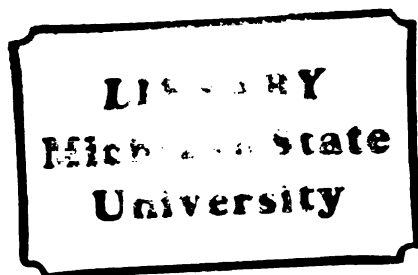


THESIS



This is to certify that the
thesis entitled

THE EFFECT OF FRAME SIZE AND DIET ON DAILY
PROTEIN ACCRETION AND FAT DEPOSITION
IN YOUNG GROWING BULLS

presented by

Steven Paul Spivey

has been accepted towards fulfillment
of the requirements for

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THE EFFECT OF FRAME SIZE AND DIET ON DAILY
PROTEIN ACCRETION AND FAT DEPOSITION
IN YOUNG GROWING BULLS

By

Steven Paul Spivey

A THESIS

Submitted to
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ABSTRACT

THE EFFECT OF FRAME SIZE AND DIET ON DAILY PROTEIN ACCRETION AND FAT DEPOSITION IN YOUNG GROWING BULLS

By

Steven Paul Spivey

Thirty-three Angus and 33 Simmental bulls were randomly allotted to either a high silage or high grain diet to determine the rates of empty body daily protein and fat gain for bulls of different frame size fed diets differing in energy density.

A 140 day feeding trial, similar to those used in beef cattle performance testing, was employed from weaning to 365 days of age. One-fourth of the bulls were slaughtered at the beginning of the trial, and approximately every 40 days thereafter. Empty body protein and fat composition was calculated from data obtained from the ninth, tenth and eleventh rib sections of the carcass.

Simmental bulls and all bulls fed high grain diets had faster ($P < .01$) live weight gains than Angus and high silage diet bulls, respectively. Rates of empty body protein gain were greater ($P < .01$) in all bulls fed high grain diets and Simmental bulls. No significant differences in fat gain were observed between frame sizes. Bulls of both breeds fed the high grain diets had greater ($P < .01$) daily empty body fat gains than bulls fed the high silage diet.

To My Grandfather

John R. Spivey

1905-1977

"...And God said 'Let the earth bring forth
living creatures according to their kinds:
cattle and creeping things and beasts of
the earth according to their kinds.' And
it was so. And God made the beasts of the
earth according to their kinds, and every-
thing that creeps upon the ground according
to its kind. And God saw that it was good.

- Genesis 1: 24-25

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INTRODUCTION

Performance information on bulls is a critical selection tool used to identify superior beef bulls for use as herd sires. Rate of live weight daily gain and live weight at one year of age are the major performance parameters used by cattle breeders to identify outstanding individuals who are superior in siring calves with the potential for faster growth rate. In order to measure the genetic potential for growth of an individual bull, a 140 day feeding trial from weaning to 365 days of age is employed to measure the performance of an individual animal. A need to compare the growth and performance potential of bulls under similar environmental conditions has been expressed by cattle breeders and animal scientists during the past 15 years. Many beef producers collect performance information on bulls at their own farm or ranch, or through a central bull test station. It is obvious that the management systems, diet formulation and environmental conditions vary widely between tests. Differences in composition of gain between young growing bulls of different frame size fed diets of different energy densities remains to be determined and applied to selection procedures at the production level. A thorough understanding of the growth, development and composition of gain of the young bull is essential if superior bulls who sire calves with more genetic potential for lean meat accretion with a minimum of fat during the growth phase are to be selected by beef producers.

The research reported herein involved bulls of two extreme genetic types. Bulls from each frame type originated from the same herd. The 140 day feeding trial was set-up as close as possible to typical performance tests used widely in the beef industry to determine the genetic potential for growth rate and yearling weight of young bulls. The growth and development of bovine muscle from weaning to one year of age was examined by analyzing the fat, moisture, protein content and nucleic acid content of the semitendinosus muscle. In addition, whole body fat, water and protein content was examined to determine the differences in composition of gain between frame types, energy density of the diets and the interaction of the two. The project also included an assessment of growth hormone and insulin levels. All the afore mentioned criteria were examined at approximately 40 day intervals.

LITERATURE REVIEW

Growth and Development

Growth and development parameters are so broad and complex they are not easily defined by animal scientists. As a result, differing definitions on the true meaning of growth and development have been proposed. Since growth and development are closely related and at times inseparable, it is important to define them as one concept.

Earlier animal scientists defined growth as the increase in total body mass. Brody (1945) proposed growth be defined as the addition of new biochemical units synthesized by either an increase in cell numbers by cell division, enlargement of existing cells, or the incorporation of new material from the outside environment into the body. Hammond (1952) and McMeekan (1959) suggested development be defined as the alterations of body confirmation until physiological maturity is reached. Furthermore, growth is the increase in body weight until mature size is reached. Maynard and Loosli (1969) proposed the most widely accepted definition of growth. True growth is defined as an increase in muscle, bone and organs to be distinguished from an increase in body mass resulting from fat deposition. True growth would include an increase of basic body building blocks, specifically body water, protein and ash of structural tissue.

The major body tissues grow and develop at different rates from conception to maturity and is sequential to the biological importance of the tissue (McMeekan, 1959). Those of greatest physiological importance, such as the nervous system, are almost completely developed at birth. Postnatally, major tissues grow at different rates exhibiting overlapping phases of development. The first phase is marked by the growth of bone, nervous tissue and the vital organs. Bone completes the primary proportion of its growth earlier in life than muscle or fat (Berg and Butterfield, 1968; Cuthbertson and Pomeroy, 1962). The second phase is the development and growth of skeletal muscle tissue and concurrent fat deposition. Fat deposition comprises most of the final phase as a result of protein synthesis having reached a maximum level (Berg and Butterfield, 1968).

The relative composition of basic chemical entities of body tissues changes during growth and development. Forbes (1968) found the fat free body of mice at birth to be composed of 82% water, 14% protein and 4% minerals. At maturity, the mineral percentage remains constant while water decreases and protein increases to 70-75% and 21-24%, respectively.

Of all the body tissues, fat is quantitatively the most variable tissue in both the total body and in muscle (Callow, 1947, 1948). Most animals have very little fat at birth but the amount of total body fat increases with an increase in live weight. However, the amount of adipose in the body is independent of the animals age (Cuthbertson and Pomeroy, 1962). Zucker and Zucker (1963) reported the accretion of adipose is related to the weight of the animal and is independent of age in growing rats under normal dietary conditions. Bergen (1974) noted the process of

protein accretion and fat deposition occurs simultaneously in early growth whereas protein accretion rate decreases and finally stops but fat deposition continues with increasing body weight. Beyond a certain body weight, fat deposition becomes a large and constant fraction of weight gain. Palsson (1955) stated adipose tissue was the latest maturing due to its being the lowest in nutrient priority of all the body tissues. Adipose tissue develops only when an animal is fed above maintenance requirements for an extended period of time. This allows more nutrients to go to the low priority tissues, such as fat. Hedrick (1968) reported fat deposition initially occurs around the vital organs progressing to intermuscular (seam fat), subcutaneous (rib fat) and finally intramuscular (Marbling). Similiar patterns of fat deposition were reported by McMeekan (1959).

Muscle Growth

Skeletal muscle is the most abundant tissue on a weight basis in mature animals (Hedrick, 1968). Skeletal muscle originates from mesoderm, the middle third of the embryonic germ layers. Myofibers (skeletal muscle cells) are the end result of mesoderm development. During the embryonic stages of development, muscle cells exist as a presumptive myoblasts originating from mesoderm. At this stage, the mononucleated cells are incapable of fusion or synthesis of contractile proteins. The presumptive myoblasts divide mitotcally into myoblasts capable of myofibril (contractile protein) synthesis and fusion to other myoblasts.

Myoblasts finally fuse to form myotubes. The myotubes are multinucleated and do not undergo further mitosis. These cells are capable of contractile protein synthesis and mature into myofibers (Stromer et al., 1974).

Myoblast fusion is nearly complete at birth in domestic farm animals. Once fusion is complete, there is no significant increase in myofiber number postnatally (Joubert, 1956; McMeekan, 1940; Smith, 1963; Staum, 1963; Strickland et al., 1975). A small increase in muscle cell numbers maybe observed after birth. However, such increases are regarded as an extension and completion of embryonic differentiation (Stromer et al., 1974).

Muscle growth and development can be divided into three phases. The first phase, hyperplasia, is marked by an increase in the number of muscle cells and occurs before fusion. This is immediately followed by a second phase of hyperplasia and hypertrophy, the increase in cell size. The final phase is almost entirely hypertrophy (Stromer et al., 1974) and hypertrophy is solely responsible for post-natal muscle growth (Joubert, 1956; Staum, 1963; Stromer et al., 1974). During muscle growth and development, the percent body weight composed of muscle tissue increases from approximately 25% in the rat and man at birth (Elliot and Cheek, 1968) to about 50% in mature animals regardless of mature size (Young, 1970).

Muscles attached to bones must lengthen in relation to post-natal longitudinal bone growth. This is accomplished by the addition of new sarcomeres at the tendons rather than increasing the length of existing sarcomeres (Bendall and Voyle, 1967; Close, 1972; Griffin et al., 1972;

Goldspin, 1968, 1972).

The proportion of lipid, protein and water in muscle tissue changes with age. Numerous researchers (Dickerson, 1960; Dickerson and Widdowson, 1960; Giovannetti and Strothers, 1975; LaFlamme et al., 1973; Sink and Judge, 1971 among others) have shown the proportion of protein and lipid in muscle increases with age and the proportion of water decreases in early growth. The decreases in percent water is less progressive with age. Eversole (1978) reported a similar decline in water and increases in lipid and protein in bovine semitendinosus muscle from the initial to terminal slaughter group.

Nucleic Acids

Tissue DNA content may be employed as an estimator of the number of nuclei present in a cell. Venderly (1955) estimated the DNA content per diploid nucleus in the bovine to be 6.4 - 7.1 picograms per nucleus. Skeletal muscle is multinucleated, it is therefore difficult to estimate cell numbers from the amount of tissue DNA. Tissue DNA content can be used to estimate cell numbers in mononucleated cells. Cheek et al., (1971), Robinson (1971), and Goldspink (1972) proposed that a meaningful measure of post-natal muscle growth is the number of nuclei per muscle cell since the amount of cytoplasm governed by one nucleus is limited. Winick and Noble (1965) and Enesco and Noble (1962) indicated cell size per given nucleus could be determined by the protein to DNA ratio or muscle weight to DNA ratio.

RNA in tissue is responsible for part of the synthesis of new proteins by the ribosomes. Hence, RNA levels in a tissue are a good measure of the cell's capacity to synthesize protein (Wannamacher, 1972). Many workers (Munro and Fleck, 1967; Powell and Aberle, 1975; Winick and Noble, 1965) have reported low protein synthesis in tissue with a low RNA to DNA ratio. Harbison et al., (1967) and Sarker (1977a) reported RNA:DNA were reliable for comparisons among different tissues, but not within a single tissue. They suggested cell function, not protein synthesizing ability was indicated by higher RNA to DNA ratios.

The changes in muscle cell RNA and DNA content and concentrations in the absence of mitosis during growth and development have been well documented in domestic livestock. Eversole (1978) noted an essentially equal increase in total RNA, DNA and protein in semitendinosus muscle over the growing-finishing period with steers of four genetic types. The increases were highly correlated to live weight gain. Similar results in pigs up to 270 days of age were noted by Powell and Aberle (1975). Robinson (1969) reported an increase in total DNA content of porcine semitendinosus muscle to four months of age whereafter the level remained constant. On the contrary, an increase in total RNA and DNA up to 104 kg and 118 kg, respectively, was reported by Harbison et al., (1976). Other researchers have documented increases in total DNA in domestic livestock during growth and development; chickens (Moss et al., 1964; Moss, 1968), cattle (LaFlamme et al., 1973), sheep (Johns and Bergen, 1976) and swine (Sarker et al., 1977b).

The concentration of both RNA and DNA per skeletal muscle unit decreases with post-natal growth and development. Eversole (1978)

reported a decrease in nucleic acid concentration during growth in the bovine. While the total RNA and DNA content also increased, increases in muscle size and weight accounted for overall decreases in RNA and DNA concentration. Similar conclusions were reported by Enesco and Puddy (1964); Moss et al., (1964) and Tsai et al., (1973) in laboratory rodents. Powell and Aberle (1975) observed that RNA and DNA concentrations in porcine skeletal muscle declined during the first 100 days post-natally and remained constant thereafter. Reports by Gilbreath and Trout (1973), Harbison et al., (1976) , and Robinson (1969) and Tsai et al., (1973) showed similar results. Tasi et al., (1973) observed a decline in RNA concentration from birth to 16 weeks of age. A rapid increase in RNA concentration followed by a decline to a constant level was also reported. Decline in RNA and DNA concentrations with advancing age have been observed in ruminants by Johns and Bergen (1976) and LaFlamme et al., (1973).

