ASPECTS OF LACTATION ENDOCRINOLOGY: I. LACTOGENIC RECEPTORS IN BOVINE MAMMARY TISSUE AT DIFFERENT STAGES OF LACTATION II. GROWTH HORMONE CONCENTRATIONS IN HOLSTEIN CATTLE OF DIFFERING GENETIC MERIT

by

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(ABSTRACT)

Mammary tissue from nine Holstein cows was collected within one week of parturition, at 60 and 180 days postpartum. Blood samples were collected at 6-hr intervals from two days prior to until two days after surgery. A membrane-enriched fraction of tissue homogenates was prepared by differential centrifugation. Displacement curve data was analyzed by a microcomputer program. Mean prolactin (Prl) during the periparturient period was greater than either postpartum period, but not prior to biopsy. Dissociation constants (Kd) estimated with NIH-bPRL-6 as competitor were not different among stages of lactation, and averaged 8.97×10^{-8}M. Receptor concentrations were less during the periparturient period than later lactation. The Kd was 100-fold greater when estimated with human growth hormone as competitor. It is concluded that lactogenic hormone receptor concentrations in bovine mammary tissue increase with the onset of lactaiton, following a pattern similar to that observed in non-ruminants.

Three experiments were conducted to investigate endocrine metabolic hormone profiles in Holstein cattle of differing genetic merit at several ages. Control animals were randomly bred to non-AI sires originiating in the Virginia Tech Dairy herd.

Selected animals were offspring of commercially available AI sires. In one experiment, mean plasma Prl was greater in control animals after feeding and insulin injection, while growth hormone (GH) was greater in selected animals at all ages. Free fatty acids were greater in selected animals at 6 and 24 months of age, while glucose (Glc) and urea were unaffected by genetic merit. In a second experiment, Holstein bull calves were administered Glc and thyrotropin releasing hormone (TRH) on different days. Plasma GH was greater in selected animals. Plasma Prl was greater in control animals after TRH. In the third experiment, Holstein cows received TRH at 30, 90 and 200 days postpartum (DPP). Net energy balance was negative at 30, while positive at 90 and 200 DPP. Plasma GH before and after TRH was greater in selected animals, and greater during early than later lactation. Thus, the results of the three experiments indicate that incrased plasma GH may be associated with selection for increased milk yield.

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Chapter I

INTRODUCTION

Two topics of current interest to lactation endocrinologists are 1) the role of the lactogenic hormone receptor in mammary tissue, and 2) the endocrine mechanisms which regulate substrate availability to the mammary gland, thereby influencing total milk production. The thrust of the studies reported herein is directed to answering questions which surround those two topics.

In mammals, prolactin is necessary for differentiation of the mammary epithelium at parturition into the highly active gland which characterizes lactation (Nickerson & Akers, 1984). The existence of a lactogenic hormone receptor in mouse mammary tissue was first demonstrated by Turkington (1970) and confirmed by Falconer (1972) and Birkinshaw & Falconer (1972) in rabbit tissue using autoradiographic techniques. These authors demonstrated that radiolabelled prolactin could be found bound to the plasmalemma of the mammary epithelial cell, presumably involving a cell surface receptor. The lactogenic hormone receptor in rats, mice and rabbit mammary tissue has been characterized through the work of many researchers and recently reviewed by Cowie et al. (1980). Dissociation constants (Kd) fall in the 10^{-10}M

range, while receptor concentrations are generally in the fentomolar range. However, little information is available concerning lactogenic hormone receptors in ruminant mammary tissue, as only two reports involving ruminant tissue are presently available. Akers & Keys (1984) characterized a lactogenic hormone receptor present in membrane preparations of ovine mammary gland. The Kd was 4.5 and 3.0 $x10^{-9}$M in early lactation and 100 days of gestation ewes, respectively, and concentrations of binding sites were greater in tissue from lactating than from pregnant ewes. Gertler et al. (1984) reported fentomolar concentrations of specific prolactin binding sites in membranes and solubilized fractions prepared from bovine mammary tissue exhibiting Kd's in the nanomolar range. Presently, no information concerning the relationship between mammary lactogenic hormone receptor concentrations and stage of lactation in cows is available.

Once lactation is established, endocrine mechanisms regulate in part substrate availability to the mammary gland. Bauman and Currie (1980) reviewed endocrine mechanisms involved in homeorhesis, ie. the orchestrated changes which meet the demands of a particular physiological state. Growth hormone, on one hand, and insulin, on the other, seem to be involved in the partitioning of nutrients among the various body tissues. The adiposite plays a pivotal role in

providing energy for production. Insulin stimulates adipocyte uptake of energy and subsequent lipogenesis. Growth hormone antagonizes insulin stimulated lipolysis and is lipolytic, resulting in the release of free fatty acids from adipocytes into the blood plasma. Several studies have documented the practical value of growth hormone's lipolytic qualities, reporting increased milk yield concurrent with increased feed efficiency in cows administered growth hormone (Hutton, 1957; Machlin, 1973, Peel et al., 1983). It is of further interest to note here that Bines and Hart (1977) reported greater growth hormone concentrations in dairy than in beef cows, while insulin was greater in beef than dairy cattle. Centuries of selection, perhaps only recently with a strong scientific base, have resulted in breeds of cattle which are physiologically adapted to produce different products, thus the possibility that different metabolic profiles between breeds has a genetic source is quite compelling. Further, one might suppose that gradations in the endocrine profiles associated with production of either greater or lesser quantities of milk may be found among animals of the same breed. Accordingly, the objectives of the present study were to:

1. characterize the lactogenic hormone receptor in bovine mammary tissue,

2. investigate whether the affinity of or concentrations of the lactogenic receptor are influenced by stage of lactation, and
3. investigate possible differences in metabolic endocrine patterns and responses to physiological challenge in Holstein cattle of differing genetic merit.

Chapter II

REVIEW OF LITERATURE

LACTOGENIC RECEPTORS

Prolactin

Prolactin is a polypeptide hormone synthesized in the anterior pituitary, with a molecular weight of about 25,000 daltons (Turner and Bagnara, 1971). Prolactin has many physiological effects across species, ranging from nest-building behavior and fin-fanning in teleosts to lactogenesis in mammals. Prolactin may even be involved in growth in sheep and cows, as several studies have suggested (McAtee and Trenkle, 1971; Ohlson et al., 1981; Forbes et al., 1975,). Thus, prolactin may be the most multifunctional hormone known. Indeed, Nicoll (1974), in listing eighty-five actions of prolactin, suggested that the name be changed to 'versatilin'. However, the myriad functions of prolactin are beyond the scope of this review; thus, only the regulation of prolactin secretion, the effects of prolactin on the mammary gland in ruminants and laboratory species, and the characteristics and regulation of the lactogenic hormone receptor present in mammary tissue of laboratory animals will be examined.

Regulation of Prolactin Secretion

Release of prolactin from the adenohypophysis is regulated by factors of hypothalamic origin. Whereas secretion of most anterior hypophyseal hormones is regulated by releasing factors, Everett (1954) reported that pituitaries transplanted to the rat kidney capsule actually secreted more prolactin than prior to transplantation. This suggested that the major hypothalamic influence on pituitary prolactin secretion must be inhibitory. The concept of a prolactin inhibiting factor (PIF) has been substantiated by reports of Woolf et al. (1974) and Vaughn et al. (1980) in which sectioning of the pituitary stalk, increased plasma prolactin concentrations.

In addition to surgical studies, characterization of the prolactin regulatory mechanisms has been attempted employing pharmacologic agents. Although previously discovered hypothalamic releasing factors were proteins, evidence began to accumulate that PIF was not necessarily of a similar structure. Meites (1957) demonstrated that injections of reserpine into estrogen-primed rabbits initiated lactogenesis and lactation. Further experimentation yielded a long list of pharmacologic agents the administration of which affected prolactin secretion and subsequent lactation, but morphine sulfate and seratonin were the most effective

(Meites, 1963). Iproniazid, a monoamine oxidase inhibitor, was reported to suppress postpartum lactation in rats (Mizuno et al., 1964) and induce regression of carcinogen-induced mammary cancers (Nagasawa and Meites, 1970).

The effectiveness of these agents, which alter catecholaminergic activity, pointed to dopamine as the endogenous PIF. Fuxe and Hokfelt (1966) had demonstrated the existence of a dopaminergic pathway, the cell bodies of which were located in the arcuate nucleus, with projections to the median eminence terminating in close approximation to the hypothalamic-hypophyseal portal system. The authors named these neurons the tuberoinfundibular pathway. The study was of further importance because it was the first to demonstrate the existence of a pathway which contained dopamine in the absence of norepinephrine. Dopamine had previously been considered only a precursor of norepinephrine in the central nervous system, thus lacking transmitter status.

MacLeod et al. (1970) compiled convincing evidence by adding dopamine to rat pituitaries in vitro. Dopamine administration decreased prolactin secretion in a dose-dependant manner, and addition of dopamine antagonists inhibited this effect. More recently, Ben-Jonathan et al. (1977) have reported that dopamine can be measured in the portal blood of rats, while Gibbs et al. (1979) reported inhibition of

prolactin secretion by dopamine infusions into alpha-methyl-p-tyrosine treated rats. Additionally, binding of labelled haloperidol to pituitary mammotrophs using immunocytological techniques has been described by Goldsmith et al. (1979), and Calabro and MacLeod (1978) demonstrated the existence of a high-affinity, low-capacity binding site for dopamine in bovine anterior pituitaries. Thus, evidence for dopamine as an endogenous PIF is overwhelming.

Some evidence exists for another hypothalamic inhibiting factor. Enjalbert et al. (1977) reported PIF-activity in a dopamine-free fraction of rat hypothalamic tissue, while Schally et al. (1977) reported that gamma-aminobutyric acid (GABA) was present in porcine hypothalami and was effective in decreasing pituitary secretion both in vivo and in vitro. However, GABA must be administered in doses approximately 100-fold greater than dopamine, and prolactin concentrations in plasma are only reduced half as much as with dopamine administration (Enjalbert et al., 1977). Further, there is no evidence of detectable quantities of GABA in the portal blood. Thus, the existence and identity of other hypothalamic factors as physiological prolactin inhibitors is still in the speculative stage and demands further research.

There is somewhat more concrete evidence for the existence of a hypothalamic prolactin releasing factor (PRF). Meites et al. (1960) reported that hypothalamic extracts could induce lactation, as did Mishkinsky et al. (1968). Weiner and Bethea (1981) list several lines of evidence supporting the notion of a hypothamic PRF, including: 1) presence of PRF-like activity in hypohtalamic extractions, 2) increased PRF-like activity in plasma concurrent with increased plasma prolactin concentrations, 3) increases in plasma prolactin during conditions of maximal dopaminergic inhibition and 4) stable portal concentrations of dopamine concurrent with increases in plasma prolactin concentrations. Thyrotropin releasing hormone (TRH) is a hypothalamic releasing factor which stimulates the release of pituitary thyroid stimulating hormone. In addition, TRH stimulates the release of prolactin and growth hormone from the pituitary in cows (Convey et al., 1973) and sheep (Fell et al., 1973; Davis et al., 1976). Koch et al. (1977) have reported that specific TRH antibodies decrease plasma prolactin concentrations in rats, and specific TRH receptors have been discovered on prolactin secreting pituitary cells (Tashjian et al., 1971). However, whether TRH functions as a physiological PRF is debatable, because in suckled rats, prolactin is released before TSH appears in plasma, and TSH

is not released during the proestrous prolactin surge (Blake, 1974).

Another nomination for an endogenous PRF is vasoactive intestinal polypeptide (VIP), a small peptide present in the hypothalamus (Larsson et al., 1976). Administration of VIP into the ventricles of the rat increases peripheral prolactin concentrations (Kato et al., 1978), and does so in a dose-dependant manner in vitro (Shaar et al., 1979). As yet, however, direct information regarding the possible role of VIP as a physiological regulator of prolactin is lacking. Studies investigating the portal concentrations of VIP and their relationship with pituitary prolactin release and the existence of specific VIP receptors in the plasmalemma of the pituitary mammotrophs are needed to provide the information critical to the assignment of VIP as an endogenous physiological PRF.

In summary, the evidence strongly suggests that the major hypothalamic control of prolactin secretion is inhibitory in nature, and that the endogenous PIF is dopamine. Some evidence points to the existence of a PRF, but the chemical composition of the hypothesized compound is still unknown. Further research is necessary to elucidate the exact nature of the neuroendocrinological regulation of pituitary prolactin secretion.

Prolactin and the Mammary Gland

Lactogenesis, or the onset of copius milk secretion, is accompanied by marked histological and biochemical changes in the mammary epithelium Cowie et al. (1980) recently reviewed the ultrastructural changes which occur in mammary tissue at lactogenesis. Prepartum, the mammary epithelial cell ultrastructure is characterized by an irregular nucleus, few mitochondria, small amounts of rough endoplasmic reticulum and poorly developed Golgi vesicles. In contrast, the actively secreting alveolar cell is polarized, with an extensive rough endoplasmic reticulum basally located, a large, round nucleus located mediobasally, and Golgi dictyosomes located apically. The basal lamina is convoluted, inferring active transport of components into the cell. The apical membrane is dotted with microvilli, and secretory products, ie. casein micelles, Golgi vesicles containing lactose, and free fat droplets, are present in the apical region of the cytoplasm. Marked changes in biochemical parameters also occur during the periparturient period. As expected, activity of those enzymes associated with energy production, ie. enzymes of glycolysis and the citric acid cycle, is increased. Additionally, enzymes involved in synthesis of fatty acids and lactose become active at lactogenesis (Baldwin and Yang, 1974). Thus, distinct and dis-

cernable alterations in cell morphology and biochemistry accompany the onset of lactogenesis.

Several hormones are involved in the initiation of lactation, and are referred to as the lactogenic complex. Forsyth (1983) has reviewed the endocrine control of lactogenesis and cites two conditions for the onset of lactation in most species, these being 1) increases in plasma glucocorticoids and prolactin concentrations and 2) decreases in plasma progesterone concentrations. Glucocorticoids are necessary for lactogenesis in all species tested (guinea pig: Nelson et al., 1943; mouse: Nandi and Bern, 1961; rat: Bintarningsih et al., 1958; goat: Cowie and Tindal, 1961; cow: Cowie, 1969), except the rabbit (Cowie and Watson, 1966), perhaps by virture of the stimulatory effect of glucocorticoids on rough endoplasmic reticulum formation in the epithelial cell (Mills and Topper, 1969; Banerjee and Banerjee, 1971). In the rabbit, prolactin alone is capable of inducing lactogenesis (Cowie, 1969). Progesterone may compete with the glucocorticoids for its receptor in the epithelial cell (Cowie et al., 1980). The increased progesterone concentrations during pregnancy serve to delay lactogenesis until parturition, and, as the inhibitory effects of progesterone decrease with luteolysis at termination of pregnancy, circulating glucocorticoids are more able to stimulate endomembrane formation.

Recently, Akers et al. (1981a, 1981b) have investigated the involvement of prolactin during the periparturient period in bovine lactogenesis. Administration of CB154, an ergot alkaloid derivative and dopamine agonist posessing specific prolactin-release inhibiting properties, was employed as a tool to assess the specific action of prolactin on cytological and biochemical parameters during lactogenesis. Cytologically, postpartum mammary tissue from cows treated with CB154 lacked the degree of differentiation noted in control cows and in cows treated with CB154 that received exogenous prolactin. Both rough endoplasmic reticulum and Golgi formation was depressed in mammary epithelial cells of cows treated with CB154 only, as compared to control cows and those receiving prolactin. Further, total mammary RNA was decreased by CB154 administration, as was the RNA/DNA ratio. Enzymes responsible for fatty acid (fatty acid synthetase and acetyl CoA synthetase) and lactose (alpha-lactalbumin) synthesis were also depressed by CB154 treatment. Prolactin, then, is an essential component of the hormonal complex responsible for the initiation of lactogenesis in the cow.

This in vivo data agrees well with available information gathered in vitro. Collier et al. (1977) recently investigated the hormonal requirements for lactogenesis in bo-

vine mammary explant tissue. Thirty to forty day prepartum tissue was incubated with insulin, cortisol or prolactin, or permutations of the three. Insulin was necessary for tissue survival in vitro, but did not induce any alterations in cell structure or activity. The addition of cortisol to the incubation media resulted in cytological changes associated with lactogenesis, but did not induce synthesis of milk components. Insulin and prolactin added to culture media induced limited synthesis, but, cytologically, alveolar integrity was not consistently maintained. Maximal lactogenic response resulted from the addition of insulin, cortisol and prolactin to culture media, as assessed by cytological parameters and by ^{14}C-labelled acetate into fatty acids. Goodman et al. (1983) have recently reported that incubation of bovine mammary explant tissue in media containing insulin and prolactin stimulates alpha-lactalbumin production, while the further addition of cortisol to the media potentiates the stimulatory effect of prolactin. Therefore, in the cow, both cortisol and prolactin are required in the absence of progesterone to initiate lactation.

In non-ruminant animals such as rats, mice and rabbits, prolactin is necessary to support established lactation. Shaar and Clemens (1972) reported that CB154 administration to rats reduced plasma prolactin concentrations and abo-

lished milk synthesis. Sinha et al. (1974) reported a similar occurrence in mice, while Utian et al. (1975) reported the same phemomena in women. Rabbits, too, are dependent on prolactin for continued lactation, as Taylor and Peaker (1975) reported complete cessation of milk production in animals treated with CB154. Thus, in these laboratory animals, prolactin is necessary not only for lactogenesis, but also to support established lactation.

In ruminants, the situation may be different. Once lactation is established in cows and goats, inhibition of prolactin secretion by CB154 administration has little effect on milk production (goats: Hart 1973; cows: Smith et al., 1974). Thus, in ruminants, prolactin may not be necessary to support lactation once established; but necessary only for differentiation of the mammary epithelium and subsequent induction of lactation. However, Cowie et al. (1980) cautioned that although CB154 reduces plasma prolactin concentrations, some basal concentrations can still be detected, and may be sufficient to influence the postpartum mammary gland. Further, those authors suggest that growth hormone, which is not supressed by CB154 administration, may be serving a lactogenic role in the mammary gland. However, demonstration of specific human growth hormone binding to mammary lactogenic hormone receptors has been reported, so-

matotrophic binding sites have been demonstrated (R.M. Akers, unpublished data). Therefore, although it seems unlikely that growth hormone is substituting for prolactin in the mammary glands of CB154-treated ruminants, the suggestion that basal concentrations of prolactin may be involved in postpartum support of lactation in ruminants cannot be discarded.

