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THE EFFECTS OF BOVINE SOMATOTROPIN, ISO-PLUS AND A COMBINATION OF THE TWO IN EARLY LACTATING HOLSTEINS

By

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ABSTRACT

THE EFFECTS OF BOVINE SOMATOTROPIN, ISO-PLUS AND A COMBINATION OF THE TWO IN EARLY LACTATING HOLSTEINS.

BY

Natalie A. Kik

The objective of this study was to investigate the action of Iso-Plus (ISO), bovine somatotropin (BST) and a combination (ISO-BST) of the two in early lactating Holsteins. Treatments included control, 100 g/cow/day of ISO, 25 mg/cow/day BST and a combination of the two. Treatments were administered for a period of 60 days. Increases in milk production over the controls were 2%, 16% and 12% for the ISO, BST and ISO-BST treated cows, respectively. There were no significant differences found in milk composition, dry matter intake, body weight changes or net energy balance across all treatments. No adverse effects on health were seen in any of the treatments.

No significant differences were found for insulin, glucose or urea nitrogen levels for either of the sampling days. Plasma somatotropin was significantly higher for the BST and ISO-BST treated animals for both sampling days.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Enhancement of lactational performance in dairy cattle has been of great interest. Research in agriculture to increase milk production has led to the development of two new products, isoacids and bovine somatotropin (BST). Somatotropin acts to alter body metabolism, whereas isoacids act to alter fermentation in the rumen for better animal performance.

Isoacids have been test marketed by Eastman Kodak and are now manufactured and sold commercially as a nutritional supplement for dairy cattle. The commercial name given to the isoacid mixture is Eastman Iso-Plus Nutritional Supplement. Iso-Plus contains the four isoacids: isobutyric acid, isovaleric acid, 2-methyl-butyric acid, and valeric acid. University and field trials have shown an 8 to 10% increase in milk production and feed efficiency (Papas et al., 1984; Pierce Sandner et al., 1985).

When bovine somatotropin is administered, increases in milk yield have ranged from 10 to 50% (Brumby and Hancock, 1955; Machlin, 1973; Peel et al., 1985; Bauman et al., 1985). Somatotropin has also been reported to improve feed efficiency (Bauman and Collier, 1985). Until quite recently the cost of extracting the hormone from pituitary glands has always precluded the application of bovine somatotropin to practical dairy farming. However, the recently developed technique of mammalian protein synthesis by bacteria has permitted production of BST in large enough amounts for a commercial application. With this new development in recombinant DNA technology, industries are in the process of testing recombinant-derived BST as a potential marketable product in the dairy industry. Milk production responses to BST have been reported at all stages of lactation and over a wide range of production levels. A limited number of studies have been performed showing increases in milk production in sheep, goats and pigs (Bauman and Collier, 1985). In the dairy cow there have been concerns about administering the hormone in early lactation. Concerns have revolved around the fact that during early lactation, response to the hormone may be limited by nutrient availability. During early lactation, cows are usually in negative energy balance. Negative energy balance is a stressful situation. Therefore, the administration of the hormone may be an added stress factor. This situation may complicate certain functions such as reproduction, and cows may not be bred back soon enough. The work presented here was designed to evaluate the effects of Iso-Plus, bovine somatotropin, and a combination of the two during early lactation.

LITERATURE REVIEW

BOVINE SOMATOTROPIN

Bovine somatotropin is a peptide hormone produced by the anterior pituitary gland located at the base of the brain. It has a molecular weight of approximately 22,000 daltons. When somatotropin is released into the blood, it exerts a growth promoting action on many tissues of the body such as cartilage, skeletal muscle, heart, liver, kidney, adipose tissue and lymphoid organs. It controls production functions, growth, milk production, nutrient partitioning and lipid metabolism (Isaksson et al., 1985). Somatotropin secretion is also under hormonal control. Thyroid hormones, androgen, estrogens, glucocorticoids, circulating growth factors, possibly insulin, prolactin and vitamin D metabolites all have an effect on somatotropin (Scanes, 1984).

The effects of exogenous BST on milk production have been a subject of scientific interest for 50 years. Early research involving the injection of crude bovine pituitary extracts into laboratory animals during growth and lactation (Riddle, 1933) led others to study effects on milk production. In 1937 Asimov and Krouse first demonstrated that crude pituitary extracts increased milk production in dairy cows. A series of studies by Folley, Young, and colleagues using pituitary- derived somatotropin were aimed at increasing food production in Britain during World War II. Yet availability of anterior-pituitary tissue was insufficient to allow for a substantial increase in the nation's milk and food supply. Limitations in the supply and purification of somatotropin have resulted in relatively slow progress in exploring and identifying the mechanism of action. However this situation has improved in the past few years with the advances in recombinant DNA technology. The general consensus seems to be that somatotropin is galactopoietic. Its effects are indirect, and nutritional factors have major impact on its secretion. Researchers are still trying to understand the mechanism of action of somatotropin but the main research effort is in exploring and developing its potential for commercial application.

Hypothalamic control of somatotropin secretion

The secretion of the single-chained polypeptide pituitary originated-hormone, somatotropin, is controlled by peptide releasing factors (hypothalamo-hypophyseotropic factors) (Scanes,1984). These factors stimulate processes such as cell division, protein metabolism and mineral metabolism. They also inhibit fat synthesis and fat cell size. There seem to be two peptide releasing factors. One is a stimulatory polypeptide, the growth hormone releasing factor (GRF). The other is an inhibitory peptide, somatostatin (SRIF). Both GRF and SRIF exert their actions at unique pituitary receptors.

When GRF has been given to cattle and sheep, there has been stimulation of somatotropin release (Moseley et al., 1983 ;Plouzek et al., 1983 ;Baile et al., 1983). Trials done with SRIF in sheep and domestic fowl have shown decreases in somatotropin secretion (Gluckman et al., 1980 ;Davis, 1975 ;Bicknell et al., 1979). Another factor thought to have somatotropin releasing functions is thyrotropin releasing hormone (TRH). TRH is considered to be a potentiator of GRF (Scanes, 1984). Considerable increases of plasma somatotropin ST have been reported when TRH has been administered to sheep (Davis, 1975 ;Thompett et al., 1980).

It has been concluded that the amount of somatotropin secreted is due to the relative concentrations of the three releasing factors in the hypophyseal portal blood along with the sensitivity of the somatotrophs to each releasing factor. Other factors that have been involved in controlling GRF and SRIF secretion include several neurotransmitters (dopamine, norepinephrine, seratonin), neuropeptides and biogenic amines (Guckman et al., 1987). These substances act by regulating the balance of actions between the two hypothalamic hormones, resulting in a specific somatotropin secretory pattern (Baile et al., 1986).

Somatotropin and its receptors

Hormones such as somatotropin almost never directly act on the intracellular machinery to control chemical reactions. Instead they invariably first combine with hormone receptors on the surfaces of the cells or inside the cells. When the hormone combines with its receptor, there is an additional cascade of reactions that occur in the cell. Receptors are under active endocrine and metabolic control. In fact, nutritional factors may play a large role in the control of the mediation of somatotropin through its receptors. Somatotropin receptors have been solubilized from liver (Collier et al., 1984). A generally accepted hypothesis on how somatotropin exerts its growth-promoting effects involves the action of somatomedins. This hypothesis states that the growth-promoting effects are stimulated by the release of insulin like growth factor-I (IGF-I) from the liver and perhaps other tissues. From this observation it might be concluded that somatotropin's primary target organ is the liver. In fact specific binding sites for somatotropin exist on the plasma membranes of many organs: cartilage, skeletal muscle, heart, liver, kidney, adipose tissue and lympoid organs (Kostyo, 1985). Evidence for control of somatotropin receptors by nutritional states is limited to studies using the rodent. Starvation and malnutrition in the young rat have reduced the binding of somatotropin to hepatic membranes. In conjunction with this decrease there was a fall in circulating IGF-I concentration (Gluckman et al., 1987).

Studies of the chemical nature of somatotropin binding sites on plasma membranes suggest that the receptor is a glycoprotein complex. It has a molecular weight of approximately 200-300K daltons. Under reducing conditions it can be dissociated into subunits of approximately 110-130K daltons. It seems to be that the subunits are covalently linked by disulfide bonds (Donner, 1983).

To understand the physiological function of the somatotropin cellular binding sites, it should be determined whether there is a correlation between the amount of hormone bound to a cell and the hormone response. It has been difficult to do this because somatotropin binding sites do not usually exhibit relevant in vitro biological responses. An experiment done by Eden et al. (1982) showed an increase in somatotropin binding to adipocyte cells of normal and hypophysectomized rats. To establish the presence of physiological receptors for somatotropin there have been attempts to determine whether the factors known to influence the responsiveness of the organism to the hormone influence binding or number of binding sites. It appears that as the animal ages the receptor binding sites increase. Studies in both the rat

(Maes et al., 1983) and the sheep (Gluckman et al., 1983) have shown that binding sites for somatotropin on liver membranes do increase from fetal to adult life. States of hormone deficiency or excess also have affected binding affinity and number of binding sites. There is a reduction of hepatic somatotropin receptors when lambs and rabbits are hypophysectomized. When somatotropin treatment is administered these receptors are restored (Gluckman et al., 1987). A report by Maes et al. (1985) suggests that state of nutrition may affect post receptor changes in addition to somatotropin receptor affinity or number.

Many researchers have proposed that a chemically heterogeneous population of receptors exists on target cells for somatotropin. While it has been known for many years that BST preparations exhibit other biological properties in addition to growth-promoting activity, it is difficult to associate one common mechanism with all activities. Most BST preparations have been diabetogenic in species such as the dog, cat and man (Kostyo, 1985). Today, preparations are highly purified. Still, it is questioned whether the diabetogenic and insulin-like properties are actually intrinsic properties of the somatotropin molecule. It may be that somatotropin possesses multiple active sites for the several activities.

Effects of exogenous somatotropin on growth

For sometime it has been known that somatotropin encourages the deposition of protein at the expense of fat. This concept of altering protein and fat deposition has been termed repartitioning of nutrients. Early experiments with rats injected with somatotropin showed increased protein synthesis in muscle. There have been limited studies conducted with meat animals. In the few studies there has been an increase in growth rate, feed efficiency and carcass composition. Evans and Long (1922) and Evans and Simpson (1931) were first to demonstrate that a substance in the anterior pituitary increased the growth of rats. In later years it was discovered that this substance was somatotropin. Further studies with swine, sheep and cattle showed promising results in a change of carcass composition. In an experiment conducted by Machlin (1972), when 13 mg per kg body weight per day of porcine somatotropin was given to swine a 13-16% increase in growth rate was found. Another experiment conducted by Chung and colleagues (1983), also in swine, showed a 9-10% increase in growth rate and a 4% increase in feed efficiency. Machlin (1972) also found an increased muscle area and decreased backfat thickness up to 20%.

Exogenous BST has been known to stimulate lypolysis and to have anabolic effects in ruminants. A study conducted with sheep, given an injection of 15mg ovine somatotropin twice daily, showed a 20% increase in average daily gain

(ADG), a 14% increase in feed efficiency and an increase in protein gain with a decrease in fat gain (Wagner and Veinhuizen, 1978). However, another study conducted in 1983 by Muir et al. did not find such dramatic increases in ADG and feed efficiency. The conclusion drawn from this experiment stated that the primary effect of exogenous somatotropin in sheep was to decrease fat deposition. A recent experiment using BST in sheep found significant increases ($p<0.01$) in ADG, feed efficiency and muscle composition similar to the increases found by Wagner and Veinhuizen (1978). The decrease in fat was not significant (Johnson et al., 1985).

In the bovine, the dramatic effects seen with the ovine are not found. The number of bovine studies are limited. One report showed an increase of 13% in ADG for dairy heifers, but no data was reported for carcass composition (Sjersen et al., 1983). A trial conducted with Holstein steers involved both infused and injected BST. The effect of BST on nitrogen (N) metabolism was measured. The trial lasted for 10 days. There were no differences in N metabolism short term (day 3 to 6). But during days 7 to 10 digestion coefficients for dry matter, dietary N, percentage of N retained and the retention of metabolized N in BST treatments were increased (Moseley et al., 1983). Eisemann et al. (1986) conducted a study of the effect of BST on metabolism in growing Hereford heifers. Dietary digestibility, energy and N balance were measured.

N retention was higher and urinary N were lower in the BST groups. This demonstrated an effect on post-absorptive metabolism of nitrogen. Total energy balance was not altered. Yet, energy retained as protein was higher after treatment was given. This implied that less energy was retained as fat.

As the industry now demands a lean carcass and it is known that the ruminant hormone is biologically inactive in humans , the anabolic properties of somatotropin provides a scheme to repartition nutrients from less fat to more protein. Therefore, somatotropin has potential as a growth promotant and as an improver of carcass quality. However, much more work is needed to understand the mechanism of action.

