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thesis entitled

INFLUENCE OF DIETARY PROTEIN ON
PREPUBERTAL MAMMARY GLAND DEVELOPMENT
IN RAPIDLY-GROWN DAIRY HEIFERS

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

Major professor

Date 8-2-99

INFLUENCE OF DIETARY PROTEIN ON PREPUBERTAL MAMMARY GLAND DEVELOPMENT IN RAPIDLY-GROWN DAIRY HEIFERS

By

Brian Keith Whitlock

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1999

ABSTRACT

INFLUENCE OF DIETARY PROTEIN ON PREPUBERTAL MAMMARY GLAND DEVELOPMENT IN RAPIDLY-GROWN DAIRY HEIFERS

By

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My hypothesis was that high dietary protein would enhance mammary development in prepubertal Holstein heifers fed a high-energy diet for rapid body growth (1.2 kg/d). Heifers (n = 54) were fed a totally mixed ration of 2.85 Mcal ME/kg with low (14% CP; LP), standard (16% CP; SP), or high (19% CP; HP) protein from 3.5 mo of age until slaughter at ~6 wk after puberty. Average daily BW gain for heifers on the low, standard, and high-protein treatments were 1130, 1170, and 1180 g/d, respectively. Dietary protein did not affect age or BW of heifers at puberty or slaughter, carcass composition, or mammary development. Average parenchymal DNA content for heifers on the low, standard, and high-protein treatments was 947, 1005, and 1054 mg/hemigland, respectively. In conclusion, increasing dietary CP from 14 to 19% does not have a major effect on mammary development of rapidly-grown prepubertal heifers.

ACKNOWLEDGEMENT

First, I would like to thank the many dedicated people that provided friendship, help and guidance throughout my M.S. program. I thank my major professor, Dr. Michael VandeHaar, for his guidance and patience as my mentor and my friend.

To the members of my guidance committee: Drs. H. Allen Tucker, Margaret Benson and Ronald Erskine, I am grateful for their contributions to my research project as well as my graduate education as a whole.

I would like to thank Mr. Jim Liesman for his invitations to football games and his help in the laboratory with the analysis of my data. I also thank Dr. Roy Fogwell and Mr. Roy P. Radcliff for their friendship, humor, insight and ovary fondling expertise. I thank Dr. Joe Domecq, Mr. Tom Pilbeam and Mr. Doug Mashek for assigning body condition scores to my heifers. I thank Ms. Kristin Perkins, Mr. Luis Silva and Ms. Maria Zavala for their friendship and assistance at the barn and the meats laboratory. To Ms. Tasha Lapinski, Ms. Theresa Piotter, Ms. Charity Koontz and Mr. Jason Horgan, I am thankful for their hard work and long hours at the barn and in the laboratory. I would like to thank Mr. Larry Chapin and Dr. Chris McMahon in the Animal Reproduction Laboratory and Mr. Dave Main and Mr. Dewey Longuski in the Forage Nutrition Laboratory for their help and patience with various lab techniques. I thank the crews at the beef cattle research center and the meats laboratory for their friendship and hard work.

I wish to thank Michigan Soybean Promotion Committee and Michigan Agricultural Experimental Station for the financial support of this experiment.

I would like to express my deepest gratitude to my family. To my parents, Rex and Mona Whitlock, I am grateful for the unconditional love, strong Christian guidance, and friendship you have always shown me. Also, thanks to my brother, Jon, and my grandmothers, Nonie and Mammaw, for their love and support. Finally, thanks to my wife, Lynette, for all her encouragement, love, and patience.

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INTRODUCTION

Fifteen to twenty percent of the overall expense of milk production is incurred by heifer replacement programs (Heinrichs, 1993). Many farmers have focused on trying to decrease the cost of rearing heifers as a means to increase farm profitability. One way to lower rearing cost is to reduce the age at first calving (AFC). Since gestation is ~284 d, reducing age at conception is the only way to decrease AFC. To achieve an earlier conception age, body growth rate must increase or body size at breeding must decrease. A smaller breeding size results in a smaller calving weight and a reduction in subsequent milk production (Keown and Everett, 1986; and Heinrichs and Hargrove, 1987). A faster rate of growth will enable earlier calving and optimal body size at calving.

To achieve an optimal postpartum body weight, at the recommended AFC of 24 mo, a heifer must gain ~800 g of BW/d. Body weight gains of ~1100 g/d must be attained during the middle of the growth curve if earlier calving (~20 mo) is to be achieved. Whereas growing prepubertal heifers at a faster rate of gain with higher energy diets solves the size problem, it creates another problem: namely, it decreases mammogenesis and subsequent milk production (Swanson, 1960; Gardner et al., 1977; Little and Kay, 1979; Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984; Valentine et al., 1987; Gardner et al., 1988; Peri et al., 1993; and Capuco et al., 1995).

However, in some studies (Capuco et al., 1995; Radcliff et al., 1997; and Van Amburgh et al., 1998), growing heifers faster did not reduce mammary development or subsequent milk production. In these studies, prepubertal dairy heifers were fed diets that were not only high in energy but also protein.

The objective of this thesis was to determine the effect of dietary protein concentration on body growth, carcass composition, and mammary development in prepubertal heifers fed high-energy diets for rapid body weight gain.

REVIEW OF LITERATURE

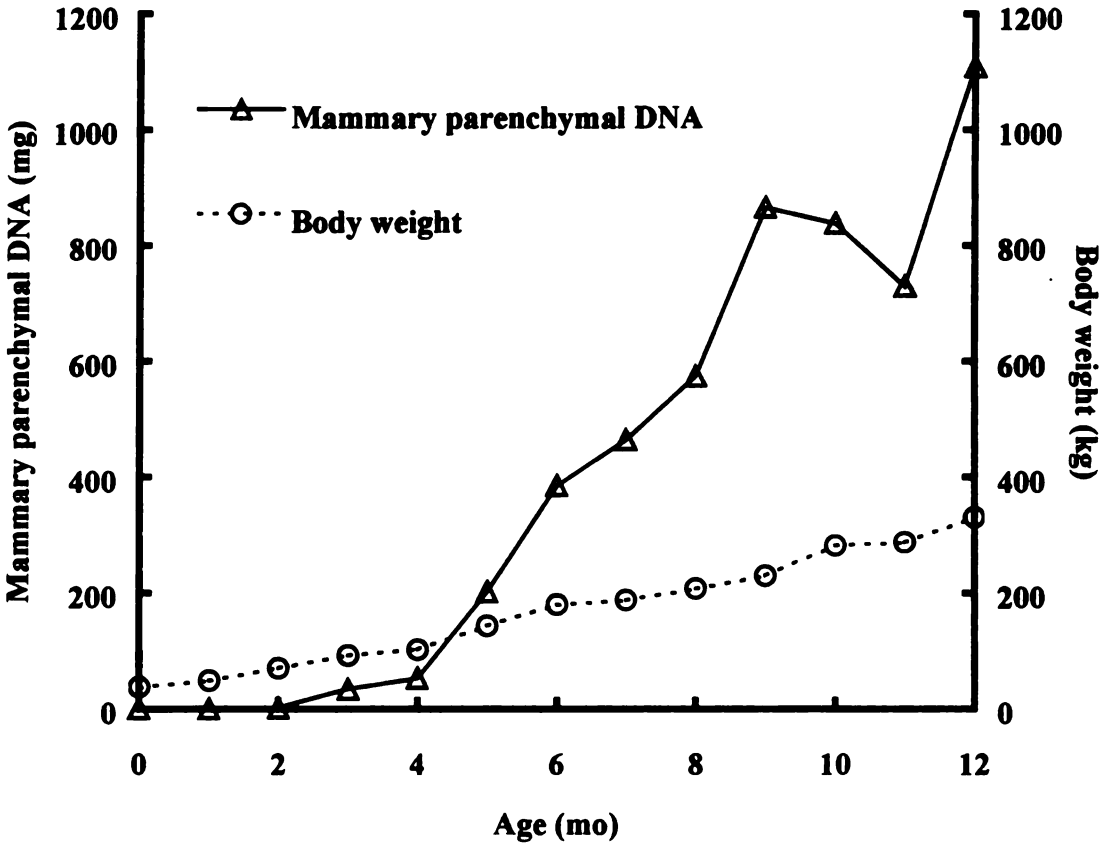
Mammogenesis from birth to conception

Cells, tissues, and structures

In contrast to most other organs, growth and differentiation of normal mammary glands occurs mostly in the postnatal state in a discontinous fashion. The mammary glands of newborns contain rudimentary primary and secondary sprouts (parenchyma) derived embryologically from ectoderm, wheras surrounding stroma and adipose tissue are from mesoderm. At birth the parenchyma consists of a restricted duct system, whereas the nonglandular stroma is proportionately larger and in mature form (Tucker, 1969).

During the first 2 to 3 months after birth, development of the heifer mammary gland is mainly due to an increase in connective tissue and deposition of fat (Schmidt, 1971). The increase in mass parallels the increase in body growth, and this phase is therefore referred to as isometric growth. At 2 to 3 months of age, well in advance of the first estrous cycle, the mammary gland begins to grow as much as three times faster than general body growth, and this phase is referred to as allometric growth (Figure 1) (Sinha and Tucker, 1969). Allometric mammary growth is characterized by ductal elongation and canalization into growing stroma (Reece, 1958). Allometric growth of the mammary

Figure 1. Mammary development in Holstein heifers from birth through 12 months of age. Modified from Sinha and Tucker (1969).



gland continues for several estrous cycles after onset of puberty, at which time it returns to an isometric growth rate until conception (Sinha and Tucker, 1969).

Prior to puberty in the rodent, expansion of the mammary ducts is the result of rapid proliferation of the end buds. These club-shaped structures occur at the elongating distal ends of the mammary ducts. In the mouse, end buds extend rapidly into the fat pad, occasionally bifurcating to form new growing ducts. The end buds push between the adipocytes until they encounter the margin of the fat pad, at which time rapid growth ceases and the end buds regress (Williams and Daniel, 1983). After puberty, the gland consists of an extended duct system, but alveolar development does not occur until after conception. Alveoli are small almost spherical structures located at the end of ducts and are made up of a single layer of epithelial cells that line the lumen of the alveoli; these are the cells that will synthesize and secrete milk (Tucker, 1969). Although most mammary growth occurs during pregnancy, the daughter cells that make alveolar formation possible depend on the ducts that arise during the peripubertal period (Smith and Medina, 1988).

Mammary development is less understood in ruminants, but some distinct differences with rodent mammary development exists. Based on Ellis and Akers (1995), the mammary glands of prepubertal lambs lack the end buds seen in the mouse mammary gland. The quantitative study of mammary slices of heifers (Sejrsen, et al., 1986) and lambs (McFadden et al., 1990) demonstrate that most of the mammary fat pad of peripubertal ruminants is devoid of epithelial ducts and the ducts in the parenchyma are closely packed. This suggests a more complex tubular structure than in the rodent. To use an analogy, peripubertal rodents have widely spaced mammary ducts that fill the

mammary fat pad like the bare branches of a tree. In contrast, peripubertal ruminants have closely packed ducts that radiate from the gland cistern in broccoli-like fashion, but the ducts fill only a fraction of the mammary fat pad. Therefore, these differences suggest that rodent mammary development should not be assumed to accurately describe ruminant mammary development.

Regulation of mammogenesis

Somatotropin and estrogen are essential for normal mammary development (Lyons et al., 1958; and Cowie et al., 1966). Ovariectomy in the first week of life abolishes mammary growth (Wallace, 1953), and removal of the ovaries of heifers at 2.5 months of age also inhibits mammary growth (Purup et al., 1993b). This dramatic effect of ovariectomy on mammary growth likely is due to removal of estrogen secreted by the ovaries. Estrogen can restore growth of the mammary glands in ovariectomized heifers (Wallace, 1953). Furthermore, Woodward and others (1993b) showed that exogenous estradiol stimulates proliferation of mammary epithelial cells in gonadally intact prepubertal heifers. However, differences between circulating levels of estradiol in intact (0.41 pg/ml) and ovariectomized (0.31 pg/ml) prepubertal heifers are small (Purup et al., 1993b). This difference in estradiol concentration seems too small to be responsible for the complete block of mammary growth in ovariectomized heifers. In addition, mammary explant cultures are more likely to proliferate in response to estradiol than isolated mammary epithelial cells (Purup et al., 1993b). Woodward and others (1993a) observed that treatment of bovine fibroblasts with estradiol induced secretion of a substance that stimulates proliferation of mammary cells. The idea that estrogen's

stimulation of epithelial cell proliferation is mediated by autocrine and/or paracrine secretion of growth factors is referred to as the estromedin hypothesis (Sirbasku, 1978). Asynchronous secretion of estrogen and progesterone is a possible cause of the return of mammary growth to an isometric rate after puberty (Tucker, 1981). However, immunization of heifers against gonadotropin-releasing hormone, which decreases serum estrogen and progesterone, does not alter mammary growth (Sejrsen, 1994).

