982), coupled with those presented from this study, indicate that anabolic treatment does not seem to decrease skeletal muscle FBR. This result is in direct contrast to data reported by other researchers. Sinnett-Smith et al. (1983) showed that lamb cathepsin D activities, utilized as an indicator of muscle protein breakdown, decreased after TBA+E2 treatment. Similarly, Bohorov et al. (1987) reported that Revalor (TBA+E2) decreased estimated skeletal muscle

FBR in lambs, whereas, Vernon and Buttery (1976; 1978b) showed that TBA and TBA+E2 treatment in rats, decreased rates of skeletal muscle protein breakdown, as measured by an isotopic sodium bicarbonate technique and urinary 3MH excretion, respectively.

Skeletal Muscle Protein Synthesis Rates. Similar to the graphical depiction in figure 5, muscle protein FSR in the steers utilized in this present study declined over time. Average FSR ranged from 1.62 to 1.77%/d between C and TBA+E2 groups (table 15). These synthesis values are markedly lower than those reported by McCarthy et al. (1983), which averaged approximately 3.26%/d for large-framed crossbred steers. Again, the results obtained from this study may be indicative of animals that are fattening, or are physiologically more mature than those used by McCarthy et al. (1983).

A trend noted in table 15 is an increase in steer FSR (4 and 9%), caused from E2 and TBA+E2 implantation. Similar results were reported by Gopinath and Kitts, (1982), showing that zeranol, DES and Synovex-S increased muscle protein synthesis 18, 11, and 15%, over C. These studies are in contrast, however, to experiments reporting decreased skeletal muscle protein FSR in both lambs (Sinnett-Smith et al., 1983) and steers (Lobely et al., 1985) after TBA+E2 implantation.

Serum Concentrations of 17 beta-Estradiol and Trenbolone. Basically, there are two types of ear implants currently

utilized to deliver anabolic treatments to cattle. First, representing TBA, is a compressed pellet implant, which is usually effective for 90-120 d (Heitzman, 1979). Commonly the pellet type implant usually gives a relatively high release of treatment into the blood within the first 30 d after implantation, followed by subsequent decrease in release rates. The second implant, a silastic rubber implant (Compudose), is partially impregnanted with E2. This type of implant, in contrast to the pellet type, functions by releasing slow and continuous rates of E2 for extended periods (Wagner, 1983).

Analysis of serum concentrations for TB and E2 are given in table 21. These values were determined in this experiment to ensure that implants delivered efficacious amounts of both E2 and TBA. During period 2, TB concentrations averaged 652 and 1672 pg/ml for TBA and TBA+E2 treatments. Henricks et al. (1982) reported that heifers administered with 300 mg of TBA showed increased plasma TB levels over 900 pg/ml after implantation. These researchers also demonstrated that TB concentrations declined over 99 d to approximately 400 pg/ml. Similar findings to these of Henrick et al. (1983) were presented by Heitzman and Harwood (1977). In contrast to both of these studies, collective serum concentration of TB in this experiment were maintained at comparable concentrations through the entire implant period. It is interesting to note that TB concentration in the TBA+E2 treated steers is almost 2-fold higher than

those demonstrated by the single TBA group (table 21). Henricks (1987) stated that the presence of circulating E2 (together with TB) may compete with TB metabolism at the liver. This occurrence would then decrease the formation of 17 alpha-hydroxy trenbolone, which is the major form excreted in the urine and feces (Pottier et al., 1981).

Some reseachers reported that TB may induce an E2 saving affect in the circulation (Riis and Suresh, 1976; Heitzman et. al., 1977b; Heitzman, 1983; Donaldson et al., 1981). Heitzman et al. (1981) also demonstrated that this response only occurs when TBA and E2 are combined together, while seperate implants of both compounds, in different ears would demonstrate similar absorptive rates as those of a single E2 treatment.

Serum E2 concentrations resulting from E2 treatment in this study, followed a similar time-trend as those presented by Heitzman (1983), this being a fairly rapid decline over time. A 5 pg/ml increase in E2, as noted in period 2 (table 21), has been demonstrated by Wagner (1983), however, in contrast to these results, the TBA+E2 implant treatment used in this trial delivered lesser quantities of E2 past 70 d after implantation. This result is in agreement with data presented by Sawyer (1987) who showed that Compudose gave an anabolic response in steer calves for only 66 d. Although E2 concentrations did decline through period 3 a numerical increase in serum GH concentration from E2 treatment (Grigsby and Trenkle, 1986) was maintained into period 3

(figure 2). Additionally, since circulating E2 levels decline with time, this may suggest that E2 may be acting through another estradiol metabolite such as 17 alpha-estradiol (Hoffman, 1980).

