ELSEVIER

Advanced Drug Delivery Reviews 38 (1999) 123–138

advanced drug delivery reviews

Hormone containing growth promoting implants in farmed livestock

R.L. Preston[1]

Animal Science and Food Technology Department, Texas Tech. University, Lubbock, TX, USA

Abstract

Growth promoting implants have been used in the production of cattle and sheep for over 40 years. Implants improve growth rate (+ 10 to 30%), feed efficiency (+ 5 to 15%) and carcass leanness (+ 5 to 8%). The history of this technology is mainly one of optimizing dose and hormone combinations, although matrices to optimize delivery rates of hormones from implants has received some attention. Estrogens are the first requirement for the growth response and in combination with androgens, growth is further enhanced. Several implant matrices are used, affecting pay-out rate and delivery time. The delivery time of most compressed implants is approximately 120 days and reimplantation after 60–120 days gives an additional response. Blood concentrations of implant hormones are increased and there appears to be a threshold blood level below which a growth response is not observed. Several proposed mechanisms are reviewed. The somatotropic axis appears most plausible for estrogens. Androgens may occupy muscle corticosteroid receptors. Regulated and proper use of implants assures their safety.

Keywords: History; Compounds; Implant matrices; Release patterns; Pay-out times; Mechanisms; Safety; Research needs

Contents

[1]Emeritus Thornton Endowed Chair and Horn Distinguished Professor. Present address: 191 Columbia Court, Pagosa Springs, CO 81147-7650, USA.

0169-409X/99/$ – see front matter
PII: S0169-409X(99)00012-5

1. Introduction

1.1. History

The earliest use of hormone enhancers in farm animal production included iodinated proteins fed to dairy cows for increased milk production and estrogen implants (diethylstilbestrol (DES) and dienestrol) in growing chickens (broilers) for enhanced fat deposition (''caponettes'') [1]. The first ''steroid-like'' hormone used in beef cattle and sheep for growth, efficiency and lean meat promotion was DES in 1954 [2]. Because of potential carcinogenicity from the use of DES in humans, not in farm animals, this compound was banned for use in cattle and sheep in 1979 [2] by the US Food and Drug Administration (FDA) as required by the Delaney Amendment [3–5].

Since growth promoting implants are commercially used only in cattle (steers and heifers) and to a lesser extent in sheep, this review will be confined to these two farm animals. Intact male cattle (bulls) also respond but to higher doses than are commonly used in steers and heifers [6]. A chronology of hormone approvals by the FDA for cattle and sheep in the USA is as follows:

- 1954 Oral DES approved for cattle
- 1956 DES implants approved for cattle
- 1956 DES implants approved for sheep
- 1956 Estradiol benzoate (EB)/progesterone implants approved for steers
- 1957 Oral DES approved for sheep
- 1958 EB/testesterone implants approved for heifers
- 1968 Oral melengestrol acetate (MGA) approved for heifers
- 1969 Zeranol implants approved for cattle
- 1969 Zeranol implants approved for lambs
- 1970 Oral DES dose range increase approved for cattle
- 1979 All use of DES banned in cattle and sheep production
- 1982 Silicone rubber–estradiol implant approved for cattle
- 1984 EB/progesterone implants approved for calves
- 1987 Trenbolone acetate (TBA) implants approved for cattle
- 1991 TBA/estradiol (5:1) implants approved for steers
- 1993 Bovine somatotropin approved for lactating dairy cows
- 1994 TBA/estradiol (10:1) implants approved for heifers
- 1995 Zeranol implant dose increase approved for cattle
- 1996 TBA/estradiol (10:1) implants approved for steers
- 1996 Estradiol/TBA (5:1) implants approved for stocker (growing) cattle

This chronology is primarily a record of attempts to optimize dose and combinations of anabolic agents. The approval of a silicone based implant in 1982 represents a significant development, modifying the implant matrix to achieve a more uniform pay-out. Approvals in other countries are in some cases difficult to determine. Approximately 30 other countries have approved one or more implants but

several indicate that they are not actually used. Unapproved use is widespread in many countries making regulatory oversight and proper use difficult to control.

1.2. Reviews

The most recent and comprehensive review of the applied and carcass effects of growth promoting implants in beef cattle was published by Oklahoma State University [7]. History, mechanisms, growth, intake and feed efficiency, behavioral, carcass yield and quality, safety and economic topics are well covered in 26 papers; specific reference to some of these papers will be made throughout this review. Many other reviews have also been published [8–53]. These leave no doubt about the growth (+ 10 to 30%), feed efficiency (+ 5 to 15%) and carcass leanness (+ 5 to 8%) enhancing effects of growth promoting implants and an economic benefit to cattle producers of $20–75 per head over the cost of implanting [44,45,54].

2. Compounds

2.1. Estrogens

Estrogens are the major class of compounds used in growth promoting implants [55]. As shown in the chronology, estradiol, its benzoate ester (EB) and zeranol are the estrogen compounds used commercially. All implant products are estrogen based, with one exception, and this seems to be the first requirement for a growth response. Combinations with other compounds often enhance the growth response, including TBA, testosterone (as the propionate ester) and progesterone. Estrogenic activity is an apparent requirement since alpha-estradiol and *cis*-DES (nonestrogenic isomers), and stilbene, estriol and estrone do not result in growth promotion [56–58]. Also, diets containing DES lost estrogenic potency and growth promoting ability in parallel during storage [59]. Several other synthetic estrogens (polydiethylstilbestrol, hexestrol, diallylhexestrol and dienestrol) give responses comparable to DES [13].

Few nonestrogenic analogs have been studied which may prove to be a fruitful research endeavor. Zeranol is a nonsteriodal macrolide, a compound in a class of natural products known as β-resorcylic acid lactones isolated originally from corn infected with the fungus, *Fusarium* [60]. The estrogenic activity of this class of compounds (natural and synthetic) has been characterized [60]. There are also many plant estrogens but these have not been well characterized for their growth promotion potential. Coumestrol has only weak growth potentiation properties [61,62]. Smilagenin, a nonestrogenic plant steroidal sapogenin, gave a growth response in lambs and cattle similar to DES [63].

2.2. Androgens

Early research with testosterone was generally disappointing regarding growth promotion [1,21,36]. However, the synthetic anabolic steroid TBA has been shown to increase growth and nitrogen balance in rats as well as cattle and sheep [21,27]. The relative androgenic and anabolic activity of TBA is 3–5 and 8–10 fold greater, respectively, compared to testosterone [27]. In combination with an estrogen, gain, efficiency and leanness are increased by TBA over an estrogen alone in steers [27,45,64–68], bulls [69] and wether lambs [70,71]. In heifers, TBA alone results in significant increases perhaps in combination with endogenous estradiol [43,67,68]. The dichotomy between the anabolic and androgenic activity for this class of compounds is very apparent [27,72,73].

2.3. Somatotropin, releasing hormone, somatostatin

The anabolic effects of the somatotropic axis in ruminants have been reviewed [34,74,75]. The first research on the effects of somatotropin (growth hormone; GH) in growing ruminants showed greater growth in cattle [76] and nitrogen retention in lambs [77]. Later research using daily injections, sustained release injections or pellets containing recombinant GH has generally shown increased gain and feed efficiency, no effect or decreased feed intake, no effect on wool growth, equivocal effects on carcass weight (dressing percentage), increased carcass protein and decreased fat, decreased plasma or serum urea, and increased blood GH and markedly increased insulin-like growth factor-1 (IGF-1) con-

centrations in cattle and sheep [78–88]. In cattle, required daily amounts of injected GH for maximum plasma urea-N (PUN) depression and increased gain ranged between 16 and 64 μg GH/kg body weight [79,87] whereas carcass leanness effects were observed through 300 μg GH/kg body weight [87].

Of particular interest is the effect of GH in combination with steroidal growth implants. All results to date indicate that the growth response is additive [42,89–93] including studies where the optimal dose of each was employed.

Growth hormone releasing factor (GRF) has also been shown to promote growth in lambs and steers [80,94–97] Daily doses required (1–10 μg/kg body weight), however, are not that much lower compared to GH. Of interest is the conclusion that the effects of GRF and steroidal implants on plasma IGF-1 and PUN in steers were additive [97].

Immunizing lambs against their own somatostatin has been shown to increase growth rate in most studies [98–102]. Immunizing steers against their own GRF decreased gain and feed efficiency, increased carcass fat, decreased serum GH, IGF-1, insulin and glucose, and increased serum urea-N [103].

