This is to certify that the

thesis entitled

ADMINISTRATION OF SOMATOTROPIN BEFORE PARTURITION
ELICITS A SHORT-TERM INCREASE
IN SUBSEQUENT MILK PROTEIN SYNTHESIS

presented by

Christine Ruth Simmons

has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

H. Allen Tucker
Major professor

Date June 3, 1993

ADMINISTRATION OF SOMATOTROPIN BEFORE PARTURITION ELICITS A SHORT-TERM INCREASE IN SUBSEQUENT MILK PROTEIN SYNTHESIS

By

Christine Ruth Simmons

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

ADMINISTRATION OF SOMATOTROPIN BEFORE PARTURITION
ELICITS A SHORT-TERM INCREASE
IN SUBSEQUENT MILK PROTEIN SYNTHESIS

By

Christine Ruth Simmons

During early lactation, cows mobilize body protein and fat because nutrients derived from feed are insufficient for body maintenance and milk production. An objective of this thesis was to determine if bovine somatotropin (bST) administered daily beginning 46 d before parturition would increase body protein and subsequent yield of milk. A second objective was to determine if bST treatment during the dry period would affect fat metabolism. A third objective was to determine if bST treatment during the dry period would alter the concentrations of somatotropin and insulin-like growth factor-I.

Bovine somatotropin increased protein reserves during the dry period. The 14-mg dose of bST increased yield of milk protein during the first week of lactation when protein degradation was elevated. Furthermore, bST treatment during the dry period did not alter fat metabolism. Lastly, energy balance is a major regulator of the responsiveness of insulin-like growth factor-I to ST.

ACKNOWLEDGEMENTS

I wish to express appreciation to my major professor, Dr. H. Allen Tucker, for the guidance and expertise that he has provided me during the completion of my masters program. I also extend thanks to my committee, Drs. Werner Bergen, Thomas Herdt, and Michael VandeHaar for their time and insight. In addition, I would like to thank Dr. Charles Sniffen for his advice throughout my project.

I thank Drs. Edward Stanisiewski and James Lauderdale from the The Upjohn Company for support and critical evaluation of my research. I also thank Mr. Bud Krabill for his assistance with sample analysis.

I would like to thank Mr. Larry Chapin for his help in all aspects of my research. I also thank Miss Miriam Weber for her help in the execution of my experiment, which included the collection of many samples. I thank Mr. Thomas Pilbeam for assigning body condition scores to my cows. I thank Dr. David Sprecher for his expertise regarding ultrasonography. In addition, I thank the graduate students in the Animal Reproduction Laboratory for their assistance with my project. I also would like to thank Mr. Jeff West for his friendship and patience.

Lastly, I express deepest gratitude to my parents, Steve and Ruth Simmons, for their encouragement, support, and love throughout not only my masters program but also for the last 25 years.

TABLE OF CONTENTS

LIST OF TABLES vi

LIST OF FIGURES vii

INTRODUCTION 1

LITERATURE REVIEW 3

- Protein Metabolism 3
- Effects of Pregnancy and Lactation on Protein Metabolism 6
- Hormonal Control of Protein Metabolism 10
- Lipid Metabolism 13
- Effects of Pregnancy and Lactation on Lipid Metabolism 15
- Hormonal Control of Lipid Metabolism 16

MATERIALS AND METHODS 18

- Design and Management 18
- Blood Collection and Analysis 20
- Urine Collection and Analysis 21
- Body Composition Measurements and Calculated Energy Balance ... 24
- Reproductive Characteristics and Health Disorders 24
- Statistical Analysis 25

RESULTS 26

- Measurements of Protein Metabolism 26
- Measurement of Fat Metabolism 29
- Measurements of Body Composition 33
- Concentrations of Serum Hormones 37
- Milk Production and Components 41
- Dry Matter Intake (DMI) and Calculated Energy Balance 44
- Reproductive Characteristics and Health Disorders 47

DISCUSSION 50

SUMMARY AND CONCLUSIONS 59

APPENDICES 61

Appendix A 61
Appendix B 62
Appendix C 63
Appendix D 64
Appendix E 66

LIST OF REFERENCES 67

LIST OF TABLES

Table 1. Reproductive characteristics of cows receiving no treatment (n=14), 5 mg bST/d (n=14), or 14 mg bST/d (n=13) for the last 46 d before parturition 48

Table 2. Incidences of health disorders of cows receiving no treatmemt (n=14), 5 mg bST/d (n=14), or 14 mg bST/d (n=13) for the last 46 d before parturition 49

Table 3. Chemical composition of diets fed during the first 5 weeks of the dry period (early dry) and the remainder of the dry period until parturition (late dry) 61

Table 4. Chemical composition of the diet during the lactation period (d 0-69) 62

Table 5. Comparison of urinary N-$^{\tau}$methylhistidine/creatinine (nmol/mg) in samples collected at 0700 h and 1900 h 66

LIST OF FIGURES

Figure 1. Concentrations of serum urea nitrogen of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .8 mg/dl during the dry period and .9 mg/dl during the lactation period. 27

Figure 2. Concentrations of urinary creatinine of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 8.4 mg/dl during the dry period and 5.2 mg/dl during the lactation period. 28

Figure 3. Ratio of urinary N^{τ}-methyl histidine/creatinine of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 9.0 nmol/mg during the dry period and 7.9 nmol/mg during the lactation period. 30

Figure 4. Yield of milk protein of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .1 kg/d. 31

Figure 5. Concentrations of serum NEFA of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 57.7 μEq/L during the dry period and 43.3 μEq/L during the lactation period. 32

Figure 6. Bodyweights of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means) adjusted by covariance with initial BW. Standard error of the difference among treatments was 7.0 kg during the dry period and 9.2 kg during the lactation period... 34

Figure 7. Body condition scores of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .5 during the dry period and .2 during the lactation period. 35

Figure 8. Subcutaneous fat between the 12th and 13th ribs of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .08 cm during the dry period and .07 cm during the lactation period. 36

Figure 9. Concentrations of serum ST of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 1.8 ng/ml during the dry period and .7 ng/ml during the lactation period. 38

Figure 10. Concentrations of serum IGF-I of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 9.1 ng/ml during the dry period and 2.7 ng/ml during the lactation period. 39

Figure 11. Concentrations of serum cortisol of cows receiving no treatment (•; n=6), 5 mg bST/d (▲; n=6), or 14 mg bST/d (▪; n=5) for the last 46 d before parturition. Average time of parturition is indicated by the arrow. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .13 ng/ml. 40

Figure 12. Concentrations of serum prolactin of cows receiving no treatment (•; n=6), 5 mg bST/d (▲; n=6), or 14 mg bST/d (▪; n=5) for the last 46 d before parturition. Average time of parturition is indicated by the arrow. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 19.3 ng/ml. ... 42

Figure 13. Solids corrected milk yield of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Each point represents the average of a treatment group (least squares means) within each 7-d period. Standard error of the difference among treatments was 2.3 kg/d. 43

Figure 14. Dry matter intake of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line. Each point represents the average of a treatment group (least squares means) within each 7-d period. Standard error of the difference among treatments was .9 kg/d during the dry period and 1.9 kg/d during the lactation period. 45

Figure 15. Calculated energy balance of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line. Each point represents the average of a treatment group within each 7-d period. Standard error of the difference among treatments was 1.0 Mcal/d during the dry period and 1.2 Mcal/d during the lactation period. 46

Figure 16. Representative trace from HPLC analysis of N^{τ}-methylhistidine in a urine sample spiked with pure N^{τ}-methylhistidine. The fluorescent excitation wavelength was 340 nm and the fluorescent emmission wavelength was 440 nm. The elution time of the N^{τ}-methylhistidine peak was 14.13 minutes. 64

Figure 17. Representative trace from HPLC analysis of N^{τ}-methylhistidine in an experimental urine sample. The fluorescent excitation wavelength was 340 nm and the fluorescent emmission wavelength was 440 nm. The elution time of the N^{τ}-methylhistidine peak was 13.90 minutes. 65

INTRODUCTION

The major function of livestock production is to provide protein, energy, minerals, and vitamins for the human diet. Ruminant animals can obtain much of their energy and protein from feeds not directly suitable for human consumption. Milk supplies a significant portion of the animal protein consumed in the United States as well as in other countries. Dairy cattle are efficient converters of feed protein and energy into milk protein and energy.

Numerous demands are placed on the dairy cow throughout lactation. Protein and energy must be available for maximal milk production as well as embryonic and fetal development. In cows producing large quantities of milk, the mammary gland takes priority in protein and energy utilization over other organs and systems.

In high producing dairy cows during early lactation, protein and energy derived from feed are less than that required for body maintenance and milk yield. Thus, utilization of body reserves, which results in a loss of body condition and body weight, is necessary to meet protein and energy requirements of peak milk production. Therefore, cows must calve with an adequate amount of stored tissue reserves for mobilization to help meet the protein and energy requirements of early lactation. Body tissue reserves need to be restored during late lactation or during the interval of nonlactation between consecutive lactations (dry period) in

preparation for the subsequent lactation. It is also important that dairy cows do not become overconditioned during the dry period because overconditioning during this interval makes them more susceptible to metabolic diseases in the subsequent lactation.

Efficiency of utilization of metabolizable energy for body reserve repletion is greater for lactating cows than for nonlactating cows. This is of concern, because average milk production per cow has increased in the dairy industry so there are more dairy cattle that enter the dry period with fewer body reserves. As a result, a large proportion of the repletion of body reserves must occur during the inefficient dry period.

Administration of bovine somatotropin (bST) increases feed conversion efficiency in growing beef steers. Furthermore, bST treatment increases protein accretion and reduces fat accretion in growing cattle and lactating cows. Therefore, bST administered to dairy cattle during the dry period may increase efficiency of feed utilization and facilitate restoration of body protein reserves, yet prevent excessive fattening prior to parturition. These effects could be beneficial in prepartum body conditioning of dairy cattle.

An objective of this thesis was to determine if bST administered to dry cows would enhance body protein reserves and thereby stimulate subsequent milk production. A second objective was to determine if bST treatment during the dry period would affect fat metabolism. A third objective was to determine if bST treatment during the dry period would alter the concentrations of somatotropin and insulin-like growth factor-I.

LITERATURE REVIEW

Protein Metabolism

Protein is one of the major components of the body's lean tissue mass, and it is metabolized continuously (Black et al., 1990). Physiological processes responsible for maintenance of body protein and amino acid homeostasis are protein synthesis, protein degradation, oxidation and intestinal absorption of amino acids, and synthesis of dispensable amino acids (Young and Marchini, 1990). Cooperative participation of the intestines, liver, kidney, and muscle determines the fate of free amino acids entering and leaving the body pools from exogenous and endogenous sources (Young et al., 1991).

Protein synthesis and protein degradation occur at all developmental stages and during all physiological conditions. Most cellular proteins are in a state of continuous turnover. In protein turnover, protein synthesis is opposed by simultaneous degradation (Sugden and Fuller, 1991). Therefore, an imbalance between synthesis and degradation rates will lead to a change in the size of the tissue protein pool. Thus, a loss of tissue protein does not necessarily entail an increase in protein degradation nor does a gain necessarily entail an increase in protein synthesis.

The amino acids that are released during intracellular breakdown of proteins

can be extensively reutilized for protein synthesis within the cell (intracellular recycling) or they may be transported to other organs where they enter pathways of protein anabolism (intercellular recycling; Young and Munro, 1978). In addition, amino acids released during intracellular breakdown may be catabolized within the cell or transported to the liver and kidneys to serve as substrates for gluconeogenesis. Eighty percent of amino acids derived from intracellular protein degradation are reutilized for protein synthesis (Millward et al., 1975). As a result, total whole body protein synthesis is two to four times greater than one would expect dietary intake of protein to support (Sugden and Fuller, 1991; Manatt and Garcia, 1992). This seeming inconsistency illustrates the body's ability to reuse amino acids made available during protein degradation (Manatt and Garcia, 1992). The intensities of amino acid recycling, protein synthesis, and protein degradation change when dietary protein intake changes (Young and Pellet, 1987). The body's first responses to dietary protein restriction are to increase its efficiency of amino acid recycling and to reduce its rate of amino acid oxidation (Young and Pellet, 1987). This recycling and decreased oxidation will conserve nitrogen in the body needed for vital protein synthesis.

In a steady state, the size of the protein pool remains constant and protein synthesis and degradation are in equilibrium. However, body protein pool size is rarely in a steady state in livestock utilized for food production (Swick and Benevenga, 1977). In a growing or lactating animal, rates of protein synthesis are higher than rates of degradation resulting in a net accumulation or production of protein. Skeletal muscle is the largest protein pool in the body.

Skeletal muscle has an unique and highly organized structure. Individual muscles are composed of a variable number of elongated, multinucleated fibers that are parallel to each other. The variation in size between similar muscles in different species is a function of the number and length of these fibers (Dayton and Hathaway, 1989). Similarly, differences in fiber number, length, and diameter are partially responsible for variations in muscle mass observed between animals of the same species. Muscle proteins are generally grouped into three categories based on their solubility: sarcoplasmic proteins, myofibrillar proteins, and stromal proteins with myofibrillar proteins constituting 50-55% of total muscle protein (Dayton and Hathaway, 1989). The myofibrillar proteins actin and myosin are found in greatest abundance in myofibrils, which are highly organized contractile threads of 12-14 proteins. The relative synthesis rate of sarcoplasmic and myofibrillar proteins is not fixed; it varies according to the nutritional state, age, and growth rate of the animals.

N^{τ}-methylhistidine (N^{τ}-MH) is an amino acid that arises from posttranslational modification of histidine in myofibrillar proteins. Release of N^{τ}-MH after the degradation of actin and some types of myosin is widely used as a method for studying muscle protein degradation (Bates and Millward, 1983). Harris and Milne (1981) validated that N^{τ}-MH is quantitatively excreted in the urine and not reincorporated into myofibrillar protein in cattle. However, controversy remains over the extent to which tissues other than skeletal muscle contribute to daily excretion of N^{τ}-MH because turnover of protein-bound N^{τ}-MH in skeletal muscle is much slower than the overall rate of protein turnover (Millward et al., 1980; Harris, 1981; Bates et al., 1983). This is not a great concern in large animals such

as cattle because the relative amount of N^{τ}-MH from non-skeletal muscle sources (7%) contributed to the total excretion of N^{τ}-MH is minimal (Nishizawa et al., 1979).

In certain tissues, protein is degraded so the resulting amino acids can be transported to other tissues and utilized in various metabolic pathways. These deposits of protein are called "protein reserves." Under a variety of physiological and pathological conditions, animals depend on skeletal muscle as a primary source of amino acids for protein synthesis in other tissues and organs (Champredon et al., 1990). Because ruminants absorb only limited quantities of glucose, they rely heavily on gluconeogenesis in the liver (about 85%) and kidneys (about 15%) for glucose production (Bergman and Heitman, 1978). Amino acids are precursors for gluconeogenesis which allows fed ruminants to derive a minimum of about 15% of their glucose from amino acids (Bergman and Heitman, 1978). Furthermore, amino acids are important for detoxifying ammonia to urea, as well as shuttling nitrogen and carbon between organs.

Effects of Pregnancy and Lactation on Protein Metabolism

The ratio of protein deposition to protein synthesis is sensitive to several factors such as nutritional and hormonal states. Adaptations of protein metabolism during pregnancy and lactation are not clearly understood, but there is a marked increase in the rate of whole body protein synthesis in both physiological states (Millican et al., 1987). This is mainly due to rapid rates of protein synthesis in the fetus, uterus, and placenta during pregnancy and mammary gland during lactation.

Pregnancy greatly increases the nutrient requirements of cows, especially

during the last trimester of gestation (Bauman and Elliot, 1983). Total requirements for nutrients at the end of pregnancy are about 75% greater than those of a nonpregnant animal of the same bodyweight (Bauman and Currie, 1980). The bulk of accumulation of fetal mass occurs late in pregnancy when dairy cows are in the dry period. During this time, amino acids are not only used as precursors for protein synthesis but are extensively metabolized within the fetus. As in the adult animal, many amino acids function as metabolic fuels in the fetus and nonessential amino acids are interconverted from one form to another serving as important pathways for shuttling nitrogen and carbon between organs (Battaglia and Meschia, 1978). The placenta is an organ that exchanges substances between the fetal and maternal circulations. Therefore, the placenta regulates the transport of metabolic substrates to the fetus. During the last 2 months of gestation, fetal requirements for glucose and amino acids are equivalent to those required by a mammary gland producing 3 to 6 kg milk/day (Bauman and Currie, 1980).

As pregnancy progresses, the uterus undergoes gradual enlargement to accommodate the growing fetus. The mechanisms that induce the enormous increase in size of the uterus are unknown but are probably hormonal (Jainudeen and Hafez, 1987). Uterine growth is comprised of muscle hypertrophy and an extensive increase in connective tissue. In experiments measuring amino acid turnover, it was apparent that a decrease in the rate of protein degradation was primarily responsible for the compensatory growth of the uterus during gestation (Morton and Goldspink, 1985).