The anterior pituitary hormones are absolutely critical for mammary development. Utilizing ablation/replacement experiments, Talwalker and Meites (1961) reported that hypophysectomy reduces mammary development in rats and exogenous prolactin and somatotropin restores mammary development. In addition, exogenous somatotropin increases mammary growth in ruminants (Sejrsen, 1986; Sandles and Peel, 1987; McFadden et al., 1990; Glasser et al., 1991; and Radcliff et al., 1997), but there is little evidence that somatotropin has a direct effect on the mammary gland. Although somatotropin receptor mRNA as been detected in bovine mammary tissue (Glimm et al., 1990), research has failed to detect somatotropin receptors in ruminant mammary tissue (Keys and Djianne, 1988; McFadden et al., 1990; and Purup et al., 1995). In addition, somatotropin does not stimulate proliferation of isolated bovine epithelial cells (Collier et al., 1993). Most evidence suggests that somatotropin does not act directly but rather it acts indirectly on the mammary gland by factors such as insulin-like growth factor-I (IGF-I) (Akers, 1990).

Administration of somatotropin in cattle increases serum IGF-I concentrations (Bauman and Vernon, 1993; Dahl et al., 1993; Purup et al., 1993a; Sharma et al., 1994;

Vanderkooi et al., 1995; Sharma et al., 1996; and Yung et al., 1996), and liver IGF-I mRNA abundance (Sharma et al., 1994; Vanderkooi et al., 1995; and Sharma et al., 1996) but has no effect on IGF-I mRNA abundance in the mammary gland (Sharma et al., 1994). Receptors for IGF-I are present in mammary tissue (Purup et al., 1995) and in isolated bovine mammary cells (Romagnolo et al., 1994), and IGF-I stimulates proliferation of isolated bovine mammary epithelial cells (Zhao et al., 1992; Romagnolo et al., 1992; and Collier et al., 1993). In addition, there are at least six different IGF-I binding proteins (IGFBPs) that modulate the biological activity of IGF-I (Clemmons, 1997). Sharma et al. (1997) reported that administration of somatotropin to prepubertal heifers decreased hepatic mRNA and serum concentrations of IGFBP-2 ~70 and ~54%, respectively. In addition, these changes were associated with increases in prepubertal mammary development (Radcliff et al., 1997). Thus, local production of IGF-I and/or IGFBPs in the mammary gland may modify the relationship between somatotropin, IGF-I and mammary growth.

Biology of body growth

Growth is the increase in the physical size of the body and increase in the total weight of the muscle and various internal organs. Growth results from an increase in both size and number of cells. An increase in number of cells is called hyperplasia; an increase in cell size is called hypertrophy. The various body parts grow in an orderly manner, with the various tissues growing at different rates from birth to maturity. One organ or tissue may begin rapid growth at a time when the growth rate of other organs or

tissues is slower. For example, the maximum rate of growth of the nervous system occurs early in development, and is followed by the skeleton, then by muscle, and finally by adipose tissue.

Shortly after birth, bone grows more rapidly than other tissues in proportion to total body weight. This rapid rate of skeletal growth decreases dramatically by 8 to 10 months of age in cattle (Berg and Butterfield, 1968). However, new bone is constantly being laid down and reabsorbed by the body throughout life. An equilibrium between new growth and reabsorption maintains mature bone size.

Maximum muscular development occurs later than maximum skeletal growth. Growth of muscle as a function of time is a sigmoidal curve: i.e., at first growth is slow, then increases dramatically before slowing again. Postnatal muscle growth is a process of hypertrophy and not hyperplasia (Burleigh, 1974; and Goldspink, 1974). As a myofiber grows, nuclei are added from mitosis of satellite cells, which reside between the basement membrane and the sarcolemma. Proteins are continually being synthesized and degraded and the balance between these two processes dictates the rate of protein deposition and muscle growth. When protein synthesis is greater than protein degradation, protein accretion or net muscle growth occurs.

Accretion of adipose tissue increases as the rate of muscle growth declines. The major function of lipid in an animal is long-term storage of energy (Leat and Cox, 1980). Thus, as net growth of bone and muscle stops, adipose accretion may continue as long as nutrient availability permits (Hammond, 1960). Similar to muscle, accretion of adipose

tissue is dependent upon rates at which lipid is deposited (lipogenesis) and removed (lipolysis).

Effects of prepubertal diet

Mammogenesis and milk production

Previous studies indicate that rapid body growth in prepubertal heifers impairs mammary development (Table 1) (Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984; Valentine et al., 1987; and Capuco et al., 1995) and subsequent milk production (Table 2) (Gardner et al., 1977; Little and Kay, 1979; Valentine et al., 1987; Peri et al., 1993; and Van Amburgh et al., 1998). This decrease in mammogenesis and milk production associated with accelerated rates of gain is often attributed to truncated parenchymal development and excess fat deposition in the mammary gland (Swanson, 1960). Prepubertal growth rate is inversely related to age at puberty (Schillo et al., 1992). Therefore, rapidly-grown prepubertal heifers will attain puberty before conventionally-reared heifers. Since mammary growth returns to an isometric rate shortly after puberty (Sinha and Tucker, 1969), reducing age at puberty by rapid prepubertal growth will truncate the allometric growth phase and thus may impair mammary development. For example, Sejrsen et al. (1982) fed pre- and postpubertal heifers a 15% CP diet for ad libitum or restricted intake to produce 1200 and 600 g of BW gain/d, respectively. All prepubertal heifers were slaughtered at 320 kg BW and heifers fed for ad libitum intake had ~30% less mammary parenchymal DNA compared with heifers on restricted feeding. However, treatment did not effect mammary development of postpubertal

Table 1. The influence of prepubertal feeding level on mammary development in dairy heifers.

Study	BW gain g/d	CP:ME[1]	% of control[2]
Sejrsen et al., 1982	1270	52	68%
Harrison et al., 1983	1180	43	59%
Petitclerc et al., 1984	1030	48	75%
Valentine et al., 1987	950	52	59%
Stelwagen and Grieve, 1990	1000	58	115%
Capuco et al., 1995	1010	54	52%
Capuco et al., 1995	970	83	94%
Radcliff et al., 1997	1190	68	102%

[1] g crude protein/Mcal metabolizable energy.
[2] Mammary development of rapid gain group relative to control group.

Table 2. The influence of prepubertal feeding level on future milk production in dairy heifers.

Study	BW gains g/d	CP:ME[1]	% of control[2]
Gardner et al., 1977	1100	55	82%
Little and Kay, 1979	1090	43	48%
Valentine et al., 1987	950	52	72%
Peri et al., 1993	1090	51	84%
Radcliff et al., 1998	1200	68	88%
Van Amburgh et al., 1998	950	64	95%

[1] g crude protein/Mcal metabolizable energy fed during prepubertal treatment period.
[2] First lactation milk production of cows grown rapidly during the prepubertal period relative to cows grown at conventional rates during the prepubertal period.

heifers. Thus, the critical period in which excessive rate of gain reduces mammary development and future milk production is before puberty.

As Table 1 shows, responses of mammary growth to rapid prepubertal BW gains in the literature have been inconsistent and ranges from no impairment to 60% less mammary tissue than conventionally grown heifers. Some of the variation might be explained by differences in laboratory techniques used to quantify mammary development. For example, Stelwagen and Grieve (1990) measured total mammary DNA instead of DNA in parenchyma. The different responses might also be the result of differences in length of treatment periods or the dietary methods implemented to achieve rapid gains. For example, some reports indicate that the ratio of protein to energy in the diet may explain some of the variation in effects of accelerated prepubertal growth on mammary development and subsequent milk production (Capuco et al., 1995; Pirlo et al., 1997; Radcliff et al., 1997; and VandeHaar, 1997).

Capuco et al. (1995) fed prepubertal Holstein heifers either an alfalfa-based high-protein diet (83 g CP/Mcal ME) or corn silage-based low–protein diet (54 g CP/Mcal ME) to achieve rapid (~1000 g/d) or control (~700 g/d) BW gains. Heifers were killed when they weighed at least 325 kg and had completed two or more estrous cycles. Mammary development was impaired 48% when accelerated gains were produced in heifers fed the corn silage-based diet but no impairment of mammary development when accelerated gains were produced by the alfalfa-based diet. Radcliff et al. (1997) fed prepubertal Holstein heifers a high-energy, high-protein (68 g CP/Mcal ME) diet to produce ~1200 g of BW gain/d or a control diet to produce ~800 g of BW gain/d from 4

mo of age until the fifth estrous cycle. Compared with control heifers, heifers fed the high-energy, high-protein diet reached puberty and were killed 1.6 mo earlier. However, the accelerated prepubertal growth rate did not impair mammary development. In a study by Pirlo et al. (1997), heifers were fed diets that were 10% above or below NRC (1989) recommendations for protein and energy from 100 to 300 kg BW. Heifers fed the high energy with high protein produced 5% more fat-corrected milk than heifers fed the high energy with low protein, and they produced 95% as much fat-corrected milk as control heifers. VandeHaar (1997) examined the relationship between mammary development or milk yield and the dietary protein to energy ratio from 11 studies in which rapid gain heifers exceeded 900 g BW gain/d. Estimated dietary protein to energy ratios varied considerably among the studies, from 43 to 83 g CP/Mcal ME. Across studies, mammary development of rapidly-grown heifers relative to their controls was positively correlated with the protein to energy ratio of the diets they were fed. Furthermore, the protein to energy ratio accounted for 51% of the variation in mammary parenchyma responses and 78% of the variation in milk yield responses to rapid growth rate.

A major mediator of dietary effects on mammary development may be differences in endogenous hormone secretions. For example, Sejrsen et al. (1983) reported that serum somatotropin concentrations and mammary development were reduced in prepubertal heifers fed for ad libitum intake compared with heifers fed restricted intake. Sejrsen hypothesized that the decreased somatotropin concentrations were responsible for the impaired mammary parenchymal growth observed for heifers fed a high-energy diet for ad libitum intake. However, when Capuco et al. (1995) gave prepubertal heifers free

access to an alfalfa-based diet to produce rapid BW gains, serum somatotropin concentrations only tended to be decreased and mammary development was not impaired. Whereas, accelerated growth from ad libitum intake of a corn silage-based diet decreased serum somatotropin concentrations and mammary development 25 and 48%, respectively. Similarly, when Radcliff et al. (1995) fed prepubertal heifers a high-protein diet to produce ~1200 g of BW gain/d, serum somatotropin concentrations and mammary development were unaffected compared with control heifers. These studies suggest that inadequate protein might have been responsible for the impaired mammary development and subsequent milk production of heifers grown rapidly before puberty in previous literature.

MATERIALS AND METHODS

Management of animals and treatments

Sixty-four Holstein heifers [approximate age = 11 wk and mean BW (±SEM) = 101 ± 1 kg] were purchased in 3 consecutive weeks between May 4 and May 19 (~20 heifers/wk): each week being a different age block. All animals were allowed 30 d to acclimate to new surroundings and were monitored for illnesses at the Michigan State University Dairy Teaching and Research Center. Heifers were grouped by age block and allowed free access to an outside paddock during the acclimation period. On the first acclimation day all heifers were injected with 10 mg/kg BW of Micotil® (Elanco Animal Health, A Division of Eli Lilly and Company) as a prophylactic. Rectal temperatures were determined for the first 5 d of the acclimation period. Heifers were injected with Micotil® a second time if rectal temperatures were above 39.7°C. During the first half of the acclimation period heifers were fed the same 15% CP complete feed and alfalfa-orchard grass hay they received prior to purchase. During the second half of the acclimation period heifers were fed an adjustment ration that initially contained 75% of the previously fed complete feed and 25% of a total mixed ration similar to the treatment diets. The percentage of complete feed was decreased over 1 wk until the diet was solely

the total mixed ration. During the last wk of acclimation heifers were fed the total mixed ration.

Following the acclimation period, heifers were transported to the Michigan State University Beef Cattle Research Center. Within each age block, the eighteen heifers with the greatest rate of BW gain during the acclimation period were blocked by BW into groups of three and randomly assigned to one of three treatments. All heifers for a given treatment within each age block were housed in the same pen. Thus, three pens of six heifers (one pen per age block) represented each of the three treatments. Treatments began at 106 d of age and continued until the early luteal phase of the fourth estrous cycle.

