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MAMMARY DEVELOPMENT IN RELATION TO PLANE OF NUTRITION AND SERUM HORMONE CONCENTRATIONS IN PRE- AND POSTPUBERTAL HEIFERS

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ABSTRACT

MAMMARY DEVELOPMENT IN RELATION TO PLANE OF NUTRITION AND SERUM HORMONE CONCENTRATIONS IN PRE- AND POSTPUBERTAL HEIFERS

By

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The objective of the experiment was to investigate the effect of plane of nutrition on mammary development in heifers during the allometric and isometric periods of mammary growth before and after onset of puberty. It was further examined whether a change in mammary growth was related to serum concentrations of hormones which regulate mammary development.

Eleven prepubertal and eleven postpubertal Holstein-Friesian heifers assigned to either restricted or ad libitum feeding completed the experiment. Dry matter intake of the pre- and postpubertal heifers on restricted feeding was 55 and 62% respectively of heifers fed ad libitum. The prepubertal heifers on restricted and ad libitum feeding gained 637 and 1272 g per day from 175 to 320 kg bodyweight, respectively, while the postpubertal groups gained 588 and 1164 g from 300 to 440 kg bodyweight.

In the prepubertal heifers ad libitum feeding resulted in a 23-40% decrease in growth of mammary secretory tissue measured as amount ($P<.13$) and percent ($P<.01$) mammary parenchyma, mammary DNA ($P<.11$) and percent epithelial cells ($P<.10$). In contrast, there was no difference in growth of secretory tissue in the postpubertal heifers. In agreement, body growth rate was negatively correlated with amount of parenchyma ($r = -.48$, $P<.15$), percent parenchyma ($r = -.75$, $P<.01$), mammary DNA ($r = -.56$, $P<.10$) and percent epithelial cells ($r = -.49$, $P<.15$) in the prepubertal heifers, while the relationship between mammary growth and body growth rate was low in the postpubertal heifers.

The composition of the secretory tissue measured as DNA, RNA, hydroxyproline and lipid per g of parenchyma and as relative amount of epithelial cells, connective tissue and fat cells were unaffected by plane of nutrition in prepubertal and postpubertal heifers.

In the prepubertal heifers on restricted intake serum growth hormone concentrations were elevated from 8 hours after feeding until next feeding, and showed positive correlations with mammary parenchyma ($r = .55$, $P<.10$), percent parenchyma ($r = .75$, $P<.01$), mammary DNA ($r = .54$, $P<.10$) and percent epithelial cells ($r = .48$, $P<.15$). In the postpubertal heifers serum growth hormone concentrations were unaffected by plane of nutrition, and

the relationship of growth hormone concentration with mammary growth was lower than observed for the prepubertal heifers.

Prolactin concentrations in serum were higher in heifers on ad libitum than restricted feeding, but the difference between planes of nutrition diminished with length of time on ad libitum feeding. Serum prolactin was, in contrast to growth hormone, negatively correlated to measures of mammary secretory tissue in the prepubertal heifers. Correlation coefficients with amount and percent of parenchyma, DNA and epithelial cells were -.47 ($P<.15$), -.69 ($P<.01$), -.53 ($P<.10$) and -.24 ($P>.20$), respectively. Correlations were low in the postpubertal heifers.

Serum insulin was higher in heifers raised on high plane of nutrition, but showed no close relationship with mammary growth. Serum glucocorticoids were also elevated in the heifers raised on high plane of nutrition ($P<.01$), and were not closely correlated with mammary development.

In conclusion, raising heifers on high planes of nutrition resulted in decreased growth of mammary secretory tissue in the period before and around puberty, while there was no influence of feeding intensity in postpubertal heifers. This supports the existence of a critical period for mammary development, during which mammary growth is adversely affected by high planes of nutrition. Changes in mammary development were correlated with serum

concentrations of growth hormone and prolactin suggesting that the negative influence of high planes of nutrition on mammary growth may be mediated by changes in these hormones.

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CHAPTER I

INTRODUCTION

The purpose of rearing dairy heifers is to produce a cow capable of milking to its genetic potential and giving birth to a viable calf each year. The rearing expenses constitute a major cost in the dairy industry and any management or feeding system that can reduce the costs of raising heifers, without having a negative effect on lactational and reproductive performance, will therefore improve efficiency of production.

The normal age at first calving is 27-30 months in most breeds, but it is possible to breed heifers to calve as early as 16-18 months of age. There is therefore great potential for a considerable reduction in the costs of raising heifers by decreasing the age at first calving. Unfortunately, experiments indicate that heifers calving at an age considerably lower than normal have poor milk production in first as well as later lactations (Larsen et al, 1975; Little & Kay, 1979). Recent results suggest that the low milk production of heifers, calving below the normal age, to a large extent is due to high planes of nutrition during rearing rather than age at first calving (Brannang & Lindkvist, 1978; Little & Kay, 1979).

The reason for the decrease in milk production in heifers fed a high feeding intensity during rearing is not known, but inhibition of mammary development and persistent changes in the endocrine system regulating mammary development and function as well as the metabolism have been suggested (Swanson, 1960; Hansson et al, 1967; Swanson, 1978).

With this background it was the objective of this experiment to examine the effect of plane of nutrition on mammary development and hormones involved in regulation of mammary growth and metabolism.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The number of milk synthesizing cells is limiting for the amount of milk produced (Tucker, 1969; Ceriani, 1974), and factors affecting mammary development therefore have great bearing on milk production. Mammary development is often divided into different stages. The quality and size of the parenchyma at the end of each stage determines, in part, the capabilities of the future parenchyma (Mayer & Klein, 1961). Lactation, the final stage of mammary development, is therefore dependent on all preceding stages, and incomplete development at any stage will negatively affect lactational performance. This is in agreement with the proposal that poor milk production of heifers raised on high planes of nutrition is due to inhibition of mammary development (Swanson, 1960).

In mammals, lactation is part of the reproductive process, hence the mammary gland is part of the reproductive system (Cowie, 1974). However, the mammary gland is a metabolic organ as well as a secondary sex organ and the development of the gland is controlled by different combinations of reproductive and metabolic hormones (Schams, 1976). It is therefore possible that a decrease

in mammary development due to high feeding intensity can be caused by changes in hormones involved in regulation of mammary growth (Sejrsen, 1978).

Milk production, apart from the number of milk synthesizing cells is related to mammary function during lactation and the ability of the cow to partition nutrients towards milk synthesis. Mammary function as well as the metabolism of the animals is regulated by hormones. It is therefore possible that persistent changes in the endocrine system as suggested by Hansson et al (1967) is the reason that cows raised on high planes of nutrition have lower milk yield than cows raised on a normal feeding intensity resulting in daily gains of 6-800 g.

After outlining normal mammary development before pregnancy, hormonal control of mammary growth and the role of hormones in regulation of metabolism, it is the objective of this review to investigate the effect of plane of nutrition during rearing on milk production, mammary growth and serum hormone concentrations. Finally, an attempt will be made to relate planes of nutrition to milk production, mammary development and hormonal changes.

Mammary Growth from Birth to Pregnancy

Growth of the mammary gland starts early during embryonic development and continues after birth with accelerated allometric growth around puberty and during pregnancy.

The development of mammary gland from birth to pregnancy is characterized by progressive proliferation of the duct system, and the epithelium of the ducts is the sole constituent of the secretory tissue in virgin heifers (Turner, 1952; Mayer & Klein, 1961).

At birth, mammary gland structures are rudimentory and the duct system with its epithelial buds are confined to a very limited zone adjoining the gland cistern. In contrast, nonglandular structures such as adipose and connective tissue are almost mature and the vascular and lymphatic systems are laid down (Mayer & Klein, 1961). The external shape of the udder is formed.

The period just after birth is a quiescent phase during which mammary growth is minimal and there is no stimulation of the duct system. Sinha & Tucker (1969b) found that the mammary gland develops at the same rate as the body from birth to about 2 months of age.

Well in advance of onset of puberty the mammary gland shows accelerated allometric growth. The allometric growth has been observed for many years. The onset was believed to coincide with the start of puberty (Myers, 1916; Turner & Schulze, 1931; Astwood et al, 1937). However, studies in rats (Cowie, 1949; Silver, 1953a) and in mice (Flux, 1954a), using the relative growth technique by Huxley & Tessier (1936), showed that allometric growth started as early as 23 days of age, well in advance

of puberty in both species. Korfmeier (1979) found in mice that rate of mitosis in the epithelial cells increased from 15/1000 cells in the second week of life to 100/1000 cells at 25 days of age. This level of about 10% mitotic cells was maintained for the following 2 weeks. Sinha & Tucker (1966, 1969b) confirmed the existence of the allometric growth period in rats and heifers using the relative growth technique with mammary DNA.

The allometric growth phase is characterized by growth of the mammary stroma, formation of a delicate and highly vascularized tissue around the ducts, and growth of ducts and their epithelial buds. The ducts branch as they develop to form a system of lobes and lobules. Collecting ducts leave the gland cistern and branch into interlobar ducts. The interlobar ducts branch off to become intralobarducts, interlobular ducts, intralobular ducts and finally terminal ducts. These end where alveoli are formed during pregnancy (Mayer & Klain, 1961). The lobes and lobules are surrounded by connective stroma.

The structure of the ductular epithelium varies according to its location within the gland. The larger ducts consist of a two layered epithelium, while the terminal ducts consist of one layer of epithelial cells (Turner, 1952). The epithelial cells rest on a basement membrane. The connective tissue surrounding the small ducts is thin and delicate and quite different from the

coarser more fibrous connective tissue surrounding the larger duct (Lenfers cit., Turner, 1952).

The allometric growth period continues until after puberty. Sinha & Tucker (1969b) observed allometric growth 2-3 months after onset of puberty in heifers, while the allometric growth of the ductular epithelium in rats was found to continue during the first 4 estrous cycles (Sinha & Tucker, 1969a). This is supported by milk production data from heifers (Gardner et al, 1977; Sejrsen, 1978).

After 2-3 months or 4 estrous cycles, mammary growth becomes isometric. Sinha & Tucker (1969b) found similar amounts of DNA per 100 kg body weight in 12 and 16 month old non-pregnant heifers.

Hammond (1927) found cyclic changes in the mammary gland of virgin heifers during the estrous cycle. The changes, however, were small compared to those before and around puberty and during pregnancy. Sinha & Tucker (1969b) found that the amount of epithelial and connective tissue measured as DNA and hydroxyprolin was highest during estrous and decreased during the luteal phase.

Lenfers (1907 cit, Turner, 1952) studied the histology of virgin heifers between 12 and 36 months of age and found little relationship between age and udder development. The gland consisted of excretory ducts and fine ducts. No alveoli were present and the appearance of

the epithelium did not indicate secretory activity. In older heifers, however, some secretion was at times present in the end branches of the duct system. Holm (1937) found that the mammary gland of nonpregnant heifers was composed primarily of fat and connective tissue, blood and lymph vessels, nerves and collecting ducts. The ducts were found in the center of the gland.

Hormonal Control of Mammary Development

Ovarian steroids have long been known to stimulate mammary development. Ovariectomized rats (Cowie, 1949; Silver, 1953a), and mice (Flux, 1954a) show reduced ductular development. Wallace (1953) found that ovariectomy in calves resulted in almost complete cessation of mammary gland growth and that free-martins had development similar to what is found in males and ovariectomized females.

The ductular development is stimulated by estrogen. Silver (1953a) in rats and Flux (1954a) in mice showed that administration of estrogen to ovariectomized animals induced normal ductular growth. In agreement, implantation of diethylstilbestrol, a synthetic estrogen, increased mammary development and promoted spreading of the ducts into the fat pad of ovariectomized heifer calves (Wallace, 1953). Estrogen implantation in free-martins induced development of the gland exactly as in normal heifers.

Progesterone is not required for ductular development, but stimulates lobuloalveolar development in combination with estrogen (Topper & Freemann, 1980).

Stricker & Grueter (1928, 1929) first showed the involvement of the pituitary in mammary function. Turner and coworkers (Turner, 1939, cit.; Elias, 1980) showed that estrogen had no effect on mammary development when the animals were hypophysectomized. This led Turner (1939) to suggest the existence of specific mammogens from the anterior pituitary. However, in a classical series of experiments Lyons and coworkers (1958) showed that ovarian hormones synergize with the pituitary hormones, growth hormone and prolactin to induce ductal and lobuloalveolar growth of the mammary gland. They further showed in ovariectomized, adrenoectomized and hypophysectomized rats, that maximal ductular development required growth hormone + estrogen + glucocorticoids. The requirement for alveolar development was estrogen + progesterone + growth hormone + prolactin + glucocorticoids. In triple operated mice, Nandi (1959) found that estrogen + growth hormone or estrogen + growth hormone + corticoid were required for maximal ductular development, but concluded that estrogen + growth hormone + corticoids is the combination most likely to work in intact animals. Alveolar development was induced by several hormone combinations, and the most effective was the same as that

found by Lyons in rats. Cowie et al (1966) found the hormonal requirement for lobuloalveolar development to be the same in goats as in mice and rats. The requirement for duct development could not be determined, however, growth hormone + estrogen did induce duct development plus a small amount of alveolar growth.

Lyons et al (1958), Nandi (1959) and Cowie et al (1966) found estrogen and progesterone ineffective when given without the pituitary hormones. This was also observed by Talwalker & Meites (1961), who further showed that high doses of growth hormone + prolactin induced mammary growth without the presence of estrogen and progesterone. This indicates that the anterior pituitary hormones are of major importance, but it does not mean that ovarian and adrenal hormones are not needed under normal conditions (Talwalker & Meites, 1961).

Lyons et al (1958), Nandi (1959) and Cowie et al (1966) reported no requirement for prolactin for ductular development. Talwalker & Meites (1961), however, showed that prolactin, like growth hormone, induced extensive ductular development with many branches and end buds. Three out of 7 animals also showed limited lobuloalveolar development. Prolactin has also been suggested to be involved in the mammary development during recurring estrous cycles (Sinha & Tucker, 1966, 1969b). Injection of prolactin and transplantation of the pituitary to the

kidney capsule of rats increased serum prolactin and mammary DNA (Sinha & Tucker, 1968). When the rats also were ovariectomized the increase due to prolactin was reduced, indicating a synergistic effect of pituitary and ovarian hormones. Eventhough growth hormone and prolactin are required for mammary growth, they may not be rate limiting (Tucker, 1981). The reasoning is that during gestation, when there is rapidly increased mammary growth no change in serum growth hormone and serum prolactin occur (Oxender et al, 1972; Koprowski & Tucker, 1973a).

The effect of corticoids seems to be stimulatory or permissive rather than direct, and extensive mammary development can occur without glucocorticoids (Topper & Freeman, 1980). Furthermore, the effect of corticoids is dose-dependent. Flux (1954b) found that high doses of cortisol inhibited the stimulating effect of estrogen in mice. Munford (1957) also found that high doses of cortisol inhibited mammary growth, but low doses, in agreement with Lyons et al (1958) and Nandi (1959), had a stimulating effect. It is therefore difficult to predict the effect of a change in serum glucocorticoids on mammary growth.

Studies investigating the effect of thyroid hormone on mammary development have shown varying results. Jacobsohn (1960) and Moon & Turner (1960) showed a positive relationship, while Meites & Kargt (1964) found a negative

effect. Recent results by Vonderhaar & Greco (1979) showed that hypothyroidism in rats leads to decreased mammary growth, but removal of thyroid hormone did not lead to regression of pre-existing mammary structures and the cells maintained their full functional capability. Hyperthyroidism, in contrast, resulted in more rapid and extensive proliferation of the mammary epithelium. Thyroid hormone, therefore may be rate limiting for mammary growth (Tucker, 1981), however, it is not absolutely necessary for ductal growth (Topper & Freeman, 1980).

Insulin is needed for mammary gland growth *in vitro* (Elias, 1957). *In vivo* studies showed that rats given estrogen + progesterone + alloxan had 18% less DNA after 19 days than rats given only estrogen + progesterone (Jacobsohn, 1961). Alloxan destroys insulin secreting cells in the pancreas and therefore caused decreased insulin secretion. Results by Kumaresan and Turner (1965, 1966) also indicated a stimulatory role for insulin. Topper & Freeman (1980) nevertheless concluded in their review that insulin is not required for ductal and alveolar development *in vivo*. Tucker (1981) concluded that insulin is unlikely to be rate-limiting for normal mammary development in cattle during pregnancy, since serum insulin decreased during pregnancy (Grigsby et al, 1974).

The experiments by Cowie (1949), Wallace (1953), Silver (1953a) and Flux (1954a) showed that the onset of

the allometric growth period is linked with secretion of estrogen from the ovaries. Cowie (1949) suggested that the most likely explanation for the onset of allometric growth is an increase in estrogen concentrations above a certain threshold. The reason that allometric growth does not occur at an earlier age may also be due to lack of sensitivity of the mammary gland to estrogen. Silver (1953b) found that the mammary gland was practically insensitive to estrogen before normal onset of allometric mammary growth.

Silver (1953b) investigated the possibilities of inducing allometric growth even earlier and found that the age at onset could be decreased when estrogen was administered with pituitary extract. This suggests that functional activity of the pituitary as well as endogenous levels of estrogen are important in determining onset of allometric growth.

The reason for the return to isometric growth after puberty is not known, but evidence suggests that the ramifications of the duct system are predetermined by the boundries of the stroma (Mayer & Klein, 1961). Outgrowing ducts do not touch except at their point of origin, and there seems to be a cylinder of adipose tissue around each duct, where adjacent ducts do not enter and there is a distinct duct-free zone at the border of adipose tissue at the end of the allometric growth period (Faulkin &

DeOme, 1960). The failure of the ducts to grow further is not due to loss of hormonal signal or growth potential, but appears to be a local "stop-signal" associated with the border of the fat pad or the adipose tissue (Faulkin & DeOme, 1960). Topper & Freeman (1980) suggest that the "stop signal" is of epithelial origin.

Faulkin and DeOme (1960) described the regulation of development of the duct system by the analogy of a "stop" and "go" system. Systemic hormones known to effect mammary growth are the "go" signals, while the local growth inhibiting phenomenon provides the, "stop" signal. The "go" signal must be present before growth can occur, but the "go" signal can be superseded by the "stop" signal acting at the local level.

Tucker (1981) suggested that the failure of allometric mammary growth to continue after puberty may be related to the asynchronous secretion of progesterone and estrogen during recurring estrous cycles. Progesterone is found to decrease the number of estrogen receptors (Leung & Sasaka, 1973) and may therefore reduce the sensitivity of the mammary gland to estrogen.