Factors Influencing Growth Rate, Body Composition and Muscle Growth

Many factors influence the growth and development of an animal. The animal's genetic background, nutrition, hormone secretion and a host of other environmental factors influence the growth rate, development and carcass composition of an individual animal. For the purpose of this study three factors; genetics, nutrition and hormone effects will be reviewed.

Genetics

The phenotypic variation between animals within a species is the result of the interaction between the animals heredity and environment (Hedrick, 1975). Heredity provides the potential for growth while environmental factors such as nutrition govern the amount of potential that is actually achieved. The results and effects of selecting for larger framed domestic animals is well documented.

Stonaker et al., (1952) reported larger framed cattle from herds where parents were selected for larger size grew faster than smaller framed cattle when similiar beginning and end points were used. Similar findings were reported by Willey et al., (1951). Brown et al., (1973, 1974) in a study using Angus and Hereford bulls reported final weight, total weight gain and feed intake were more predictable from overall body size than feed efficiency. Guenther (1974) also reported faster gains and heavier live weights with larger framed cattle. Eversole (1978) using unselected Hereford, selected Hereford, Angus x Hereford x Charolais and Angus x Hereford x Holstein steers reported an increase in average daily gain with increasing frame size. Similar results were reported by Byers and Parker (1979b), Harpster et al., (1978) and Smith et al., (1976a). Larger framed exotic cattle have more efficient live weight gains than smaller British breeds (National Academy of Science, 1975) and tend to be leaner at a given body weight (Bond et al., 1972). However, research by Klosterman et al., (1968) and Brungardt (1972) comparing British breeds with Charolais steers showed no difference in feed efficiency when fed to equal finish. According to the energy

metabolism model developed by Fox and Black (1977), larger framed cattle can be expected to gain at a faster rate. Holstein steers have a higher net energy for maintenance and gain requirement (Garrett, 1971).

Luff and Goldspink (1967, 1970) reported mice of larger body size have larger muscles as a result of increased fiber number. They concluded fiber number, not fiber size is genetically determined. Aberle and Doolittle (1976) supported this conclusion. However, other workers (Byrne et al., 1973; Ezekwe and Martin, 1975; Hanrahn et al., 1973) suggested some differences in fiber size as well as fiber number were genetically determined.

Byers (1979a) observed a faster rate of protein growth with an increase in mature size of cattle. Rate of protein accretion increased at a decreasing rate as rate of gain increased until an upper limit was achieved. Aberle (1975) reported greater muscle development and larger loin eye area in heavy muscled pigs compared to light muscled one. Total DNA content of the biceps femoris was higher in the heavier muscled pigs. Heavier semitendinosus muscles and more total RNA, DNA and muscle protein in faster growing pigs was reported by Martin and Ezekwe (1975) who concluded that faster growing pigs have more and larger muscle cells as indicated by greater protein to DNA ratios and greater DNA content. Harbison et al., (1976) comparing genetically muscular and obese pigs reported that the muscular pigs had more muscle and less separable fat on a total weight and percent carcass basis at 45 kg live weight. Muscular pigs had more total DNA in the longissimus muscle with only total DNA correlated with the total muscle mass. In contrast, Bergen et al., (1975) using genetically lean and obese mice observed no difference in hind-limb DNA content up to 18 weeks or age.

Nutrition

Nutrition during post-natal growth has a profound effect on body composition and growth (Hedrick, 1975). For an animal to achieve its maximum genetic potential for muscle growth, nutrition must play a significant role (Goldspink, 1964). Dietary energy and protein levels have a significant impact on protein accretion and fat deposition. The effect of protein in the diet above levels required for maintenance and growth depends upon the genetic potential of the animal and composition of the protein used (Clausen, 1959). Three concepts of lean and fat accretion in the pig suggested by Clausen (1959) support the above conclusion. First, a pig cannot grow and produce muscle to its genetic limits unless a sufficient amount of protein of high biological value is provided in the diet. Second, no pig can produce more muscle beyond its genetic potential by excessively high levels of dietary protein. Finally, the protein and energy requirements for maintenance and muscle production are met first, with the remainder used for fat deposition. Smith et al., (1967) reported a linear increase in percent lean cuts in pigs as the crude protein percent in the ration increased up to 17.2%.

The effect of diet energy density above levels required for maintenance also depends on the genetic potential of the animal. Jessee et al., (1976) and Prior et al., (1977) observed an increase in rate of gain in cattle with an increase in diet energy density when protein levels were adequate. Numerous workers have reported an increase in growth rate with an increase in diet energy density. In a study using Polled Hereford bulls, Geuns and Hawkins (1978) reported bulls fed an 80% (dry matter

basis) high moisture corn diet gained faster ($P < .01$) and required less feed on a dry matter basis ($P < .01$) than bulls fed high corn silage diets. Eversole (1978) using steers of different frame types reported steers on high energy diets (90% high moisture corn) gained faster in all frame types than steers on a 90% corn silage diet.

The concentration of dietary energy and energy intake during the growth phase has a significant impact on the composition of gain in the bovine. Numerous researchers have reported increases in carcass fat and protein gains in steers fed diets with a high energy density (Byers and Parker, 1977b; Crickenberger, 1977; Eversole, 1978; Newland, 1979a, 1979b). Byers (1979a, 1980) suggested daily protein gain increases as live weight gain increases. Maximum protein daily gain is reached at approximately 1.0 kg live weight gain per day. Increases in live weight daily gain above 1.0 kg are almost entirely deposited as fat.

It can be concluded that increases in dietary energy will result in greater fat and protein daily gain until the maximum rate of protein synthesis is achieved. Increases in energy beyond this level are then deposited as a depot adipose.

Dietary energy content also has a dramatic influence on body composition and overall growth. Geuns and Hawkins (1978) reported significant ($P < .01$) increases in body length, heart girth circumference, testicle circumference, rib eye area and rib fat in Polled Hereford bulls fed high grain diets. Similar increases in rib eye area and rib fat in steers have been reported by Crickenberger (1977), Harpster (1978) and Woody et al., (1978).

Dietary energy content also has a dramatic influence on body composition.

Schemmel et al., (1970) suggested diet was responsible for 40% of the total variation in body weight and 74% of the variation in body fat for rats of the same age and sex. Numerous workers have reported increases in carcass fat in cattle fed high energy diets compared to cattle on low energy diets when fed for equal lengths of time. Cattle on high energy diets also have higher rib eye marbling scores (Bond et al., 1972; Garrigus et al., 1967; Johnson et al., 1967; Leander et al., 1978; Oltjen et al., 1971; Richardson et al., 1971; Utley et al., 1975).

Henrickson (1965) and Waldman et al., (1971) proposed that dietary energy density in the last half of the feedlot gain period dictated differences in carcass composition. Cattle on high energy diets during the feedlot period showed a decrease in percent bone and lean and an increase in fat percentage. Harpster (1978) reported significant ($P < .05$) differences in marbling score, quality grade, rib eye area, and kidney, heart and pelvic fat percentage in steers from four genetic types when compared at similar carcass fat content. Smaller framed steers were fatter while larger steers were leaner and more muscular. Similar differences were reported by Crickenberger (1977) and Woody et al., (1978). Woody et al., (1978) observed no influence on marbling, quality grade, rib eye area and kidney, heart and pelvic fat percentage in Hereford steers. On the contrary, Garrett and Hinman (1971) reported significant ($P < .01$) relationships between increased body fat and higher quality grade, higher marbling score and higher yield grade score ($P < .05$). Ferrell et al., (1978) noted heavier carcass weight and increased fat with high grain diets. Byers and Parker (1976b) reported more body fat at similar empty body weights on high grain diets. Work by Ferrell et al.,

(1978) achieved similar results. Byers and Parker (1979b) demonstrated higher rates of protein accretion and fat deposition in steers fed high energy diets. This is in contrast to Ferrel et al., (1978) who noted no increase in carcass protein on high energy diets. Leander et al., (1980) reported a decrease in water and increase in lipid but no change in protein in semitendinosus muscle of steers. Eversole (1978) reported no effect on muscle protein content with high energy diets.

No differences in feed efficiency have been observed in cattle taken from similar beginning and ending compositions across all frame sizes (Brungardt, 1972; Klosterman, et al., 1968; Stonaker et al., 1952).

Byers and Parker (1979a) suggested forage feeding as being more appropriate for smaller framed cattle and high grain as best suited for larger framed, faster growing cattle to best express full genetic potential for protein growth. Larger framed cattle benefit from increased rate of fat deposition allowing desired carcass fat at a lighter, more desirable weight. Furthermore, larger framed cattle were more efficient on high grain diets and smaller framed cattle were more efficient on forage diets. Earlier observations by Klosterman and Parker (1976) agreed with this last point. Smaller framed cattle require less feed per unit of gain than cattle of larger frame size (Byers and Parker, 1979b; Eversole, 1978; Ferrell et al., 1978; Harpster, 1978). However, larger frame cattle produce more protein per unit of feed than cattle of smaller frame size.

Energy and protein intake has a significant role in muscle growth and development. Hill et al., (1970) observed a decrease in protein

synthesis, total protein, total DNA, protein:DNA and RNA:DNA in rats fed a protein deficient diet. They also observed a decrease in total RNA and DNA with a caloric restricted diet. Howarth (1972) using diets of 6%, 12%, 18% and 24% crude protein observed a decrease in gastrocnemius muscle weight as protein levels decreased in the diet fed to rats. When fed low protein diets, DNA accumulation was not observed but a small increase in muscle protein was noted. A loss of total RNA accumulation was also observed. Trenkle (1974) reported a decreased muscle growth, RNA, DNA and muscle protein with diets deficient in protein or calories when fed to rats. Winick and Noble (1966) demonstrated a decrease in DNA synthesis in laboratory rodents with undernutrition. They concluded DNA synthesis is more permanently affected by under nutrition than RNA or protein synthesis. Permanent retardation of growth could occur if restrictions are imposed during the time DNA accumulation is most rapid.

Eversole (1978) reported heavier semitendinosus muscle weights with steers fed high silage diets. Leander et al., (1978) reported no differences in percent protein of semitendinosus muscles with high grain diets.

Hormones

Hormones, secreted by various endocrine glands, control many metabolic functions in the body. Released into the circulatory system, hormones travel to various parts of the body where they alter or regulate organ function and/or cell function (Goodman, 1974). Since there are many hormones in the body that have an impact on growth and development, a discussion of all of them is not warranted here. The role of growth

hormone and insulin will be discussed below.

Growth Hormone

Growth hormone and insulin are both responsible alone and/or in combination for increasing protein synthesis rate and nitrogen balance (Althen, 1975; Rabinowitz and Zierler, 1963). Weil (1965) proposed a synergistic effect on protein synthesis by growth hormone and insulin.

It is well documented that hypophysectomy decreases content and synthesis of protein, RNA (Manchester, 1970) and DNA content and synthesis in skeletal muscle (Cheek and Hill, 1970; Trenkle, 1974). Injection of growth hormone in hypophysectomized rats restored protein, RNA and DNA content and synthesis to near normal levels (Cheek and Hill, 1970; Manchester, 1970; Trenkle, 1974). Growth hormone has been shown to increase and promote peptide bond formation (Kostyo and Rillema, 1971), increase RNA synthesis (Garren et al., 1967) and enhance protein synthesis by promoting amino acid transport (Jefferson and Korner, 1967; Kostyo, 1964; Snipes, 1967). Turner et al., (1967) reported that growth hormone sustained protein synthesis when substrate amino acid levels declined. Growth hormone does not have an effect on muscle protein turnover (Goldberg, 1969).

Conflicting results on the relationship between growth hormone and body growth and development have been reported by workers. In a study involving 40 Holstein heifers, Purchas et al., (1971) reported a negative correlation ($r = -0.37$) between growth hormone and growth rate from 4 to 10 months of age. Siers and Sweiger (1971) also reported a negative relationship between growth hormone and growth rate. Weiss et al., (1974) and Siers and Hazel (1970) found a negative relationship between

growth hormone levels and percent lean cuts in swine.

Lower pituitary and serum levels of growth hormone were reported by Althen and Gerritts (1976a) in swine genetically selected for high backfat when compared to swine selected for low backfat. They concluded selection for low backfat resulted in selection for higher growth hormone levels. This conclusion was supported by Wangsness et al., (1977). However, Toppel et al., (1972) and Weiss et al., (1974) noted higher plasma growth hormone levels in genetically obese pigs when compared to stress prone muscular pigs. Weiss et al., (1974) suggested the differences were due to differences in body composition, not stress susceptibility.