Lactogenic Receptors

Turkington (1970) is credited with the first report of a specific mammary prolactin receptor, demonstrating that Sepharose bound prolactin in the presence of insulin and cortisol stimulated the incorporation of tritiated uridine into RNA in rat mammary tissue. Since the Sepharose bound hormone is presumably too large to diffuse through the cell membrane, the existence of a surface receptor is implied. Flaconer (1972) and Birkinshaw and Falconer (1972) using autoradiographic techniques demonstrated that, in rabbit mammary tissue, the prolactin receptor was located in the plasmalemma of the epithelial cell ajacent to the vascular supply. Costlow and McGuire (1977), also using autoradiography, concurred with the previous reports in demonstrating the existence of a surface receptor in the mammary glands of suckled and weaned lactating rats. Of course, the existence

of cell surface receptors for protein hormones has been well documented with other hormones, and has become central to the dogma regarding the mechanism of action of protein hormones (Catt and Dufau, 1976).

The characteristics of a solubilized prolactin receptor from rabbit mammary glands have been reported by Shiu and Friesen (1974). The specific prolactin receptor exhibited a molecular weight of 220,000 daltons, and Scatchard analysis indicated a dissociation constant (Kd) of .63 $x10^{-10}$M. However, when the Kd was estimated using a membrane preparation of the same tissue, a five-fold decrease in affinity was noted. In contrast, Dufau et al. (1973) reported that the affinity constant was greater when assessed in a membrane preparation as compared with a solubilized preparation.

Binding parameters in lactating mammary tissue from several non-ruminant species have been reviewed by Cowie et al. (1980). Dissociation constants of the lactogenic receptor from mouse, rat and rabbit mammary glands ranged from 9 to 90 $x10^{-10}$M, while numbers of receptor sites ranged from 10 to 100 fmol/mg membrane protein in membrane preparations. Thus, the lactogenic receptor in non-ruminant mammary tissue displays the necessary charateristics of sufficient binding affinity and limited binding capacity as discussed by Ryan and Lee (1976).

Recently, some information regarding lactogenic hormone binding sites in ruminant mammary tissue has become available. Akers and Keys (1984) reported the Kd of the ovine mammary receptor to be 4.5 $x10^{-9}$M, while Gertler et al. (1984) reported the Kd of the lactogenic receptor present in lactating bovine tissue to be 8.1 $x10^{-10}$M, as determined by Scatchard analysis. However, these two reports represent the extent of the literature presently available which offers any information about lactogenic receptors present in ruminant mammary tissue.

Several reports have indicated that prolactin binding to mammary tissue is affected by the physiological state of the animal. Prolactin binding is greater after than prior to parturition in mice (Frantz et al., 1974), rats (Holcomb et al., 1976; Hayden et al., 1979) and rabbits (Djiane et al., 1977). Thus mammary prolactin binding increases with the onset of lactation, and remains elevated until weaning (Hayden et al., 1979). Akers and Keys (1984) reported a similar phenomenon in ovine mammary tissue. Binding in 20-day postpartum mammary tissue was increased by 10-fold as compared with 50-day prepartum tissue. This report is unique in the literature, however, as no other information concerning alterations in lactogenic receptors during different physiological states in ruminant mammmary tissue is available.

Concentrations of lactogenic hormone receptors are regulated by several endocrine factors. Studies have indicated that self-regulation may be particularly important. The earliest study investigating self-regulation of prolactin receptors employed liver tissue. Posner et al. (1974) demonstrated that hepatic prolactin receptors decline after hypophysectomy, and that the administration of prolactin to hypophysectomized rats induced an increase in prolactin binding to hepatic tissue (Posner et al, 1975). Ranke et al. (1976) reported similar increases in prolactin receptors in isolated rat hepatocyes. Using mammary tissue, Bohnet et al. (1977) and Djiane and Durand (1977) both used CB154 administration to reduce endogenous prolactin concentrations and observed a reduction in prolactin binding. Hayden et al. (1979) reported that the maintenance of mammary prolactin binding was pituitary dependent, and that binding was also somewhat inhibited by ovariectomy, adrenalectomy or thyroparathyroidectomy. Thus, in both liver and mammary tissue from laboratory animals, prolactin is necessary to maintain concentrations of its own receptor in the target tissue, thus fullfilling a self-stimulatory role.

Glucocorticoids are also involved in the induction of prolactin receptors in the mammary gland. Kohmoto and Sakai (1978) reported that the addition of insulin to cultured

mouse mammary explants increased specific prolactin binding two-fold over controls before culture, while the addition of cortisol and insulin increased specific binding six-fold over controls. Using ovariectomized mid-pregnant mice, Harigaya et al. (1982) reported that adrenalectomy reduced specific mammary prolactin binding sites, while an injection of corticosterone or cortisol restored the number of binding sites to preoperative concentrations in ovariectomized-adrenalectomized mice. Aldosterone, deoxycorticosterone and estradiol did not affect the number of receptors present in ovariectomized-adrenalectomized mice, and the dissociation constant of the prolactin receptor was not affected by any of the hormone treatments or surgical manipulations. Plasma prolactin concentrations were not affected by hormonal manipulations with the exception of estradiol administration, and were not correlated with the concentrations of specific prolactin receptors.

Progesterone is another steroid which may be involved in regulation of prolactin receptor concentrations in the mammary epithelial cell. Djiane and Durand (1977) reported that while administration of prolactin to pseudopregnant rabbits increased mammary lactogenic hormone receptor concentrations, progesterone treatment concurrent with prolactin inhibited prolactin-dependent increases in its own re-

ceptor. The inhibitory characteristics of progesterone may be related to its ability to bind with the mammary glucocorticoid receptor, thus interfering with the stimulatory effect of glucocorticoids on prolactin binding (Shyamala, 1973; Capuco and Tucker, 1980).

Thus, concentrations of mammary lactogenic hormone receptors is regulated by endocrine factors which, not surprisingly, resemble endocrine patterns in the periparturient animal, ie., decreasing plasma progesterone concentrations coupled with increasing plasma prolactin and glucocorticoid concentrations. As the inhibitory influence of progesterone is removed by luteolysis at termination of pregnancy, glucocorticoids are able to stimulate lactogenic receptor concentrations, thus increasing the epithelial cell's sensitivity to prolactin. Under the influence of prolactin, mammary epithelial cells differentiate and begin to produce the unique secretory products characteristic of lactation. Lactogenesis, therefore, is most easily understood in terms of the increases in lactogenic hormone receptor concentrations which occur in the mammary gland of periparturient animals.

GROWTH HORMONE IN CATTLE

Growth hormone administration to cattle

Many studies have involved the administration of growth hormone (GH) to dairy cattle. Asimov and Krouze (1937) reported a rather extensive study in which cattle at various ages and stages of lactation were administered GH, with an increase in milk yield. However, the yields, while perhaps impressive in 1937, were less than one third the yield of an average cow in 1984. Brumby and Hancock (1955) reported that not only was milk production increased in cattle given GH, but that the extra milk was produced without an increase in feed intake, resulting in more efficient milk production. Hutton (1957) also reported that milk production was increased as was production efficiency in identical twins given GH as compared to the twin receiving saline injections. Machlin (1973) reported an increase of 18% in milk yield from Holstein cows receiving GH for a 10 day injection period. Most recently, Peel et al. (1981) have reported an increase of 9.5% milk yield, 22.7% in milk fat, 14.5% in lactose and 17.1% in milk energy secretion during an 11 day GH injection regimen. During this time, feed intake was not increased, resulting in a more efficient production of milk. Further, Peel et al. (1982) reported 15.2% increases in milk yield in Holstein cattle administered GH, again with no in-

crease in feed intake. Most recently, Peel et al. (1983) administered GH to early and late lactation cows. Early lactation cows administered GH produced 15% more milk, 17% more fat, 14% more protein and 21% more lactose than those receiving saline, while late lactation animals given GH produced 31% more milk, 42% more fat, 18% more protein and 35% more lactose than other late lactation cows not given GH. Thus, administration of GH has consistently resulted in an increase in milk yield, and in studies that recorded feed intake the increases in milk produced have not been accompanied by an additional consumption of feed.

Physiological effects of growth hormone

With such consistent results through the years, there has been a great deal of speculation regarding the mechanism of action by which the galactopoietic effects of GH may be mediated. Early researchers suggested that GH was exerting a direct effect on the mammary tissue, inducing increases in the number of secretory cells comprising the mammary epithelium (Brumby and Hancock, 1955). However, to suppose a direct effect of GH on mammary tissue is to suppose that the tissue is sensitive to plasma GH concentrations, which implies the existence of specific GH receptors in the mammary epithelium. However, although attempts have been made, to

date no demonstration of specific GH (somatotrophic) binding to mammary tissue has been reported. Thus, the galactopoietic effects of GH must be assumed to be indirect.

Bassett (1978) reviewed endocrine factors in the control of nutrient utilization in ruminants. Growth hormone administration results in lipolysis and increased plasma free fatty acid concentrations. Bauman and Currie (1980) suggested that GH serves to partition energy toward the mammary gland by virtue of its lipolytic and anti-lipogenic properties. Vernon (1981) reported that fatty acid synthesis in sheep adipose tissue in vitro was stimulated by the addition of insulin to the culture media, while the addition of GH had antagonistic effects. Trenkle (1981) also reviewed the endocrine regulation of energy metabolism in nutrients, and reported that GH partitions energy toward skeletal muscle and the mammary gland, while insulin partitions energy toward adipose tissue. Thus, the galactopoietic effects of growth hormone appear to be mediated by its lipolytic and anti-lipogenic properties, and not by any direct effect on the mammary gland.

Growth hormone concentrations and genetics

Bines and Hart (1977) reported that several hormones and metabolites differed between Holstein and Hereford-cross cows. Plasma GH concentrations were greater in Holstein than Hereford-cross cows, while insulin concentrations were greater in the beef breed. Further, beta-hydroxybutyric acid was greater in the dairy breed. Thus, the possibility exists that these endocrine and metabolite differences may have a genetic origin. The authors cautioned, however, that the effects of the greater milk production in the Holstein cows as compared with the Hereford-cross cattle may be the cause of the observed differences, instead of the effect. Because GH concentrations increase in underfed animals, perhaps the negative energy state of the high producing animals was inducing increased GH release. In support, they (Bines and Hart, 1977) reported that differences in GH concentrations between Holstein and Hereford-cross cattle were not apparent during the dry period. Hart et al. (1978) reported hormone and metabolite concentrations in high (Holstein) and low (Hereford-cross) producing cattle. Again, plasma concentrations of GH, along with free fatty acids and beta-hydroxybutyrate, were greater in the higher yielding Holstein cattle as compared with the lower yielding Hereford-cross cattle. Insulin was greater in the Hereford-cross than in

the Holstein cows. However, no differences in either GH or insulin were noted during the dry period. Thus some question still exists as to whether GH is the genetic mediator of, or the physiological result of increased milk production in dairy cattle.

Chapter III

LACTOGENIC RECEPTORS IN BOVINE MAMMARY TISSUE

INTRODUCTION

In mammals, prolactin is necessary for differentiation of the mammary epithelium at parturition into the highly active gland which characterizes lactation (Nickerson & Akers, 1984). The existence of a lactogenic receptor in mouse mammary tissue was first demonstrated by Turkington (1970) and confirmed by Falconer (1972) and Birkinshaw & Falconer (1972) in rabbit tissue using autoradiographic techniques. These authors demonstrated that radiolabelled prolactin could be found bound to the plasmalemma of the mammary epithelial cell, presumably involving a cell surface receptor. The lactogenic hormone receptor in rats, mice and rabbit mammary tissue has been characterized through the work of many researchers and recently reviewed by Cowie et al. (1980). Dissociation constants (Kd) fall in the 10^{-10}M range, while receptor concentrations are generally in the femtomolar range. However, little information is available concerning lactogenic hormone receptors in ruminant mammary tissue, as only two reports involving ruminant tissue are presently available. Akers & Keys (1984) characterized a lactogenic hormone receptor present in membrane preparations

of ovine mammary gland. The Kd was 4.5 and 3.0 $x10^{-9}M$ in early lactation and 100 days of gestation ewes, respectively, and concentrations of binding sites were greater in tissue from lactating than from pregnant ewes. Gertler et al. (1984) reported femtomolar concentrations of specific prolactin binding sites in membrane preparations and solubilized fractions of bovine mammary tissue. Presently, no information concerning the relationship between mammary lactogenic receptor concentrations and stage of lactation in cows is available. Accordingly, the objectives of the present study were to:

1. characterize the lactogenic hormone receptor in bovine mammary tissue, and
2. investigate whether the affinity of or concentrations of the lactogenic receptor are influenced by stage of lactation.

MATERIALS AND METHODS

Tissue samples

Nine primaparous Holstein cows, calving between April and July, 1983, were used in the experiment. Tissue was excised from the mammary gland within one week of expected parturition, at 60 and again at 180 (n=8) days postpartum. One animal was culled from the experiment between 60 and 180

days postpartum due to illness. All animals exhibited normal estrus cycles but were not rebred during the lactation. Animals were anesthetized with 100 mg intramuscular injection of xylazine (Rompun). Another 50 mg injection was administered intravenously when animals were not sufficiently sedated after the first dose. Approximately 15 gm of tissue was removed at each of the three stages of lactation from an area about 15 cm dorsal to the teat base from any of the four quarters. An incision (approximately 10 cm) was made, avoiding discernable surface vessels, in the skin and through the connective tissue. Tissue samples were collected from the exterior of the parenchyma to a depth of approximately 5 cm, yielding tissue relatively free of large ducts. Tissue was placed in .3 M sucrose on ice and transported to the laboratory for further processing.

Blood samples

Blood samples were collected by puncture of the coccygeal vein or artery at 6 h intervals. Evacuated 12 ml tubes with 100 units sodium heparin/tube were used to collect blood. Sampling was begun 2 days prior to and continued through 2 days following the day of surgery. Additionally, 15 min samples were collected during the hour immediately preceeding biopsy. Samples were centrifuged immediately and the plasma stored at -20C until assayed for hormone content.

Minced tissue preparation

Five g of mammary tissue was minced with a razor blade in Hank's balanced salt solution (Gibco, Inc.) with 10mM sodium acetate, 10mM glucose and 10mM Hepes buffer added (HBSS-Hepes). Increasing amounts of tissue were added to culture tubes, and specific binding of prolactin was estimated by displacement of radiolabelled human growth hormone during a 3 h incubation at 37C.

Membrane preparations

A membrane-enriched fraction was prepared from tissue homogenate by differential centrifugation (Akers & Keys, 1984). The mammary tissue was weighed and diluted 1 to 4 in ice-cold .3M sucrose containing .1% sodium azide, minced and homogenized with a Brinkman Polytron Homogenizer, model PCU-2-110, using the PT 10/35 probe (three 30 s burst at 90% of full power spaced by one min cooling periods on ice). The homogenate was then centrifuged at 1,000g for 10 min in a Beckman J-6B with the 4.2 rotor. The supernatant fraction was removed, avoiding the lipid layer, and centrifuged at 13,000g for 20 min in a Sorvall RC-5 using the SS-34 rotor. The resulting supernatant was subsequently centrifuged at 100,000g for 1 h in a Beckman L-75 Ultracentrifuge using the 50.2 Ti rotor. The pellet from this high-speed spin was

then resuspended in 4 ml of buffer containing 25 mM trizma hydrochloride with .1% sodium azide (Tris), pH 7.5. The membrane preparations were stored at -70C until assayed for lactogenic receptor concentrations.

Receptor assay

Lactogenic hormone receptors were assayed by methods of Akers and Keys (1984). Each assay tube contained, in order of addition, 300 ul of Tris buffer plus 0.1% bovine serum albumin (Tris-BSA) with 40 mM calcium chloride and magnesium chloride, 100 ul of unlabelled competitor in Tris-BSA buffer, 100 ul of radiolabelled human growth hormone (approximately 2 ng at a specific activity of 20 ug/uCi) in Tris buffer containing 1% BSA, and a volume of membrane preparation equivalent to 600 ug of membrane protein, diluted to 100 ul. Assay incubation was at room temperature in a shaker bath for 24 h, after which 2 ml of ice-cold Tris-BSA buffer were added to each tube, and the assay was centrifuged at 1000g for 30 min. The supernatant was aspirated and the bound fraction was counted by a Beckman Gamma 4000. Displacement curves were generated for each cow at each stage of lactation with the addition of unlabelled increasing quantities of bovine prolactin (NIH-bPRL-6). The standard curve ranged from 50 to 4000 ng bovine prolactin, and each

point was determined in triplicate. Additionally, displacement curves with membrane preparations pooled by stage of lactation were generated with both ovine prolactin (NIH-oPRL-15) and unlabelled human growth hormone. The human growth hormone standard curve ranged from .5 to 1000 ng. Generation of displacement curves for experimental samples was accomplished in six assays within one week using an equal mass of tracer per assay tube from a single iodination of human growth hormone. Membrane preparations from different stages of lactation were equally dispersed within the six assays. The coefficients of variation of percent specific binding as assessed by displacement of tracer with 4000 ng bovine prolactin in two membrane preparation pools not related to the experimental samples averaged 12.8%. Receptor-ligand affinities and receptor concentration were estimated with a microcomputer version of LIGAND, a program providing Scatchard analysis of displacement curve data (Munson and Rodbard, 1980) (see Appendix A for brief explanation of Scatchard analysis). Protein content of membrane preparations was determined according to the method of Lowery et al. (1951), using bovine serum albumin as standard.

Hormone assay

Plasma concentrations of prolactin were quantified using a double antibody radioimmunoassay with bovine prolactin antiserum, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:50,000, bound 40% of labelled prolactin in the absence of unlabelled hormone. With bovine prolactin as standard, the assay system exhibited 7.0% cross-reactivity with thyroid stimulating hormone (NIH-oTSH-9), but less than 2% with any other pituitary hormones added to the assay system in amounts up to 1,000 ng. The ovine TSH did contain .1% prolactin, as the preparation was not highly purified. The standard curve ranged from .25 to 5 ng per assay tube, 1.25 ng/ml being the least detectable dose. The intraassay coefficient of variation averaged 9.5% in two plasma pools. All samples were quantified in a single assay.

Data analysis

Mean plasma prolactin concentrations were compared by split-plot analysis of variance procedures of the General Linear Model option (GLM) of the 1982 release of the Statistical Analysis System (SAS, 1982). Samples collected prior to biopsy were designated as period 1, while those collected after biopsy were designated as period 2. The model ac-

counted for variation due to cow, stage of lactation, cow x stage, period, period x cow, period x stage, period x cow x stage and error. The cow x stage interaction was used to test for differences due to stage of lactation. To test for differences among stages prior to the time of tissue removal, period 1 samples were compared among the stages of lactation. Dissociation constants and receptor concentrations were compared by analysis of variance procedures of GLM (SAS, 1982). The model accounted for variation due to cow, stage of lactation and error. The error term was used to test for differences due to stage of lactation. The correlation coefficient between mean plasma prolactin concentrations prior to biopsy and receptor concentrations in tissue was tested for significance by the CORR procedure of SAS (SAS, 1982).