Each of the three treatment diets was 40% alfalfa-grass haylage and 60% grain and contained ~2.85 Mcal ME/kg. This energy density was expected to produce 1200 g of BW gain/d when diets were fed ad libitum. Diets were low (14% CP; n = 18; LP), standard (16% CP; n = 18; SP), or high (19% CP; n = 18; HP) protein. The alfalfa-grass haylage was the first-cutting from a single field and harvested and stored in a bag during the early bloom period. Haylage samples were collected twice a week to assess dry matter content and haylage was collected every other week to assess protein and fiber content. Samples of ground corn, soybean meal-48, and expeller soybean meal were collected upon purchase to assess protein and energy content. Composition of diets based on actual analysis is described in Table 3. Diets were fed as a TMR fresh every day between 0900 and 0930 h and heifers had free access to water and the respective diet. Orts for each pen were collected at 0700 h and weighed daily. Mean dry matter intake

Table 3. Composition of low, standard, and high protein diets.

	Low	Standard	High
Ingredients, % of DM			
Alfalfa-grass haylage[1]	40.0	40.0	40.0
Ground corn[2]	54.0	48.1	42.2
Soybean meal[3]	5.0	5.0	5.0
Expeller soybean meal[4]	0.0	5.9	11.8
Minerals and vitamins[5]	1.0	1.0	1.0
Nutrient composition			
NDF[6], % of DM	25.1	25.4	25.6
ADF[7], % of DM	13.9	14.3	14.7
ME[8], Mcal/kg	2.85	2.85	2.85
NEm[9], Mcal/kg	1.90	1.91	1.91
NEg[10], Mcal/kg	1.26	1.27	1.27
CP, % of DM	13.7	16.2	18.8
RUP[11], % of CP	33.4	36.0	37.9
AP[12], % of DM	10.6	11.6	12.7
CP:ME, (g CP/Mcal ME)	48.0	57.0	66.0

[1] Haylage was analyzed every 2 wk and contained 15.3% CP (± 0.8 SD), 47.1% NDF (± 1.6 SD) and 30.0% ADF (± 0.8 SD) and energy values were estimated to be 2.43 Mcal ME/kg, 1.55 Mcal NEm/kg, and 0.95 Mcal NEg/kg.

[2] Ground corn was analyzed twice and contained 9.5% CP (± 0.5 SD), 11.4% NDF (± 0.1), and 3.0% ADF (± 0.0) and energy values were estimated to be 3.18 Mcal ME/kg, 2.17 Mcal NEm/kg, 1.50 Mcal NEg/kg.

[3] Soybean meal-48 was analyzed once and contained 53.2% CP, 6.9% NDF, and 5.2% ADF and energy values were estimated to be 3.26 Mcal ME/kg, 2.24 Mcal NEm/kg, 1.55 Mcal NEg/kg.

[4] Expeller soybean meal was all from the same batch and was analyzed once. It contained 53% CP, 8.0% NDF, and 4.2% ADF and energy values were estimated to be 3.21 Mcal ME/kg, 2.20 Mcal NEm/kg, 1.52 Mcal NEg/kg.

[5] Mineral and vitamin mix contained 23.8% white salt, 10.4% trace mineral-vitamin premix and 4.0% DECCOX-10 Etts (Purina Mills; .5% decoquinate) and was formulated so the diet provided 100% mineral and vitamin requirement (NRC 1989).

[6] Neutral detergent fiber.

[7] Acid detergent fiber.

[8] Metabolizable energy.

[9] Net energy for maintenance.

[10] Net energy for gain.

[11] Rumen-undegraded protein using book values of 21%, 50%, 30%, and 50% of CP for alfalfa-grass haylage, ground corn, soybean meal, and expeller soybean meal.

[12] Absorbed protein = .8[.8(MCP g/d) + (%RUP as %CP)(CP g/d)], MCP = (38 g/Mcal)(ME intake Mcal/d).

for a pen was recorded. Heifers were exposed to ambient temperatures and photoperiod from the time of purchase until slaughter.

All heifers were weighed at ~0800 h before feeding on 2 consecutive d each week to monitor BW gain. The mean of the two weights was then assigned as a heifer's weekly weight. Weekly weights were used to calculate average daily BW gains. The height at the withers was measured every 2 wk. Body condition score (BCS) was assessed using a five-point scale (1 = thin, 5 = fat; Wildman et al., 1982) every 4 wk by three experienced examiners. The three scores for each heifer were averaged and assigned to that heifer as her monthly score.

Commencing when a heifer attained 215 kg of BW or 7 mo of age, which ever was first, her reproductive status was examined weekly to determine onset of puberty. A heifer was considered pubertal when a corpus luteum (CL) was detected on either ovary by palpation through the rectum. If a CL was present again 21 d following the first CL, heifers were injected with 25 mg of $PGF_2\alpha$ (Lutalyse®, Pharmacia & Upjohn Inc.) 3 d later. Eleven d after their first injection of $PGF_2\alpha$, heifers were rectally palpated again. If a CL was present they received another injection of $PGF_2\alpha$. Eleven d after the second injection of $PGF_2\alpha$, heifers were rectally palpated again. If a CL was present, heifers were killed on the following day or d 47 after detection of the first CL.

On the day of slaughter, heifers were transported to the Michigan State University Meats Laboratory at ~0630 h. All heifers were weighed, stunned by captive bolt, and killed by exsanguination between 0700 and 1000 h. The number of heifers killed each week depended on the date for detection of the first corpus luteum and ranged from 1 to 6 heifers. All heifers were slaughtered at a mean of 46 ± 0.7 d after detection of the first corpus luteum.

Blood collection and analysis

Blood samples (~10 ml) were collected every 4 wk at ~0800 h via jugular venipuncture with Vacutainers® (Becton Dickinson & Co., Rutherford, NJ). All samples were stored at room temperature (~21°C) for ~6 h and then at 4°C ~15 h. Serum was harvested after centrifugation at 1550 x *g* and 4°C for 25 min and then frozen at -20°C. Serum samples were assayed for insulin-like growth factor-I concentrations using

ethanol/acid extraction (Bruce et al., 1991) and radioimmunoassay according to GroPep Pty Ltd (Growth Factors Products and Protocols, Adelaide, Australia) with Gro*Pep*'s IGF-I standard and primary antibody but modified as in Sharma et al. (1994) with Staphylococcus *aureus* used in place of the secondary antibody.

For slaughter dates in which each treatment was represented by at least one heifer, intensive sampling of blood for assessment of the profile of somatotropin concentration was completed 4 d before slaughter. Heifers were fitted with sterile indwelling jugular catheters (18 gauge; Ico-Rally, Palo Alto, CA) 5 d before slaughter. On the following d, serial blood samples were collected at 20-min intervals for 12 h (0700 to 1900 h). Twenty-one heifers, seven from the low-protein diet, six from the standard-protein diet, and eight from the high-protein diet, were bled for assessment of the profile of somatotropin concentration. Catheter patency was maintained between samples by flushing the catheter with 3.5% sodium citrate in sterile water. Serum was harvested and samples were stored as previously mentioned until serum concentrations of somatotropin were quantified using radioimmunoassay as in Gaynor et al. (1995).

Tissue collection

Mammary glands were quickly removed from the carcass and placed on a table with the ventral surface up. A metric ruler was used to measure from the base of each teat to its tip. The glands were bisected along the median suspensory ligament 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 hemiglands were stored at -20°C until analyzed.

The digestive tract was removed from the carcass, the gallbladder was removed from the liver, and the liver was weighed. The intestines were separated from the upper gastrointestinal tract at the pylorus. Approximately 80 to 90% of the fat was removed from the upper gastrointestinal tract and saved. Digesta were flushed from the intestines with water, and the intestines were cut several times to remove water. The intestines with omental fat were combined with the fat from the upper gastrointestinal tract and stored at -20°C until grinding and analysis of lipid content.

After the hide was removed, the carcass was split into halves along the vertebral column. 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 carcass was washed and stored at 2°C.

Carcass composition and analysis

Twenty-four h after slaughter, pelvic area was calculated from two linear measurements of the left half of the carcass, one from the ventral edge of the third coccygeal vertebrae to the symphysis pubis and a second at 90° from the midsagittal plane of the carcass to the middle of the pelvic wall. The second measurement was multiplied by two to represent the total width of the pelvic opening and then multiplied by the first measurement to estimate total pelvic area (Radcliff et al., 1997).

The left half of each carcass was cut between the 7th and 8th ribs and between the 12th and 13th ribs. The rib section, including ribs 8 through 12, was removed. The section containing ribs 9, 10, and 11 was then dissected (Hankins and Howe, 1946), weighed, and deboned. In addition to the rib sections from each carcass, the entire right half of 12 animals was deboned. Bone and soft tissue were weighed. Soft tissue was ground, mixed and subsampled for analyses of protein, fat, and water content. Crude protein content was determined in fresh samples by combustion and subsequent measurement of thermal conductivity using the Combustion method (AOAC, 1990, Method 990.03) utilizing the combustion nitrogen analyzer LECO® FP-2000 (Leco Corporation, St. Joseph, MI). Fat was determined by Soxhlet ether extraction of fresh samples (AOAC, 1990). Water was determined by the difference in weight after drying fresh samples in an oven at 110°C for 24 h. Carcass protein, fat, and water contents were estimated using equations based on the ninth-tenth-eleventh-rib cut (Hankins and Howe, 1946). Equations, were $Y = 5.64 + .69X$, $Y = 2.73 + .78X$, and $Y = 14.28 + .78X$, for the protein, fat, and water, respectively, where Y was the edible portion of the dressed heifer carcass, and X was the edible portion of the heifer three-rib cut. These estimates were compared with the actual measurements of the right half of 12 animals.

Ruminal fat and intestines were weighed and ground at the Michigan State University Poultry/Mink Farm. The ground tissue was mixed and subsampled for analyses of fat content. Fat was determined by Soxhlet ether extraction of fresh samples (AOAC, 1990).

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 both the anterior and posterior ends of the gland that did not contain parenchymal tissue were discarded. Slices were allowed to thaw slightly, and skin, teats, and supramammary lymph nodes were dissected. 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 was referred to as parenchymal tissue. Frozen parenchymal tissue was weighed and ground with liquid nitrogen into a fine powder with a blender. The powder was mixed and subsampled for subsequent analysis of DNA and RNA content (Tucker, 1964), dry matter, protein, and fat. Dry matter was determined by the difference in weight after drying fresh samples in an oven at 110°C for 24 h. Crude protein content was determined in fresh samples by combustion and subsequent measurement of thermal conductivity using the Combustion method (AOAC, 1990, Method 990.03) utilizing the combustion nitrogen analyzer LECO® FP-2000 (Leco Corporation, St. Joseph, MI). Fat was determined by Soxhlet ether extraction of fresh samples (AOAC, 1990).

Statistical analysis

Data for mean live body growth from d 0 until slaughter, carcass composition, mammary composition, and mammary nucleic acid content were analyzed by the GLM procedure of SAS® (1996). The model included treatment as a fixed effect and group as a random effect using the following equation:

Y = treatment + group + group*treatment + residual.

treatment = low, standard, and high protein diet

group = A, B, and C age group

residual = animal(group*treatment)

Differences were determined using orthogonal contrast for the linear and quadratic effects of the diet using the CONTRAST statement in PROC GLM of SAS® (1996). Least squares means and standard errors of least squares means are presented.

Mean concentrations of somatotropin and IGF-I were transformed by natural logarithm to eliminate heterogenous variance. Natural logarithm transformed concentrations of somatotropin and IGF-I were analyzed by the GLM procedure of SAS® (1996) using the following equation:

Y = treatment + group + group*treatment + animal(group*treatment) + time + time*treatment + residual.

treatment = low, standard, and high protein diet

group = A, B, and C age group

residual = time*group + time*group*treatment + animal(time*group*treatment)

Values presented for concentrations of somatotropin and IGF-I are means of untransformed data.

RESULTS

Eight heifers, three from both the low and standard protein diets and two from the high protein diet, were removed from the experiment. Four heifers were removed because they were freemartins'; one from each of the low and high protein diets and two were from the standard protein diet. Two heifers were removed due to complications from rectal palpation; both were from the low protein diet. Two heifers were removed from the experiment because of late onset of puberty; one was from both the standard and high protein diets. Treatments continued for 165, 164, and 160 ± 8 d for heifers receiving the low, standard and high protein diet, respectively (Table 4).

