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thesis entitled

Effects of Recombinant Bovine Somatotropin (rbST) and rbST Releasing Factor on Somatotropin Secretion, Mammary Function and Body Growth Adaptations in Lactating Dairy Cows

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

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Date November 24, 1993

EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN (rbST) AND rbST RELEASING FACTOR ON SOMATOTROPIN SECRETION, MAMMARY FUNCTION AND BODY GROWTH ADAPTATIONS IN LACTATING DAIRY COWS

By

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A THESIS

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

MASTER OF SCIENCE

Department of Animal Science

1993

ABSTRACT

EFFECTS OF RECOMBINANT BOVINE SOMATOTROPIN (rbST) AND rbST RELEASING FACTOR ON SOMATOTROPIN SECRETION, MAMMARY FUNCTION AND BODY GROWTH ADAPTATIONS IN LACTATING DAIRY COWS

By

Mario Binelli

The objective of this thesis was to compare the effects of recombinant bovine somatotropin releasing factor (rbGRF) and recombinant bovine somatotropin (rbST) on somatotropin secretion, mammary function and body growth adaptations in lactating, primiparous dairy cows. Cows (118 d of lactation) were infused for 63 d with doses of rbGRF and rbST that similarly elevated ST concentration in serum. Both rbGRF and rbST increased yield of milk, yield of milk components, weight of organs, mobilization of adipose tissue, accretion of carcass lean tissue and metabolic activity of mammary tissue. There was no difference between rbGRF- and rbST-treated cows in the variables measured. Development of refractoriness to either rbGRF or rbST did not occur in terms of ST secretion, mammary function or body growth.

None of the variables analyzed in this thesis provided strong evidence for galactopoietic effects of rbGRF independent of ST.

I dedicate this thesis to four women that have, in different ways and to different extents, influenced my way of thinking and the way I look at the world today.

Ms. Carmem Lucia Rezende de Oliveira, my mother;

Ms. Maria Estela Machado Binelli, my other mother;

Ms. Marisia Anna Violante (in Memoriam), my friend;

Ms. Eliana Kampf Binelli, my wife.

ACKNOWLEDGMENTS

I would like to acknowledge some people for their help in the accomplishment of the goals I set forth for myself during my Master's program at Michigan State University.

First I would like to thank the members of my committee, Drs. Roy Fogwell, Thomas Herdt and Michael VandeHaar for their advice and expertise during the execution of my project. Most specially, I would like to thank my advisor, Dr. Allen Tucker, for the guidance, patience and strong commitment to excellence in graduate student training.

I would like to thank Bill VanderKooi for all his help throughout the experiment, but most important, for his strong friendship.

I thank Mr. Larry Chapin for his help in the execution of the project and for instruction in computer matters and statistical analysis.

I also thank Dr. Paula Gaynor and Mr. Jim Liesman for the applied and philosophical discussions about animal biology and experimental design.

I want to thank the dairy barn crew, the meats laboratory crew and the dairy nutrition laboratory crew for their hard work and cooperation.

I express my gratitude to fellow graduate students for their support and assistance throughout graduate school, especially Brent Buchanan, Christine Simmons, Amy Terhune and Fermin Jimenez from the Animal Reproduction Laboratory.

From the Upjohn Company I thank Drs. Mike Moseley and Jim Lauderdale for providing the economic support for the realization of this project. Glenn Alaniz and

Bill Claflin for performing the Vetport surgeries and Bud Krabill for performing the ST assay.

I am especially thankful to CNPq, my sponsor from Brazil, for providing the funding for my Master's program. I am indebted to Dr. João Lucio de Azevedo, Dean of the College of Agriculture (ESALQ) of the University of São Paulo (USP), for all the support and advice before and after I came to the US.

Finally I thank my family and friends back in Brasil for all the support and also my family in the US, which is my wife Nana, for her love, patience and unending support throughout my graduate studies.

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LIST OF ABBREVIATIONS

b	bovine
BCS	body condition score
BW	body weight
DMI	dry matter intake
DNA	deoxyribonucleic acid
EB	energy balance
GRF	somatotropin releasing factor
GT	glucose transporter
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
mRNA	messenger RNA
NEFA	non-esterified fatty acids
NS	not significant
r	recombinant
RNA	ribonucleic acid
s.c.	subcutaneous
SCC	somatic cell count
SCM	solids corrected milk
ST	somatotropin
STBP	somatotropin binding protein

INTRODUCTION

The world's human population is increasing at a fast rate, and consequently the need for basic nutrients, including carbohydrates, lipids, proteins and minerals, is also increasing. Animal agriculture has historically been one of the most important sources of nutrients for humans. Dairy cows efficiently metabolize feed nutrients and synthesize milk, which provides protein and energy in a suitable form for human consumption. Therefore, use of techniques that increase milk production efficiency is of vital importance to minimize input of resources and maximize output of high quality nutrients available to humans.

Recombinant (r) bovine (b) somatotropin (ST) is a product of biotechnology that has a dramatic influence on the productivity of dairy cows. For example, compared with non-injected controls, injections of bST increase milk yield an from 10 to 25% without affecting concentration of protein, lactose or fat in milk (Bauman, 1992).

Several possible mechanisms contribute to the galactopoietic effects of rbST. For example, rbST may increase mammary epithelial cell numbers and(or) metabolic activity. Also, rbST treatment is associated with elevated plasma concentration of non-esterified fatty acids (NEFA) in serum and reduced carcass fat percentage (McDowell et al., 1987a, Solderholm et al., 1988; Dahl et al., 1990; Dahl et al., 1991; Dahl et al., 1993), indicating reduced accumulation and increased mobilization of fat in adipose tissue. Thus, rbST may increase energy available for milk production. Thirdly, changes in organ size after rbST treatment have been reported (Johnsson et al., 1985 and Brown

et al., 1989), which are indicative of homeorhetic adaptation of the whole organism to support lactation.

An alternative to administration of exogenous rbST is to regulate endogenous secretion of ST. Somatotropin releasing factor (GRF) is a neurohormone that stimulates secretion of ST from the anterior pituitary gland (Barinaga et al., 1985). Injections of GRF acutely and specifically increase serum concentrations of ST several fold in lactating dairy cows (Johke et al., 1984; McCutcheon et al., 1984; Enright et al., 1986; Enright et al., 1987; Dahl et al., 1990 and Dahl et al., 1993). Furthermore, concentrations of ST in serum remained elevated throughout 60 d of intravenous infusions of GRF and milk yield of cows was increased as much as 46% in relation to controls (Dahl et al., 1993). Moreover, Dahl et al. (1993) showed that rbGRF-infused cows produced more milk than cows infused with rbST, despite the same concentration of ST in serum for both groups. Therefore, the galactopoietic effects of GRF may involve mechanisms other than increased secretion of ST.

Specific objectives of this thesis were to achieve similar concentrations of ST in serum of cows by constant infusion of proper doses of rbGRF or rbST and then: 1) compare the effects of rbGRF and rbST on organ weights, carcass composition, and adipose tissue mobilization, 2) compare the effects of rbGRF and rbST on mammary function, and 3) compare the effects of rbGRF and rbST on anterior pituitary size and ST content.

REVIEW OF LITERATURE

Generalities about ST

Somatotrophs are a population of specialized cells in the anterior pituitary gland (Frohman et al., 1992) that secrete the peptide hormone, ST. Bovine ST produced by the pituitary can have either 191 or 190 amino acids, with either a leucine or valine at position 127. Thus, there are four variants of bST produced endogenously (Wood et al., 1989). Synthesis and release are the two main events involved in secretion of ST. Both events are regulated primarily by two hypothalamic peptides, GRF and somatostatin. Somatotropin releasing factor stimulates and somatostatin inhibits secretion of ST (Frohman et al., 1992). Somatotropin releasing factor stimulates transcription of the gene coding for ST (Barinaga et al., 1983 and Gick et al., 1984) and also stimulates release of ST from somatotrophs into the circulation (Barinaga et al, 1985; Cella et al., 1985). In contrast to GRF, somatostatin inhibits synthesis of ST (Tanner et al., 1990 and Sugihara et al., 1993) and GRF-stimulated release of ST (Lussier et al., 1991 and Chen and Clarke, 1992). ST is released in a pulsatile manner. This secretory pattern is dependent on interactions between GRF and somatostatin at the somatotrophs (Frohman et al., 1992), but such interactions are currently not well understood. Somatotropin itself regulates its own secretion by negative feedback mechanisms. In rats, both peripheral and intracerebroventricular administration of ST inhibit endogenous secretion of ST (Tannenbaum, 1980 and Abe et al., 1983).

Upon release from the anterior pituitary cells, ST enters the blood stream where it forms a complex with a specific carrier protein, ST binding protein (STBP)

(Baumann, 1993). The STBP binds from 45 to 60 % of all circulating ST (Baumann, 1993), and as a consequence it lowers metabolic clearance as well as degradation of ST (Baumann et al, 1987 and Bauman et al, 1989). Somatotropin is cleared from the circulation by glomerular filtration and degradation in the proximal tubule and also by receptor-mediated internalization into cells and subsequent lysosomal degradation in target tissues (Baumann, 1993).

The receptor for ST is a single peptide containing an external, a transmembrane and an internal domain. The external domain is similar to STBP (Waters et al., 1990). The receptor apparently belongs to a family of receptors which include the prolactin receptor and a number of interleukin receptors (Mathews, 1991 and Kelly et al., 1992). The ST receptor may be internalized following binding of ST, but the role of internalization in signal transduction is unclear (Roupas and Herington, 1989).

Somatotropin exerts specific actions in a variety of tissues, such as skeletal muscle, adipose tissue, liver and mammary gland (Thorner et al., 1992). There are two theories regarding the actions of ST: the ST hypothesis, where ST actions occur by specific binding of ST to a receptor on the cell surface of a target tissue and triggering of a specific response, and the somatomedin hypothesis (Underwood and Wyk, 1992), where ST effects in vivo are not direct, but mediated by other ST-dependent factors. It now appears that most biological activity in tissues attributed to these ST-dependent factors are due to two distinct peptides secreted in an endocrine fashion from the liver and in an autocrine/paracrine fashion from other tissues. These peptides are insulin-like growth factor-I (IGF-I) and IGF-II, which are also called somatomedins. The ST and somatomedin hypotheses are not mutually exclusive; therefore, both ST and somatomedins may affect a given target tissue by different mechanisms (Underwood and Wyk, 1992). Insulin-like growth factor I also plays a role in regulation of ST secretion,

exhibiting feedback effects at both the pituitary and hypothalamic levels to decrease ST secretion (Berelowitz et al., 1981).

Production responses of cows to ST

Asdel (1932) was the first to report that injections of pituitary extracts were galactopoietic in goats. Asimov and Krouze (1937) confirmed such an effect in dairy cows. Studies of effects of ST on lactation of dairy cows were performed using pituitary-derived ST until 1982, when Bauman et al. published results of the first experiment utilizing recombinantly-derived ST. Biotechnology made available a large quantity of recombinant ST, and recently many studies have been performed to clarify effects of ST on the biology of lactation of dairy cows and to develop ST as a galactopoietic tool for the dairy producers.

Administration of rbST causes a gradual increase in milk yield from cows over the first few days of treatment. Milk yields reach a maximum during the first week and remain elevated until treatment is stopped (Bauman and Vernon, 1993). Concentration of fat, protein and lactose in milk is not substantially altered during ST treatment (Bauman, 1992). Therefore, total yield of components increase in the same proportion as milk yield increases.

Production efficiency of lactating dairy cows is increased with the use of rbST. Cows receiving rbST utilize a smaller proportion of consumed nutrients for body maintenance, and therefore utilize a greater proportion for milk synthesis compared with untreated controls (Peel and Bauman, 1987).

Homeorhesis is defined as the coordinated changes in metabolism of body tissues necessary to support a physiological state such as lactation. (Bauman and Currie, 1980). Somatotropin is a homeorhetic controller that shifts the partitioning of nutrients so more nutrients are available for use in milk synthesis (Bauman, 1992). These

orchestrated changes likely involve direct effects of ST on some tissues such as adipose and liver, as well as indirect effects, mediated by IGF-I for example, in other tissues such as mammary gland (Bauman, 1992).

Two main theories have been formulated to explain the sequence of events that lead to the galactopoietic effects of rbST. A "pull" theory states that rbST first acts directly or indirectly at the mammary gland providing the stimulus for milk synthesis. Increased extraction of nutrients for milk synthesis by the mammary gland would cause a shift in metabolism of other organs and tissues to support the increased milk synthesis. A second theory , the "push " theory, suggests that body organs and metabolism of tissues are first modified by rbST to direct more nutrients to the mammary gland, which in turn would respond with increased milk synthesis.

In the discussion that follows I will review the main effects of rbST on tissues and organs that are critically involved in galactopoiesis.

Somatotropin effects on whole body metabolism

To the animal scientist and also to the dairy farmer, the most interesting aspect of the homeorhetic phenomenon directed by rbST is increased milk yield. Milk yield is increased as an immediate consequence of increased uptake of milk precursors by the mammary gland (Collier, 1985). Increased uptake of milk precursors must be a result of increased availability of nutrients to the mammary gland , increased ability of the mammary gland to extract milk precursors from the circulation or both (Miller et al., 1991a and Miller et al., 1991b).