Role of Hormones in Regulation of Nutrient Utilization

Growth Hormone

Growth hormone has long been recognized as important for growth (Evans & Long, 1921; cit Philips,

1979), and growth hormone treated animals have improved growth rate (Brumby, 1959; Machlin, 1972; Lee et al, cit Machlin, 1976). Growth hormone stimulates many aspects of protein synthesis. Nitrogen retention, amino acid uptake and incorporation into protein as well as enzymes involved in regulation of protein synthesis are stimulated by growth hormone.

Growth hormone also stimulates milk production and injection of growth hormone has resulted in increased milk production (Hutton, 1957; Machlin, 1973; Bines et al, 1980; Peel et al, 1980). The increased milk yield may be due at least in part to an altered partitioning of nutrients between milk and body tissue, since the cows given growth hormone were not allowed to consume extra food. Bines & Hart (1978) suggested that growth hormone may play a role in mobilizing body fat and diverting the energy away from body tissue synthesis; and Lee et al (1974) has shown that bovine growth hormone has lipolytic activity. In agreement, Trenkle (1978) suggested that when nutrient intake is inadequate to meet demands, growth hormone acts to facilitate transfer of energy from adipose to lean tissue or to the mammary gland for milk synthesis in early lactation.

Another possible explanation for the higher milk production is that growth hormone stimulates the capacity of the mammary gland for nutrient uptake and milk synthesis. This has been proposed by Riis (1981).

The proposed roles of growth hormone in stimulation of growth and fat mobilization are supported by several experiments. Hart et al (1978) found growth hormone to be positively correlated with milk yield and negatively correlated with body weight changes; and the serum concentrations were elevated in early lactation, which agrees with experiments by Koprowski & Tucker (1973b), Hove & Blom (1976), and Smith et al (1976). Trenkle & Irvin (1970) and Trenkle & Topel (1978) found that serum growth hormone concentration was positively correlated with percent lean meat of the carcass and negatively correlated with carcass fat.

Prolactin

The role of prolactin in regulation of nutrient utilization is not well understood. McAtee & Trenkle (1971b) suggested an anabolic effect of prolactin, since increased concentration of prolactin in the circulation coincides with the absorption of nutrients from the digestive tract. Forbes et al (1975) proposed that the increased body weight gain observed with 16 hours photoperiod was due to increased prolactin. The increase in daily gain, however, occurs even if prolactin is depressed by low temperatures (Peters & Tucker, 1978; Peters et al, 1978). It is therefore likely that the higher growth rate of animals on 16 hours photoperiod is due to factors other

than increased serum prolactin; and the role of prolactin in regulation of growth is uncertain.

Prolactin's role in regulation of metabolism also is unknown. Bauman & Currie (1980) suggested that prolactin is involved in the homeorhetic control of lactation by decreasing synthesis of lipid reserves and increasing mobilization of lipid stores, as observed in late pregnancy and early lactation (Sidhu & Emery, 1972; Metz & van den Bergh, 1977).

The proposal is based on findings showing that blocking of prolactin release during early lactation increases adipose lipid synthesis and reduces rates of lipid mobilization (Zinder et al, 1974; McNamara & Bauman, 1978; Agins et al, 1979). Furthermore, they found that exogenous prolactin reversed these effects. Zinder et al (1974) reported that increased prolactin at parturition decreased lipoprotion lipase (LPL) activity in adipose tissue and increased LPL activity in the mammary gland. Shirley et al (1973) also found that prolactin stimulated mammary gland LPL activity.

An alternative role of prolactin in control of lipid metabolism during the onset of lactation was proposed by Swan (1976). He suggested that prolactin stimulates accumulation of body lipid reserves. The proposal was based on negative correlations between basal prolactin and serum free fatty acids during the first 3 months of

lactation. Koprowski & Tucker (1973a) found that basal serum prolactin (5-20 mg/ml) was lower in early than late lactation, but that the milking induced release of prolactin (>100 ng/ml) which decreased as lactation advances.

Insulin

Insulin plays a central role in regulation of metabolism. Insulin has important anabolic effects (Bassett, 1978) and is positively correlated with growth rate (Trenkle, 1970; Eversole et al, 1980). Glucose uptake and utilization by peripheral tissues is stimulated by insulin (Bassett, 1975), whereas the mammary gland is insensitive to the stimulatory effect of insulin on glucose uptake (Hove, 1978). Insulin also stimulates peripheral tissue uptake of amino acids, incorporation of amino acids into protein (Bassett, 1978) and utilization of acetate by peripheral tissues (Yang & Baldwin, 1973). In adipose tissue, insulin stimulates lipogenesis and inhibits mobilization of fat (Bauman, 1976). All these actions of insulin support the flow of nutrients towards deposition of body tissue and away from the mammary gland.

In line with the above-mentioned actions of insulin, Hart et al (1978) found that serum insulin was negatively correlated with milk yield. Injection of insulin in lactating cows depressed milk production

(Kronfeld et al, 1963). Serum insulin has been observed to be low in early lactation and increase as lactation advances and milk production decreases (Hove & Blom, 1973; Koprowski & Tucker, 1973b; Smith et al, 1976; Hart et al, 1978).

Glucocorticoids

Glucocorticoids participate in minute to minute regulation of metabolism. Glucocorticoids increase availability of glucose for glucose-requiring tissues by increasing gluconeogenic precursors through promoting catabolism of protein and adipose tissue (Schulz, 1974). The availability of glucose is also promoted by decreasing peripheral glucose utilization in muscle and adipose tissues (Reilly & Black, 1973; Schulz, 1974). These actions divert nutrients away from body deposition and are negatively related to growth rate in growing heifers (Purchas et al, 1971b), bulls (Obst, 1974) and steers (Trenkle & Topel, 1978).

The glucocorticoids presumably have a permissive rather than a direct effect. They act by increasing the responsiveness of the target tissue to other hormones affecting metabolic processes (Riis, 1981).

Adrenalectomy impairs milk production due to impaired electrolyte, protein and carbohydrate metabolism; and milk secretion was partially restored by gluco- and

mineralocorticoids (Cowie & Tindal, 1958; cit Tucker, 1974). This suggests a stimulatory effect of corticoids on milk production, but several reports indicate that glucocorticoid causes a decrease in milk production in cattle (Shaw et al, 1955; cit Tucker, 1974). Koprowski & Tucker (1973b) found that overall serum glucocorticoid concentrations at various stages of laction, sampled 1 hour after milking, were positively correlated with milk production (r = .19). However, within stage of lactation, there were no consistent relationships.

Effect of Plane of Nutrition on Milk Yield, Mammary Development and Nutritional Regulation of Serum Hormone Concentrations

Milk Production

Early experiments investigated the need for feeding heifers high planes of nutrition. They showed little effect of raising plane of nutrition on subsequent milk production. Hansen and Steensberg (1950) compared 3 planes of nutrition and found that the heifers fed a low plane of nutrition had slightly lower production in first lactation. In later lactations, however, these heifers had slightly higher yields. It was concluded that there was no need for feeding heifers a high plane of nutrition during the rearing period. Reid et al (1964) reached the same conclusion in an experiment comparing 3 planes of nutrition. They observed no difference in milk production through

4 lactations between heifers fed medium and high feeding levels. Similarly, Chrichton et al (1960) and Thomas et al (1959) showed no difference in milk production between heifers fed different planes of nutrition throughout the rearing period. In contrast, Eskedal and Klausen (1958) found a small decrease in subsequent milk production in heifers reared at the highest intensity compared to the lowest.

In the experiments mentioned above, differences in planes of nutrition were small. When higher feeding intensities were practiced, a decrease in subsequent milk production was observed for heifers raised on high planes of nutrition. Swanson (1960) compared heifers fed a standard plane of nutrition with heifers fed a fattening ration. Milk yields in first lactation were 15% lower for the fattened heifers and differences were maintained during subsequent lactations. Similar results were found in other experiments comparing milk yield of heifers raised on different planes of nutrition (Herman & Ragsdale, 1946; Amir et al, 1968; Brannang & Lindkvist, 1978; Little & Kay, 1979). Hansson et al (1967) compared heifers raised on several feeding levels and showed decreased milk production as plane of nutrition increased from 60% of standard to ad libitum. The differences were maintained during all lactations.

Several experiments have been conducted where high feeding intensity during rearing combined with early calving was compared with normal feeding intensity and a normal calving age. The increased plane of nutrition was combined with the decreased calving age for the following reasons: Firstly, onset of puberty is more closely related to body weight than age (Reid et al, 1964; Sejrsen & Larsen, 1978); hence an increased feeding intensity decreases age at puberty and makes a further decrease in age at calving possible. Secondly, heavy feeding was used to obtain sufficient size at parturition to avoid calving difficulties, since smaller cows have more calving problems (Philipsson, 1976; Larsen & Sejrsen, 1979). Thirdly, a positive relationship between body weight and milk production has been observed using data from Dairy Herd Improvement Association and progeny tests (Miller & McGilliard, 1959; Clark & Touchberry, 1962; Nielsen, 1962). The positive effect of size on production, however, seems to be related to genetically determined mature size rather than actual body weight at a given age. Miller & McGilliard (1959) found that most of the difference in first lactation milk yields related to body weight, was associated with herd differences. After correction for age and herd differences, body weight only could explain 2% of the total variation in first lactation yield.

In most of the experiments, comparing early calving and high feeding intensity during rearing with normal calving and normal feeding intensity, the heifers of early calving age had lowest milk production in the first as well as later lactations (Swarck & Lippmann, 1971; Amir, 1974; Sejrsen et al, 1976; Gardner et al, 1977; Brannang & Lindkvist, 1978; Foldager et al, 1978; Little & Kay, 1979). Larsen et al (1975), and Sejrsen & Larsen (1978) did not find significantly lower milk production in early calving cows raised on high plane of nutrition. However, in the experiment by Larsen et al (1975), the difference in feeding intensity was small and the lowest calving age was 24 months. In the experiment by Sejrsen & Larsen (1978), the heifers on the lowest feeding level probably had too high plane of nutrition for maximum milk production.

Only in a few experiments was it possible to differentiate between the effect of age and level of feeding. Little & Kay (1979) compared heifers calving at 19 months of age on a high plane of nutrition with two groups calving at 27 months on either a high or normal feeding intensity. The group calving at 19 months yielded 500 kg less milk in the first lactation than the group calving at 27 months raised on a high plane of nutrition, but 1900 kg less than the group raised on a normal feeding intensity calving at 27 months. Brannang & Lindkvist (1978) found that ad libitum-fed heifers calving at 22 or

27 months of age yielded the same, while heifers fed 50-67% of ad libitum during the rearing period and calving at 27 months of age yielded about 600 kg more milk. Amir et al (1978) examined the literature and found that milk production decreased 51 kg per month as calving age was lowered when the heifers calving at the early age was raised on a higher feeding intensity than those calving at older ages. When heifers of the different calving ages were raised on the same plane of nutrition, the decrease in milk per month of decrease in calving age was only 31 kg. These results show that the high plane of nutrition during rearing is a major reason for the lower milk production observed in heifers calving at an early age.

Mammary Growth

Mammary development depends on reproductive maturity, but sexual development is related more to body weight than to age (Reid et al, 1964; Sejrsen & Larsen, 1978). Comparisons of heifers at the same age but raised on different feeding intensities will, therefore, be confounded by differences in stage of mammary development. Also, changes in the size of the total gland not necessarily reflect changes in the parenchyma or secretory tissue, but can be due to differences in adipose tissue. Comparisons that are based on the size or weight of the total gland or on external assessment may be misleading.

In heifers of similar ages, Sorensen et al (1959) observed a positive effect of plane of nutrition on mammary development as assessed by the palpation method of Swett et al (1955). However, at 16 weeks the secretory tissue comprised only 9% of the total gland in the heifers fed the highest feeding intensity, compared to the 63% and 31% on medium and low feeding level respectively. Four heifers from each feeding intensity were slaughtered and the mean body weights on low, medium and high plane of nutrition were 77, 104 and 127 kg respectively.

Amir et al (1968) slaughtered heifers fed a medium and a high plane of nutrition at 6, 9, 12 and 16 months of age. Significantly more secretory tissue was found in udders from the heifers fed the high feeding intensity. There was an increased deposition of fatty tissue in the heavy-reared heifers, but a comparison of lipid and protein in the secretory tissue indicated that infiltration of fat into the glandular tissue was not extreme. Histological examination showed more developed tissue in heifers on the high plane when compared at same age. Amir et al (1968) concluded that high plane of nutrition did not impair the development of the mammary gland and did not cause harmful fattening. Furthermore, they suggested that fat deposition and development of secretory tissue are independent processes, one related to plane of nutrition and the other to sexual maturity.

Pritchard et al (1972) compared mammary gland development at first estrus and at 120 cm withers height of heifers fed .9 or 4.5 kg concentrate and corn silage ad libitum either with or without melengestrol acetate (MGA). There was no effect of plane of nutrition on amount of mammary parenchyma and feeding intensity had no influence on amount of mammary DNA in the heifers given MGA. However, the heifers given 4.5 kg grain and no MGA had significantly less DNA at first estrus than the heifers given 0.9 kg grain. The same trend was found at 120 cm withers height, but differences were not significant.

Herman & Ragsdale (1946) observed that heifers raised on a high feeding intensity had heavy, meaty udders and with a great deal of fat deposition at freshening. Swanson & Span (1954) reported that mammary glands of heifers and rats fed a high plane of nutrition were not fully developed. Swanson (1960) examined the udders after completion of second lactation of cows raised on high and normal feeding intensity. The udders from the cows raised on a high plane of nutrition contained areas of incompletely developed parenchyma at the periphery of the gland. Little & Kay (1979) also observed that udders of heifers raised on a high feeding intensity were different from those of heifers fed a normal plane of nutrition. They appeared smaller with a deep cleft between front and hind quarters

and between the left and right sides. The differences persisted throughout lifetime.

From the presented reports it is not possible to clearly determine if mammary growth is affected by the feeding intensity during the rearing period. However, the experiments where the comparisons are made at a comparable stage of development (Swanson, 1960; Pritchard et al, 1972; Little & Kay, 1979) the growth of the secretory tissue of the mammary gland appears impaired by high feeding intensities during rearing.

Nutritional Regulation of Serum Hormone Concentrations

The digestion and absorption of nutrients in ruminants, compared to monogastrics, is a prolonged process with continued flow of digesta for long periods after feeding. There are, however, diurnal variations in the flow of digesta and many of the observed changes in hormone concentrations are related to feeding (Bassett, 1978).

Growth hormone.--The secretion of growth hormone in ruminants is episodic (Bassett, 1974; Trenkle, 1978) making it impossible to obtain reliable estimates from a single sample (Trenkle & Topel, 1978). In meal-fed animals and during frequent sampling, growth hormone usually decreases after feeding and increases to prefeeding values within a few hours (Wallace & Bassett, 1970; Bassett, 1971,

1973, 1974a,b; McAtee & Trenkle, 1971a). In studies with sheep and cattle, prolonged periods of fasting resulted in no increase in growth hormone serum concentrations (Machlin et al, 1968; McAtee & Trenkle, 1971a), while increases were observed in pregnant ewes (Bassett & Madill, 1974). Fasting increases the sensitivity of the pituitary to other stimuli, such as stress or arginine infusion, which cause GH release (McAtee & Trenkle, 1971a; Trenkle, 1978).

Serum growth hormone concentrations are negatively related to energy level. Bassett (1974b) found less serum growth hormone in sheep fed ad libitum than at lower intakes. Also, concentrations were lower in sheep fed hay and grain ad libitum than in sheep fed only hay. Hart et al (1978) reported higher serum growth hormone in dairy cows on a negative energy balance than in beef cows on a positive balance. Reflecting the postpartum energy deficit growth hormone concentrations were higher in the dairy cows in early than late lactation. In beef cows, which maintained their body weights, growth hormone serum concentrations did not change between early and late lactation. Smith et al (1976), Koprowski and Tucker (1973b) and Halse et al (1976) also reported that growth hormone decreased as the lactation progressed in dairy cows. A negative relationship between serum growth hormone and energy intake has also been reported by Bassett et al

(1971), Hove and Blom (1973), Blum et al (1979), and Forbes et al (1979). Carstairs (1978), Trenkle & Topel (1978), and Keller et al (1979) did not find an effect of nutrient intake on serum growth hormone. However, in the experiment by Trenkle & Topel (1978), samples were taken in the postabsorptive state more than 12 hrs after the feed was removed. Therefore, growth hormone concentrations would be expected to be elevated even if the animals were fed a high plane of nutrition. In the experiments by Carstairs (1978) and Keller et al (1979) only one sample was taken per day by venipuncture. The known episodic release pattern and possible stress probably made it impossible to detect an effect of feeding level.

The mechanism by which feeding regulates growth hormone concentrations is not known (Trenkle, 1978). Glucose utilization has been suggested as an important stimuli and injection of glucose increases growth hormone serum levels (McAtee & Trenkle, 1971a; Reynaert et al, 1975). However, the normal increases in growth hormone are probably not caused by glucose, since raised serum growth hormone has been shown both when glucose concentrations were increasing and decreasing. Moreover, inhibition of glucose utilization did not affect growth hormone (McAtee & Trenkle, 1971a).

Injection of amino acids also increased growth hormone concentrations (McAtee & Trenkle, 1971a; Davis,

1972), but plasma amino acid (PAA) concentration probably does not regulate growth hormone secretion, since growth hormone was negatively correlated with PAA and ingested protein (Bassett et al, 1971). Butyrate, insulin and glucagon injections also increased growth hormone concentrations in the blood (Bassett, 1971; Reynaert et al, 1975).

All metabolites that have been shown to cause an increase in growth hormone also decrease free fatty acids (FFA) in serum. This rapid drop in FFA or their metabolites (ketone bodies) may be the signal for growth hormone secretion in the ruminant (Hertelendy & Kipnis, 1973; Reynaert et al, 1975). Hertelendy and Kipnis (1973) showed that the increase in growth hormone caused by the drop in FFA, can be eliminated by infusion of volatile fatty acids.

It has been suggested that the decrease in growth hormone after feeding is caused by release of stomach, intestinal and/or pancreatic somatostatin (Trenkle, 1978). In support, somatostatin-suppressed growth hormone secretion in sheep (David & Anfinson, 1975) and somatostatin infusion inhibited growth hormone secretion caused by several factors (Bryce et al, 1975; cit. Trenkle, 1978).

