A STUDY OF GROWTH PARAMETERS OF BONE

IN HEREFORD AND CHAROLAIS

CROSSBRED CATTLE

 $\mathbf{B}\mathbf{y}$

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CHAPTER I

INTRODUCTION

The beef cattle industry of today plays an important role in the world economy. The beef industry in the United States ranks third, behind steel and rubber in total dollars invested. The major goals of beef producers and beef cattle scientists are to increase the total production of high quality red meat, as well as to improve production efficiency, in order to supply an increasing population with an adequate amount of high quality animal protein.

Recently, beef cattle scientists have explored the possibility of establishing the growth patterns and potential of various breeds of cattle. Today, with the high cost of feed-stuffs, highly flexible market prices and tight economic management practices, the growth potential of animals must be established early in life. The major reason for establishing the growth potential early in life is to be able to control and direct the growth patterns in later life for maximum production. If indices of future growth and performance were established before weaning time, the beef producer could cull the calves with poor potential and this would result in considerable savings of time and money. If calves with excellent growth potential were identified in

early life, the most suitable method of finishing (such as on grass, grain plus grass, full feed, etc.) could then be determined.

The growth patterns of bone, lean and fatty tissue must be observed, studied and measured to evaluate the growth potential for a particular breed of cattle. Apparently, beef cattlemen in the past were only interested in muscle and fatty tissue production. Today, however, the beef scientists have become interested in evaluating bone growth and skeleton development because more recent research works have established a strong muscle to bone relationship in beef cattle. Light boned calves tend to be smaller framed and produce less total pounds of muscle tissue than large boned calves.

The purpose of this study was to identify and establish some growth parameters of bone and muscle tissue in beef cattle slaughtered at different weights and to establish the time at which long bone attained physiological maturity. Also, the rate of growth of bones in the thoracic limb, pelvic limb, forequarter, and hindquarter of Hereford and Charolais Crossbred cattle was investigated.

CHAPTER II

LITERATURE REVIEW

The following review presents a general discussion on bovine growth and development. The review takes into account bovine growth patterns and relationships, normal bone growth and development, serum alkaline phosphatase activity in bovine and some factors affecting bone-muscle development in bovine.

Bovine Growth Patterns

Growth Relationships

During growth and development an animal changes in form and composition. There is no complete explanation as to why growth starts, how it is regulated, or why it stops at the definite point which characterizes adult development (Maynard and Loosi, 1962). Development has been referred to as changes in body shape or conformation until the body structure and its various parts reach maturity (Hammond, 1952 and McMeekan, 1956). Growth has been defined by some investigators to include development, whereas others define development to include growth and no distinction is made among increases of bone, muscle or fat.

A fundamental law of growth is that the shape of the growth curve is similar in all species (Brody, 1945 and McMeekan, 1959). The growth curve of the rat as is shown in Figure 1 is, supposedly, typical of the growth curve of all mammals (Brody, 1928). Different species of animals differ with regard to the time when the curve shows the largest increase or inflection, but the general shape of the curve is the same in all animals (Brody, 1928). There is a tendency for species of animals that have a larger mature size to require a longer period of time to reach maturity than species of smaller mature size (Taylor, 1965). The order in which the anatomical parts and their composite tissues develop is similar in all species. This is based on the relative importance of the functions and the parts of tissues for survival of the animal (Hammond, 1960). The order of tissue growth follows a sequential trend starting with the central nervous system and progressing to the bone, tendon, muscle, intermuscular fat and subcutaneous fat (Palsson and Verges, 1952 and McMeekan, 1959).

In the time of growth, an animal increases in size and experiences changes in form due to differential growth rates of constituent parts (Palsson, 1955). Changes in body proportions are brought about by the different parts growing at different rates; for example, the head grows rapidly early in life and is proportionally larger than other parts such as the pelvic and thoracic limbs. These latter parts grow more rapidly in later life and form a larger proportion of





final body weight (Hammond, 1932 and Huxley, 1932). The shape, size and composition of an animal varies continuously during growth and in the most subtle ways. During normal growth, changes occur in various parts at a rate that is somehow in equilibrium, harmoniously adjusted and controlled in some way by the genetic make up (Hirsch, 1941). The major body tissues exhibit differential growth patterns, with the skeleton, muscle and fat tending to develop in the order listed (McMeekan, 1940a).

Growth patterns of the tissues can be established by dissection of animals slaughtered over the appropriate ranges of ages or weights. Several researchers in England and New Zealand (Hammond, 1921 and 1932, McMeekan, 1940a, b, c, and 1941 and Palsson, 1939) have completely dissected many meat animals, separating, weighing and measuring each muscle and each bone in the body. The graphs in Figure 2 illustrate the growth patterns of beef-type (Herefords) and dairy-type (Friesians) cattle slaughtered at 6 month intervals. Carcass weight, muscle and fat exhibited a sigmoid shaped curve, while bone increased in weight at a constant rate. It is only by weighing dissected tissues that the bone-weight distribution, muscle-weight distribution and fat-weight distribution of individual animals can be shown (Butterfield and Berg, 1966).

Thus, from data accumulated by the dissection of many animals slaughtered at different points along the growth curve of the species, the approximate growth patterns of



Figure 2. Carcass and Tissue Weights From Hereford and Friesian Steers Slaughtered at Six Month Intervals

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muscle can be derived.

Guenther et al. (1965) described how beef calves deposit lean, fat and bone from weaning to slaughter weight, when fed for moderate or rapid gain and slaughtered on weight-and age-constant bases. They established that lean deposition reached its maximum rate during the early part of the feedlot test. The fat accumulation was most rapid during the latter half of the feeding period and showed a sharp increase after lean production began to subside. Skeletal development was accomplished early in life and was related more to animal age and duration of feeding time than to the nutritional treatments imposed.

Meyer et al. (1965) fed steers on high, medium and low planes of nutrition. They demonstrated that the high-plane resulted in a greater proportion of the net energy being used for growth than either the medium or low planes.

Breed and type influences growth and development in cattle. Hankins, Knapp and Phillips (1943) proposed musclebone ratio as an index of merit in beef cattle and demonstrated a significant difference in this trait in favor of beef-type vs. dual purpose Shorthorns slaughtered at similar live weights of approximately 410 kg. Berg and Butterfield (1966) extended this concept and suggested that muscle-bone ratio adjusted for carcass weight might be amenable to improvement by breeding and selection.

Branaman et al. (1962) compared beef and dairy cattle at nearly equal carcass weights and found the beef carcasses higher in percentage of fat and also higher in muscle-bone ratio. Breed effect growth studies published by Cole et al. (1964) compared 133 steers of six breeds and one crossbred group. They concluded that the fat percentage difference and muscle-bone ratio appear to be quite large among all the breeds.

Sex influence on growth is known to have a marked effect. Bradley et al. (1966) established that sex influences the onset of fattening, with heifers fattening at lighter weights than steers, and steers at lighter weights than bulls. Prescott and Lamming (1964) compared steers castrated at 7 months with bulls. The bulls had heavier carcass weights, less fat cover and a higher muscle-bone ratio.

Some general conclusions were pointed out about growth and development by Berg and Butterfield (1966). The differential growth rate of bone, muscle and fat in the carcasses of cattle results in changes in the proportions of these tissues as growth and development proceed. The immediate post-natal period is characterized by relatively rapid muscle growth and an increasing proportion of muscle in the carcass. At a later stage, fat deposition assumes major importance and as the percentage of fat increases, the percentage of muscle decreases. Bone growth proceeds at a slow rate relative to muscle and fat.

Other environmental and genetic factors influence the normal differential growth patterns, changing the expected

tissue proportions at given slaughter weights. Plane of nutrition has a marked influence of relative tissue growth, with high-plane nutrition causing the fattening phase to be earlier relative to muscle and bone development and a lowplane resulting in a delayed fattening phase (Guenther et al., 1965). Genetic influences play a major part in carcass Their effects are related in a large degree to composition. differences which occur in the growth curve, in physiological maturity or in mature weight. The British beef breeds have been shown to be early fattening; that is, the inflection in their growth curve occurs at relatively light weights. The larger European breeds enter the fattening phase at heavier weights and their muscular development seems to be somewhat delayed in comparison with the British beef breeds. Sex has a major effect on beef carcass composition through its influence during the onset of the fattening phase. Heifers normally fatten at lighter weights than bulls. Major influences on growth and development of bone, muscle and fat can be brought about by altering slaughter weights or by shifting the onset of the fattening phase by either genetic or environmental means.

Normal Growth and Development of Bone

General

The skeleton completes a greater portion of its growth earlier in post-natal life than muscle or fat (McMeekan, 1959). The growth processes with which we are concerned in

the skeleton of bovine are usually considered to be of three types. The first is endochondral ossification responsible for the growth of bones in length, in skeleton "frame" development and in repair of bone damage due to fractures. Membranous ossification is the second type and is responsible for periosteal bone growth and for bone growth in other situations where the bone tissue is not preceded by cartilage. The third type is bone remodeling, a process of structural change which occurs in the adult, as well as in the developing and early post-natal skeleton. Each of these processes involves the formation of new bone tissue by osteoblasts and each process occurs in a basically similar fashion.

In the endochondral ossification the bone is deposited on a scaffolding of calcified cartilage, while in the other types the new bone is deposited on the surfaces of preexisting bony structures. The anatomical arrangement of bone tissue, and of its constituent collagen fibers, varies widely in different parts of the skeleton, with age and with specie (Enlow, 1966).

It is important to realize that calcified bone is a rigid material, it does not show the interstitial expansion which occurs with other types of tissue. Thus an increase in size is brought about only by the addition of new tissue to existing surfaces. Sissons (1953a), using bone markers, showed that the increase in length of growing bones was due to the addition of new tissue at the ends and not to interstitial growth of the shaft. More recently, similar information on the sites of bone growth, and on the rate at which new tissue is formed, has been obtained by radiological measurement following the insertion of metal markers (Sissons, 1956).