Increased availability of nutrients to the mammary gland can occur as a consequence of increased dry matter intake (DMI), increased feed digestibility, altered synthesis and degradation of milk precursors, restricted uptake of precursors by other tissues, increased mobilization of body reserves or combinations of these events.

Dry matter intake gradually increases in cows treated with rbST. Cows increase their voluntary feed intake proportionately to the increased need for extra nutrients required for increased production of milk (Bauman, 1992). However, adaptations in metabolism, such as use of alternative fuels to provide energy for metabolic processes and sparing glucose for milk production, are critical during the initial period of rbST treatment, when milk yield increases but feed intake does not (Bauman and Vernon, 1993). Digestibility of feed does not change for lactating dairy cows treated with rbST (Peel and Bauman, 1987).

Glucose in blood is the sole precursor for lactose synthesis in milk. Peel and Bauman (1987) demonstrated that metabolic adaptations in glucose turnover and oxidation provided the additional glucose required for increased lactose synthesis during rbST administration. Such adaptations include a reduction in glucose oxidation which accounts for 30% of the additional glucose necessary for lactose synthesis. In addition, there is increased hydrolysis of triacylglycerides providing glycerol that may be transformed into glucose through the gluconeogenic pathway. Recently, Cohick et al. (1989) reported increased hepatic production of glucose for cows treated with rbST. McDowell et al. (1987a) reported decreased use of glucose by hind limb muscle in multiparous dairy cows treated with rbST, which suggests a shift in the priority of glucose distribution among tissues favoring the mammary gland.

Amino acids are a common source of carbon for gluconeogenesis, but due to the increased demand for amino acids used in synthesis of milk protein in cows treated with rbST, this is an unlikely source of glucose (Bauman, 1992). Similarly, increased capacity of liver slices of cows treated with rbST to synthesize glucose from propionate did not account for the additional glucose necessary for lactose synthesis, as rates of conversion of propionate to CO^2 (i.e., oxidation of glucose) were proportionately elevated (Pocius and Herbein, 1986).

Mobilization of fat from adipose depots is commonly observed in cows treated with rbST, especially if they are in negative energy balance (EB). Such mobilization is indicated by elevated levels of NEFA in serum (Bauman and Vernon, 1993). The mammary gland may directly utilize mobilized fatty acids for milk fat synthesis or fatty acids may undergo β-oxidation and provide energy for metabolic processes in organs and tissues of the animal, including the mammary gland (Emery and Herdt, 1991). Utilization of NEFA as metabolic fuel facilitates the reduction of glucose oxidation previously mentioned (Bauman and Vernon, 1993). During positive EB, the major effect of rbST is to inhibit lipid synthesis (Bauman and Vernon, 1993). As a consequence, decreased utilization of nutrients for body fat stores would allow redirection of nutrients towards the mammary gland to support increased milk synthesis (Bauman and Vernon, 1993).

Changes in size of organs may be part of the physiological response of the lactating dairy cow to the homeorhetic changes promoted by rbST treatment. For example, Brown et al. (1989) reported increased foregut tissue mass for cows treated with rbST for 18 weeks in relation to untreated controls although there were no differences in weights of liver, kidney, heart, lung or spleen. Increase in foregut tissue mass was probably associated with increased total metabolic activity of the tissue and increased intake of feed.

Somatotropin effects on the mammary gland

Now it is clear that rbST increases the ability of the mammary gland to extract milk precursors from the circulation, thereby increasing milk yield (Miller et al. 1991a, Miller et al. 1991b and Bauman and Vernon, 1993). However, the putative effector of rbST actions is undetermined and both direct action of rbST on the mammary gland and indirect action through IGF-I mediation have been proposed.

Mammary growth can occur in goats and rodents during established lactation (Glimm, 1992). However, adding ST to lactating bovine mammary explants in culture does not stimulate cellular proliferation and generally does not alter rates of synthesis of casein, fat or α-lactalbumin (Gertler et al., 1983 and Baumrucker and Stemberger, 1989). McDowell et al. (1987b) tested for a direct effect of rbST on the mammary gland using a closed arterial infusion technique, but milk yield was not altered. Attempts to detect ST receptors in bovine mammary tissue have not been successful (Gertler et al., 1984; Akers, 1985). Furthermore, concentrations of ST in milk are very low and not appreciably altered by rbST treatment (Juskevich and Guyer, 1990). A recent study reported the presence of messenger ribonucleic acid (mRNA) for ST receptors in mammary alveolar cells from lactating sows (Magri et al., 1990), but the message level was low relative to what is normally found in liver (Bauman and Vernon, 1993). In addition, Keys and Djiane (1988) found no functional ST receptor protein in mammary tissue. Although the message for ST receptor is present , either it is not translated or the number of receptors produced is too low to be detected by conventional techniques. Therefore, ST seems to play an indirect role in galactopoiesis, acting as a homeorhetic controller of metabolism (Glimm, 1992) and through another hormonal mediator (IGF-I) acting at the mammary gland.

As a result of the evidence that ST does not act directly on mammary epithelial cells, IGF-I has been implicated as a possible mediator of ST actions. Administration of rbST to lactating dairy cows increases concentration of IGF-I in serum (Bauman and Vernon, 93; Dahl et al., 93). Also, rbST elevates abundance of IGF-I mRNA in the liver and mRNA for the receptor of IGF-I in mammary tissue (VanderKooi, 1993) and binding of IGF-I to mammary tissue increases during lactogenesis (Dehoff et al., 1988). In vitro, IGF-I stimulates casein synthesis in mammary cells from lactating cows (Hanigan et al., 1992) and increases both casein and

glucose transport in mammary explants of mid-pregnant mice (Prosser et al., 1987). However, research on the effects of IGF-I in vivo has generated inconsistent results. For example, Davis et al. (1989) reported no effect of a jugular infusion of IGF-I on milk yield of goats, even though serum concentrations of IGF-I were comparable with those of a second group of animals infused with ST, which showed an increase in milk yield. In contrast, Prosser et al. (1990) showed that infusion of IGF-I into the pudendal artery of lactating goats for 6 h increased milk production by 30 %, as compared with the non-infused hemigland of the same animal. Interpretation of such results, however, must take into account the role of IGF binding proteins (BP). The IGFBP are soluble, large-molecular-weight proteins found in blood. The IGFBP have been postulated to transport IGF in blood, to retard IGF degradation, to facilitate transvascular movement of IGF and /or to modulate IGF actions at specific sites (Bauman and Vernon, 1993). Moreover, rbST treatment increases IGFBP-3 and decreases IGFBP-2 concentrations in serum (VanderKooi, 1993). Therefore, it is possible that increased concentration of ST is necessary for full expression of IGF-I actions at the mammary gland.

Increased ability of the mammary gland to extract milk precursors from the circulation may be due to increased mammary secretory cell numbers and/or decreased cell loss. Total mammary deoxyribonucleic acid (DNA) is a measure of cell numbers. Capuco et al (1989) and Baldwin (1990) reported that rbST did not affect total mammary DNA content of cows treated during mid-lactation. In contrast, Knight et al. (1990) reported that ST prevented the decline that normally occurs in mammary cell numbers during lactation in goats. Politis et al. (1990) observed a lower level of plasmin in milk, a serine protease associated with mammary gland involution in cows treated with rbST. This agrees with a possible role of rbST in increasing maintenance / decreasing loss of mammary cells.

Increased synthetic capacity of mammary cells is another possible explanation for increased ability of the mammary gland to synthesize milk when cows are treated with rbST. Increased synthesis of enzymes involved in milk production or increased activity of such enzymes may increase synthetic capacity of mammary cells. Baldwin (1990) demonstrated that rbST-treated cows had increased total RNA per mammary gland. Since RNA is an index of metabolic activity of cells and protein synthesis ability, an increase in RNA may increase translation of key enzymes involved in milk synthesis and proteins involved in nutrient uptake. Thyroxine-5'-monodeiodinase is an enzyme that converts thyroxine to triiodothyronine in several tissues. Capuco et al. (1989) reported an elevation in activity of thyroxine-5'-monodeiodinase in mammary tissue of cows receiving rbST. Thus, it is likely that rbST increases availability of triiodothyronine in the mammary gland.

A family of mammary gland specific glucose transporters is responsible for mammary cell uptake of glucose from the circulation for milk synthesis. Fawcett et al. (1991) investigated the effect of ST and prolactin in numbers of type 1 glucose transporters (GT-1) on mammary epithelial cell membranes of rats. An antiserum against ST specifically decreased the number of GT-1 on mammary cell membranes, but concurrent injections of ST with ST-antiserum returned GT-1 levels to normal. They concluded that ST up-regulates GT-1 numbers in mammary tissue of rats.

Rate of milk synthesis and secretion depends on availability of milk precursors, which in turn depends on rate of mammary blood flow and uptake by the mammary gland (Collier, 1985). Changes in mammary synthetic activity in response to rbST are supported by increased nutrient availability induced by the homeorhetic effect of rbST and also by increased mammary blood flow (Fullerton et al., 1989). Davis et al. (1988) measured cardiac output and mammary gland blood flow in lactating Jersey cows in response to rbST. Injections of rbST increased cardiac output and mammary gland

blood flow. Forty eight percent of the increased cardiac output could be accounted for by the increased blood flow perfusing the mammary gland. Such changes would permit the mammary gland to receive more nutrients per unit time and a greater share of the nutrients than other tissues. These authors speculated that stimulation of mammary metabolism increased output of a local vasodilator, which decreased mammary vascular resistance, thereby increasing blood flow.

Somatotropin effects on lipid metabolism

The lactation cycle of dairy cows may be divided into two distinct phases: an initial phase in early lactation that is characterized by negative EB, and a subsequent phase during mid/late lactation where the cow is in positive EB. In early lactation, high producing dairy cows cannot consume sufficient energy to meet the energy demand for milk production (NRC, 1989). Therefore, nutrients are partitioned from peripheral tissues, mainly adipose tissue, to the mammary gland to support elevated milk synthesis (Miller et al., 1991b). In fact, cows frequently mobilize 30 to 50 kg of fat during the first few weeks of lactation (Emery and Herdt, 1991). As lactation progresses, cows increase their DMI while milk production eventually declines, leading to a state of positive EB, where intake of feed provides nutrients in excess of those needed for milk production. As a result, body reserves are replenished.

Somatotropin has direct effects on adipose tissue. For example, in rats treated with ST, adipocytes had decreased number of glucose transporter proteins (Louveau et al., 1991) and decreased activities of key lipogenic enzymes, such as fatty acid synthase (Mildner and Clarke, 1991). Collectively, increased number of glucose transporters and decreased activity of fatty acid synthase would cause a net reduction in lipid accretion by adipose tissue. But, the effects of rbST on adipose tissue of lactating dairy cows depends on EB. For example, when rbST treatment induces negative EB,

lipid mobilization is increased, as reflected by decreased body fat and chronic elevation of circulating NEFA; therefore, lipolysis is stimulated (Barbano et al., 1992). However, when rbST-treated cows are in positive EB, lipogenesis in adipose tissue is decreased , but mobilization of body fat (i.e., lipolysis) is unaffected (Bauman, 1992).

Miller et al. (1991b) treated cows with rbST from d 71 to d 126 of lactation and observed that patterns of nutrient delivery and uptake by the mammary gland were similar between cows in early lactation (i.e., negative EB) and cows treated with rbST. For example, concentration of NEFA in serum and uptake of NEFA by the mammary gland were elevated for both groups. Therefore, rbST treatment decreased EB leading cows to an energy status similar to that of cows in early lactation.

Increased mobilization of NEFA is consistently observed in animals in negative EB as a result of rbST treatment (Peel and Bauman, 1987 and Dahl, et al., 1993). Peripheral tissues utilize NEFA as an alternative to glucose as a source of energy and for milk fat synthesis (Bauman, 1992). In fact, oxidation of NEFA increased 93 % while glucose oxidation decreased 19 % during rbST administration to dairy cows.

Somatotropin effects on carcass composition

Effects of rbST on carcass lipid content are dependent on EB status of the animal. If animals are in positive EB, rbST reduces lipogenesis rate, whereas the effects on lipolysis are minimal (Etherton and Louveau, 1992). This represents the typical situation for growing animals treated with rbST, but is also observed with rbST treatment of lactating cows in substantial positive EB (Etherton and Louveau, 1992). In contrast, when animals are in negative EB, rates of lipogenesis are already low and rbST treatment increases rates of lipid mobilization (Bauman et al., 1988 and Etherton and Louveau, 1992). A large number of studies with animals treated with rbST in various physiological states consistently show decreased carcass fat (Enright, 1989; Moseley, et

al., 1992 and Vestegaard et al. 1993). Carcass lipid content is decreased more when rbST treatment is given to animals in lower EB status.

In early lactation and during negative EB, less protein than fat is mobilized. Moreover, after mobilization ceases, protein is replaced sooner than fat. Therefore, negative protein balance is not as severe and duration is shorter than negative EB (Ferguson and Otto, 1989). In addition, rbST treatment increases lean tissue growth in growing animals (Eisemann, et al., 1989; Maltin et al., 1990 and Vestegaard et al. 1993) because rbST increased net protein synthesis rate to overcome a smaller increase in protein degradation rate (Simmons, 1993). Because protein is continuously recycled, the increase in protein synthesis, both in muscle and whole body may exceed protein accretion by as much as two fold (Simmons, 1993). Therefore, increased protein synthesis is not directly proportional to increased protein accretion. Conversely, for mature Holstein cows, rbST treatment did not alter total amount of lean tissue (Solderholm et al., 1988 and Brown et al., 1989). It is possible that in the overall scheme of nutrient partitioning promoted by rbST, skeletal muscle has a lower priority than the mammary gland, especially in mature cows, where skeletal growth has stopped.