Prolactin.--Prolactin concentration in serum increases to a peak 6-8 hrs after feeding in cattle and goats, while fasting causes a decrease (Bryant et al, 1970; Schams & Karg, 1970; McAtee & Trenkle, 1971b). Hart et al

(1975) reported an increase in prolactin after feeding in beef cows which were in positive energy balance. No increase was observed in dairy cows in negative energy balance.

Growing sheep fed ad libitum had significantly higher serum prolactin concentrations than sheep fed a restricted (Forbes et al, 1975; Forbes et al, 1979). Swan (1976) reported data by Oltjen et al which showed a decrease in prolactin concentration in steers fed below maintenance after a period of ad libitum feeding. After refeeding, prolactin concentrations in serum increased to above previous levels. Swan (1976) also reported an increase in prolactin in serum of lactating cows with advancing lactation. Similar results were found by Hart et al (1978). Koprowski and Tucker (1973a) reported increase in basal prolactin concentration until week 16 of lactation. In contrast, they reported that prolactin released after milking decreased as lactation advanced after peak milk yield.

Plasma glucose does not seem to regulate prolactin secretion, since neither glucose infusion or inhibition of glucose utilization affected prolactin concentrations (McAtee & Trenkle, 1971b). Furthermore, prolactin injection has no effect on glucose or insulin concentrations (Manns & Boda, 1967). Infusion of amino acids causes a rapid increase in serum prolactin (McAtee &

Trenkle, 1971b; Davis, 1972), but prolactin injection, in contrast to GH, did not diminish plasma amino nitrogen (Manns & Boda, 1965).

Insulin.--Insulin levels generally increase, when supply of nutrients is high and decrease when supply is low. Insulin was found to be increased after feeding once a day as well as twice a day (McAtee & Trenkle, 1971c; Hove & Blom, 1973; Bassett, 1974b). The increase in insulin is biphasic with a rapid peak after feeding followed by a slower increase that is maintained for a longer time (Trenkle, 1978). The length of time insulin was elevated was directly related to the amount fed in a single meal (Bassett, 1974b). That nutrient intake is more important than volume of feed, is supported by experiments showing greater increases in insulin concentration after feeding grain than hay (Lofgren & Warner, 1972; Ross & Kitts, 1973). Bassett (1975) attributed the difference to slower digestion and a smaller amount of digestible nutrients provided by the hay diet, rather than to a specific difference between diets. Fasting causes a decrease in insulin levels (Trenkle, 1970, 1971c; Bassett et al, 1971; McAtee & Trenkle, 1971c; Bassett, 1974a).

The findings that dairy cows in early lactation have lower insulin levels than cows in late lactation (Hove & Blom, 1973; Koprowski & Tucker, 1973b; Smith et al,

1976; Hart et al, 1978) suggest that insulin concentration is related to energy balance. Hart et al (1978) reported that dairy cows in negative energy balance had lower serum insulin than beef cows in positive energy balance.

The mechanism by which diet regulates insulin concentration in ruminants is not completely understood. Glucose is probably not important for the increase after feeding, since little glucose is present in absorbed digesta and glucose concentration in the blood is more likely the end result of metabolic regulation (Bassett, 1975). Furthermore, Bassett (1975) suggests that increases in glucose 4 to 8 hrs after feeding may be involved in maintaining increased insulin concentrations.

Stimulation of insulin secretion in ruminants by intravenous or portal infusion of propionate or butyrate was reported by Manns and Boda (1967), Manns et al (1967), Horino and Machlin (1968), Trenkle (1970), McAtee and Trenkle (1971c), and Bassett (1972). Intraruminal infusion of physiological concentrations of propionate or butyrate into ad libitum-fed animals did not affect serum insulin (Stern et al, 1970), but in meal-fed or fasted animals lower amounts of VFA significantly increased serum insulin (Bassett, 1972; Overfield, 1975, cit. Trenkle, 1978). Moreover, diets causing higher concentrations of VFA's have resulted in increased insulin, and insulin and VFA's have been shown to be positive correlated (Trenkle, 1970;

Ross & Kitts, 1973). Trenkle (1978) suggests that the reason Stern et al (1970) did not find increased insulin concentration is that the animals were fed ad libitum and insulin therefore was already elevated.

Infusion of large amounts of amino acids elevated insulin secretion (McAtee & Trenkle, 1971c; Davis, 1972). Bassett et al (1971) found a positive correlation between insulin concentrations and concentrations of several amino acids; however, insulin was more closely related to amount of protein digested in the intestine. Plasma amino acids do not usually rise after feeding in ruminants (Purser et al, 1966; Fenderson & Bergen, 1975) and the removal of amino acids by peripheral tissue may actually exceed the rate of absorption from the small intestine (Bergen, 1979). The net result of feeding may therefore be a decrease in plasma amino acids. The reason for this may be that an increase in VFA's after feeding causes release of insulin (Trenkle, 1970), which stimulates removal of amino acids from the circulation by peripheral tissue (Bergen, 1979). It is therefore unlikely that amino acids play a major role in stimulating insulin secretion after feeding. In agreement, Baile et al (1971) found no important role for amino acids in regulating serum insulin.

Neither VFA's, glucose nor amino acids cause the first rapid release of insulin after feeding, but it probably results from the release of gastrointestinal

hormones due to passage of feed into the intestine, since cholecystokinin and secretin stimulate insulin secretion (Baile et al, 1969; Trenkle, 1972). Bassett (1978) suggested that stimulation of the vagus nerve is involved in the early changes in insulin concentrations after feeding cattle.

Glucocorticoids.--The effect of nutritional state on serum glucocorticoids is not clear (Trenkle, 1978). There was no change in corticoids with time after feeding in sheep fed once daily (Bassett, 1974b) or in cows fed twice a day (Hudson et al, 1975). Trenkle and Topel (1978) found higher concentrations of glucocorticoids in steers fed a restricted plane of nutrition than in steers fed ad libitum. The feed was removed from both groups the day before sampling. The difference between the restricted and ad libitum fed animals increased with increasing body weight. In contrast, Mills and Jenny (1979) found elevated serum glucocorticoids in dairy heifers fed a high energy ration ad libitum (70% concentrate:30% corn silage) compared with heifers fed a lower energy ration ad libitum (20% concentrate:80% corn silage). Glucocorticoids were higher in the heifers fed high energy before, as well as 1, 12, 24, 48 and 72 hrs after feeding.

Discussion of Suggested Causes for Low Subsequent Milk Production in Heifers Raised on High Plane of Nutrition

The reason for the low milk production in heifers raised on high planes of nutrition is not known, but impaired mammary development has been suggested (Swanson, 1960; Amir, 1975; Little & Kay, 1979). The available data are not conclusive, but indicate that high planes of nutrition negatively influence development of secretory tissue (Swanson, 1960; Pritchard et al, 1972; Little & Kay, 1979).

Swanson (1960) suggested that poor mammary development in heifers raised at high feeding intensities may be caused by fat infiltration in the mammary gland preventing development of the secretory tissue. In contrast, Amir et al (1968) stated that the fat infiltration into the secretory tissue was not extreme and concluded that harmful fattening did not occur. Amir et al (1968) suggested that secretory tissue development was related to the stage of development and not to fat deposition.

Sejrsen (1978) proposed that impaired mammary development could be due to changes in hormones involved in regulation of mammary growth. This possibility cannot be ruled out, since hormones known to regulate mammary growth are affected by plane of nutrition. Growth hormone, known to stimulate ductular development in goats (Cowie et al, 1966) is inversely related to energy intake

(Hove & Blom, 1973; Bassett, 1974b). It is therefore possible that decreased mammary growth in heifers fed high planes of nutrition could be related to lower serum growth hormone concentrations. The role of prolactin in regulation of ductular development is not clear, but it has stimulated some ductular development in hypophysectomized goats (Cowie et al, 1966). However, prolactin serum concentrations were highest in sheep fed high planes of nutrition (Forbes et al, 1979), indicating a negative relationship between serum prolactin and growth of mammary ductular tissue. Insulin concentration in the circulation is also positively related to plane of nutrition (Bassett, 1974b) but probably has no effect on mammary growth since it does not regulate mammary development (Topper & Freeman, 1980; Tucker, 1981). The effect of plane of nutrition on glucocorticoid concentrations in the blood is unclear. Eventhough glucocorticoids are involved in regulation of mammary growth (Lyons et al, 1958; Nandi, 1959; Cowie et al, 1966), the effect of a change in serum glucocorticoids is difficult to predict since low doses of glucocorticoids increased and high doses decreased mammary growth (Flux, 1954b; Munford, 1957).

Hansson et al (1967) observed that the metabolic rate was increased in heifers fed high planes of nutrition, and suggested that the decreased milk production was due to persistent changes in the part of the endocrine system which

regulates the activity of the mammary gland. Swanson (1978) suggested that the total metabolic integration of the fattened heifers may be less suited to meet the demands of lactation.

Little, however, is known about hormonal differences between heifers raised on different planes of nutrition (Little & Kay, 1979), and no reports are available on the hormonal status during lactation of heifers raised at different feeding intensities. If the observed negative relationship between plane of nutrition and serum growth hormone and the positive relationship between plane of nutrition and serum insulin persists into the lactation the higher serum insulin and lower serum growth hormone in heifers raised on high feeding intensity would favor body deposition of nutrients over partitioning the nutrients for milk synthesis (Hart et al, 1978).

It is not known, if a particular stage during the rearing period is especially sensitive to high planes of nutrition with respect to subsequent milk production (Amir & Kali, 1975; Little & Kay, 1979). Based on the accelerated allometric mammary growth before and around puberty (Sinha & Tucker, 1969b), Sejrsen (1978) suggested that this period is critical for mammary development and subsequent milk yield. Results from Danish experiments support this idea, since milk production in first lactation was inversely related to growth rate before and around

puberty and not related to daily gain later in the rearing period (Sejrsen, 1978). In agreement, Brannang & Lindkvist (1978) found a strong negative relationship between daily gain 6 months before puberty and milk production. Results by Chrichton et al (1960), Swanson (1960), and Hansson et al (1967) also indicate that the negative effects of high planes of nutrition on subsequent milk production takes place early in life.

The optimal feeding intensity during rearing is not established, but breed differences exist. Swanson (1967) suggested an optimal growth rate for Holstein-Friesian heifers of 500 to 600 g per day, and 450 g for Jerseys. Amir (1974) proposed a critical upper limit for daily gain of Israeli Friesians of 800 g per day. Sejrsen (1978) examined results from Danish experiments and suggested an upper limit for daily gain of 700-750 g for Danish Red and Danish Friesians. Brannang & Lindkvist (1978) found no indication of a critical upper limit for daily gain in an experiment with Swedish breeds, but observed a continuous gradual decline in milk production as daily gain increased.

Swanson (1975) discussed future research priorities in the area of calving age and feeding of heifers. He underlined the need for research into fundamental reasons for poor mammary development and milk production in heifers raised on high feeding intensities. Such experiments should determine whether heifers can be raised on high

planes of nutrition without a negative effect on mammary growth and milk production. Tucker (1981) stated that the hormonal implications between feed intake and mammary development need to be established.

CHAPTER III

MATERIAL AND METHODS

Experimental Objective and Design

The objective of the experiment was to examine suggested reasons for the negative effect of raising heifers on high feeding intensity on subsequent milk production.

Specific questions the experiment attempted to answer were:

1) Do high planes of nutrition negatively affect mammary development?

2) Does plane of nutrition have the same effect on mammary development in the allometric and the isometric periods?

3) Does plane of nutrition affect serum concentrations of hormones, which regulate mammary growth and function, and, if so, are the changes related to changes in mammary development?

4) Does plane of nutrition affect the ability of the heifer to respond to a metabolic challenge?

The experiment was carried out as a 2 x 2 factorial design with plane of nutrition and stage of development as main factors (Table 1). The two feeding intensities were ad libitum (A) and restricted feeding (R). Heifers on

TABLE 1.--Experimental design.

Stage of development	Plane of nutrition	No. of animals
Prepubertal	Restricted (R)	5
	Ad libitum (A)	6
Postpubertal	Restricted (R)	5
	Ad libitum (A)	6

group R were fed to gain approximately 600 g per day, which is 100-200 below the growth rate recommended by National Research Council (1978), but in agreement with Danish recommendations (Foldager et al, 1980).

Stages of development compared were prepubertal heifers in the allometric phase of mammary growth and postpubertal heifers in the isometric growth period.

Heifers in each stage of development started the experiment and were slaughtered at same body weight rather than same age. The reason being that the mammary gland is part of the reproductive system (Cowie, 1974), and that reproductive development, and therefore mammary development, is more closely related to body weight than age (Reid et al, 1964).

The challenges applied were fasting and injection of thyrotropin releasing hormone (TRH). The challenges

and collection of blood after feeding were also carried out at similar body weights.

Animals

Twelve prepubertal and 12 postpubertal Holstein-Friesian heifers were grouped by body weight and randomly assigned to the different planes of nutrition. Two heifers were lost during the course of the experiment. Cause of death of one reported at autopsy was bloat. The other heifer that was lost was unknowingly bred by a young bull at about 200 kg body weight.

The heifers were purchased at a livestock market, so genetic background, apart from breed, and age were unknown. The age at the start of the experiment was estimated from the average growth curve for Holstein-Friesian heifers published by National Research Council (1978).

Age and body weight at the beginning of the experiment and body weight at slaughter is shown in Table 2. The prepubertal heifers started the experiment at approximately 175 kg body weight and were slaughtered at 320 kg. The postpubertal started around 300 kg body weight and were slaughtered at approximately 440 kg. The prepubertal heifers were 7 months of age at the beginning of the experiment, and postpubertal were 13 months.

TABLE 2.--Age and body weight at beginning and body weight at slaughter.

	Prepuberty		Postpuberty	
	Restricted	Ad libitum	Restricted	Ad libitum
Age at beginning, months	7.4±.3	7.0±.3	13.1±.3	13.1±.3
Body weight at beginning, kg	180±7	172±7	302±7	304±7
Body weight at slaughter, kg	320±10	321±9	440±10	438±9

The heifers were weighed for three consecutive days after arrival, at 14 day intervals throughout the experiment, and the day before slaughter.

At the beginning of the experiment, only the postpubertal had reached puberty. The prepubertal heifers, reached puberty at an average age of 10.8±.8 and 9.7±.3 months for the R and A groups, respectively. The heifers in group A reached puberty at 278±11 kg body weight compared to 258±19 kg in group R. Thus, heifers that started the experiment before puberty and referred to as the prepubertal animals actually reached puberty well in advance of the planned time of slaughter.

Feeding

All heifers were individually fed the same ration once a day. The heifers of group A received sufficient feed for a 10% weighback each day, while the heifers on group R usually had eaten their feed within two hours. The amount offered to group R was adjusted biweekly according to intake during the previous two weeks.

During fasting, feed was removed two hours after feeding when group R, under normal conditions, had eaten their daily allotment. Heifers were then kept without feed until the normal feeding time (10:00 a.m.) 2 days later.

A complete mixed ration, which, on a dry matter basis, consisted of 55% corn, 6% soybean meal, 39% alfalfa

haylage and trace mineralized salt, was fed. During part of the experiment high moisture corn replaced dry corn. The crude protein averaged 15%, which was higher than originally planned. The increase was made by adding more soybean meal to the ration, because the haylage appeared to have been subjected to some heat damage.

Feed was sampled on alternate weeks and analysed for crude protein, acid detergent fiber (ADF), and dry matter according to AOAC (1975). The average percent dry matter, crude protein, and ADF in dry matter of the individual feeds are shown in Table 3.

TABLE 3.--Composition of individual feedstuffs.

	No. of analysis	% dry matter	% crude protein	% ADF
Haylage	25	52.4±1.9	16.1±.3	43.7±1.0
Corn grain	13	85.3±.6	10.4±.1	4.1±.5
High moisture corn	12	67.9±.7	9.9±.3	3.6±.2
Soybean meal	22	87.7±.1	47.5±.7	6.4±.2

Blood Sampling

Blood samples were collected during 2 periods. During each collection period blood was sampled twice 9 days apart. This was done to assure that in heifers which had

reached puberty at least one bleeding was carried out during the luteal phase of the estrus cycle. Only samples taken in the luteal phase were analysed. At each sampling, blood was collected at 30 minute intervals from 1 hour before feeding on one morning to 1 hour after feeding the following morning. At each sampling 52 samples were collected from each heifer. Age and body weight at each sampling period (bleed 1 and 2) and at the TRH challenge are shown in Table 4.

The first blood collection period (bleed 1) was carried out 5-7 weeks after heifers on group A started ad libitum feeding. At this time the growth rate was very high (13-1400 g per day), but the heifers did not appear fat. However, at the second collection period (bleed 2) 13-14 weeks after the start of ad libitum feeding, group A heifers were extremely fat.

The TRH challenge was carried out in prepubertal heifers at about 275 kg body weight (Table 4), and at approximately 400 kg in the postpubertal. The dosage used was 15 ug TRH per 100 kg body weight. The TRH was injected into each animal through a catheter inserted into the jugular vein 1½ hours after feeding. Blood was collected at -15, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 minutes after the challenge.

The heifers were fasted after bleed 2. During fasting "windows" of 4 samples were collected at 30 minute

TABLE 4.--Age, body weight and length of time on ad libitum feeding at blood sampling.

Plane of nutrition	Stage of development			
	Prepuberty		Postpuberty	
	Restricted	Ad libitum	Restricted	Ad libitum
Bleed 1				
Age, months	11.0±.9	8.6±.5	16.2±.2	14.3±.1
Body weight, kg	251±14	247±15	360±3	363±5
Weeks on ad libitum feeding[u]	-	6.7±.5	-	5.4±.4
TRH Challenge				
Age, months	12.6±.9	9.2±.5	17.9±.9	15.2±.1
Body weight, kg	281±11	270±14	392±6	398±5
Weeks on ad libitum feeding	-	9.4±.2	-	9.4±.1
Bleed 2				
Age, months	14.1±.9	10.3±.3	20.1±.5	16.2±.1
Body weight, kg	307±10	304±11	429±7	425±6
Weeks on ad libitum feeding[u]	-	13.5±.8	-	13.7±.4

intervals. The "windows" were taken after 22 and 45 hours of fasting and immediately after refeeding.

Ambient temperatures were recorded at regular intervals during blood collection.

Throughout the experiment, weekly blood samples were collected from a tail vessel from all heifers. The samples were assayed for progesterone for determination of puberty and stage of the estrous cycle at bleeding and slaughter.

