CHAPTER II

BACKGROUND INFORMATIONS

The structure of Growth hormone

Growth hormone or somatotropin is a protein hormone synthesized by the anterior pituitary gland. The secretion of growth hormone from the pituitary gland is regulated by two peptides: the growth hormone-releasing factor (GRF), which stimulates the release and the somatostatin, which inhibits the release. In addition to these two peptides, a third as yet unidentified hormone binds to the growth secretagogue receptor to stimulate growth hormone release using a signal transduction pathway distinct from that of GRF is reported (Etherton and Bauman, 1998).

Growth hormone contains 191 amino acids, and bovine somatotropin (bST) and porcine somatotropin (pST) share a high degree of amino acid sequence similarity (~90%). In contrast, the amino acid sequence of both bST and pST is appreciably different from that of human somatotropin (hST) (~35% of the amino acids in hST differ from these of bST and pST). Because of this difference, bST and pST have no effect on human growth, which is consistent with their binding affinity to the hST receptor being several orders of magnitude lower than that of hST.

It is noted that there are variant forms of growth hormone. For example, bST is released from the pituitary as four variants. These variants have either a leucine or valine substitution at position 127 and an alanine (191 amino acid sequence) or a phenylalanine (190 amino acid sequence) at the NH2 terminus. The variation in the NH2 terminus is due to differences in the cleavage of the signal peptide. The frequency of these gene alleles differs between dairy breeds.

Recombinantly derived forms of bST that have been used experimentally can differ slightly from the bST produced by the pituitary gland. Depending on the manufacturing process, from 0 to 8 extra amino acids are attached to the N-terminus of the bST molecule. However, when the same purification techniques are used, recombinantly derived and pituitary-derived bST have similar potency in various biological test systems (Etherton and Bauman, 1998).

Exogenous growth hormone must be injected to be biologically active. The digestive tract secretes enzymes that break proteins down to amino acids to be absorbed. If exogenous growth hormone is given orally, it is broken down to amino acids in the digestive process just like other dietary proteins. These reason is that bST is cleared rapidly from the blood stream and is not stored in the body. Clearance of bST occurs via normal body mechanisms and involves breaking the protein down to amino acids. An achievement for sustaining increase in milk yield needs to give daily injections or use a prolonged-release formulation of bST. Several prolonged-release formulations have been developed with small volumes that are administered by subcutaneous injection at time intervals ranging from 2 to 4 weeks (Bauman, 1992).

Mechanisms of growth hormone action

Growth hormone is a homeorhetic control that affects numerous target tissues in ways that are highly coordinated to affect marked changes in nutrient partitioning among these tissues. The biological effects of growth hormone can be broadly classified as either somatogenic or metabolic. The somatogenic effects are those in which ST stimulates cell proliferation. These effects are mediated by IGF-I. Many of the metabolic effects are a direct action of ST that involve a variety of tissues and the metabolism of all nutrient classes: carbohydrate, lipid, protein, and minerals. These coordinated changes in tissue metabolism alter nutrient partitioning and thus play a

key role in increasing growth performance or milk yield. (Etherton and Bauman, 1998).

Milk responses for bST administration

For all species, milk yield follows the lactation curve, increasing to peak yield and then declining at weaning or the cessation of milking. For dairy cows, genetic selection has greatly increased two interrelated factor, peak yield and lactation persistency, declined as the change of yield with time in mid-lactation. Broster and Broster (1984) calculated that peak yield of dairy cows accounts for 66 to 80% of the variance in total yield compared with 8 to 12% for persistency. Peak yield is in turn determined by secretory cell number and by secretory activity per cell. Studies in goats by Knight and Wilde (1993) show that parenchyma cells increase in number during pregnancy and early lactation. Between parturition and peak lactation secretory cells increase in size and become more fully differentiated. After peak, cell loss is largely responsible for decline in milk yield, but the activity per cell is maintained.

Milk yield response to bST administration has been observed for all dairy breeds and in animals of different parity and genetic potential (Burton et al., 1994). In general, response is negligible in early lactation before peak yield, so bST use is over the last 80% of the lactation cycle. Typical milk yield responses are increase of 10-15% (~4-6 kg/day), although even greater increase occur when the management and care of the animals are excellent (Bauman, 1992). The pattern of response is one where milk yield gradually increase over the first few days of bST treatment and reaches a maximum during the first week. If treatment is terminated, milk yield gradually returns to pretreatment leveles over a similar time period. However, when treatment is continued, the increased milk yield is maintained. Thus bST

administration results in greater peak milk yield and an increased persistency in yield over the lactation cycle. (Etherton and Bauman, 1998).