Effects of exogenous somatotropin on lactation

The mammary gland is one of the most highly differentiated and metabolically active tissues in the body. Its physiological function is milk synthesis. The mammary gland is metabolically activated by the increased use of nutrients. BST has increased cardiac output by 10% and mammary blood flow by 35% in the bovine. Along with these increases, there has been a 21% increase in milk production in the dairy cow. Increases in blood flow during administration of BST to dairy goats have also been reported (Peel and Bauman, 1987). BST and its role in lactation has been examined primarily by three groups of scientists.

These include Machlin, Hart, Bines and Morant, Bauman and co-workers and Peel and colleagues. Performance responses on milk yield with exogenous administration of recombinant BST have been examined usually in short term trials. Most investigators administer BST to cattle when they are beyond 60 days postpartum. At this time, the cows are typically beyond peak production and in positive energy balance. Studies conducted when the animals were in the stage of early lactation has not been as consistent when compared to mid-and late-lactation. When exogenous BST was administered at 20 weeks postpartum Bines and Hart (1982) found increases in milk production. In this same experiment no response was found when treatment of cows began at 7 weeks of lactation. Richard et al. (1985) administered BST during two 10-day periods in early lactation (beginning 20 and 60 days post partum). During the first injection period milk yield was 6% greater than the controls, while during the second period there was a 12% increase over the controls. The researches speculated that during early lactation the ability of cows to increase milk yield in response to exogenous BST may be limited by the supply of glucose for lactose synthesis rather than by the ability to mobilize body reserves in support of lactation. During the first period, treated cows were substantially in more negative energy balance than the controls. Also when milk composition was analyzed, yield of milk fat was increased 25%. There was no significant difference during the second period in milk composition. However, the treated cows were still in negative energy balance. Peel et al. (1983) compared high yielding Holsteins in early and late lactation when exogenous BST was given. Although milk yield increase was similar (early lactation 4.3 kg increase, late lactation 3.9 kg increase), the percentage of increase over controls was quite different (early lactation 14%, late lactation 31% increase). Plasma ST increases were also different (early lactation 400%, late lactation 700% increase). This may suggest that the exogenous administration of BST acts substantially more on lower, endogenous plasma concentrations (Bines and Hart, 1982).

Before the use of recombinant somatotropin, natural (pituitary-derived) somatotropin was relatively scarce. This prevented administration of BST to animals for an entire lactation. The short term studies have shown a 10-50% increase in yield (Bauman and Collier, 1985). Very few long term studies have been conducted. One of the earliest trials, conducted by Brumby and Hancock (1955) administered BST over a 12 week period. Cows were at weeks 6 to 8 of lactation when treatment began. They observed a 45% increase over the control cows in milk yield. There was no alteration in milk composition. A 5kg per day increase was observed by Machlin (1973). He treated cows for a 10 week period. The stage of lactation for these animals was not reported. In another long term trial cows were treated for a 22 week period beginning 5 weeks postpartum. The BST treatment resulted in an 18% increase in milk yield (Peel et al., 1985). The longest trial reported so far was conducted for 188 days. These cows began treatment 84 ± 10 days postpartum. Three levels of recombinantly-derived somatotropin (rbST) and one level of pituitary-derived somatotropin (pbST) was administered. A 23 to 41% increase in milk yield was observed when three levels of the rbST (13.5, 27 and 40.5 mg/day) was given, while the pbST (27 mg/day) resulted in an increase of 16% (Bauman et al., 1985). In both the long term and short term trials when milk composition was monitored, the yields of milk fat, protein and lactose followed changes in milk production (Peel et al., 1983, 1985; Hart et al., 1985; Richard et al., 1985). However, if BST treatment causes the cows to be in negative energy balance, the milk fat content increases so that the response in milk fat yield exceeds the milk yield response. In the same respect milk protein percent decreases (Peel et al., 1981; Eppard et al., 1985; McCutcheon and Bauman, 1986). These types of responses are typical for cows in early lactation. At this time cows are usually in negative energy balance.

High-yielding dairy cows will divert dietary energy and mobilize body tissue to meet the high metabolic demands during early and mid-lactation. However, low-yielding cows will divert nutrients to the body tissues for weight gain. Hart et al. (1978) reported a positive correlation between changes in plasma ST concentration and milk yield. High yielding cows have a higher concentration of plasma ST. Barnes et al. (1985) stated that high-yielding, underfed dairy cows have higher plasma concentrations of ST and NEFA and lower concentrations of insulin than do lower-yielding, overfed cows. This can be considered to be associated with the changes in body metabolism, especially at the onset of lactation. The high-yielding cows have a greater demand for increased need of energy and protein for higher milk production. Hart et al. (1980b) compared high-yielding Friesian dairy cows with low-yielding Hereford and Hereford x Friesian cows. During the first part of lactation the high-yielding cows lost weight, while the low-yielders gained weight. These two types of cows are in distinctly different metabolic states and most likely utilize and metabolize hormones at different rates. Examining the responses to BST during different stages of lactation, reports have concluded that plasma ST concentrations during lactation are related to the stage of lactation (Koprowski and Tucker, 1973). Concentrations decrease as lactation progresses. Despite the large increase in milk production, most short term trials reported no significant change in feed consumption between the controls and treated animals (Peel et al., 1981, 1982 ; Fronk et al., 1983). This suggests that there may be an improvement of feed utilization. The increases in production could be caused by improvement in the digestibility of the feed, alterations of the bioenergetic efficiencies of maintenance or milk synthesis, or partitioning of nutrients away from body tissues to the mammary gland for milk synthesis. Improvement in the digestibility of the feed would be related to the partial efficiency with which energy is used for improvement in milk synthesis during the treatment period (Peel and Bauman, 1987). A 10 day trial conducted by Eppard et al. (1985) showed a decrease in feed intake when cattle were given 100IU per day of BST. During the long-term trials, intake was not different during the first part of the experiment but did significantly increase by the 8th to the 10th week of the trial to support the higher milk production (Peel et al., 1985; Bauman et al., 1985). Not much information has been reported in reference to reproductive performance and health considerations when BST is administered. What has been reported has shown no apparent health problems, normal pregnancies and normal calvings (Bauman et al., 1985; Eppard et al, 1985; Peel et al., 1983). Eppard et al., (1987) reported that cows receiving BST (27 mg/cow/d of pituitary ST and 13.5, 27, & 40.5 mg/cow/d of recombinant ST) averaged 96% conception rate, 2.0 services per conception and 116 days open. These data were comparable to controls. There were no discernible effects on mammary health, no subclinical or clinical evidence of ketosis or milk fever. Overall no serious health problems were noted. As more research is performed, more data on reproduction and health will become available, and better conclusions can be made.

Somatotropin and metabolic interactions

Potential for the mammary gland to synthesize milk to its fullest capability relies on adequate and correct proportions of nutrients channeled to the mammary gland. Substrates required for the synthesis of milk are glucose, amino acids, acetate, 3-hydroxybutyrate and long-chain fatty acids (LCFA). Exogenous BST administration has been reported to increase metabolic heat production, altering the partitioning of absorbed nutrients but having no apparent effect on the maintenance requirement or on the energy costs associated with the synthesis of each increment of milk (Bauman and Collier, 1985; Bauman and McCutcheon, 1986). There seems to be an increase in efficiency, and the responses are primarily related to the post-absorptive use of nutrients. The most obvious shift in nutrient partitioning involves carbohydrate metabolism. The carbon source for milk lactose is glucose. To define the role of somatotropin in glucose metabolism in ruminants is difficult. Lactose is the major osmoregulator of milk volume. In the dairy animal, glucose is derived from propionate and synthesized in the liver. Pocius and Herbein (1986) took liver biopsies from cows treated with somatotropin. The liver had a greater capacity to produce glucose from propionate when BST was given. About 60 to 80% of total glucose turnover is utilized by the mammary gland (Bauman and Collier, 1985). When BST is administered an even greater portion is required for the increase in milk lactose secretion. Bines et al. (1980) observed increases in plasma glucose when BST was given to beef cows but no increase in dairy cows. It is suggested that BST may have increased the availability of glucose to the mammary gland. However, the relatively low capacity of the beef cow gland for milk synthesis prohibited full utilization. This was the opposite for the dairy cow gland. The rate of irreversible loss of glucose has been measured by Bines and Hart (1982) during somatotropin administration. There was no difference despite the increased output of lactose in milk. The implication here may be that the increased availability of glucose to the mammary gland by the reduction in glucose utilization elsewhere, leads to a partitioning of glucose utilization toward the mammary gland. Peel and Bauman (1987) did report increased rates of glucose irreversible loss and reduced rates of glucose oxidation. These adaptations in glucose turnover and oxidation accommodate the additional glucose required for the increased synthesis of lactose.

Partitioning of amino acids also seems to be altered by the administration of BST. When the cows are in positive energy balance milk crude protein is unchanged. The percent increase is identical to the increase in milk production (Bauman et al., 1982; Peel et al., 1982, 1983; Fronk et al., 1983; Eppard et al., 1985). In contrast, when cows are in negative energy and nitrogen balance, protein content of milk decreases (Peel et al., 1981,1983; Tyrrell et al., 1982). This reflects the limited quantity of labile protein reserves available to the cow. In beef cattle when irreversible loss and oxidation rates of leucine were measured during BST treatment there was no change in the irreversible loss of leucine. However, the oxidation of leucine was reduced. This sparing of amino acids could be an additional source of production capacity (Eisemann et al, 1985).

Mineral needs such as calcium increase 2-3 times from late gestation to early lactation. This increase is met by the increase in absorption of dietary calcium mobilized from bone. The partitioning of minerals is altered by BST treatment. However, the change in mineral flux is perfectly coordinated with the increased secretion of milk. To meet the increased energy need for higher milk production the animals must either alter the rate of alimentary absorption, the rate of accretion or the mobilization from tissue reserves (Peel and Bauman, 1987). Calcium and phosphorous concentrations in milk and blood are unchanged during BST treatment (Bauman and Collier, 1985).

In the dairy animal, body energy reserves are predominately in the form of lipids stored in adipose tissue. Adipose functions in the synthesis of lipids and in mobilization of lipids. A major effect of BST treatment involves lipid metabolism. Specific adaptations differ in states of energy balance. During late gestation and early lactation investigators have demonstrated increased rates of lipid mobilization and decreased rates of pathways of lipid synthesis in adipose tissue (Bauman and Currie, 1980). Investigations have demonstrated that chronic BST administration acts on the enzymes involved in lipid metabolism by increasing the activity of hormone sensitive lipase and decreasing the activities of lipoprotein lipase, acetyl coenzyme-A carboxylase and palimate synthetase (Peel and Bauman, 1987). Fat is a source of energy and preformed long chain-fatty acids (LCFA). It seems that in early lactation there is an increase in fat mobilization caused by dietary energy supply not meeting the cow's needs. When cattle are in negative energy balance, there is an increase in nonesterfied-fatty acids (NEFA) in the blood. Earlier reports of BST administration proved somatotropin to be lipolytic and diabetogenic in ruminants (Bauman and Collier, 1985). Now it appears that these effects were chemical and conformational variants relating to the purification techniques and purity of the BST preparations (Bauman and McCutcheon, 1986). When the state of negative energy balance already exists, especially in early lactation, BST treatment tends to cause the animal to be in more negative energy balance. When BST-treated cows are in negative energy balance milk fat increases. The response in milk fat content exceeds the milk yield response. Increases of free-fatty acids (FFA) correlates with the degree of negative energy balance (Peel et al., 1982). The composition of milk shifts to a greater proportion of LCFA which are characteristic of body fat reserves (Eppard et al., 1985). In contrast, when cows are in positive energy balance none of these changes are observed. Milk fat percentage is not altered. The increase in milk fat parallels the increase in milk yield. It may be possible that somatotropin exerts indirect effects on adipose tissue by influencing the circulating concentrations of nutrients or the release of other hormones including somatomedins and insulin. Somatotropin may act on lipid metabolism by altering homeostatic signals in adipose tissue. For example, *in vitro* studies have been conducted using bovine, ovine and porcine adipose tissue when incubated with somatotropin. These studies showed that somatotropin treatment antagonized the responsiveness of the tissue to insulin. Alterations could consist of changes in receptor number, binding kinetics or intracellular expression of the signal (Peel and Bauman, 1987). Whether the cows are in positive or negative energy balance, the net effect of the treatment is to reduce lipid accretion, allowing for greater partitioning of nutrients to the mammary gland in support of milk synthesis.

Somatotropin administration and other hormones

Other hormones such as insulin (INS) and glucagon (GLN), may possibly be involved in the process of milk synthesis. INS can be characterized as a storage hormone through its action of promoting the movement of glucose, acetate and amino acids into peripheral tissues. GLN can be considered as an energy mobilizer since it increases hepatic glucose output and lipolysis. During gluconeogenesis amino acids are used. GLN has a net effect of reducing amino acid supply for nonhepatic tissues by enhancing the hepatic uptake of amino acids. Somatotropin effects and interaction with these hormones are important aspects in gaining some insight on the mechanism of action. Both INS and GLN play important roles in regulating ruminant metabolism. Peripheral sites such as the muscle and adipose tissue appear to be the primary regulatory sites for INS. GLN appears to act directly at the liver to increase glucose production (Brockman, 1978).