Handling of the Blood

After collection, blood was allowed to clot at room temperature. After clotting, it was stored at 4°C until centrifuged at 2500 xg for 20 minutes. This usually occurred 24 to 36 hours after collection. Blood serum was poured off and stored at -20°C until hormone assay.

Hormone Assays

Serum concentrations of growth hormone, prolactin and insulin were determined by double antibody radioimmunoassay (R.I.A.) procedures described by Purchas et al (1970), Tucker (1971) and Grigsby et al (1974), respectively. Total serum glucocorticoids were determined by competitive protein binding according to procedure described by Smith et al (1972).

Methods of Measuring Mammary Growth

Histometric procedures for measuring mammary development are relevant to all stages of mammary growth (Cowie & Tindal, 1971), however, quantification is on a per field basis; therefore total mammary development is not estimated (Tucker, 1981).

The gross weight of the mammary gland does not indicate the amount of secretory tissue. However, when trimmed for surrounding adipose and connective tissue, the weight of the parenchyma is an objective measure of mammary development (Cowie & Tindal, 1971).

Determination of mammary DNA is a commonly used technique for quantitating total cell numbers in the mammary gland. The procedure is based on the assumption that DNA per cell is constant in somatic cells for a given species (Mirsky & Ris, 1949). The method was first used for assessment of mammary growth by Kirkham & Turner (1953). Measurement of DNA does not quantify individual classes of cells. Therefore, a change in mammary DNA may not necessarily reflect changes in secretory cells.

To overcome possible errors in assessment of amount secretory tissue, determination of DNA has been combined with determination of fat and hydroxyproline (Paage & Tucker, 1969). Hydroxyproline is present in collagen in a fixed percentage; thus, it measures the amount of collagen in tissue. If no changes in fat and collagen

are observed, changes in DNA are likely to be due primarily to changes in secretory tissue.

Histological procedures, in contrast to DNA, identify relative changes in individual cell classes. Munford (1964) therefore concluded that the biochemical and histological methods should not be considered as alternatives, but as complementary methods for assessing mammary gland growth and development.

Weight of Parenchyma and Adipose Tissue

There is recurrent growth and regression of the mammary gland during estrous cycles (Sinha & Tucker, 1969b). Changes are especially dramatic just before and after estrus. Therefore, heifers were all slaughtered in the luteal phase of the estrous cycle. The stage of the cycle was checked the day before slaughter by palpation. After slaughter, the size of the follicles and the corpus luteum were examined.

Mammary glands were removed immediately after slaughter and stored at -20°C until dissection. Frozen glands were sawed into 2-3 cm thick steaks with a band saw. Skin and teats were removed and the remainder of the gland was separated into glandular parenchyma and adipose tissue. The separation of adipose and parenchyma was by the color of the tissue. Adipose tissue is white and parenchyma orange, yellow (Mayer & Klein, 1961). Adipose tissue

and the parenchyma were weighed separately and weights recorded.

Biochemical Measures of Mammary Growth

After dissection and weighing, the glandular tissue and the adipose tissue were minced separately in a meat grinder. From the ground tissue 100-200 g were randomly taken for determination of DNA, RNA, lipid and hydroxyproline. Samples were kept at -20°C until analysed.

Two g of tissue were suspended in 38 ml of distilled water and homogenized. Then DNA, RNA and lipid were determined according to the methods described by Tucker (1964). Hydroxyproline was measured using the method of Prockop & Udenfriend (1960).

Histological Assessment of Mammary Development

At slaughter, three slices were taken from the left, rear quarter of the mammary gland for histological determination of development. Slices were taken from 3 zones. Zone 1 was 1 cm above the base of the gland, adjacent to the gland cistern. Zone 2 was centered between the base of the gland and the edge of the parenchyma, and zone 3 was 1 cm below the edge of the parenchyma. The distance from the base of the gland to the edge of the parenchyma was measured and recorded.

The slices were cut into several blocks approximately 1 mm^3 in size. The blocks were fixed for 2 hrs in Karnovsky fixative (Karnovsky, 1965, cit Akers, 1980), washed three times at hourly intervals in 0.1 M phosphate buffer containing 8% sucrose, and then stored at 4°C. Before embedding, the blocks were dehydrated by washing in increasing concentrations of ethanol solutions (30, 50, 70, 90 and 100%), and 3 times in prophyleneoxide. Three tissue blocks from each zone were then embedded in a 1:1 mixture of epon and araldite (Geisleman & Burke, 1973, cit Akers, 1980).

A minimum of 5 sections were cut from the 9 blocks taken from each animal at a thickness of 10-20 u on an ultramicrotome. The sections were mounted on glass slides and stained with Asure II (Feon, 1965, cit Akers, 1980).

Five randomly selected sections from each of 9 slides per animal were used to estimate relative amounts of epithelial cells, connective tissue, ductular lumen and fat cells. The connective tissue was classified into 2 classes. Class 1 was the intralobular smaller cells; and class 2 the larger, more fibrous cells found between clusters of ducts. Cell counts were made using an 8x8 square grid with 64 intersections placed in the ocular of the microscope. The method is modified after that of Chalkey (1943, cit Akers, 1980). At each of the 64 intersections in the grid the underlying type of cell

was determined and the number of each of the 5 classes computed and expressed as percent of total tissue.

For each animal, the mean cellular composition was based on 2,880 determinations (3 zones x 3 replicates x 5 sections x 64 intersections). In the complete experiment a total of 63,360 classifications were made. Before the classifications were made, the animal and treatment number on the slides were covered and a random number was assigned. Therefore, the animal and treatment were unknown to the person counting the slides.

Statistical Methods

Effects of plane of nutrition and stage of development on age, body weights, growth rate and feed intake, as well as mammary gland weights and biochemical constituents were analysed by a 2 x 2 factorial analysis of variance. Effect of plane of nutrition within stage of development was also analysed. Histological determinations were analysed by a split-plot design, using variance between animals within treatment as the error term for the effect of plane of nutrition and stage of development. The effect of zone within animals and interactions between zone and stage and plane were tested against the residual mean square.

Serum hormone concentrations were tested for normality and heterogeneity of variance. The results

showed that the serum concentrations of prolactin and insulin had to be transformed to natural logarithms (ln) in order to achieve normality and minimize heterogeniety of variance.

Each hormone was tested for effect of temperature. Results showed that serum prolactin and insulin concentrations were significantly related to ambient temperature. The effect of temperature was tested within treatments and at varying times after feeding, however, no significant differences were observed. An overall regression was used to adjust for ambient temperatures. The statistical comparisons for serum prolactin and insulin were then carried out on ln values adjusted for ambient temperature. The temperature used was the maximal temperature observed at each 24 hour sampling period.

The slope and level of the curves of serum hormone concentrations after feeding and TRH challenge were tested by multivariate analysis of variance according to Gill & Hafs (1971) and Volund (1980). The multivariate analysis is used due to high correlation between samples, which makes it more sensitive than the univariate F-test (Gill & Hafs, 1971). The number of values per animal was restricted to 12 in order to obtain appropriate degrees of freedom for the test (Anderson, 1980, personal communication). For serum concentrations between feedings, values obtained at 2 hour intervals were used.

The effect of plane of nutrition within stage of development and period of bleeding on serum hormone concentration at varying times after feeding was tested by split-plot analysis of variance. Plane of nutrition was tested against variation between animals within treatment, while the effect of bleeding was tested using residual variance as the error term. The values used at the different times after feeding were the average of 4 samples collected 30 minutes apart. The value 1 hour after feeding was the mean of hormone concentrations 30 and 60 minutes after both feedings.

The statistical comparisons between treatments for serum insulin and prolactin concentrations were carried out on the ln transformed values. However, the data presented in the figures and tables are the observed values adjusted for temperature. Therefore, there existed no standard errors pertaining to the comparisons made for the observed values. However, the mean ln values at varying times after feeding with the appropriate standard errors are shown in Tables A1 and A2.

CHAPTER IV

RESULTS

Feed Intake and Daily Gain

The ad libitum fed pre- and postpubertal heifers gained 1272 g and 1164 g per day, respectively, compared to corresponding average daily gains of 637 g and 588 g in heifers in group R (Table 5). Since the heifers were slaughtered at same body weight the heifers of group R required approximately 233 days to reach the slaughter-weight compared with about 117 days needed for the heifers of group A. The age at slaughter was therefore 14.9 and 20.9 months for pre- and postpubertal heifers of group R. This was 4 months older than the pre- and postpubertal heifers of group A, which were 10.9 and 16.9 months old at slaughter.

Prepubertal heifers of group A had a daily dry matter intake of 7.4 kg, while group R was given 4.1 kg or 55% of ad libitum. The postpubertal heifers of group R received in average 5.7 kg dry matter per day. This was 62% of the 9.2 kg eaten by group A.

Mammary Gland Development

In the prepubertal heifers the mammary glands were heavier for the heifers fed ad libitum ($P < .01$) (Table 6).

TABLE 5.--Daily dry matter intake and growth rate of heifers fed restricted or ad libitum before or after puberty.

	Stage of development			
	Prepuberty		Postpuberty	
	Restricted (R)	Ad libitum (A)	Restricted (R)	Ad libitum (A)
Dry matter intake, kg per day	4.1±.3[a]	7.4±.3	5.7±.3	9.2±.3
Daily gain, g	637±72[a]	1271±66	588±72	1164±66

[a]Group R significantly different from group A (P<.001).

The greater weight, however, was entirely due to an increase in the amount of adipose tissue ($P<.01$), since 23% less parenchyma was observed for heifers fed the high plane of nutrition ($P<.13$).

TABLE 6.--Weight of mammary parenchyma and adipose tissue of heifers fed restricted or ad libitum before or after puberty.

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
Parenchyma, g	642±65[a]	495±60
Adipose tissue, g	1040±125[b]	1708±113
Total gland, g	1683±114[b]	2203±113
% parenchyma	38±.03[b]	23±.03
% adipose tissue	62±.03[b]	77±.03
Postpuberty		
Parenchyma, g	987±110	957±100
Adipose tissue, g	1751±297	2113±271
Total gland, g	2739±321	3020±293
% parenchyma	37±.1	32±.1
% adipose tissue	63±.1	68±.1

Significance of difference between means of group R and A.

[a] $P<.13$

[b] $P<.01$

In the postpubertal heifers high feeding intensity also increased weight of the total gland, and the higher weight was totally accounted for by increased adipose tissue. However, only a 3% difference in the amount of parenchyma was observed between the two feeding levels in the postpubertal heifers.

In the prepubertal heifers of group R, the parenchyma constituted 38% of the gland compared to 23% in group A ($P<.01$). In the postpubertal heifers there was no significant difference in the relative amounts of parenchyma and adipose tissue.

Mammary epithelial cells, estimated by DNA content of the parenchyma (Table 7), in the prepubertal heifers were 32% lower in the animals fed ad libitum than in those fed restricted ($P<.11$). In contrast, plane of nutrition did not affect DNA content in postpubertal heifers (2524 vs. 2566 mg). Protein synthesis machinery measured by RNA content was lower in the heifers of group A than of group R, and the difference approached significance in the prepubertal heifers ($P<.12$). Connective tissue measured as parenchymal hydroxyproline followed the same trend as DNA with decreased amounts in prepubertal heifers of group A ($P<.05$) and no difference between groups in postpubertal heifers. The lower lipid content in heifers of group A is a reflection of less parenchyma.

TABLE 7.--DNA, RNA, hydroxyproline and lipid in the parenchyma of heifers fed restricted or ad libitum before or after puberty.

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
DNA, mg	1562±204[a]	1061±186
RNA, mg	1909±300[b]	1026±274
Hydroxyproline, mg	2288±244[c]	1466±223
Lipid, g	314±37[d]	234±33
Postpuberty		
DNA, mg	2524±426	2566±385
RNA, mg	2603±422	2298±385
Hydroxyproline, mg	3647±549	3797±501
Lipid, g	526±52	456±47

Significance of difference between means.

[a]P<.11 [b]P<.12 [c]P<.05 [d]P<.14

Epithelial cells in the parenchyma, expressed as % of the total gland (Table 8), agreed with biochemical measurements. There were 37% lower in the prepubertal heifers of group A than those of group R (P<.10). Similarly, parenchymal connective tissue was decreased 33% in the prepubertal heifers of group A. In contrast, there was little difference in % epithelial cells and connective tissue between feeding intensities in post-pubertal heifers.

TABLE 8.--Epithelial cell, parenchymal connective tissue and ductular lumen, as percent of the total gland, in heifers fed restricted or ad libitum before or after puberty.

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
Epithelial cells, %	3.74±.55[b]	2.36±.50
Connective tissue, %	18.1±1.6[a]	12.3±1.5
Ductular lumen, %	.82±.39	.83±.36
Postpuberty		
Epithelial cells, %	3.94±1.06	4.58±.97
Connective tissue, %	17.6±2.4	17.5±2.2
Ductular lumen, %	1.15±.30	.77±.27

Significance of difference between means.

[a] $P<.05$

[b] $P<.10$

The effect of plane of nutrition on the composition of the secretory tissue or parenchyma is shown in Tables 9 and 10. DNA, RNA, hydroxyproline and lipid per g of parenchyma was unaffected by feeding intensity in prepubertal or postpubertal heifers. Similarly, there was no significant difference between groups in % epithelial cells, connective tissue, fat cells and ductular lumen. The parenchyma consisted of approximately 10%

epithelial cells, 50% connective tissue, 30-40% fat cells and 2-3% ductular lumen.

TABLE 9.--DNA, RNA, hydroxyproline and lipid, per g of parenchyma, in heifers fed restricted or ad libitum before or after puberty.

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
DNA mg/g	2.47±.28	2.09±.26
RNA mg/g	2.66±.35	1.99±.32
Hydroxyproline mg/g	3.51±.31	3.07±.29
Lipid mg/g	4.84±.29	4.75±.26
Postpuberty		
DNA mg/g	2.53±.34	2.70±.31
RNA mg/g	2.62±.36	2.40±.33
Hydroxyproline mg/g	3.99±.52	3.82±.47
Lipid mg/g	5.38±.24	4.82±.22

No significant differences between means.

TABLE 10.--Epithelial cells, connective tissue, fat cells and ductular lumen, as percent of the parenchyma, in heifers fed restricted or ad libitum before or after puberty.

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
Epithelial cells, %	9.7±1.1	10.4±1.0
Connective tissue, %	47.9±3.6	54.3±3.3
Fat cells, %	40.4±4.3	32.1±3.9
Ductular lumen, %	2.1±1.1	3.1±1.0
Postpuberty		
Epithelial cells, %	10.7±2.2	13.8±2.0
Connective tissue, %	48.6±4.0	53.6±3.6
Fat cells, %	37.4±5.8	29.9±5.3
Ductular lumen, %	3.2±.9	2.6±.9

No significant differences between means.

The composition of the parenchymal tissue varies depending on its position in the gland (Table 11). The relative area occupied by epithelial cells and connective tissue decreases as the sampling site moves away from the base of the gland ($P<.01$ and $P<.001$). The decrease in connective tissue is due to similar decreases in fine and coarse connective tissue. The coarse makes up about 60% of the total connective tissue at the base of the gland as well as at the edge of the parenchyma. Fat cells constitute a larger proportion of the gland as distance

TABLE 11.--Effect of distance from the base of mammary gland on the relative area of epithelial cells, connective tissue, fat cells and ductular lumen in the parenchyma.

	Distance from base, cm		
	1.0	3.8	7.6
Epithelial cells, %	11.9±.6[b]	12.1±.6	9.7±.6
Fine connective tissue, %	24.5±1.0[a]	21.3±1.0	17.5±1.0
Coarse connective tissue, %	36.1±1.2[a]	30.2±1.2	24.3±1.2
Total connective tissue, %	60.6±1.6[a]	51.6±1.6	41.9±1.6
Fat cells, %	25.4±2.1[a]	33.0±2.1	45.4±2.1
Ductular lumen, %	2.0±.6	3.1±.6	3.0±.6

Significance of difference between zones.

[a] $P<.001$

[b] $P<.01$

from the gland cistern or the base of the gland increases ($P<.001$). There was no significant difference in the relative amount of ductular lumen in the tissue taken at different positions in the gland. This, of course, does not include the lumen of the gland cistern. The mean values for each zone within treatments are shown in Table A3.

A difficulty in assessing growth of mammary secretory tissue lies in separation of secretory tissue from adipose tissue. Since epithelial cells are considerably smaller than fat cells and the DNA content per cell is the same, the DNA content per g of tissue would be expected to be considerably higher in the parenchyma than in the adipose tissue if the two tissues are separated correctly, without leaving epithelial cells in the adipose tissue. Furthermore, if the DNA content per g of adipose tissue is the same there is less likelihood that bias in the separation of the tissues has affected the comparison between treatments. The DNA and lipid content per g of adipose tissue is shown in Table 12. Neither DNA nor fat was affected by feeding intensity. The DNA content per g of adipose is only 10-20% as high as in the parenchyma ($P<.001$). The adipose tissue contains 70-80% lipid compared to approximately 50% lipid in the parenchyma ($P<.01$). The fact that the DNA content per mg of adipose tissue is so low compared to the parenchyma makes the separation of the parenchyma from the adipose tissue less critical as long as the epithelial cells remain with the parenchyma. A 10% increase in the weight of the parenchyma because too much adipose tissue is included in the parenchyma will only increase the amount of DNA 1-2%, and a doubling of the weight of the parenchyma will only increase DNA 10-20%.

TABLE 12.--DNA and lipid, per g of adipose tissue, in pre- and postpubertal heifers fed restricted or ad libitum.

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
DNA mg/g	.17±.04	.24±.04
Lipid mg/g	765±61	701±56
Postpuberty		
DNA mg/g	.39±.06	.47±.06
Lipid mg/g	716±32	811±30

No significant differences between means.

The average daily gain of prepubertal heifers was positively correlated with mammary gland weight, weight of the adipose tissue and % adipose tissue, with correlation coefficients of .65, .75 and .75, respectively (Table 13). However, growth rate showed high negative correlations with measures of epithelial cells and connective tissue in the parenchyma. Growth rate was correlated -.48 with parenchymal weight, -.75 with percent parenchyma and -.56, -.54 and -.64 with mg DNA, RNA and hydroxyproline, respectively. Percent epithelial cells of the total gland was negatively correlated with growth rate in the prepubertal heifers (r = -.49), but positively correlated

TABLE 13.--Correlation between growth rate and mammary growth measurements in pre- and postpubertal heifers fed 2 planes of nutrition.