The endochondral ossification of bone has a general arrangement of cartilage cells that are arranged longitudinally in columns in the growing epiphyseal cartilage plate (Giese, 1962). Cells are continually being added by mitotic cell division in a distinguished arrangement. The hypertrophic cartilage cells are deposited from the diaphysis extremity towards the epiphysis extremity. After growth and maturation of the cells, the hypertrophic cartilage is invaded by blood vessels and connective tissue cells from both the extreme metaphysical surfaces. The uncalcified cartilage of the plate grows by interstitial expansion. As the cartilage cells multiply and enlarge, vascularization and bony replacement of the cartilage keep pace with its longitudinal growth (Young, 1962). In normal endochondral ossification, the hypertrophic cartilage cells do not persist, but disintegrate as calcification of the surrounding matrix and vascular invasion of the cartilage columns occurs. The proliferating cells in bone-forming tissue actively invade the metaphysical surface of the plates. Active proliferating cells are not differentiated osteoblasts, but an unspecialized osteoprogenitor cell form which the osteoblast and osteoclasts are both derived.

Under conditions of normal growth, there is a close linear relationship between the rate of growth of an epiphyseal cartilage plate and its thickness (Fahmy, 1956). The rate of growth of the plate clearly depends on the rate of formation of cartilage cells in the plates and on the final size attained by each of these cells at the metaphysical surface of the plate. The thickness of the plate is also influenced by the time that elapses during the growth and maturation of the cartilage cells, as they progress from one side of the plate to the other. There is a linear relationship between the growth rate and thickness of the plate that reflects a relatively constant life-span for the cartilage cells, despite considerable variation in their rate of formation and final size.

The growing epiphyseal cartilage plate is affected by a variety of nutritional, hormonal and other circumstances (Sissons, 1956). Its rate of growth is accelerated by increased amounts of pituitary growth hormone (Greenspan et al., 1949) and virtually ceases in the absence of this hormone (Asling et al., 1950). The hormone, thyroxin, also is required for normal endochondral bone growth (Ray et al., 1950) and particularly for the closure of the epiphyses during skeletal maturation (Ray et al., 1954). The gonadal and adrenal hormones also influence the growth and maturation of the epiphyseal plate.

The restriction of dietary intake retards endochondral bone growth. An excellent example of this is a vitamin D

deficiency which causes rickets. Rickets are associated with the failure of calcification of the hypertropic cartilage of the epiphyseal plate, which in turn, is associated with failure of vascularization and bony replacement of the abnormal cartilage. The cartilage plate consequently becomes thickened and irregular, its growth being slowed but its thickness increased due to an increase in the lifespan of the cartilage cells in the absence of normal calcification (Fahmy, 1956). Interference with the vascularization of the growing plate can also be produced by surgical incision on the metaphysical surface. As in the case of rickets, the hypertrophic cartilage cells continue to accumulate and the thickness of the plate increases.

During membranous ossification, the osteoblasts deposit bone on the periosteum or endosteum surfaces without the intervention of a cartilage model (Sissons, 1956). Periosteal bone formation results in the increase in thickness of the shaft of long bone during growth.

Bone remodeling during growth occurs via the deposition of calcium, phosphorus, and new bone tissue accompanied by resorption of existing bone by osteoclasts during growth (Hancox, 1956). It is by the control of these two processes that the size and shape of the developing skeleton is changed. The same processes of bone deposition and bone resorption continue during adult life and are responsible for a continuous remodeling (turnover) of bone tissue. Normally the two opposed processes are balanced in any part

of the skeleton. It is possible, by the use of chemicals and different drugs, called markers, to determine the rate at which bone is deposited on individual surfaces as well as the rate of turnover for the part of the skeleton concerned. Such information is of interest in bone metabolic activity, mineral turnover and normal bone growth condition. Also, the skeletal turnover of calcium, as opposed to that of actual bone tissue can be measured by these markers. The schematic shown in Figure 3 illustrates the remodeling that occurs during long bone growth. Both resorption and deposition are exhibited in the illustration, with (A) representing the size of young bone and (B) the size of mature bone.

Epiphyseal Cartilage

During development of the fetus, the majority of the bones of the skeleton have a cartilage pattern formed which is gradually replaced by bone. This replacement process is known as endochondral ossification (Ham and Leeson, 1961). After several centers of ossification form in the cartilage model of a long bone, it continues to grow in length at the epiphyseal plates, the junction of the diaphysis and the epiphyses. The epiphyseal plate is also called the epiphyseal line. It is evident that as long as the epiphyseal line (cartilage) persists and grows, new bone may continue to be formed at the expense of the cartilage, and an increase in length is possible. When the epiphyseal cartilage ceases to grow it undergoes ossification, the bone is



Figure 3. Remodeling that Occurs During Long Bone Growth. A, Size of Young Bone. B, Size of Mature Bone Growth consolidated and no further increase in length is possible. The fusion of the diaphysis and epiphyses takes place at fairly definite periods in the various bones and it is of value to know the usual times at which this phenomenon occurs in the larger bones of the limbs (Sisson, 1953b).

Each epiphyseal plate or epiphyseal line may be divided into several zones which are classified according to the activity occurring in each. In Figure 4, the zones of an epiphyseal plate are illustrated. The cartilage-cell multiplication zone will force the epiphysis away from the diaphysis, thus lengthening the epiphyseal plate. Subsequent removal of cartilage and deposition of bone will lengthen the diaphysis (shaft) of the bone. Increase in diameter of the bone results from activity of the osteogenic layer of the periosteum (Ham and Lesson, 1961).

Emara (1937) used the advent of epiphyseal union of long bones in young Egyptian cattle to estimate the age of the animal. The estimation of the age of an animal is sometimes of importance (1) for purposes of identification, (2) in cases where age is not known, and (3) in relation to different breeding purposes such as fattening, milk production, work, etc. Emara (1937) pointed out facts about growth patterns of certain bones in young Egyptian heifers. His experiment illustrated the age of epiphyseal closure of the long bones (humerus, radius, ulna, metacarpal, femur and tibia). The data was collected from radiographs taken from live cattle.



Figure 4. Zones of Epiphyseal Plate

Fusion of the epiphyses of the limb bones of sheep was undertaken as one of the first steps in general research into the skeletal development of the domestic animals (Smith, 1956). There were 12 different sheep involved in the above experiment. Radiographs were taken from all bones of the pelvic and thoracic limbs including the phalanges. The data collected by Smith (1956) indicated that a very wide range of "times of fusion" is possible. Many factors are involved which might produce discrepancies, among them being the criterion of fusion, the breed and sex of the sheep and the method of husbandry.

Grant et al. (1972) observed the fusion of the epiphyseal plate (distal epiphysis) in cattle to determine the physiological age (maturity). The radius was the only bone measured and no measurement could be made on the proximal end of the radius, as all proximal epiphyses were completely ossified. Emara (1937) and Sisson (1953a) indicated that the proximal epiphysis ossifies when cattle are 18 to 24 months of age. In sheep, proximal radii epiphyses were shown to ossify before the distal epiphyses by Smith (1956). Grant et al. (1972) collected data by two different techniques (1) the epiphyseal plate was x-rayed and the radiographs were measured with a ruler and (2) the epiphyseal plate from the bone itself was measured with a filar micrometer on a dissecting microscope. Grant et al. (1972) concluded that measurements on bones were easier to obtain than radiographic measurements.

Some researchers routinely radiographed the epiphyses of the long bones in the legs of young race horses to estimate physiological maturity (Meyers, 1965 and Monfort, 1967). This maturity measure is a guide used in training or racing schedules of horses. The concern is whether or not the epiphyseal cartilage has ossified and a decision is based on the subjective evaluation of radiographs.

Metacarpal and Metatarsal

Characteristics

The percent and amount of bone in meat animal carcasses have been indicated as relatively accurate criteria for prediction of animal growth and carcass meatiness (McMeekan, 1941, Hankins, Knapp and Phillips, 1943, Aunan and Winters, 1949, Callow, 1962, Harrington and King, 1963 and Jackson, 1967).

The metacarpals and metatarsals, by-products of the meat industry which are easily obtained, may also provide acceptable estimates of animal growth, carcass meatiness and carcass quality measures. The cannon bones have been studied as to their possible relationship to early maturity and degree of muscling (Hammond, 1932). Palsson (1939, 1940) reported that circumference of the fore cannon (metacarpus) bone in live beef animals was directly associated with increased meat production and early maturity. Hirzel (1939) reported that shortening and thickening of the fore cannon was associated with a thickening and shortening of muscle in

sheep. McMeekan (1956) stated that the weight of muscle could be determined within one percent if the weight of the fore cannon bones are known. He also stated that fine boned animals have a smaller amount of lean tissue and a larger amount of fat than the heavy boned animals. Data from Wythe (1958) on 28 steers indicated that a significant positive relationship existed between fore cannon bone thickness and muscling. Orme et al. (1959) demonstrated the usefulness of using various measurements of cannon bones and those taken from radiographs of the lumbar region of the vertebrae as possible indexes of total muscling in beef cattle. Orts, King and Butler (1969) reported significant relationships of metacarpal and metatarsal weights or lengths with muscling characteristics.

The metacarpals and metatarsals can be cleaned after slaughter and evaluated by weighing, measuring the length, width, outside and inside circumference, thickness and specific gravity. Also, the cannon bones can be x-rayed and the radiographs used for evaluation of bone growth. Scarth (1966) and Stout (1970) reported significant relationships between live metacarpal circumference and metatarsal length with growth and carcass muscling characters. There is an abundant amount of information available concerning the relationship of certain limb bone measures (linear, area, density, etc.) with growth and carcass characters.

Bones as Indicators of Muscling

General

Many research workers in carcass evaluation have reported on the relationship of bone characteristics to muscling in beef cattle. This activity has increased greatly with the advent of muscled, large boned "exotic" bovine breeds. In the past fifteen years many research workers have explored this area with the bovine. Prior to this time, however, most of the work had been conducted on lambs, hogs and poultry.