Exogenous BST may also affect the increase of milk production efficiency by increasing the supply of milk precursors. Most important is the availability of glucose for lactose synthesis. This is one of the primary factors limiting milk production. Concentrations of plasma ST , INS and GLN appear to be related to glucose availability. The relative changes in the secretion of INS and GLN are important for maintenance of metabolic homeostasis. High ST relative to INS could result in fat mobilization, thus affecting the availability of energy precursors for milk production to be increased (Hart et al., 1980a; de Boer, 1985). It has been hypothesized that the ST:INS ratio may play an important part in glucose availability and the supply of other nutrients for milk synthesis (Herbein et al., 1985). Many have reported higher concentrations of plasma ST in high-yielding dairy cows than in low-yielding cows (Bines and Hart, 1982; Barnes et al., 1985). Changes in plasma ST appear to be positively correlated with changes in milk yield and negatively related to body weight changes. Hart et al. (1980a) reported a 0.40 correlation coefficient (CC) between milk yield and plasma ST and a -0.43 CC between live weight gain and plasma ST. It has also been shown that the ratio of ST to INS may be influenced by body weight. Hart et al. (1980a) calculated a 0.45 CC between plasma INS and live weight gain.

No change in plasma INS was reported by Peel et al. (1983) or Eppard et al. (1985) when BST was given. INS is positively correlated with stage of lactation but negatively correlated with daily milk yield (Walsh et al., 1980; Hart et al., 1980a). Insulin also promotes the storage of metabolites in peripheral tissues. Gluconeogenesis and lipolysis are inhibited by INS (de Boer, 1985). Even though a glucoregulatory effect has not been demonstrated with the direct effects observed with BST administration in ruminants, the increase in peripheral ST relative to INS during early lactation or the increase of peripheral ST due to the administration of exogenous BST might increase glucogenic substrates, amino acids and glycerol. In fact, glycerol could be a potential source for glucose. Peel and Bauman (1987) reported that up to 27% of additional glucose could be accounted for by hydrolysis of adipose triglycerides to glycerol. Glucose requirements for a lactational response would be met from a lower INS:GLN ratio. The decrease in INS and the increase in GLN would facilitate substrate utilization to meet nutrient requirements for lactation. This would be especially prevalent during negative energy balance. Later into lactation the ST:INS ratio and INS:GLN ratio would favor deposition of nutrients in peripheral tissues (Herbein et al., 1985). Perhaps injections of BST prolongs this action by acting on the ST:INS ratio.

Reports based on the rodent and human clinical studies appear to show somatotropin acting not directly at the tissue level but rather acting on mediating factors at the tissue level (Scanes, 1984) ;thereby, interacting with other hormones and metabolites. A very important mediating factor includes the insulin-like-growth factors (IGF) or the somatomedins. IGFs are a family of polypeptides related to proinsulin. These polypeptides have important growth-promoting effects which have been shown in vitro and in vivo. A prevailing viewpoint is that somatotropin acts by increasing IGFs (Scanes, 1984) from liver and other tissues. When the pituitary gland is removed from normal sheep there is a decrease in plasma somatomedin C. However in acromegaly sheep (excessive somatotropin secretion) there is an increase in plasma somatomedin C (Scanes, 1984). In muscle somatomedin C increases protein synthesis. Somatotropin receptors have not been found in mammary epithelial cells (Bauman et al., 1985; Bauman and McCutcheon, 1986). Yet, IGF type 1 receptors have been found in mammary epithelial cells of pigs and cows (Bauman and McCutcheon, 1986). After several hours of BST administration to sheep and dairy cows, IGF-1 concentrations were increased. When BST was administered to dairy cattle, IGF-I and IGF-II concentrations in plasma increased two-to-three fold. Milk production increased 17% (Davis and Bass, 1984). In humans there is a threefold increase in plasma IGF-1 concentration after exogenous somatotropin administration. In vitro studies on rat liver suggest that the effect of exogenous somatotropin is on the stimulation of de novo synthesis of IGF-1 (Gluckman et al., 1987). It seems to be conclusive that somatotropin very strongly influences plasma IGF-1 concentrations. IGF-I could very well be a secondary mediator in the mechanism of action of somatotropin.

In summary, the action of somatotropin in the ruminant animal still is inconclusive. A single biochemical or physiological event to account for the increases in production efficiency in the animal is elusive. Since coordinated changes in many tissues and physiological processes occur during administration of BST, the mechanism of action can not be related to one particular event. Direct and indirect effects on the mammary gland, other body tissues, blood flow, lipid metabolism, carbohydrate metabolism and protein metabolism are probably all interactive to accommodate the alterations induced by exogenous BST.

ISOACIDS

The isoacids or branched-chain VFA (isobutyric, isovaleric, and 2-methyl-butyric acids) and the straight chain 5-carbon valeric acid are essential nutrients for cellulolytic bacterial growth in the rumen (Allison et al., 1962; Bryant and Robinson, 1963; Felix et al., 1980b; Papas et al., 1984). Stanton and Canale-Parola (1980) reported interaction of a saccharolytic spirochete, *Treponema bryantii*, with cellulolytic bacteria. This spirochete enhanced the cellulose breakdown by *B. succinogenes*. *T. bryantii* also required the branched short-chain fatty acids, DL-2-methyl butyrate and isobutyrate for growth. Isobutyrate, isovalerate and 2-methyl-butyrate are produced in the rumen by oxidative deamination and decarboxylation of the amino acids valine, leucine and isoleucine, respectively. Valeric acid is produced from either carbohydrates or from the amino acid proline (Papas et al., 1984). The fermentation of dietary protein is the source of isoacids and other fatty acids (Bentley et al., 1955 and Bryant and Robinson, 1963). *In vitro* work has shown that these acids can enhance microbial growth and cellulolysis by mixed rumen cultures (Bentley et al., 1955 and Takahashi et al., 1978). Over the years investigators have shown improvement in milk production, feed intake, cellulose digestion, nitrogen retention, microbial growth and weight gain in ruminants when isoacids are fed (Felix et al., 1980a; Papas et al., 1984; Russell and Sniffen, 1984; Deetz et al., 1985; Pierce-Sandner et al., 1985). This work has led to the development of a nutritional supplement for dairy cows. The product is now manufactured by the chemical division of the Eastman Kodak Company. The trade name for the product is Eastman Iso-Plus nutritional supplement. Its chemical composition consists of an 84% minimum of calcium salts of isobutyric and a mixed 5-carbon VFA, 3% maximum of calcium hydroxide, 10% maximum fat, water maximum 3% and artificial and natural flavors of 0.5% maximum (Eastman Animal Nutrition Supplement).

The mechanism by which isoacids or Iso-Plus affects different aspects of animal performance is still inconclusive. This review will address some of the effects isoacids have on the rumen and possible effects on extra-ruminal tissues.

Ruminal effects of isoacids

Cellulolytic bacteria such as Bacteroides succinogenes and noncellulolytic bacteria such as a strain of Rumminococcus albus require or are stimulated by 4-carbon and 5-carbon VFA (Allison et al., 1962). These acids when added to the diets of ruminants have enhanced animal performance. These effects have been attributed to the enhancement of microbial growth and cellulose degradation in the rumen (Russell and Sniffen, 1984 and Hoover, 1986). Bacteria use the acids as carbon skeletons for the synthesis of branched-chain amino acids, higher fatty acids and aldehydes. Bacteria provide isobutyrate, isovalerate 2-methyl-butyrate, acetate and phenylacetate by deaminating and decarboxylating the branched-chain amino acids valine, leucine, isoleucine, alanine and phenylalanine. Investigations by Bryant and Robinson (1963) have shown that a substantial amount of the bacterial population in the rumen requires ammonia nitrogen as well as higher VFA for synthesis of their cellular constituents. When carbohydrates and amino acids are fermented by these bacteria, they provide the 5-carbon acid, valerate. While dietary proteins are the primary source of VFA, recycling of bacterial protein may be a source as well (Gorosito et al., 1985). Reviews addressing branched-chain VFA sources in the rumen are discussed in previous literature (El-Shazly, 1952; Annison, 1954; Bryant, 1973; Brondani, 1986). It appears that the VFA and rumen bacteria are involved in amino acid synthesis. This aspect has been reviewed by Allison (1969, 1970) Bryant (1973) and Felix (1976). Increased bacterial growth and protein synthesis suggest that isoacids could cause an improvement in dry matter digestibility. Reports indicate that there is cellulose digestion by mixed rumen bacteria when urea or ammonia is used as the sole source of nitrogen (Burroughs et al., 1951). Rumen microorganisms were reported to have a higher rate of cellulose digestion and of urea nitrogen incorporation into protein when isoacids were added to an *in vitro* medium (Bentley et al., 1955).

The degradation of carbohydrates and the synthesis of microbial protein results in ruminal fermentation. Isoacids enhance these two processes. Therefore, ruminal fermentation, the production of acetate, propionate and butyrate, is also improved. Isoacids have increased acetate production up to 28% (Cook, 1985). The production of acetate is a measure of the extent of feed fermented in the rumen.

As stated previously, isoacids are important sources of amino acids. The ruminant receives 40 to 80% of its daily amino acid requirements from microbial protein flowing to the small intestine (Sniffen and Robinson, 1987). Many researches have shown that supplementation of VFA can increase microbial growth, synthesis and efficiency from NPN (Chalupa and Bloch, 1984; Russell and Sniffen, 1984; Deetz et al., 1985). Russell and Sniffen (1984) showed a 35% increase in rumen bacterial synthesis efficiency (gram per 100g OM digested in the rumen) when VFA were added to a high-forage diet. Treatment did not affect whole-tract digestion of DM, OM or any NDF component. It is suggested that VFA stimulate microbial synthesis and rumen digestion of hemicellulose and compounds soluble in neutral detergent. Perhaps a shift of OM digestion to the lower tract represents a beneficial occurrence toward a positive production response in animal performance. Urea utilization and ruminal fermentation also appear to be stimulated when isoacids are given.

Cummins and Papas (1985) evaluated the effect of an ammonium salt mixture of isocarbon-4 (31.5%) and isocarbon-5 (69.5%) (AS-VFA) on microbial nitrogen incorporation and in vitro dry matter digestibility (IVDDM) to batch-culture fermentation systems receiving corn silage diets with varying concentrations and sources of protein. Dietary protein supplements were cottonseed, corn gluten meal and urea. The concentrations of crude protein varied from 13 to 16%. When isoacids were added to the diets, dry matter digestibility and microbial growth were increased in vitro.

Russell and Sniffen (1984) studied the effects of 4-carbon and 5-carbon VFA on growth of mixed rumen bacteria in vitro. The substrates used included mixed carbohydrates (equal parts glucose, maltose, sucrose, cellobiose and soluable starch), timothy hay inoculum and an inoculum of 60% concentrate and mixed hay. When VFA were added to the carbohydrate, average bacterial growth was slow. Addition of VFA (isovalerate and 2-methyl butyrate) to timothy hay inoculum increased protein synthesis by 11.2% and 16.4%. There was no effect with the addition of isobutyrate and valerate alone. When all four acids were added, there was an increase of 18.7%. When the VFA were added to the 60% concentrate diet, bacterial growth and utilization of ammonia were similar to incubations not receiving the acids. Microbial protein concentration, feed intake, total ruminal VFA and ammonia concentrations were all increased when hay diets fed to sheep were supplemented with isobutyric, valeric and isovaleric acids (Hemsley and Moir 1963). Cline et al. (1966) found a positive correlation between the level of protein in the diet and level of branched-chain VFA in the rumen fluid, concluding that the primary source of branched-chain VFA was proteins.

A high-producing animal may be in negative nitrogen balance, especially when 30% or more of the total nitrogen is from urea. The rapid hydrolysis of urea to ammonia is responsible for nitrogen loss (Bloomfield et al., 1960 and Chalupa, 1968). The excess ammonia is absorbed into the blood, converted to urea in the liver and urea is excreted in the urine. This results in a significant loss of nitrogen (Umunna et al., 1975). When ammonia is used for microbial synthesis, there is a decrease in ammonia absorption through the rumen. The conversion of ammonia nitrogen to microbial protein is dependent upon a large supply of energy and carbon skeletons. Isoacids have been shown to increase nitrogen retention in ruminants (Cline et al., 1966; Miron et al., 1968; Oltjen et al., 1971; Umunna et al., 1975; Felix et al., 1980b). Felix (1976) fed two mixtures of isoacids (mixture 1: 20g of isobutyrate, isovalerate, methyl-butyrate, n-valerate and phenylacetate; mixture 2: 20g of the four isoacids) to lactating cows. When the mixtures were added to the diets there was an increase in retention of absorbed nitrogen and a decrease in urinary nitrogen. It was suggested that there may be an increase in the supply of leucine, valine and isoleucine which would be especially important in urea diets. These diets tend to decrease the amino acids aforementioned (Hemsley and Moir, 1963).