Live body growth and DMI

Initial measures of age, BW, height at the withers, and BCS were not different among treatment groups (Table 4). Figure 2 illustrates that dry matter intake and BW were unaffected by increasing dietary protein. Diet did not influence body weight at slaughter or final height at the withers. Since age at slaughter was similar for treatment groups, rates of BW and withers height gain were unaffected by the differences in dietary protein (Table 4). Because changes in dietary protein to energy did not influence DMI or BW gain, no difference in feed efficiency resulted with the gain:feed ratio for the low,

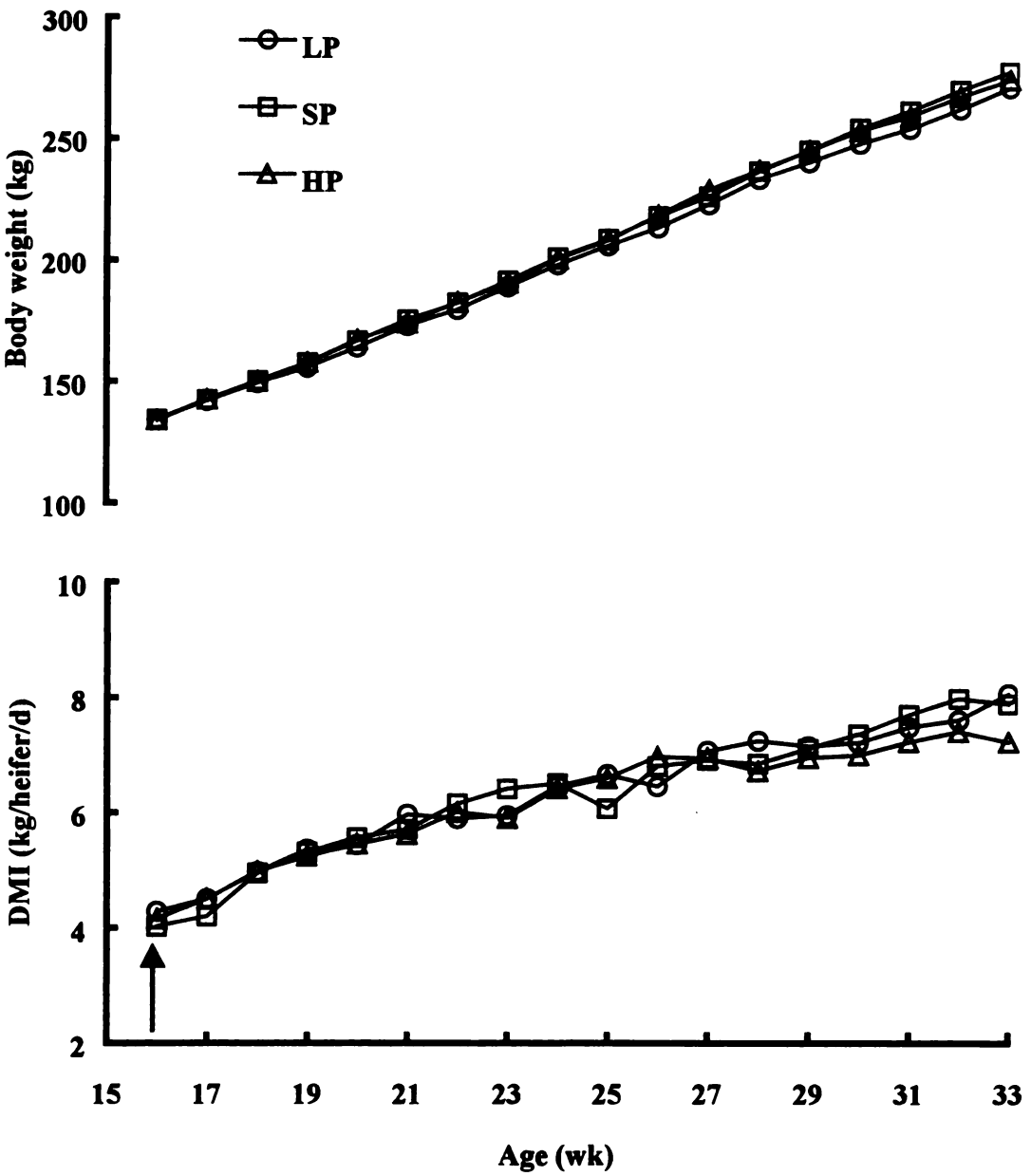
Table 4. Least squares means for body growth.

	Protein to Energy Level				Contrast	
	Low	Standard	High	SEM[1]	Linear	Quadratic
n	15	15	16		P	
Initial BW, kg	134	135	134	2	0.82	0.80
Initial BCS	2.7	2.7	2.8	0.1	0.46	0.69
Initial withers height, cm	96.9	96.9	96.8	0.6	0.95	0.98
Time on treatment, d	165	164	160	8	0.66	0.83
Age at slaughter, mo	8.9	8.9	8.8	0.2	0.66	0.83
BW at slaughter, kg	321	326	321	8	0.97	0.55
Final BCS	3.6	3.7	3.6	0.1	0.58	0.22
Final withers height, cm	118	119	118	1	0.90	0.67
BW gain, kg/d	1.13	1.17	1.18	0.03	0.25	0.62
BCS gain	0.9	1.0	0.8	0.1	0.19	0.16
Withers height gain, cm/d	0.15	0.15	0.16	0.01	0.32	0.74
Age at first corpus luteum, mo	7.5	7.5	7.4	0.2	0.90	0.76
BW at first corpus luteum, kg	266	274	271	6	0.61	0.51
Pelvic area, cm^2	206[a]	219[b]	204[a]	4	0.61	0.01

[1] Pooled standard error of means as calculated with n = 15

[a,b] Least squares means in rows with different superscripts differ quadratically ($p<0.05$).

Figure 2. Weekly body weights (top panel; n = 18 heifers/treatment) and dry matter intakes (bottom panel; n = 3 pens/treatment) of heifers fed low protein (LP), standard protein (SP), or high protein (HP). For BW, SEM = 3 and for DMI, SEM = 0.14. The arrow represents the time treatment started.



standard and high protein diet being 0.19, 0.20, and 0.20 kg BW gain/kg DMI, respectively (Figure 3). Similarly, change in body condition score throughout the experiment was unaffected by treatment. Dietary protein also did not affect age or body weight at the time of observed first corpus luteum. In contrast, diet altered pelvic area at slaughter. Heifers fed the standard-protein diet had greater pelvic areas than the other groups.

Carcass composition

Carcass weights and carcass weights as a percentage of live BW were similar among treatment groups (Table 5). Diet did not influence total liver weight or liver weight as a percentage of BW. As assessed using equations based on the edible portion of the ninth-tenth-eleventh-rib cut, percentage of carcass protein and total protein in the carcass were not affected by treatment. The standard diet decreased percentage of carcass fat relative to the low and high protein diets. Diet did not affect total perirenal or omental-intestinal fat.

In addition, diet did not influence the percentage of carcass protein, fat, or water as assessed by actual carcass analysis of 12 animals. Using equations based on the edible portion of the ninth-tenth-eleventh-rib cut (Hankins and Howe, 1946), the estimated mean percentage of carcass protein, fat, and water of 12 heifers was 17.9 ± 0.3, 20.0 ± 0.9, and 60.3 ± 0.7, respectively. The mean percentage of carcass protein, fat, and water determined from the edible portion of the right half carcass of the same 12 heifers was 18.9 ± 0.3, 17.9 ± 1.1, and 63.8 ± 1.0, respectively. The correlation coefficients between

Figure 3. Weekly gain to feed ratio (n = 3 pens/treatment) of heifers fed low protein (LP), standard protein (SP), or high protein (HP). SEM = 0.01. The arrow represents the time treatments started.

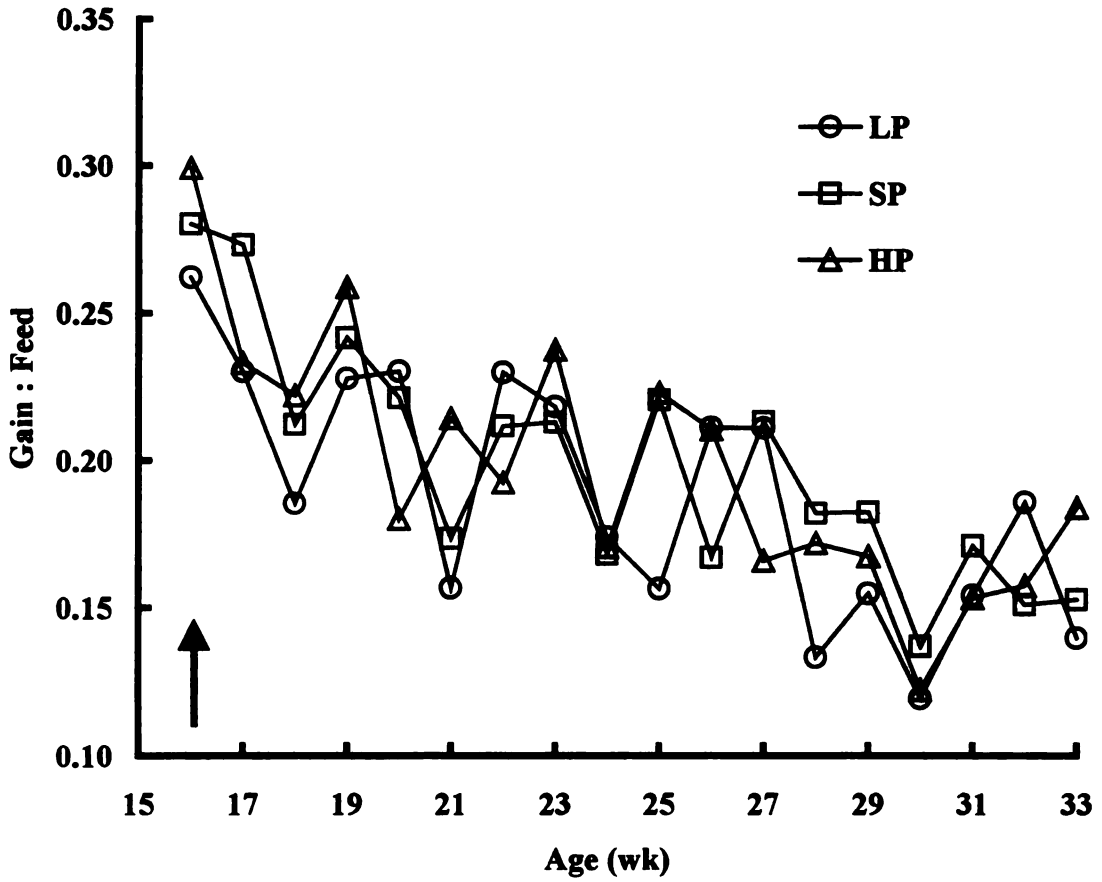


Table 5. Least squares means for carcass composition.

	Protein to Energy Level				Contrast	
	Low	Standard	High	SEM[1]	Linear	Quadratic
n	15	15	16		P	
Carcass weight, kg	175	179	175	5	0.99	0.44
Carcass weight as a % of live BW	54.6	55.0	54.5	0.3	0.78	0.27
Liver weight, kg	5.3	5.5	5.5	0.1	0.30	0.55
Liver weight, kg/100 kg BW	1.7	1.7	1.7	0.04	0.26	0.91
Carcass protein[2], % of carcass	17.7	18.0	18.0	0.2	0.39	0.55
Carcass fat[3], % of carcass	21.5[a]	18.8[b]	20.4[a]	0.8	0.32	0.03
Carcass water, % of carcass	59.5	61.5	60.7	0.6	0.16	0.07
Carcass protein[2], kg	25.8	27.4	26.5	0.9	0.57	0.26
Carcass fat[3], kg	31.7	28.8	30.1	1.5	0.48	0.27
Perirenal fat[4], kg	5.1	5.4	4.8	0.5	0.66	0.53
Omental-intestinal fat[5], kg	13.3	13.9	11.7	0.9	0.22	0.22
Internal fat[5], kg/100 kg BW	5.5	5.8	5.1	0.3	0.35	0.11

[1] Pooled standard error of means as calculated with n = 15
[2] Low, n = 12, Standard, n = 15, High, n = 16
[3] Low, n = 15, Standard, n = 15, High, n = 15
[4] Low, n = 14, Standard, n = 14, High, n = 16
[5] Low, n = 12, Standard, n = 13, High, n = 15
[a,b] Least squares means in rows with different superscripts differ quadratically ($p<0.05$).

the percentage of carcass protein, fat, and water estimated from the edible portion of the ninth-tenth-eleventh-rib cut (Hankins and Howe, 1946) and from the edible portion of the right half carcass were: r = 0..29 for percentage of carcass protein, 0.71 for fat and 0.46 for water.

