ABSTRACT

EFFECTS OF PREPUBERTAL DIET AND INJECTION OF bST ON AGE AT PUBERTY, BODY GROWTH, CARCASS COMPOSITION, AND MAMMARY DEVELOPMENT OF DAIRY HEIFERS

By

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Reducing the age at first calving would increase a farm's profit margin. One way to reduce age at first calving is to reduce the age at puberty and breeding. Previous experiments have demonstrated that a high energy diet will decrease age at puberty and breeding, but such diets are detrimental to mammary development and future milk production. The objective of this thesis was to determine the effects of feeding dairy heifers either a high-protein, high-energy diet or a standard diet, both with and without injection of bovine somatotropin on body growth, carcass composition, age at puberty, and composition and metabolic activity of the mammary gland.

The high diet increased growth rate by increasing growth of soft tissue. Bovine somatotropin increased the growth of muscle and bone, while decreasing adipose deposition. Diet had no effect on mammary cell numbers or metabolic activity, whereas bST increased both variables. Thus, a high-protein, high-energy diet combined with bST potentially may decrease age at first calving without reducing future milk yield.

I dedicate this thesis to my closest family, who have given me more love and support than anyone could ever ask for.

Jack and Joan Radcliff, my parents;

Ed and Mary Norman, my uncle and aunt;

Jackie and Linda Gorbey, my uncle and aunt.

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INTRODUCTION

Rearing replacement dairy heifers accounts for approximately 20% of total production costs on a dairy farm (Annexstad, 1986). During the time between birth and first calving expenses are generated in the form of feed, housing, and labor, while contributing nothing to the income of the farm. Any way for the farmer to reduce the expense of rearing replacement heifers without impairing productivity after calving would increase overall profitability of the farm.

Current recommendations suggest that 24 mo is an optimal age at first calving for dairy heifers. Calving at a later age increases the total cost of rearing replacement heifers by increasing the amount of time they are supported by the farm without generating income. However, calving at an earlier age could increase costs by increasing the incidence of dystocia and metabolic disorders at calving if the heifer has not attained the proper skeletal size and weight by the time of calving. While growing prepubertal heifers at a higher rate of gain with high energy diets could solve the size problem, it produces another problem: namely, it decreases milk production (Swanson, 1960; Gardner et al., 1977; Sejrsen, 1978, and Little and Kay, 1979).

During the period between 3 mo of age and puberty the mammary

gland grows allometrically, in other words, more rapidly than the rest of the body (Sinha and Tucker, 1969). The decrease in milk production associated with feeding high energy diets during this allometric growth phase has been attributed to a decrease in growth of mammary parenchyma and a concurrent increase in deposition of mammary adipose tissue. Van Amburgh and Galton (1994) fed dairy heifers a diet high in energy and balanced with elevated protein. As in other studies with diets formulated for high rates of gain, age at first calving was reduced. However, subsequent milk production was not reduced.

The practical recommendation to dairy farmers to grow replacement heifers at 800 g/d during the prepubertal period comes from calculating the growth required to ensure adequate size and body weight for breeding by 15 mo of age. This growth rate allows the heifer to attain an adequate size for breeding by 15 mo of age, while at the same time allowing for greater parenchymal development without excess adipose deposition in the mammary gland (Sejrsen, 1994).

Administration of bovine somatotropin (bST) increases body growth rate while decreasing carcass fat (McShane et al., 1989; Hufstedler et al., 1991; Moseley et al., 1992 and Vestergaard et al., 1993). In addition, injection of bST to dairy heifers during the prepubertal period increases mammary parenchymal tissue while decreasing mammary adipose tissue (Sejrsen et al., 1986 and Glasser et al., 1991). I hypothesized that a high-protein, high-energy diet combined with administration of bST would increase body growth rate and

mammary development, and thereby allow heifers to calve earlier than 24 mo of age without decreasing milk production.

Specific objectives of this thesis were to determine effects of bST on body growth rates, carcass composition, age at puberty, and growth and metabolic activity of mammary parenchyma in heifers fed to gain either .8 kg/d or 1.2 kg/d. Information about body growth as well as mammary growth and metabolic activity could then be used to choose those treatments with the greatest potential to maximize body and mammary growth, thereby shortening the interval between birth and calving without reducing subsequent milk production.

REVIEW OF LITERATURE

Mammary development from birth to conception

At the time of birth, the mammary gland of a heifer is an immature duct system consisting of primary and secondary sprouts and end buds (parenchyma), which are derived from ectoderm. This duct system is surrounded by a combination of mature smooth muscle, adipose, connective, blood, and lymph tissue (stroma), which are derived from mesoderm (Tucker, 1969). During the first 2 to 3 mo after birth, the mammary gland grows at a rate similar to the rest of the body (isometric growth). At 2 to 3 mo of age, the mammary gland begins to grow as much as three times faster than the rest of the body (allometric growth). This allometric growth continues through several estrous cycles after onset of puberty, at which time it returns to an isometric growth rate (Sinha and Tucker, 1969).

Prepubertal growth of parenchymal tissue is characterized by extension of the ducts into the stroma in the form of a solid cord of cells, followed by canalization (lumen formation). Along with growth of parenchyma, there is concurrent growth of stroma (Reece, 1958). Extension of the ducts is the result of rapid proliferation of the end buds. At the tip of the end bud, a layer of undifferentiated cuboidal epithelial cells (cap cells) engage in intense mitotic

activity (Williams and Daniel, 1983). The progeny of these cells, not the parents, differentiate into epithelial cells, forming ducts and myoepithelial cells (Smith and Medina, 1988). After puberty, the gland consists of an extended duct system but alveoli have not yet formed. Alveoli are small almost spherical structures located at the end of ducts, and are made up of a single layer of epithelial cells. Milk synthesis and secretion occur in the alveoli. Alveolar development usually occurs after conception during a second allometric growth phase (Tucker, 1969).

Effect of diet on body growth

Growth can be defined as an increase in size or mass. Growth of an animal can be characterized by two processes, hypertrophy and hyperplasia. Different body tissues grow and mature at different rates throughout the life of an animal. Although the rate of growth can be controlled, the sequence of tissue maturation remains the same regardless of the growth rate of an animal (Batt, 1980). Tissues mature in the following order: neural tissue, bone, muscle, and finally adipose tissue. Because neural tissue is relatively mature at birth, in this review I will focus only on postnatal growth of bone, muscle, and adipose tissue, and how altering dietary nutrient content affects their growth.

Normal bone growth throughout the life of cattle can be described as an increase in growth rate beginning soon after birth. However, the growth rate slows dramatically by 8 to 10 mo of age in cattle (Berg and Butterfield, 1968). However, new bone is constantly being laid down and reabsorbed by the

body throughout an animal's life. Mature bone size is maintained by an equilibrium between new growth and reabsorption.

As bone growth slows, muscle growth rate increases. If muscle growth is graphed as a function of time after birth, it is a sigmoidal curve: i.e., at first muscle growth is slow, then it increases dramatically before slowing again by 15 mo of age in Holstein steers (Berg and Butterfield, 1968). Net muscle growth is the difference between total protein synthesis and degradation by the body. During rapid muscle growth, total protein synthesis is 6 to 10 times greater than protein accretion (Eisemann et al., 1989b and Bergen and Merkel, 1991). Postnatal muscle growth is a process of increasing myofiber cross-sectional area and length, but fiber numbers do not change (Burleigh, 1974 and Goldspink, 1974). As a muscle fiber grows, nuclei are added from mitosis of satellite cells which reside between the basement membrane and the plasma membrane of the muscle fiber.

Although the functions of lipid in an animal are numerous, the major role of adipose tissue is long-term storage of energy (Leat and Cox, 1980). As the rate of muscle growth decreases, the rate of adipose accretion increases. As net growth of the body increases after 100 to 200 d of age in cattle both hypertrophy and hyperplasia occur in the carcass fat depots, whereas hypertrophy alone occurs in the perirenal depots of steers (Robelin, 1981 and Truscott, Wood, and Denny, 1983). After net growth of bone and muscle stop, adipose accretion may continue by hypertrophy of quiescent preadipocytes, as well as mature

adipocytes (Vernon, 1986) as long as nutrient availability permits. Accretion of adipose tissue is the difference between lipogenesis and lipolysis. When rates of lipogenesis and lipolysis become equal, lipid accretion stops.

