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Are the Galactopoietic Effects of Growth Hormone-Releasing Factor in Dairy Cattle Mediated Solely Through Somatotropin?

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Geoffrey Eliot Dahl

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ARE THE GALACTOPOIETIC EFFECTS OF GROWTH HORMONE-RELEASING FACTOR IN DAIRY CATTLE MEDIATED SOLELY THROUGH SOMATOTROPIN?

by

Geoffrey Eliot Dahl

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ABSTRACT

ARE THE GALACTOPOIETIC EFFECTS OF GROWTH HORMONE-RELEASING FACTOR IN DAIRY CATTLE MEDIATED SOLELY THROUGH SOMATOTROPIN?

By

Geoffrey Eliot Dahl

Three studies were conducted in lactating Holstein cows to determine if growth hormone-releasing factor (GRF)-induced increases in milk yield and serum concentrations of somatotropin (ST) and insulin-like growth factor-I (IGF-I) could be sustained over a 60-d period, and to compare the effects of GRF and bovine ST (bST) on milk yield and serum concentrations of ST and IGF-I.

In Experiment 1, relative to controls, 1, 3 or 12 mg GRF/d increased milk yield and serum ST and IGF-I in a dose dependent manner for 60 d. Furthermore, response of serum ST to 3 and 12 mg GRF/d was sustained over 60 d, thus there was no evidence of refractoriness to GRF. Following withdrawal of 12 mg GRF/d, milk yield remained elevated for at least 15 d.

In Experiment 2, the galactopoietic effects of GRF (12 mg/d) and bST (14 mg/d) were compared. In previous independent studies these doses of GRF and bST optimally increased milk yield. Relative to controls, bST increased milk yield and serum concentrations of ST and IGF-I. Relative to bST, GRF increased milk yield

and serum concentrations of ST and IGF-I. The pattern of response of milk yield and serum ST and IGF-I support the hypothesis that GRF induced increases in milk yield are mediated by increased serum concentrations of ST and IGF-I.

In Experiment 3, the galactopoietic effects of i.v. infusion of GRF (12 mg/d) and bST (29 mg/d), which elicited similar increases in serum ST, were compared. Relative to controls, GRF and bST increased serum concentrations of ST and IGF-I. Serum concentrations of ST and IGF-I did not differ between GRF- and bST-treated cows. Relative to controls, bST and GRF increased milk yield 28 and 41%. Relative to bST, GRF increased milk yield by 10%, despite similar concentrations of serum ST and IGF-I in both groups.

In conclusion, GRF is galactopoietic and maintains increased secretion of ST in dairy cattle for at least 60 d. GRF-induced increases in milk yield were greater than increases induced by bST. The galactopoietic action of GRF is not mediated solely by elevation of total radioimmunoassayable ST and IGF-I.

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Introduction

During the 20th century, American agriculture experienced revolutions in mechanical, biological and chemical technologies (Cochrane, 1979). As a microcosm of agriculture, the dairy industry has been at the forefront of the technological revolution. Indeed, bulk tanks (mechanical), artificial insemination (biological) and improved sanitation (chemical) provide examples of advancements in technology in the dairy industry. Combined, these and other technologies have facilitated an increase in yearly milk production from 2400 kg/cow/yr in 1950 to over 6440 kg/cow/yr in 1988 (USDA, 1989).

Currently, the dairy industry is on the verge of a fourth revolution involving biotechnology. Already, dairy processors are using recombinantly-derived rennet for the manufacture of cheese (Pfizer Informational Bulletin). However, the product from biotechnology of greatest immediate potential impact to the dairy industry is recombinantly-derived bovine somatotropin (bST). Since 1981, results from a plethora of studies indicate that bST increases milk yield in cattle from 10 to 41%. Thus, bST would markedly enhance the rate of increase in productivity of milk production. Mix (1987) estimated that adoption of bST by the dairy industry would cause the yearly yield of milk per cow to increase to 9280 kg by the 21st century. For the purposes of this dissertation I will refer to endogenous growth hormone as ST and exogenous growth hormone as bST.

An alternative approach to administration of bST to stimulate milk secretion rates in dairy cows would be to regulate endogenous secretion of ST. ST secretion is under the dual control of growth hormone-releasing factor (GRF) and somatostatin (SRIF). GRF increases concentrations of ST in serum and milk yield in a dose dependent manner for up to 20 d in lactating dairy cows (Enright et al., 1988). However, whether various doses of GRF increase ST and milk yield over a long time is unknown. Therefore, the objective of the experiment described in Chapter 1 was to determine the effects of 60-d infusions of various doses of GRF on milk yield and serum hormone and metabolite concentrations in lactating cows.

One characteristic of the response of bST-treated dairy cows is a rapid decline in milk yield following cessation of treatment (Eppard et al., 1985; Peel et al., 1985; Peel et al., 1982). In contrast, GRF-treated cows maintain elevated yield of milk following cessation of treatment (Enright et al., 1988; Lapierre et al., 1988a). The reason for these differences in milk yield in response to GRF and bST are unknown. However, the galactopoietic response to GRF and bST has not been compared in the same study. Therefore, the objective of the experiments described in Chapters 2 and 3 was to compare the response of milk yield, dry matter intake (DMI) and digestibility, and serum concentrations of ST and insulin-like growth factor-I (IGF-I) to bST and GRF in lactating cows.

Review of Literature

The review of literature is divided into three sections. In the first section, I reviewed the literature which pertains to the effects of bST on lactation and mammary gland function. In the second section, I reviewed the literature which pertains to bST-induced alterations in lipid, carbohydrate and protein metabolism that support lactation. In the third section, I reviewed the literature which pertains to GRF, particularly in cattle. Chapter 1 of this dissertation has been published (Dahl et al., 1990) and Chapter 2 has been accepted for publication (Dahl et al., 1991).

Section 1: Effects of bST on Lactation and Mammary Gland Function

In 1937, Asimov and Krouze (1937) reported that extracts of the anterior pituitary gland increased milk yield in cattle. ST was later identified as the active galactopoietic agent in extracts of the anterior pituitary gland (Young, 1947). From 1937 to 1980, pituitary-derived ST increased milk yield in a number of studies (Bines et al., 1980; Machlin, 1973; Brumby and Hancock, 1955; Young, 1947). However, a limited supply of pituitary ST precluded commercial use in dairy cattle.

The advent of recombinant DNA technology allowed bST to be made in E.

coli (Seeburg et al., 1983), thus the supply of bST became unlimited. From 1981 to the present, a plethora of studies indicate that bST increases milk yield from 10 to 41% (reviewed by Peel and Bauman, 1987). Moreover, bST increases milk yield to a greater extent than pituitary ST (Bauman et al., 1985). The form of bST used by Bauman et al. (1985) contained an extra methionine residue at the amino terminus which may increase its stability in vivo. Increased stability of bST relative to pituitary ST may explain the greater galactopoietic response. In general, bST treatment does not affect milk composition (Peel and Bauman, 1987). Thus, bST increases milk yield and milk component yield.

Administration of bST to lactating cows over multiple lactations increased milk yield in a dose dependent manner (8 to 36%) relative to excipient-treated cows (Annexstad et al., 1990; McBride et al., 1990). In addition, bST treatment had no effect on the incidence of ketosis, mastitis or other health-related problems. In one study (Burton et al., 1990) a decrease in reproductive efficiency was observed in cows receiving the highest dose of bST. However, similar decreases in reproductive efficiency are noted in genetically superior, high-yielding cows (Peel and Bauman, 1987). Thus, whether the effects on reproductive performance are due to bST per se or high milk yield is unclear. Nevertheless, the results of these studies indicate that bST is efficacious and safe over multiple lactations in dairy cows.

Administration of bST to lactating cows increases the efficiency of milk production. This occurs because bST treatment reduces the proportion of consumed nutrients used for body maintenance (Peel and Bauman, 1987). However, bST treatment does not affect the partial efficiency of milk synthesis (Peel and Bauman, 1987). Thus, the actual synthesis of milk is not more efficient, rather cows produce more milk for each unit of feed consumed.

While it is well established that bST increases milk yield and productive efficiency, how this is accomplished is less clear. Direct action of bST on the bovine mammary gland during lactation has been largely discounted due to the absence of specific ST receptors in mammary tissue (Keys and Djiane, 1988; Akers, 1985). Recently of two studies reported the expression of ST-receptor mRNA in bovine mammary gland from lactating (Glimm et al., 1990) and pregnant (Hauser et al., 1990) cows. However, receptor protein expression was not found. Theoretically then, ST may act directly at the mammary gland during lactation.

An alternative to direct action of bST at the mammary gland is an indirect mediation of bST action by IGF-I (Gluckman et al., 1987). Secretion of IGF-I has long been known to mediate the effects of ST on skeletal muscle growth (Daughaday, 1982). Indeed, administration of bST to cows increases serum concentrations of IGF-I (Gluckman et al., 1987). Moreover, bST treatment increases IGF-I binding to mammary epithelial cells (Glimm et al., 1988). However, Shamay et al. (1988) reported that IGF-I has no galactopoietic action in vitro. Furthermore, in a comparison of the effects of IGF-I and bST on lactation in goats, IGF-I did not increase milk yield (Davis et al., 1989). Although IGF-I may mediate bST action at the mammary gland, galactopoietic action of bST requires coordination of metabolism in tissues that support milk synthesis such as the liver, muscle and adipose tissue.

Milk yield is a function of mammary cell number and the metabolic activity of each cell (Knight and Wilde, 1987). During early lactation mammary cell numbers increase, but eventually decline as lactation advances (Tucker, 1987). In cows, milk yield parallels the increase and decline in mammary cell numbers such that milk yield increases for 6 to 8 weeks following parturition, attains a peak, then gradually declines as lactation advances. Thus, increased mammary cell numbers during lactation could increase total milk yield. Indeed, the increased yield of milk in goats milked thrice daily versus twice daily is associated with increased mammary cell numbers (Knight and Wilde, 1987). Theoretically, bST could increase milk yield by increasing mammary cell numbers.

It is clear that bST increases mammary growth in heifers. For example, bST treatment increased mammary parenchyma in pre-pubertal (3.5 mo; Sandles et al., 1987) and pubertal heifers (8 mo; Sejrsen et al., 1986). Possibly, IGF-I mediates the effects of bST on mammary growth. Indeed, IGF-I increases ^{3}H-thymidine incorporation into differentiated mammary tissue from heifer calves (Shamay et al., 1988). However, bST treatment of pre-pubertal calves did not affect subsequent milk yield. But, regression of bST-induced growth of the mammary gland could have occurred between treatment at 3.5 mo and parturition at 24 mo (Sandles et al., 1987).

During lactation, the effects of bST on mammary growth are equivocal. For example, bST treatment did not increase total mammary DNA in lactating cows, although total liver DNA did increase with bST treatment (Capuco et al., 1989). In contrast, IGF-I increases ^{3}H-thymidine incorporation into mammary tissue from lactating cows (Baumrucker and Stemberger, 1989). However, I am not aware of any reports of bST or IGF-I-induced increases in mammary cell numbers in vivo.

Concentrations of plasmin, a serine-protease, increase in milk as lactation advances and mammary cell numbers decline (Politis et al., 1989). Bovine ST has been postulated to decrease plasmin production within the mammary gland and thereby maintain mammary cell numbers (Politis et al., 1990). However, if bST-induced increments in milk yield are mediated by retardation of mammary cell number losses, a slow decline in milk yield would be expected following withdrawal of bST. To my knowledge, no such residual elevation of milk yield following cessation of bST treatment has been reported. Increased mammary epithelial cell metabolism could account for the bST-induced increase in milk yield. The enzyme thyroxine (T_4)-5'-monodeiodinase catalyzes the conversion of T_4 to triiodothyronine (Capuco et al., 1989). Triiodothyronine is a more active thyroid hormone relative to T_4, thus increased T_4-5'-monodeiodinase activity is an index of increased cellular metabolism. Recently, Capuco et al. (1989) reported that bST increased T_4-5'-monodeiodinase activity in mammary tissue of cows. Furthermore, bST treatment did not change T_4-5'-monodeiodinase activity in liver or kidney. These results suggest a specific action of GH to increase mammary cell metabolism. However, no cause-effect relationship between milk yield and cellular metabolism could be determined, because increased cellular metabolism could be an effect of increased milk yield rather than the reverse.

Section 2: Alterations of Lipid, Protein and Carbohydrate Metabolism in bST-treated Cows

Effects of bST on Lipid Metabolism

Early in lactation, high producing dairy cows do not consume energy at a rate sufficient to meet the energy demand of milk production (NRC, 1989; Bauman and Currie, 1980). Thus, cows enter a phase of negative energy balance, characterized by loss of body weight (BW), elevated concentrations of non-esterified fatty acids (NEFA) in blood, slightly increased basal lipolysis and markedly increased norepinephrine-stimulated lipolysis (Bauman and Currie, 1980). As lactation advances milk yield declines and DMI is maintained or increased. After these two forces converge, cows enter positive energy balance. Thus, in the latter stages of lactation cows gain weight (i.e., adipose tissue) to provide energy reserves for a subsequent lactation.

A change in energy balance emerges in cows treated with bST similar to that of early lactation. Thus, Bauman et al. (1985) noted an initial dose- dependent loss of BW and decreased energy balance in cows treated with bST. However, as duration of bST treatment progressed, DMI and energy balance increasd. Within adipose tissue of lactating cows, treatment with bST increases lipolysis and decreases lipogenesis (Peel and Bauman, 1987). These shifts in lipid metabolism are associated with increased concentrations of NEFA in serum (Peel and Bauman, 1987). Although bST alters lipid metabolism in lactating cows, the specific enzymes involved have not been identified.

Elevation of blood concentrations of NEFA occurs when bST-treated cows are in negative energy balance (Sechen et al., 1990; Sechen et al., 1989; Solderholm et al., 1988; Peel and Bauman, 1987). In addition to elevations in NEFA in bST-treated cows, Bauman et al. (1988) reported increased (74%) irreversible loss of NEFA as well as a doubling of NEFA oxidation to CO_2. Consistent with these data, McDowell et al. (1987) reported that mammary gland uptake of NEFA increases with bST treatment. Moreover, the increased uptake of NEFA was in excess of fatty acid requirements for increased milk fat synthesis, leading to speculation that the increased oxidation of NEFA might spare glucose metabolism at the mammary gland (Bauman et al., 1988; McDowell et al. 1987).

The mechanism of mobilization of lipid reserves in cows treated with bST is not fully understood. In vitro, Etherton et al. (1987) reported that chronic administration of bST antagonized insulin action in bovine adipose tissue. Sechen et al. (1989) observed that the responsiveness of bST-treated cows to insulin, glucose and epinephrine was altered. For example, bST reduced removal of glucose from plasma following an insulin challenge. Also, bST increased plasma concentrations of NEFA in response to epinephrine (Sechen et al., 1989). These alterations of lipid metabolism were manifested as increased concentrations of NEFA in blood. Recently, Sechen et al. (1990) observed that bST increased the maximal response of NEFA and glycerol to epinephrine, whereas the sensitivity (i.e., half-maximal responsive dose) was unchanged. If the number of epinephrine receptors in adipose tissue was increased by bST, a decrease in the half-maximal responsive dose would be expected. However, if bST alters a post-receptor event in epinephrine-stimulated lipolysis, the half-maximal responsive dose would be unchanged. Thus, the evidence suggests that bST exerts its action on post-epinephrine receptor events. In addition, the NEFA response to epinephrine of cows treated with bST is independent of energy balance (Sechen et al., 1990). Based on this evidence, Sechen et al. (1990) proposed that hormone sensitive lipase (HSL) may be the lipolytic enzyme affected by bST, because HSL activity and ST are elevated in early lactation as compared with activities during pregnancy and HSL activity is unaffected by energy balance. Thus, HSL is a possible site for bST control of lipolysis in adipose tissue. To the best of my knowledge, which lipogenic enzyme(s) bST affects in lactating cows is unknown. Nonetheless, it is apparent that bST treatment alters lipid metabolism, partitioning nutrients from energy reserves toward the mammary gland.

Effects of bST on Carbohydrate Metabolism

The principle carbohydrate of milk, lactose, is the primary osmotic regulator of milk production (Linzell and Peaker, 1971). Thus, any increase in lactose yield is associated with an increase in milk yield. Generally, there is no effect of bST treatment on concentrations of glucose or insulin in blood of lactating cows (Bauman et al., 1988; Peel and Bauman, 1987). However, an increase in glucose anabolism or a decrease in glucose catabolism must occur to provide increased glucose precursor for increased lactose synthesis. In bST-treated cows, a combination of increased synthesis of glucose and decreased oxidation of glucose provides the increased glucose precursor necessary for increased lactose synthesis at the mammary gland.