Increased nitrogen retention has been found in cellulose-urea diets fed to lambs, calves, steers and dairy heifers (Cline et al., 1966; Miron et al., 1968; Oltjen et al., 1971; Umunna et al., 1975). Umunna et al. (1975) fed 0.37% isobutyrate, 0.37% isovalerate, and 0.74% isobutyrate and isovalerate to sheep on whole corn pellets and urea diets. These diets resulted in a 37%, 110%, 110% increase in nitrogen retention for each diet, respectively. There appeared to be an increased utilization of ruminal NPN, resulting in increased N retention. Changes in N balance have also been shown when isoacids are added to soybean meal diets (Oltjen et al., 1971 and Umunna et al., 1975). When steers were fed starch-glucose-wood pulp plus soy protein with an addition of a mixture of isobutyrate, 2-methyl-butyrate, isovalerate and valerate at 1% of the diet, there was a 23% increase in N retention. No effect on number of bacteria, on cellulose digestion or on the amino acids in blood was reported (Oltjen et al., 1971). Addition of branched-and straight-chain VFA to purified lamb diets demonstrated increases in N digestibility, N retention, cellulose digestibility and dry matter digestibility (Cline et al., 1966).

Isoacid effects on growth and lactation

The improved ruminal effects of isoacids discussed so far lead to an overall improvement in animal growth and production. Early work by Beeson and Perry (1952) reported an improvement in the growth rate of beef cattle when isoacids were fed. Other work conducted by Lassiter et al., (1958a) showed that a combination of valerate and isovalerate improved the growth rate of dairy heifers fed corncob roughage. Hungate and Dryer (1956) showed no effect on cellulose digestion when steers were fed straw supplemented with .04% valerate and .01% isovalerate.

Felix et al. (1980a) fed isoacids to eighteen dairy heifers over ninety days. The diet consisted of timothy hay ad libitum. The treated group was fed 10g of isobutyrate, isovalerate, 2-methyl-butyrate and phenylactetate. Addition of the acids to the diets increased the growth in the younger animals 15.9% over initial weight. An effect in the older animals was not reported.

The possibility of using isoacids as a supplement for feedlot cattle has been demonstrated by Deetz et al., (1985). Two trials were done using a mixture of ammonium salts of isovalerate, 2-methyl-butyrate, isobutyrate and valerate (AS-VFA). Variables studied included feedlot performance and carcass characteristics of growing and finishing Angus, Hereford and Angus x Hereford steers. Three levels of the AS-VFA were fed. These included .14%, .28% and .42% AS-VFA of the diet DM. During the growing period there were no differences reported for ADG or feed conversion. However, during the finishing period, gains increased 3.5% ($p<.10$), and feed conversion improved 5.9% ($p<.07$) in the steers fed 0.28% AS-VFA. Carcass characteristics in general did not show a decrease in backfat thickness and kidney, heart and pelvic fat. Little research has been reported for the growing and finishing animal. However, potential for improving the carcass characteristics of meat animals appears to exist.

Most of the work done with isoacids has been conducted with lactating dairy cows. Many studies have shown increases in milk production with no adverse effects on health or reproduction (Felix et al., 1980b; Papas et al., 1984; Pierce-Sandner et al., 1985). Some of the early work was conducted by Felix (1976). Four short term, early-lactation studies were conducted to study the effects of isoacids when added to high-urea, corn-silage and corn-grain diets in 98 dairy cows. The first trial

examined the effects of a mixture of isoacids and phenylacetic acid on milk production. Cows were fed only corn silage as the basal feed . When cows were given 20g of each acid, milk production and persistency of lactation were higher than the control cows, which were fed an urea-supplemented diet. In a second trial there were four treatments: (1) negative control (no N supplement) (2) positive control (SBM) (3) urea and (4) urea plus isoacids. Milk yields and persistency of lactation were similar for treatments 2,3 and 4 but higher than treatment 1. There was a tendency for increased body weight and feed intake when isoacids were fed. The third trial tested two blends of isoacids. Both were compared against a urea control. Blend one consisted of a 28, 24, 24, and 24 molar % of isobutyrate, isovalerate, 2-methyl-butyrate, and valerate acids, respectively. The corresponding percent for blend two was 36,17,17 and 30 molar% for each acid respectively. fat-corrected milk (FCM) was significantly higher ($p<.05$), and persistency was significantly greater for cows fed blend two of the isoacids. Finally, in the last trial 28 cows were fed either a urea control diet or 80g of the isoacids. The treatment showed an increase in persistency of lactation and feed intake but not body weight.

A study involving three universities examined effects of the ammonium salts of VFA on dairy cows for a full lactation cycle. Six blends of ammonium 5-carbon acids and ammonium isobutyric acids were tested. The optimum blend or the center-point blend, defined from response surfaces based on milk yield, was composed of 61g ammonium salts of 5-carbon acids plus 28g ammonium isobutyrate per cow per day. Yields for 305-day milk and 4% FCM for controls were 20.5 and 19.7 kg, while the center-point blend yields were 23.2 and 21.6 kg, a significant increase over the controls. The effect of isoacids was more pronounced in early lactation. During early lactation the cellulose content of the diet and urea N as a % of total N intake were low (Papas et al., 1984). The higher response of the AS-VFA during early lactation was also reported by Rogers and Cook (1986). There was a 2.7 kg per day increase when 120g AS-VFA were fed. No differences were found in the protein and solids-non-fat content of the milk. Dry matter (DM) intake values during full lactation for controls and the center point blend were 17.3 and 17.5 kg, respectively. The difference in the intake was less than the increase in milk yield, resulting in an improved utilization of feed. In the three-university study no adverse effects on reproduction or health were detected (Papas et al., 1984).

An efficacy study conducted by Pierce-Sandner et al (1985) examined a blend of the ammonium salts of isobutyrate, 2-methyl- butyrate, isovalerate and valerate at 120g per cow per day. The cows were fed from 3 weeks prepartum through a complete lactation. They found a 7%

increase in milk yield along with an improvement in feed utilization. Again there were no adverse effects on health or reproduction.

Along with increases in 4% FCM, researchers have found better feed efficiency and positive responses on body weight changes when isoacids are fed (Sweeney et al., 1984 and Papas et al., 1984). Sweeney et al. (1984) reported a 1.33 feed conversion for controls and 1.52 feed conversion for cows fed 120 g per cow per day of AS-VFA. Pierce-Sandner et al. (1985) reported similar body weight gain for controls and treatments. This suggests that the increase in milk yield with the 120 g per cow per day AS-VFA was most likely associated with an apparent increase in feed efficiency.

Results from milk composition changes when AS-VFA are fed showed basically no difference in milk fat%. When milk protein% was measured, researchers found either no difference or a lower percentage (Sweeney et al., 1984; Papas et al., 1984; Newman et al., 1986; Pierce-Sandner et al., 1985). Pierce-Sandner et al. (1985) found a significant decrease ($p<.10$) in protein percent during the first 105 days of lactation. However, the yields of protein and fat generally paralleled milk production (Papas et al., 1984 ; Pierce-Sandner et al., 1985).

Any direct influence of the AS-VFA on metabolism of the mammary gland is not clear. Since short-chain VFA are important intermediates of tissue metabolism (Clark et al., 1977; Raurama et al., 1977; Bergman and Heitmann, 1978; Walser and Williamson, 1980), they may affect metabolism and health of humans and animals. In combination with leucine, isovalerate can cause hypoglycemia (Woolf, 1960). Papas and Sniffen (1984) determined the concentrations of isobutyric, isovaleric, 2-methyl-butyric and valeric acids in milk from dairy cows supplemented with 94g AS-VFA and 376 g AS-VFA per day. The concentrations they found were low (isobutyrate: 37+/- 15ppb, isovalerate and 2-methyl-butyrate: 58+/- 8ppb, and valerate: 39+/- 6ppb). Feeding the diets supplemented with AS-VFA did not increase their concentrations as free acids in milk.

The isoacids are normal components of ruminal fluid and occur in blood and tissues (Allison and Bryant, 1963; Clark et al., 1977; Walser and Williamson, 1980; Huntington, 1983). A portion of these acids is absorbed from the digestive tract. When diets are fed which increase the ruminal concentration of these acids, higher rates of absorption might be expected. Yet feeding the higher-concentrate diets failed to result in increased concentrations of isoacids in milk (Papas and Sniffen, 1984). Metabolism of these acids by liver, kidney, muscle and mammary gland has been shown by Clark et al. (1977), Raurama (1977), Bergman and Heitmann (1978) and Walser and Williamson (1980). Chang et al. (1985) studied the effects of the branched-chain fatty acids on the metabolism of their respective precursor amino acids in lactating bovine mammary tissue. Their results suggested that *in vitro* the isoacids and ketoacids conserve their precursor amino acids by decreasing the catabolism of the amino acids. The pool of isoleucine was conserved by 2-methyl-butyrate. Isobutyrate and isovalerate were similar with regard to valine and leucine. However, their activity was lower.

Effects of isoacids on peripheral concentrations of hormones and metabolites

Accompanying with the changes in animal performance, changes in plasma concentrations of hormones and metabolites have been reported. Few studies have been published that discuss the post-ruminal responses of isoacids. Further research needs to be conducted. Towns and Cook (1984) found lower glucose levels in dairy cows fed 80g of an equal weight mixture of isobutyrate, 2-methyl-butyrate, isovalerate and valerate. In contrast, Brondani (1986) found no change in plasma levels of glucose in sheep fed 0.2g per kg body weight per day of isoacids. As discussed previously, isoacids are thought to contribute to the improved utilization of urea in the rumen and to increase the synthesis of microbial protein. However, changes in BUN vary from one extreme to the other. Towns and Cook (1984) found an increase in BUN plasma levels. These cows were on a ration of high quality protein. Felix et al., (1980a) found a decrease in BUN levels. These cows were on a ration that included NPN. The decrease found in this experiment could have been attributed to the isoacid-stimulated trapping of recycled N by rumen microbes, the synthesis of microbial protein (Towns and Cook, 1984). In sheep fed 0.1g and 0.2g per kg body weight per day of isoacids, no changes in BUN were reported (Brondani and Cook, 1985).

Measurements of hormones have demonstrated some controversial results. In addition to the increase in milk production reported by Towns and Cook in 1984, there was a two- fold increase in plasma ST. Fieo et al. (1984) found an increase in plasma ST in cows fed 120g of AS-VFA per cow per day (8.42 ng/ml versus 5.68 ng/ml). Yet, no increase in milk yield or FCM was reported. The duration of feeding the isoacids was only six weeks. Perhaps the shortness of the exposure could be an explanation of no response in milk production. Recent reports have found no changes in peripheral ST levels when isoacids were fed to lactating dairy cows and growing sheep (Klusmeyer et al., 1986; Brondani, 1986). Both Brondani (1986) and Towns and Cook (1984) found no changes in cortisol levels. Plasma levels of insulin have been reported to be lower (Brondani 1986; Kik et al., 1987) or unchanged (Towns et al., 1987) when isoacids are fed.

Production performance of the ruminant when isoacids are present in the diet appears to involve the interaction of ruminal, metabolic and endocrine regulation to increase milk production and to improve carcass characteristics and efficiency.

Materials and Methods

Forty-eight lactating Holsteins were divided into four groups of twelve each. Level of milk production ranged from 26.1 to 48 kg/day while days in milk ranged from 7 to 80. Groups were balanced on level of milk production prior to treatment and days in milk. Average milk production for each group pretreatment was approximately 30.45 kg/day, and cows averaged 45 days postpartum prior to treatment. Cows were housed in a free stall barn at the Kellogg Biological Station Dairy Center in Battle Creek, Michigan. The four treatments administered included control, bovine somatotropin at 25 mg per cow per day Iso-Plus at 100 g per cow per day and a combination of BST and Iso-Plus. The duration of the trial was 60 days. Daily subcutaneous injections were administered at approximately 0900 hours. The site of injection was at two alternating sites on the left and right sides of the tail-head. The recombinant bovine somatotropin was solubilized with sterile saline at a concentration of 12.5 mg/ml. Thus, the daily injection volume was 2.0 ml. Controls received 2.0 ml of sterile saline. American Cyanamid supplied the recombinant BST and sterile saline. Solubilized hormone was stored at 4 degrees

Celsius for seven to fourteen days. The Iso-Plus nutritional supplement was mixed thoroughly with the soybean meal. The Eastman Kodak Company supplied the Iso-Plus.

Daily milk production was recorded. Cows were milked twice a day at 0400 and 1530 hours. Cows were fed ad libitum three times a day at 0700, 1200 and 1900 hours. Feed refusal was measured once a day at 0600 hours. A total mix ration (TMR) was fed consisting of haylage, corn silage, high moisture corn and soybean meal. The ingredient composition and nutrient content of the diet is given in Table 1. Body weights were recorded weekly. Cows were observed carefully for indications of toxicity and any adverse effects on health. Data on reproductive performance were summarized at the end of the trial from Michigan State University Farmex Herd Inventory Program.