To live and grow, animals must obtain nutrients from outside the body. A certain amount of these nutrients are required just to provide energy for body functions to proceed normally. This is the amount required for maintenance. Once the quota for maintenance is met, excess nutrients are used for growth and production, or excreted. If the level of nutrient intake is not adequate for maintenance, the body then calls on its own reserves to meet these needs and growth is reduced.

When level of nutrition is adequate, all growing tissues are served according to their needs. As an animal ages these needs change. For example, early in life bone will require more nutrients for growth. However, as the animal ages, bone requires less and muscle requires more nutrients. As muscle matures, more nutrients are available for storage in the form of adipose tissue. Nutrient intake and age of the animal affect how nutrients are partitioned to bone and muscle growth, adipose tissue accretion, or excreated (Koch et al., 1979). By increasing nutrient density of a diet, growth rates of body tissues are increased. When nutrition is limiting for a growing animal, the earlier developing tissues take priority for the supply of available nutrients. Thus, nerve and bone will grow normally while growth of muscle and adipose tissue are hindered. If nutrients are severely limited, growth of bone will slow and that of muscle and adipose will stop

(Hammond, 1960). Pomeroy (1941) demonstrated that if pigs weighing 136 kg were fed a low level of nutrition, bone growth continued normally but muscle growth and adipose accretion stopped. If nutrients were further restricted, muscle and adipose tissue even atrophied. Similar results have been reported in lambs. For example, Palsson and Verges (1952) reported that severe restriction of nutrients early in life would decrease bone growth. Lambs fed adequate nutrition early in life followed by a period of restricted nutrition had the same amount of bone, but less muscle growth and adipose tissue accretion than nonrestricted lambs. These data indicate that to impair bone growth, dietary nutrients have to be limiting during the time bone growth rate is highest; i.e., early in life.

Effects of bST on body growth

In 1959, Brumby first showed the effects of growth hormone (bST) on growth of cattle. Since then, many researchers have investigated many different doses and many different periods of administration. During this time bST has been shown to increase average daily gain (ADG) in lambs (Pell and Bates, 1987), crossbred heifers and steers (Hufstedler et al.,1991 and Moseley et al.1992), beef heifers (McShane et al. 1989 and Vestergaard et al. 1993) and dairy heifers (Sejrsen et al., 1986; Sandles et al., 1987a and Gringes et al., 1990). This increase in ADG can be attributed to an increase in bone and muscle growth. Increased bone growth is indicated by an increase in withers or hip height (Brumby, 1959 and Gringes et al. 1990), and pelvic area (McShane et al., 1989

and Gringes et al. 1990). Injection of bST increases muscle growth, as indicated by protein content in the 9-10-11 rib section (Peters, 1986; Hufstedler et al., 1991; Moseley et al., 1992 and Schwarz et al., 1993); DNA content of semimembranosus and tricep muscles (Malton et al., 1990); and rate of protein synthesis (Pell and Bates, 1987 and Eisemann et al., 1989A), and lean tissue mass (Vestegaard et al., 1993). The increase in growth of bone and muscle is accompanied by a concurrent decrease in adipose accretion (McShane et al., 1989; Hufstedler et al., 1991; Moseley et al., 1992; Schwarz et al., 1993 and Vestergaard et al., 1993). Thus exogenous bST will not only increase the growth rate of cattle but will also improve carcass composition by increasing muscle and reducing adipose accretion. The increase in growth from bST may prove to be economically beneficial to the dairy farm by decreasing the time required to bring a heifer into milk production and insuring that the heifer has attained an adequate body weight and skeletal size for ease of calving, and to support milk production after calving.

Effects of prepubertal diet on mammary development

There have been mixed reports of the effect of diet on mammary development. Early reports suggest that rapid body growth in heifers decreases subsequent milk production. This decrease in milk production associated with accelerated rates of gain was attributed to incomplete parenchymal development and excess fat deposition in the mammary gland (Swanson, 1960). Since then, there have been many reports that increased body growth rates are associated

with impaired mammary development (Gardener et al., 1977; Little and Kay, 1979; Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984 and Stelwagen and Grieve, 1990). Sejrsen et al. (1982) concluded from comparison of animals fed ad libitum and restricted diets before and after puberty, that dietary impairment of mammary development occurred during the prepubertal allometric growth phase. This conclusion is supported by a report from Harrison et al. (1983), in which heifers reared at higher rates of gain for the first year of life had less mammary secretory tissue at 12 mo of age as well as after two lactations, and Lacasse et al. (1993) who reported that plane of nutrition after 1 year of life had no effect on first lactation milk yield.

Sejrsen (1978 and 1994) attributed impairment of mammary development almost entirely to level of energy intake, and recommends feeding dairy heifers to gain no more than .6 to .7 kg/d before puberty. However, reports cited by Sejrsen to support this conclusion used feed restriction to reduce the level of energy intake. Impairment of prepubertal mammary development in dairy heifers fed ad libitum compared with heifers that are restricted-fed may be due to differences in endogenous hormone secretions. Sejrsen et. al. (1983) reported that restricted-fed dairy heifers had higher growth hormone concentrations than heifers fed ad libitum. These restricted heifers also had more parenchymal tissue and less adipose tissue in the mammary glands. Another explanation for increased mammary development in restricted-fed dairy heifers is that puberty is delayed and these heifers have more time for allometric growth of mammary parenchymal

tissue to occur. However, there have also been reports of accelerated body growth without impairment of mammary development (Peri et al., 1993 and Van Amburgh and Galton, 1994). In both of these reports the accelerated groups of heifers were fed a diet that was elevated in both protein and energy. Furthermore, Peri et al. (1993) reported no difference in serum growth hormone concentrations. Perhaps the difference in mammary development in earlier experiments as compared with these recent studies is that the former had a dietary imbalance of protein and energy that in some way is inhibitory to mammary growth.

Effects of prepubertal administration of bST on mammary development

Somatotropin as well as other pituitary hormones are necessary for mammary development (Tucker, 1969). Effects of administration of exogenous somatotropin on mammary development have been investigated by several people since the observation of reduced somatotropin concentrations in serum of heifers reared at an accelerated growth rate (Sejrsen et al., 1983). Sejrsen et al. (1986) administered either pituitary-extracted somatotropin or vehicle for 15.6 wk beginning at 8 mo of age, to either Danish Friesian or Red Danish milkbreed monozygotic twins. Heifers treated with somatotropin had 18% more parenchyma and 26% less fat in the mammary gland than control heifers. Sandles and Peel (1987b) reported similar results. Administration of somatotropin, for 21 wk beginning at 3.5 mo of age, increased mammary DNA 20% and reduced the

amount of adipose tissue in the mammary gland 16% compared with controls. Injection of bST, for 15 wk beginning at 45 d of age, increased mammary development in lambs (McFadden et al., 1990). Glasser et al. (1993) reported that bST administered for 10 mo, beginning at 6 mo of age, increased parenchymal tissue 45% and decreased extra-parenchymal tissue 36% in Angus x Holstein heifers reared at either a constant growth rate of .8 kg/d or an intermittent growth rate. Intermittent growth was produced by two consecutive cycles. In each cycle, feed was restricted to produce .2 kg/d gain for 3 mo followed by a period of unrestricted gain. These results demonstrate the ability of exogenous somatotropin to enhance mammary development in cattle. However, the effect of somatotropin on mammary development is not independent of other hormones. For example, Purup et al. (1993) reported that bST failed to increase mammary development in ovariectomized heifers when compared with ovariectomized, vehicle-treated heifers, indicating that the increase in mammary development produced by somatotropin is also dependent on ovarian hormones such as estrogen or progesterone.

Somatotropin receptor mRNA has been identified in both rat and bovine mammary glands by in situ hybridization (Glimm et al., 1990). Therefore, somatotropin may bind to mammary cells and act locally to stimulate mammary development. However, mouse mammary glands incubated in whole organ culture with either rat, ovine, or bovine somatotropin failed to develop until supraphysiological concentrations were used (Plaut et al., 1993). Collier et al.

