EFFECTS OF GENDER ON PROTEIN REQUIREMENTS AND THE SOMATOTROPIC AXIS IN FEEDLOT CATTLE

by

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To my grandparents Loy and Mildred Bailey, for their love, support, and always being a positive role model.

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ABSTRACT

Two trials were conducted to evaluate the effects of gender on optimal CP concentrations (TRIAL 1) and gender and anabolic implants on the somatotropic axis in feedlot cattle (TRIAL 2). More specifically, the objective of TRIAL 1 was to examine the effects of 3 dietary CP concentrations on performance, carcass characteristics, and serum urea nitrogen (SUN) in finishing steers and heifers and the objective of TRIAL 2 was to evaluate the effects of ovariectomy (OVX) and implantantation (200 mg of trenbolone acetate and 28 mg of estradiol benzoate; Synovex-Plus) on performance, serum urea nitrogen (SUN), serum IGF-1, and mRNA expression of hepatic IGF-1, GH receptor, and E receptor-α as well as pituitary GH, E receptor-α and GHRH receptor in feedlot heifers. TRIAL 1 results indicated that ADG was optimized when both steers and heifers were fed 12.5% CP and G:F was optimized for steers fed 12.5% CP but heifer G:F was optimal at 14.0% CP. Feeding diets containing 11.0% CP appears to cause a protein deficiency in both steers and heifers. TRIAL 2 data indicated that gender had no influence on performance or SUN in feedlot heifers. Serum IGF-1 is increased more in OVX heifers than intact heifers due to a greater response to implantation from the OVX heifers. However, the reason for the extra increase in serum IGF-1 is not clear, although trends in gene expression analysis suggest the possibility that the increased serum IGF-1 may be controlled outside of the somatotropic axis. Further research is warranted to examine the effects of OVX and anabolic implants on the somatotropic axis.

CHAPTER 1

INTRODUCTION

Environmental issues such as the evolution of N, P, and CH_4 into the environment offer the greatest challenges facing the feedlot industry today, and in the future. This is specifically true in areas of the southwest where urbanization is beginning to surround large commercial feedlots. Additionally, due to more efficient production practices in today's cattle industry there are larger numbers of heifers consistently entering United States feedlots than any time in recent history. This is also of specific importance in the southwest, where many of the crossbred heifers originate in Mexico and, according to United States law, must either be ovariectomized upon entry into the U.S. or have originated in a tuberculosis free herd and test negative for brucellosis. Furthermore, recent scientific advances have led to the production of more efficacious growth promotants (anabolic implants), feed additives (β-adrenergic agonists; ionophores; melengesterol acetate) and genetic selection, which, in turn, has produced more rapid changes in the cattle industry than ever before. These changes have drastically influenced finish weight, muscularity, frame size, and adipose deposition; all of which have a direct influence on the concentrations of nutrients necessary to maximize ADG, G:F, and carcass quality, not the least of which is CP.

Common sense tells us that profits in the feedlot industry not only depend on cattle prices, but also are a result of feed prices. On a per unit basis, CP is the costliest macro-nutrient added to feedlot diets. It is also well known that overfeeding CP leads to increased N secretion due to decreased utilization; thereby not only harming the

environment, but also unnecessarily increasing feed costs. However, underfeeding CP can adversely affect ADG, G:F and even carcass merit. All of which, not only lead to reduced profits, due to increased days on feed and reduced packer premiums, but also lead to increased re-introduction of nutrients into the environment, due to increased time on feed. Therefore, given today's growing environmental concerns and ever increasing feed costs, feeding proper CP concentrations to maximize gains, due to advances in production, realized through genetic improvement and technological advances in feed additives and anabolic implants, is of the utmost importance in the feedlot industry.

Since the development of diethylstilbestrol (DES), in 1938, anabolic implants, along with saturated fat, antibiotic feed additives and rBST, have garnered a great amount of negative publicity for the beef and dairy industry. In the beef industry, everyone from the packer to the consumer has expressed concern about the use of anabolic implants. Consumer concerns primarily focus on the existence of hormones in the final product. While it is true that meat products contain hormones, it is also true these hormones are present in all meat products, due to endogenous production, not the use of anabolic implants. Packer concerns are more understandable, in that anabolic implants have been shown to have deleterious effects on carcass quality. Still it is well known, but poorly advertised, that anabolic implants, the most powerful non-nutritional management tool available to feedlot managers, improve growth, increase muscularity, improve nitrogen utilization and reduce days on feed; thereby enhancing efficiency and reducing N evolution into the environment. Furthermore, until larger premiums are paid by the packer, for non-implanted beef, feeders will continue to utilize anabolic implants.

While it is well known that anabolic implants increase muscle and decrease adipose tissue, the exact mechanism through which they work has yet to be elucidated. Certainly, anabolic implants increase both circulating IGF-1 and the expression of IGF-1 genes, not only in the hepatocyte, but also in the periphery. Interestingly, this appears to be a direct effect on IGF-1 production, and not one mediated through the somatotropic axis, as there is usually no effect on GH in circulation.

The purpose of the studies presented herein, was to first examine the effects of increasing CP concentrations in feedlot diets on performance, carcass characteristics and serum urea nitrogen (SUN) in growing and finishing steers and heifers. Secondly we examined the effects and interactions of ovariectomization and a Synovex-plus implant (200 mg trenbolone acetate; 28mg estradiol) on growth performance, SUN, serum IGF-1, and the expression of genes associated with implanting and the somatotropic axis. Target genes included IGF-1, total GH receptor and E receptor-α in the liver as well as GH, E receptor-α, and GHRH receptor in the pituitary.

CHAPTER 2

LITERATURE REVIEW

United States Feedlot Industry

If a single book existed covering all aspects of feedlot management it would have to be a living document. From 1995 to 2000 there was a substantial swing in the numbers of steers and heifers in feedlots throughout the United States, which amounts to a 5% decline in steer numbers, and a 5% increase in heifer numbers (USDA, 2000a). In the southwest, many of those heifers are coming out of Mexico and are now required, by federal law, to be ovariectomized upon entry into the United States, therefore, in essence adding a completely new gender to commercial feedlots.

Dr. Mike Galyean at Texas Tech University has completed two separate surveys of consulting nutritionists within the past ten years. The first was conducted at New Mexico State University and was a survey of 6 consultants that serviced an average of 600,000 hd/year/consultant (Galyean, 1996). The second was conducted at Texas Tech University and included responses from 19 consulting nutritionists servicing an average of 735,278 hd/year/consultant (Galyean and Gleghorn, 2001).

Galyean (1996) reported that 50 to 80% of the cattle serviced by consultants were considered yearlings with the balance considered calf-fed cattle. Yearlings were those cattle in the feedlot less than 180 d and calves were those in the feedlot for more than 180 d. In both studies corn was the predominant grain fed in the feedlots surveyed, with milo being second and other grains consisting of wheat and barley. The 2001 study reported

that the average grain concentration in the diet was 78%. With the exception of 3 consulting nutritionists reporting in the 2001 study, all nutritionists for both studies reported that steam-flaking, while possibly used in combination with dry rolled or steam high moisture grains, was the primary method of grain processing.

Roughage levels were similar in both Galyean (1996) and Galyean and Gleghorn (2001) with the 1996 study reporting levels to vary from 3 to 11% and from 4.5 to 13.5% in the 2001 study. The 1996 study reported that the roughage sources used by the consultants included wheat straw, corn silage, alfalfa and sudangrass hay, and the 2001 study reported roughage sources of alfalfa, corn silage, cottonseed hulls and sudangrass hay.

Galyean and Gleghorn (2001) reported on the use of fat in the diet, and indicated that 75% of the consultants' clients added fat to the diet at a rate of 2.5 to 6.5% with the mean being 3.68 and the mode being 2.5%.

The 1996 study discussed the importance of bunk management. Among the six consulting nutritionists, they all used a variation of "clean" bunk management and agreed that consistency in the time of feed delivery was a critical feature of bunk management. Additionally, they all agreed that clean bunk management ultimately led to greater intake by cattle than those programs that allowed cattle feed to accumulate in the bunk. For a more in depth review of bunk management, utilizing a clean bunk approach, readers are referred to Duff (2001).

Five of 6 consultants, surveyed by Galyean (1996), recommended an aggressive implant program, which consisted of an estrogen implant at the start of the feeding program followed by a combination of an estrogen + trenbolone acetate (E + TBA) implant at 80 to 90 d pre-slaughter for yearling steers. For yearling heifers, an aggressive program consisted of an initial E implant followed by a TBA implant 80 to 90 d pre slaughter. It should be noted that with the approval of Synovex-Plus for both heifers and steers, heifers could now also receive the same implant program as the steers. The remaining consultant in this study used a moderate implant program, which consisted of an initial E implant followed by another E implant. More recently, 98% of feedlots reported using at least one anabolic implant (USDA, 2000b). Today there are numerous implants on the market that can be used in cattle of all ages (Table 2-1).

Galyean (1996) reported that all 6 consultants surveyed added an ionophore to the finishing diet at or slightly less than maximum legal concentration and 5 of the 6 fed melengesterol acetate (MGA) to the heifers.

Feedlot nutritionists have reported that formulated values for the percentage CP (DM basis) in finishing diets ranged from 12.5 to 14.4% with a large portion of supplemental protein supplied by urea, which ranged from 0.5 to 1.5% of the diet, and only 1 of 6 consultants polled formulated for escape protein (Galyean, 1996). In another survey, 19 consulting nutritionists reported feeding a high of 14%, a low of 12.5% and a mean of 13.3% CP with the addition of 0.78 to 1.35% urea, as supplemental protein, was recommended to clients (Galyean and Gleghorn, 2001) These findings are very similar to

those reported by Galyean (1996). Differently, however, in the 2001 study 14 of the 19 consulting nutritionist reported formulating diets for DIP and UIP. Furthermore Galyean (1996) reported that the CP concentrations reported in the questionnaires were greater than that called for by the NRC (1984). However, the majority of consultants reporting in the 2001 study indicated that they either exclusively used the 1984 NRC (8) or used it in combination with the 1996 NRC (4). This is interesting, given the similar protein concentrations fed by both sets of nutritionists, and indicates that, likely, recommendations made with respect to CP concentrations, were based on personal knowledge of different cattle types and their CP requirements.

Growth and Development

Growth is perhaps the most important measure related to profitability in the feedlot industry. Growth may be defined as the progressive increase in the size (volume, length, height, or girth), or weight of the animal that results from the accretion of nutrients over time (Marple, 2003) and is usually measured as the change in live weight or mass (Owens, et al., 1995). However, given the emphasis placed on producing a lean product for consumers, and reducing waste trim, the composition of growth becomes more important than rate per se. Ultimately the weights of closely trimmed retail product are preferable to live or carcass weights as an index of the commercial value of a carcass (Owens et al., 1995). Therefore, it is important for nutritionists to have an understanding of classical growth curves and the factors which affect them.

Growth is allometric or proportional to other tissues and organs in the body (Marple, 2003). Additionally, Marple (2003) reported on a number of classical growth and development studies. Two of interest are: Huxley (1932) who reported that skeletal growth proceeds from the cranium to the posterior and from the distal portion of the limbs to the medial portion of the body and Hammond (1932) who provided the first observation of the orderly progression of growth of tissues being nervous tissue, bone, muscle and fat, respectively. For the purpose of this review I will focus on the latter two of those two tissues as the economically important tissues to meat animal production.

Much literature is available indicating the difference in fat and protein accretion rates of growing animals. In a compilation of data from previous literature, Owens et al.

(1995), generated growth curves that showed protein to increase in a linear manner with empty body weight (EBW), while fat increased in a quadratic manner compared to EBW and attributed this to change in body composition to a change in degree of maturity and not rate of gain. Additionally, nutrition can not only affect rate of gain, but also affect composition of gain. When energy does not limit growth, empty body contains an increasingly smaller percentage of protein, an increasingly larger percentage of fat, and reaches chemical maturity when additional weight contains little additional protein (NRC, 1996). Conversely, when net energy intake is restricted, rate of fat accretion often is reduced although protein growth may continue at nearly normal rates if protein intake is adequate (Anderson et al., 1988). Moreover, the weight at which cattle reach the same chemical composition differs depending on mature size and sex; hence the composition is different even when the weight is the same (Fortin et al., 1980). Furthermore, other variables such as BW, frame size, breed type, and the use of growth stimulants, all affect body composition (Fox and Black, 1984).