Production responses of cows to GRF

As mentioned previously, GRF is the primary endogenous stimulator of synthesis and release of ST (Frohman et al., 1992). Therefore, it is not surprising that administration of rbGRF to lactating dairy cows is galactopoietic (Dahl et al., 1993). In fact, among a wide variety of experimental conditions regarding stage of lactation, dose, route, frequency and duration of administration, a consistent increase in milk yield (9.6 to 46 %) was reported (Dahl et al., 1990 and Dahl, 1991).

It was first postulated that the galactopoietic effects of GRF were attributed solely to increased ST secretion. In a series of experiments conducted by Dr. G.E. Dahl

at Michigan State University, the galactopoietic effects of rbGRF and rbST were compared in dairy cows. In an initial experiment, 12 mg per d of rbGRF was continuously infused i.v. for 60 d, and milk production of cows increased an average of 46 % in relation to controls (Dahl et al., 1990). Next, doses of rbGRF and rbST that were reported to maximize the galactopoietic response were administered for 60 d in lactating dairy cows. Both rbGRF- and rbST-treated cows had increased solids-corrected milk (SCM) yield as compared with controls, but average SCM for rbGRF-treated cows was greater than for rbST-treated cows (Dahl et al., 1991). However, ST concentrations in serum were also greater in the rbGRF- than in the rbST-treated cows. Therefore, a third experiment was designed where cows were infused for 60 d with doses of rbGRF and rbST that elicited similar increases in ST concentrations in serum (Dahl et al., 1993). Concentrations IGF-I in serum were also similar throughout the experiment. Solids-corrected milk-yields were 10 % higher for rbGRF- than for rbST-infused cows during the last 20 d of the experiment (Dahl et al., 1993). Results from these experiments confirmed that rbGRF is clearly galactopoietic and the authors suggested that galactopoietic effects of GRF may not be mediated solely by increased serum ST concentrations.

The objective of my thesis was to investigate possible explanations for the greater ability of rbGRF to stimulate milk production as compared with rbST, when doses of rbGRF and of rbST that elicited a similar concentration of ST in serum of lactating cows were administered. The first objective was to examine the effects of rbGRF and rbST on homeorhetic adaptation. To this end, body weights, body condition scores, NEFA concentrations in serum, organ weights, adipose depot weights and carcass composition were measured; The second objective was to examine the effects of rbGRF and rbST on mammary function. Therefore, mammary parenchymal cell number, mammary parenchymal cell metabolic activity and lactose synthesis in mammary tissue

were measured. Since the animals were killed, there was an opportunity to examine effects of rbGRF and rbST on the pituitary gland with respect to refractoriness mechanisms. Therefore, a third objective was to determine the effects of rbGRF and rbST on concentration of ST in anterior pituitaries.

MATERIALS AND METHODS

Animals and treatments

Forty primiparous cows were used in a completely randomized block design with repeated measurement. Cows were blocked by day of parturition into 10 blocks of four cows each. Within each block, three cows started the experiment at 118 ± 1 d after parturition (experimental d 0) and were randomly assigned to one of three treatment groups: one cow received 12 mg/d of rbGRF (1-45) homoserine lactone (The Upjohn Co., Kalamazoo), one cow received 29 mg/d of rbST (Somavubove®, The Upjohn Co., Kalamazoo, MI) and one cow served as an untreated control. Doses were selected based on previous experiments by Dahl et al. (1993), where the same doses elicited a similar concentration of ST in serum for cows continuously infused with rbGRF or rbST for 60 d. These three cows were slaughtered after 63 d of treatment, which corresponded to 181 ± 1 d of lactation. A fourth cow in each block was slaughtered at 118 ± 1 d after parturition and was used as a pre-treatment control.

Cows were housed in tie stalls and exposed to 24 h of light per day. Cows were milked three times per day : 0545, 1430 and 2200 h (AM, midday and PM milkings, respectively), in a parlor at the Michigan State University Dairy Cattle Teaching and Research Center. Cows were fed a total mixed ration ad libitum. Feed was offered twice daily and orts were recorded once daily. Feed samples were collected once per week and analyzed for dry matter content. Dry matter values were used for calculations of DMI (VanderKooi, 1993).

On experimental d -8, VETport® (Thermedics, Worburn, MA) infusion catheters were surgically implanted in cows assigned to rbGRF and rbST groups (Alaniz and Claflin, 1993). Before surgery, neck and shoulder regions of cows were carefully

scrubbed with a .5% Betadine solution (The Purdue Frederick Co., Norwalk, CT), and a path 8 cm wide connecting the point of the shoulders to the right jugular vein was locally anesthetized by s.c. injections of Lidocaine (Vedco, St. Joseph, MO). The cow was then placed on a surgical table and a sedative was administered (Rompun, Mobay Co., Shawnee, KN.). The surgery consisted of the s.c. routing of the catheter from a 5 cm incision at the point of the shoulder to another 5 cm wide incision over the jugular vein at the neck. Five cm caudally to the incision at the point of the shoulders, a .5 cm diameter hole was cut and the plastic "top-hat"-shaped VETport® device was slid underneath the skin from the incision. The protruding top face of the plastic device was pushed from underneath the skin through the hole. The catheter had previously been inserted through and firmly secured to the plastic device. The external end of the catheter was exposed and was later connected to a syringe for infusion. The other end of the catheter was inserted into the jugular vein with the aid of a needle covered with a split plastic sheath. After insertion into the jugular vein, the internal end of the catheter was secured in place by a plastic tab which was sutured to the connective tissue fascia approximately 2 cm behind the point of insertion of the catheter in the jugular vein. Each catheter was filled with heparin (heparin sodium injection, 1000 U/ml, The Upjohn Co., Kalamazoo, MI) and plugged at the external end. Incisions were closed with staples. Post-surgery care consisted of cleansing the incision with hydrogen peroxide and wrapping of cows necks with a stretch wrap (Elastikon, Johnson and Johnson Medical Inc., Arlington, TX). Catheters were flushed with 5 ml of heparin every other day until pumps were connected. Staples were removed 10 d after surgery.

The external end of the catheter was attached to a .20 μm pore sterile Acrodisk syringe filter (Gelman Sciences, Ann Arbor, MI) which was connected to a 20 cc plastic sterile disposable syringe (Becton Dickinson and Co., Rutherford, NJ) containing solutions of rbGRF or rbST treatments. Syringes were installed on AS-2BH

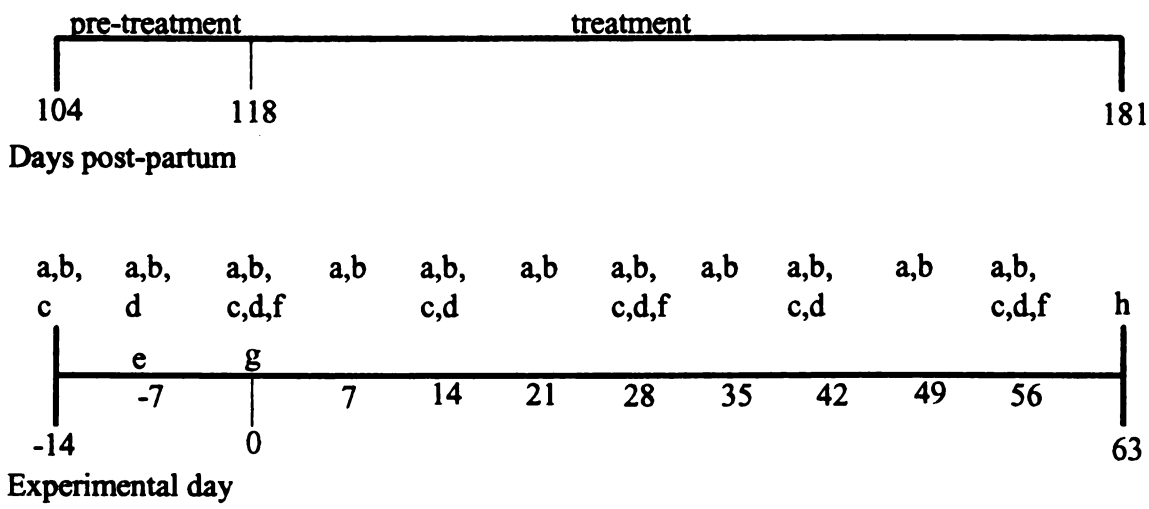
Autosyringe infusion pumps (Autosyringe Inc., Hooksett, NH) . Autosyringe pumps were affixed to an aluminum and plastic carrier that was firmly secured to elastic straps. The elastic straps encircled the cow behind the shoulders. This system allowed cows to be moved from stall to milking parlor without interruption of infusion. Pumps delivered .5 ml of solution per h, in pulses every 3.75 min, 24 h per day. Infusions were initiated at 1700 h on experimental d 0. Hormone solutions were made fresh daily and syringes were changed once every 24 h. Infusions were continued until approximately 10 min before cows were slaughtered on experimental d 63.

All four cows of each block were slaughtered each week. On the day of slaughter cows were milked in the AM and then transported to the abattoir at approximately 0630h. Cows were weighed, stunned with a captive bolt and killed by exsanguination beginning at 0700 h. Order of slaughter was: control or pre-treatment control first and second, rbST-treated third and rbGRF-treated last. There was an average interval of 40 min between the slaughter of each cow. After slaughter, tissues and organs of interest were removed and handled as described in section "Tissue removal: general".

The timetable for the experiment is depicted in Figure 1.

Milk collection and analysis

Milk yields were recorded at each milking and totaled daily. Milk samples, combined from three consecutive milkings, were collected and analyzed for composition once a week, between experimental d -17 to 60. Fat, protein and lactose in milk were quantified using an infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA (East Lansing). Somatic cell count (SCC) was measured by fluorescence microscopy (Somacount 300, Bentley Instruments, Chaska, MN). Yields of SCM and energy output in milk (EOM) were calculated for each week (Tyrrell and Reid, 1965).



a: milk samples
b: feed samples
c: body weight, body condition score
d: calculated energy balance
e: surgery
f: blood samples
g: start of infusions
h: slaughter

Figure 1. Timetable of data collection during the experimental period

Blood collection and analysis

On experimental d -1, 27 and 55 cows were fitted with an ethylene oxide-sterilized indwelling catheter (16 gauge; Ico-Rally, Palo Alto, CA) in the left jugular vein. After insertion , catheters were filled with a 3.5% Na citrate solution to prevent coagulation of blood in the catheter.

Blood samples were collected every 20 min for 6 h, starting at 0800 h on experimental d 1, 29 and 57. Blood samples were stored at room temperature for approximately 6 h, and then were stored at 4 C for another 24 h. Serum was harvested by centrifugation at 1550 x g for 30 min and subsequently stored at -20 C until assayed. Somatotropin concentration in serum was quantified as described by Moseley et al., (1982), and NEFA concentrations were measured using the NEFA-C kit (Wako Chemicals USA, Dallas, TX; as modified by McCutcheon and Bauman, 1986).

Body measurements

Cows were weighed at 1000 h on experimental d -12, -11,-5, -4, 2, 3, 16, 17, 30, 31, 44, 45, 58, and 59. Weights for two consecutive days were averaged and the value obtained was used for statistical analysis. One experienced examiner scored body condition (BCS) (Wildman et al., 1982) of cows on a 1 to 5 scale on experimental d -11, -5, 3, 17, 30, 44 and 59.

Energy balance was calculated by the expression: EB (Mcal net energy of lactation (NE_l /d) = [feed energy input (Mcal NE_l/d, NRC, 1989) minus maintenance energy (Mcal NE_l/d, NRC, 1989) minus milk energy output (Mcal NE_l/d, Tyrrell and Reid, 1965)]. The NE_l of feed used was reported by VanderKooi (1993).

Tissue removal: general

Immediately following exsanguination, heads were removed, sawed open horizontally, anteroposteriorly, at the forehead region above the eyes to expose brain tissue. Pituitaries were subsequently removed and separated into anterior and posterior lobes. Anterior pituitaries were weighed, bisected and frozen by submersion in liquid nitrogen. Frozen anterior pituitaries were stored at -80 C until assayed for ST content (Moseley, 1982). The procedure for ST extraction from the anterior pituitary gland is described in Appendix A (Krabill, L.F., 1993 - personal communication).

Mammary glands were quickly removed, and separated into right and left halves. The left half was weighed and immediately frozen by submersion in a tub containing dry ice and 70% ethanol. Frozen half udders were then stored at -20 C until analyzed as described in section "**Mammary tissue analysis - 1) Tissue composition**". A 10 to 15 g sample of mammary tissue was removed from the middle region of the right-rear mammary gland, placed in a plastic bag containing .25 M sucrose solution at 5 C and delivered to the laboratory for slicing and incubations as described in section "**Mammary tissue analysis - 2) Lactose synthesis**".