On day 30 and day 60 of the trial, jugular vein cannulas were established in the cattle. The following day blood samples were taken every 20 minutes over an eight hour period, beginning at 1020 hours. Blood was added to tubes containing EDTA at a concentration of 1mg per 1ml of blood and centrifuged immediately to recover plasma. Plasma was stored frozen until assayed for somatotropin, insulin, glucose and blood urea nitrogen. Plasma ST was analyzed by radioimmunoassay, validated by the National Hormone and Pituitary Program of NIH. The bovine somatotropin for iodination was donated by NIH. A commercial radioimmunoassay kit by micromedic Systems, Inc., Horsham,

TABLE 1. COMPOSITION OF THE DIET

Ingrediant & Nutrient	Amount Present(1)
Haylage	32%
Corn Silage	13%
High Moisture Corn	45%
Soybean Meal	9%
Mineral, Salt, Buffer(2)	1%

Nutrient Content	Amount
NEl (Mcal/kg DM)	1.60
Crude Protein	16.48%
Neutral Detergent Fiber	26.35%
Phosphorus	0.33%
Calcium	0.69%
Magnesium	0.23%
Sulfur	0.22%
Sodium	0.51%
Manganese	62 ppm
Iron	201 ppm
Copper	9 ppm
Zinc	74 ppm

1- all values presented on a dry matter basis
2- supplements were added to meet National Research Council recommendations (NRC)

PA was used for insulin determination. The commercial kit utilized a treated tube procedure which allowed a rapid and complete separation of antibody-bound-from free-radioactive-antigen. Glucose was determined by using the quantitative glucose oxidase and perioxidase method. BUN was determined by the diacetyl monoxime quantitative colorimetric method. Methods for glucose and BUN were kits purchased from Sigma Chemical Company, St. Louis, MO.

Statistical analysis was conducted using Statistical Analysis System (SAS, 1985) using a split-plot, repeated-measurement design (Gill, 1986).

The model used was:

Yijkl= u + Ai + Bj + (AB)ij + D(ij)k + Tl + (AT)ij + (BT)jl + (ABT)ijl + Eijkl

Where:

Y= variable measured (milk production, hormone and metabolite concentrations)

u= overall mean

Ai= BST effects (treatment)

Bj= Iso-Plus effects (treatment)

(AB)ij= interaction of both factors

D(ij)k= cows per treatment

Tl= effect of time

(AT)ij= interaction of time and BST treatment

(BT)jl= interaction of time and Iso-Plus treatment

(ABT)ijl= interaction of BST and Iso-Plus combination treatment and time

Pre-treatment milk production was used as a covariate in the analysis of milk yield. Specific differences between means were tested for significance by using Bonferroni t-tests. Body weights, feed intake, net energy balance and milk composition were analyzed by analysis of variance, using a reduced model without time effects.

RESULTS AND DISCUSSION

A. Effect of Iso-Plus, bovine somatotropin and a combination of the two on milk production, milk composition, feed intake, body weight and net energy balance.

It has been suggested that exogenous BST enhances the ability of mammary tissues to synthesize milk components. Other tissues are also affected such that nutrients are preferentially partitioned to the mammary galnd. Most of the work done with lactating cows has been during mid to late lactation. Very little research has been conducted when cows were in the stage of early lactation. Richard et al. (1985) found 6% and 12% increases in milk yield when 50IU of BST was injected 20 and 60 days postpartum. Peel et al. (1983) studied the effects of exogenous somatotropin in both early and late lactation. Milk yields were increased by 15% during early lactation and 31% during late lactaion. On an absolute yield basis, increases for milk yields were similar for early and late lactating Holsteins (4.3 kg/day vs.3.9 kg/day).

It was the objective of this experiment to study the effects of bovine somatotropin and Iso-Plus individually when administered to cows in early lactation, and to

TABLE 2. Effect of ISO, BST and ISO-BST on milk production.(1)

	TREATMENT				COMPARISONS (2)			
WEEK	CON	ISO	BST	ISO-BST	1	2	3	4
1	30.53	31.42	33.08	31.74	NS	NS	NS	NS
2	31.18	31.80	34.80	33.71	NS	p<0.10	NS	NS
3	31.86	31.74	36.11	35.04	NS	p<0.025	NS	NS
4	31.31	32.65	36.20	35.84	NS	p<0.005	NS	NS
5	31.86	31.56	37.20	35.87	NS	p<0.005	p<0.025	NS
6	31.31	30.90	36.17	34.57	NS	p<0.005	p<0.10	NS
7	30.10	31.06	36.45	34.86	NS	p<0.001	p<0.05	NS
8	29.79	30.80	35.73	35.14	NS	p<0.001	p<0.025	NS
9	30.18	30.88	36.74	35.70	NS	p<0.001	p<0.01	NS

1-Milk production is average kg/cow/day. SED (among treatments)=3.087. SED (among weeks)=1.533.
2-Bonferroni comparisons made were 1(control vs. Iso-Plus), 2(control vs. BST), 3(ISO-BST vs. ISO), and 4(ISO-BST vs. BST).

determine whether there was an additive effect of the combined treatments when administered in early lactation (45 days postpartum).

Effects of treatment on milk production are represented graphically in Figures 1-5. Weekly means for each treatment are presented in Table 2 (kg/cow/day). Cows fed Iso-Plus (ISO) gave an overall 2% increase in milk production over the control group (CON), whereas cows given exogenous bovine somatotropin (BST) gave an overall increase of 16% over CON. When the combination (ISO-BST) was administered, cows gave a 12% increase in milk production over CON. Significant responses were not apparent for the cows given ISO. When cows were given exogenous BST a significant response was first seen during the second week of treatment. Cows of the BST treatment were not significantly different from the cows of the ISO-BST treatment. Milk production of the ISO-BST cows was not significantly different from milk production of the ISO cows until the 5th week of treatment. In Figures 1-5 weeks 10-13 depict the response after treatment withdrawal. Milk production for the cows fed ISO continued to stay above CON (Figure 1). In contrast, once BST was withdrawn milk production declined rapidly. Before the 13th week milk production for the BST treated cows was below that of the CON cows (Figure 2). Also when the ISO-BST treatment was withdrawn milk production declined rapidly (Figure 4). Throughout the treatment period, milk production by the ISO-BST animals was

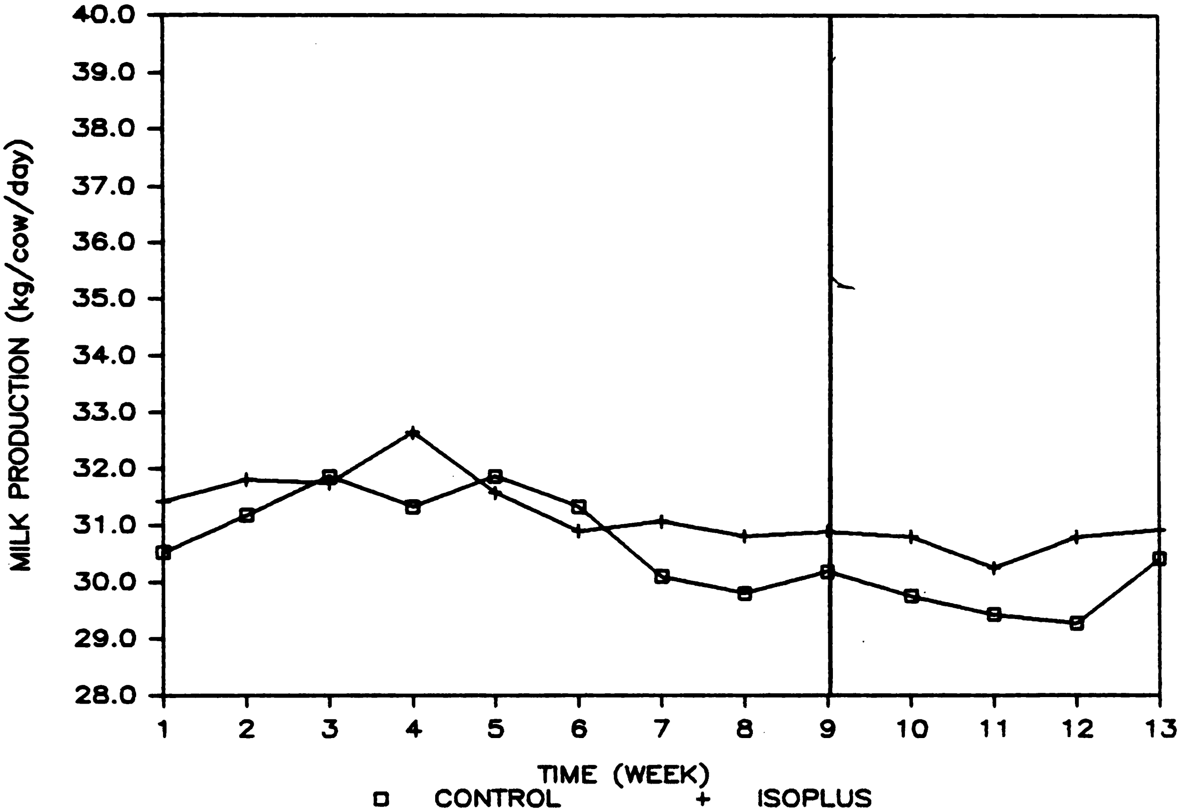

Figure 1. Effect of Iso-Plus on milk production in lactating dairy cows. Treatment period consists of week 1 to week 9. Milk production data represent adjusted weekly averages.

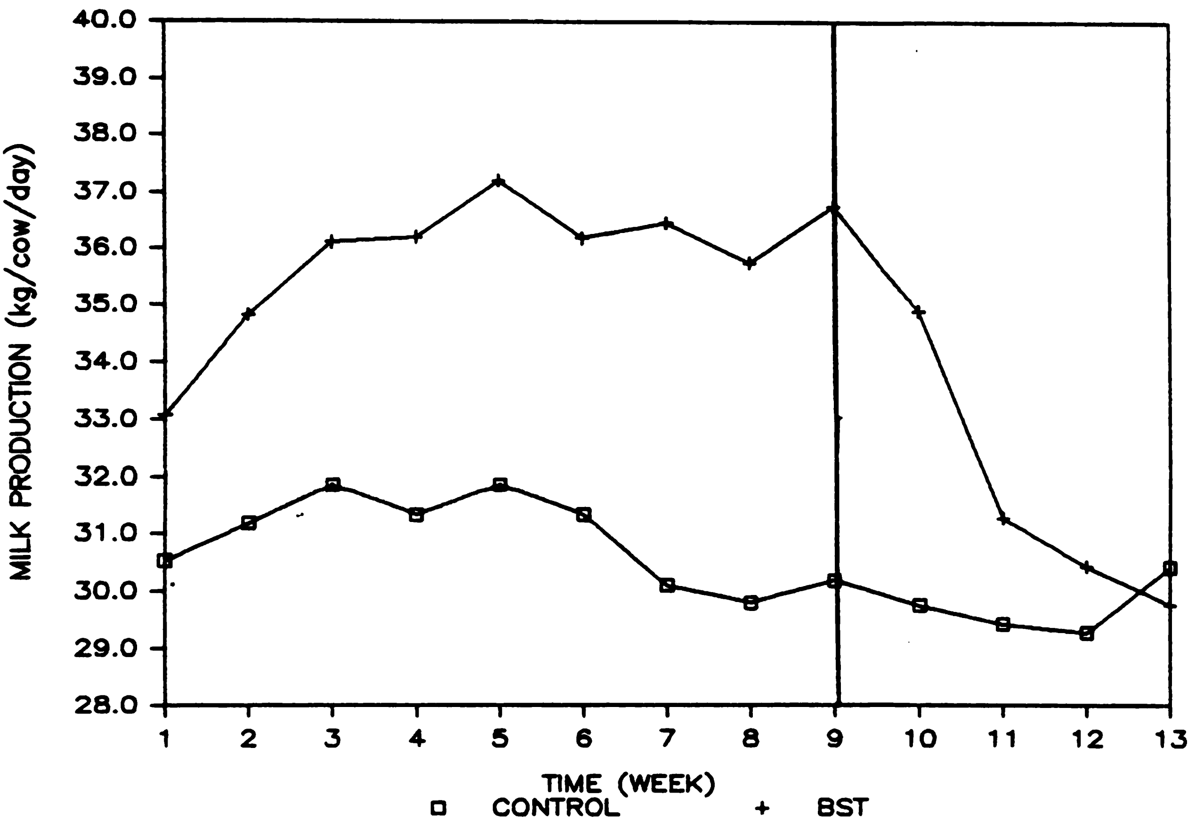

Figure 2. Effect of exogenous bovine somatotropin on milk production in lactating dairy cows. Treatment period consists of week 1 to week 9. Milk production data represent adjusted weekly averages.

