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EFFECTS OF ANABOLIC AGENTS ON BONE GROWTH IN CATTLE AND SWINE

by

ZAFAR IQBAL CHAUDHARY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

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Dedicated to

My son Asif Iqbal

ABSTRACT

Three experiments were conducted to study the effects of anabolic agents on growth traits especially on linear bone growth in cattle and pigs.

In the first experiment thirty-two cull cows aged one to 13 years were allocated to five age groups. Half of the cows within each group were implanted with 36 mg zeranol. The cows were fed individually for 82 days, then slaughtered. Bone growth was monitored by oxytetracycline labelling of the epiphyseal growth plates. Zeranol had no significant effect on average daily gain, feed efficiency or backfat thickness, but caused a decrease in radio-ulna mass ($P<0.03$). Rate of bone growth at the distal end of the radio-ulna decreased with increasing age and was zero at 4-5 years.

The second experiment was performed on forty-eight steers and bulls starting at an average weight of 200 kg to look for the effects of zeranol for a trial period of 183 days. Cattle treated with zeranol showed higher average daily gain than controls ($P<0.05$). There was no significant interaction between gender and treatment. Castration increased carcass fatness ($P<0.01$) and decreased dressing percentage ($P<0.01$), ribeye area ($P<0.01$), shank muscle ($P<0.01$) and shank muscle:bone ratio ($P<0.01$). Implantation with zeranol had no effect on the carcass traits. The weights of the radio-ulna, metacarpal and metatarsal bones were not significantly affected by gender or treatment.

Growth of the radius at the distal end during the last two months of the experiment was significantly less in treated cattle than in controls ($P<0.01$) and in steers than in bulls ($P<0.01$). Treatment with zeranol resulted in larger pelvic openings ($P<0.01$) in bulls and steers and reduced libido ($P<0.05$) in treated bulls.

In the last experiment the effects of estradiol-17β, testosterone and castration were studied in male pigs, from birth to six months of age. Treatment had no effect on average daily gain, feed efficiency or backfat thickness ($P>0.05$). The growth coefficients of individual organs relative to body weight or carcass weight were also not affected by treatment. Weights of limb bones (radio-ulna, humerus, scapula, tibio-fibula, patella, femur and pelvis) did not differ significantly among the treatment groups. A similar trend was noticed in the length measurements of the radio-ulna, humerus, tibio-fibula and femur. The boars had thicker ($P<0.05$) radio-ulna bones as compared to the other three treatment groups. Treatment did not influence the rate of growth at the epiphyseal growth plates of the radius, humerus, tibia and femur as determined by oxytetracycline infusion or the rate of glycosaminoglycan synthesis in the epiphyseal growth cartilage of the distal radius and proximal tibia as determined by $^{35}SO_4$ incorporation.

It is concluded from the three experiments that of the anabolic agents used in this work only zeranol increased the rate of growth in male cattle; zeranol had negative effect

on linear bone growth in cattle.

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GENERAL INTRODUCTION

The efficient production of lean meat is a major goal of the livestock industry. Animal growth, particularly the rate and efficiency of weight gain and the composition of the animal or carcass, is a critical component of this goal. The use of growth promoting agents can have a profound influence on the efficiency of meat production. New substances are constantly entering the livestock industry, though in many cases their mode of action and influence on body composition is not clear.

The substances used in meat animals (animals raised for the production of meat) to increase growth rates and efficiency of production can be divided into two broad classes according to the mode of action. One class acts to increase the quantity or quality of nutrients made available to the animal tissues (biostimulators). The second increases the efficiency with which nutrients reaching the animal tissue are utilized for productive purposes (anabolic agents).

The endocrine system is known to regulate animal metabolism, but the manner in which hormones interrelate to control the efficiency of meat production is not fully understood. The differences in growth rate and mature body size of male and female animals suggest that sex hormones play an important role in the control of growth.

The precise mechanism by which estrogens influence skeletal muscle metobolism is unclear, and fundamental studies of their actions have not been conducted in farm animals. However, a number of theories to explain the mode of action of estrogens have been proposed on the basis of changes in the weights of endocrine glands, concentration of plasma hormones and metabolites noted in animal growth trials (Wiggins et al., 1976). One widely held hypothesis suggests that estrogens act primarily on the hypothalamus or anterior pituitary to increase growth hormone (somatotropin) secretion and that this hormone is responsible for the growth response. Somatomedin and somatostatin are now considered more important than somatotropin in this regard (Spencer, 1984).

The actual mechanism by which androgens increase net muscle protein synthesis has been the subject of debate. One school of workers (Michel and Baulieu, 1976) favours the concept that fibres are the direct target of androgens in skeletal muscles, where they act by binding onto specific cytosol receptors, to increase protein synthesis. An alternate hypothesis has been proposed by Mayer and Rosen (1977) that androgens inhibit the binding of glucocorticoids to their specific receptors in muscle cytosol. Some glucocorticoids exert a catabolic effect on muscle protein. In cultured myoblasts, testosterone produces a modest stimulation of mitotic proliferation, and reduces the time spent by the myoblast in the G_1 phase (phase of DNA

synthesis) of mitosis (Powers and Florini, 1975).

Fears have been expressed to the likelihood of consumers being affected by traces of anabolic agents remaining in the edible carcass. In the case of estradiol-17β and testosterone, convincing evidence has been presented, that following recommended treatments and based on the tissue-hormone levels measurable, no differentiation could be made at the time of slaughter between treated and untreated animals. Thus a residue problem does not exist and no questions in respect to public health have to be answered (Hoffmann, 1984). As regards zeranol, Heitzman et al. (1984) have shown the presence of residues throughout and beyond the recommended withdrawal period. However, from results concerning affinity for DNA, zeranol appears to be a non genotoxic compound (Rico and Sacaze, 1984).

The use of anabolic agents in human beings is mainly therapeutic. They are used to improve nitrogen balance in debilitating diseases and old age, in hypogenital men in an aim to restore or bring to normal male secondary sexual characteristics and male sexual behaviour and to mimic the hormonal effects on somatic developement. Anabolic agents are also used by athletes in a belief that they would improve their athletic performance. The most commonly used anabolic agents for this purpose are the derivatives of testosterone (Gribbin and Matts, 1976).

Wilson and Griffin (1980) suggested that the effect of anabolic agents in undernourished, debilitated, or elderly

individuals was probably due to enhanced appetite as in controlled studies, there was no effect on weight and strength following treatment. According to Wynn (1968) androgens have proved disappointing as therapeutic aids to promote anabolism in acute illness, containing trauma, and chronic illness associated with protein depletion.

The administration of androgens to hypogonadal men induces a retardation in urinary excretion of nitrogen and causes a gain in weight. In contrast, in normal men given exogenous androgen retention is only half that of hypogonadal men (Wilson and Griffin, 1980). In man muscles of the pectoral and shoulder region were most responsive. Histologically, the enlargement of the responsive muscles was due to an increase in the diameter of muscle fibers and fibrils (Szirmai, 1962).

Any positive effect of anabolic agents on lean body mass and muscular growth is presumed to be beneficial to atheletic performances. There is still controversy whether or not anabolic steroids increase muscle size and strength in athletes (Hill et al., 1983). According to Ryan (1981) anabolic agents did not contribute significantly to gain in muscle strength.

The use of the anabolic agent zeranol is widespread in beef cattle production but not in pork production. Zeranol is derived from the compound zearalenone produced by the maize mold Gibberella zea (Stob et al., 1962). The efficacy of zeranol has been reviewed by Sharp and Dyer (1971). These

workers demonstrated stimulation of live weight gain in heifers and steers. Zeranol has little application in the swine industry since it causes an estrogenic syndrome i.e. hypertrophy of vulva, vaginal eversion, and interruption of estrus in sows and gilts and mammary hyperplasia and testicular atrophy in boars and barrows (Kiessling, 1982).

The substances most commonly used as growth promotants in pigs are antibiotics. These substances are not classified as anabolic agents. The anabolic agents used (only in experimental trials) in pigs are the estrogens and testosterone (sex steroids). Reports regarding the effects of steroid hormones in pigs are confusing and so far there are no clear recommendations which can be made from the literature available. The effect of estrogens and testosterone in pigs is slightly different from that in cattle. The principal effect in pigs is an increase in the lean:fat tissue ratio. Backfat thickness is reduced and carcass quality improved in favour of larger hams and loins (van Weerden and Gradadam, 1976) in castrated male pigs receiving either a combination of trenbolone acetate (synthetic androgen) and estradiol or oral administration of trenbolone acetate plus diethylestradiol.

The bone tissue constitutes a frame on which the other body tissues are supported. Little work has been done in farm animals regarding the effect of anabolic agents on the growth of this tissue, although there is voluminous literature in which the effects of sex steroids on bone

growth have been studied in laboratory animals.

The individual constituents of the skeleton begin as mesenchymal condensations during the embryonic period. These mesenchymal cells are derived from the primary germ layers, usually under the mechanical or chemotactic influence of other tissue structures. Some of these cellular condensations ossify directly to form the membrane-derived or intramembranous bones. However, the rest of the skeleton is derived from cartilage models mainly by endochondral ossification. This consists of two discrete processes: (1) the formation of a primary osseous collar and subsequent vascular invasion to form the primary ossification center, which will become the diaphysis and metaphyses, and (2) a later (usually postnatal) vascularly mediated ossification in the epiphysis to form the secondary ossification center (Figure 1). Particular areas of cartilage, termed growth plates, capable of rapid growth longitudinally and latitudinally, are left between the primary and secondary centres (Matthews, 1980).

The effects of estrogens on the skeleton have been reviewed by Silberberg and Silberberg (1971). Estrogens promote maturational changes in the epiphyseal growth plates, and thus modify the pace of linear bone growth. The skeletal responses to the hormone appear strongly dose and species dependent. It has been shown that large doses inhibit the bone growth, while small doses resulted in increased growth in chicks (Budy et al., 1952; Negulesco and

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Figure 1. The development of long bones.

(a) cartilaginous model, (b) appearance of subperiosteal bone, (c) appearance of primary ossification centre, (d) extension of the latter towards the epiphysis, (e) appearance of secondary ossification centres, (f) ossification complete except the epiphyseal plates, (g) disappearance of the latter.

(Taken from Hafez, E.S.E. and Dyer, I.A. 1969. Animal Growth and Nutrition. Lea and Febiger. Philadelphia.)

Kossler, 1978).

Testosterone, in the epiphyseal growth plates, also increases maturational changes which lead to epiphyseal-diaphyseal union. These changes include stimulation of age changes in the cells to promote the formation of hypertrophic chondrocytes (Howard, 1962). In castrated animals, the period of longitudinal growth of bone is increased due to delayed maturation of the epiphyses and the skeleton as a whole (Silberberg and Silberberg, 1971). The retardation of maturational changes in the cartilage and bone of gonadectomized rats is due to diminished glycosaminoglycan synthesis (Kowalewski and Gouws, 1957). These maturational changes can be reinitiated and even accelerated by testosterone therapy (Salomon et al., 1973).

In the present work the effects of castration and the anabolic agents zeranol, estradiol-17β, and testosterone as implants on the growth, particularly on the growth of long bones, were explored in cattle and swine.

I. EFFECTS OF ZERANOL ON SKELETAL GROWTH OF CULL COWS AND HEIFERS

INTRODUCTION

Body growth is a function of bone, muscle and fat growth; a superior carcass being one with maximum muscle, minimum bone and optimum fat for the particular market being supplied. Zeranol, a resorcylic acid lactone used commercially as a growth stimulant, has been found to have no influence on carcass muscle and fat characteristics of cows (Price and Makarechian, 1982). It has however been suggested by Staigmiller et al. (1983) that the substance may increase the pelvic opening in heifers, suggesting an influence on bone growth. This would have commercial importance by changing the ratio of edible to non-edible portion in the carcass. The following experiment investigated the effect of zeranol in the form of the commercial implant Ralgro® (I.M.C.: Terre Haute, Indiana.) on the growth of the radio-ulna bones of five groups of cull cows ranging in age from one to 13 yr. to study the nature and extent of any influence of zeranol on skeletal growth.

MATERIALS AND METHODS

A total of 32 April and May born cull cows and heifers from the University of Alberta herd were used in this experiment. 6 one year old and 8 two year old heifers were selected at random. Older females were selected from those

which had been culled for inferior reproductive performance (failure to become pregnant, difficult birth, mammary gland problems). Cows were categorized as beef-type or dairy-type, and subjectively scored for fatness by an experienced cattleman through physical palpation of the subcutaneous fat at the brisket, flank and tailhead. They were then divided into five age groups: less than two, two to three, three to four, four to five and greater than 5 yr (see Table I.1). Within each age group they were balanced with respect to the numbers of beef (which are early maturing cattle having small frames) and dairy-types (which are late maturing cattle having large frames), and the fattest and thinnest cows were excluded.