In ruminants, propionate is the major precursor for gluconeogenesis which occurs primarily in the liver. Thus, increased propionate supply or rate of hepatic gluconeogenesis increases blood glucose and supports increased milk production. Indeed, Pocius and Herbein (1986) reported that bST-treatment increases the rate of conversion of propionate to glucose in the liver. However, rates of propionate conversion to CO_2 were similarly elevated. In the absence of a shift of propionate flux toward glucose at the expense of CO_2, no net increase in glucose production can occur without an increase in propionate supply to the liver. But, an increase in propionate supply from the rumen to the liver is unlikely because bST does not affect DMI (at least early in treatment) or preabsorptive efficiency of nutrient uptake (Bauman et al., 1988). Demand for amino acids for milk protein synthesis increases in response to bST. Thus, amino acids are an unlikely source of increased precursor for hepatic gluconeogenesis, (Peel and Bauman, 1987). However, glycerol, derived from adipose tissue following degradation of triglycerides is the most likely source of the increased precursor for glucose production in response to bST. Indeed, Bauman et al. (1988) estimated that glycerol accounts for up to 27% of the increased glucose demand with bST treatment. Thus, increased production of glucose partially offsets increased demand for glucose in cows treated with bST.

In lactating cows, the major proportion of glucose is used for lactose synthesis and for oxidation to CO_2 (Bauman et al., 1988). Thus, a shift from oxidation of glucose to CO_2 toward lactose synthesis might be expected with bST treatment. McDowell et al. (1987) provided indirect evidence of such a shift in bST-treated lactating cows, where bST reduced muscle glucose uptake (i.e., glucose oxidation to CO_2), but did not increase mammary glucose uptake (i.e., glucose to lactose synthesis). Recently, Bauman et al. (1988) provided direct evidence that bST treatment decreases overall glucose oxidation to CO_2 in lactating cows. Therefore, it appears that bST shifted glucose metabolism from muscle tissue toward the mammary gland. This shift is concurrent with increased hepatic gluconeogenesis from glycerol to meet the increased demand for glucose as a precursor for lactose in the mammary gland.

Effects of bST on Protein Metabolism

Treatment with bST does not affect percentage of protein in milk of cows in positive nitrogen balance; however, concentrations of protein decrease in milk of bST-treated cows in negative protein balance (Tyrrell et al., 1988; Peel and Bauman, 1987). Nevertheless, a bST-induced increase in milk production dictates increased milk protein secretion. Therefore, bST treatment increases demand for amino acids in the mammary gland. IGF-I is the putative mediator of bST action at the mammary gland (Gluckman et al., 1987). Thus, a possible mechanism of bST action on amino acid metabolism would be an indirect effect at the mammary gland, mediated through IGF-I. Consistent with this hypothesis is the observation that uptake of essential amino acids at the mammary gland is stimulated by bST (Fullerton et al., 1989). However, this response was not sustained throughout bST treatment. There have been no reports of IGF-I stimulation of amino acid uptake at the mammary gland, but IGF-I directly affects muscle cell protein metabolism. For example, IGF-I stimulated differentiation and proliferation of bovine satellite cells in vitro (Greene and Allen, 1989).

Absorption of nitrogen from feed is unaffected by bST treatment in lactating cows (Tyrrell et al., 1988; Peel et al., 1982). Thus, in a fashion similar to carbohydrate and lipid metabolism, bST affects postabsorptive aspects of nitrogen metabolism. To the best of my knowledge, the effects of bST treatment on amino acid metabolism in lactating cows are unknown. However, Eisemann et al. (1989) reported that bST treatment decreases overall leucine oxidation to CO_2 in growing steers. Also, bST treatment increased body protein, while plasma concentrations of essential amino acids and protein degradation (i.e., collagen and myofibrillar) were unchanged (Eisemann et al., 1989). Thus, bST alters protein metabolism in growing steers to increase nitrogen retention. In lactating cows, bST treatment does not affect the total amount of lean tissue (Brown et al., 1989; Soderholm et al., 1988). Therefore, bST does not alter nitrogen retention in lactating cows. However, bST-treatment increases amino acid demand for synthesis of milk protein in lactating cows. Thus, decreased overall amino acid oxidation could accomodate the increased demand for amino acids in the mammary gland of bST-treated cows.

Section 3: Effects of GRF in Cattle

Discovery and Characterization of GRF

Deuben and Meites (1964) first reported that hypothalamic extracts stimulated ST secretion from cultured rat anterior pituitary glands. However, the structure of the active compound in the extracts (GRF) eluded researchers until 1982, when two groups simultaneously isolated peptides from human pancreatic tumors (hpGRF; Guillemin et al., 1982; Rivier et al., 1982) that increase ST secretion. Subsequently, hpGRF was shown to be identical to human hypothalamic GRF (hGRF; Ling et al., 1984), a 1-40-NH_2 polypeptide. Bovine GRF (bGRF) was first characterized by Esch et al. (1983). The structure of bGRF differs by five amino acid residues from that of hGRF, although only one replacement occurs in the biologically active 1-29-NH_2 fragment (Esch et al., 1983). Recently, bGRF has been expressed in E. coli (Kirschner et al., 1989), and this 1-45 (Leu^{27}, Hse^{45}) bGRF was used in the experiments described in Chapters 1, 2 and 3.

Effects of GRF on ST Secretion

Secretion of ST is episodic in mammals, and cattle are no exception (Millard, 1989; Gluckman et al., 1987). In rats, the pulsatility of secretion of ST is putatively a result of intermittent and asynchronous release of GRF and SRIF, with GRF responsible for pulses and SRIF responsible for troughs in ST secretion (Tannenbaum et al., 1990). Similarly, GRF and SRIF have a reciprocal effect on secretion of ST from bovine anterior pituitary cells (Tanner et al., 1990; Padmanabhan et al., 1987; Glenn, 1986). In vivo, hypophysial stalk transection (HSTX) abolishes episodic secretion of ST in calves (Plouzek et al., 1988). However, a GRF challenge increases ST secretion in HSTX calves (Plouzek et al., 1988). Furthermore, passive immunization against GRF decreases serum concentrations of ST in steers, and GRF-immunization reduces BW gain in steers (Trout and Schanbacher, 1990). Results to passive immunization against SRIF are more variable. For example, Vicini et al. (1988) found that SRIF-immunization increases serum concentrations of ST and average daily gain in dairy heifers. In contrast, Trout and Schanbacher (1990) found no effect of SRIF-immunization on serum concentrations of ST or IGF-I, or any variable associated with growth. However, negative results to passive immunization should be interpreted with caution because the epitopic site on the hormone molecule is not always an active site. Thus, binding of an antibody to a hormone does not guarantee interference between the antibody, hormone and receptor in vivo. Nevertheless, the bulk of the evidence supports the hypothesis that ST secretion in cattle is characterized by the reciprocal secretion of GRF and SRIF.

Mechanism of GRF Action at the Somatotrope

An exocytotic event is associated with ST release from somatotropes. GRF stimulates and SRIF inhibits this exocytotic event (Draznin et al., 1988). In cultured rat somatotropes, GRF acts at a specific receptor coupled to a stimulatory G-protein subunit (G_s), which activates adenylate cyclase (AC; Narayanan et al., 1989). AC catalyzes the conversion of ATP to the intracellular second messenger, cyclic AMP (cAMP). Elevated levels of cAMP within somatotropes elicit increased intracellular concentrations of Ca^{++}, which is associated with ST release from the somatotrope (Ohlsson and Lindstrom, 1990). The evidence for this mechanism is: (1) cAMP agonists (e.g., 8-br-cAMP) and phosphodiesterase inhibitors (e.g., IBMX) increase intracellular concentrations of cAMP ($[cAMP]_i$) and Ca^{++} ($[Ca^{++}]_i$) and stimulate secretion of ST from rat somatotropes with kinetics identical to those of GRF; (2) cholera toxin (CT) a G_s activator and forskolin (FK) an AC activator increase $[cAMP]_i$ and $[Ca^{++}]_i$ and stimulate secretion of GH; furthermore, multiple doses of FK and CT are not additive with the maximal dose of GRF; and (3) Ca^{++} channel blockers (e.g., diltiazem, nifedipine) inhibit secretion of ST (Lussier et al., 1988). Recently, Tanner et al. (1990) reported that this mechanism for GRF stimulation of ST release exists in bovine somatotropes.

In contrast to CT, pertussis toxin (PT) activates the inhibitory G-protein subunit (G_i; Cronin et al., 1984). PT binds to G_i and inhibits the G_i interaction with G_s. Thus, PT potentiates AC activity through inhibition of G_i. SRIF activates G_i. Inhibition of secretion of ST by SRIF is attenuated by PT (Cronin et al., 1984). Thus, GRF activates G_s to stimulate secretion of ST whereas SRIF activates G_i to inhibit secretion of ST. As with GRF, the SRIF mechanism was recently confirmed in the bovine somatotrope (Tanner et al., 1990).

GRF Regulation of ST Gene Expression

Stimulation of somatotropes of cattle (Tanner et al., 1990; Silverman et al., 1988) and rats (Barinaga et al., 1985) with GRF not only increases ST secretion, but also increases ST gene transcription and GH mRNA synthesis. GRF-induced accumulation of cAMP is associated with stimulation of type I and II cAMP-dependent protein kinases in rat anterior pituitary cells (Bilezikjian et al., 1987). Bilezikjian et al. (1987) speculate that the two cAMP-dependent protein kinases each mediate specific effects (i.e., one increases ST secretion, while the other increases ST gene transcription). Indeed, the kinetics of activation of the two protein kinases are dissimilar which suggests that each enzyme is active in separate intracellular pathways. In addition, Copp and Samuels (1989) recently identified a cAMP-responsive region within the rat ST gene. Thus, elevated levels of cAMP mediate GRF-induced transcription of the ST gene as well as secretion of ST.

Effects of GRF on Secretion of ST in Cattle

Administration of GRF and GRF analogs elicits a rapid increase in serum concentrations of ST in fetal calves (Coxam et al., 1988), prepubertal bulls (Enright et al., 1987) and heifers (Scarborough et al., 1988), steers (Moseley et al., 1984) and lactating cows (Enright et al., 1988; Lapierre et al., 1988). Furthermore, the response to GRF is dose dependent. In cattle, 20 d administration of GRF does not affect secretion of other anterior pituitary hormones (Enright et al., 1989). Thus, GRF specifically increases serum concentrations of ST in cattle.

As previously mentioned, secretion of ST is controlled by the interaction of GRF and SRIF. However, ST can inhibit GRF action through a negative feedback mechanism, suggesting that GRF-induced increases in serum concentrations of ST would diminish with time. Indeed, 5 months of daily injections of ST diminishes the response of ST secretion to GRF in heifers (Grings et al., 1988). In contrast, lactating cows exhibited no evidence of a diminished response of ST secretion to GRF after 57 days of treatment with GRF (Lapierre et al., 1988b). These apparent differences can be reconciled with consideration of the mechanisms of the two hormones. Negative feedback induced by exogenous ST diminishes GRF secretion and increases SRIF secretion, thereby down-regulating somatotrope function. On the other hand, GRF treatment would increase ST gene expression and secretion of ST (Tanner et al., 1990) thereby up-regulating somatotrope function. This hypothesis is further supported in that the diminished response of bST-treated heifers to GRF is transient because GRF-induced secretion of ST returned within 5 d of cessation of bST treatment (Grings et al., 1988).

In addition to GRF, thyrotropin-releasing hormone (TRH) is a ST secretagogue in cattle (Bourne et al., 1977). Indeed, combined exogenous GRF and TRH are synergistic to secretion of ST (Lapierre et al., 1987). Furthermore, GRF and TRH have additive galactopoietic effects (Lapierre et al., 1990a). However, the synergy of GRF and TRH on secretion of ST in calves is only present during the lighted portion of the photoperiod (Lapierre et al., 1990a). Nonetheless, it is interesting to speculate that this synergy on ST secretion may be involved in the stimulatory effects of photoperiod on lactation (Peters et al., 1978). Possibly, cows on a long-day photoperiod (e.g., 16 h of light:8 h of dark) might have higher amplitude pulses of ST which, in turn, would stimulate increased milk production. However, in numerous studies of photoperiod and lactation, photoperiod did not effect secretion of ST (Tucker, 1985; Peters et al., 1981). Thus, the relationship between GRF, TRH, photoperiod and lactation is complex and presently unclear.

Effects of GRF on Lactation

Purification of GRF immediately stimulated interest in research of its potential as a galactopoietic agent in cattle. However, early attempts to increase serum concentrations of ST (and in turn milk yield) in lactating cows were unfavorable in comparison with bST (McCutcheon et al., 1984). In contrast, Hart et al. (1985) observed that GRF increased milk yield in sheep 27% which is comparable to the milk yield response obtained with ST treatment. A summary of studies on the effects of GRF in lactating cows is in Table 1.

In comparison with ST, the effects of GRF on lactation in dairy cattle are strikingly similar. Indeed, exogenous GRF increased concentrations of ST and IGF-I in serum (Hodate et al., 1990; Lapierre et al., 1990b; Enright et al., 1989). Furthermore, administration of GRF increased milk production in a dose dependent manner for 10 to 20 d (Lapierre et al., 1990b; Enright et al., 1988). The evidence strongly supports the hypothesis that GRF is galactopoietic in cattle. Moreover,

Table 1. Summary of effects of growth hormone-releasing factor on lactation in cattle.

Reference	No. of cows per trt.[1]	Stage of lactation	Type of GRF	Dose of GRF/ admin.	Frequency of admin.	Route of admin.	Duration of trt.	Period of trt. analyzed	Increase in milk yield	Comments
Enright et al., 1986	16	178 d	hGRF 1-44-NH$_2$	.2 μg/kg[5]	6/d	iv	10 d	d 8-10	11.0%	
Pelletier et al., 1987	36	158 d	hGRF 1-44-NH$_2$ hGRF 1-29-NH$_2$	.2 nmol/kg	6/d	iv	10 d	d 6-10	16.7% 13.2%	No difference in milk yield response to GRF analog versus hGRF
Enright et al., 1988	5	114 d	bGRF 1-44-NH$_2$	1 mg/d 3 mg/d	continuous	iv	20 d	d 16-20	11.0% 23.0%	Residual effect at 3 mg/d dose.
Lapierre et al., 1988a	6	209 d	hGRF 1-29-NH$_2$	16 μg/kg	1/d	sc	10 d	d 6-10	14.3%	Residual effect of elevated milk yield following cessation of treatment.
Lapierre et al., 1988c	9	252 d	hGRF 1-29-NH$_2$	10 μg/kg	1/d	sc	57 d	d 1-57	13.7%	DMI not affected. GRF increased BW.
Lapierre et al., 1990a	8	196 d	hGRF 1-29-NH$_2$	10 μg/kg	1/d	sc	10 d	d 6-10	10.1%	GRF with TRH gave additive increase in milk yield.
Lapierre et al., 1990b	8	191 d	hGRF (1-29) NH$_2$ GRF-A	10 μg/kg 0.6 μg/kg 1.8 μg/kg	1/d	sc	10 d	d 6-10	9.6% 11.7% 17.6%	GRF analog 15X the potency of hGRF.
Hodate et al., 1990	4	182 d 185 d	DAhGRF 1-29-NH$_2$ A15 DAhGRF 1-29-NH$_2$	2 mg/d	1/d	sc	14 d	d 1-14	10.0% 15.4%	

[1] trt. = treatment [2] h = human [3] b = bovine [4] admin. = administration [5] kg = kg BW

results of these studies suggest that the galactopoietic action of GRF is mediated by increased secretion of ST and IGF-I. However, at the time of initiation of the experiments contained in this dissertation, several questions concerning the galactopoietic effect of GRF were unresolved. Specifically:

1. Was the long term response of ST and milk yield to GRF dose dependent?
2. Were the galactopoietic effects of GRF mediated solely through increases in serum concentrations of ST?
3. Were differences (if present) in galactopoietic response to GRF versus bST associated with differences in indices of energy metabolism such as DMI, DM digestibility, energy balance and/or serum concentrations of NEFA?