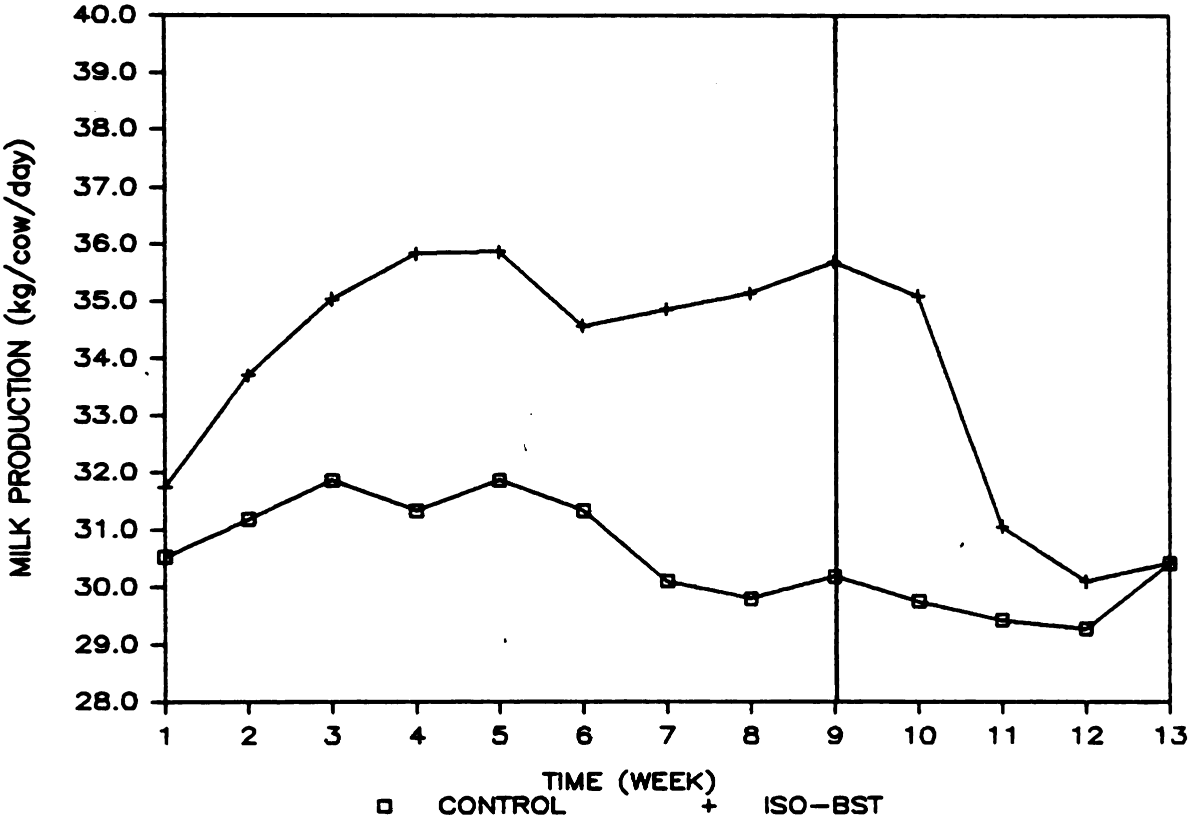

Figure 3. Effect of ISO-BST on milk production in lactating dairy cows. Treatment period consists of week 1 to week 9. Milk production data represent adjusted weekly averages.

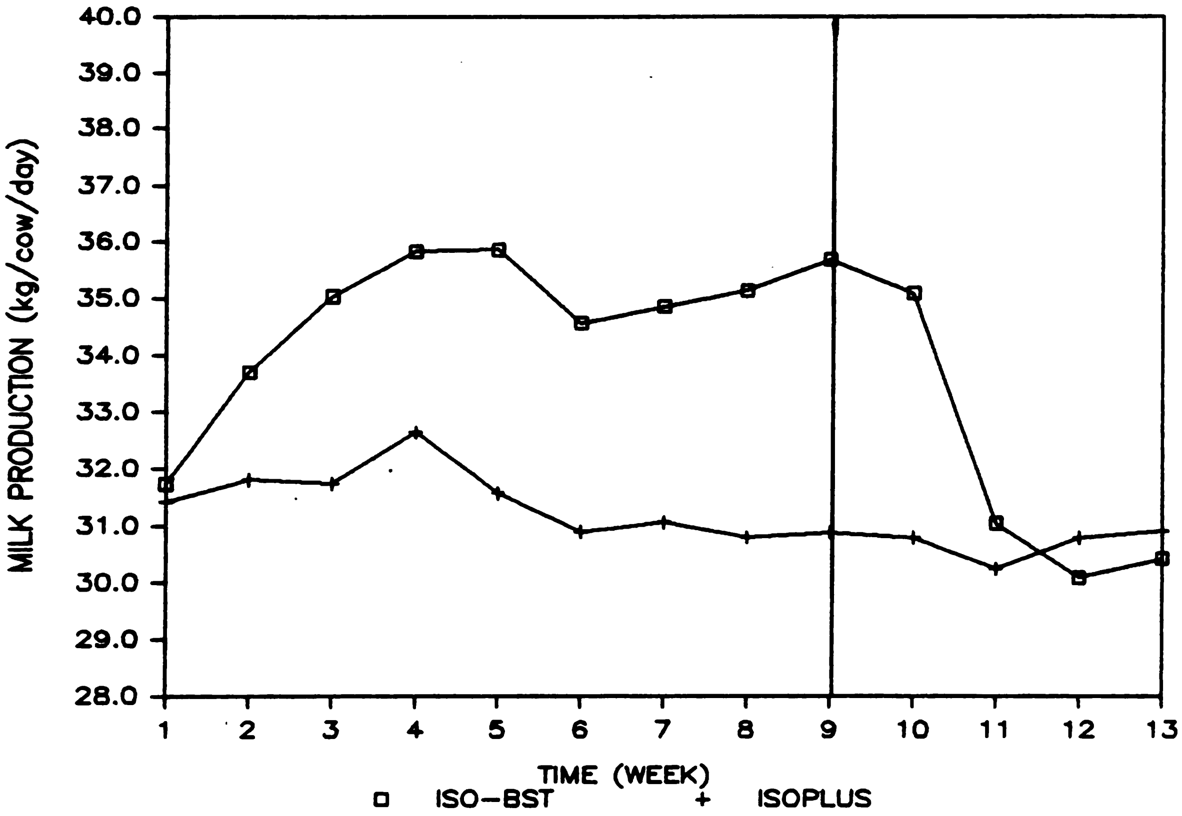

Figure 4. Effect of ISO-BST and Iso-Plus on milk production in lactating dairy cows. Treatment period consists of week 1 to week 9. Milk production data represent weekly averages.

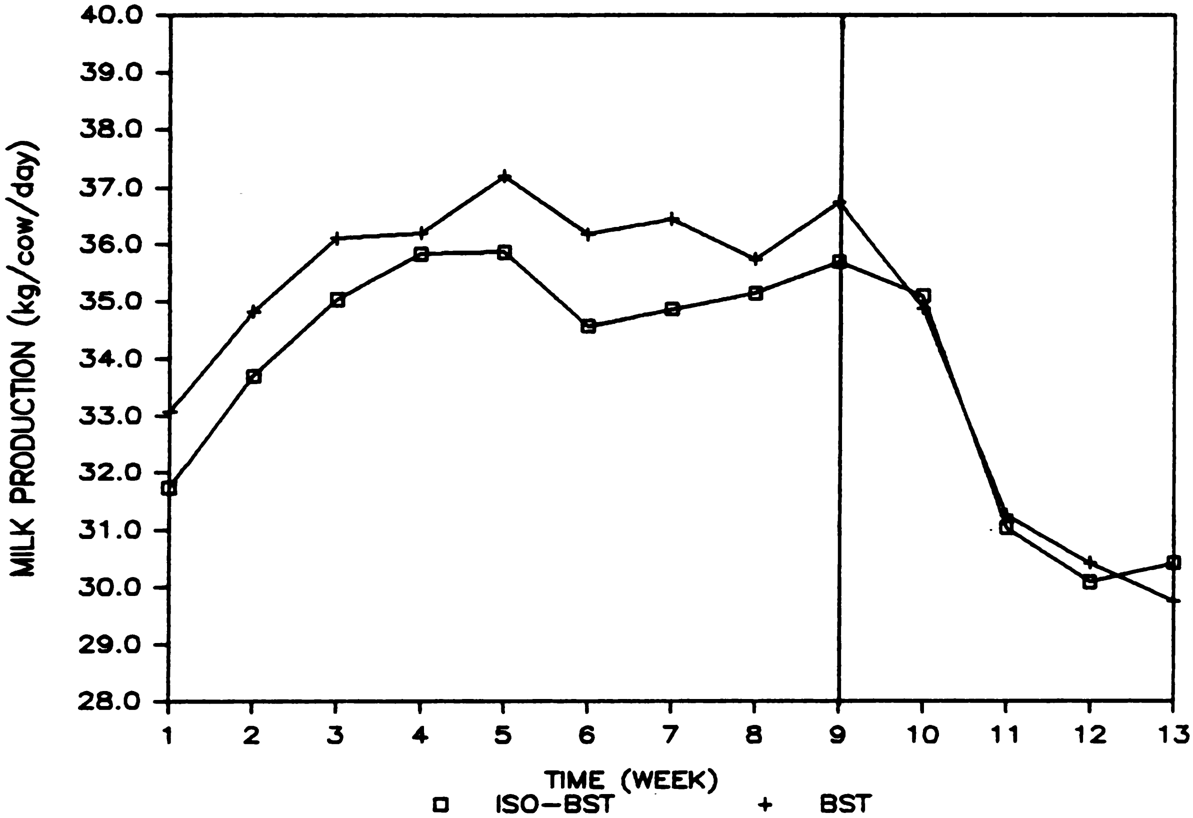

Figure 5. Effect of ISO-BST & BST on milk production in lactating dairy cows. Treatment period consists of week 1 to week 9. Milk production data represent adjusted weekly averages.

consistently lower than for the BST animals (Figure 5). When the treatments are withdrawn milk production declined at the rate until the middle of the 12th week (Figure 5).

Milk composition and somatic cell count was not affected by treatment (Table 3). Increases in the yield of milk protein (kg) and fat (kg) paralleled the increases in milk yield observed for BST and ISO-BST treatments.

The effect of treatment on dry matter intake is shown in Table 4. Dry matter intake was numerically higher for both the BST and ISO-BST animals. No difference was observed across treatments. Treatment effects on body weights are shown in Figure 6 and Table 5. Prior to treatment the animals were not balanced according to body weight. Each treatment group gained on the average 21 kg throughout the experimental period. Percent average change over the entire treatment period was not significantly different across treatments. Net energy balance per week during the pre-treatment and treatment period is shown in Figure 7. Overall means for the treatment period are in Table 6. All treatments were in negative energy balance until the 4th or 5th week treatment. Since animals were in early lactation, this was expected. Average net energy balance for the treatment period for the CON and ISO treated cows was positive. In contrast, the BST and ISO-BST treated animals were in negative energy balance. However, there was no significant difference in net energy balance means across treatments (Table 6). It appears that the increase

TABLE 3. Effect of Iso-Plus, BST and ISO-BST on milk composition.

VARIABLE (1)	CONTROL	ISOPLUS	BST	ISO-BST	SEM
Somatic Cell Counts (x10 5 cells/ml)	1.833	1.909	3.5	4.5	2.453
Milk protein (%)	3.413	3.377	3.429	3.338	0.0738
Milk protein (kg/cow/d)	1.092a	1.110a	1.286b	1.243ab	0.097
Milk fat (%)	3.433	3.386	3.508	3.513	0.1084
Milk fat (kg/cow/d)	1.082a	1.109a	1.253b	1.185ab	0.0873

1-Somatic cell counts are for one month during the treatment period due to loss of data during the month of February.
Milk protein and milk fat data are for the two months of the treatment period.
a,b-Means in the same row with different superscripts differ ($p<0.025$).

in milk production by BST treated cows during early lactation affected energy balance. Still the animals were able to replenish body stores as all animals gained weight (Figure 6).

Even though a stronger response in milk yield was not seen when 100 g of (Iso-Plus) was fed, a positive response was still present. By the 7th week of the trial ISO cows consistently produced more milk than the control cows. The significant responses in milk yield found in other reported studies were from cows fed 100 or 120 grams of the ammonium salt volatile fatty acids (AS-VFA) per day. In these studies the AS-VFA were also fed for much longer periods of time than compared to the length of feeding time in this trial (Papas et al., 1984 ; Pierce-Sandner et al., 1985 ; Rogers and Cook, 1986 ; Rogers et al., 1986). Fieo et al. (1984) fed 120 grams AS-VFA per cow per day for 6 weeks and saw no effect of treatment on milk production. In this present study an effect was not seen until after the 6th week. Researchers have also observed that the effect is higher when the AS-VFA or calcium salt volatile fatty acids (CA-VFA) are fed right after parturition (Rogers and Cook, 1986; Clark et al.,1986). The cows in this trial were in early lactation, but averaged 45 postpartum. The varied response reported by researchers may be dependent upon the salt form (calcium or ammonium) of the isoacids fed. Rogers et al. (1986) found a lower response when the calcium salts were fed less than two months.