	Stage of development	
	Prepuberty	Postpuberty
Total gland, g	.65[b]	.05
Adipose tissue, g	.75[a]	.04
Parenchyma, g	-.48[d]	.02
% adipose tissue	.75[a]	-.01
% parenchyma	-.75[a]	.01
DNA, mg	-.56[c]	.15
RNA, mg	-.54[c]	-.13
Hydroxyproline, mg	-.67[b]	.22
Epithelial cells, % of total gland	-.49[d]	.45[e]

Significance of r = 0.

[a]P<.01 [b]P<.05 [c]P<.10 [d]P<.15 [e]P<.20

in postpubertal heifers (r = .45). Low correlations were observed for all other measures in the postpubertal heifers.

Serum Hormone Concentrations After Feeding, Fasting and TRH-Challenge

Growth Hormone (GH)

The mean GH concentrations in serum of pre- and postpubertal heifers fed ad libitum or restricted planes of nutrition are shown in Figure 1. Serum GH concentrations are similar for both planes of nutrition during the first 6 to 8 hours after feeding. At 8 hours there was

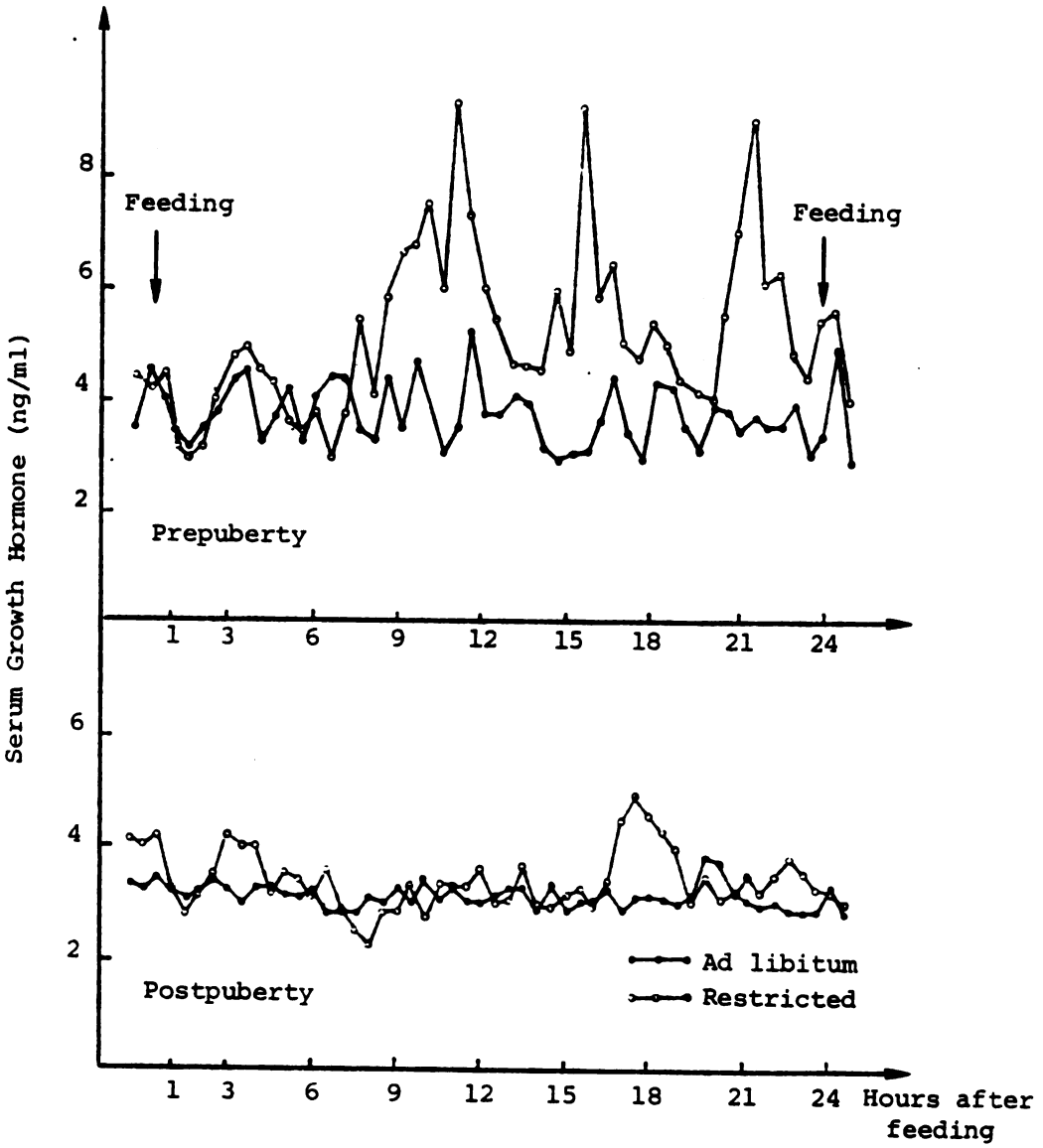

Figure 1.--Serum growth hormone concentration in heifers fed restricted or ad libitum before or after puberty. Bleed 1 and 2 combined.

an increase in GH concentration in the prepubertal heifers fed the restricted plane of nutrition, which was maintained until next feeding. The increase in the serum GH concentration on the restricted plane of nutrition was not observed in the postpubertal heifers. The effect of plane of nutrition ($P<.07$) and stage of development ($P<.12$) approached significance. There was no significant difference between the two bleeding periods.

A comparison within stage of development of mean serum GH concentrations at varying times after feeding is shown in Table 14. As noted above, higher serum concentrations of GH were observed in prepubertal heifers on group R after 8 hours post-feeding. In contrast, only small differences due to plane of nutrition were noted in the postpubertal heifers.

Growth hormone release in response to TRH-challenge is shown in Figure 2 after removal of changes due to random episodic releases. The results show greater release in the heifers of group R ($P<.05$), with mean concentrations at 15, 30 and 45 minutes being higher ($P<.01$, $<.03$, $<.03$) than in group A. A comparison without removal of episodic spikes also shows a significant effect of plane of nutrition ($P<.02$). Serum concentrations after TRH-challenges were similar in pre- and postpubertal heifers.

Serum GH remained low after removal of the feed in heifers of group A (Table 15). In contrast, GH was higher

TABLE 14.--Serum growth hormone concentrations at different times after feeding in heifers fed restricted or ad libitum before or after puberty (ng/ml).[1,2,3]

	Hours after feeding	Plane of nutrition	
		Restricted (R)	Ad libitum (A)
Prepuberty	1[2]	4.3±.7	3.8±.6
	2	3.8±.9	3.7±.8
	4	4.4±1.0	4.0±1.0
	6	3.5±1.0	4.1±.9
	8	5.8±1.4	3.2±1.3
	10	7.4±1.7[a]	3.8±1.6
	12	5.9±1.2	4.2±1.1
	14	5.0±.8[b]	3.3±.8
	16	6.5±1.2[a]	3.6±1.1
	18	4.9±.9	3.7±.8
	20	5.2±.3[a]	3.5±.3
	22	6.6±1.1[a]	3.6±1.0
Postpuberty	1	3.3±.5	3.2±.5
	2	3.4±.8	3.2±.7
	4	3.7±.8	3.2±.7
	6	3.2±.6	3.0±.6
	8	2.6±.7	3.0±.6
	10	3.2±.8	3.2±.7
	12	3.2±.7	3.1±.7
	14	3.1±.6	3.1±.6
	16	3.5±.6	3.0±.6
	18	3.7±.1	3.0±1.0
	20	3.2±.7	3.5±.7
	22	3.3±.6	3.0±.6

[1]Value for each animal mean of 4 samples taken 30 minutes apart 1 hour after feeding mean of 2 samples after the 2 feedings.

[2]Bleed 1 and Bleed 2 combined.

[3]Significance of difference between means.

[a]$P<.05$ [b]$P<.10$

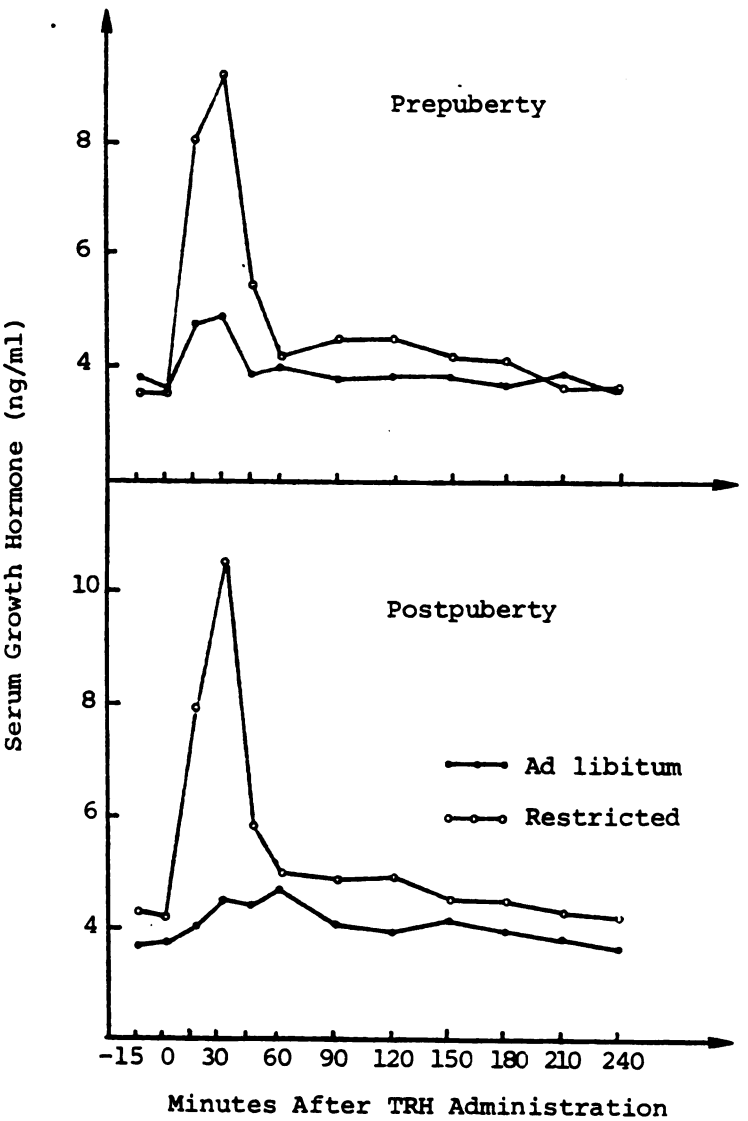

Figure 2.--Serum growth hormone after TRH-challenge in heifers fed restricted or ad libitum before or after puberty.

TABLE 15.--Serum growth hormone concentration after fasting and refeeding in heifers fed restricted or ad libitum before or after puberty (ng/ml).

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
24 hours fasting	7.4±1.0[a]	3.9±.9
46 hours fasting	4.3±1.0	4.0±.9
Refed	5.5±1.0	6.8±.9
Postpuberty		
24 hours fasting	3.7±.2[b]	3.2±.2
46 hours fasting	3.9±.2	3.5±.2
Refed	4.2±.2	4.1±.2

[a]Significant plane of nutrition x hours fasting interaction.

[b]Significant differences between means at different hours after fasting (24, 46 and refeeding).

in the prepubertal heifers of group R. The mean serum GH concentration in the prepubertal heifers of group A 24 hours after removal of the feed was 3.9 ng/ml vs 7.4 ng in the R heifers. At 46 hours of fasting GH concentrations were similar in both groups, thus causing a significant interaction between plane of nutrition and hours fasting for prepubertal heifers ($P<.05$). In contrast to what was observed at the normal feeding interval, there

was an increase in GH serum concentrations immediately after feeding in pre- and postpubertal heifers of both groups ($P<.12$ and $P<.01$, respectively).

Prolactin

Serum prolactin was strongly related to ambient temperature, and a cubic regression explained 65% of the variation in prolactin serum concentration (Figure 3). There was a gradual increase in serum prolactin from below 10 ng/ml at 5°C to a plateau of 18-20 ng/ml beginning at about 12°C. As ambient temperature rose above 20°C, serum prolactin increased rapidly from 20 ng/ml at 20°C to 32 ng at 25°C and at 30°C the predicted serum prolactin was above 80 ng/ml.

Figure 4 shows the effect of plane of nutrition on serum prolactin adjusted for ambient temperature. At 1st bleed, the high plane of nutrition resulted in a large increase in serum prolactin concentrations in pre- and postpubertal heifers. At the second bleed, prepubertal heifers in group A had higher serum prolactin concentrations than prepubertal heifers of group R. The increases, however, were considerably less than at 1st bleed. In the postpubertal heifers there was no sustained difference in serum prolactin due to plane of nutrition at bleed 2 after 14 weeks on treatment. The decrease, or lack of increase in prolactin after 14 weeks of ad libitum feeding caused

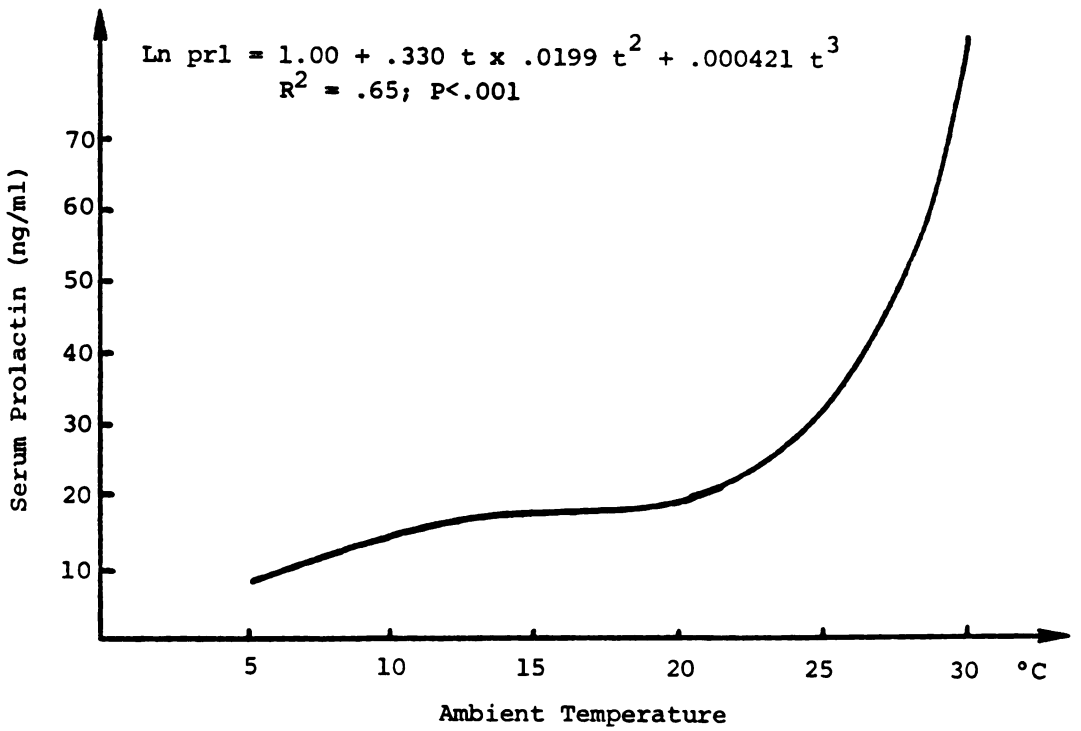

Figure 3.--Effect of ambient temperature on serum prolactin.

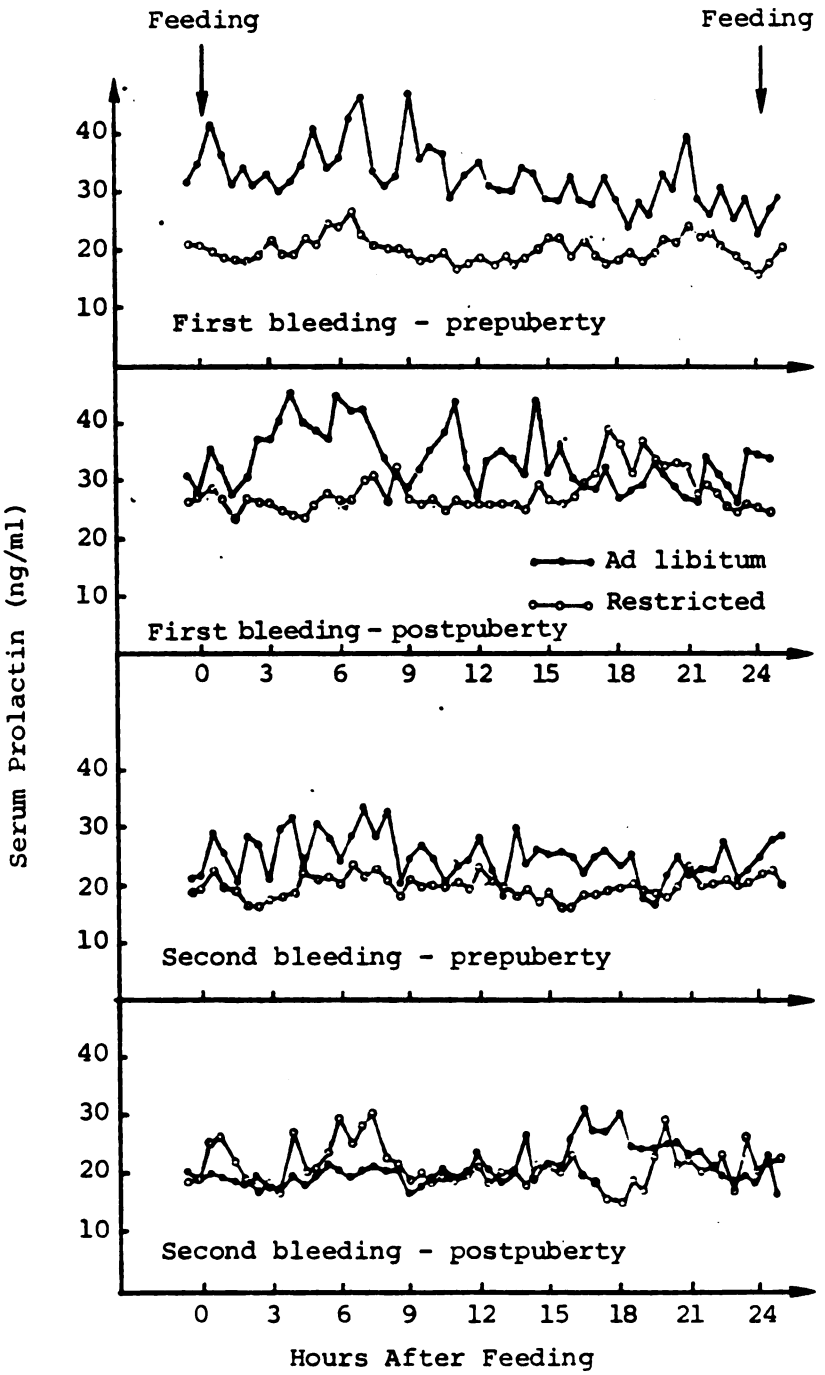

Figure 4.--Serum prolactin concentration in heifers fed restricted or ad libitum before or after puberty. Adjusted for ambient temperature.

significant interaction between plane of nutrition and bleeding period ($P<.05$).