Half the cows in each age group were implanted subcutaneously at the base of the ear with 36 mg of zeranol (in the form of three 12 mg pellets of Ralgro®) by a specially designed trocar and canula and all of the cows were infused intravenously with oxytetracycline (Terramycin® 100 mg/ml, Pfizer Co. Ltd.). The oxytetracycline was diluted 4 times with sterile normal saline and was administered as a single injection (15 mg/kg body weight) on day 1 of the experiment to label the calcifying zone at the epiphyseal growth plates and subsequently to measure the rate of longitudinal bone growth (Graham and Price, 1981). The cows were placed in individual pens and fed a high en diet containing 60% grain, (rolled barley:rolled oats, 3:1), 35% hay, and 5% of a protein-vitamin-mineral supplement at the

rate of 120 gm feed/kg$^{0.75}$ of body weight (metabolic size of the animal). This feeding regime which is close to the appetite of the cattle (Webster, 1980) was preferred because: (i) if the animals were fed ad libitum those having smaller appetites would have been at a disadvantage, and (ii) if the animals were fed on the basis of body weight (W') the smaller animals who have greater maintenance requirement per unit body weight would have been at a disadvantage (Parks, 1982). Basal metabolism has been measured for animals of different sizes, and from such data two facts have been established. Firstly the basal heat production is affected by the weight of the animal, and secondly the metabolism of the smaller animals is greater than that of large animals per unit of body weight. Theoretical considerations suggest that heat loss may be related to the surface area of the body. If the heat losses were affected by radiation, the surface area might be a factor, and the 2/3 power of body weight is a better index of surface than is weight to the first power. However, it must be noted that the exterior body surface is not constant in living animals, nor can it be measured satisfactorily. Furthermore, the causal factors of heat production, and hence of basal heat loss, are not dependent on external body surface. Basal metabolism data for adult animals of species ranging from mice to elephant were plotted on log-log paper and the regression fitted by the method of least squares. If weight (W) is in kg and metabolism (C) in Kcal. The slope of

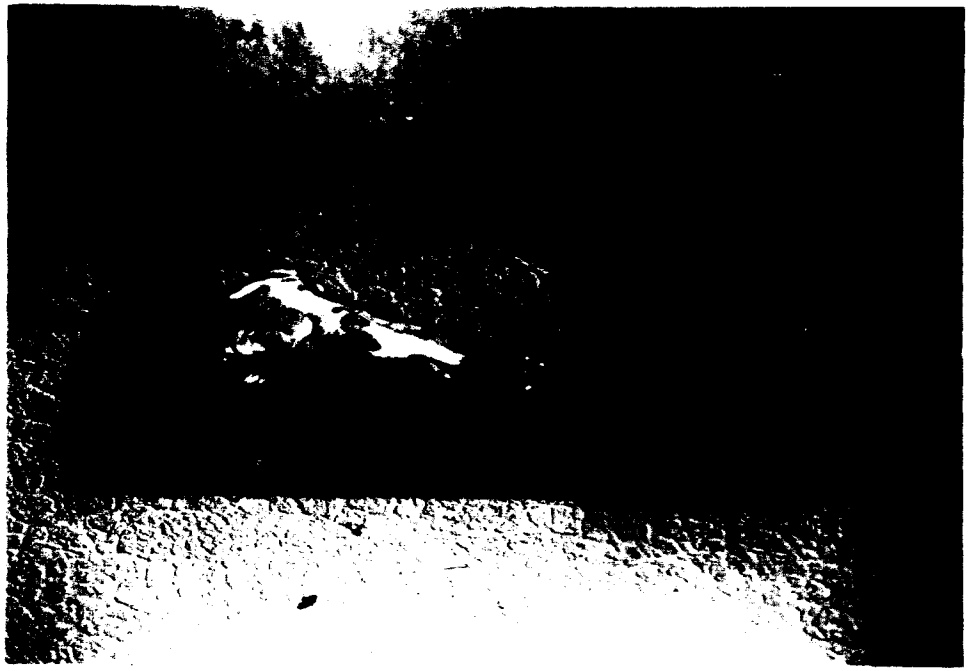

Plate I.1. Method of measuring the length of bones

Plate I.2. Method of measuring the circumference of bones

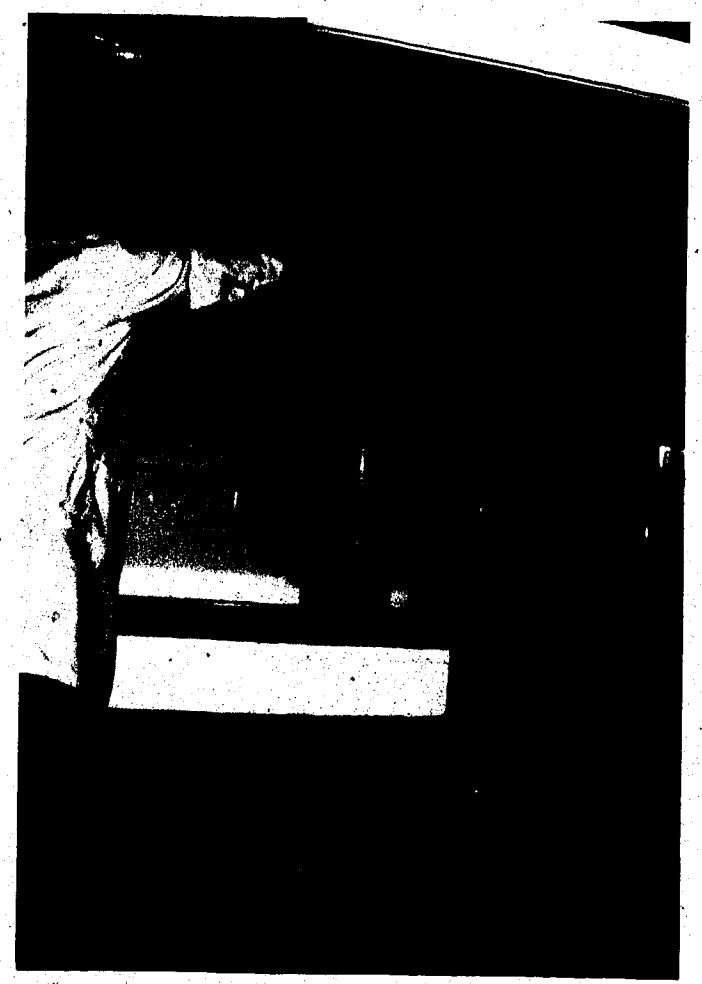

Plate I.3. Method of measuring the specific gravity of bones

the curve proved to be 0.73, and the value of b, the ratio $C/W^{0.73}$, was 70.5 (Crampton and Harris, 1969).

The cows were weighed and feed allowance recalculated weekly. Following 82 days of feeding the cows were trucked to a commercial packing plant 150 km away in Edmonton for slaughter. After the routine procedures of killing, dressing and grading by Agriculture Canada personnel at the packing plant both fore-shanks were removed by a cut above the elbow (radio-ulna to humerus) joint and taken to the meat research laboratory where the shanks were trimmed of tissue distal and proximal to the radio-ulna and then dissected under the supervision of the laboratory technician to record the amount of muscle, fat, and bone. Length, circumference and specific gravity of the radio-ulnae were also recorded. Length measurments were the greatest distance between two parallel lines at the proximal and distal points of each bone (Plate I.1). The circumference was measured at the isthmus of the bones (Plate I.2). For calculating the specific gravity the bones were weighed in air and their volume was measured (Plate I.3) by water displacement in a measuring cylinder (Jones et al., 1978). The bones were then split longitudinally and photographed under ultraviolet light to measure linear growth during the 82 days since oxytetracycline infusion. Oxytetracycline, commonly used as an antibiotic, when injected into animal's body is absorbed by newly forming bone tissue. Bone which is formed within 12 hours, the time duration which oxytetracycline remains in

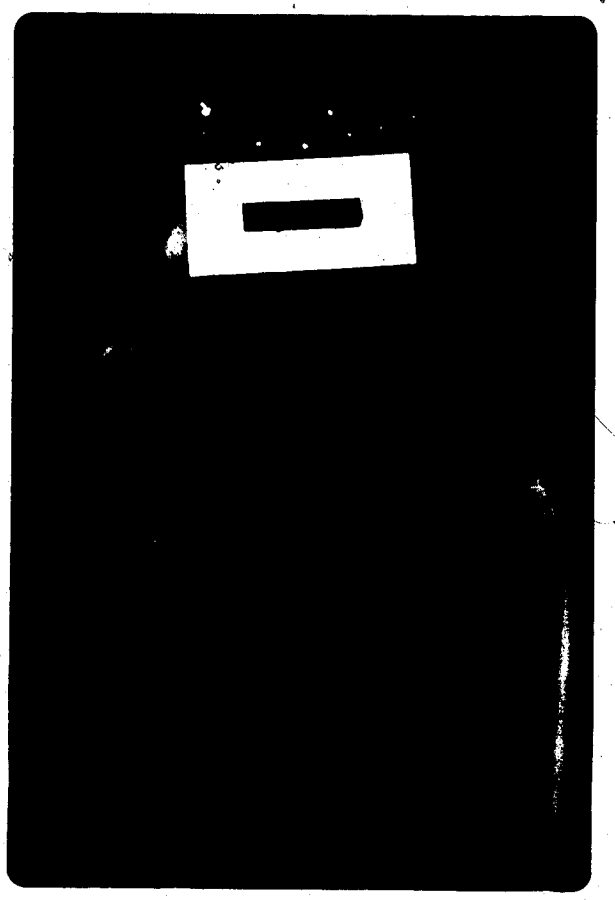

Plate I.4. Photograph of distal extremity of radius showing yellow fluorescent band

the body (Milch et al., 1957), will be "labelled" and all bone formed subsequently will appear normal. The label can be easily detected as a yellow fluorescent band when examined under ultraviolet light (Plate I.4). The growth of bone can thus be recorded by measuring the distance between the fluorescent band formed at the time of infusion and the current position of the growth plate. The ultraviolet lamp used was long wave u.v. lamp 366 nm, 7,000 $\mu w/cm^2$ at 38cm. Kodacolor ASA 400 film was used for taking pictures of the bones. A medium yellow filter to block visible blue light emmitted by the lamp was fitted to the camera. A 1 cm scale was placed on each bone at the time of exposure. After processing the slides were projected and the distance between the growth plate and fluorescent band developed due to absorption of oxytetracycline in the calcifying matrix of the bone was measured at three different places. Data were analyzed for the effects of treatment, age and their interaction by least squares analysis of variance for unequal subclass numbers (Harvey, 1976). Accepted level of significance was $P<0.05$.

RESULTS

Zeranol implantation had no significant effect on average daily gain, feed efficiency (kg of feed required to gain 1 kg live weight), or backfat thickness (Table I.1). There was no effect of age on average daily gain and feed efficiency. The 1-2 year old heifers had less backfat

Table 1.1. Least squares means and standard errors for the traits studied in 32 cull cows and heifers[1]

	Treatment			Age groups (years)					
	Zeranol	Control	Probability	1-2	2-3	3-4	4-5	>5	Probability
No. of Animals	16	16		6	8	6	6	6	
ADG, kg	1.06 ± 0.05	1.14 ± 0.05	P=0.24	1.08ab ± 0.08	1.08ab ± 0.07	1.13ab ± 0.08	1.29a ± 0.08	0.92b ± 0.08	P=0.04
Feed/kg wt gain, kg	10.88 ± 0.71	11.04 ± 0.71	P=0.87	7.25a ± 1.15	10.65b ± 0.99	11.93c ± 1.15	9.70b ± 1.15	15.28d ± 1.15	P=0.01
Backfat, mm	13.69 ± 0.84	12.70 ± 0.84	P=0.42	7.87a ± 1.37	14.30b ± 1.17	14.61b ± 1.37	14.55b ± 1.37	14.53b ± 1.37	P=0.01
SHANK DISSECTION									
Muscle wt., kg	1.83 ± 0.04	1.82 ± 0.04	P=0.77	1.25a ± 0.06	1.81b ± 0.05	1.95bc ± 0.06	2.01c ± 0.06	2.11c ± 0.06	P<0.01
Bone wt., kg	1.11 ± 0.02	1.16 ± 0.02	P=0.10	0.80a ± 0.03	1.14b ± 0.03	1.24b ± 0.03	1.16b ± 0.03	1.34c ± 0.03	P<0.01
Fat wt., kg	0.46 ± 0.03	0.40 ± 0.03	P=0.17	0.27a ± 0.05	0.42b ± 0.04	0.38ab ± 0.05	0.52b ± 0.05	0.52b ±0.05	P<0.01
Muscle:bone ratio	1.65 ± 0.03	1.58 ± 0.03	P=0.08	1.58 ± 0.04	1.59 ± 0.04	1.59 ± 0.04	1.73 ± 0.04	1.59 ± 0.04	P=0.08
RADIO-ULNA MEASUREMENTS									
Length, mm	385.51 ± 1.91	391.39 ± 1.91	P=0.03	346.00a ± 3.10	388.75b ± 2.69	401.58c ± 3.10	398.00c ± 3.10	407.92c ± 3.10	P<0.01
Circumference, mm	125.04 ± 1.31	126.72 ± 1.31	P=0.37	110.33a ± 2.12	126.50b ± 1.84	130.25bc ± 2.12	127.92bc ± 2.12	134.42c ± 2.12	P<0.01
Linear growth at the distal end, mm/82 days	2.67 ± 0.21	2.77 ± 0.21	P=0.74	9.81a ± 0.34	3.36b ± 0.29	0.41c ± 0.34	0.00c ± 0.34	0.00c ± 0.34	P<0.01
Specific gravity	1.45 ± 0.02	1.43 ± 0.02	P=0.35	1.37a ± 0.02	1.42ab ± 0.02	1.49b ± 0.02	1.46b ± 0.02	1.48b ± 0.02	P=0.01

Means having different letters in the same row (a,b,c) are different at P<0.05.
[1]No significant treatment x age interaction was found for any of the traits analyzed.