After the ISO treatment was discontinued in this trial, there was a clear carry-over effect from treatment. Rogers et al. (1986) reported a minimal carry-over effect when 100 g of Iso-Plus was removed from the diet. In contrast, Clark et al. (1986) found no significant carry-over milk response after Iso-Plus supplementation was discontinued. In this study the ISO treated animals had a 3% higher milk yield than the control animals after the treatments had ceased. In addition, throughout the entire treatment period of 9 weeks, milk production for the BST treated cows was consistently higher over the ISO-BST treated cows. During treatment BST animals were 3% higher than ISO-BST animals. When treatments were withdrawn, the combination treatment (ISO-BST) showed a carry-over effect. By the 13th week the ISO-BST treated cows were producing 30.35 kg of milk and the BST cows were producing 29.67 kg of milk. Although there was no significant difference between the BST and ISO-BST treated animals , the BST treated cows were numerically higher. Even after the treatments were discontinued the same trend should have been maintained. Yet it appears as if the ISO-BST cows held on to their production level better than the BST treated cows. It is postulated that this response could be due to a carry-over effect from feeding Iso-Plus.

The mechanism of action for the increases in milk production seen when isoacids are fed is still not fully understood. Early work has shown that isoacids enhance growth of cellulolytic bacteria (Lassiter et al., 1958a, 1958b; Hemsley and Moir, 1963; Cline et al., 1966; Van Gylswyk, 1970). These acids are known to function as carbon skeleton for the biosynthesis of branched- chain amino acids (El-Shazly, 1952 ;Allison et al., 1962; Allison and Bryant, 1963; Bryant, 1973). The level of free amino acids in rumen fluid is quite low, this may be a factor in determining the amounts of VFA used in the biosynthesis of amino acids for increased protein synthesis leading to better animal performance. Many reports have concluded that isoacids improve nitrogen balance (Hemsley and Moir, 1963; Cline et al., 1966; Felix, 1976). Rumen ammonia has been found to be lower for animals fed isoacids suggesting higher utilization of ammonia converted into microbial protein. Isoacids have been reported to decrease urinary nitrogen loss and increase utilization of absorbed nitrogen (Hemsley and Moir, 1963 and Felix, 1976). Ammonia utilization and nitrogen balance was not specifically measured in this study. In those studies where performance was improved and utilization of ammonia was higher, the dietary protein was low and the protein requirement for animal performance was high. This type of situation is seen especially when urea or other non-protein nitrogen sources are used. When there is lack of dietary preformed protein ,a source of carbon skeletons, supplemental branched-chain acids are needed. Therefore, when there is supplementation of the isoacids an enhanced performance is readily seen. In this study soybean

TABLE 4. Effect of Iso-Plus, BST and ISO-BST on dry matter intake.(1)

TREATMENT	DM INTAKE (kg/cow/day)
CONTROL	17.789
ISOPLUS	17.794
BST	18.434
ISO-BST	18.121

1-Cows were pen fed. DM intake is average per treatment for entire treatment period.

TABLE 5. Body weight and body weight change of cows as affected by ISO, BST and ISO-BST (1).

VARIABLE	CON	ISO	BST	ISO-BST	SEM
Body weight					
Prior to treatment, (kg)	554	531	542	569	25
Average during treatment, (kg)	575	548	566	590	25
Change during treatment, % (2)	4.6	3.6	4.9	3.9	0.7809

1-Overall (unadjusted) treatment means are presented.
2-% change is the average change during the treatment period.

meal was the primary source of protein fed to the animals. Soybean meal is a higher quality protien. Any effects of the isoacids enhancing the utilization od nitrogen would be less readily seen.

Reports have also indicated an improvement in feed efficiency (Papas et al., 1984 ; Pierce-Sandner et al., 1985 ; Deetz et al., 1985). In this study there was no difference in feed intake. However, when efficiency (kg of milk/ kg of DM intake) was calculated the ISO cows did have a numerical feed utilization higher than the control cows (1.8 (ISO) vs. 1.7 (CON)). Felix (1976) reported that isoacid addition to an urea- corn silage diet improved animal body weight gain over the control animals which were fed an urea-corn silage diet with no addition of isoacids. There was no significant difference in % of body weight change between the CON and ISO treated cows in this trial (Table 5). Looking at net energy balance (Table 6) the CON animals were in a more positive energy balance than the ISO animals. This may suggest that the ISO treated animals were using more energy (Mcal/day) for better production. This may also support a better performance in feed efficiency since there was no significant difference in dry matter intake. The increases in animal performance seen by other researchers seem to reflect the interaction of rumen microbes and isoacids. This interaction improves characteristics such as feed intake, cellulose digestion, nitrogen retention, microbial growth, microbial synthesis of

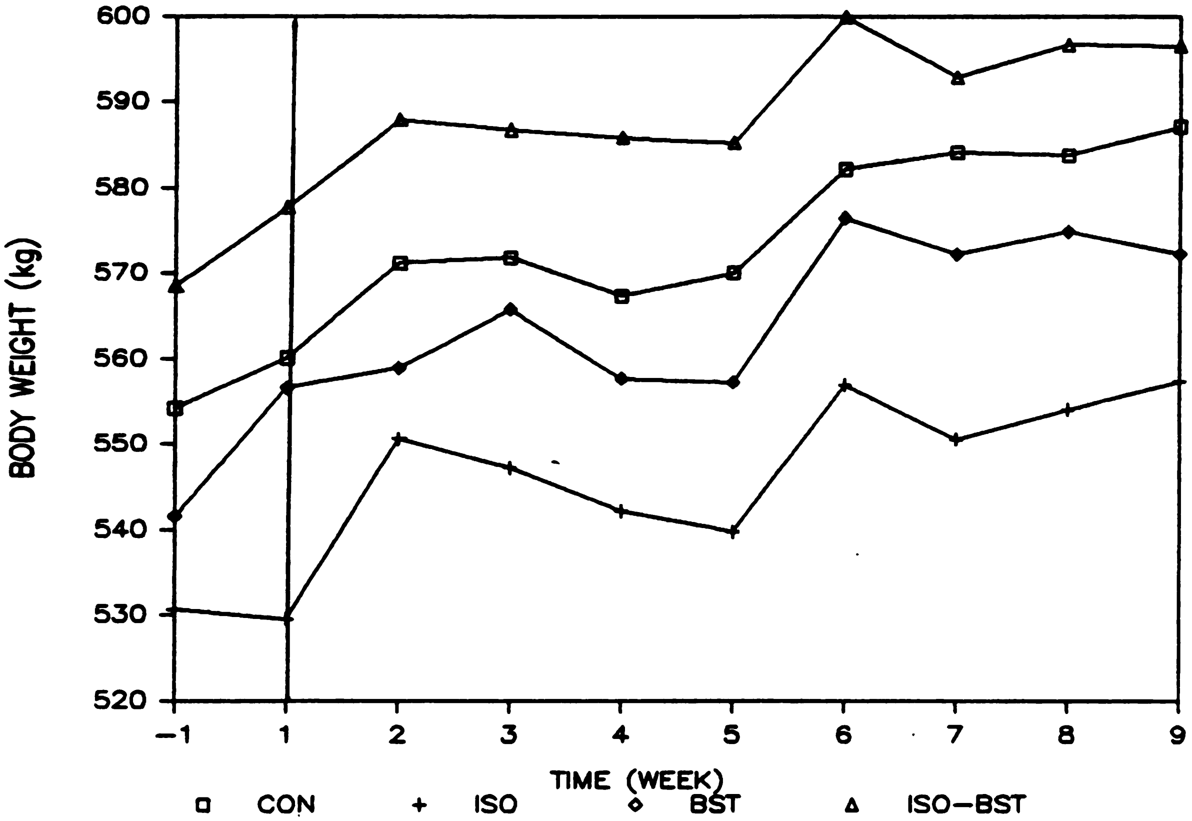

Figure 6. Effect of ISO, BST, & ISO-BST on body weight in lactating dairy cows. Treatment commenced at week 1 and continued for 9 weeks. Body weights represent (unadjusted) weekly averages.

protein and milk yield. When the cellulolytic activity is increased there is an increase in dietary energy produced from the feed stuffs.

The carry-over effect found in this experiment in both groups fed isoacids (ISO and ISO-BST) has not been reported. As previously reported, isoacids stimulate growth of cellulolytic bacteria. When the exogenous supplementation is discontinued the increased numbers of microorganisms may still be present. If dietary protein is available to meet the needs for adequate deamination of amino acids, sources of isoacids are still available to rumen microorganism. Recycling of bacterial protein has been a source of some C4 and C5 acids (Annison, 1954; Miura et al., 1980). If sources are still available then the effects of the isoacids on production performance can still be attained. It has been reported that changes in the rumen environment or microbial population and type may influence the rate at which NH3-N is taken up by microbes (Smith, 1979). Miura et al. (1980) has shown that sufficient isoacids for growth of Ruminococcus albus were produced through sequential growth of other organisms. When Bacteroides ampylophilus was lysed amino acids released were subsequently deaminated by Megashpaera elsdenii, to isoacids. Further examination into the carry-over effect of feeding isoacids to the ruminant is needed before a conclusive statement can be made.

Effects of BST and ISO-BST on lactational performance were dramatically seen. This study clearly demonstrates that short-term administration of bovine somatotropin causes remarkable increases in milk production when dairy cows are in early lactation. Significant differences from control were not seen until the second week of treatment (Table 2). However, BST treated cows gave 8% more milk during the first week of treatment. Bullis et al. (1965) and Machlin (1973) have shown that the response to daily injections of BST may take at least up to seven days before a significant response is seen. A study conducted by Hart et al. (1985) also did not find a significant response in milk production until the second week of treatment. The cattle in that experiment were given 30ug/kg of body weight and were 239 ± 114 days into lactation. When BST was given in combination with Iso-Plus there was no statistically significant difference seen until the fifth week of treatment when compared with the ISO animals. The BST and ISO-BST treatments were not significantly different as were the CON and ISO treatments. Failure to find significant differences earlier between the ISO-BST and ISO treatments could be due to animal or environmental variation. During early lactation, changes in milk yield is very rapid. Therefore, it is difficult to detect significant differences, which was the situation when ISO-BST treated animals was compared against the ISO treated animals.

The action of bovine somatotropin is still under intensive investigation. Further research in understanding the mechanism of action during lactation and regulation of metabolism is still needed. Many researches have classified BST as a nutrient partitioning agent (Bauman and Currie 1980; Bauman et al., 1982; Collier et al., 1984; Gluckman et al., 1987). Production responses of dairy cows to exogenous BST during early lactation have been few. Results have also varied considerably. When cows are treated beyond 60 days postpartum results are more consistent (Collier et al., 1984; Bauman and McCutcheon, 1985). Early work done by Fawns et al. (1945) reported no response to exogenous bovine somatotropin pituitary extracts during the first 7 weeks of lactation. A few years later Brumby (1956) did find increases in milk production when first-lactation heifers in early lactation were treated with BST. These animals were administered exogenous bovine somatotropin 2 weeks prior to and after freshening. Bines and Hart (1982) reported no response of BST when administered 35 days postpartum. More recent studies conducted by Peel et al. (1983) administered 51.5 IU/day of BST commencing 12 weeks postpartum and found a 15% increase in milk yield. Richard et al. (1985) reported a 6% increase in milk production when 50 IU of BST was administered 20 days postpartum, and a 12% increase when treatment was administered 60 days postpartum. The responses seen by both Peel et al. (1983) and Richard et al. (1985) are similar to those found in this study when

TABLE 6. Net energy balance as affected by Iso-Plus, BST and ISO-BST (1).

TREATMENT	NET ENERGY BALANCE (Mcal/cow/d) (2)
CONTROL	1.0174
ISOPLUS	0.3314
BST	-1.3976
ISO-BST	-1.2629
SEM	1.3639

1-Treatment period was 9 weeks commencing 45$\pm$22 days post partum. Data are not adjusted for pretreatment response.
2-NEB (Mcal/cow/d) is the average net energy balance for the entire treatment period.