(1993) also reported no effect of bST on proliferation of isolated bovine mammary epithelial cells. Furthermore, many efforts to demonstrate somatotropin binding to mammary epithelial cells have failed (Gertler et al., 1984; Akers, 1985; and Kazmer et al., 1986). The results of these studies suggest that somatotropin does not directly affect mammary growth.

An alternative to direct stimulation of mammary growth by somatotropin could be indirect stimulation through growth factors. It has been postulated that the effect of somatotropin on mammary development is mediated through insulin-like growth factor-I (IGF-I). Injecting dairy cattle with bST increases mammary development (Sejrsen, 1986; Sandles and Peel, 1987; McFadden et al., 1990, and Glasser et al., 1993), as well as serum IGF-I concentrations (Bauman and Vernon, 1993 and Dahl, 1993). Injection of bST also increases IGF-I mRNA and IGF-I receptor mRNA in the liver (VanderKooi, 1993). Incubating isolated bovine mammary epithelial cells in collagen gel with IGF-I alone or combined with epidermal growth factor increased cell proliferation (Collier et al., 1993). In contrast, incubation of mouse mammary glands from 34 to 37 d old BALB/c mice in whole gland culture with concentrations of IGF-I ranging from 10 ng/ml to 1 ug/ml did not stimulate mammary development (Plaut et al., 1993). Determining the role of IGF-I in mammary development is even more complicated when data from in vivo experiments are considered. For example, restricting dietary intake not only increases mammary development and serum somatotropin concentration, (Sejrsen et al., 1982), but decreases

concentrations of IGF-I in serum (Breier, 1988). Thus, the roles of somatotropin and IGF-I are an enigma. A direct effect of somatotropin on mammary development, when increased by either dietary manipulation or injection of bST. is doubtful due to the fact that somatotropin receptors are absent from the mammary gland (Gertler et al., 1984; Akers, 1985, and Kazmer et al., 1986). The indirect role of somatotropin mediated through IGF-I on mammary development is questionable. Injection of bST increases mammary growth as well as concentrations of IGF-I in serum; however dietary restriction increases mammary development and serum somatotropin concentrations, but decreases concentrations of IGF-I in serum. Thus, results concerning the role of IGF-I on mammary development are not clear, and more research is needed to help clarify this question. Although somatotropin's mechanism of action is still not known, somatotropin's effect on, and importance to, mammary development are well documented.

MATERIALS AND METHODS

Management of animals

Thirty-eight Holstein heifers, born between June 15 and July 15 were purchased and housed at the Michigan State University Dairy Teaching and Research Center located on College Road, East Lansing, Michigan. All animals were allowed 30 d to acclimate to new surroundings and monitored for illnesses resulting from shipping or commingling.

Heifers were blocked by weight into groups of four. Within each block, heifers were randomly assigned to one of 4 treatments. Heifers were fed one of two diets from 4 mo of age until the early luteal phase of their fifth estrous cycle. Nine heifers were fed a total mixed ration that met the current recommended amounts of protein and energy formulated to produce .8 kg gain per day (standard control diet; SC). Nine heifers were fed the standard control diet and injected daily with recombinant bovine somatotropin (bST; SB). Ten heifers were fed a total mixed ration containing elevated protein and energy formulated to produce 1.2 kg gain per day (high control diet; HC). Ten heifers were fed the high control diet and injected daily with bST (HB). Feed samples were collected every other week to assess protein and fiber content. Nutrient composition of diets is described in Table 1. Feed was offered ad libitum from

TABLE 1. Composition of Standard and High diets.

	Standard	High
Ingredients		
Grain, %	10%[1]	75%[2]
Haylage, %	90%[3]	25%[4]
Nutrient composition		
NDF, % of DM	49.6	19.4
NE_m,[5] Mcal/kg	1.17	1.83
NE_g,[6] Mcal/kg	.57	1.20
CP, % of DM	16.3	19.4
Absorbable protein, % of DM [7]	8.8	13.3
Rumen-undegraded protein [8], % of CP	26.9	38.0
Absorbable protein/NE_m	7.5	7.3

[1] On a DM basis, contained 93.4% ground corn, .8% monocalcium phosphate, 1.5% white salt, .9% trace mineral-vitamin premix and 3.9% DECCOX-10x (Purina Mills; .5 % decoquinate) and was formulated so that diet provided 100% of protein, mineral, and vitamin requirements (NRC, 1989).

[2] On a DM basis, contained 73.6% ground corn, 20.0% soybean meal, 3.7% animal protein supp., .9% limestone, 3.7% white salt, ..89% trace mineral-vitamin premix, and .5% DECCOX-10x (Purina Mills; .5% decoquinate) and was formulated so that diet provided 100% mineral and vitamin requirements (NRC, 1989).

[3] Haylage contained 1.05 Mcal NE_m/kg, .45 Mcal NE_g/kg, 17.3% CP, 25% rumen-undegraded protein, and 55.8% NDF.

[4] Haylage contained 1.28 Mcal NE_m/kg, .65 Mcal NE_g/kg, 18.8% CP, 24% rumen-undegraded protein, and 47% NDF.

[5] Net energy for maintenance.

Table 1 (cont'd)

[6] Net energy for gain.

[7] as calculated by Spartan Dairy ration balancing program.

[8] Estimated from NRC

experimental d 0 to slaughter. Fresh feed was offered every day between 0845 and 0930 h. Orts for each pen were weighed daily and recorded to calculate average daily dry matter intake for each treatment group. At each daily feeding (0845 to 0930 h) all heifers were restrained in gang-lock stanchions and heifers that received bST were injected i.m. with bST (25 μg/kg BW, Somavubove, The Upjohn Co., Kalamazoo, MI) in the semitendinosus muscle using a 3-ml disposable syringe with a 23-gauge, 1.9-cm needle. This dosage was determined to be an optimal dose to improve carcass composition in crossbred steers (Moseley et al., 1992). Every 72 h, bST was reconstituted in sterile bottles with sterile water to a concentration of 14 mg/ml, diluted with sodium monobasic buffer (2 mg/ml, pH 11.2), and stored at 4°C. The concentration of bST was adjusted throughout the experiment so that all doses could be delivered in a volume of 1 to 2 ml for every heifer. Heifers were grouped by treatment and allowed free access to an outside paddock. They were exposed to ambient temperatures and 16 h of light (0600 to 2200 h), a photoperiod that stimulates mammary development (Petitclerc et al., 1985).

Serum concentrations of progesterone were monitored as an

indicator of puberty as described in "**Blood collection and analysis**". Estrus detection commenced twice daily for 30 min per pen after the first heifer attained puberty. Heifers were injected with Lutalyse• (The Upjohn Co., Kalamazoo, MI) during their third luteal phase, and again 11 d later. Nine d after their second injection of Lutalyse•, heifers were transported in a trailer to the abattoir at the Michigan State University Meats Laboratory at 0630 h for slaughter.

Body measurements

All heifers were weighed before feeding on two consecutive days each week to monitor body weight gain. The average of the two weights was then assigned as the weekly weight. Weekly weights were used to calculate average daily gain (ADG). Diets were adjusted to maintain desired BW gains (Appendix A). Withers heights were measured every 2 wk while heifers were restrained in the gang lock stanchions. Commencing at 250 kg BW, body condition was scored (BCS) on a scale of 1 to 5 (Wildman et al., 1982) every 2 wk by three experienced examiners. The three scores for each heifer were then averaged and assigned to that heifer as her score. Twenty-four h after slaughter, pelvic area was calculated from two linear measurements of the left half of the carcass; one from the third coccygeal vertebrae to the pubis symphysis, and a second from the midline of the carcass to the pelvic wall. The second measurement was multiplied by 2 to represent the total width of the pelvic opening, and then multiplied by the first to produce the total pelvic area.

Blood collection and analysis

All blood samples were stored at room temperature for approximately 6 h and then 4°C for approximately 15 h. Serum was harvested after centrifugation at 1550 x g for 25 min, and frozen at -20°C until assayed.