Mammary development

The mass of dissected parenchyma and parenchyma as a percentage of BW was ~10% greater for heifers fed the high protein diet than those fed low protein; however, this difference was not statistically significant. The total mass of extra-parenchymal fat as well as extra-parenchymal fat as a percentage of BW was similar among treatment groups. In addition, diet did not affect lipid, dry-fat-free tissue, or protein in the parenchymal tissue (Table 6).

Similar to the total mass of dissected parenchyma, the parenchymal DNA and RNA content was ~10% greater, but not significantly, in heifers fed the high protein diet relative to heifers fed the low protein diet (Table 7). Similarly, parenchymal DNA and RNA content as a percentage of BW was greater for heifers fed the high protein diet compared with heifers fed the low protein diet, but this was not statistically significant. Diet did not affect concentrations of DNA or RNA in parenchymal tissue, and the RNA/DNA ratio was similar among treatment groups.

Table 6. Least squares means for mammary hemigland composition.

	Protein to Energy Level				Contrast	
	Low	Standard	High	SEM[1]	Linear	Quadratic
n	15	15	16		P	
Parenchyma[2], g	302	308	331	24	0.39	0.76
Parenchyma[2], g/100 kg BW	95	94	104	8	0.40	0.59
Extra-parenchyma[2], g	717	796	733	48	0.81	0.24
Extra-parenchyma[2], g/100 kg BW	222	244	229	13	0.68	0.25
Parenchymal lipid, g	142	137	147	13	0.77	0.62
Parenchymal DFFT[3], g	24.0	25.5	26.8	2.1	0.37	0.96
Parenchymal CP[2], g	24.0	24.8	25.6	2.1	0.66	0.53
Teat length						
Front, mm	33	30	33	1	0.88	0.12
Rear, mm	30	28	32	2	0.47	0.19

[1] Pooled standard error of means as calculated with n = 15
[2] Based on wet matter
[3] DFFT = dry fat-free tissue

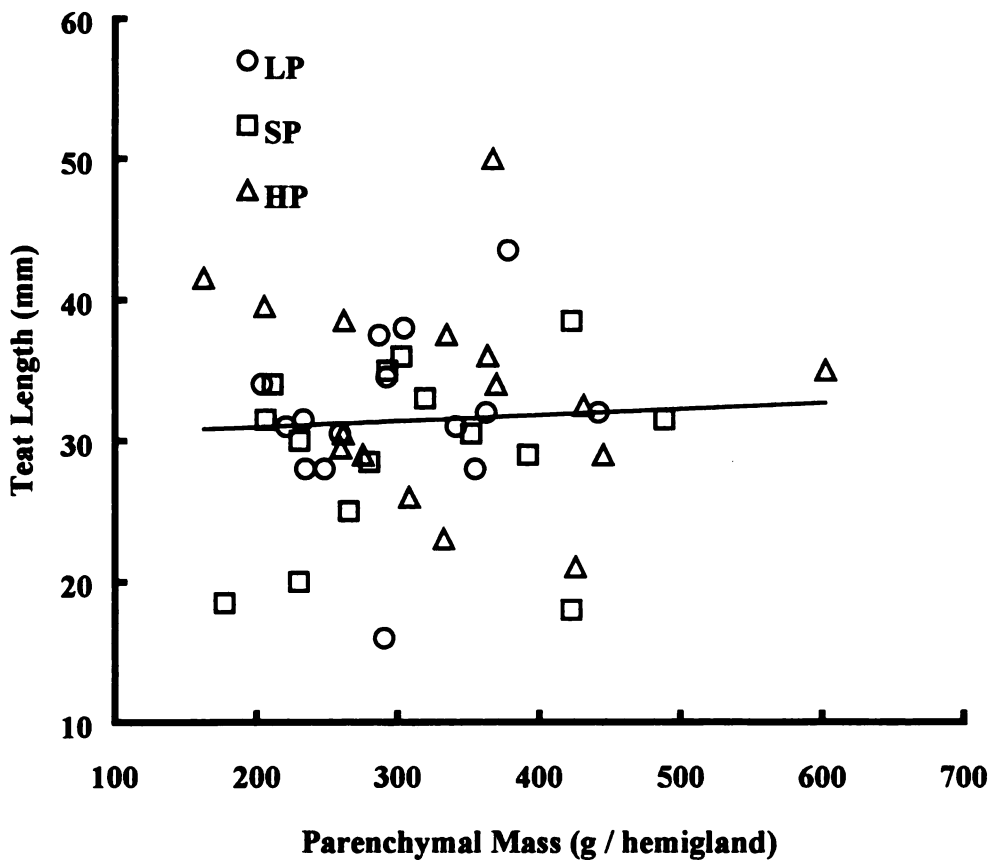
Table 7. Least squares means for mammary hemigland nucleic acid content.

	Protein to Energy Level				Contrast	
	Low	Standard	High	SEM[1]	Linear	Quadratic
n	15	15	16		P	
Parenchymal DNA, mg	947	1005	1054	89	0.40	0.97
DNA, mg/100 kg BW	298	309	335	31	0.39	0.85
Concentration DNA, mg/g	3.11	3.25	3.22	0.14	0.60	0.65
Parenchymal RNA, mg	557	612	648	66	0.33	0.91
RNA, mg/100 kg BW	175	189	207	23	0.33	0.96
Concentration RNA, mg/g	1.83	1.97	1.95	0.11	0.43	0.57
RNA : DNA	0.59	0.60	0.61	0.02	0.65	0.84

[1] Pooled standard error of means as calculated with n = 15

In addition to measuring dissected parenchyma mass, mammary cell numbers and metabolic activity, teat length was measured as a possible gauge of mammary development. Diet did not influence front or rear teat length (Table 6), and variation in teat length accounted for only 1% of the variation in mammary parenchymal mass (Figure 4).

Figure 4. Teat length mean for all four teats versus parenchymal mass of heifers fed low protein (n = 15; LP), standard protein (n = 15; SP), or high protein (n = 16; HP). r^2 = 0.01.



Serum profiles of somatotropin and IGF-I

Profiles of somatotropin concentration in serum for 12 h, at 4 d before slaughter are presented in Figure 5. Increasing the ratio of dietary protein to energy did not affect average somatotropin concentrations during the day or the feeding induced decrease in somatotropin conentrations. Profiles of IGF-I concentrations in serum are presented in Figure 6. Concentrations of IGF-I were not different among treatment groups.

Figure 5. Untransformed concentrations of serum somatotropin in heifers fed low protein (n = 7; LP), standard protein (n = 6; SP), or high protein (n = 8; HP) for 12 h, at 4 d before slaughter. SEM = 0.54. The arrow represents when fresh feed was offered.

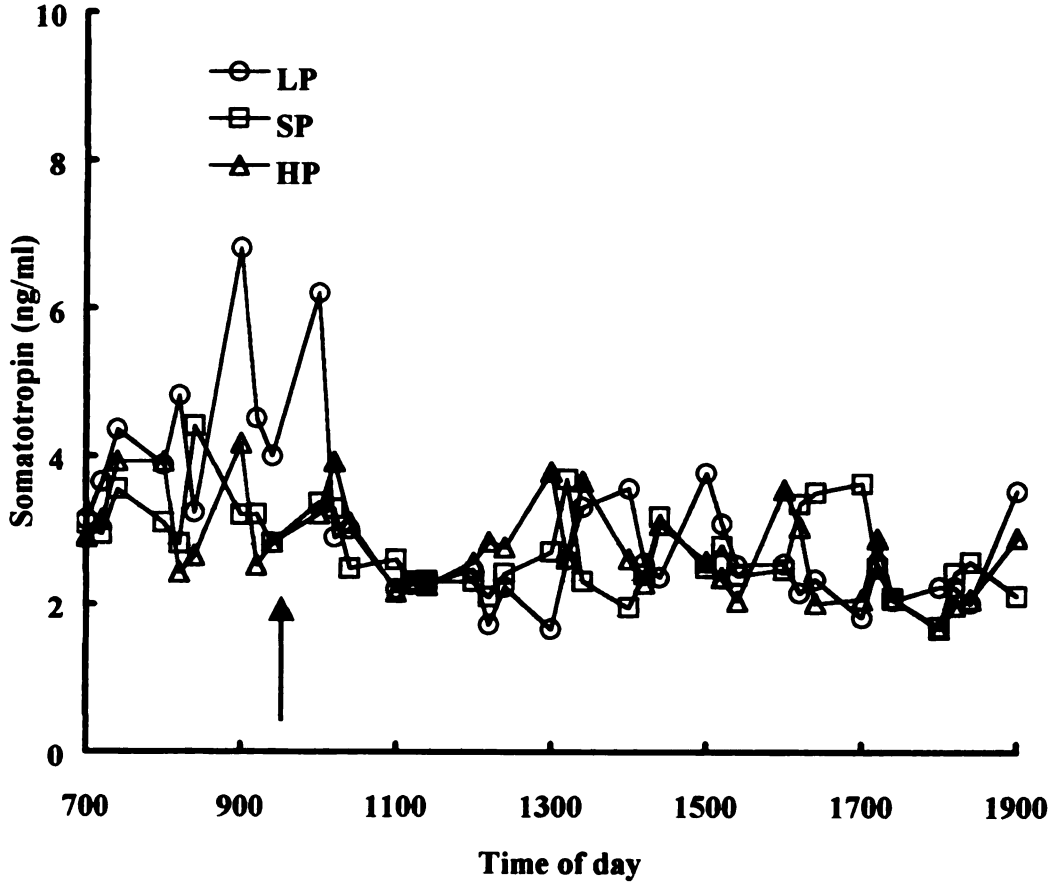
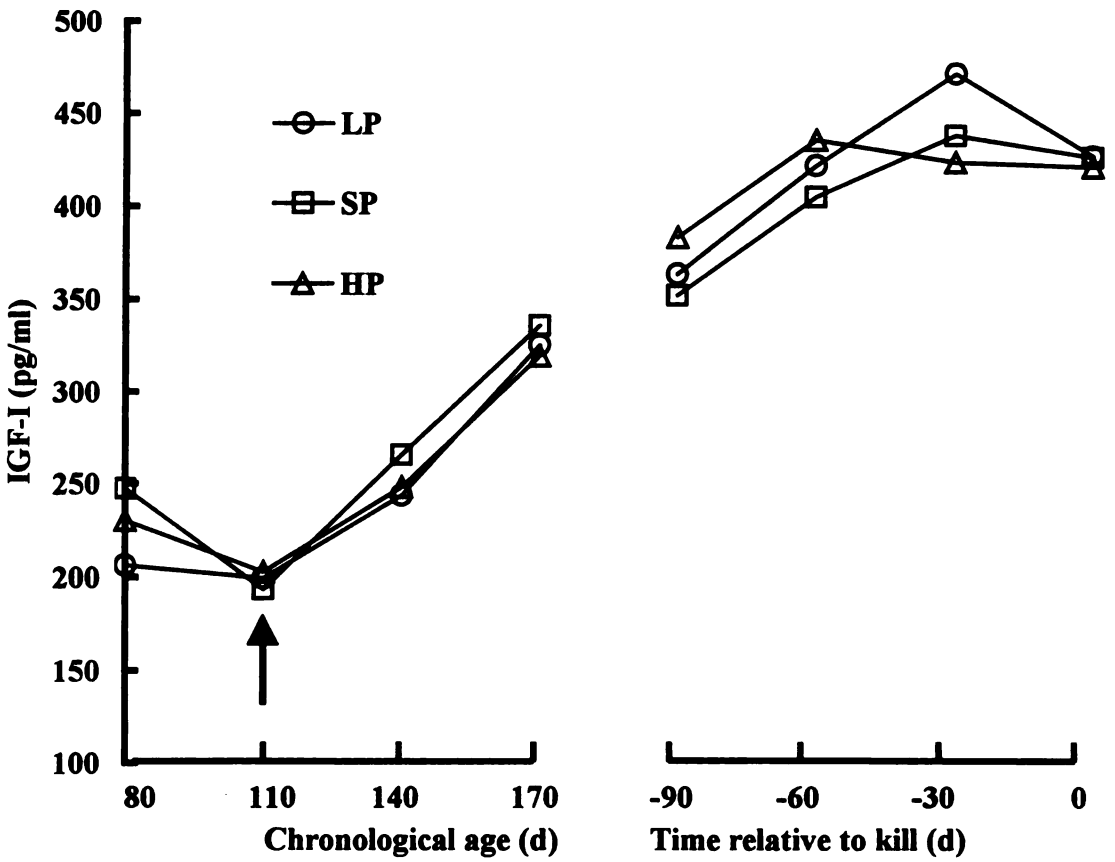


Figure 6. Untransformed concentrations of serum IGF-I in heifers fed low protein (n = 15; LP), standard protein (n = 15; SP), or high protein (n = 16; HP). SEM = 15. The arrow represents the time treatments started.