The placenta just prior to parturition contains a large variety of enzymes that are capable of metabolizing amino acids through pathways such as gluconeogenesis,

glycogen synthesis, protein synthesis, amino acid oxidation, and formation of ammonia (Hay, 1989). Amino acids are also required by the placenta for synthesis of protein hormones. Mammary development is another metabolic burden of pregnancy. During pregnancy, the flow of nutrients into the placenta as well as into the developing mammary gland are both under control of hormones from the conceptus (Bauman and Currie, 1980).

At the initiation of lactation, marked differences in partitioning of nutrients and metabolism of the whole animal must occur to accommodate demands of the mammary gland (Baracos et al., 1991). For example, whole-body phenylalanine flux was 52% greater in lactating goats than in dry, non-pregnant goats (Baracos et al., 1991). Increases in feed intake and milk production as well as uterine involution associated with early lactation increase protein turnover. The rate of protein synthesis in the alveolar mammary cell exceeds that of any other cell type in the body with the exception of the regenerating liver cell (Blaxter, 1964).

In the cow immediately after parturition, the uterus undergoes a drastic reduction in size and reorganization of tissues. Reduction in uterine size progresses in a decreasing logarithmic scale, with the greatest change occurring during the first few days after parturition (Gier and Marion, 1968). The rapid uterine involution is due to both a decrease in the rate of protein synthesis and a complementary increase in the rate of protein degradation (Morton and Goldspink, 1985). Cathepsin D, a lysosomal protease that degrades myofibrillar protein, increases 200% in the uterus of rats after parturition (Morton and Goldspink, 1985).

The cow's ability to produce milk frequently exceeds her ability to consume

sufficient energy and protein to meet nutrient demands, therefore she is in negative energy and nitrogen balance (Baracos et al., 1991). To support milk output, maternal sources of protein and energy are necessary to supplement the diet (Lormore et al., 1990). An increased reserve of protein within the body of high producing cows in early lactation could supplement dietary protein further and thereby increase milk production. It has been estimated that up to 27% of the total body protein of a lactating cow could be degraded to provide precursors for metabolic pathways. (Botts et al., 1979).

Decreases in muscle fiber diameter, as a result of myofibrillar protein degradation, occur during the early postpartum period. Mobilization of tissue protein during the first 2 months of lactation varies from 0 to 15 kg (Chilliard et al., 1991). Therefore, during early lactation muscle tissue can serve as a protein reserve and substantial losses can be replaced with adequate nutrition later in lactation (Swick and Benevenga, 1977). The rate of protein turnover in cows is large enough, relative to milk protein production, to counteract short-term deficiencies in amino acid absorption by altering relative rates of synthesis and degradation (Oldham, 1984).

Protein metabolism in muscle of high-producing, lactating cows is modified to support the increased nutrient demands for milk production. During the initial stages of lactation, muscle protein synthesis is suppressed below degradation rates and the amino acids thereby released are channeled into milk protein production (Champredon et al., 1990). This condition persists until either amino acid absorption from the diet increases or milk production decreases, allowing the cow to return to positive nitrogen balance. Throughout the entire pregnancy-lactation cycle, nitrogen

intake must be high enough to support skeletal muscle protein synthesis so that protein reserves are large enough to make this adaptation again during the next lactation. However, it appears that the labile protein reserve can be expanded only to a limited extent (Swick and Benevenga, 1977). This is particularly true in the ruminant where a portion of dietary protein which is in excess of requirements is generally degraded to ammonia in the rumen and then excreted from the rumen, not incorporated into muscle protein.

Hormonal Control of Protein Metabolism

Hormonal control of protein metabolism is complex and multifaceted. There are numerous hormones as well as a variety of growth factors which have hormone-like activity that affect the extent and rate of protein metabolism. The endocrine system influences protein metabolism by regulating nutrient intake and partitioning of nutrients among various metabolic pathways (Young, 1980). Major cellular effects of many hormones are due to the recognition and interaction of the hormone with receptors located either on the cell membrane or within the cell. Concentrations of receptors, circulating hormones, and their associated affinities can affect the interaction of the hormone and receptor. Hormones may exert their actions via endocrine, autocrine, and/or paracrine mechanisms (Evers, 1989).

In general, insulin is the major hormone that controls basal metabolism and energy balance. In addition, one peptide hormone, growth hormone, also called somatotropin (ST), exhibits regulatory effects on metabolism and partitioning of absorbed nutrients used for growth and lactation. For example, treatment of animals

with exogenous ST increases lean tissue growth (Eisemann et al., 1989; Maltin et al., 1990). This results because administration of exogenous ST causes a net increase in protein synthesis rates which exceed the concomitant, minimal increase in protein degradation rates (Boyd and Bauman, 1989; Eisemann et al., 1989; Pell and Bates, 1987). Because protein is continually recycled, the increase in protein synthesis, both in muscle and whole body, may exceed protein accretion by as much as twofold (Lobley et al., 1987; Eisemann et al., 1989).

Somatotropin administration increases RNA concentration in muscle (Pell and Bates, 1987). Furthermore, veal calves treated with ST have elevated DNA concentrations in both semimembranous and triceps muscles (Maltin et al., 1990). The source of new nuclei in growing muscle of postnatal animals is satellite cells (Boyd and Bauman, 1989). Therefore, ST treatment of postnatal animals appears to result in satellite cell proliferation and incorporation of daughter nuclei into the adjacent myofibers (Boyd and Bauman, 1989). Thus, increases in muscle hypertrophy appear to be a result of an increase in muscle DNA, RNA, and protein.

Polypeptide hormones called somatomedins mediate the major growth-promoting actions of ST, including stimulation of protein synthesis (Crooker et al., 1990; McDowell and Annison, 1991; Moseley et al., 1992). Insulin-like growth factors I and II (IGF-I and IGF-II) are the primary somatomedins and are produced by the liver and other tissues. Insulin-like growth factors have the ability to act in an endocrine, autocrine, and(or) paracrine fashion. Bovine ST treatment increases serum concentrations of IGF-I in steers (Moseley et al., 1992). These increases are mainly due to increased production by the liver but may also have been due to

increased local production of IGF in other tissues (McGuire et al., 1992). Circulating concentrations of IGF(s) and the hypothalamic-pituitary-IGF-I axis not only vary according to concentrations of ST but also with physiological state and nutritional status (Hannon and Trenkle, 1991; McGuire et al., 1992).

Glucocorticoids, which are formed in the adrenal cortex under the influence of the hypothalamus and anterior pituitary, usually exert net catabolic effects on protein metabolism and decrease protein synthesis in muscle (Evers, 1989; Florini, 1987). Cortisol is the dominant glucocorticoid in cattle, but corticosterone also has glucocorticoid activity (Genuth, 1993). Acute physical or psychological stress activates the hypothalamic-pituitary-adrenal axis, resulting in increased levels of plasma cortisol. Cortisol enhances the mobilization of muscle protein for gluconeogenesis by accelerating protein degradation and inhibiting protein synthesis (Genuth, 1993).

Serum glucocorticoids increase three-fold at parturition (Edgerton and Hafs, 1973). The adrenal of the fetus contributes greatly to elevated concentrations of cortisol and provides a means for the fetus to initiate parturition (Jainudeen and Hafez, 1987). In addition, labor and delivery of the fetus are traumatic experiences which are likely to induce a stress reaction and, therefore, increase cortisol. In cattle, abdominal and uterine contractions occur and are coincidental with the increased glucocorticoids observed around parturition (Gillette and Holm, 1963).

Lipid Metabolism

Adipose tissue is composed of cells (adipocytes) that contain primarily lipids and is found concentrated in depots (Byers and Schelling, 1988). Lipids are predominately triacylglycerols consisting of three fatty acid molecules linked to a glycerol molecule by ester bonds. The fatty acid portion of the triacylglycerol can be supplied from three sources: the uptake of fatty acids from plasma lipoproteins, from circulating free fatty acids, or from de novo synthesis of fatty acids in the cytosol of adipocytes (Emery, 1979; Hood, 1982).

The adipose depots include subcutaneous sites, inter- and intramuscular sites, bone marrow, visceral and omental fat, and fat associated with vital organs. In general, 40-50% of the fatty acids in adipose tissue of cattle are saturated (Byers and Schelling, 1988). Dairy cattle preferentially deposit internal fat as opposed to subcutaneous fat (Byers and Schelling, 1988). This likely reflects the relative importance of the dairy cow's ability to mobilize lipids rapidly from highly vascularized internal sites on demand to support high levels of milk production.

Adipose and lactating mammary tissues are the major sites of fatty acid synthesis in cattle, which is in contrast to rats and humans where 50% of fatty acid synthesis occurs in the liver (Bauman, 1976). Glucose is essentially not used for fatty acid synthesis in ruminants. Rather, in ruminants, fatty acids are synthesized from acetate, which is the most abundant energy source (Hanson and Ballard, 1967; Hood, 1982; Herdt, 1992). In lactating animals, fatty acids produced in the mammary gland for milk fat are synthesized from either acetate or ketone bodies (Herdt, 1992).

The release of fatty acids from fat depots, or lipolysis, is an important

mechanism to control the net deposition of triacylglycerols in adipose tissue. Triacylglycerols are hydrolyzed to free fatty acids and glycerol. This cleavage is catalyzed by hormone-sensitive lipase which is stimulated by epinephrine (Genuth, 1993). Stimulation of hormone-sensitive lipase leads to the release of fatty acids from adipose tissue into blood.

Some fatty acids in blood are reversibly bound to albumin and are referred to as nonesterified fatty acids (NEFA) to distinguish them from triacylglycerol fatty acids in lipoproteins. Nonesterified fatty acids in blood may be used directly for energy by many tissues (Herdt, 1992). Most plasma fatty acids are transported to tissues of the body as triacylglycerols bound to very low density lipoproteins (Emery, 1979; Genuth, 1993). However in rats and humans, chylomicrons also transport plasma fatty acids to capillary endothelium for hydrolysis (Genuth, 1993).

Lipoprotein lipase in the capillary endothelium is responsible for uptake of plasma lipoprotein-bound fatty acids by adipose and mammary tissue. Adipose tissue lipoprotein lipase is stimulated by insulin; thus, during the absorptive phase of nutrients, fatty acids from blood are selectively transferred to adipose tissue (Herdt, 1992). Lipoprotein lipase also exists in muscle tissue, but it is not dependent on insulin stimulation for activity. Thus, during periods of low glucose availability, adipose tissue lipoprotein lipase is inhibited because of a lack of insulin, but muscle tissue lipoprotein lipase is fully active. In nonlactating animals, this leads to the selective direction of very low density lipoproteins (VLDL) fatty acids to muscle tissue during times of adipose mobilization (Herdt, 1992).

Effects of Pregnancy and Lactation on Lipid Metabolism

The cycle of pregnancy and lactation has a profound effect on lipid metabolism in adipose and mammary tissue. In ruminants, lipid reserves are usually accumulated in adipose tissue during much of pregnancy, lost during early lactation and then replenished during the declining phase of lactation (Bauman and Elliot, 1983; Vernon et al., 1981). Although both synthetic and lipolytic pathways are affected, the timing and magnitude of changes in these pathways during gestation and lactation are still unidentified.

Lipogenesis is lower in adipose tissue of cows in early lactation versus those that are either in late lactation or in the early dry period because adipose tissue is the site for more than 90% of fatty acid synthesis in non-lactating cows (McNamara and Hillers, 1986). During the peripartum period in cows, de novo lipogenesis, esterification, and lipoprotein lipase activity decrease, whereas lipolysis increases (McNamara and Hillers, 1986). This catabolic shift precedes the energy demand for lactation. As a result of these changes, adipose tissue mass and adipocyte size decrease during early lactation (Vernon, 1980). Presumably these fatty acids are mobilized to supply fatty acids for energy and for milk fat synthesis. During early lactation, the supply of fatty acids to the liver and mammary gland is increased as a result of a rise in NEFA concentrations in blood (due to increased lipolysis) and an increase in blood flow to the liver (Vernon, 1988; Bauman and Currie, 1980). As lactation proceeds and milk production declines, anabolic activity increases in adipose tissue to replete the lipid reserves (Vernon, 1980).

Hormonal Control of Lipid Metabolism

Pregnancy and lactogenic hormones initiate adaptations in adipose tissue metabolism prior to the increased energy demand associated with early lactation. The sharp prepartum drop in lipogenesis, the functionally nonexistent rates during early lactation, and the rebound to high rates during midlactation suggest this pathway is highly regulated (McNamara and Hillers, 1986). Lipid synthesis in adipose tissue decreases and lipolysis increases because concentrations of serum insulin are low during early lactation (Vernon, 1989). In a growing animal, insulin suppresses lipid mobilization and increases re-esterification rates in adipose tissue (Bergman, 1968; Vernon et al., 1985; Brockman and Laarveld, 1986). However, during early lactation, the response of adipocytes to insulin is diminished relative to the response during late lactation. The mechanism for this insensitivity to insulin around parturition and during early lactation is not known (Brockman and Laarveld, 1986).

Somatotropin's metabolic effects on lipid metabolism are dependent on the energy balance of the animal. If animals are in positive energy balance, ST decreases lipogenesis in adipose tissue and does not appreciably affect lipolysis (Bauman, 1992; Etherton and Louveau, 1992). Exogenous ST does not affect circulating levels of NEFA, percentage of fat in milk, or composition of milk fatty acids in animals in positive energy balance (Peters, 1986; Bauman, 1992). However, if the animal is in negative energy balance, lipid mobilization increases, resulting in decreases in body fat, chronic elevation of circulating NEFA, and in lactating cows, increases in percentage of milk fat and proportion of long-chain fatty acids in milk (Vicini et al.,

1991; Bauman, 1992; Etherton and Louveau, 1992). In this situation, peripheral tissues utilize NEFA mobilized by ST as an alternative to glucose for a source of energy and for milk fat synthesis (Bauman et al., 1988; Gallo and Block, 1990).

Chronic exposure to ST increases the number of β-adrenergic receptors as well as the response and sensitivity of ruminant adipose tissue to β-agonists (Watt et al., 1991). The signalling mechanism of these receptors is via a G_s protein and the adenylate cyclase pathway. Somatotropin interferes with an adipose tissue membrane G_i protein and prevents the inhibition of cAMP production, thereby enhancing the lipolytic effects of G_s-stimulating agonists (Bergen and Merkel, 1991). This mechanism offers an explanation for the increase in sensitivity of adipose tissue to catecholamine-enhanced lipolysis after chronic ST administration (Bergen and Merkel, 1991).

Cortisol alone is weakly lipolytic, however its presence is necessary for maximal stimulation of fat mobilization by ST and other lipolytic peptides (Genuth, 1993). Cortisol permits accelerated release of energy stores as fatty acids for gluconeogenesis.

MATERIALS AND METHODS

Design and Management

Forty-one Holstein cows (all had at least one calf) were used in a split block design with repeated measurement. For all cows, milking was stopped (dried off) after 305-365 d of lactation which was approximately 60 d before the expected date of parturition. The dry-off routine included immediate and total cessation of milking and intramammary infusion of a dry period antibiotic (Cefa-dri; Aveco Company Inc., Fort Dodge, IA). Cows were blocked at drying off in groups of three in order of expected date of parturition (14 blocks). Within each block, cows were randomly assigned to one of three treatment groups: 1) non-injected controls, 2) injected intramuscularly (IM) with 5 mg bST/d, and 3) injected IM with 14 mg bST/d. Treatments began 46 d before the expected date of parturition (2 weeks after dry-off), and continued until parturition occurred. Injections were administered in the semitendinosus muscle once daily (1600 h) using a 3-ml disposable syringe with a 23-gauge, 1.9-cm needle. Bottles of bST were stored at 4 °C and solubilized on the day of use with 21 ml of sterile distilled water. Solubilized bST was used for no more than 2 d. Final concentration was 8.6 mg of bST/ml. Cows of group 3 were injected with 1.6 ml of sterile distilled water containing 13.8 mg of bST (hereafter referred to as 14 mg), and cows of group 2 received 5.2 mg of bST in .6 ml distilled water

(5mg).

Cows were housed in tie stalls, exposed to 24 h of light per day, and milked at 0530, 1330 and 2200 h during lactation. Milk production was recorded daily, and milk was sampled weekly for composition analysis. Fat, protein and lactose in milk were measured using an infrared analyzer (Multispec, Wheldrake, UK) at Michigan DHIA (East Lansing). Yield of solids-corrected milk (SCM) was calculated.

During the beginning of the dry period, cows were fed a diet (ED; Appendix A) which was estimated to be in 10% excess of National Research Council (NRC) requirements for energy and the rumen undegradable protein (UIP) portion of the diet. Rumen undegradable protein is not broken down in the rumen and therefore passes to the small intestine and is degraded and absorbed there. We fed excess amounts of UIP to ensure adequate metabolizable amino acids were available for fetal growth and for additional body gain of the cow that may have occurred as a result of bST treatment. Three weeks after treatment began, cows were changed to a diet (LD; Appendix A) that was higher than ED in density of energy and protein to reflect the increased rate of fetal growth and mammary cell differentiation and decreased feed intake during the periparturient period. Because of the difficulty in predicting actual parturition date, there was a period in the middle of the dry period when some cows were eating diet ED and some diet LD (diet transition). In the analysis of dry matter intake (DMI) during the dry period, all daily feed intakes were averaged by week, within treatment, regardless of the diet that was consumed. During lactation, the cows were fed a diet balanced to meet NRC requirements for crude protein, energy and minerals during early lactation (EL; Appendix B). Feed

was offered ad libitum as a total mixed ration (TMR) twice per day (0330 and 1530 h) at an amount so 10% of the feed offered was refused.