Hammond (1921, 1932), McMeekan (1940a, 1940b, 1940c, 1941) and Palsson (1939) have completely dissected numerous meat animals, separating, weighing and measuring each individual muscle and each individual bone in the body. These researchers found a strong, positive correlation between the weight of bone and the weight of muscle tissue.

Relationships Between Long Bones and

Carcass Composition

The most practical indicator of the percentage of bone in dressed carcasses seemed likely to be the weight of the four legs below the knees and hocks (Lush, 1926). As fatness increased this tended to decrease bone percentage; while a decrease in fatness tended to increase bone percentage. Lush (1926) indicated that the percentage of bone was not too accurate a predictor of composition when comparing animals uniform in age and fatness. Lush concluded that there is no relationship between dressing percent and percentage of bone in dressed meat, when the degree of fatness is held constant.

McMeekan (1956) made some very strong statements about bone-muscle relationships. He stated that the muscle weight could be determined within one percent if the weight of metacarpals and metatarsals were known. He concluded that on the average most fine boned animals will dress out a smaller percentage of lean meat and a larger percentage of fat, at the same weight, than large boned, heavy muscled animals. Regarding shape and weight, McMeekan established that the shorter and thicker the bone, the greater the depth or thickness of muscle lying around the bone.

The cannon bones in pigs, make a relatively smaller amount of growth after birth than do any of the other bones of the skeleton (McMeekan, 1940a). In addition to an anterior-posterior direction of development, the pig in common with other vertebrates exhibits a well defined centripetal gradient in the rate and order of development of the bones of the limbs. McMeekan (1940a) concluded that, as one proceeds up the limb, the rate of increase of the individual bone weight and length over their respective birth weight increases.

Working with weights of bone and muscle, Callow (1948) ascertained that the ratio between the weight of muscle tissue and that of bone increased with an increased

percentage of fatty tissue in the carcass, because during fattening the muscle tissue grows more rapidly than bone. He reported that there was nearly three times as much muscle as bone in lean carcasses that were carrying around 10 percent fatty tissue. In carcasses with 20 percent fatty tissue, there was four times as much muscle as bone and in carcasses with 40 percent fatty tissue there was nearly five times as much muscle as bone. Callow concluded that carcasses containing 20 percent fatty tissue had slightly more fatty tissue than bone; while carcasses with 40 percent fatty tissue had about four times as much fatty tissue as Hankins et al. (1943) observed a difference between bone. the muscle-bone ratios of dual-purpose and beef-type shorthorns as well as a difference between muscle-bone ratios of the progeny of sires within each type. The data demonstrated that muscle-bone ratios and certain live animal measurements give little indication that selection for these particular characters could be based on the conformation of the live Hankins found significant correlations between some animal. of the carcass measurements and the muscle-bone ratio, but none were high enough for predicting muscle-bone ratios. The highest relationships were with leg bones, as a percentage of live weight, and the average thickness of meat over the ribs.

Many animal science researchers have collected data and tried to sustain a method for predicting rib-eye area from live animal measurements and measurements of bone, muscle

and fat in the beef carcass. Wesili, Good and Holland (1958) reported a non-significant relationship between metacarpal and metatarsal circumference and rib-eye area. Also, they discovered a non-significant relationship between muscles in the round (biceps femoris, semitendinosus and semimembranosus) and metacarpal and metartarsal circumference. Good (1958) reported very low correlations between live measurements of front and rear cannon bone circumference and length and area of the rib-eye in the beef carcass. Wesili, Good and Holland (1958) and Good (1958) concluded that live measurements are not too useful for predicting actual bone measurements.

Butler, Warwick and Cartwright (1956) concluded that bones tend to develop proportionally in beef animals. Muscles in most cases are attached to bones at their origin and at their insertion, therefore muscle weight in different wholesale cuts tends to be proportionate. This concept was supported by the work of Wythe (1958).

Wythe (1958) established some strong correlations between the weight and length ratio of trimmed metacarpal, tibia, femur, metatarsal, radius and ulna bones. This highly repeatable data presents further evidence that bones develop proportional in both length and weight. Wythe reported highly significant relationships between long bone weight and weight of the loin, rib, and wholesale round; weight trimmed rib, chuck, loin plus trimmed boneless cushion round and the area of the rib-eye muscle. Wythe also found a

highly significant relationship between bone weight-length ratio and the above named cuts.

Research with chickens showed live shank length to be correlated with the live body weight, when the comparison was between chickens of the same age (Lerner, 1939). This author also stated that the correlation between length of shank and body weight are higher for growing chicks than for mature chickens.

Bird (1944) reported a "fleshing index" as the deviation actually observed from expected body weight relative to skeletal size, as indicated by length of tibia. This fleshing index is obtained by logarithmic regression of body weight on tibia length. Bird's results showed the calculated deviations to be almost perfectly correlated to the actual weight of combined breast and leg muscle dissected from mature chicken carcasses.

Deans (1959) reported data from 130 lamb carcasses in which the relationships between certain bone lengths, cross sections and weights and certain carcass muscle cross sections were determined and used as indexes of total muscling. Close associations were observed between the weight of the metacarpals and combined area of lean at the shoulder, thirteenth rib and leg.

Orts (1959) reported significant relationships between trimmed cannon bone weight, specific gravity, area of cannon bone and cushion round plus rump weight, loin weight, rib
weight, sum of loin, rib, cushion round, rump weight and rib eye area.

Serum Alkaline Phosphatase Activity

Blood serum contains a mixture of small amounts of phosphatases of which the principal components are the phosphomonoesterases. Alkaline phosphatase or phosphomonoesterase I is present in high concentration in the blood serum (Sumner and Myrback, 1950). It is found in all animal tissue except hyaline cartilage and the walls of the blood vessels. However, the tissues which contain relatively large amounts of this enzyme are the zones of growth of the bones of young animals, the intestinal mucosa, the kidney cortex, and lactating mammary gland. To a lesser extent, liver and brain also contain large amounts of alkaline phosphatase. The alkaline phosphatases from various tissue sources are distinguishable from one another with some degree of acceptance. Serum alkaline phosphatase and bone alkaline phosphatase have similar properties and are not distinguishable from one another. It has been suggested that the bulk of the alkaline phosphatase in the serum is derived from osseous sources (Moog, 1946). It has been further postulated that the serum alkaline phosphatase is excreted in the bile (Gutman et al., 1940). Fell and Robinson (1929, 1930, 1934) demonstrated that there was elevated serum alkaline phosphatase activity immediately preceding the calcification of bone. Further studies

established that bone alkaline phosphatase was one of the most important elements in the mineralization of bones and teeth, especially during periods of growth. Furthermore, serum alkaline phosphatase has been found in high concentration wherever rapid calcification was in progress.

The blood phosphates are believed to be fixed as esters in the bones where the phosphatase can readily liberate them by means of hydrolysis. This permits the rapid and abundant formation of tricalcium phosphate, with the renewal of Ca^{++} ions in the osseous fluids being assured by diffusion of calcium from the blood into the intercellular osseous fluids.

There are a number of methods available for the quantitative estimation of alkaline phosphatase activity. In all the techniques, a phosphoric ester serves as the substrate and colorimetric determinations are made of the organic substrate released by enzymatic hydrolysis. Of the original determinations for serum phosphatase that proved to be satisfactory for experimental and clinical work was that of Bodansky (1933). This method is satisfactory for many uses, but is rather time consuming when large numbers of determinations are required. Bessey, Lowry and Brock (1946) developed a method for the rapid determination of alkaline phosphatase with a relative small amount (five milliliters) of serum.

Many researchers have examined serum alkaline phosphatase activity in cattle for various reasons. Kunkel et al. (1953) examined European and Brahman cross cattle and found a lowering of phosphatase activity with age. In studies

with Brahman male and female cattle carried out over a period of three years, Fletcher et al. (1956) could find no consistent relationship between serum alkaline phosphatase activity and subsequent gain. Roubicek and Ray (1974) indicated that environmental factors affected serum alkaline phosphatase in range cattle. They also concluded that heifers had significantly higher serum alkaline phosphatase than steers and bulls, but the enzyme is not associated with growth as measured by weight.

CHAPTER III

MATERIALS AND METHODS

General

The experimental units for this study were sixteen Hereford and sixteen crossbred Charolais steer calves. The crossbred calves were of two types, with some being Angus x Charolais and the remaining Hereford x Charolais.

The calves were selected and grouped into four different feeding pens per breed. The four Hereford pens and four Charolais cross pens each had four steers. The steers were grouped according to their intended slaughter weight groups. Slaughter weight groups were 227.3 kgs., 318.2 kgs., 409.1 kgs. and 500.0 kgs. All calves were of weaning age when brought to the feedlot and fed, <u>ad libitum</u>, a standard feedlot ration. The feedlot ration maintained a protein content based on the body weight of the calves.

Procedures

Slaughtering and Cutting Procedure

Upon attaining the desired weight, the steers were transported to the meat laboratory and slaughtered according to the recommended procedure outlined by Deans (1951). The

carcasses were chilled at $2^{\circ}-3^{\circ}$ C. for approximately three days prior to cutting.

The right side of the carcass was cut into standard wholesale cuts following the method recommended by Wellington (1953), as ammended by King, Butler and Wythe (1959) and Orts (1962). Each wholesale cut was physically separated into bone, lean and fat and the weight of each of these tissues was recorded.

Bone Weight and Length Determinations

The limb bones were evaluated by several different procedures to determine the amount of bone growth. After physical separation from the carcass, the limb bones were cleaned of extraneous material with a knife and scissors. The following bones were used in this study: the metacarpus, ulna, radius, humerus, scapula, metatarsus, tibia and femur. Individual bones were weighed on an electrical Mettler balance to the nearest gram.