RESULTS

Mean plasma prolactin concentrations overall (figure 1) were greater ($P<.01$) during the periparturient period than at 60 or 180 days postpartum (15.5 vs. 4.7 and 3.8 ng/ml, respectively). However, in period 1 samples, no differences in mean prolactin concentrations were found among stages of lactation ($P>.05$) (7.1, 5.0 and 3.7 ng/ml, respectively). Further, pre-surgery mean prolactin concentrations were not correlated with lactogenic receptor concentrations ($P>.05$).

Specific prolactin binding sites in minced tissue preparations did not increase as amount of tissue added to culture tubes increased. This result may have been due to a concealment of specific sites by the relatively greater availability of non-specific binding sites. Thus, generation of displacement curves for Scatchard analysis was not attempted.

Least squares means of lactogenic receptor concentrations and affinity constants in membrane preparations of mammary tissue from periparturient, 60 and 180 days postpartum Holstein cows are depicted in table 1. Dissociation constants ($x10^{-8}M$) were 8.32, 9.62 and 9.47, respectively, and no differences ($P>.05$) in Kd's among stages of lactation were found. However, receptor concentrations in membrane preparations (fmol/mg membrane protein) were less ($P<.01$) just prior to parturition than at 60 or 180 days postpartum (.55 vs. 1.34 or 1.25, respectively). Residuals associated with the LIGAND model fit of the data at each stage of lactation were not different ($P>.05$).

Least squares means of lactogenic receptor concentrations in membrane preparations of bovine mammary tissue from periparturient, 60 and 180 days postpartum Holstein cattle with the dissociation constant fixed at 8.97 $x10^{-8}M$ are depicted in table 2. Again, receptor concentrations were less

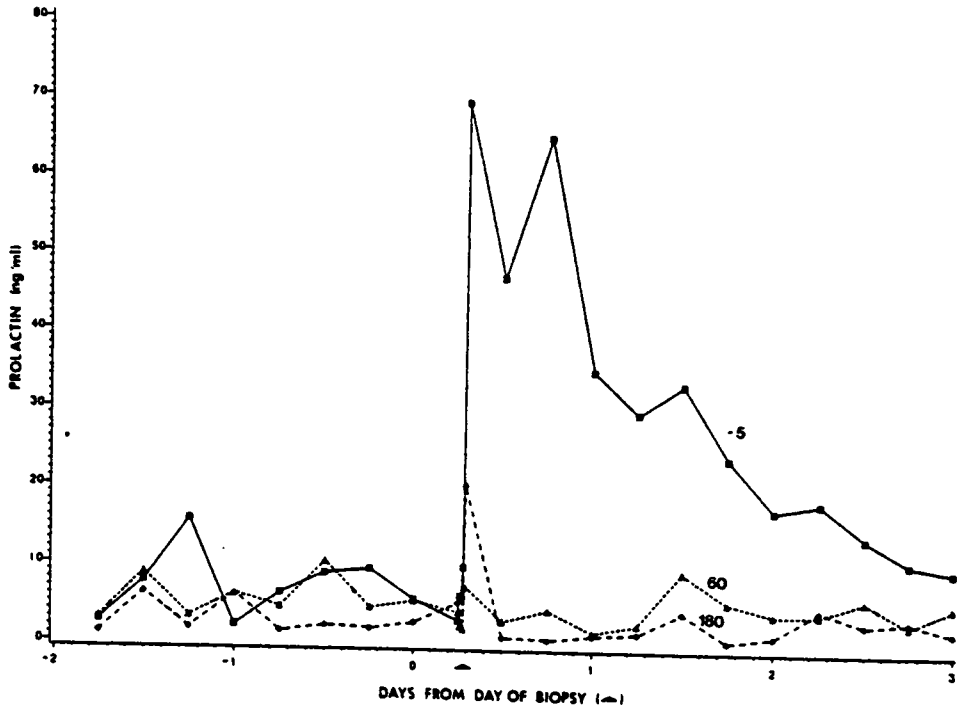

Figure 1: Prolactin concentrations during the surgery period in periparturient, 60 and 180 dayspostpartum Holstein cattle

TABLE 1

Least squares means of lactogenic receptor charateristics in -5, 60 and 180 days postpartum Holstein cattle

Days postpartum	Kd ($x10^{-8}$)	Sites (fmol/mg mem. prot.)	Residual
-5	8.326±.496[a]	.574±.115[b]	28.0±4.1
60	9.625±.496	1.337±.115[c]	25.1±4.1
180	9.470±.698	1.253±.127[c]	30.9±4.4

[a]mean ± standard error of the mean

[bc]means with different superscripts differ ($p<.01$)

($P<.01$) prior to parturition than at 60 or 180 days postpartum, while residual variance associated with the best fit curves of the data were not different ($P>.05$).

Lactogenic receptor concentrations and dissociation constants in membrane preparations of bovine mammary tissue pooled by stage of lactation as assessed by competition with ovine prolactin and human growth hormone are depicted in tables 3 and 4, respectively. Since the data from these pooled samples represent only one determination, statistical analysis is not possible. The standard error shown in the table is the error associated with LIGAND's attempt to estimate the Kd and the receptor concentrations from the triplicate determination of the points on the standard curve. Even so, receptor concentrations in membrane pooled preparations followed the same pattern as previously described with the means of the individual assessments with bovine prolactin competition, ie. receptor concentrations were less at parturition than at 60 or 180 days postpartum. Mean Kd as assessed by ovine prolactin was 2.78 $x10^{-8}$M. Additionally, competition of radioiodinated human growth hormone by unlabelled human growth hormone from the same preparation yielded a similar pattern in receptor concentrations with respect to stage of lactation as described with bovine and ovine prolactin. However, affinity estimates averaged 9.78

TABLE 2

Least squares means of lactogenic receptor concentrations with dissociation constant fixed at 8.97 $x10^{-8}M$

Days postpartum	Sites (fmol/mg mem. prot.)	Residual
-5	.647±.084[a,b]	24.9±3.24
60	1.199±.084[c]	24.0±.2.96
180	1.145±.093[c]	26.7±.3.28

[a]mean ± standard error of the mean

[b,c]means with different superscripts differ ($p<.01$)

TABLE 3

Least squares means of lactogenic receptor characteristics using ovine prolactin as unlabelled competitor

Days postpartum	Kd ($x10^{-8}$)	Sites (fmol/mg mem. prot.)	Residual
-5	3.248±.410	.237±.033	10.21
60	2.622±.322	.347±.013	9.79
180	2.573±.413	.341±.059	20.21
Holding Kd constant at 2.783:			
-5	2.783	.206±.007	10.42
60	2.783	.367±.011	9.01
180	2.783	.365±.016	18.56

$x10^{-10}M$, approximately a 100-fold increase as compared with bovine prolactin displacement.

DISCUSSION

Mean plasma prolactin concentrations were greater during the periparturient period than at 60 or 180 days postpartum, agreeing with studies which have demonstrated increases in prolactin concentrations during the periparturient period in cattle (Ingalls et al., 1971; Ingalls et al., 1973). Ingalls et al. (1973) reported that prolactin concentrations in 34 heifers averaged less than 100 ng/ml 5 days prior to calving, but were increased to greater than 200 ng/ml on the day of calving. Plasma prolactin concentrations began to increase from 48 to 36 hours prior to calving, and returned to pre-calving concentrations by 72 hours post-calving. This periparturient surge of prolactin is also observed in other species including laboratory animals (Cowie et al., 1980). Akers et al. (1981a) and Akers et al. (1981b) have demonstrated that reduction of the periparturient surge of prolactin by administration of CB154 reduces the degree of differentiation in bovine mammary tissue as measured by cytological and biochemical parameters. Thus, the peripaturient surge of prolactin is necessary for complete transformation of the quiescent preparturient tis-

TABLE 4

Least squares means of lactogenic receptor characteristics using human growth hormone as unlabelled competitor

Days postpartum	Kd ($x10^{-9}$)	Sites (fmol/mg mem. prot.)	Residual
-5	.784±.104	.904±.101	22.98
60	1.089±.079	1.757±.112	6.64
180	1.188±.086	1.683±.103	6.13
Holding Kd constant at .9775:			
-5	.9775	1.073±.036	26.41
60	.9775	1.623±.028	7.07
180	.9775	1.456±.029	8.84

sue into the actively secreting gland. However, mean prolactin concentrations during the 2 days of sampling prior to removal of tissue (period 1) were not different, indicating that subsequent measurements of available binding sites would not be biased by the exposure of the tissues to different hormonal environments prior to biopsy. Furthermore, mean prolactin concentrations prior to biopsy and subsequent receptor concentrations were not correlated. This is of importance to note because of results of studies which have investigated the relationship between plasma prolactin concentrations and concentrations of mammary prolactin binding sites. Barash et al. (1983) reported that infusion of prolactin or human growth hormone into virgin rats resulted in a down-regulation of apparent prolactin binding sites within 30 min of commencement of infusion. This short-term down-regulation contrasts with the well documented long-term stimulatory effects of prolactin on concentrations of its own receptor in mammary tissue (Cowie et al., 1980). Djiane et al. (1982) examined prolactin receptor turnover in explants of mammary tissue from pseudopregnant rabbits by treatment with transcription, translation, metabolic and lysosomal degradation inhibitors. Apparent mammary binding sites were increased by treatment with chloroquine, an inhibitor of lysosomal degradation, while decreased by treatment with cy-

cloheximide, an inhibitor of translation. On the other hand, treatment with actinomycin D or 5,6-dichloro-1,B,D-ribofuranoslybenzimidazole, both potent transcrition inhibitiors or with ouabain, a metabolic inhibitor, had no effect on concentrations of mammary lactogenic binding sites. These authors concluded that the concentrations of prolactin receptors in the mammary gland at any given time is the result of a dynamic equilibrium between receptor synthesis and lysosomal degradation, and that the most frequent modulations occur at the level of translation and lysosomal degradation. Thus, although the short-term regulation of mammary lactogenic receptors is without a doubt an extremely complex subject, and apparent binding sites are assuredly influenced by circulating prolactin concentrations, no evidence was found in the present study to suggest that comparisons of the three stages of lactation would be effected by plasma prolactin concentrations prior to surgery, since plasma prolactin concentrations at that time were not different among stages of lactation.

Little information concerning characteristics of the lactogenic receptor or receptors in bovine mammary tissue is available. Only the report of Gertler et al. (1984) provides an estimate of the Kd (8.2 $x10^{-8}$M) of a lactogenic receptor in mammary tissue from lactating cows using ovine

prolactin (NIH-oPRL-14) as a reference standard. However, since it is not indicated that those researchers used the LIGAND program to calculate the receptor parameters, direct comparisons with the present study are unjustified. Akers and Keys (1984) reported the Kd and binding capacity of a lactogenic receptor in membrane preparations of ovine mammary tissue. The apparent Kd was not different between lactating and pregnant ewes, but lactating tissue exhibited an 8-10 fold increase in binding capacity. The present data depict a similar pattern of changes in lactogenic receptor concentrations with the onset of lactation in cattle. Further, Hayden et al. (1979) described a similar pattern of changes in receptor concentrations during pregnancy and lactation in rats. Studies of the regulation of lactogenic receptor sites in non-ruminants have concluded a self-regulating role for prolactin, enhanced by glucocorticoids and triiodothyronine, and antagonized by progesterone (Cowie et al., 1980). Therefore, given the hormonal milieu of the periparturient cow, the lesser mammary lactogenic hormone receptor concentrations just prior to parturition are to be expected. However, in the rat and other non-ruminants, established lactation is also dependant on circulating prolactin, as administration of CB154 has demonstrated (rats: Schaar & Clemens, 1972; mice: Singa et al., 1974; women:

Utian et al., 1975). In the cow, prolactin may be only necessary for lactogenisis (Akers et al. 1981a; Akers et al., 1981b) as administration of CB154 during established lactation has little effect on milk yield (Karg et al., 1972; Smith et al., 1974). Exactly how this contrasting information between ruminants and non-ruminants relates to a similarity in patterns of receptor concentrations with regard to stage of lactation remains to be investigated.

Almira & Reddy (1979) have reported that both a high ($Kd=10^{-9}M$) and a low ($Kd=10^{-7}M$) affinity insulin receptor exists in rat hepatocytes. Additionally, Oscar et al., (1984) reported the existence of two classes of insulin receptor sites in bovine mammary microsomes and smooth membrane preparations. In order to investigate the possibility that two classes of lactogenic hormone receptors might exists in the mammary gland, an attempt was made to fit a two-site model to the present data using that option of the LIGAND program. However, residuals associated with two-site models were greater by at least 50-fold when compared with the residuals from the one-site models. Thus, little supporting evidence of the existence of two classes of lactogenic hormone receptors in bovine mammary tissue was found. Likewise, there was no evidence suggesting that the Kd of the lactogenic receptor varies either among or within cows

at different stages of lactation (table 1). Accordingly, if we assume that there is indeed only one class of lactogenic receptor in bovine mammary tissue, and that this receptor exhibits a uniform Kd among and within cows, then the best estimate of the true Kd is the mean of the individual observations. Least square means of the receptor concentrations with the Kd held constant at 8.97 $x10^{-8}M$, then, are presented in table 2. Again, lactogenic receptor concentrations are less ($P<.01$) during the periparturient period than at 60 or 180 days postpartum. However, the relationship in receptor concentrations among stages of lactation as depicted in table 2 may be more accurate than those in table 1 due to reduction of error associated with estimating the Kd, which is now held constant.

Due to a lack of sufficient quantities to produce additional curves on each individual at each stage of lactation, membrane preparations were pooled by days postpartum prior to generation of displacement curves with ovine prolactin and human growth hormone. Mammary lactogenic receptor parameters in membrane preparations pooled by stage of lactation as assessed by competition with ovine prolactin and human growth hormone yielded results similar to those just discussed (tables 3 and 4, respectively). It is of interest to note that displacement of tracer with unlabelled human

growth hormone produced Kd estimates 100-fold greater than those produced with bovine prolactin competition, agreeing with Gertler et al. (1984) who reported that human growth hormone displacement produced Kd estimates of greater affinity than did either bovine or ovine prolactin. Thus, the relative nature of displacement studies is emphasized, implying that the absolute values of affinity constants and receptor concentrations may not be as informative as the relative changes in mammary receptor concentrations during various stages of lactation.

In summary, plasma prolactin concentrations were greater at parturition than at 60 or 180 days postpartum, but not different among stages of lactation prior to surgery. Thus, surgery was evidently scheduled sufficently in advance of the periparturient surge of prolactin to avoid the possibility that measurable lactogenic receptors in the mammary gland would be masked by pre-surgery plasma prolactin concentrations. Scatchard analysis of displacement binding data revealed that, using bovine prolactin as competitor, the average dissociation constant was 8.97 $x10^{-8}$M, and was not different among stages of lactation. However, Scatchard analysis also revealed that lactogenic receptor concentrations in membrane preparations of bovine mammary tissue were less at parturition (.55 fmol/mg membrane protein) than at

60 or 180 days postpartum (1.34 or 1.25 fmol/mg membrane protein, respectively). Additionally, while estimates of the dissociation constant in pooled membrane preparations were similar using bovine or ovine prolactin as competitor, estimates of the dissociation constant were 100-fold different when human growth hormone was used as competitor in displacement curves. Furthermore, no evidence was found that suggested that two classes of lactogenic receptors were present in bovine mammary tissue.

From the results of the present study, we conclude that the dissociation constant of bovine mammary lactogenic receptors is not affected by stage of lactation, but that the concentrations of these receptors in mammary tissue is greater during lactation than just prior to parturition. Additionally, care must be taken when comparing information from studies conducted at different locations employing differing assay systems, as the use of different tracers or competitiors may have noticeable effects on values reported. Further studies regarding the regulation of mammary lactogenic receptors may provide insights into mechanisms regulating lactogenesis, lactation and involution.

Chapter IV

HORMONES AND METABOLITES IN COWS AND HEIFERS OF DIFFERING GENETIC MERIT

INTRODUCTION

High milk-yielding, underfed dairy cows have greater plasma concentrations of growth hormone (GH), nonesterified fatty acids and lesser concentrations of insulin than do lower yielding, overfed cows (Hart et al., 1978). Changes in GH concentrations were positively correlated with changes in milk yield and negatively related to changes in body weight (Hart et al., 1979). In addition, nutritional studies have shown that high yielding cows preferentially partition energy toward milk production while low yielding cows direct energy toward deposition of body tissues (Broster et al, 1969). Prolactin (Prl), GH, insulin and glucagon have all been implicated or shown to play a role in metabolic regulation in the ruminant (Forbes et al., 1975; Hart et al., 1978; Trenkle, 1981). Tilakaratne et al. (1980) showed that calves with different potentials for milk production were different in aspects of energy and nitrogen metabolism and suggested the possibility of using physiological traits that affect milk yield as a basis of selection for increased milk yield. There is a paucity of information on the resulting

changes in physiological traits result from selection for increased milk yield.

This experiment was designed to investigate the effect of genetic selection for increased milk yield in Holstein cattle on concentrations of endogenous hormones and metabolites in response to feeding. In addition, exogenous insulin administration was employed as a means to investigate possible differences in response of these cattle to metabolic challenge. Determination of physiological response to these treatments may provide useful information on inherent differences in metabolic control mechanisms between two groups of dairy cattle with different genetic potential for milk yield.

MATERIALS AND METHODS

Animals

Twelve female Holstein calves (117.0 kg body weight (BW)), 12 yearlings (273.8 kg BW), 12 bred heifers (434.5 kg BW) and 12 mid-lactation primiparous cows (541.3 kg BW) were utilized in a 2x4 factorial experiment (n=6). The factors included were genetic merit and age group. One-half of the cattle in each age group were daughters of selected sires (average predicted difference (PD_{82})=+1543lbs.) available through artificial insemination organizations (selection

group). The remaining cattle were second to fourth generation daughters of cows random bred to non-AI unselected sires originating in the Virginia Tech dairy herd (control group). At the time of the experiment, control heifers weighed an average of 8.3 kg more than selected heifers, while control group milk cows outweighed there selection group counterparts by 10.1 kg. All animals were considered to be in positive energy balance and were gaining weight prior to the initiation of the study. Mean daily milk production at the time of the study was 19.5 and 20.5 kg for the control and selection group animals, respectively. Based on the average PD of sires, the selection group daughters would be expected to outproduce breed average herdmates by approximately 701 kg during the first lactation. Subsequent mature equivalent 305-d lactation records averaged 6,885 and 7,890 kg for the control and selection group animals, respectively, suggesting that differences in daily milk yield became greater with advancing lactations. The study was conducted in an open-sided freestall barn during the month of May.