DISCUSSION

Mammary development

The present study is the first to directly show that dietary protein does not have a significant affect on mammary growth in rapidly growing prepubertal dairy heifers. Level of dietary protein did not alter mammary growth regardless of how it was measured: parenchymal mass, protein, dry-fat-free tissue, DNA, or RNA.

Several experiments (Gardener et al., 1977; Little and Kay, 1979; Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984; Valentine et al., 1987; Gardner et al., 1988; and Peri et al., 1993) demonstrated that rearing prepubertal dairy heifers at accelerated growth rates reduced mammary development and subsequent milk production. However, other studies, in which high protein diets were fed, found that rearing dairy heifers at accelerated rates of BW gain did not impair mammogenesis (Capuco et al., 1995; and Radcliff et al., 1997) or subsequent lactation (Van Amburgh et al., 1998). Sejrsen et al. (1982) fed prepubertal heifers a 15% CP (52 g CP/Mcal ME) diet at ad libitum or restricted intake to produce 1200 and 600 g of BW gain/d, respectively. Heifers were slaughtered at 320 kg BW and those fed ad libitum had ~30% less mammary parenchymal DNA than those on restricted feeding.

Capuco et al. (1995) fed prepubertal Holstein heifers either an alfalfa-based high protein diet (83 g CP/Mcal ME) or corn silage-based low protein diet (54 g CP/Mcal ME)

to achieve rapid (~1000 g/d) or control (~700 g/d) BW gains. Heifers were killed when they weighed at least 325 kg and had two or more estrous cycles. Mammary development was impaired 48% when accelerated gains were produced in heifers fed the corn silage-based diet but no impairment of mammary development occurred when accelerated gains were produced by the alfalfa-based diet. Although the authors speculated that the different responses were caused by the difference in bulk densities of the corn silage-based and alfalfa-based diets, the low protein of the corn silage diet seems a more likely explanation. In addition, Radcliff et al. (1997) fed prepubertal Holstein heifers a high-energy, high-protein (68 g CP/Mcal ME) diet to produce ~1200 g of BW gain/d or a control diet to produce ~800 g of BW gain/d from 4 mo of age until the fifth estrous cycle after puberty. Heifers fed the high-energy, high-protein diet had mammary development similar to that of control heifers.

VandeHaar (1997) examined the relationship between mammary development or milk yield and the dietary protein to energy ratio from 11 studies in which gains of heifers exceeded 900 g of BW/d. Estimated dietary protein to energy ratio varied considerably among the studies, from 43 to 83 g CP/Mcal ME. Across the studies, mammary development of rapidly-grown heifers relative to their controls was positively correlated with the protein to energy ratio of the diets. Furthermore, the protein to energy ratio accounted for 51% of the variation in mammary parenchyma responses and 78% of the variation in milk yield responses to rapid growth rate. These reports suggest that inadequate protein might be responsible for the impaired mammary development and subsequent milk production of rapidly-grown prepubertal heifers. However, this is the

first study to directly investigate the effect of dietary protein on mammary development in heifers fed isocaloric diets to achieve rapid rates of BW gain.

Based on previous literature (Table 1), I expected mammary development to be impaired 30 to 50% in rapidly-grown prepubertal heifers fed 14% CP (48 g CP/Mcal ME) compared with 19% CP (66 g CP/Mcal ME). Although parenchymal DNA was 10% greater in heifers fed 19% CP compared with heifers fed 14% CP, this difference was not close to statistical significance. Thus, these data show that protein is not a major regulator of mammary growth in rapidly-grown prepubertal heifers provided protein is adequate for structural growth.

How might this lack of effect be explained? First of all I suggest that it can not be explained by differences in laboratory techniques. Differences in techniques exists in the literature for example, Stelwagen and Grieve (1990) measured total mammary DNA instead of DNA in parenchyma. The current study used laboratory techniques similar to Sejrsen et al. (1982), Capuco et al. (1995), and Radcliff et al. (1997) to evaluate mammary development. Thus, difference in laboratory techniques used to quantify mammary development does not explain the lack of a treatment effect on mammary growth in the present study.

Another factor that might be important in considering effects of dietary protein on rapidly growing heifers is the actual rate of gain. Rate of prepubertal BW gain in the present experiment was ~1200 g/d and typical of those associated with inhibition of mammary development (Sejrsen et al., 1982; Harrison et al., 1983; Petitclerc et al., 1984; Valentine et al., 1987; and Capuco et al., 1995) and subsequent milk production (Gardner

et al., 1977; Little and Kay, 1979; Valentine et al., 1987; Gardner et al., 1988; Peri et al., 1993; Capuco et al., 1995; and Van Amburgh et al., 1998). Therefore, my model was well suited to test if increasing dietary protein from 14 to 19% alters mammary development in rapidly-grown heifers.

Furthermore, the current study was conducted during the critical phase of mammary development. The existence of a critical period for mammary development, where growth of the secretory tissue is sensitive to high planes of nutrition, is widely supported (Foldager and Sejrsen, 1987; and Johnsson, 1988). For example, Sejrsen and coworkers (1982) reported that mammary development was less when prepubertal heifers had ad libitum access to feed relative to restricted-fed heifers, but postpubertal mammary development was the same regardless of feed intake. This sensitive period seems to coincide with the allometric growth phase of the heifer mammary gland, which occurs from 2 until 9 mo of age or shortly after puberty (Sinha and Tucker, 1969). In my experiment, treatments were initiated at ~3.5 mo of age and continued for ~5.5 mo. Level of dietary CP had no effect on age at puberty, with the mean age of first corpus luteum being 7.5 months. All heifers were slaughtered at ~9 months of age. Therefore, treatments were imposed during most of the critical allometric growth phase of the mammary gland when impairment is normally seen.

In the current experiment mammary parenchymal DNA content at slaughter as a measure of mammary development was ~1000 mg and BW at slaughter was ~320 kg. In experiments by Sejrsen et al. (1982) and Capuco et al. (1995) mammary development of rapidly-grown heifers relative to their controls was impaired ~30 and ~48%, respectively.

In both of these studies, heifers were killed at ~320 kg BW and rapidly-grown heifers with impaired mammary development had ~1000 mg of parenchymal DNA. Comparing mammary parenchymal DNA content in the present experiment to these previous studies (Sejrsen et al., 1982 and Capuco et al., 1995) which used the same methodology for measuring mammary parenchymal DNA indicates that impairment occurred in all treatments. Therefore, results from this experiment dispute the theory that extra protein will alleviate the detrimental effects of high energy on mammary development.

Hormones

Several studies have implicated somatotropin as a major mediator of dietary effects on mammary development (Sejrsen et al., 1983; Johnsson et al., 1985; and Sejrsen et al., 1986). Mammary growth is increased by exogenous somatotropin in lambs (Johnsson et al., 1986; and McFadden et al., 1990), dairy heifers (Sejrsen, 1986; Sandles and Peel, 1987; and Radcliff et al., 1997) and beef heifers (Glasser et al., 1991). Sejrsen et al. (1983) reported that serum somatotropin concentrations were reduced in heifers with ad libitum access to feed relative to restricted-fed heifers. Sejrsen also hypothesized that decreased somatotropin concentrations were responsible for impaired mammary parenchymal growth observed for heifers consuming a high-energy diet. Capuco and coworkers (1995) reported that somatotropin concentrations and mammary development were reduced ~25% and ~48%, respectively when rapid BW gains were achieved from high intake of a corn silage-based diet but neither were reduced from high intake of an alfalfa-based high protein diet. In the current experiment all heifers had ad libitum access

to their respective high-energy diet, and there was no difference in serum somatotropin concentrations. Thus, results from the current experiment disagree with the idea that higher protein may offset the reduction in somatotropin concentration caused by high-energy diets. Therefore, one explanation for level of dietary protein not having an effect on mammary development is that serum somatotropin concentrations were not different among treatment groups.

In spite of its documented effect on mammary growth, the mechanism of action of somatotropin is not clear. Although somatotropin receptor mRNA as been detected in mammary tissue (Glimm et al., 1990), a direct effect of somatotropin on the mammary gland is questionable. It has not been possible to demonstrate somatotropin binding to mammary epithelial cells (Gertler et al., 1984; Akers, 1985; and Kazmer et al., 1986) and somatotropin does not stimulate proliferation of isolated bovine epithelial cells (Collier et al., 1993). Most evidence suggests that somatotropin does not act directly but rather it acts indirectly on the mammary gland by other factors such as insulin-like growth factor-I (IGF-I) (Akers, 1990).

Administration of somatotropin increases serum IGF-I concentrations (Bauman and Vernon, 1993; Dahl, 1993; Purup et al., 1993a; Sharma et al., 1994; Vanderkooi et al., 1995; Sharma et al., 1996; and Yung et al., 1996) and IGF-I receptors are present in mammary tissue (Purup et al., 1995) and in isolated bovine mammary cells (Romagnolo et al., 1994). Insulin-like-growth-factor-I also stimulates proliferation of isolated bovine mammary epithelial cells (Zhao et al., 1992; Romagnolo et al., 1992 and Collier et al., 1993). In the current study level of dietary CP did not affect serum IGF-I. Perhaps,

similar serum IGF-I concentrations not being different among treatment groups is another explanation for the lack of a treatment effect on mammary development.

External measure of mammary development

There is a high correlation (r = 0.50 to 0.85) between mammary epithelial cell number and milk yield, suggesting that increased mammary epithelial cell number results in increased milk yield (Tucker, 1969). However, the terminal nature of mammary gland dissection and analysis prevents researchers from extrapolating the effects of a treatment on parenchymal growth to future milk production. Thus, it would be useful if there were other methods of determining mammary development in young heifers so that the heifers could be carried through lactation. Unfortunately, it is very difficult, if not impossible, to get a reliable estimate of mammary growth in a live animal. Palpation of mammary tissue has been used to assess mammary development in heifers, but this often gives misleading results because the parenchyma constitutes a relatively small part of the total mammary gland and parenchymal composition is not always constant (Sorensen et al., 1964). Stelwagen and Grieve (1990) reported that morphometric evaluation of mammary biopsies correlated poorly with chemical analysis of dissected heifer mammary glands. The correlation coefficient between ultrasonic area measurement and amount of parenchymal DFFT is also poor (Niezen et al., 1996). However, the correlation coefficients (r) between measures of parenchymal tissue by dissection and computer tomography were 0.80 for fat-free parenchyma and 0.62 for total parenchyma (Sorensen

et al., 1987). However, measuring parenchymal tissue by computer tomography is expensive.

Teat length has been proposed as an additional parameter for assessing mammary development in heifers (Moran et al., 1991). There are three advantages to measuring teat length as a parameter for assessing mammary development: it is relatively easy, it is not expensive, and it is non-terminal. There was a large variation in mammary parenchymal mass (~200 to ~600 g/hemigland) and teat length (~20 to ~50 mm) in the animals on the present study to test the relationship (Figure 3). In the current experiment, variation in teat length accounted for only 1% of the variation in mammary parenchymal mass. Thus, my results indicate that teat length is not a valid external estimate of mammary development.

Growth and DMI

In the current experiment increasing dietary protein from 14 to 19% increased mean BW gain and feed efficiency ~5% but the increase was not statistically significant. Previous experiments indicated that feed efficiency in heifers is improved as much as 7% when dietary protein is increased from 11 to 16% (Bagg et al., 1985; and Heinrichs and Lammers, 1998). Heinrichs and Lammers (1998) reported that DMI increased 2.6% and BW gain increased 9.5% in rapidly-grown heifers fed 16% CP relative to heifers fed 12% CP. Results from the current experiment seem inconsistent with those results because the high level of dietary protein in my study did not alter DMI, BW gain or feed efficiency.