A set of calipers and tape measure were used to determine bone length to the nearest centimeter. All bone length measurements were made from the proximal epiphyseal line to the distal epiphyseal line. The metacarpus, radius, and humerus bones were measured at the anterior, medial surface to determine bone growth. As the ulna is fused to the posterior, lateral border of the radius, its measurement was taken at the lateral face. The scapula was measured on the medial face so that the spine of the scapula would not

interfere with the measurement.

The metatarsus, tibia and femur were the bones measured in the pelvic limb. The metatarsus, tibia and femur bones were measured at the anterior, medial surface to determine bone growth.

The above bone length determinations were actual measurements of the diaphyses. This would be a more accurate measurement than total bone length which includes the proximal and distal epiphysis.

Compact or Cortical Bone Area

Determination

The metacarpus and metatarsus bones were used to quantitate the area of compactor cortical bone. This was accomplished by obtaining a cross section through the middiaphysis of these bones.

A "printing" of these cross sections was made on a 5 inch by 8 inch note card. The cross sectional surface of the bone was cleaned and smoothed with a metal file to remove any rough edges so the print would be sharp and represent a true cross section of compact bone. It was found that the bones must be filed so the print will not leave a distorted impression. Black India ink was used as the marking material.

The following measurements were made after the impressions had dried using a compensating polar planimeter, and the results were expressed in square centimeters. The total (outside) bone area was determined. The total (inside) bone marrow area was determined. Subtracting the total (inside) bone marrow area from the total (outside) bone area, gave the total area of the compact bone.

Epiphyseal Cartilage Measurement

Many research workers have used the "tibia test" on hypophysectomized rats (Evans et al., 1943, Greenspan et al., 1949, Lostroh and Li, 1957, Simpson, Ashling and Evans, 1950, Becks et al., 1941, Kibrick et al., 1941, Ray, Evans and Becks, 1941, Ingalls, 1941 and Huggins and Smith, 1937). This test was used to determine age and the amount of skeleton growth by measuring the stained epiphyseal cartilage. The original procedure was established by Evans et al. (1943).

The metacarpus, metatarsus, tibia and femur were used to estimate the amount of long bone growth by the epiphyseal staining procedure. The bones were prepared as outlined earlier cleaned and freed of tissue during the quantiative bone growth evaluation procedure. The distal epiphyses of the metacarpus, metatarsus, tibia and femur were sliced on an electric Biro band saw. The saw was equipped with a stainless steel side arm gage to allow even slices to be cut, one-fourth inch thick. The bones were sliced about one inch dorsal to the epiphyseal cartilage, in a sagittal plane.

The bone slices were washed with warm water and soap to

remove excess bone dust and other extraneous matter. The slices were fixed for twenty-four hours in neutral, ten percent formalin. After fixing they were placed in running tap water for one hour, to remove the excess formalin and then placed into acetone for one hour. The acetone was not washed off the slices. The slices were placed in a fresh two percent mixture of silver nitrate for two and one-half minutes and rinsed once with distilled water to remove the excess silver nitrate. The bone slices were exposed to a strong light (two - 300 watt spot light bulbs) until the calcified areas turn dark brown in color. After developing, the bones were washed and cleaned in a ten percent solution of sodium thiosulfate for one-half minute and placed in running water for one hour. All slices were stored at 4° C in eighty percent ethanol, in dark glass jars. The jars of bone slices must be stored in the dark to prevent excessive developing and darkening of the calcified areas.

The calcified tissue stains dark brown and the proliferating zone of epiphyseal cartilage appears as a white band. The width of the cartilage was measured via a dissecting microscope fitted with an ocular micrometer. Three measurements, evenly spaced (anterior - posterior) were obtained.

Serum Alkaline Phosphatase Assay

Serum alkaline phosphatase was determined on all thirtytwo steers. The blood sample was collected at two different

times; prior to loading the steers for shipment to the meat laboratory and when the animals were bled during slaughter. The sample obtained prior to loading at the feedlot was collected by the tail bleeding method. The sample obtained at the meat laboratory was collected by sticking at time of slaughter.

All blood samples were held at room temperature in fifty milliliter centrifuge tubes for twenty-four hours. The blood was centrifuged at 5000 RPM in a Sorvall RC-2B refrigerated centrifuge at 2° C for 30 minutes. The serum fraction was then decanted into plastic freezer test tubes, fitted with caps and frozen at -20° C until assays could be accomplished.

The procedure employed for the serum alkaline phosphatase assay uses the compound p-nitrophenol which is liberated. Maximum absorption occurs at 400 nm. Thus, the hydrolysed substrate indicates the amount of phosphate ester splitting and, hence, is a measure of phosphatase activity, as indicated by the following reaction:

P-nitrophenyl phosphate (colorless in alkali) + H_2^{0} P-nitrophenol (yellow in alkali) + $H_3^{P0}_4$

The serum is incubated with the buffered substrate and the reaction is stopped by the addition of alkali and without further treatment the amount of color developed is measured. Serum alkaline phosphatase assay procedure requires an

alkaline glycine buffer solution which should be adjusted to a pH of 10.5 with 1.0 N NaOH or 1.0 N HCL. The substrate solution was prepared by adding 100 milligrams of Sigma phosphatase substrate (p-nitrophenyl phosphate) to 25 ml of deionized, glass distilled water. Each tube contained 0.50 ml of alkaline buffer, 0.50 ml of substrate and 0.10 ml of serum. The blank tubes contained the same quantity of buffer and substrate with 0.10 ml of deionized, glass distilled water in place of the serum. The tubes are incubated in a thermostatically controlled water bath at 37° C for 30 minutes. Exactly 30 minutes after adding the serum, 10 ml of 0.20 N NaOH was added to each tube and the tubes were stoppered and mixed by inversion. The yellow color formation is stable for several hours after the NaOH is added.

Three replicate tubes were used per serum sample. All tubes were read at 401 nm on a Gilford Spectrophotometer (Model 240) and the results recorded. To each tube 0.10 ml concentrated HCL was added and mixed to remove the yellow color. Again the tubes are read at 401 nm and the results were recorded. The latter reading was subtracted from the first reading to correct for the solution density. The corrected readings were used to determine serum alkaline phosphatase activity from the standard curve.

The standard curve is prepared by pipetting 0.50 ml p-nitrophenol standard working solution into a 100 ml volumetric flask. The flask is filled with 0.20 N NaOH to

100 ml and mixed thoroughly. The solution is pipetted into six test tubes as shown in Table I.

TABLE I

(1)	(2)	(3)	(4)	(5)
Tube No.	ml. Working Standard	ml. 0.20 N NaOH	Insert your O.D. Readings	Equiv. to Sigma units/ml.
1	1.0	10.0		1.0
2	2.0	9.0		2.0
3	4.0	7.0		4.0
4	6.0	5.0		6.0
5	8.0	3.0		8.0
6	10.0	1.0		10.0

SERUM ALKALINE PHOSPHATASE CALIBRATION TABLE

After reading, then record the optical density (0.D.) of each of the above mixtures at 410 nm using 0.02 NaOH in the reference (blank) tube. The serum alkaline Phosphatase calibration curve is prepared by plotting the six 0.D.'s in column (4) vs. the serum alkaline units in column (5). The serum alkaline phosphatase activity from the curve is reported in Sigma Units. A Sigma Unit is one micro mole of activity per hour per milliliter of serum. Also a Sigma Unit is the same as a Bodansky Unit or Bessey-Lowry-Brock Unit of phosphatase. The calibration curve is prepared each day the assays are analyzed.

Fiber Diameter Measurement

Muscle samples were obtained from the right Longissimus dorsi, at the twelfth thoracic vertebra. The samples were wrapped in heavy aluminum foil in order to insure a tight seal, then frozen in liquid nitrogen. After freezing the samples were placed in Cry-O-Vac plastic bags and stored at -20° C until fiber diameter determinations could be obtained.

Small pieces of tissue were removed from the frozen muscle sample and cut transversely, in order to obtain the longest possible fibers and to minimize shrinkage. These small tissue pieces were placed in a 10 percent solution of formalin fixative and stored in glass containers for 24 hours. The glass containers were maintained in an environment at one to four degrees centigrade in order to prevent shrinkage and fiber damage.

After twenty-four hours, the small pieces of muscle tissue were placed in a Waring blender (blades reversed) and 100 ml of cold 10 percent formalin fixative were added. Samples were blended for 2 minutes at a rheostat setting of 40 and omni-mixer speed control setting of ten.

An aliquot was taken from the blender with a disposable pipette and placed into a two-inch diameter petri dish. The petri dish was placed on an American Optical microscope equipped with an ocular micrometer and the fibers were allowed to settle to the bottom of the dish. Those fibers which appeared steady, were measured at their widest point. Fifty fibers were measured on each muscle sample.

Statistical Analysis

Statistical analysis of the experimental data consisted of computing the mean values, standard deviations and range values for all observations. The analysis of variance was determined for breed, weight and breed x weight interaction.

CHAPTER IV

RESULTS AND DISCUSSION

General

The data obtained from this experiment exhibits some quantitative derivation of skeletal and muscular growth in Hereford and Charolais Crossbred steers. Growth parameters cited in this section reflects the changes in epiphyseal cartilage growth, cortical (compact) bone growth, serum alkaline phosphatase activity, muscle fiber and tissue growth and bone-muscle comparison in the two groups of steers. In this study several problems were encountered when trying to evaluate the results, on a comparative basis, between the two breed types.

The chronological age of the beef cattle created a problem because the Charolais Crossbred cattle came from a source that did not keep accurate age records. Hence, but the ages for these cattle had to be approximated from information gained from the herdsman. The Hereford cattle were raised at the Oklahoma Agricultural Experiment Station and accurate birthdates were obtained on these cattle. As may be observed in Figure 5, the variation in chronological age (Birth to Slaughter) reflects the overall problem. As expected, the Hereford cattle were older than the Charolais



Crossbred at the 700, 900 and 1100 pound slaughter weight groups.