Blood samples

Serial blood samples were collected beginning 24 h after last feeding from 0900 to 1700 h. Lactating animals were milked at 0500 and 1700 h after completion of sampling. All animals were feed the same mixed complete diet (blended corn silage, hayladge and concentrate) ad libitum at 1100 h. The diet consisted of 15.5% crude protein, 10.8% digestible protein, 22.2% acid detergent fiber and 73% total digestible nutrients (TDN) on a dry matter basis. Feed was removed at 1200 h and animals were administered insulin (.6 IU/100 kg BW) at 1400 h. Serial blood samples were collected via indwelling jugular cannula into heparinized tubes at 15-min intervals, except between 1100 and 1200 h, and again between 1400 and 1500 h when samples were collected at 10-min intervals. Blood samples were centrifuged immediately and plasma stored at -20 C until assayed for hormone or metabolite content.

Hormone and metabolite assay

Plasma Prl concentrations were quantified using a double antibody radioimmunoassay (RIA) with bovine prolactin antiserum, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:50,000, bound 40% of labelled prolactin in the absence of unlabelled

hormone. With bovine prolactin (NIH-bPRL-6) as reference standard, the assay system exhibited 7.0% cross-reactivity with thyroid stimulating hormone (NIH-oTSH-9), but less than 2% with any other pituitary hormones added to the assay system in amounts up to 1,000 ng. The ovine TSH did contain .1% prolactin, as the preparation was not highly purified. The standard curve ranged from .25 to 5 ng per assay tube, 1.25 ng/ml being the least detectable dose. Standards added to plasma pools were recoverable and produced parallel inhibition curves with NIH-bPRL-6. All samples were assayed in duplicate, and intra- and interassay coefficients of variation averaged 9.5 and 12.4%, respectively, in two plasma pools.

Plasma GH concentrations were quantified using a double antibody RIA with bovine GH antiserum, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:1,000, bound 35% of labelled GH in the absence of unlabelled hormone. With NIH-bGH-18 as reference standard, the assay system did not cross-react (<2%) with other pituitary hormones. The standard curve ranged from .5 to 20 ng per assay tube, 1.5 ng/ml being the least detectable dose. Standards added to plasma pools were recoverable and produced parallel inhibition curves with NIH-bGH-18. All samples were assayed in duplicate, and intra- and inter-

assay coefficients of variation averaged 7.9 and 5.2%, respectively, in two plasma pools.

Plasma insulin concentrations were quantified using a double antibody RIA with bovine insulin antiserum, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:10,000, bound 30% of labelled insulin in the absence of unlabelled hormone. With highly purified bovine insulin (Lot No 615-70-80, Eli Lily Co., Indianapolis, IN.) as a reference standard, there was no cross-reactivity with (<2%) with glucagon. The standard curve ranged from .125 to 4 ng per assay tube, .5 ng/ml being the least detectable dose. Standards added to plasma pools were recoverable and produced parallel inhibition curves with the purified insulin. All samples were assayed in duplicate, and intra- and interassay coefficients of variation averaged 9.9 and 13.4%, respectively, in two plasma pools.

Plasma glucagon concentrations were quantified in benzamadine preserved samples using a double antibody RIA, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:50,000, bound 25% of labelled glucagon in the absence of unlabelled hormone. With highly purified bovine glucagon as standard, the assay system did not cross-react (<2%) with insulin. The standard

curve ranged from .06 to 2 ng per assay tube, .2 ng/ml being the least detectable dose. Standards added to plasma pools were recoverable and produced parallel inhibition curves with the purified glucagon. All samples were assayed in duplicate, and intra- and interassay coefficients of variation averaged 15.4 and 18.2%, respectively, in two plasma pools.

Determination of plasma urea concentrations were accomplished by reaction with diacetlymonoscomine by the method of Coulombe and Favreau (1963) and glucose by the O-toluidine reaction (Feteris, 1965). Plasma free fatty acid (FFA) concentrations were determined in pooled samples by the method of Ko and Royer (1967).

Data analysis

Blood hormone and metabolite data were analyzed as a 2x4 factorial design by the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS, 1982). Hormone and metabolite response were analyzed within the three periods investigated: pre-feeding (Per 1), after feeding (Per 2) and after insulin administration (Per 3). The model accounted for variation due to age, genetic group, genetic group x age, cow within genetic group x age, period, age x period, genetic group x period, genetic group x age x period and error. The first three factors were tested for

significant effect using cow within genetic group x age as the denominator. Similar analyses were conducted on logarithmic transformation of the hormonal and metabolite data and resulted in no changes in significances detected.

RESULTS

Dry matter intake data collected before the study began was available on cows but not on heifers. Control and selection group cows were consuming the complete diet ad libitum at a rate of 118 and 120%, respectively, of their calculated daily TDN requirement based on body weights and quantity of milk produced. Heifers and milk cows were gaining weight at the time of the study. During the 1 h feeding period on the day of the study, heifer groups consumed an average of 82.9% of their calculated daily TDN requirement while lactating animals consumed an average of 72.3%. Intakes did not differ between genetic groups on the day of sampling.

Mean Prl differed ($P<.01$) with age group and was lowest in 6-mo-old calves and greatest in the 2-yr-old cows (table 5). Prolactin was lowest prior to feeding (Per 1), increased after feeding (Per 2) and remained increased after insulin injection (Per 3). While Prl did not differ overall due to genetic group, an interaction indicated that control

TABLE 5

Plasma prolactin concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			ng/ml				
1	Selection	6	6.0	7.9	12.1	15.2	±1.3
	Control	6	5.7	9.3	11.8	10.7	±1.3
2	Selection	6	8.4	16.6	15.9	23.0	±1.1
	Control	6	10.3	19.2	13.5	24.1	±1.1
3	Selection	6	9.7	8.0	16.4	18.0	±1.1
	Control	6	13.5	28.8	15.6	24.4	±1.1

[a] Least-squares means.

[b] Effects (P<.05 or greater) include age, period age x period and genetic group x period.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

animals had greater ($p<.01$) Prl concentrations after insulin injection than did selected animals (figure 2).

In selected animals, Prl was positively related to peripheral GH and FFA concentrations and negatively related to glucagon and glucose concentrations (table 12). In control animals, plasma Prl concentrations were also negatively related to peripheral glucagon and overall were positively related to GH, insulin and urea concentrations (table 12).

Plasma GH concentrations differed ($p<.01$) with age and were greatest in 6-mo-old and 2-yr-old animals while lowest in 18-mo-old heifers (table 6). Selection group animals had greater ($p<.01$) mean GH concentrations overall. A genetic group x period interaction indicated that while GH was greater during Per 2 as compared with Per 1 or 3 in selected animals, GH in control animals was greater during Per 2 or 3 than during Per 1 (figure 3). Overall, GH was increased ($p<.01$) during Per 2 and then declined during Per 3, but remained greater than during Per 1. An age x period interaction indicated that 18-mo-old heifers differed ($p<.001$) from the other three age groups, as GH did not decrease during Per 3 as it did in the 6-, 12- and 24-mo-old animals. In selection group animals, peripheral GH concentrations and were positively related to glucagon negatively related to insulin and glucose concentrations (table 12). However, in

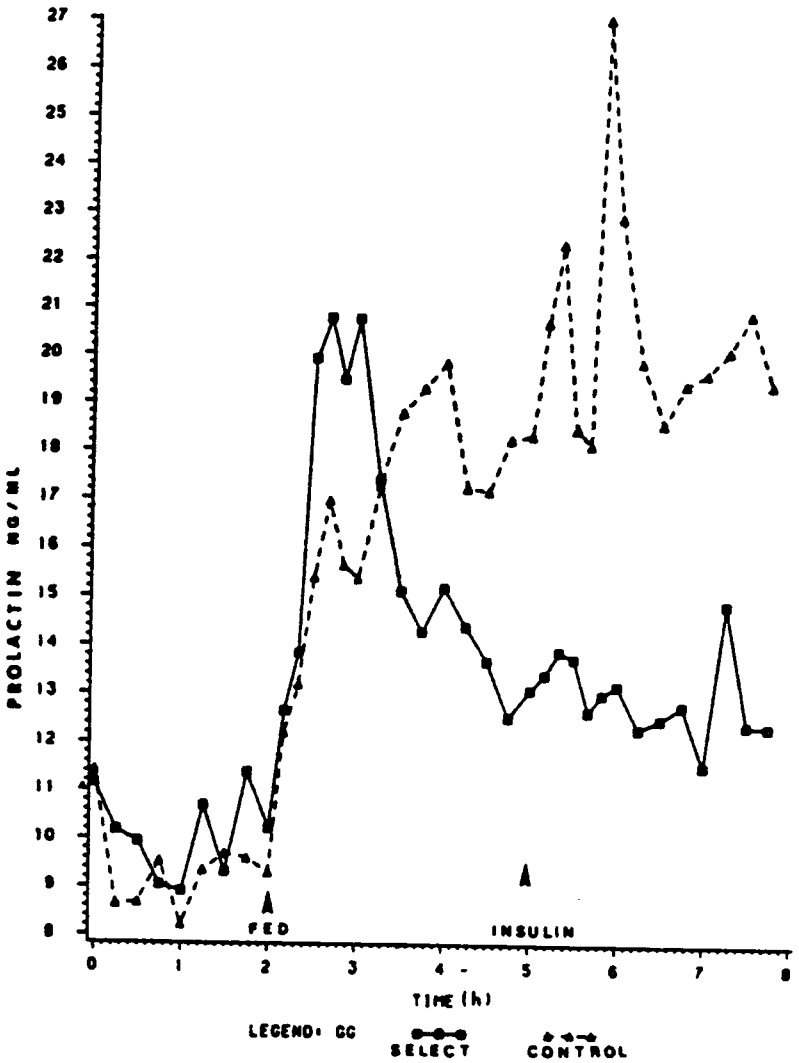

Figure 2: Mean plasma prolactin concentrations in selection and control animals before and after feeding and insulin injection

TABLE 6

Plasma growth hormone concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			ng/ml				
1	Selection	6	8.2	8.5	4.3	9.9	±.6
	Control	6	6.0	5.8	3.5	5.5	±.6
2	Selection	6	13.0	11.4	5.3	17.5	±.5
	Control	6	10.9	9.8	5.6	10.6	±.5
3	Selection	6	12.3	10.7	5.9	10.5	±.5
	Control	6	10.3	8.5	7.9	7.7	±.5

[a] Least-squares means.

[b] Effects ($P<.05$ or greater) include age, period, genetic group, age x period and genetic group x period.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

the control group, GH concentrations were not related ($p>.05$) to glucagon but positively related to insulin and negatively related to glucose and FFA concentrations (table 12).

Plasma insulin concentrations were different ($p<.01$) with age, was greater in the selection group ($p<.05$) and did not increase significantly after feeding (table 7 and figure 4). Peripheral insulin was greatest in 18-mo-old heifers and least in 2-yr-old cows. As expected, plasma insulin was increased ($p<.01$) during Per 3 due to insulin injection. Changes in plasma insulin concentrations were not associated ($p>.05$) peripheral glucagon concentrations, and were associated differently with changes in plasma GH between groups (table 12). Plasma insulin concentrations were also negatively associated with FFA and positively with glucose concentrations in the control group (table 12).

Glucagon was greater ($p<.01$) overall in the 6-mo-old animals than in the other age groups and was greater in 12-mo-old animals than in 18-mo- or 2-yr-old animals (table 8). Plasma glucagon did not differ ($p>.05$) between Per 1 and 2, but was decreased ($p<.05$) overall during Per 3. An age x period interaction ($p<.01$) indicated that the decrease during Per 3 was entirely attributable to the 6-mo-old group. Similarily, a genetic group interaction (p,>01) in-

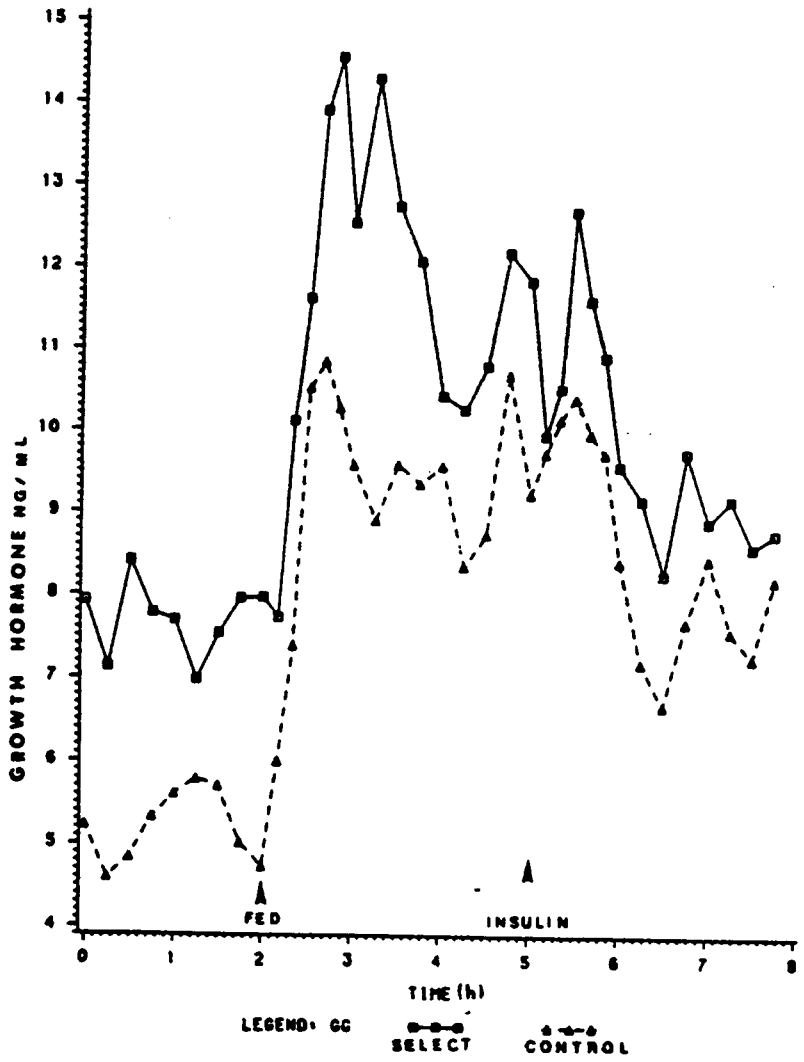

Figure 3: Plasma growth hormone concentrations in selection and control animals before and after feeding and insulin injection

TABLE 7

Plasma insulin concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			ng/ml				
1	Selection	6	1.1	1.0	1.3	1.0	±.3
	Control	6	.9	.9	1.1	.7	±.3
2	Selection	6	1.3	1.1	1.6	.9	±.3
	Control	6	1.0	1.2	1.4	.8	±.3
3	Selection	6	2.3	3.2	6.0	1.7	±.3
	Control	6	1.9	2.7	2.8	1.7	±.3

[a] Least-squares means.

[b] Effects ($P<.05$ or greater) include age, genetic group, period, age x period and genetic group x period.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

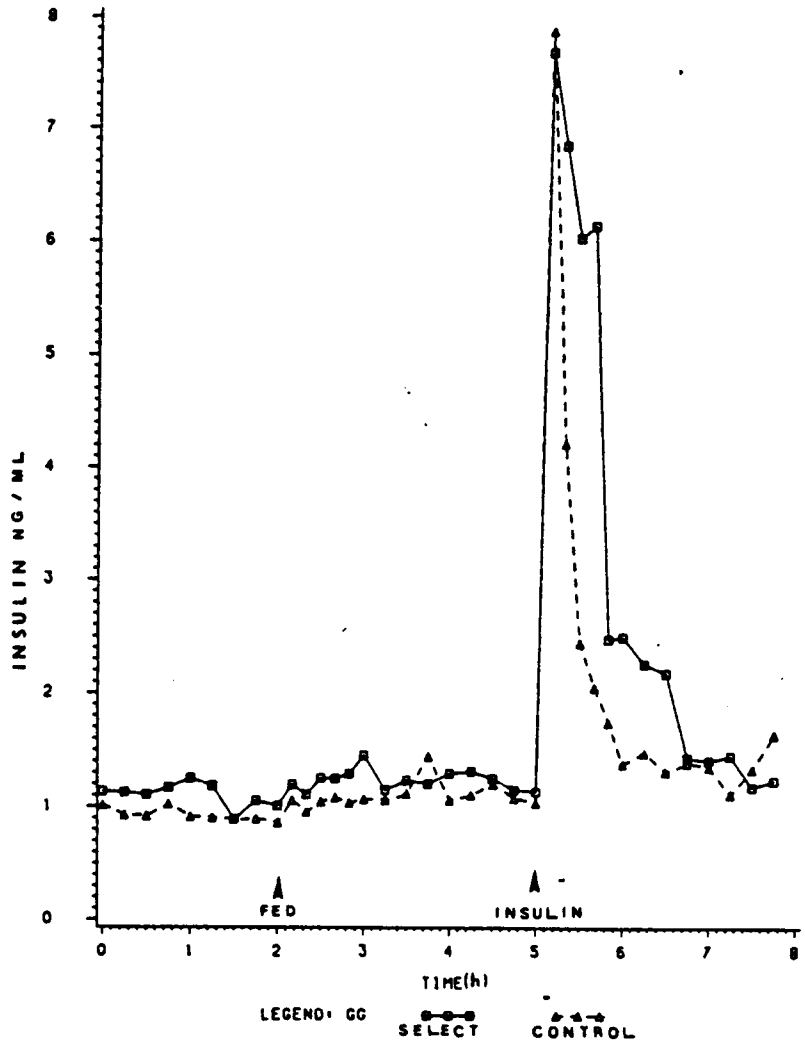

Figure 4: Plasma insulin concentrations in selection and control animals before and after feeding and insulin injection

dicated that glucagon was not different among periods in selected animals, but was greater during Per 1 and 2 than Per 3 (figure 5). Plasma glucagon concentrations were negatively related to peripheral Prl and urea in both selection and control animals (table 12). In selection group animals, changes in glucagon were positively related to changes in plasma GH concentrations (table 12).

Plasma metabolites also varied with treatment. Plasma FFA concentrations were determined in pooled samples for periods 1, 2 and 3. Plasma FFA differed ($p<.01$) among ages and was increased from 6 to 12 mo to 2 yr of age, but least at 18 mo of age (table 9). Plasma FFA concentrations were greatest ($p<.01$) during Per 1, decreased during Per 2 and then increased ($p<.05$) during Per 3 in all age groups except 18-mo-old heifers, in which concentrations during Per 3 were not different ($p<.05$) than those during Per 2. An age x genetic group interaction ($p<.05$) indicated that plasma FFA concentrations were greater in 6-mo and 2-yr-old selected animals as compared with controls, but no similar differences were found between the 12- and 18-mo-old selected and control animals. Plasma FFA concentrations were positively associated with peripheral Prl in the selection group and negatively related with insulin and glucose concentrations (table 12). Plasma GH and FFA were negatively related in

TABLE 8

Plasma glucagon concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			ng/ml				
1	Selection	6	.8	.5	.2	.2	±.07
	Control	6	2.1	.5	.1	.2	±.05
2	Selection	6	.8	.4	.2	.2	±.05
	Control	6	1.9	.5	.1	.2	±.05
3	Selection	6	.9	.4	.2	.2	±.05
	Control	6	1.4	.6	.1	.2	±.05

[a] Least-squares means.