These questions were the basis for the experiments described in Chapters 1, 2 and 3.

CHAPTER 1

The Effects of Sixty Days of Infusion of rbGRF on Milk Production in Dairy Cows

INTRODUCTION

Administration of GRF to cattle for either 10 and 20 d increased milk production 3 (Lapierre et al., 1988a) and 6.2 kg (Enright et al., 1988), respectively. However, GRF-induced increases in yield of milk throughout the 20-d period did not plateau, and the maximal response was not established (Enright et al., 1988). Thus, one objective of the present study was to infuse GRF for 60 d to more precisely describe the pattern of the milk yield response.

Galactopoietic effects of GRF have been associated with concomitant increases in serum concentrations of ST (Enright et al., 1988; Lapierre et al., 1988b). Indeed, our previous selection of doses of GRF to test for galactopoietic activity was based on the ability of i.v. infusions of GRF to increase serum concentrations of ST over 24 h (Enright et al., 1988). When infused for 24 h, doses of GRF between 3.25 and 50 mg increased ST similarly; thus, 3 mg was chosen previously as the high dose to test the galactopoietic effects of GRF over a 20-d period (Enright et al., 1988). In the present study, we chose to evaluate the direct galactopoietic effects of a daily dose of 1, 3, and 12 mg of GRF infused over a 60-d period. A second objective was to determine if the 1, 3, and 12 mg doses of GRF similarly affected serum concentrations of ST.

Effects of chronic (60 d) infusion of exogenous GRF on other hormones and metabolites in blood are unknown. Therefore, the third objective was to determine blood concentrations of IGF-I, insulin (INS), prolactin (PRL), triiodothyronine (T_3), thyroxine (T_4), glucose and NEFA in response to a 60-d infusion of 1, 3 and 12 mg of GRF.

MATERIALS AND METHODS

Design and Management

Fourteen primiparous and 10 multiparous Holstein cows averaging 191 d (SD = 80.7 d) of lactation were used in a randomized, complete block design with repeated measurement. Twenty one days before treatment began, cows were fitted surgically with Dermaport infusion catheters (Thermedics, Woburn, MA). A path approximately 6 cm wide from the top of the shoulder to a jugular vein was anesthesized by s.c. injections of lidocaine (Vedco, St. Josephs, MO). Subsequently, a 3 to 5 cm incision was made at the top of the shoulder and over the jugular vein. Catheters were inserted aseptically at the shoulder, routed s.c. and inserted into a jugular vein.

Six blocks of four cows each were formed based on pre-infusion milk yield between -11 and -7 d. Within each block, cows were assigned randomly to treatment (six cows/treatment). Treatments were 0 (placebo, sterile water) or 1, 3, or 12 mg/d of recombinant bovine GRF (1-45) homoserine lactone (rbGRF; Kirschner et al., 1989). Cows received pulses of placebo or rbGRF every 3.75 min from AS-2BH

Autosyringe infuAutosyringe, Inc., Hooksett, NH), a procedure that results in serum c of ST that are indistinguishable from those obtained with continuous RF (Moseley et al., 1987). Doses of rbGRF were prepared daily in n-free water. Infusion volume was 12.8 ml/d for all doses of rbGRFtheters were coated initially with 1% bovine serum albumin dissolve er. A .22-μm pore, Millex-GV filter (Millipore Corp., Bedford, MA) ween the syringe containing rbGRF and the infusion catheter. Infusiere encased in plastic coiled hoses (Re-koil nylon air hose, Milton InChicago, IL) as described by Enright et al. (1988). Infusions were i0 h at 0 d. Cows were housed in tie stalls, exposed to 24 h of light per:d at 0600 and 1700 h. Milk production was recorded daily, and milk for 5 consecutive days every 14 d for composition analysis beginni5 to -1 pre-infusion period. Fat, protein, and lactose in milk were man infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA). Yield of solids corrected milk (SCM) and output of energy in miere calculated (Tyrrell and Reid, 1965).

A total TMR) was fed ad lib. The TMR was formulated (Appendix A, ovide adequate nutrition for a cow (612 kg BW) producing 38.6 ntaining 3.5% fat and assuming an intake of 23.9 kg dry matter (DNC, 1989). Feed was offered daily at 0300 and 1200 h. Orts were recily. Data for feed offered were lost for 14 cows; therefore, feed ion was not available for all cows. Samples of TMR and orts were c4 d and chemically analyzed for DM, CP, ADF, crude fiber, Ca, and P (Midwest Feed Test, Farwell, MI). Cows were weighed for 3 consecutive days every 14 d. Two experienced examiners scored body condition (BCS) 1 to 5 (Wildman et al., 1982) at -11, 27, and 56 d. Scorers were unaware of treatment assigned to individual cows.

Blood Collection and Analysis

Blood was collected from an indwelling jugular catheter at 20-min intervals for 8 h (0900 to 1700 h) at 1, 30, 59, 60 and 65 d. Catheters were inserted into the jugular contralateral to the rbGRF-infused vein on the day before sampling. Blood samples were stored at room temperature for 2 to 6 h then at 4 °C for approximately 15 h. Blood samples for collection of plasma were treated with NaF-EDTA and placed on ice immediately after collection. Plasma or serum was harvested by centrifugation for 30 min at 1550 x g and frozen at -20 °C until assayed for ST (Moseley et al., 1982), INS (Villa-Godoy et al., 1990), PRL (Koprowski and Tucker, 1971), T_3 (Refsal et al., 1984), T_4 (Gerloff et al., 1986), and IGF-I (Dahl et al., 1990). Three plasma samples collected at 0900, 1300, and 1700 h on each day of sampling were analyzed for glucose (Sigma kit No. 305 [Trinder], Sigma Chemical Co., St. Louis, MO) and NEFA (NEFA-C kit, Wako Chemicals USA, Dallas, TX; as modified by McCutcheon and Bauman, 1986b).

Statistical Analysis

The experiment comprised 10 periods: one pre-infusion period (-10 to -1 d), six infusion periods (0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, and 50 to 59 d), and three post-infusion periods (60 to 64, 65 to 69, and 70 to 74 d). Characteristics of ST in serum (which included mean, baseline, pulse frequency, pulse amplitude, and pulse duration) during each 8-h sampling period were determined using a pulse analysis program (PULSAR; Merriam and Wachter, 1982).

All data were subjected to split block ANOVA with repeated measurement (Gill, 1986). Mean comparisons within periods were tested using Dunnett's t test (Gill, 1978).

RESULTS

Pre-infusion milk yields (-10 to -1 d; Figure 1) were not different among treatment groups (avg = 25.1 ± .7 kg/d). During infusion of 3 and 12 mg of rbGRF, milk production increased to a mean of 28.8 ($P<.05$) and 33.3 ($P<.01$) kg/d, relative to placebo (22.8; SE of difference = 1.6 kg/d). At 1 mg rbGRF, milk averaged 27.5 kg/d during infusion but was greater than placebo ($P<.10$) only through 39 d.

During the first 5 d following withdrawal of rbGRF, milk production of cows previously given 1 and 3 mg of rbGRF was not different from production of cows previously infused with placebo. In contrast, after infusion of 12 mg rbGRF ended, milk yield remained above controls at 60 to 64 ($P<.01$), 65 to 69 ($P<.05$) and 70 to 74 d ($P<.10$). Interpretation of effect of treatments on yield of milk was not altered by correction for solids.

Percentages of protein, lactose, fat, and solids in milk were similar among treatments throughout the study with averages of 3.40, 4.96, 3.67, and 12.73%, respectively. Yields of milk components were not different among groups during the pre-infusion period (Table 2). At 1 mg rbGRF, yield of protein, lactose, fat, and

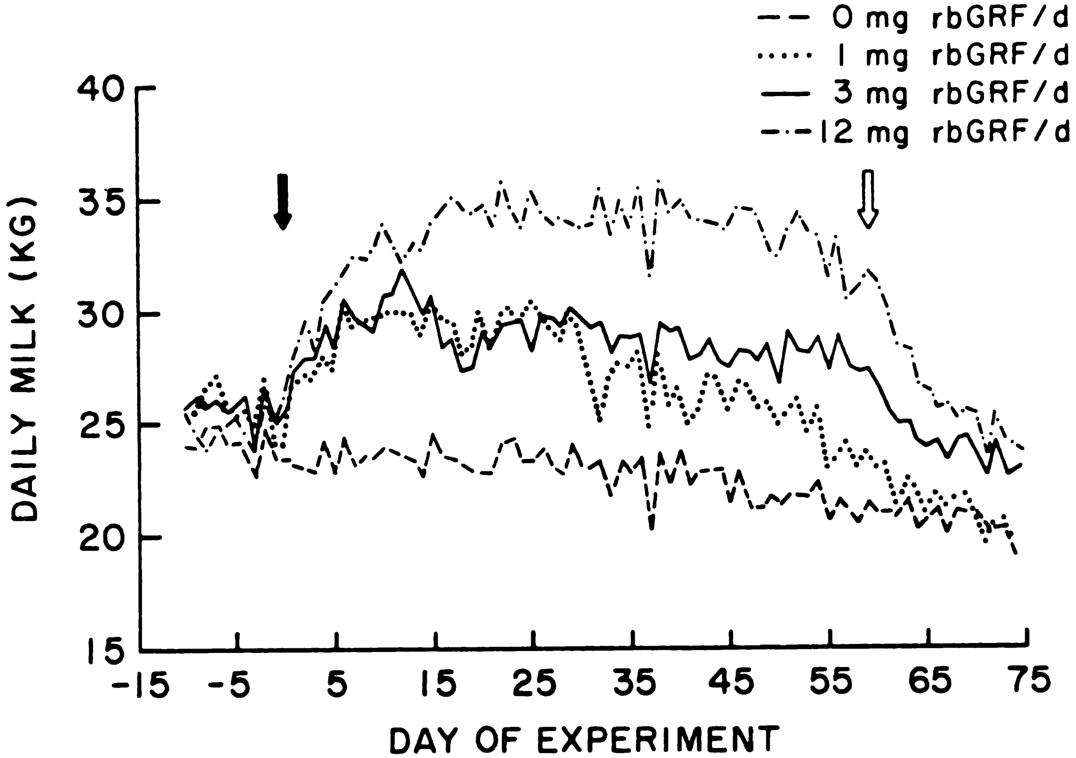

Figure 1. Daily milk yield of cows (six/treatment) continuously infused for 60 d with 0, 1, 3, and 12 mg rbGRF/d. Beginning and end of rbGRF infusion indicated by the solid and open arrows, respectively. The SE of the difference among treatments was 1.6 kg/d.

TABLE 2. Yield of milk (kg/d) components from cows treated with 0, 1, 3, or 12 mg rbGRF/d for 60 d.

	Experimental period	Dose of rbGRF (mg/d)				SE of difference
Variable	(d)	0	1	3	12	
Protein, kg/d	-10 to -1	.79	.89	.89	.85	.06
	0 to 14	.80	1.01[b]	1.01[b]	1.10[a]	
	15 to 29	.81	1.02[b]	.99[c]	1.15[a]	
	30 to 44	.77	.90	.97[b]	1.15[a]	
	45 to 59	.71	.80	.89[c]	1.10[a]	
	60 to 74	.74	.73	.83	.86	
Lactose, kg/d	-10 to -1	1.14	1.32	1.26	1.29	.09
	0 to 14	1.12	1.49[b]	1.42[c]	1.70[a]	
	15 to 29	1.15	1.52[b]	1.42	1.83[a]	
	30 to 44	1.12	1.35	1.44[c]	1.85[a]	
	45 to 59	1.02	1.19	1.34[c]	1.60[a]	
	60 to 74	1.03	1.08	1.18	1.34[c]	
Fat, kg/d	-10 to -1	.82	.95	.96	.86	.08
	0 to 14	.89	1.18[b]	1.16[c]	1.32[a]	
	15 to 29	.87	1.14[c]	1.10	1.30[a]	
	30 to 44	.81	.94	1.02	1.17[a]	
	45 to 59	.78	.89	.99	1.12[a]	
	60 to 74	.76	.79	.82	.82	
Solids, kg/d	-10 to -1	2.92	3.35	3.29	3.18	.23
	0 to 14	2.97	3.90[b]	3.79[b]	4.35[a]	
	15 to 29	2.99	3.89[b]	3.72[c]	4.52[a]	
	30 to 44	2.86	3.36	3.63[c]	4.41[a]	
	45 to 59	2.66	3.04	3.42[c]	3.94[a]	
	60 to 74	2.67	2.75	2.99	3.19	

[a,b,c] Treatments differ from placebo within a period (Dunnett's t test).

[a] P<.01.

[b] P<.05.

[c] P<.10.

solids increased above that of placebo from 0 to 29 d. Generally, 3 mg of rbGRF increased protein, lactose, and solids yield throughout infusion, but fat yield only increased from 0 to 14 d. At 12 mg rbGRF, protein, lactose, fat, and solids yields increased above controls from 0 to 59 d. After infusion ended, there was no difference in yields of milk components between 0 and 12 mg rbGRF, except for lactose which remained elevated.

During infusion, 3 ($P<.1$) and 12 mg ($P<.01$) of rbGRF increased energy output in milk, but 1 mg of rbGRF increased ($P<.05$) energy output in milk only through 29 d (data not shown). Energetic efficiency of milk production, or energetic efficiency adjusted for BW change were not affected by rbGRF treatment (data not shown). Initial BW averaged 590.6 ± 17.0 kg. Body weight of cows treated with rbGRF did not differ from that of cows infused with placebo during any period (data not shown). Initial BCS were not different among rbGRF and placebo cows, averaging 2.3 ± .6 at -11 d. The BCS did not differ between placebo and rbGRF-treated cows during infusion averaging 2.1 and 2.0 at 27 d and 2.2 and 1.8 at 56 d, respectively.

Compared with placebo, all doses of rbGRF increased mean serum ST concentration at 1 d (Figure 2). Although numerical differences were apparent at 30 and 59 d, there was no significant difference in mean serum ST concentrations between 0 and 1 mg of rbGRF at 30 and 59 d. Infusions of 3 and 12 mg rbGRF increased ($P<.01$) mean serum concentrations of ST above placebo at 30 and 59 d. Concentrations of ST declined 27 to 67% within 1 h after cessation of the rbGRF infusion at 59 d and were similar to the concentration of placebo-infused cows at 17

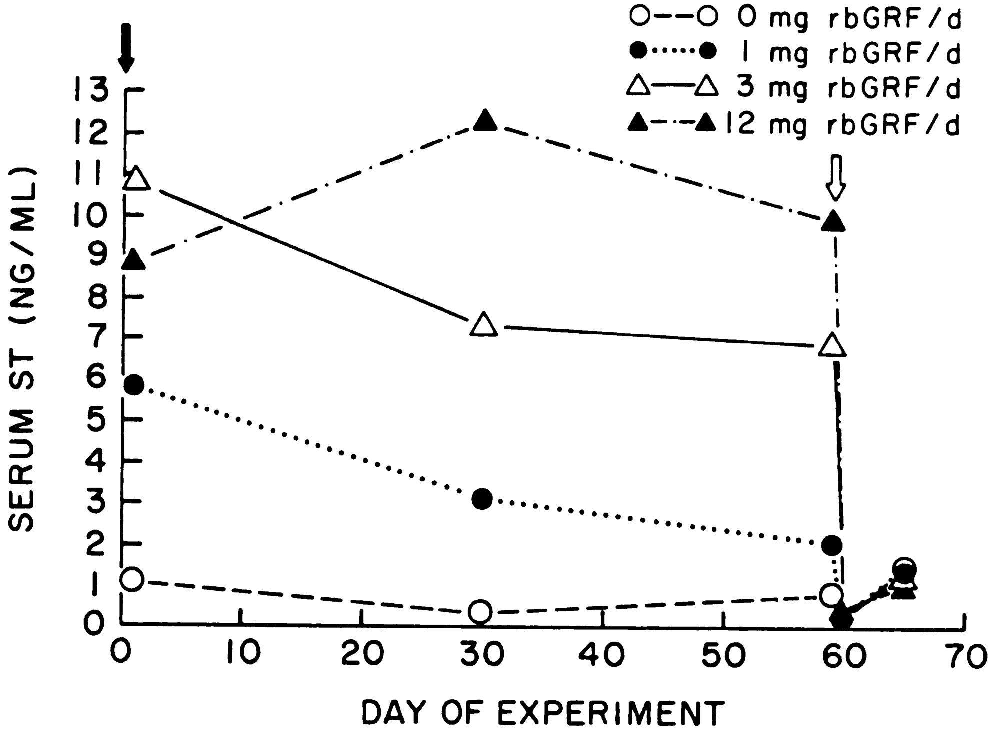

Figure 2. Serum concentrations of ST of cows (six/treatment) continuously infused for 60 d with 0, 1, 3, and 12 mg rbGRF/d. Beginning and end of rbGRF infusion indicated by the solid and open arrows, respectively. The SE of the difference among treatments was 1.56 ng/ml of serum.

h after infusions ended. At 60 and 65 d serum concentrations of ST were not different among cows previously infused with rbGRF and placebo. Based on Pulsar analysis (Merriam and Wachter, 1982) baseline ST increased ($P<.01$) above placebo an average of 6.0 and 7.1 ng/ml for the 3 and 12 mg doses of rbGRF, respectively at 1, 30, and 59 d. In contrast, baseline concentrations after 1 mg rbGRF were similar to those of placebo. Number of pulses of ST increased ($P<.01$) above placebo (.8/8 h) at 1 d for the 1 (2.7/8 h) and 12 mg (2.4/8 h) treatments, but not for 3 mg (1.8/8 h) of rbGRF. Relative to placebo, at 30 d all doses of rbGRF increased ($P<.01$) the number of pulses of ST (2.2/8 h). At 59 d, only the 12 mg dose of rbGRF increased ($P<.05$) pulse number (1.38/8 h). Pulse amplitude increased ($P<.01$) above that of placebo (.5 ng/ml) at all doses at 1 and 30 d to an average of 10.7 and 9.1 ng/ml, respectively. At 59 d pulse amplitude was greater than that of placebo (.14 ng/ml) for the 3 (6.77 ng/ml) and 12 mg (9.32 ng/ml) doses of rbGRF. Placebo-infused cows had no pulses of ST at 30 d, whereas all cows infused with rbGRF had pulses ($P<.01$) with an average duration of 53.1 min. At 59 d, 1 and 3 mg rbGRF increased ($P<.05$) pulse duration an average of 35.8 min relative to placebo. Pulse duration was not different for placebo and rbGRF doses at 1 d. No difference was observed between rbGRF doses and placebo in any characteristic of serum ST at 60 and 65 d.