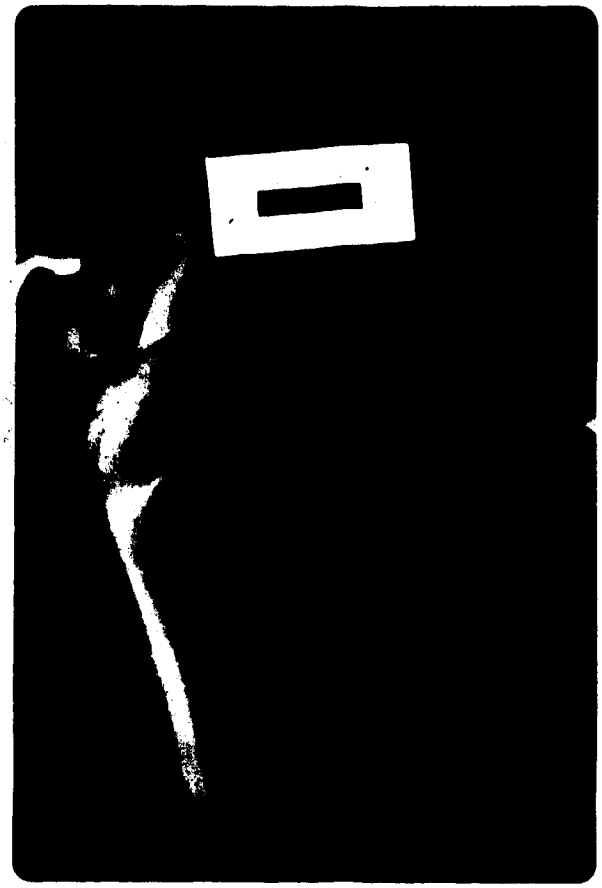

Plate I.5. Photograph of distal end of radius of three years old cow exposed under ultraviolet light (The epiphyseal growth cartilage is about to ossify)

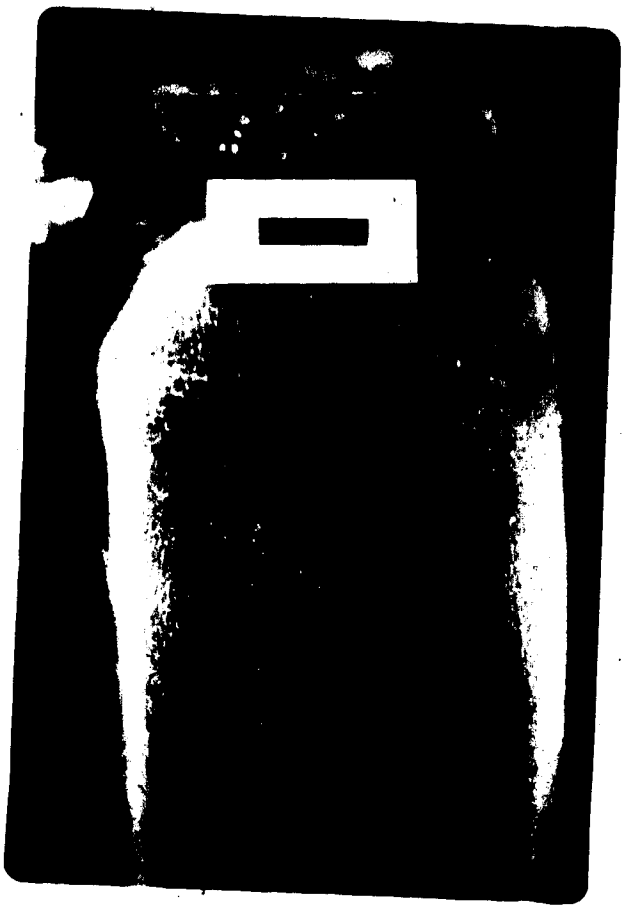

Plate I.6. Photograph of distal end of radius of four years old cow exposed under ultraviolet light (The epiphyseal growth cartilage is replaced by bone)

(P=0.01) than the four older age groups.

The amount of muscle, bone, and fat dissected from the shank and muscle:bone ratio was not affected by zeranol treatment. There was a significant increase in both muscle and bone weight with age. The heifers (1-2 yr) had less dissectable fat ($P<0.05$) than the oldest cows. The muscle:bone ratio of the shank was not affected by age (Table I.1).

The radio-ulnae of zeranol treated cattle were shorter (Table I.1) than the controls ($P<0.01$). There was no significant effect of zeranol implantation on the rate of growth at the distal end of the radius in the 82 days prior to slaughter, in circumference or specific gravity of radio-ulnae.

The length, circumference, growth rate at the distal end of radius, and specific gravity of the radio-ulna were all affected by the age ($P<0.01$). The significant increase in the length of the bone stopped at the age of 3-4 years (Plates I.5,I.6).

No significant treatment x age interaction was found for any of the traits analysed.

DISCUSSION

Zeranol had no significant effect on average daily weight gain (Table I.1). This agrees with the results of Price and Makarechian (1982), though they reported an interaction between age and zeranol treatment for average

daily gain. There was no effect of zeranol on feed efficiency or backfat thickness. Age had a variable effect on growth and efficiency. The 1-2 year old heifers had 7-9 mm backfat which was less ($P<0.05$) than the 14.5 mm average of the older cows. The shank dissection indicated that the zeranol had no significant effect ($P>0.05$) on the amount of muscle, bone, and fat dissected from the shank or on the shank muscle:bone ratio. However, the oldest cows (>5 yr) had more dissectable fat ($P<0.05$) than the heifers (1-2 yr). There was no effect of age on shank muscle to bone ratio, but there was a significant increase in both muscle and bone weight with age.

The length of the radio-ulna was less ($P<0.05$) in zeranol treated cattle than in controls (Table I.1). However, no significant effect of zeranol treatment on growth rate of the bone during the 82 days before slaughter could be detected by the oxytetracycline method. The bone growth at this epiphyseal plate during the trial period of 82 days could have been too small to detect significant differences among the treatments or the growth might have been occuring in the hyaline articular cartilage at the ends of the bones. Zeranol had no effect on the circumference or specific gravity of the radio-ulnae. The treatment differences in bone length may have been caused by zeranol binding with the estrogen receptors in the epiphyseal growth plates and invoking a similar response to that of naturally occurring estrogens as suggested by Katzenellenbogen et al.

(1979). It is known that estrogens reduce linear bone growth and hasten closure of epiphyseal growth plates (Silberberg and Silberberg 1971).

The length and circumference of the radio-ulna were affected by age ($P<0.01$) up to 3-4 yr (Table I.1), after which no difference in length was evident. The rate of growth detected at the distal end of the radius confirmed that active growth was occurring in the 1-2 and 2-3 yr old females, that some growth was occurring in the 3-4 yr old group and that no growth was occurring at this site in the two oldest groups. The radio-ulna grows at four separate epiphyseal plates, corresponding to one plate each at the distal and proximal end of the radius and the ulna. The distal radius plate was reported by Sisson and Grossman (1964) to ossify at approximately 39 to 48 mo of age which corresponds to the results of the present study. The rates of growth found at the distal radius in the present experiment also confirm those reported by Graham and Price (1981). The specific gravity of the radio-ulna bones was significantly less in the youngest group than in the three oldest groups. Jones et al. (1978) reported an increase in bovine radio-ulna density during the first two years. Field et al. (1974) indicated that increase in density with age was due to gradual dehydration, presumably as a result of an increase in fat and ash in bones.

It is concluded that zeranol has an inhibitory effect on the growth of the radio-ulna bone in young cows, but that

It has no influence on the gain and carcass traits in cows and heifers.

II. EFFECTS OF ZERANOL ON CARCASS TRAITS AND IN PARTICULAR BONE GROWTH IN STEERS AND BULLS

INTRODUCTION

Zeranol, in the commercial form Ralgro® (IMC:Terre Haute), is used as a growth stimulant for feedlot cattle (Perry et al., 1970; Price and Makarechian, 1982; Ford and Gregory, 1983). Although its effects on muscle and fat deposition have been studied (Ford and Gregory, 1983), little is known of its effects on bone growth. It was reported in the previous chapter that zeranol-treated culled cows had significantly shorter radio-ulna bones than untreated cows. Staigmiller et al. (1983) reported that zeranol increased the pelvic openings in a group of heifers.

It has been suggested by Wiggins et al. (1979) that zeranol may reduce thyroid function and hence metabolic rate. If this is true, it could be anticipated that the substance would reduce the level of physical activity in implanted animals. In the present experiment the effect of zeranol on linear bone growth, size of pelvic opening and physical activities such as fighting, bunting, mounting, flehmen and libido were studied in steers and bulls.

MATERIALS AND METHODS

Forty eight male Hereford crossbred calves from The University of Alberta ranch at Kinsella, Alberta were used for this study. They were weaned in October 1982 at 5-6

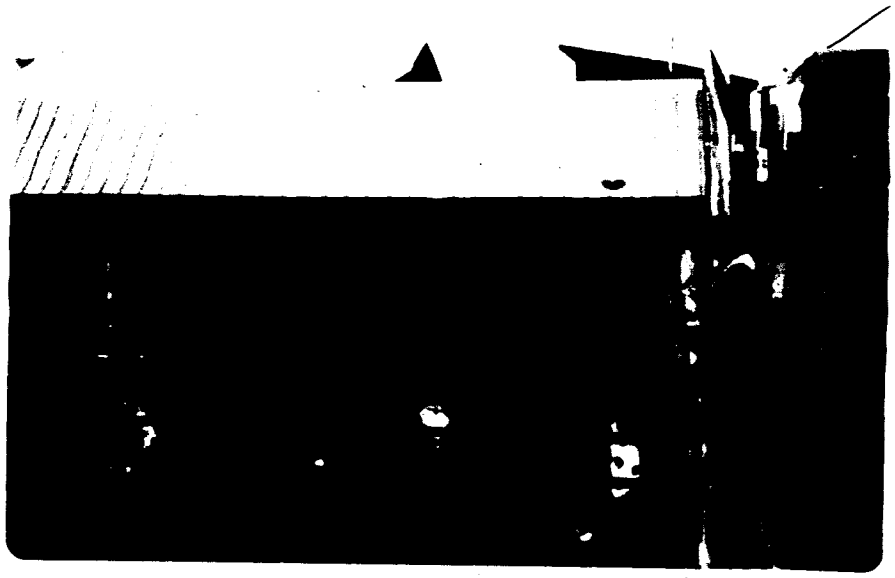

plate II.1. Pens where the cattle were kept during the experiment

months of age, were fed on long hay and gradually (concentrate diet was supplemented to hay in 1:5 ratio and then concentrate portion of the diet was increased 20% every 4 days) introduced to a diet of 64% barley, 21% oats, 10% pelleted dehydrated alfalfa hay and 5% of a protein/mineral/vitamin supplement, until after about three weeks, it was being offered ad libitum. The animals were ranked for body weight and each four animals with the least weight differences were randomly allocated to 4 groups of 12 calves each. On day 1 (1982 11 10), animals in two of the groups (24 of the calves), were castrated, and one group of bulls and one group of steers was implanted with 36 mg zeranol (one steer was mistakenly implanted instead of a bull). They were allocated to 4 pens (Plate II.1), one for each gender x treatment combination. The animals were weighed monthly.

The implanted cattle were reimplanted on day 62 of the experiment as the blood levels of the compound start dropping after 2 months (Sharp and Dyer, 1972). All the cattle were infused with oxytetracycline (20 mg/kg body weight) 64 days before slaughter to monitor linear bone growth as described in chapter 1.