Beginning when calves weighed 205 kg, blood samples were collected twice weekly via jugular venipuncture with Vacutainers® (Becton Dickenson & Co., Rutheford, NJ). To monitor for the onset of puberty, this serum was assayed to quantify progesterone (P_4) concentrations (Spicer et al., 1981). A heifer was considered pubertal when P_4 concentrations were $\geq$ 1 ng/ml in three consecutive serum samples.

Two days before slaughter, each heifer was fitted with a sterile indwelling jugular catheter (18 gauge; Ico-Rally, Palo Alto, CA). Twenty four hours later, blood samples were collected at 20-min intervals for 6 h (0800 h to 1400 h). Catheter patency was maintained between samples by flushing the catheter with 3.5% sodium citrate in sterile water. Heifers in the SB and HB groups were injected with bST immediately after collection of the 0900 h sample. Serum concentrations of growth hormone were quantified according to Gaynor et al. (1995). Nonesterified fatty acids (NEFA; NEFA-C kit, Waco Chemicals USA, Dallas, TX; as modified by Johnson, et al., 1993) were quantified in a serum sample collected via venipuncture of each heifer at 0930 h on experimental d 10 and 58, ≈ 14 d before puberty, ≈ 30 d after puberty, and at the time of slaughter. Because animals were slaughtered according to date of puberty, the

amount of time between experimental d 58 and ≈ 14 d before puberty ranged from 37 to 187 d.

Tissue collection

All heifers were weighed, stunned by captive bolt and killed by exsanguination at the Michigan State University Meats Laboratory. The number of heifers killed each week depended on date of first ovulation, and ranged from 1 to 6 heifers. Heifers were slaughtered an average of 74 d after first ovulation. Immediately after exsanguination, heads were removed, sawed open along the coronal plane from immediately above the eyes to the top of the ears, to expose the brain tissue. Pituitary glands were subsequently removed and separated into anterior and posterior lobes. Anterior pituitaries were weighed and frozen by submersion in liquid nitrogen.

Mammary glands were quickly removed and bisected into right and left halves. The left half was weighed, placed in a plastic bag, and frozen by submersion in a tub of dry ice and 95% ethanol. Frozen half udders were stored at -20°C until analyzed as described in section **"Mammary tissue analysis"**.

Internal organs were removed soon after the carcass was split open. The gall-bladder was removed from the liver and the liver was weighed. The rumen was emptied of it's contents and visually examined for lesions. After the hide was removed, the carcass was then hoisted to the rail and split into halves along the spine. The carcass halves were then weighed. Perirenal fat was

removed from the left half beginning at the 4th lumbar vertebra and proceeding forward to the adrenal gland and then weighed. The carcasses were washed, wrapped with wet drapes, and hung in chambers at 2°C.

Carcass composition analysis

Twenty-four hours after slaughter, the left half of each carcass was cut between 7th and 8th, and the 12th and 13th ribs. The rib section including the 8th through 12th ribs was removed. The 9-10-11th rib section was then dissected according to the methods of Hankins and Howe (1946). The 9-10-11th rib section was then weighed and deboned. Next, bone and soft tissue were weighed. Soft tissue was ground, mixed and subsampled for analysis of protein, fat, and water content. Protein was determined in fresh samples by the macro-Kjeldahl procedure (AOAC, 1984). Fat was determined by Soxlet ether extraction of fresh samples. Water was determined by the difference in weight after drying in an oven at 110°C for 24 h.

Mammary tissue analysis

The frozen left half of the udder was cut transversely with a band saw into 5- to 10-mm thick slices. All slices from the anterior and posterior ends of the gland that did not contain parenchymal tissue were discarded. Slices were placed on a cutting board and allowed to thaw slightly. Skin, teats, and

supramammary lymph nodes were dissected from the parenchyma with a scalpel and discarded. Fat located beyond the border of the parenchyma (in those slices that contained parenchyma) was removed and weighed. This fat was defined as extra-parenchymal fat. The remaining tissue will be referred to as mammary parenchymal tissue. The frozen mammary parenchymal tissue was weighed and then ground with dry ice into a fine powder with a blender. The powder was mixed and subsampled for subsequent analysis for DNA and RNA content (Tucker, 1964), dry matter, and fat by Soxlet ether extraction.

Statistical analysis

The data for total DNA, DNA adjusted for body weight, total parenchyma, total extra-parenchymal fat, extra-parenchymal fat adjusted for body weight, total intra-parenchymal fat, intra-parenchymal fat adjusted for body weight, percentage of carcass fat, percentage of carcass water, total carcass fat, total perirenal fat, and perirenal fat adjusted for body weight were transformed by natural logarithm to eliminate heterogeneous variance. For the variable of body weight at puberty, one heifer tested positive as an outlier using a standardized residual test and was removed from the data set. All data were analyzed by ANOVA. Least squares means of main effects, diet and bST, and for any diet × bST interactions were compared using an f test (Gill, 1978).

Overall mean serum somatotropin concentrations were calculated from samples that were collected on the day before slaughter at 20-min intervals

supramammary lymph nodes were dissected from the parenchyma with a scalpel and discarded. Fat located beyond the border of the parenchyma (in those slices that contained parenchyma) was removed and weighed. This fat was defined as extra-parenchymal fat. The remaining tissue will be referred to as mammary parenchymal tissue. The frozen mammary parenchymal tissue was weighed and then ground with dry ice into a fine powder with a blender. The powder was mixed and subsampled for subsequent analysis for DNA and RNA content (Tucker, 1964), dry matter, and fat by Soxlet ether extraction.

Statistical analysis

The data for total DNA, DNA adjusted for body weight, total parenchyma, total extra-parenchymal fat, extra-parenchymal fat adjusted for body weight, total intra-parenchymal fat, intra-parenchymal fat adjusted for body weight, percentage of carcass fat, percentage of carcass water, total carcass fat, total perirenal fat, and perirenal fat adjusted for body weight were transformed by natural logarithm to eliminate heterogeneous variance. For the variable of body weight at puberty, one heifer tested positive as an outlier using a standardized residual test and was removed from the data set. All data were analyzed by ANOVA. Least squares means of main effects, diet and bST, and for any diet × bST interactions were compared using an f test (Gill, 1978).

Overall mean serum somatotropin concentrations were calculated from samples that were collected on the day before slaughter at 20-min intervals

from 0800 to 0900 h, and from 0900 to 1400 h , and for serum NEFA concentrations from d 10 to d 58, and from 14 d before puberty to slaughter. Overall mean concentrations of somatotropin were transformed by natural logarithm to eliminate heterogeneous variance. Overall mean NEFA and natural logarithm transformed somatotropin concentrations were analyzed by ANOVA. Main effects were compared using an f test. Values presented for mean concentrations of somatotropin are least square means of untransformed data. Student's t test was used for independent comparisons between treatments (Gill, 1978). The criterion for statistical significance was $P < .05$; therefore, comparisons in which the P value was greater than .05 were considered not significant.

RESULTS

General

Treatments commenced for an average of 219, 220, 262, 288 d for high diet, high diet + bST, standard diet, and standard diet + bST, respectively. There were no significant diet × bST interactions for any variable examined in this thesis. Because there were no significant diet × bST interactions, the appropriate data were pooled and results presented as main effects of diet and bST. Neither diet nor bST affected anterior pituitary weight. There were no visible lesions in the rumen epithelium of any of the heifers. None of the livers from these heifers were condemned due to abscesses or fat.

Body growth

Initial body weight was not different among treatment groups (Table 2). Compared with the standard diet, the high diet increased body weight and BCS at slaughter. Compared with noninjection, injection of bST increased body weight at slaughter but did not affect BCS at slaughter. Feeding the high diet to heifers increased ADG over that of heifers fed the standard diet. Similarly, injection of bST increased ADG over that of noninjected heifers.

Compared with the standard diet, the high diet decreased age at

TABLE 2. Least squares means of individual treatments and significance of pooled main effects (diet and bST) and interaction of main effects on body growth.