Relative to placebo, 3 and 12 mg rbGRF/d increased ($P<.01$) serum concentrations of IGF-1 at 59 d from 115.8 to 204.7 and 261.4 (SE of difference = 13.3) ng/ml. At 65 d, serum IGF-1 was similar among groups and averaged 93.7 ng/ml. Overall, serum concentrations of INS, T_4, T_3, free T_4, free T_3, and PRL were not different among the treatment groups (data not shown).

Although plasma NEFA at 1, 30, and 59 d were numerically and consistently greater for animals given rbGRF than for placebo-treated cows (Figure 3), differences from placebo were significant ($P<.01$) only at 30 d of infusion of 3 and 12 mg of rbGRF. NEFA were similar among all treatments after infusions ended. Plasma glucose was not affected by rbGRF treatment and averaged 70.4 mg/dl.

DISCUSSION

The increase of 10.5 kg/d (46%) in yield of milk during a 60-d infusion of 12 mg of rbGRF exceeded the milk yield response observed during 2 mo of daily injection of hGRF (1-29)-NH_2 (Lapierre et al., 1988c). Increases in average total daily yield of milk at 1 and 3 mg of rbGRF for 60 d in the present study were similar to previous responses (Enright et al., 1988) using identical doses of GRF for 20 d. Previously, interval to maximal yield of milk with GRF treatment was unknown since yield was still increasing through 20 d of treatment (Enright et al., 1988). Data from the present study indicate maximal yield of milk occurred by 15 to 30 d for the three doses of rbGRF infused. Treatment with GRF did not affect energetic efficiency of milk production. However, energetic efficiency was available for only 10 of the 24 cows. Thus, the power to detect significant differences among treatment groups was reduced.

Enright et al. (1988) reported that the increase in serum ST was similar between 3.25 and 50 mg GRF during 24-h infusion. In agreement, on the first day

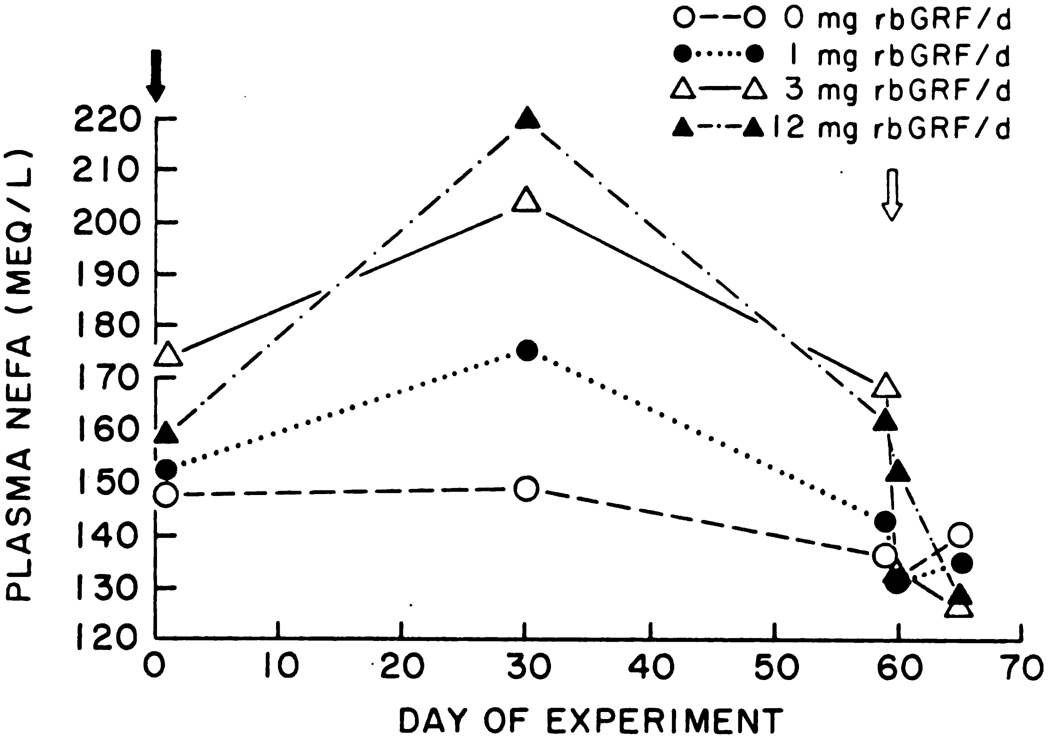

Figure 3. Plasma concentrations of NEFA of cows (six/treatment) continuously infused for 60 d with 0, 1, 3, and 12 mg rbGRF/d. Beginning and end of rbGRF infusion indicated by the solid and open arrows, respectively. The SE of the difference among treatment was 18.5 meq/l of plasma.

of the present study, the ST responses to 3 and 12 mg of rbGRF were virtually identical. However, ST response to 12 mg was greater than the response to 3 mg at 30 and 59 d, indicating subsequent development of a dose response to rbGRF. Also, average yield of milk over the 60-d infusion increased in a dose-dependent manner. The dose response of ST and milk yield to rbGRF confirmed the earlier report of Enright et al. (1988). Therefore, ST response to a 24-h infusion of rbGRF is not indicative of longer term responses of milk yield and ST.

Infusion of 3 and 12 mg rbGRF increased ST in serum throughout 60-d, whereas, the ST increment in response to 1 mg of rbGRF decreased during the 60-d infusion. Thus, in confirmation of previous data (Enright et al., 1988; Lapierre et al., 1988b), appropriate doses of rbGRF will increase secretion of ST for long periods. Our results are consistent with the hypothesis that GRF stimulates synthesis (Silverman et al., 1988) and release (Padmanabhan et al., 1987) of ST. Following withdrawal of rbGRF, ST concentrations in serum declined within 17 h to concentrations found in placebo-treated cows. Although the milk yield curves appeared to converge over the 15 d following withdrawal of rbGRF, milk and lactose yields of cows previously infused with 12 mg of rbGRF averaged 5.8 kg/d and .31 kg/d, respectively, more than those of placebo cows. Hart et al. (1985) and Lapierre et al. (1988a) reported similar sustained elevations of yield of milk in sheep and cows following withdrawal of GRF. In contrast, increased yield of milk in cows treated with exogenous ST is not sustained after withdrawal of treatment (Pocius and Herbein 1986; Eppard et al., 1985; Peel et al., 1982). If differences between milk yield response after cessation of exogenous ST and rbGRF can be confirmed, this may suggest that rbGRF has a non-ST mediated component in its galactopoietic action.

Administration of exogenous bST (Davis et al., 1987; Peel et al., 1985) or GRF (Enright et al., 1989) to dairy cows increased serum concentrations of IGF-1. Indeed, the galactopoietic mechanism of action of ST probably is mediated through IGF-1 (Bauman Gluckman et al., 1987). In the present study, infusion of 3 or 12 mg rbGRF/d increased serum concentrations of IGF-1 relative to controls. Therefore, during rbGRF infusion, it is likely that rbGRF is acting similarly to ST to increase milk production (i.e., mediation by IGF-I). However, after infusions of rbGRF ended, there was no difference in serum IGF-I among treatment groups at 65 d. Thus, milk production remained elevated following cessation of rbGRF treatment, although serum concentrations of ST and IGF-1 declined in cows previously infused with 12 mg rbGRF/d.

Generally, rbGRF treatment had little affect on concentrations of other blood hormones or metabolites. Enright et al. (1989) reported increased serum concentrations of INS on the last day of a 20-d infusion of GRF, and suggested that GRF or ST treatment greater than 10 d may be necessary before increases in serum INS are observed. However, 60-d infusion of rbGRF did not affect serum concentrations of INS in the present study. Exogenous ST does not affect serum concentrations of INS (Pocius and Herbein, 1986; Eppard et al., 1985; Peel et al., 1982) during 10- to 11-d treatments, but in a longer study ST increased INS (Soderholm et al., 1988). Thus, effects of ST and GRF on serum INS are variable.

Administration of rbGRF for 60 d did not affect serum concentrations of

PRL which agrees with previous reports of short-term infusions of GRF (Enright et al., 1989; Moseley et al., 1985). In contrast to Enright et al. (1989), in the present study rbGRF infusion did not increase serum concentrations of T_3 or T_4. The reason for these differences between experiments is unknown.

Exogenous ST does not affect blood glucose concentrations in cows (Bauman et al., 1988; Pocius and Herbein, 1986; Peel et al., 1982). Similarly, exogenous rbGRF had no effect on plasma glucose concentrations. In contrast, in lactating ewes (Hart et al., 1985) and cows (Enright et al., 1989), blood glucose concentrations increased at 4 and 20 d of GRF treatment, respectively. Possibly, any rbGRF-induced increase in blood glucose is transient and had disappeared before blood sampling at 30 d.

Plasma concentrations of NEFA increase with exogenous GRF (Enright et al., 1989; Lapierre et al., 1988c) and ST (Bauman et al., 1988; McDowell et al., 1987). In the present study, increased serum NEFA at 30 d is in agreement with previous work.

In summary, i.v. infusion of rbGRF (12 mg/d) for 60 d increased yield of milk an average of 10.5 kg/d above controls. A concomitant increase in serum concentration of ST was observed. Mediation of rbGRF effects is most likely through increased secretion of ST and IGF-I. However, the mechanism remains to be determined whereby yield of milk remains elevated following cessation of rbGRF infusion while serum ST and IGF-I returned to basal concentrations.

CHAPTER 2

Comparison of rbST and rbGRF on Milk Yield, Serum Hormones and Energy Status of Dairy Cows

INTRODUCTION

Exogenous GRF and bST increase milk production in dairy cattle (Dahl et al., 1990; Lapierre et al., 1988a; Bauman et al., 1985; Eppard et al., 1985). However, the relative galactopoietic potencies of GRF and bST have not been compared directly. Moreover, among studies, milk production responses to GRF or bST are dissimilar following withdrawal of treatment. For example, elevation of milk yield in sheep (Hart et al., 1985) and cows (Dahl et al., 1990; Lapierre et al., 1988a) is sustained following withdrawal of GRF. In contrast, milk production declines rapidly after withdrawal of bST from ewes (Hart et al., 1985) and cows (Gluckman et al., 1987). The first objective was to compare the effects of GRF and bST on milk yield during and after treatment. Our approach was to use doses and routes of administration of GRF and bST that in independent studies optimized their respective galactopoietic responses (Dahl, et al., 1990; Ash et al., 1989).

Potentially, differences in galactopoietic potency between GRF and bST may be explained by differences in response of serum concentrations of ST or IGF-I. Both GRF and bST increase serum concentrations of ST (Dahl et al., 1990; Lapierre et al., 1988a; Gluckman et al., 1987; Bauman et al., 1985). Indeed, the galactopoietic action of GRF is attributed to increased serum concentrations of ST (Dahl et al., 1990; Enright et al., 1989; Lapierre et al., 1988b). However, whether GRF and bST each at doses and routes of administration that optimize yield of milk elicit similar responses in serum concentrations of ST is unknown. Thus, the second objective was to compare the effects of GRF and bST on serum concentrations of ST. The putative mediator of bST galactopoietic action at the mammary gland is IGF-I (Gluckman et al., 1987). To our knowledge, there has been no comparison of the abilities of GRF and bST to increase serum concentrations of IGF-I. Therefore, a third objective was to compare effects of GRF and bST on serum concentrations of IGF-I.

Differences in nutrient partitioning between GRF and bST may affect their relative galactopoietic potency. Increased milk production requires increased nutrient availability at the mammary gland (Bauman and Currie, 1980). Potentially, increases in DMI, DM digestibility, or mobilization of tissue stores are sources of energy to support increased milk production (Peel and Bauman, 1987). Thus, the final objective was to identify the source or sources of energy that support increased milk yield in response to GRF and bST.

MATERIALS AND METHODS

Design and Management

Fifteen multiparous and nine primiparous Holstein cows averaging 77.8 ± 7.3 d of lactation were used in a randomized complete block design with repeated measurement. Eight blocks of three cows each were formed based on parity and pretreatment milk yield between -21 and -17 d of the experiment. Within each block, cows were assigned randomly to treatment (eight cows/treatment). Treatments were continuous i.v. (jugular) infusion of recombinant bovine GRF (1-45) homoserine lactone (rbGRF; 12 mg/d; Kirschner et al., 1989); single daily i.m. injection of recombinant bST (rbST; 14 mg/d); and uninjected, uninfused controls. Fourteen days before treatment began, VETport® infusion catheters (Thermedics, Woburn, MA) were implanted surgically into cows assigned to rbGRF treatment as described previously (Chapter 1; Dahl et al., 1990). Doses of rbGRF and rbST were prepared daily in sterile pyrogen-free water. Infusions of rbGRF were as previously described (Chapter 1; Dahl et al., 1990). Injections of rbST were made in the left or right flank region, alternating each day. Treatments were initiated at 0900 h on d 0. Each day of treatment consisted of the 24 h period following 0900 h. Cows were housed in tie stalls, exposed to 24 h of light per day, and milked at 0600 and 1700 h. Milk production was recorded daily, and milk was sampled for 3 consecutive days every 20 d for composition analysis beginning with the -3 to -1 d pretreatment period. Fat, protein, solids, and lactose in milk were measured using an infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA (East Lansing). Yield of SCM and output of energy in milk (Mcal/d) were calculated (Tyrrell and Reid, 1965). A TMR was fed ad libitum. The TMR was formulated (Appendix B) to provide adequate nutrition for a 612.2 kg cow producing 40.8 kg of milk/d containing 3.6% fat and assuming an intake of 24.2 kg DM per day (NRC, 1989). Feed was offered daily at 0300 and 1200 h. Weight of orts was recorded once daily. Cows were weighed for 3 consecutive days every 20 d beginning on -3 to -1 d. Two experienced examiners scored body condition (BCS) 1 to 5 (Wildman et al., 1982) at -1, 59, and 79 d.

During the study, three cows were removed after they contracted coliform mastitis and ceased to lactate. The three cows were in the rbGRF treatment group although one of the three contracted the mastitis and was removed from study prior to receiving rbGRF. The data of these three cows were deleted from all statistical analyses. It should be noted that the incidence of coliform mastitis was elevated in the entire Michigan State University Dairy herd at the time of the study.