One month before slaughter the internal horizontal and vertical diameters of the pelvic openings were measured through rectum using a Rice pelvimeter (Figure II.1) as described by Wiltbank and LeFever (1961) and at the same time scrotal circumferences were measured at the widest part

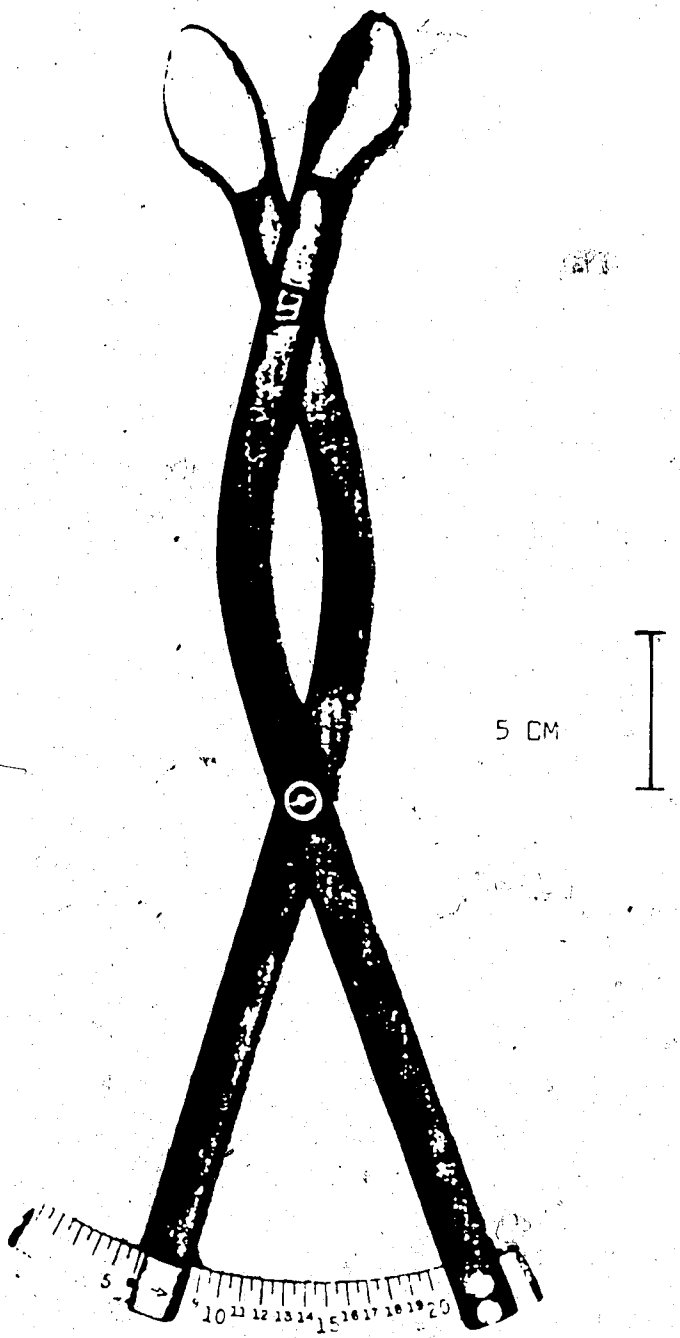

Figure II.1. Rice Pelvimeter

of the testes and the bulls were scored for libido from minimum 0 (no interest) to maximum 10 (two services and continued interest).

Pelvic measurements

The Rice pelvimeter, developed at the Animal Science Department of Colorado State University, consisted of two cast aluminum arms and a stainless steel scale graduated in cm. The measurements were read on the inside of the measuring arm marked with an arrow (see Figure II.1).

Two measurements were made via the rectum, a vertical measurement and a horizontal measurement. The vertical measurement was taken by holding the pelvimeter in a vertical position, spreading the jaws slowly while placing the end of one jaw on the symphysis pubis and the other jaw on the sacral vertebrae (Figure II.2). The horizontal measurement was taken at the level of psoas tubercles. The psoas tubercles form a depression point in the shaft of the ilium.

Scrotal Circumference

The scrotal circumference was measured by a scrotal tape (Figure II.3a) made from a vinyl-coated fiberglass tape, formed into a sliding loop. The slider (S) in Figure II.3a was made from a rectangular piece of cork (slightly wider than the tape) and nylon-reinforced strapping tape.

Before measuring the scrotal circumference the scrotal contents were thoroughly palpated and the testes were brought into the lower part of scrotum so that they were

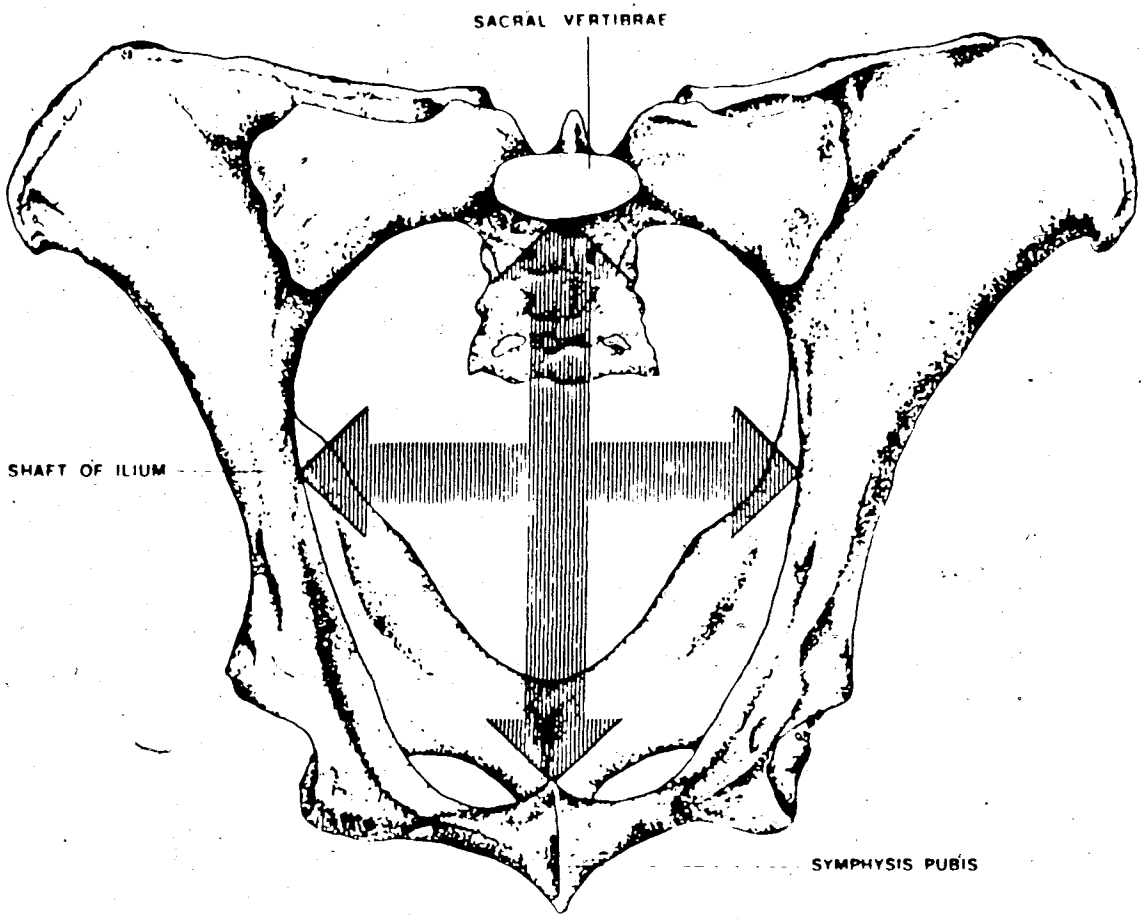

Figure II.2. Sites for taking vertical and horizontal pelvic measurements

side by side and wrinkles were eliminated. The fingers and thumb of one hand were then placed on the sides of the scrotum so as to cradle the testes (Figure II.3b). The scrotal tape was next slipped over the testes-scrotum and contracted around the largest circumference. Tension on the sliding tape with the thumb was placed until moderate resistence was met and the circumference in mm was recorded.

Libido Scoring

For libido scoring, the reaction of each bull, to cows in estrus restrained by the head in the corner of a pen, for a period of 15 minutes was observed (Chenoweth, 1981). The following scoring system was adopted:

0 = bull showed no sexual interest.

1 = sexual interest (e.g. flehmen) showed only once.

2 = positive sexual interest in female on more than one occasion.

3 = active pursuit of female with persistent sexual interest.

4 = one mount or mounting attempt. No service.

5 = two mounts, or mounting attempts. No service.

6 = more than two mounts or mounting attempts. No service.

7 = one service followed by no further sexual interest.

8 = one service followed by sexual interest including mounts or mounting attempts.

9 = two services followed by no further sexual interest.

10 = two services followed by sexual interest, including mounts, mounting attempts or further services.

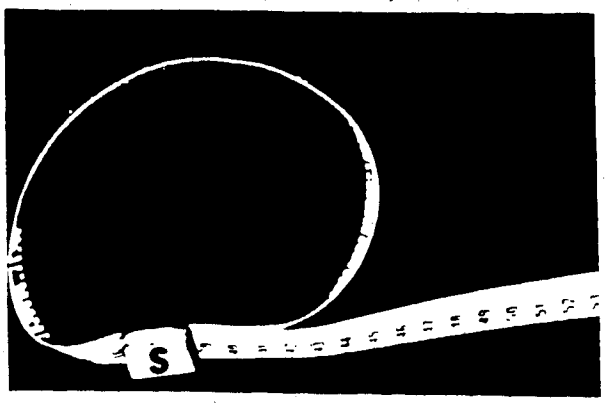

Figure II.3a. Scrotal circumference measuring tape

Figure II.3b. Method of measuring the scrotal circumference

Sexual and Agressive Behaviour

The bulls were observed for 15 min in their pens on each of 22 consecutive evenings during the last month of the trial; three classes of behaviour were recorded: flehmen (a lip-curl response, preceded by licking or smelling the urine of another animal), mounting another animal and bunting (hitting any part of another animal with the head). In addition the total time involved in head to head bunting and pushing was recorded as fighting (Tennessen, 1983).

At the end of the trial period (day 183) the cattle were trucked to a commercial packing plant 150 km away in Edmonton for slaughter. After slaughter the left fore and hind feet were cut off at the knee and hock respectively and brought to the meat laboratory. The metacarpal and metatarsal bones were dissected out, weighed and length-measured.

After dressing and overnight chilling at the packing plant the carcasses were graded by Agriculture Canada personnel and the left fore shank was removed with a cut above the 'elbow' (humerus to radio-ulna) joint. The shank was trimmed to include only the radio-ulna, its associated flexor and extensor muscles and fat, and then dissected into bone, muscle and fat. The length, the minimum circumference and the density of each radio-ulna were recorded as described in chapter 1. They were then split longitudinally and their distal extremities were photographed under ultraviolet light to expose the oxytetracycline fluorescence

and record longitudinal growth (chapter 1).

All data were analyzed by least squares analysis of variance (Harvey, 1976). The behaviour data were transformed before statistical analysis to more closely approximate a normal distribution. The log transformation was used for the fighting and bunting data (after adding 1.0 to allow the transformation of scores = 0); the square root transformation was used for the mounting and flehmen data (Steel and Torrie, 1980).

RESULTS

There were no significant gender x treatment interaction effects for any of the traits shown in Table II.1. Average daily gain (ADG), estimated by the linear regression of liveweight on time did not differ between genders ($P>0.05$). It was greater ($P=0.01$) in the zeranol treated cattle than in the controls. The backfat thickness of the carcass at the grading position (minimum fat thickness at the lateral quarter of longissimus dorsi, between 11th and 12th ribs) was greater ($P<0.01$) in steers than in bulls, and dressing percentage and the longissimus dorsi area were greater ($P<0.01$) in bulls than in steers. Neither of the two traits were significantly influenced by zeranol treatment. The fore-shank dissection showed that bulls had more muscle ($P<0.01$), less fat ($P<0.05$) and a higher muscle to bone ratio ($P<0.01$) in this region than steers, but zeranol implantation had no significant effect on these traits. The

Table II.1. Least squares means of live animal and carcass data from zeranol implanted and control bulls and steers

	Effect of Gender			Effect of Zeranol				
	Bulls	Steers	Probability	Implanted	Control	Probability	SEM‡	Gender x Zeranol Interaction
Number	24	24		24	24			
ADG kg/day	1.67	1.56	P=0.07	1.69	1.54	P=0.01*	0.04	P=0.09
Grade fat thickness mm	9.9	12.6	P<0.01*	11.2	11.3	P=0.88	0.68	P=0.54
Slaughter weight, kg	499	474.0	P=0.14	478	495	P=0.32	11.80	P=0.15
Dressing percentage	60.8	58.8	P<0.01*	60.1	59.5	P=0.20	0.36	P=0.72
Longissimus dorsi area sq.cm.	89.2	72.4	P<.01*	84.0	77.7	P=0.18	2.57	P=0.10
Shank dissection								
Muscle kg	2.07	1.77	P<0.01*	1.93	1.92	P=0.30	0.05	P=0.95
Fat kg	0.64	0.77	P=0.03*	0.71	0.70	P=0.90	0.04	P=0.40
Bone kg (Radio-ulna)	1.25	1.19	P=0.17	1.22	1.22	P=1.00	0.03	P=0.44
Muscle:Bone	1.67	1.51	P<0.01*	1.59	1.59	P=0.87	0.03	P=0.25
Metacarpal kg	0.52	0.51	P=0.54	0.51	0.52	P=0.81	0.01	P=0.66
Metatarsal kg	0.59	0.58	P=0.81	0.58	0.59	P=0.72	0.01	P=0.66

* Levels of probability considered to be significant.
‡SEM = Standard error of means

weights of the radio-ulna, metacarpal and metatarsal bones were not influenced by either castration or zeranol treatment.