When the source of the feed ingredients changed, the feedstuffs were analyzed for protein fractions, neutral detergent fiber, and minerals so adjustments could be made in order to maintain similar diets throughout the entire experiment. Individual feed intake was recorded daily. Samples of the offered feed and samples of individual orts were collected weekly. At the end of the experiment, the samples were composited within treatment on a monthly basis for dry matter, protein, and fiber analysis.

Blood Collection and Analysis

Blood samples were collected by tail vessel puncture twice weekly from 46 to 8 d before expected date of parturition and twice weekly from 7 through 70 d after parturition. In addition, blood was collected daily from 7 d before expected date of parturition through 6 d after parturition. Blood was collected at a constant time (1700 h) relative to feeding and milking during the lactation period.

In six selected blocks of cows (blocks 7, 8, 9, 11, 12, and 14), blood was collected from an indwelling jugular catheter (16 gauge; Ico-Rally, Palo Alto, CA) inserted approximately 72 h before the day of expected parturition. In the 14 mg bST group, only five cows were sampled because the sixth cow calved early and a catheter was not inserted. The catheters were coated with a heparin complex to reduce fibrin accumulation (TDMAC Heparin, Polysciences Inc., Warrington, PA) and sterilized with ethylene oxide. Catheter patency was maintained by flushing with 3.5% Na

citrate and 50% sucrose in sterile water after collection of each blood sample. Blood was sampled at 6-h intervals beginning 48 h before expected parturition through 12 h after parturition.

All blood samples were stored at room temperature for 6 h and then at 4°C for approximately 15 h. Serum was harvested by centrifugation for 30 min at 1550 x g and frozen at -20°C until assayed. NEFA (NEFA-C kit, Wako Chemicals USA, Dallas, TX; as modified by McCutcheon and Bauman, 1986), urea nitrogen (SUN; Sigma kit No. 535, Sigma Chemical Co., St. Louis, MO), IGF-I (Kamdar, 1992), and ST (Moseley et al., 1982) were quantified in serum from blood collected twice weekly or daily. Concentrations of progesterone (Spicer et al., 1981) were measured in the samples collected after parturition. In addition, prolactin (Newbold et al., 1991) and cortisol (Kamdar, personal communication) were measured in serum from blood collected at 6-h intervals in the periparturient period.

Urine Collection and Analysis

Once per week, two samples (0700 and 1900 h) of urine were collected from each cow. A 100-ml sample was collected and immediately frozen at -20°C until processed further. Urine samples were thawed and centrifuged (3000 rpm) for 15 minutes to remove sediments, and 15 ml of urine was refrozen. Samples for N^{τ}-MH analysis were thawed again and deproteinized with 50% sulfosalicylic acid (SSA; .9 ml urine and .1 ml 50% SSA). The samples were centrifuged at 13,000 x g for 15 minutes at 4°C. The supernatant was diluted 1:1 with sodium hexane sulfonic acid because this served as the mobile phase in the chromatograph. The appropriate

amount of sodium hexane sulfonic acid needed for dilution was based on the size of the peaks that were eluted after different dilutions of the samples.

After dilution, the mixture was vortexed and placed into a high-performance liquid chromatography (HPLC) vial. The samples were chilled at 4°C and then analyzed by reversed-phase HPLC separation using ion-pairing and post-column derivatization with *o*-phthalaldehyde and fluorescence detection (Friedman and Smith, 1980). N^{τ}-MH peaks in the samples were identified on the basis of retention time as compared with N^{τ}-MH standards. Concentrations of N^{τ}-MH in samples were calculated by comparing the peak area of N^{τ}-MH in standards (50 μg/ml) and samples (Appendix C). Representative traces of the elution profile are located in Appendix D.

A stock solution of 5 mM sodium hexanesulfonate with a pH 3.2 was used as the mobile phase in the chromatograph. Sodium hexanesulfonate increases the retention of N^{τ}-MH and allows for good resolution of N^{τ}-MH. The solution consisted of 1 l of HPLC-quality H_20 and .94 g 1-hexane sulfonic acid, sodium salt (Aldrich, Milwaukee, WI; F.W. 188.22, 98%). Approximately 1 ml glacial acetic acid was added dropwise to lower the pH to 3.2. The solution was stirred for 30 to 45 minutes and then was allowed to stand for 30 to 45 minutes. Next, the solution was filtered through a .45 μm aqueous filter (47 mm diameter; Millipore Corp., Bedford, MA) with a glass prefilter (Whatman GF/C, 4.25 cm; Whatman, Inc., Clifton, NJ) on top of a Millipore-type all glass vacuum filtration system with a 1 l flask. Lastly, a vacuum was applied for about 1 minute to degas the solution. The solution was stirred rapidly with a magnet while it was being degassed.

The *o*-phthaladehyde reagent was prepared by dissolving 30 g of boric acid in 1 l of HPLC-quality H_20. To adjust pH, 20 g of potassium hydroxide was added to the solution first, then approximately 5 g of potassium hydroxide was added slowly until a pH of 10.4 was achieved. The solution was stirred for at least 30 minutes and then it was allowed to stand for at least 30 minutes. Next, the solution was filtered in the same manner as the mobile phase; however, the solution was not degassed. Separately, a solution which contained 600 mg fluoraldehyde (Pierce OPA crystals, catalog no. 26015; Pierce Chemical Co., Rockford, IL), 10 ml ethanol, 200 μl of β-mercaptoethanol, and 1 ml of 30% (w/v) aqueous solution of Brij 35 (Pierce Chemical Co., Rockford, IL) was prepared. The *o*-phthalaldehyde solution and the borate solution were mixed in a dark glass bottle just before use and stored under N_2 gas. The borate solution was prepared one day ahead of time, but the *o*-pthalaldehyde solution was prepared just prior to use.

Injections of 25 μl of sample were added to a 25 cm by 4.6 mm internal diameter, Vydac C-18 peptide/protein column (Rainin Instrument Co. Inc., Woburn, MA). The flow rate of the mobile phase was .8 ml/min and isocratic conditions were used to separate N^{τ}-MH from other urinary components. The column was periodically washed with acetonitrile to remove non-polar contaminants which were retained on the column.

Raw urine samples (not deproteinized) were analyzed for creatinine using Sigma kit number 555-A (Sigma Chemical Co., St. Louis, MO) based on the Jaffe' reaction. Urine samples from all cows in two randomly chosen blocks were analyzed to determine if the ratio of N^{τ}-MH to creatinine was affected by the time when the

urine samples were collected (0700 h vs. 1900 h). Because a difference was not detected (P>.2; Appendix E), the sample collected at 1900 h was used for the urine analysis.

Body Composition Measurements and Calculated Energy Balance

Cows were weighed for 2 consecutive days every week. Energy balance was calculated daily according to NRC (1989) and averaged by week within treatment. Body condition scores (BCS) were measured every 2 weeks on a given day of the week using a system with a scale from 1 (thinnest) to 5 (fattest; Wildman et al., 1982). Scorers were unaware of the treatment assigned to individual cows.

Real-time linear array ultrasound measurements of subcutaneous fat were recorded every 2 weeks (Faulkner et al., 1990). An Aloka real-time ultrasound device in the B-mode with a 7.5 MHz linear array probe was used. Light mineral oil was used to obtain adequate acoustic contact. Hair in the area to be scanned was clipped to help increase the contact. Fat thickness was measured 10 cm from the dorsal process of the thoracic vertebrae between the 12^{th} and 13^{th} ribs by freezing the image on the screen and measuring it to the nearest .1 cm with an internal electronic caliper.

Reproductive Characteristics and Health Disorders

Records were kept on incidence of dystocia, retained placenta, metritis, milk fever, ketosis, displaced abomasa, mastitis, and other health related abnormalities. Birthweight and sex of calves was recorded.

Statistical Analysis

Separate analyses were used for the prepartum period and the postpartum period of the experiment. All data were subjected to split block ANOVA with repeated measurement over time (Gill, 1986). Mean comparisons within periods were tested using the Bonferroni-t test (Gill, 1978). When significant ($P<.1$), initial body weight was included in the model as a covariate in the analysis of body weight. To minimize heterogeneous variance, kg of milk protein was transformed to logarithms for analysis (Gill, 1978). When making comparisons between the last sample of the dry period and the first sample of the lactation period, a paired t-test was used (Gill, 1978). Chi-square analysis was used to test for treatment differences in occurrence of health disorders.

RESULTS

Measurements of Protein Metabolism

Concentrations of SUN did not change ($P > .20$) from d -46 to d -23 of the dry period in any of the treatment groups, but it declined ($P < .01$) an average of 25% in all cows from d -23 to d -1 of the dry period (Figure 1). During the dry period, 14 mg bST reduced overall SUN to 9.7 mg/dl compared with 12.2 mg/dl in controls ($P < .03$). In addition, concentrations of SUN tended to be lower in the 5 mg dose when compared with controls ($P < .12$). Between d -1 and d 0, concentrations of SUN increased in all three groups ($P < .001$). Concentrations of SUN increased ($P < .01$) in all cows from d 0 to d 69 of lactation ($P < .01$). However, concentrations of SUN were not different among groups after parturition ($P > .20$).

During the dry period, average urinary creatinine in the bST groups was 144 mg/dl, which was greater than the 120 mg/dl average of the controls ($P < .05$; Figure 2). From the end of the dry period to the beginning of lactation, urinary creatinine in the bST-treated cows decreased an average of 30% ($P < .001$). Similarly, concentrations of creatinine in the controls decreased 10% over this interval ($P < .12$). During the lactation period, there were no treatment differences ($P > .20$), but urinary creatinine in all three groups decreased ($P < .01$) from an average of 105 mg/dl 6 d after parturition to 81 mg/dl at 62 d after parturition.

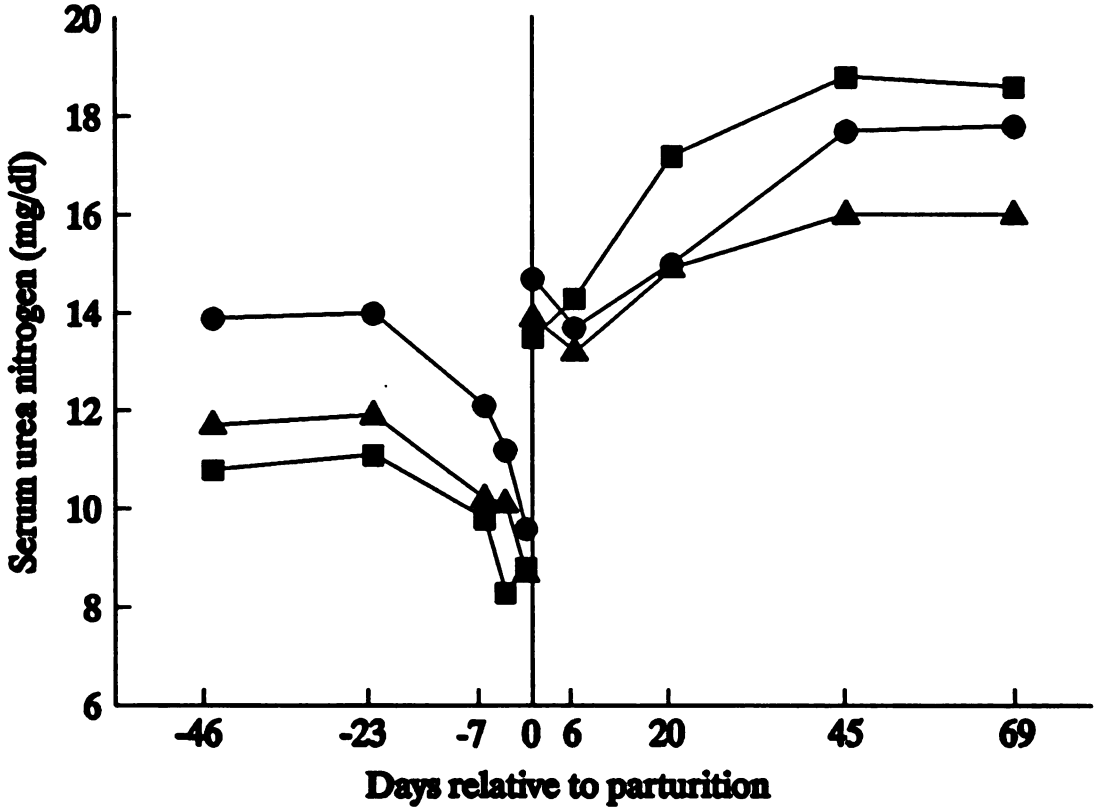

Figure 1. Concentrations of serum urea nitrogen of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .8 mg/dl during the dry period and .9 mg/dl during the lactation period.

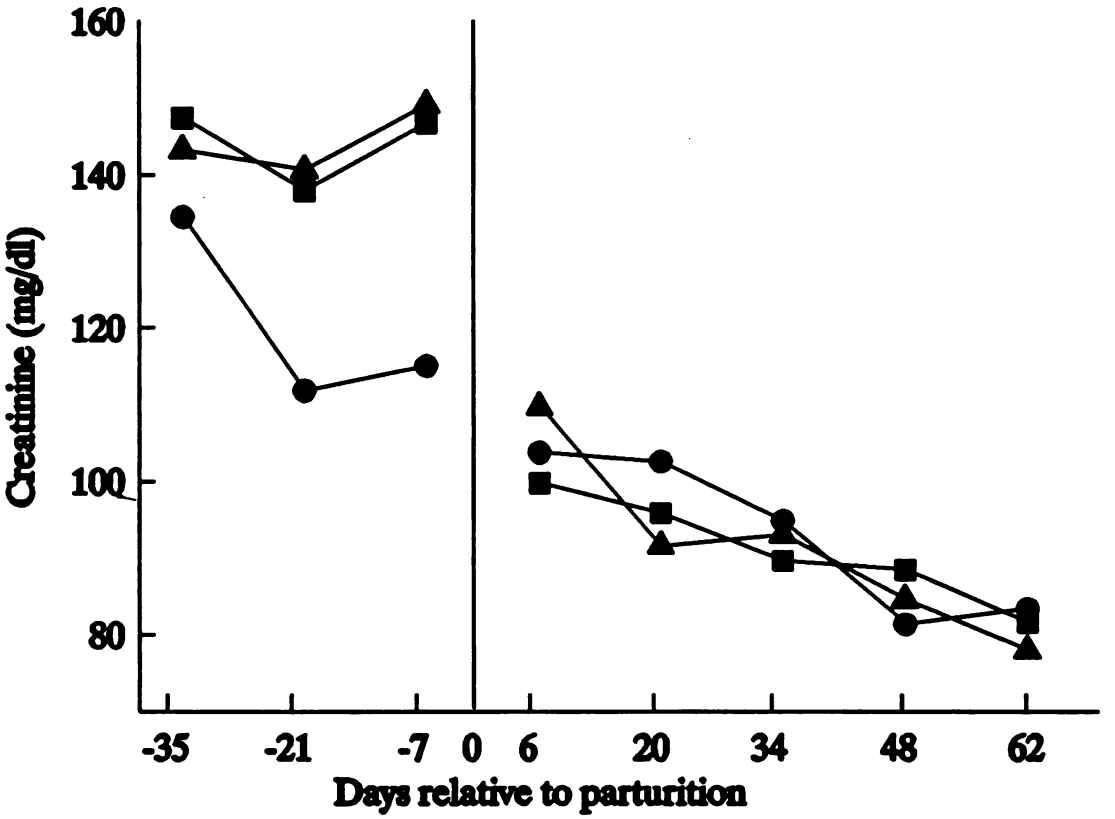

Figure 2. Concentrations of urinary creatinine of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 8.4 mg/dl during the dry period and 5.2 mg/dl during the lactation period.

Before parturition there were no differences in the ratio of urinary N^{τ}-MH/creatinine ($P>.20$; Figure 3). Between d -1 and d 0, the ratio of N^{τ}-MH/creatinine increased an average of 84 nmol/mg in the bST-treated cows ($P<.01$). The ratio of controls increased 34 nmol/mg during this period ($P<.10$). During the first 62 d after parturition, treatment with 5 and 14 mg bST during the dry period increased mean N^{τ}-MH/creatinine 22 and 16% relative to controls ($P<.01$; $<.07$). More specifically, there was a 30% increase in N^{τ}-MH/creatinine 6 d after parturition in both the 5 and 14 mg groups relative to controls ($P<.01$). The ratio decreased in all cows between 6 and 20 d after parturition ($P<.01$). However, from 20 to 62 d after parturition, urinary N^{τ}-MH/creatinine did not change within a group and was not different among treatments ($P>.20$).