Feed Digestibility Determination

Between -5 to -1, 55 to 59, and 75 to 79 d, fecal samples were collected every 15 h. Also, on each day during fecal collections, feed and orts were sampled from each cow. All fecal, feed, and orts samples were dried at 55°C, ground through a Wiley Mill (1mm screen) and each was composited for each cow. Neutral detergent fiber (NDF) was determined in duplicate according to Goering and Van Soest (1970) with the omission of decahydronapthalene, sodium sulfite (which was added to fecal samples), the substitution of trimethylene glycol for 2-ethoxyethanol (Cherney et al., 1989), and the inclusion of α-amylase (Robertson and Van Soest, 1977). Acid detergent fiber (ADF) was determined sequentially on the NDF residue according to Goering and Van Soest (1970). Lignin content was quantified by treating the ADF residue with 72% H_2SO_4 (Goering and Van Soest, 1970). Crude protein was determined using the method of Hach et al. (1987). Dry matter (DM) content was determined gravimetrically after drying samples at 100°C for 24 h. Samples were ignited at 500°C for 5 h to determine ash content. Apparent digestibility was calculated using lignin as an intrinsic marker.

Blood Sampling and Analysis

Blood was sampled hourly from an indwelling jugular catheter for 25 h at 1 and 59 d, and for 8 h on 60 and 64 d. Also, single samples were collected by tail vessel puncture on d -1, 19, 39, and 79. Blood samples collected for serum were stored at room temperature for 2 to 6 h then at 4°C for approximately 15 h. Blood samples collected for plasma were treated with NaF-EDTA and placed on ice immediately after collection. Serum or plasma was harvested after centrifugation for 30 min at 1550 x g and frozen at -20°C until assayed for ST (Moseley et al., 1982), IGF-I (Dahl et al., 1990) and NEFA [NEFA-C kit, Wako Chemicals USA, Dallas, TX; as modified by (McCutcheon and Bauman, 1986b)].

Statistical Analysis

The experiment had nine periods: one pre-treatment period (-10 to -1 d), six treatment periods (0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, and 50 to 59 d), and two post-treatment periods (60 to 69, and 70 to 79 d). All data were subjected to split block ANOVA with repeated measurement (Gill, 1986). Within period means were examined using the Bonferroni t test (Gill, 1978).

RESULTS

Pre-treatment milk yield (-10 to -1 d; Figure 4) was significant when tested as a covariate, therefore, subsequent milk yields were adjusted by covariance for pretreatment milk yield. Compared with controls (31.6 ± .6 kg/d), rbST and rbGRF increased milk production to 34.2 ± .6 ($P<.06$) and 37.0 ± .7 ($P<.01$) kg/d during

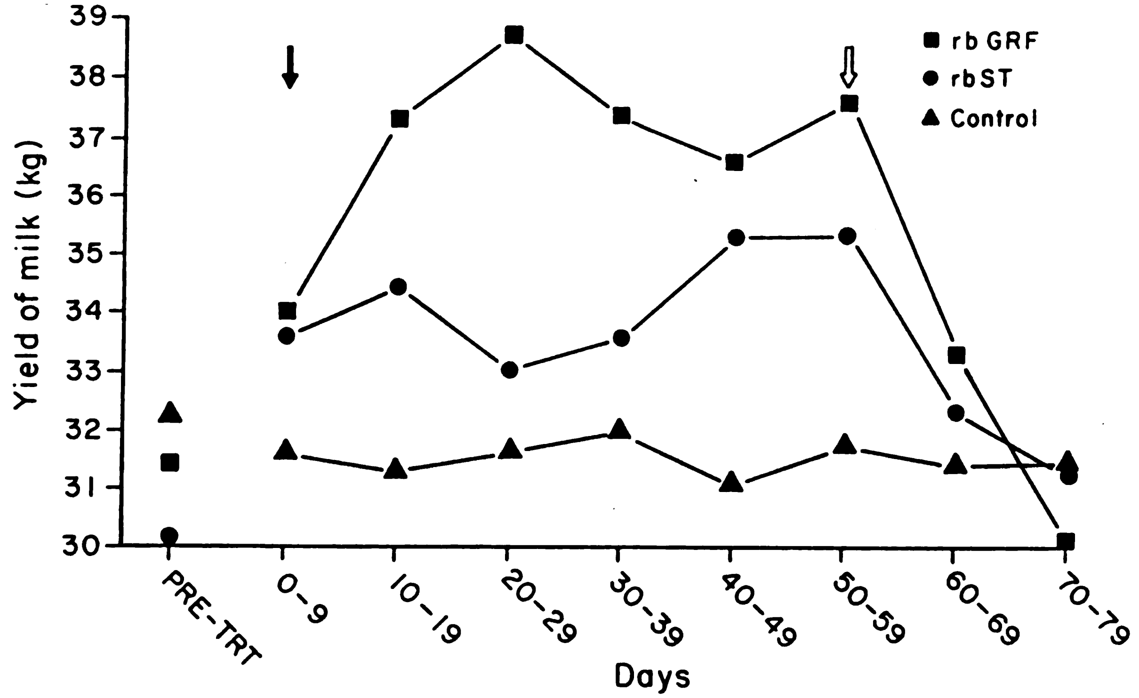

Figure 4. Milk yield (solids corrected) of cows receiving 12 mg rbGRF/d, 14 mg rbST/d, or no treatment for 60 d. Each connected point represents the average of a treatment group (least squares means) within each 10-d period, adjusted by covariance with pre-treatment milk yield. Beginning and end of treatment indicated by the solid and open arrows, respectively. SE of difference within a period for control versus rbST was 1.25 kg/d. SE of difference for all other comparisons within a period was 1.43 kg/d.

treatment. Following cessation of treatment (60 to 79 d), there was no difference in milk yield among treatment groups. Yield of SCM of rbGRF-treated cows (38.0 k/d) increased ($P<.01$) relative to control cows (32.3 kg/d). However, yield of SCM of rbST-treated cows (34.8 kg/d) was not different ($P>.10$) from that of rbGRF-treated or control cows. Average percentages of protein (3.2), lactose (4.8), fat (4.2), and solids (12.9) in milk were similar among treatments throughout the study.

Relative to controls, cows receiving rbGRF increased ($P<.01$; Figure 5) energy output in milk during all treatment periods, but cows receiving rbST had increased ($P<.05$) energy output in milk only from 40 to 59 d. Neither rbGRF nor rbST affected energetic efficiency of milk production adjusted for BW differences (data not shown). During pretreatment, DMI was not different among treatment groups (Figure 6). Relative to control, neither rbST or rbGRF affected DMI (Figure 6) or DM digestibility (data not shown) during the treatment and post-treatment periods. Initial BW of all cows averaged 531.7 ± 9.2 kg, and BW of cows treated with rbGRF or rbST did not differ from that of controls within any period (Table 3). However, control and rbST-treated cows gained ($p<.01$) 28.6 ± 4.6 and 22.3 ± 4.6 kg of BW from -1 to 79 d, while BW of rbGRF-treated cows was unchanged. Initial BCS was not different among treatments (Table 4). However, BCS of control and rbST-treated cows increased ($p<.05$) from d -1 to 79, while BCS of rbGRF-treated cows was unchanged. Control and rbST-treated cows sustained positive calculated energy balance throughout the study (Table 5). Calculated energy balance was negative for rbGRF-treated cows from 0 to 59 d.

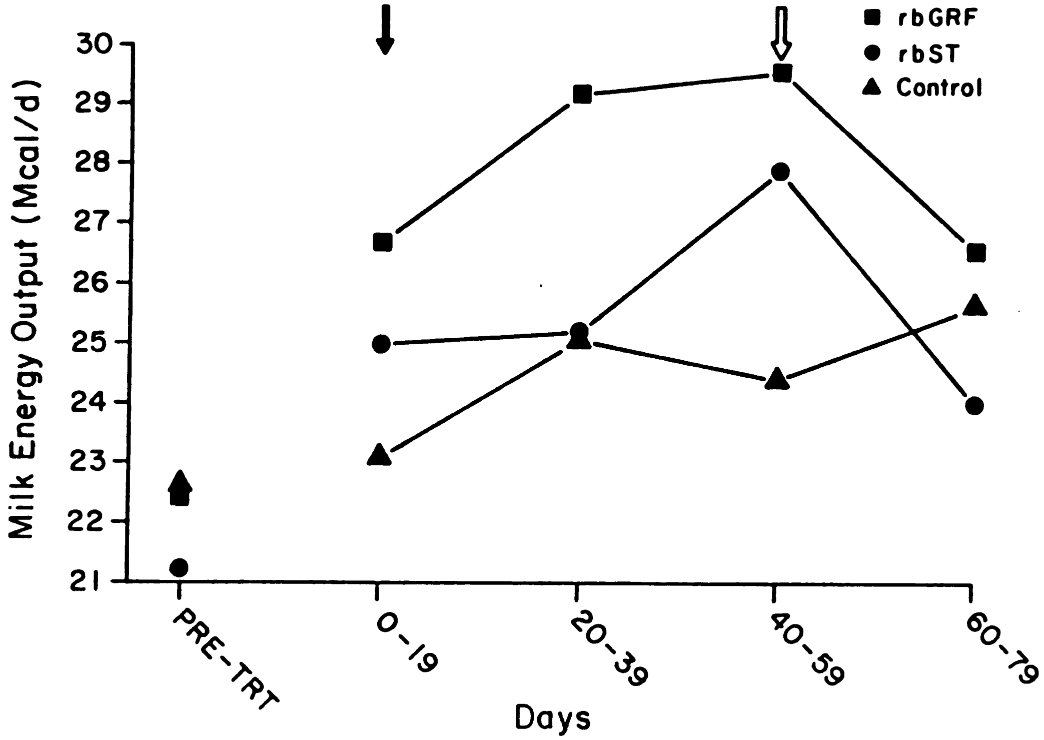

Figure 5. Milk energy output of cows receiving 12 mg rbGRF/d, 14 mg rbST/d, or no treatment for 60 d. Each connected point represents the average of a treatment group (least squares means) within each 20-d period, adjusted by covariance with pretreatment milk energy output. Beginning and end of treatment indicated by the solid and open arrows, respectively. SE of difference within a period for control versus rbST was 1.17 Mcal/d. SE of difference for all other comparisons within a period was 1.33 Mcal/d.

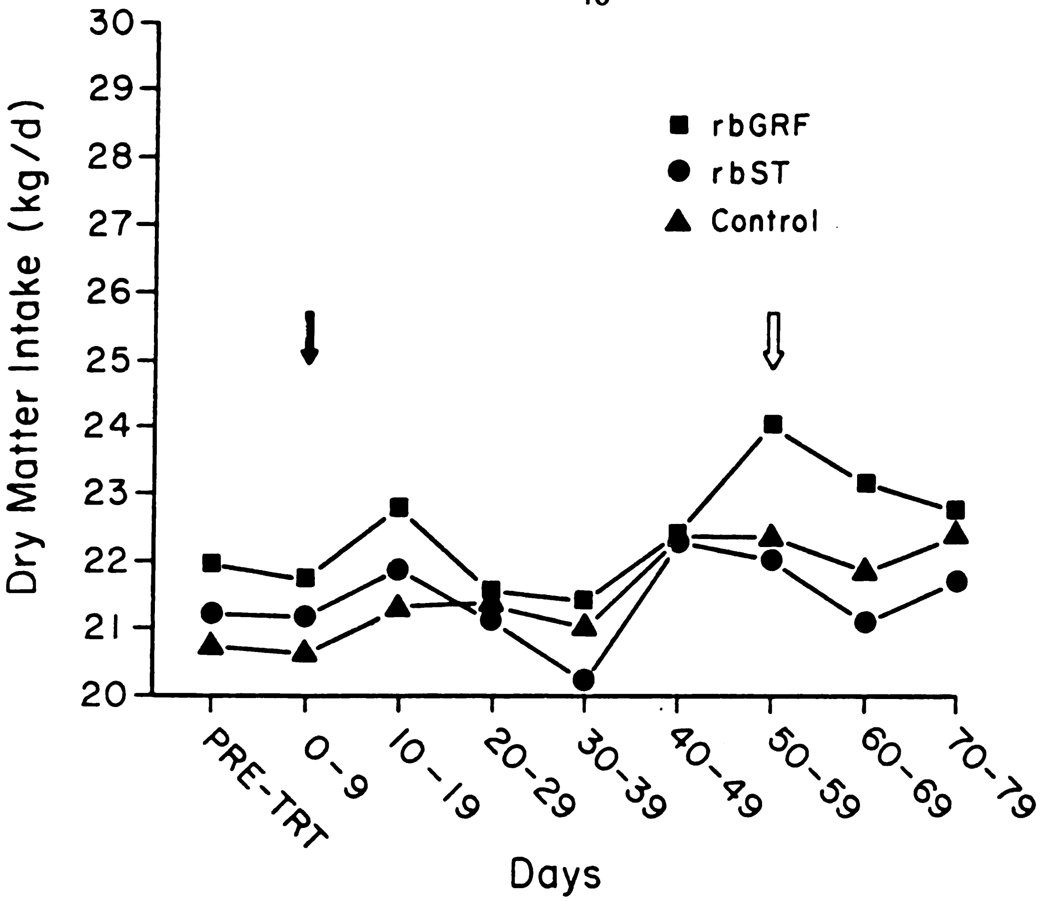

Figure 6. DMI of cows receiving 12 mg rbGRF/d, 14 mg rbST/d, or no treatment for 60 d. Each point represents the average of a treatment group (least squares means) within each 10-d period, adjusted by covariance with initial BW. Beginning and end of treatment indicated by the solid and open arrows, respectively. SE of difference within a period for control versus rbST was .90 kg/d. SE of difference for all other comparisons within a period was 1.02 kg/d.

Table 3. Body weights (kg) of cows receiving 14 mg rbST/d or 12 mg rbGRF/d or serving as controls for 60 d.

Treatment[1]	Body weight				
	d -1	d 19	d 39	d 59	d 79
Control	511.8	513.8	524.3	532.9	540.4[2]
rbST	535.6	536.0	545.8	556.5	558.0[2]
rbGRF	557.2	561.8	556.8	579.9	569.2

[1] SE of difference within a day for control versus rbST was 22.3 kg. SE of difference for all other comparisons within a day was 25.4 kg.

[2] Gains from -1 to 79 d were significant ($P<.01$) for these groups. SE of difference for gain for these groups was 4.6 kg. SE of difference for gain for rbGRF-treatment cows was 5.8 kg.

Table 4. Body condition scores (1-5 scale) of cows receiving 14 mg rbST/d or 12 mg rbGRF/d or serving as controls for 60 d.

Treatment[1]	Body Condition Score		
	d -1	d 59	d 79
Control	1.66	1.72	1.94[2]
rbST	1.70	1.61	1.97[2]
rbGRF	1.76	1.58	1.82

[1] SE of difference within a day for control versus rbST was .14. SE of difference for all other comparisons within a day was .16.

[2] Increases in BCS from -1 to 79 d were significant ($P<.05$) for these groups. SE of difference for increases in BCS across day for these groups was .09. SE of difference for increases in BCS across day for rbGRF-treated cows was .11.

Table 5. Calculated energy balance (Mcal/d) of cows receiving 14 mg of rbST/d 12 mg rbGRF/d or serving as controls for 60 d.

Treatment	Energy balance (Mcal/d)				
	d -10 to -1	0 to 19	20 to 39	40 to 59	60 to 79
Control	4.8	3.5	1.4	3.2	1.3
rbST	6.3	3.9	1.8	2.3	3.6
rbGRF	4.4	0.9	-1.4	0	4.1

Compared with control, rbGRF and rbST increased mean serum concentrations of ST at 1 and 59 d (Figure 7). Furthermore, rbGRF increased serum concentration of ST relative to rbST on 1 and 59 d. At 60 d, cows previously treated with rbGRF had increased ($P<.05$) serum concentrations of ST relative to rbST and control cows. But by 64 d serum concentrations of ST were not different among controls and cows previously treated with rbGRF or rbST.

On -1 d there was no difference in serum concentrations of IGF-I among treatment groups (Figure 8). Compared with controls, rbGRF and rbST increased ($P<.05$) serum concentrations of IGF-I during treatment. Furthermore, concentrations of IGF-I in rbGRF-treated cows were greater ($P<.05$) than in rbST-treated cows. Serum concentrations of IGF-I remained elevated at 24 h after cessation of treatment with rbGRF and rbST (60 d); however, there was no difference in serum IGF-I among treatment groups on 64 or 79 d.