Exogenous growth hormone enhances milk production in dairy cows by coordinating a complex series of adaptations within the body (Richard et al., 1985). Treatment with bST increases the rate of milk production within the mammary gland and provides the necessary nutrients in support of this enhanced rate of milk synthesis. Voluntary intake increases in bST supplemented dairy cows. This increase in voluntary intake occurs after a few weeks of bST supplementation, persists throughout the interval of bST use, and has been consistently observed across a wide range of diets. The magnitude of increase in feed intake is dependent on the response in milk yield and the energy density of the diet (Soderholm et al., 1988). Overall, cows supplemented with bST adjust their voluntary intake in a predictable manner related to the extra nutrients required for increased production of milk (Bauman, 1992).

Additional nutrients required to maintain augmented rates of milk production in bST-treated cows appear to be of endogenous origin early in the treatment period (Peel and Baunman, 1987). Examination of nutrient uptake in cows treated with bST demonstrated that arterial-venous differences of glucose decreased across the leg muscles and that udder NEFA arterial-venous differences increased (McDowell et al., 1987). Subsequently, investigations of the response of lactating ewes to short- and long-term bST administration indicated that NEFA and glucose arterial-venous differences across the leg muscle decreased, whereas mammary gland NEFA arterial-venous differences increased, after 3 d of bST administration. In the same study, glucose and NEFA uptakes by leg muscles and mammary glands did not differ after 45 d of bST treatment. Irreversible losses (IL) of glucose and NEFA increased with bST administration (McDowell et al., 1988). The increase in glucose IL was in spite of a decrease in oxidation rate and due to the increase in conversion to lactation. Higher

rates of NEFA IL in bST-treated animals were observed in spite of increased oxidation rates in the mammary glands and were correlated with plasma concentrations and energy balance. Additional data are still required to improve an understanding of the effects of physiological state, genetic potential, and exogenous bST administration on nutrient arterial concentrations and uptake by the mammary glands (Miller et al., 1991).

Increased milk yield should reflect increased flow of blood carrying milk precursors to the mammary gland. Control of mammary blood flow (MBF) may be a way to control nutrient partitioning. Cardiac output was reported to be 10% higher and MBF increased by 35% in bST-treated cows studies by Davis et al. (1988).

The daily outputs of major milk constituents (lactose, fat, protein, minerals and vitamins) are elevated by an amount comparable to milk volume in bST-treated cows (Burton et al., 1994). The concentrations of fat and protein in milk normally vary as a result of factors such as genetics, breed, stage of lactation, season, diet and nutritional status. These similar factors also affect the composition of milk from bST-treated cows (Etherton and Bauman, 1998).

Effects of bST administration on lipogenesis and lipolysis

Propionate and acetate are the main energy sources in ruminant animals because of their availablility and high are rate of uptake by the lactating mammary gland, acetate and to a lesser extent, β-hydroxybutyric acid are considered the most important energy metabilites in mammary gland metabolism of ruminants. Two of the most significant functions of acetate are to supply carbon atom for *de novo* synthesis of fatty acids and to generate adenosin triphosphate through the tricarboxylic acid acid cycle and the electron transport system. Growth hormone has dramatic effects on

adipose tissue and lipid metabolism. Both lipogenesis and lipolysis are altered by growth hormone treatment, with effects on lipid synthesis being of major importance if animals are in positive energy balance, whereas effects on lipolysis predominate when animals are at an energy balance near zero or negative (Peel and Bauman, 1987).

When bST treatment causes cows to be in negative energy balance, lipid decreases in body fat, chronic elevation of circulating NEFA, and increases in milk fat percentage and the proportion of long-chain fatty acids in milk. Under such conditions, use of body fat reserves is increased to an extent related to the degree of negative energy balance and quantitatively equal to increases in fatty acid oxidation and secretion of long-chain fatty acids in milk. This increased reliance on NEFA as metabolic fuel facilitates the reduction in glucose oxidation. When bST-treated cows are in positive energy balance, lipogenesis by adipose tissue is decreased, and body fat mobilization, milk fat percentage, and milk fatty acid composition are unaffected. The magnitude of the reduction in lipogenesis is a function of amount of excess energy available. With chronic bST use, lipid metabolism gradually readjusts as voluntary intake increase. Thus, typical replenishment of body reserves can occur during lactation under a wide variety of dietary conditions (Chalupa and Galligan, 1989).