							Contrasts		
	HC[1]	HB[1]	SEM[2]	SC[1]	SB[1]	SEM[3]	Diet	bST	Int[4]
n	10	10		9	9				
Initial weight (kg)	126	124	3	127	128	3	.46	.52	.87
Weight at slaughter (kg)	396	411	12	336	379	12	.001	.04	.2
BCS at slaughter	3.9	3.9	.1	2.9	2.8	.1	.001	.36	.99
ADG (kg/d)	1.22	1.30	.025	.79	.87	.026	.001	.004	.9
Age at puberty (d)	266	269	9	313	337	10	.001	.17	.26
Weight at puberty (kg)	305	329	11	296	326	11	.55	.02	.79
Withers height at puberty (cm)	115	119	1	117	119	1	.45	.03	.67
Pelvic area (cm^2)	216	239	7	210	241	7	.74	.001	.56

[1] HC = high control diet, HB = high diet + bST, SC = standard control diet, SB = standard diet + bST.
[2] Pooled standard error of means as calculated with n = 10.
[3] Pooled sandard error of means as calculated with n = 9.
[4] Diet × bST interaction.

puberty, but the high diet did not affect body weight or withers height at puberty, or pelvic area at slaughter (Table 2). In contrast, injection of bST did not affect age at puberty but increased body weight and withers height at puberty and pelvic area at slaughter.

Carcass composition

Compared with the standard diet, the high diet increased carcass weight as well as dressing percentage (Table 3). Compared with noninjection, injection of bST increased carcass weight but did not affect dressing percentage. Compared with the standard diet, the high diet increased total liver weight as well as liver weight adjusted for differences in body weight. Compared with noninjection, injection of bST also increased total liver weight and liver weight when adjusted for differences in body weight.

Compared with the standard diet, the high diet decreased percentage of carcass protein and water, but increased percentage of carcass fat (Table 3). In contrast, injection of bST increased percentage of carcass protein and water, but injection of bST decreased percentage of fat. The high diet increased total amounts of protein and fat in the carcass. Injection of bST increased total protein, but did not affect total fat in the carcass. The high diet increased total perirenal fat from the left half of the carcass, as well as perirenal fat when adjusted for differences in body weight. Injection of bST did not affect total perirenal fat from the left half of the carcass, but tended to decrease

TABLE 3. Least squares means of individual treatments and significance of pooled main effects (diet and bST) and interaction of main effects on carcass composition.

							Contrasts		
	HC[1]	HB[1]	SEM[2]	SC[1]	SB[1]	SEM[3]	Diet	bST	Int[5]
n	10	10		9	9				
Carcass weight (kg)	223	233	8.1	171	195	8.5	.001	.02	.74
Dressing percentage	53.6	56.6	2.9	50.7	45.7	3.0	.001	.39	.76
Liver weight (kg)	6.2	7.1	.25	4.8	6.0	.26	.001	.001	.54
Liver weight (kg/100 kg BW)	1.6	1.7	.04	1.4	1.5	.04	.001	..001	.94
Anterior pituitary weight (g)	1.40	1.57	.08	1.30	1.38	.09	.10	.16	.61
Carcass protein (%)	16.3	17	.17	17.4	17.9	.18	.001	.001	.57
Carcass fat (%)	24.8	21.6	.84	16.6	14.1	.88	.001	.002	.72
Carcass water (%)	58.1	60.5	.67	64.6	66.6	.71	.001	.004	.71
Total carcass protein (kg)	36.2	39.4	1.1	29.7	34.9	1.1	.001	.001	.38
Total carcass fat (kg)	56.1	50.4	3.1	28.4	27.5	3.3	.001	.41	.70
Perirenal fat (kg)[4]	3.7	2.9	.30[2]	1.5	1.5	.3	.001	.40	.32
Perirenal fat (g/100kg BW)	907	706	57[2]	462	412	57	.001	.09	.47

[1] HC = high control diet, HB = high diet + bST, SC = standard control diet, SB = standard diet + bST.
[2] Pooled standard error of means as calculated with n = 10.
[3] Pooled standard error of means as calculated with n = 9.

Table 3 (cont'd)

[4] n = 9 per treatment.
[5] Diet × bST interaction.

perirenal fat adjusted for differences in body weight.

Mammary development

Compared with the standard diet, the high diet did not affect total mammary DNA content or DNA content adjusted for differences in body weight, total mammary RNA content or mammary RNA content adjusted for differences in body weight, RNA/DNA ratio or total weight of dissectable parenchyma (Table 4). In contrast, injection of bST increased total mammary DNA content, as well as mammary DNA content adjusted for differences in body weight, total mammary RNA content, as well as mammary RNA content adjusted for differences in body weight, the RNA/DNA ratio, and total weight of dissectable parenchyma. Neither diet nor injection of bST affected the concentration of DNA in the mammary gland. However both the high diet and injection of bST increased the concentration of RNA in the mammary gland. Compared with the standard diet, the high diet increased the total amount of extra-parenchymal fat as well as extra-parenchymal fat adjusted for differences in body weight. The high diet not affect the total amount of intra-parenchymal fat, but decreased the amount of intra-parenchymal fat when adjusted for differences in body weight. Injection of bST did not affect the total amount of extra-parenchymal fat, but tended to decrease the amount of extra-parenchymal fat when adjusted for

TABLE 4. Least squares means of individual treatments and significance of pooled main effects (diet and bST) and interaction of main effects on mammary development.

							Contrasts		
	HB[1]	HB[1]	SEM[2]	SC[1]	SB[1]	SEM[3]	Diet	bST	Int[4]
n	10	10		9	9				
Total DNA (mg)	1824	2696	361	1469	2219	381	.47	.006	.91
DNA (mg/100 kg BW)	439	644	73	428	581	77	.81	.003	.82
Concentration DNA (mg/g)[5]	4.24	4.19	.24	3.76	4.23	.26	.38	.41	.31
Total RNA (mg)	1634	3156	358	1394	2226	378	.12	.003	.35
RNA (mg/100 kg BW)	401	757	77	401	581	81	.27	.001	.27
Concentration RNA (mg/g)[5]	3.97	4.84	.23	3.36	4.18	.24	.01	.001	.91
RNA/DNA	.94	1.18	.07	1	.9	.07	.12	.01	.32
Total parenchyma (g)	408	661	77	401	520	81	.69	.007	.55
Total extra-parenchymal fat (g)	955.9	850.7	63.0	391.7	366.8	66.4	.001	.55	.88
Extra-parenchymal fat (g/100kg BW)	237.7	207.2	12.6	115.1	96.7	13.3	.001	.08	.73
Total intra-parenchymal fat (g)	176.1	165.8	25.2	187.7	197	26.5	.17	.74	.60
Intra-parenchymal fat (g/100 kg BW)	42.6	39.9	5.1	54.7	51.9	5.4	.02	.6	.99

[1] HC = high control diet, HB = high diet + bST, SC = standard control diet, SB = standard diet + bST.
[2] Pooled standard error of means as calculated with n = 10.
[3] Pooled standard error of means as calculated with n = 9.

Table 4 (cont'd)

[4] Diet × bST interaction.
[5] mg/g = mg nucleic acid per g of parenchymal tissue.

differences in body weight (P = .07). Injection of bST had no effect on total intra-parenchymal fat or intra-parenchymal fat adjusted for differences in body weight.