Internal organs were removed soon after the carcasses were slit open. Weights of liver, spleen, lungs, heart ventricles and kidneys were recorded. Small intestines were dissected from the rest of the gastrointestinal tract, cut into sections approximately 1 m long, the lumen was flushed internally with water to remove ingesta, placed on hangers and allowed to air dry for approximately 1 h and then weighed. Adipose tissue was removed from the omental and perirenal depots and individually weighed.

After removal of the hide, carcasses were sawed in half, weighed, washed and hung in refrigerated chambers at 2 C.

Carcass composition analysis

The morning following slaughter, the left half of each carcass was cut between the 8th and 13th ribs, the fat depth at the 12th rib was obtained and the 9-10-11th rib sections were dissected according to the method of Hankins and Howe (1946). Rib sections were weighed, deboned, ground, mixed and sub sampled for analysis of lipid, water and protein content. Fat was determined in dried samples by ether extraction and protein was determined in fresh samples by the macro-Kjeldahl procedure (AOAC, 1965).

Average of carcass water, protein and fat for pre-treatment controls were calculated and accretion of water, protein and fat (kg/d) were estimated by subtracting each rbGRF, rbST or control value from the pre-treatment group average for each parameter and dividing the difference by 63 d.

Mammary tissue analysis

1) Tissue composition

The frozen left half of the mammary gland was sliced into 10 mm sections using a band saw. Skin, teats, extraparenchymal fat and supramammary lymph nodes were removed and discarded. The remaining mammary tissue will be called mammary parenchyma for the purpose of this thesis. Mammary parenchyma was weighed, and ground by a vertical mixer-cutter (The Hobart Manufacturing Co., Troy, OH) into approximately 2 to 5 mm^3 particles. For each cow, a composited sample was collected by randomly subsampling directly from the mixer-cutter bowl. Parenchyma was never allowed to thaw during tissue handling. Samples were stored at -20 C until analyzed for DNA and RNA content (Tucker, 1964; as modified in Appendix B), dry matter content and ether extractable fat (soxlet analysis). Concentrations of nucleic acids in mammary

tissue were expressed on a dry, fat free, solids-non-fat-corrected tissue weight basis. Calculations used to adjust tissue weights are described in Appendix C.

Average of total DNA and total RNA for pre-treatment controls were calculated. Accretion of total DNA and of total RNA (g/d) were estimated by subtracting each rbGRF, rbST or control value from the pre-treatment group average for each parameter, and dividing the difference by 63.

Yield of SCM from d 56 to 62 was expressed per kg of parenchyma and per g of DNA by dividing the average of the half-daily SCM for that period by the weight of dry, fat free, solids-non-fat corrected parenchyma of the 1/2 gland and by total DNA of the 1/2 gland, respectively.

2) Lactose synthesis

Upon delivery to the laboratory, 150 mg mammary tissue slices were prepared with a Stadie-Riggs microtome (Arthur H. Thomas Co., Philadelphia, PA). Slices were rinsed in a .25 M sucrose solution, blotted and individually transferred to a 25 ml Erlenmeyer flask containing 3 ml of Krebs-Ringer bicarbonate buffer (Deluca and Cohen, 1964) supplemented with acetate, glucose and insulin, as described by Akers et al., (1981). Flasks were subsequently stoppered and incubated for 3 h in a shaking water bath (60 cycles per min) at 37 C. Lactose synthesis was determined by measurement of lactose (Coffey and Reithel, 1969; Kurz and Wallenfels, 1974, as modified in Appendix D) released into the medium during the 3-h incubation. Additional tissue slices were incubated for 30 min, and concentration of lactose in the medium was used to adjust for lactose present in the tissue before incubations started.

Statistical analysis

All data were analyzed using ANOVA, with repeated measurements when applicable (Gill, 1986). Average SCM, milk composition, BW and BCS were measured

2 wk prior to initiation of infusions (pre-treatment period) and, when significant, were used as covariates for SCM, milk components, BW and BCS, respectively. Average DMI was measured and average EB was calculated 1 wk prior to initiation of infusions (pre-treatment) and were used as a covariate for DMI and calculated EB, respectively, in subsequent periods. Carcass weight was used as a covariate for organ weights, adipose tissue weights, carcass protein, fat and water weights and carcass protein, fat and water accretion. Adjustment of weights of organs and tissues was performed because relative weight of an organ in relation to the carcass, rather than absolute weight, is generally a better indication of function of the organ. Adjustment of means by covariance was performed when the appropriate covariate was significant ($P<.1$) and treatment by covariance interaction was not significant ($P>.1$) (Gill, 1978). Means were compared using Student's t test applied to a set of orthogonal contrasts (controls vs. rbGRF and rbST; rbGRF vs. rbST) for both treatment averages and within-period comparisons, (Gill, 1978). Bonferroni t test was used when more specific mean comparisons were needed. The criterion for statistical significance was $P<.1$; therefore, any comparisons in which P value was greater than .1 was designated "not significant" (NS).

RESULTS

Somatotropin and pituitary measurements

Somatotropin concentrations in serum averaged across d 1, 29 and 57 were 18.9, 19.2 and 3.1 ng/ml for rbGRF, rbST and controls, respectively (Figure 2). There was a treatment by time interaction (P<.01); therefore, comparisons between treatment means were performed within each sampling day (d 1, 29 or 57). Serum ST concentrations were increased for rbGRF and rbST in comparison with controls on d 1, 29 and 57 (P<.01). On experimental d 1, ST concentration in serum for rbST-infused cows was higher than for rbGRF-infused cows (P<.01), but rbGRF-infused cows had elevated ST concentration in serum on d 29 (P<.05) as compared with rbST-infused cows. There was no difference in ST concentration in serum between rbGRF- and rbST-infused cows on experimental d 57 (P=.13).

Pituitaries from rbGRF-infused cows were heavier than those of controls (P<.01) and those of rbST-infused cows (P<.02; Table 1). Somatotropin content of pituitaries from rbGRF-infused cows was numerically lower than rbST-infused cows, but it was significantly lower than controls (P<.01). Somatotropin concentrations in pituitaries from both control (P<.01) and rbST-treated cows (P<.01) were greater than that of rbGRF-treated cows.

Milk yield and composition

Solids-corrected-milk yield from d -14 to 0 (pre-treatment) was significant when tested as a covariate (P<.1); therefore, SCM yields were adjusted using

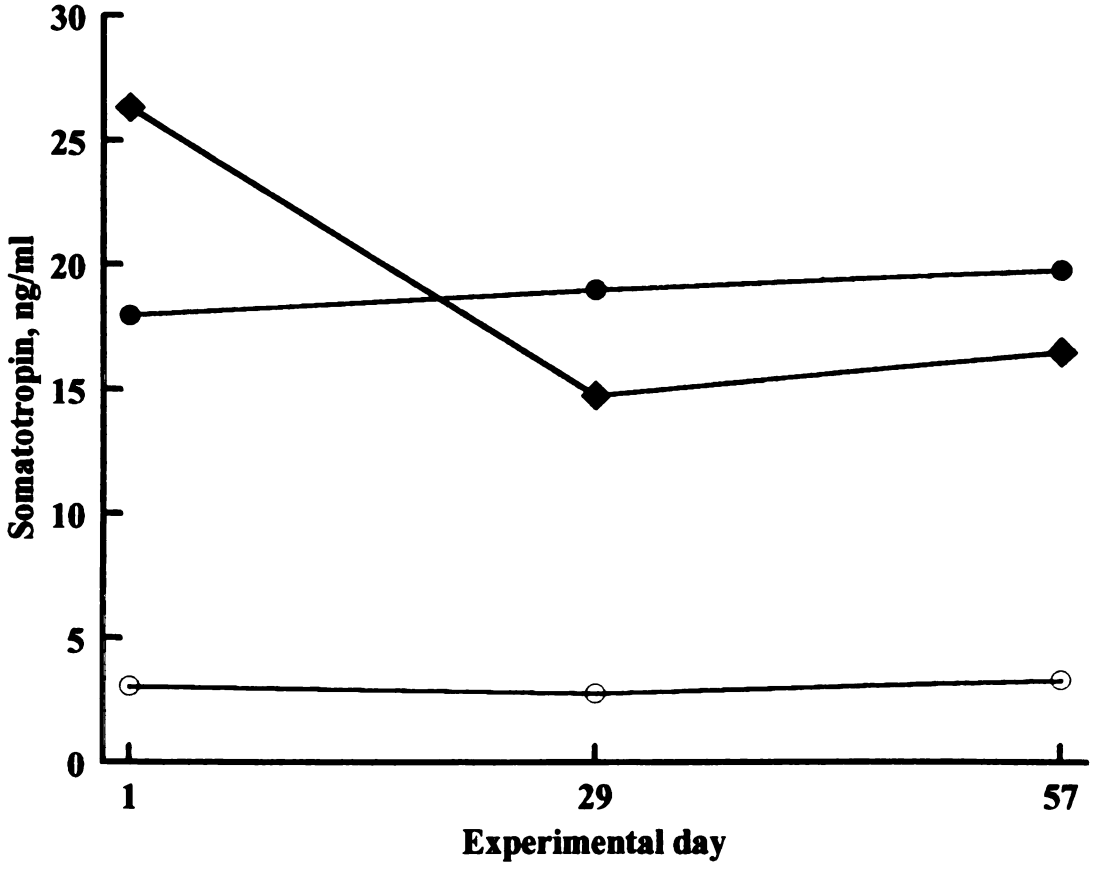


Figure 2. Least squares means of concentrations of somatotropin in serum of cows receiving no treatment (—○—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Standard error of the difference among treatments was 1.97 ng/ml on d 1 and 29, and 2.07 ng/ml on d 57, standard error of the difference of periods within a treatment was 1.6 ng/ml. Mean comparisons of periods within a treatment were performed using Bonferroni t test (n=9 or 10).

Table 1. Least squares means of weight, ST content and ST concentration in anterior pituitary glands of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST). Mean comparisons were performed using Bonferroni t test (n=10).

					P values		
	CON	GRF	bST	SED	CON vs. bST	CON vs. GRF	GRF vs. bST
Pituitary weight, g	2.0	2.6	2.1	.2	NS	.01	.02
ST content, mg	35.0	21.3	30.2	4.1	NS	.01	NS
ST concentration, mg/g	17.9	8.3	14.7	1.9	NS	.01	.01

pre-treatment milk yields as a covariate. Averaged throughout the experiment, SCM yields of rbGRF (33.3 kg/d) and rbST (34.1 kg/d) were increased (P<.01) relative to controls (29.1 kg/d) (Figure 3). There was no difference in SCM yields between rbGRF- and rbST- infused cows .

Pre-treatment milk fat, lactose, and protein percentages were significant when tested as covariates (P<.1); therefore, each milk component was adjusted using their respective pre-treatment values as a covariate. There was no difference in percentage of any milk component between any of the treatments (Table 2). Somatic cell count values were converted to natural logarithms (log SCC) in order to minimize heterogeneous variance. However, there was no difference in log SCC among treatment groups.

Body weight, dry matter intake, calculated energy balance, organ weights and carcass composition measurements

Body weight values were adjusted using pre-treatment body weight as a covariate. There were no differences in average BW throughout the experiment among rbGRF (539.3 kg), rbST (542.1 kg) and control cows (536.5 kg) (Figure 4). However, slopes for BW throughout the experiment were positive and significant for controls (P<.01; adjusted r^2 = .97), and rbGRF-treated cows (P<.03; adjusted r^2 = .78). The body weight of one rbST-treated cow decreased from 564.5 (d 44,45) to 508.5 kg (d 58,59) due to lack of feed intake associated with a case of acute mastitis. Although that animal did not test as an outlier (Gill, 1978), if she was removed from the analysis, BW average for the rbST group increased from 542.1 to 546.1 kg on d 58,59, and the slope for BW throughout the experiment approached significance (P=.06; adjusted r^2 = .67).

Means for DMI were adjusted using pretreatment DMI as a covariate. Averaged throughout the experiment, DMI was not different among treatments

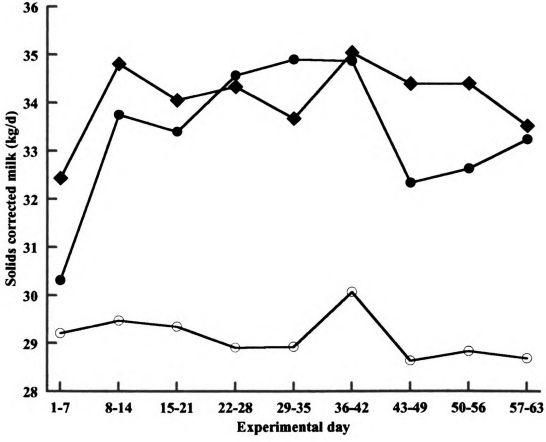


Figure 3. Least squares means of solids corrected milk yield of cows receiving no treatment (—○—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Each point represents the average of a treatment group within each 7-d period, adjusted by covariance using pre-treatment SCM as a covariate. Standard error of the difference among treatments was 1.0 kg/d (n=10).

Table 2. Least squares means of percentages of milk components of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST). Values represent the average of cows sampled once per week in each treatment group, adjusted by covariance using pre-treatment milk composition as a covariate (n=10).