[b] Effects ($P<.05$ or greater) include age, period, age x period and genetic group x period.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

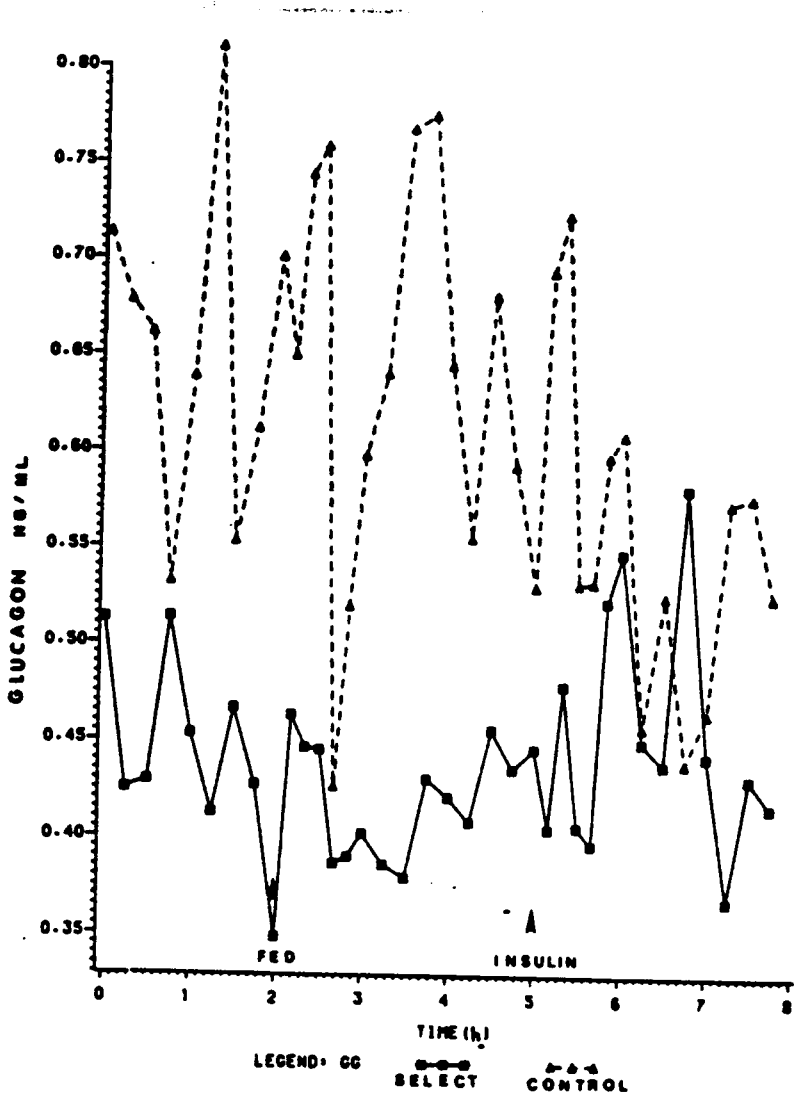

Figure 5: Plasma glucagon concentrations in selection and control animals before and after feeding and insulin injection

the control group (table 12). Plasma urea concentrations also differed ($p<.05$) among ages and was greatest in 2-yr-old cows and least in 12-mo-old heifers (table 10). Urea also tended ($p<.01$) to be greater in selected animals overall. Plasma urea concentrations were different ($p<.01$) among periods, being least during Per 1 and greatest during Per 3. Plasma urea was negatively related to peripheral glucagon in both genetic groups (table 12). In control animals, plasma urea concentrations were also negatively related to changes in glucose, while in selected animals the reverse was true (table 12).

Plasma glucose concentrations differed ($p<.01$) overall among ages and was greatest in 18-mo-old heifers and least in 2-yr-old cows (table 11). Glucose was also increased ($p<.01$) during Per 3 compared with Per 1 and 2. An age x period interaction ($p<.01$) indicated that in 6- and 18-mo-old animals, glucose concentrations were greater during Per 2 than during Per 1, but not in 12-mo- and 2-yr-old animals. Plasma glucose concentrations did not differ ($p>.05$) between genetic groups. Plasma glucose concentrations were positively correlated with insulin concentrations during Per 1 and 2 in both genetic groups, but not related after insulin injection (table 12). Plasma glucose concentrations were negatively related with GH concentrations in both genetic groups.

TABLE 9

Free fatty acid concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			----------μEq/ml----------				
1	Selection	6	.42	.44	.39	.80	±.03
	Control	6	.33	.53	.41	.73	±.03
2	Selection	6	.27	.28	.18	.34	±.03
	Control	6	.20	.29	.22	.26	±.03
3	Selection	6	.30	.30	.15	.39	±.03
	Control	6	.27	.43	.19	.28	±.03

[a] Least-squares means.

[b] Effects ($P<.05$ or greater) include age, period, age x period and age x genetic group.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

TABLE 10

Plasma urea concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			mg/dl				
1	Selection	6	17.7	8.9	13.6	17.1	±1.6
	Control	6	10.3	11.1	14.0	16.6	±1.6
2	Selection	6	20.2	11.7	17.8	19.4	±1.3
	Control	6	12.1	13.5	15.0	19.6	±1.3
3	Selection	6	24.9	15.2	17.1	31.3	±1.3
	Control	6	12.2	18.6	16.3	23.6	±1.3

[a] Least-squares means.

[b] Effects ($P<.05$ or greater) include age and period.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

TABLE 11

Plasma glucose concentrations in selection and control animals before and after feeding and insulin injection

Period[c]	Genetic group	No.	Age, mo				SE[d]
			6	12	18	24	
			mg/dl				
1	Selection	6	65.8	65.9	74.6	58.5	±1.0
	Control	6	57.9	64.7	79.7	56.6	±1.0
2	Selection	6	70.1	62.9	75.3	58.3	± .8
	Control	6	60.6	65.7	82.3	54.3	± .8
3	Selection	6	73.5	64.9	78.7	60.3	± .9
	Control	6	63.2	67.0	82.7	60.6	± .9

[a] Least-squares means.

[b] Effects ($P<.05$ or greater) include age, period and age x period.

[c] Period 1 = pre-feeding; Period 2 = post-feeding; Period 3 = post-insulin injection.

[d] Standard error of the mean.

TABLE 12

Correlation coefficients among endogenous hormone and metabolite concentrations in control and selected animals

Hormone or metabolite	Genetic group	Hormone or metabolite						
		PRL	GH	Insulin	Glucagon	Glucose	Urea	FFA
PRL	Control		.21***	.08**	-.11***		.17***	
	Selection		.22***		-.19***	-.17***		.25*
GH	Control			.12***		-.18***		-.38***
	Selection			-.08**	.21***	-.09**		
Insulin	Control					.12***		
	Selection							-.34**
Glucagon	Control						-.21***	
	Selection						-.07*	
Glucose	Control						-.09**	
	Selection						.16***	-.39***
Urea	Control							
	Selection							
FFA	Control							
	Selection							

[a] Includes all three periods; only significant (P<.05 or greater) correlations are presented.

* P<.05.

** P<.01.

***P<.001.

DISCUSSION

The elevated plasma GH in selection group as compared with control animals was the most uniform difference among the hormones and metabolites investigated. This increased GH in selected animals was consistent after feeding and insulin, however, increases relative to basal GH concentrations were similar between genetic groups under the conditions studied. Further, this capacity to maintain greater blood GH concentrations is present in the Holstein calf by 6-mo of age. Others have reported increased GH in the blood of high yielding as compared with low yielding cows (Hart et al., 1978, 1979; Bines et al., 1982). The increased GH is likely one physiological factor responsible for the genetically transmitted capacity of offspring of high PD milk sires to yield greater amounts of milk than herdmates from sires with less genetic transmitting ability for milk production. Injection of GH has been reported to increase both glucose and insulin in ruminants (Davis et al., 1970), but more recently research has shown that more highly purified preparations of GH did not increase glucose or insulin (Peel et al., 1981, 1982). Exogenous GH has increased both the efficiency of and quantity of milk produced (Brumby and Hancock, 1955; Machlin, 1973; Peel et al., 1983).

While the main purpose of this study was to investigate differences in hormone and metabolite concentrations plasma of animals of various ages which could be attributed to genetic merit, some differences due to age are of interest. Caution must be exercised in interpretation of age results because age is confounded with pregnancy and lactation, and the cattle in the four age groups certainly differ metabolically. Animals with a greater nutrient requirement per metabolic body size will be relatively more nutrient deficient after a 24 h fast. Thus, the lactating cows and the pregnant heifers would represent extremes, while the calves and yearlings would be intermediate in terms of nutrient depletion just prior to the feeding period.

Plasma GH did change with age. Sejrsen (1983) recently reported similar basal GH concentrations in prepuberal and postpuberal heifers that were approximately 6 mo different in age. The differences in GH due to age in the present study may have resulted from the treatments administered because all hormone values were determined in samples collected before or after feeding or after insulin injection. Results indicate that the 6-mo and 2-yr-old animals had greater GH concentrations than did the 12- or 18-mo-old animals. Because changes in plasma GH are associated with changes in FFA concentrations (Hertelendy and Kipnis, 1973;

Reynaert et al., 1975), the increased GH in these age groups may indicate a greater need or capacity for increased metabolic energy source. These two groups have the greatest metabolic requirement on a body weight basis.

There is a paucity of information concerning GH response to feeding in dairy cattle. It is generally accepted that feeding supresses GH in ruminants and that GH is negatively related to nutrient intake and plasma insulin and positively related to circulating FFA concentrations (Basset, 1978). Much of the previous work has been done with sheep (Basset, 1974a, 1974b) and GH response to feeding was not always consistent. Growth hormone increased in lambs after feeding of milk, but GH decreased after feeding of hay or grain to older sheep. Blom et al. (1976) reported increased GH in young bulls suffering from energy deficit and decreased GH during feeding. Growth hormone did not change after feeding or a fasting period, but did increase after infusion of arginine in fasted beef heifers (McAtee and Trenkle, 1971) and in lambs (Godden and Weeks, 1981). The reason that GH increased after feeding in this study is not known, but may be a reflection of species differences or other effects, such as inherent diurnal variation. The increase in GH after feeding in the present study was uniform and may indicate an increased tendancy toward utilization of

energy source for protein synthesis at the expense of lipid deposition because GH is lipolytic in action and does increase protein depostiton in the rumnant (Trenkle, 1981). This action of GH may account for one endogenous factor that contributes to greater efficiency of production in daughters of sires with high PD milk.

Although insulin was greater overall in the blood of selection compared with the control group animals, this difference resulted from the fact that selection group cows had elevated insulin concentrations for a longer period of time after insulin injection than control animals. Insulin was not different between groups before or after feeding. These results are rather surprising because others have shown that basal insulin is greater in lower than in higher yielding cows (Hart et al., 1978). Feeding increases insulin in ruminants (Bassett, 1978), and the increase is greater after feeding concentrates than after feeding hay. Feeding of hay or oat grain to sheep caused increased insulin (Bassett, 1974a, 1974b). However, Blom et al., (1976) reported both declines and increases in plasma insulin concentrations in young bulls during different feeding periods. The slight mean increase in insulin after feeding in the present study was not significant. The failure of feeding to elicit a detectable insulin increase is likely due to the forage-based

nature of the ration fed with no separate concentrate. This lack of dramatic insulin response to feed intake in these dairy cattle may suggest a low priority for lipogenesis. Insulin is both lipogenic and antilipolytic in cattle (Bauman, 1976), and is low in energy deficient animals (Schwalm and Schultz, 1976). The similar insulin concentrations before and after feeding between groups and the increased GH in the selection group should allow for increased conversion of adipose tissue to energy source and reduced lipogenesis in this group. The increased insulin in the selection group animals during Per 3 may have resulted from a reduced blood clearance rate as compared with control animals because maximum concentrations were similar. A second possibility is that adipose insulin binding may have been greater in the control animals. There are conflicting reports concerning adipose tissue binding. Yang and Baldwin (1973) reported insulin enhanced adipose tissue glucose uptake. However, Vasilatos et al. (1983) have recently reported a lack of specific insulin binding to bovine adipocytes in vitro.

The lesser glucagon concentrations in selection group animals may be a reflection or result of the increased efficiency of lipolysis caused by greater GH concentrations in that group. Selection group cattle had a reduced glucagon:insulin ratio in comparison with control animals, but

this was associated with increased GH in the selection group. The increased plasma glucagon in younger animals led to a greater glucagon:insulin ratio in those groups and may suggest greater stimulation of hepatic gluconeogenic pathways. Glucagon appears to be primarily involved with stimulation of gluconeogenesis in the ruminant (Trenkle, 1981). The combined effect of increased glucagon and GH in the 6-mo-old animals should increase availability of energy source in the form of glycogen and lipid while exerting minimal effect on protein degradation.

Prolactin increased in response to feeding in both groups as expected from previous reports (McAtee and Trenkle, 1971; Sejrsen et al., 1983). There are no reports to indicate that lower yielding cattle secrete greater Prl in response to feeding or insulin injection (Hart et al., 1978). Forbes et al. (1975), however, suggested an anabolic role for Prl, because lambs with increased growth rates had greater Prl concentrations. In agreement, the lower yielding control group in this study did have increased growth rates compared with the selection animals. The lack of a positive relationship of plasma Prl and genetic selection for milk yield is not surprising because others have been unable to correlate basal Prl with increased milk production (Hart et al., 1978, 1979). While Prl has been shown to be

essential for full lactogensis in cattle (Akers et al., 1981a), its effect is greatly dependent upon synergism with other lactogenic hormones (Tucker, 1981).

Free fatty acid concentrations were within the range cited by Phillips and Athanasiou (1978) for Holstein cattle. The difference in FFA concentrations among age groups may reflect different rates of catabolic activity or differences in available adipose tissue lipid to supply energy source in these animals. Overall, GH was least during Per 1, concurrent with the greatest circulating FFA concentrations. However, plasma insulin concentrations were also decreased during this same period, and this may have permitted mobilization of lipid and protein stores (Baird, 1981). Athanasiou and Phillips (1978) have reported increased peripheral FFA in cattle during a 48 h fast. These researchers also reported decreased GH concentrations during the initial stages of the fast; GH increased only after 24 h of fasting. The age x genetic group interaction that was noted due to increased FFA in 6-mo and 2-yr-old animals was associated with elevated GH in these same animals. Increased GH concentrations have been associated with elevated FFA concentrations in milk cows (Hart et al., 1978).

In spite of differences in several of the metabolic hormones studied, plasma glucose was not different between

genetic groups. However, despite lack of change in peripheral glucose, partitioning of glucose may vary in high and low yielding cattle (Bines and Hart, 1982). Availability of glucose to the mammary gland may be increased with increased GH. Growth hormone injections increased lactose in milk in cows without affecting irreversible loss of glucose (Bines and Hart, 1982) and exogenous GH increased blood flow to the mammary gland in goats (Hart et al., 1980). The increased glucose after the administration of insulin was unexpected. Several studies have shown that larger doses of exogenous insulin given over time decrease blood glucose concentrations in the ruminant (Kronfeld et al., 1963; Hove, 1978a, 1978b). Results of this study indicate that the single injection elevated blood insulin for only about 1 h with little effect on blood glucose, since glucose concentration actually was slightly increased for Per 3. Apparently, either the tissues could use no more blood glucose or exogenous insulin must be administered over a longer period of time to produce any dramatic alterations in blood metabolites or metabolic hormones in cattle. The increased plasma glucose during Per 3 may have resulted from the increase in GH after feeding. Davis et al. (1970) reported increased plasma glucose and insulin and decreased plasma urea in lambs given exogenous GH. A chronic increase in the GH:insulin ratio

may be responsible for increased glucose availability (Bines and Hart, 1982) and decreased deposition of lipids in adipose tissue by promoting insensitivity to the effects of insulin (Goodman and Coiro, 1981; Muir et al., 1983). Whether the acute changes occurring in the GH:insulin ratio in this study affect glucose availability is not known. In retrospect, exogenous insulin administration should have been delayed to allow longer term monitering of post-prandial endocrine changes. However, the fact that neither blood insulin, glucagon or glucose was changed after feeding compared to before feeding probably indicates little carryover effect of feeding on the response of the endocrine system to the exogenous insulin.

Plasma urea concentrations presumably are a reflection of amino acid catabolism in the liver. Thus, blood urea would be expected to be decreased 24 h after feeding due to decreased ingested protein. Because the complete diet fed contained soybean oil meal, protein catabolism was apparently accomplished quickly to elevate blood urea during Per 2 and 3. The tendancy for blood urea to be greater in selection group animals may indicate increased efficiency of catabolism of ingested protein in that group.

In conclusion, under the conditions of the study, differences in response of all hormones investigated could be

attributed to differences in genetic merit. Increased plasma GH appears to be characteristic of daughters of high PD milk sires when compared with progeny of unselected sires. In addition, altered response of Prl, glucagon and insulin may also be involved in regulation of increased efficiency of production in these offspring. Further, findings indicate that genetic differences in several physiological traits may not be apparent at all ages in dairy cattle, and investigation of differences in these traits under various metabolic challenges will be necessary if they are to be of value as predictors of genetic merit.

Chapter V

HORMONES AND METABOLITES IN BULL CALVES OF DIFFERING GENETIC MERIT

INTRODUCTION

Calves with different genetic potentials for milk production were different in aspects of energy and nitrogen metabolism (Tilakaratne et al., 1980). The possibility of using other genetically influenced physiological traits that affect milk yield as a basis of selection for increased milk yield has been suggested (Land, 1981; Osmond et al., 1981). In the lactating cow the energy demand of the mammary gland is considerable, yet some animals meet this demand more readily and efficiently than others. The principal physiologic component in variation of milk producing ability may be genetically influenced variation in partitioning of nutrients (Land, 1980; Hart, 1983). Calves of superior genetic merit for milk production appear to preferentially mobilize fat as an energy source in comparison to calves of lesser genetic merit (Hart, 1983). On the other hand, single measure of peripheral triiodothyronine (T3), thyroxine (T4) and insulin were not related to genetic merit for milk yield in bulls (Osmond et al., 1981).