Beef cattle research has indicated that bulls deposit more lean tissue than steers (Vanderwert et al., 1985; Anderson et al., 1986), and steers deposit a greater amount of lean tissue than heifers (Fox and Black, 1984). Crouse et al. (1985) indicated that intact males had a greater ADG and DMI compared to castrates and they were also larger and more muscular than castrated males at a given rib fat percentage. Field (1971), reported that intact males had a 2.6% average advantage in cutability compared to castrates, while

Crouse et al. (1985) showed no advantage in leanness of castrates compared to intact males.

Data for differences in heifer growth and composition after ovariectomy is also variable. Dinusson et al. (1950) and Horstman et al. (1982) reported that ovariectomy had an adverse influence on rate and efficiency of growth. Conversely, Ray et al. (1969) showed that, in the feedlot, steers outperformed intact heifers, which, in turn outperformed ovariectomized heifers. However, Klindt and Crouse (1990) and Hamernik et al. (1985) indicated that ovariectomy did not influence ADG compared to intact heifers, and Field et al. (1996) indicated that there was no difference in total gain when ovariectomized heifers were compared to intact heifers. Klindt and Crouse (1990) also indicated that ovariectomized heifers were not as lean as intact heifers and Field et al. (1996) reported that intact virgin heifers had greater LM area and less KPH compared to ovariectomized heifers, but they reported no differences in fat depth.

In addition to management practices concerning gender, the use of anabolic implants also affects growth and development and in some cases may even completely rescue the loss of growth associated with gender alteration. Certainly, due to their positive effects on protein deposition, anabolic implants have revolutionized the feedlot industry. The effects of these implants on growth and development will be given in depth consideration later in this review.

Crude Protein Requirements

Due to environmental concerns, more stringent EPA regulations and the cost of CP supplements, feeding optimal concentrations of CP is of the utmost importance in animal agriculture. Unique features of ruminant N digestion and metabolism require not only consideration of tissue protein and amino acid needs of the ruminant animal, but also of the microbial population inhabiting the digestive tract, particularly the rumen (Galyean, 1996). Overfeeding CP may be detrimental to the environment by increasing nitrogen excretion and consequently increasing the evolution of NH_3 into the environment (Klemesrud et al., 2000), but underfeeding CP may result in decreased ADG, DMI, G:F and carcass quality (Galyean, 1996). Furthermore, prediction of body composition in cattle is necessary to predict net nutrient requirements and BW, ADG, frame size, breed type, sex, use of growth stimulants, special dietary effects and the nutritional management system, all affect body composition (Fox and Black, 1984). For the purpose of this review the focus will be on the effects of gender and implant status on the prediction of net nutrient requirements followed by a review pertaining to the prediction of CP requirements in beef cattle based on NRC models and contemporary literature.

In examining gender related differences in protein requirements it is important to look at advances that have been made in the swine industry. Unruh et al., (1996) indicated that when gilts and barrows were fed identical diets, to similar weights, gilts had a greater percentage of lean than barrows, and increased dietary lysine enhanced

cutability in high lean growth gilts fed to 104 kg but not 127 kg, while additional lysine produced fatter carcasses in barrows finished to both 104 and 127 kg. Friesen et al. (1994) reported that, within genotype, gender differences result in alterations in growth performance, protein accretion, and lysine requirements in growing-finishing swine. Additionally, Cromwell et al. (1993) indicated that gilts require higher levels of dietary protein (lysine) to maximize rate and efficiency of gain and carcass muscle than do barrows. They further speculated that penning barrows and gilts separately and feeding diets that more closely met their nutrient requirements might provide an economic advantage to producers.

In beef cattle, Anderson et al. (1986) indicated that bulls consume less feed per unit of body weight or metabolic body size than steers, but excelled steers in the proportion of dry matter gained as protein. These data suggest, that, on a body weight basis, bulls utilize protein more efficiently than steers, and therefore, when compared to steers, may not have an increased CP concentration requirement.

Estimating CP requirements in growing and finishing beef cattle and other ruminants is 2-fold. First there is a N requirement for proper microbial fermentation in the reticulo-rumen and secondly there is the need for post ruminal amino acids for tissues of the host ruminant (NRC, 1984). Still the NRC (1984) estimated CP requirements in growing and finishing cattle in terms of CP without considering the two requirements separately, but in 1985 the Subcommittee on Nitrogen Usage in Ruminants presented a rationale for expressing protein requirements in terms of absorbed protein, which has

since become synonymous with metabolizable protein (MP). Metabolizable protein accounts for rumen degradation of protein and separates requirements into needs of microbes and needs of the animal (NRC, 1996). Metabolizable protein is then defined as true protein absorbed by the intestine, supplied by microbial protein and undegraded intake protein (UIP; NRC, 1996). However, although the cattle feeding industry has modified dietary CP over time, by increasing dietary CP concentrations above that predicted to be required by the factorial equations of the 1984 NRC, feedlot diets are still typically formulated on the basis of percentage CP, with little or no effort to consider ruminant N transactions and/or the protein/amino acid requirements of the host animal (Galyean, 1996). Furthermore, it is interesting to note that out of 19 feedlot nutrition consultants, 8 solely use the 1984 NRC as their information source and an additional 4 more use the 1984, in combination with the 1996 NRC (Galyean and Gleghorn, 2001). However, this still presents a problem, in that dietary protein requirements may be over estimated due to an imbalance of DIP and UIP in the diet, when utilizing the CP system. Thus, not only does the MP system allow for more accurate prediction of bacterial CP synthesis (BCP) and UIP than the 1984 model, but it also alleviates the invalid assumption that all feedstuffs have an equal extent of protein degradation in the rumen, with CP being converted to MP with equal efficiency in all diets (NRC, 1996).

This discrepancy is evidenced in a number of research projects conducted in the 1990's and early in the new millennium. Galyean (1996) used data from a study by Malcolm-Callis et al. (1995) to evaluate and compare protein requirements calculated

with factorial and metabolizable protein systems. These results indicated, that using the factorial model (NRC, 1984), for a 91% concentrate steam-flaked corn based diet (CP, 14.5%; MP, 6.58%;), the CP requirement of large-framed British x Continental steers was calculated to be 10.49% of the actual DMI of 9.24 kg/d, that 3.41% of the dietary CP could be supplied by NPN (1.19% urea) and that 7.08% should be intact protein. However, when using the MP system of the NRC (1996), which calculates the dietary CP need as CP needed from UIP + CP needed for BCP synthesis, it was found that the animal requirement was 767.34 g, in the Malcolm-Callis et al. (1995) study, and that the BCP synthesis for the given diet was 839.87 g/d, which is considered the microbial requirement. However, it must be recognized that this latter number is not indicative of the total MP supplied from the microbes and that microbial MP supplied is calculated as 64% of BCP synthesis or 537.52. Therefore, microbial MP supplied all but 229.82 g of MP needed by the animal which is considered the UIP MP requirement. This number is then divided by 0.8, or the efficiency of conversion of MP to net protein, which yields a requirement for an additional 287.28 g of MP needed from UIP. Therefore, final calculations reveal a protein requirement of 1127.15 g (839.87 + 287.28) or 12.2% CP based on a DMI of 9.24 kg/d. Even so, this assumes that DIP and UIP are balanced, which is not the case in high grain diets. Due to the relatively high UIP in grains, the actual calculated DIP for the Malcolm-Callis diet was only 608.14 leaving a deficit of 231.73 g, which could be derived from the addition of 0.87% urea. This, however, would adjust that diet to a total of 13.7% CP, in order to meet BCP requirements. Consequently,

an oversupply of total CP, relative to the total requirements, would be fed. These calculations by Galyean (1996) were in agreement with earlier work that indicated the actual CP requirement, in finishing steers, to be 12% due to an overfeeding of UIP (Shain et al., 1994) and it was later suggested that the CP requirement could be as low as 11.1%, if the proper amount (6.4% of dietary DM) of DIP was fed (Shain et al., 1998), but Gleghorn et al. (2004), suggested the optimal CP concentration, for finishing steers, fed a steam-flaked corn based diet, to be approximately 13%. The differences between the Shain et al. (1998) data and that provided by Gleghorn et al. (2004) were likely associated with dietary differences. In the former study, the diet was a dry rolled corn based diet, and in the latter, the grain source was steam-flaked corn. Therefore, the further processing of the corn likely increased the DIP requirement, because of increased starch digestion in the rumen (Cooper et al., 2002). The results of Gleghorn et al. (2004) reported a DIP concentration of 6.07%, in a diet containing 11.5% CP derived with cottonseed meal as the sole supplemental CP source, which would be below the DIP requirement indicated by Shain et al. (1998), and a DIP concentration of 8.2% for a diet containing 13% CP and urea as the sole supplemental CP source. While the latter is well in excess of the 6.4% DIP requirement, reported by Shain et al. (1998), for a dry rolled corn based diet, it is very much in agreement with the 8.2% DIP proposed by Cooper et al. (2002) and, again, is likely a reflection of grain processing. In conclusion, recent research indicates that the factorial model of CP inclusion may be accurate as long as there is a balance of DIP and UIP. However, as these variables are not included in the

factorial model; the models provided in the NRC (1996) should be used when estimating CP requirements, and particular attention should be paid to the balance between DIP and UIP. This is supported by research that places optimal CP concentrations between 11.0 and 13.0% CP, which is ultimately associated with the balance between DIP and UIP. It should also be noted that this balance is particularly influenced by grain source and processing as well as by protein source. Research indicates that CP inclusion above 13%, that shows an improvement in ADG, is likely confounded by an underfeeding of DIP and may indicate a wasting of resources.

Anabolic Implants:

The development of the anabolic implant is the single most important technological advance to the cattle feeding industry to date. Certainly, implants have been associated with negative effects such as decreased quality grade, dark cutting beef, decreased meat tenderness, rectal and vaginal prolapse, and buller steer syndrome. However, the advantages still out weigh the disadvantages. The advantages of label use of implants include improved ADG, G:F, HCW and lean tissue accretion. These advantages ultimately lead to significant economic benefits. When steers were fed for 197 d, the benefit of re-implanting, compared to implanting only once, was $15.04, whereas the loss from never implanting is $19.73; therefore, the total cost of not implanting is $34.77 (Pritchard, 1999). Furthermore, the USDA (2000b) indicated that the utilization of anabolic implants returned an estimated $15 to $40 per animal.

In 1938, diethylstilbestrol (DES), the first synthetic estrogen, was created by Sir E. Charles Dodds in England. In 1940, a French medical journal reported that DES caused mammary tumors in male mice. Then, in 1943, Lorenz (as cited by Montgomery et al., 2001) noted the benefits of implanting poultry with DES, when they showed that DES implants increased the muscle and fat content of the legs and breast by 300% in cockerels. It was not until five years later that the effects of DES on cattle were reported by Dinusson et al. 1948; 1950 (as cited by Raun and Preston, 1997). These authors showed, in a pair of studies conducted at Purdue University in 1947 and early 1948, DES improved ADG, G:F, increased length of leg and back, width of back and appetite.

However, DES carcasses were slightly “hooky”, or more mature in appearance, and DES caused vulva swelling, extended estrus, produced a nymphomaniac stance, elevated tail heads and pronounced mammary and teat development. However, at the time, these side effects were considered to be very negative and without any immediate, apparent solution. This, combined with the apparent carcass fat reduction, undoubtedly resulted in the considerable delay in the commercial application of this technology (Raun and Preston, 1997). Even so, in 1947, just prior to the Dinusson studies, the FDA formally granted approval of DES to be used as a miscarriage preventative, and a husband and wife team, George and Olive Smith, a physician and a biochemist, published a report extolling the use of high doses of DES during pregnancy, which launched wide scale use of the pseudoestrogen. Interestingly, in 1953, the physician William Dieckmann, of the University of Chicago, conducted the first controlled, double-blind study on the use of DES during pregnancy and revealed that DES did not reduce miscarriages but actually increased them, yet physicians continued to prescribe DES to women until 1971.