Serum profiles of somatotropin and NEFA

Profiles of somatotropin concentrations in serum for 6 h on the day before slaughter are presented in Figure 1. Neither diet nor injection of bST affected the mean somatotropin concentration in serum before injection (0800 to 0900 h). After injection of bST (0900 h) the mean concentration of somatotropin in serum from 0900 to 1400 h was higher in injected heifers compared with noninjected heifers (17.5 vs. 2.8 ± .7 ng/ml respectively; P < .001). Compared with the standard diet, the high diet decreased the mean somatotropin concentration from 0900 to 1400 h (12.4 vs. 7.9 ± .8 ng/ml respectively; P < .001). To determine the effect of diet on somatotropin concentrations within injected and noninjected heifers, the mean somatotropin concentrations from 0900 to 1400 h were compared within injected or noninjected heifers using a t-test. Compared with the standard diet, the high diet decreased the mean somatotropin concentration from 0900 to 1400 h in injected heifers (21.5 vs. 13.5 ± 1.1 ng/ml

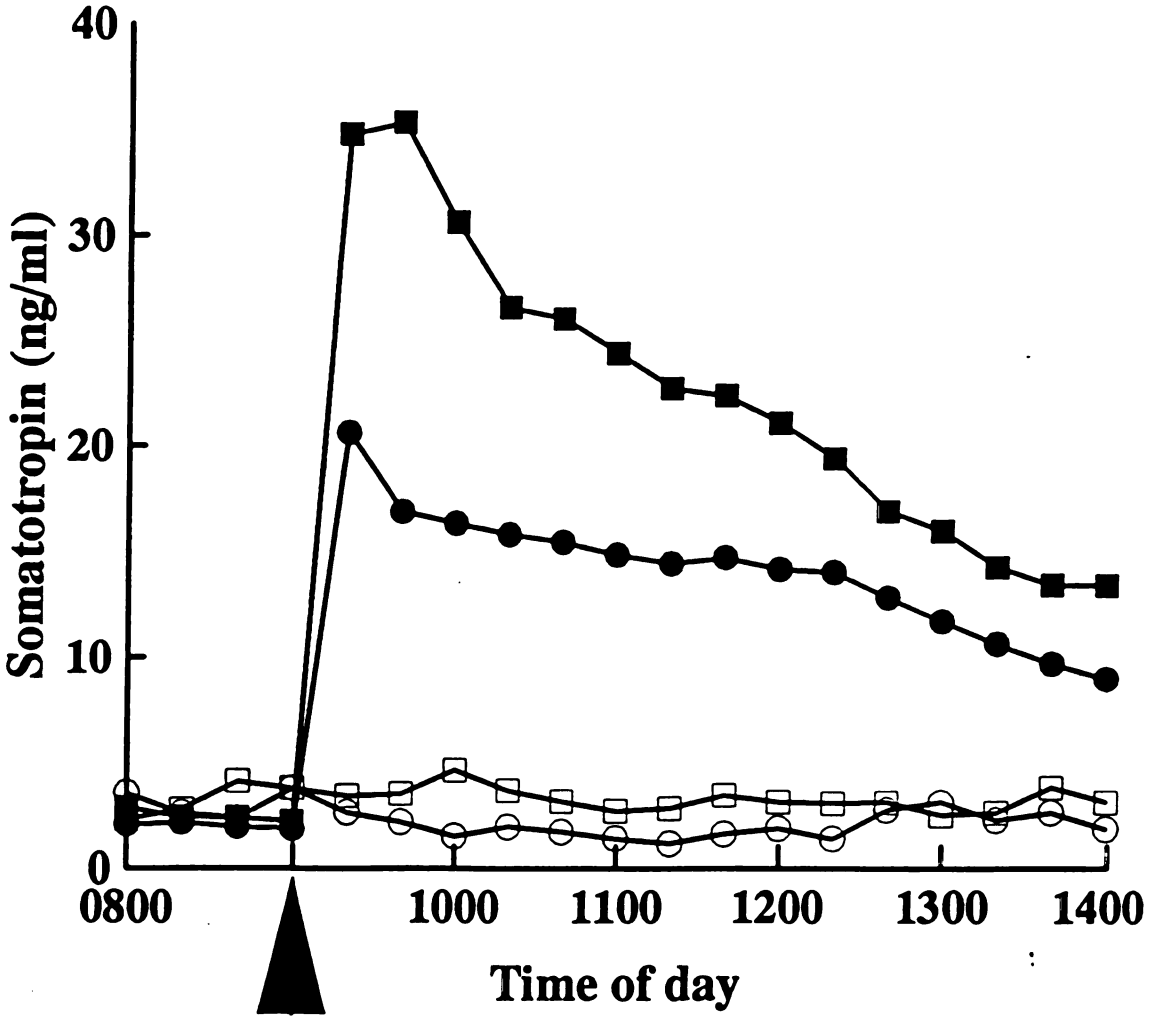

Figure 1. Concentrations of serum somatotropin in heifers fed high diet (○; n=10), high diet and injected with bST (●; n=10), standard diet (□; n=9), standard diet and injected with bST (■; n=9) for 6 h on the day before slaughter. Each point represents the average of a treatment group. Arrow represents time of injection.

respectively; P = .001) and tended to decrease mean somatotropin concentrations in noninjected heifers (3.3 vs. 2.3 ± 1.1 ng/ml respectively; P = .09).

Profiles of NEFA concentrations in serum are presented in Figure 2. Compared with the standard diet, the high diet did not affect mean concentrations of NEFA in serum from d 10 to d 58 of the experiment (94.9 vs. 90.1 μEq/L respectively; P = .68). Compared with noninjection, injection of bST did not affect mean concentrations of NEFA in serum from d 10 to 58 of the experiment (95.9 vs. 89.9 μEq/L respectively; P = .53). However, heifers fed the high diet had lower mean concentrations of NEFA in serum from 14 d before puberty to slaughter than heifers fed the standard diet (94.7 vs. 118.9 μEq/L respectively; P<.01). Injection of bST did not affect mean NEFA concentrations in serum from 14 d before puberty to slaughter compared with noninjection (100.6 vs. 113.1 μEq/L respectively; P =.14).

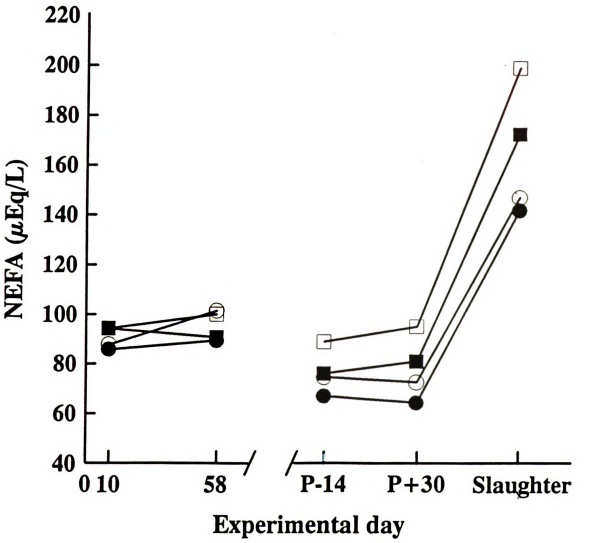

Figure 2. Concentrations of serum nonesterified fatty acids of heifers fed high diet (○; n=10), high diet and injected with bST (●; n=10), standard diet (□; n=9), or standard diet and injected with bST (■; n=9) from d 0 to slaughter. Each point represents the average of a treatment group. P=puberty, P-14 = 14 days before onset of puberty, P+30 = 30 days after onset of puberty.

DISCUSSION

Currently, the average age at first calving for dairy heifers is 27 mo (Thelen, 1995). The period from birth to first parturition is expensive to the dairy farm because heifers are generating expenses (feed, housing, labor) without providing income. In the current experiment, I hypothesized that feeding a diet high in protein and energy in combination with injection of bST would increase the growth rate of dairy heifers as well as mammary development, thereby decreasing age at puberty without having detrimental effects on mammary development.

Previous review articles have reported the effects of prepubertal growth rate on age at puberty (Schillo et al., 1992; Adam et al., 1994 and Sejrsen, 1994). Briefly, age at puberty is inversely related to prepubertal growth rate. Previous experiments have shown that injection of bST increases growth rate (Sejrsen, 1986; Pell and Bates, 1987; Sandles et at., 1987a; McShane et al., 1989; Gringes et al., 1990; Hufstedler et al., 1991; Moseley et al., 1992 and Vestergaard et al., 1993). In the current experiment, the high diet increased ADG, and decreased age at puberty. Injection of bST also increased ADG, but in contrast to the high diet, bST had no effect on age at puberty, which is in agreement with other reports by McShane et al. (1989), Hall et al. (1994), and Stanko et al

(1994). The data from the current experiment contrast with those of Hawkins et al., (1991), who reported that injection of bST delayed onset of puberty by 38 d. The discrepancy between the current experiment and the report by Hawkins et. al., (1991) concerning onset of puberty may be explained by the dose of bST administered. Hawkins et al. injected 500 mg bST, in a slow release vehicle, every 14 d regardless of body weight. This dose is far greater than the dose I used in the current experiment (25 μg/kg BW daily). During a 2-wk period in my experiment the heaviest heifer would have received a total of only 168 mg bST.