Fourteen milligrams of bST increased total yield of milk protein 33 and 44% during the first week of lactation relative to that of controls and cows treated with 5 mg bST ($P<.01$; Figure 4). By the second week of lactation, milk protein was similar among groups and remained so throughout the remainder of the experiment ($P>.20$).

Measurement of Fat Metabolism

There were no treatment differences in concentrations of serum NEFA before or after parturition ($P>.20$; Figure 5). Even though a treatment difference was not observed, all groups exhibited a similar pattern relative to parturition. Serum NEFA levels increased from an average of 268 μEq/L on d -23 to 803 μEq/L 1 d before parturition ($P<.01$). The extremely high levels of NEFA were maintained ($P>.20$)

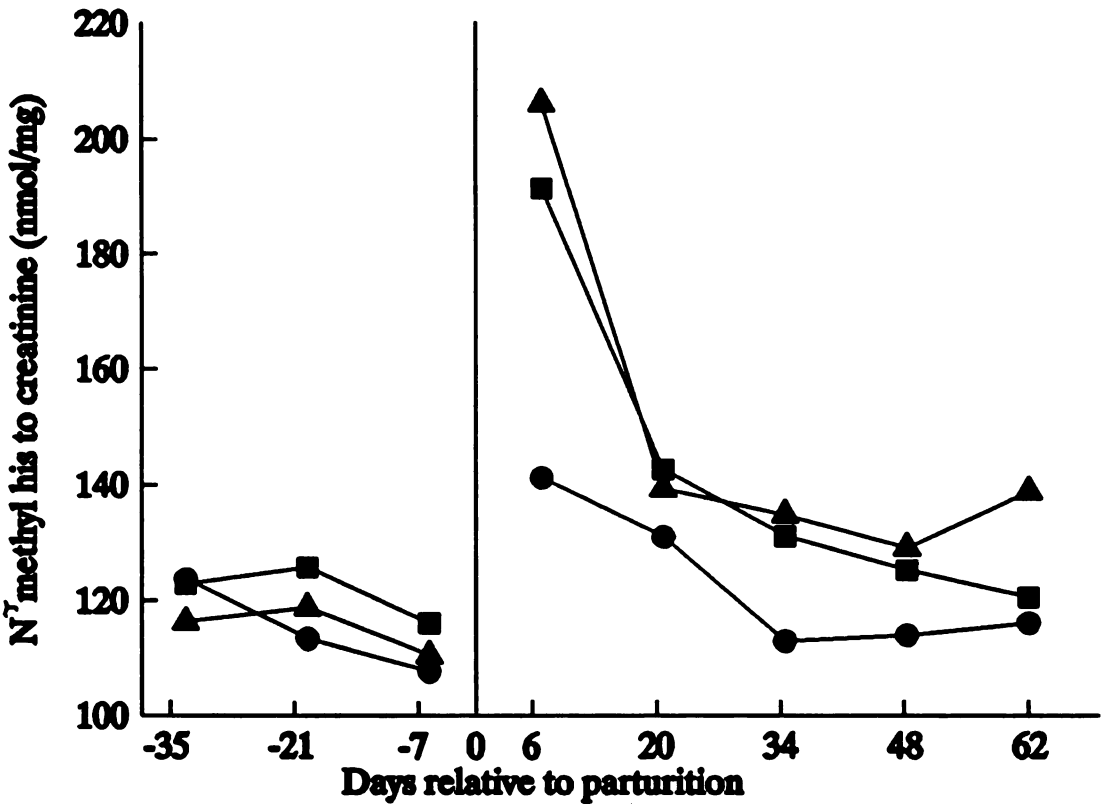

Figure 3. Ratio of urinary N^{τ}-methyl histidine/creatinine of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 9.0 nmol/mg during the dry period and 7.9 nmol/mg during the lactation period.

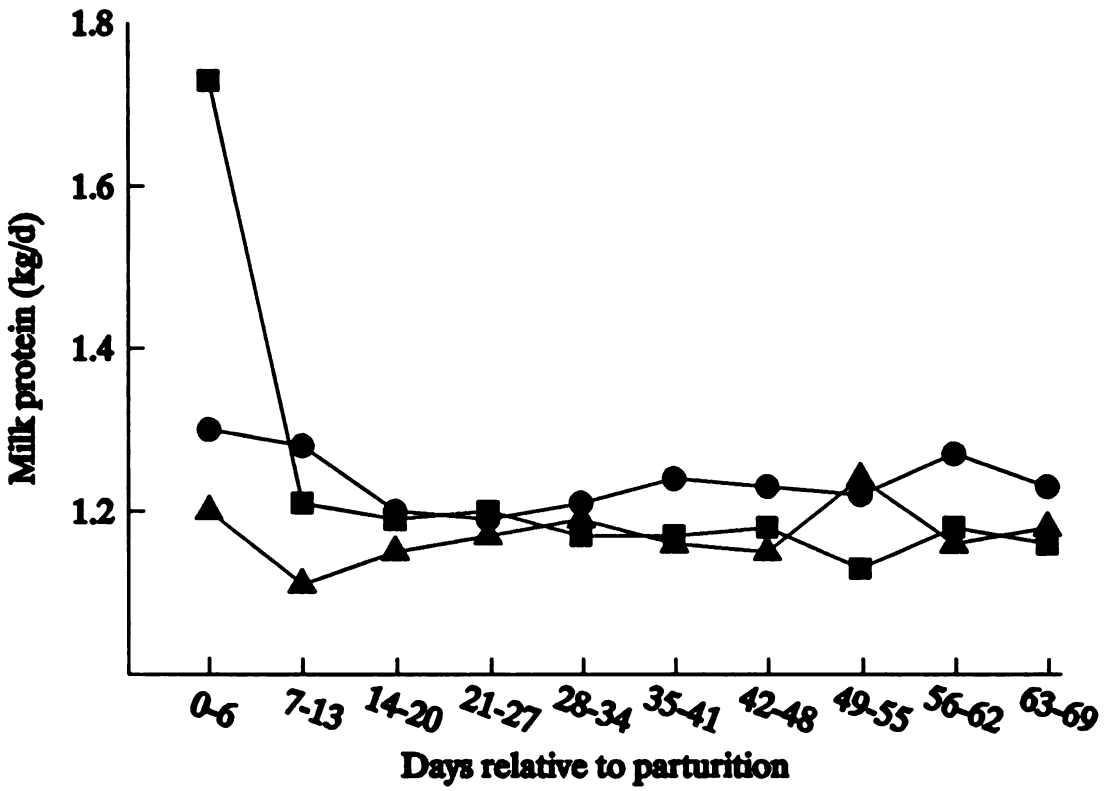

Figure 4. Yield of milk protein of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .1 kg/d.

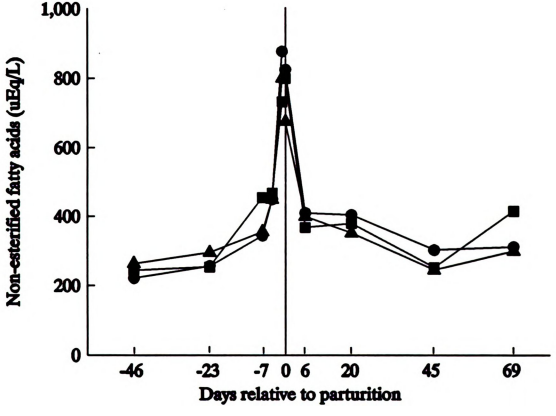

Figure 5. Concentrations of serum NEFA of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 57.7 μEq/L during the dry period and 43.3 μEq/L during the lactation period.

on the day of parturition, but NEFA concentrations decreased ($P<.05$) by 6 d after parturition to levels similar to those observed before parturition.

Measurements of Body Composition

Initial body weight (BW) was measured for every cow 2 d after treatment began and was not different among groups ($P<.20$). However, initial BW was significant when tested as a covariate; therefore, subsequent BW were adjusted by covariance for initial BW. Before parturition, overall treatment means for BW were similar ($P>.20$; Figure 6). However, when comparing treatment means at specific periods, BW were lower at d -7 in both the 5- and 14-mg groups relative to controls ($P<.05$). In association with parturition, BW decreased an average of 84 kg in all three groups ($P<.001$). During the first 3 weeks after parturition in all three groups, BW decreased ($P<.01$) 37 kg and then plateaued ($P>.20$) for the remainder of the experiment. The overall mean BW in the 5- and 14-mg groups were about 25 kg less than that of controls during lactation ($P<.05$).

Before parturition, the average BCS for all cows was 3.25, and there were no overall treatment differences ($P>.20$; Figure 7). Between d -7 and d 0, BCS decreased in all cows and declined 25% throughout the first 63 d of lactation ($P<.05$). After parturition, BCS were not different among groups ($P>.20$).

There were no overall treatment differences in subcutaneous fat over the 12th and 13th ribs measured by ultrasound before or after parturition ($P>.20$; Figure 8). However during the dry period, overall subcutaneous fat increased from an average of .36 cm on d -35 to .43 cm on d -7 ($P<.01$). In all three groups, subcutaneous fat

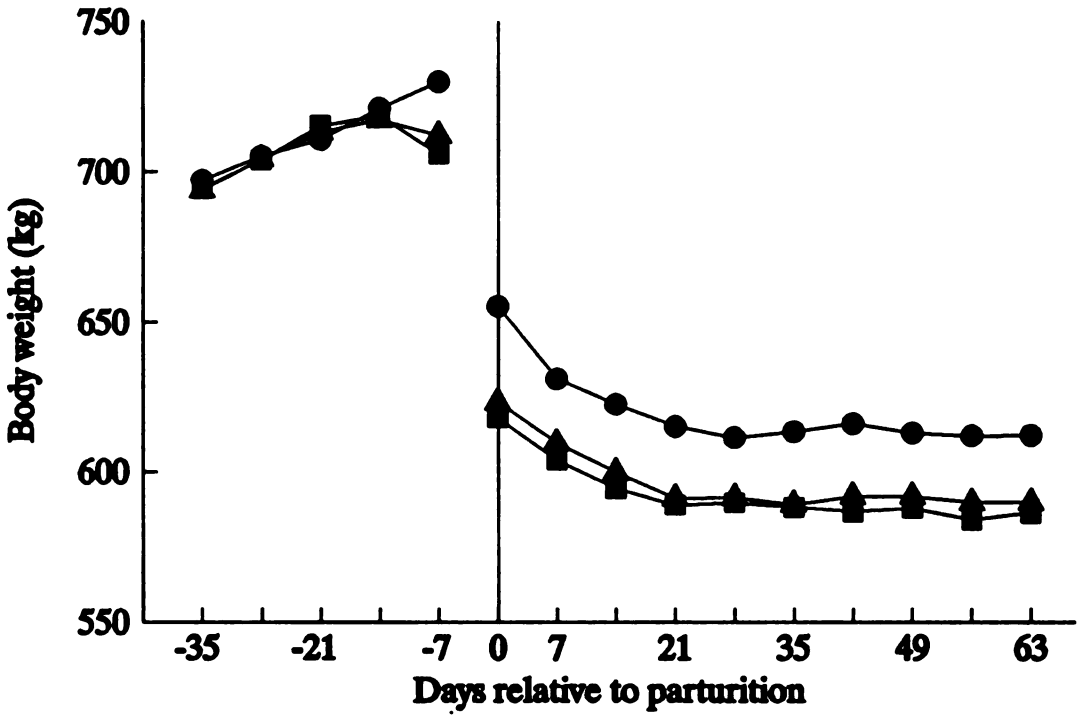

Figure 6. Bodyweights of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means) adjusted by covariance with initial BW. Standard error of the difference among treatments was 7.0 kg during the dry period and 9.2 kg during the lactation period.

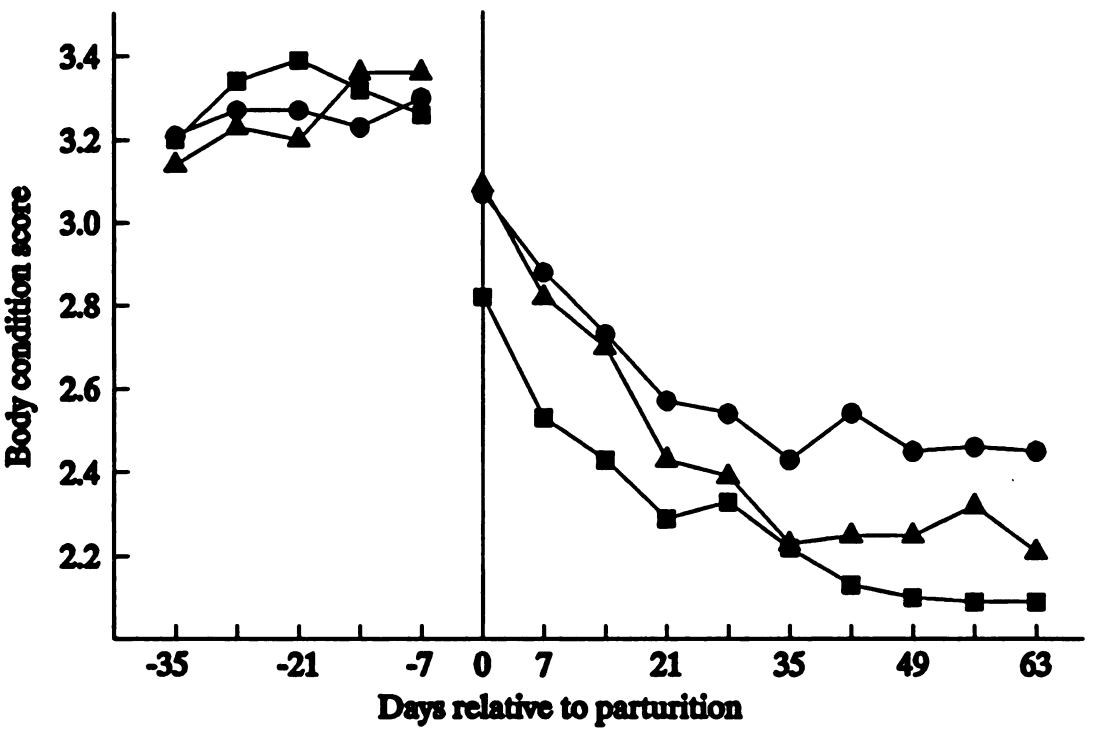

Figure 7. Body condition scores of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .5 during the dry period and .2 during the lactation period.

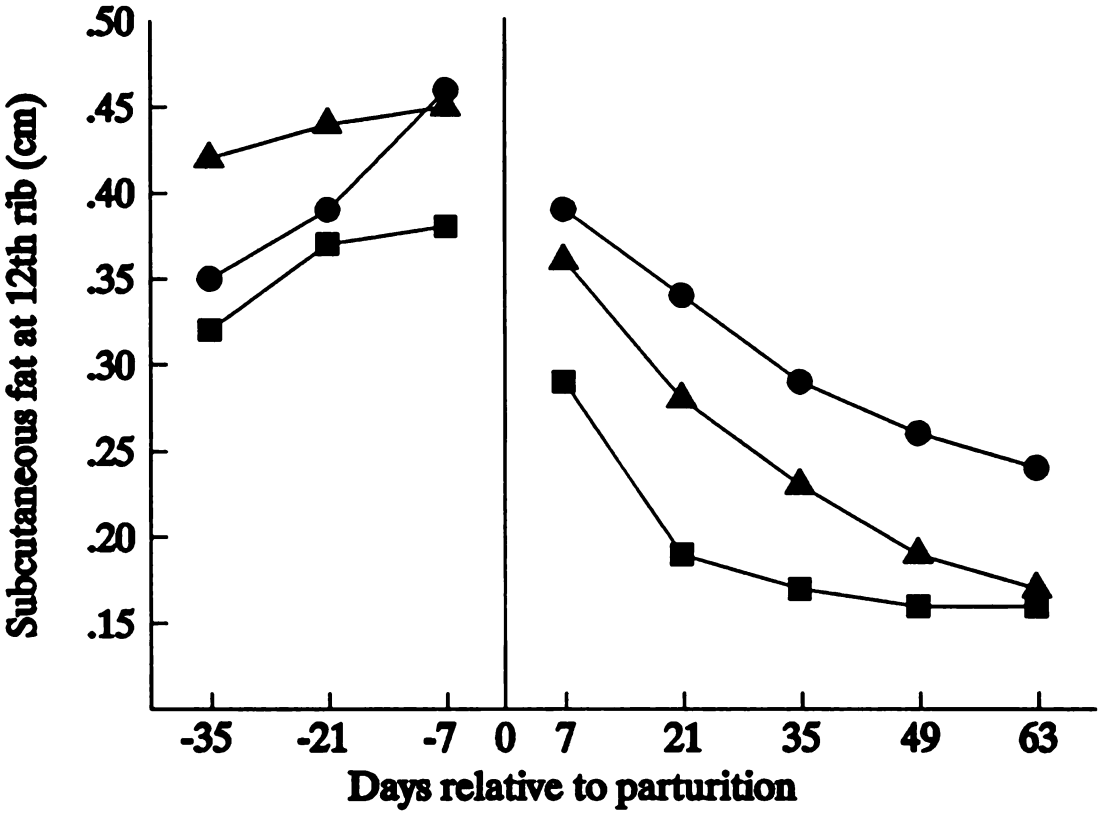

Figure 8. Subcutaneous fat between the 12th and 13th ribs of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .08 cm during the dry period and .07 cm during the lactation period.

decreased 19% from 7 d before parturition to 7 d after parturition (P<.05). Subcutaneous fat continued to decrease as lactation progressed from an average of .35 cm on d 7 to .19 on d 63 (P<.05).