	CON	GRF	bST	SED
Fat, %	3.2	3.3	3.4	.15
Lactose, %	5.0	5.0	5.0	.03
Protein, %	3.0	3.1	3.0	.04
log SCC	4.7	4.4	4.5	.3

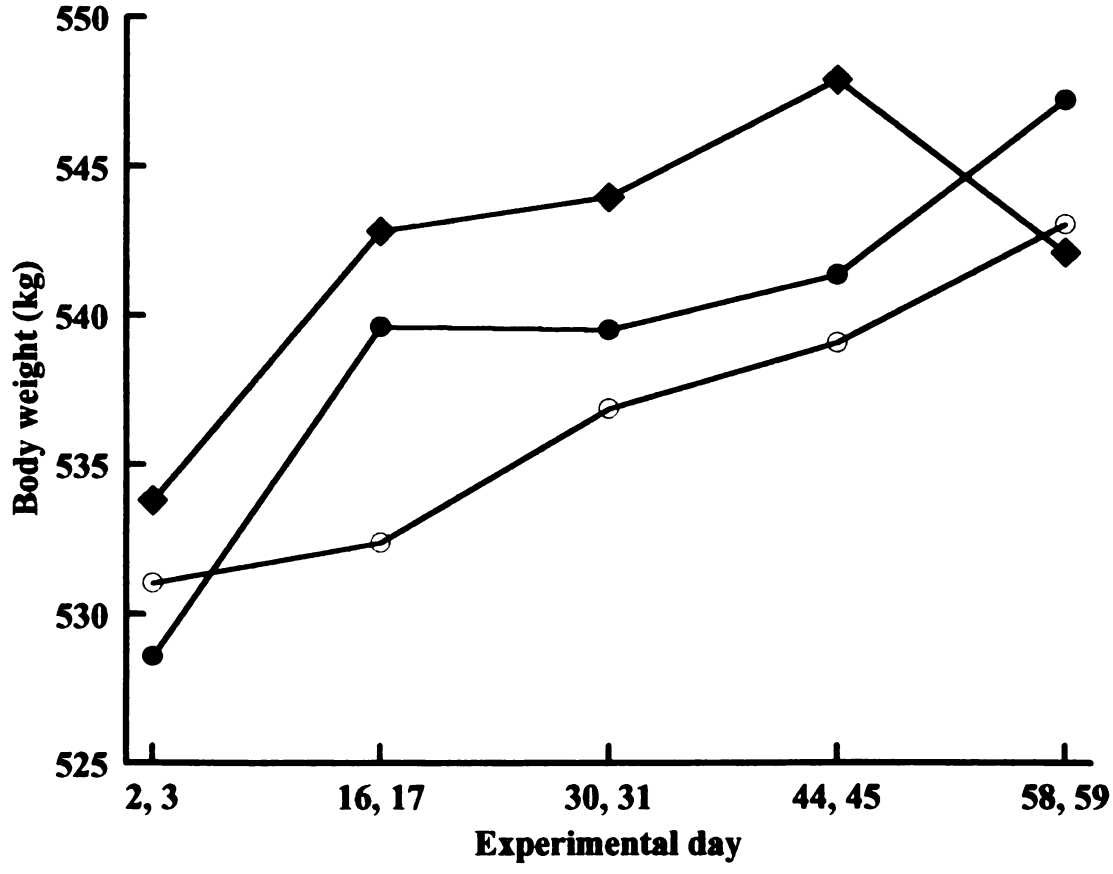


Figure 4. Least squares means of body weight of cows receiving no treatment (—○—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Standard error of the difference among treatments was 4.98 kg. Means were adjusted by covariance using pre-treatment body weight as a covariate (n=10).

(Figure 5). However, slopes for DMI throughout the experiment were positive and significant for controls ($P<.01$; adjusted $r^2 = .86$), rbGRF-treated cows ($P<.01$; adjusted $r^2 = .80$) and rbST-treated cows ($P<.01$; adjusted $r^2 = .73$). Within each treatment group, means of each experimental period were compared with means from d 1-7. For rbGRF-treated cows DMI was increased in relation to d 1-7 on all other days ($P<.1$). For rbST-treated cows DMI tended to increase on d 43-49 and 57-63 ($P<.1$). For controls, DMI was elevated on d 43-49 and 57-63 ($P<.05$). On d 57-63 DMI tended to be greater for rbGRF than for rbST-treated cows ($P<.1$). Change in DMI from d 1-7 to d 8-14 were not statistically different among treatments ($P>.1$).

Means for calculated EB were adjusted using pre-treatment calculated EB as a covariate. There was a treatment by time interaction on calculated EB means ($P<.01$); therefore, comparisons between treatments were performed within each period. Calculated energy balance was lower for rbGRF- and rbST-treated cows in comparison with controls from d 17 to d 59 ($P<.01$); (Figure 6). On d 59 calculated EB was greater ($P<.05$) for rbGRF- as compared with rbST-treated cows. For rbGRF-treated cows, calculated EB on d 31 was lower ($P<.01$) than on d 59. Cows treated with rbST tended to have lower calculated EB from d 17 to d 45 ($P<.1$) as compared with d 3. Controls had elevated calculated EB on d 45 and 59 ($P<.01$) as compared with d 3.

Carcass weight was significant ($P<.1$) when tested as a covariate for weights of heart ventricles, intestine, kidney, liver, lung and spleen; therefore, weights of these organs were adjusted by covariance using carcass weight. Weights of heart ventricles, intestine, kidney, lung and spleen were greater for rbGRF- and rbST-infused cows as compared with controls (Table 3). Because liver weight means of rbGRF and rbST groups were dissimilar, means were not compared as orthogonal contrasts. Instead non-orthogonal comparisons were performed by Bonferroni t test. Cows infused with rbST

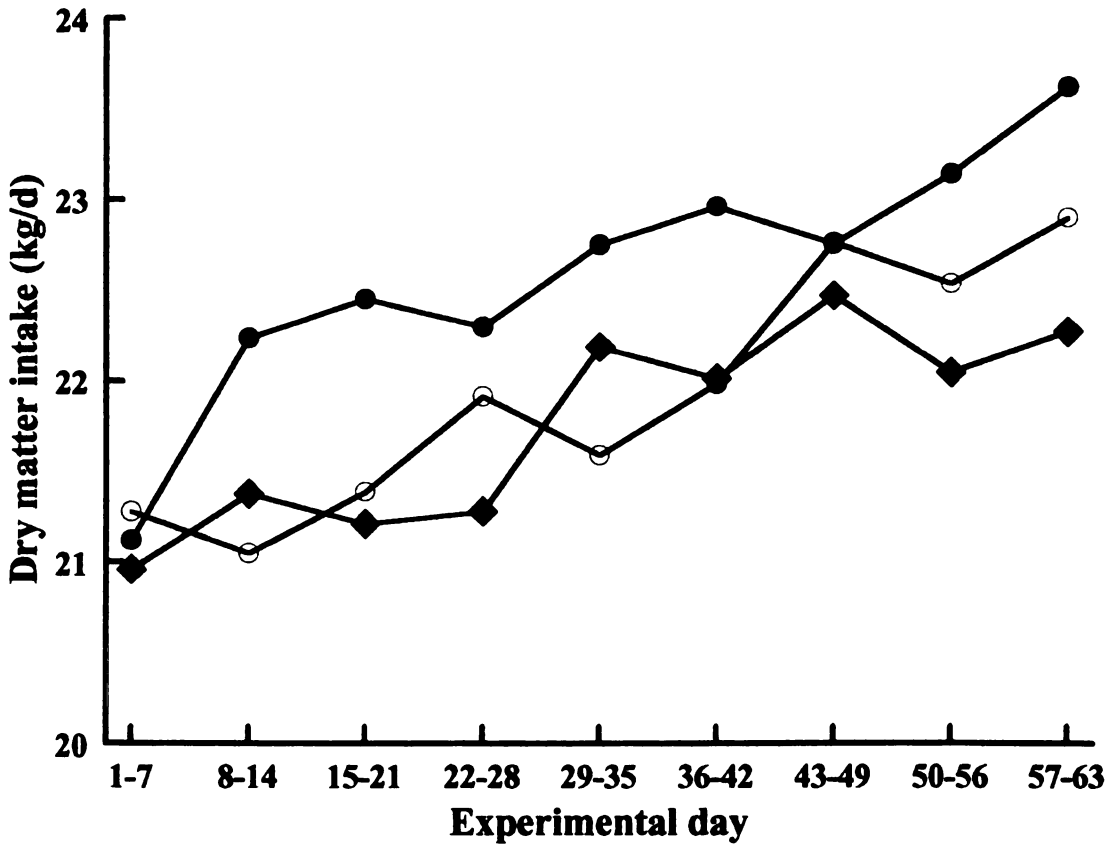


Figure 5. Least squares means of dry matter intake of cows receiving no treatment (—○—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Standard error of the difference among treatments was .42 kg/d, standard error of the difference of periods within a treatment was .51 kg/d, standard error of the difference of treatments within a period was .6 kg/d. Mean comparisons of periods within a treatment and treatments within periods were performed using Bonferroni t test (n=10).

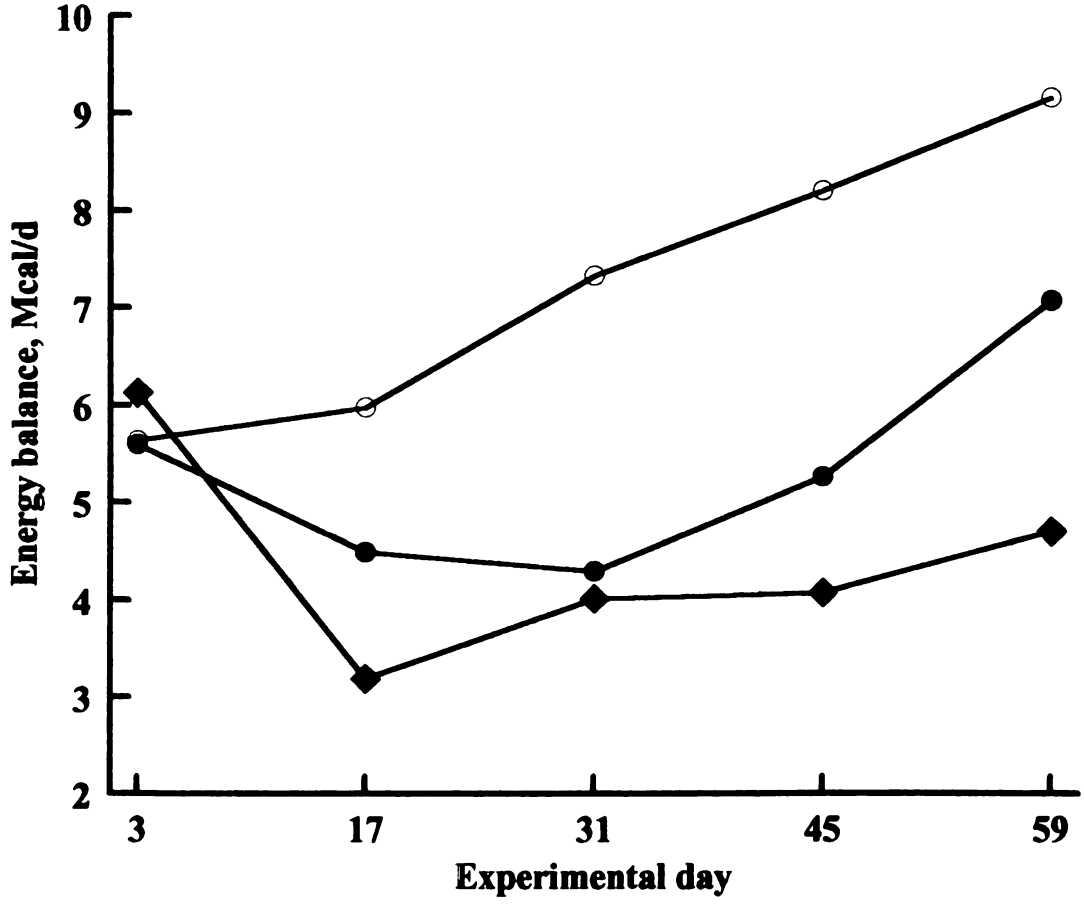


Figure 6. Least squares means of calculated energy balance of cows receiving no treatment (—○—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Standard error of the difference among treatments within a period was 1.5 Mcal/d, standard error of the difference of periods within a treatment was .87 Mcal/d. Mean comparisons of periods within a treatment were performed using Bonferroni t test (n=10).

Table 3. Least squares means of organ weights of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST). Means were adjusted by covariance using carcass weight as a covariate (n=10).

					P values	
	CON	GRF	bST	SED	CON vs. GRF and bST	GRF vs. bST
Heart ventricles , kg	2.0	2.1	2.1	.08	.05	NS
Intestine, kg	8.8	10.1	9.9	.48	.02	NS
Kidney, kg	1.5	1.6	1.6	.06	.05	NS
Liver, kg	9.3	9.7	10.6	.28	*	*
Lung, kg	3.5	3.9	4.0	.14	.01	NS
Spleen, kg	1.0	.9	1.0	.05	NS	NS

* Liver weight means were compared using Bonferroni t test. The comparisons performed were: controls vs. rbGRF (NS), controls vs. rbST (P<.01) and rbGRF vs. rbST (P<.02).

had greater liver weights than those of rbGRF-infused and control cows (P<.01), but rbGRF treatment did not increase liver weight in relation to untreated control cows.