The use of DES was not considered beneficial in cattle until 1953 (Montgomery et al., 2001), when W. H. Hale and Wise Burroughs discussed the idea of feeding DES to cattle (Raun and Preston, 1997). After a number of experiments, beginning in 1953, Burroughs and co-workers (as cited by Raun and Preston, 1997), indicated that DES increased gains up to 35% and reduced feed costs up to 20% without a reduction in fatness or meat quality. Additionally, none of the undesirable effects previously reported with DES implants were noted. Therefore, through subsequent studies and the efforts of

Wise Burroughs, Iowa State College and Eli Lilly, FDA clearance for DES came only one year after the first report on the results of feeding DES to cattle on November 5, 1954. This approved DES for feeding to beef cattle at a rate of 10 mg/head/day, and it was on the market within 4 weeks as a premix called STILBOSOL. STILBOSOL ultimately provided the foundation for the animal product business of ELANCO Animal Health (Raun and Preston, 1997). By the end of 1955 it was estimated that 6 million cattle were being fed DES (Montgomery et al., 2001) and later estimates showed that between 80 and 95% of all feedlot cattle were fed DES in their lifetime (Cheatham, 2005).

Bill Wick and Henry Fry, two formulation chemists for Eli Lilly and Co., Inc., who did the formulation work on DES implants for use in poultry, approached George Varnes, the President of Lilly Industrial and Agriculture Products Division, to determine if Lilly had an interest in developing DES implants for cattle. Varnes declined the offer however (Raun and Preston, 1997); citing personal communications with T. M. Means, 1996). The same two men then cooperated with Chas. Pfizer, Inc., (Terre Haut, IN) in the development of DES implants for use in cattle. Pfizer then obtained the FDA approval for DES and implantation of feedlot cattle began in 1957 (Raun and Preston, 1997). From the time it hit the shelves, DES continually struggled with poor press and misconceptions related to its effect on animal sexual behavior and complaints and discounts from packers regarding reduced carcass quality (Montgomery et al., 2001). In 1959, DES was banned in chicken and lambs after high DES levels in these animals

produced side effects such as male breast growth in humans. Despite this, the FDA maintained that there were no detectable residues (> 2 – 3 ppb) found in meat from beef animals, and only 0.5% of beef livers had detectable DES residues, and the use of DES continued to increase until 1972 (Montgomery et al., 2001).

In the early 1970's, a number of blows hit DES including: Arthur Herbst, M.D. and co-workers (1971) published a report in the New England Journal of medicine linking in utero DES exposure to vaginal cancer in young women; the FDA issued a drug bulletin to physicians, stating that DES is contra-indicated for use in pregnant women; the incidence of detectable residues in beef livers rose from 0.5 to 2.0 to 2.5%; (likely due to off label administration). Then in 1972 an article in Science reported that DES was a spectacularly dangerous carcinogen, the FDA was accused of political manipulation in an election year for its support of DES, and congress began pressuring the FDA to enact the Delany (zero residue) amendment. Consequently, in 1972, the FDA banned oral DES, instituted a 120 d withdrawal on DES implants, prosecuted feeders who did not use it correctly and in 1973 the use of DES implants was also banned. Still, it should be noted that the report of adenocarcinoma in daughters of mothers who had taken DES was likely influenced by the size of the dose given to the women during pregnancy. These doses, up to 125 mg/d, were massive, 12.5 fold greater than that approved for cattle and if considered on a BW basis over 100 times that approved for cattle. Additionally, in order for a person to consume that amount of DES, they would have to eat almost 42 kg (91.6 lbs) of beef daily, if the residue was at 3 ppb.

Following the ban of DES in 1973 the FDA began prosecuting cattle feeders with DES contaminated cattle, but, in 1974, the U.S Court of appeals overturned the ban, citing the FDA had failed to hold proper hearings. All the while, in 1976, Dr. Tom Jukes and many others maintained that the cancer risk associated with DES was infinitesimal and the USDA continued to detect presumed residues at < 2–3 ppb, based on total radioactivity using ^{14}C-labeled DES (Raun and Preston, 1997). In 1977, the FDA held further hearings and DES was banned for good in 1979.

Even so, the findings of these of these cases are still considered to not only be unclear, but to also lack objectivity (Montgomery et al., 2001). Certainly, it would seem the risk of getting struck by lightning was greater than getting cancer from consuming meat products from animals that had been fed or implanted with DES, even if one could consume 42 kg of meat daily. Raun and Preston (1997) share a similar outlook, and maintain that, if it were still on the market, DES would likely still rank as one of the most effective growth promotants, and, that in the feed form, DES offered dosage and withdrawal control not available with implant products. However, both Raun and Preston (1997) and Montgomery et al. (2001) agree that its banning likely paved the way for the development of the large number of anabolic implants available today, which will be discussed heretofore.

Interestingly, prior to the approval of DES, estradiol benzoate/progesterone implants were approved for steers, in 1956. Then estradiol benzoate/testosterone implants were approved for use in heifers (1958); MGA was approved for oral use in

heifers (1968); zeranol implants (36 mg) were approved for cattle (1969); silastic estradiol implants were approved for cattle (1982); estradiol/progesterone implants were approved for calves (1984); trenbolone acetate (TBA) implants were approved for cattle (1987); estradiol/TBA implants were approved for steers (1991); BST injections were approved for lactating dairy cows (1993); estradiol/TBA implants were approved for heifers (1994); zeranol (72 mg) implants were approved for cattle (1995); estradiol/TBA implants were approved for stocker cattle (1996). As of 2001 there were 24 different anabolic implants that could be classified by their active ingredient profile (single or combination) and their respective dosage registered with the FDA as suitable for use, through all stages of growth, in beef cattle (Montgomery et al., 2001) and as of 2005 their was one additional implant available bringing the total now available for use in beef cattle to 25.

These implant active ingredients can be split into three major groups: estrogens (estradiol; zeranol; estradiol benzoate), androgens (TBA; testosterone propionate) and progestins (progesterone). Of these ingredients, estradiol, zeranol, and TBA may be given alone as single ingredient implants and estradiol may be combined with TBA in a combination implant, while estradiol benzoate may be combined with either, TBA or progesterone. Each of these 24 implants are listed in Table 2-1 according to their active ingredient, and including their dosage, trade name, category, relative potency, and the group of cattle for which they are approved. The remainder of this section will discuss

the effects of each of the active ingredients in these implants on performance and the somatotropic axis

In a 249 d trial Apple (1989) in his Master's research at Kansas State University examined the effects of implanting Holstein steers with zeranol (36mg; Ralgro), TBA (140 mg; Finaplix), estradiol benzoate (20 mg) plus progesterone (200 mg; Synovex-S), a combination of zeranol plus Synovex-S, or a combination of Finaplix plus Synovex-S at 56 d intervals from d 0 to 168 but not 224, due to the lack of a d 0 withdrawal for Ralgro. Implants were compared to each other, in addition to an unimplanted control at d 56, 112, 168, 205 and 249 and for each period (0 to 56,1 ; 56 to 112, 2; to 168, 3; 168 to 205, 4). Steers implanted with Ralgro had greater ADG than their unimplanted counterparts at d 56, 112, 168, 205 and 249, but only outgained control steers in period 1. Steers implanted with Finaplix out gained controls at d 56, 112 and 205 d of the study but did not differ from control steers at d 168 or for the entire feeding period (249 d). Synovex-S steers outgained control steers at every date and for the first two sampling periods. However, in this trial, no differences were noted between Finaplix, Ralgro or Synovex-S treatments at any date, implant period or for the overall study. Holsteins implanted with Finaplix + Synovex S had greater ADG than those implanted with Finaplix alone at each date with the exception of d 56 and for the second and overall feeding periods. Additionally, they had greater ADG than steers implanted with Ralgro or Synovex S alone at d 112, 168 and 205 and greater ADG than those steers implanted with Ralgro alone during the second feeding period. Furthermore they outgained those steers

implanted with Finaplix + Ralgro for the overall feeding period (d 249). Steers implanted with Finaplix + Ralgro had greater ADG than controls at each date and during periods 1 and 2 but did not differ from those steers implanted with Finaplix, Ralgro, or Synovex-S at any sampling date or sampling period. Dry matter intake in this study did not differ from controls or as a result of treatment for any date; however Finaplix + Synovex-S implanted steers consumed more than controls and steers implanted with Finaplix during the second, third and fourth feeding period and more than Ralgro implanted steers during the second feeding period. Steers implanted with Finaplix + Ralgro consumed more than controls and steers implanted with Finaplix during the second, third and fourth feeding period and more than those implanted with Ralgro or Synovex-S during the second feeding period. Feed efficiencies, however, did not differ as much. No feed efficiency differences were observed at d 56, 112, 168 or 249. Steers implanted with Finaplix or Ralgro alone did have better feed efficiencies than those implanted with Finaplix + Ralgro during period 4 and steers implanted with Finaplix had better feed efficiencies than control steers during period 4.

Cheatham (2005) in a trial conducted at the University of Arizona, consisting of a non-implanted control or one of three treatments: 1) animals implanted with zeranol (36mg; Ralgro) on d 0 followed by a combination of estradiol benzoate (20 mg) and progesterone (200 mg; Synovex S) at d 84 and 168; 2) animals implanted with Ralgro on d 0, Synovex S on d 84 and combination of estradiol (28 mg) and trenbolone acetate (200 mg; Synovex Plus) on day168; 3) animals implanted with Ralgro on d 0, and Synovex-

Plus on d 84 and 168. This study found that implanted Holstein steers had greater final BW, ADG (23%) and DMI (18%) than control steers, but found no differences between treatment groups and that G:F did not differ between implanted and control steers.

Research reported for beef steers returns similar results. Preston et al. (1995) reported that Continental x British steers, implanted with a combination of Synovex-S and Finaplix-S, exhibited a 28% increase in ADG, a 5% increase in DMI and 21% better gain efficiency than non implanted steers. Perry et al. (1991) indicated when compared to non-implanted controls, E (28 mg) + TBA (140 mg) implanted animals had greater ADG and G:F in both Angus and Angus x Simmental steers. Additionally, crossbred steers implanted with either Revalor-S (120 mg TBA, 24 mg estradiol) or Synovex-Plus (200 mg TBA, 28 mg estradiol benzoate) showed improved ADG and G:F compared to non-implanted controls and that animals implanted with Synovex-Plus tended to have greater ADG than those implanted with Revalor-S (Hermesmeyer et al., 2000). Furthermore, in a review of literature, Duckett et al. (1997) reported that, while steers implanted with either an estrogenic or a combination implant performed better than unimplanted steers; steers implanted with combination estrogen and androgen compounds had the highest gains and feed efficiency. Similarly, Gardner et al. (1999) reported that steers fed for maintenance only and implanted with TBA either singularly or in combination with estradiol 17β had greater weight retention than non-implanted steers or those that received an estradiol 17β implant only.

Implanting beef heifers reaps much the same rewards as implanting either beef or Holstein steers. Implanting with androgen based or combination implants results in improved performance, but implanting with a single dose of an estrogen based implant does not improve performance in intact heifers (Duckett et al., 1997; Montgomery et al., 2001). Research on the effects of anabolic implants in OVX heifers is limited. Duckett et al. (1997), in a review of the literature, reported that, despite the paucity of the data present, implanting OVX heifers with androgen plus estrogen appeared to result in increased gain, feed intake, carcass weight, and dressing percentage and reduced feed:gain. Adams et al., (1990) reported that implanting OVX heifers with Synovex-H resulted in weight gains similar to those in intact heifers implanted with Synovex-H and Garber et al. (1990) indicated that OVX heifers exhibited a fourfold greater response to implantation than intact heifers.

These data indicate that combination implants are more effective than single ingredient implants in steers, intact heifers, and OVX heifers. Additionally, it appears that anabolic implants may restore performance losses associated with ovariectomization in feedlot heifers.