Table 16 shows mean serum prolactin concentration at different times after feeding. Plane of nutrition at the first bleed 1 significantly affected prolactin from 1 to 14 hours after feeding (5 weeks on ad libitum feeding). At bleed 2 the mean values for the prepubertal heifers in group A are higher than for group R. However, in the older heifers no consistent pattern was noted.

Prolactin release after TRH-challenge (Figure 5) was greater in heifers in group A than in group R ($P<.02$). In Figure 5, serum concentrations of prolactin are corrected by removing the episodic spikes. The multivariate analysis of variance which tested the level and slope of curves also showed that stage of development significantly affected prolactin concentrations ($P<.01$). This, however, was due to the effect of plane of nutrition on baseline prolactin, since stage of development had no influence on prolactin concentration the first hour after challenge.

Neither plane of nutrition nor stage of development significantly influenced serum prolactin measured after 24 and 46 hours of fasting or after refeeding (Table 17).

TABLE 16.--Serum prolactin concentration at different times after feeding in heifers fed restricted or ad libitum before or after puberty (ng/ml).[1] Adjusted for ambient temperature.

		Stage of development						
		Prepuberty		Postpuberty		Significance of		
Plane of nutrition		Restricted	Ad libitum	Restricted	Ad libitum	Plane	Stage	PxST
Bleed 1:	1	19.3	34.0	29.9	33.4	P<.13	P>.20	P>.20
Hours after	2	17.9	32.4	25.1	30.5	P<.04	"	"
feeding	4	19.9	35.2	24.3	40.9	P<.01	"	"
	6	24.1	40.3	26.8	41.5	P<.05	"	"
	8	19.9	36.1	28.7	30.6	P<.15	"	"
	10	18.2	35.0	25.2	37.1	P<.03	"	"
	12	18.1	32.7	24.9	35.4	P<.05	"	"
	14	18.9	31.9	26.0	34.7	P<.18	"	"
	16	20.2	29.3	28.0	31.2	P>.20	"	"
	18	18.2	28.6	34.8	29.0	P>.20	"	"
	20	22.7	33.4	32.7	30.2	P>.20	"	"
	22	21.0	28.1	26.7	28.9	P>.20	"	"
Bleed 2:	1	21.3	27.9	25.8	20.9	P>.20	P>.20	P<.07
	2	17.2	26.4	19.0	19.8	P<.18	"	P>.20
	4	20.5	29.9	22.4	20.3	P>.20	"	P<.14
	6	22.2	29.3	27.5	22.1	P>.20	"	P<.05
	8	21.3	27.5	24.0	21.5	P>.20	"	P>.20
	10	20.9	24.5	19.8	20.1	P>.20	P<.14	"
	12	21.9	23.8	20.6	21.9	P>.20	P<.20	"
	14	19.2	26.6	21.3	23.3	P<.13	"	"
	16	18.2	25.0	22.2	27.5	P<.04	"	"
	18	19.6	23.8	22.1	27.6	P<.20	"	"
	20	20.8	21.8	24.5	26.0	P>.20	"	"
	22	19.9	24.0	20.9	21.2	P>.20	"	"

[1]Value for each animal mean of 4 samples taken 30 minutes apart 1 hour after feeding mean of 2 samples after the 2 feedings.

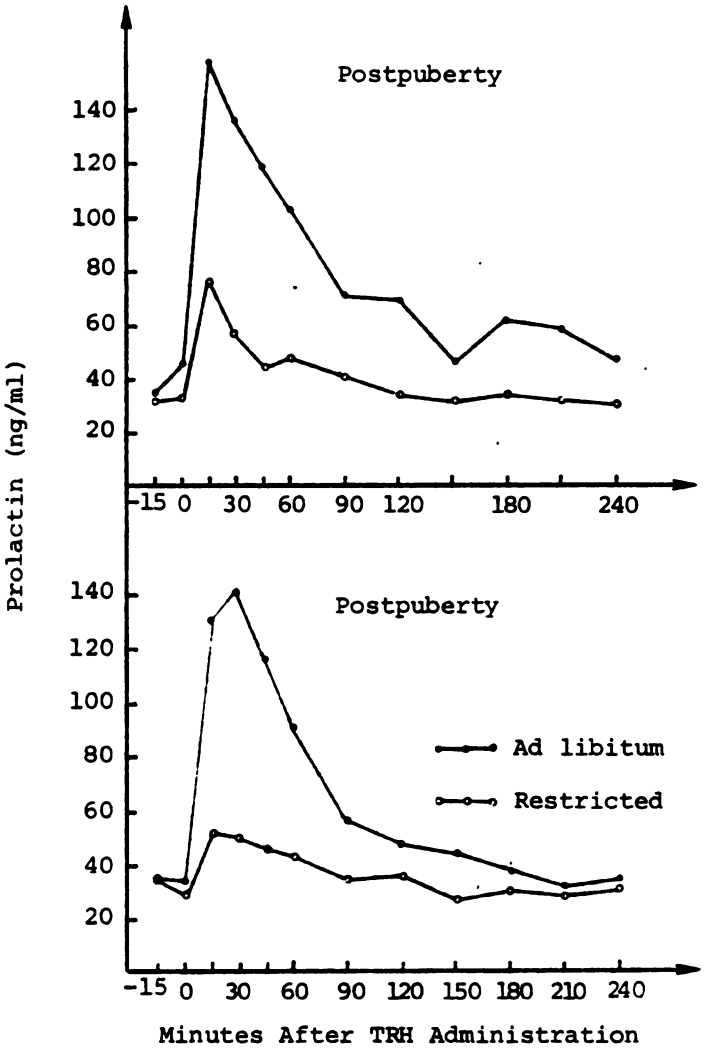

Figure 5.--Serum prolactin after TRH-challenge in heifers fed restricted or ad libitum before or after puberty. Adjusted for ambient temperature.

TABLE 17.--Serum prolactin after fasting and refeeding in heifers fed restricted or ad libitum before or after puberty. Adjusted for ambient temperature (ng/ml).[1]

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
24 hours fasting	15.5	18.6
46 hours fasting	14.2	18.0
Refed	14.4	15.6
Postpuberty		
24 hours fasting	14.3	14.1
46 hours fasting	13.9	13.5
Refed	15.6	14.0

[1]No significant difference between means of group R and A, or of pre- and postpubertal heifers and at different hours fasting.

Insulin

Serum insulin concentrations declined gradually as ambient temperature increased (Figure 6). This regression, however, described only 13% of the variation ($P<.05$), in contrast to the 65% explained by temperature for prolactin. Group A had higher serum insulin concentrations (Figure 7) than group R ($P<.05$), especially among prepubertal heifers. Serum insulin concentrations were higher ($P<.02$) in

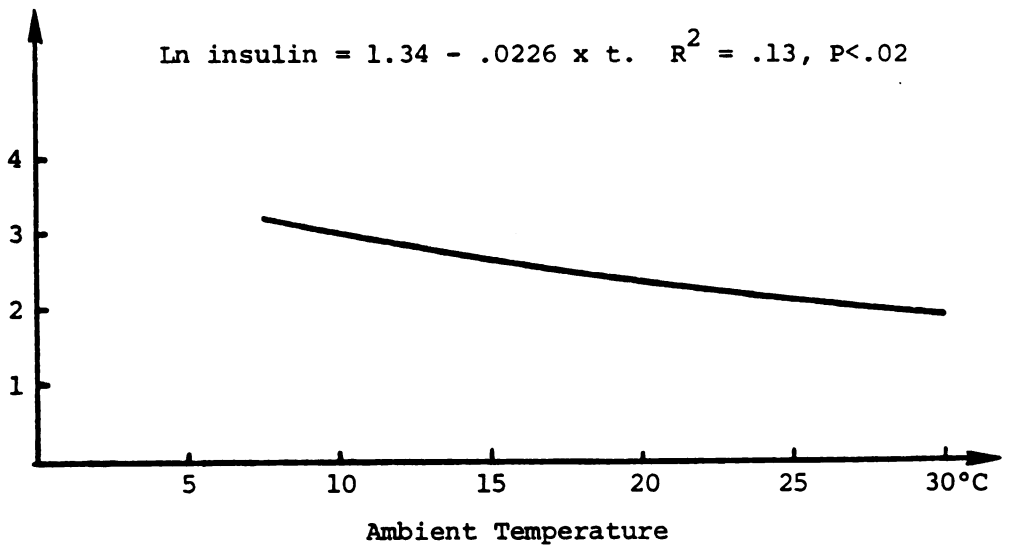

Figure 6.--Effect of ambient temperature on serum insulin concentration.

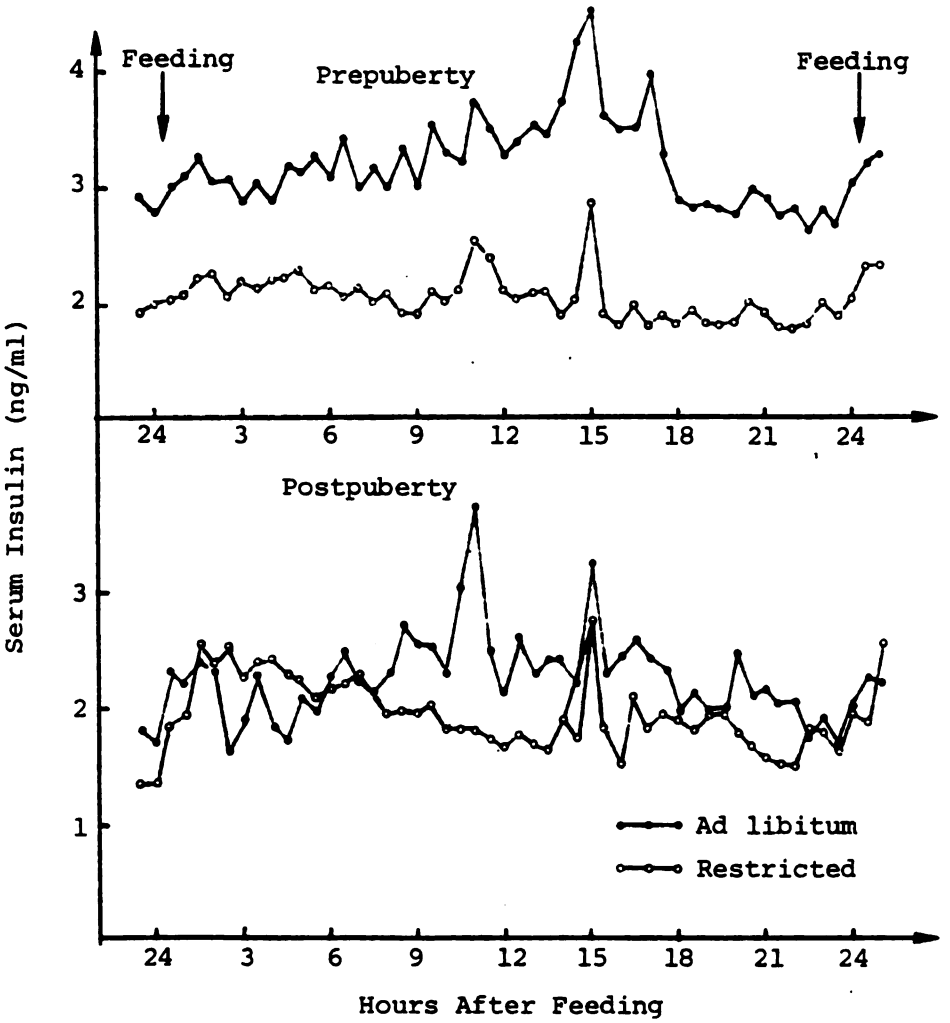

Figure 7.--Serum insulin in heifers fed restricted or ad libitum before or after puberty. Bleed 1 and 2 combined. Adjusted for ambient temperature.

postpubertal than prepubertal heifers, but was not affected by time on treatment (bleed 1 vs bleed 2).

High plane of nutrition caused larger increases in serum insulin in the pre- than postpubertal heifers (Table 18), particularly from 8 to 22 hours after feeding. There were no significant differences between the two feeding levels in the postpubertal heifers, even though the mean values were higher in heifers of group A. Serum insulin concentrations in the meal-fed animals (group R) showed an increase until about 2 hours after feeding. These increased insulin concentrations were maintained until 6-8 hours after feeding, after which there was a gradual decline until the next feeding.

The decline in serum insulin continued during fasting until 48 hours after feeding ($P<.001$; Table 19), and was not affected by previous plane of nutrition.

Glucocorticoids

Mean serum glucocorticoid concentrations 2 and 23 hours after feeding are shown in Table 20. Values for each animal are the average of 2 samples taken 30 minutes apart. Glucocorticoid concentrations were higher for animals fed ad libitum ($P<.01$) and for heifers at 23 hours than 2 hours after feeding ($P<.05$). Higher serum concentrations were also observed after 14 weeks on ad libitum feeding than after 5-6 weeks ($P<.001$). This latter

TABLE 18.--Serum insulin concentrations at different times after feeding in heifers fed restricted or ad libitum before or after puberty (ng/ml). Adjusted for ambient temperature.[1,2]

	Hours after feeding	Plane of nutrition[3]	
		Restricted (R)	Ad libitum (A)
Prepuberty	1	2.2	3.2
	2	2.2	3.1
	4	2.2	3.1
	6	2.1	3.2
	8	2.0[a]	3.1
	10	2.2[b]	3.4
	12	2.0[b]	3.4
	14	2.2[b]	4.0
	16	1.9[a]	3.7
	18	1.9[a]	3.0
	20	1.9[a]	2.9
	22	1.9[a]	2.8
Postpuberty	1	3.1	3.3
	2	3.5	3.2
	4	3.4	3.0
	6	3.2	3.3
	8	3.0	3.4
	10	2.9	3.9
	12	2.7	3.4
	14	3.0	3.6
	16	2.8	3.4
	18	2.9	3.1
	20	2.8	3.2
	22	2.6	2.9

[1]Value for each animal at each bleed is mean of 4 samples collected 30 minutes apart. One hour after feeding is mean of 2 values after the feeding in the beginning and end of the collection period.

[2]Bleed 1 and 2 are combined.

[3]Significance of difference between means of group R and A.

[a]$P<.05$ [b]$P<.10$

TABLE 19.--Serum insulin concentration after fasting and refeeding in heifers fed restricted or ad libitum before or after puberty. Adjusted for ambient temperature (ng/ml).[1]

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
24 hours fasting	3.2	4.4
46 hours fasting	2.7	3.1
Refed	2.6	2.8
Postpuberty		
24 hours fasting	4.5	3.1
46 hours fasting	3.0	2.4
Refed	2.9	2.4

[1]Significant difference between means at different hours fasting.

TABLE 20.--Serum glucocorticoid concentration in heifers fed restricted or ad libitum before and after puberty (ng/ml).[1]

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
Bleed 1:		
2 hours after feeding	1.0	6.7
23 hours after feeding	3.5	4.7
Bleed 2:		
2 hours after feeding	2.1	9.4
23 hours after feeding	13.0	10.6
Postpuberty		
Bleed 1:		
2 hours after feeding	1.1	3.6
23 hours after feeding	3.3	6.0
Bleed 2:		
2 hours after feeding	4.5	8.7
23 hours after feeding	7.9	10.7
Standard error	±1.8	±1.6

[1]Significant difference between group R and A ($P<.01$), bleed 1 and 2 ($P<.001$) and 2 and 23 hours after feeding ($P<.05$).

difference appeared due to time on treatment and not increase in body weight, since there was no difference in serum glucocorticoid concentrations between pre- and postpubertal heifers.

A significant 3-way interaction ($P<.05$) was observed between hours fasting, plane of nutrition and stage of development (Table 21).

TABLE 21.--Serum glucocorticoid concentrations after fasting and refeeding in heifers fed restricted or ad libitum before or after puberty (ng/ml).[1]

	Plane of nutrition	
	Restricted (R)	Ad libitum (A)
Prepuberty		
24 hours fasting	6.5	9.6
46 hours fasting	8.8	11.5
Refed	7.5	10.5
Postpuberty		
24 hours fasting	6.3	8.7
46 hours fasting	5.8	7.5
Refed	15.6	5.0
Standard error	±2.0	±2.0

[1]Significant 3-way interaction between plane of nutrition, stage of development and hours fasting ($P<.05$).

Correlations Between Serum Hormone Concentrations and Feed Intakes, Growth Rates and Measures of Mammary Development

In order to see, if the relationship between serum hormone concentrations and other measures were influenced by time after feeding, correlations were calculated using mean serum hormone concentration of 4 samples taken at 4, 10, 16 and 22 hours after feeding, within each bleeding period. The results showed that the closest relationships corresponded to the periods where the individual hormones were affected most by plane of nutrition and that the correlations were similar using hormone values from bleed 1 and 2. Overall correlations were therefore calculated using combined values for bleed 1 and 2. For growth hormone the mean of values at 10, 16 and 22 after feeding were used, for prolactin values at 4 and 10 hours were used, and for insulin values taken at 10 and 16 hours after feeding were averaged. For glucocorticoids the overall means were used.

Table 22 shows the correlations between feed intake and serum hormone concentrations calculated within stage of development across planes of nutrition. Growth hormone was negatively correlated with feed intake, and values were -.66 and -.14 in the pre- and postpubertal heifers, respectively. In contrast, prolactin was positively correlated with feed intake in young (r=.75) as well as

older (r=.78) heifers. Feed intake was also positively correlated with serum glucocorticoids (r=.29 and .34), while very low correlations were observed between feed intake and serum insulin.

TABLE 22.--Correlations between feed intake and serum hormone concentrations within stage of development across plane of nutrition.