Radio-ulna length was not significantly affected by castration (Table II.2), but its circumference and density were both greater ($P<0.01$) in bulls than in steers. None of these traits was significantly influenced by zeranol treatment. There was, however, a significant interaction between gender and zeranol treatment for bone density ($P=0.05$). Treatment resulted in an increased bone density in steers and decreased bone density in bulls. Neither gender nor zeranol treatment had any significant effect on the lengths of the metacarpal or metatarsal bones.

Growth at the distal end of the radius during the last 64 days of the experiment (from about 10 months to one year of age) was significantly greater in bulls than in steers ($P<0.01$) and in control than in zeranol treated cattle ($P<0.01$). There was also a significant interaction ($P=0.02$) between gender and treatment for this trait. The linear bone growth was affected more in steers (22.4%) than bulls (4.5%). Zeranol treated cattle had significantly larger horizontal ($P<0.01$) pelvic diameters than control (Table II.2). Castration had no significant effect on pelvic diameters.

Libido among the bulls was significantly ($P<0.01$) reduced by zeranol treatment (Table II.3) but treatment had no significant effect on the other sex related behaviour

Table II.2. Least squares means of skeletal data from zeranol implanted and control bulls and steers

	Effect of Gender			Effect of Zeranol				
	Bulls	Steers	Probability	Implanted	Control	Probability	SEM‡	Gender x Zeranol interaction
Number	24	24		24	24			
Radio-ulna:								
Length mm	373	370	P=0.40	369	374	P=0.20	2.8	P=0.96
Circumference mm	167	158	P<0.01*	164	160	P=0.12	1.8	P=0.35
Density g/cc	1.51	1.42	P<0.01*	1.47	1.46	P=0.80	0.02	P=0.05*
Metacarpal length mm	206	207	P=0.57	206	207	P=0.50	1.67	P=0.82
Metatarsal length mm	232	233	P=0.78	231	234	P=0.20	2.66	P=0.85
Growth at distal radius mm/60 days	9.17	8.11	P<0.01*	8.03	9.26	P<0.01*	0.23	P=0.02*
Pelvic diameter:								
Horizontal mm	123	123	P=0.60	126	120	P<0.01*	1.20	P=0.42
Vertical mm	141	143	P=0.30	144	140	P=0.07*	1.12	P=0.74

* Levels of probability considered to be significant.
‡SEM = Standard error of means

traits recorded, or on scrotal circumference.

DISCUSSION

Castration had no significant effect on average daily gain in this trial as compared to 17% normally reported (Field, 1971). This was probably due to the fact that animals were relatively young and the the effects of castration on growth are not fully exhibited before puberty (Price and Yeates, 1969).

The positive effect of zeranol on growth in male cattle of both genders is well known (Perry et al., 1970; Greathouse et al., 1983). Although the gender x treatment interaction was not statistically significant ($P=0.09$), the effect of zeranol was greater in steers (17.4%) than in bulls (3.1%). Price et al. (1983) and Perry et al. (1970) have reported that zeranol implanted bulls gain 5-10% faster than controls. Zeranol implantation had no effect on backfat thickness, dressing percentage or longissimus dorsi area (Table II.1), which is in agreement with previous reports (Greathouse et al., 1983; Price et al., 1983).

Shank muscle to bone ratio was greater in bulls than in steers ($P<0.01$), a phenomenon which was associated with an increase in shank muscle weight rather than a decrease in radio-ulna weight. The increase in shank muscle to bone ratio is likely to correspond to a greater muscle to bone ratio in the carcass as a whole (Butterfield, 1965). The greater muscularity of entire males is well known (Field,

Table II.3. Least squares means of sexual development data in zeranol implanted and control bulls

	Control	SEM	Implanted	SEM‡	Probability
No. of animals	13		11		
$\log_{10}$ (Fights +1)[1]	.17	.015	.26	.015	P = .59
$\log_{10}$ (Bunts +1)[1]	.37	.015	.40	.105	P = .87
Mounts[1]	.08	.005	.25	.005	P = .12
Flehmen[1]	1.06	.036	.82	.36	P = .37
Libido[2]	4.38	.384	3.18	.418	P = 0.01*
Scrotal circumference (cm)	25.38	.284	24.91	.308	P = 0.43

[1]Data transformed as shown, see text for details
[2]0 = no interest, 10 = maximum interest
‡SEM = Standard error of means

1971).

The thicker ($P<0.01$) and denser ($P<0.01$) radio-ulnae of the bulls (Table II.2) are attributed to testicular androgens which are known to stimulate matrix formation, and to promote calcium retention and positive nitrogen balance in the skeleton (Simmons, 1971). Jones et al. (1978) observed no effect of castration on the density of radio-ulnae but they reported denser femurs in bulls than steers. Silberberg and Silberberg (1971) reported thicker bones in intact males than in castrates in all the mammals they studied.

Bone growth at the distal epiphyseal cartilage plate of the radius was significantly less than control ($P<0.01$) in zeranol treated animals (Table II.2). An explanation for this observation has been suggested by Katzenellenbogen et al. (1979) that zearalenones (zeranol is a derivative of zearalenone) bind with estrogen receptors and evoke an estrogenic response. It has been reported that estrogens retard linear bone growth by interfering with chondrocytic proliferation and matrix synthesis in the epiphyseal growth plates (Simmons, 1971). Linear bone growth was more ($P=0.01$) in bulls than in steers. Androgens have been shown to stimulate bone growth in many species of mammals, including man (Silberberg and Silberberg, 1971).

Ralston (1978) reported that zeranol, implanted at birth or at 90 days, retarded the development of masculine characteristics such as curly hair on the neck and head,

width of head, and crest development in cattle. In the present experiment pelvic diameters were significantly larger in zeranol treated animals, which is presumed to be a feminine characteristic. It has been reported that spaying in female animals results in smaller pelvic diameters and exogenous estrogens cause pubic relaxation (Silberberg and Silberberg, 1971). Staigmiller et al. (1983) observed a significant increase in "pelvic area" (vertical x horizontal diameter) in zeranol treated yearling heifers.

Zeranol significantly (P=0.01) lowered the libido scores in treated bulls. This contrasts with the study by Price et al. (1983) which reported no difference in the libido of zeranol treated and control bulls. In the present study all the bulls showed low level of libido but this is normal among such young and inexperienced bulls (Chenoweth, 1981). Scrotal circumference in bulls was not affected by zeranol which is in agreement with Price et al. (1983) for bulls implanted at about puberty. There is considerable evidence that implanting before puberty will inhibit sexual development (O'Lamhna and Roche, 1984).

Although bulls produce beef more efficiently than steers (Price and Yeates, 1969; Field, 1971), one disadvantage of bulls is their aggressive behaviour and the consequent increase in incidence of dark, firm, dry (DFD) meat compared to steers (Price et al., 1983). It has been suggested by Wiggins et al. (1979) that zeranol implantation would reduce the secretion of thyroid hormones and hence may

reduce the metabolic rate and physical activity. Zeranol has also been claimed as a tranquilizing agent in cattle (Brown et al., 1975). Hence, it might be anticipated that zeranol implantation would reduce the physical activity in bulls. In this study zeranol had no effect on behavioural traits, other than libido, which confirms the report of Price et al. (1983) who found no significant effect of zeranol implantation on behaviour traits of bulls which had been mixed together from several pens.

It is concluded from this work that zeranol implanted at weaning has a negative effect on bone growth but increases pelvic diameters in steers and bulls and reduces libido in bulls. It does not appear to have any other influence on feedlot behaviour.

III. EFFECTS OF CASTRATION AND SEX STEROIDS ON GAIN, FEED EFFICIENCY, CARCASS TRAITS, AND BONE GROWTH IN PIGS

INTRODUCTION

The endocrine system plays a fundamental role in growth. The endocrine glands secrete hormones that influence metabolic activities and most hormones, directly or indirectly, affe he growth processes.

Androgens in the male and estrogens and progesterone in the female are the principal steroid secretions from the gonads. Steroids are organic molecules which have in common a perhydroxy-cyclo-pentano-phenan-threne nucleus. They are so named because they are related to, and in most cases derived from, sterols which are found abundantly in nature, usually in the non-saponifiable fraction of animal and plant fats (Applezweig, 1962). The adrenal cortex also produces limited quantities of androgens, estrogens and progesterone (McDonald, 1980).

Hormones (both endogenous and exogenous) are transported to the target organs through the blood stream. In the target organs they attach to the receptor sites and accelerate or decelerate normal biochemical processes (Roth et al., 1979).

The influence of endogenous sex steroids on the body can be studied by castrating the male animal or spaying the female animal, and comparing them with intact animals. One effect of the removal of the gonads is a marked reduction in

secretion and subsequent low blood levels of androgens and estrogens in males and females respectively. Comparative studies of growth and body composition of entire and castrated male and female animals are therefore an indication of the action of androgens and estrogens on the rate of growth and body composition.

A few workers (Dinusson et al., 1950; Robertson et al., 1969; Wise and Ferrel, 1984) have examined the effects of spaying female animals (heifers) but the procedure showed no improvement in the growth traits studied.

One of the effects of androgens is to increase the synthesis of proteins in the muscles. This is mediated by androgen receptors in the muscle fiber cytosol (Michel and Baulieu, 1976; Snochowski et al., 1981). Estrogens also increase muscle growth, not by deposition of muscle protein but perhaps due to changes in the secretion of other hormones (Rabkin and Frantz, 1965; Davis et al., 1977).

In the epiphyseal cartilage of bones, testosterone increases maturational changes which lead to epiphyseal-diaphyseal fusion. These changes include stimulation of age changes in the cells to promote the formation of hypertrophic chondrocytes (Howard, 1963). The estrogens also promote maturational changes (changes leading to epiphyseal-diaphyseal union) in the epiphyseal growth apparatus (Simmons, 1963).

Gain and Carcass Traits

Numerous studies have shown that entire males of all species of farm animals grow more rapidly, utilize feed more efficiently and produce a high yielding carcass with less fat than castrated males (Turton, 1969; Field, 1971; Seideman et al., 1982). The disadvantages of the intact male to the farmer and consumer include aggressive behaviour (bulls and boars) and occassionlly undesirable odors (boars) due to the presence of androstenone (5α-androst-16-ene-3-one) in the fat (Patterson, 1968).

A great deal of work has been undertaken to fully elucidate the differences in production characteristics and body composition between boars and castrates but certain quantitative aspects remain unclear. According to Galbraith and Topps (1981), "unlike cattle, when entire and castrated male pigs are compared under conditions of ad libitum feeding the latter tend to grow more quickly mainly because they eat more feed. If feed is restricted however, the reverse trend may be seen. Irrespective of level of nutrition, boars are more efficient than castrates in converting feed to live weight and the difference is even greater if the efficiency of production of lean meat is considered".

Most workers studying growth characteristics of male and castrate pigs, have ignored bone growth. In the present work an attempt has been made to address this neglected aspect of growth.

The development of synthetic estrogenic compounds (Dodds et al., 1938) resulted in their use for growth promotion in poultry (Lorenz, 1943), beef (Dinusson et al., 1948), lamb (Andrews et al., 1949) and pig production (Braude, 1947). However, the potential health hazards associated with the use of some synthetic estrogenic substances, especially diethylstilbestrol, were recognized and during the 1970s several countries banned the use of this drug. This resulted in an intensive search for alternatives

Estrogenic compounds e.g. hexoestrol, dienoestrol (Perry et al., 1954), estradiol-17β (Hale and Ray, 1973), polydiethylstilbestrol phosphate (Angeo and Gassner, 1966), and stilbene (Preston et al., 1978) have been successfully used as feed additives and implants in improving liveweight gain and feed efficiency in ruminants.