Also it should be noted that variation in age existed among the four individual animals in each slaughter weight group. Variation in the animals chronological age could affect nutritional requirements, overall metabolic activity, ossification of bones, muscle fiber type (red-white), tissue growth patterns and etc.

Another problem encountered in this study was the variation in amount of Charolais blood in the crossbred cattle. In was pointed out in Chapter III that part of the cattle were Angus x Charolais and others were Hereford x Charolais Crossbreds. The amount of Angus and Hereford breeding in the crossbred calves was not known and this could reflect some of the variation encountered in the growth patterns of the skeletal and muscular systems.

The Hereford cattle displayed less variation in body weight, breed type and age than was exhibited by the Charolais Crossbred cattle.

The technique in allotting the cattle to their slaughter weight groups could have resulted in some of the variation noted in the experimental results. The Charolais Crossbred cattle were shipped from the weaning pasture to the feedlot and the calves weighing nearest 500 pounds were placed in the first (500 pound) slaughter group. Whereas the smaller, lighter crossbred calves were allotted at random to the remaining slaughter groups.

The Hereford cattle were allotted at random to the various slaughter groups, thus reducing animal variation to a minimum.

As stated above, the larger amount of variation in the Charolais Crossbred cattle would not establish a true representation of growth to enable a two breed comparison. Thus, the results and discussion to follow will be presented on an individual breed basis and only general trends will be presented on some growth parameters comparing the Hereford and Charolais Crossbred steers.

Epiphyseal Cartilage

The data given in Table II point out the general trend in epiphyseal cartilage growth of the Herefords' right metacarpal bones. The 500 pound group exhibited the thicker cartilage, with a mean value of 712.87 microns. Also, this group showed the largest amount of variation, with a standard deviation of 129.16. The analysis of variance (Table X, Appendix) showed the right metacarpus epiphyseal line thickness to exhibit a highly significant (P < .01) decrease with increased age or weight.

These data reflect the overall physiological changes in epiphyseal cartilage growth, thus the mean values decrease with an increase in body weight and age. As age increases the epiphyseal cartilage decreases in thickness and as maturity is reached the cartilage will disappear and endochondral ossification will cease. Also bone growth in

TABLE II

Variable	Mean ^a	Std. Dev.	Range	
Metacarnus				
500	712.87	129.16	546 1	861 4
700	589.15	97.89	452 3	683 3
900	649.35	45.01	595 0	602.6
1100	494.30	95.76	426.2	603.8
Metatarsus				
500	739.26	118.70	563.9	825.8
700	621.60	165.90	475.1	834.7
900	769.23	102.29	643.8	892.4
1100	581.63	266.85	377.4	825.8
Tibia				
500	594.95	30.98	559.4	634.9
700	665.98	133.96	475.1	759.2
900	897.98	354.76	559.4	1345.3
1100	970.13	313.57	590.5	1345.3
Femur				
500	789.23	201.99	572.8	967.9
700	771.43	139.80	590.5	892.4
900	844.70	155.61	612.7	945.7
1100	765.90	46.22	701.5	803.6

EPIPHYSEAL LINE MEASUREMENTS FOR THE RIGHT METACARPUS, METATARSUS, TIBIA AND FEMUR IN HEREFORD STEERS

^aMeasurements made in microns

length will cease due to the ossification of the epiphyseal cartilage. At this stage in an animal's life span, several tissue changes take place. Bone growth is stabilized or plateaus and a steady increase in muscle and fatty tissue occurs. The point at which bone growth is completed, except for bone maintainance and remodeling, occurs at a definite chronological age in a particular animal. The point where bone tissue ossification stops (growth) and muscle tissue growth increases is a very dynamic phase of growth and development. Many researchers have tried to establish this point or phase in an animals life.

Epiphyseal cartilage growth of the Hereford metatarsal bones is present in Table II. Again, these results indicate considerable variation in this measurement as indicated by the large standard deviations and ranges. Nevertheless, the mean values were non-significant and do indicate a decreasing trend with advanced age except for the 900 pound weight group. In comparing the group mean values, it would be noted that these mean values were larger than those of the metacarpal bones. This suggests that the metatarsal bones are later maturing, based on epiphyseal cartilage standpoint, than the metacarpal bones.

Tibia bones (Table II) had an increase in epiphyseal cartilage with increased body weight and age. The 500 pound Hereford group was 594.95 microns and the 1100 pound group was 970.13 microns. It should be noted that the 900 and 1100 pound groups had standard deviations of 354.76 and

313.57, respectively, suggesting considerable variation in these measurements. The analysis of variance (Table X, Appendix) showed the tibia epiphyseal line thickness to have a highly significant (P < .01) increase with increased age or weight. In general, the tibia bones are later maturing, lengthwise, than the metacarpal and metatarsal bones. Perhaps this is a natural result, required to keep pace with the increased muscle tissue mass of the hind limb.

Results indicated by the mean values of the femur bones are in line with the bone growth trends of the metatarsal bones. The cartilage being the thickest or widest in the 900 pound group but, all other groups showing a small but steady decline in cartilage thickness which was nonsignificant.

In Table III are presented the epiphyseal line measurement data for the Charolais Crossbred steers. The mean values for the right metacarpal bones indicate a decrease in cartilage thickness with increased body weight and age. The mean value for the 500 pound crossbred group was 657.13microns and 573.85 microns for the 1100 crossbred group. As may be observed in Table III, the standard deviation for all the metacarpal weight groups demonstrated little variation within each range. In Table X (Appendix), differences in the right metacarpus epiphyseal line thickness was highly significant (P < .01).

Metatarsal epiphyseal cartilage thickness (Table III) increased during the course of the experiment, except at the

TABLE III

Variable	Mean ^a	Std. Dev.	Range					
Metacarpus								
500	657.13	50.36	586 .1	697.1				
700	631.58	49.63	581.6	688.2				
900	613.83	42.48	572.8	652.7				
1100	573.85	80.93	479.5	670.4				
Metatarsus								
500	607.18	201.53	426.2	861.4				
700	552.78	33.54	523.9	586.1				
900	622.73	158.87	435.1	821.4				
1100	758.13	284.43	492.8	1150.0				
Tibia								
500	540.55	54.53	492.8	603.8				
700	709.29	142.03	541.7	848.1				
900	698.18	159.61	515.0	901.3				
1100	809.18	121.20	705.9	963.5				
Femur								
500	626.05	83.05	555.0	745.9				
700	721.50	59.07	674.9	808.1				
900	795.88	133.31	617.2	928.0				
1100	896.88	117.65	799.2	1052.3				

EPIPHYSEAL LINE MEASUREMENTS FOR THE RIGHT METACARPUS, METATARSUS, TIBIA, AND FEMUR IN CHAROLAIS CROSSBRED STEERS

^aMeasurements made in microns

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700 pound weight group. The mean value was 607.18 microns at the 500 pound weight group and increased to 758.13 microns in the 1100 pound group. The metatarsal cartilage thickness was non-significant for all weight groups. The 1100 pound crossbred group displayed the most variation with a range of 492.8 microns to 1150.0 microns and a standard deviation of 284.43.

The tibia bones tended to increase in epiphyseal cartilage thickness as the steers increased in weight and age. The epiphyseal cartilage of the tibia bone made its largest increase between the 500 to 700 pound weight groups. The mean value for the 500 pound group was 540.55 microns and 709.29 microns for the 700 pound group. These results also suggest that the tibia bone was later maturing than the metatarsal bones. The analysis of variance (Table X, Appendix) showed the tibia epiphyseal line thickness to have a highly significant (P < .01) increase with increased age and weight.

Femur bones demonstrated a steady increase in the amount of epiphyseal cartilage with increased weight and age which was non-significant. The mean values for the 500 pound group is 626.05 microns and 896.88 microns for the 1100 pound group. In viewing these mean values and the increase in epiphyseal cartilage in each weight group a relationship between epiphyseal cartilage growth and muscle tissue growth might be gleaned. The Charolais Crossbred cattle were heavier muscled than the Hereford cattle. Thus, bone growth

would be expected to proceed for a longer period of time in order to support the additional tissue. Hence, these cattle were "later maturing".

General trends in epiphyseal cartilage growth of the right metacarpal bones of both breeds are illustrated in Figure 6 (Appendix). The Charolais Crossbred steers showed a steady decline in epiphyseal cartilage and, hence, linear bone growth, throughout the test. Except for the 900 pound group, the Hereford steers followed a similar trend in cartilage growth. On a "percent of final measurement" basis, the graphs for both breeds exhibited the same growth patterns.

Figure 7 (Appendix) compares the right metatarsal epiphyseal cartilage growth patterns in the two breeds of cattle. The metatarsal bones of the Charolais Crossbred steers were later maturing than those of the Herefords.

The epiphyseal cartilage growth patterns of the tibia bones of both breeds are illustrated in Figure 8 (Appendix). These growth patterns, in both breeds point out that the tibias are late maturing bones in the bovine.

The femur bones of Charolais Crossbred steers follow the same general trends as the tibia bones in epiphyseal cartilage growth. Herefords femur bones were relatively static, except for the sharp increase noted in the 900 pound weight group. The percent of final graphs (Figure 9, Appendix) establish a steady increase in the crossbred steers and points out the sharp decrease in the Herefords

after 900 pounds.

As may be noted from the statistics reported above, rather large variations were encountered in measuring the epiphyseal cartilages. This variation may be attributed to several sources, which are discussed below.

In addition to the variation due to the age factors, identified at the outset of this chapter, variation was also encountered with the epiphyseal line staining process. The distal epiphyses of the right metacarpus, metatarus, tibia and femur were sliced one-fourth inch thick with an electric Biro band saw. As the bone was being sliced, the very fine saw blade would smear and spread the soft epiphyseal cartilage. This could cause the cartilage to stain thicker at some areas and result in wider measurements. Also, the distal epiphyses slices must be cut as close as possible to one-fourth inch in thickness, as differences in thickness will change the microscopic viewing distance and result in measurement error. Thus, bone slicing technique created some variations in the cartilage measurements.