Similar results in ruminants have been documented. Johns and Bergen (1976) observed higher levels of growth hormone (4.8-8.0 ng/ml) in serum to 90 days of age and decrease to a lower level (2.4 ng/ml) at four months of age in sheep. However, Trenkle and Irvin (1970) observed no differences in growth hormone levels between 18 day old calves and 13 month old cattle. Furthermore, growth hormone levels were positively related to carcass weight, rib eye area and daily gain, but negatively related to growth rate and percent lean cuts in cattle. Dev and Lasley (1969) showed no relationship between growth hormone and preweaning gains, 210 day weight, 392 day weight or rate of gain in cattle.

Hafs et al., (1971), Seirs and Hazel (1970) and Siers and Sweiger (1971) proposed rapidly growing animals utilize growth hormone more rapidly, resulting in lower serum levels. However, Trenkle and Irvin (1970) suggested mature animals were not as responsive as young growing ones because of lower growth hormone levels resulting in decreased growth rate. Trenkle and Topel (1978) reported plasma concentrations of growth hormone, pituitary concentration per unit body weight, secretion of growth

hormone per unit body weight and metabolic clearance rate (MCR) of growth hormone per unit body weight decreased with increasing body weight.

Earlier work by Gerrits (1976a) and Swaitek (1968) reported serum growth hormone levels in swine to be highest at birth. Similar results in sheep were observed by Bassett et al., (1970) and Johns and Bergen (1976).

Purchas et al., (1970) proposed that the growth hormone status of an animal should be based on plasma growth hormone levels, anterior pituitary content, rate of turnover, hypothalamic growth hormone releasing factor, tissue responsiveness to growth hormone or a combination of these. In addition, the metabolic clearance rate (MCR) of growth hormone is a more accurate method to describe the growth hormone status of an animal (Seirs and Hazel, 1970; Purchas et al., 1970). Trenkle (1976) suggested concentration of growth hormone in the blood is a function of its clearance rate and secretion rates from the anterior pituitary. Trenkle and Topel (1978) reported growth hormone secretion from the anterior pituitary and MCR increased with increased body weight but significantly ($P < .01$) decreased per unit body weight. Furthermore, plasma growth hormone concentration was positively correlated to MCR ($P < .05$) and secretion by the anterior pituitary ($P < .01$). The higher growth hormone secretion rates per unit body weight in young animals was significantly associated ($P < .01$) with greater MCR and anterior pituitary growth hormone concentration per unit body weight. This concurs with Althen and Gerrits (1976b) who reported a decrease in MCR in larger frame swine.

Curl et al., (1968) reported an increase in total growth hormone content of the anterior pituitary with age as a result of increased overall gland size. However, the concentration of hormone per unit

of gland and per unit body weight decreased with age. Johns and Bergen (1976) also reported an increase in total growth hormone content with age in sheep.

Studies on the effect of diet on the growth hormone status of an animal has produced variable results. Trenkle (1970) reported no change in plasma growth hormone levels in steers fed high energy diets. These results were later confirmed by Trenkle (1971b) and McAtee and Trenkle (1971) who found no effect on plasma growth hormone levels from feeding, fasting or nutrient intake in sheep or cattle, respectively. On the contrary, Trenkle (1978) reported a decrease in plasma growth hormone after feeding. It was further suggested the growth hormone levels increased when nutrient intake is limiting to mobilize energy from adipose tissue (Turner et al., 1976).

Machlin (1968, 1970) reported a negative relationship between growth hormone and blood glucose levels. Similar findings were reported by Siers and Trenkle (1973) and Davis et al., (1970). Eversole (1978) reported no relationship between frame size or diet and plasma growth hormone levels in steers. He did note more fluctuations in plasma growth hormone levels in steers fed high grain diets. Bassett et al., (1971) reported a negative correlation between organic matter and crude protein digested and plasma growth hormone of $r = -0.62$ and $r = -0.63$, respectively. Rabolli and Martin (1977) showed no effect of diet on serum growth hormone concentrations in rats.

Conflicting data on secretion patterns of growth hormone have been reported. Anfinson et al., (1975) and Trenkle (1977, 1978) have reported an episodic secretion pattern of growth hormone in cattle. The later worker reported frequent peaks in concentration during a 24 hr period.

An increase in environmental temperature or extreme cold temperature increased plasma growth hormone levels. Other investigators have reported different patterns of hormone release. Miller et al., (1970) reported a circadian release of growth hormone in a 24 hr. period but Dunn et al., (1973, 1974) demonstrated bimodal release over the same time period. Tannenbaum and Martin (1976) suggested an endogenous circadian rhythm not dependent upon feeding or serum glucose levels. Furthermore, they suggested light and dark periods probably act as a cue to the secretory rhythm but were not necessary to the basic circadian rhythm.

Insulin

Insulin is an important growth promoting hormone that increases the rate of protein synthesis and nitrogen balance (Manchester, 1970). Manchester (1959) suggested insulin increased the incorporation of amino acids into intracellular protein. The effect of insulin on protein synthesis is independent of amino acid transport into the cell (Manchester, 1959). Wool and Krah1 (1959) reported an increase in protein synthesis and amino acid uptake in the rat diaphragm in vitro with insulin. Goldstein and Reddy (1970) proposed insulin increased amino acid transport thereby increasing muscle protein synthesis. Later work by Manchester (1972) proposed that insulin has three principal effects that directly effect protein synthesis in skeletal muscle: 1) increases the ribosome to polysome ratio, 2) increase total number of ribosomes present, and 3) increase ribosome movement along the mRNA e.g. increase protein synthesis.

Numerous researchers have reported on the effect of diet on insulin

levels. Trenkle (1970) reported an increase in serum insulin as the cereal grain content of finishing diets increased in ruminants. Similar plasma insulin concentration in sheep increased as the energy density of the diet increased. (Trenkle, 1966). Siers and Trenkle (1973) and Davis et al., (1970) reported a positive correlation between serum glucose and insulin concentrations immediately after feeding. In addition, serum concentrations of insulin increase immediately after feeding in ruminants (Chase et al., 1977a; Machlin, 1968; Trenkle, 1978), in rats (Rabolli and Martin, 1977), and in swine (Grigsby et al., 1972). Chase et al., (1977a) suggested the insulin release after feeding is the result of a vagus nerve reflex and not in response to metabolite levels. However, Stern et al., (1971) and Feldman and Jackson (1974) reported increases in serum insulin concentrations with intravenous injection of glucose in ruminants.

Trenkle and Topel (1978) using steers reported a significant ($P < .01$) positive relationship between plasma insulin concentration and body size (live weight), age, carcass adipose and percent lipids in the M longissimus muscle. A significant ($P < .05$) negative relationship between plasma insulin concentration and carcass muscle and DNA concentration of the M longissimus muscle was also observed. Johns and Bergen (1976) also reported an increase in serum insulin concentration with age in sheep.

MATERIALS AND METHODS

Experimental Design

A 2 x 2 factorial design was employed in a 140 day feeding trial similar in design to those used in actual bull tests. The trial was conducted from mid-December, 1978, to mid-May, 1979. Bulls of different frame types were compared to evaluate the role of skeletal size and diet energy concentration on protein accretion, fat deposition, rate of gain, semitendinosus muscle proximate analysis, nucleic acids, serum growth hormone and serum insulin levels.

Sixty-six bull calves representing small and large frame types were used. The small frame type was represented by 33 straightbred Angus bulls originating from a commercial Angus herd in South Dakota. Larger frame type was represented by 33 3/4 Simmental bulls all originating from the same ranch also located in South Dakota. All bulls used in the trial were born within a 35 day period from early April to early May, 1978 as verified by records supplied by the two ranches.

All bulls were shipped to the Michigan State University Beef Cattle Research Center during late November, 1978. All bulls were in good condition upon arrival. Within 12 hours after arrival, the bulls were tattooed, ear tagged and vaccinated for pasteurella and IBR, PI₃ and BVD in a three-way vaccine. All bulls were injected with 2 million I.U.

of vitamin A and 150,000 I.U. of vitamin D. A pour-on insecticide for lice and grubs was used as needed. Bulls were allowed to roam in open dirt lots until the start of the trial. During this adaptation period the bulls were fed corn silage and a soybean meal-mineral supplement to provide 13% crude protein, 0.25% salt, 0.45% calcium, 0.34% phosphorus on a dry matter basis. The bulls showed no signs of sickness during the adaptation period. One bull was treated for lameness and recovered satisfactorily. All bulls were consuming expected amounts of dry matter based on live weight when the trial began.

The 66 bulls were stratified by weight within each type and then randomly allotted to one of two treatment groups resulting in nine bulls per pen (Appendix A.). Six bulls from each frame type were randomly allotted to an all silage treatment. These bulls represented the initial slaughter group. In order to facilitate the working schedule of the Meats Laboratory, these 12 bulls were not slaughtered until day 26 of the trial.

Average weight differences between pens was less than 45 kg. One-third of the bulls in each type were allotted to a high corn silage diet and remaining two-thirds to a high concentrate diet. All diets were supplemented with soybean meal, calcium, phosphorous and salt. The high corn silage diet contained 38% dry matter, 15% protein, 0.40% calcium, 0.31% phosphorous, 1.11% potassium and 0.25% salt. On a dry matter basis, the diet contained 16.6% soybean meal-mineral supplement, 74.2% corn silage and 9.2% high moisture corn. This provided 1.67 megacalories per kg for net energy maintenance and 1.08 megacalories per kg for net energy gain. The high concentrate diet contained 64.4%

dry matter, 15.0% crude protein. 0.50% calcium, 0.42% phosphorous, 0.82% potassium and 0.25% salt. On a dry matter basis the diet was composed of 14.4% soybean meal-mineral supplement, 9.4% corn silage and 76.2% high moisture corn. The diet provided 2.11 megacalories per kg for net energy maintenance and 1.39 megacalories per kg for net energy gain.

Feeding, Weighing and Management

Diets were mixed in a horizontal batch mixer and fed once daily. A sufficient amount was fed to just keep ahead of voluntary feed consumption with any unconsumed feed removed, weighed and recorded as necessary (approximately every 15 days). Feed records were maintained daily on each individual pen.

The bulls were weighed individually at the beginning of the trial and every 40 days thereafter until the completion of the trial. Bulls were kept off water for a 16 hr shrink prior to weighing and were not fed in the morning until after weighing. The bulls were housed in partially covered concrete slab pens, open to the south and bedded with straw. All bulls remaining after the second slaughter were treated for grubs and lice during March.

Slaughter of Animals

All bulls were slaughtered at the Michigan State University Meats Laboratory to facilitate removal of the semitendinosus (ST) muscle and the taking of carcass measurements. Bulls were trucked to the

laboratory the afternoon prior to slaughter. Three animals were randomly selected from each pen for the 40 day slaughter.

In order to accomodate the capacity of the Meats Laboratory, the 40 day slaughters were done in three groups over a seven day period. Animals were slaughtered as soon after a 40 day period as practical.

Carcass data, with the exception of hot carcass weight, was collected the day after slaughter. Estimates of marbling score, quality grade; kidney, heart and pelvic fat were made and actual measurements of rib eye area and rib fat at the eleventh rib were taken on each carcass. Hot carcass weight was measured on each side to the nearest one-half pound. Carcasses were tagged immediately after the hide was removed from the animal. Rib eyes were exposed for thirty minutes before the estimate of marbling was made.

Collection and Preparation of Samples

Blood Serum

Blood samples were taken on each bull prior to shipment to the Meats Laboratory. Blood was collected in two, 10 ml vacutainer tubes using 18 gauge needles, 2.5 cm in length. The bulls were haltered and secured in a squeeze chute during the collection of the samples from the jugular vein.

The blood was allowed to stand for 30 minutes at room temperature in the vacutainer then stored overnight in a cold room at 4 C. The clots were rimmed and the serum collected by centrifugation at 2,500 x g for 20 minutes. Serum was transferred by disposable pasteur pipettes

into clean test tubes, labeled and stored at -10 C until the hormone analysis was performed.

Semitendinosus Muscle

Immediately after exsanguination, the hide on the left hind quarter of the animal was peeled back and the semitendinosus muscle removed. After removal from the carcass, the muscle was trimmed of excessive connective tissue, fat and other muscle. The muscle was then weighed to the nearest gram and sliced into four to six sections approximately 2.5 cm in diameter. Three alternating sections were quick frozen in a solution of isopentane and dry ice in a styrofoam container. The samples were then transferred to plastic bags with tongs, labeled and stored in a walk-in freezer at -30 C.

The muscle samples were pulverized with dry ice in a Waring blender, manually mixed and stored in a Whirlpak plastic bag. The entire process took place in a walk-in freezer. The bags were allowed to remain open overnight to allow evaporation of the dry ice and then closed. The samples remained in the walk-in freezer until analyzed for fat, protein, nucleic acids and moisture.