	Stage of development	
	Prepuberty	Postpuberty
Growth hormone	-.66[b]	-.14
Prolactin	.75[a]	.78[a]
Insulin	.16	-.06
Glucocorticoids	.29	.34

Significance of r=0.

[a]P<.01 [b]P<.05

Correlations between serum hormone concentrations and growth rate, calculated within stage of development across planes of nutrition as well as within plane of nutrition, are shown in Table 23. Growth hormone showed a high negative correlation with growth rate in the prepubertal (R=-.82), but not postpubertal heifers (r=-.06). In heifers of group R, a correlation of -.35 was found, in contrast to the positive correlation of .22

TABLE 23.--Correlations between growth rate and serum hormone concentrations within stage of development across plane of nutrition and within plane of nutrition across stage of development.

	Stage of development		Plane of nutrition	
	Prepuberty	Postpuberty	Restricted	Ad libitum
Growth hormone	-.82[a]	-.06	-.35	.22
Prolactin	.75[a]	.74[a]	-.08	.43[e]
Insulin	.06	.09	-.42	-.11
Glucocorticoids	.30	.39	.52[c]	-.33

Significance of r=0

[a]P<.01 [b]P<.05 [c]P<.10 [d]P<.15 [e]P<.20

observed for group A. Prolactin was positively correlated with growth rate when calculated across planes of nutrition (r=.75 and .75) and in ad libitum heifers (r=.43); but in restricted heifers the correlation was -.08. Insulin showed low positive correlations with growth rate when calculated across planes of nutrition; but correlations were negative within planes of nutrition. Serum glucocorticoids were correlated positively with growth rate in pre- and postpubertal heifers and in group R. In contrast, the correlation found in group A was -.33.

Growth hormone was negatively correlated with prolactin (-.58) and glucocorticoids (-.43) in the prepubertal heifers (Table 24), but in the postpubertal heifers the correlations were low. Growth hormone showed positive correlations of .21 and .67 with serum insulin in pre- and postpubertal heifers, respectively. Positive correlations were also observed between prolactin and insulin, between prolactin and glucocorticoid and between insulin and glucocorticoids.

In the prepubertal heifers growth hormone was negatively correlated with total weight of the mammary gland (r=-.54), and amount (r=-.67) and percent (r=-.75) of adipose tissue (Table 25). In contrast, growth hormone showed positive correlations with amoung and % parenchyma as well as DNA, RNA, hydroxyproline and % epithelial cells.

TABLE 24.--Correlations between serum hormone concentrations in heifers fed 2 planes of nutrition.

	GH	PRL	INS	GC
Prepuberty				
GH		-.58[c]	.21	-.43
PRL			.21	.61[b]
INS				.09
GC				
Postpuberty				
GH		.06	.67[b]	.02
PRL			.26	.34
INS				.14
GC				

[a]P<.05 [c]P<.10

In the postpubertal heifers correlations between measures of mammary development and serum growth hormone concentrations were much lower.

Prolactin was positively correlated with adipose tissue (r=.63) and the total weight of the mammary gland (r=.53) in prepubertal heifers; whereas, negative correlations with measures of epithelial cells and connective tissue were observed. Correlation coefficients ranged from -.20 for percent epithelial cells to -.69 for RNA. In the postpubertal animals, correlations found

TABLE 25.--Correlations between serum hormone concentrations and measures of mammary growth in pre- and postpubertal heifers fed 2 planes of nutrition.

	GH	PRL	INS	GC
Prepuberty				
Total gland	-.54[c]	.53[c]	-.23	.63[b]
Adipose tissue	-.67[b]	.63[b]	-.16	.54[c]
Parenchyma	.55[c]	-.47[d]	-.11	.01
% adipose	-.75[a]	.69[a]	.01	.33
% parenchyma	.75[a]	-.69[a]	-.01	-.33
DNA, mg	.54[c]	-.53[c]	.07	-.05
RNA, mg	.75[a]	-.58[b]	.34	-.22
Hydroxyproline, mg	.52[d]	-.77[a]	-.33	-.14
Epithelial cell, % total	.48[d]	-.24	.09	-.21
Postpuberty				
Total gland	-.27	-.10	-.51[d]	.17
Adipose tissue	-.40	.03	-.44[e]	.22
Parenchyma	.31	-.37	-.29	-.11
% adipose	-.47[d]	.20	-.17	+.21
% parenchyma	.47[d]	-.20	.17	-.21
DNA, mg	.00	-.23	-.09	.29
RNA, mg	.40	-.44[c]	.26	.13
Hydroxyproline, mg	.45[e]	-.15	.41	.17
Epithelial cell, % total	.11	.02	-.29	.03

Significance of r=0

[a] $P<.01$ [b] $P<.05$ [c] $P<.10$

[d] $P<.15$ [e] $P<.20$

between serum prolactin and measures of epithelial and connective tissue were considerably lower; although, in most cases, the trend was the same.

Insulin showed no close relationship with measures of mammary development in the prepubertal or postpubertal heifers. The negative correlations with total weight of the gland and adipose tissue approached significance in the postpubertal heifers ($P<.15$ and $P<.20$).

Glucocorticoids in serum or prepubertal heifers was positively correlated with adipose tissue ($r=.63$) and weight of the total gland ($r=.54$). Relationship with other measures were generally low, as was the case for all measures in the postpubertal heifers.

CHAPTER V

DISCUSSION

Secretory tissue and mammary epithelial cells measured as amount and percent parenchyma, parenchymal DNA and percent epithelial cells in the whole gland was 20-40% lower in heifers fed ad libitum gaining 1200 g per day from 175 to 320 kg body weight, than in restricted heifers gaining only 600 g per day during the same weight interval. This supports the suggestion by Swanson (1960) that the observed negative effect of plane of nutrition during rearing on subsequent milk production is due to impaired development of mammary secretory tissue.

Pritchard et al (1972) also showed that a high plane of nutrition reduced mammary DNA in heifers not given melengestrol acetate (MGA). In heifers fed MGA, mammary DNA was not reduced by high feeding intensity. Amir et al (1968) found larger amounts of secretory tissue in heifers fed a high than low plane of nutrition. This comparison, however, was made at the same age and was therefore biased by stage of development. When the data published by Amir et al (1968) were compared at first heat (Sejrsen, 1978), there was agreement with this study and reports by Swanson & Span (1954), Swanson (1960) and Little & Kay

(1979), which all showed a negative relationship between plane of nutrition and mammary secretory tissue.

The existence of a critical period where the mammary gland is especially sensitive to high feeding intensity, as suggested by Sejrsen (1978), is supported by these results. In contrast to the depressed secretory growth at the high feeding intensity during the 175 to 320 kg body weight period, there was no difference in mammary parenchymal growth between restricted or ad libitum heifers from 300 to 440 kg. The effects of plane of nutrition on mammary development during the allometric and the isometric periods of mammary growth have not previously been determined. Our results, however, are supported by other data indicating that the negative effect on subsequent milk production of raising heifers on a high plane of nutrition occurs early in life (Swanson, 1960; Hansson et al, 1967; Brannang & Lindkvist, 1978; Sejrsen, 1978; Little & Kay, 1979).

The observed correlations between growth rate and measures of mammary secretory tissue strongly support the existence of a critical period for mammary development. There was a strong negative correlation between growth rate of heifers fed different planes of nutrition and measurements of secretory tissue during the allometric period of mammary growth but not during the isometric growth period.

The amount of parenchyma or glandular tissue observed in this experiment corresponds well with that observed by Amir et al (1968) and Sinha & Tucker (1969b) at comparable body weights. Amir et al (1968) and Sinha & Tucker (1969b) reported that heifers reared on a normal plane of nutrition contained 630 and 673 g, respectively. The comparable number from this experiment was 642 g. In contrast, Pritchard et al (1972) found that heifers reared on a standard plane of nutrition contained 1414 g parenchyma at 350 kg. However, at 250 kg Pritchard et al (1972) reported a mean value of 656 g.

Sinha & Tucker (1969b) observed a period of rapid allometric mammary development before and around puberty. After that, the mammary gland grows at a rate similar to that of the remainder of the body. Their findings are supported by the present experiment, since the amount of parenchyma and DNA expressed per 100 kg body weight were not statistically different at 320 kg and 440 kg. Using the mean amount of parenchyma found by Sinha & Tucker (1969b) and Pritchard et al (1974) at 175 kg BW as starting value, the secretory tissue in heifers fed restricted grew 2.4 times faster than the body from 175 kg to 320 kg, while the parenchyma of the ad libitum fed heifers grew only 1.8 times faster. Sorensen et al (1959) and Amir et al (1968) also reported rapid ductular development around puberty.

The decrease in amount of secretory tissue in heifers fed the higher plane of nutrition before puberty occurred in spite of greater weight of the total gland. The increase in the weight of the total gland was due alone to an increase in amount of adipose tissue. The amount of adipose tissue was also greater in the post-pubertal heifers fed the high feeding intensity. In agreement, Sorenson et al (1959) and Amir et al (1968) found greater weights of the whole gland in heifers fed the high plane of nutrition than in heifers raised at standard feeding intensity. Palpation grades have also been observed to increase with increasing feeding intensity (Swett et al, 1955; Sorenson et al, 1959). However, Sorenson et al (1959) slaughtered some heifers and observed a considerable decrease in percent secretory tissue in the heifers fed a high plane of nutrition. They, therefore, questioned the usefulness of palpation in assessing mammary development. Cowie & Tindal (1971) state that gross weight of the mammary gland is not a reliable measure of glandular tissue. These conclusions are strongly confirmed by the results in this experiment.

The relative amounts of adipose and secretory tissue observed in this experiment agree with the values reported by Amir et al (1968). They found that the gland contained 15 and 33% parenchyma in heifers fed a high and standard plane of nutrition, respectively. Percent

parenchyma in the ad libitum and restricted heifers in this experiment was 22 and 38%, respectively.

The composition of the parenchyma was unaffected by plane of nutrition in the prepubertal and postpubertal heifers. Neither number of cells, protein synthesis machinery, connective tissue collagen nor lipid per gram of parenchyma was significantly affected by plane of nutrition. The relative area of the parenchyma constituted by different cell types was also unaffected by the feeding level. In agreement, Pritchard (1970) found no effect of feeding level on DNA and RNA per gram of glandular tissue; and mean values for heifers not receiving MGA corresponded closely to means observed in our experiment. Amir et al (1968) assessed the composition of the secretory tissue in heifers on different feeding levels by measuring nitrogen and lipid content and found no difference due to ration. Their observed 40 to 47% lipid content of parenchyma agrees with the 50% shown in this experiment.

Sinha & Tucker (1969b) found considerably higher DNA per mg parenchyma, while the lipid and hydroxyproline were lower than observed in this experiment or by Pritchard (1970). Differences are probably due to the method used for separating parenchyma from fat.

No other quantitative histological assessment of cell types in heifers reared on different planes of nutrition has been reported. Amir et al (1968) examined

mammary tissue from heifers fed different feeding levels and reported that the tissue was more developed in heifers fed the high plane of nutrition. However, these comparisons were made at similar ages; hence, the heifers on high feeding intensity were heavier and in a later stage of mammary development. Pritchard (1970) also examined sections from heifers fed different planes of nutrition, but did not report differences in composition of mammary tissue between heifers fed the high and standard feeding intensities.

Swanson (1960) proposed that poor mammary development in heifers raised on high planes of nutrition is caused by excessive fattening with fat infiltration preventing development of the secretory tissue, while Amir et al (1968) suggested that development of the secretory tissue was unrelated to the fat deposition. The results of our experiment cannot resolve this conflict. The increased amount of adipose tissue and decreased amount of secretory tissue in the prepubertal heifers is in agreement with the proposal by Swanson (1960). However, the composition of the secretory tissue was unaffected by plane of nutrition. The postpubertal heifers fed high plane of nutrition had increased adipose tissue, but there was no decrease in amount of secretory tissue, supporting the proposal by Amir et al (1968).

Many of the daily patterns in serum concentrations of hormones in the ruminant are closely related to feeding times (Trenkle, 1978). Growth hormone concentrations in serum of meal-fed ruminants usually decreases shortly after feeding and returns to prefeeding values within a few hours (McAtee & Trenkle, 1971a; Hove & Blom, 1973; Bassett, 1974a; Bassett, 1974b). This pattern was confirmed in our prepubertal heifers fed the restricted diet where serum growth hormone remained low until 7-8 hours after feeding. In contrast, serum growth hormone remained low throughout the day and night in the ad libitum fed heifers. This trend was also observed by Bassett (1974).

The gradual increase in serum prolactin until 6-8 hours after feeding and the gradual decline thereafter agrees with Bryant et al (1970); Schams & Karg (1970) and McAtee & Trenkle (1971b).

Serum insulin increases after feeding in meal-fed ruminants (Bassett et al, 1971; McAtee & Trenkle, 1971c; Lofgren & Warner, 1972; Hove & Blom, 1973; Ross & Kitts, 1973) and the length of time insulin remains elevated depends on the quantity of feed (Bassett, 1974b). This pattern was also found in our experiment, especially in the postpubertal heifers.

The higher serum growth hormone concentration in the prepubertal heifers on the restricted diet and the negative correlation between feed intake and serum growth

hormone were also observed by Bassett et al (1971), Purchas et al (1971a), Hove & Blom (1973), Bassett (1974b), Smith et al (1976) and Blum et al (1979). That serum growth hormone is related to energy balance is supported by several reports showing elevated levels during early lactation in dairy cows, when cows are generally deficient in energy relative to needs (Koprowski & Tucker, 1973b; Halse et al, 1976; Smith et al, 1976; Hart et al, 1978).

In the postpubertal heifers there was no difference in growth hormone concentrations between the two planes of nutrition. Similar results were observed by Trenkle & Topel (1978) and Keller et al (1979) in steers and by Carstairs (1978) using first calf heifers.

Ad libitum-fed heifers had higher serum prolactin than restricted heifers. Supporting data were not found for cattle, but Forbes et al (1979) reported similar results in sheep. It is not known why the increase in serum prolactin at the high plane of nutrition was lower after 3 months of ad libitum feeding, but it may be due to a greater degree of fatness in heifers fed ad libitum after 3 months of treatment than after 1 month. Fatness might also explain the interaction between stage of development and plane of nutrition observed after 3 months of ad libitum feeding. The postpubertal heifers weighing 425 kg obviously are further into the fat depositing phase than the prepubertal heifers weighing 300 kg. An

alternative explanation is that the smaller differences in serum prolactin after 3 than 1 month of ad libitum feeding was due to an adaptation of the animals to the high plane of nutrition.

Serum insulin concentrations were higher in the heifers fed the high plane of nutrition reported previously by Trenkle (1970), Bassett (1974), Walker & Elliot (1975) and Carstairs (1978). That serum insulin is related to energy intake is also supported by findings showing serum insulin to be low in early lactation in dairy cows and increase as lactation advances (Hove & Blom, 1973; Koprowski & Tucker, 1973b; Hart et al, 1978).

Serum glucocorticoid concentrations were higher in ad libitum than restricted heifers. These results agree with Mills & Jenny (1979). In contrast, Trenkle & Topel (1978) found that steers fed a restricted diet had higher serum glucocorticoids than steers fed ad libitum. Hudson et al (1975) also found a negative relationship between plane of nutrition and serum glucocorticoid concentrations. Purchas et al (1971) showed a slight decrease in plasma cortisol and corticosterone concentrations in heifers fed a high plane of nutrition; whereas Bassett (1974b) found no effect of plane of nutrition on serum glucocorticoids in sheep.

During fasting growth hormone increases to prefeeding concentrations and remains at this level or

becomes even further elevated (Machlin et al, 1968; McAtee & Trenkle, 1971a; Bassett & Madill, 1974). In this experiment growth hormone concentrations remained elevated at 24 hours after feeding in the prepubertal heifers fed the restricted diet; but in heifers fed ad libitum, serum growth hormone remained low. This may indicate that heifers raised on a high plane of nutrition are less capable of responding to a low energy intake by increasing mobilization from energy stores.

The observed increase in growth hormone after refeeding in all groups is in contrast to the normally observed decrease in serum concentrations after feeding. Stress or excitement of being fed after fasting may have caused a growth hormone release (McAtee & Trenkle, 1971a; Blom et al, 1976; Wagner cit Trenkle, 1978). Blum et al (1979) reported that steers subjected to a period of restricted feeding had higher serum growth hormone concentrations after refeeding than steers fed the same amount of feed but not subjected to a period of restricted feeding.

The decrease in serum insulin and prolactin during fasting agrees with results in cattle by McAtee & Trenkle (1971b and c). An increase in serum glucocorticoids while fasting was earlier reported by Mills & Jenny (1979).

Thyrotropin releasing hormone (TRH) challenge resulted in higher serum growth hormone concentrations in heifers fed restricted diets than in those fed ad libitum.

This agrees with Baumann et al (1979), who showed an inverse relationship between feed intake and growth hormone in cows fed above, at or below their energy requirement and is supported by the greater release of growth hormone after TRH challenge in early than in late lactation (Bourne et al, 1977). The positive effect of plane of nutrition on serum prolactin after TRH-challenge is in conflict to results by Bauman et al (1979), who found that prolactin after TRH-challenge was unaffected by level of intake. Vines et al (1977) and Peters (1980) found that prolactin release after TRH challenge in cows was the same in early and late lactation. A possible reason for the conflicting results between energy balance or feed intake and serum prolactin may be due to a lower TRH dose used in this experiment (15 vs 33 ng/100 kg BW), or differences between lactating cows and heifers. Kesner et al (1977) found that prolactin released after TRH challenge was higher in heavier, older heifers than in those that were lighter and younger whose body weights ranged from 115 to 290 kg. In our experiment no differences in prolactin release were observed between heifers of 275 to 325 kg body weight.

The observed relationship between ambient temperature and serum prolactin corresponds closely to results by Peters & Tucker (1978). Wettemann & Tucker (1974) and Tucker & Wettemann (1976) also reported increasing serum prolactin with rising temperatures. The observed negative

effect of temperature on serum insulin concentrations agrees with Kamal et al (1970, cit Johnson & Vanjonack, 1976), who reported that thermal stress lowered serum insulin.

The observed positive correlations between serum growth hormone concentrations and growth of secretory tissue indicate that the negative effect of high plane of nutrition on mammary growth can, at least in part, be mediated through changes in hormones, as suggested by Sejrsen (1978). Serum growth hormone was positively correlated with the measures of secretory tissue in the prepubertal heifers. This is in full agreement with experiments elucidating hormonal requirements for mammary development in rats (Lyons et al, 1958), mice (Nandi, 1959) and goats (Cowie et al, 1966).