Much of the information regarding the use of sex steroids on growth and feed conversion efficiency in pigs is conflicting and inconclusive. Braude (1947) first reported that feeding 50 mg of diethylstilbestrol daily to young castrated male pigs produced a slight increase in growth rate. Later investigation also indicated a small improvement in liveweight gain due to diethylstilbestrol feeding (Beeson et al., 1955; Sewell et al., 1957). The subcutaneous implantation of stilbestrol either as a single implant (Dinusson et al., 1951; Murphree et al., 1951; Gorrill et al., 1964) or as more than one implant (Woehling et al.,

1951; Pearson et al., 1952) did not increase rate of gain in barrows or gilts. Sleeth et al. (1953) studied the effect of weekly intramuscular injections for 6 weeks of estradiol benzoate on the growth rate of barrows and gilts. This treatment, likewise, did not significantly affect average daily gain. Heitzman and Clegg (1957), Beacom (1963) and Cahill et al. (1960) have indicated that greater production efficiency (kg of weight gained per kg of feed consumed) and leaner carcasses may be obtained by the use of this compound. Elliot and Fowler (1974) demonstrated that oral diethylstilbestrol and ethylestradiol stimulated the growth of lean tissue by 3 percent and 11 percent respectively.

As regards the effect of androgens on gain and feed efficiency some studies have shown that oral treatment (Norland and Burris, 1956; Perry et al., 1954), subcutaneous implanting (Woehling et al.,1951; Bratzler et al.,1954) or injection (Sleeth et al., 1953) of testosterone propionate had no effect on growth or carcass characteristics of females or castrated males. However, Beeson et al. (1955) while recording variable effects on growth, noticed an increase in the percentage of lean cuts (hams, loins, picnics, and boston butts) and a reduction in the percentage of fat cuts (fat backs, bellies, and jowls) through the oral administration of 20 mg of methyltestosterone daily. Other workers have also demonstrated an increase in the lean content of pig carcasses following treatment with oral methyltestosterone (Elliot and Fowler, 1974; Whiteker et

al., 1959; Thrasher et al., 1959; Fowler et al., 1978) while obtaining either small or zero effects on live weight gain. Robinson and Singleton (1966) reported that castrated male pigs given a basal diet containing 12 percent crude protein and incorporating the anabolic steroid norbolethone had faster growth rates and leaner carcasses than pigs fed on a diet containing 18 percent crude protein, although live weight was depressed. In contrast, Williams and Dunkin (1975) observed that treatment of gilts and barrows with injections at 4 weekly intervals of the anabolic steroid 17-β-hydroxyestr-4-en-3-ene-4 methyl bicyclo (2.2.2) oct-2-ene-1-carboxylate (RS-3268-R) resulted in less backfat, but only in pigs fed on a diet high in crude protein.

The effects of castration on weight gain, feed efficiency and carcass traits have recently been reviewed by Seideman et al. (1982). These authors have concluded that differences in growth rate between boars and barrows are not great. Differences in feed efficiency are also small, but boars are more efficient. Sixteen of the research papers quoted by Walstra and Kroeske (1968) stated that boars grew faster than barrows but in twelve cases the differences were not significant. Nine references show increased gains in barrows.

In all of the studies regarding the effect of anabolic steroids on growth and carcass composition of swine the substances were used as injections, feed additives or slow

release subcutaneous implants. Injections of steroids provide neither a suitable treatment regime nor stable blood levels. The effect of implants in the form of pellets is of short duration (Schake et al., 1979) and the absorption by the animal is variable over time (Utley et al., 1980).

Silastic rubber implants either impregnated or filled with steroids deliver a constant and predictable dose of hormone (Stratton et al., 1973). The effects of testosterone and estradiol-17β in the form of silastic rubber implants have not been explored extensively in pigs.

Bone Growth

Bone growth consists of two parts; increase in length and increase in cross section. In the limb bones increase in length takes place at the epiphyseal cartilage plates and growth ceases when these plates ossify; continued increase in cross section, however, appears to be a life long possibility (Garn, 1981).

The rigid nature of skeletal tissue makes its interstitial growth impossible, and in all situations increase in the size of a bone is brought about by the deposition of new tissue on an existing surface (McLean and Budy, 1964).

The formation of bone is the function of specialized cells, the osteoblasts. In situations such as the periosteal region of the diaphysis in long bones these cells form bone by surface accretion, while at the ends of the shaft they

form bone on a scaffolding provided by the continuous proliferation of the cartilaginous epiphyseal plate. The first type of bone formation is known as membranous ossification and the second as endochondral ossification (Sissons, 1971).

In the mammalian skeleton, endochondral ossification consists of a coordinated sequence of cellular processes. These are the multiplication, growth, and degeneration of the cartilage cells in the cartilage plate; the invasion of the hypertrophic cartilage by blood vessels and connective tissue from the adjacent shaft of the bone; the formation of a network of bone trabeculae on the framework of unresorbed cartilage by the ingrowing connective tissue cells; and finally the remodelling, or progressive structural modification of the bony tissue (Figure III.1). Under normal circumstances all of these proceed in equilibrium, with the result that the trabeculae of the metaphysis extend continuously into a receeding zone of hypertrophic cartilage, thus extending the length of the bony shaft (Ogden, 1980).

Long bone growth in most species of mammals and birds is inhibited by estrogens, but their effects vary considerably depending upon a number of modifying factors, and the individual bone under investigation.

Exogenous estrogen administration results in the suppression of longitudinal bone growth and premature aging of cartilage in guinea pigs, mice, and rats (Silberberg and

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Figure III.1. Schematic of the zones of the growth plate concerned with longitudinal and latitudinal ossification. PC=perichondrium, PO=periosteum, M=undifferentiated mesenchymal tissue, ORL=ossification ring. (Taken from Urist, M.R. 1980. Fundamental and Clinical Bone Physiology. J.B. Lippincott Company. Philadelphia.)

Silberberg, 1939, 1941a, 1941b). The latter report also indicated that under the influence of a moderate dose (not described) of estrogen the growth inhibition was transitory followed by thickening of the epiphyseal plates associated with an increased proliferation of chondrocytes in guinea pigs, which was not true with rats and mice. Administration of 12.5 μgm estradiol every day for 8-10 days caused significant advance in the skeletal age of female but not of male, rats (Talbot, 1939).

Prolonged subcutaneous administration of 10,000-20,000 i.u. estradiol benzoate per week for 5-6 months caused inhibition of the growth of the skeleton and earlier closure of epiphyseal growth plates of proximal humerus, femur and tibia in dogs (Sutro and Pomerantz, 1942). Berntsen (1968) observed a reduction in bone growth of rabbits (4 females and 2 males) by injecting 2-5 mg of estradiol monobenzoate every other day for 30 days.

Linear growth of the femur was increased in orchidectomized and ovariectomized (at 2-3 months of age) mice given small doses (0.2-20 μg) of estrogen (Suzuki, 1958). There was no effect of small doses (2-10μg) of estrogen on the tail and body length of hypopituitary mice. However, higher doses (50μg) administered with growth hormone resulted in reduced tail length as compared to mice treated with growth hormone alone (Holder et al., 1983).

Budy et al. (1952) reported that with large doses (4mg/week) of estrogen the growth of the long bones was

greatly retarded in rats, but with moderate doses (2mg/week), the long bones were only slightly shorter than the controls and the metaphyses were elongated to four times the length of those in nontreated rats. In the same study it was observed that compared to the mouse, the rat appeared to be more resistant to the skeletal effects of estrogen.

According to Noback et al. (1949) new-born rats treated from the day of birth were more resistant to the skeletal effects of estrogen than were weanling rats.

Negulesco and Kossler (1978) showed that the administration of daily estrone levels of 0.4 mg for 14 days to chicks resulted in a significant depression of growth in the thickness of the hypertrophic cartilage zone of the distal radius. The mean thickness of the remaining zones (resting, proliferating, and calcified cartilage) and all cartilage zones of the distal epiphyses of chicks receiving hormonal levels of 0.2 mg were similar to controls. Daily administration of 0.2 mg estrone resulted in a significantly increased growth in height of the resting and hypertrophic cartilage zones of the proximal epiphyses.

It has also been shown by Gardner (1943) that the effect of estrogen varies from one part of the skeleton to another. Studies with human beings have indicated that estrogen treatment of excessively tall girls (van den Bosch et al., 1981) and boys (van den Bosch et al., 1982) can curb the linear growth.

Little research has been conducted into the effects of estrogens or androgens on various aspects of bone growth in farm animals, particularly pigs.

Sobel et al. (1956) have reported that exogenous testosterone stimulates linear growth only in hypogenital boys. Simpson et al. (1944) observed the same effect in hypophysectomized and castrated rats. According to Bergstand (1950) growth of long bones was inhibited in rabbits by exogenous androgens. Howard (1963) has shown that androgens enhance skeletal age in mice. All these reports suggest that adequate testosterone levels are essential for optimum skeletal growth of the species studied.

There are a few recent reports regarding the effect of castration on bone growth. In cattle, castration delays closure of the epiphyseal plates, which is most noticeable in the distal bones of the legs and in the vertebral column (Swatland, 1984). It is reported in chapter 1 of this writing that the long bones were thinner and the rate of growth at the distal end of the radius was less in steers than in bulls. Work with rodents has indicated that the body length, tail length, and body weight was less in rats castrated just after birth (van Wagenen, 1928) compared to late castrated ones. Tang (1941) observed that on the average normal male albino rats had 2% longer tibiae and 5% longer femurs than their castrated litter mates.

The epiphyseal growth plate demonstrates a highly organized pattern of cellular orientation. Proximally,

beneath a zone of more or less randomly organized cells, the cartilage cells of the growth plate become lined up in narrow, well separated columns. The upper most cells of each column, by a process of continual cell division, are responsible for longitudinal growth of the bone (Kember, 1960). Toward the lower (more metaphyseal) ends of the columns the cells become progressively larger, contain increasing amounts of glycogen and alkaline phosphatase, and are presumably concerned, in a manner as yet not completely understood, with matrix mineralization (Neuman, 1980). Throughout their lifespan both of these groups of specialized cells synthesize a matrix composed primarily of collagen and protein-polysaccharide (Shatton and Schubert, 1954; Guri et al., 1965).

Exogenous sulfur, as a sulfate, through ester linkages, is incorporated in the mucopolysacchrides of the ground substance of cartilages and bones (Rang, 1969). The radionuclide $^{35}SO_4$ has proved useful as a tracer element in studying this incorporation. It has been shown by Greer et al. (1968) that the amount of $^{35}SO_4$ incorporated in vitro is directly proportional to the rate of protein-polysaccharide synthesis by the chondrocytes.

There are a few reports in the literature about the effects of steroids on cartilage formation using $^{35}SO_4$ incorporation to study protein-polysacchrides (proteoglycans) synthesis. Most of the work in this field has been done on laboratory animals, particularly rodents,

and the cartilages used were the articular cartilages. It is known that physical composition and metabolic activity of the epiphyseal cartilage differs in many respects from articular or costal cartilage e.g. collagen content of epiphyseal cartilage is lower than that of costal cartilage and chondroitin sulfate is considerably higher (Greer et al., 1968). Burnett and Redi (1983) have reported that estrogens in combination with progesterone did not influence sulfate incorporation into proteoglycans. Holder et al. (1983) have shown no effect of estrogen on the uptake of $^{35}SO_4$ by proteoglycans in dwarf mice. Priest et al. (1960) and Herbai (1971) demonstrated estrogen induced inhibition of sulfate incorporation in vivo in mice. Kowalewski (1958) observed that 17-ethyl-19-nortestosterone promoted the uptake of $^{35}SO_4$ by growing bone in chicks. Salmon et al. (1963) reported that castration did not significantly affect sulfate uptake by the cartilage and administration of testosterone propionate had a slightly positive effect on sulfate uptake.

In the present experiment the effects of castration, estradiol-17β and testosterone on the growth, particularly linear bone growth and rate of $^{35}SO_4$-incorporation in the epiphyseal growth cartilage of selected long bones of pigs were studied.

Plate III.1. Indoor pens where the pigs were kept during the experiment

MATERIALS AND METHODS

For this experiment sixty newborn, crossbred (Yorkshire x Landrace) male pigs were selected at random out of 15 litters between 1984 04 07 and 1984 04 13. The pigs were ranked for birth weight and starting from the top of the list each four animals were randomly allocated to four treatment groups (fifteen animals per treatment).

Treatment 1 - Surgically castrated on day 1 (1984 04 14) and implanted subcutaneously on the left side of the neck just behind the base of the ear with a silastic rubber product which gave a sustained release of estradiol-17β. The product used was Compudose® manufactured by Elanco Products Division of Eli Lilly Co. The implants were 3 cm long and 4.76 mm in diameter and contained 24 mg of estradiol.