The largest source of variation in the staining technique was in trying to establish a standard location to measure on each bone slice. The epiphyseal cartilage changes in thickness moving from anterior to posterior and also from medial to lateral in each one-fourth inch slice. In addition the error in measuring when dealing with small units like microns will create a source of variation.

In trying to eliminate measurement variation,

radiographs were taken of the whole bones and also the distal slices. The radiographs appeared to create another source of variation. The radiographs would not separate the two epiphyseal lines, rather the radiographs resulted in a superimposing of the anterior to posterior and the medial to lateral epiphyseal lines, giving a much thicker cartilage than was actually present. The cartilage does not grow in an uniform manner so, the radiographs resulted with a large amount of variation too. Thus, the lack of uniformity in the growth of the epiphyseal line presents formidable difficulties in the measurement of this entity.

Cross Sectional Compact Bone Area

The following discussion will be directed towards some changes in compact or cortical bone of the Hereford and Charolais Crossbred experimental animals, as influenced by weight and age. Also, some general compact bone growth trends within the two breeds will be made. The data obtained from this phase of the experiment are exhibited in Table IV for the right metacarpal and metatarsal bones.

As one would expect the 500 pound Hereford group displayed the smallest amount of compact bone in the right metacarpus. The average mean value being 0.4371 square centimeters with a range of 0.3871 to 0.5032 square centimeters. The compact bone area increased systematically from 500 to the 1100 pound weight groups in Herefords and was significant (P < .001), the averages for the 700, 900 and

TABLE IV

Variable	Mean ^a	Std. Dev.	Range	
Metacarpus				
H-500	0.4371	0.0484	0.3871	0,5032
H -700	0.4807	0.0478	0.4258	0.5419
H -900	0.5984	0.0454	0.5613	0.6645
H -1100	0.6581	0.0303	0.6129	0.6774
Metatarsus				
Н-500	0.5113	0.0432	0.4516	0.5548
H -700	0.5452	0.0845	0.4387	0.6452
H -900	0.6726	0.0359	0.6387	0.7226
H -1100	0.6516	0.0713	0.5871	0.7226
Metacarpus				
CX-500	0.4581	0.0535	0.3935	0.5161
CX-700	0.4952	0.0412	0.4645	0.5548
CX -900	0.6355	0.0396	0.5871	0.6839
CX -1100	0.6984	0.0675	0.6194	0.7742
Metatarsus	· .			
CX-500	0.5323	0.0597	0.4839	0.6129
CX-700	0.5484	0.0519	0.4710	0.5806
CX-900	0.6532	0.0495	0.6000	0.7097
CX -1100	0.7452	0.0771	0.6323	0.8065

DENSE COMPACT BONE CROSS SECTIONAL AREA OF THE RIGHT METACARPAL AND METATARSAL BONES IN HEREFORD AND CHAROLAIS CROSSBRED STEERS

 $a_{\mathrm{Measurements}}$ made in square centimeters

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H - Hereford

CX - Charolais Crossbred

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1100 pound Herefords were 0.4807, 0.5984 and 0.6581 square centimeters, respectively. It should be noted that the variation from 500 to 1100 pounds was minimal as indicated by the ranges and standard deviations listed in Table IV.

The metatarsal bones follow the same systematic growth patterns or trends in each weight group as the metacarpal bones and are significant at the .001 level (P < .001). The average mean values are displayed in Table IV with the 500 pound group showing the least amount of compact bone, 0.5113 square centimeters. The 1100 pound weight group had an average mean value of 0.6516 square centimeters. The 700 and 1100 pound Herefords illustrated the most variation with standard deviations of 0.0845 and 0.0713, respectively. Ascan be observed from the mean values, the metatarsal bones are larger than the metacarpal bones at the same weight group. This may be due to the fact that the metatarsal bones support more body weight and are under more continual stress. A practical example of this is the stress imposed when the calf stands up after it has been laying down.

The data obtained for the right metacarpal compact bone areas of the Charolais Crossbred steers are also listed in Table IV. The crossbred steers followed the same general trends in this variable as did the Hereford steers. However, the mean values were larger in all weight groups.

The 500 pound crossbred steers had an average mean value for the metacarpal bones of 0.4581 square centimeters and for the 1100 pound steers, 0.6984 square centimeters.

The 1100 pound group showed thirty percent more compact bone area over the 500 pounders. As can be observed from the data, the "percent of Charolais" in the crossbred steers appeared to increase metacarpal bone thickness and bone substance.

The metatarsal bones in the crossbred steers followed the same pattern of compact bone area as did the Hereford steers. The average mean value for the 500 pound group was 0.5323 square centimeters and for the 1100 pound group was 0.7452 square centimeters. The metatarsal bones in the crossbred steers were larger than the Hereford steers in all weight groups. As stated earlier, the metatarsal bones in the hind limb are under more weight and stress than the metacarpal bones in the fore limb. The pelvic limb contains three long bones (metatarsus, tibia and femur) and the thoracic limb contains five long bones (metacarpus, ulna, radius, humerus and scapula). The general weight load per bone would be more in the pelvic limb; thus, the metatarsal bones would carry more weight per bone and be under more stress. This could be one reason why the metatarsus has more compact bone area than the metacarpus.

Table X (Appendix) shows the differences in metacarpus and metatarsus compact bone area to increase with increased weight and age (P < .001) in both Hereford and Charolais Crossbred cattle. The breed effect and breed-weight interaction did show a non-significant difference.

A graphic comparison of the compact bone area of the

metacarpal bones in the two breeds is shown in Figure 10 (Appendix). In viewing the graph it may be seen that the Charolais Crossbred steers exhibited the most compact bone in all weight groups as compared to the Hereford steers. These findings are in close accord with other research reports.

The general growth trends for metatarsal compact bones of the two breeds are demonstrated in Figure 11 (Appendix). The crossbred steers established a steady increase in compact bone in all weight groups. However, the Hereford steers showed a steady increase from 500 pounds to 900 pounds, thus decreased in compact bone from 900 to 1100 pounds.

Serum Alkaline Phosphatase

As stated in Chapter III the serum for the alkaline phosphatase assay was collected at the feedlot and also on the slaughter floor. The serum alkaline phosphatase results ascertained on the feedlot samples are illustrated by the line graphs in Figure 12 (Appendix). The graph for the Hereford cattle shows tremendous variation in the course of phosphatase activity and was significant (P < .001). Several reasons for this variation may be advanced, one being the different methods used in handling and working the steers. Also, the blood was collected by several different laboratory technicians which might cause some variation. It would appear from the data (Figure 12, Appendix) that the steers

must be handled with a very minimal amount of movement to keep stress at a low level. In Table X (Appendix) the variation between breeds is significant (P < .01) and the weight groups are significantly different (P < .001). Also, the breed x weight interaction was significant at the .05 level (P < .05).

The data in Table V represent the serum alkaline phosphatase activity in Hereford steers. Collection of blood was done on the slaughter floor, as uniformly as possible. The mean value for the 500 pound group was 5.97 Sigma units and these values decreased systematically to 3.23 Sigma units in the 1100 pound group. The 700 and 900 pound cattle showed the most variation, with standard deviation values of 2.01 and 1.88, respectively. In general, decreasing phosphatase activity tended to decrease as the rate of linear bone growth subsided. In the Herefords, as the epiphyseal cartilage and the compact bone area matured, the serum alkaline phosphatase activity decreased proportionately. This is demonstrated graphically in Figure 13 (Appendix).

The bone forming cells (osteoblasts) are responsible for the formation of osteoid tissue and almost immediately secrete the enzyme phosphatase (alkaline) which is necessary for deposition of calcium salts in the osteoid tissue. After bone growth has stopped or maturity is reached the osteoblasts are changed to osteocytes (bone cells), which cause a sharp decline in serum alkaline phosphatase activity.

As discovered by the data in Table V most of the phosphatase activity in the Herefords was prior to the 500 pound weight group (based on pilot work with lighter calves) and showed a steady decline throughout the experiment.

As may be observed in Table V the Charolais Crossbreds followed the same general trends as the Hereford steers, except for the 700 pound crossbred group. The 700 pound group exhibited an average mean value of 7.33 Sigma units. This group also displayed the highest standard deviation (2.03) and range (4.30 to 8.70 Sigma units). The average mean values for the 500, 900 and 1100 pound weight groups exhibited a decrease in activity, the mean values being 5.78, 4.90 and 4.30 Sigma units, respectively. The analysis of variance (Table X, Appendix) showed the serum alkaline phosphatase activity to have a highly significant (P < .01) difference. As the cattle increased in body weight and age the serum alkaline phosphatase activity decreased significantly (P < .01). The breed difference and breed-weight interaction did not effect the activity significantly.

It should also be noted (Figure 13, Appendix) that the Charolais Crossbred steers exhibited more serum alkaline phosphatase activity in each weight group. This trend would support epiphyseal cartilage and compact bone results. As stated earlier, the Charolais Crossbred cattle were later maturing and their skeletal development proceeded over a longer span of time than the Herefords, hence the alkaline

TABLE V

AND CHAROLAIS CROSSBRED STEERS (Slaughter Groups)					
Variable	Mean ^a	Std. Dev.	Ra	nge	
Phosphatase Activity					
H -500	5.97	0.84	5.18	7.00	
H -700	5.78	2.01	2.90	7.70	
Н–900	4.06	1.88	2.90	6.90	
H -1100	3.23	0.35	2.80	3.60	
CX -500	5.78	1.45	4.70	7.90	
CX -700	7.33	2.03	4.30	8.70	

SERUM ALKALINE PHOSPHATASE ACTIVITY IN HEREFORD

^aSerum Alkaline Phosphatase Activity reported in Sigma Units or micro moles per hour per milliliter.