The regulation of lipolysis involves cAMP and a signal transduction system that includes stimulatory G proteins (G_s) and inhibitory G proteins (G_i). Catecholamines effect lipolysis through the G_s system, and growth hormone treatment dramatically increases the lipolytic respond to catecholamines in lactating cows (Sechen et al., 1990). This change in the response to catecholamines is evident within 15 h after the initiation of growth hormone treatment and in observed regardless of whether animals are in a positive or negative net energy balance (Etherton and Bauman, 1998).

Effects of bST administration on carbohydrate metabolism

Growth hormone has numerous tissue effects related to carbohydrate metabolism. This is of particular importance in the dairy cow in which glucose originates almost exclusively from gluconeogenesis and typically 60-80% of the glucose turnover is used for milk synthesis. Treatment of cows with bST increases the rate of glucose irreversible loss and reduces whole body glucose oxidation (Bauman et al., 1988). These adaptations in glucose production and oxidation in bST-treated cows are quantitatively equal to the extra glucose required for the increased milk synthesis. Hepatic rate of gluconeogenesis are increased with treatment of dairy cows as demonstrated by in vivo and in vitro studies (Knapp et al., 1992). Mechanism include a decreased ability of insulin to inhibit gluconeogenesis. Thus the reduction in hepatic response to insulin in bST-treated cows allows the liver to sustain an increased rate of gluconeogenesis that is critical to support the increase in the synthesis of milk components. In contrast, growth hormone treatment had no effect on liver glycogen concentration in lactating cattle in positive energy balance, although growth hormone treatment did include a small decrease in cows in negative energy balance (Knapp et al., 1992). Liver glycogen reserves are too limited to sustain increased glucose output by the liver in lactating cows.

Effects of bST administration on protein metabolism

The effects of growth hormone on growth and protein metabolism depend on an interaction between growth hormone and somatomedins, which are polypeptide growth factor (70 amino acids) secreted by liver and other tissues in response to stimulation by growth hormone. Little is known about the effects of growth hormone on protein metabolism of domestic animals compared to lipid or carbohydrate metabolism. It is clear that growth hormone treatment increases muscle protein

accretion in growing animals and milk protein synthesis in lactating cows. However, the precise mechanism are not clear, and the extents to which the effects of growth hormone on protein metabolism are direct or mediated by insulin-like growth factor-1 (IGF-1) remain unclear.

Effects of bST administration on blood flow

In dairy cows growth hormone has increased cardiac output 10% and mammary blood flow 35% concurrent with a 21% increase in milk yield. Similarly, blood flow has increased during growth hormone administration in goats and on some occasions blood flow increased without a concomitant increase in milk synthesis, which implied a direct effect of growth hormone on blood flow to the mammary gland. Although the precise mechanism by which blood flow to the mammary gland is controlled is uncertain, it is generally thought that autoregulation via increased metabolic activity in the mammary gland is a key component. To do this will require elegant experiments in which the time sequence of events is measured in relation to administration of growth hormone. This will determine if growth hormone is having an effect on mammary blood flow that is independent of the autoregulation that occurs as a result of the increased metabolic activity in the mammary gland (Peel and Bauman, 1987).

Effects of bST administration on mammary gland function

Treatment with bST cause a dramatic increase in the uptake and utilization of nutrients for the synthesis of milk. However, it has proven to be difficult to document specific mechanisms. At the cellular level, the magnitude of the biochemical changes would likely be small, and mammary epithelial cells, which are actively secreting milk component, are difficult to maintain in vitro because of their high rates of metabolic

activity. Nevertheless, the pattern of response to exogenous bST and the change in the shape of the lactation curve indicate that bST effects involve both an increase in the rates of milk component synthesis per cell and an improved maintenance of secretory cell (Etherton and Bauman, 1998).

Maintaining higher rates of milk synthesis requires a greater nutrient support. Some have suggested that the increase in mammary rate of milk synthesis was merely the consequence of growth hormone effects on nonmammary tissues that allowed for a greater supply of nutrients to the mammary gland (Keys et al., 1997). However, it is clear that simply increasing nutrient available by itself dose not mimic the effect of bST on lactation performance (Burton et al., 1994); rather, bST is a homeorhetic control that results in a coordinated series of changes involving both nutrient supply and mammary utilization. This coordination includes a diversion of cardiac output and an increase in blood flow to the mammary gland that parallels the magnitude of the milk response to exogenous bST.