Body weight alone can be misleading when describing the size of an animal. An animal can be short and fat and weigh just as much as an animal that is tall and thin. Skeletal size could be important at the time of parturition. Heifers that are short and fat could potentially have increased incidence of dystocia, or postpartum metabolic disorders. In the current experiment, I measured withers height at puberty and pelvic area at slaughter to estimate skeletal size. Similar to reports by Koch et al., (1979), McShane et al., (1989), Stelwagen and Grieve (1990) and Hawkins et al., (1991), diet did not affect withers height at puberty in the current experiment. Similar to the report by Hawkins et al., (1991) pelvic area was not affected by diet in the current experiment. The lack of effect of diet on withers height at puberty is plausible because severe dietary restriction is required to detrimentally affect skeletal growth (Pomeroy, 1941; and Palsson and Varges, 1952). However, rate of skeletal growth was increased considering all heifers were similar in height at puberty but

the heifers fed the high diet attained puberty 58 d earlier. In the current experiment, bST increased withers height at puberty, similar to reports by Brumby et al., (1959) and Gringes et al., (1990). The bST-induced increase in pelvic area agrees with reports by McShane et al., (1989) and Gringes et al., (1990). The data from the current experiment indicate that the high diet in combination with injection of bST can be used to decrease age at puberty and actually increase skeletal size at puberty. If the bST-induced increase in skeletal size persists through gestation, I speculate there could be a decrease in the incidence of dystocia.

Feeding ruminants a high concentrate diet can cause acidosis, which could result in ruminal ulcers and hepatic abscesses (Huntington, 1988). Disorders such as ruminal ulcers and hepatic abscesses could be detrimental to future growth and milk production. For this reason, the rumens and livers were visually examined at slaughter. We did not observe any lesions in the rumens of these heifers, and none of the livers were condemned due to abscesses or fat. It should be noted that the amount of concentrate in our high diet was less than that reported to cause these ruminal ulcers or hepatic abscesses. Thus, the high diet described in the current experiment could be fed to growing heifers without fear of causing these disorders.

Previous experiments indicate that rearing dairy heifers at accelerated growth rates by feeding high energy diets causes excessive fattening and reduces subsequent mammary development and milk production (Swanson,

1960; Gardener et al., 1977; Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984 and Stelwagen and Grieve, 1990). Injection of bST decreases fat deposition in crossbred steers (Moseley et al., 1992), beef heifers (McShane et al., 1989; Hufstedler et al., 1991 and Schwartz et al., 1993) dairy heifers (Vestergaard et al., 1993) and lactating dairy cows (Binelli, 1993). Results from the current experiment are similar to earlier work in that feeding the high diet increased fat deposition in the carcass and mammary gland. Injection of bST tended to decrease the amount of fat in both the mammary gland, and the carcass. However, when comparing the amount of fat in the carcass, heifers fed the high diet and injected with bST were still fatter than noninjected heifers fed the standard diet.

The effects of rapid growth of dairy heifers on mammary development or milk production has been studied extensively. Rearing heifers at an accelerated rate of gain with high energy diets reduces mammary development and milk production (Swanson, 1960; Gardener et al., 1977; Little and Kay, 1979; Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984 and Stelwagen and Grieve, 1990). Results from the current experiment contrast with those of previous experiments. For example, feeding the high diet in the current experiment did not reduce mammary cell numbers or metabolic activity. One difference between previous experiments and the current experiment is the high diet fed in the current experiment had a similar protein to energy ratio as the standard diet; ie., protein and energy were elevated similarly. A report by Park et

al. (1987) indicated that elevating protein in the diet may increase mammogenesis in rats. Peri et al. (1993) and VanAmburgh and Galton (1994) both reported rearing heifers at accelerated rates of gain using a high protein diet without any detrimental effects on subsequent lactation. Thus, results from the current experiment add support to the theory that extra protein may offset the detrimental effects of high energy on mammary development.

Sejrsen et al. (1983) reported that restricted-fed heifers had higher serum somatotropin concentrations than heifers with ad libitum access to feed. Sejrsen also reported that restricted-fed heifers had greater numbers of mammary cells. Sejrsen suggested that the negative effects of a high energy diet on mammary development were mediated through endogenous somatotropin concentrations. In the current experiment all heifers had ad libitum access to their respective diet. When comparing noninjected heifers fed the high diet vs standard diet, there was no difference in serum somatotropin concentrations or numbers of mammary cells. The fact that endogenous somatotropin was not affected by diet may be another explanation for diet not having an effect on mammary development. As expected, injection of bST increased concentrations of somatotropin in serum that peaked at 25 to 30 ng/ml within 20 min after injection and slowly declined toward concentrations similar to noninjected controls. Because the dose was based on body weight, the fact that heifers fed the standard diet attained higher concentrations than heifers fed the high diet is intriguing. One possible explanation for this may be the method of delivery.

Injections were made with a 23-gauge, 1.9 cm needle. Samples analyzed for somatotropin were collected at the end of the experiment. Thus the heifers on the high diet were much fatter and it could be possible that the bST was injected into subcutaneous fat and not muscle. This could result in a slower release into the blood stream thus explaining the lower mean somatotropin concentrations after injection.

Prepubertal administration of bST increases mammary development in dairy heifers (Sejrsen, 1986 and Sandles and Peel, 1987b), beef heifers (Glasser et al., 1991), and lambs (McFadden et al., 1990). In the current experiment, bST increased cell numbers as well as metabolic activity of the mammary gland. The increase in cell numbers was similar in both diets. These data indicate that heifers can be reared at higher rates of gain without detriment to mammary development if allowed ad libitum access to a diet that contains elevated protein as well as energy. Furthermore, injection of bST to prepubertal heifers will increase numbers of mammary cells as well as their metabolic activity regardless of ADG.

Concentrations of NEFA in serum can be used as an indicator of lipid mobilization. In the current experiment, NEFA concentrations were not affected by diet or bST from d 14 to 58. This would seem reasonable since heifers were growing during this time. If lipid mobilization was occurring during this time growth should have been hindered. Bauman et al. (1988) and Etherton and Louveau (1992) reported that bST alters lipid synthesis not lipid mobilization.

Because the heifers in this experiment were growing, lipid mobilization would have been minimal, and bST should have decreased adipose deposition. This was confirmed because percentage of carcass fat decreased in heifers injected with bST. From approximately 2 wk before puberty to slaughter, serum NEFA concentrations were higher in heifers fed the standard diet. The reason for this dietary effect is unknown because heifers fed the standard diet were still growing at .8 kg/d; therefore, they were most certainly in a positive energy balance. All heifers showed a dramatic increase in serum NEFA concentrations on the day of slaughter. Agnes et al. (1990) and Sartorelli et al. (1992) reported that simulated transport for 30 min increases plasma NEFA concentrations. The increase in serum NEFA concentrations on the day of slaughter may be attributed to the stress of the trailer ride to the abattoir as well as the stress of an unfamiliar environment.

SUMMARY AND CONCLUSIONS

The objective of the experiment described in this thesis was to determine the effects of rearing dairy heifers at a constant growth rate of either .8 kg/d or 1.2 kg/d with and without daily injection of bST on body growth rates, carcass composition, age at puberty, and growth and metabolic activity of mammary parenchyma from 4 mo of age until the slaughter at the fifth estrous cycle after puberty. Weekly weights of the heifers indicate that both the high diet and injection of bST increased body growth. Percentages of protein, water, and fat in the carcass was calculated from the 9-10-11 rib section to determine effects on body composition. Withers height at puberty and pelvic area at slaughter were measured as indices of bone growth. Heifers fed the high diet had greater percentages of protein, water and fat as well as greater total protein and fat than heifers fed the standard diet. However, heifers were of similar heights and had similar pelvic areas regardless of diet. Thus, the high diet increased growth of soft tissue, while not affecting growth of bone. Injection of bST increased the percentages of protein and water, while decreasing fat in the carcass. Injection of bST increased withers height and pelvic area. These data indicate bST not only increased growth of muscle but increased growth of bone and decreased fat deposition. Feeding the high diet decreased age at puberty, but did not affect

weight or withers height at puberty. Injection of bST did not affect the age at puberty, but increased both weight and withers height at puberty. Thus, all animals were of adequate size and weight to breed at the time of, or shortly after puberty. The major objection to rearing dairy heifers at a high growth rate is compromised mammary development and decreased subsequent milk production. Mammary glands were dissected, and parenchyma was analyzed for DNA and RNA content as indices of mammary development and metabolic activity, respectively. Feeding heifers the high diet did not reduce the total amount of dissectable parenchyma, mammary cell numbers, or metabolic activity. Furthermore, injection of bST increased total dissectable parenchyma, mammary cell numbers, and metabolic activity. Thus, injection of bST increased mammary development regardless of diet.