High-yielding, underfed dairy cows had greater plasma concentrations of growth hormone (GH) and nonesterified fatty acids and lower insulin than did lower-yielding, overfed cows (Hart et al., 1978). Changes in GH concentrations were positively correlated with changes in milk yield and negatively related to body weight changes (Hart et al., 1979). In addition, high-yielding cows preferentially partition energy toward milk production while low-yielding cows direct energy toward depostion of body tissues (Broster et al., 1969). These findings suggest possible physiological differences in the regulation and secretion of prolactin (PRL), GH and insulin, all of which have been implicated in playing a role in metabolic regulation in the ruminant (Forbes et al., 1975; Hart et al., 1978; Bauman and Currie, 1980; Trenkle, 1981).

The purpose of this study was to investigate the response of several metabolites and hormones which are altered by or directly affect partitioning of nutrients in young dairy bulls of different genetic merit for milk production. Studies such as this may lead to a more complete understanding of the role which increased genetic selection pressure for milk yield plays in the alteration of physiological regulation of metabolism.

MATERIALS AND METHODS

Animals

Fourteen young Holstein bulls housed at the Virginia Tech Dairy Cattle Center were paired by age and allotted to the trial. Eight animals (control group) were offspring of second to fourth generation daughters of cows randomly bred to non-AI unselected sires originating in the Virginia Tech dairy herd (mean age=24 wk; mean body weight (BW)=139 kg). The remaining animals (n=6; selection group) were offspring of cows bred to selected sires (average predicted difference (PD_{82})=+36 kg milk) available through artificial insemination (AI) organizations (mean age=24 wk; mean BW=131 kg). Selection animals had a mean calculated estimated breeding value ([PD_{82}+CI82]/2) of +371 kg for milk production based on the January 1984 and cow evaluations. Animals were housed in separate stalls in an open-sided barn during March and fed a forage-based complete diet at 0600 and 1700 h daily with free access to hay and water. The mixed complete diet (blended corn silage, haylage and concentrate) consisted of 15.5% crude protein, 10.8% digestible protein, 22.2% acid digestible fiber and 73% total digestible nutrients. Animals were fed at a rate to meet National Research Council nutrient requirements and feed weigh back information was not recorded. All animals were gaining weight at the time of the trial.

Blood samples

All animals were fitted with jugular cannulae prior to the initiation of the trial and haltered for 24 h before sampling. Serial blood samples were collected on alternate days, with a day of rest in between, into heparinized tubes at 20 min intervals from 0900 to 1600 h or 1700 h except between 1100 and 1200 h when samples were collected every 10 min. On the first and second alternate days, respectively, all animals were administered glucose (.1g/kgBW) or thyrotropin releasing hormone (TRH) (.33ug/kgBW) at 1100 h via jugular cannulae.

Hormone assay

Plasma PRL concentrations were quantified by a double antibody radioimmunoassay (RIA) with bovine PRL antiserum by the method of Barnes et al. (1985). The specific antiserum was used at 1:50,000 dilution and bound 40% of radiolabelled PRL in the absence of unlabelled hormone. With NIH-bPRL-6 as a reference standard, the antiserum showed 7.0% cross-reactivity to thyroid stimulating hormone (TSH) and <2% cross-reactivity to other pituitary hormones when added to the assay media in amounts up to 1,000 ng. The ovine TSH did contain .1% PRL. Reliable estimates of PRL activity were obtained over a range from .25 to 5 ng per assay tube.

Standards added to plasma pools were recoverable and produced parallel inhibition curves with NIH-bPRL-6. All samples were assayed in duplicate and intra- and interassay coefficients of variation calculated for plasma pools averaged 8.4 and 11.5%, respectively.

Plasma GH concentrations were quantified by a homologous double antibody RIA with bovine GH antiserum by the method of Barnes et al. (1985). The specific antiserum used at a dilution of 1:2,000 bound 35% of labelled GH. With NIH-bGH-18 as a reference standard, the antiserum did not cross-react with any other pituitary hormones. Reliable estimates of GH activity were obtained over a range of 1 to 20 ng per assay tube. All samples were assayed in duplicate and intra- and interassay coefficients of variation calculated for plasma pools averaged 7.6 and 6.1%, respectively.

Plasma insulin concentrations were determined using a double antibody RIA (Barnes et al., 1985) with specific antiserum which, when used at a dilution of 1:10,000, bound 30% of radiolabelled insulin. With highly purified bovine insulin (Lot No 615-70-80, Eli Lily Co., Indianapolis, IN.) as a reference standard, there was no cross-reactivity with glucagon (<2%). Reliable estimates of insulin activity were obtained over a range of .25 to 4 ng per assay tube. All samples were assayed in duplicate and intra- and interassay

coefficients of variation calculated for plasma pools averaged 8.7 and 12.5%, respectively.

Determination of plasma urea concentrations were accomplished on pooled samples for each h by reaction with diacetylmonoscamine by the method of (Coulombe and Favreau, 1963) and glucose by the O-toluidine reaction (Feteris, 1965).

Data analysis

Logarithmic transformations of blood hormone and metabolite data were analyzed by least-squares analysis of variance. Data were segregated into three periods, these being a 2 h basal period (Per 1), a 2 h response period following glucose or TRH injection (Per 2), followed by another collection period either 3 (day of glucose) or 4 h (day of TRH) long (Per 3). The main treatment effects were genetic selection group and period, and the model accounted for variation among selection groups, periods, bulls within selection groups, and selection group x period and bulls within selection group x period interactions. The variance associated with bulls within selection group was used as the error term to test for presence of selection group effects, while the variance associated with bulls within selection group x period was used to test for presence of period and selection x period interactions.

RESULTS

Mean plasma concentrations of hormones and metabolites before and after glucose injection are depicted in table 13 and figure 6. Plasma insulin increased ($P<.05$) after glucose injection in all bulls and a selection group and period interaction ($P<.05$) indicated that peripheral insulin was greater after glucose injection in control than in selection group animals. Increased plasma insulin concentrations were apparently induced by stimulation of pancreatic insulin release by exogenous glucose. Plasma glucose tended ($P<.1$) to decline after glucose infusion and the decline appeared to be more marked in the control group than in the selection group animals. A selection group and period interaction ($P<.05$) indicated that plasma GH was greater in selection group bulls before glucose, greater in control bulls after glucose, and then reversed again during Per 3.

Mean hormone and metabolite concentrations before and after TRH injections on the second trial day are depicted in table 14 and figure 7. The overall increase in PRL on day 2 compared to day 1 was presumably due to increased ambient temperature on the second trial day. Peripheral PRL was increased ($P<.01$) by TRH injection and showed a biphasic response to this stimulus. Prolactin remained elevated with respect to Per 1 though Per 2 and 3 and a selection group

TABLE 13

Least squares means of hormones and metabolites in control and selection bulls before and after exogenous glucose

Period[a]	Genetic group	Insulin		Growth hormone		Prolactin		Glucose		Urea	
		$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
1	Control	1.5	.2	9.0	1.1	5.9	.6	83.7	1.7	5.9	.3
	Selection	1.4	.2	16.9	1.2	4.7	.6	90.4	2.0	8.2	.4
2	Control	2.9	.2	14.2	.9	5.1	.5	81.0	2.1	5.4	.4
	Selection	2.0	.2	13.0	1.1	5.1	.5	90.6	2.4	8.3	.5
3	Control	1.3	.2	11.5	.9	2.5	.5	90.9	2.1	5.7	.4
	Selection	1.2	.2	14.7	1.1	4.3	.5	92.8	1.9	9.8	.5

[a] Period 1 = before exogenous glucose; Period 2 = 2h period immediately after exogenous glucose; Period 3 = period from 2h to 5h after exogenous glucose.

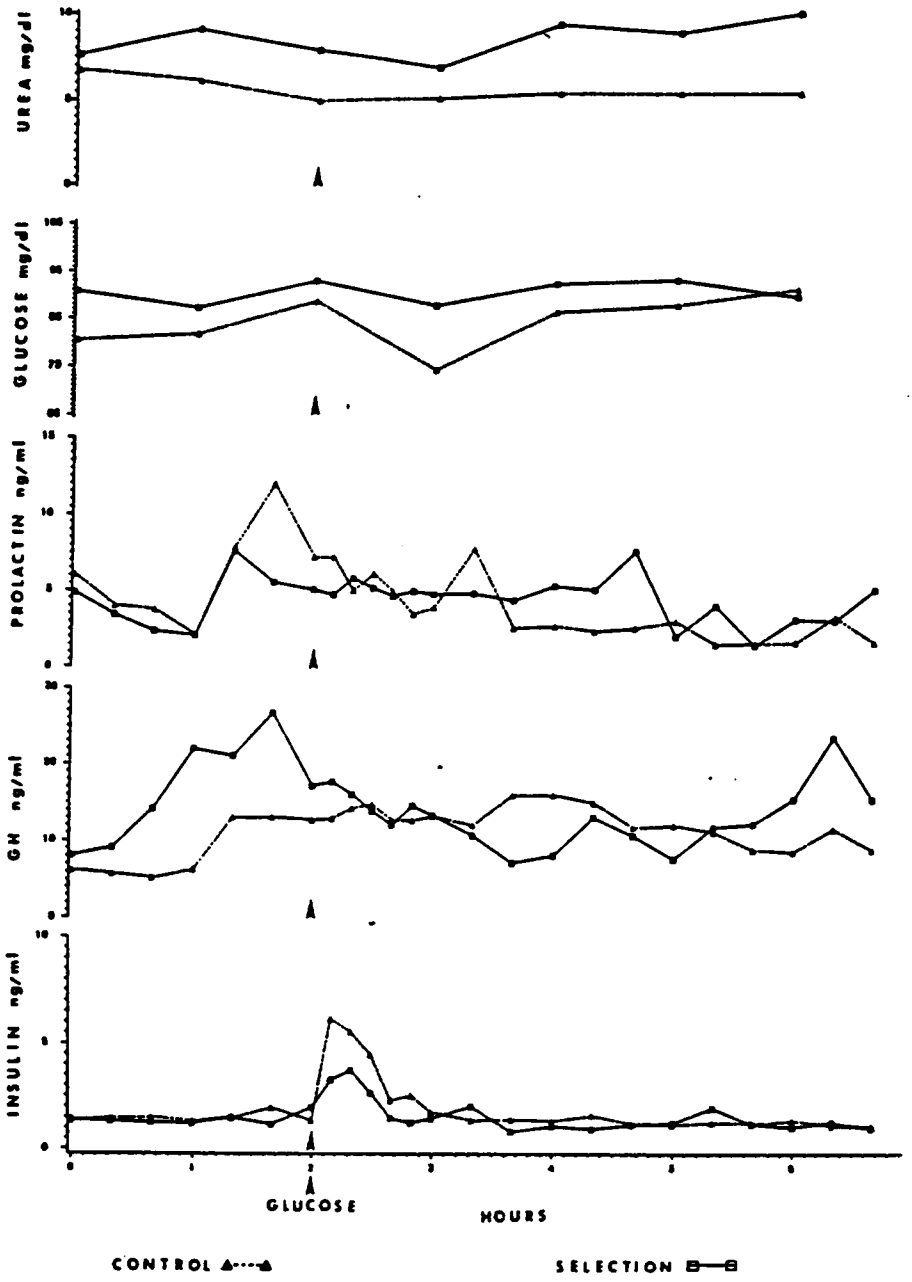

Figure 6: Plasma hormones and metabolites before and after glucose infusion in control and selection bulls

and period interaction ($P<.01$) indicated that PRL was increased in the selection group bulls after TRH but not before TRH compared to control group bulls. Growth hormone also increased ($P<.01$) after TRH but did not differ ($P>.05$) between genetic selection groups either before or after TRH, even though absolute values for GH were larger after TRH in selection as compared to control animals. On the other hand plasma glucose declined ($P<.01$) after TRH evidently as the result of increased ($P<.05$) plasma insulin in the control group animals. Insulin was unchanged in selection bulls after TRH resulting again in a greater insulin to GH ratio in the control group and a trend towards increased blood glucose uptake. Plasma urea concentrations did not differ ($P<.05$) in response to TRH injection or in response to genetic selection.

DISCUSSION

Plasma glucose tended to decline after glucose infusion and the decline appeared to be more marked in the control group than in the selection group animals. The steeper decline in glucose in the control group was presumably due to the increased insulin concentration after glucose infusion in that group. The decreased peripheral glucose during Per 2 can be attributed to the increased endogenous release and

TABLE 14

Least squares means of hormones and metabolites in control and selection bulls before and after exogenous TRH

Period[a]	Genetic group	Insulin		Growth hormone		Prolactin		Glucose		Urea	
		$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
1	Control	1.4	.1	8.7	1.2	19.3	2.7	89.2	2.0	5.9	.2
	Selection	1.6	.1	9.9	1.3	13.3	2.9	88.1	2.2	6.4	.2
2	Control	2.2	.1	13.8	1.1	32.4	2.3	78.0	2.4	5.7	.2
	Selection	1.3	.1	20.1	1.1	48.6	2.6	84.2	2.6	6.5	.2
3	Control	1.2	.1	12.3	.9	16.2	2.0	85.0	2.0	5.9	.3
	Selection	1.2	.1	11.6	1.0	39.1	2.2	93.6	2.2	7.3	.2

[a] Period 1 = before exogenous TRH; Period 2 = 2h period immediately after exogenous TRH; Period 3 = period from 2h to 6h after exogenous TRH.

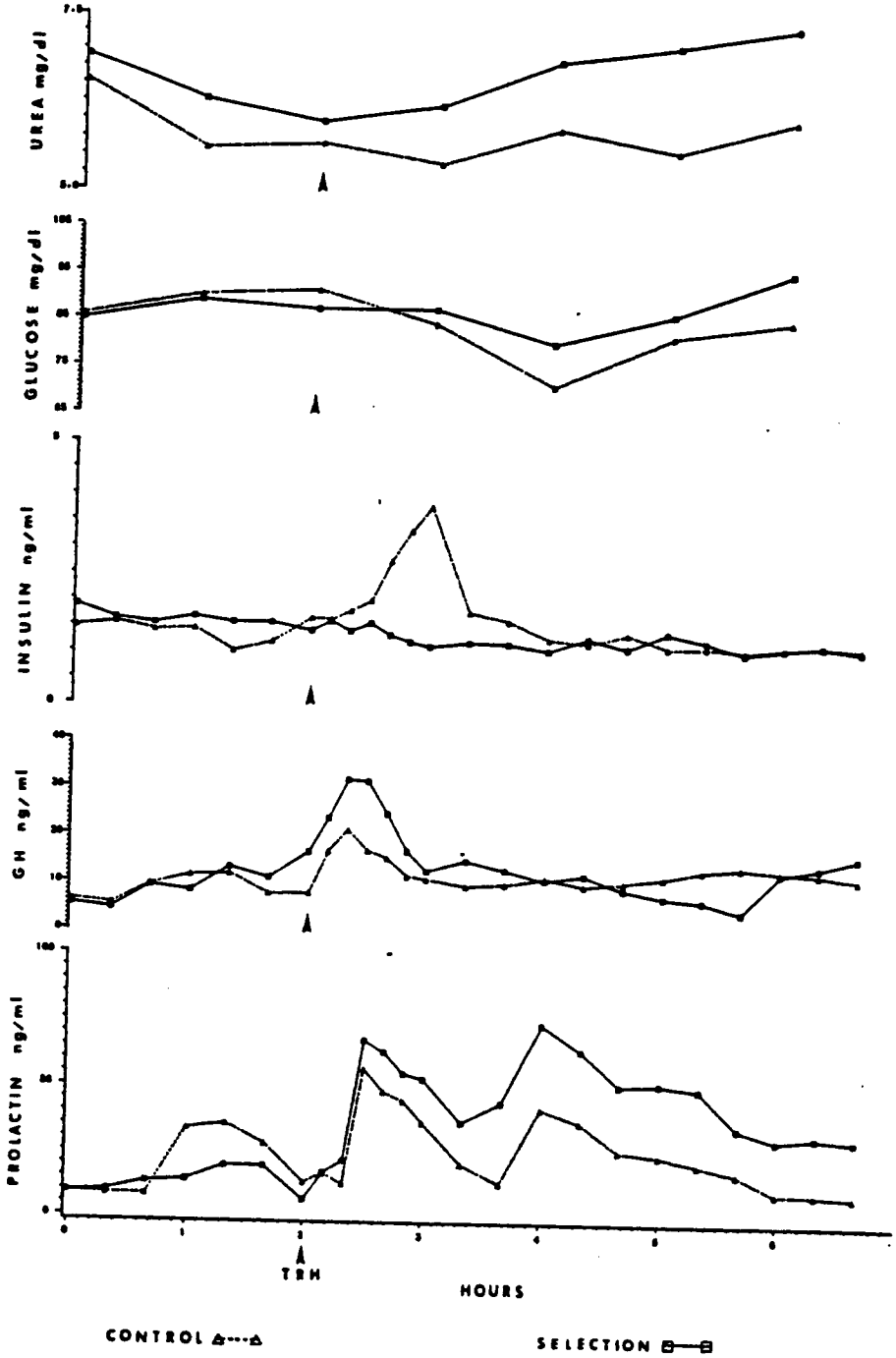

Figure 7: Plasma hormones and metabolites before and after TRH injection in control and selection bulls

the lipogenic action of pancreatic insulin (Bauman, 1976; Hart, 1983).

The increased GH in selection animals during Per 1 and 3 and the increased insulin in the control group after glucose contributed to a greater GH to insulin ratio in the selection group which would suggest a greater tendency towards lipolysis and decreased lipogenesis in that group compared to the control group. Growth hormone is lipolytic in the ruminant and can inhibit insulin-stimulated removal of glucose from the blood (Hart, 1983). Neither plasma PRL nor urea was different between genetic selection groups nor were either affected by exogenous glucose administration.

Glucose administration causes transient hyperglycemia and increases blood insulin in lactating cows (Baird, 1981) and sheep (Trenkle, 1981). The fact that insulin was increased in the control group bulls compared to the selection animals may indicate a greater tendency towards more rapid tissue uptake of glucose and an increased glucose utilization for lipogenesis in that group. Insulin is both lipogenic and antilipolytic in cattle (Bauman, 1976; Hart, 1983). The increased insulin response to exogenous glucose challenge in these young bulls of low genetic merit for transmitting milk production would tend to agree with studies which indicated that peripheral insulin was greater in

low yielding cows compared to high-yielding cows (Hart et al., 1978; Hart et al., 1979; Hart, 1983). It is important to realize that nutritional allowance and energy balance may play a role in the ability to measure this phenomena, since this difference in insulin between high and low-yielding cows is not present when cattle are dry or when they are fed to similar weight change (Hart, 1983). On the other hand, calves of superior genetic merit have decreased insulin response and, based on differences in blood urea nitrogen and free fatty acids, preferentially mobilize fat and not protein compared to calves of lower genetic merit for milk production (Tilakaratne et al., 1980; Land, 1981).