Growth Hormone. The response in mean steer GH concentration from the varied implant treatments is presented in figure 2. A general trend noted within treatments C, TBA and TBA+E2 is a linear decline of serum GH concentration over time. For the C group the decline in GH is similar to patterns noted in other steer studies (Trenkle, 1970; Trenkle and Topel, 1978).

While not statistically different, E2 in this present study increased GH concentration 29 and 46% over C during periods 2 and 3 (figure 2). Similar results have been reported for wethers and steers, where E2 increased overall plasma GH concentration (Davis et al., 1977; Donaldson and Hart, 1981. Trenkle 1970; Grigsby and Trenkle, 1986). Treatment of steers with TBA+E2, in contrast to E2, resulted in lower serum GH concentrations. Donaldson and Hart, (1981) reported similar findings in sheep, where TBA blocked the increased GH response from E2, when both compounds were implanted simultaneously together.

The increase of overall GH concentration by E2 in this study seem to be related to increased GH pulse amplitude and pulse duration (tables 24 and 25). Although not statistically different from C, E2 enhanced amplitude and duration during periods 2 and 3, 25 and 68%, and 115 and 98%,

respectively (table 24). These data are comparable to those presented by Davis et al. (1977) who reported that DES treatment in wethers tended to increase blood GH amplitude. In contrast to these results, Grigsby and Trenkle (1986) attributed greater GH concentrations to increased GH pulse frequency in steers. After treating wethers with DES, Davis et al. (1977) demonstrated that a significant increase in serum GH basal mean was prevalent. This result is similar to this present study, where E2 treatment increased serum GH baseline concentrations during period 2 (table 22).

Although TBA+E2 treatment in this study did elicit a marked increase in steer growth, this response does not seem to be a function of increased GH concentration in the blood, for TBA+E2 effectively decreased GH amplitude ($P<.05$), when compared to E2. Overall, both TBA+E2 and single TBA treatment exhibited similar decreases in serum mean GH concentration over both periods 2 and 3, which were similar to C. This response in GH concentration has also been reported in steers by Heitzman et al. (1977a). Another trend (table 25) noticed upon TBA administration in steers, was that this treatment decreased both GH pulse amplitude (table 24) and duration time. This result is directly in contrast to those presented by Davis et al. (1977) who showed that testosterone propionate tended to increase blood GH pulse amplitude.

Upon reviewing the GH data from this present trial, E2 and TBA seem to be functioning at the level of the pituitary

gland. This is especially noted by the action of both of these steroids on GH pulse amplitude and duration (tables 24 and 25). If the site of action of these anabolic steroids were at the hypothalmus, a treatment response involving pulse frequency should be evident. In support of this hypothesis, Carlsson et al. (1987) recently reported that E2 can directly bind to receptors on rat pituitary. Extensive reports have been presented showing that synthetic estrogen treatment in ruminants successfully increases pituitary weight, (Preston, 1975). Recently, this result was repeated in sheep, where Muir et al. (1983) demonstrated that 0.1 mg/d of subcutaneously administered DES increased pituitary weights 26%, as compared to C. Additionally, this increase in pituitary weight upon E2 treatment seems to be due to an increase in cell size and number in this gland (Martin and Lamming, 1958). Furthermore, Trenkle (1983) proposed that E2 action may modulate pituitary action by actually increasing the sensitivity of varied receptor types on the pituitary gland.

Insulin. Although reported treatment differences were not different (P>.10), some interesting numerical trends are worth noting about INS response to anabolic treatment. Concentrations of INS in the C group increased between periods 1 and 2, and remained fairly constant through period 3 (figure 3). This response is partially supported by results reported by Trenkle (1970) and Trenkle and Topel (1978), showing that blood INS concentrations increase

concomittantly with maturity.

Estradiol implantation of the steers in this study did not increase INS concentration, as was reported in sheep (Donaldson et al., 1981). The results from this present study are, however, in agreement with those reported by Grigsby and Trenkle (1986) who utilized steers implanted with E2. Donaldson and Hart (1981) suggested that increased blood INS concentrations in sheep, implanted with E2, may be related to an increase in concentration of GH, which is indirectly causing a diabetogenic effect.