Body weights at slaughter were not different among the three treatment groups (Table 4). Carcass weights tended to be greater (P<.08) for control cows as compared to rbGRF- and rbST-infused cows. Similarly, dressing percentage was greater (P<.02) for controls than for rbGRF- and rbST-treated cows. Carcass water percentage, kg of carcass water and carcass water accretion were greater for rbGRF- and rbST-infused cows as compared with controls (P<.01). Similarly, carcass protein percentage was greater (P<.02), and kg of carcass protein and carcass protein accretion tended to be greater (P=.06) for rbGRF- and rbST- infused cows in relation to controls. In contrast, carcass fat percentage, kg of carcass fat and carcass fat accretion were greater (P<.01) for controls as compared with rbGRF- and rbST-treated cows. There were no differences in any of the carcass parameters between rbGRF- and rbST-treated cows (P>.1).

Fat mobilization measurements

Pre-treatment BCS was significant (P<.1) when tested as a covariate for BCS, but there was a significant treatment by covariate interaction (P<.1); therefore, no adjustment was performed on treatment means. There were no overall treatment differences in BCS, although BCS tended to be lower (P<.1) for rbGRF- and rbST-treated cows in relation to controls on d 59 of the experiment (Figure 7).

Both rbGRF (279.5 uEq/L) and rbST (284.7 uEq/L) treatments increased NEFA concentration in serum of cows as compared with controls (196.9 uEq/L) (P<.01) (Figure 8).

Carcass weight was significant (P<.1) when tested as a covariate; therefore, means of omental and perirenal fat weights were adjusted by covariance. Both rbGRF- and rbST-infused cows had lower omental fat weights and omental fat accretion as

Table 4. Least squares means of body weight at d 63 and carcass parameters of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST). Means of kg and accretion of carcass water, protein and fat were adjusted by covariance using carcass weight as a covariate (n=10).

					P values	
	CON	GRF	bST	SED	CON vs. GRF and bST	GRF vs. bST
Body weight, kg	547.2	539.9	536.9	14.3	NS	NS
Carcass, kg	241.5	233.5	226.5	7.1	.08	NS
Dressing, %	44.14	43.31	42.18	.65	.02	NS
Carcass water, %	67.9	71.5	71.8	.6	.01	NS
Carcass water, kg	159.1	167.2	167.2	1.4	.01	NS
Carcass water accretion, kg/d [a]	-.01	.12	.12	.022	.01	NS
Carcass protein, %	18.2	19.1	19.4	.4	.02	NS
Carcass protein, kg	42.8	44.6	45.1	1.1	.06	NS
Carcass protein accretion, kg/d [a]	.01	.04	.05	.02	.06	NS
Carcass fat, %	11.9	7.8	6.6	.9	.01	NS
Carcass fat, kg	27.5	18.4	16.4	2.1	.01	NS
Carcass fat accretion, kg/d [a]	-.008	-.15	-.18	.03	.01	NS

[a] Value at d 181 minus value at d 118 divided by 63 d.

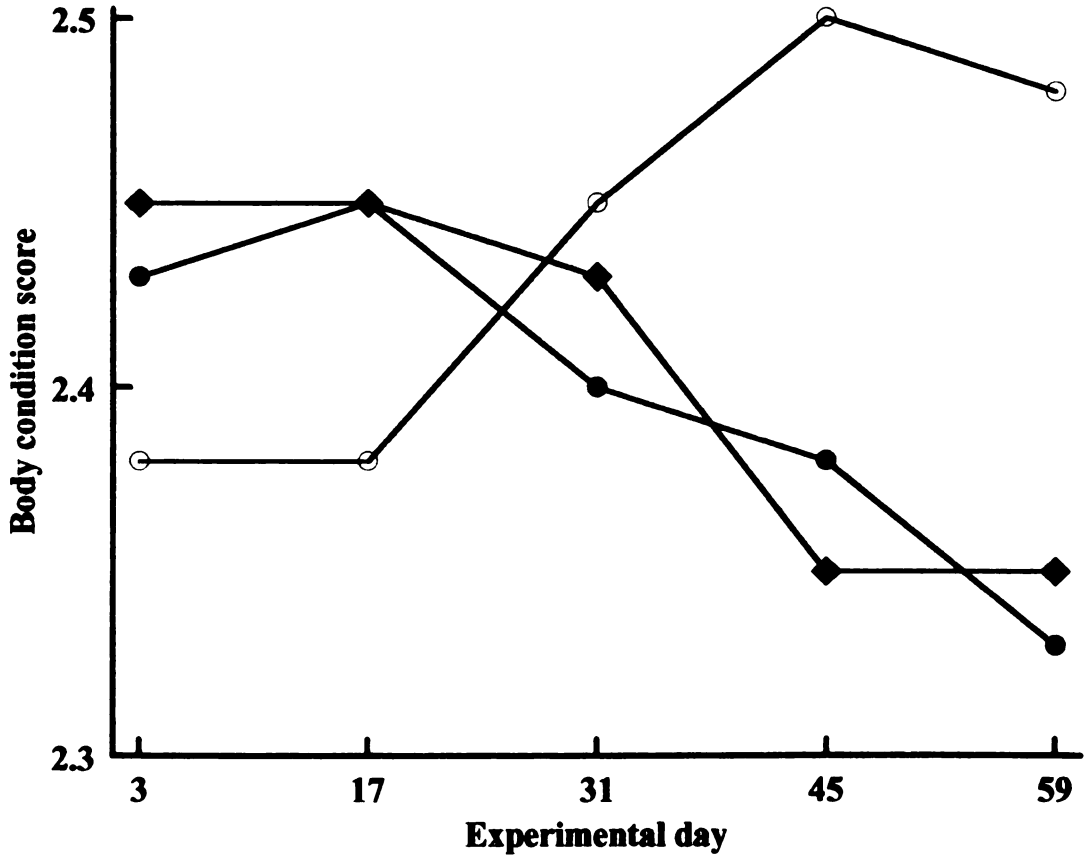


Figure 7. Least squares means of body condition score of cows receiving no treatment (—⊖—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Standard error of the difference among treatments was .1. Standard error of the difference among treatments within d 45 and within d 59 was .1 (n=10).

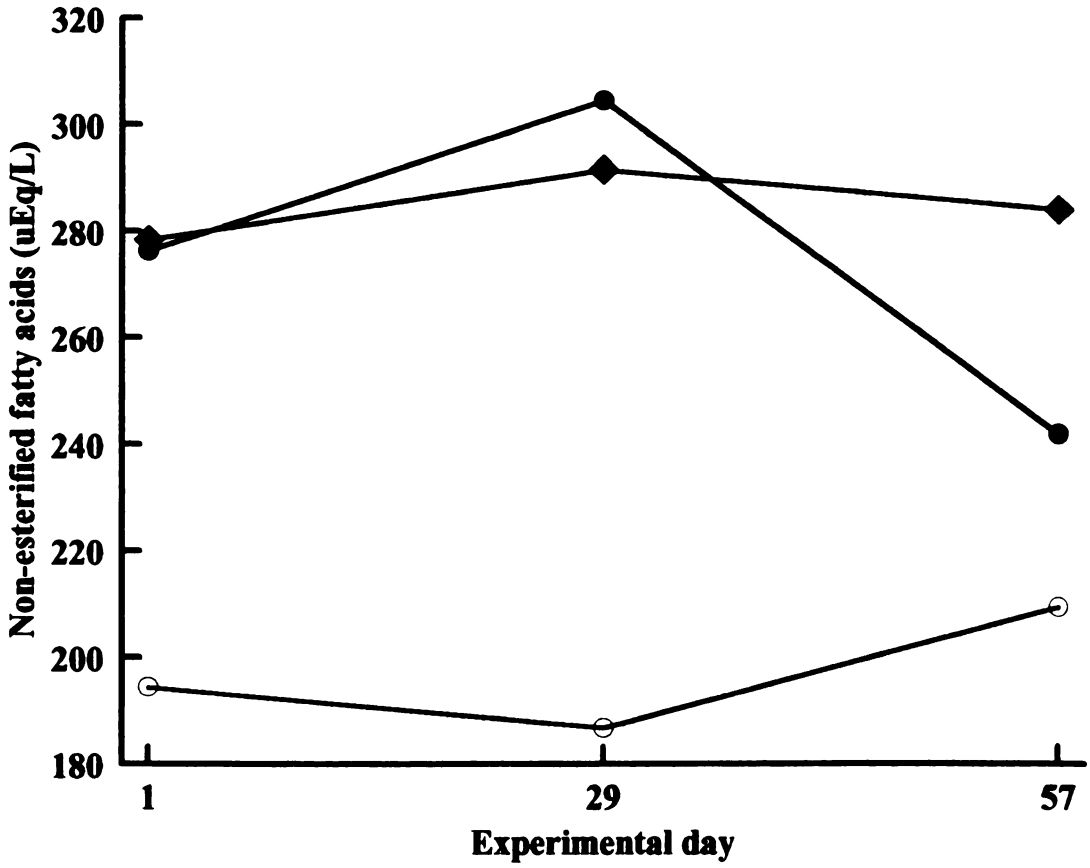


Figure 8. Least squares means of concentration of serum NEFA of cows receiving no treatment (—○—), 12 mg rbGRF/d (—●—) or 29 mg rbST/d (—◆—). Standard error of the difference among treatments was 27.7 uEq/L (n=10).

compared with controls (P<.01; Table 5). Similarly, perirenal fat weight and perirenal fat accretion were lower for rbGRF- and rbST-infused cows relative to controls. In contrast, there was no difference among treatment groups in the 12th rib fat depth of cows.

Mammary function measurements

Mammary parenchymal weight was not different among the three treatment groups (Table 6). However, when expressed per 100 kg of carcass weight, parenchymal weight tended (P=.07) to be greater for rbGRF- and rbST-treated cows as compared with controls. The rbGRF- and rbST-infused cows had 34% less intraparenchymal fat than controls (P<.01). There was no difference in mammary parenchymal weight between rbGRF- and rbST-infused cows.

Total DNA, DNA concentration, and DNA accretion were not different among treatment groups (Table 7). In contrast, total RNA (P<.05), RNA concentration (P<.01), RNA accretion (P<.01), and RNA to DNA ratio (P<.05) were greater for rbGRF- and rbST- infused cows as compared with controls. There was no difference in any of the measures of RNA between rbGRF- and rbST-infused cows.

There was no difference in the ratios of SCM per kg of parenchyma or per g of DNA among the three treatment groups in this experiment (Table 8).

Lactose synthesis rate of mammary parenchymal tissue slices was not different among treatment groups (Figure 9).

Table 5. Least squares means of adipose tissue weight of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST). Means of kg and accretion of omental and perirenal fat were adjusted by covariance using carcass weight as a covariate (n=10).

					P values	
	CON	GRF	bST	SED	CON vs. GRF and bST	GRF vs. bST
Omental fat, kg	5.2	2.9	2.4	.42	.01	NS
Omental fat accretion, g/d [a]	2.21	-33.8	-42.4	6.05	.01	NS
Perirenal fat, kg	3.5	1.6	1.4	.42	.01	NS
Perirenal fat accretion, g/d [a]	.75	-30.4	-33.05	6.5	.01	NS
12th rib fat depth, cm	1.2	1.1	1.1	.2	NS	NS

[a] Value at d 181 minus value at d 118 divided by 63 d.

Table 6. Least squares means of mammary parenchymal weight and intraparenchymal fat of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST) (n=10).

					P values	
	CON	GRF	bST	SED	CON vs. GRF and bST	GRF vs. bST
Mammary parenchymal weight [a]	.77	.83	.85	.07	NS	NS
Mammary parenchymal weight [b]	.32	.36	.37	.03	.07	NS
Intraparenchymal fat [c]	31.8	20.1	21.8	2.5	.01	NS

[a] kg dry, fat-free, solids-non-fat-corrected parenchyma per 1/2 gland

[b] kg dry, fat-free, solids-non-fat-corrected parenchyma per 1/2 gland per 100 kg carcass wt.

[c] g/g dry, fat-free, solids-non-fat-corrected mammary parenchyma.

Table 7. Least squares means of mammary parenchymal nucleic acid composition of cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST) (n=10).

					P values	
	CON	GRF	bST	SED	CON vs. GRF and bST	GRF vs. bST
Total DNA[a]	22.7	24	23.6	2	NS	NS
Total DNA[b]	9.4	10.3	10.5	.8	NS	NS
DNA concentration[c]	29.2	29.0	28.1	1.0	NS	NS
DNA accretion[d]	-.008	.006	.008	.01	NS	NS
Total RNA[a]	60.8	77.7	73.7	6.4	.02	NS
Total RNA[b]	25.3	33.4	32.5	2.5	.05	NS
RNA concentration[c]	79.4	93.6	88.1	4.6	.01	NS
RNA accretion[d]	-.02	.1	.09	.04	.01	NS
RNA/DNA	2.7	3.2	3.2	.2	.05	NS

[a] g per 1/2 gland.

[b] g/100 kg carcass wt per 1/2 gland.

[c] mg/g dry, fat-free, solids-non-fat-corrected mammary parenchyma.

[d] Value at d 181 minus value at d 118 divided by 63 d (g/d).

Table 8. Least squares means of solids corrected milk yields from d 56 to 62 expressed per kg of mammary parenchyma and per kg of mammary parenchymal DNA for cows receiving no treatment (CON), 12 mg rbGRF/d (GRF) or 29 mg rbST/d (bST) (n=10).