While there is ample research proving that modern anabolic implant programs improve feedlot performance, the biological mechanisms responsible for this improvement in performance is still poorly understood. Implanting with E implants alone causes an increase in circulating IGF-1 (Grigsby and Trenkle, 1986; Breier et al., 1988). Circulating IGF-1 can be much as 53% and 85% greater at d 14 and 28,

respectively, in steers implanted with an E + TBA implant compared to non-implanted controls (Dunn et al., 2003). Additionally, serum IGF-1 has been shown to increase when E implants were followed by E + TBA implants (Cheatham, 2005). Others have shown that TBA alone or implanted synergistically with an E implant also increases IGF-1 (Lee and Henricks, 1990; Hunt et al., 1991). Mader and Kreikemeier (2006) found IGF-1 concentrations to be higher in the E + TBA implanted heifers compared to controls, but found no differences in IGF-1 between controls and those animals implanted only with E or TBA.

Given this data, one would expect that implantantation would also cause an increase in circulating GH, as IGF-1 is a GH dependent peptide that stimulates cell proliferation and differentiation (Florini et al., 1991). However, data has been conflicting and the majority of data reveals the possibility that the effects of anabolic implants may be independent of the somatotropic axis. Trenbolone acetate alone or implanted synergistically with an estrogenic (E) implant has been shown to have no effect on GH (Hunt et al., 1991; Hayden et al., 1992). Even more interesting, Cheatham (2005) reported that, in unimplanted Holstein steers, the GH response to a GRF challenge was actually greater than that of implanted steers and that GH response did not differ due to the aggressiveness of the implant program. On the other hand, in a study conducted at Texas Tech University, using a 2 x 3 factorial design to examine the effectiveness of anabolic implants and somatotropin on feedlot steers, both serum GH and plasma IGF-1 was increased by anabolic implants, and exogenous administration of somatotropin

increased plasma IGF-1 and decreased serum GH (Preston et al., 1995). Furthermore, the same study showed that the effects of anabolic implants and somatotropin on ADG and G:F were additive, while they were opposing in relation to DMI, indicating the possibility that their modes of action may be independent of one another. All data combined reveals a dilemma, if compared to the central dogma of the somatotropic axis, and actually points to some other mechanism triggering increased circulating IGF-1.

Gene expression data has shown that implanting lambs with E (8 mg) + TBA (40 mg) increased steady-state hepatic IGF-1 expression by 150% in implanted wethers compared to non-implanted wethers and suggests that the liver may be the source of at least part of the increased circulating IGF-1 in steroid implanted animals (Johnson et al., 1996). Additionally, the same authors demonstrated, in a companion study, that implanting steers with Revalor-S (24 mg E_2 + 120 mg TBA) increased IGF-1 mRNA in the longissimus muscle by 68%. In vitro research indicates that treating bovine satellite cell (BSC) cultures with either E or TBA increases IGF-1 mRNA level and cell proliferation and causes an increase in both E receptor-α and androgen receptor mRNA when added at 0.001 nM each. However, neither E receptor-α, nor androgen receptor mRNA levels were affected by E or TBA, compared to non-treated controls, when the steroids were added at greater concentrations (Kamanga-Sollo et al., 2004). The timeline for measurable effects of implantation with E + TBA on gene expression is not consistent, as reports have varied from as early as 7 d post implantation (Pampusch et al., 2003) to as late as 28 d post-implantation (Dunn et al., 2003). It should be noted

however, that the Pampusch et al. (2003) study was a comparison within steer (ie. d 0 to d 7) and the Dunn et al. (2003) study compared implanted to non implanted steers. Which could also explain differences in final expression between the studies, as the IGF-1 expression on d 26, in the Pampusch et al. (2003) study, was reported to have tripled compared to d 0 expression, while Dunn et al., (2003) showed that, although IGF-1 in circulation increased as early as 14 d post-implantation, mRNA expression of IGF-1 in the muscle did not increase until d 28 and was 2.4 fold greater in implanted calves than non-implanted calves at that time.

Table 2-1 Anabolic implants approved for use in U.S. Beef Cattle.[a]

Trade name	Active ingredient[b]	Category	Approval[c]
Elanco Animal Health (Greenfield, IN)			
Compudose 200	E (25.7 mg)	Estrogen	S, H
Schering Plough (Madison, NJ)			
Ralgro	Z (36 mg)	Estrogen	Cattle
Ralgro Magnum	Z (72 mg)	Estrogen	S
Ivy Laboratories (Overland Park, KS)			
Encore	E (43.8 mg)	Estrogen	S, H
Component T-S	TBA (140 mg)	Androgen	S
Component T-H	TBA (200 mg)	Androgen	H
Component E-C	EB (10 mg), P (100 mg)	Estrogen, Progestin	S, H
Component E-S	EB (20 mg), P (200 mg)	Estrogen, Progestin	S
Component E-H	EB (20 mg), TP (200 mg)	Estrogen, Androgen	H
Component TE-S	E (24 mg), TBA (120 mg)	Estrogen, Androgen	S
Component TE-G	E (8 mg), TBA (40 mg)	Estrogen, Androgen	S, H
Implus-C	EB (10 mg), P (100 mg)	Estrogen, Progestin	S, H
Implus-S	EB (20 mg), P (200 mg)	Estrogen, Progestin	S
Implus-H	EB (20 mg), TP (200 mg)	Estrogen, Androgen	H
Fort Dodge Animal Health (Overland Park, KS)			
Synovex-C	EB (10 mg), P (100 mg)	Estrogen, Progestin	S, H
Synovex-S	EB (20 mg), P (200 mg)	Estrogen, Progestin	S
Synovex-H	EB (20 mg), TP (200 mg)	Estrogen, Androgen	H
Synovex-Plus	EB (28 mg), TBA (200 mg)	Estrogen, Androgen	S, H
Intervet Inc. (Flemmington, NJ)			
Finaplix-H	TBA (200 mg)	Androgen	H
Revalor-S	E (24 mg), TBA (120 mg)	Estrogen, Androgen	S
Revalor-H	E (14 mg), TBA (140 mg)	Estrogen, Androgen	H
Revalor-IS	E (16 mg), TBA (80 mg)	Estrogen, Androgen	S
Revalor- IH	E (8 mg), TBA (80 mg)	Estrogen, Androgen	H
Revalor-G	E (8 mg), TBA (40 mg)	Estrogen, Androgen	S, H
Revalor-200	EB (20 mg), TBA (200 mg)	Estrogen, Androgen	S

[a] Adapted from Cheatham (2005) and Montgomery et al. (2001)

[b] E = estradiol, Z = zeranol, TBA = trenbolone acetate, EB = estradiol benzoate, P = progestin

[c] S = steers; H = heifers

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CHAPTER 3

EFFECTS OF INCREASING CRUDE PROTEIN CONCENTRATIONS ON PERFORMANCE AND CARCASS CHARACTERISTICS OF GROWING AND FINISHING STEERS AND HEIFERS

Abstract

A 2x3 factorial design was utilized to ascertain the effects of three dietary protein concentrations on performance, carcass characteristics, and serum urea nitrogen (SUN) in finishing steers and heifers. Animals were blocked by gender (n=9) and randomly assigned to a diet containing 11.0, 12.5 or 14.0% CP (n=6). Animals were weighed and bled at 28 d intervals for 84 d, overall daily DMI, ADG, and G:F were calculated and SUN was analyzed as a repeated measure throughout the study. Following slaughter, carcass data was collected for HCW, dressing percent (DP), KPH, 12th rib backfat (BF), LM area, marbling score (MS), and yield grade (YG). Heifers consumed less and gained less than steers ($P < 0.01$) and had lighter HCW ($P < 0.01$), less KPH ($P = 0.08$), lower MS ($P = 0.02$) and YG ($P < 0.01$) and numerically had less BF ($P = 0.15$). Dry matter intake ($P = 0.02$), ADG ($P = 0.05$), HCW ($P = 0.08$), and YG ($P = 0.09$) increased quadratically with increasing dietary protein. Hot carcass weight ($P = 0.02$), BF ($P = 0.04$) and YG ($P = 0.09$) increased linearly with increasing dietary protein. Gain:feed, DP, KPH, LM area and MS was not affected by dietary protein concentration and G:F, DP, and LM area did not differ between genders. However, there was a gender x dietary protein interaction ($P = 0.01$) for G:F with steers being the most efficient at 12.5% CP, while heifers were most efficient at 14.0% CP. This could be a reflection of the heifers'

overall advantage in leanness. Gender had no effect on SUN concentrations, but SUN increased linearly ($P < 0.01$) with respect to dietary protein concentrations and both linearly ($P < 0.01$) and quadratically ($P = 0.01$) increased with respect to time. In conclusion, quadratic responses in DMI and ADG indicate that a 12.5% dietary CP concentration is optimal for either steers or heifers during the finishing period.

Introduction

Considering today's growing environmental concerns, and increasing feed costs, feeding appropriate CP concentrations is of the utmost importance in the feedlot industry. Feeding excess CP may be detrimental to the environment by increasing nitrogen excretion; thereby, increasing the evolution of NH_3 into the environment (Klemesrud et al., 2000), whereas underfeeding CP may result in decreased ADG, DMI, G:F and carcass quality (Galyean, 1996). Formulated values for percentage CP (DM basis), in commercial feedlot diets, range from 12.5 to 14.4% (Galyean, 1996) and university studies evaluating optimal CP concentrations, for finishing steers, have found varying results. Shain et al. (1994) reported that diets containing 12% CP were optimal for feedlot steers and Gleghorn et al. (2004) suggested 13% CP to be optimal for finishing steers. Fox and Black (1984) indicated that prediction of body composition is necessary to estimate net nutrient requirements, and steers deposit more lean tissue than heifers. Additionally, heifers now make up a larger percentage of cattle in feedlots than in the past. From 1995 to 2000, United States feedlots reported that the percentage of heifers on feed rose from 35 to 40% and the percentage of steers on feed declined from 65 to 60% (USDA, 2000). In Canada, a similar change was observed from 1996 to 2004, as the percentage of heifers on feed rose from 36 to 41%, and the percentage of steers on feed fell from 64 to 59% (OCA, 2005). Given these findings it is important to maintain a close watch on optimal CP concentrations for growing steers and heifers. The focal point of this project was to evaluate the effects of varying concentrations of CP on performance, carcass characteristics and SUN in finishing steers and heifers.

Materials and Methods

All procedures were approved by the University of Arizona Institutional Animal Care and Use Committee.

Fifty-two English x Continental steers and 54 heifers, were acquired from the University of Arizona V-V ranch following a post-weaning (83 d) preconditioning period at the University of Arizona feedlot.

A 2 x 3 factorial design was used to ascertain the effects of increasing dietary CP concentrations on growth performance and carcass characteristics in growing and finishing steers and heifers. On January 14, 2003, a preliminary un-shrunk BW was taken prior to feeding, using a digital scale while animals were restrained individually in a hydraulic squeeze chute, and calves were subsequently blocked by gender (n = 9 pens/gender) and, due to large weight variations within gender (steers 243 ± 33 kg; heifers 221 ± 38 kg), weight class (n = 3/gender). Calves, within each weight class, were stratified by weight and randomly assigned to one of 18 pens (6.1 x 18.3 m; 5 or 6 calves/pen) and one of three isocaloric, steam-flaked corn based diets (Table 3-1; n = 6 pens/CP treatment), balanced according to NRC (1996) tabular values, to provide 11.0, 12.5 or 14.0% CP. On January 15, 2003, steer calves were implanted with Synovex-S (20 mg estradiol benzoate and 200 mg progesterone; Fort Dodge Animal Health, Overland Park, KS) and heifer calves were implanted with Synovex-H (20 mg estradiol benzoate and 200 mg testosterone propionate; Fort Dodge Animal Health) and re-weighed, to obtain an initial BW (Table 3-2). Each weight class was subsequently subjected to a growing period of 56, 84, or 112 d for heavy, intermediate and light weight

calves, respectively, in order to obtain a similar initial BW for the beginning of the finishing period (84 d).

Diets were mixed approximately every other day and feed samples were collected at 2 week intervals to be analyzed for: DM (oven drying at 55°C until no further weight loss), CP (% N x 6.25; LECO Corporation, St. Joseph, MI, USA), ADF (ANKOM filter bag system; ANKOM Technology, Fairport, NY, USA) and ash (combusted 6 h in a muffle furnace at 500°C).