After viewing figure 3, a common trend noted between collective TBA treatments, is an initial decline in serum INS levels. This response was not maintained, however, for INS concentrations rebounded back towards those of C during period 3. To date, nothing has been published in the literature to support these findings. In the past, however, both TBA and TBA+E2 treatments do not seem to effect blood INS concentration (Donaldson and Hart, 1981).

Collective insulin results (table 26), show that INS pulse, amplitude and duration increase among all treatments, with respect to time. This increase may be a direct reflection on mean serum INS concentrations, however, mean INS concentrations during period 2 remained depressed as a result of TBA treatment. Together, these data do not support any distinct mechanism of TBA action on the pancreas, which warrents further experimentation to elucidate a possible mode of action of this xenobiotic on

the pancreatic beta-islets.

Cortisol. Total serum CT concentrations during the pre-implant period were not different (P>.10) and averaged 3.5 ng/ml. Upon E2 treatment, steer CT levels rose markedly in comparison to C, TBA and TBA+E2. Similarly, Thomas and Rodway, (1983a) reported that E2 administration increased plasma CT concentrations in lambs. Collectively, these data agree with those reported by Seal and Doe (1963) who showed that the effect of pregnancy induced an increase in circulating E2 concentration, which subsequently increased plasma CT levels in a variety of mammalian species. In contrast to the above reports, Sharpe et al. (1986) presented data showing that zeranol increased CBG, which was positively correlated to growth rate. Estradiol treatment in the present study did not maintain an increase in CT concentration through period 3 (figure 4), which may be a direct function of declining blood E2 levels (table 21).

TBA treatment in both single and dual implanted steers resulted in a decline of CT concentrations over both periods 2 and 3 (figure 4). These results agree with work done in rats (Thomas and Rodway, 1982 a,b) and lambs (Thomas and Rodway, 1982a; Sillence et al., 1987) treated with TBA. Additionally, similar results demonstrating circulating CT suppression were reported in lambs after being implanted with combined TBA and E2 (Singh et al., 1983; Thomas and Rodway, 1983a; Sillence et al., 1987).

Experiments involving the effect of GC on muscle protein

synthesis and degradation have been limited primarily to the rat. Overall, glucocorticoids (GC) have proven to decrease protein synthesis by effecting peptide transcription and translation rates (Baxter et al., 1972; Rannels and Jefferson, 1980). Presently the response of GC on skeletal muscle degradation still remains unclear, however, recent reports do show that daily GC administration to rats, produces a considerable increase in 3MH excretion (Santidrian et al., 1981; Odedra et al., 1983). In addition to Santidrian et al. (1981) and Odedra et al. (1983), Rannels and Jefferson, (1980) reported that degradation in perfused rat hemicorpus muscle was not affected by cortisone acetate treatment. Together, these data suggest that during normal physiological conditions GC action is antianabolic and mediates its effect by decreasing muscle protein synthesis rates. Results from this present study seem to agree with this hypothesis, especially with regard to the results obtained from the TBA treatment groups. Overall, the decreased blood concentrations of CT in these groups do not effect skeletal muscle FBR, as compared to C. Conversely, increased synthesis rates have been noted upon TBA+E2 administration to steers. Since TBA does not demonstrate any differences in FSR when compared to C, it seems that TBA in conjunction with E2 is needed to increase muscle protein synthesis. Overall, as derived from this present study, it seems that the E2 effect on muscle anabolism is mediated through increasing GH concentration in

the blood, while TBA is working primarily through decreasing circulating CT levels. Why differences in growth rates occur between TBA and TBA+E2, although their hormonal profiles are similar, remains to be resolved. One aspect is clear, however, and that is the anabolic action on steer muscle protein metabolism is directly affected by a hormonal milieu.

Glucose. Within each collection period, plasma glucose concentrations did not differ among treatments. Collectively, these data are in agreement with research reported from steers implanted with TBA (Gailbraith and Watson, 1978; Heitzman et al., 1977a) and TBA+E2 (Heitzman et al., 1977a). Overall, steer glucose values declined over time, possibly in association with the demonstrated increase in serum INS concentration.

SUMMARY AND CONCLUSIONS

This present study examined the effects of a 300 mg TBA, 24 mg E2 and combined implant (TBA+E2) treatment on growth, skeletal muscle protein turnover, specific serum hormone concentrations, plasma glucose concentration and selected urinary metabolites, in steers.