4.90

4.30

0.93

0.52

4.00

3.60

6.20

4.70

H - Hereford

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CX-900

CX**-1100**

CX - Charolais Crossbred

phosphatase activity of the crossbreds would be expected to remain elevated for a longer period of time.

Muscle Fiber Diameter

The muscle fiber diameter data of the Hereford and Charolais Crossbred steers are shown in Table VI. As may be observed from the Hereford data, a general muscle fiber growth pattern was established. The mean value for the 500 pound group was 64.83 microns and this increased in each weight group. The mean values for the 700, 900 and 1100 pound Hereford steers were 67.65, 69.98 and 76.48 microns, respectively. In general, as age increased in each weight group so did the muscle fiber diameters. Muscle fiber diameter in the Hereford calves made its largest increase in growth (about 10%) between the 900 and 1100 pound weight groups. The 700 pound Herefords showed the most variation in fiber diameter, with a standard deviation of 6.32 and a range of 62.7 to 76.7 microns.

The crossbred steers followed the same pattern of muscle fiber growth as did the Hereford steers. The 500 pound crossbred group displayed less muscle fiber diameter (61.00μ) than the 500 pound Herefords (64.83μ) . There was very little difference between breeds at the 700 and 900 pound weight groups. The Charolais Crossbred steers, being later maturing, made a larger increase in muscle fiber diameter at 1100 pounds.

In Table X (Appendix) the analysis of variance showed

TABLE VI

Std. Dev.	Ran	Range	
3.54	60.5	68.6	
6.32	62.7	76.7	
3.19	66.8	74.4	
3.02	74.9	81.0	
6.17	53.5	68.6	
5.33	62.3	73.7	
1.66	68.7	72.5	
4.70	75.3	85.2	
	3.54 6.32 3.19 3.02 6.17 5.33 1.66 4.70	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	

MUSCLE FIBER DIAMETER IN HEREFORD AND CHAROLAIS CROSSBRED STEERS

^aMeasurements made in microns

H - Hereford

CX - Charolais Crossbred

the muscle fiber diameter thickness to have a highly significant (P < .001) increase with increased weight and age. The breed and breed-weight interaction did not effect the muscle fiber diameter measurement significantly as did the different weight groups.

Total Lean and Bone Weights

The data in Table VII reflects the right side total lean tissue in the test cattle. As one would expect, there was a steady increase in lean tissue weight with age. The average mean value for the 500 pound group was 41.590 kg. and for the 1100 pound group, 81.648 kg. Lean tissue increased approximately fifty percent, by weight, from the 500 to 1100 pound weight groups.

Charolais Crossbred steers followed the same general lean tissue growth patterns as the Herefords. The 500 pound group had a mean average value of 46.381 kilograms as compared to 88.509 kilograms in the 1100 pound weight group. This represents an increase of approximately fifty percent in total lean tissue growth during the course of the experiment. These data also point out that crossbred steers have a larger amount of tissue in all four weight groups as compared to Herefords. The variation is greater in the Charolais Crossbred steers than in the Herefords as pointed out by the standard deviation and range values (Table VII).

The breed comparison in total lean tissue growth is illustrated graphically in Figure 14 (Appendix) and

TABLE VII

Variable	Mean ^a	Std. Dev.	Ra	Range	
Right Side Lean					
H-500	41.590	1.115	40.624	42.729	
H-700	54.489	1.782	52.482	56.745	
H-900	67.280	1.170	65.999	68.630	
H-1100	81.648	3.197	78.291	85.820	
CX-500	46.381	4.496	42.911	52.981	
CX-700	55.521	2.073	53.616	58.378	
CX-900	73.245	6.062	65.001	79.607	
CX-1100	88.509	4.764	84.914	95.483	
Right Side Bone					
H-500	10.761	0.633	10.251	11.658	
H-700	13.438	1.315	12.474	15.377	
H-900	16.137	0.440	15.604	16.012	
H-1100	18.234	1.159	17.191	19.686	
CX-500	11.964	0.495	11.295	12.338	
CX-700	14.062	1.112	12.701	15.014	
CX-900	17.237	1.670	15.785	19.641	
CX-1100	19.743	0.578	19.097	20.503	

TOTAL RIGHT CARCASS SIDE LEAN AND BONE IN HEREFORD AND CHAROLAIS CROSSBRED STEERS

^aMeasurements made in kilograms

H - Hereford

CX - Charolais Crossbred
illustrates little difference in breed effect. All Charolais Crossbred weight groups had more total lean tissue than the Herefords. The rate of increase in lean tissue, however, was approximately the same in both breeds. These results are similar in trends to the muscle fiber diameter data. The lean tissue and muscle fiber diameter values increased in all weight groups. Also, the Charolais Crossbred steers had larger muscle fibers and more total lean tissue than the Hereford in all weight groups.

Total bone growth, as shown in Table VII, follows the same pattern of growth as muscle. The 500 pound Herefords displaying the least total bone and 1100 pound Herefords the largest amount of total bone. The variation in total carcass bone is low and can be noted by the low standard deviations and ranges.

Charolais Crossbred steers follow the same general trends as the Herefords. The total bone data for the Charolais Crossbred steers may be observed in Table VII. The crossbred steers indicate more total bone in all weight groups than the Herefords. The breed comparison trends may be observed in Figure 15 (Appendix).

In Figures 16 and 17 (Appendix), it may be seen that the Charolais Crossbred steers had slightly more total bone in the thoracic limb and pelvic limb than the Hereford steers at all weight groups. The total change in bone of both limbs was very little for either breed.

The analysis of variance in Table X (Appendix) showed

total right side bone and muscle tissue to have a highly significant (P < .001) increase caused by the differences due to breed and weight. The total bone in the thoracic and pelvic limbs exhibited the same increase (P < .001) due to breed and weight effect.

Bone - Muscle Comparison

In Table VIII are listed the ratios of total right side bone to total right side muscle for both breeds of steers. The bone-muscle ratios increased systematically with body weight and age in the Herefords and Charolais Crossbred steers. The total ratio change from 500 pounds to 1100 pounds was 0.613 for the Herefords and 0.606 for the Charolais Crossbred cattle. Also, the total percent change of the ratios from 500 to 1100 pounds was similar, with the Herefords showing a 6.8% change and the Charolais Crossbreds, 8.3%.

The Charolais Crossbred cattle illustrated a larger bone-muscle ratio in each weight group than the Hereford cattle. Bone-muscle ratio patterns increased throughout the test and suggested that the crossbred steers were larger boned and heavier muscled than the Hereford steers at each weight group.

The data in Table VIII point out that additional bone tissue was being deposited throughout the test, though it tended to diminish substantially during the 700 - 900 pound periods. Subsequent to this time, total muscle, as well as

TABLE VIII

MEAN AND RATIOS OF TOTAL RIGHT SIDE BONE TO TOTAL RIGHT SIDE LEAN IN HEREFORD AND CHAROLAIS CROSSBRED STEERS

Variable	Ratio (Bone:Lean)	
H-500 H-700 H-900 H-1100	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	
CX-500 CX-700 CX-900 CX-1100	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	·.
Overall Mean	1: 4.141	

H - Hereford

CX - Charolais Crossbred

fiber diameter, exhibited maximal rate of deposition. The overall mean value for the bone to muscle ratio was 1:4.141 for the cattle used in this experiment.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to attempt to identify and establish some growth parameters of bone and muscle tissue in beef cattle slaughtered at different weights and to establish the time at which long bone attained physiological maturity. Also the rate of growth of bones in the thoracic limb, pelvic limb, forequarter, and hindquarter of Hereford and Charolais Crossbred cattle was investigated.

Experimental materials for this study were sixteen Hereford steer calves and sixteen Charolais Crossbred steer calves. The crossbred calves were of two types, either Angus x Charolais or Hereford x Charolais.

The calves were randomly selected and grouped into four different pens per breed. The four Hereford pens and four Charolais cross pens each had four steers. The steers were grouped according to their intended slaughter weight groups. Slaughter weight groups were 227.3 kgs., 318.2 kgs., 409.1 kgs. and 500.0 kgs. All calves were of weaning age when brought to the feedlot and fed, <u>ad libitum</u>, a standard feedlot ration. The feedlot ration maintained a protein content based on the body weight of the calves.

Upon attaining the desired weight, the steers were

transported to the meat laboratory and slaughtered. The carcasses were chilled at $2.22^{\circ}-3.33^{\circ}$ C. for approximately three days prior to cutting. The right side of the carcass was cut into standard wholesale cuts and each wholesale cut was physically separated into bone, lean and fat and the weight of each of these tissues were recorded.

The bones were evaluated by several different procedures to determine the amount of bone growth. After physical separation from the carcass, all bones were cleaned of extraneous material. The following bones were used in this study: the metacarpus; ulna; radius; humerus; scapula; metatarsus; tibia and femur. The individual bones were weighed on an electrical Mettler balance and these data were recorded in grams. The above bones were measured with a ruler for diaphyses length determinations in centimeters.

The metacarpus and metatarsus bones were used to quantitate the area of compact space or cortical bone. This was accomplished by obtaining a cross section through the middiaphysis and measuring the printed area with a polar planimeter, and the results were expressed in square centimeters.

The metacarpus, metatarsus, tibia and femur were used to establish the amount of long bone growth by the epiphyseal staining procedure (Tibia Test) and evaluated in microns.

Serum alkaline phosphatase was determined on all thirtytwo steers. The blood sample was collected at two different times; prior to loading the steers for shipment to the meat laboratory and when the animals were bled during slaughter.

The serum alkaline phosphatase activity was reported in Sigma Units.

Muscle samples were obtained from the right Longissimus dorsi, at the twelfth thoracic vertebra. The muscle fiber diameter measurement was taken from fifty fibers and results were expressed in microns.

Epiphyseal cartilage growth of the right metacarpus in both breeds of steers exhibited the thicker cartilage at 500 pounds than at 1100 pounds. The data reflect the overall physiological changes in epiphyseal cartilage growth, thus the mean values decreased with an increase in body weight and age.