During the growing period, steers were programmed to gain 1.02 kg/d and heifers to gain 0.91 kg/d, according to NRC (1996) guidelines. Steers were programmed to gain more than heifers due to initial differences in BW and traditional beliefs that heifers reach an ideal compositional endpoint at lighter weights than steers. Additionally, unshrunk BW was taken, before feeding, at 28 d intervals to determine ADG, G:F and the amount of feed required to achieve proper growth during the subsequent period.

Upon completion of the growing period, animals were re-implanted with Synovex-Plus (28 mg estradiol benzoate and 200 mg trenbolone acetate; Fort Dodge Animal Health) and maintained in the same pens. Feed offered was increased at a rate of 0.91 kg/calf d^{-1} until feed remained in the bunk, and a slick bunk management system, similar to that described by Duff (2001), was subsequently applied. During the finishing period, unshrunk BW was taken, before feeding, and bunks were swept every 28 d for determination of ADG, DMI and G:F. Blood samples were drawn via jugular puncture, allowed to clot at room temperature and serum was collected via centrifugation at 1,000 x g for 20 min and stored (-20º C) for later analysis of SUN (mg/dL). On d 84, calves were

transported to Sun Land Beef (Tolleson, AZ) and humanely slaughtered, via captive bolt and exsanguination. Hot carcass weight was immediately collected and KPH, LM area, BF, and MS data were acquired after a 48 h chill. Yield grade was calculated using criteria set forth by the USDA (1997). Additionally, following completion of the study optimal MP concentrations were calculated for steers and heifers, using the equations set forth by the NRC (1996).

Statistical analysis was performed using the PROC MIXED procedures of SAS (SAS Inst. Inc., Cary, NC, USA). The model included gender (steers and heifers), treatment (CP; 11.0, 12.5 and 14.0%) and the gender x treatment interaction as the fixed effects. The random effect was block and the subject was id within pen (experimental unit). Serum urea nitrogen was analyzed in the same way, except sampling date was considered a repeated measure. Therefore the model also included gender x day, treatment x day and gender x treatment x day interactions as fixed effects; autoregressive of order one was used for a covariance structure. Due to CP treatment differences in G:F and ADG during the growing period, initial finish BW was used as a covariate in the analysis of performance data during the finishing period. Carcass characteristics were analyzed using the same model as performance data, with one exception, due to gender differences in HCW, carcass merit was analyzed using HCW as a covariate and quality grad distributions for animals grading choice or better were analyzed using the PROC FREQ procedures of SAS (SAS Inst. Inc., Cary, NC, USA) for Chi-Square analysis. Final treatment responses were measured for linear and quadratic orthogonal contrasts, and LS means were reported for each variable, with significance considered to be $P < 0.10$.

Results and Discussion

Initial growing period BW (Table 3-2) did not differ with respect to CP treatment ($P > 0.76$); however, steers were heavier than heifers ($P < 0.01$). As per the intention of the growing program, restricted DMI in both steers and heifers produced no CP treatment effect ($P > 0.10$), heifers consumed significantly less feed than steers and steers gained more than heifers ($P < 0.01$; Table 3-2). However, increasing CP did result in a quadratic ($P = 0.04$) increase in ADG. Accordingly, with respect to increasing CP concentrations, end BW and G:F increased quadratically ($P = 0.06$) and linearly ($P = 0.08$), respectively. Even so, there was no efficiency difference observed between steers and heifers, during the growing period.

For the finishing period, heifer DMI ($P < 0.01$; Table 3-2) was less than that of steers. The DMI response was quadratic ($P = 0.02$) with respect to treatment with the greatest DMI observed at the 12.5% CP level. Average daily gain (Table 3-2) increased quadratically ($P = 0.05$), with CP treatment, and steers exhibited greater ADG than heifers ($P < 0.01$). Final body weight (Table 3-2) was greater for steers than heifers ($P < 0.01$) and was quadratic ($P = 0.01$), with respect to CP treatment. No difference in G:F (Table 3-2) was observed with respect to gender ($P = 0.13$) or CP treatment ($P = 0.94$). However, a gender x CP treatment interaction ($P = 0.01$) was observed for G:F, as indicated by the following LS interaction means: steers (0.195; 0.205; 0.188), heifers (0.191; 0.181; 0.198) for 11.0, 12.5, and 14.0% CP treatments, respectively.

Carcass characteristics (Table 3-3) were analyzed using HCW as a covariate, due to steers exhibiting a markedly heavier HCW than heifers ($P < 0.1$); thereby, in effect,

adjusting carcass merit measures for HCW. Additionally, HCW increased both linearly ($P = 0.02$) and quadratically ($P = 0.08$) with CP concentrations. Following covariate analysis CP treatment had no effect on KPH, BF, LM area, MS, or YG ($P > 0.13$). A quadratic effect was observed for DP ($P = 0.06$) as cattle consuming the 12.5% CP had the lowest DP. Interestingly, following covariate analysis, heifers exhibited a greater ($P = 0.01$) DP than steers, which was likely due to their advantage in LM area ($P = 0.01$). However, KPH, BF, MS or YG were not different between genders ($P > 0.15$). Furthermore, Chi-Square analysis, of the distribution of animals grading choice or better, indicated no differences as related to treatment ($P = 0.75$), with percentages of 14, 11 and 13 for the 11.0, 12.5 and 14.0% CP diets, respectively, or gender ($P = 0.18$) with percentages of 23 and 15 for steers and heifers, respectively.

Serum urea nitrogen (Table 3-2) was not affected by gender ($P = 0.52$) and there were no two or three way interactions due to gender ($P > 0.14$). However, SUN increased linearly ($P < 0.01$) with respect to CP treatment. Additionally, we observed a treatment x day interaction ($P < 0.01$; data not shown), however the interaction was in magnitude and not direction.

Although cattle were programmed for equal ADG, within gender, we still observed small increases in ADG and G:F with increasing CP concentrations. However this is not surprising as it has been well documented that increasing dietary CP increases both ADG and G:F during times of reduced feed intake (Cole and Hutcheson, 1990; Fluharty and Loerch, 1995; Rossi et al., 2001).

Data reporting the optimal CP concentration to achieve maximal performance varies. Shain et al. (1998) suggested that the optimal CP concentration to achieve maximal growth could be as low as 11.1%, Gleghorn et al. (2004) suggested the optimal CP inclusion to be 13% and consulting nutritionists have reported ranges in recommended CP concentrations to be between 12.5 to 14.4% (Galyean, 1996; Galyean and Gleghorn, 2001). For the present study, utilizing the metabolizable protein system equations from the NRC (1996), calculations of optimal CP concentrations were 11.25% for steers and 11.23% for heifers. While this is below the optimal CP concentrations reported by the previously mentioned authors, it certainly offers an explanation as to why we did not see further improvement in performance past the 12.5% CP diet. However, it is likely the variations reported for optimal CP concentrations are due to dietary differences and the need to balance UIP and DIP. Galyean (1996) used data from a study by Malcolm-Callis et al. (1995) to illustrate this very point. Galyean reported that for a 91% concentrate, steam-flaked corn based diet, the optimal CP concentration for large-framed British x Continental steers was calculated to be 12.2% of the actual DMI of 9.24 kg/d, using the MP system of the NRC (1996). Even so, Galyean goes on to report that the MP system assumes that DIP and UIP are balanced, but due to the relatively high UIP in grains, the actual calculated optimal CP for that diet was 13.7%, due to an overfeeding of UIP and the need to add larger quantities of DIP, in order to meet optimal bacterial CP concentrations. Others have since reported that optimal CP concentrations are affected by grain processing, as further processing may increase the optimal DIP concentration, because of increased starch digestion (Cooper et al., 2002), which likely explains the

discrepancy between reports by Shain et al. (1998) and Gleghorn et al. (2004) for optimal CP concentrations. In the Shain et al. (1998) study, a dry rolled corn based diet was fed, which contained 11.1% CP and 6.4% DIP, and focused on the 6.4% DIP being the key to feeding such low amounts of CP. The results of Gleghorn et al. (2004) reported a DIP concentration of only 6.07% in a steam-flaked corn based diet containing 11.5% CP and a DIP concentration of 8.2% for a diet containing 13% CP. While the latter is well in excess of the 6.4% optimal DIP concentration reported by Shain et al. (1998) for a dry rolled corn based diet it is very much in agreement with the 8.3% DIP proposed by Cooper et al. (2002), which is likely a reflection of grain processing. Calculations of DIP (Table 3-1), in the present study, indicate that DIP concentrations for the 11.0% and 12.5% diets were below and equal to, respectively, the 6.4% DIP reported as optimal by Shain et al. (1998). This could also explain the reduced DMI for the 11.0% diet, as it could be a reflection of reduced microbial efficiency. It should also be noted that concentrations in all diets were below the 8.2% reported to be optimal by Gleghorn et al. (2004) and the 8.3% reported to be optimal by Cooper et al. (2002). Additionally, when dietary DIP concentrations are pooled and tabular values are used for calculations of DIP, there is a DIP deficit of 250 g/d for steers and 227 g/d for heifers in the present study. However, whether or not increasing DIP to 8.2% of the diet would have resulted in increased performance is beyond the scope of this study.

Only HCW and DP were affected by CP treatment in the present study, which is not surprising. Gleghorn et al. (2004) reported that increasing CP concentration increased both HCW and DP, but did not have any effect on MS, LM area or BF. While the same

authors did report a linear increase in YG with increasing CP concentrations the YG range in their study was almost identical to the present study, and, like the present study, YG across treatments was below 3.0.

In agreement with previous studies (Gleghorn et al., 2004; Huntington et al., 2001), SUN increased with increasing amounts of CP. Additionally, Johnson and Preston (1995) suggested that plasma urea N values in excess of 5 to 8 mg/dL are indicative of excessive N intake and N wastage; consequently, one could consider that SUN measurements below or even between 5 – 8 mg/dL could signify a CP deficiency, which would explain the reduced ADG for calves fed the 11.0% CP diet in the present study.

Performance differences between steers and heifers, during the growing period, in the present study, should be disregarded as it was the intention of the programmed rate of gain to induce such differences. However, for the finishing period, DMI was greater for steers than heifers. This is likely a function of BW, because as a percentage of BW, DMI for steers and heifers is almost identical.

Average daily gain and consequently end BW was also greater for steers than heifers, which is in agreement with previous research. Ray et al. (1969) showed that, in the feedlot, steers outperformed heifers. Feed efficiency was not different between steers and heifers. However, given the fact that heifers consumed as much per unit of BW as did steers, and had reduced ADG compared to steers, one could speculate that if heifers were finished to a common end BW with steers they would be less efficient.

Because of greater start weight, greater ADG and greater final weight, steers had a greater HCW than heifers. Interestingly, heifers exhibited a greater DP than steers, which

is not surprising given that heifers also had a greater LM area than steers. This, in itself, is also surprising in that previous research has indicated either no differences in LM area (Hassen et al., 1998) or that steers deposit a greater amount of lean tissue than heifers (Fox and Black, 1984; Hassen et al., 1998). Even so, no differences were seen between steers and heifers for KPH, BF, MS or YG. While much of this data is antithetical to traditional thought it should be noted that, in the present study, there was a gender x CP interaction and heifers were actually more efficient than steers at the 14.0% CP level, which could possible serve as an explanation for the larger LM area and the lack of a difference in YG between steers and heifers.

Conclusion

Results indicated that ADG was optimized when both steers and heifers were fed 12.5% CP. However, G:F was optimized for steers fed 12.5% CP, and heifers fed 14.0% CP. Feeding diets containing 11.0% CP appears to cause a protein deficiency in finishing steers and heifers.