Plasma concentrations of NEFA were not different among treatment groups on -1 d. Although plasma NEFA throughout treatment were numerically and consistently greater for animals given rbGRF or rbST than for control cows (Figure 9), differences from control were significant ($P<.05$) only at 19 and 39 d of treatment with rbGRF. Plasma concentrations of NEFA did not differ among cows after treatment ended.

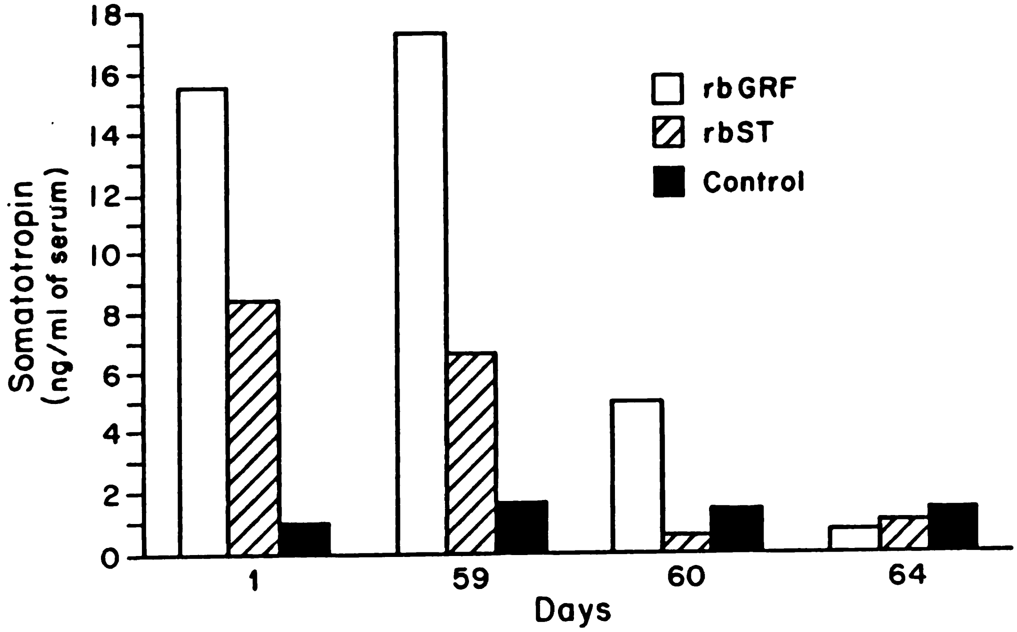

Figure 7. Serum concentrations of ST of cows receiving 12 mg rbGRF/d, 14 mg rbST/d, or no treatment for 60 d. SE of difference within a day for control versus rbST was 1.38 ng/ml. SE of difference for all other comparisons within a day was 1.57 ng/ml.

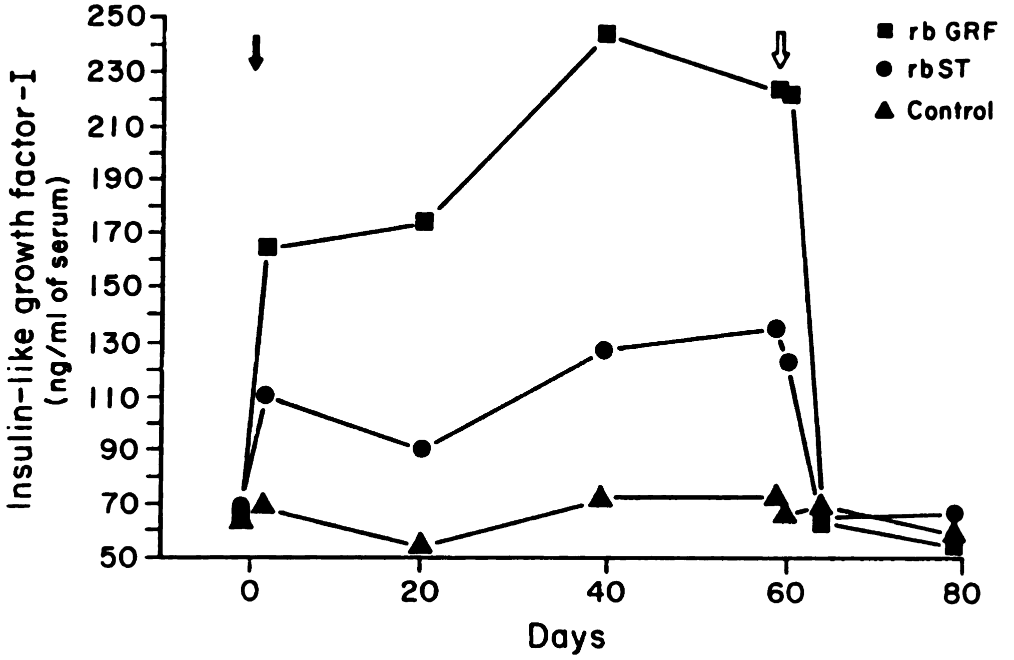

Figure 8. Serum concentrations of IGF-I of cows receiving 12 mg rbGRF/d, 14 mg rbST/d, or no treatment for 60 d. Beginning and end of treatment indicated by the solid and open arrows, respectively. SE of difference within a day for control versus rbST was 14.6 ng/ml. SE of difference for all other comparisons within a day was 16.7 ng/ml.

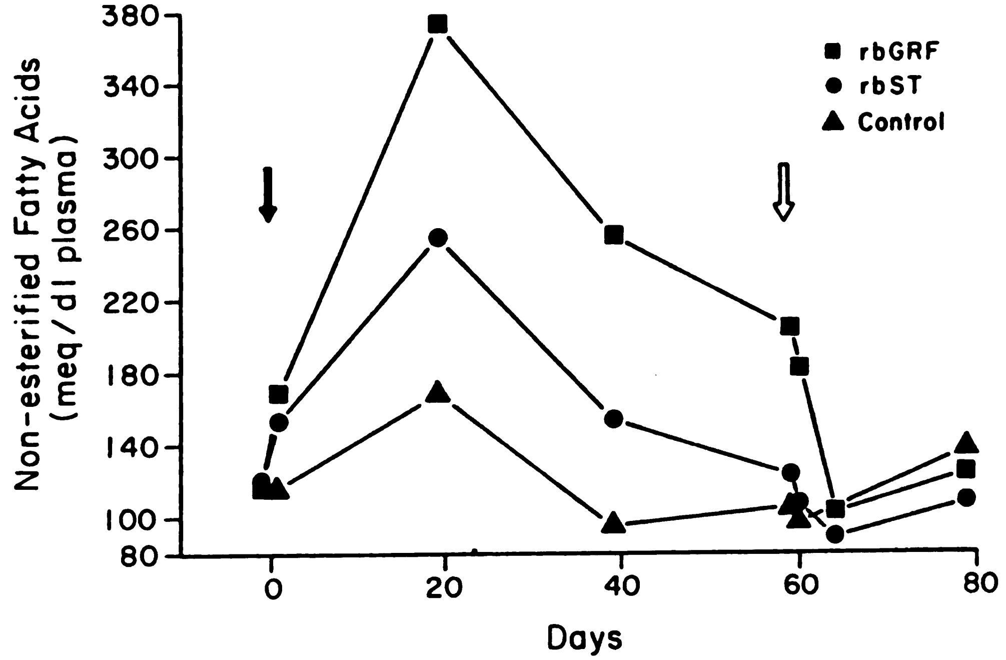

Figure 9. Plasma concentrations of NEFA of cows receiving 12 mg rbGRF/d, 14 mg rbST/d, or no treatment for 60 d. Beginning and end of treatment indicated by solid and open arrows, respectively. SE of difference within a day for control versus rbST was 44.7 meq/dl. SE of difference for all other comparisons within a day was 51.0 meq/dl.

DISCUSSION

Increased milk yield in response to rbGRF or rbST during the 60-d treatment are within the range of responses previously reported (Dahl et al., 1990; Lapierre et al., 1988a; Lapierre et al., 1988c; Eppard et al., 1985; Richard et al., 1985). Indeed, Peel et al. (1981) reported similar increases in milk yield in cows treated with bST at the same production level and stage of lactation as rbST-treated cows of the present study. Milk yield did not remain elevated following the cessation of rbGRF or rbST, whereas in a previous study milk yield was elevated for at least 15 days following the end of rbGRF treatment (Dahl et al., 1990). Possibly, the earlier stage of lactation and(or) lower BCS of cows in the present study relative to cows in our previous study accounts for the discrepancy in milk yield response after withdrawal of treatment.

Increasing doses of GRF or bST increases milk yield in association with increases in serum concentrations of ST and IGF-I (Dahl et al., 1990; Enright et al., 1989; Kerr et al., 1988). The doses of rbGRF and rbST used in the present study were previously shown to optimize the galactopoietic response, albeit in independent experiments (Dahl et al., 1990; Ash et al., 1989). In the present study, rbGRF elicited a greater increase in serum concentrations of ST and IGF-I than rbST. Thus, the ability of rbGRF to increase serum concentrations of ST and IGF-I relative to the rbST treatment probably explains the larger increases in milk yield with rbGRF treatment. Another hypothesis is that the pattern of ST response to rbGRF (pulsatile) versus rbST (single daily pulse) affects the subsequent galactopoietic response. Indeed, body growth was greater in rats that received ST via a continuous infusion versus single daily injections (Cotes et al., 1980). However, different routes of bST administration did not affect the galactopoietic response in dairy cows (McCutcheon and Bauman, 1986a; Fronk et al., 1983). Furthermore, increases in nitrogen retention in steers were not different when identical amounts of ST were administered in various patterns each day (Moseley et al., 1982). Therefore, pattern of ST administration does not appear to affect lactational or growth responses in cattle. Rather, the absolute amount of serum ST per day appears to determine the lactational or growth response. One approach to further study differences in galactopoietic action of GRF and bST would be to match serum concentrations of ST. In a preliminary study, we found that continuous infusion of 29 mg rbST/d was necessary to attain an increase in serum concentration of ST of 15 ng/ml, thus matching the average serum concentration of ST with 12 mg rbGRF/d (Dahl, Chapin, Moseley, and Tucker, unpublished observations; Chapter 3). However, the galactopoietic effects of similar serum concentrations of ST induced by rbST and rbGRF treatments are unknown.

In agreement with previous reports (Dahl et al., 1990; Bauman et al., 1985), milk composition in the present study was unaffected by rbGRF or rbST treatment. Thus, the pattern of rbGRF- and rbST-induced increases in milk energy output was similar to those for milk yield. Relative to controls DMI was unaffected by rbGRF or rbST treatment during any period. Previous reports indicate that bST has no effect on DM digestibility (Winsryg et al., 1989; Peel et al., 1981). In agreement, rbST had no effect on any aspect of DM digestibility that were examined in the present study. In contrast to bST, Tyrrell et al. (1989) reported that GRF reduced losses of energy and nitrogen in feces and urine of steers. In the present study, rbGRF had no effect on DM digestibility. Thus, neither DMI or DM digestibility contributed to the increased energy required to support increased milk production. Exogenous GRF (Dahl et al., 1990; Enright et al., 1989; Lapierre et al., 1988c) and bST (Bauman et al., 1988) increase plasma concentrations of NEFA. In the present study, plasma concentrations of NEFA were generally increased by rbGRF or rbST, suggesting mobilization of lipid stores (Gluckman et al., 1987). Moreover, the negative energy balance experienced by rbGRF-treated cows supports the hypothesis that cows in negative energy balance mobilize adipose reserves to sustain increased milk production (Peel and Bauman, 1987). Conversely, control- and rbST-treated cows gained BW and BCS in the present study especially after treatments ceased. The lack of increase in BCS and BW in rbGRF-treated cows coupled with increased concentrations of NEFA indicates that mobilization of adipose tissue is the likely source of energy to support increased milk production.

It is concluded that the galactopoietic response to continuous i.v. infusion of 12 mg rbGRF/d is greater than that of once daily i.m. injection of 14 mg rbST/d. The greater galactopoietic effects of rbGRF relative to rbST are probably mediated via increased secretion of ST and IGF-I. Mobilization of adipose tissue reserves likely provided the energy to support increased milk production in rbGRF-treated cows.

CHAPTER 3

Galactopoietic Effects of Doses of
rbST and rbGRF
that Elicit Similar Increases in Concentrations
of ST and IGF-I in Serum of Dairy Cows

INTRODUCTION

It is now well established that bST and bGRF increase milk yield in dairy cows (Dahl et al., 1990; Peel and Bauman, 1987). Indeed, the galactopoietic action of bGRF is attributed to its ability to increase endogenous secretion of ST. However, whether this is the sole mediator of the galactopoietic action of bGRF is unknown. Previously, we compared the effects of bST and bGRF treatment on milk yield in dairy cows, selecting doses which had optimally increased milk yield in independent studies (Dahl et al., 1991). Treatment with bGRF increased milk production to a greater extent than bST treatment (Dahl et al., 1991). However, serum concentrations of ST and IGF-I were also greater with bGRF treatment as compared with bST treatment. Thus, whether GRF galactopoietic action is mediated solely through increases in serum concentrations of ST remains unknown. In the present study, the first objective was to compare the effects on milk yield of doses of bST and bGRF which elicit similar increases in concentrations of ST in serum.

IGF-I is a putative mediator of galactopoietic action of bST at the mammary gland (Gluckman et al., 1987). Dissimilar responses of IGF-I to increases in concentrations of ST in serum induced by bST or bGRF could account for differences in milk yield response. Therefore, the second objective was to compare effects of bST and bGRF on concentrations of IGF-I in serum when serum concentrations of ST were similar.

Cows producing large quantities of milk often mobilize adipose tissue reserves or increase DMI to meet energy demands (Bauman and Currie, 1980). Indices of adipose mobilization include serum concentrations of NEFA and BCS. Potentially, differences in nutrient partitioning may explain differences in relative galactopoietic potency between GRF and bST. Thus, the third objective was to compare the effects of GRF and bST on DMI and adipose tissue mobilization when serum concentrations of ST in both groups were similar.

MATERIALS AND METHODS

Design and Management

Eighteen multiparous and six primiparous Holstein cows averaging 175 ± 33 d of lactation were used in a randomized complete block design with repeated measurement. Eight blocks of three cows each were formed based on parity and pre-infusion milk yield between -30 and -21 d of the experiment. Within each block, cows were assigned randomly to treatment (eight cows/treatment). Treatments were continuous i.v. (jugular) infusion of recombinant bovine GRF (1-45) homoserine lactone (rbGRF; 12 mg/d) recombinant bST (rbST; 25-29 mg/d); and uninfused controls. Fourteen days before treatment began, VETport® (Thermedics, Woburn, MA, USA) infusion catheters were implanted surgically into cows assigned to rbGRF and rbST treatment as described previously (Dahl et al., 1990). Doses of rbGRF and rbST were prepared daily in sterile pyrogen-free water. Infusions of rbGRF and rbST were as described previously (Dahl et al., 1990). Cows were housed in tie stalls, exposed to 24 h of light per day, and milked at 0500 and 1530 h. Milk production was summed daily, and milk was sampled for 3 consecutive days every 10 d for composition analysis beginning with the -3 to -1 d pre-infusion period. Fat, protein, solids, lactose, and somatic cell count (SCC) in milk were measured using an infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA (East Lansing). Yield of SCM and output of energy in milk (Mcal/d) were calculated (Tyrrell and Reid, 1965). A TMR was fed ad libitum. The TMR was formulated (Appendix C) to provide adequate nutrition for a 612.2 kg cow producing 40.8 kg of milk/d containing 3.6% fat and assuming an intake of 24.2 kg DM per day (NRC, 1989). Feed was offered daily at 0300 and 1400 h. Weight of orts was recorded once daily. Cows were weighed for 3 consecutive days every 20 d beginning on -3 to -1 d. Two experienced examiners scored body condition on a 1 to 5 scale (Wildman et al., 1982) at -1, 19, 39, 59, and 79 d.