Treatment 2 - Also surgically castrated on day 1 and similarly implanted subcutaneously with testosterone packed in silastic capsules, at the same site as the estradiol-17β implants. The testosterone implants were prepared by tightly filling 3 cm lengths of silastic tubing (DOW Corning) having 3.35 mm inside diameter and 4.65 mm outside diameter with crystalline testosterone (Sigma Chemical Co. Louis, Mo.)and sealing both ends with silastic adhesive type A. Since all capsules were of similar size the dose rate was expected to be constant (Legan et al.; 1975). All the capsules were incubated for thirty minutes in water at 21° C just before insertion to minimize the peak in circulating testosterone. The implants were inserted through a sterile trocar and

canula.

Treatment 3 - Surgically castrated on day 1.

Treatment 4 - Uncastrated controls.

Each treatment group was divided randomly into three subgroups (five animals per subgroup). Each subgroup was allocated to one pen. The animal from different treatment treatments were not mixed because of the behavioural problems amongst genders. The pens (Plate III.1) were built indoors, and were well ventilated. The floor of the pens was made of concrete. Adequate straw bedding was provided. Drinking water was provided through an automatic watering system 24 hours a day.

Gain and Carcass Traits

The pigs were weaned at 17 days of experiment and were given a starter diet (Table III.1) until 52 days when they were transfered to a grower diet. They remained on that until 102 days of experiment when they were placed on a finisher diet. The changing of diet is a commercial practice which was followed in this experiment. One subgroup per treatment was killed between 1984 06 04 and 1984 06 18 (slaughter group 1), a second subgroup between 1984 08 06 and 1984 08 20 (slaughter group 2), and the last subgroup between 1984 10 03 and 1984 10 17 (slaughter group 3).

Feed intake on a pen basis and weight of individual animals were recorded on day 1, 52, 102 and 167 of experiment. Backfat thickness of slaughter group 3 animals

was measured at the tenth rib ultrasonically by Scanogram model 722 (Ithaca, New York). The backfat measurements were taken on day 122, 143, and 164 of experiment. This measurement could only be taken on slaughter group 3 pigs, since only they had sufficient fat to be measured ultrasonically.

The basis of the ultrasonic method is that high frequency sound waves are generated by a trasducer on the animal's back. Since the velocity of the waves through the tissue is unknown, the time taken for the echoes to return from tissue boundary layers can be used to determine their depth. Strong echoes are returned from the muscle-fat boundary over the longissimus dorsi muscle, but other echoes are also retuned from connective tissue septa in the subcutaneous adipose tissue and from the axial skeleton ventral the longissimus dorsi. By moving the transducer in an arc over the animal's back, depth measurements can be assembeled into a map of the carcass section. Wallace et al. (1977) reported that ultrasonic fat thickness measurements were highly correlated with their corresponding carcass measurements and the repeatability was high (Campbell and Herve, 1971).

Bone Growth

Slaughter group 3 was infused with oxytetracycline (100 mg/ml) at the rate of 20 mg per kg body weight intramuscularly at the lateral aspect of neck, using

Table III.1. Composition of diets fed to the pigs during the experiment

Ingredients (kg)	Starter	Grower	Finisher
Wheat	250.0	250.0	
Barley	136.0	551.0	845.0
Oat Groats	255.0		
Tallow	30.0		
Soyabean Meal (44% Cl)	130.5	160.0	115.0
Fish Meal	64.0		
Dried Whey	100.0		
Iodined Salt	4.0	4.0	5.0
Cal. Phosphate	10.0	15.0	15.0
Lime Stone	8.0	10.0	10.0
Min. Vit. Mix*	10.0	10.0	10.0
Lysine	2.5		
Total	1000.0	1000.0	1000.0

* Provides per kg of diet: 60.0 mg zinc, 6.0 mg manganese, 75.0 mg iron, 6.0 mg copper, 0.1 mg selenium, 2500 iu vitamin A, 250 iu vitamin D, 11 iu vitamin E, 6 mg riboflavin, 22.5 mg niacin, 12.5 mg calcium pantothenate, 15 microgram vitamin B_{12}, 250 mg choline.

hypodermic syringe with gauge 18 needle. This operation was done 16-26 days before slaughter to label the calcifying zone of the epiphyses and subsequently to measure the rate of linear growth in selected long bones (Graham and Price, 1981). The pigs were slaughtered by stunning with a captive bolt pistol and cutting the throat (jugular veins and carotid arteries were severed). The head and viscera were then removed and weighed. The carcass was then split in the middle longitudinally by a hand saw.

From the right side of the carcasses the radio-ulnae and tibio-fibulae were removed aseptically (the skin was thoroughly cleaned and sterilized with absolute ethanol and sterile instruments were used) to dissect out the distal radial and proximal tibial growth plates. Aseptic precautions were taken to prevent possible bacterial contamination of culture medium. For dissecting out the growth plates all the periosteum and non-growth plate cartilage that was contiguous with growth plate was removed with scalpel. At this point in the dissection, the specimen consisted of metaphyseal and epiphyseal bone with a plate of cartilage between them. The tip of the scalpel was then inserted under one edge of the growth plate on the metaphyseal side and the knife was twisted so that the epiphysis with the growth plate popped off. In the older animals this process was repeated several times at different places. The fracture line ran along the border between the hypertrophic zone of calcifying cartilage. The epiphyseal

plate cartilage was removed from the underlying epiphyseal bone with a razor blade (Seinsheimer and Sledge, 1981). The growth plates were then brought to the laboratory in cold sterile normal saline. A small piece (3-4 mm) of the cartilage cut into one mm slices was incubated in Eagle's medium containing 15 microcuries of $^{35}SO_4$ (obtained from New England Nuclear) per ml, 100 i.u. penicillin per ml, and 100µg streptomycin per ml for twenty four hours at 37° C. The cartilage was then soaked in a saturated solution of sodium sulfate for four hours and washed in running tap water at room temperature for twenty four hours. The cartilage was then blotted on filter paper, weighed and hydrolyzed in 23N formic acid for ten to fifteen minutes. An aliquot of 0.2 ml was added to ten ml of Aquasol (New England Nuclear) shaken well, allowed to cool for more than four hours and counted in a liquid scintillation counter.

After cooling, the left sides of the carcasses were dissected to record the total muscle, fat, and bone. Length and circumference measurements were made on the humerus, radio-ulna, femur, and tibio-fibula. Length measurements were the greatest distance between proximal and distal points of each bone. These bones from slaughter group 3 were split longitudinally on a band saw and photographed under ultravoilet light as described in Chapter 1 to record the growth at each end of the bones.

The data were analyzed by least squares analysis of variance (Harvey, 1976).

One pig from treatment 1 and two from treatment 2 died during the experiment. Their data were not included in the analysis.

RESULTS

Gain and Carcass Traits

Average daily weight gain and feed efficiency of the pigs during both the periods when they were on the starter and grower diets, were not affected by the treatments (Table III.2).

The backfat thickness measured ultrasonically on day 122, 143, and 164 of the experiment was not significantly affected by the treatment.

The treatment did not affect the growth coefficients (b in the allometric equation $y = ax^b$) of individual tissues and organs relative to body weight or carcass weight. (Table III.3). The weights of limb bones i.e. radio-ulna, humerus, tibio-fibula, patella, femur and pelvis were not influenced by the treatment. There was also no effect of treatment on the length measurements of the radio-ulna, humerus, tibio-fibula and femur (Table III.4). The circumference of the radio-ulna was significantly ($P<0.05$) larger in boars than in implanted and non-implanted barrows.

All of the traits studied in this experiment were significantly ($P<0.05$) affected by age (Tables III.3,III.4).

Table III.2. Least-squares means and standard errors of gain, feed efficiency, and ultrasonic back fat thickness in 57 experimental pigs

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Significance
No of animals	14	13	15	15	
ADG on starter diet kg	0.32±0.02	0.35±0.01	0.33±0.02	0.36±0.01	P=0.66
Feed Efficiency kg gain/kg feed on starter diet	0.67±0.01	0.66±0.01	0.67±0.02	0.70±0.02	P=0.29
No of animals	10	8	10	10	
ADG on grower diet kg	0.67±0.03	0.78±0.03	0.74±0.04	0.77±0.02	P=0.31
Feed Efficiency kg gain/kg feed on grower diet	0.30±0.01	0.29±0.01	0.29±0.01	0.32±0.01	P=0.14
No of animals	5	3	5	5	
Back Fat Thickness at day 122 mm	19.0±1.90	21.7±1.70	19.0±3.30	19.0±3.30	P=0.92
Back Fat Thickness at day 143 mm	24.0±3.70	28.3±1.70	26.0±3.30	24.0±2.90	P=0.80
Back Fat Thickness at day 164 mm	38.0±5.10	38.3±4.40	38.0±4.90	30.0±3.50	P=0.50

Treatment 1 = Estrogen-implanted barrows
Treatment 2 = Testosterone-implanted barrows
Treatment 3 = Non-implanted barrows
Treatment 4 = Non-implanted boars

Table III.3. Growth coefficients (log10 y = log10 a + b. log10 x) and standard errors of tissues and organs for 57 experimental pigs

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Sig.
No of animals	14	13	15	15	
Growth coefficients relative to body weight:					
Head weight	0.84±0.04	0.87±0.04	0.82±0.04	0.87±0.04	P>0.05
Alimentary tract weight	0.93±0.06	0.84±0.08	0.82±0.07	0.86±0.06	P>0.05
Liver weight	0.70±0.03	0.71±0.04	0.70±0.03	0.70±0.03	P>0.05
Spleen weight	0.70±0.03	0.71±0.04	0.70±0.03	0.70±0.03	P>0.05
Kidneys weight	0.66±0.04	0.68±0.05	0.62±0.04	0.63±0.04	P>0.05
Heart weight	0.70±0.05	0.82±0.06	0.70±0.05	0.71±0.05	P>0.05
Lungs weight	0.44±0.08	0.74±0.10	0.62±0.09	0.59±0.09	P>0.05
Growth coefficients relative to carcass weight:					
Muscle weight	0.89±0.02	0.84±0.03	0.86±0.03	0.91±0.03	P>0.05
Fat weight	1.59±0.06	1.62±0.07	1.60±0.06	1.48±0.06	P>0.05
Bone weight	0.63±0.03	0.71±0.03	0.65±0.03	0.71±0.03	P>0.05
Tendon, ligaments etc. weight	0.55±0.23	1.23±0.27	0.48±0.24	0.73±0.24	P>0.05

Treatment 1 = Estrogen-implanted barrows
Treatment 2 = Testosterone-implanted barrows
Treatment 3 = Non-implanted barrows
Treatment 4 = Non-implanted boars
Sig. = Level of significance

Table III.4. Least-squares means and standard errors of weight (g), length (mm), circumference (mm), and rate of sulfate-35 incorporation (DPM/mg cartilage) in the long bones studied

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Sig.	Slaughter group 1	Slaughter group 2	Slaughter group 3	Sig.
No of animals	14	13	15	15		19	20	18	
Weights of:									
Tibio-fibula	174±7.7	190±7.8	176±7.1	197±7.1	P=0.09	81±6.3a	192±6.3b	278±6.6c	P<0.01
Femur	234±10.3	253±10.6	243±9.6	263±9.6	P=0.20	105±8.5a	261±8.5b	378±8.9c	P<0.01
Patella	21.0±1.5	24.0±1.5	24.0±1.4	26.0±1.4	P=0.08	9.0±1.2a	24.0±1.2b	38.0±1.3c	P<0.01
Pelvis	273±16.8	304±17.2	280±15.5	304±15.5	P=0.42	108±13.9a	297±13.9b	465±14.5c	P<0.01
Radio-ulna	162±8.0	172±8.2	160±7.4	187±7.4	P=0.06	69±6.6a	178±6.6b	262±6.9c	P<0.01
Humerus	228±8.6	240±8.8	226±8.0	253±8.0	P=0.08	93±7.1a	248±7.1b	370±7.4c	P<0.01
Scapula	161±9.4	176±9.6	158±8.7	182±8.7	P=0.20	67±7.8a	169±7.8b	271±8.1c	P<0.01
Length of:									
Tibio-fibula	158±2.2	163±2.3	160±2.00	164±2.0	P=0.24	118±1.8a	170±1.8b	195±1.9c	P<0.01
Femur	165±2.7	172±2.7	169±2.5	172±2.5	P=0.19	124±2.2a	179±2.2b	205±2.3c	P<0.01
Radio-ulna	167±2.5	173±2.6	168±2.3	174±2.3	P=0.08	128±2.1a	179±2.1b	204±2.2c	P<0.01
Humerus	159±2.2	162±2.3	160±2.1	160±2.1	P=0.77	119±2.1a	168±2.1b	194±2.2c	P<0.01
Circumference of:									
Femur	77±1.5	78±1.5	77±1.4	78±1.4	P=0.85	67±1.2a	79±1.2b	90±1.3c	P<0.01
Radio-ulna	98±1.8a	97±1.8a	97±1.7a	103±1.7b	P=0.03	79±1.5a	102±1.5b	115±1.6c	P<0.01
Humerus	78±1.5	77±1.5	78±1.3	80±1.4	P=0.46	62±1.2a	80±1.2b	92±1.3c	P<0.01
Rate of sulfate-35 incorporation in growth cartilage of:									
Proximal Tibia	216±35	171±35	190±32	225±32	P=0.66	178±30	198±30	226±30	P=0.80
Distal Radius	163±29	158±30	140±27	192±27	P=0.59	151±24	173±24	165±25	P=0.51

Treatment 1 = Estrogen-implanted barrows
Treatment 2 = Testosterone-implanted barrows
Treatment 3 = Non-implanted barrows
Treatment 4 = Non-implanted boars
Means followed by different letters in the same row are significantly different (P<0.05)
Sig. = Level of significance

Bone Growth

The rate of growth at the epiphyseal plates of the long bones as measured by the oxytetracycline infusion technique during the last 16-26 days of the experiment was not affected by castration, estradiol-17β or testosterone implantation (Table III.5). The growth recorded during this period was greatest at the proximal tibia and least at the proximal radius.