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Table 3-1. Ingredient and nutrient composition of diets (DM basis)

	CP[1]		
Ingredient	11.0%	12.5%	14.0%
Sudangrass hay	10.00	10.00	10.00
Steam-flaked corn	78.95	75.20	71.50
Soybean meal	-	3.75	7.45
Urea	0.55	0.55	0.55
Tallow	3.00	3.00	3.00
Cane molasses	5.00	5.00	5.00
Mineral supplement[2]	2.50	2.50	2.50
Dry matter[3]	86.74	86.44	87.03
Ash[3]	5.34	5.41	6.12
CP[3]	11.14	12.75	14.99
ADF[3]	4.82	5.61	5.90
DIP[4]	5.51	6.41	7.66
NE_m, Mcal/kg	2.20	2.19	2.18
NE_g, Mcal/kg	1.51	1.50	1.49
Effective NDF	9.88	9.85	9.81
TDN[5]	88.87	88.52	88.18

[1] Diets were formulated to contain 11.0, 12.5, and 14.0% CP (DM basis)

[2] Mineral supplement composition (DM basis): limestone, 47.059%; dicalcium phosphate, 1.036%; potassium chloride, 8.000%; magnesium oxide, 3.448%; ammonium sulfate, 6.667%; salt, 12.000%; cobalt carbonate, 0.002%; copper sulfate, 0.157%; iron sulfate, 0.133%; calcium iodate, 0.003%; manganese sulfate, 0.500%; selenium premix (0.16%), 0.125%; zinc sulfate, 0.845%; vitamin A (30,000 IU/g), 0.264%; vitamin E (500 IU/g), 0.540%; Rumensin-80, 0.675%; Tylan 40, 0.450%.; ground corn, 18.096%.

[3] Analyzed values

[4] Calculated using tabular values (NRC, 1996)

[5] Calculated from dietary NE_m (Mcal/kg) concentration: TDN, % = 23.8573 x NE_m + 2.3974 x NE_m^2 + 24.7761 (Galyean, 1996)

Table 3-2: Effect of CP concentration on performance and serum urea nitrogen of steers and heifers fed a high concentrate diet.

	CP			Contrasts			Gender			
Item	11.0%	12.5%	14.0%	SEM[1]	L[2]	Q[2]	Steers	Heifers	SEM[1]	*P*
No. of pens	6	6	6	-	-	-	9	9	-	-
Growing period										
Initial weight, kg	227	230	228	6	0.91	0.77	241	215	5	< 0.01
Final weight, kg	322	339	334	4	0.06	0.06	350	313	4	< 0.01
DMI, kg/d	5.0	5.1	5.1	0.18	0.28	0.56	5.4	4.7	0.18	< 0.01
ADG, kg	1.1	1.2	1.2	0.03	< 0.01	0.04	1.2	1.1	0.03	< 0.01
G:F	0.22	0.25	0.24	0.02	0.08	0.14	0.23	0.24	0.01	0.33
Finishing period										
Initial weight, kg	322	339	334	5	0.06	0.06	350	313	4	< 0.01
Final weight, kg	489	517	504	6	0.08	0.01	532	474	5	< 0.01
DMI, kg/d	10.0	10.8	10.2	0.24	0.42	0.02	10.9	9.9	0.20	< 0.01
ADG, kg	1.9	2.1	2.0	0.05	0.53	0.05	2.1	1.9	0.04	< 0.01
G:F[3]	0.2	0.2	0.2	0.01	0.94	0.94	0.2	0.2	0.01	0.13
SUN, mg/dL[4]	5.8	8.6	11.5	0.24	< 0.01	0.76	8.7	8.5	0.20	0.52

[1] Standard error of the mean
[2] L = linear, Q = quadratic
[3] Gender x CP treatment interaction $P = 0.01$ (See text for LS interaction means)
[4] Linear ($P > 0.01$) and quadratic ($P = 0.01$) day effect (See text for LS means)

Table 3-3: Effect of CP concentration on carcass characteristics of steers and heifers fed a high concentrate diet.

	CP			Contrasts			Gender			
Item	11.0%	12.5%	14.0%	SEM[1]	L[2]	Q[2]	Steers	Heifers	SEM[1]	*P*
No. of pens	6	6	6	-	-	-	9	9	-	-
Carcass Characteristics										
Hot carcass weight, kg	289	304	302	4	0.02	0.08	316	281	3	< 0.01
Dressing percentage, %	59.6	58.6	59.8	0.5	0.83	0.06	58.5	60.2	0.4	0.01
KPH, %	2.2	2.3	2.3	0.1	0.73	0.82	2.3	2.2	0.1	0.42
Backfat, mm	112	121	123	5	0.16	0.55	116	122	5	0.38
Loin muscle area, cm^2	77.4	74.8	76.4	1.2	0.54	0.14	74.0	78.4	1.1	0.01
Marbling score[3]	506	495	502	13	0.81	0.55	509	493	12	0.38
Yield grade	2.72	2.95	2.90	0.09	0.19	0.17	2.94	2.76	0.1	0.15

[1] Standard error of the mean

[2] L = linear, Q = quadratic

[3] Marbling score: 400, slight; 500, small; 530, modest.

CHAPTER 4

EFFECTS OF OVARIECTOMY AND ANABOLIC STEROID IMPLANTATION ON THE SOMATOTROPIC AXIS IN FEEDLOT HEIFERS

Abstract

A 2 x 2 factorial arrangement was used to evaluate effects of ovariectomy (**OVX**) and implantantation (200 mg of trenbolone acetate and 28 mg of estradiol benzoate; Synovex-Plus) on performance, serum urea nitrogen (**SUN**), serum IGF-1, and mRNA expression of hepatic IGF-1, GH receptor, and E receptor-α (**HERA**) as well as pituitary growth hormone (**GH**), E receptor-α (**PERA**) and GHRH receptor in feedlot heifers. Thirty-two British x Continental heifers were randomly assigned to 1 of 2 gender groups (OVX or intact) and then either a non-implanted control or to receive a Synovex-Plus implant and fed a 90% concentrate steam-flaked corn based diet for 42 d. Liver biopsies were taken prior to OVX for baseline expression of HEPATIC IGF-1, hepatic GH receptor and HERA, which did not differ ($P > 0.45$). Blood and BW were taken on d 0, 28, and 42 and 1 heifer/pen was slaughtered on d 42 for liver and pituitary samples. Initial and final BW did not differ ($P > 0.19$) due to OVX or implant. No gender x treatment interaction ($P > 0.24$) was observed for ADG or final BW. Neither OVX nor implant affected ADG for the final 14 d of the feeding period ($P > 0.48$), but 28 ($P = 0.03$) and 42 d ADG ($P = 0.02$) were greater in implanted than control. No 2 or 3 way interactions with day were observed for SUN ($P > 0.26$) and SUN was greater in control heifers than in implanted heifers ($P < 0.01$), but gender had no effect ($P = 0.31$). Both implanted and OVX increased serum IGF-1 ($P < 0.01$) and a gender x treatment interaction ($P < 0.01$) was observed. Neither gender nor implant treatment affected HERA, hepatic IGF-1, hepatic

GH receptor, GHRH receptor, GH, or PERA ($P > 0.06$). Data indicate that reductions in performance of OVX heifers can be eliminated through use of Synovex-Plus implants. This is likely due to the implant treatment related increase of serum IGF-1, but the reason for this increase cannot be explained by mRNA expression of key somatotropic genes in the present study.

Introduction

United States law stipulates that all heifers entering from Mexico must either be ovariectomized or have originated in a tuberculosis-free herd and test negative for brucellosis. Therefore, research concerning the effects of ovariectomy **(OVX)** is of particular importance in the southwest. Early research showed that OVX decreased ADG and G:F (Ray et al., 1969), but more recent research has indicated that OVX does not reduce ADG (Klindt and Crouse, 1990). Specifically, when implanted, OVX heifers have exhibited a four-fold greater response to implantation than intact heifers (Garber et al., 1990); and, when implanted with Synovex-H, OVX heifers had similar ADG to intact heifers implanted with Synovex-H (Adams et al., 1990). However, the physiological and molecular explanation for this performance recovery, via implantation, is poorly understood.

Vestergaard et al. (1995) found that OVX did not affect circulating IGF-1 in unimplanted Holstein heifers, and Mader and Kreikemeier (2006) found IGF-1 concentrations to be higher in estradiol (E) + trenbolone acetate (TBA) implanted heifers compared with unimplanted heifers, but found no differences in IGF-1concentrations between controls and those animals implanted only with E or TBA. However, TBA alone or implanted synergistically with an estrogenic implant had no effect on GH (Hunt et al., 1991). Implanting lambs with E + TBA increased hepatic mRNA expression of IGF-1 and implanting steers with E + TBA increased expression of IGF-1 in the muscle (Johnson et al., 1998). These data suggest that serum IGF-1 may be responsive to other mechanisms in addition to increased GH.

The purpose of this study was to utilize quantitative RT-PCR technology to examine the physiological mechanisms by which anabolic implants improve performance of intact and OVX heifers in the feedlot.

Materials and Methods

All procedures were approved by the University of Arizona Institutional Animal Care and Use Committee.

A 2 x 2 factorial treatment arrangement was used to evaluate the effects of OVX and implantantation with Synovex-Plus [200 mg of TBA and 28 mg of E benzoate (EB); Fort Dodge Animal Health, Overland Park, KS] on ADG, serum urea nitrogen (**SUN**), serum IGF-1 and mRNA expression of hepatic IGF-1, total GH receptor and E receptor-α as well as GH, E receptor-α, and GHRH receptor in the pituitary.

Cattle Management: Thirty-two British x Continental heifers with an initial, unshrunk BW of 374 ± 3.8 kg (mean ± SEM) were stratified by BW and assigned to 1 of 2 gender groups (OVX or intact). Heifers entering this study were heavier than heifers normally entering the feedlot; however, this is typical for cull heifers of Mexican origin entering feedlots in the Southwest United States. Heifers were then blocked by gender (n = 8 pens/gender) and randomly assigned to treatment [implanted (implant; n = 8) or non-implanted (control; n = 8)] and then to 1 of 16, partially shaded, soil surfaced pens (6.1 x 21.3 m; 2 heifers/pen), with concrete feed bunks and a shared water source for a 42-d feeding period. On d 0, cattle assigned to the implanted group were implanted with Synovex-Plus. Prior to implanting, the right ear was cleaned with an anti-microbial disinfectant, sprayed with 70% ethanol and wiped dry to prevent infection. Additionally, before use and between each animal, the implant gun was cleaned and sterilized using a sponge immersed in antimicrobial disinfectant and then wiped dry. Following implanting, the ear was thoroughly inspected to ensure that all pellets were inserted and securely in place. Heifers were fed a 90% concentrate, steam-flaked corn based diet

(Table 4-1), mixed daily, immediately before feeding (0700) and feed samples were collected at 2-wk intervals to be subjected to all of the following analyses: DM, CP, soluble protein (% of CP), ADF, ash, NEg and NEm (Dairy One, Forage Testing Laboratory, Dairy One, Inc., Ithaca, NY 14850). Ort samples were taken at d 28, 42 and when inclement weather so required. Feed refusals were weighed and a representative sample from each pen was taken and mixed with samples from all other pens to form a composite sample that was then sub sampled and subjected to DM analysis.

Surgical Procedures and Sample Collection: All surgical procedures were performed by, or under the direct supervision of a licensed veterinarian.

Liver biopsies were taken on d -14 between the 11^{th} and 12^{th} ribs on the perceptual line from the tuber coxae to the point of the scapula. The area was shaved, cleaned and anesthetized by local infiltration of 5 to 10 mL of 2% lidocaine (lidocaine hydrochloride, injectable, 2%; Vedco Inc., St. Joseph, MO). A puncture incision was made through the skin with a scalpel blade and a biopsy needle was subsequently inserted through the underlying muscle layers and into the liver. Two biopsies (~100 mg/each) were taken and combined on the anterior side of a sterile collection glove, split equally into 2 screw top tubes, and subsequently snap frozen in liquid N. Upon completion of all biopsies, samples were transported to the University of Arizona Ruminant Nutrition Laboratory and stored at -80˚C for later mRNA expression analysis.

Surgical procedures for animals assigned to the OVX group were performed on d -7 and -5. Heifers were restrained in a hydraulic working chute and bi-lateral ovariectomies were performed via entrance through the left para lumbar fossa. Prior to surgery, the area was shaved, cleaned and anesthetized by local infiltration of 25 to 30

mL of 2% lidocaine. A 10 to 15 cm vertical incision was made through the skin, underlying muscle layers were separated via blunt dissection and the abdominal cavity was entered. Each ovary was located and removed from the broad ligament by hand and allowed to drop into the abdominal cavity. The skin incision was then closed by using a horizontal mattress suture pattern of #1 Vetafil. Heifers were subsequently maintained in individual pens on the previously mentioned diet until the initiation of the study.