Blood Sampling and Analysis

Blood was sampled every 30 min from an indwelling jugular catheter for 8 h at 1, 10, 20, 30, 45, 59, 60 and 65 d. Blood samples were stored at room temperature for 2 to 6 h then at 4°C for approximately 15 h. Serum was harvested after centrifugation for 30 min at 1550 x g and frozen at -20°C until assayed for ST (Moseley et al., 1982), IGF-I (Dahl, et al., 1990) and NEFA (NEFA-C kit, Wako Chemicals USA, Dallas, TX; as modified in McCutcheon and Bauman, 1986b). Assays of ST in serum were conducted within 2 to 3 d of a blood collection day. Using this blood sample collection and assay protocol, adjustment (if necessary) could be made in the amount bST infused during the subsequent sampling period.

Determination of Doses

Previously, we determined that infusion of 12 mg rbGRF/d increases serum concentrations of ST by 15 ng/ml (Dahl et al., 1991). In a preliminary experiment we determined that infusion of 25 mg rbST/d increased serum concentrations of ST by 15 ng/ml, thus, we initially chose 25 mg rbST/d for use in the present study. However, based on assays of ST on d 1, the rbST dose was increased to 29 mg/d on d 9 to more closely approximate the serum concentrations of ST quantified in the cows infused with rbGRF.

Statistical Analysis

The experiment had nine periods: one pre-infusion period (-10 to -1 d), six infusion periods (0 to 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, and 50 to 59 d), and two post-infusion periods (60 to 69, and 70 to 79 d). Characteristics of ST in serum (mean, baseline, pulse frequence, pulse amplitude, and pulse duration) during each 8-h sampling period were determined using a pulse analysis program (PULSAR; Merriam and Wachter, 1982). All data were subjected to split block ANOVA with repeated measurement (Gill, 1986). Within period means were compared using the Bonferroni t test (Gill, 1978).

RESULTS

Compared with controls (1.2 ng/ml), rbGRF and rbST increased mean serum concentrations of ST to 12.5 ± 2.7 and 12.9 ± 2.7 ng/ml during infusion, respectively (Figure 10). Average concentrations of ST were not different ($p > .20$) between rbGRF- and rbST-treated cows at any time during the study. Infusion of rbGRF increased ($p < .05$) the number of peaks (0.9/8 h), peak amplitude (11.4 ng/ml), peak length (38.2 min), peak length (38.1 min), and peak frequency (.002/8 h) of ST above that of rbGH on d 10 and 20, but not on d 1, 30, 45 or 59. On d 60 and 65 serum concentrations of ST were not different among controls or cows previously infused with rbGRF or rbST.

Compared with controls (60.7 ± 15.8 ng/ml), rbGRF and rbST increased ($p < .01$) serum concentrations of IGF-I to 129.4 ± 15.8 and 144.4 ± 15.8 ng/ml during infusion (Figure 11). On d 60, relative to controls, serum concentrations of IGF-I remained elevated in cows previously infused with rbGRF or rbST. However, by d 65 serum concentrations of IGF-I had declines such that there was no difference among controls and cows previously infused with rbGRF or rbST. Serum concentrations of IGF-I did not differ ($p < .20$) between rbGRF and rbST infused cows at any time during the study. Serum concentrations of IGF-I declined following cessation of rbGRF- and rbGH-infusion such that there was no diffrence among control, rbGRF-, or rbST-infused cows on d 65.

Pre-infusion milk yield (-10 to -1 d) milk yield corrected for solids content (SCM; Figure 12) was significant when tested as a covariate; therefore, subsequent

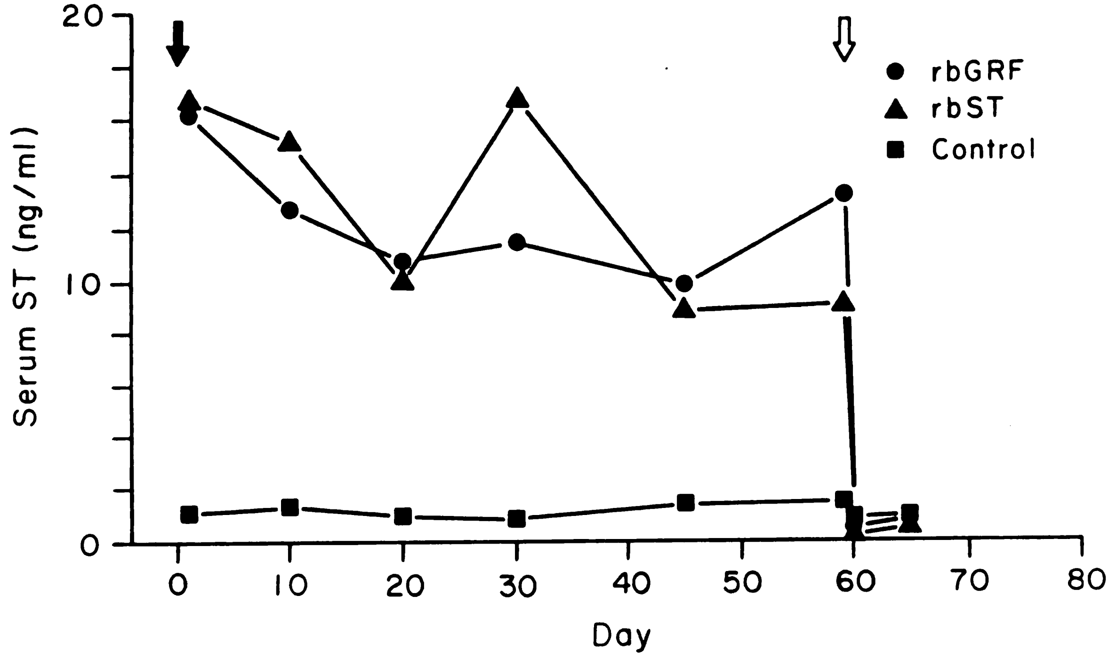

Figure 10. Serum concentrations ST of cows receiving 12 mg rbGRF/d, 29 mg rbST/d, or no treatment for 60 d. Pooled SE of difference within a day was 2.7 ng/ml.

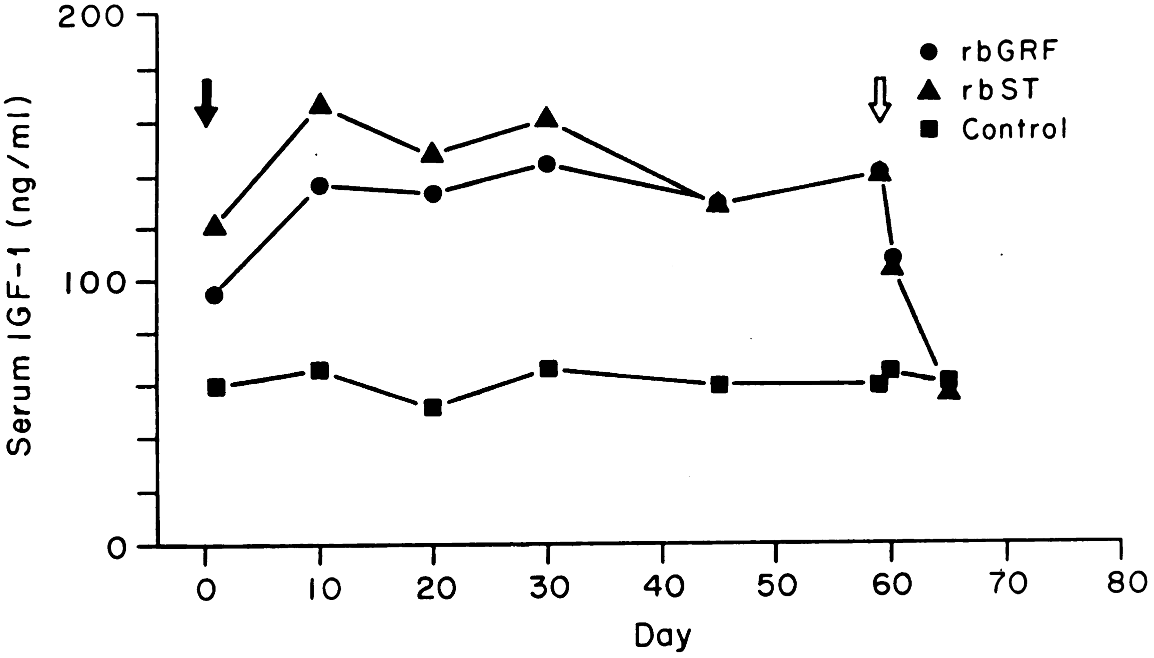

Figure 11. Serum concentrations of IGF-I of cows receiving 12 mg rbGRF/d, 29 mg rbST/d, or no treatment for 60 d. Beginning and end of treatment indicated by the solid and open arrows, respectively. Pooled SE of difference within a day was 15.8 ng/ml.

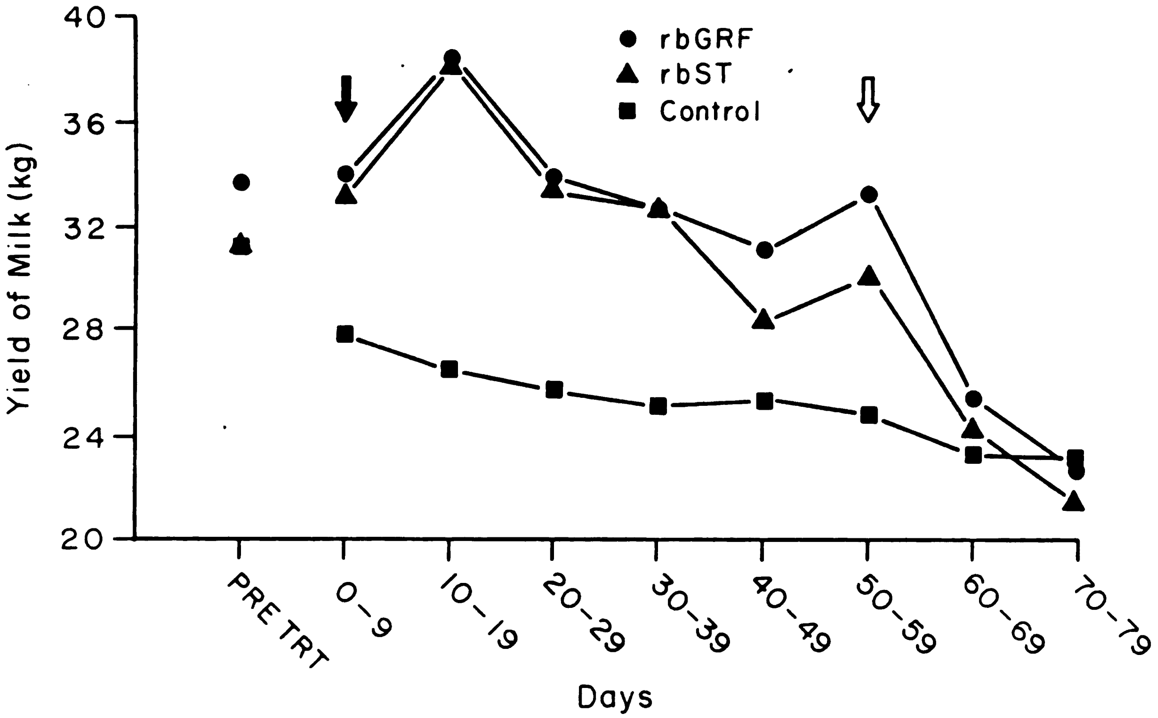

Figure 12. SCM yield of cows receiving 12 mg rbGRF/d, 29 mg rbST/d, or no treatment for 60 d. Each connected point represents the average of a treatment group (least squares means) within each 10-d period, adjusted by covariance for differences in pre-infusion milk yield. Beginning and end of treatment indicated by the solid and open arrows, respectively. Pooled SE of difference within a period was 1.4 kg/d.

SCM yields were adjusted by covariance for pre-infusion milk yield. During infusion with rbGRF and rbST, milk yield increased ($P<.01$) from control values of 25.1 ± 1.1 kg/d to 32.2 ± 1.1 and 35.5 ± 1.1 kg/d, respectively. The SCM yield of bGRF infused cows was greater ($P<.05$) than that of bGH infused cows. This difference was associated with the milk yield response between d 40 and 59. Following cessation of infusion (60 to 79 d), milk yield remained elevated for the first 10 d ($P<.06$) in cows previously receiving rbGRF compared with control and rbST-infused cows. Relative to controls (3.8 ± .15), infusion of rbGRF (4.4 ± .15) and rbST (4.2 ± .15) increased ($P<.05$) the percentage of milk fat from d 0 to 59. Relative to controls (12.5 ± .19) rbGRF (13.0 ± .19) and rbST (13.0 ± .19) increased ($P<.05$) the percentage of total solids in milk from d 0 to 59. Average percentages of protein (3.1 ± .05) and lactose (4.8 ± .08) in milk were similar among treatments throughout the study. Somatic cell count was unaffected by treatment and averaged 329,000 ± 179,000 cells/ml.

During pre-infusion (-10 to -1d) and the first 40 d of infusion (0 to 39d), DMI was not different among groups (Figure 13). From d 40 to 79 of the experiment, rbGRF tended to increase ($P<.08$) DMI relative to that of control cows. DMI was not different between cows treated with rbST or rbGRF during 40 to 79 d. Initial BW of all cows averaged 588.9 ± 52.0 kg, and BW of cows treated with rbGRF or rbST did not differ from that of controls within any period (data not shown). Initial BCS was not different among treatments (Figure 14). During infusion and post-infusion (d0 to 79), BCS of rbGRF- and rbST-treated cows were lower ($p<.01$) than those of control cows.

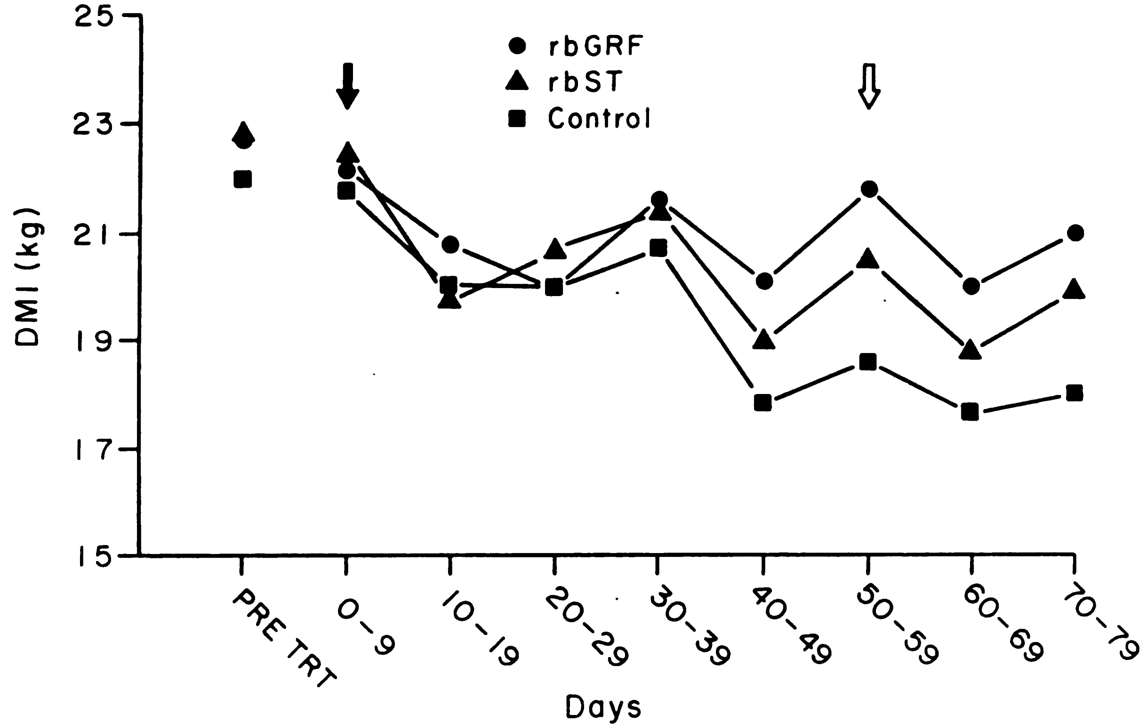

Figure 13. DMI of cows receiving 12 mg rbGRF/d, 29 mg rbST/d, or no treatment for 60 d. Each point represents the average of a treatment group (least squares means) within each 10-d period, adjusted by covariance for differences in initial BW. Beginning and end of treatment indicated by the solid and open arrows, respectively. Pooled SE of difference within a period was .99 kg/d.