When evaluating heifer growth, an important parameter is skeletal growth. Body weight as the sole criterion to define optimum size of an animal has limitations. Markusfeld and Ezra (1993) demonstrated that withers height of Holstein replacement heifers at first calving was a better determinant of peak and 305-d first lactation milk yield than BW. An animal can be short and fat and weigh just as much as an animal that is tall and thin. Skeletal size at parturition is important. Pelvic area is negatively correlated to dystocia (Stevenson and Call, 1988). Thus, heifers that are short are more likely to have increased incidence of dystocia. Heinrichs and Lammers (1998) reported that increasing dietary CP from 11 to 16% increased the gain in hip height and width of rapidly grown heifers 0.008 and 0.017 cm/d, respectively. In contrast, increasing dietary CP from 14 to 16% did not influence indices of skeletal growth (Heinrichs and Lammers, 1998). In the current experiment, height at the withers and pelvic area at slaughter were used to estimate skeletal size. In support of previous reports (Bagg et al., 1985; Kertz et al., 1987 and Heinrichs and Lammers, 1998), the current study indicates that skeletal growth is not improved in rapidly-grown dairy heifers by increasing dietary CP above 14%. Thus, feeding 19% instead of 14% CP to rapidly-grown prepubertal heifers did not improve skeletal growth.

Higher-energy diets that produce accelerated BW gains increased body protein deposition, but body protein deposition becomes proportionately less than that of fat accretion (Radcliff et al., 1997 and Waldo et al., 1997). Previous studies suggest that excessive fattening as a result of accelerated growth rates may increase incidence of postpartum metabolic disorders (Grummer et al., 1995) and decrease first lactation milk

yield (Hoffman et al., 1997). These problems have led investigators to seek replacement heifer management strategies that increase growth without increasing body fat. One strategy is to increase absorbed protein, which increases body protein accretion (Hoffman, 1997). I was concerned that any effects of diet on carcass composition would make it difficult to interpret dietary effects on mammary development. However, Waldo et al. (1997) reported that carcass composition was not different for rapidly-grown heifers regardless of protein to energy ratio (54 or 83 g CP/Mcal ME) fed. Results from the current study add support to Waldo et al. (1997) in that heifer BCS and carcass composition were unaffected by the level of dietary CP. Therefore, our model was well suited to test the idea that dietary protein affects mammary development of rapidly grown heifers independent of effects on carcass composition.

In the current experiment, the correlation coefficients between the percentage of carcass protein, fat, and water estimated from the edible portion of the ninth-tenth-eleventh-rib cut (Hankins and Howe, 1946) and from the edible portion of the half carcass of 12 heifers were: r = 0.29, 0.71, and 0.46, respectively. In contrast, Hankins and Howe (1946) found that correlation coefficients between the percentage of carcass protein, fat, and water estimated from the edible portion of the ninth-tenth-eleventh-rib cut and from the edible portion of the dressed carcass were much higher: r = 0.94, 0.81, and 0.94, respectively. The differences in correlation coefficients may be the result of fewer animals used and a more narrow range of protein, fat, and water in my experiment than in Hankins and Howe (1946). Regardless, in the present study estimated

percentages of carcass protein, fat, and water were only 1 to 3 percentage points different than the actual half carcass values.

Implications

The objective of feeding and management of replacement dairy heifers is to produce the best possible cows. Success should not be measured solely in terms of average rates of gain or feed efficiency but rather by the milk production potential of the heifer once she becomes a cow. However, the period from birth to first parturition is expensive and many farmers have focused on trying to decrease the cost of rearing heifers as a means to increase farm profitability.

The most effective way to reduce rearing costs is to reduce the age at first calving without compromising BW at parturition. Age at onset of puberty is inversely related to growth rate. Therefore, heifers need to be reared at accelerated growth rates (> 1000 g/d) until puberty to obtain a substantial reduction of age at first calving. Unfortunately, rapid growth rates before puberty can have a negative influence on mammary growth and future milk production.

Recent experiments suggest indirectly that type of diet or protein level can modify the effect of accelerated growth rates on mammary development (Capuco et al., 1995, Radcliff et al., 1997, VandeHaar, 1997; and Van Amburgh et al., 1998). The current study, in which I directly examine the effect of dietary protein, indicates that high protein perhaps could increase mammogenesis as much as 10% but the impairment of mammogenesis may be as great as 30 to 50%. I strongly suggest that feeding high

dietary protein will not prevent the impaired prepubertal mammary development that occurs in heifers fed for rates of gain > 1000 g/d. Therefore, decreasing the time to AFC could increase profits further but only if milk production is not impaired. To date, data indicates this risk is not worth taking.

CONCLUSIONS

Level of dietary CP did not effect body growth, DMI or feed efficiency of heifers. Height at the withers was similar for all treatments. Heifers fed the standard-protein diet had greater pelvic area at slaughter than heifers fed the low or high-protein diets. Diet did not alter total carcass protein and fat content as estimated by 9-10-11 rib section or half carcass. The level of dietary CP did not affect age or weight at puberty. Serum concentrations of somatotropin and IGF-I were not influenced by diet. The major objection to rearing dairy heifers at a high growth rate is compromised mammary development and decreased subsequent milk production. Increasing dietary CP fed to rapidly-grown prepubertal heifers from 14 to 19% produced 10% greater mammary development but was not statistically significant. I conclude that dietary CP does not have a major effect on mammary development of rapidly grown prepubertal heifers. Comparing measurements of mammary development in the present experiment to previous studies indicates that impairment probably occurred. Thus, I suggest that feeding high protein will not prevent the commonly observed impairment of mammary development when prepubertal heifers are grown rapidly.

APPENDIX

APPENDIX

Table 8. The percentage of carcass lipid, protein, and water estimated from the three-rib cut compared with the actual values from the right half carcass.

	Estimated from three-rib cut			Actual from right half carcass		
Heifer ID	% Lipid	% Protein	% Water	% Lipid	% Protein	% Water
1	20.3	MV[1]	61.2	13.9	MV[1]	66.4
2	21.2	18.1	61.9	23.5	17.0	62.7
6	18.6	18.1	60.1	18.7	19.5	60.1
11	20.1	17.8	60.6	15.6	18.5	60.1
13	16.9	18.1	57.9	13.4	19.1	67.5
17	19.9	17.9	60.2	19.4	20.5	68.2
20	18.3	18.6	59.2	17.7	18.2	63.6
21	16.8	19.5	57.5	18.8	20.0	60.6
23	15.3	18.8	56.1	11.4	20.1	58.9
30	26.5	16.4	64.0	23.7	18.7	68.1
53	25.1	16.3	62.9	21.0	17.0	66.6
56	21.5	17.9	62.1	17.1	18.7	62.3
Average	20.0	17.9	60.3	17.9	18.9	63.8

[1]MV = Missing value

BIBLIOGRAPHY

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Akers, R.M., 1985. Lactogenic hormones: Binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. J. Dairy Sci. 68:501.

Akers, R.M. 1990. Lactation physiology: A ruminant animal perspective. Protoplasma 159:96.

Association of Official Analytical Chemists International. 1990. Official methods of analysis. 15th ed. 1st Supplement. AOAC. Arlington, VA.

Bagg, J.G., D.G. Grieve, J.H. Burton, and J.B. Stone. 1985. Effect of protein on growth of Holstein heifer calves from 2 to 10 months. J. Dairy Sci. 68:2929.

Bauman, D.E., R.G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. Annu. Rev. Nutr. 13:437.

Berg, R.T., and R.M. Butterfield. 1968. Growth patterns of bovine muscle, fat and bone. J. Anim. Sci. 27:611.

Bruce, L.A., T. Atkinson, J.S.M. Hutchinson, R.A. Shakespear, and J.C. MacRae. 1991. The measurement of insulin-like growth factor I in sheep plasma. J. Endocrinol. 128:R1.

Burleigh, I.G. 1974. On the cellular regulation of growth and development in skeletal muscle. Biological Reviews. 49:267.

Capuco, A.V., J.J. Smith, D.R. Waldo, and C.E. Rexroad, Jr. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709.

Clemmons, D.R. 1997. Insulin-like growth factor binding proteins and their role in controlling IGF-I action. Cytoline and growth factor reviews. 8:45.

Collier, R.J., M.F. McGrath, J.C. Byatt, and L.L. Zurfluh. 1993. Regulation of bovine mammary growth by peptide hormones: involvement of receptors, growth factors and binding proteins. Livest. Prod. Sci. 35:21.

Cowie, A.T., J.S. Tindal, and A. Yokoyama. 1966. The induction of mammary growth in the hypophysectomized goat. J. Endocrinol. 15:185.

Dahl, G.E., L.T. Chapin, M.S. Allen, W.M. Moseley, and H.A. Tucker. 1993. Galactopoietic effects of recombinant somatotropin and growth hormone releasing factor in dairy cows. J. Dairy Sci. 76:1550.

Ellis, S. and R.M. Akers. 1995. Morphological and histological analysis of the prepubertal ovine mammary gland. J. Dairy Sci. 78(Suppl.1):202.

Foldager, J. and K. Sejrsen. 1987. Mammary gland development and milk production in dairy cows in relation to feeding and hormone manipulation during rearing. Page 102-116 *in*: Research in Cattle Production Danish Status and Perspectives 1st ed., Frederiksberg: Landhusholdningsselskabets Forlag.

Gardner, R.W., J.D. Schuh, and L.G. Vargus. 1977. Accelerated growth and early breeding of Holstein heifers . J. Dairy Sci. 60:1941.

Gardner, R.W., L.W. Smith, and R.L. Park. 1988. Feeding and management of dairy heifers for optimal lifetime productivity. J. Dairy Sci. 71:996.

Gaynor, P.J., K.J. Lookingland, and H.A. Tucker. 1995. 5-Hydroxytryptaminergic receptor-stimulated growth hormone secreation occurs independently of changes in peripheral somatostatin concentration. Proc. Soc. Exp. Bio. Med. 209:79.

Gertler, A., A. Ashkenazi, and Z. Madar. 1984. Binding sites of human growth hormone and ovine and bovine prolactins in the mammary gland and in the liver of lactating dairy cows. Mol. and Cell Endocrinol. 35:51

Glasser, D.E., G.E. Carstens, F.M. Byers, and L.W. Greene. 1991. The effects of bovine somatotropin and intermittent growth on heifer development: Mammary gland development. PR-4966 *in* The Texas Agriculture Experiment Station Progress Report. E.A. Hiter Dir. The Texas A&M University System, College Station, Texas.

Glimm, D.R., V.E. Baracos, and J.J. Kennelly. 1990. Molecular evidence for the presence of growth hormone receptors in the bovine mammary gland. J. Endocrinol. 126:R5.

Goldspink, G. 1974. Development of muscle. Page 69 *in* Differentiation and growth of cells in vertibrate tissue. G. Goldspink ed. Chapman and Hill, London.

Grummer, R.R., P.C. Hoffman, M.L. Luck, and S.J. Bertics. 1995. Effect of prepartum and postpartum dietary energy on growth and lactation of primiparous cows. J. Dairy Sci. 78:172.

Hankins, O.G., and P.E. Howe. 1946. Estimation of the composition of beef carcasses and cuts. USDA Tech. Bull. 926. USDA, Washington, DC.

Hammond, J. 1960. Growth in size and proportions in farm animals. Page 321 in Growth in living systems. M.X. Zarrow ed. Basic Books Inc., New York.

Harrison, R.D., I.P. Reynolds, and W. Little. 1983. A quantitative analysis on mammary glands of heifers reared at different rates of live weight gain. J. Dairy Res. 50:405.

Heinrichs, A.J. 1993. Raising dairy replacements to meet the needs of the 21st century. J. Dairy Sci. 76:3179.

Heinrichs, A.J., and G.L. Hargrove. 1987. Standards of weight and height for Holstein heifers. J. Dairy Sci. 70:653.

Heinrichs, A.J., and B.P. Lammers. 1998. The response of additional dietary protein fed to prepubertal heifers on feed efficiency and growth. J. Dairy Sci. 81(Suppl.1):325.

Hoffman, P.C. 1997. Optimum body size of Holstein replacement heifers. J. Anim. Sci. 75:836.

Johnsson, I.D. 1988. The effect of pubertal nutrition on lactation performance by dairy cows. Page 171-192 *in*: Nutrition and lactation in the dairy cow. P.C. Garnsworthy, ed. Butterworth, London.

Johnsson, I.D., I.C. Hart, A.D. Simmonds, and S.V. Morant. 1985. Pre-pubertal mammogenesis in the sheep. 2. The effect of level of nutrition on the plasma concentrations of growth hormone, insulin and prolactin at various ages in female lambs and their relationship with mammary development. Anim. Prod. 41:333.