Ninth, Tenth and Eleventh Rib Sections

After all carcass measurements were taken, the ninth through eleventh rib sections were removed intact from the carcass. The ribs were cut-off approximately 5.0 cm below the rib eye muscle. The sections were tagged, placed in plastic bags and stored at -30 C. At a later

date the sections were removed from the freezer, placed in a large stainless steel vat and allowed to thaw for five days in a walk-in cooler at 5 C. When thawed, the sections were separated into bone and soft tissue. The soft tissue was then ground in a Hobert grinder three times using a 0.47 cm plate. The sample was manually mixed and a 1 kg subsample taken and stored in a plastic Whirlpak bag and sealed. The bag was stored at -30 C until analyzed for fat, protein and water.

Proximate Analysis

Each powered sample of ST muscle and ground ninth through eleventh rib sample were analyzed for moisture, ether extract and crude protein (N x 6.25). Moisture content was determined by drying approximately 5 g of freshly thawed sample in a forced air oven at 85 C for 24 hrs. The dried sample was then cooled and weighed. Ether extract was determined using the dried samples obtained from the moisture determination. The Goldfish apparatus and procedure was employed in the ether extraction. Crude protein levels were determined using approximately 1 g of fresh sample with the Technicon Block Digestion Auto-Kjeldahl System using HgO as the digestion catalyst.

Nucleic Acid Analysis

A modified version of the Munro and Fleck (1966) method was used to determine the RNA and DNA content of the ST muscle. Two samples of fresh, powered muscle tissue were placed into two glass centrifuge tubes.

Five ml of cold 2.5% perchloric acid (PCA) was dispensed into each tube. The tubes were stoppered, vortexed and let stand on ice for 15 minutes, vortexed again and centrifuged at 34,800 x g for 15 minutes. The supernatant was decanted and discarded. The pellet in each tube was broken with a wooden applicator stick and 5 ml of 1.0% PCA added to each tube. All tubes were vortexed and centrifuged at 34,800 x g for 15 minutes.

After the second centrifugation, the supernatant was decanted, discarded, the pellet broken apart and 4 ml of 0.3 N potassium hydroxide were added to all tubes. The tubes were gently vortexed, a marble placed on top of each, and incubated for approximately 2.5 hr at 37 C in a water bath. The tubes were agitated several times during the incubation. After all the samples were digested, the tubes were removed from the water bath and placed on ice for 10 minutes. Five ml of cold 5.0% PCA were added to each tube, the tubes vortexed, stoppered and placed on ice for 20 minutes after which the tubes were again vortexed and centrifuged at 34,800 x g for 15 minutes. The supernatant was decanted into 25 ml graduated tubes and saved. The pellet was washed in 5.0% PCA, vortexed and centrifuged twice at 34,800 x g for 15 minutes each time the two pellet washings were added to the original supernatant. The collected supernatants were made up to 20 ml volume with 5.0% PCA. The remaining pellet was stored in 4.9 ml of 10% PCA. This was a convenient place for overnight storage. The RNA fraction in the 25 ml tubes and the remaining pellet were stored in a walk-in cooler at 4 C.

The DNA was extracted by incubating the pellet in 4.9 ml of 10% PCA, after vortexing, in a 70 C water bath for 25 minutes. A marble was placed on top of each tube during the incubation and the tubes were gently agitated near the beginning, middle and end of incubation and digestion. After digestion, the tubes were stoppered, vortexed and placed on ice. When cold, the samples in the tubes were centrifuged for 15 minutes at 34,800 x g. The supernatant containing DNA were decanted into 10 ml calibrated tubes. The pellet was washed with 4.9 ml of 10% PCA, vortexed and centrifuged at 34,800 x g for 15 minutes. The supernatant was combined with the original 10% PCA extracts in 10 ml calibrated tubes. The volume was brought up to 10 ml by adding 10% PCA. This represented the DNA fraction. The pellet was then discarded. PCA extracts containing RNA and DNA were stored on ice in a walk-in cooler until nucleic acid concentrations were determined.

A colorimetric procedure using orcinol (Mejbaum) was used to determine RNA levels. Two ml of the 20 ml supernatant were pipetted in duplicate into clean test tubes. A reagent blank of 2 ml 5.0% PCA in place of sample and RNA standards of 12.5, 25.0, 37.5, and 50.0 mg/ml for the standard curve were pipetted into test tubes in duplicate. Two ml of a 1.0% orcinol reagent were added to each tube and vortexed. A marble was placed on top of each tube and the tubes incubated in boiling water for 30 minutes. After boiling, the tubes were removed and cooled in cold running water. The optical densities on all tubes were determined at room temperature using a Gilford Spectrophotometer

at a 680 nm wavelength.

DNA concentration was colorimetrically determined using diphenylamine and acetaldehyde (Burton, 1956, 1968). Two ml of the 10% PCA extracts in the 10 ml tubes were pipetted in duplicate into clean test tubes. A reagent blank of 2 ml 10% PCA in lieu of sample and DNA standards of 12.5, 25.0, 37.5, and 50.0 mg/ml for the standard curve were pipetted into tubes in duplicate. Two ml of 4.0% diphenylamine in glacial acetic acid and 0.1 ml of acetaldehyde solution were added to the tubes and vortexed. Marbles were placed on each tube and the samples were incubated in a water bath at 30 C for 16 hours. Tubes were removed from the water bath and cooled to room temperature. A Gilford Spectrophotometer was used to determine the optical density on all tubes at a wavelength of 595 nm.

Growth Hormone Determination

Serum growth hormone concentrations were determined by using the double antibody radioimmunoassay technique (Purchas, 1970). Composition of all reagents used are shown in Appendix C. The assay employed sheep anti-guinea pig gamma globulin (SAGPGG) and a guinea pig antiovine growth hormone serum (GPABGH) to form an insoluble complex which is precipitated when centrifuged at 2,500 x g for 30 minutes.

Disposable 12 x 75 mm culture tubes were used for all samples and standards. The serum samples were thawed to room temperature immediately prior to use and maintained at 4 C until sampling was completed. All samples and standards were pipetted and diluted using an automatic pipette.

Standards were prepared from NIH-GH-B2 with 100 μ l of each standard containing 0.1, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 ng of growth hormone. One hundred or 150 μ l of serum sample were pipetted and dispensed simultaneously with 400 or 350 μ l of PBS-1% BSA into each tube. Four hundred μ l of PBS-1%-BSA were simultaneously added into each 100 μ l of growth hormone standard. Assay tubes 1 and 2 (the non-specific binding or background tubes) received 500 μ l of the above buffer and tubes 3 and 4 (total count tubes) contained only 100 μ l of I 125 growth hormone.

On the first day, 200 μ l of GPABGH diluted 1:3200 were added to each tube except background and total count tubes, gently vortexed and incubated at 4 C for 24 hr.

On day two, 100 μ l of I 125-GH containing 20,000 cpm were added to each tube, gently vortexed and incubated at 4 C for 24 hr.

On the third day, 200 μ l of SAGPGG were added to all tubes, except total count tubes, gently vortexed and incubated at 4 C for 72 hr.

At the end of the 72 hr incubation, 3 ml of 0.05 M phosphate-buffered saline (PBS) were added to all tubes, except total count, and centrifuged with a swinging bucket rotor at 2,500 x g. Supernatants were decanted and the tubes inverted on absorbant paper for 30 minutes. Excess liquid was wiped from the upper half of the tube and the precipitate was counted for four minutes or 4,000 counts whichever came first, in a Nuclear-Chicago Model 43-4230 Auto-Gamma Scintillation Counter.

Insulin Determination

A double antibody radioimmunoassay technique (Grigsgy, 1973) was

used to determine the serum insulin concentration. The equipment, materials and reagents used in the growth hormone determination were also used in the insulin determination.

Standards were prepared from highly purified bovine insulin with 100 μ l of each standard containing 0.1, 0.2, 0.3, 0.4, 0.6, 1.0, 1.2, 1.6, 2.0, 2.5, and 3.0 ng of insulin.

Either 150 or 250 μ l of serum sample were pipetted and dispensed simultaneously with 350 or 250 μ l of 0.05 M phosphate-buffered saline, 1% bovine serum albumin (PBS-BSA, pH 7.4) into each tube. Standards and serum samples were handled in the same manner as the preparation of the growth hormone assay.

On the first day, 200 μ l of GPABI diluted 1:105,000 in normal guinea pig serum (NGPS) were added to each tube, except background and total count tubes. Tubes 1 and 2 received 200 μ l of 1:400 HGPS. All tubes were gently vortexed and incubated for 24 hr at 4 C.

On day two, 100 μ l of I 125-insulin of approximately 15,000 cpm were added to all tubes, gently vortexed and incubated for 24 hr at 4 C.

On the third day, 200 μ l of SAGPGG were added to the tubes, except total count, vortexed gently and incubated for 96 hr at 4 C. At the end of the incubation, the same handling procedures and additions previously described for the growth hormone assay were used.

Statistical Methods

Least squares analysis of variance about the mean was used to determine the statistical significance of all the variables analyzed.

The statistical analysis was performed by the CDC 6500 computer in the Michigan State University Computer Laboratory.

The fat and protein composition of the empty body were computed using the following equations.

From the data collected from the rib samples, the following equations (Hankins and Howe, 1946) were used to estimate the carcass composition from the composition of the ninth, tenth and eleventh rib cut:

$$y = .66 + 5.98x$$

where: y = carcass protein (%) and
 x = rib cut protein (%).

$$y = .77 + 2.82x$$

where: y = carcass fat (%) and
 x = rib fat (%).

Empty body composition was calculated from carcass composition using the following equations (Garrett and Hinman, 1969):

$$y = .772x + 4.456$$

where: y = empty body protein (%) and
 x = carcass protein (%).

$$y = .9246x - .647$$

where: y = empty body fat (%) and
 x = carcass fat (%).

Empty body weight was determined by the equation developed by Garrett and Hinman (1969):

$$y = 1.362 + 30.30x$$

where: y = empty body weight and
 x = carcass weight (kg hot).

Initial slaughter group body composition was
determined by the following equation:

$$y = (\text{empty body weight})x$$

where: y = empty body protein in kg and
 x = empty body protein (%).

$$y = (\text{empty body weight})x$$

where: y = empty body fat in kg and
 x = empty body fat (%).

The rate of protein and fat gain was determined
using the following equation:

$$y = \frac{(\text{empty body protein}\%)x - (\text{initial group body protein})}{\text{days on feed}}$$

where: y = daily protein gain and
 x = empty body weight at slaughter.

$$y = \frac{(\text{empty body fat}\%)x - (\text{initial group body fat})}{\text{days on feed}}$$

where: y = daily fat gain and
 x = empty body weight at slaughter.

RESULTS

Feeding Trial Performance

Averaged across both frame types, high grain diet bulls gained faster than bulls on the high silage diet. Simmental bulls also gained live weight faster than Angus bulls when averaged across the two diets. Differences in daily gain were consistent with the differences in frame size (Table 1).

No significant differences in rate of gain were observed at 62 day or 99 day slaughter groups. However, in the 140 day slaughter group, Simmental bulls and bulls fed the high grain diet gained weight at a significantly ($P < .01$) faster rate. Simmental bulls and all bulls on the high grain diet did have significantly ($P < .01$) heavier live weights at 62 day, 99 day and 140 day groups when compared to Angus and all bulls fed the high silage diet, respectively. In addition, significant ($P < .15$ or better) interactions between breed and diet were observed in all slaughter groups. This resulted in Simmentals fed the high grain diets having heavier live weights than Angus bulls fed the high silage diet.

Slaughter weight (Table 1), carcass weight, rib eye area, rib fat; kidney, pelvic and heart fat percentage; and rib eye marbling scores all increased significantly ($P < .01$) with advancing age in all bulls (Table 2). When averaged across all four slaughter groups, Simmental

Table 1. THE EFFECT OF CATTLE TYPE AND DIET ON LIVE WEIGHT AND LIVE WEIGHT DAILY GAIN.^a

Period	Breed and Treatment ^b								EMSC
	AHG		AHS		SHG		SHS		
	Sl.Wt.	ADG	Sl.Wt.	ADG	Sl.Wt.	ADG	Sl.Wt.	ADG	
	kg								
Initial	278	0.96	274	1.02	270	0.94	281	0.91	0.041
62 Day	331	1.16	327	1.13	333	1.04	334	1.22	0.170
99 Day	393	1.42	389	1.40	385	1.50	396	1.41	0.074
140 Day	429	1.56	396	1.31	489	1.87	571	1.53	0.018
Mean	339	1.28	368	1.22	370	1.34	415	1.27	
EMS ^d	1206.8		1206.8		1206.8		1206.8		

Period	Breed and Treatment								EMS ^c
	Angus		Simmental		High Grain		High Silage		
	Sl.Wt.	ADG	Sl.Wt.	ADG	Sl.Wt.	ADG	Sl.Wt.	ADG	
Initial	276	0.73 ^e	276	0.36 ^f	278	0.54	374	0.56	0.041
62 Day	306	1.15	352	1.13	339	1.10	319	1.18	0.170
99 Day	370	1.41	412	1.46	416	1.46	336	1.41	0.074
140 Day	463	1.43 ^e	530	1.70 ^f	534 ^E	1.71 ^f	459 ^F	1.42 ^e	0.018
Mean	354 ^e	1.18	393 ^f	1.16	392 ^e	1.21	335 ^f	1.14	
EMS ^d	1206.8		1206.8		1206.8		1206.8		

^aLeast square means.^bAHG= Angus fed high grain diet, AHS= Angus fed high silage diet, SHG= Simmentals fed high grain diet, SHS= Simmentals fed high silage diet.^cError mean square for ADG for the period.^e^fMeans in rows with different subscripts differ significantly = P < .01, EF= P < .05.

bulls and all bulls on high grain diet had significantly ($P < .01$) heavier slaughter weights than Angus bulls and all bulls on the high silage diet, respectively. Bulls on high grain had significantly ($P < .05$) heavier slaughter weights with advancing age. Simmentals tended to have similar increases but the differences were not significant.