2.4. Others

As shown in the chronology, progesterone and testosterone propionate are included in some implants. References citing reasons for these inclusions are nonexistent. They were probably included to potentially reduce side effects of estrogen, since early research with DES at implant doses much higher than eventually approved recorded many of these side effects. As will be discussed later, the presence of these compounds may result in a more ideal estrogen release from the implant than acting as an additional growth stimulant.

MGA is a synthetic progestogen that is 30 to 125 times more potent that progesterone and is used in the diet as an estrus suppresser in feedlot heifers; MGA also improves rate of gain in heifers [11,29,104–107], presumably because of greater follicular development and therefore greater endogenous estradiol secretion, which is supported by the observation that serum estradiol concentrations were increased 29–277% (not significant) after 21 to 140 days of MGA feeding compared to controls [107]. A long-lasting formulation (DEPO-MGA™) injected subcutaneously in the ear suppressed estrus for up to 325 days [108,109] but effects on gain were equivocal. Recently, implants containing increasing doses of norgestomet, a potent synthetic progestogen, reduced pregnancy rate in heifers on pasture for 154 days and increased rate of gain in a dose dependent manner [110]. The growth response of steers to MGA at doses commonly fed to heifers is equivocal [104–106].

Cortisol administration in cattle and sheep increased weight gain but in contrast to estrogen administration, carcass fat was increased [13].

3. Implant matrices

Early research studied the release of DES in vitro and in vivo; the only mention of formulation variables was ‘‘percent solvent in the formula granulation at the time of pelleting and the compression applied at time of pelleting’’ [111]. Implants used today contain either lactose, cholesterol or a large polymer of polyethylene glycol as a matrix (carrier) for compressed implants or a silicone rubber matrix [112]. Lactose-based implants are ‘‘short-acting’’ whereas cholesterol-based implants are ‘‘long-acting’’ [69] and when compared in terms of feedlot cattle performance, the response to cholesterol-based implants was sustained for 84 but not 126 days compared to lactose-based implants given every 42 days. When feedlot performance was compared over 140–168 days, no difference was observed between lactose- versus cholesterol-based implants [66]. Imbedding estradiol in a silicone rubber matrix provides some theoretical advantages such as modulating the dose rate over time and tailoring the dose rate simply by the length of implant [113]. Another approach to modulating the dose rate over time is to encapsulate the implant in an osmotic membrane which resulted in improved gain in steers that was dose related [114,115].

In addition to matrix variations, compression pressures used in the manufacture of implants can vary but apparently are confidential since data are not available in the literature.

4. Release patterns and pay-out times

Steroidal implant release rates and resulting blood hormone levels have been briefly reviewed recently [112].

4.1. Implant removal

The most thorough research on release patterns from growth implants excised at various times after implantation in cattle was conducted with DES. First order kinetics described this release pattern. Using three lots of DES implants from one manufacturer, half-lives of 80, 73 and 96 days were observed, or 78, 56 and 63% remaining 60 days after implantation [111]. When a DES implant from another manufacturer was similarly characterized [116], a half-life of 31 days (not 50 days as stated) or 33% remaining 60 days after implantation was observed. Thus differences exist between manufacturers in release patterns from compressed implants.

When the release pattern of an EB + progesterone implant was studied following excision 60–150 days after implantation [117], similar kinetics were observed (although not mathematically expressed). At 60 days, 32% of the estradiol and 27% of the progesterone remained in the implant. Palpation of the ears for the presence of implants is often carried out to assess the presence or absence of implants and to estimate release patterns. In this study, the authors state ''some implants were not palpable through the skin of the ear at slaughter but were readily located by dissection'' similar to a previous observation [116].

These results demonstrate a greater release of implant hormone during the first 60 days after implantation with measurable hormone (14–41%), depending on the implant formulation, remaining at the implant site 120 days after implantation, a time commonly assumed to be the effective pay-out period for compressed implants [118].

For the silicone rubber implant containing estradiol, pay-out periods up to 392 days have been observed; after an initial release (''burst'') over the first 14 days (approx. 200 μg/day), the release rate averaged 55 μg/day, declining at a rate of 0.058 μg/day [113].

Only one study has reported the release of TBA based on excised implants [119]; over the first 62 days, the average release rate was 4.36 compared to 0.56 mg/day over the next 37 days.

When implants (lactose base) containing radiolabeled estradiol with or without TBA were placed in calves, 95% of the radioactivity was excreted in 20 days when estradiol was implanted alone whereas 107 days were required to excrete most of the radioactivity when estradiol was combined with TBA [120].

4.2. Blood levels

Blood, plasma or serum concentrations of implant hormones have provided useful data but because of considerable variation, their meaning has limitations. Steers and bulls implanted with either estradiol alone or in combination with TBA had elevated levels of serum estradiol and trenbolone (TB) that declined with time after implantation [121]. It is generally thought that there is a ''biphasic'' concentration pattern (two or more curve components) over time that result in an initial high concentration followed by a declining concentration [112]. It is also generally assumed that there is a threshold concentration below which there is no further growth response. Definition of the optimum concentration in relation to the observed growth response is not clear. Based upon three studies, blood concentrations in steers were highest within a few days after implantation with estradiol + TBA (estradiol: 60–80 pg/ml; TB: 290–310 pg/ml); the threshold concentration of estradiol appeared to be 3–5 pg/ml above controls (2–5 pg/ml) 120 days after implantation when the growth response approached zero; the threshold concentration of TB was unclear since the concentration at 120 days was still 45 pg/ml [122,123]. In heifers implanted with TBA, plasma TB increased within 1 week and declined with time after implantation [119]. In more recent research [124], heifers implanted with TBA alone or in combination with estradiol had peak serum TB concentration 1 day after implantation which decreased through 42 days followed by a minor peak at 56 days and then a decrease through 140 days; serum estradiol increased 1 day after implantation with estradiol + TBA but did not peak until 56 days after implantation. A similar serum estradiol concentration pattern was

reported in steers implanted with estradiol + TBA [125]. When these two hormones were implanted as a single implant, serum concentrations of estradiol were elevated for 91 days whereas serum concentrations were increased for only 60 days if implanted in opposite ears [126]. Serum concentrations of TB were higher in steers implanted with estradiol + TBA than with TBA alone 31 and 72 days after implantation [127]. The half-life of one steroid has been shown to be increased by the simultaneous implantation of another steroid [128].

When steers were implanted with an EB + progesterone implant encapsulated in an osmotic membrane [114,115], serum estradiol concentrations 7 days after implantation were elevated in proportion to the number of implants used which declined somewhat with time after implantation through 108 days; serum from the ipsilateral jugular vein was higher in estradiol concentration (2–7 pg/ml) than serum from the contralateral vein indicating partial clearance from the blood, probably by the liver.

While not a controlled experiment [129], plasma estradiol concentrations were elevated (12 pg/ml above that expected for nonimplanted steers) 11 months after steer calves (2–3 months of age) were implanted with EB + progesterone. Additionally, contralateral vein concentrations were lower than ipsilateral vein concentrations indicating partial clearance of the implant hormone. This may be the explanation for an apparent extended growth promotion (150–210 days) in young steer calves following implantation [118,131].

4.3. Reimplantation

Many studies confirm additional growth and efficiency responses when cattle are implanted again (reimplanted) 60–120 days after previously receiving a compressed implant [45,68,118,122,123], providing practical support for the idea that release from these implants decreases with time after implantation to a point that is below the optimum for growth stimulation. The magnitude of the reimplantation response is variable (5–20%) depending on the previous implant, time to reimplantation and the implant used at reimplantation. Of practical concern is the effect of a previous implant on the subsequent performance of cattle. If the pay-out period of the previous implant has been exceeded and the cattle are not reimplanted, gains less than those observed in nonimplanted cattle may be observed. However, if the cattle are reimplanted, perhaps with a more ''aggressive'' or potent implant, positive growth responses are observed [68,118].

5. Mechanisms of growth promoting implants

Several reviews have discussed the many proposed mechanisms of growth promoting implants [8,9,13,16–25,30,31,34–37,42,50,52,132]. At this point, there is not a definitive mechanism that explains all observations, especially the fact that growth promotion by steroidal compounds outlined above in the chronology is limited to growing ruminants, not monogastric animals [13,42].

5.1. Synthesis/release of GH

The early explanation was that these compounds caused an increased synthesis and secretion or release of endogenous GH, based on increased anterior pituitary size [13], increased proportion of acidophilic cells in the anterior pituitary [8], increased GH secretion [133] or release [134], and increased circulating concentrations of GH and insulin [13,52,135]. However, many of these same changes have been observed in vitro and in vivo, primarily in rats [42]. Estrogens (DES) depress the growth of rats, in both intact and castrate male rats [136]. This lead to the comment ''If GH release explains the anabolic response in ruminants, why do estrogens depress the growth of rats?'' [36]. Volatile fatty acids are the major energy substrate in ruminants whereas in monogastric animals, glucose is the major energy substrate, which has been speculated to be the explanation for the difference [137]. In one experiment, the growth of guinea pigs was increased by low doses of DES [138] and since there is significant fermentation in the large intestine and therefore absorption of volatile fatty acids in guinea pigs, energy substrate may be involved in the differential response. Calves prior to significant rumen function do not respond to anabolic steroids [15].