The mechanism by which growth hormone affects mammary gland function is still uncertain but appears to be indirect, involving the IGF system. Several lines of evidence indicate that exogenous somatotropin dose not act directly on the mammary gland (Peel and Bauman, 1987). Bovine research has been focused on the association between bST and IGF-1 primary. Implicating IGF-1 in bovine galactopoiesis includes observations of chronically elevated IGF-1 concentrations in blood and lactating mammary tissue during periods of bST administration. Administration of bST to lactating cows causes an increase in concentration of IGF-1 in blood (Davis et al., 1987).

There are abundant type I and type II IGF receptors in bovine mammary tissue, and IGF-1 addition to bovine cell culture systems increases casein synthesis (Burton et al., 1994). In contrast, attempts to detect growth hormone receptors in bovine mammary tissue have been unsuccessful, and only a very low level of expression of growth hormone receptor mRNA can be detected. Close arterial infusion of the mammary gland with bST had no effect on milk yield , whereas close arterial infusion of IGF-1 or IGF-II stimulates milk yield. A role for IGF-1 is also consistent with observations that IGF-1 dramatically increases blood flow to the mammary gland, and this effect appears to be mediated by local production of nitric oxide (Etherton and Bauman, 1998).

Growth hormone and water metabolism of dairy cattle

Water is the most important nutrient for dairy cattle. It is required for all of life's processes-transport of nutrients and other compounds to and from cells; digestion and metabolism of nutrients; elimination of waste materials (urine, feces, and respiration) and excess heat (perspiration) from the body; maintenance of a proper fluid and ion balance in the body; and provision of a fluid environment for the developing fetus (Murphy, 1992). A loss of 20 percent of the body water is fatal.

The total body water content of dairy cattle is 56 to 81% of their body weight (Murphy, 1992). Physiologic stage and body composition affect the body's water content. Cows in early lactation have more body weight in water (69%) than cows in late lactation (62.4%) with late-gestation dry cows intermediate in body water content (64.7%) (Andrew et al., 1995). Fat cows have a lower water content than thin lactating cows, and younger, leaner animals have a higher water content than older animals (Murphy, 1992). Reid and co worker (1955), described the constancy of the fat-free

matter (FFM) in growing cattle. The FFM contains water, protein and ash and these proportions remain relatively constant.

Body water is divided into intracellular and extracellular compartments. Intracellular water is the largest compartment, accounting for about two-thirds of the water in the body. The extracellular fluid comprises water around cells and connective tissue, water in plasma, and transcellular water or water in the gastrointestinal tract. Intestinal water accounts for 15-35% of body weight (Woodford et al., 1984). Cows in early lactation had about 15% of their body weight in gastrointestinal water (Arnold and Trenkle, 1986), while cows in late lactation and in gestation had 10 to 11% (Andrew et al., 1995). Resident time of a water molecule in the rumen was estimated to be 61 minutes in sheep and 62 minutes in lactating dairy cattle (Woodford et al., 1984).

Loss of water from the body occurs through milk production, urine excretion, fecal excretion, sweat, and vapor loss from the lungs. Water losses through milk of cows producing 33 kg/day were about 34% (Holter and Urban, 1992), 29%, and 26% of total water intake (feed plus free water consumed).

It has been reported that injection with bST significantly increased body water of animals due to lower body lipids (Chiliard et al., 1991.). The study in human, several studies have recently shown that growth hormone deficiency (GHD) in adults is associated with reduction in total body water (TBW) and extracellular water (ECW). Suggestion a low TBW could be explained by a lower ECW compartment (Binnerts et al., 1992). Several other studies directly measured ECW, but not TBW, with variable reports of low ECW, a proportional decrease in ECW corresponding to a decrease in fat-free soft tissue mass (FFSTM). In GHD adults, GH therapy appears to increase fat-free mass (FFM) and decrease fat mass, thereby improving body composition. A study

the effect of GH replacement therapy for 4 and 52 weeks on body water distribution by Janssen and co-worker (1997) showed patients with GHD had significantly lower ECW and TBW than healthy controls. Four weeks of GH treatment significantly increased body weight, TBW and ECW. A further increase in TBW, but not ECW, was found after 52 weeks of treatment.