Changes in carcass weight followed trends similar to changes in slaughter weight. Simmental bulls fed the high grain diet had significantly ($P < .01$) heavier carcass weights than Angus bulls when averaged across all four slaughter groups, and especially in the terminal group ($P < .05$). Simmental bulls had increasingly heavier carcass weights with advancing age. The Angus bulls did have heavier carcass weights in the initial group.

Averaged across the four slaughter groups, all bulls on the high grain diet had significantly ($P < .01$) heavier carcass weights than all bulls on the high silage diet. All bulls on the high grain diet had increasingly heavier carcass weights with each succeeding slaughter group, and were significantly ($P < .05$) heavier in the terminal group.

Rib eye area tended to increase more in the Simmentals fed the high grain diet. The Angus bulls and high silage diet bulls had significantly ($P < .01$) smaller rib eye areas when averaged over the entire trial. In the 99 day group, Simmental bulls on the high grain diet had significantly ($P < .05$) larger rib eyes.

Angus bulls had increasingly more rib fat with increased time on trial ($P < .05$). Averaged across all slaughter groups, Angus bulls had significantly ($P < .01$) more rib fat. High grain diet bulls also tended to have more rib fat but the differences were not significant. Bulls

Table 2. THE EFFECT OF CATTLE TYPE AND DIET ON CARCASS WEIGHT, RIB EYE AREA, RIB FAT, KIDNEY, PELVIC AND HEART FAT PERCENTAGE, AND MARBLING SCORE.^a

Period	Angus High Silage						Angus High Grain						Simmental High Silage						Simmental High Grain						
	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR ^b	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR ^b	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR ^b	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR ^b	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR ^b
26 Day	160	55.3	.46	.6	31	152	50.5	.44	.7	124	149	54.8	.2	.4	-0-	152	56.6	.27	.6	31	152	56.6	.27	.6	31
62 Day	193	58.9	.50	.8	139	186	54.1	.49	.9	232	183	58.4	.25	.5	77	186	60.2	.31	.8	139	186	60.2	.31	.8	139
99 Day	244	74.0	.76	1.4	231 ^E	237	69.3	.74	1.5	324	234	73.6	.51	1.2	169 ^G	238	75.3	.57	1.4	213 ^E	238	75.3	.57	1.4	213 ^E
140 Day	257	72.1 ^E	1.04	1.8	271 ^E	301	77.6	1.27	2.0	326 ^F	301	81.7	.27	1.5	242 ^G	355	93.8 ^H	.58	1.9	266 ^E	355	93.8 ^H	.58	1.9	266 ^E
Mean	200	61.5	.70	1.2	168	217	63.4	.78	1.3	22	219	66.6	.26	.9	113	246	75.0	.42	1.2	166	246	75.0	.42	1.2	166

Period	Angus						Simmental						High Grain						High Silage						
	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR	Cr.Wt. Kg	REA cm ³	RF cm	KPH %	MAR
26 Day	156 ^E	52.9	.45	.7	76	151	55.7	.23	.5	-0-	152	53.6	.35	.7	78	154	55.1	.33	.5	-0-	154	55.1	.33	.5	-0-
62 Day	174	52.6	.48	.9	200	200	63.2	.29	.6	93	195	60.5	.51	.9	182	179	55.3	.26	.6	111	179	55.3	.26	.6	111
99 Day	224	69.6	.89	1.4	265	253	76.5	.40	1.4	212	250	77.2	.61	1.4	282	277	68.9	.68	1.3	195	277	68.9	.68	1.3	195
140 Day	279 ^E	74.8	1.15	1.9	298	328 ^F	87.8	.42	1.7	254	328 ^F	85.7	.92	1.9	296	279 ^E	76.9	.66	1.7	257	279 ^E	76.9	.66	1.7	257
Mean	209 ^e	62.5 ^E	.72 ^e	1.2	210 ^e	234 ^f	71.0 ^F	.33 ^f	1.1	142 ^f	232 ^f	69.3 ^e	.58	1.2	210 ^e	210 ^e	64.2 ^f	.46	1.0	142 ^f	210 ^e	64.2 ^f	.46	1.0	142 ^f
ENS ^C	583	56.7	.08	.21	4292	583	56.7	.08	.21	3292	583	56.7	.08	.21	4292	583	56.7	.08	.21	4292	583	56.7	.08	.21	4292

^aLeast Square Means

^bMarbling Scores: 0 = Devoid, 1-99 = Practically Devoid, 100-199 = Traces, 200-299 = Slight, 300-399 = Modest.

^cError Mean Square for column heading.

^eMeans in rows with the same column heading differing in subscripts differ significantly = $P < .01$, EFGH = $P < .05$.

in the initial group had the least ($P < .01$) external finish. Rib fat increased at 62 day ($P < .05$) and 140 day ($P < .01$) periods. Angus bulls tended to have more rib fat than Simmentals in each slaughter group, especially in the 140 day group ($P < .01$).

Kidney, pelvic and heart fat percentage (KPH) increased with age ($P < .05$). The initial group had the lowest KPH ($P < .01$). All bulls fed the high grain diet and Angus bulls tended to have a higher KPH in the 62 day and 99 day groups but differences were not significant. However, there were no differences in KPH ($P > .1$) between Angus and Simmentals in the 140 day group. There was no interaction between breed and diet ($P > .05$) nor between breed and time on feed ($P > .15$).

Rib eye marbling scores increased significantly ($P < .01$) with time on trial. Angus and all bulls on the high grain diet had significantly ($P < .01$) higher marbling scores when averaged across all four groups. Angus bulls fed the high grain diet tended to have higher marbling scores in each slaughter group, but differences were significant ($P < .05$) only in the initial slaughter group.

Abscess livers were observed in the 62 day, 99 day and 140 day groups. One Simmental on the high grain diet had a condemned liver in the 62 day group. In the 99 day group, two high grain diet Simmentals and two Angus fed the high grain diet had abscess livers. One Angus and one Simmental, both fed the high grain diet had condemned livers in the 140 day group.

Ninth, Tenth and Eleventh Rib Proximate Analysis

When averaged over the entire feeding trial, Angus and all bulls fed high grain diets had significantly ($P < .01$) higher rib dry matter percentages. In addition, dry matter percentages increased significantly ($P < .01$) with increased age. All bulls in the initial slaughter group had the lowest dry matter percentages ($P < .01$) and percent dry matter increased significantly in the 62 day ($P < .01$) and 140 day ($P < .01$) groups. No other significant interactions were observed.

Percent lipids also increased significantly ($P < .01$) over time. In addition, Angus and all bulls fed the high grain diet had significantly ($P < .01$) higher rib fat percentages when averaged over the entire trial. However, there were no interactions ($P > .05$) between breed and diet. Fat percentage was lowest in the initial group ($P < .01$) and highest in the terminal group ($P < .01$) when averaged across both breeds and both diets.

Rib section crude protein percent decreased significantly ($P < .01$) in both breeds and all bulls on both diets with advancing age. Additionally, a significant ($P < .05$) breed x diet x time interaction was observed resulting in the high silage diet Simmentals in the initial group having the highest crude protein percentage and Angus bulls on the high grain diet in the terminal group having the lowest percentage. When averaged across all slaughter groups, Simmental bulls had a significantly ($P < .01$) higher protein percentage than the Angus bulls and all bulls on the high silage diets had a significantly ($P < .05$) higher protein percentage when compared to all bulls fed the high grain diet. Protein percentages were lowest in the 140 day group ($P < .01$) and highest in the initial

Table 3. THE EFFECT OF CATTLE TYPE AND DIET ON NINTH, TENTH AND ELEVENTH RIB PROXIMATE ANALYSIS.^a

Period	Angus High Silage				Angus High Grain				Simmental High Silage				Simmental High Grain			
	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%
Initial	38.42	17.60	18.15	39.13	19.31	17.94	31.14	9.05	19.83	32.48	10.87	19.82				
62 Day	39.59	19.08	18.32	40.31	20.79	18.02	32.31	10.53	19.91	33.65	12.35	19.90				
99 Day	42.10	22.54	16.67	42.81	24.25	16.47	34.82	13.99	18.36	36.16	15.81	18.35				
140 Day	45.43	27.67	16.00	47.89	30.23	15.60	38.57	18.24	18.23	41.65	20.92	18.04				
Mean	40.13	20.62	17.33	43.15	24.42	16.59	33.60	12.18	19.50	37.19	16.09	18.95				
EMS ^b	10.03	15.19	1.06	10.03	15.19	1.06	10.03	15.19	1.06	10.03	15.19	1.06				

Period	Angus				Simmental				High Grain				High Silage			
	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%	FAT%	PRT.%	DM%
Initial	38.78	18.64	18.05	31.81	9.96	19.83	36.80	15.09	18.88	34.78	13.33	18.99				
62 Day	39.82	19.94	17.88	33.11	11.43	20.14	38.56	18.27	18.30	34.36	13.10	19.72				
99 Day	41.40	22.74	16.13	36.55	15.55	18.90	41.53	22.08	17.08	36.41	16.21	17.85				
140 Day	46.66	28.95	15.80	40.11	19.58	18.14	44.77	25.58	16.82	42.00	22.96	17.11				
Mean	41.62 ^c	22.48 ^c	16.96 ^c	35.37 ^d	14.09 ^d	19.24 ^d	36.85 ^d	16.34 ^d	18.43 ^c	40.15 ^c	20.23 ^c	17.78 ^d				
EMS ^b	10.03	15.19	1.06	10.03	15.19	1.06	10.03	15.19	1.06	10.03	15.19	1.06				

^aLeast Square Means

^bError Mean Square for Column Heading.

^{cd} Means in rows with the same column heading differ significantly = $P < .01$,
CD = $P < .05$.

group ($P < .01$). Rib cut proximate analysis results are listed in Table 3.

Carcass Fat and Protein

The carcass fat and protein composition was calculated using the data from the rib cut proximate analysis and the equations previously mentioned under Statistical Methods. The results are listed in Table 4.

Averaged across all slaughter groups, Simmental bulls and all bulls on the high silage diet had significantly ($P < .01$) less total carcass fat when compared to Angus and all bulls on the high grain diet, respectively. Total carcass fat increased ($P < .05$) with each succeeding slaughter group with the exception of the 99 day group where the increase was not highly significant. There was no interaction ($P > .1$) observed between breed and diet.

Total carcass protein increased significantly ($P < .01$) with age. Angus bulls had significantly ($P < .01$) more total protein in the initial group, but significantly ($P < .01$) less in the terminal group when compared to Simmental bulls. All bulls fed the high silage diet tended to have more protein in the initial group but all bulls on the high grain diet did have significantly ($P < .01$) more protein in the terminal group. A significant ($P < .05$ or better) interaction between breed and diet was observed in the 26 day and 140 day groups. This resulted in Angus bulls on the high silage diet having the most total protein in the initial group and Simmentals fed the high grain diet having the most total protein in the terminal group.