Recently [139], we have shown that steers im-

planted with EB + TBA had a larger proportion of somatotrope cells (28%) in the anterior pituitary gland by 24 days after implantation compared to controls (10%), adding support for the endogenous GH enhancing hypothesis.

5.2. Independent action

If enhancement of endogenous GH is the mechanism for growth stimulation, then there should be no additional growth response to GH in the presence of an anabolic steroid, assuming both are given at their optimum dose. Early research with Zeranol and GH indicated there was an additive response [35,89,90]. Using PUN reduction as a measure of anabolic effect, an additive response was observed using optimum doses of estradiol and GH for maximum PUN reduction [129,130]. Subsequent feedlot experiments confirm that the response to GH and either estradiol + progesterone + TBA [91] or estradiol + progesterone [92] is additive. Additionally, there was an opposite response in feed intake and magnitude differences in plasma IGF-1 and carcass fat changes.

Thus it seems that these two growth promoter class of compounds have additive and independent actions in the growth of ruminants and therefore argues against enhancement of endogenous GH secretion as the mechanism for anabolic steroids.

5.3. Cell receptors

Estrogen receptors are present in cattle and sheep muscle although their concentration is many fold less than in uterine tissue [42]. Estrogen receptors, however, are also present in rat skeletal muscle. Androgen receptors are present in the cytosol of skeletal muscle from sheep treated with TBA [140] and TBA alters the responsiveness of skeletal muscle satellite cells to fibroblast growth factor and IGF-1 [141]. Corticosteroids have catabolic effects on muscle protein metabolism and androgens (e.g. TBA) compete for corticosteroid receptors thereby decreasing muscle protein degradation [42]. Therefore, implant hormones could have direct effects on skeletal muscle cells but this has not been demonstrated in vitro.

Binding characteristics of liver membranes in young steers when implanted with estradiol revealed increased GH receptor capacity compared to nonimplanted controls [142]; rate of weight gain was significantly correlated with ''high affinity'' GH receptor capacity. Perhaps because of this increased GH binding capacity, wether lambs implanted with estradiol + TBA had 150% higher hepatic levels of ''steady-state'' IGF-1 mRNA compared to controls and implanted steers had 68% higher ''steady-state'' IGF-1 mRNA in the longissimus muscle compared to nonimplanted controls [143]; circulating levels of IGF-1 were increased 32%. Thus increased local production of IGF-1 following implantation may play a role in increasing circulating IGF-1 as well as stimulating muscle growth through autocrine and/or paracrine mechanisms.

5.4. Muscle protein turnover and cellular response

The anabolic effect of growth promoting steroids in ruminants occurs very fast, within 2–7 days for PUN reduction [114,115,134,144], by 3–5 days for decreased urinary N excretion [145], 2–3 days for increased concentrations of circulating IGF-1 [143,146], by 24 days for cellular changes in the anterior pituitary gland [139] and 7–40 days for increased growth and carcass protein deposition [122,123,125,127,147] that ''declined in concert with decreasing concentration of serum estradiol'' [127]. Initially this increase in muscle protein was attributed to a decrease in muscle protein degradation together with a lesser reduction in muscle protein synthesis [31]. Subsequent research failed to confirm a reduction in muscle protein degradation during a period (0–30 days) when muscle protein accretion was increased 21 and 82% in steers implanted with estradiol or estradiol + TBA, respectively [127].

We have investigated in vitro bovine muscle protein synthesis and degradation, and muscle cell (rat C2 cells) proliferation using serum from steers implanted with estradiol + TBA [148]. Protein synthesis was enhanced without effects on protein degradation indicating muscle protein metabolism is affected in implanted steers indirectly via growth factors in the serum since direct application of the implant hormones in vitro had no effect [149]. Muscle cell proliferation was increased additively when serum was added in vitro from steers implanted with estradiol + TBA, GH or the combina-

tion [148]. In a subsequent experiment [150], however, serum taken from steers that received several implant treatments 28 days earlier did not result in enhanced in vitro protein synthesis or degradation and variable effects on cell proliferation. Using cloned sheep satellite cells, serum from steers implanted with estradiol + TBA showed enhanced mitogenic activity 21, 40, 115 and 143 days after implantation compared to controls [146]. Serum IGF-1 and serum IGF binding protein-3 were also markedly increased in the implanted steers. Furthermore, growth factor responsiveness (IGF-1 and basic fibroblast growth factor) of bovine satellite cells isolated from steers implanted with estradiol + TBA is enhanced in vitro compared to controls [151].

These results show that implantation with anabolic steroids in cattle enhance muscle growth factors (e.g. IGF-1, IGF-2) in the serum, and the responsiveness and proliferation of muscle satellite cells.

5.5. Other observations

Mature size, both weight and height, of steers was increased by continuous implantation every 84 days with DES compared to controls [152], an observation confirmed using Zeranol and estradiol + progesterone [153]. While these observations do not explain the mechanism of action of implants, they do provide rationale for some of the observed effects such as higher growth rate and increased leanness at a given body weight.

Cloned steers implanted with either estradiol + progesterone, TBA, or estradiol + TBA had less empty gastrointestinal tract weight (estradiol implant), larger livers, greater hide mass and greater daily protein accretion (129, 137 and 163 g/day, respectively) compared to controls (101 g/day) with no change in the rate of fat deposition (452g/day) [154]. Energy requirement for body gain was estimated to be reduced 19% by implants compared to controls. Estrogenic implants increase the maintenance energy requirements of steers [13,155–157] whereas androgenic (TBA) implants may reduce maintenance energy requirements [158].

When steers were implanted with estradiol + TBA for 24 days and then slaughtered for anterior pituitary cell differentiation [139], marbling (intramuscular fat deposits) was decreased one full marbling score compared to controls [159], perhaps indicating fat mobilization following implantation. This is supported by the observation that plasma nonesterified fatty acid concentration 3 weeks after steers were implanted with estradiol + progesterone is increased 12% compared to controls [160].

Catechol estrogens are estrogen metabolites formed in many tissues and structurally resemble catecholamines [42]. When injected on an equal molar dose, a catechol estrogen (4-hydroxyestradiol) had similar PUN depressing activity as estradiol in steers [42,129].

When steers were ''primed'' by injecting progesterone at a dose that inhibits the estrus response to injected estradiol in cows, no effect was observed on the anabolic ability of estradiol, based on PUN depression [129]. Therefore, the anabolic activity of estrogens may not require estrogenic activity.

Thus there are several possible mechanisms by which growth promoting implants improve growth and efficiency in cattle and sheep, several with supporting data but none appear to exclude all others. The IGF-1 axis appears to be the most plausible mechanism, either as a direct result of increased GH secretion or through enhanced GH receptor activity in the liver (and skeletal muscle?) leading to increased IGF-1 mRNA.

6. Safety

Implant products have been used safely in cattle and sheep for over 40 years. The safety aspects of implants have been reviewed and discussed many times [11,13,38–40,42,49,53,54,161–167]. In fact, the last European Agriculture Commission Scientific Conference on Growth Promotion in Meat Production held in 1995 [162] was largely devoted to this issue. Dealing specifically with ''anabolic agents with sex hormone-like activities'', it was concluded [163] that based on ''data available it seems most unlikely or even impossible that the residues following the use of these compounds according to good agriculture practice will ever exceed the set tolerance levels'', similar to a previous conclusion [38].

Implant products properly administered are placed subcutaneously in the center third of the posterior side of the ear of cattle and sheep. In this position,

the implants remain in place during their pay-out period and at slaughter, the ear is removed thereby eliminating any possibility of human consumption of implant material at the site of implantation.

In general, regulatory approvals require the determination of the no hormonal effect level in the most sensitive animal [53] which is then divided by a safety factor of 100 to give an acceptable daily human intake (ADI) per unit of body weight. Using average daily intake of animal tissues by humans multiplied times any residue found in these animal tissues following implantation gives a potential daily intake (PDI). An implant product will not be approved if the PDI exceeds the ADI. For TBA, depending on the metabolite, the ADI is 46 to 1193 times the PDI providing a very wide safety margin. Similar safety margins exist for the other implant products. Additionally, endogenous production in humans of ''natural'' hormones used in implant products greatly exceeds any potential intake from beef produced using implants [167].