Blood was taken via jugular puncture on d 0, 28, and 42 and allowed to clot at room temperature before serum was harvested via centrifugation at 1,000 x g for 20 min and stored (-20º C) for later analysis of SUN and serum IGF-1.

On d 42, following a 12-h fast, 1 heifer/pen was slaughtered, via captive bolt and exsanguination, beginning at 0700, at the University of Arizona Meats Laboratory, to obtain liver and pituitary samples. Pituitary glands were excised, trimmed and divided midsagitally and each half was placed in a separate screw top tube and snap frozen in liquid N within 30 min after exsanguination. Liver samples (5 to 10 g) were taken immediately following USDA inspection and further processed into 2 sub-samples (1 to 2 g), divided equally into 2 screw-top tubes and snap frozen in liquid N, within 30 min after exsanguination. All pituitaries and liver samples were kept frozen in liquid N until they were transported to the University of Arizona Ruminant Nutrition Laboratory and stored at -80˚C for later mRNA expression analysis.

Serum Analysis: Serum urea N was determined using a direct colorimetric determination method (TECO Diagnostics, Anaheim, CA 92807) and serum IGF-1 (ng/mL) was determined by RIA as described by Berrie et al. (1995).

Gene Expression Analysis: Pituitary (0.2 to 0.3g; as-is basis), liver biopsy (0.1g; as-is basis) and post-mortem (PM) liver (0.1g as-is basis) samples were homogenized (Polytron, Brinkmann Instruments, Inc., Westbury NY) and total RNA was extracted with TRIzol (Invitrogen; Carlsbad, CA), according to manufacturers guidelines. Pelleted RNA was then re-suspended in molecular biology grade H_2O and stored at -80˚C. Total RNA was DNase treated (DNase I – Amplification grade, Invitrogen) and final sample concentration and quality was determined through nano-drop analysis using undiluted total RNA. Aliquots of DNase-treated RNA, taken from pituitary and liver, were normalized to a concentration of 1μg/μL or 0.40μg/μL, respectively and cDNA was created using Super Script III (Super Script III First-Strand Synthesis System for RT-PCR; Invitrogen), according to product guidelines. Synthetic oligonucleotide primers (Table 4-2) were generated from published primer sequences for GH (Chen et al., 1997) and E receptor-α (Lamote et al., 2006). Primers were designed with Primer Express 2.0 software for the GHRH receptor nucleotide sequence in GenBank (Accession # AF1848960) and un-published nucleotide sequences for IGF-1, HPRT and GH receptor (M. Rhodes, The University of Arizona, Tucson, AZ, personal communication).

Quantitative real time PCR was performed at the University of Arizona Genomic Analysis and Technology Core facilities on an ABI PRISM 7300 Sequence Detection System (Applied Biosystems; Foster City, CA) and ABI PRISM 7300 Sequence Detection System Software (Applied Biosystems) was used to visualize and interpret data. Real time PCR conditions for amplification of all gene products of interest were 1 cycle at 50˚C (2 min), 1 cycle at 95˚C (3min), followed by 40 cycles of 95˚C (15 s) and 66˚C (60s), followed by 1 cycle at 95˚C (15 s) and 60˚C (30 s). Disassociation curves

were run on every plate to ensure single product amplification per primer set. A reaction volume of 25 µL was identical for each sample well on every plate and all samples were ran with iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories, Hercules, CA), in triplicate, alongside triplicate standard curves, in 96 well optical reaction plates with an optical reaction cover (Applied Biosystems).

Following each run, primer efficiencies were calculated using the equation: Efficiency = $1 - 10^{(-1/\text{slope of the standard curve})}$. Average of all triplicates, across all genes, resulted in efficiencies of 98, 94 and 97% for liver biopsy, post-mortem liver, and pituitary plates, respectively. Standard deviation and CV values were calculated for cycle threshold values and all values had a CV of lower than 1.0 %. Cycle threshold values for HPRT were statistically analyzed to ensure no treatment or gender differences existed, thereby quantifying the proper choice for an endogenous reference gene. The mathematical model used to quantify the relative expression of each target gene is described by Pffafl (2001).

Statistical Analysis: Due to insufficient RNA yield, 3 animals were excluded from liver biopsy gene expression analysis, 2 animals were excluded from post-mortem liver gene expression analysis and 1 animal was excluded from pituitary gene expression analysis. Therefore these data were analyzed as missing values and statistical analysis was performed using the PROC MIXED procedures of SAS (SAS Inst. Inc., Cary, NC, USA). The model for all data included gender (intact vs. OVX), treatment (implant vs. control), pen (experimental unit) and the gender x implant treatment interaction as fixed effects and the random effect was heifer. The model for SUN and serum IGF-1 also included day as a repeated measure and tested all 2 and three way interactions with day and the

gender x implant treatment x day interaction was considered the residual. Additionally, d 0 SUN was analyzed as a covariate, within sampling date, due to initial differences observed between gender groups; autoregressive of order 1 was used for a covariance structure. Significance was considered $P < 0.05$ and LS means along with the most conservative estimate for SEM were reported for each variable.

Results and Discussion

Performance: No gender x treatment interaction ($P > 0.24$) was observed for any performance parameters. No differences in d 0 BW ($P > 0.49$) were detected for either gender or treatment group and d 42 BW was not changed as a result of gender or treatment ($P > 0.19$; Table 4-3). Likewise, ADG (Table 4-3) for the final 14 d of the feeding period was not affected ($P = 0.94$) by gender or implant treatment. However, for the initial 28 d ($P = 0.03$) and for the overall feeding period ADG was greater ($P = 0.02$) in implanted than control heifers. These data agree with previous research conducted in our laboratory, which indicated that long-fed Holstein steers implanted initially (d 0) with 36 mg of zeranol followed by 2 subsequent implants of E + TBA on d 84 and 168 had greater ADG than non-implanted controls (Cheatham, 2005). Perry et al. (1991) reported that both Holstein and beef steers implanted with Revalor (28 mg E + 140 mg TBA) had greater ADG than non implanted controls. Furthermore, Herschler et al. (1995) showed that implanting E + TBA ratios equal to those used in the present study improved ADG in both steers and heifers.

Gender did not affect ADG (Table 4-3) for the initial 28 d ($P = 0.29$) or the final 14 d ($P = 0.49$) of the study, although it was numerically greater ($P = 0.07$) in intact than OVX heifers for the overall feeding period. While these data support research showing that OVX does not reduce ADG (Hamernik et al., 1985; Klindt and Crouse, 1990; Field et al., 1996), it is in contrast to early research that indicated that OVX decreased ADG (Dinusson et al., 1950; Ray et al., 1969; Horstman et al., 1982). However, heifers studied by Horstman et al. (1982) were implanted with zeranol only, a much less aggressive implant than used in the present study. Additionally, OVX heifers in that study only

exhibited reduced ADG for the first 24 d of the study and surgeries were performed between d 8 and 10, whereas, in the present study, OVX surgeries were performed on d -5 and -7 and heifers were allowed to recover before the initiation of the study. Furthermore, although we did not see a gender x implant treatment interaction, this lack of significance, in relation to gender, could have been due to a numerically greater ADG in implanted OVX heifers than in intact controls and the equality of ADG between intact implanted heifers and OVX implanted heifers for the initial 28 d of the study (data not reported). This is in agreement with Garber et al. (1990), as they indicated that OVX heifers exhibited a fourfold greater response to implantation than intact heifers, and Adams et al. (1990) reported that implanting OVX heifers with Synovex-H resulted in BW gains similar to those in intact heifers implanted with Synovex-H.

Neither gender nor implant treatment affected DMI ($P > 0.30$) and gender did not affect G:F ($P > 0.05$). The lack of a gender effect on DMI agrees with early research that indicated that OVX did not have any affect on DMI (Adams et al., 1990). On the other hand, reports on the effects of anabolic implants on DMI vary. Adams et al. (1990) reported that implanting heifers with Synovex-H increased DMI; but Apple (1989) reported no difference in DMI between implanted and control steers. The lack of an affect of Synovex-Plus on DMI, in the present study, could also be attributed to the short time that these heifers were on feed. The lack of a gender effect on G:F disagrees with early research that indicated that OVX had an adverse influence on efficiency of growth (Dinusson et al., 1950; Horstman et al., 1982), but in agreement with later research that indicated that OVX did not have any effect on efficiency of growth (Klindt and Crouse, 1990). Due to the lack of difference in DMI and the increased ADG in implanted heifers

compared to controls, G:F was greater for implanted heifers compared to controls at both d 28 ($P = 0.03$) and 42 ($P = 0.01$), but did not differ between d 28 and 42 ($P = 0.97$). This finding agrees with previous reports that indicate implantation improves feed efficiency (Adams et al., 1990; Garber et al., 1990).

Serum Urea Nitrogen: Serum urea N (Table 4-3) was lower ($P > 0.01$) in intact than OVX heifers on d 0 (data not reported), thus baseline SUN was analyzed as a covariate, within sampling date, to alleviate any pre-treatment effects at d 28 and 42. This is not surprising as OVX operations were completed on d -7 and -5 and OVX heifers were likely still recovering from the surgical procedures and therefore N utilization would have been reduced. Following covariate analysis, no two or three way interactions with day were observed for SUN ($P > 0.26$); thus only overall means are reported for implant treatment and gender. These data indicate a trend for a gender x implant treatment interaction was present for SUN ($P = 0.09$; SEM = 0.87) with LS means of 10.41 (intact implanted), 12.64 (intact control), 8.10 (OVX implanted) and 13.10 mg/dL (OVX control). Additionally, SUN was greater in control heifers than in implanted heifers ($P < 0.01$). Considering the greater performance observed in implanted heifers, one would expect that they utilized N better than control heifers. Supporting the present study, previous research indicated heifers implanted with E + TBA had reduced plasma urea N compared to non-implanted controls or heifers implanted with either E or TBA alone (Mader and Kreikemeier, 2006). On the other hand, Cheatham (2005) indicated that SUN did not differ between implanted and non-implanted Holstein steers. In that study, SUN concentrations were much lower (near 5 mg/dL) than in the present study (> 8.0 mg/dL). Johnson and Preston (1995) reported that plasma urea N concentrations greater than 5.0

to 8.0 mg/dL were indicative of excessive N intake and N wasting. This would indicate that heifers in the present study were fed at, or in excess of, their CP requirement, whereas Holstein steers in the previous study were fed at, or possibly somewhat below, their CP requirement, which would explain the reason for treatment differences in the present study and not in the previous study. Finally, gender had no effect on SUN (P = 0.31; Table 4-3); however, this could be expected, considering the lack of change in ADG due to OVX, the trend for a gender x treatment interaction observed for SUN, and previous research reported in this dissertation indicated gender did not affect SUN when steers were compared to heifers.

Serum IGF-1: Implants increased ($P < 0.01$) serum IGF-1 (Table 4-3), which was greater ($P < 0.01$) in OVX than intact heifers. This is explained by the gender x treatment interaction ($P < 0.01$; SEM = 25.5) observed for serum IGF-1 with LS means of 267.4 (intact implanted), 170.9 (intact control), 434.4 (OVX implanted) and 167.0 ng/mL (OVX control) indicating that the greatest response in serum IGF-1 was in the OVX implanted heifers. This agrees with many previous reports indicating that implanting steers increases circulating IGF-1 (Lee and Henricks, 1990; Hunt et al., 1991; Cheatham, 2005). Furthermore, Mader and Kreikemeier (2006) found IGF-1 concentrations to be greater in E + TBA implanted heifers compared to controls, but found no differences in IGF-1 between controls and those animals implanted only with E or TBA. Moreover, serum IGF-1 is generally considered to be a GH dependent peptide (Florini et al., 1991). However, implants do not increase circulating GH (Hunt et al., 1991; Hayden et al., 1992). In fact, Cheatham (2005) reported that, in non-implanted Holstein steers, the GH response to a GHRH challenge was actually greater than that of implanted steers.