					P values	
	CON	GRF	bST	SED	CON vs. GRF and bST	GRF vs. bST
SCM / kg parenchyma [a]	22.7	24	23.6	2	NS	NS
SCM / g DNA [b]	9.4	10.3	10.5	.8	NS	NS

[a] kg/kg dry, fat-free, solids-non-fat-corrected parenchyma per 1/2 gland.

[b] kg/g total DNA per 1/2 gland.

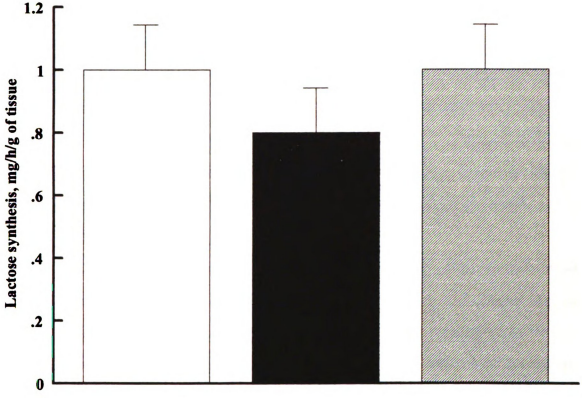


Figure 9. Least squares means of lactose synthesis of cows receiving no treatment (open bar), 12 mg rbGRF/d (solid bar) or 29 mg rbST/d (dashed bar). Standard error of the mean for each treatment is depicted on top of each bar (n=6 to 9).

DISCUSSION

Galactopoietic effects of rbGRF and rbST have been profusely documented for dairy cattle (Dahl et al., 1991; Dahl et al., 1993; Bauman, 1992; Bauman and Vernon, 1993). Therefore, as expected, in the current study both rbGRF and rbST treatments increased SCM yield of cows compared with non-infused controls. Elevated milk yield was probably largely due to elevated ST concentration in serum, caused by rbGRF-stimulation of endogenous ST secretion from the anterior pituitary gland, or by exogenous rbST itself.

Dahl et al. (1993) infused lactating dairy cows for 60 d with the same doses of rbGRF and rbST used in the current experiment and reported a 10 % greater increase in SCM yield for rbGRF- as compared with rbST-treated cows during the last 20 d of infusion. This disagrees with data from the current experiment, where SCM yields were similar between rbGRF- and rbST-infused cows throughout the experiment. Several reasons could possibly explain this discrepancy. For example, animals in the present experiment were milked three times daily which is by itself galactopoietic (DePeters et al., 1985). Moreover, Bauman (1992) stated that milk yield response to increasing doses of rbST increases linearly until a plateau is reached at a dose of 30 to 40 mg of rbST/d. Thereafter, milk yield is only increased marginally, even with several fold higher doses of rbST. Speicher et al. (1993) reported the additive effects of thrice (vs. twice) daily milkings and rbST injections on milk production of cows. Therefore, it is possible that effects of three daily milkings added to the effects of elevated ST in serum maximized the galactopoietic response of cows in the present experiment to ST (i.e., a greater

concentration of ST in serum would not further stimulate milk production). In contrast, cows in the experiment of Dahl et al. (1993) were only milked twice daily. In addition, at the beginning of the experiment their cows were in a later stage of lactation (175 d). Furthermore, the majority of their cows were multiparous. Collectively, the cows used in the experiment of Dahl et al. (1993) may not have been as close to maximum production of milk as those used in the present study. I speculate, therefore, that their cattle may have been more suitable to show differences in milk yield response to rbGRF vs. rbST as compared with the cattle in my experiment.

Treatment of dairy cows with rbST does not affect percentage of milk components (Bauman, 1992). Similarly, Dahl et al. (1990) reported that rbGRF infusions did not change gross composition of milk. We also found no difference in percentages of any milk component among treatments. Therefore, since total yield of milk increased, total yields of lactose, fat and protein were elevated for rbGRF- and rbST-treated cows relative to controls.

An immediate result of rbST administration is increased radioimmunoassayable levels of ST in serum (Dahl et al., 1991 and Dahl et al., 1993). Similarly, administration of rbGRF acutely and specifically increases concentration of ST in serum (Enright et al., 1986). Moreover, prolonged continuous infusion of rbGRF maintains elevated concentrations of ST in serum for at least as long as 90 d (Tucker et al., 1993; personal communication). Dahl et al. (1993) noticed a greater increase in milk production for cows infused with rbGRF as compared with cows infused with rbST, although the concentrations of ST in serum for both groups were similar. Possibly, the galactopoietic effects of rbGRF are not solely mediated by ST. In the present experiment, my aim was to increase concentrations of ST in serum to similar levels for both rbGRF and rbST treatment groups. I wanted to examine potential ST-independent effects of rbGRF on variables involved in galactopoiesis. Both rbGRF and rbST

treatments increased concentrations of ST in serum of cows in relation to untreated controls on d 1, 29 and 57 of the experiment. However, ST concentrations in serum were greater for cows treated with rbST than for cows treated with rbGRF on d 1. In contrast, ST concentrations were greater for cows treated with rbGRF on d 29 and there was a tendency for ST concentrations to be greater on d 57 as compared with cows infused with rbST. This does not agree with the results of Dahl et al. (1993). In their experiment they infused the same doses of rbGRF and rbST as used in the present study and achieved similar concentrations of ST in serum between both groups. Concentrations of endogenous ST in serum of cows decrease with age (Lapierre et al., 1992), probably reflecting a progressive diminution of pituitary synthetic capacity (Sadow and Rubin, 1992). Indeed, Dahl et al. (1993) worked mostly with multiparous cows (75 % of the animals in that experiment were multiparous) and average concentrations of ST in serum of both rbGRF-treated cows (12.5 ng/ml) and also control cows (1.2 ng/ml) were numerically smaller than concentrations in cows of the current experiment (18.9 and 3.1 ng/ml, for rbGRF-treated and controls, respectively). It should be noted that the assay used for ST was the same for both experiments. Therefore, a possible explanation for the difference in serum concentrations of ST between rbGRF- and rbST-infused cows of the present study may be that primiparous cows in the present experiment were more responsive to stimulation by rbGRF than the predominantly multiparous cows used in the experiment conducted by Dahl et al. (1993). As a result, ST concentrations in serum were elevated for rbGRF- relative to rbST-infused cows on d 29 and 57. Therefore, if a smaller dose of rbGRF had been used in the present experiment, probably similar concentrations of ST in serum would have been achieved throughout the experiment for both rbGRF- and rbST-infused cows. But whether such differences in ST concentrations in serum would be biologically significant is questionable. Eppard et al. (1985) showed that the milk yield response increased in a curvilinear fashion with linear increases in ST

concentration in serum, tending to plateau between 15 and 20 ng/ml of ST. Moreover, as indicated previously, cows in the experiment conducted by Dahl et al. had lower concentrations of ST in serum than cows in the current experiment, even though the doses used in both experiments were the same. However, milk production response to hormone treatments in their experiment were within the expected range (approximately 35% above the controls), suggesting that those lower concentrations of ST in serum sufficed to evoke significant galactopoiesis. I speculate that ST concentration in serum of rbGRF- and rbST-treated cows in the present experiment were at the plateau region of the curve of milk response to ST concentration in serum; therefore, the fact that ST concentrations in serum were not similar throughout the experiment should not cause different milk production responses between these two groups of cows. In fact, milk production was similar between rbGRF- and rbST-infused cows, therefore, differences in ST concentration in serum were probably not biologically significant, at least in terms of milk production.

Concentration of ST in serum is not the sole indication of stimulation of the cascade of events promoted by infusions of rbGRF and rbST. In the same experiment described in my thesis, VanderKooi (1993) reported that both rbGRF and rbST treatments increased concentrations of IGF-I and IGFBP-3 in serum and IGF-I mRNA abundance in liver in relation to controls. However, IGF-I and IGFBP-3 concentrations in serum and IGF-I mRNA abundance in liver were greater for rbST- relative to rbGRF-treated cows. Despite the above described dissimilarities in variables influencing the somatotropic cascade, milk yields were not different between rbGRF- and rbST-infused cows. Therefore, VanderKooi (1993) suggested that rbGRF exerted at least part of its galactopoietic effects through processes not mediated by IGF-I nor IGFBP-3. Another interpretation for the similar milk production for rbST- compared with rbGRF-treated cows, despite the greater levels of IGF-I and IGFBP-3 for rbST-treated cows, is that,

possibly, levels of IGF-I and IGFBP-3 were above the threshold for milk synthesis stimulation in rbGRF- and rbST-treated cows. Therefore, greater stimulation of such variables by rbST did not result in increased milk yield compared with rbGRF.

Frohman et al., (1992) reported in rats that treatment with exogenous GRF increased anterior pituitary weight, and this was associated with hyperplasia of somatotrophs. Such an increase in anterior pituitary weight was also observed in rats transgenic for GRF (Asa et al., 1990). The increased anterior pituitary weight of rbGRF-treated cows in the current experiment, relative to controls, could indicate proliferation of somatotrophs, but I do not have a direct measure of this possibility. Effects of GRF on synthesis and release of ST from somatotrophs have been widely documented (Padmanabhan et al., 1987 and Frohman et al., 1992). Elevated concentrations of ST in serum of cows infused with rbGRF are probably a result of increased synthesis and release of ST from somatotrophs. Indeed, content of ST in anterior pituitaries of rbGRF-treated cows was smaller than that of controls, which I speculate supports the notion that release of ST from somatotrophs may have been increased. However, rbGRF-stimulated synthesis of ST from somatotrophs was probably also elevated because serum concentration of ST remained elevated throughout the experiment. Thus, I have no evidence that rbGRF caused the pituitary to become refractory to rbGRF treatment over the 63-d period of this experiment.

The similarities between rbST-infused and control cows for pituitary weight and ST content and concentrations suggests that rbST did not cause refractoriness to inhibit synthesis or release of ST from the anterior pituitary gland.

Knight et al. (1990) reported increased mammary gland volume in goats treated with ST. They attributed such results to decreased mammary cell loss and increased mammary cell volume. In contrast, Capuco et al. (1989) reported no changes in mammary DNA in lactating dairy cows in response to rbST. In the current

experiment, rbGRF and rbST treatments each tended to increase mammary parenchymal weight relative to controls. However, this result could not be explained by a change in mammary cell number because treatments did not affect total DNA nor DNA concentration in mammary tissue. I suggest that increased cell size may have accounted for the increased parenchymal weight.

Total RNA is an index of cell metabolic activity. Baldwin (1990) reported increased total RNA per mammary gland in lactating cows treated with rbST. In the current experiment there was an increase in mammary tissue total RNA, RNA concentration, RNA accretion between d 118 and 181 of lactation and RNA to DNA ratio for cows treated with rbGRF and rbST. Data from Baldwin (1990) suggest that rbST increased the secretory capacity of the mammary gland. These findings support the argument that at least part of the action of rbGRF and rbST on galactopoiesis is through an effect on metabolism within the mammary gland. However, I was unable to confirm such an increase in metabolic efficiency in the mammary tissue when SCM was expressed on the basis of kg of parenchyma or per g of DNA. Perhaps, calculation of these ratios diluted effects of treatments previously observed.

In contrast to the evidence of increased secretory activity in mammary cells was the finding that rbGRF or rbST treatments did not affect lactose synthesis in incubated mammary tissue slices. Increased RNA levels per mammary cell reflects increased levels of transcription and eventually translation of proteins involved in milk synthesis. Therefore, cells possessing more synthetic machinery should have had greater ability to transform glucose into lactose. The reason for this lack of effect of rbGRF and rbST on lactose synthesis is unknown.

An important characteristic of the lactating cows used in the present experiment is the fact that these animals were still growing. This situation is of interest because in the overall scheme of nutrient partitioning, body growth was not impaired as a

consequence of the great demand of nutrients by the mammary gland as a result of hormone treatments. These growing cows treated with rbGRF and rbST may have adopted different strategies to support milk synthesis than mature cows that are not growing. Some examples are discussed below.

Dry matter intake gradually increases as a result of rbST treatment (Bauman, 1992). This is in contrast to the data from Dahl et al. (1993), in which DMI decreased within time in rbGRF-infused, rbST-infused and control cows. In the current experiment, DMI increased for all groups throughout the 63-d of the experiment. Two possible explanations for this finding are that in the experiment of Dahl et al. (1993) the majority of the cows were multiparous (i.e., skeletal growth had probably ceased), and they started the experiment in a more advanced stage of lactation (175 d). Therefore, their cows may not have had as great a demand to consume feed as did the growing and high producing cows of the present study. I also found that DMI began to increase by the second week after treatment started for cows treated with rbGRF, and gradually increased for both rbST-treated and control cows. However, this apparently quicker increase in DMI for rbGRF-treated cows was not statistically significant. I speculate that rbGRF-treated cows may have relied more on increased DMI to support increased milk production than rbST-treated cows.