Table 4. THE EFFECT OF CATTLE TYPE AND DIET ON CARCASS
AND EMPTY BODY PROTEIN AND FAT COMPOSITION^a

Angus High Silage								Angus High Grain			
Period	Cr.Pt. %	Cr.Ft. %	E.Bd.Pt. %	E.Bd.Ft. %	Cr.Pt. %	Cr.Ft. %	E.Bd.Ft. %	Cr.Pt. %	E.Bd.Pt. %	Cr.Ft. %	E.Bd.Ft. %
Initial	17.96	16.37	18.41	14.49	17.82	17.69	18.31	17.82	17.69	18.31	15.71
62 Day	18.01	17.51	18.45	15.54	17.87	18.83	18.35	17.87	18.83	18.35	16.76
99 Day	16.98	20.18	17.66	18.01	16.85	21.49	17.55	16.85	21.49	17.55	19.22
140 Day	16.53	24.12	17.30	21.66	16.28	26.10	17.11	16.28	26.10	17.11	23.48
Mean	17.42	18.70	18.00	16.64	16.93	21.63	17.61	16.93	21.63	17.61	19.35
EMSb	.464	9.00	.280	7.70	.464	9.00	.280	.464	9.00	.280	7.70

Simmental High Silage								Simmental High Grain			
Period	Cr.Pt. %	Cr.Ft. %	E.Bd.Pt. %	E.Bd.Ft. %	Cr.Pt. %	Cr.Ft. %	E.Bd.Ft. %	Cr.Pt. %	E.Bd.Pt. %	Cr.Ft. %	E.Bd.Ft. %
Initial	19.07	9.97	19.28	8.40	19.06	11.19	19.27	19.06	11.19	19.27	9.70
62 Day	19.12	10.93	19.32	9.46	19.11	12.33	19.31	19.11	12.33	19.31	10.75
99 Day	18.10	13.59	18.52	11.92	18.09	14.99	18.51	18.09	14.99	18.51	13.22
140 Day	18.01	16.87	18.46	14.95	17.89	18.93	18.36	17.89	18.93	18.36	16.86
Mean	18.85	12.19	19.11	10.63	18.49	15.21	18.82	18.49	15.21	18.82	13.42
EMSb	.464	9.00	.280	7.70	.464	9.00	.280	.464	9.00	.280	7.70

Table 4. - Continued

Period	Angus				Simmental			
	Cr.Pt. %	Cr.Ft. %	E.Bd.Pt. %	E.Bd.Ft. %	Cr.Pt. %	Cr.Ft. %	E.Bd.Pt. %	E.Bd.Ft. %
Initial	17.89	17.03	18.36	15.10	19.07	10.49	19.27	9.05
62 Day	17.78	18.17	18.28	16.16	19.28	11.62	19.44	10.10
99 Day	16.62	20.33	17.38	18.15	18.38	14.80	18.74	13.03
140 Day	16.40	25.11	17.21	22.57	17.95	17.90	18.41	15.90
Mean	17.80 ^c	20.13 ^c	17.80 ^c	17.96 ^c	18.68 ^d	13.67 ^d	18.97 ^d	11.99 ^d
EMS ^b	.484	9.00	.280	7.70	.484	9.00	.280	7.70

Period	High Grain				High Silage			
	Cr.Pt. %	Cr.Ft. %	E.Bd.Pt. %	E.Bd.Ft. %	Cr.Pt. %	Cr.Ft. %	E.Bd.Pt. %	E.Bd.Ft. %
Initial	18.44	14.44	18.79	12.70	18.51	13.08	18.84	11.50
62 Day	18.06	16.88	18.49	14.97	19.00	12.90	19.22	11.28
99 Day	17.25	19.82	17.86	17.68	17.76	15.30	18.26	13.50
140 Day	17.08	22.51	17.73	20.17	17.27	20.50	17.88	18.30
Mean	17.71 ^c	18.40 ^e	18.22 ^c	16.38 ^e	18.14 ^d	15.40 ^f	18.55 ^d	13.59 ^f
EMS ^b	.464	9.00	.280	7.70	.464	9.00	.280	7.70

aLeast Square Means.

bHigh Grain Diet.

cHigh Silage Diet.

dError Mean Square for period.

eMeans in rows with the same column heading differ significantly =
P < .01, CD = < .05.

Empty Body Fat and Protein

The empty body fat and protein composition was calculated from the data obtained from the rib cut proximate analysis and using the series of equations previously mentioned under Statistical Methods. Results are listed in Table 4.

Empty body fat increased significantly ($P < .01$) with time on trial, with the exception of the 99 day group. No interaction between breed and diet ($P > .01$) was observed overall.

Total empty body protein increased ($P < .05$) with age. Averaged over all four slaughter groups, Angus and all bulls on the high silage diet had less total protein ($P < .01$) when compared to Simmental bulls and all bulls fed the high grain diets, respectively. In the initial group, bulls on the high silage diet and Angus bulls had the lowest total protein in the initial group ($P < .01$ and $P < .1$, respectively) but bulls on the high grain diet had the most total protein in the terminal group ($P < .01$ and $P < .05$, respectively).

Empty Body Fat and Protein Gain

Empty body fat and protein gain was calculated using the data from the proximate analysis of the rib cut and a series of equations listed under Statistical Methods. The results of empty body fat and protein gain are listed in Table 5.

Simmental bulls and all bulls on the high grain diet had significantly ($P < .01$) greater daily protein gain than Angus and all bulls

Table 5. THE EFFECT OF BREED AND DIET ON DAILY
EMPTY BODY PROTEIN AND FAT GAIN^a

Period	Angus Hgb			Angus HSC			Simmental HGB			Simmental HSC			EHSD ^d		
	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.
	----- Kg/day -----														
62 Day	.12	.30	.10	.08	.42	.44	.32	.09	.010	.042					
99 Day	.18 ^C	.37	.18 ^C	.21	.40 ^D	.57	.28 ^C	.21	.002	.022					
140 Day	.25	.57	.20	.37	.45	.55	.29	.40	.004	.013					
Mean	.18	.51	.16	.22	.42	.52	.30	.23	-	-					

Period	Angus			Simmental			High Grain			High Silage			EHSD		
	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.	Fat	Prt.
	----- Kg/day -----														
62 Day	.11 ^C	.11	.37 ^d	.27	.21	.37 ^C	.27	.01 ^d	.010	.042					
99 Day	.18 ^C	.30	.34 ^d	.39	.23 ^C	.47 ^C	.29 ^D	.21 ^d	.002	.022					
140 Day	.22 ^C	.47	.37 ^d	.48	.25 ^C	.56 ^C	.35 ^d	.39 ^d	.004	.013					
Mean	.17 ^C	.29	.36 ^d	.38	.23	.47 ^C	.30	.20 ^d	-	-					

^aLeast Square Means.^bHigh Grain Diet.^cHigh Silage Diet.^dError Mean Square for period.^{cd} Means in rows with the same column heading differ significantly =
P < .01, CD = P < .05.

on high silage diets, respectively. Simmental bulls, averaged across both diets, had greater ($P < .01$) daily protein gains in the 62 day, 99 day and 140 day groups. Bulls of both breeds on high grain diets had higher rates of protein gain in the 99 day ($P < .05$) and 140 day ($P < .01$) groups when compared to bulls of both breeds on high silage diets. An interaction ($P < .05$) between breed and diet was observed in the 99 day group resulting in Simmental bulls on the high grain diet having the highest rate of protein gain and Angus bulls on the high silage diet having the lowest daily protein gain. A similar trend was observed in the 140 day group, but the differences were not highly significant.

There were no significant differences in the daily fat gain between breeds in the 62, 99 and 140 day groups. All bulls fed high grain diets did have significantly ($P < .01$) greater fat gains in the 62, 99 and 140 day groups. No interaction between breed and diet were observed.

Semitendinosus Muscle Data

The semitendinosus muscle (ST) weights and proximate analysis results are listed in Table 6.

ST weights increased significantly ($P < .01$) with each succeeding slaughter group. Significant ($P < .01$) increases were observed with each group. Averaged across the four slaughter groups, Angus bulls had significantly ($P < .01$) lighter ST weights when compared to Simmentals. Angus bulls also had a lower percentage of ST weight to empty body weight. Although bulls on the high grain diet tended to have heavier ST weights over the four slaughter groups, differences were not significant.

Additionally, Simmentals on the high grain diet tended to have the heaviest ST weights, but differences were significant ($P < .01$) only in the final group.

Water composed about 75% of the ST on a fresh tissue basis. Overall dry matter percentages were highest in bulls fed the high grain diet ($P < .01$) and Angus bulls ($P < .01$) when compared to bulls on high silage diets and Simmental bulls, respectively. Dry matter percentages increased significantly ($P < .01$) from 26 day to 140 day groups. In the 140 day group, all bulls on the high grain diet had significantly ($P < .05$) higher dry matter percentages than all bulls fed high silage.

The fat content of the ST increased with time ($P < .05$) resulting in terminal group bulls having the highest fat percentage ($P < .01$). However, differences between other slaughter groups were not significant. Differences tended to become greater with each successive group, but did not reach significant levels. When averaged across all four slaughter groups, Angus bulls and all bulls fed the high grain diet did have higher ST fat percentages ($P < .01$).

Simmental bulls and all bulls on the high grain diet had greater ST crude protein percentages ($P < .01$) for the entire trial. Percentages tended to increase with each progressing slaughter group, but the differences were not significant. A breed x diet x time interaction favored Simmentals on high grain diets with a higher protein percentage, but the differences were not significant.

Total ST protein increased significantly ($P < .01$) during the trial. Simmentals had the most total protein ($P < .01$) for the entire trial. Bulls on the high grain diet tended to have more total protein when compared

Table 6. THE EFFECT OF CATTLE TYPE AND DIET ON SEMITENDINOSUS
MUSCLE WEIGHT AND PROXIMATE ANALYSIS.^a

Period	Angus High Silage					Angus High Grain				
	Wt. g	DM%	Fat%	Prt.%	Wt. g	DM%	Fat%	Prt.%		
Initial	1272	24.12	2.11	20.58	1120	24.51	2.36	20.67		
62 Day	1609	24.54	2.38	20.90	1457	24.84	2.62	20.99		
99 Day	1981	24.81	2.42	20.69	1829	25.11	2.66	20.73		
140 Day	1972	25.04	2.96	20.65	1956	27.23	4.65	20.91		
Mean	15.81	24.31	2.25	1.33	1545	25.68	3.23	21.01		
EMS ^b	51911.15	0.89	1.03	0.26	51911.15	0.89	1.03	0.26		

Period	Simmental High Silage					Simmental High Grain				
	Wt. g	DM%	Fat%	Prt.%	Wt. g	DM%	Fat%	Prt.%		
Initial	1203	23.83	1.64	21.17	1266	23.55	1.34	1.58		
62 Day	1540	24.16	1.91	21.50	1604	23.89	1.61	21.66		
99 Day	1912	24.43	1.95	21.29	1975	24.15	1.65	21.46		
140 Day	2660	23.94	1.50	21.60	2860	25.56	2.64	21.94		
Mean	1875	23.84	1.59	21.22	2054	24.63	2.02	21.73		
EMS ^b	51911.15	0.89	1.03	0.26	51911.15	0.89	1.03	0.26		

Table 6. - Continued.

Period	Angus					Simmental				
	Wt. g	DM%	Fat%	Prt.%	Wt. g	DM%	Fat%	Prt.%		
Initial	1196 ^c	24.36	2.23	20.62	1235 ^d	23.70	1.49	21.26		
62 Day	1363	24.71	2.59	20.95	1743	24.00	1.67	21.58		
99 Day	1728	24.76	2.35	20.82	2120	24.50	1.99	21.29		
140 Day	1964	26.13	3.80	20.78	2760	24.75	2.07	21.77		
Mean	1564 ^c	24.98 ^c	2.74 ^c	20.78 ^c	1970 ^d	24.22 ^d	1.78 ^d	21.47 ^d		
EMS ^b	51911.15	0.89	1.03	0.26	51911.15	0.89	1.03	0.26		

Period	High Grain					High Silage				
	Wt. g	DM%	Fat%	Prt.%	Wt. g	DM%	Fat%	Prt.%		
Initial	1193	24.03	1.85	21.00	1238	24.02	1.88	20.88		
62 Day	1640	24.87 ^C	2.50	21.58	1466	23.84 ^D	1.76	20.94		
99 Day	1957	25.32	2.52	21.47	1891	23.93	1.82	20.64		
140 Day	2408	26.39	3.65	21.43	2316	24.49	2.23	21.12		
Mean	1802	25.15 ^e	2.62 ^c	21.37 ^d	1732	24.06 ^f	1.91 ^d	20.89 ^c		
EMS ^b	51911.15	0.89	1.03	0.26	51911.15	0.89	1.03	0.26		

^aLeast Square Means.^bError Mean Square for column heading.^{cdef} Means in rows with the same column heading differ significantly
P < .01, CDEF = P < .05.

to bulls fed the high silage diet but differences were not significant.

The Angus bulls did have more total protein in the initial group ($P < .01$), but the Simmentals had significantly ($P < .01$) more in the 140 day group.