Implant response on steer weight gain performance during period 1 indicated that both E2 and TBA+E2 administration increased steer growth rates. However, during period 2 only TBA+E2 implanted animals maintained an increase in live body weight gain. Feed efficiency throughout the entire experimental period, showed that only steers implanted with TBA+E2 demonstrated an increase in feed efficiency (+18%) when compared to C.

Steer EBH2O, EBPROT and skl prot deposition, were all increased by both TBA and E2 treatments over the entire experimental period. Additionally, further treatment constrasts revealed that TBA+E2 treatment, during the entire treatment period, increased these compositional parameters when compared to both E2 and TBA implanted steers. These results suggest that a synergistic mechanism prevails when both treatments are implanted concurrently.

During the entire treatment period, steer average daily skeletal muscle protein deposition ranged from 65 - 120 g/d. TBA+E2 treatment increased steer protein accretion, as

compared to both E2 and TBA through d 40, however, this response was not maintained over the following sampling period interval. Again an additive effect on skl prot deposition was demonstrated by utilizing combined implant treatments.

Similarly to the skeletal muscle protein accretion results, steer skeletal muscle FAR was accelerated upon TBA+E2 treatment. Unlike FAR, skeletal muscle FBR was unaffected by implant treatment, whereas, skeletal muscle FSR was increased by collective E2 treatment.

Steer urinary 3MH excretion, increased over the entire treatment period, as a result of increasing skeletal muscle mass. Urinary 3MH excretion was not different among treatments, except for TBA+E2 treated steers, who demonstrated increased urinary 3MH concentrations, as compared to TBA treated animals.

Similarly to 3MH, urinary creatinine concentration increased over time indicative of increasing steer muscle mass. Overall, TBA+E2 treated steers demonstrated increased urinary creatinine concentrations, as compared to single TBA administered animals during d 80. In addition, 3-methylhistidine-to-creatinine ratios, utilized as a index of muscle protein breakdown per unit muscle mass, were unaffected by implant treatments during the total experimental period.

Serum concentrations of TB and E2, indicated that implants were effective towards suppling efficacious amounts of

treatment to the steers during the entire treatment period.

Steer serum GH concentration was numerically increased by E2 treatment, as a result of increases in both GH pulse amplitude and duration. In contrast, collectively treated TBA steers demonstrated decreases in both GH pulse amplitude and duration.

Steer mean serum INS concentrations among the experimental treatments did not differ. Serum INS pulse amplitude, frequency and duration all demonstrated to increase over time.

Steer serum CT concentration, upon E2 administration, increased during d 30, then declined back towards C levels during d 60. After collective TBA administration to steers, however, CT levels declined dramatically and were maintained at this repressed level for the remainder of the experiment.

Plasma glucose concentrations did not differ among the experimental animals within any given collection period, however, values did increase with respect to time.

In conclusion, the results from this present experiment demonstrated that increased skeletal muscle protein deposition in steers, as a result of both E2 and TBA+E2 implantation, is not a consequence of decreased rates of skeletal muscle protein breakdown. Conversely, implant action seems to be mediated through enhancement of skeletal muscle protein synthetic rate, where anabolic action is related to changes within the hormonal milieu affecting muscle protein metabolism.

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APPENDICES

APPENDIX A

Table 1. Source of Variance Table for Steer Dry Matter Intake and Feed Efficiency Values[a]

Source	df[b]
E2	1
TBA	1
TBA*E2	1
(pens/trt) = $error_1$	4

[a] Values were analyzed by 2^2 factor ANOVA.
[b] Degrees of freedom for variables of DMI and F/G, with pen representing the experimental unit.

Table 2. Source of Variance Table for Steer Average Daily Gain

Source	df[a]	df[b]
Main-plot		
E2	1	1
TBA	1	1
TBA*E2	1	1
(animals/trt) = $error_1$	4	39
Sub-plot		
Per		1
Per*E2		1
Per*TBA		1
Per*E2*TBA		1
(animals/per) = $error_2$		39

[a] Degrees of freedom for variables of ADG over the entire treatment period. Values were analyzed by 2^2 factor ANOVA with animal representing the experimental unit.

[b] Degrees of freedom for variables of ADG representing days 0-40 and 40-80 after implantation. Values were analyzed by 2^2 factor ANOVA with split-plot structure. Animal is representative of the experimental unit.