Lyons et al (1958), Nandi (1959) and Cowie et al (1966) also agree that prolactin is not involved in regulation of ductular development. An increase in mammary development occurs around estrus in cattle (Sinha & Tucker, 1969b) with no corresponding increase in serum prolactin (Rzepkowski et al, 1980). Our data suggest negative relationship between serum prolactin and growth of mammary secretory tissue. Pritchard et al (1972) supported such a negative relationship. They showed that an increase in mammary DNA in heifers given melengestrol acetate occurred

simultaneously with a decrease in serum prolactin concentration.

Lyons et al (1958) reported that glucocorticoids are required in combination with growth hormone and estrogen for maximal ductular development in rats. In mice, Nandi (1959) found that growth hormone and estrogen gave similar duct growth with or without glucocorticoids. Flux (1954b) and Munford (1957) found that low levels of glucocorticoids stimulate mammary growth while high doses have an inhibitory effect. These results indicate that it is difficult to predict the effect of a change in serum glucocorticoid concentration, and in the present experiment no close relationship was observed between serum glucocorticoid and amount of secretory tissue.

Serum insulin was not closely correlated with measures of secretory tissue. This is in agreement with the conclusion by Tucker (1981) stating that insulin is unlikely to be rate-limiting for mammary development. Topper & Freeman (1980) also concluded that there is no evidence that insulin is required for ductular development.

It is not possible to determine whether the observed correlations between serum hormone concentrations and mammary development are true cause and effect relationships. Tucker (1981) stated that eventhough growth hormone and prolactin undoubtedly are required for mammary development they may not be rate-limiting. This implies

that a further increase in these hormones above a certain level probably will have no effect on mammary development. However, a decrease in the concentration below a certain threshold would be important. This suggests that the observed negative correlation between growth hormone and mammary development in the prepubertal heifers may be a true positive cause and effect relationship, since the heifers fed high plane of nutrition had lower serum growth hormone than the heifers fed the restricted diet. This is also supported by the fact that in the postpubertal heifers, where serum growth hormone was the same at both feeding intensities, there was no effect of feeding intensity on mammary development.

Since prolactin is not involved in regulating ductular growth (Lyons et al, 1958; Nandi, 1959) and is probably not rate-limiting for mammary development (Tucker, 1981), the observed correlation between serum prolactin and mammary growth should not imply a direct cause and effect relationship. If, however, the development of the secretory tissue is inhibited by fat deposition in the mammary gland, there may exist an indirect cause and effect relationship, since serum prolactin has been suggested to stimulate lipogenesis in adipose tissue (Swan, 1976) and serum prolactin was positively correlated with adipose tissue. Serum growth hormone was negatively correlated with adipose

tissue, it may stimulate development of secretory tissue through its negative effect on adipose tissue.

If the observed correlations between growth of secretory tissue and serum growth hormone and prolactin are true cause and effect relationships, the possibility exists that the negative effect of high plane of nutrition on mammary development may be overcome by manipulation of the endocrine system. A further stimulation of ductular growth may also be possible by endocrine manipulation.

Administration of exogenous growth hormone is still not possible on a commercial scale, but new techniques in genetic engineering could make it available in the future. Already we can stimulate endogenous growth hormone secretion by pharmacological and nutritional means (Bines & Hart, 1978). Pharmacological stimulation, however, might have undesirable effects (Reynaert et al, 1975). Administration of TRH and infusion of individual amino acids, mixtures of amino acids and casein also stimulate growth hormone secretion (McAtee & Trenkle, 1971; Davis, 1972; Convey et al, 1973; Bines & Hart, 1978). The usefulness of these to stimulate ductular development may be minimal, since TRH administration and amino acid infusion simultaneously increase prolactin release (McAtee & Trenkle, 1971b; Davis, 1972; Convey et al, 1973). In contrast, it is possible by administration of Ergot Alkoloids or their derivates to suppress prolactin release

without affecting serum growth hormone concentration (Smith et al, 1974).

A negative relationship between serum prolactin and development of mammary secretory tissue may limit the usefulness of an increased photoperiod in stimulating body growth of dairy heifers, since the increased photoperiod elevates serum prolactin (Peters & Tucker, 1978). The effect of photoperiod on mammary development has been investigated by Petitclerc & Tucker (1981), but results are not yet available.

The observed high negative correlation between growth hormone and growth rate agrees with findings by Purchas et al (1971b), Trenkle & Topel (1978) and Forbes et al (1979), who also showed negative correlations in animals fed two planes of nutrition but conflicts with the known effects of growth hormone (Machlin, 1976). However, Trenkle (1978) suggests that the elevated growth hormone at lower intakes, when nutrient supply is limited, is involved in mobilization of energy from adipose tissue. This would account for the negative correlations observed between growth rate, growth hormone and mammary adipose tissue. The growth-stimulating effect of growth hormone is supported by the positive correlation between growth hormone and growth rate when based only on heifers fed ad libitum.

An alternative explanation for the observed negative correlation between growth rate and growth hormone is given by Riis (1981). He suggests that serum somatomedin, under inadequate feeding conditions, is low and cannot exert negative feedback on growth hormone.

The positive relationship observed between serum prolactin and daily gain, supports the anabolic role of prolactin suggested by McAtee & Trenkle (1971b) and Forbes et al (1975). However, Forbes et al (1979) found no positive correlation between serum prolactin and growth rate. Furthermore, Peters (1980) showed that the increased growth rate and milk production due to increased photoperiod occurred even if prolactin concentrations were depressed by low temperatures. Further evidence that prolactin is not involved in regulation of growth in ruminants was reported by Brown et al (1976). They suppressed serum prolactin by the Ergot Alkaloid CB 154 in lambs kept on 16 hours of light and 8 hours of dark and found daily gains were similar to lambs not given CB 154.

The positive correlation between serum prolactin and mammary adipose tissue, together with the higher serum prolactin in the ad libitum fed heifers support Swan (1976) who suggested that prolactin stimulates lipogenesis in adipose tissue. Hart et al (1975) found increased serum prolactin after feeding, in cows in positive energy balance,

but no increase in cows in negative energy balance. These results also suggest a role for prolactin in stimulating storage of nutrients when nutrient supply is in excess and agree with a lipogenic role of baseline prolactin. Baumann & Currie (1980) suggested a lipolytic role of milking-induced prolactin, agreeing with the finding that milking-induced prolactin is high in early lactation and declines as lactation advances (Koprowski & Tucker, 1973a). This, together with increasing basal prolactin with advancing lactation (Koprowski & Tucker, 1973a) suggests that the proposed lipolytic role of milking-induced prolactin could operate in coordination with the lipogenic role suggested for baseline prolactin. In periods of positive energy balance, a low milking-induced release and a high basal level will favor energy storage. In periods of energy deficit a low basal prolactin and a high milking-induced release would favor mobilization of energy stores.

The effect of plane of nutrition on serum insulin concentrations and the change observed after feeding supports the suggested metabolic roles that insulin stimulates nutrient uptake by peripheral tissues (Bassett, 1975; Bassett, 1978; Trenkle, 1978) and lipogenesis in adipose tissue, but inhibits mobilization of fat (Bauman, 1976). Insulin is also believed to stimulate growth (Trenkle, 1970; Eversole et al, 1980; Trenkle, 1980), but only a low positive correlation between serum

insulin and growth rate was observed in this experiment. Trenkle & Topel (1978) also failed to show a significant relationship between insulin and growth rate.

The observed positive correlations between growth rate and serum glucocorticoids are in disagreement with negative correlations reported by Trenkle & Topel (1978) and Purchas et al (1971). Serum glucocorticoids were positively correlated with mammary adipose tissue agreeing with the positive correlation with carcass adipose tissue reported by Trenkle & Topel (1978). The reason for the discrepancy is not evident, but it might possibly be related to interactions with serum insulin, since the actions of glucocorticoids in regulation of growth seems to be related insulin (Bassett & Wallace, 1967; Bassett, 1968; Trenkle & Topel, 1978).

Hansson et al (1967) and Swanson (1978) suggested that the lower milk production in heifers reared on high feeding intensity could be due to persistent changes in the endocrine system regulating the activity of the mammary gland and the metabolic integration of the animal. That persistent changes in the endocrine system may be developed by long time ad libitum feeding is supported by the higher growth hormone and lower prolactin release after TRH challenge and the lack of increase in serum growth hormone during fasting in heifers fed ad libitum. The lower increase in serum prolactin after 3 than 1 month of

ad libitum feeding also suggests that long time feeding of a high plane of nutrition alters the response of the endocrine system. For this experiment, however, it is not possible to know, if the observed differences persist into lactation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The objective of the experiment was to investigate the influence of raising heifers on a high plane of nutrition on mammary growth and development. It was also our purpose to investigate the sensitivity of the mammary gland to high feeding intensity in its allometric and isometric phases of growth to determine whether there exists a critical feeding period with respect to mammary development. Furthermore, the influence of plane of nutrition on mammary growth was related to changes in serum concentrations of hormones involved in regulation of mammary development.

Eleven prepubertal and eleven postpubertal heifers assigned to either restricted or ad libitum feeding completed the experiment (Table 1). The prepubertal heifers in the allometric period of mammary growth started the experiment at approximately 175 kg body weight and were slaughtered at about 320 kg (Table 2). The post-pubertal heifers in the isometric phase of mammary growth entered the experiment at 300 kg and were slaughtered at 440 kg. The pre- and postpubertal heifers on restricted feeding gained 637 and 588 g per day, respectively, while

the ad libitum fed heifers had average daily gains of 1272 and 1167 g, respectively (Table 5).

Growth of mammary secretory tissue, measured as amount and percent parenchyma, amount of DNA and mammary epithelial cells was lower in the prepubertal heifers fed a high plane of nutrition and growth of mammary secretory tissue was negatively correlated with growth rate (Tables 6, 7, 8, 13). In contrast, there was no influence of plane of nutrition on growth of secretory tissue in the post-pubertal heifers and measures of secretory tissue were unrelated with growth rate.

The composition of the secretory tissue measured as DNA, RNA, hydroxyproline and lipid per g of parenchyma, and as percent epithelial cells, connective tissue, and fat cells in the parenchyma was unaffected by plane of nutrition in prepubertal as well as postpubertal heifers (Tables 9, 10).

Serum growth hormone concentrations were increased in the prepubertal heifers on restricted intake from 8 hours after feeding until the next feeding (Figure 1, Table 14). Serum growth hormone was positively correlated with measures of secretory tissue and negatively correlated with amount of adipose tissue (Table 25). In the post-pubertal heifers serum growth hormone concentrations were unaffected by plane of nutrition, and the relationship

with mammary growth was lower than observed for the prepubertal heifers.

Serum prolactin was increased in heifers raised on the high plane of nutrition from 2 to 12 hours after feeding, when measured after approximately 1 month of ad libitum feeding (Figure 4, Table 16). After 3 months of ad libitum feeding the difference in serum prolactin concentration between planes of nutrition was considerably smaller and there was significant interaction between plane of nutrition and length of time on ad libitum feeding. Serum prolactin was, in contrast to growth hormone, negatively related to growth of mammary secretory tissue and positively correlated to amount of adipose tissue in the prepubertal heifers (Table 25).

Serum insulin was elevated in heifers raised on high plane of nutrition (Figure 7) and serum insulin showed no close relationship with mammary growth (Table 25).

The heifers on ad libitum feeding had higher serum glucocorticoid concentrations than the heifers on restricted feeding, and serum glucocorticoids were higher 23 hours after feeding than at 2 hours (Table 20). Serum glucocorticoid concentrations were also higher after 3 than 1 month of ad libitum feeding. No close relationships were observed between serum glucocorticoid concentrations and mammary growth (Table 25).

Challenge with thyrotropin releasing hormone resulted in greater release of growth hormone in the heifers on restricted feeding than in those fed ad libitum (Figure 2), while the release of prolactin was highest in the heifers fed ad libitum (Figure 5).

It can be concluded that the negative effect of raising heifers on a high plane of nutrition on subsequent milk production is, at least partly, caused by impaired mammary development. The results also support the existence of a critical period for mammary growth and show that mammary growth is especially sensitive to a high plane of nutrition during the allometric period of mammary development before and around puberty. It is also indicated that the negative effect of plane of nutrition on mammary growth may be mediated through changes in serum concentrations of growth hormone and prolactin, and that growth hormone seems to stimulate growth of mammary secretory tissue, while prolactin either directly or indirectly inhibits development of the secretory tissue.

The practical conclusions are that heifers, in order to maintain their maximal milk production potential, should not be fed a high plane of nutrition during the allometric period of mammary development and that a high plane of nutrition after that period has no negative effect on subsequent milk production due to impaired mammary development.

APPENDIX

TABLE A1.--Serum prolactin in pre- and postpubertal heifers fed ad libitum for varying length of time compared to heifers fed restricted plane of nutrition--in values adjusted for ambient temperature (ng/ml).

		Stage of development			
		Prepuberty		Postpuberty	
Plane of nutrition		Restricted	Ad libitum	Restricted	Ad libitum
Bleed 1					
Hours after feeding	1	2.87±.25	3.36±.23	2.11±.25	3.38±.23
	2	2.80±.21	3.41±.19	3.06±.21	3.34±.19
	4	2.92±.20	3.43±.18	3.09±.20	3.64±.18
	6	3.12±.21	3.50±.19	3.18±.21	3.67±.19
	8	2.92±.23	3.42±.21	3.19±.23	3.36±.21
	10	2.85±.22	3.39±.20	3.07±.22	3.51±.20
	12	2.84±.23	3.34±.21	3.06±.23	3.45±.21
	14	2.89±.25	3.31±.23	3.11±.25	3.36±.23
	16	2.92±.24	3.24±.22	3.13±.24	3.34±.22
	18	2.86±.25	3.21±.23	3.32±.25	3.27±.23
	20	3.01±.28	3.30±.26	3.29±.28	3.23±.26
	22	2.94±.25	3.19±.23	3.12±.25	3.25±.23
Bleed 2	1	3.02±.13	3.27±.12	3.19±.13	2.97±.12
	2	2.81±.14	3.15±.13	2.92±.14	2.96±.13
	4	2.93±.15	3.31±.14	3.03±.15	2.97±.14
	6	3.05±.12	3.32±.11	3.26±.12	3.03±.11
	8	3.00±.15	3.20±.14	3.13±.15	2.96±.14
	10	3.02±.09	3.16±.08	2.95±.09	2.97±.08
	12	3.02±.12	3.13±.11	3.00±.12	3.03±.11
	14	2.93±.12	3.24±.11	3.02±.12	3.08±.11
	16	2.87±.12	3.20±.11	3.05±.12	3.23±.11
	18	2.95±.14	3.10±.13	3.02±.14	3.25±.13
	20	3.00±.12	3.03±.11	3.14±.12	3.19±.11
	22	2.95±.12	3.11±.11	3.00±.12	3.01±.11

*For statistical comparisons see Table 16.

TABLE A2.--Effect of plane of nutrition on serum insulin--in values adjusted for ambient temperature (ng/ml).

	Stage of development			
	Prepuberty		Postpuberty	
Plane of nutrition	Restricted	Ad libitum	Restricted	Ad libitum
Hours after feeding				
1	.75±.14	1.07±.13	1.02±.19	1.04±.17
2	.74±.15	1.07±.14	1.08±.21	1.02±.19
4	.74±.15	1.06±.14	1.07±.20	.98±.19
6	.70±.16	1.08±.14	1.02±.23	1.02±.21
8	.66±.12[a]	1.07±.11	0.96±.21	1.07±.20
10	.71±.17[b]	1.13±.15	.94±.21	1.16±.19
12	.68±.16[b]	1.12±.15	.90±.21	1.07±.19
14	.76±.16[b]	1.24±.15	1.00±.19	1.14±.17
16	.59±.16[a]	1.17±.15	.94±.19	1.11±.17
18	.60±.12[a]	1.02±.11	.95±.17	1.02±.15
20	.62±.12[a]	.99±.11	.93±.17	1.02±.16
22	.59±.11[a]	.97±.10	.89±.16	.98±.14

Significant effect of plane of nutrition with stage of development.

[a]P .05 [b]P .10

TABLE A3.--Effect of plane of nutrition on relative area of different cell types in pre- and postpubertal heifers.

Plane of nutrition		Stage of development				Effect of zone
		Prepuberty		Postpuberty		
		Restricted	Ad libitum	Restricted	Ad libitum	
	Zone					
Distance from base, cm	1	1.0	1.0	1.0	1.0	1.0
	2	3.6	2.6	4.5	4.8	3.8
	3	7.2	5.1	9.0	9.3	7.6
	x	3.9	2.9	4.8	5.0	
Epithelial cells, %	1	11.3±1.1	10.5±1.0	10.8±1.2	14.9±1.1	11.9±.6
	2	9.8±1.1	11.2±1.0	12.3±1.2	14.9±1.1	12.1±.6
	3	8.1±1.1	9.5±1.0	9.1±1.2	11.7±1.1	9.7±.6
	x	9.7±1.1	10.4±1.0	10.7±2.2	13.8±2.0	
Connective tissue, %	1	60.9±3.6	65.3±3.3	54.0±3.2	61.2±2.9	60.6±1.6
	2	46.2±3.6	52.2±3.3	56.5±3.2	51.4±2.9	52.6±1.6
	3	36.5±3.6	45.4±3.3	35.4±3.2	48.1±2.9	41.9±1.6
	x	47.9±3.6	54.3±3.3	48.6±4.0	53.6±3.6	
Fat cells, %	1	25.4±4.7	22.2±4.3	33.4±4.3	21.8±3.9	25.4±2.1
	2	42.6±4.7	33.3±4.3	27.1±4.3	29.6±3.9	33.0±2.1
	3	53.2±4.7	40.7±4.3	51.8±4.3	38.4±3.9	45.4±2.1
	x	40.4±4.3	32.1±3.9	37.4±5.8	29.9±5.3	
Ductular lumen, %	1	2.4±1.5	1.9±1.4	1.6±.8	2.2±.7	2.0±.6
	2	1.8±1.5	3.0±1.4	4.1±.8	3.6±.7	3.1±.6
	3	2.1±1.5	4.4±1.4	3.7±.8	1.9±.7	3.0±.6
	x	2.1±1.1	3.1±1.0	3.2±.9	2.6±.9	

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