Concentrations of Serum Hormones

Throughout the dry period, serum ST was increased and maintained at 6.5 and 22.7 ng/ml in the 5- and 14-mg treated cows when compared with 1.6 ng/ml in controls (P<.05; Figure 9). In the 14 mg bST group, serum ST decreased 15 ng/ml from d -1 to d 0 (P<.05). After treatment ceased and lactation began, concentrations of serum ST were not different among groups (P>.20). Average serum ST decreased (P<.01) from 7.8 ng/ml on d 0 to 2.5 ng/ml on d 3 and then increased (P<.05) to 5.5 ng/ml by d 6. Throughout the rest of the lactation period, concentrations of serum ST decreased 3.6 ng/ml (P<.05).

Serum IGF-I in 14 mg bST-treated cows increased 23% between d -46 and d -23 of the dry period, while no increase occurred in either the 5 mg bST-treated cows or controls during this period (P<.05; Figure 10). From d -23 to d -1 before parturition, concentrations of serum IGF-I decreased in all groups to an average of 68.8 ng/ml (P<.05). As the physiological state of the cows changed from dry to lactating, concentrations of serum IGF-I decreased in both bST-treated groups (P<.01). After parturition, concentrations of serum IGF-I were similar among groups (P>.20).

Concentrations of serum cortisol were not different among groups (P>.20; Figure 11). However, there was a period by treatment interaction (P<.05). In 5 mg

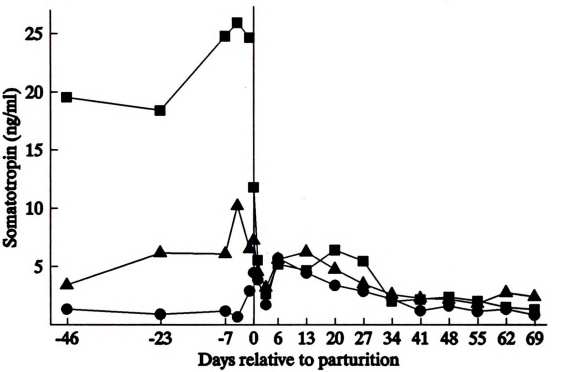

Figure 9. Concentrations of serum ST of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 1.8 ng/ml during the dry period and .7 ng/ml during the lactation period.

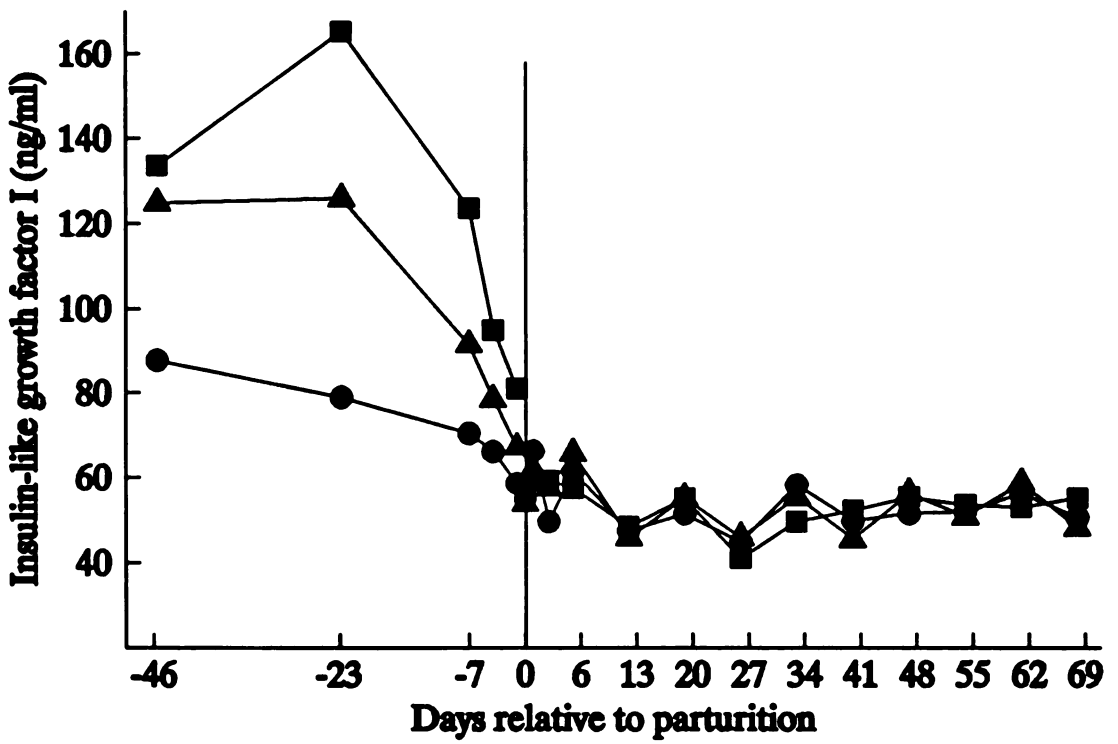

Figure 10. Concentrations of serum IGF-I of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line at d 0. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 9.1 ng/ml during the dry period and 2.7 ng/ml during the lactation period.

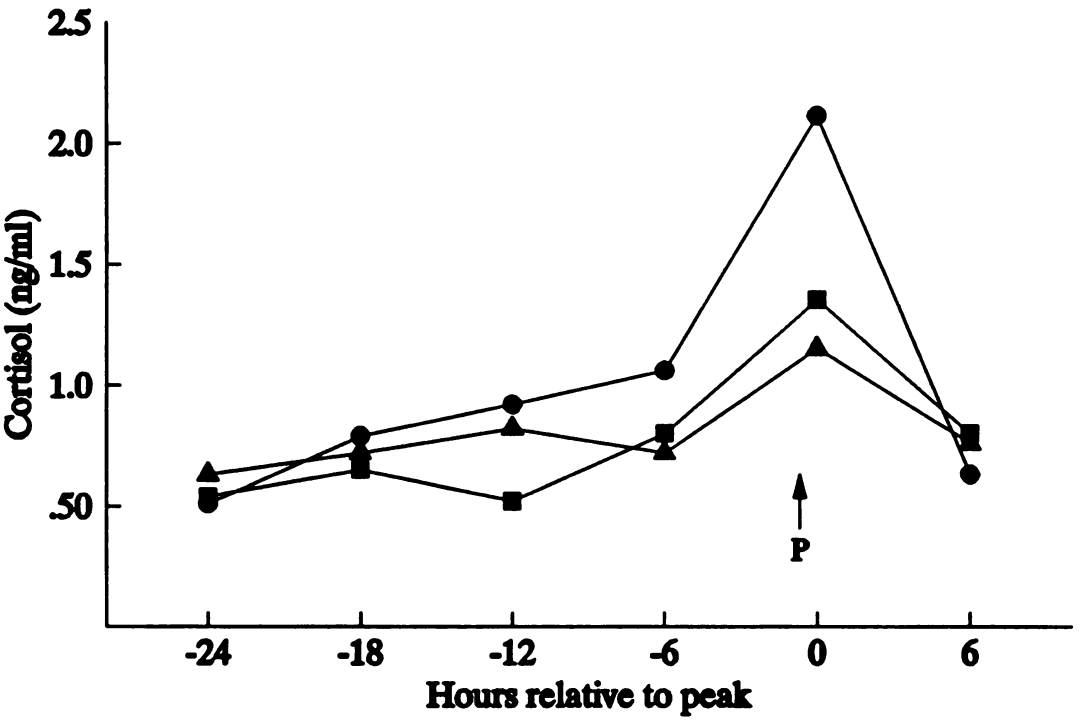

Figure 11. Concentrations of serum cortisol of cows receiving no treatment (•; n=6), 5 mg bST/d (▲; n=6), or 14 mg bST/d (▪; n=5) for the last 46 d before parturition. Average time of parturition is indicated by the arrow. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was .13 ng/ml.

bST-treated cows, concentrations of serum cortisol did not increase (P>.20) from 24 h to 6 h prior to the peak value (.7 h after parturition), while in the controls concentrations increased 120% (P<.05). The peak values of serum cortisol were an average of .8 ng/ml lower in both the 5- and 14 mg groups when compared with the peak value of controls (P<.01).

Prolactin concentrations began to increase (P<.05) approximately 18 h before peak values were reached (Figure 12). One cow treated with 5 mg bST did not exhibit a periparturient surge of prolactin. Between 30 and 6 h before peak concentrations of prolactin were reached, 5-mg bST decreased overall serum prolactin to 70 ng/ml when compared with controls or 14-mg treated cows which averaged 113 ng/ml (P<.05). Peak concentration of prolactin (10 h before parturition) was lower in the 5-mg group relative to either controls or the 14-mg group. When the values from the cow that did not exhibit a surge of prolactin were not included in the treatment averages, there were no differences among groups (P>.20).

Milk Production and Components

There were no overall treatment differences in solids corrected-milk yield (SCM; P>.20; Figure 13). However, when considering individual periods and treatments an interaction was observed (P<.05). The 14 mg bST-treated cows had increased SCM yields during the first week of lactation when compared with controls and the 5-mg group (P<.07; .01). In the 14-mg group, SCM yield did not increase (P>.20) during the first 70 d of lactation whereas, in both the controls and 5 mg

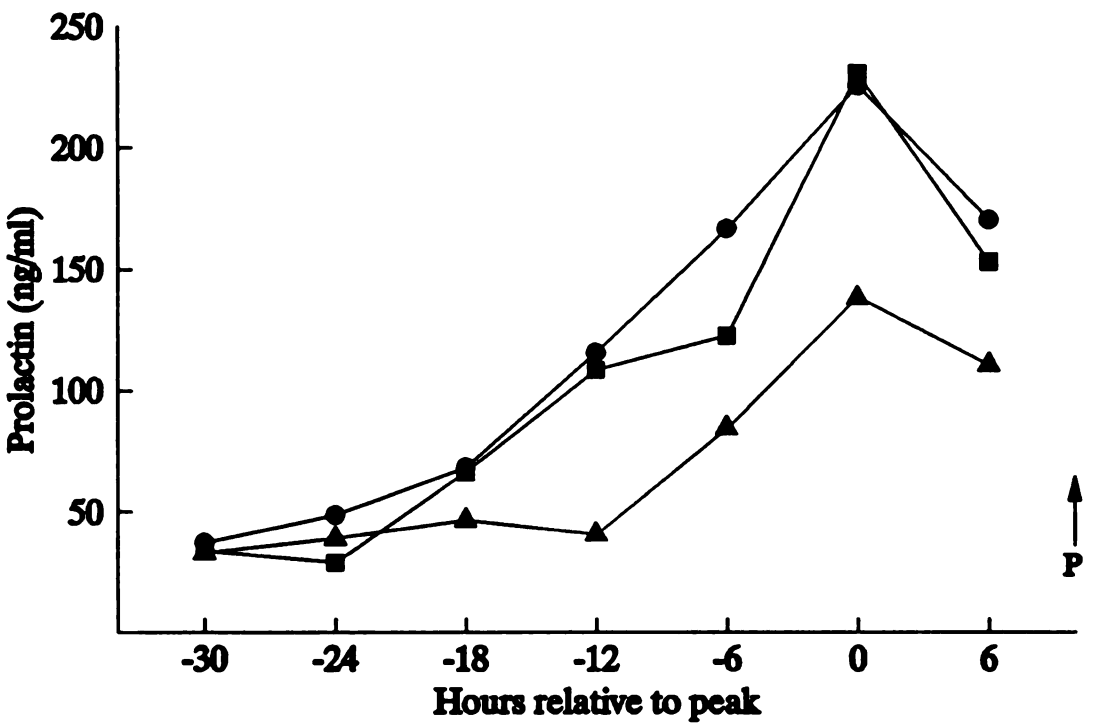

Figure 12. Concentrations of serum prolactin of cows receiving no treatment (•; n=6), 5 mg bST/d (▲; n=6), or 14 mg bST/d (▪; n=5) for the last 46 d before parturition. Average time of parturition is indicated by the arrow. Each point represents the average of a treatment group (least squares means). Standard error of the difference among treatments was 19.3 ng/ml.

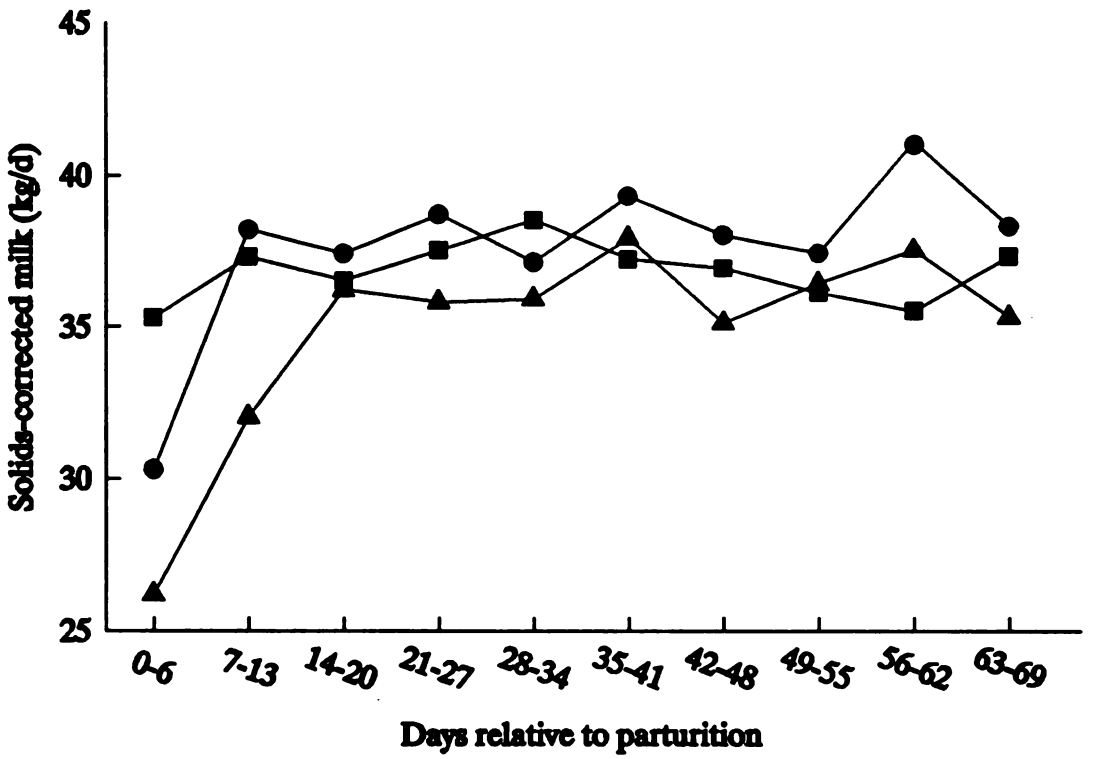

Figure 13. Solids corrected milk yield of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Each point represents the average of a treatment group (least squares means) within each 7-d period. Standard error of the difference among treatments was 2.3 kg/d.

bST-treated cows, SCM yield increased ($P<.05$) 28% from the first week to the third week of lactation and then plateaued ($P>.20$) for the remainder of the experiment. There were no differences among treatments in the yield of milk fat, lactose, total solids or somatic cell count ($P>.20$).

Dry Matter Intake (DMI) and Calculated Energy Balance

Before parturition, overall DMI was not different among groups ($P>.20$; Figure 14). However, DMI in the 5- and 14-mg groups averaged 2.1 kg/d less than that of the controls during the last week of the dry period ($P<.07$). Dry matter intake decreased ($P<.05$) in all cows as parturition approached, but the decline was 1.9 kg/d larger ($P<.06$) in the 14-mg group than in controls. From the last week of the dry period to the first week of lactation, DMI increased an average of 3 kg/d in the bST-treated groups ($P<.01$). After parturition, DMI was not different among groups ($P>.20$). But, DMI increased an average of 65% in all cows from the first week to the 10^{th} week of lactation ($P<.01$).