Nucleic Acids

The results of nucleic acid determination of the ST muscle are reported in Table 7. Averaged across all slaughter groups, all bulls on the high grain diet had a significantly ($P < .05$) higher concentration of RNA per unit of fresh ST muscle tissue when compared to the bulls fed the high silage diet. Angus bulls also tended to have a higher concentration of RNA when compared to Simmentals, but differences were not significant. Overall, the RNA concentration of the ST decreased significantly ($P < .01$) as the time on trial increased, especially in the Angus and all bulls fed the high silage diet ($P < .05$). Additionally, the bulls in the initial group had the highest concentration of RNA and the bulls in the terminal group had the lowest ($P < .01$) when averaged across both breeds and both diets. As a result, Angus bulls fed the high silage diet tended to have a lower concentration as time progressed when compared to Simmentals fed the high grain diet, but the interaction was not highly significant. In the terminal group, the RNA concentration in Angus bulls was significantly ($P < .01$) higher than in the Simmentals.

Differences in RNA concentration due to diet were observed in three of the groups. All bulls fed high silage tended to have a lower RNA

Table 7. THE EFFECT OF BREED AND DIET ON SEMITENDINOSUS
MUSCLE NUCLEIC ACIDS AND PROTEIN.^a

Angus High Grain						Angus High Silage				
Period	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA
Initial	1704	352	4.8	232	0.67	2016	321	6.2	264	0.81
62 Day	1807	493	3.7	308	0.66	2120	462	4.6	340	0.80
99 Day	2414	586	4.1	383	0.67	2726	555	4.9	415	0.81
140 Day	3058	611	5.0	410	0.68	2227	512	4.3	406	0.82
Mean	2308	502	4.6	325	0.67	2029	444	4.6	325	0.75
EMS ^b	33157	12639	1.39	2529	.018	33157	12639	1.39	2529	.018

Simmental High Grain						Simmental High Silage				
Period	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA
Initial	1983	485	4.1	270	0.54	2024	444	4.6	253	0.60
62 Day	2086	626	3.3	346	0.53	2127	585	3.6	329	0.59
99 Day	2693	719	3.7	421	0.54	2734	678	4.0	404	0.60
140 Day	3101	800	3.9	629	0.79	1999	690	2.9	575	0.84
Mean	2466	676	3.6	448	0.66	2158	608	3.5	398	0.67
EMS ^b	33157	12639	1.39	2529	.018	33157	12639	1.39	2529	.018

Table 7. - Continued.

Angus						Simmental				
Period	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA
Initial	2003	465	4.3	262 ^c	0.57	1860	337	5.5	248 ^d	0.74
62 Day	1890	454	4.2	286	0.67	2180	629	3.5	376	0.63
99 Day	2281	539	4.2	359	0.68	3002	730	4.1	452	0.68
140 Day	2643	561	4.7	408 ^c	0.75	2550	745	3.4	602 ^d	0.82
Mean	2169	473 ^c	4.6 ^c	325 ^c	0.71	2434	642 ^d	3.8 ^d	423 ^d	0.66
EMS ^b	33157	12639	1.39	2529	.018	33157	12639	1.39	2529	.018

High Grain						High Silage				
Period	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA	RNA mg/ST	DNA mg/ST	RNA:DNA	Prt. g	Prt:DNA
Initial	1843	419	4.4 ^c	251	0.61	2020	383	5.3 ^d	259	0.70
62 Day	2461 ^c	605	4.1	354	0.61	1609 ^d	478	3.4	308	0.68
99 Day	2650	625	4.2	421	0.69	2633	644	4.1	391	0.63
140 Day	3080 ^c	705	4.4	520	0.74	2113 ^d	601	3.5	490	0.83
Mean	2509 ^c	589 ^c	4.3	386	0.66	2094 ^d	526 ^d	4.0	362	0.71
EMS ^b	33157	12639	1.39	2529	.018	33157	12639	1.39	2529	.018

^aLeast Square Means^bError Mean Square for column heading.^{c,d} Means in rows with the same column heading differ significantly = $P < .01$, $CD = P < .05$.

concentration at 62 day, and 99 day periods when compared to all bulls on high grain in the same slaughter group. In the 140 day group a significant ($P < .05$) interaction between breed and diet resulted in high grain diets fed to Angus bulls having a higher concentration of RNA than the Simmentals fed high silage.

Overall, the total ST RNA increased significantly ($P < .05$) with each succeeding slaughter group. Averaged across the four groups, Angus bulls and all bulls on high silage diets had less total RNA ($P < .1$ and $P < .01$, respectively) when compared to their counterpart Simmentals and all bulls fed the high grain diet. In addition, the Angus and all bulls fed high silage diets in the terminal slaughter group had significantly ($P < .05$) less total DNA than the Simmental bulls and bulls of both breeds on the high grain diet, respectively.

Differences in DNA per unit of ST were not as varied as those for RNA. The DNA concentration decreased significantly ($P < .05$) with time when averaged over the entire trial. Averaged across both breeds and both diets, DNA concentration tended to be highest at the 62 day period and the lowest ($P < .01$) in the 140 day group. Differences between breeds and diets were not significant.

Total DNA levels were lowest in Angus ($P < .01$) and bulls of both breeds fed high silage diets ($P < .05$) when compared to Simmentals and all bulls fed the high grain diet, respectively, for the entire trial. When slaughter groups were compared, DNA was lowest in the initial group ($P < .01$) and increased significantly at 99 day ($P < .01$) and 140 day ($P < .01$) intervals. In addition, there was no difference in total DNA between breeds in the 62 day group ($P > .02$). There was no interaction

($P > .15$) between breed and diet when four slaughter groups were averaged, nor in the terminal group when compared to the other groups ($P > .15$).

When averaged over all four groups, the Angus bulls had significantly ($P < .01$) higher RNA to DNA ratios than the Simmental bulls. Bulls in the initial group had a significantly ($P < .05$) higher ratio when compared to bulls in the other three groups. In addition, bulls fed high silage in the initial group had a significantly ($P < .05$) higher ratio when compared to bulls on the high grain diet in the same group.

Protein to DNA ratios were significantly ($P < .01$) higher in the 140 day group. In this terminal group, Angus bulls had a lower ratio compared to the Simmentals. Ratio differences between breeds were not different ($P > .1$) in the 62 day and 99 day groups. No other differences or trends were observed.

Growth Hormone

Serum growth hormone levels did not differ ($P > .1$) between diets when averaged across all slaughter groups, especially at 62 day ($P > .05$) and 140 day ($P > .05$) groups. Time on feed had no effect ($P > .1$) on hormone levels. There was no interaction ($P > .01$) between breed, diet and time in the 140 day group. Results of the growth hormone assay are listed in Appendix B.

Insulin

Bulls of both breeds fed the high grain diet had significantly

($P < .01$) more serum insulin than bulls fed the high silage diet across all slaughter groups. There was no difference ($P > .05$) in serum insulin levels between breeds in the 99 day group. There also was no interaction ($P > .05$) between time on feed, breed and diet in the terminal group. The insulin assay findings are listed in Appendix B.

DISCUSSION

Feeding Trial Performance

The increases in live weight daily gain with increased dietary energy and/or frame size increases reported in this study are in agreement with the results previously reported by Crickenberger (1977), Eversole (1978), Harpster (1978) Stonaker et al., (1952), and Willey et al., (1951). The results substantiate the ideas and projections of the model developed by Fox and Black (1977), which predicts larger framed cattle will grow increasingly faster as frame size increases. The larger framed Simmentals grew at a faster rate from day 26 to the termination of the trial. The faster growth rate of the Angus bulls during the first 26 days of the trial was probably the result of compensatory gains.

Diet also accounted for differences in rate of gain. Bulls fed the high grain diet gained significantly ($P < .01$) faster than bulls on the high silage diet when taken the full 140 days of the trial. This agrees with the work of Geuns and Hawkins (1978) who reported faster growth rates in Polled Hereford bulls fed a high grain diet than high silage diets. The results also concur with the previous work involving steers by Eversole (1978), Harpster (1978), and Crickenberger (1977) among others.

The data would indicate that the Simmental bulls required a higher

diet energy density to achieve their maximum genetic potential for rate of gain when dietary crude protein levels are adequate. Since both the high grain and high silage diets contained the same crude protein percentage which was assumed to be in excess of amounts normally needed by young bulls, the differences in rate of gain for the Simmentals probably is the result of the increased energy content. Furthermore, since bulls were randomly allotted to treatments (diets) and the Simmentals were from the same herd, genetic differences between diets should have been minimal. It can also be concluded from the results that differences in genetic potential can also be determined using high silage diets. While the differences in rate of gain would probably be significant, the average differences will not be as great as would be achieved using higher concentrate levels in the diet.

Angus bulls were better suited to the high silage diet since the increases in gain from high grain were not significantly greater within the breed. This agrees with Byers and Parker (1979a) who suggested forage feeding as being more appropriate for smaller framed cattle and high grain diets as best suited for larger framed cattle.

The lack of significant differences in performance at the 62 day period may be the result of extremely cold environmental temperatures and heavy snow cover in the open part of the pens, resulting in more feed being required for metabolism and heat production and less available for gain. The rate of gain during this period (day 26 to day 62) was less than what might normally be expected of young bulls of that age and weight.

Carcass Traits

The data presented in this study concurs with the previous findings of Bond et al., (1972), Garrigus et al., (1967), Johnson et al., (1967) and Schemmel et al., (1970) who reported increased carcass fat in steers fed high energy diets. The increases in rib eye area and rib fat are in agreement with work done by Crickenberger (1977) and Harpster (1978) using steers and Geuns and Hawkins (1978) with bulls. The increases in marbling scores with high energy diets supports work done by Leander et al., (1978), Oltjen et al., (1971), Richards et al., (1961) and Utley et al., (1975) all using steers.

The differences in carcass traits between frame types found in this study are inconcurrence with previous work by Crickenberger (1977), Harpster (1978) and Woody et al., (1978) who reported lower marbling scores and quality grades, larger rib eye areas and less kidney, pelvic and heart percentage in large frame steers fed for equal length of time with small frame steers.

Empty Body Composition and Empty Body

Daily Fat and Protein Gain

Little work has been done on the composition of gain and empty body composition of young growing bulls. The increases in carcass and empty body fat with high grain diets were reported by Bond et al., (1972), Garrigus et al., (1967), (1970) in the rat. The results of this study supports those previous findings. In addition, the increase in total

protein reported in this study with high grain diets is in agreement with the work of Byers and Parker (1979b) but disagrees with Ferrell et al., (1978) who found no increase in carcass protein with high energy diets.

The results show that larger framed bulls have a higher rate of protein gain and slower rates of fat gained when compared to smaller framed bulls. The increase in protein gains with increased frame size is in agreement with the work of Byers (1979a). The larger framed Simmentals also had a higher protein percentage and lower fat percentage in both the carcass and empty body.

Energy density of the diet also had a dramatic effect on fat and protein gains. The increases in daily fat and protein gains on high grain diets across both breeds agrees with the findings of Byers and Parker (1977b), Crickenberger (1977) and Newland (1979).

It can be concluded that the larger framed Simmental bulls did not reach the upper limit of protein accretion at one year of age since the rate of protein gain did not decrease significantly from beginning to end of the trial. The decrease in protein daily gain in Angus bulls indicates they were near the peak of their growth curve at one year of age. In addition, the lower protein gains on high silage diets, especially in the Simmentals, was the result of lower energy intakes when compared to bulls on the high grain diet. As a result, they could not attain their full genetic potential for protein accretion as described by Bergen (1974) because of insufficient dietary energy.

The results of this study would indicate that larger framed bulls have the ability to use the high energy in high grain diets for protein accretion with a minimum of fat deposition when crude protein levels are

adequate. On the contrary, smaller framed bulls cannot use the energy of high grain diets for protein synthesis. As a result, a significant proportion of the energy is deposited as fat. This concurs with the conclusions of Byers and Parker (1979a) who suggested high forage diets as being best suited for smaller framed cattle and high grain diets as best suited for larger framed cattle.

Semitendinosus Muscle Data

The increases in semitendinosus muscle (ST) weights of bulls from both breeds and both diets with advancing age can be defined as true growth, the increase in muscle, bone and organs as defined by Maynard and Loosli (1969). The heavier ST weights in the larger framed Simmentals is the result of greater muscle mass both totally and within individual muscles in the larger framed cattle. This conclusion concurs with previous work with steers by Eversole (1978). The lack of significant differences between diets differs from the findings of Eversole (1978) who reported significantly lighter ST weights in steers fed high grain diets. It should be noted however, the Eversole (1978) study was longer, involved steers and used animals which were older than the bulls used in this study.

Results of this study indicate bulls fed high grain diets tend to have heavier ST weights. This may be the result of bulls having a greater propensity to convert feed into lean muscle as opposed to steers. Furthermore, the interaction ($P < .076$) between frame type and diet resulting

in heavier ST weights for the Simmentals fed high grain diets and lighter weights for Angus bulls fed the high silage diet indicates larger framed bulls can effectively use higher dietary energy densities and convert the additional energy into lean muscle. It can therefore be concluded that dietary energy density and animal frame size determine the extent of muscle mass in bulls.