In conclusion, allowing dairy heifers ad libitum access to a diet high in both protein and energy combined with injection of bST has the potential to decrease age at first calving without reducing future milk production.

APPENDIX A

Adjustment of dietary ingredients

Nutrient content of the diets were adjusted during the first 30 d of the experiment to produce the desired body weight gains. After experimental d 30 any adjustments to the diets were to compensate for either a change in haylage DM or new haylage. Due to the length of time the heifers were on the experiment, haylage was obtained from one of several different silos. When one silo was empty we would switch to another that contained a haylage of similar quality. Both the high and standard diets contained a grain mix. The grain mix for the standard diet was used to supply vitamins and minerals. The grain mix for the high diet was used to supply supplemental energy and protein (soybean meal and Purina 19A6 protein blend). The protein to energy ratios were similar between the two diets.

Table 1 summarizes the composition of the diets. The experiment was divided into periods. A period was defined as a length of time that a diet was fed in which there was no change in diet DM or silo used. Average daily intake per animal on an as-fed basis was calculated for each treatment (pen) as follows. Total weight of orts was subtracted from total weight of feed offered and divided by the number of animals in the pen. Average as-fed intake was multiplied by the

DM of the diet to calculate average daily DMI per animal. Total DMI per animal for each period was calculated by summing all of the average daily DMIs for that period. For example, if we fed from the same silo from Nov 1 to Jan 15, and DM of the silo did not change, average daily DMI per animal was summed from Nov 1 to Jan 15 to get the total average DMI per animal for each period. Using this estimate of total average DMI per animal for each period, average nutrient intake per animal was calculated for each nutrient for each period. Nutrient intakes were then totaled across periods for each diet and used to estimate the composition of the diets.

APPENDIX B

Dry matter intake and body weights

The average dry mater intake per animal for each treatment over the course of this experiment (Figure 3) gradually increased as the body weights of the animals (Figure 4) increased. Dry matter intake was not statistically analyzed because each treatment was group fed, thus producing an n of 1 per treatment

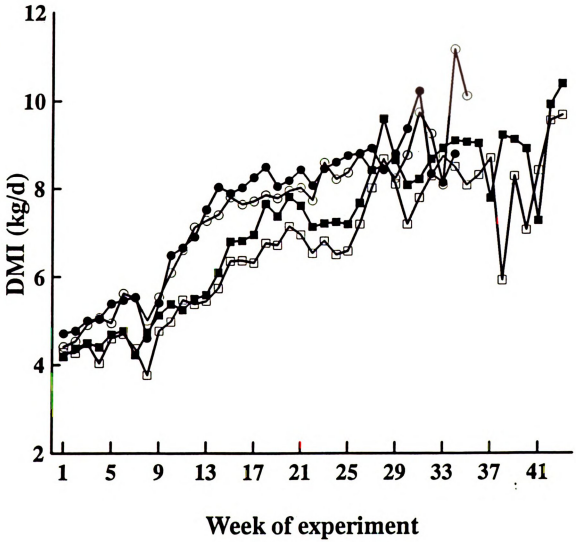

Figure 3. Dry matter intake heifers fed high control diet (○; n=10), high diet and injected with bST (●; n=10), standard control diet (□; n=9), standard diet and injected with bST (■; n=9). Each point represents the average weekly DMI of a treatment group.

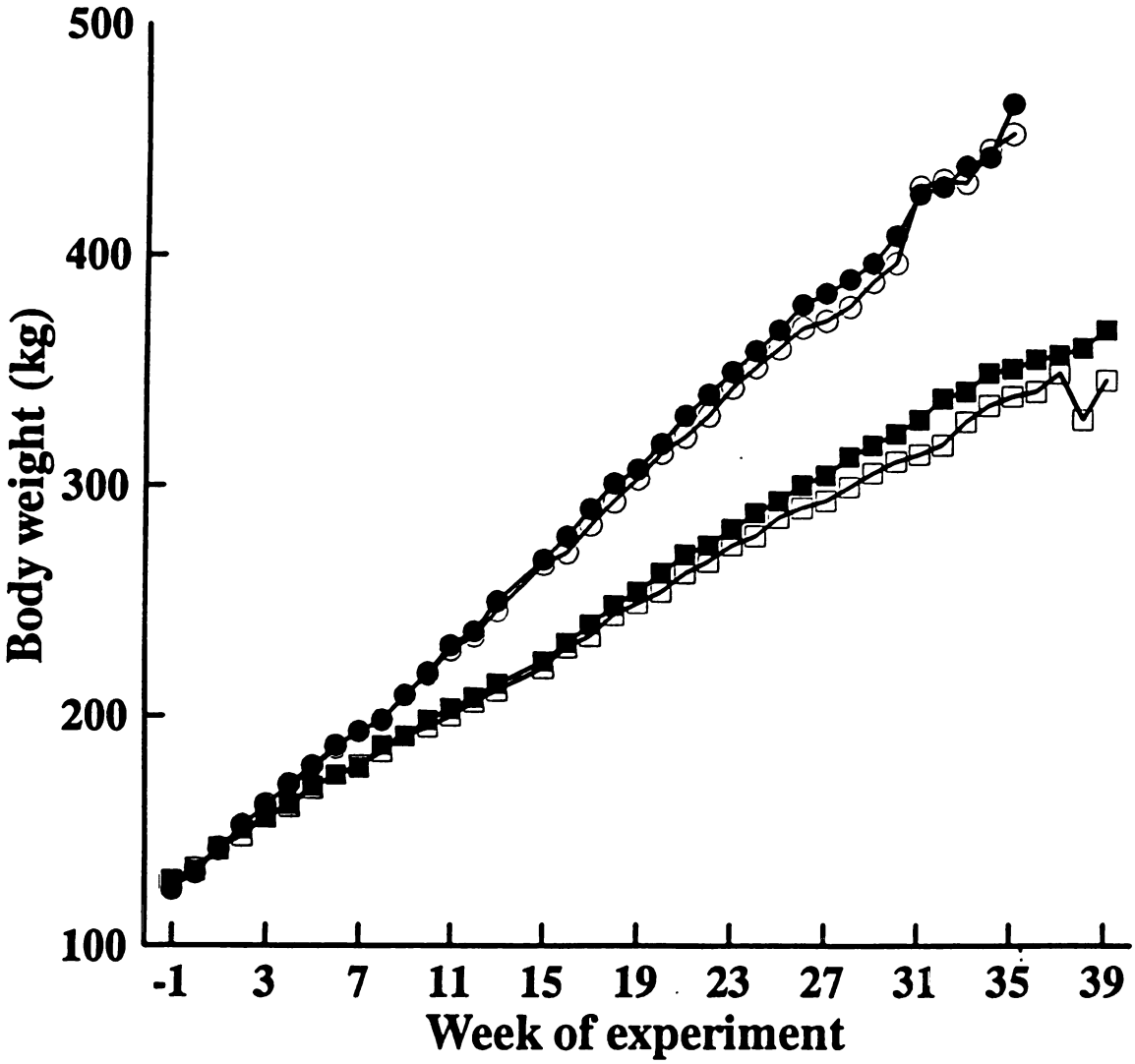

Figure 4. Weekly body weights of heifers fed high control diet (○; n=10), high diet and injected with bST (●; n=10), standard control diet (□; n=9), standard diet and injected with bST (■; n=9). Each point represents the average weekly weight of a treatment.

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