Table 3. Source of Variance Table for Varied Metabolism Data Obtained Over 3 Periods

Period	df[a]
Main-plot	
E2	1
TBA	1
E2*TBA	1
(animals/trt) = $error_1$	39
Sub-plot	
Per	2
Per*E2	2
Per*TBA	2
Per*E2*TBA	2
(animals/per) = $error_2$	78

[a] Degrees of freedom for variables pertinent to the metabolism parameters measured. Values were analyzed by 2^2 factor ANOVA with split-plot structure. Animal is representative of the experimental unit.

Table 4. Source of Variance Table for Serum Hormone Concentrations and Plasma Glucose Levels

Period	df[a]
Main-plot	
E2	1
TBA	1
E2*TBA	1
(animals/trt) = $error_1$	12
Sub-plot	
Per	2
Per*E2	2
Per*TBA	2
Per*E2*TBA	2
(animals/per) = $error_2$	24

[a] Degrees of freedom for variables of serum hormone parameters and mean plasma glucose concentration. Values were analyzed by 2^2 factor ANOVA with split-plot structure. Animal is representative of the experimental unit.

APPENDIX B

Preparation of Physiological Fluids for Free Amino Acid Analysis Utilizing the Waters Pico-Tag HPLC System

A) Ultrafiltration

1) Aliquot 1 ml of sample (plasma filtered urine) into a 16 x 100 mm test tube containing 1 ml of 0.1 N HCl previously spiked with a Nle internal standard (after final dilution Nle concentration should equal 625 pm)
2) Vortex solution briefly to insure a homogeneous mixture
3) Pipette approximately 1 ml into the stalk of the ultrafiltration assembly and centrifuge at 1500 xg for 20 min
4) Transfer filtered solution into etched 6 x 50 mm glass culture tubes (Pyrex no. 1820) via Hamilton syringe. Between transfer, flush the Hamilton syringe with sequential washes of H20, H20:MeOH (50:50), MeOH, H20:MeOH (50:50), and finally H20

Type of sample	Amount added to 6 x 50 mm tube
urine	25 ul
plasma	50 ul

B) Preparation of Two Seperate Standards

Add together the following in 6 x 50 mm tubes:

10 ul Custom A/N (Waters, Assoc.)	10 ul Pierce A/N
10 ul Custom B (Waters, Assoc.)	10 ul Pierce B
5 ul 1.25 mM NLE	5 ul 1.25 mM NLE

C) Prederivation of Standards and Samples

1) Put standards and sample tubes into the Millipore reaction vessel (max. 14 tubes) and connect to work station
2) Slowly turn on vacuum (open vac. knob approximately 2.5 turns). Allow preparations to dry completely (approximately 60-70 m Torr)
3) Treat samples and standards with a redry agent

Redry Agent Preparation:

Std's and plasma by volume	Urine by volume
2 0.6 m Na Acetate	1 0.5 m Na Acetate
2 MeOH	2 MeOH
1 Triethylamine (TEA)	1 Triethylamine
	1 H20

-20 ul of the redry agent is added per tube.
-Redry this preparation.

D) Derivatization

1) Make solution previous to addition:

by volume: 1 H20
1 TEA
7 MeOH
1 phenylisothiocyanate (PITC)
*add 20 ul/tube (use EDP multipipette or repeating Hamilton)

2) Additions should be made within a 1 min time interval
3) Vortex all tubes and allow to incubate for 20 min at room temperature
4) Connect rxn. vessel to work station and dry tubes for 1 hour

E) Additional Washes (approx. 20 ul/tube) In Sequential Order

1) MeOH
2) MeOH:H20
3) MeOH

F) Final Dilution

1) Add:

-200 ul of diluent (Waters, Assoc.) to standards and plasma samples
-100 ul of diluent to urine samples

2) Pipette into volume restriction inserts (Waters, Assoc.). Standards and samples are ready to be added on the WISP carousel

G) Calculation of Daily Excreted Urinary 3-Methylhistidine

1) 3MH (pM/1.25 ul sample) = peak area of unknown 3MH x response factor (rf) x correction factor (cf)

$$rf = \frac{\text{3MH (pM) in external std. 1250 pM}}{\text{response of external std (peak area)}}$$

$$cf - \frac{\text{response of Nle in external std (peak area)}}{\text{response of Nle in unknown (peak area)}}$$

2) $$\text{3MH (mM/d)} = \text{3MH}\frac{(\text{pM})}{1.25\ \text{ul}} * (1.25) * \frac{1000\ \text{ul}}{\text{ml}} * \text{daily urine excretion (ml/d)}$$

$$\div \quad (1 \times 10^{9}\ \text{pM/mM})$$