Effects of rbGRF and rbST on carcass composition also support the concept that these hormones were simultaneously affecting growth and lactation of cows in the current experiment. Several studies indicate that rbST treatment changes carcass composition of growing animals: increasing lean (protein and water) and decreasing fat in the carcass (Enright, 1989; Moseley et al., 1992; Vestegaard et al., 1993). In fact, Moseley et al. (1992) showed that an important effect of rbST injected to feedlot steers was that it promoted changes in the composition of gain (i.e., increased lean, decreased fat tissue) rather than improving average daily gain. In contrast, for mature lactating

Holstein cows, rbST treatment did not affect lean tissue growth (Solderholm et al., 1988). Treatments with rbGRF and rbST increased carcass water and protein and decreased carcass fat of cows in the current experiment. Accretion of lean tissue is consistent with the fact that animals in the present experiment were still growing, as evidenced by increasing BW in all groups. Therefore, primiparous cows in the present experiment responded to the effects of rbGRF and rbST more like growing steers than like mature cows. Mobilization of adipose depots probably provided the extra energy needed for milk synthesis and lean tissue growth.

Another possible strategy adopted by rbGRF- and rbST-treated cows in order to cope with simultaneous processes of growth and lactation could involve alterations in organ weights. Increased organ weights possibly play a role in increasing availability of nutrients to the mammary gland and thereby contribute to the galactopoietic effects of rbGRF and rbST. For example, Davis et al. (1988) reported an increase in cardiac output and mammary gland blood flow in cows treated with rbST. They interpreted this finding as a mechanism influenced by rbST that directed more nutrients to the mammary gland for milk synthesis. Increased cardiac output may explain increased weight of hearts (increased mass to support increased activity), lungs (more blood had to be oxygenated per unit of time) and kidneys (more blood had to be filtered per unit of time) in rbGRF- and rbST-treated cows in the current experiment. This contrasts with findings of Brown et al. (1989), who reported that rbST treatment did not affect weight of the above mentioned organs, but the cows used in their study were mature. In contrast, they reported that rbST treatment increased foregut mass, which was also observed in the current experiment. Increased weights of intestines of cows in the current experiment may reflect an increased ability of rbGRF- and rbST-treated animals to ingest and absorb nutrients. It is possible that increased organ weights were associated with the ability of

rbGRF- and rbST-infused cows in the present study to repartition nutrients towards the processes of growth and lactation.

In addition to increased organ weights in response to rbGRF and rbST treatments, liver weights were increased in rbST-infused cows relative to controls. VanderKooi (1993) reported increased mRNA abundance for IGF-I in liver tissue of cows treated with rbST in relation to cows treated with rbGRF in the same experiment described in this thesis. Perhaps the recombinant bST used in this experiment stimulated liver tissue more than did endogenous ST secreted by the anterior pituitary gland of cows treated with rbGRF, resulting in greater liver weights for rbST- than for rbGRF-treated cows.

Despite greater milk production for rbGRF- and rbST-treated cows compared with controls, DMI and BW were similar among the three groups of cows throughout the entire experiment. The fact that control cows were ingesting as much feed as hormone-treated cows suggests that control animals were using feed nutrients in processes other than lactation. In fact, carcass weight tended to be greater and dressing percentage was also greater for controls relative to rbGRF- and rbST-infused animals, indicating that carcass growth was occurring in control cows whereas non-carcass growth was occurring in hormone-treated animals. Such carcass growth was mostly explained by increased fat deposition, as control cows had 12 kg more fat in the carcass than the average for hormone-treated cows (kg of carcass fat, unadjusted; data not shown). Unadjusted weights of organs were not different among the three groups of cows (data not shown), therefore, weights of organs measured did not account for non-carcass growth of hormone-treated animals. I speculate that weight of ingesta was greater in the gastrointestinal tract of both rbGRF- and rbST-treated cows relative to controls. Therefore, the similar body weights observed among the three treatment groups in this experiment had different origins; control cows increased their body weight mainly

because of increased deposition of fat in the carcass, whereas hormone-treated cows probably increased their body weight because of increased weight of ingesta in the gastrointestinal tract.

Somatotropin is a homeorhetic regulator that influences metabolic processes by promoting a repartitioning of nutrients that favors preferential uptake of nutrients by the mammary gland during lactation (Peel and Bauman, 1987). Homeorhetic changes include increased mobilization and decreased accumulation of adipose tissue. However, these effects on adipose tissue are dependent on EB. For example, when lactating cows are in negative EB, lipid mobilization (lipolysis) is increased as reflected by decreased body fat and chronic elevation on serum concentration of NEFA (Barbano et al., 1992). In contrast, when cows are in positive energy balance, effects of ST on adipose tissue are on inhibition of lipogenesis, not on stimulation of lipolysis (Bauman, 1992). Data from the current experiment disagrees with such findings of Bauman. Calculated EB was always positive for all groups in all periods of the current experiment, but lipolysis was clearly stimulated in rbGRF- and rbST- infused cows. For example, there was increased NEFA concentration in serum, and decreased weights of omental, perirenal and carcass fat depots and decreased percent fat in mammary gland. Moreover, while control cows gained adipose tissue in both omental and perirenal depots, rbGRF- and rbST-treated cows mobilized fat from those same depots at a much greater rate than the gain of adipose tissue in control cows. These findings suggest that mobilization of fat was occurring. There are two possible explanations for the occurrence of lipolysis in cows in positive calculated EB. First, calculated EB does not necessarily reflect fat balance status. In fact, McNamara et al. (1986) discussed that although in positive EB, cows may be in negative fat balance, where mobilization of fat is greater than accretion of fat. Second, I speculate that it is not absolutely necessary that cows reach negative calculated

EB in order to initiate lipolysis. Perhaps, merely decreasing calculated EB is sufficient to evoke a lipolytic state.

Liesman and Emery (1993, personal communication) collected adipose tissue from the omental depot of cows used in the current experiment and observed that lipoprotein lipase activity (an enzyme involved with accumulation of fat in adipose tissue) in rbGRF- and rbST-treated cows was reduced on d 63 (d of slaughter) as compared with controls. Moreover, they found less protein (i.e., more fat) per g of adipose tissue (omental depot) in rbGRF-infused cows in comparison with rbST-infused cows. Therefore, I postulate that although lipogenesis was equally impaired for both rbGRF- and rbST-treated cows, rbGRF-treated cows were mobilizing less fat from the omental depot compared with rbST-treated cows. In fact, weight of the omental fat depot was numerically greater for rbGRF- than for rbST-treated animals and concentration of NEFA in serum on d 57 was numerically smaller for rbGRF- relative to rbST-treated cows. Moreover, greater DMI and calculated EB on d 59 for rbGRF- compared with rbST-treated animals, leads me to speculate that towards the end of the experiment, rbGRF-treated cows were relying less on mobilization of adipose to maintain elevated milk production compared with rbST-treated animals.

One main thrust of this thesis was to determine possible ST-independent effects of rbGRF on variables involved in galactopoietic responses. However, except for liver weight, there was no evidence for such effects in the variables that I measured.

SUMMARY AND CONCLUSIONS

The overall goal of this thesis was to examine potential mechanisms whereby rbGRF and rbST exert their galactopoietic effects in dairy cows. Infusions of rbGRF and rbST each increased concentrations of ST in serum of cows relative to non-infused controls. Milk yields were stimulated by rbGRF and rbST compared with no treatment.

The first objective of this thesis was to compare the effects of rbGRF and rbST on homeorhetic adaptation. All three groups of cows increased BW, but composition of gain was different. For example, rbGRF- and rbST-treated animals had a greater percentage of lean tissue and smaller percentage of adipose tissue in the carcass relative to control animals. Increased organ weights, presumably to support increased cardiac output and repartitioning of nutrients, was also observed for both rbGRF and rbST groups relative to controls. Despite positive calculated EB for both rbGRF- and rbST-treated cows throughout the whole experiment, adipose tissue mobilization occurred as indicated by increased NEFA concentrations in serum and reduced weight of carcass, omental and perirenal adipose weights and mammary gland fat percentage. Mobilized fat probably provided extra energy necessary for increased milk production of cows treated with rbGRF or rbST. Altogether, homeorhetic changes set forth by rbGRF and rbST probably increased availability of nutrients to the mammary gland and skeletal muscle. My data support the notion that cows receiving rbST have a greater reliance on lipolysis as a source of energy for extra milk production than cows receiving rbGRF.

A second objective was to examine the effects of rbGRF and rbST on mammary function. Relative to controls, rbGRF and rbST treatments did not affect

mammary cell numbers, but both treatments elevated metabolic activity of mammary cells. This indicates that both rbGRF and rbST treatments increase the ability of the mammary gland to take up milk precursors from the circulation and to synthesize milk. In contrast, lactose synthesis in vitro was unmodified by any of the hormone treatments in relation to controls.

My third objective was to examine the effects of rbGRF and rbST on anterior pituitary function (i.e., synthesis and release of ST). Anterior pituitary weight, ST concentration in anterior pituitary and ST concentration in serum of rbGRF-treated cows support greater synthesis and release of ST than in controls. Both anterior pituitary weight and ST concentration in the anterior pituitary were similar when rbST-treated and control cows were compared. Development of refractoriness to either rbGRF or rbST did not occur. In addition, neither mammary function or body growth become refractory to either rbGRF or rbST.

In conclusion, rbGRF and rbST increased the ability of dairy cows to repartition nutrients towards the mammary gland, and also increased the ability of the mammary gland to take up nutrients from the circulation and synthesize milk. None of the variables analyzed in this thesis provided strong evidence for galactopoietic effects of rbGRF independent of ST.

APPENDICES

APPENDIX A

Extraction of ST from anterior pituitary glands

Extraction of ST was performed on one half of each anterior pituitary gland of each animal. Frozen tissue was weighed, placed in a 16 x 100 mm plastic tube, minced with scissors, mixed with 1.0 ml of .01 N NaOH solution, and homogenized by sonication (Polytron, Brinkmann, Westbury, NY). The pellet was rinsed with 2.0 ml of .01 N NaOH, centrifuged for 15 min at 1800 x g, and the supernatant was poured off and refrigerated. The steps were repeated with the remaining pellet, and the two supernatants were combined for subsequent quantification of ST.

APPENDIX B

Quantification of mammary parenchymal nucleic acids

One part of frozen ground parenchyma (approximately 10 g) was placed with two parts of dry ice in the cup of a blender (Junke & Kunkel, West Germany) and blended until powdered, at 4 C. Powdered parenchyma was sifted through a flour sifter, and the resulting material was stored at - 20 C for 6 to 8 h to allow the dry ice to sublimate. Approximately 1.0 g of powdered parenchyma was transferred to a 50 ml conical polypropylene tube (Corning Inc., Corning, NY). Forty ml of 100% ethanol was immediately added to the tube that was sealed with a screw cap. Tubes were placed horizontally in a rack and shaken vigorously for 24 h. Tubes were subsequently centrifuged for 20 min at 1000 x g and the supernatant was poured off. The same sequence was repeated using 40 ml of 2:1 methanol:chloroform followed by 40 ml of ethyl ether. After the ethyl ether was poured off, tubes were recapped and stored for 36 h at 4 C, so the residual ether would evaporate. Dry matter content of the resulting fat extracted powder was measured for use in calculations. A .14 g sample of frozen, fat extracted powder was used for DNA and RNA determinations, following the procedure of Tucker (1964).

APPENDIX C

Adjustment of mammary parenchymal weight

Dry, fat-free tissue weights of cows were normalized by estimating the amount of milk solids non-fat present per g of tissue (TSP/g) for cows killed at different times and subtracting that amount from dry, fat-free tissue weights. Calculations were performed for each cow as follows:

total milk synthesized per h (TM/h):

TM/h = (milk yield from AM milking for 3 d prior to slaughter) / 3 / 8.75 h (time elapsed between AM and midday milkings);

total solids non-fat synthesized per h (TS/h):

TS/h = (TM/h) x solids non-fat percentage from milk composition analysis (obtained 3 d before slaughter);

total solids non-fat present in half udder at time of slaughter (TSS):

TSS = (TS/h) x time elapsed between AM milking and slaughter time / 2;

total solids non-fat per g of mammary parenchyma at time of slaughter (TSP/g):

TSP/g = (TSS) / g of dry, fat free mammary parenchyma weight of half udder.

APPENDIX D

Quantification of lactose synthesis

In order to deproteinize samples, 500μl of media were mixed with 500μl of 1 N perchloric acid and centrifuged for 10 min at 1550 x g, and immediately neutralized with 138μl of 4 N KOH solution. Tubes were than centrifuged for 10 min at 1550 x g to precipitate the salt, and placed in an ice bath for 15 min. Supernatants were saved for lactose quantification. Three hundred microliters of deproteinized samples were mixed with 1500 μl of potassium phosphate buffer (pH 7.5) and 200μl of .1 % NAD (Sigma Chemical Co., St. Louis, MO) solution. Absorbance (A1) at 340 nm wavelength was measured in a Beckman DU-64 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) blank-calibrated with air. Galactose dehydrogenase (Sigma Chemical Co., St. Louis, MO) (1.2 U) and β-galactosidase (Sigma Chemical Co., St. Louis, MO) (10 U) were mixed in the tubes, and tubes were incubated in a shaking water bath (60 cycles per min) at 37 C for 1 h. Absorbance was measured again (A2), and the difference of A2 minus A1 was used in an equation developed from the lactose standard curve (mg of lactose / unit of absorbance). All lactose values were corrected for tissue differences in weight. Lactose synthesis per h per g of tissue was calculated, within a given cow, by subtracting the lactose produced by mammary tissue slices incubated for 3 h from lactose produced by mammary tissue slices incubated for .5 h, divided by 2.5 h.

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