Although circulating GH was not measured in our study, data reported for serum IGF-1 agrees with previous research, which may indicate that anabolic implants stimulate IGF-1 via a different mechanism than through GH. Therefore, to evaluate effects of implanting OVX and intact feedlot heifers on the somatotropic axis, mRNA expression of genes with the somatotropic axis were analyzed

Relative mRNA expression: An initial liver biopsy was taken on d -14 to quantify expression of hepatic IGF-1, E receptor-α, and GH receptor. Relative gene expression analysis revealed no pre-treatment differences ($P > 0.45$; data not reported) for any of the genes examined.

No difference was observed in post mortem hepatic IGF-1 ΔCT values (Table 4-4) as a result of OVX ($P = 0.31$) or implant ($P = 0.69$). Previous gene expression data has showed that implanting wethers with E (8 mg) + TBA (40 mg) increased steady-state hepatic IGF-1 expression by 150% and suggested that the liver may be the source of at least part of the increased circulating IGF-1 in steroid implanted animals (Johnson et al., 1998). Additionally, the same authors reported that implanting steers with Revalor-S (24 mg E_2 + 120 mg TBA) increased IGF-1 mRNA in the longissimus muscle by 68%. Furthermore, in vitro research indicates that treating bovine satellite cell cultures with either E or TBA increases IGF-1 mRNA level (Kamanga-Sollo et al., 2004). However, the timeline for measurable effects of implantation with E + TBA on gene expression is not consistent, as reports have varied from as early as 7 d post implantation (Pampusch et al., 2003) to as late as 28 d post-implantation (Dunn et al., 2003). It should be noted however, that the Pampusch et al. (2003) study was a comparison within steer (i.e. d 0 to d 7) and the Dunn et al. (2003) study compared implanted to non implanted steers, which

could explain differences in the final expression between studies. Pampusch et al. (2003) observed that IGF-1 expression on d 26 tripled compared to d 0 expression. Dunn et al. (2003) showed that, although IGF-1 in circulation increased as early as 14 d post-implantation, mRNA expression of IGF-1 in the muscle did not increase until d 28 and was 2.4 fold greater in implanted calves than non-implanted calves at that time. This latter study demonstrates that an increase in serum IGF-1 may be observed without an increase in hepatic IGF-1 expression, as noted in the present study. In our study, a trend ($P = 0.11$) for a gender x treatment interaction was observed with ΔCT values of 0.70 (intact implanted), 1.18 (intact control), 1.80 (OVX implanted) and 0.89 (OVX control). This is similar to the response we observed for serum IGF-1 in that the difference in the relative hepatic IGF-1 expression between intact implanted and OVX implanted heifers ($P = 0.07$) could explain the reason for an increased response of OVX heifers to anabolic implants.

Hepatic E receptor-α expression (Table 4-4) was not affected by treatment ($P = 0.72$) and no gender x treatment interaction ($P = 0.38$) was observed. In vitro research indicated that when bovine satellite cell cultures were treated with either E or TBA, both E receptor-α and androgen receptor mRNA increased when added at 0.001 nM each. However, neither E receptor-α nor androgen receptor mRNA levels were affected by E or TBA, compared to non-treated controls, when the steroids were added at greater concentrations (Kamanga-Sollo et al., 2004). Notably, a trend for intact heifers to have a greater hepatic E receptor-α expression than OVX heifers ($P = 0.06$) was observed. A more in depth analysis revealed that this trend may be influenced by numerical differences in the relative expression of hepatic E receptor-α between OVX implanted

and intact implanted heifers with ΔCT values of 0.43 compared to 0.76 (SEM = 0.16; P = 0.06), respectively. This could allow for E supplied by the implant to anabolically act through the IGF-1 receptor, as was demonstrated in the mouse (Klotz et al., 2002). However, further research should be conducted analyzing IGF-1 receptor expression to potentially validate this hypothesis.

Pituitary E receptor-α expression results are different, in that treatment (P = 0.07) and not gender (P = 0.28), decreased the ΔCT value of pituitary E receptor-α. Additionally, differences observed among interaction means showed that OVX implanted heifers had lower ($P \leq 0.05$) pituitary E receptor-α expression (ΔCT = 0.39) than either intact or OVX controls which had ΔCT values of 0.82 and 0.81, respectively and they also tend to express lower (P = 0.13) pituitary E receptor-α than intact implanted heifers (ΔCT = 0.69). These effects suggest that implantation with E + TBA implants may also suppress pituitary E receptor-α more in OVX heifers than in intact heifers, which could also suggest an increase in binding to the IGF-1 receptor. However, further analysis is warranted to examine this response.

Neither gender ($P > 0.65$) nor treatment had ($P > 0.65$) any affect on the ΔCT values of GHRH receptor, GH, hepatic GH receptor, and there were no gender x treatment interactions ($P > 0.55$) observed for GHRH receptor, GH or hepatic GH receptor. Lack of differences in the relative ΔCT values in the expression of mRNA from these genes strengthens the hypothesis that E + TBA implants are acting outside the somatotropic axis to increase IGF-1.

Conclusion

In conclusion, data indicate that heifer gender (OVX vs. intact) has no influence on performance or SUN in feedlot heifers. However, serum IGF-1 is increased more in OVX heifers than intact heifers due to a greater response to implantation from the OVX heifers. However, the reason for the extra increase in serum IGF-1 is not clear, although trends in gene expression analysis suggest the possibility that the increased serum IGF-1 may be controlled outside of the somatotropic axis. Further research is warranted to examine the effects of OVX and anabolic implants on the somatotropic axis.

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Table 4-1. Ingredient and nutrient composition of diet (DM basis)

Item	Concentration
Ingredient	
Alfalfa Hay, %	10.00
Steam-flaked corn, %	78.25
Urea, %	1.25
Tallow, %	3.00
Cane molasses, %	5.00
Mineral supplement[1], %	2.50
Nutrient	
DM, %	87.03
Ash, %	4.73
CP, %	13.00
Soluble protein, % of CP	45.67
NEm, Mcal/kg	2.11
NEg, Mcal/kg	1.44
ADF, %	6.70

[1] Mineral supplement composition (DM basis): limestone, 47.059%; dicalcium phosphate, 1.036%; potassium chloride, 8.000%; magnesium oxide, 3.448%; ammonium sulfate, 6.667%; salt, 12.000%; cobalt carbonate, 0.002%; copper sulfate, 0.157%; iron sulfate, 0.133%; calcium iodate, 0.003%; manganese sulfate, 0.500%; selenium premix (0.16%), 0.125%; zinc sulfate, 0.845%; vitamin A (30,000 IU/g), 0.264%; vitamin E (500 IU/g) 0.540%; Rumensin-80, 0.675%; Tylan 40, 0.450%.; ground corn, 18.096%.

Table 4-2: Forward primers, reverse primers and the corresponding reference or GenBank accession number for genes of interest

Gene	Primer Sequence (5′ – 3′)	Reference
Total Growth Hormone Receptor (GHR)		Radcliff et al., 2003
Forward	GGTATGGATCTCTGGCAGCTG	
Reverse	CTCTGACAAGGAAAGCTGGTGTG	
Insulin-like Growth Factor -1 (IGF-1)		Radcliff et al., 2003
Forward	TTGGTGGATGCTCTCCAGTTC	
Reverse	GCACTCATCCACGATTCCTGT	
HPRT		M. L. Rhoads 2006 (Unpublished Data)
Forward	GAGAGTCCGAGTTGAGTTTGGAA	
Reverse	GGCTCGTAGTGCAAATGAAGAGT	
Growth Hormone (GH)		Chen et al., 1997
Forward	CCGGAGGGACAGAGATACTC	
Reverse	GAGTGGCACCTTCCAGGGTC	
Estrogen Receptor-α (ERA)		Lamote et al., 2006
Forward	AGGGAAGCTCCTATTTGCTCC	
Reverse	CGGTGGATGTGGTCCTTCTCT	
GHRH receptor		GenBank (AF1848960)
Forward	TGTCATCACTCAGCTGCGAGA	
Reverse	TCG GCA GCTTGTAGACATGCT	

Table 4-3: Effects of OVX and Synovex-Plus implants on performance and serum metabolites in feedlot heifers

	Gender[1]				Implant Treatment[2]			
Item	Intact	OVX	SEM[3]	*P*-value	+	–	SEM[3]	*P*-value
No. of pens	8	8	–	–	8	8	–	–
BW, kg								
Day 0	378	371	5.5	0.49	374	375	5.5	0.86
Day 42	436	423	6.8	0.19	433	426	6.8	0.45
ADG, kg								
Day 0 – 28	1.3	1.2	0.1	0.29	1.4	1.1	0.1	0.03
Day 28 – 42	1.6	1.4	0.2	0.49	1.5	1.5	0.2	0.94
Day 0 – 42	1.4	1.2	0.1	0.07	1.4	1.2	0.1	0.02
DMI, kg/d								
Day 0 – 28	11.0	11.0	0.03	0.31	11.1	11.0	0.03	0.25
Day 28 – 42	10.6	10.4	0.18	0.54	10.5	10.5	0.18	0.98
Day 0 – 42	10.9	10.8	0.07	0.43	10.9	10.9	0.07	0.74
G:F								
Day 0 – 28	0.118	0.105	0.009	0.32	0.127	0.097	0.009	0.03
Day 28 – 42	0.149	0.142	0.018	0.62	0.142	0.142	0.018	0.97
Day 0 – 42	0.128	0.114	0.004	0.06	0.131	0.114	0.004	0.01
Serum Analysis								
SUN mg/dL	11.5	10.6	0.6	0.31	9.3	12.9	0.5	< 0.01
IGF-1[4] ng/mL	219.2	300.7	17.7	< 0.01	350.9	169.0	17.7	< 0.01

[1] Intact (Intact heifers); OVX (Ovariectomized heifers)
[2] + (implanted with 28 mg E Benzoate and 200 mg TBA); – (non-implanted)
[3] Standard error of the mean (Most conservative estimate reported).
[4] Gender x treatment interaction ($P < 0.01$; LS means listed in text)

Table 4-4: Mean ΔCT values classified by gender and Synovex-Plus implant treatment and normalized to the housekeeping gene used for each target

	Gender[1]				Implant Treatment[2]			
Item	Intact	OVX	SEM[3]	*P*-value	+	−	SEM[3]	*P*-value
Liver post-mortem ΔCT								
IGF-1	0.94	1.34	0.30	0.33	1.24	1.03	0.28	0.60
GH receptor	0.92	0.83	0.14	0.63	0.78	0.98	0.13	0.29
E receptor-α	0.73	0.50	0.08	0.06	0.60	0.64	0.08	0.72
Pituitary ΔCT								
GH	2.06	2.04	0.38	0.97	2.33	1.77	0.38	0.31
GHRH receptor	0.78	0.73	0.10	0.70	0.85	0.66	0.10	0.21
E receptor-α	0.75	0.60	0.10	0.28	0.54	0.82	0.10	0.07

[1] Intact (Intact heifers); OVX (Ovariectomized heifers)
[2] + (implanted with 28 mg E Benzoate and 200 mg TBA); – (non-implanted)
[3] Standard error of the mean (Most conservative estimate reported).

CHAPTER 5

CONCLUSION

In conclusion, with the exception of a slight advantage in feed efficiency, optimal protein concentrations in feedlot diets were not influenced by gender. Results indicated that ADG was optimized when both steers and heifers were fed 12.5% CP and G:F was optimized for steers fed 12.5% CP, and heifers fed 14.0% CP. Feeding diets containing 11.0% CP appears to cause a protein deficiency in finishing steers and heifers. Furthermore, data indicate that heifer gender (OVX vs. intact) has no influence on performance or SUN in feedlot heifers. However, serum IGF-1 to is increased more in OVX heifers than intact heifers due to a greater response to implantation from the OVX heifers. However, the reason for the extra increase in serum IGF-1 is not clear, although trends in gene expression analysis suggest the possibility that the increased serum IGF-1 may be controlled outside of the somatotropic axis.

With today's ever-changing feedlot industry, research concerning optimal CP concentrations, in relation to changing cattle types and new and improved implants and feed additives, will be of the utmost importance. Additionally, further research is warranted to examine the effects of these same implants and feed additives, as well as CP concentrations, on OVX cattle and the somatotropic axis in all feedlot cattle.

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