The metatarsus epiphyseal cartilage mean values for the Hereford steers indicated a decreasing trend with advanced age except for the 900 pound weight group. In comparing the group mean values, it was noted that the metatarsus mean values were larger than those for the metacarpal bones. Results showed that the metatarsus epiphyseal cartilage thickness increased during the course of the experiment, except at the 700 pound weight group in the Charolais Crossbred steers.

Tibia bones in both breeds showed an increase in epiphyseal cartilage mean values with increased body weight and age. In general the tibia bones were later maturing, lengthwise, than the metacarpal and metatarsal bones. Perhaps this was a natural result, required to keep pace with the increased muscle tissue mass of the hind limb.

Results indicated by the mean values of the Hereford femur bones were in agreement with the bone growth trends of the metatarsal bones, the cartilage being the thickest or widest in the 900 pound group but, all other groups showing a decline in cartilage thickness. The Charolais Crossbred femur bone data demonstrated a steady increase in the amount of epiphyseal cartilage with increased weight and age.

The cross sectional compact or cortical bone data for both breeds indicated that the 500 pound weight group displayed the smallest amount of compact bone in the right metacarpus. The compact bone area increased, systematically, from 500 to the 1100 pound weight groups in Hereford and Charolais Crossbred steers.

The metatarsal bones followed the same systematic growth patterns or trends in each weight group as the metacarpal bones for both breeds of steers.

Serum alkaline phosphatase activity in Hereford steers at 500 pounds had a mean value of 5.97 Sigma Units and these values decreased, systematically, to 3.23 Sigma Units in the 1100 pound group. In the Herefords, as the epiphyseal cartilage and the compact bone area matured, the serum alkaline phosphatase activity decreased.

Serum alkaline phosphatase results for the Charolais Crossbreds followed the same general trends as that of the Hereford steers, except for the 700 pound group. The 700 pound group exhibited an average mean value of 7.23 Sigma Units and this group also displayed the highest standard

deviation (2.03) and range (4.30 to 8.70 Sigma Units).

A general muscle fiber growth pattern was established in the Hereford steers. The mean value for the 500 pound group was 64.83 microns and this increased in each weight group. In general, as age increased in each weight group so did the muscle fiber diameters. Muscle fiber diameter in the Hereford calves made its largest increase in growth (about 10%) between the 900 and 1100 pound weight groups.

The crossbred steers followed the same pattern of muscle fiber growth as did the Hereford steers. The 500 pound crossbred group displayed less muscle fiber diameter (61.00μ) than the 500 pound Herefords (64.83μ) . There was very little difference between breeds at the 700 and 900 pound weight groups. The Charolais Crossbred steers, being later maturing, made a larger increase in muscle fiber diameter at 1100 pounds.

Total right side muscle tissue in Herefords followed the same trend as fiber diameter throughout all weight groups. As one would expect, there was a steady increase in muscle tissue weight with age. Charolais Crossbred steers followed the same general lean tissue growth patterns as the Herefords, but the Charolais displayed more total pounds of lean tissue.

Total right side bone tissue growth followed the same pattern of growth as muscle, the 500 pound Herefords displaying the least total bone and 1100 pound Herefords the largest amount of total bone.

Results for the Charolais Crossbred steers followed the same general trends as the Herefords, but the crossbred steers had more total bone in all weight groups than the Herefords. The Charolais Crossbred steers had slightly more total bone in the thoracic limb and pelvic limb than the Hereford steers at all weight groups. Also, the crossbred steers contained more total bone in the forequarter than the Hereford steers. The same trend was found in the hindquarter in all weight groups.

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APPENDIX

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Bones by Weight Group and Breed





Bones by Weight Group and Breed





















Figure 16. Total Right Side Carcass Bone in Thoracic Limb by Weight Group and Breed








































Group and Breed







Group and Breed



Group and Breed

TABLE IX

Variable	Mean ^a	Std. Dev.	Range	
Right Metacarpus				
H-500	18,590	0.677	17 68	10 02
H-700	19,780	0.949	18 73	20 76
H-900	22,885	0.204	22 74	20.70
H-1100	24.653	1.579	22.77	26.62
CX-500	19.003	0.742	18.41	20.04
CX-700	21.020	1.314	19.37	20.04 22 43
CX-900	24.568	2.536	20 93	26.70
CX-1100	25.580	1.237	24.11	26.98
Right Metatarsus				
H-500	19,698	0.928	18 02	20 85
H-700	19.805	0.667	10.92	20.05
H -900	23,340	0.428	22 36	20.91
H-1100	24.173	1.051	23.05	25.57
CX -500	19.788	1.372	17.95	21.23
CX-700	20.403	1.134	19.85	22,68
CX -900	24.145	1.336	22.83	25,98
CX -1100	26.545	2.475	22.96	28.50
Right Scapula				
H-500	21.160	0.706	20.20	21.86
H - 700	22.710	2.560	20.70	26.38
н–900	28.290	1.720	25.83	29.83
H -1100	32.070	3.616	26.76	34.64
CX-500	20.328	1.214	18.87	21.48
CX-700	23.383	3.277	18.83	26.61
CX-900	29.790	3.962	24.00	32.63
CX-1100	30.838	0.813	29.86	31.85
Right Humerus)		
H - 500	58.668	4.408	53.36	63.10
H - 700	77.148	17.189	55.21	92.48
H - 900	95.850	7.169	87.71	103.64
H -1100	95.563	2.898	92.60	99.03
CX-500	67.065	4.417	62.57	72.64
CX-700	87.930	7.150	81.04	97.73
CX-900	94.908	4.286	91.02	99.03
CX -1100	95.438	8.669	84.00	102.82

WEIGHT-LENGTH RATIOS OF LIMB BONES IN HEREFORD AND CROSSBRED CHAROLAIS STEERS

Variable	Mean ^a	Std. Dev.	Range					
Right Ulna-Radius								
H-500	29.310	0.643	28.73	29.93				
H - 700	30.798	2.643	28.67	34.60				
H - 900	35.545	2.118	33.04	37.65				
H -1100	43.598	0.633	42.72	44.07				
CX-500	33.280	3.948	28.36	37.80				
CX-700	32.845	3.768	28.11	36.57				
CX-900	38.258	2.218	35.83	41.20				
CX - 1100	43.858	5.150	36.61	48.73				
Right Tibia								
H-500	57.498	6.160	53.02	66.44				
H -700	59.825	9.116	47.39	69.27				
Н –900	71.475	6.784	65.33	81.17				
H -1100	74.733	1.289	73.71	76.56				
CX -500	63.650	0.956	62.78	65.01				
CX-700	68.568	7.188	60.17	75.60				
CX - 900	75.633	11.609	61.02	88.61				
CX-1100	82.850	8.591	70.20	89.17				
Right Femur								
H-500	30. 488	2.210	27.46	32.68				
H -700	35.018	4.565	30.24	41.16				
Н–900	42.430	2.914	40.02	46.67				
H -1100	43.358	1.620	42.17	45.71				
CX - 500	35.513	1.434	33.99	37.44				
CX -700	37.005	3.764	33.07	41.85				
CX -900	45.375	8.348	38.62	57.50				
CX -1100	47.955	4.608	41.69	52.34				

TABLE IX (Continued)

^aMeasurements made in grams per centimeters.

H - Hereford

CX - Charolais Crossbred

TABLE X

ANALYSIS OF VARIANCE OF THE EPIPHYSEAL LINE THICKNESS, COMPACT BONE AREA, SERUM ALKALINE PHOSPHATASE ACTIVITY, FIBER DIAMETER MEASUREMENTS AND TOTAL MUSCLE AND BONE TISSUE IN THE CARCASS^a

Source of Variation	D.F.	MCELT ^b	MTELT	TBELT ^b	FBELT ^b
Total	31			: 	
Breed	1	471.245	14603.405	69136.211	8573.951
Weight Group	3	31320.652**	18188.483	154959.010**	28198.041
Breed x Weight Group	3	8175.033	44995.656	24060.725	29579.961
Animals in Breed and Weight	24	5969.778	29283.177	38299.265	16087.096

**Significant level (P < .01)

^aAll values denote mean square

^bMCELT - Metacarpus epiphyseal line thickness MTELT - Metatarsus epiphyseal line thickness TBELT - Tibia bone epiphyseal line thickness FBELT - Femur bone epiphyseal line thickness

TABLE X (Continued)	
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Source of Variation	D.F.	мссва	мтсва ^b	SAPFB ^b	SAPSB ^b
Total	31	••••••••••••••••••••••••••••••••••••••			
Breed	1	0.00637	0.00484	36.722**	5.427
Weight Group	3	0.09342***	0.05964***	49.223***	12.201**
Breed x Weight Group	3	0.00031	0.00477	12.030*	1.011
Animals in Breed and Weight	24	0.00229	0.00376	3.749	2.041

*Significant level (P < .05)

**Significant level (P < .01)

***Significant level (P < .001)

^bMCCBA - Metacarpus compact bone area

MTCBA - Metatarsus compact bone area

SAPFB - Serum alkaline phosphatase feedlot bleeding

SAPSB - Serum alkaline phosphatase slaughter bleeding

Source of Variation	D.F.	MFD ^b	TMRS ^b	TBRSb	TBTL ^b	TBPL ^b
Total	31					
Breed	1	1.125	188.132***	9.713**	0.461***	0.641***
Weight Group	3	290.325***	2558.225***	88.801***	3.645***	2.536***
Breed x Weight Group	3	11.636	14.711	0.268	0.016	0.031
Animals in Breed and Weight	24	20.356	12.443	1.034	0.026	0.042

TABLE X (Continued)

**Significant level (P < .01)

***Significant level (P < .001)

^bMFD - Muscle fiber diameter TMRS - Total muscle right side TBRS - Total bone right side TBTL - Total bone thoracic limb

TBPL - Total bone pelvic limb

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