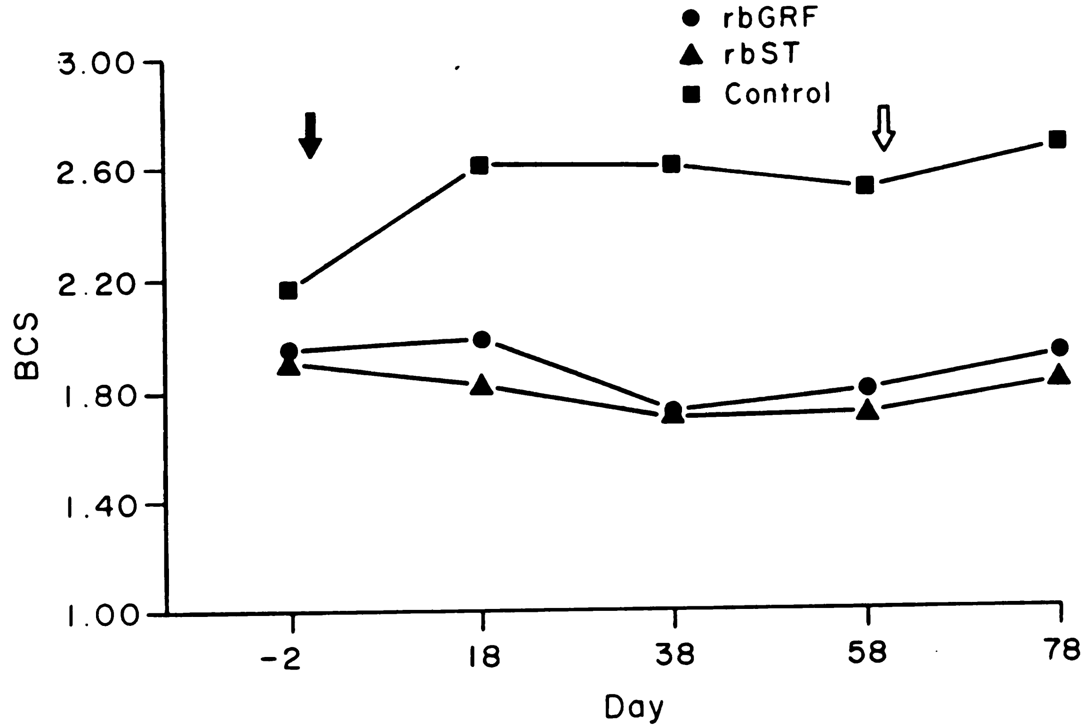

Figure 14. BCS of cows receiving 12 mg rbGRF/d, 29 mg rbST/d, or no treatment for 60 d. Each point represented the average of a treatment group (least squares means) on d -2, 18, 38, 58, and 78. Beginning and end of treatment indicated by the solid and open arrows, respectively. Pooled SE of difference within a period was 0.2.

Relative to controls, serum concentrations of NEFA (Figure 15) were increased ($p<.01$) by rbGRF on d 10, 20, 30 and 45, and by rbST on d 10, 20, and 45. Between rbGRF and rbST, differences ($p<.05$) in serum concentrations of NEFA were noted only at d 30. Serum concentrations of NEFA did not differ among cows on d 59 or after infusions ended.

DISCUSSION

Results of the present study clearly support the hypothesis that rbST and rbGRF are galactopoietic (Dahl et al., 1990; Peel and Bauman, 1987). However, the 10% greater response of milk yield to rbGRF relative to rbST is intriguing, in light of the fact that no differences in serum concentrations of ST or IGF-I were noted. Furthermore, following cessation of treatment, milk yield remained elevated in cows previously infused with rbGRF relative to rbST-infused and control cows. This evidence suggests that the galactopoietic action of rbGRF is not due solely to increases in total radioimmunoassayable concentrations of ST.

The increase in serum concentrations of NEFA and elevated milk fat percentage seen in the present study are often associated with bST and bGRF treatment (Dahl et al., 1990; Peel and Bauman, 1987). Indeed, this indicates that both rbGRF and rbST cows were mobilizing lipid reserves to meet the increased demand for energy at the mammary gland. Although rbGRF-infused cows tended to increase DMI in the latter portion of the study, this increase was likely due to their greater milk production relative to rbST-infused and control cows. Thus,

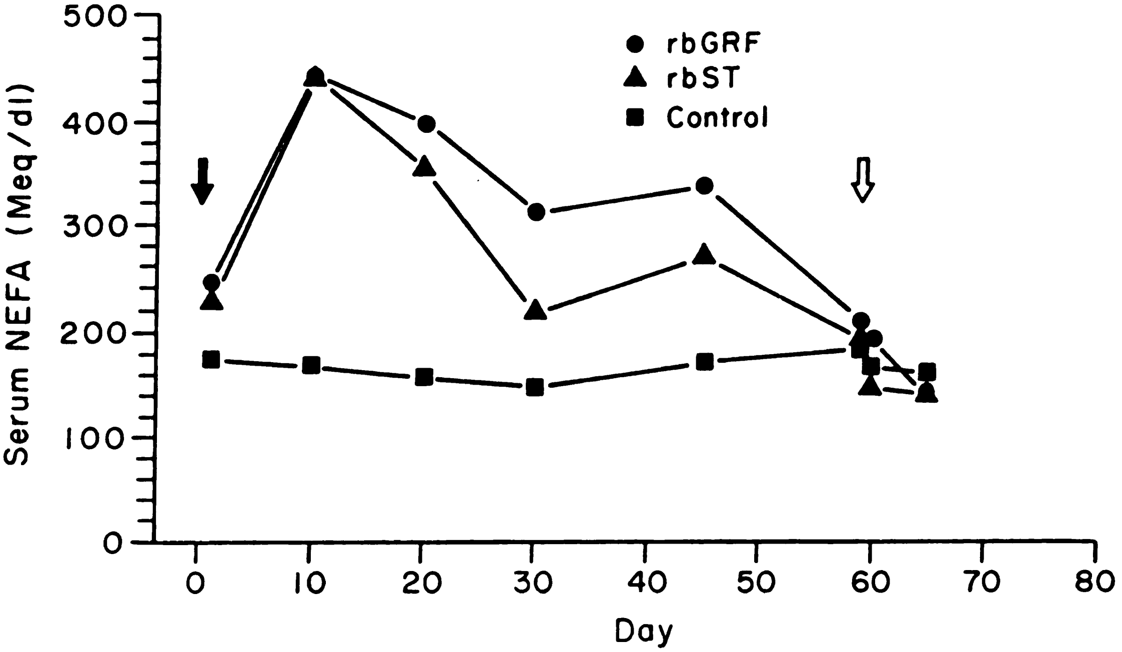

Figure 15. Serum concentrations of NEFA of cows receiving 12 mg rbGRF/d, 29 mg rbST/d, or no treatment for 60 d. Beginning and end of treatment indicated by solid and open arrows, respectively. Pooled SE of difference within a day was 40.7 meq/dl.

differences in gross energy metabolism do not explain the differences in milk production between rbGRF- and rbST-infused cows.

In some systems, the pattern of ST administration affects the response to ST. For example, serum concentrations of cholesterol, apoenzyme-E (Apo-E) and high density lipoproteins (HDL) in hypophysectomized rats given continuous infusions of ST are similar to those in intact controls, while hypophysectomized rats receiving twice daily injections of the same quantity of ST had depressed serum concentrations of cholesterol, Apo-E, and HDL (Oscarsson et al., 1989). Also, body growth is greater in hypophysectomized rats given ST by continuous infusion relative to single daily injections (Cotes et al., 1980). However, the galactopoietic response to ST in dairy cattle is unaffected by pattern of administration (McCutcheon and Bauman, 1986a; Fronk et al., 1983). In addition, pattern of administration of bST does not affect the increases in nitrogen retention observed with bST-treatment in steers (Moseley et al., 1982). Therefore, pattern of administration of bST does not appear to affect lactational or growth responses in cattle. Rather, the absolute amount of serum ST administered per day appears to determine the lactational or growth response.

Production of antibodies to exogenous bST has been reported in dairy cows (Zwickl et al., 1990). Antibodies to bST might be expected to decrease the galactopoietic response by interfering with the ligand-receptor interaction. In contrast, others report potentiation of the biological action of bST with concurrent administration of an antibody to bST (Bomford and Aston, 1990; Pell et al., 1990). Zwickl, et al. (1990) reported no adverse effect of antibodies formed to bST on the galactopoietic response to bST. In the present study, using IGF-I as an index of response to ST, there was no difference in response to exogenous (bST) or endogenous (rbGRF) ST. Thus, immunological depression of rbST activity is an unlikely explanation for the differences in milk yields.

Although GRF is considered to be a hypothalamic releasing factor, it was initially isolated from a pancreatic tumor (Rivier et al., 1982). Therefore, it is not surprising that GRF stimulates insulin secretion from pancreatic islets and islet cells of the rat in vitro (Green et al., 1990). In the intestine, GRF binds to vasoactive intestinal polypeptide (VIP) receptors stimulating adenylate cyclase activity in epithelial cells (Laburthe et al., 1983). In addition, GRF-like immunoreactivity has been isolated from duodenal tissues (Bruhn et al., 1985). Combined, this evidence suggests a direct action of GRF, possibly in the gastrointestinal tract. Indeed, GRF treatment increases digestibility of DM in growing steers (Lapierre et al., 1991) but GH does not (Peel and Bauman, 1987). Thus, differences in nutrient metabolism between rbGRF and rbST cannot be excluded when considering differencces in milk production. However, there is no difference in energy or protein digestibility in rbGRF- or rbST-treated lactating cows (Dahl et al., 1991).

Variant forms of ST have been reported in the cattle (Krivi et al., 1989; Hampson and Rottman, 1987). Indeed, Krivi et al. (1989) reported differences in galactopoietic activity of ST variants in lactating cows. Several ST-related peptides have been identified in the anterior pituitary gland (Sinha and Jacobson, 1988). Furthermore, somatomammotroph cells secrete a factor with a mitogenic effect on mammary epithelium (Chomzynski and Brar, 1989). It is possible that GRF causes secretion of many ST variants and(or) other factors, as opposed to the single ST variant supplied by rbST. Thus, I speculate that differences in the proportion of variants of ST in serum may explain the differences in milk yield response in the present study.

In summary, milk yield was greater in cows infused with rbGRF versus rbST, despite similar increases in serum concentrations of ST and IGF-I. It is concluded that the galactopoietic action of GRF is not associated solely with increases in serum total radioimmunoassayable concentrations of ST in serum. However, the mechanism remains to be determined whereby rbGRF stimulates milk yield to a greater extent than rbST.

SUMMARY AND CONCLUSIONS

The objectives of the experiments described in this dissertation were to examine the long term galactopoietic effects of GRF and to compare those effects with those of bST. Results presented in Chapter 1 indicate that GRF increased milk yield for up to 60 d during treatment, and for 15 days following cessation of treatment. Also, GRF increases serum concentrations of ST and IGF-I. The effects of GRF are dose related. Responsiveness of ST secretion to GRF did not diminish over a 60 d period. Thus, refractoriness to GRF at the anterior pituitary gland did not occur. In general, the effects of GRF in lactating cows are similar to those of bST. Indeed, GRF increased yield of milk components and serum concentrations of NEFA. Treatment with GRF did not affect serum concentrations of PRL, INS or glucose, nor did GRF affect DMI, BW, or BCS.

The study presented in Chapter 2 involved comparison of the galactopoietic effects of GRF and bST. Optimal doses and routes of administration of GRF and bST were selected based on results of previous independent studies. GRF increased milk yield to a greater extent than bST which increased milk yield above that of control cows. However, a similar pattern (i.e., GRF > bST > control) of response of serum concentrations of ST and IGF-I was observed. Thus, whether GRF-induced increases in milk yield were totally a function of increased serum concentrations of

ST remained unknown. Treatment of cows with GRF or bST had no effect on DM digestibility, although GRF tended to increase DMI late in the experiment.

The study presented in Chapter 3 was a repeat of that in Chapter 2, however, by use of appropriate doses of bST on GRF, similar serum concentrations of ST were obtained in the GRF and bST treatment groups. In addition, GRF- and bST-treatment induced similar increases in serum concentrations of IGF-I. Milk yield, however, was 10% greater during GRF treatment compared with bST treatment. Thus, it is concluded that GRF has galactopoietic effects not solely mediated by increases in serum concentrations of total radioimmunoassayable ST or IGF-I.

I hypothesize that the greater galactopoietic effect of GRF results from GRF-induced secretion of various isoforms of ST, versus the single isoform delivered by bST. I speculate that the combination of isoforms coordinates to a greater degree than bST the response of liver, adipose and muscle tissue to support lactation. Therefore, one future area of research is to identify and characterize ST variants in cattle, specifically, those induced by GRF treatment. It is possible that differences in binding proteins affect activity of ST and IGF-I. Thus, a parallel area of research is investigation of effects of GRF treatment on serum binding proteins of ST and IGF-I in lactating dairy cattle.

Another area of future research is that of the effects of GRF and in turn, ST on mammary gland function. Reports of work in this area of research are scarce. A combination of techniques could be used to determine the effects of GRF treatment on mammary function. For example, plasmin concentrations in milk could be used as an index of mammary cell loss, RNA/DNA ratios of mammary biopsies and 5'-monodeiodinase activity could be used as indices of mammary cell metabolic capacity. Subsequent experiments that involve sacrifice of animals during treatment would allow assessment of GRF-induced effects on mammary growth.

In conclusion results of this research demonstrate that GRF is galactopoietic in cattle for up to 60 d. There is no evidence of pituitary refractoriness to GRF for up to 60 d. Furthermore, GRF is more galactopoietic than bST. As an alternative to bST, exogenous GRF can manipulate endogenous ST secretion and increase the efficiency of milk production in dairy cattle.

APPENDICES

APPENDIX A

TABLE 6. Feed composition (DM basis) of the diet fed to lactating Holstein cows infused with 0, 1, 3, or 12 mg rbGRF/d[1].

Ingredient	Percentage
Alfalfa haylage	24.0
Corn silage	24.0
Ground shell corn	31.9
Soybean meal	17.9
Mineral mix[2]	2.0
Salt	.2

[1] Diet contained calculated values of 17.8% CP, 1.68 Mcal/kg NE_L, 15.0% crude fiber, 18.4% ADF, 30.3% NDF, .78% Ca, and .46% P.

[2] Mineral and vitamin premix contained .89% S, 5709 ppm Zn, 3101 ppm Cu, 7819 ppm Mn, 181 ppm I, 132 ppm Se, 1.74×10^6 IU vitamin A, 5.11×10^5 IU vitamin D, and 7.3×10^3 IU vitamin E per kg DM.

APPENDIX B

TABLE 7. Feed composition (DM basis) of the diet fed to lactating Holstein cows treated with 14 mg rbST or 12 mg rbGRF/d[1].

Ingredient	Percentage
Alfalfa haylage	30.2
Corn silage	11.2
Ground shell corn	37.3
Soybean meal	9.9
Whole cotton seed	8.7
Mineral mix[2]	2.4
Salt	.3

[1] Diet contained calculated values of 17.0% CP, 1.72 mcal/kg NE_L, 15% crude fiber, 19.3% ADF, 32.4% NDF, .97% Ca, and .49% P.

[2] Mineral and vitamin premix contained .89% S, 5709 PPM Zn, 3101 PPM Cu, 7819 PPM Mn, 181 PPM I, 132 PPM Se, 1.74×10^6 IU Vit. A, 5.11×10^5 IU Vit. D, and 7.3×10^3 IU Vit. E per kg DM.

APPENDIX C

TABLE 8. Feed composition (DM basis) of the diet fed to lactating Holstein cows treated with 29 mg rbST or 12 mg rbGRF/d[1].

Ingredient	Percentage
Alfalfa haylage	30.2
Corn silage	15.1
Ground shell corn	28.1
Soybean meal	15.3
Whole cotton seed	8.7
Mineral mix[2]	2.6

[1] Diet contained calculated values of 17.3% CP, 1.68 mcal/kg NE_L, 15% crude fiber, 19.8% ADF, 27.9% NDF, .91% Ca, and .45% P.

[2] Mineral and vitamin premix contained .89% S, 5709 PPM Zn, 3101 PPM Cu, 7819 PPM Mn, 181 PPM I, 132 PPM Se, 1.74×10^6 IU Vit. A, 5.11×10^5 IU Vit. D, and 7.3×10^3 IU Vit. E per kg DM.

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