The treatment did not influence the rate of proteoglycans synthesis as determined by radio-sulfate incorporation into the proximal tibia and distal radial growth cartilage (Table III.4).

DISCUSSION

Gain and Carcass Traits

Table III.2 shows average daily weight gain (estimated by linear regression of liveweight on time) of the pigs during the period when they were on the starter and grower diets. There were no significant differences among the treatment groups. The feed efficiency (kg body weight gain per kg feed) during this period was also not affected by treatments (Table III.2).

This is in agreement with the findings of Dinusson et al. (1951) and Gorrill et al. (1964) who reported no effect

Table III.5. Least-squares means and standard errors of growth at the epiphyseal growth plates of long bones micro m/day studied by oxytetracycline infusion technique

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Signifinance	Grand means
No of animals	5	3	5	5		18
Proximal Humerus	133±10	126±26	127±8	128±27	P=0.99	129±8
Distal Humerus	34±6	34±20	35±5	34±9	P=0.99	34±4
Proximal Radius	29±3	17±9	18±5	27±7	P=0.44	23±3
Distal Radius	139±9	115±21	107±11	121±23	P=0.54	121±8
Proximal Femur	104±6	108±10	76±13	99±17	P=0.34	96±7
Distal Femur	183±	142±18	141±28	158±33	P=0.62	158±12
Proximal Tibia	177±	151±16	136±72	187±33	P=0.43	163±51
Distal Tibia	81±24	62±10	72±11	79±14	P=0.89	75±8

Treatment 1 = Estrogen-implanted barrows
Treatment 2 = Testosterone-implanted barrows
Treatment 3 = Non-implanted barrows
Treatment 4 = Non-implanted boars

of implanting stilbestrol in barrows and gilts. Woehling et al. (1951) and Bratzler et al. (1954) demonstrated no effect of testosterone implants on growth and carcass traits of pigs.

The effects of castration on weight gain, feed efficiency and carcass traits have recently been reviewed by Seideman et al. (1982). These authors have concluded that difference in growth rate between boars and barrows is not great. Difference in feed efficiency are also small, but boars are more efficient.

Table III.2 also shows back fat thickness measured ultrasonically on day 122, 143, and 164 of the experiment. Neither implantation nor castration had any significant effect on this trait. This is in agreement with Beacom (1963), who reported no effect of hormonal (estrogen or testosterone) treatment on backfat thickness.

The growth coefficients of the head, alimentary tract, liver, spleen, kidneys, heart, and lungs relative to body weight are given in Table III.3. None of these traits were significantly affected by treatment.

The growth coefficients of the side muscle, side fat, side bone and side tendon relative to the carcass weight were also not significantly affected ($p>0.05$) by treatment (Table III.3).

There are numerous contradicting reports about the effect of estradiol and testosterone on the growth of muscle, bone and fat in pigs. Heitzman and Clegg (1957),

Beacom (1963), Cahill et al. (1960), and Elliot and Fowler (1974) have reported that treatment with diethylstilbestrol produces leaner carcasses in castrated pigs. Dinusson et al. (1951) and Woehling et al. (1951) however, noticed no improvment in carcass quality as a result of treatment with estrogenic compounds. Robinson and Singleton (1966) and Williams and Dunkin (1975) observed that androgen treatment resulted in a significant improvement in the lean content of pork carcasses. One of the most marked effects of castration is its effect on fatness of the carcass. Barrows have thicker backfat than boars (Turton, 1969; Carroll et al., 1979). The present study confirms those reports.

The weights of radio-ulna, humerus, scapula, tibio-fibula, patella, femur, and pelvis were not affected by any of the treatments (Table III.4), but it was observed that all of the bones were nonsignificantly heavier in testosterone treated and entire male pigs than in the other two groups. This is in agreement with Martin (1969) who has reported 2% heavier bones in boars than in barrows. Most of the bones in estradiol treated pigs were non-significantly lighter than the other three treatment groups. The same trend was noticed in the length measurment of radio-ulna, humerus, tibio-fibula, and femur. The length of radio-ulna was more sensitive to treatment than any of the other bones. The radio-ulna was not significantly longer ($P=0.08$) in testosterone implanted and intact pigs.

Silberberg and Silberberg (1971) have reviewed the effect of estrogens on skeletal growth, particularly in rodents. They concluded that estrogens inhibit the growth of long and flat bones. However, in contrast, Woehling et al. (1951) reported no effect of stilbestrol on femur growth in growing fattening pigs. Suzuki (1958) noticed that femurs were longer in estrogen treated male or orchidectomized rats. Recent studies of van den Bosch et al. (1981 and 1982) have confirmed most of the earlier reports regarding the action of estrogens on skeletal growth in humans. These authors treated excessively tall girls and boys with estrogen and were sucessful in reducing the growth rate of long bones in both sexes. In the present study with pigs estrogen implanted castrated males had slightly shorter bones than the other three groups.

The influence of androgens, mainly testosterone and its esters, on bone growth has been studied repeatedly, especially in rodents. The results however, vary considerably. It has been shown that administration of androgens to young castrated or normal animals inhibits linear growth (Rubinstein and Solomon, 1941a; Simpson et al., 1944; Reiss et al. 1946). In other experiments androgens have stimulated growth (Rubinstein and Solomon, 1941b; van Wagenen, 1949; Sobel et al. 1956; Velle, 1977). Others have found no effect (Turner et al., 1941; Woehling et al., 1951). These divergent results might be due to species variation, dose, route of administration, or

duration of treatment. In most of the reports large doses inhibited the growth, while small doses stimulated the growth. The rats were more sensitive to the treatment compared to other species of animals studied.

There is no specific study regarding the effect of sex steroids and castration on the development of the radio-ulna in pigs. Turton (1969) reported that development of the fore leg was significantly greater in boars than barrows. Brannang (1971) has shown that castration causes a marked decrease in growth of the scapula in cattle.

The circumference of the radio-ulna was significantly larger ($P=0.03$) in boars than in estradiol implanted, testosterone implanted, and non-implanted barrows. This trait was not significantly affected by any of the treatments in the other bones studied. However, the long bones tended to be thicker in boars and testosterone treated animals.

Bone Growth

The rate of growth at the epiphyseal plates of the long bones during the last 16- 26 days of the experiment was not affected by treatment (Table III.5).

In this experiment the magnitude of growth at the epiphyses may have been too small to detect significant differences among treatments, although the growth retardation effect of estrogen is well documented (Josimovich et al., 1967). The fact that estrogen is capable

of slowing growth while the epiphyses remain open, yet acts to enhance growth hormone secretion, constituted a paradox not resolved until the late 1960s. Salmon and Daughaday (1957) demonstrated that serum from normal animals contains a factor differing from growth hormone that stimulates the uptake of sulfate by the sensitive cartilage obtained from young hypophysectemized rats. They called this substance sulfation factor, and noted that it is almost absent from the serum after removal of the pituitary but was restored after treatment with growth hormone. The exact role of sulfation factor was described by Laron et al. (1966),and Daughaday et al. (1969) while explaining "Laron's dwarfism". This is the familial syndrome in which children have the phenotype of growth hormone deficiency yet exhibit high levels of plasma growth hormone and fail to respond to exogenous hormone. It is now understood that Laron's dwarfism is a genetically determined failure on the part of the affected child to respond to growth hormone by elaboration of sulfation factor (somatomedin).

Wiedemann and Schwartz (1972) are credited with finally elucidating how estrogen, while elevating plasma growth hormone concentration, could curb longitudinal growth of long bones. Working with acromegalics, they showed that elevated serum levels of sulfation factor, but not of growth hormone, fell when estrogen was given, and rose again when estrogen therapy was discontinued. They also noted that in growth hormone deficient individuals, estrogen aborted the

abrupt rise in somatomedin that otherwise regularly followed treatment with growth hormone.

With regard to androgens, Silberberg and Silberberg (1971) have indicated that testosterone promotes all phases of endochondral ossification, but stimulation of proliferation of the chondrocytes is usually less consipicuous than the intensification of hypertrophy, calcification, and ossification. The latter changes predominate particularly after administration of large doses of hormone.

In the present study it was found (Table III.5) that growth was greatest (163 μm/day) at the proximal tibia, followed by the distal femur (158 μm/day), proximal humerus (129 μm/day), distal radius [illegible] μm/day), proximal femur (96 μm/day), distal tibia (75 [illegible]), distal humerus (34 μm/day), and proximal radius 23 μm/day). According to Moss-Salentijn (1974) there is general agreement that in mammals the proximal end of the humerus and the distal end of the radius grow at a more rapid rate than the other ends of the same bones. In the hind limb, the plates of the distal end of the femur and the proximal end of the tibia grow more rapidly than the plates at the other ends. This was confirmed in the present experiment.

The rate of synthesis of proteoglycans as determined by radiosulfate incorporation into the proximal tibial and distal radial growth cartilage (Table III.4) was not influenced by any of the treatments. There are conflicting

reports regarding sulfate incorporation into the proteoglycans of cartilage. This variation may be due the amount of steroid administered, the duration of the treatment, or the route of administration. According to Burnett and Redi (1983), estrogens do not influence sulfate incorporation. Priest et al. (1960) however, stated that estrogen in large amounts reduces the incorporation of sulfate into the cartilage. Berntsen (1968) observed that uptake of sulfate was depressed in the epiphyseal cartilage of rabbits. Herbai (1971) found that sulfate incorporation into costal cartilage was inhibited in the presence of estrogen in the incubation medium.

The capacity of growing bones to bind labe[illegible]ate in 17-ethyl-19-nor testosterone treated cocker[illegible] increased (Kowalewski, 1958). Salmon et al. (1[illegible] observed a slight effects of testosterone on sulfate uptake by costal, nasal, and xiphoid cartilage in rats. They also reported no effect of castration on sulfate uptake.

It is concluded from this experiment that implantation of pigs with estradiol-17β and testosterone had no significant effect on average daily gain, feed efficiency, backfat thickness, carcass traits and bone growth. Boars had leaner carcasses than implanted and non-implanted barrows.

In conclusion the growth traits, carcass characteristics, and linear growth of limb bones is not affected by castration and exogenous estradiol-17β and testosterone implantation in male pigs from birth to 6

months of age.

IV. CONCLUSIONS

It is concluded from these three experiments, in which the effects of commonly used anabolic agents (zeranol in cattle; estradiol-17β and testosterone in pigs) were observed on the growth traits in cattle and male pigs, that:

Zeranol had little effect on average daily gain in cull cows aged 1- 13 years, however, it increased average daily gain in male cattle. This effect was greater in steers than bulls. In boars implantation with estradiol-17β and testosterone has no effect on ADG. Entire cattle gained more weight per day than castrates, whereas the rate of gain was not so clearly affected by castration in pigs.

Treatment had no effect on backfat thickness or the weight of fat dissected from the shank in cull cows. Similarly this trait was not affected in either gender of male cattle by zeranol implantation. In boars backfat thickness and the weight of fat dissected from the half carcass was not influenced by the exogenous hormonal treatment. Entire males of both species were leaner than castrates.

Bone growth was significantly affected by zeranol implantation both in young cull cows and bovine males as reflected by shorter radio-ulnae in the cull cows, reduced linear bone growth at the distal epiphyseal growth plate of radius and enlarged pelvic openings in steers and bulls. Estradiol-17β and testosterone treatment had no significant effect on the growth of proximal limb bones (radio-ulna,

humerus, tibio-fibula and femur) in barrows. Castration reduced linear bone growth at the distal radius in cattle as determined by the oxytetracycline infusion method, whereas it had no effect on the growth of any of the epiphyses of the proximal limb bones of pigs.

It is also concluded from this study that zeranol decreased libido in bulls implanted at about puberty.

In young actively growing pigs in this experiment the hormone level had no effect on growth traits probably because as they approach puberty the hormone receptors could mature enough to respond to the respective hormone, but before puberty they were too immature to have any response. Since the pigs were slaughtered at about puberty, stimulation of growth by exogenous hormone administration is unlikely to be successful.

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