During the dry period, overall calculated energy balance was positive for all cows and there were no treatment differences among groups ($P>.20$; Figure 15). However, during the last week of the dry period, calculated energy balance averaged 1.5 Mcal/d in the 5-mg group and 1.1 Mcal/d in the 14-mg group, and these were lower than the 3.8 Mcal/d average of the controls ($P<.07$). From the last week of the dry period to the first week of lactation, calculated energy balance decreased an average of 10 Mcal/d in all cows ($P<.001$). During lactation there were no differences in overall calculated energy balance among groups ($P>.20$). Except for

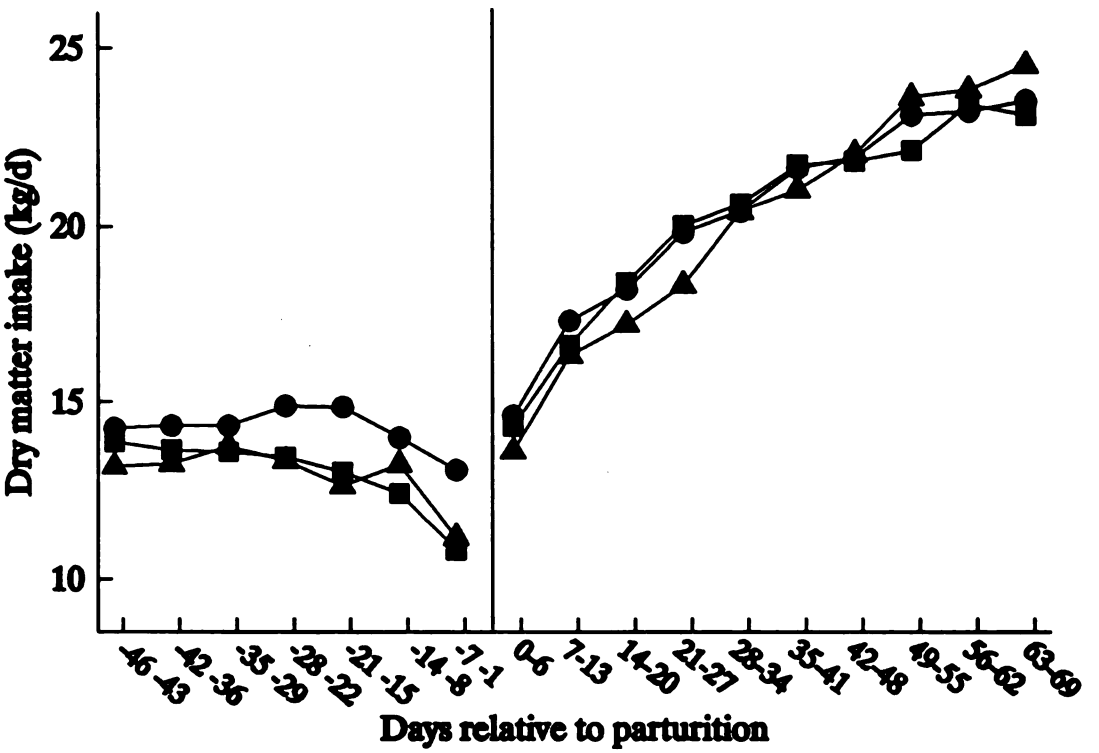

Figure 14. Dry matter intake of cows receiving no treatment (•; n=14), 5 mg bST/d (▲; n=14), or 14 mg bST/d (▪; n=13) for the last 46 d before parturition. Parturition is indicated by the vertical line. Each point represents the average of a treatment group (least squares means) within each 7-d period. Standard error of the difference among treatments was .9 kg/d during the dry period and 1.9 kg/d during the lactation period.

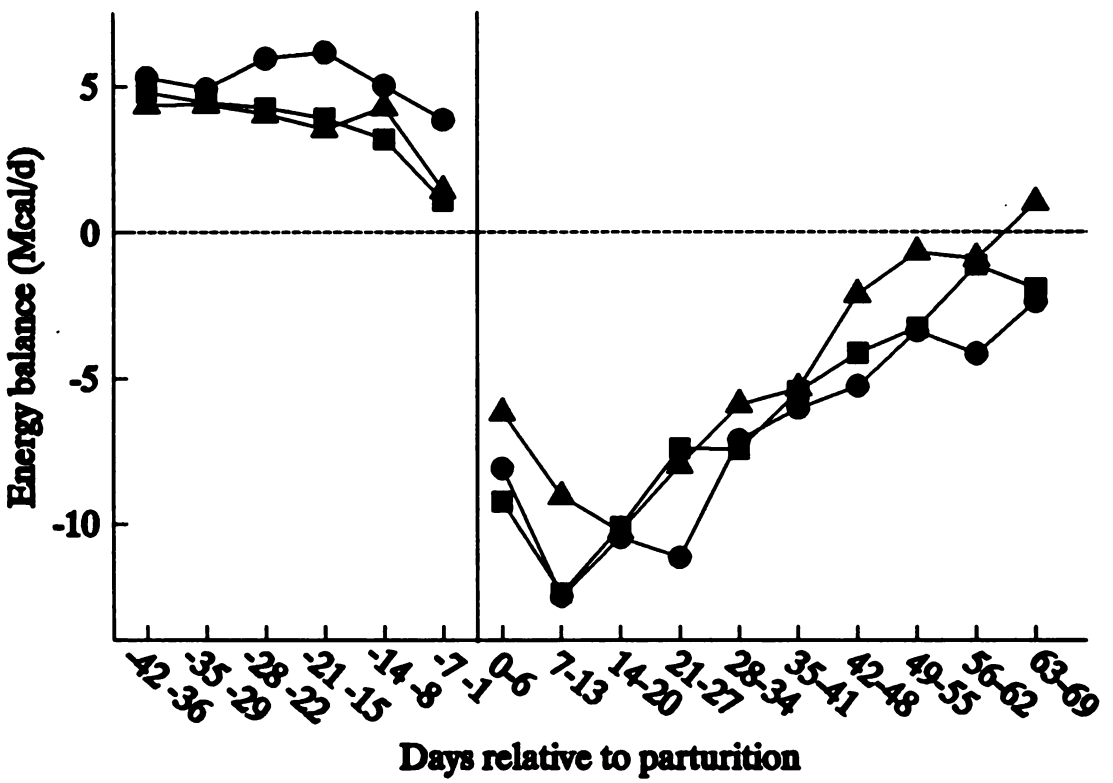

Figure 15. Calculated energy balance of cows receiving no treatment (•; n = 14), 5 mg bST/d (▲; n = 14), or 14 mg bST/d (▪; n = 13) for the last 46 d before parturition. Parturition is indicated by the vertical line. Each point represents the average of a treatment group within each 7-d period. Standard error of the difference among treatments was 1.0 Mcal/d during the dry period and 1.2 Mcal/d during the lactation period.

the 5 mg bST-treated cows during the 10th week of lactation, calculated energy balance was negative for all cows during the first 70 d of lactation.

Reproductive Characteristics and Health Disorders

There were no treatment differences in the reproductive characteristics or birthweights of the calves among groups (P>.20; Table 1). In addition, there were no treatment differences in health-related abnormalities among groups (P>.20; Table 2).

Table 1. Reproductive characteristics of cows receiving no treatment (n=14), 5 mg bST/d (n=14), or 14 mg bST/d (n=13) for the last 46 d before parturition[a].

	Treatment		
	0 mg bST/d	5 mg bST/d	14 mg bST/d
Gestation length	279±6 d	277±5 d	277±6 d
Incidence of dystocia[b]	4/14	4/14	6/13
Birthweight of calf[c]	46±1.6 kg	45±1.9 kg	43±1.6 kg
Incidence of twins	2/14	2/14	1/13
Incidence of retained placenta	1/14	4/14	2/13
Incidence of metritis	1/14	2/14	1/13
Return to estrous cyclicity	38±11 d	37±13 d	39±15 d
No. of cows returning to estrous cyclicity within 70 d after parturition	12/14	9/14	12/13

[a] No treatment differences were detected in any reproductive characteristics (P>.20).

[b] Required use of chains to remove calf.

[c] Birthweights from single births only.

Table 2. Incidences of health disorders during the first 70 d of lactation of cows receiving no treatment (n=14), 5 mg bST/d (n=14), or 14 mg bST/d (n=13) for the last 46 d before parturition[a].

	Treatment		
	0 mg bST/d	5 mg bST/d	14 mg bST/d
Milk fever	1/14	1/14	0/13
Ketosis	3/14	3/14	4/13
Displaced abomasum	1/14	2/14	0/13
Kidney/bladder infection	0/14	1/14	1/13
Pneumonia	1/14	1/14	1/13
Mastitis	3/14	3/14	0/13

[a] No treatment differences were detected in any health disorders (P>.20).

DISCUSSION

Protein status of the dairy cow during early lactation is extremely important in allowing the cow to achieve her genetic potential for milk production (McGuffey et al., 1990). Generally, the capacity of the cow to consume enough protein during early lactation is not adequate to support the demands of milk production. Therefore, during early lactation, cows must rely on limited body protein reserves to maintain the increase in milk synthesis. As a result, protein rapidly becomes a potentially limiting nutrient for milk production (McGuffey et al., 1990). Since bST increases body protein in growing steers and heifers (Eisemann et al., 1989; Crooker et al., 1990), an objective of the present study was to determine if bST administered during the dry period would increase body protein reserves and subsequent yield of milk.

In the present study, 14-mg bST increased yield of milk protein during the first week of lactation, a period when nutrients are partially derived from body reserves. The increase in milk protein in the cows treated with 14-mg bST was responsible for the increase in solids-corrected milk yield during the first week of lactation, since milk protein was the only component affected by bST treatment. I speculate that the 14-mg bST-treated cows may have had larger protein reserves available for degradation immediately after parturition, thereby resulting in increased milk protein

secretion.

Lower concentrations of SUN and increased concentrations of urinary creatinine during the dry period in the 14-mg group indirectly support my hypothesis. Concentrations of SUN reflect the blood's supply of nitrogen for urea synthesis (Hayase et al., 1991). Creatinine is an end-product of creatine metabolism in muscle and is an index of total muscle mass (Hayden et al., 1992).

Reduced concentrations of SUN are consistent with observations in bST-treated steers (Moseley et al., 1982; Eisemann et al., 1989; Early et al., 1990; Hancock and Preston, 1990) and bST-treated, growing Holstein heifers (Crooker et al., 1990). In steers and heifers treated with bST, lower concentrations of SUN are associated with increased nitrogen retention (Eisemann et al., 1989; Crooker et al., 1990). Thus, the lower concentrations of SUN during the dry period in the bST-treated cows probably reflected increased amino acid uptake and increased nitrogen retention. When steers are treated with a synthetic androgen, trenbolone acetate, excretion of urinary creatinine increases as a result of increased whole-body protein mass (McCarthy et al., 1983; Hayden et al., 1992). During the dry period of the current study, concentrations of urinary creatinine were increased in both bST-treated groups supporting the concept that bST increased body protein reserves.

In all cows, regardless of treatment, during the transition from the dry period to the lactation period, concentrations of SUN increased, concentrations of urinary creatinine decreased, and the ratio of N^{τ}-MH/creatinine increased. The ratio of urinary N^{τ}-MH/creatinine is used as an estimate of myofibrillar protein degradation which is standardized for muscle mass. In the current experiment, ratios of urinary

N^{τ}-MH/creatinine indirectly suggest protein degradation increased after parturition. In order to maintain milk output, utilization of maternal sources of protein is necessary to supplement the dietary supply, resulting in increased protein degradation (Bryant and Smith, 1982; Baracos et al., 1991).

Additional evidence supporting the concept that the 14-mg group had a larger protein reserve to mobilize is that, relative to controls, cows treated with bST had a larger decrease in urinary concentrations of creatinine and a larger increase in the ratio of urinary N^{τ}-MH/creatinine immediately after parturition. However, increased urine volume during early lactation may have diluted the concentrations of urinary creatinine. Regardless, results from the current study related to protein degradation could indicate that the loss of muscle mass and the rate of protein degradation was increased in bST-treated cows, thereby suggesting that they had more protein available for degradation.

While the 5-mg bST-treated cows seemed to have increased nitrogen retention during the dry period and increased protein degradation after parturition relative to controls, as did the 14-mg bST-treated cows, the 5-mg dose of bST did not result in increased yield of milk protein. There does not seem to be an obvious explanation, but I speculate that the 5-mg dose of bST was too low to greatly increase the protein reserve and result in an increase of milk protein that was measurable. In addition, cows treated with 5-mg bST had numerically more incidences of retained placenta which could have resulted in lower milk production and thereby masked the effects of increased protein reserves on synthesis of milk protein.

In ruminants, a current hypothesis regarding ST mechanism of action on

muscle is that ST increases protein synthesis while having little or no effect on protein degradation (Hart and Johnsson, 1986; Pell and Bates, 1987; Buttery and Dawson, 1990). For example, Pell and Bates (1987) administered ST to lambs and observed increased rates of protein synthesis in muscle; therefore, net deposition of protein was increased because the increase in rates of protein synthesis exceeded the concomitant increase in rates of protein degradation. In the present study, similar ratios of urinary N^{τ}-MH/creatinine among cows treated with bST and controls agree with reports in bST-treated steers and heifers which have indicated that ST does not affect protein degradation (Eisemann et al., 1986, 1989). Furthermore, SUN and creatinine results from the current experiment support the presented hypothesis that ST stimulates protein synthesis in muscle.

Somatotropin's effects on lipid metabolism depend on the energy balance of the animal at the time of treatment (Bauman et al., 1988). When animals are in positive energy balance, the shift in nutrient partitioning associated with bST treatment appears to involve a reduction in energy deposition in tissue to accommodate increased nutrient utilization for milk synthesis or lean tissue accretion (Tyrrell et al., 1988). Somatotropin decreases lipogenic enzymes, decreases glucose uptake and oxidation, and decreases the sensitivity of adipose tissue to insulin (Bergen and Merkel, 1991). Concentrations of NEFA in serum are indicative of the interaction between rates of lipolysis from adipose tissue and uptake of NEFA by liver and other tissues (Veenhuizen et al., 1991). Tissue utilization of NEFA increases with increasing concentrations of serum NEFA (Eisemann et al., 1986). When bST-treated animals are in positive energy balance, concentrations of NEFA

in serum are not changed because bST treatment alters lipid synthesis in adipose tissue rather than rates of lipid mobilization (Bauman et al., 1988; Etherton and Louveau, 1992). In contrast, there is a chronic elevation of concentrations of plasma NEFA and an increase in rates of NEFA turnover and oxidation when bST is administered to animals in negative energy balance (Bauman et al., 1988). Thus, in bST-treated, negative energy balance animals, the additional energy needed for milk synthesis or lean tissue accretion is derived from enhanced lipid mobilization (Sechen et al., 1989).

In the current study, all cows were calculated to be in positive energy balance during the dry period based on 1989 NRC specifications. However, if an adjustment of more than 3 Mcal/d of energy was made for fetal and maternal development during the dry period, the bST-treated cows may have been in a negative energy balance during the last week of the dry period. Regardless of the value of the adjustment made for late pregnancy, the relative differences between treatment groups would not be different. If one accepts that all cows were in positive energy balance, the lack of treatment effect on concentrations of serum NEFA is in agreement with previous reports that indicate bST treatment does not increase lipolysis. In addition, BCS and measurements of subcutaneous fat over the 12th and 13th ribs were similar among treatment groups during the dry period, offering further support that rates of lipolysis were not increased by bST treatment. Because dairy cattle preferentially deposit fat in internal depots and I did not measure this, bST treatment may have resulted in less lipogenesis in the internal depots and it would not have been detected by my methods. In any case, bST treatment during the dry

period did not appear to prevent replenishing of subcutaneous body fat reserves during the dry period. This is a significant finding, because not only do dairy cows utilize body protein reserves during early lactation, they also mobilize body fat reserves to support milk production during early lactation (McNamara and Hillers, 1986). In the present experiment, concentrations of serum NEFA increased 1.5 fold at parturition, and during lactation, BCS and subcutaneous fat over the 12th and 13th ribs declined. In agreement with the literature, these data indicate that lipolysis was increased at parturition to provide energy for lactogenesis and milk production and that the cows continued to utilize stores of body fat during early lactation.

Physiological adaptations which occur during the dry period, such as involution and remodeling of mammary tissue, lactogenesis, and repletion of body reserves are carefully orchestrated and controlled by complex interactions among several hormones. Somatotropin is a key somatotropic hormone and homeorhetic controller (Boyd and Bauman, 1989). It exhibits regulatory effects on metabolism and consequently provides control over how absorbed nutrients are partitioned for growth and lactation (Boyd and Bauman, 1989). Insulin-like growth factor-I is a primary endocrine mediator of ST action (Hua et al., 1993). It is now well established that variation in plasma IGF-I depends on ST status and energy balance (Clemmons, 1984; Bass et al., 1991; Hua et al., 1993). For example, in sheep and cattle, starvation decreases concentrations of plasma IGF-I, and ST increases concentration of plasma IGF-I but, only in fed animals (Elsasser et al., 1989; McShane et al., 1989; Hua, et al., 1993). Very little is known about the effects of administering exogenous bST during the dry period on concentrations of serum IGF-I.

In the present experiment, as expected, from d -46 to -23, concentrations of ST increased with bST dose, and serum concentrations of IGF-I increased concomitantly. However, from d -23 to -1 concentrations of serum IGF-I no longer responded to bST treatment. For example, concentrations of serum ST remained elevated in cows treated with bST until parturition, while concentrations of serum IGF-I decreased from d -23 to parturition. In agreement with my data, Vicini et al. (1991) administered bST during the dry period and observed a decrease in concentrations of serum IGF-I about 2 weeks before parturition. The uncoupling between concentrations of ST and IGF-I possibly could be attributed to the decrease in energy balance which was observed from d -21 to parturition. These data agree with reports of low circulating concentrations of IGF-I during early lactation which are also associated with low nutrient balance (Vicini et al., 1991; McGuire, et al., 1992).