Human safety of implant products properly used in beef production has been confirmed by the US FDA, World Health Organization, Food and Agricultural Organization, European Economic Community Scientific Working Group on Anabolic Agents (1981) and the European Community Scientific Conference on Growth Promotion in Meat Production (1995) [167]. An important component to successful regulatory control is a monitoring program that tracks any residues in the commercial meat supply [166].

''There is evidence from many European countries and from elsewhere for the illegal use of growth-promoting substances, often in the form of mixtures of recognized substances or of others which are not at present approved for use in any countries'' [162]. This is a major human health risk in these countries [53] where there are no regulatory protocols or residue monitoring programs.

7. Research needs

That implants for cattle and sheep are effective and safe are no longer issues. Research needs have been previously addressed [50]. The following areas would seem to be the most important in terms of implant effectiveness.

7.1. Compounds

As mentioned previously, estrogens seem to be the first requirement for the growth response to implants in ruminants. Most of the known estrogenic compounds have been tested and two are used commercially (estradiol and Zeranol). Estrogen analogs, however, have been little studied. Because of the possible dichotomy between estrogenic and anabolic activity, further research is needed to define to what degree estrogenic activity is required to elicit an anabolic response. There may be structurally similar compounds with limited estrogenic activity that promote the growth of ruminants.

The potential for dichotomy of action is more apparent for androgens. The synthetic androgen, TBA, is a good example of greater anabolic relative to androgenic potency compared to testosterone. Other androgenic compounds may be even better.

Of the protein type hormones, GH has the greatest potential especially since its growth and lean meat promotion appear to be additive and independent to that of the steroidal implants.

Thyroid active compounds would seem useful because of the known role the thyroid plays in basal metabolism and development. Earlier research, however, has not been encouraging in this regard. New research approaches and new thyroid analogs, however, may offer new insight into the possible role of this class of compounds in the growth of ruminants.

7.2. Dose

The chronology above is a record of continuing application of dose optimization. This will no doubt continue. Optimum implant dose is closely tied to pay-out rates. In one study [67], statistical treatment of the data indicated the optimum EB implant dose for steers was about 36 mg (26 mg estradiol) whereas more than 250–300 mg of TBA was required; optimum dosages for heifers were similar but less clear. The number of data points on which to base optimum doses was limited, however. With a silicone matrix, the optimum estradiol dose was determined to be 50–60 μg/day in steers [113], which is near to the dose (33 μg/day) determined using PUN reduction [129]. However, with an EB + progesterone implant encapsulated in an osmotic

membrane, a growth response in steers was observed through an average estradiol and progesterone delivery of 174 and 3720 μg/day, respectively, over 108 days [114,115]. This estradiol delivery is similar to that observed (164 μg/day) during the first 60 days after implanting EB + progesterone [117].

Therefore the optimum dose required daily and therefore the amount of estrogen and androgen required in an implant for maximum growth and efficiency promotion is still an uncertainty.

7.3. Pay-out pattern

The optimum pay out pattern is unknown. Combinations of estradiol + TBA or EB + TBA always give large growth responses in steers (30 to 60%) during the first 28–35 days after implantation which then diminishes over a 120 day period to a lesser final growth response (15 to 20%). On the other hand, estradiol + progesterone implants result in a smaller initial response (5 to 10%) that increases, especially with reimplantation at 60–80 days, to a similar response by 120 days. Therefore, is the optimum pay-out an initial burst (primer?) followed by a declining or steady pay-out, a steady pay-out or an increasing pay-out? Other than research using blood levels to estimate effective ranges in concentrations of implant hormones for growth stimulation, little is known about optimum pay-out patterns. Furthermore, since it appears that the pay-out of one implant component can be affected by the presence of another, the optimum pay-out of combinations is also unknown.

7.4. Delivery time

The optimum delivery time is closely tied to the phase of the beef production cycle when implants are administered. For short term finishing periods, a delivery time of 60–90 days is sufficient whereas pasture or growing (stocker) plus finishing periods could benefit from a delivery time of 300–350 days. The observation that implantation of young steers may result in an extended delivery time raises the possibility that a properly designed implant given at this time might suffice for the entire production cycle of an individual.

Pay-out of protein type anabolic hormones has been extended from 2 to 4 weeks for commercial use in lactating dairy cows. For practical application in growing beef cattle, a pay-out period of at least 8 weeks is required.

7.5. Mechanisms

As stated above, the mechanism of implants used commercially is still unclear. New results again point to the somatotropic axis as the mechanism for estrogens, perhaps affecting the liver and skeletal muscle by ‘‘upregulating’’ the sensitivity of these tissues to an increase in circulating levels of GH resulting in an increase in serum concentrations of tissue growth factors such as IGF-1. Such a postulation has been presented [132]. Androgens appear to occupy corticosteroid cell receptors in muscle thereby decreasing muscle protein breakdown. These are tenuous hypotheses at the moment especially considering they somehow apply only to ruminants. The observation that GH injection does not increase IGF-1 mRNA in the longissimus muscle of pigs whereas estradiol + TBA implantation does in steers [143] may be the first clue to the difference between ruminants and monogastrics in their response to anabolic steroids.

Newer research approaches are better clarifying events at the cellular level [50,132] which may give optimum dose rates and lead to alternate approaches to growth promotion in cattle and sheep.

References

[1] Natl. Acad. Sci.—Natl. Res. Council. Hormonal relationships and applications in the production of meats, milk, and eggs, Pub. 266, 714 and 1415. Washington DC, 1953, 1959 and 1966.

[2] A.P. Raun, R.L. Preston, History of hormonal modifier use, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Exp. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 1–7.

[3] Delaney Amendment, Federal Food, Drug and Cosmetic Act, US Congressional Record, 85th Congress, 2nd session, 1958, Sections 409(c)(3)(A), 512(d)(1)(H) and 512(e)(1)(B).

[4] Anonymous, New additives law, J. Agric. Food. Chem. 6 (1958) 725.