The decrease in percent water in the ST and increased percentage of protein and lipid with advancing age is in agreement with the previous work of Eversole (1978), Giovannetti and Strothers (1975), Harpster (1978), LaFlamme et al., (1973) and Sink and Judge (1971). The decrease in percent water is the result of increased protein and lipid in the muscle which displaces moisture.

The ST fat content on a percent of muscle weight basis was greater in the bulls fed the high grain diet compared to bulls on the high silage diet. This supports the earlier findings of Eversole (1978), Harpster (1978) and Leander et al., (1978) with steers. The workers all reported decreased moisture and increased ether extract with high grain diets when compared to high silage diets.

The differences in ST lipid content between breeds maybe the result of the Simmentals having the ability to use the extra energy of the high grain diets in the high priority tissues like muscle instead of going to depot adipose cells, and eventually to intramuscular adipose.

In contrast to the findings of Eversole (1978) and Leander et al., (1978), crude protein percentage of the ST did differ significantly between diets with all bulls fed the high silage diet and Angus bulls having a

lower percent. Bulls in the terminal group had a higher protein percentage than bulls in the initial group. In comparison to steers, bulls may have a greater capacity to use high grain diets for muscle and protein production. There maybe an interaction between breed, diet and time ($P < .2$) resulting in Simmentals fed the high grain diet having the highest percentage as time increases. The significance of this interaction may be determined by using larger numbers.

Nucleic Acids

The DNA concentration of a muscle is one of the most widely used and best available indicators of muscle cell number. However, the use of DNA concentrations as an indicator of muscle cell numbers remains suspect since muscle cells are multinucleated (Bergen et al., 1975). Robison (1971) and Cheek et al., (1971) have suggested the protein to DNA ratio is a good indicator of cell size, and hence number.

Muscle RNA and DNA concentrations decreased with age. This is in agreement with the work of Harbison et al., (1976), Powell and Aberle (1975), Robison (1959), and Tsai et al., (1973) using swine, Eversole (1978) and LaFlamme et al., (1973) in cattle, and Johns and Bergen (1976) in sheep. The increased RNA concentration from day 62 to day 99 in the Simmentals was probably the result of inadequate numbers to give good statistical analysis. In addition, the feed intake of two of the Simmentals on the high silage diet was decreased due to respiratory infection. Lower protein synthesis and less hypertrophy may have resulted in RNA and DNA concentration increases from day 62 to day 99 slaughter

groups.

The lower RNA to DNA ratio in the 62 day group in comparison to the 26 day or 99 day groups ratios may have been the result of cold environmental temperatures causing less protein synthesis in favor of metabolism for heat, or the previously mentioned decrease in feed intake. Since RNA levels are a good estimator of protein synthesizing machinery (Wanamacher, 1972) high RNA to DNA ratios are a good indicator of high protein synthesis capacity (Munro, 1967; Powell and Aberle, 1975; Winick and Nobel, 1965).

Averaged across the two frame types, bulls fed the high grain diet had a higher concentration of RNA and a higher RNA to DNA ratio. This is in contrast to the data reported by Eversole (1978) using steers. Since dietary protein was presumed to be not limiting in this study, dietary protein should not have been an influencing factor. Decreases in protein synthesis with diets low in protein have been observed by Gilbreath and Trout (1973), Johns (1974), Trenkle (1974) and Young et al., (1971) who showed a decreased RNA content with decreased dietary protein.

The bulls on the high grain diet in this study were able to utilize the high protein and energy levels to synthesize more muscle protein. This was especially true in the Simmentals.

Growth Hormone

Results from this study show no effect of diet on serum growth hormone concentrations. This agrees with the findings of McAtee and Trenkle (1971), Rabolli and Martin (1977) and Trenkle (1971b). As was

previously reported by Dev and Lasley (1969), Grigsby, (1973) and Trenkle (1971a), there was no consistent relationship between growth hormone concentration and breed.

The single sampling of blood prior to slaughter yielded little significant information on growth hormone and its relationship to growth and development in young growing bulls. The importance of multiple samples during a 24 hr period to assure repeatability is obvious because of diurnal variation, episodic surges and different environmental conditions from one slaughter group to the next (Trenkle, 1978).

Insulin

The increased concentration of serum insulin with high grain diets was the result of the increased amount of glucose, other carbohydrates and volatile fatty acids being absorbed from the digestive tract when compared to bulls fed the high silage diets (Trenkle, 1966, 1970).

The lack of other significant differences may be the result of taking only one sample prior to slaughter. In addition, since feed was available to the bulls at all times, there may have been variation between bulls in the same pen since insulin is released immediately after the consumption of feed (Chase et al., 1977a; Machlin, 1968; Trenkle, 1978).

CONCLUSIONS

1. Larger framed bulls have a higher rate of live weight gain than smaller framed bulls when fed the same diet.
2. Bulls fed high grain diets have a higher daily live weight gain than bulls fed high silage diets when comparing bulls of the same frame type.
3. Larger framed bulls fed high grain diets have the heaviest live weights and small frame size bulls fed high silage diets have the lightest weights.
4. Larger frame bulls fed high grain diets gain weight faster than all bulls on high silage diets and large frame bulls on high silage diets.
5. Large frame bulls have leaner, more muscular carcasses than small frame bulls.
6. Bulls fed high grain diets have more carcass fat than bulls fed high silage diets.
7. Rib cut moisture percentage decreases with advancing time on feed.
8. Rib cut fat percentage is higher in bulls fed high grain diets and small frame size bulls.
9. Rib cut protein percent is higher in larger frame bulls and all bulls fed high silage diets.
10. Daily protein gains are greater in large frame bulls and in all bulls fed high grain diets.
11. Daily protein gain is highest in larger frame bulls fed high grain

diets and lowest in small frame bulls fed high silage diets.

12. Small frame size bulls have a higher rate of daily fat gain than larger frame bulls.
13. Bulls fed high grain diets have higher daily fat gains than bulls fed high silage diets.
14. Large frame bulls do not reach the upper limit of protein accretion at one year of age, but small frame bulls appear to be near the peak of their growth curve at a year.
15. Large frame bulls can use high dietary energy for protein synthesis as opposed to fat deposition in small frame bulls.
16. High grain diets are most appropriate for large frame bulls and high silage is best suited for small frame bulls.
17. High grain diets are necessary to achieve maximum genetic potential in large frame bulls.
18. Differences in genetic potential for growth rate can be achieved with high silage diets, but differences are less than those achieved with high grain diets.
19. Large frame bulls have heavier ST weights.
20. ST fat content is higher in all bulls fed high grain and the small frame bulls.
21. ST protein content is higher in large frame bulls and bulls fed high grain diets.
22. Large frame bulls have greater protein synthesis capacity in the ST.
23. Serum insulin concentrations increase with high grain.

APPENDIX A
TREATMENTS AND ANIMALS

Table A. 1 Experimental Design

Breed	Initial Slaughter (all silage diet)	Diet		Total
		High Silage	High Grain	
Angus	6	9	18	33
Simmental	6	9	18	33
	—	—	—	—
Total	12	18	36	66

APPENDIX B

GROWTH HORMONE AND INSULIN CONCENTRATIONS

Table B.1. THE EFFECT OF CATTLE TYPE AND DIET ON SERUM GROWTH HORMONE AND INSULIN CONCENTRATIONS.^a

Period	Angus High Grain		Angus High Silage		Simm. High Grain		Simm. High Silage	
	GH	Insulin	GH	Insulin	GH	Insulin	GH	Insulin
	-----		-----		-----		-----	
	ng/ml		ng/ml		ng/ml		ng/ml	
Initial	6.93	4.76	4.13	1.40	8.91	6.72	6.11	2.04
62 Day	8.27	5.44	5.47	2.08	10.25	7.40	7.45	2.73
99 Day	9.73	5.65	6.93	2.29	11.71	7.62	8.91	2.94
140 Day	4.01	3.78	6.80	2.27	9.07	3.23	11.86	0.40
Mean	7.24	5.00	5.83	2.19	9.99	6.06	8.58	1.93
EMS ^b	46.42	6.84	46.42	6.84	46.42	6.84	46.42	6.84

Period	Angus		Simmental		High Grain		High Silage	
	GH	Insulin	GH	Insulin	GH	Insulin	GH	Insulin
	-----		-----		-----		-----	
	ng/ml		ng/ml		ng/ml		ng/ml	
Initial	5.53	3.08	7.54	4.38	7.92	5.74	5.12	1.72
62 Day	11.10	4.25	4.62	4.58	9.12	5.54	6.61	3.28
99 Day	10.89	4.04	7.75	5.21	7.71	7.33	10.92	1.92
140 Day	5.40	3.03	10.46	1.82	6.54	3.51	9.33	1.33
Mean	8.23	3.60	7.59	4.00	7.82	5.53	8.00	1.33
EMS ^b	46.42	6.84	46.42	6.84	46.42	6.84	46.42	6.84

^aLeast Square Means^bError means Square for Column Heading

cd

Means in rows with the same column heading differ significantly = $P < .01$, $CD = P < .05$.

APPENDIX C
COMPOSITION OF REAGENTS

Table C.1 Orcinol Reagent

Make a stock solution of 0.1% $\text{FeCl}_2 \cdot 6 \text{H}_2\text{O}$ in concentrated HCl. Before each use, dissolve 1 g orcinol in 100 ml of stock solution.

Table C.2 Diphenylamine Reagent

Dissolve 4 g diphenylamine reagent in 100 ml of glacial acetic acid.

Table C.3 Acetaldehyde Solution

Add .4 ml of acetaldehyde to a 250 ml volumetric flask. Dilute with deionized distilled water and bring volume to 250 ml. Store at 4°C.

Table C. 4 Composition of Reagents for Radioimmunoassays

A. Buffer A ₁	
NaH ₂ PO ₄ · 2 H ₂ O	6.2 g
Merthilate (thimersal)	0.25 g
Bovine Serum Albumin (BSA; Fraction V, Sterile, 35% solution serological, NBC, Cleveland, Ohio)	14.6 ml
Deionized distilled water	950 ml
Adjust pH to 7.5 with 5 N NaOH.	
Bring final volume to 1 liter.	
Store at 4°C (up to 3 months).	
B. 0.05 M PBS-1% BSA, pH 7.4	
NaCl	9.0 g
Dissolve with 1 liter of Buffer A ₁ .	
Store at 4°C (up to 3 months).	
C. Guinea Pig Anti-Bovine Insulin (GPABI) and Guinea Pig Anti-Bovine Growth Hormone (GPABGH; hereafter referred to as antibody 1.	
Antisera diluted 1:400 with 0.05 M PBS-EDTA, pH 7.0.	
On day of use, dilute 1:400 antisera to required concentration using 1:400 NGPS as diluent.	
D. 0.05 M Disodium Ethylenediamine Tetraacetate (EDTA)-PBS, pH 7.0	
Disodium EDTA	18.612 g
Add 0.01 M PBS	950 ml
Adjust pH to 7.0 with 5 N NaOH.	
Dilute to 1 liter.	
Store at 4°C.	
E. 0.01 M Phosphate Buffered Saline (PBS), pH 7.0	
NaCl	143 g
Monobasic phosphate	120 ml
Dibasic phosphate	240 ml
Merthiolate (Thimersal)	1.75 g
Dissolve in deionized distilled water and transfer to large container.	
Dilute to 17.5 liters with deionized distilled water.	
Adjust pH to 7.0 with NaOH if necessary.	
Store at 4°C.	

Table C.4 - Continued

F. Phosphate Buffered Saline-1% Bovine Serum Albumin (PSB-1% BSA).

BSA (Fraction V, Sterile, 35% solution serological, NBC, Cleveland, Ohio) 50 ml
 Add 1,750 ml PBS; mix.
 Store at 4°C.

G. Monobasic Phosphate (0.5 M)

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 69.05 g
 Dissolve in deionized distilled water and dilute to 1 liter.

H. Dibasic Phosphate (0.5M)

Na_2HPO_4 70.98 g
 Dissolve in deionized distilled water, heat to dissolve and dilute to 1 liter.

I. 1:400 Normal Guinea Pig Serum (NGPS)

Obtain blood from guinea pigs not used for antibody production.
 Clot the blood, harvest serum and store (-20°C).
 Add 2.5 ml of serum to 1 liter volumetric flask and dilute to 1 liter with 0.05 M PBS-EDTA and store (-20°C).

J. Anti-Gamma Globulin

Use sheep anti-guinea pig gamma globulin (SAGPGG) obtained from sheep injected with guinea pig gamma globulin.
 Dilute antisera 1 + 25 on day of use (one part SAGPGG serum plus 25 parts 0.05 M PBS-EDTA, pH 7.0).
 Store at 4°C.

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