Hua et al. (1993) suggested that the sensitivity of the ST-IGF-I axis to changes in nutrition is mediated by ST receptors on the liver. In order for ST to increase concentrations of serum IGF-I, it must first bind to a specific cell receptor, primarily on the liver. The high-affinity, hepatic-ST receptor, which may regulate the ST control of IGF-I, is particularly sensitive to nutritional manipulation in ruminants (Breier et al., 1988). The capacity of the high-affinity ST receptor decreases with malnutrition and correlates positively with growth rate and concentrations of plasma IGF-I (Breier et al., 1988). In the present study, reduced DMI and increased nutrient demands prior to parturition may have resulted in fewer ST receptors on the liver and in turn less IGF-I release.

Another possible explanation for the uncoupling of ST and IGF-I is that energy balance began to decline prior to parturition and this may have decreased various IGF-binding proteins which then would increase the clearance of IGF-I. Insulin-like growth factor-I in serum and other biological fluids is bound to multiple, specific-binding proteins which alter its distribution and biological activity (Davenport et al., 1990). In serum, IGF-binding proteins prolong the half-life of IGFs from 10 minutes to 15 hours (Suikkari and Baxter, 1992). For example, in states of IGF-binding protein deficiency, the rate of IGF-I efflux from the vascular compartment is increased (Davenport et al., 1990).

In subsequent experiments utilizing the samples collected for the present experiment, we examined the response of IGF-binding proteins to ST administration during the dry period to see if the results would give insight as to a possible reason for the uncoupling of ST and IGF-I (Simmons et al., 1993). Western ligand blots were used to measure IGF-binding proteins. Two forms of IGF-binding protein-3 on d -23 were increased in cows injected with 14-mg bST when compared with either the 5 mg-group or controls. Somatotropin treatment also increases IGF-binding protein-3 in lactating cows (Cohick et al., 1992). However, in the present study, in all cows IGF-binding protein-3 decreased during the last 22 d of the dry period and was not different among groups. The disappearance of IGF-binding protein-3 from serum has been demonstrated in rats and mice during late pregnancy (Suikkari and Baxter, 1992). The current concept regarding the decrease of IGF-binding protein-3 late in gestation in rodents is that a specific protease converts IGF-binding protein-3 into smaller fragments that are undetectable by Western ligand blotting (Ocrant et al.,

1992). To my knowledge, this protease has not been identified in ruminants thus far. Therefore, the decrease in IGF-binding protein-3 observed in my study may or may not have been due to degradation by a protease. The physiological significance of this phenomenon is unknown. In addition, IGF-binding protein-2 was decreased in the 5- and 14-mg bST-treated cows during the last 23 d of the dry period. Vicini et al. (1991) and Cohick et al. (1992) observed decreases in IGF-binding protein-2 in lactating dairy cows treated with bST. Based on data from the present study, we speculate that decreased energy balance associated with lower DMI during the last 2 weeks of the dry period reduced IGF-binding protein-2 and decreased concentrations of serum IGF-I.

Results from the current study demonstrate the complex interactions between metabolism and the ST-IGF-I axis. To date, studies in which modulation of IGF-binding proteins, IGF receptors, and ST receptors were examined are limited. However, the nutritional status of the cow seems to be a major modulator of concentrations of IGF-I in serum and the ST-IGF-I axis (Ronge and Blum, 1988; Vicini et al., 1991).

SUMMARY AND CONCLUSIONS

The first objective of the experiment described in this thesis was to determine if bST administered to cows during the dry period would enhance body protein reserves and thereby stimulate subsequent milk production. Measures of protein metabolism presented in this study indirectly indicate that bST increased protein synthesis and presumably increased protein reserves during the dry period. Furthermore, exogenous bST during the dry period increased apparent protein degradation during the first week after parturition. The 14-mg dose of bST increased the yield of milk protein during the first week of lactation when protein degradation was elevated. Collectively, these data support the concept that bST treatment during the dry period increased body protein reserves, which then were mobilized during early lactation to increase milk protein synthesis. While bST treatment during the dry period did not increase subsequent overall milk production, it is of interest that bST increased the protein reserve. Although the resulting increase in milk protein was short-lived, it does indicate that the protein reserve can be enhanced.

The second objective of my thesis was to determine if bST treatment during the dry period would affect fat metabolism. Because no treatment differences were observed in concentrations of serum NEFA, BCS, or subcutaneous fat between the 12^{th} and 13^{th} ribs, I conclude that bST during the dry period did not alter fat

metabolism.

The third objective of my thesis was to study hormonal changes associated with bST treatment during the dry period. Data from the current study illustrated the impact of the nutrient status of cows on the ST-IGF-I axis. Prior to parturition, DMI decreased in all cows as nutrient demands were beginning to increase. Therefore, energy balance of the cows began to decline as parturition approached. As a result, concentrations of IGF-I no longer increased concomitantly with concentrations of ST. The physiological impact of the uncoupling of ST and IGF-I is unknown thus far.

In conclusion, results of this research demonstrated that administration of bST during the dry period can increase protein reserves and thereby stimulate milk protein production for 1 week. Furthermore, bST treatment during the dry period did not alter fat metabolism. Lastly, energy balance was a major regulator of the responsiveness of IGF-I to exogenous ST during the dry period.

APPENDICES

APPENDIX A

Table 3. Chemical composition of diets fed during the first 5 weeks of the dry period (early dry) and the remainder of the dry period until parturition (late dry).

	Diet	
	Early dry[a]	Late dry[b]
Dry matter, %	69.5	63.1
Crude protein, % of DM	9.7	11.6
Available protein, % of DM	8.7	10.6
Unavailable protein, % of DM	1.0	1.0
Acid detergent fiber, % of DM	37.3	29.9
Neutral detergent fiber, % of DM	60.5	47.5
Net energy-lactation (Mcal/kg DM)	1.3	1.5

[a] Diet contained approximately 66% grass hay, 16% corn silage, 8% ground shell corn, 9% soybean meal, .3% urea, .3% vitamin-mineral mix, and .3% salt. The estimated DIP was 68% of crude protein and the estimated UIP was 32% of crude protein.

[b] Diet contained approximately 44% grass hay, 26% corn silage, 15% ground shell corn, 9% distillers grain, 4% soybean meal, .4% urea, .4% vitamin-mineral mix, and .4% salt. The estimated DIP was 61% of crude protein and the estimated UIP was 39% of crude protein.

APPENDIX B

Table 4. Chemical composition of the diet during the lactation period (d 0-69)[a].

Dry matter, %	57.1
Crude protein, % of DM	16.3
Available protein, % of DM	15.3
Unavailable protein, % of DM	.9
Acid detergent fiber, % of DM	23.9
Neutral detergent fiber, % of DM	34.6
Net energy-lactation (Mcal/kg DM)	1.5

[a] Diet contained approximately 38% ground shell corn, 23% corn silage, 23% alfalfa haylage, 14% soybean meal, .7% calcium carbonate, .5% salt, .4% dicalcium phosphate and .2% vitamin-mineral mix. The estimated DIP was 65% of crude protein and the estimated UIP was 35% of crude protein.

APPENDIX C

Calculation of N^{τ}-methylhistidine (nmoles/ml urine) concentrations:

1) $$\textit{nmoles } N^{\tau}\textit{-MH standard injected} = \textit{ng } N^{\tau}\textit{-MH injected} \times \frac{1\ \textit{nmole}}{169.2\ \textit{ng}}$$

2) $$\frac{\textit{nmoles } N^{\tau}\textit{-MH}}{\textit{injection}} = \textit{sample } N^{\tau}\textit{-MH area} \times \frac{\textit{nmoles } N^{\tau}\textit{-MH standard injected}}{\textit{standard } N^{\tau}\textit{-MH area}}$$

3) $$\frac{\textit{nmoles } N^{\tau}\textit{-MH}}{\textit{ml urine}} = \frac{\textit{nmoles } N^{\tau}\textit{-NM}}{\textit{injection}} \times \frac{1\ \textit{injection}}{\mu\textit{l sample}} \times \frac{1000\ \mu\textit{l}}{\textit{ml}}$$

APPENDIX D

Figure 16. Representative trace from HPLC analysis of N$^{\tau}$-methylhistidine in a urine sample spiked with pure N$^{\tau}$-methylhistidine. The fluorescent excitation wavelength was 340 nm and the fluorescent emmission wavelength was 440 nm. The elution time of the N$^{\tau}$-methylhistidine peak was 14.13 minutes.

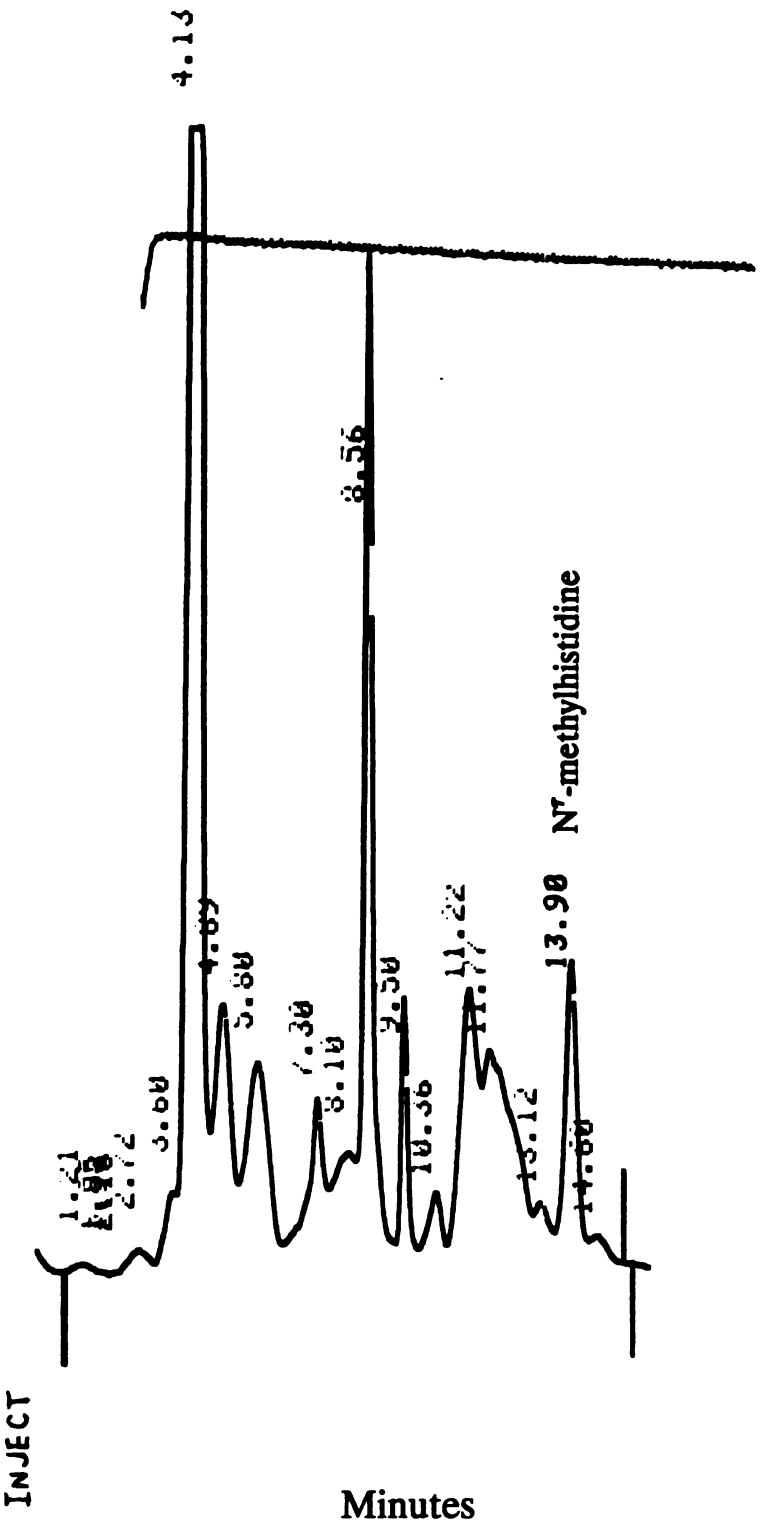

Figure 17. Representative trace from HPLC analysis of N^{τ}-methylhistidine in an experimental urine sample. The fluorescent excitation wavelength was 340 nm and the fluorescent emmission wavelength was 440 nm. The elution time of the N^{τ}-methylhistidine peak was 13.90 minutes.

APPENDIX E

Table 5. Comparison of urinary N-$^{\tau}$methylhistidine/creatinine (nmol/mg) in samples collected at 0700 h and 1900 h[a].

	Time	
Days relative to parturition	0700 h	1900 h
-35 d	142.0	135.2
-21 d	110.3	85.8
-7 d	118.5	92.9
6 d	256.4	223.7
20 d	199.8	168.0
34 d	130.6	140.4
48 d	126.4	172.6
62 d	155.4	134.6

[a] A difference was not detected between 0700 h and 1900 h ($P > .20$).

LIST OF REFERENCES

LIST OF REFERENCES

Baracos, V.E., J. Brun-Bellut and M. Marie. 1991. Tissue protein synthesis in lactating and dry goats. British J. of Nutr. 66:451-465.

Bass, J.J., J.M. Oldham, S.C. Hodgkinson, P.J. Fowke, H. Sauerwein, P. Molan, B.H. Breier, and P.D. Gluckman. 1991. Influence of nutrition and bovine growth hormone (GH) on hepatic GH binding, insulin-like growth factor-I and growth of lambs. J. Endo. 128:181-186.

Battaglia, F.C. and G. Meschia. 1978. Principal substrates of fetal metabolism. Physiological Rev. 58:499-527.

Bates, P.C., G.K. Grimble, M.P. Sparow and D.J. Millward. 1983. Myofibrillar protein turnover. Biochem. J. 214:593-605.

Bates, P.C. and D.J. Millward. 1983. Myofibrillar protein turnover. Biochem. J. 214:587-592.

Bauman, D.E. 1976. Intermediary metabolism of adipose tissue. Federation Proc. 35:2308-2313.

Bauman, D.E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432-3451.

Bauman, D.E. and W.B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D.E. and J.M. Elliot. 1983. Control of nutrient partitioning in lactating ruminants. pp. 437-468 in Biochemistry of Lactation, ed. T.B. Mepham. Elsevier Science Publishers, Amsterdam, The Netherlands.

Bauman, D.E., C.J. Peel, W.D. Seinhour, P.J. Reynolds, H.F. Tyrrell, A.C.G. Brown, and G.L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairly cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J. Nutr. 118:1031-1040.

Bergen, W.G. and R.A. Merkel. 1991. Body composition of animals treated with partitioning agents: implications for human health. FASEB J. 5:2951-2957.

Bergman, E.N. 1968. Glycerol turnover in the nonpregnant and ketotic pregnant sheep. Am. J. Physiol. 215:865-873.

Bergman, E.N. and R.N. Heitmann. 1978. Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. Federation Proc. 37:1228-1232.

Black, A.L., R.S. Anand, M.L. Bruss, C.A. Brown, and J.A. Nakagiri. 1990. Partitioning of amino acids in lactating cows: oxidation to carbon dioxide. J. Nutr. 120:700-710.

Blaxter, K.L. 1964. Protein metabolism and requirements in pregnancy and lactation. pp. 173-223 in Mammalian Protein Metabolism, eds. H.N. Munro and J.B. Allison. Academic Press, New York.

Botts, R.L., R.W. Hemken, and L.S. Bull. 1979. Protein reserves in the lactating dairy cow. J. Dairy Sci. 62:433-440.

Boyd, R.D. and D.E. Bauman. 1989. Mechanisms of action for somatotropin in growth. pp. 257-293 in Current Concepts of Animal Growth Regulation, eds. D.R. Campion, G.J. Hausman, and R.J. Martin. Plenum Publishing Co., New York.

Breier,B.H., P.D. Gluckman, and J.J. Bass. 1986. The somatotrophic axis in young steers: influence of nutritional status and oestradiol-17β on hepatic high- and low-affinity somatotrophic binding sites. J. Endocrinology 116:169-177.

Brockman, R.P. and B. Laarveld. 1986. Hormonal regulation of metabolism in ruminants; a review. Livestock Prod. Sci. 14:313-334.

Bryant, D.T.W. and R.W. Smith. 1982. The effect of lactation on protein synthesis in ovine skeletal muscle. J. Agric. Sci. 99:319-323.

Buttery, P.J. and J.M. Dawson. 1990. Growth promotion in farm animals. Proc. Nutr. Soc. 49:459-483.

Byers, F.M. and G.T. Schelling. 1988. Lipids in ruminant nutrition. pp.298-312 in The Ruminant Animal Digestive Physiology and Nutrition, ed. D.C. Church. Prentice Hall, Englewood Cliffs.