[5] R.G. Zimbelman, Approach to hormones as drugs for

animals — Relationship to Delaney Amendment, J. Anim. Sci. 38 (Suppl. 1) (1974) 68–76.
[6] F.H. Baker, V.H. Arthaud, Use of hormones or hormone active agents in production of slaughter bulls, J. Anim. Sci. 35 (1972) 752–754.
[7] F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn, Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997.
[8] M.T. Clegg, H.H. Cole, The action of stilbestrol on the growth response in ruminants, J. Anim. Sci. 13 (1954) 108–130.
[9] A.H. Trenkle, The mechanisms of action of estrogens in feeds on mammalian and avian growth, in: Use of Drugs in Animal Feeds, Natl. Acad. Sci. — Natl. Res. Council, Wash. DC, 1969, pp. 150–164, Publ. 1679.
[10] J.B. Kilkenny, J.E. Sutherland, The use of hormone administrations in commercial beef production in the UK, Vet. Rec. 87 (1970) 734–736.
[11] R.G. Zimbelman, J.W. Lauderdale, J.H. Sokolowski, T.G. Schalk, Safety and Pharmacologic evaluations of melengestrol acetate in cattle and other animals: A review, J. Am. Vet. Med. Assoc. 157 (1970) 1528–1536.
[12] H.D. Hafs, R.W. Purchas, A.M. Pearson, A review: Relationships of some hormones to growth and carcass quality of ruminants, J. Anim. Sci. 33 (1971) 64–71.
[13] R.L. Preston, Biological responses to estrogen additives in meat producing cattle and sheep, J. Anim. Sci. 41 (1975) 1414–1430.
[14] E.J. Umberger, Products marketed to promote growth in food producing animals: Steroid and hormone products, Toxicology 3 (1975) 3–22.
[15] P. Van der Wal, General aspects of the effectiveness of anabolic agents in increasing protein production in farm animals, in particular bull calves, in: F. Coulston, F. Korte (Eds.), Anabolic Agents in Animal Production, Environmental Quality and Safety, Georg Thieme, Stuttgart, 1976, pp. 60–78.
[16] A. Trenkle, The anabolic effect of estrogens on nitrogen metabolism of growing and finishing cattle and sheep, in: F. Coulston, F. Korte (Eds.), Anabolic Agents in Animal Production, Environmental Quality and Safety, Georg Thieme, Stuttgart, 1976.
[17] P.J. Buttery, B.G. Vernon, J.T. Pearson, Anabolic agents — some thoughts on their mode of action, Proc. Nutr. Soc. 37 (1978) 311–315.
[18] A. Trenkle, W. Burroughs, Physiological effects of estrogens in animal feeds with emphasis on growth of ruminants, in: Nutrition and Drug Interrelations, Academic Press, New York, 1978, pp. 577–611.
[19] R.J. Heitzman, Growth stimulation in ruminants, in: W. Haresign, D. Lewis (Eds.), Recent Advances in Animal Nutrition, Butterworths, London, 1979, pp. 133–143.
[20] R.J. Heitzman, The efficacy and mechanism of action of anabolic agents as growth promoters in farm animals, J. Steroid Biochem. 11 (1979) 927–930.
[21] H. Gailbraith, J.H. Topps, Effect of hormones on growth and body composition of animals, Nutr. Abstr. Rev. Series B 51 (1981) 521–540.
[22] R.J. Heitzman, Mode of action of anabolic agents, in: J.M. Forbes, M.A. Lomax (Eds.), Hormones and Metabolism in Ruminants, Agric. Res. Council, London, 1981, pp. 129–138.
[23] A. Trenkle, Mechanisms of action for the use of anabolics in animals, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 65–71.
[24] P. Van der Wal, P.L.M. Berende, Effects of anabolic agents on food producing animals, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 73–115.
[25] J.F. Roche, The use of natural steroids, hormonal and xenobiotics, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 119–127.
[26] J.F.S. Reid, Estradiol benzoate implants, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 143–154.
[27] J.C. Bouffault, J.P. Willemart, Anabolic activity of trenbolone acetate alone or in association with estrogens, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 155–179.
[28] R.G. Brown, Zeranol implants, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 181–192.
[29] J.W. Lauderdale, Use of MGA (melengestrol acetate) in animal production, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 193–212.
[30] P.J. Buttery, Hormonal control of protein deposition in animals, Proc. Nutr. Soc. 42 (1983) 137–148.
[31] P.J. Buttery, P.A. Sinnett-Smith, The mode of action of anabolic agents with special reference to their effects on protein metabolism — some speculations, in: J.F. Roche, D. O'Callagan (Eds.), Manipulation of Growth in Farm Animals, 1984, pp. 211–232.
[32] B.D. Schanbacher, Manipulation of endogenous and exogenous hormones for red meat production, J. Anim. Sci. 59 (1984) 1621–1630.
[33] R.L.S. Patterson, L.J. Salter, Anabolic agents and meat quality: A review, Meat Sci. 14 (1985) 191–220.
[34] L.A. Muir, Mode of action of exogenous substances on animal growth — An overview, J. Anim. Sci. 61 (Suppl. 2) (1985) 154–180.
[35] J.F. Roche, J.F. Quirke, The effects of steroid hormones and xenobiotics on growth of farm animals, in: P.J. Buttery, D.B. Linsay, N.B. Haynes (Eds.), Control and Manipulation of Animal Growth, Butterworths, London, 1986, pp. 39–51.
[36] R.L. Preston, Role of anabolic and repartitioning agents in the production of lean beef, in: Proc. SW Nutr. Mgt. Conf., Univ. Ariz. Tucson, 1987, pp. 12–25.
[37] H.A. Tucker, R.A. Merkel, Applications of hormones in the metabolic regulation of growth and lactation in ruminants, Fed. Proc. 46 (1987) 300–306.

[38] G.E. Lamming, Scientific report on anabolic agents in animal production, Vet. Rec. 121 (1987) 389–392.
[39] S.S. Collins, K.E. Belk, H.R. Cross, G.C. Smith, The EEC ban against growth-promoting hormones, Nutr. Rev. 47 (1989) 238–246.
[40] J. Kenney, D. Fallert, Livestock hormones in the United States, Natl. Food Rev. 12 (3) (1989) 21–24.
[41] W.G. Bergen, R.A. Merkel, Body composition of animals treated with partitioning agents: implications for human health, FASEB J. 5 (1991) 2951–2957.
[42] D.L. Hancock, J.F. Wagner, D.B. Anderson, Effects of estrogens and androgens on animal growth, A.M. Pearson, T.R. Dutson (Eds.), Growth Regulation in Farm Animals 7 (1991) 255–297.
[43] D.P. Hutcheson, J.P. Rains, J.W. Paul, The effects of different implant and feed additive strategies on performance and carcass characteristics in finishing heifers: A review, Prof. Anim. Sci. 9 (1993) 132–137.
[44] D.H. Beerman. (, Carcass composition of animals given partitioning agents, in: H.D. Hafs, R.G. Zimbelman (Eds.), Low-fat Meats Design Strategies and Human Implications, Academic Press, New York, 1994, pp. 203–232.
[45] S.K. Duckett, D.G. Wagner, F.N. Owens, H.G. Dolezal, D.R. Gill, Effects of estrogenic and androgenic implants on performance, carcass traits, and meat tenderness in feedlot steers: A review, Prof. Anim. Sci. 12 (1996) 205–214.
[46] D.H. Beerman, Existing and emerging strategies for enhancing efficiency and composition of meat animal growth, in: Proc. Sci. Conf. On Growth Promotion in Meat Prod., European Comm., Dir. Gen. VI Agric., Office of Official Pub. European Comm, Brussels, 1996, pp. 43–61.
[47] R. Renaville, S. Massart, A. Prandi, U. Fazzini, M. Sindic, L. Nicolay, M. Falaki, A. Burny, D. Portetelle, Aspects on the use of anabolic steroids in animal production, in: Proc. Sci. Conf. On Growth Promotion in Meat Prod., European Comm., Dir. Gen. VI Agric., Office of Official Pub. European Comm, Brussels, 1996, pp. 63–85.
[48] A.R. Peters, Effect of anabolic agents on carcase composition and meat quality: A review, in: Proc. Sci. Conf. On Growth Promotion in Meat Prod., European Comm., Dir. Gen. VI Agric., Office of Official Pub. European Comm, Brussels, 1996, p. 239.
[49] R.L. Preston, Current status on the use of steroids for growth promotion in cattle, in: Proc. Sci. Conf. On Growth Promotion in Meat Prod., European Comm., Dir. Gen. VI Agric., Office of Official Pub. European Comm, Brussels, 1996, p. 241.
[50] R.L. Preston, Estrogenic and androgenic implants — Present status and future potential in beef cattle, in: Proc. SW Nutr. Mgt. Conf., Univ. Arizona, Tucson, 1997, pp. 15–22.
[51] H.G. Dolezal, B.A. Gardner, Effects of estrogenic and androgenic implants on carcass quality and maturity, in: Proc. SW Nutr. Mgt. Conf., Univ. Arizona, Tucson, 1997, pp. 23–34.
[52] A. Trenkle, Mechanisms of action of estrogens and androgens on performance of cattle — hormonal basis, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 15–22.
[53] K.P. Lone, Natural sex steroids and their xenobiotic analogs in animal production: Growth, carcass quality, pharmacokinetics, metabolism, mode of action, residues, methods, and epidemiology, Crit. Rev. Food Sci. Nutr. 37 (1997) 93–209.
[54] R.L. Preston, The role of animal drugs in food animal production, in: G.E. Stefan (Ed.), Symposium on Animal Drug Use — Dollars and Sense, Ctr. Vet. Med., Food and Drug Admin, Rockville, MD, 1987, pp. 127–134.
[55] R. Botts, P. Anderson, K. DeHaan, Growth stimulants: Compounds, concentrations, combinations and regulations, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 10–14.
[56] A.P. Raun, C.P. Cooley, F.A. Smith, Comparative efficacy of the *trans* and *cis* isomers of DES in ruminants (Abstract), J. Anim. Sci. 31 (1970) 252.
[57] R.L. Preston, E.W. Klosterman, V.R. Cahill, Levels and isomers of diethylstilbestrol for finishing steers, J. Anim. Sci. 33 (1971) 491–496.
[58] R.L. Preston, F. Byers, K.R. Stevens, Estrogenic activity and growth stimulation in steers fed varying protein levels, J. Anim. Sci. 46 (1978) 541–546.
[59] D.P. Hutcheson, R.L. Preston, Stability of diethylstilbestrol and its effect on performance in lambs, J. Anim. Sci. 32 (1971) 146–151.
[60] B.S. Katzenellenbogen, J.A. Katzenellenbogen, D. Mordecai, Zearalenones: Characterization of the estrogenic potencies and receptor interactions of a series of fungal β-resorcylic acid lactones, Endocrinology 105 (1979) 33–40.
[61] J.E. Oldfield, C.W. Fox, A.V. Bahn, E.M. Bickoff, G.O. Kohler, Comestrol in alfalfa as a factor in growth and carcass quality in lambs, J. Anim. Sci. 25 (1966) 167–174.
[62] M. Stob, W.M. Beeson, T.W. Perry, M.T. Mohler, Effects of coumestrol in combination with implanted and orally administered diethylstilbestrol on gains and tissue residues in cattle, J. Anim. Sci. 27 (1968) 1638–1642.
[63] W.H. Hale, W.C. Sherman, W.M. Reynolds, H.G. Luther, The value of certain steroidal sapogenins in rations of fattening lambs and cattle, Proc. Soc. Exp. Biol. Med. 106 (1961) 486–489.
[64] J.A. Grandadam, J.P. Scheid, A. Jobard, H. Dreux, J.M. Boisson, Results obtained with trenbolone acetate in conjunction with estradiol 17β in veal calves, feedlot bulls, lambs and pigs, J. Anim. Sci. 41 (1975) 969–977.
[65] S.J. Bartle, R.L. Preston, D.J. Smith, Dual implantation of feedlot steers with commercial estradiol and trenbolone acetate implants, J. Prod. Agric. 3 (1990) 403–407.
[66] S.J. Bartle, R.L. Preston, R.E. Brown, R.J. Grant, Trenbolone acetate/estradiol combinations in feedlot steers: Dose–response and implant carrier effects, J. Anim. Sci. 70 (1992) 1326–1332.