Both T3 and T4 would be expected to increase after TRH in response to increased pituitary thyroid stimulating hormone release (Olhson et al., 1978). Because thyroxine enhances cellular glucose uptake (Dickson, 1982), exogenous TRH may be expected to reduce peripheral glucose. Thus, some of the observed reduction in plasma glucose concentrations may be due in fact to increases in plasma T3 or T4 concentrations.

While the initial increase in PRL after TRH injection could be attributed to TRH induced pituitary release of PRL, the cause of the biphasic response is not clear. One might speculate that the second increase in PRL may have been a

result of reduced peripheral glucose. In support of this, Godden and Weekes (1981) have reported increased PRL in response to insulin induced hypoglycemia in lambs.

The fact that peripheral GH did not differ significantly between selection groups after TRH, while PRL was increased in selection group animals after TRH is in contrast to our previous findings in lactating cows of differing genetic merit that GH but not PRL was increased after TRH in selection animals (Kazmer et al., 1983) Futher, studies in dairy heifers and cows (Barnes et al., 1985) have indicated a rather uniform increase in GH in selection group animals compared to the control group. The inconsistent difference in GH between selection groups on different days of the present study may be attributed to a stronger effect of animal variation in GH or possibly a difference in response due to sex or age of the animals. At any rate, it was evident that under the conditions of this trial, differences in GH concentrations were not always indicative of differences in genetic merit.

In conclusion, of the hormones and metabolites studied, PRL, GH and insulin did differ between genetic selection groups on different days of the study. Basal GH was increased in the selection group on the first day but not on the second day and differences in response to either exoge-

nous glucose or TRH were not evident. On the other hand, basal PRL did not differ between groups but was increased in selection animals after TRH. The most uniform difference between selection groups was the increased insulin response of the control group animals after exogenous glucose and TRH which suggests greater metabolic drive towards tissue uptake of blood glucose in animals of low estimated transmitting ability for milk production.

Chapter VI

GROWTH HORMONE IN DAIRY CATTLE OF DIFFERING GENETIC MERIT

INTRODUCTION

Several studies (Asimov and Krouze, 1937; Hutton, 1957; Machlin, 1973; Peel et al., 1983) have demonstrated that GH administration to dairy cattle during lactation increases milk yield and feed efficiency. Recent reviews (Bauman and Currie, 1980; Hart, 1983) have indicated that such increases are due to the involvement of GH in the partitioning of nutrients and energy to meet various physiological demands. Adipocyte lipolysis is stimulated by GH, while lipogenesis is inhibited. Additionally, GH antagonizes the insulin-dependant uptake of glucose and acetate into body tissues, thus directing energy and substrates toward the mammary gland to be utilized for milk production. Bines and Hart (1977) have shown that GH concentrations are greater in dairy than in beef cows. Thus, the possibility arises that changes in plasma GH concentrations may be associated with increased genetic selection pressure for increased milk yield in dairy cattle. In support of this concept, it is interesting to note that the only conformation type trait variable strongly correlated with milk yield is dairy character, ie. the lack of thick subcutaneous adipose deposits.

Studies have demonstrated that increased frequency of milking results in increased quantities of milk produced (Goff and Gaunya, 1977; Pearson et al., 1979), though the mechanism of action is not fully understood. Linzell (1974) has demonstrated that milk synthesis is decreased with increases in intramammary pressure by virtue of a reduction in blood flow. Endocrine mechanisms may be involved as Hart et al. (1980) have reported that GH increases mammary blood flow, though the possibility that other endocrine mechanisms exist is not excluded. Thus, the objectives of the present study were to measure GH and prolactin concentrations before and after thyrotropin releasing hormone (TRH) administration in dairy cows of differing genetic merit milked either twice (2x) or thrice (3x) daily.

MATERIALS AND METHODS

Animals

Fifty Holstein cows housed at the Virginia Tech Dairy Center were randomly allotted to be milked twice (2x; 0400 and 1530 h) or thrice (3x; 0000, 0600 and 1600 h) daily. Animals were either daughters of selected sires (mean PD_{84}=+368M, +8F) (selection group) or second to fourth generation daughters of cows random bred to non-AI unselected sires originating in the Virginia Tech dairy herd (control

group). Data was collected during the first and second lactations. The experiment was begun in October, 1981 and completed in August, 1984. The distribution of animals within the experimental design is shown in table 15. Serial blood samples were collected at 30, 90 and 200 days postpartum (DPP) via jugular cannulae inserted at 0700 h on the day of sampling. Samples were collected at 15 min intervals from 1030 to 1600 h and then half-hourly until 1730 h. Thyrotropin releasing hormone (TRH) was administered (.33 ug/kg BW) at 1300 h. Blood was centrifuged and plasma was stored at -20 C until assayed for hormone content.

Feed intake and daily milk yield

Feed intake data for 5 days prior to cannulation was recorded via a commercial electronic intake monitoring device (Pinpointer, Inc.) Feed samples collected during intake monitoring were analyzed at the Virginia Tech Forage Testing Lab and averaged 48.4% dry matter, 16.0% crude protein and 76.0% total digestible nutrients on a dry matter basis. Milk yield over the same 5-d period was recorded at each milking. Feed intake, body weight and milk yield (table 16) were used to calculate net energy balance (NEB) at each postpartum period. NRC figures (NRC, 1978) concerning energy requirements for maintenance, growth and for milk produc-

TABLE 15

Distribution of animals within the experimental design

First Lactation

Frequency Of Milking	Genetic Merit Selected	Control	Totals
2x	n=18	n=8	26
3x	n=16	n=8	24
Totals	34	16	50

Second Lactation

Frequency of Milking	Genetic Merit Selected	Control	Totals
2x	n=15	n=4	19
3x	n=13	n=7	20
Totals	28	11	39

tion of a particular fat test were multiplied by the body weight and milk yield of each animal. The resulting value was subtracted from the energy value of the feed consumed by each animal, yielding a NEB estimate. Fat test data was obtained from DHIA records, averaging the test day record prior to with the record just following the date of cannulation.

Hormone assay

Plasma Prl concentrations were quantified using a double antibody radioimmunoassay (RIA) with bovine prolactin antiserum, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:50,000, bound 40% of labelled prolactin in the absence of unlabelled hormone. With bovine prolactin (NIH-bPRL-6) as reference standard, the assay system exhibited 7.0% cross-reactivity with thyroid stimulating hormone (NIH-oTSH-9), but less than 2% with any other pituitary hormones added to the assay system in amounts up to 1,000 ng. The ovine TSH did contain .1% prolactin, as the preparation was not highly purified. The standard curve ranged from .25 to 5 ng per assay tube, 1.25 ng/ml being the least detectable dose. Standards added to plasma pools were recoverable and produced parallel inhibition curves with NIH-bPRL-6. All samples were assayed in

TABLE 16

Least squares means of feed intake, body weight and milk yield in experimental animals

Lactation	Genetic Merit	Frequency of Milking	Days Postpartum	Feed Intake[a]	Body Weight[b]	Milk Yield[c]
First	Selected	Twice	30	31.4[d]	504.5[d]	27.1[d]
			90	35.5	525.0	25.8
			200	34.0	550.1	22.4
		Thrice	30	29.8	497.7	28.3
			90	33.2	510.5	26.9
			200	32.9	532.3	24.1
	Control	Twice	30	29.2	592.7	22.3
			90	31.2	512.3	19.5
			200	30.8	575.9	16.0
		Thrice	30	26.2	471.8	25.7
			90	31.9	513.6	24.1
			200	30.8	534.1	20.0
Second	Selected	Twice	30	40.0	580.0	34.7
			90	39.5	591.8	29.9
			200	37.0	612.7	24.9
		Thrice	30	38.0	568.6	33.0
			90	40.5	576.8	30.7
			200	37.5	612.3	23.2
	Control	Twice	30	32.5	568.6	28.7
			90	37.7	575.9	25.6
			200	31.9	580.0	18.1
		Thrice	30	34.4	526.8	27.5
			90	35.3	543.2	26.0
			200	33.7	570.5	20.3

[a]Feed intake differs by genetic merit, lactation and days postpartum ($p < .001$)

[b]Body weight differs by lactation and days postpartum ($p < .0001$)

[c]Milk yield differs by genetic merit, lactation and days postpartum ($p < .05$)

[d]kg

duplicate, and intra- and interassay coefficients of variation averaged 10.5 and 9.3%, respectively, in two plasma pools.

Plasma GH concentrations were quantified using a double antibody RIA with bovine GH antiserum, by the method of Barnes et al. (1985). The specific antibody, used at an initial dilution of 1:1,000, bound 35% of labelled GH in the absence of unlabelled hormone. With NIH-bGH-18 as reference standard, the assay system did not cross-react (<2%) with other pituitary hormones. The standard curve ranged from .5 to 20 ng per assay tube, 1.5 ng/ml being the least detectable dose. Standards added to plasma pools were recoverable and produced parallel inhibition curves with NIH-bGH-18. All samples were assayed in duplicate, and intra- and interassay coefficients of variation averaged 8.9 and 7.2%, respectively, in two plasma pools.

Data Analysis

Hormonal data prior to (basal period) and following (response period) TRH administration were analyzed as a split-plot design by the General Linear Models (GLM) option of the Statistical Analysis System (SAS) (SAS, 1982). The model accounted for variation in genetic merit, frequency of milking, days postpartum (group 30, 90 or 200), lactation

number, period, all possible interactions, cow within genetic merit by frequency of milking and error. Additionally, the mean ambient temperature during the basal or response period was included as a covariate. The mean square for cow within genetic merit by frequency was used as the denominator to test for differences in genetic merit, frequency of milking and the interaction between the two. The mean square for error was used to test all other effects. Mean daily feed intake, milk yield and NEB data at 30, 90 and 200 d postpartum were analyzed by the GLM option of SAS (SAS, 1982). The model accounted for variation in genetic merit, frequency of milking, days postpartum, lactation number, all possible interactions and error. The mean square for cow within genetic merit by frequency was used as the denominator to test for differences in genetic merit, frequency of milking and the interaction between the two.

RESULTS

Least squares means for NEB at 30, 90 and 200 DPP in 2x and 3x selected and control cows during the first two lactations are presented in table 17. Overall, daily NEB was less positive in 3x as compared with 2x animals (.41 vs .94 kg TDN, $p<.05$), negative at 30 while positive at 90 and 200 DPP, (-.80 vs. 1.16 or 1.66 kg TDN, $p<.01$) and less positive

during the first as compared with the second lactation (.33 vs 1.01 kg TDN, $p<.01$). However, there was no difference in NEB between selected and control animals ($p>.05$). Among all cows, NEB was positively related ($p<.001, r=.45$) to daily feed intake and negatively related ($p<.001, r=-.34$) to daily milk yield. Further, daily milk yield and feed intake were positively correlated ($p<.001, r=.48$). Mean ambient temperature was not correlated to NEB or feed intake, but tended to be negatively related ($p<.1, r=-.11$) to milk yield. The inclusion of ambient temperature as a covariate in the model indicated that daily yield was affected by temperature ($p<.05$).

Mean plasma Prl concentrations in first and second lactation cows before and after TRH (figure 8) ranged between 12.2 and 102.5 ng/ml, and were affected by ambient temperature. Administration of TRH induced Prl release, since Prl concentrations during the response period averaged greater than those during the the basal period (41.0 vs 14.6 ng/ml, $p<.01$), No differences during either the basal or response periods due to genetic merit, frequency of milking, or DPP were noted ($p>.05$). However, overall Prl was greater during second as compared with first lactation (29.4 vs 26.2 ng/ml, $p<.05$).

TABLE 17

Least squares means of net energy balance in experimental animals

Lactation[a]	Frequency of Milking[b]	Days Postpartum[c]	Genetic Merit Selected	Genetic Merit Control
First	Twice	30	- .47±.4[d]	- .01±.6
		90	1.05±.4	1.56±.6
		200	.73±.4	2.24±.6
	Thrice	30	-1.92±.4	-1.94±.6
		90	.21±.4	.41±.6
		200	.50±.4	1.60±.6
Second	Twice	30	.05±.5	-1.64±.8
		90	1.68±.5	1.93±.8
		200	1.92±.5	2.27±.8
	Thrice	30	- .38±.4	- .05±.7
		90	1.09±.4	1.35±.7
		200	2.02±.4	2.01±.7

[a] First lactation cows < second ($p<.0001$)

[b] Cows milked twice > cows milked thrice daily ($p<.05$)

[c] Cows at 30 < 90 or 200 days postpartum ($p<.0001$)

[d] Least squares mean ± SE, kg TDN

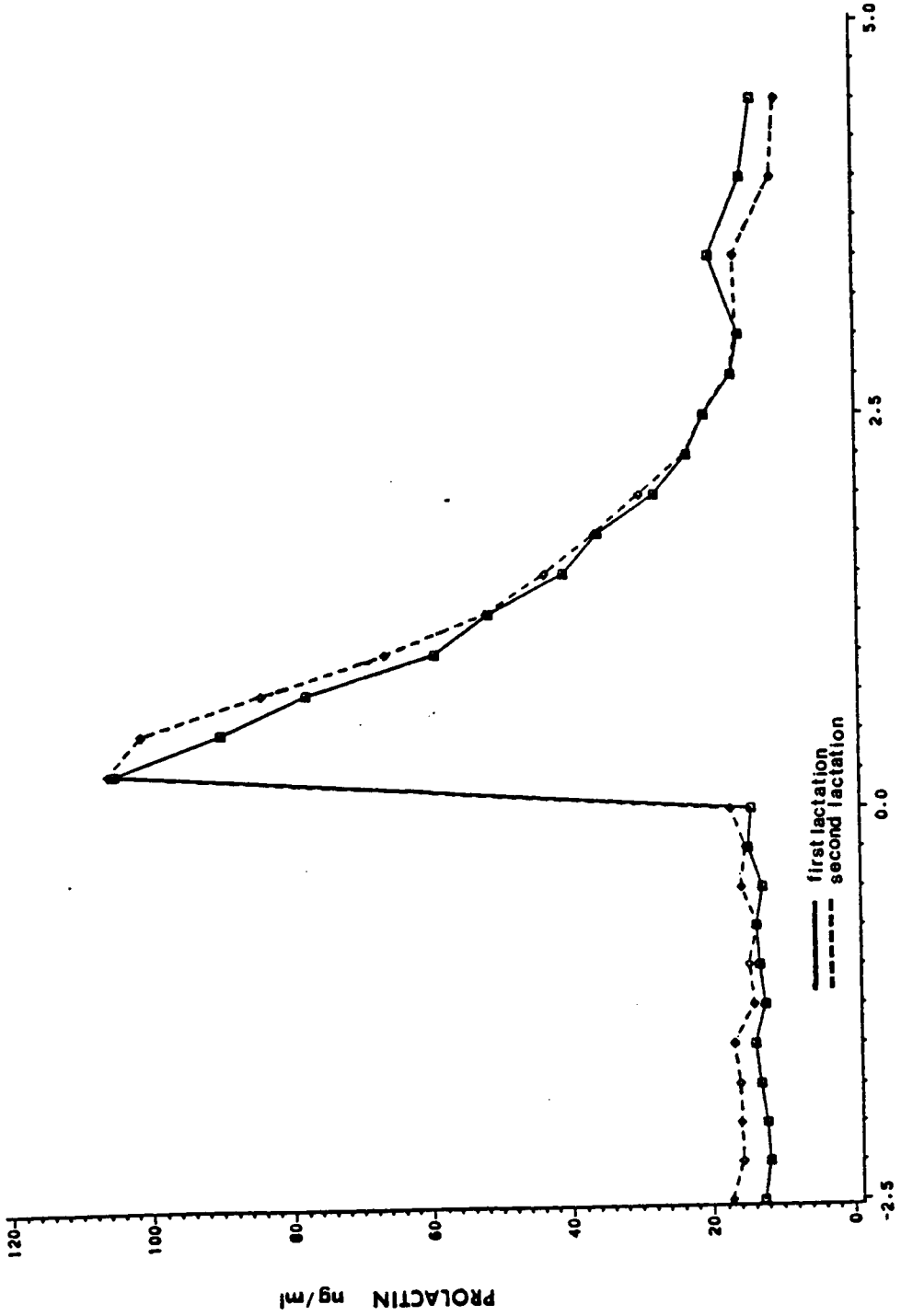

Figure 8: Prolactin concentrations in first and second lactation cows before and after TRH administration

Mean plasma GH concentrations from 2 h prior to until 4.5 h after TRH administration to selected and control animals at 30, 90 and 200 DPP during the first and second lactations are depicted in figure 9. Because no differences in GH concentrations were found between 2x and 3x cows ($p>.05$), classification of data according to frequency of milking is not shown. Mean GH concentrations during the basal and response periods in first lactation selected and control cows at 30, 90 and 200 DPP are depicted in figure 10, while those in second lactation animals are depicted in figure 11. Least squares means for GH concentrations in the experimental animals during each period are depicted in table 18. Overall, administration of TRH increased GH concentrations, since the response averaged greater than the basal period (7.45 vs 5.83 ng/ml, $p<.01$). Further, selected animals had greater mean GH concentrations than did control animals during both the basal (6.68 vs 4.99 ng/ml, $p<.01$) and response periods (8.28 vs 6.56 ng/ml, $p<.01$), and mean GH concentrations were greater at 30>90>200 DPP during the basal (7.09 vs 5.72 vs 4.69 ng/ml, $p<.01$) and response (8.98 vs 7.59 vs 5.70 ng/ml, $p<.01$) periods.