Champredon, C., E. Debras, P.P. Mirand, and M. Arnal. 1990. Methionine flux and tissue protein synthesis in lactating and dry goats. J Nutr. 120:1006-1015.

Chilliard, Y.D., M. Cisse', R. LeFaivre and B. Remond. 1991. Body composition of dairy cows according to lactation stage, somatotropin treatment, and concentrate supplementation. J. Dairy Sci. 74:3103-3116.

Clemmons, D.R. 1984. Factors controlling blood concentrations of somatomedin C. J. Clinical Endo. Metabolism 13:113-143.

Crooker, B.A., M.A. McGuire, W.S. Cohick, M. Harkins, D.E. Bauman and K. Sejrsen. 1990. Effect of dose of bovine somatotropin on nutrient utilization in growing dairy heifers. J. Nutr. 120:1256-1263.

Davenport, M.L., D.R. Clemmons, M.V. Miles, C. Camacho-Hubner, A.J. D'Ercole, and L.E. Underwood. 1990. Regulation of serum insulin-like growth factor-I (IGF-I) and IGF binding proteins during rat pregnancy. Endocrinology 127:1278-1286.

Dayton, W.R. and M.R. Hathaway. 1989. Autocrine, paracrine, and endocrine regulation of myogenesis. pp. 69-90 in Animal Growth Regulation, eds. D.R. Campion, G.J. Hausman, R.J. Martin. Plenum Press, New York.

Early, R.J., B.W. McBride, and R.O. Ball. 1990. Growth and metabolism in somatotropin-treated steers: I. Growth, serum chemistry and carcass weights. J. Anim. Sci. 68:4134-4143.

Edgerton, L.A. and H.D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid, and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451-458.

Eisemann, J.H., A.C. Hammond, T.S. Rumsey and D.E. Bauman. 1989. Nitrogen and protein metabolism and metabolites in plasma and urine of beef steers treated with somatotropin. J. Animal Sci. 67:105-115.

Eisemann, J.H., H.F. Tyrrell, A.C. Hammond, P.J. Reynolds, D.E. Bauman, G.L. Haaland, J.P. McMurtry, G.A. Varga. 1986. Effect of bovine growth hormone administration of metabolism of growing hereford heifers: dietary digestibility, energy and nitrogen balance. J. Nutr. 116:157-163.

Elsasser, T.H., I.S. Rumsey, and A.C. Hammond. 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. J Anim. Sci. 67:128-141.

Emery, R.S. 1979. Deposition, secretion, transport and oxidation of fat in ruminants. J. Dairy Sci. 48:1530-1537.

Etherton, T.D. and I. Louveau. 1992. Manipulation of adiposity by somatotropin and β-adrenergic agonists: a comparison of their mechanisms of action. Proc. Nutrition Soc. 51:419-431.

Evers, B. 1989. Hormonal effects on protein turnover. pp. 367-403 in Protein Metabolism in Farm Animals, eds. H.D. Bock, B.O. Eggum, A.G. Low, O. Simon and T. Zebrowska. Oxford Science Publications, Berlin, Germany.

Faulkner, D.B., D.F. Parrett, F.K. McKeith, and L.L. Berger. 1990. Prediction of fat cover and carcass composition from live and carcass measurements. J Anim. Sci. 68:604-610.

Florini, J.R. 1987. Hormonal control of muscle growth. Muscle & Nerve 10:577-598.

Friedman, Z. and H.W. Smith. 1980. Rapid hig-performance liquid chromatographic method for quantitation of 3-methylhistidine. J Chromatography 182:414-418.

Gallo, G.F. and E. Block. 1990. Effects of recombinant bovine somatotropin on nutritional status of dairy cows during pregnancy and of their calves. J. Dairy Sci. 73:3266-3275.

Genuth, S.M. 1993. The endocrine system. pp. 813-979 in Physiology, 3rd ed., eds. R.M. Berne and M.N. Levy. Mosby Year Book, St. Louis.

Gier, H.T. and G.B. Marion. 1968. Uterus of the cow after parturition: involutional changes. American J. Vet. Research 29:83-96.

Gill, J.L. 1978. Design and Analysis of Experimetns in the Animal and Medical Sciences. Vol. 1-3. Iowa State Univ. Press, Ames.

Gill, J.L. 1986. Repeated measurement: sensitive tests for experiments with few animals. J. Anim. Sci. 63:943-954.

Gillette, D.D. and L. Holm. 1963. Prepartum to postpartum uterine and abdominal contractions in cows. Amer. J. Physiol. 204:1115-1121.

Hancock, D.L. and R.L. Preston. 1990. Titration of the recombinant bovine somatotropin dosage that maximizes the anabolic response in feedlot steers. J. Anim. Sci. 68:4117-4121.

Hannon, K. and A. Trenkle. 1991. Relationship of thyroid status to growth hormone and insulin-like growth factor-I (IGF-I) in plasma and IGF-I mRNA in liver and skeletal muscle of cattle. Domestic Animal Endocrinology 8:595-600.

Hanson, R.W. and F.J. Ballard. 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Biochem. J. 105:529-536.

Harris, C.I. 1981. Reappraisal of the quantitative importance of non-skeletal muscle source of N^{τ}-methylhistidine in urine. Biochem. J. 194:1011-1014.

Harris, C.I. and G. Milne. 1981. The urinary excretion of N^{τ}-methylhistidine by cattle: Validation as an index of muscle protein breakdown. Br. J. Nutr. 45:411-422.

Hart, I.C. and I.D. Johnsson. 1986. Growth hormone and growth in meat producing animals. pp. 135-159 in Control and Manipulation of Animal Growth, eds. P.J. Buttery, D.B. Lindsay, and N.B. Haynes. Butterworths, London.

Hay, W.W. 1989. Placental control of fetal metabolism. pp. 33-52 in Fetal Growth, eds. F. Sharp, R.B. Fraser, R.D.B. Milner. Springer-Verlag, London, UK.

Hayase, K., G. Yonekawa, H. Yokogoshi, and A. Yoshida. 1991. Triiodothyronine administration affects urea synthesis in rats. J. Nutr. 121:970-978.

Hayden, J.M., W.G. Bergen, and R.A. Merkel. 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin, and cortisol concentrations in growing steers implanted with Estradiol-17β, trenbolone acetate, or estradiol-17β plus trenbolone acetate. J Anim. Sci. 70:2109-2119.

Herdt, T.H. 1992. Post-absorbtive nutrient utilization. pp. 345-365 in Textbook of Veterinary Physiology, ed. J.G. Cunningham. Saunders, Philadelphia.

Hood, R.L. 1982. Relationships among growth, adipose cell size, and lipid metabolism in ruminant adipose tissue. Federation Proc. 41:2555-2561.

Hua, K.M., R. Ord, S. Kirk, Q.J. Li, S.C. Hodgkinson, G.S.G. Spencer, P.C. Molan, and J.J. Bass. 1993. Regulation of plasma and tissue levels of insulin-like growth factor-I by nutrition and treatment with growth hormone in sheep. J. Endo. 136:217-224.

Jainudeen, M.R. and E.S.E. Hafez. 1987. Gestation, prenatal physiology and parturition. pp. 229-259 in Reproduction in Farm Animals, ed. E.S.E. Hafez. Lea & Febiger, Philadelphia.

Kamdar, M.B. Personal communication. Unpublished Upjohn Co. standard procedure.

Kamdar, M.B., L.F. Krabill, and M.E. Zantello. 1992. Development and validation of a sensitive RIA to measure insulin-like growth factor (IGF-I) in bovine serum. The Upjohn Co. Technical Report No. 451-7921-92-016, 23 June, 1992.

Lobley, G.E., A. Connell, and V. Buchan. 1987. Effect of food intake on protein and energy metabolism in finishing beef steers. Br. J. Nutr. 57:457-465.

Lormore, M.J., L.D. Muller, D.R. Deaver and L.C. Griel. 1990. Early lactation responses of dairy cows administered bovine somatotropin and fed diets high in energy and protein. J. Dairy Sci. 73:3237-3247.

Maltin, C.A., M.I. Delday, S.M. Hay, G.M. Innes, and P.E.V. Williams. 1990. Effects of bovine pituitary growth hormone alone or in combination with the B-agonist clenbuterol on muscle growth and composition in veal calves. British J. of Nutr. 63:535-545.

Manatt, M.W. and P.A. Garcia. 1992. Nitrogen balance: concepts and techniques. pp.9-66 in Modern Methods in Protein Nutritionand Metabolism, ed. S. Nissen. Academic Press, New York.

McCarthy, F.D., W.G. Bergen, and D.R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N^{τ}-methylhistidine excretion. J. Nutr. 113:2455-2463.

McCutcheon, S.N. and D.E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin releasing hormone in lactating cows. J. Dairy Sci. 69:44-51.

McDowell, G.H. and E.F. Annison. 1991. Hormonal control of energy and protein metabolism. pp. 231-253 in Physiological Aspects of Digestion and Metabolism in Ruminants: Proceedings of the Seventh International Symposium on Ruminant Physiology, eds. T. Tsuda, Y. Sasaki and R. Kawashima. Academic Press, New York.

McGuffey, R.K., H.B. Green, and P.R. Basson. 1990. Lactation response of dairy cows receiving bovine somatotropin and fed rations varying in crude protein and undegradable intake protein. J. Dairy Sci. 73:2437-2443.

McGuire, M.A., J.L. Vicini, D.E. Bauman, and J.J. Veenhuizen. 1992. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J Anim. Sci. 70:2901-2910.

McNamara, J.P. and J.K. Hillers. 1986. Adaptations in lipid metabolism of bovine adipose tissue in lactogenesis and lactation. J. Lipid Res. 27:150-157.

McShane, T.M., K.K. Schillo, J.A. Boling, N.W. Brandley, and J.B. Hall. 1989. Effects of recombinant DNA-dervied somatotropin and dietary energy intake on development of beef heifers. I. Growth and puberty. J Anim. Sci. 67:2230-2236.

Millican, P.E., R.G. Vernon and V.M. Pain. 1987. Protein metabolism in the mouse during pregnancy and lactation. Biochem. J. 248:251-257.

Millward, D.J., P.C. Bates, G.K. Grimble and J.C. Brown. 1980. Quantitative importance of non-skeletal-muscle sources of N^{τ}-methylhistidine in urine. Biochem. J. 190:225-228.

Millward, D.J., P.J. Garlick, R.J.C. Stewart, D.O. Nnanyelugo, and J.C. Waterlow. 1975. Skeletal muscle growth and protein turnover. Biochem J. 150:235-243.

Morton, A.J. and D.F. Goldspink. 1985. Protein turnover in the rat uterus during and after pregnancy. pp.641-643 in Intracellular Protein Metabolism, eds. E.A. Khairallah, J.S. Bond and J.W.C. Bird. Alan R. Liss, Inc., New York.

Moseley, W.M., L.F. Krabill, and R.F. Olsen. 1982. Effect of bovine growth hormone administered in various patterns on nitrogen metabolism in the Holstein steer. J. Anim. Sci. 55:1062-1070.

Moseley, W.M., J.B. Paulissen, M.C. Goodwin, G.R. Alaniz and W.H. Clafin. 1992. Recombinant bovine somatotropin improves growth performance in finishing beef steers. J. Anim. Sci. 70:412-425.

National Research Council. 1989. Nutrient requirements of dairy cattle. Sixth rev. ed. Nat'l. Acad. Sci., Washington, D.C.

Newbold, J.A., L.T. Chapin, S.A. Zinn, and H.A. Tucker. 1991. Effects of photoperiod on mammary development and concentrations of hormones in serum of pregnant dairy heifers. J. Dairy Sci. 74:100-108.

Nishizawa, N., Y. Toyoda, T. Noguchi and S. Hareyama. 1979. N -Methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N -methylhistidine. British J. Nutr. 42:247-252.

Ocrant, I., C.T. Fay, H. Pham, and R.G. Rosenfeld. 1992. Not all insulin-like growth factor-binding proetins (IGFBPs) are detectable by western ligand blotting: case studies of PC12 pheochromocytoma and rat anterior pituitary IGFBPs and proteolyzed IGFBP-3. Endo. 131:221-227.

Oldham, J.D. 1984. Protein-energy interrelationships in dairy cows. J Dairy Sci. 67:1090-1114.

Pell J.M. and P.C. Bates. 1987. Collagen and non-collagen protein turnover in skeletal muscle of growth hormone-treated lambs. J. Endocr. 115:R1-R4.

Peters, J.P. 1986. Consequences of accelerated gain and GH administration for lipid metabolism in growing beef steers. J. Nutr. 116:2490-2503.

Ronge, H. and J.W. Blum. 1988. Somatomedin C and other hormones in dairy cows around parturition, in newborn calves and in milk. J. Anim. Physiol. a. Anim. Nutr. 60:168-176.

Sechen, S.J., D.E. Bauman, H.F. Tyrrell, and P.J. Reynolds. 1989. Effect of somatotropin on kinetics of nonesterified fatty acids and partition of energy, carbon, and nitrogen in lactating dairy cows. J. Dairy Sci. 72:59-67.

Simmons, C.R., E.P. Stanisiewski, L.F. Krabill, B.K. Sharma, L.T. Chapin, C.J. Sniffen, M.J. VandeHaar, and H.A. Tucker. 1992. Somatotropin (ST), insulin-like growth factor-I (IGF-I) and IGF binding proteins (BP)in cows given Somavubove (bST) during the dry period. J. Dairy Sci.(Suppl. 1) 76:accepted.

Spicer, L.J., J.J. Ireland, and J.F. Roche. 1981. Cahnges in serum LH, progesterone and specific binding of ^{125}I-hCG to luteal cells during regression and development of bovine corpora lutea. Biol. Reprod. 25:832-841.

Sugden, P.H. and S.J. Fuller. 1991. Regulation of protein turnover in skeletal and cardiac muscle. Biochem. J. 273:21-37.

Suikkari, A.M. and R.C. Baxter. 1992. Insulin-like growth factor-binding protein-3 is functionally normal in pregnancy serum. J. Clin. Endocrinol. Metab. 74:177-183.

Swick, R.W. and N.J. Benevenga. 1977. Labile protein reserves and protein turnover. J. Dairy Sci. 60:505-515.

Tyrrell, H.F., A.C.G. Brown, P.J. Reynolds, G.L. Haaland, D.E. Bauman, C.J. Peel, and W.D. Steinhour. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: energy and nitrogen utilization as determined by respiration calorimetry. J. Nutr. 118:1024-1030.

Veenhuizen, J.J., J.K. Drackley, M.J. Richard, T.P. Sanderson, L.D. Miller, and J.W. Young. 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J. Dairy Sci. 74:4238-4253.

Vernon, R.G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19:23-106.

Vernon, R.G. 1988. The partition of nutrients during the lactation cycle. pp. 32-52 in Nutrition and Lactation in the Dairy Cow, ed. P.C. Garnsworthy. Butterworths, London, UK.

Vernon, R.G. 1989. Endocrine control of metabolic adaptation during lactation. Proc. Nutr. Soc. 48:23-32.

Vernon, R.G., R.A. Clegg, and D.J. Flint. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation. Biochem. J. 200:307-314.

Vernon, R.G., E. Finley, E. Taylor, and D.J. Flint. 1985. Insulin binding and action on bovine adipocytes. Endo. 116:1195-1199.

Vicini, J.L., F.C. Buonomo, J.J. Veenhuizen, M.A. Miller, D.R. Clemmons, and R.J. Collier. 1991. Nutrient balance and stage of lactation affect responese of insulin, insulin-like growth factor-I and factor-II and insulin-like growth factor-binding protein-2 to somatotropin administration in dairy cows. J. Nutr. 121:1656-1664

Watt, P.W., E.Finley, S.Cork, R.A. Clegg and R.G. Vernon. 1991. Chronic control of the β- and α_2-adrenergic systems of sheep adipose tissue by growth hormone and insulin. Biochem. J. 273:39-42.

Wildman, E.E., G.M. Jones, P.E. Wagner, R.L. Boman, H.F. Troutt and T.N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Young, V.R. 1980. Hormonal control of protein metabolism, with particular reference to body protein gain. pp. 167-191 in Protein Deposition in Animals, eds. P.J. Buttery and D.B. Lindsay. Butterworths, London, UK.

Young, V.R. and J.S. Marchini. 1990. Mechanisms and nutritional significance of metabolic responses to altered intakes of protein and amino acids, with reference to nutritional adaptation in humans. Am. J. Clinical Nutr. 51:270-289.

Young, V.R. and H.N. Munro. 1978. N^{τ}-Methylhistidine (3-methylhistidine) and muscle protein trunover: an overview. Federation Proc. 37:2291-2300.

Young, V.R. and P.L. Pellett. 1987. Protein intake and requirements with reference to diet and health. Am. J. Clinical Nutr. 45:1323-1343.

Young, V.R., Y.M. Yu, and M. Kremph. 1991. Protein and amino acid turnover using the stable isotopes ^{15}N, ^{13}C, and ^{2}H as probes. pp.17-72 in New Techniques in Nutritional Research, eds. R.G. Whitehead and A. Prentice. Academic Press, San Diego.