[67] R.C. Herschler, A.W. Olmsted, A.J. Edwards, R.L. Hale, T. Montgomery, R.L. Preston, S.J. Bartle, J.J. Sheldon, Production responses to various doses and ratios of estradiol benzoate and trenbolone acetate implants in steers and heifers, J. Anim. Sci. 73 (1995) 2873–2881.
[68] S.K. Duckett, F.N. Owens, J.G. Andrae, Effects of implants on performance and carcass traits of feedlot steers and heifers, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 63–82.
[69] I. Istasse, P. Evrard, C. Van Eenaeme, M. Gielen, G. Maghuin-Rogister, J.M. Bienfait, Trenbolone acetate in combination with 17β-estradiol: Influence of implant supports and dose levels on animal performance and plasma metabolites, J. Anim. Sci. 66 (1988) 1212–1222.
[70] J.F.S. Coelho, H. Galbraith, J.H. Topps, The effect of a combination of trenbolone acetate and oestradiol-17β on growth performance and blood, carcass and body characteristics of wether lambs, Anim. Prod. 32 (1981) 261–266.
[71] A.R.M. Yasin, H. Galbraith, A note on the response of wether lambs to treatment with trenbolone acetate combined with oestradiol-17β or zeranol, Anim. Prod. 32 (1981) 337–340.
[72] A. Arnold, A.L. Beyler, G.O. Potts, Andro-stanazole, a new orally active anabolic steroid, Proc. Soc. Exp. Biol. Med. 102 (1959) 184–187.
[73] C.L. Johnson, J.M. Wilkinson, An investigation of the anabolic effects of androstenedione in young ruminants, Anim. Prod. 18 (1974) 179–189.
[74] P.D. Gluckman, B.H. Breier, S.R. Davis, Physiology of the somatotropic axis with particular reference to the ruminant, J. Dairy Sci. 70 (1987) 442–466.
[75] D.H. Beerman, D.L. DeVol, Effects of somatotropin, somatotropin releasing factor and somatostatin on growth, A.M. Pearson, T.R. Dutson (Eds.), Growth Regulation in Farm Animals 7 (1991) 373–426.
[76] P.J. Brumby, The influence of growth hormone on growth in young cattle, New Zealand J. Agric. Res. 2 (1959) 683–689.
[77] L. Breuer, R.L. Preston, W.H. Pfander, Effects of growth hormone and diethylstilbestrol on nitrogen metabolism in ruminants, Fed. Proc. 20 (1961) 372.
[78] A.S. Zainur, R. Tassell, R.C. Kellaway, W.R. Dodemaide, Recombinant growth hormone in growing lambs: Effects on growth, feed utilization, body and carcase characteristics and on wool growth, Aust. J. Agric. Res. 40 (1989) 195–206.
[79] D.L. Hancock, R.L. Preston, Titration of the recombinant somatotropin dosage that maximizes the anabolic response in feedlot steers, J. Anim. Sci. 68 (1990) 4117–4121.
[80] D.H. Beerman, D.E. Hogue, V.K. Fishell, S. Aronica, H.W. Dickson, B.R. Schricker, Exogenous human growth hormone-releasing factor and ovine somatotropin improve growth performance and composition of gain in lambs, J. Anim. Sci. 68 (1990) 4122–4133.
[81] R.J. Early, B.W. McBride, R.O. Ball, Growth and metabolism in somatotropin-treated steers: Growth, serum chemistry and carcass weights, J. Anim. Sci. 68 (1990) 4134–4143.
[82] R.J. Early, B.W. McBride, R.O. Ball, Growth and metabolism in somatotropin-treated steers: Carcass and noncarcass tissue components and chemical composition, J. Anim. Sci. 68 (1990) 4144–4152.
[83] R.J. Early, B.W. McBride, R.O. Ball, Growth and metabolism in somatotropin-treated steers: Protein synthesis and tissue energy expenditures, J. Anim. Sci. 68 (1990) 4153–4166.
[84] B.A. Crooker, M.A. McGuire, W.S. Cohick, M. Harkins, D.E. Bauman, K. Sejrsen, Effect of dose of somatotropin on nutrient utilization and insulin-like growth factor-1 by growing dairy heifers, J. Nutr. 120 (1990) 1256–1263.
[85] T.R. Kasser, C.A. Baile, Effect of weekly BST pellet implantation on growth, feed efficiency and lean yield of feedlot steers, FASEB J. 5 (1991) A1295.
[86] B.S. Dalke, R.A. Roeder, T.R. Kasser, J.J. Veenhuizen, C.W. Hunt, D.D. Hinman, G.T. Schelling, Dose–response effects of recombinant bovine somatotropin implants on feedlot performance in steers, J. Anim. Sci. 70 (1992) 2130–2137.
[87] W.M. Moseley, J.B. Paulissen, M.C. Goodwin, G.R. Alaniz, W.H. Claflin, Recombinant bovine somatotropin improves growth performance in finishing beef steers, J. Anim. Sci. 70 (1992) 412–425.
[88] F.J. Schwarz, D. Schams, R. Ropke, M. Kirchgessner, J. Kogel, P. Matzke, Effects of somatotropin treatment on growth performance, carcass traits, and the endocrine system in finishing beef heifers, J. Anim. Sci. 71 (1993) 2721–2731.
[89] G.W. Wolfrom, R.E. Ivy, C.D. Baldwin, Effects of growth hormone alone and in combination with Ralgro (Zeranol) in lambs, J. Anim. Sci. 61 (Suppl. 1) (1985) 249.
[90] R.E. Ivy, G.W. Wolfrom, C.K. Edwards III, Effects of growth hormone and Ralgro (zeranol) in finishing cattle, J. Anim. Sci. 63 (Suppl. 1) (1986) 217.
[91] R.L. Preston, S.J. Bartle, T.R. Kasser, J.D. Day, J.J. Veenhuizen, C.A. Baile, Comparative effectiveness of somatotropin and anabolic steroids in feedlot steers, J. Anim. Sci. 73 (1995) 1038–1047.
[92] T.S. Rumsey, T.H. Elsasser, S. Kahl, W.M. Moseley, M.B. Solomon, Effects of Synovex-S® and recombinant bovine growth hormone (Somavubove®) on growth responses of steers: I. Performance and composition of gain, J. Anim. Sci. 74 (1996) 2917–2928.
[93] Y. Ono, M.B. Solomon, T.H. Elsasser, T.S. Rumsey, W.M. Moseley, Effects of Synovex-S® and recombinant bovine growth hormone (Somavubove®) on growth responses of steers: II. Muscle morphology and proximate composition of muscles, J. Anim. Sci. 74 (1996) 2929–2934.
[94] H. Lapierre, C.K. Reynolds, T.H. Elsasser, P. Gaudreau, P. Brazeau, H.F. Tyrrell, Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: Net hormone metabolism by portal-drained viscera and liver, J. Anim. Sci. 70 (1992) 742–751.
[95] C.K. Reynolds, H. Lapierre, H.F. Tyrrell, T.H. Elsasser, R.C. Staples, P. Gaudreau, P. Brazeau, Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: Net nutrient metabolism by portal-drained viscera and liver, J. Anim. Sci. 70 (1992) 752–763.