Johnsson, I.D., I.C. Hart, and A. Turvey. 1986. Pre-pubertal mammogenesis in the sheep. 3. The effect of restricted feeding or daily administration of bovine growth hormone and bromocriptine on mammary growth and morphology. Anim. Prod. 42:53.

Kazmer, G.W., M.A. Barnes, R.M. Akers, and W.D. Wittier. 1986. Lactogenic hormone receptors in mammary membrane preparations from prepartum and 60 and 180 day post-partum Holstein cattle. J. Endocrinol. 109:175.

Keown, J.F., and R.W. Everett. 1986. Effects of days carried calf, days dry, and weight of first calf heifers on yield. J. Dairy Sci. 69:1891.

Kertz, A.F., L.R. Prewitt, and J.M. Ballam. 1987. Increased weight gain and effects on growth parameters of Holstein heifer calves from 3 to 12 months of age. J. Dairy Sci. 70:1612.

Keys, D.J., and J. Djiane. 1988. Prolactin and growth hormone binding in mammary and liver tissue of lactating cows. J. Recent Res. 8:731.

Leat, W.M.F., and R.W. Cox. 1980. Fundamental aspects of adipose tissue growth. Page 137 *in* Growth in animals. T.L.J. Lawrence ed. Butterworths, London.

Little, W., and R.M. Kay. 1979. The effects of rapid rearing and early calving on the subsequent performance of dairy heifers. Anim. Prod. 29:131.

Lyons, W.R., C.H. Li, and R.E. Johnson. 1958. The hormonal control of mammary growth. Recent Prog. Hormone Res. 14:219.

Markusfeld, O., and E. Ezra. 1993. Body measurements, metritis and postpartum performance of first lactation cows. J. Dairy Sci. 76:3771.

McFadden, T.B., T.E. Daniel, and R.M. Akers. 1990. Effects of plane of nutrition, growth hormone and unsaturated fat on mammary growth in prepubertal lambs. J. Anim. Sci. 68:3171.

Moran, C., J.F. Quirke, D.J. Prendiville, S. Bourke, and J.F. Roche. 1991. The effect of estradiol, trenbolone acetate, or zeranol on growth rate, mammary development, carcass traits, and plasma estradiol concentrations of beef heifers. J. Anim. Sci. 69:4249.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th rev. ed. National Academy Press, Washington, DC.

Niezen, J.H., D.G. Grieve, B.W. McBride, and J.H. Burton. 1996. Effect of plane of nurtition before and after 200 kilograms of body weight on mammary development of prepubertal Holstein heifers. J. Dairy Sci. 79:1255.

Peri, I., A. Gertler, I. Bruckental, and H. Barash. 1993. The effect of manipulation of energy allowance during the rearing period of heifers on hormone concentrations and milk production in first lactation cows. J. Dairy Sci. 76:742.

Petitclerc, D., L.T. Chapin, and H.A.Tucker. 1984. Carcass composition and mammary development responses to photoperiod and plane of nutrition in Holstein heifers. J Anim. Sci. 58:913.

Pirlo,G., M. Capellette, and G. Marchetto. 1997. Effects of energy and protein allowances in the diets prepubertal heifers on growth and milk production. J. Dairy Sci. 80:730.

Purup, S., K. Sejrsen, J. Foldager, and R.M. Akers. 1993a. Effect of exogenous bovine growth hormone and ovariectomy on prepubertal mammary growth, serum hormones and acute in-vitro proliferative response of mammary explants from Holstein heifers. J. Endocrinol. 139:19.

Purup, S., K. Sejrsen, and R.M. Akers. 1993b. Influence of estradiol on insulin-like growth factor I (IGF-I) stimulation of DNA synthesis in vitro in mammary gland explants from intact and ovariectomized prepubertal heifers. Livestock Prod. Sci. 35:182.

Purup, S., K. Sejrsen, and R.M. Akers. 1995. Effect of bovine growth hormone and ovariectomy on mammary tissue sensitivity to IGF-I in prepubertal heifers. J. Endocrinol. 144:153.

Radcliff, R.P. 1995. Effects of prepubertal diet and injection of bST on body growth, carcass composition, age at puberty, and mammary development of dairy heifers. M.S. Thesis, Michigan State University, East Lansing.

Radcliff, R.P., M.J. VandeHaar, A.L. Skidmore, L.T. Chapin, B.R. Radke, J.W. Lloyd, E.P. Stanisiewski, and H.A. Tucker. 1997. Effect of diet and bST on heifer growth and mammary development. J. Dairy Sci. 80:1996.

Radcliff, R.P., M.J. VandeHaar, L.T. Chapin, T.E. Pilbeam, R.W. Ashley, S.M. Puffenbarger, E.P. Stanisiewski, D.K. Beede, and H.A. Tucker. 1998. Effects of diet and exogenous bST on growth and lactation of dairy heifers. J. Dairy Sci. 81(Suppl.1):227.

Reece, R.P. 1958. Mammary gland development and function. In The Endocrinology of Reproduction. J.T. Velardo, ed. Oxford University Press, NY.

Romagnolo, D., R.M. Akers, E.A. Wong, P.L. Boyle, T.B. McFadden and J.D. Turner. 1992. Overexpression of ovine insulin-like growth factor-I stimulates autonomous autocrine or paracrine growth in bovine mammary derived (MD-IGF-I) epithelial cells. Molecular Endocrinol. 6:1774.

Romagnolo, D., R.M. Akers, J.C. Byatt, E.A. Wong, and J.D. Turner. 1994. Regulation of expression of IGF-I induced IGFBP-3 and IGF-I-receptor by constitutive vs regulated expression of recombinant IGF-I in transfected mammary epithelial cells. Endocrine 2:375.

Sandles, L.D., and C.J. Peel. 1987. Mammogenesis and first lactation milk yields of identical-twin heifers following pre-pubertal administration of bovine growth hormone. Anim. Prod. 45:349.

SAS® User's Guide: Statistics, Version 6.12, 1996. SAS Inst., Inc., Cary, NC.

Schillo, K.K., J.B. Hall, S.M. and Hileman. 1992. Effect of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. 70:3994.

Schmidt, G.H. 1971. Biology of lactation. W.H. Freeman. San Francisco, CA.

Sejrsen, K., J.T. Huber, H.A. Tucker, and R.M. Akers. 1982. Influence of nutrition on mammary development in pre- and postpubertal heifers. J. Dairy Sci. 65:793.

Sejrsen, K., J.T. Huber, and H.A. Tucker. 1983. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. J. Dairy Sci. 66:845.

Sejrsen, K., J. Foldager, M.T. Sorensen, R.M. Akers, and D.E. Bauman. 1986. Effect of exogenous bovine somatotropin on pubertal mammary development in heifers. J. Dairy Sci. 69:1528.

Sejrsen, K., 1994. Relationship between nutrition, puberty and mammary development in cattle. Proc. Nut. Soc. 53:103.

Sharma, B.K., M.J. VandeHaar, and N.K. Ames. 1994. Expression of insulin-like growth factor-I in cows at different stages of lactation and in late lactation in cows treated with somatotropin. J. Dairy Sci. 77:2232.

Sharma, B.K., M.J. VandeHaar, R.P. Radcliff, and H.A. Tucker. 1996. Effects of diet and exogenous bST on serum insulin-like growth factor-I (IGF-I), IGF binding proteins (IGFBP), and hepatic mRNA for IGF-I of dairy heifers. J. Dairy Sci. 79(Suppl.1):177.

Sharma, B.K., M.J. VandeHaar, H.A. Tucker, R. Renaville, D. Portetelle, R.P. Radcliff, W.K. VanderKooi, M. Binelli, and J.S. Liesman. 1997. Effect of diet and bST on mRNA for IGF-I binding proteins (BP) -2 and -3 in dairy cattle. J. Dairy Sci. 80(Suppl.1):176.

Sinha, Y.N., and H.A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507.

Sirbasku, D.A. 1978. Estrogenic induction of growth factors specific for hormone-responsive mammary, pituitary, and kidney tumor cells. Proc. Natl. Acad. Sci. 75:3786.

Smith, G.H., and D. Medina. 1988. A morphologically distinct candidate for an epithelial stem cell in mouse mammary gland. J. Cell Sci. 89:173.

Sorensen, A.M., W. Hansel, W.H. Hough, D.T. Armstrong, K. McEntee, and R.W. Bratton. 1964. Causes and prevention of reproductive failures in dairy cattle. I. Influence of underfeeding and overfeeding on growth and development of Holstein heifers. Cornell Univ. Agric. Bull. 936, Ithaca, NY.

Sorensen, M.T., K. Sejrsen, and J. Foldager. 1987. Estimation of pubertal mammary development in Holstein heifers. J. Dairy Sci. 70:265.

Stelwagen, K., and D.G. Grieve. 1990. The effect of plane of nutrition on growth and mammary development in Holstein heifers. J. Dairy Sci. 73:2333.

Stevenson, J.S., and E.P. Call. 1988. Reproductive disorders in the periparturient dairy cow. J. Dairy Sci. 71:2572.

Swanson, E.W., 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. J. Dairy Sci. 43:377.

Talwalker, P.K., and J. Meites. 1961. Mammary lobulo-alveolar growth induced by anterior pituitary hormones in adreno-ovariectomized and adreno-ovariectomized-hypophysectomized rats. Proc. Soc. Exptl. Biol. Med. 107:880.

Tucker, H.A. 1964. Influence of number of suckling young on nucleic acid content of lactating rat mammary gland. Proc. Soc. Exp. Biol. Med. 116:218.

Tucker, H.A. 1969. Factors affecting mammary gland cell numbers. J. Dairy Sci. 52:721.

Tucker, H.A. 1981. Physiological control of mammary growth, lactogenesis and lactation. J. Dairy Sci. 64:1403.

Valentine, S.C., R.C. Dobos, P.A. Lewis, B.D. Bartsch, and R.B. Wickers. 1987. Effects of live-weight gain before or during pregnancy on mammary gland development and subsequent milk production of Australian Holstein-Friesian heifers. Aust. J. Exp. Agric. 27:195.

Van Amburgh M.E., D.M. Galton, D.E. Bauman, R.W. Everett, D.G. Fox, L.E. Chase,, and H.N. Erb. 1998. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J. Dairy Sci. 81:527.

VandeHaar, M.J. 1997. Dietary protein and mammary development of heifers: analysis from literature data. J. Dairy Sci. 80(Suppl.1):216.

Vanderkooi, W.K., M.J. Vandehaar, B.K. Sharma, M. Binelli, H.A. Tucker, R.M. Akers, and W.M. Moseley. 1995. Comparison of growth hormone-releasing factor and somatotropin: the somatotropic axis in lactating primiparous cows. J. Dairy Sci. 78:2140.

Waldo, D.R., H.F. Tyrrell, A.V. Capuco, and C.E. Rexroad, Jr. 1997. Components of growth in Holstein heifers fed either alfalfa or corn silage diets to produce two daily gains. J. Dairy Sci. 80:1674.

Wallace, C. 1953. Observations on mammary development in calves and lambs. J. Agric. Sci. 43:413.

Wildman, E.E., G. M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt, and T.N. Lesch. 1982. A dairy cow body condition scoring system and it's relationship to selected production characteristics. J. Dairy Sci. 65:495.

Williams, J.M., and C.W. Daniel. 1983. Mammary ductal elongation: differentiation of myoepithelium and basal lamina during branching morphogenesis. Dev. Biol. 97:183.

Woodward, T.L., R.M. Akers, T.B. McFadden, H.T. Huynh, and J.D. Turner. 1993a. Estrogen mediated bovine mammary epithelial cell proliferation: evidence of indirect action. J. Dairy Sci. 75(Suppl.1):293.

Woodward, T.L., W.E. Beal, and R.M. Akers. 1993b. Cell interactions in initiation of mammary epithelial cell proliferation by oestradiol and progesterone in prepubertal heifers. J. Endocrinol. 136:149.

Yung, M.C., M.J. VandeHaar, R.L. Fogwell, and B.K. Sharma. 1996. Effect of energy balance and somatotropin on insulin-like growth factor I in serum and on weight and progesterone of corpus luteum in heifers. J. Anim. Sci. 74:2239.

Zhao, X., B.W. McBride, I. Politis, H.T. Huynh, R.M. Akers, J.H. Burton, and J.D. Turner. 1992. Receptor binding and growth-promoting activity of insulin-like growth factor-I in a bovine mammary epithelial cell line (MAC-T3). J. Endocrinol. 134:307.