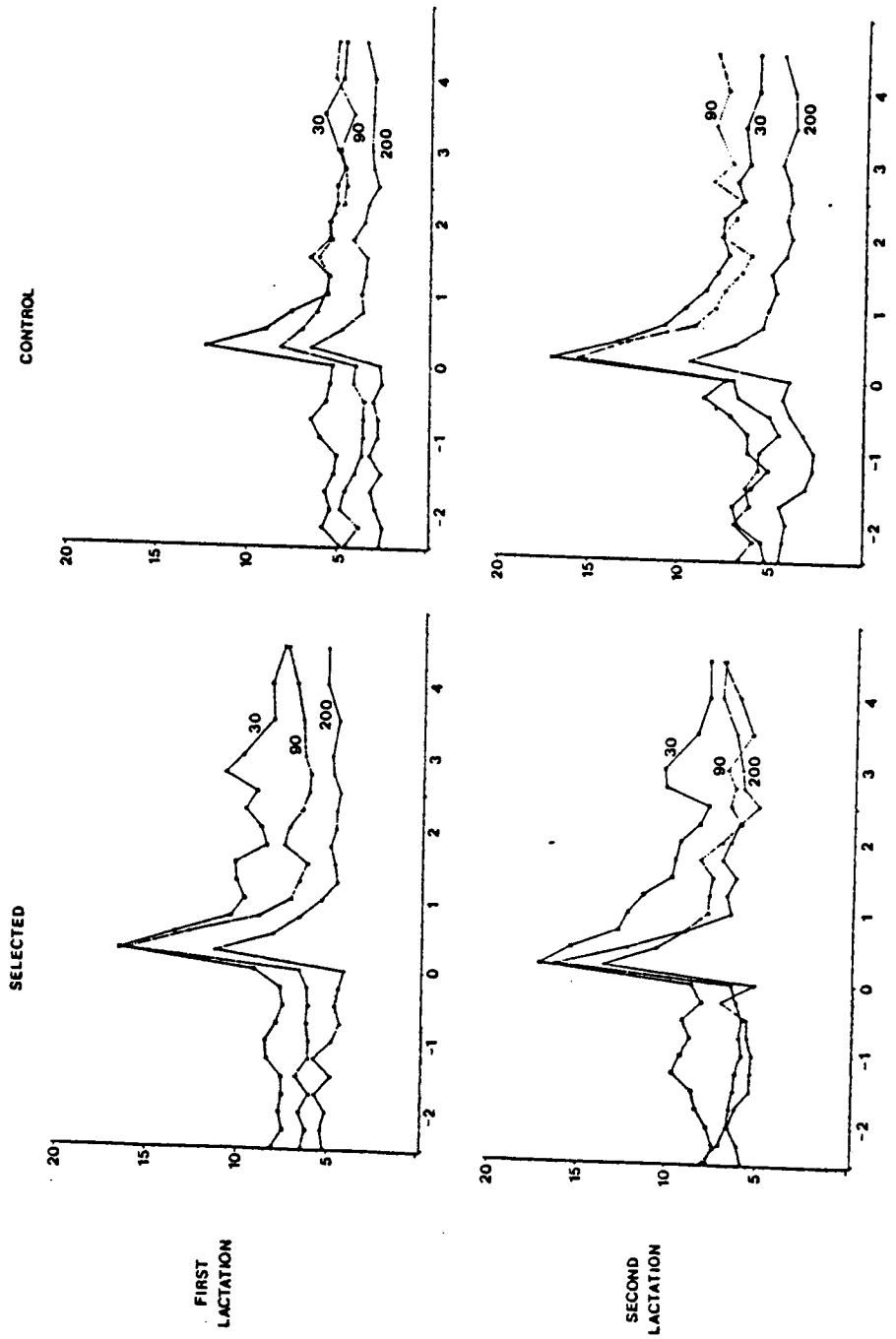

Figure 9: Mean plasma GH concentrations in experimental animals

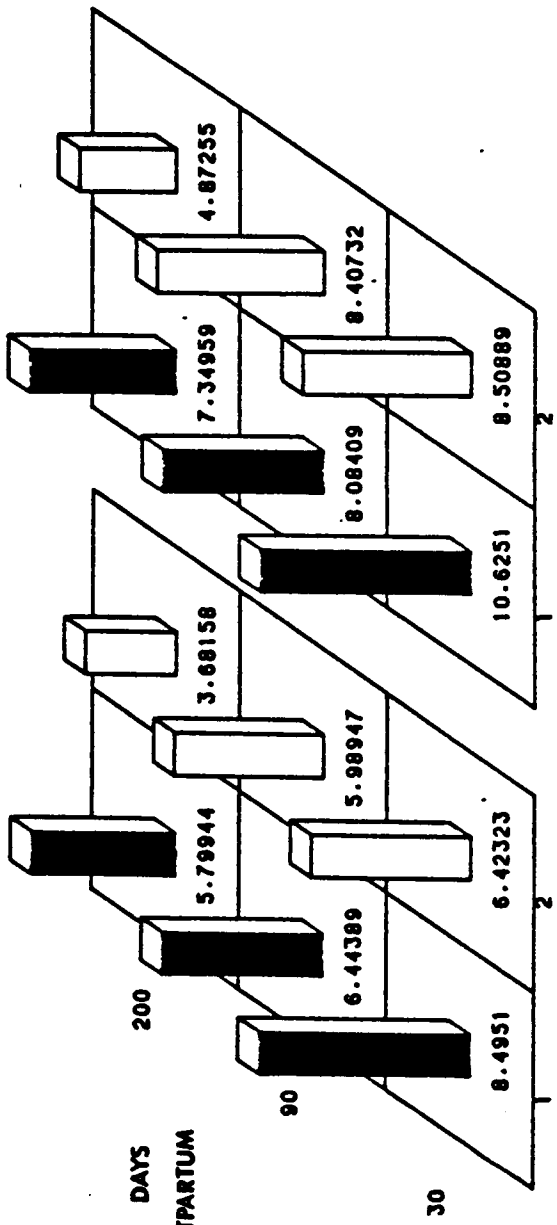

Figure 10: Mean plasma GH concentrations before (basal) and after (response) TRH administration to first lactation cows (Sel 1 = selection group cows; Sel 2 = control group cows)

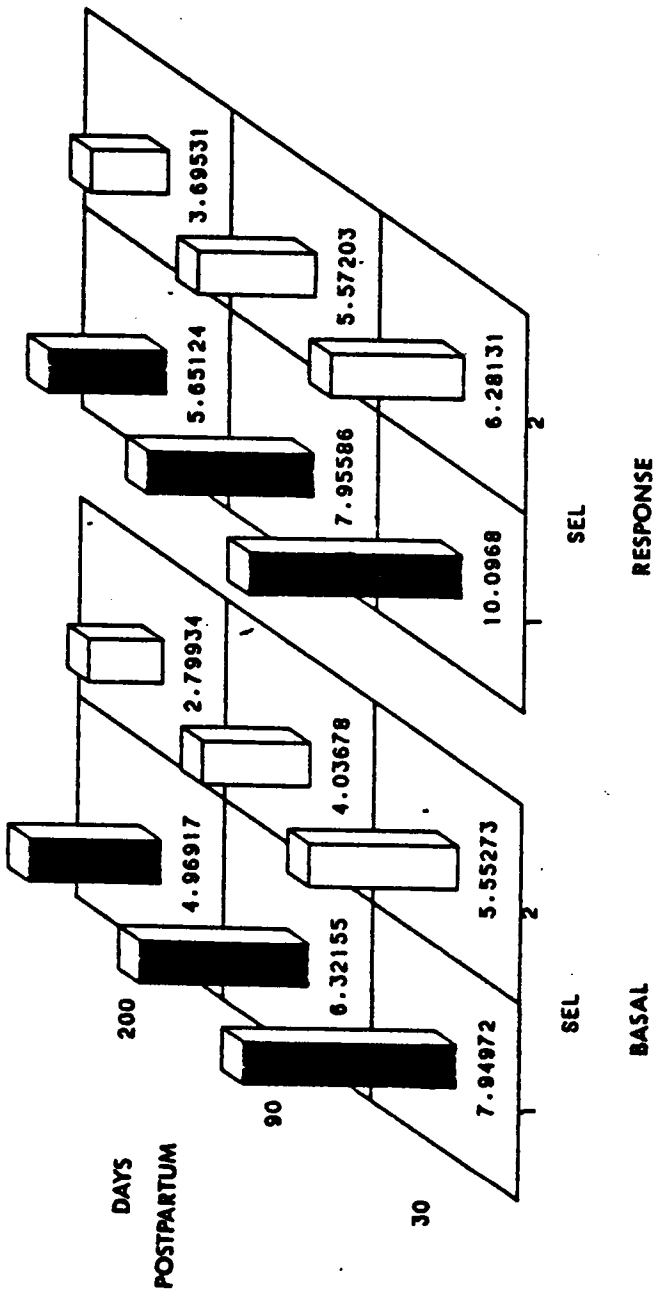

Figure 11: Mean plasma GH concentrations before (basal) and after (response) TRH administration to second lactation cows (Sel 1 = selection group cows; Sel 2 = control group cows)

TABLE 18

Least squares means of GH concentrations in experimental animals

Lactation	Period[b]	Days Postpartum[c]	Genetic Merit[a] Selected	Control
First	Basal	30	8.2±.3[d]	5.2±.4
		90	6.3±.3	4.1±.4
		200	5.9±.3	3.4±.4
	Response	30	10.4±.2	6.1±.3
		90	7.5±.3	5.7±.3
		200	7.6±.3	4.3±.3
Second	Basal	30	8.3±.3	6.7±.5
		90	6.0±.3	6.6±.4
		200	6.0±.4	4.0±.5
	Response	30	10.3±.2	9.2±.4
		90	8.0±.2	9.1±.4
		200	5.9±.2	4.9±.5

[a] Selected cows > control cows ($p<.0001$)

[b] Basal period < response period ($p<.0001$)

[c] Cows at 30 > 90 > 200 days postpartum ($p<.0001$)

[d] Least squares mean ± SE, ng/ml

DISCUSSION

Results indicate that NEB at 30 DPP was negative and became more positive as lactation progressed, was more positive during the second as compared to the first lactation, and was more negative in 3x as compared with 2x cows. Bauman and Currie (1980) reported that dairy cows were in a negative energy state for the first 15 wk of lactation. During this time, even maximal energy intake is insufficient to meet energy required for body maintenance and milk production. Thus, body lipid stores are mobilized in order to meet the energy demand. These authors (Bauman and Currie, 1980) estimated that fully one-third of the energy required for the milk produced was derived from stored adipose tissue. A more positive NEB would be expected in second as compared to first lactation cows, as first lactation cows require more energy to support a more rapid growth rate. Additionally, first lactation cows did not consume as much feed as the second lactation animals, perhaps due to their smaller size and stature. Admittedly, second lactation cows produced more milk than first lactation cows but the energy involved in the increased in milk production was not sufficient to account for the energy present in the extra feed consumed by these cows, resulting in a more positive NEB. The difference in NEB between 2x and 3x cows is more diffi-

cult to explain, because comsumption between the two groups was not different and milk production only tended to be greater in 3x cows ($p<.1$).

Administration of TRH was effective in increasing plasma Prl concentrations, as previously reported by several authors (Convey et al., 1973; Kelly et al., 1973; Vines et al., 1977). Mean plasma prolactin concentrations were not affected by frequency of milking, DPP or genetic merit. However, Prl concentrations were greater in second than in first lactation animals. This phenomenon was not due to different ambient temperatures, as the effects of temperature were removed by its inclusion in the model as a covariate. The physiological reason for such differences is presently obscure. Several studies have demonstrated that Prl increases with feeding while decreasing during fasting (McAtee and Trenkle, 1971; Serjsen et al., 1983; Kazmer et al., 1985), implying a role in regulating growth or metabolism. Thus, the observed differences between first and second lactation animals may be related to the amount of feed consumed.

Administration of TRH was also effective in increasing GH concentrations, agreeing with the previous report of Vines et al. (1977). Because no differences were found between 2x and 3x cows, it does not appear that alterations in

endocrine patterns are associated with increases in milk yield due to increased milking frequency. However, GH concentrations were affected by days postpartum and by genetic merit. Bauman and Currie (1980) suggested that these alterations in GH as lactation progresses may be an example of homeorhesis, ie. orchestrated alterations to meet the needs of a particular physiological state, and thus introduce the concept of partitioning of nutrients. In the lactating dairy cow, available energy is used for maintenance, growth or milk production. The percentage of energy that it shuttled toward any one particular need is ultimately under endocrine control. Growth hormone is lipolytic and anti-lipogenic in cattle (Bauman and Currie, 1983; Hart, 1983). Thus, increased GH during early lactation serves to mobilize energy away from body adipose stores toward the production of copious quantities of milk. As lactation progresses, homeorhetic regulatory mechanisms begin to shift, and, particularily in late lactation, energy is partitioned toward storage as adipose tissue in preparation for the next lactation. Using the same logic, the greater GH concentrations in selected as compared with control cows may be a physiological factor mediating increased milk yield in cattle selected for that trait. The greater availability of GH in selected animals serves to partition more of the available

energy away from adipose deposition and toward milk production. Bines and Hart (1977) reported that GH concentrations were greater in dairy as compared with beef cows, lending credence to the nomination of GH as a physiological mediator of selection pressures. Hart (1983), however, cautioned that differences in GH concentrations among cows of differing genetic merit may be induced by differences in energy balance, due to increased milk production in animals of superior genetic merit. In that study, GH concentrations were not different between cows of differing genetic merit when they were fed to the same weight gain. However, this could only be accomplished by restricting intake in lower producing cows so that their rates of gain were supressed in order to be similar to the gain rate of higher producing cows. The possibility exists, therefore, that the endocrine environment within the two groups was actually dissimilar, the higher producing cows consuming ad libitum, while the lower producing cows were on a resticted diet, and presumably would have consumed more feed, given the opportunity. In any case, in the present study, no differences in NEB between selected and control cows was noted, while NEB was affected by other factors, implying that NEB was not involved in the observed differences in GH concentrations between selected and control cattle.

In summary, NEB was affected by DPP, lactation number and frequency of milking, while Prl concentrations were affected only by lactation number. Growth hormone concentrations were greater in early lactation, and decreased as lactation progressed. Frequency of milking did not affect GH concentrations, implying that alterations in GH are not involved in increased milk yield from animals milked more than twice daily. Additionally, the present study supports the contention that GH may be a physiological mediator of increased milk production in cows of superior genetic merit, as GH concentrations were greater in selected than control cows. Further investigations into endogenous mechanisms involved in regulation of GH secretion in cows of differing genetic merit may provide insights and information which would be useful in increasing the efficiency of milk production in dairy cattle.

Chapter VII

SUMMARY

Several aspects of lactation endocrinology have been examined through these experiments. First, the results of the study concerning lactogenic hormone receptors in bovine mammary tissue indicated that the dissociation constant of bovine mammary lactogenic hormone receptors is not affected by stage of lactation, but that the concentrations of these receptors in mammary tissue is greater during lactation than just prior to parturition. The lactogenic receptor exhibited a dissociation constant of 8.97 $x10^{-8}$M when estimated by NIH-bPRL-6 displacement, and 9.78 $x10^{-10}$M when estimated by purified human growth hormone displacement. Lactogenic receptor concentrations were greater at 60 and 180 days postpartum than during the periparturient period. Studies regarding the regulation of mammary lactogenic hormone receptors may provide insights into mechanisms regulating lactogenesis, lactation and involution.

The series of studies concerning the possible relationship between GH and selection for increased milk production indicates that GH concentrations are greater in animals of increased genetic merit when measured at several ages. Further, GH is greater in early than in later lactation, re-

sembling the lactation curve. However, net energy balance, while negative in early lactation and positive during later lacation, was not affected at any time period by genetic merit. Thus, we suggest that altered GH secretion may indeed be a physiological mediator of selection pressure for increased milk yield in Holstein cattle.

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Appendix A

SCATCHARD ANALYSIS OF DISPLACEMENT DATA

Although the manipulation of displacement data to produce a Scatchard plot may seem quite mysterious at times, it is actually quite a simple affair. Estimates of total counts, total bound, and counts with increasing dosages of unlabelled competitor are used to produce the ratios and values depicted in figure 12. At dose 0, the bound/tracer ration (B/T) is simply the total bound counts (10,000) divided by the total counts (60,000). Multiplying the total tracer mass (2 ng in this case) by the B/T ratio yields the amount bound (.3 ng). Subtracting .3 from 2 yields the amount free. Dividing the amount bound by the amount free yields the B/F ratio. The B/F ratio is used as the y-axis co-ordinate when plotting a Scatchard, while the amount bound is used as the x-axis co-ordinate. As a further example, the calcualtions involving the 1000 ng dose are as follows. Total bound was 4,560, divided by 60,000 yields our B/T ratio of .076. Multipy .076 by 1002 to get the amount bound of 76.2. Subtract 76.2 from 1002 leaves 925.8, and a B/F ratio of .082. The data from the displacement curve described in figure 12 is plotted using the B/F ratio as the y-axis co-ordinate, and the amount bound as the x-axis co-ordinate (figure 13).

After plotting the points, a least squares line is drawn. The slope of this line represents the affinity between ligand and receptor, while the x-intercept represents the amount of hormone bound. If we know the molecular weight, we can then calculate the number of moles of the hormone bound, and from there, if we assume an equimolar relationship between receptor and ligand, we can infer molar concentrations of the receptor.

EXAMPLE:

Total counts: 60000

Total bound: 10000

Tracer mass: 2 ng

with B6 added 100 ng: 9022
500 ng: 6416
1000 ng: 4560
2000 ng: 2403
3000 ng: 1196

Dose	B/T	amt B	amt F	B/F
0	.167	.3	1.7	.176
100	.150	15.3	86.7	.176
500	.107	53.7	448.3	.112
1000	.076	76.2	925.8	.082
2000	.040	80.1	1921.9	.042
3000	.020	59.8	2942.2	.020

Figure 12: Scatchard plots: Manipulating the data

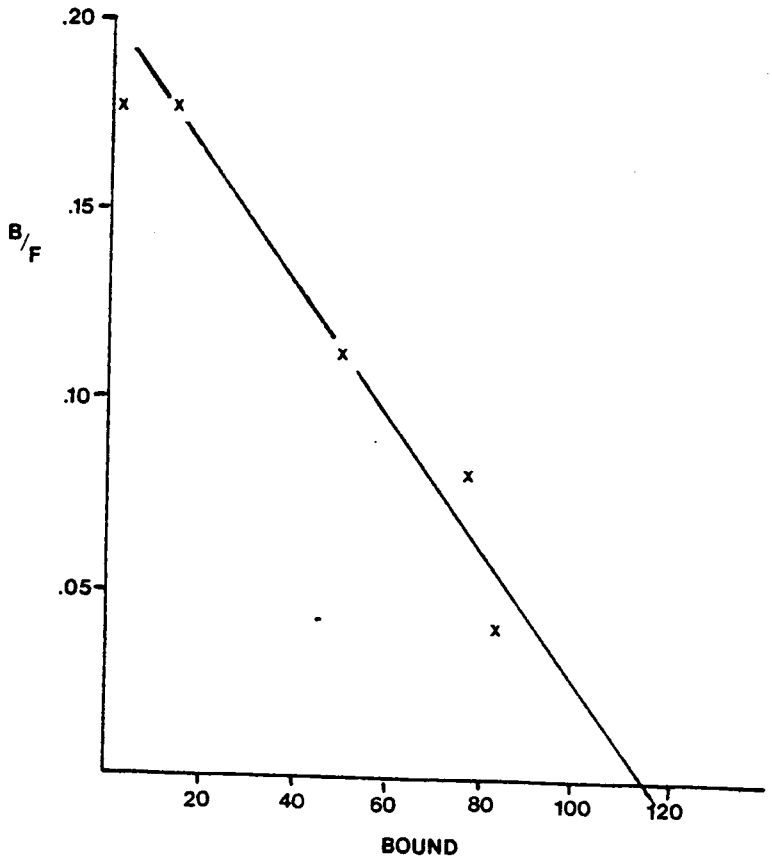

Figure 13: Scatchard plots: plotting the data

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