[96] H. Lapierre, H.F. Tyrrell, C.K. Reynolds, T.H. Elsasser, P. Gaudreau, P. Brazeau, Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: Whole-body energy and nitrogen metabolism, J. Anim. Sci. 70 (1992) 764–772.
[97] D.D. Hongerholt, B.A. Crooker, J.E. Wheaton, K.M. Carlson, D.M. Jorgenson, Effects of a growth hormone-releasing factor analogue and an estradiol–trenbolone acetate implant on somatotropin, insulin-like growth factor I, and metabolite profiles in growing Hereford steers, J. Anim. Sci. 70 (1992) 1439–1448.
[98] M.A. Varner, S.L. Davis, J.J. Reeves, Temporal serum concentrations of growth hormone, thyrotropin, insulin, and glucagon in sheep immunized against somatostatin, Endocrinology 106 (1980) 1027–1032.
[99] G.S.G. Spencer, G.J. Garssen, I.E. Hart, A novel approach to growth promotion using auto-immunization against somatostatin. I. Effects on growth and hormone levels in lambs, Livest. Prod. Sci. 10 (1983) 25–37.
[100] G.S.G. Spencer, G. Garssen, P.L. Bergstrom, A novel approach to growth promotion using auto-immunization against somatostatin. II. Effects on appetite, carcass composition and food utilization in lambs, Livest. Prod. Sci. 10 (1983) 469–477.
[101] G.S.G. Spencer, Immuno-neutralization of somatostatin and its effects on animal production, Domest. Anim. Endocrinol. 3 (1986) 55–68.
[102] R.K. Chaplin, D.E. Kerr, B. Laarveld, Somatostatin immunization and growth in lambs, Can. J. Anim. Sci. 64 (Suppl.) (1984) 312–313.
[103] R.W. Harvey, J.D. Armstrong, E.P. Heimer, R.M. Campbell, Feedlot performance, carcass characteristics, hormones, and metabolites in steers actively immunized against growth hormone-releasing factor, J. Anim. Sci. 71 (1993) 2853–2859.
[104] R.E. Bloss, J.I. Northam, L.W. Smith, R.G. Zimbelman, Effects of oral melengestrol acetate on the performance of feedlot cattle, J. Anim. Sci. 25 (1966) 1048–1053.
[105] D.E. Ray, W.H. Hale, J.A. Marchello, Influence of season, sex and hormonal growth stimulants on feedlot performance of beef cattle, J. Anim. Sci. 29 (1969) 490–495.
[106] D.R. Hawkins, H.E. Henderson, H.W. Newland, Effects of melengestrol acetate on the performance of feedlot cattle receiving corn silage rations, J. Anim. Sci. 35 (1972) 1257–1262.
[107] D.M. Hendricks, R.T. Brandt Jr., E.C. Titgemeyer, C.T. Milton, Serum concentrations of trenbolone-17β and estradiol-17β and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17β, J. Anim. Sci. 75 (1997) 2627–2633.
[108] W.M. Moseley, J.W. Lauderdale, M.C. Goodwin, D.M. Meeuwse, J.R. Chenault, Inhibition of estrus and pregnancy in heifers by depot-formulated melengestrol acetate (MGA), J. Anim. Sci. 63 (Suppl. 1) (1986) 334.
[109] G.M. Hill, K.L. Richardson, P.R. Utley, Feedlot performance and pregnancy inhibition of heifers treated with depot-formulated melengestrol acetate, J. Anim. Sci. 66 (1988) 2435–2442.
[110] T.W. Geary, J.J. Reeves, D.W. Schafer, R.R. Evans, R.D. Randel, L.M. Rutter, R.G. Sasser, R. Guardia, B. Alexander, D. Holcombe, D.R. Hanks, D.B. Faulkner, Norgestomet implants prevent pregnancy in heifers on pasture, J. Anim. Sci. 75 (1997) 3089–3093.
[111] W.H. Hale, W.C. Sherman, E.A. White, G. Kuhn, R.B. Schnell, W.M. Reynolds, H.G. Luther, Absorption of diethylstilbestrol pellets in steers, J. Anim. Sci. 18 (1959) 1201–1207.
[112] R.T. Brandt Jr., Factors affecting release rates and blood levels of hormones from steroidal implants, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 34–39.
[113] J.F. Wagner, Estradiol controlled release implants: Efficacy and drug delivery, in: E. Meissonnier (Ed.), Anabolics in Animal Production, Office International des Epizooties, Levallois, France, 1983, pp. 129–142.
[114] R.L. Preston H, R.C. Herschler, Controlled release estradiol/progesterone anabolic implant in cattle, FASEB J. 4 (1990) A505.
[115] R.L. Preston, R.C. Herschler, Controlled release estradiol/progesterone anabolic implant in cattle, in: Texas Tech Univ. Anim. Sci. Res. Rpt., Lubbock, TX, 1992, pp. 140–142.
[116] T.S. Rumsey, R.R. Oltjen, A.S. Kozak, Implant absorption, performance and tissue analysis for beef steers implanted with diethylstilbestrol and fed an all-concentrate diet, J. Anim. Sci. 39 (1974) 1193–1199.
[117] T.S. Rumsey, A.C. Hammond, J.P. McMurtry, Response to reimplanting beef steers with estradiol benzoate and progesterone: Performance, implant absorption pattern, and thyroxine status, J. Anim. Sci. 70 (1992) 995–1001.
[118] T.L. Mader, Carryover and lifetime effects of growth promoting implants, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 88–94.
[119] D.M. Hendricks, R.L. Edwards, K.A. Champe, T.W. Gettys, G.C. Skelley Jr., T. Gimenez, Trenbolone, estradiol-17β and estrone levels in plasma and tissues and live weight gains of heifers implanted with trenbolone acetate, J. Anim. Sci. 55 (1982) 1048–1056.
[120] P.M. Riis, T.P. Suresh, The effect of a synthetic steroid (trienbolone) on the rate of release and excretion of subcutaneously administered estradiol in calves, Steroids 27 (1976) 5–15.
[121] D.W. Hunt, D.M. Hendricks, G.C. Skelley, L.W. Grimes, Use of trenbolone acetate and estradiol in intact and castrate male cattle: Effects on growth, serum hormones, and carcass characteristics, J. Anim. Sci. 69 (1991) 2452–2462.
[122] R.L. Preston, J.R. Rains, Response dynamics to Revalor®-S implantation in steers. Tech Bulletin 10, Hoechst–Roussel Agri–Vet Co., Somerville, NJ, 1994.

[123] R.L. Preston, J.R. Rains, Response dynamics evaluated for estradiol/TBA implantation, Feedstuffs 65 (3) (1993) 18–20.

[124] D.M. Hendricks, R.T. Brandt Jr., E.C. Titgemeyer, C.T. Milton, Serum concentrations of trenbolone-17β and estradiol-17β and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17β, J. Anim. Sci. 75 (1997) 2627–2633.

[125] B.J. Johnson, P.T. Anderson, J.C. Meiske, W.R. Dayton, Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics, and carcass composition of feedlot steers, J. Anim. Sci. 74 (1996) 363–371.

[126] R.J. Heitzman, D.N. Gibbons, W. Little, L.P. Harrison, A note on the comparative performance of beef steers implanted with anabolic steroids trenbolone acetate and estradiol-17β, alone, or in combination, Anim. Prod. 32 (1981) 219–222.

[127] J.M. Hayden, W.G. Bergen, R.A. Merkel, Skeletal muscle protein metabolism and serum growth hormone, insulin, and cortisol concentrations in growing steers implanted with estradiol-17β, trenbolone acetate, or estradiol-17β plus trenbolone acetate, J. Anim. Sci. 70 (1992) 2109–2119.

[128] L.P. Harrison, R.J. Heitzman, B.F. Samson, The absorption of anabolic agents from pellets implanted at the base of the ear in sheep, J. Vet. Pharm. Ther. 6 (1983) 293–303.

[129] D.L. Hancock, Mechanism of estrogen action which elicits the anabolic response observed in ruminants. Ph.D. Dissertation, Texas Tech Univ., Lubbock, TX, 1989.

[130] D.L. Hancock, R.L. Preston, Interaction between 17-β estradiol (E_2) and recombinant bovine somatotropin (bST) in the anabolic response of feedlot steers, J. Anim. Sci. 67 (Suppl. 1) (1989) 215.

[131] G. Selk, Implants for suckling steer and heifer calves and potential replacement heifers, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 40–50.

[132] W.R. Dayton, B.J. Johnson, M.R. Hathaway, Effects of a combined trenbolone acetate and estradiol implant (Revalor-S) on carcass composition and biological parameters of feedlot steers, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 23–33.

[133] R. Gopinath, W.D. Kitts, Growth hormone secretion and clearance rates in growing beef steers implanted with estrogenic anabolic compounds, Growth 48 (1984) 499–514.

[134] R.L. Preston, Reduction of plasma urea-N by diethylstilbestrol in ruminants, Proc. Soc. Exp. Biol Med. 129 (1968) 250–256.

[135] M.E. Grigsby, A. Trenkle, Plasma growth hormone, insulin, glucocorticoids and thyroid hormones in large, medium and small breeds of steers with and without an estradiol implant, Domest. Anim. Endocrinol. 3 (1986) 261–267.

[136] P.E. Moffitt, G.R. Wilson, R.L. Preston, Comparative response of castrate and intact male rats to diethylstilbestrol, Proc. Soc. Exp. Biol. Med. 148 (1975) 650–652.

[137] A.A. Wright, Endocrine control of metabolism with reference to the effect of oestrogens on growth, in: D. Lewis (Ed.), Digestive Physiology and Nutrition of Ruminants, Butterworths, London, 1961, pp. 242–248.

[138] R. Preston, E. Cheng, W. Burroughs, Growth and other physiological responses to diethylstilbestrol in diet of rats and guinea pigs, Proc. Iowa Acad. Sci. 63 (1956) 423–427.

[139] D.U. Thomson, C.S. Whisnant, R.L. Preston, M.F. Miller, M.A. Carr, C.R. Kerth, Short term effect of trenbolone acetate and estradiol on finishing steers: Anterior pituitary cell transformations in finishing beef steers, in: Texas Tech Univ. Agric. Sci. Natural Res. Tech. Rpt. No. T-5-316, 1996, pp. 11–12.

[140] P.A. Sinnett-Smith, C.A. Palmer, P.J. Buttery, Androgen receptors in skeletal muscle cytosol from sheep treated with trenbolone acetate, Horm. Metab. Res. 19 (1987) 110–114.

[141] S.H. Thompson, L.K. Boxhorn, W. Kong, R.E. Allen, Trenbolone alters the responsiveness of skeletal muscle satellite cells to fibroblast growth factor and insulin-like growth factor I, Endocrinology 124 (1989) 2110–2117.

[142] B.H. Breier, P.D. Gluckman, J.J. Bass, The somatotropic axis in young steers: influence of nutritional status and oestradiol-17β on hepatic high- and low-affinity somatotropic binding sites, J. Endocrinol. 116 (1988) 169–177.

[143] B.J. Johnson, M.E. White, M.R. Hathaway, C.J. Christians, W.R. Dayton, Effect of a combined trenbolone acetate and estradiol implant on steady-state IGF-I mRNA concentrations in the liver of wethers and the longissimus muscle of steers, J. Anim. Sci. 76 (1998) 491–497.

[144] D.U. Thomson, In vitro muscle cell protein synthesis and degradation, nitrogen balance and the feedlot response to trenbolone acetate, estradiol, and somatotropin in finishing beef steers, Ph.D. Dissertation, Texas Tech Univ., Lubbock, TX, 1996.

[145] S.E. Grebing, D.P. Hutcheson, R.L. Preston, Early reduction in urinary-N by diethylstilbestrol in lambs, J. Anim. Sci. 31 (1970) 763–766.

[146] B.J. Johnson, M.R. Hathaway, P.T. Anderson, J.C. Meiske, W.R. Dayton, Stimulation of circulating insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGFBP) due to administration of a combined trenbolone acetate and estradiol implant in feedlot cattle, J. Anim. Sci. 74 (1996) 372–379.

[147] R.L. Preston, D.U. Thomson, R. Grant, W. Nichols, Trenbolone acetate and estradiol dose titration study in feedlot steers, in: Texas Tech Univ. Agric. Sci. Natural Res. Tech. Rpt. No. T-5-316, 1996, pp. 7–8.

[148] D.U. Thomson, R.L. Preston, K.J. Morrow, Effect of serum from steers treated with a steroidal implant and somatotropin alone and in combination on in vitro muscle cell proliferation, protein synthesis and degradation, in: Texas Tech Univ. Agric. Sci. Natural Res. Tech. Rpt. No. T-5-316, 1996, pp. 13–15.

[149] D.U. Thomson, R.L. Preston, K.L. Morrow, Direct effects of cholesterol, trenbolone acetate, estradiol and progesterone on beef muscle cell protein synthesis, in: Texas Tech Univ. Agric. Sci. Natural Res. Tech. Rpt. No. T-5-316, 1996, p. 16.

[150] D.U. Thomson, R.L. Preston, K.J. Morrow, Effect of serum from steers implanted with different ratios and dosages of trenbolone acetate and estradiol on muscle cell proliferation, protein synthesis and degradation, in: Texas Tech Univ. Agric. Sci. Natural Res. Tech. Rpt. No. T-5-316, 1996, pp. 9–10.

[151] R.S. Frey, B.J. Johnson, M.R. Hathaway, M.E. White, W.R. Dayton, Growth factor responsiveness of primary satellite cell cultures from steers implanted with trenbolone acetate and estradiol-17β, Basic Appl. Mycol. 5 (1995) 71–79.

[152] R.L. Preston, Possible role of DES on mature size of steers, J. Anim. Sci. 47 (Suppl. 1) (1978) 436.

[153] D.D. Loy, H.W. Harpster, E.H. Cash, Rate, composition and efficiency of growth in feedlot steers reimplanted with growth stimulants, J. Anim. Sci. 66 (1988) 2668–2677.

[154] J.P. Hutcheson, D.E. Johnson, C.L. Gerken, J.B. Morgan, J.D. Tatum, Anabolic implant effects on visceral organ mass, chemical body composition, and estimated energetic efficiency in cloned (genetically identical) beef steers, J. Anim. Sci. 75 (1997) 2620–2626.

[155] T.S. Rumsey, H.F. Tyrell, P.W. Moe, Effect of diethylstilbestrol and Synovex-S on fasting metabolism measurements of beef steers, J. Anim. Sci. 50 (1980) 160–166.

[156] J.C. Solis, F.M. Byers, G.T. Schelling, L.W. Green, Anabolic implant and frame size effects on growth regulation, nutrient repartitioning and energetic efficiency of feedlot steers, J. Anim. Sci. 67 (1989) 2792–2801.

[157] T.S. Rumsey, A.C. Hammond, Effects of intake level on metabolic response to estrogenic growth promoters in beef steers, J. Anim. Sci. 68 (1990) 4310–4318.

[158] R.A. Hunter, J.E. Vercoe, Reduction of energy requirements of steers fed on a low-quality roughage diets using trenbolone acetate, Br. J. Nutr. 58 (1987) 477–483.

[159] C.R. Kerth, M.A. Carr, M.F. Miller, D.U. Thomson, R.L. Preston, C.S. Whisnant, Short term effects of implants on carcass characteristics, sensory traits, shear values, and the calcium dependent protease system, in: Texas Tech Univ. Agric. Sci. Natural Res. Tech. Rpt. No. T-5-316, 1996, pp. 27–28.

[160] T.S. Rumsey, T.H. Elsasser, S. Kahl, Roasted soybeans and an estrogenic growth promoter affect growth hormone status and performance of beef steers, J. Nutr. 126 (1996) 2880–2887.

[161] T.H. Jukes, Estrogens in beefsteaks, J. Am. Med. Assoc. 229 (1974) 1920–1921.

[162] J. Maddox, in: Proc. Sci. Conf. on Growth Promotion in Meat Prod., European Commission, Dir.-Gen. VI. Agric., Official Pub. European Comm, Brussels, 1996.

[163] B. Hoffmann, Problems of residues and health risks of anabolic agents with sex hormone-like activities, in: Proc. Sci. Conf. On Growth Promotion in Meat Prod., European Comm., Dir. Gen. VI. Agric., Official Pub. European Comm, Brussels, 1996, pp. 271–296.

[164] B. Hoffman, P. Evers, Risks of anabolic agents with sex hormone-like activities: Problems of residues, in: A. Rico (Ed.), Drug Residues in Animals, Adv. Vet. Sci. Comp. Med, Academic Press, New York, 1986, pp. 111–146.

[165] G.C. Smith, Consumer acceptance — domestically and internationally — of beef from cattle produced with use of growth promotant implants, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 182–192.

[166] H.R. Cross, M. Pfeiffer, USDA residue tracking program for growth promotants, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 193–198.

[167] R.L. Preston, Rationale for the safety of implants, in: F. Owens, D. Gill, G. Dolezal, B. Morgan, G. Horn (Organizing Comm.), Symposium: Impact of Implants on Performance and Carcass Value of Beef Cattle, P-957, Ok. Agric. Expt. Sta., Div. Agric. Sci. Natural Res., Ok. State Univ., Stillwater, 1997, pp. 199–203.