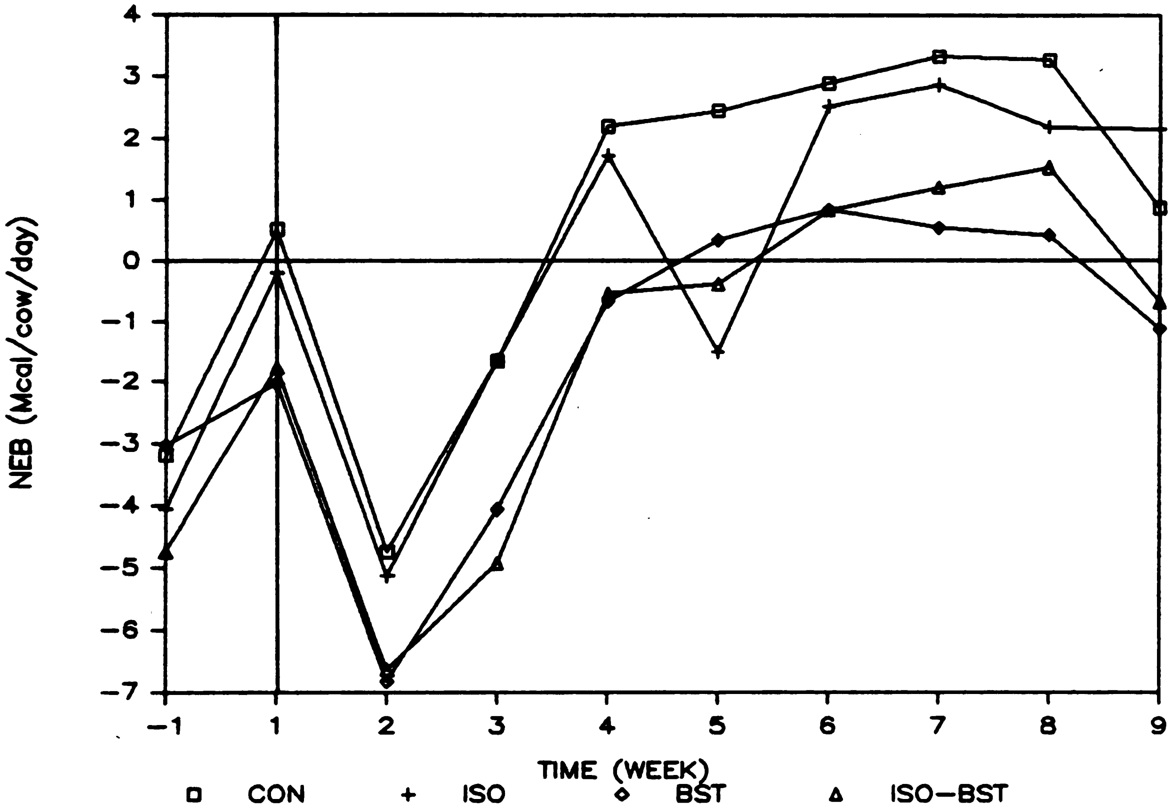

Figure 7. Effect of ISO, BST, & ISO-BST on net energy balance (Mcal/cow/day). Treatments commenced at week 1 and continued for 9 weeks. Net energy balance represents (unadjusted) weekly averages.

BST is given alone and in combination with Iso-Plus.

Since high producing animals are in negative energy balance during early lactation, absorbed nutrients are channeled towards the animal's most important physiological function. This function or state during early lactation is the function of the mammary gland. When nutrient intake is not sufficient for full tissue utilization, body reserves are mobilized to fulfill the requirement. As Mcal/day of intake is less than Mcal/day of requirement the animal is in a state of negative energy balance. Such was the situation in this study (Figure 7) for the first 4 weeks of the trial. The higher producers were in more negative energy balance than the lower producers. Endogenous BST's primary role is to preserve body protein, particularly during periods of energy deficit. BST accomplishes this by diverting glucose and fatty acids away from tissue deposition while inhibiting proteolysis and stimulating protein incorporation into muscle (Hart, 1983; McDowell, 1983; Bauman and McCutcheon, 1986). Endogenous BST's intrinsic lipolytic ability allows it to act as a mobilizer of body fat. The mobilized body fat acts as source of energy. BST acts to divert food energy away from tissue synthesis (Bines and Hart, 1982). When BST is given exogenously these properties are enhanced. In this particular study feed consumption did not change enough to meet the increasing need for more nutrients. There was no significant difference in dry matter intake across all treatments (Table 4), although both BST treated groups did have numerically higher intakes. There did seem to be a slight improvement in feed efficiency (lb milk/lb dry matter, 1.9 BST & ISO-BST vs. 1.7 CON). This is a common feature in short term studies where BST is exogenously administered. It has been postulated that production increases could be due to an improvement in the digestibility of the feed, alterations in the bioenergetic efficiencies of maintenance or milk synthesis, or partitioning of nutrients away from body tissues to the mammary gland for milk synthesis (Peel and Bauman, 1987). Both Peel et al. (1981) and Tyrell et al. (1982) reported no changes in digestibility or the partial efficiency with which energy was used for maintenance or milk synthesis during somatotropin administration. The work presented here supports the partitioning of nutrients away from body tissues to the mammary gland. The BST and ISO-BST treated cows were in negative energy balance for most of the experimental period. The cows were able to gain weight (Table 5 and Figure 6), thus, replenishing their body stores. Still the BST and ISO-BST animals did not replenish as fast when compared to the control animals. Due to the increasing demand of the mammary gland to function to its fullest capacity the BST treatments did not replenish body stores as fast (Figure 7).

Milk composition was not affected in by BST or ISO-BST treatments (Table 3). Other short term studies reported similar results (Peel et al., 1982, 1983; Fronk et al., 1983; Eppard et al., 1985). In these studies cattle were in positive energy and nitrogen balance. In contrast, in studies where cows were in negative energy and protein balance the percent milk fat increases and the percent milk protein decreases (Peel et al., 1981, 1983 ; Tyrell et al., 1982; Eppard et al., 1985; Richard et al., 1985). Even though the BST treated cows were in negative energy balance for 4 weeks of the treatment period, milk composition was unaltered. It appears that the cows were able to increase the mobilization of body reserves during the first part of the experimental period when cows were in negative energy balance. The animals were able to replenish their body stores during the rest of the treatment period with no change in milk composition. Perhaps if the treated cows had substained a longer period of negative energy balance alterations in milk composition would have appeared.

For any of the treatments, no adverse effects on health were noted. Slightly higher somatic cell counts were found in the BST and ISO-BST treatments (Table 3). However, the higher means were due to one animal in each treatment exhibiting a high count for that month. Effects of treatment on services per conception were observed (Table 7). Even though the BST treatment had four cows open at six months postpartum, it is difficult to say whether it was due to treatment. Two of the four cows were previous problem breeders. Eppard et al. (1987) reported that cows receiving

TABLE 7. Effect of Iso-Plus, BST and ISO-BST on services per conception.

TREATMENT	SERVICES/CONCEPTION	COWS OPEN AT 6 MONTHS POSTPARTUM
CONTROL	1.73	1
ISOPLUS	1.70	1
BST	2.25	4 (2 problem breeders pre-trial)
ISO-BST	2.00	2 (1 problem breeder pre-trial)

somatotropin (13.5, 27, & 40.5 mg/cow/d rBST and 27 mg/cow/d pBST) had conception rates (services per conception) and days open comparable to control animals. In contrast cows treated with 50 mg/day had less established pregnancies, higher embryo loss and longer time between parturition and established pregnancy (Thomas et al., 1987). Burton et al. (1987) found more days open for BST treated cows (12.5, 25, & 50 mg/cow/d). While Chalupa et al. (1987) cited fewer cows to become pregnant while on BST treatment (12.5, 25, & 50 mg/cow/d). Other health problems were not apparent to these researches. As the possible commercial use of somatotropin in the dairy industry continues to be pursued examination of subtle health and reproduction effects will require larger numbers of animals.

B. Effect of Iso-Plus, bovine somatotropin and a combination of the two on plasma urea nitrogen, glucose, insulin and somatotropin.

When plasma urea nitrogen was measured both at 30 and at 60 days of treatment, no significant difference was found in daily mean values across treatments (Tables 8 & 9). Figures 8 & 9 depict the concentration throughout the sampling period of each sampling day. All treatments tend to follow the same pattern throughout the day. On day 30 of the sampling period the ISO and BST animals showed significantly higher BUN levels post-feeding (Table 10). While, during the 60 day sampling period the CON, ISO and BST animals had lower post-feeding BUN levels (Table 11). Mean plasma glucose levels were also similar across treatments and across time (Table 8 & 9). When graphically shown, again all treatments follow a similar pattern on both 30 and 60 days of treatment (Figures 10 & 11). During the 30th day of treatment there was a response to feeding as all treatments showed a trend for higher plasma glucose levels after feeding (sample #6, Figure 10). Only the ISO and BST animals showed significantly ($p<0.05$) higher levels of plasma glucose post-feeding. However, no response to

TABLE 8. Effect of Iso-Plus, BST and ISO-BST on daily means of plasma glucose, urea nitrogen, somatotropin, and insulin at day 30 of sampling.

VARIABLE	CONTROL	ISOPLUS	BST	ISO-BST	SEM
Glucose (mg/dl)	68.41	69.32	69.87	71.24	3.396
BUN (mg/dl)	15.33	14.85	14.32	15.27	1.635
ST (ng/ml)	6.04a	5.59a	15.82b	15.72b	2.225
Insulin (uIU/ml)	9.03	9.59	9.43	10.84	2.586

a,b-Means in the same row with different superscripts differ ($p<0.001$).

TABLE 9. Effect of Iso-Plus, BST and ISO-BST on daily means of plasma glucose, urea nitrogen, somatotropin, and insulin at day 60 of sampling.

VARIABLE	CONTROL	ISOPLUS	BST	ISO-BST	SEM
Glucose (mg/dl)	68.19	70.77	71.25	72.36	3.396
BUN (mg/dl)	15.91	14.78	15.48	16.25	1.639
ST (ng/ml)	4.61a	3.54a	10.75b	9.61b	2.225
Insulin (uIU/ml)	13.72	13.41	15.78	15.99	2.586

a,b-Means in the same row with different superscripts differ ($p<0.0025$).

feeding was found during the 60th day of sampling (Table 11). Pre-feeding and post-feeding means were fairly similar on day 60 for all treatments. There was no effect of treatment on mean plasma insulin on day 30 or 60 (Table 8 & 9). Both sampling days showed a response to feeding. Plasma insulin levels rose after feeding (Figures 12 & 13, sample #6 represents feeding time). Pre-feeding means were numerically less than post-feeding on both 30 and 60 days of treatment (Table 10 & 11). On day 30 only the ISO and ISO-BST animals had significantly higher plasma insulin. Yet, at 60 days all treatments showed a significant response to feeding ($p<0.0001$). The effect of treatment on plasma ST levels is seen in Tables 8 & 9 and Figures 14 & 15. Mean plasma levels were significantly different at 30 days ($p<0.001$) for those animals administered exogenous BST (Table 8). No difference was found in those animals supplemented with Iso-Plus. On day 60 of treatment mean plasma levels were significantly higher ($p<0.0025$) for those animals on the BST treatments (Table 9). Again animals fed Iso-Plus had similar plasma levels to the controls. Highest peaks of plasma ST can be seen in the earliest part of the sampling period. This would correspond to the administration of BST at 0900 hours (Figures 14 & 15). During the 30th day of treatment, all treatments showed a response to feeding (Figure 14). Plasma levels tended to decrease after animals were fed but were only significant for BST treated animals.

TABLE 10. Pre and post feeding mean plasma levels of glucose, urea nitrogen, somatotropin, and insulin at day 30 of sampling within each treatment as affected by Iso-Plus, BST and ISO-BST.

	VARIABLE							
	GLUCOSE(mg/dl)		BUN(mg/dl)		ST(ng/ml)		INSULIN(uIU/ml)	
TRT	PRE	POST	PRE	POST	PRE	POST	PRE	POST
CONTROL	65.6	70.67	13.9	15.1	6.35	5.77	7.72	9.77
ISOPLUS	63.8	72.2a	12.98	15.1c	6.91	4.93	6.81	10.7b
BST	64.7	72.4a	12.8	14.5a	20.3	13.9a	7.77	9.99
ISO-BST	67.5	72.8	14.5	14.5	21.0	13.5b	7.31	12.7d
SEM	2.947		0.597		2.327		1.117	

Pre and post refer to pre-feeding and post-feeding. Means between each feeding time within each treatment differ if represented by a, $p<0.05$; b, $p<0.025$; c, $p<0.01$; d, $p<0.001$.

The increases in production performance found both in the feeding of isoacids and administering of BST has led to postulations of post-absorptive use of nutrients. While the isoacids have been shown to improve the utilization of urea nitrogen, no direct evidence for BST's effect on N recycling exists. Felix et al. (1980b) showed beneficial effects on nitrogen utilization in dairy heifers consuming a low energy diet and in lactating dairy cows when isoacids were added to the diet. Plasma urea nitrogen levels were decreased when isoacids were fed to lactating cows (Felix et al., 1980a). Hence an improvement was seen in utilization of urea nitrogen and, consequently, more microbial synthesis of protein. As it has been previously stated, the enhancement of microbial synthesis of protein is a factor in the improvement in production performance found when isoacids are fed to ruminants. An increase in plasma urea nitrogen levels when 100g of isoacids were fed was found by Towns and Cook (1984). The source of protein in this trial consisted of a high quality protein. This is in contrast to the diet fed by Felix et al. (1980a) which included NPN. To account for these differences of plasma urea nitrogen, speculation has led to the feeding of different sources of protein. If NPN was included in the ration, there was a decrease in plasma urea due to the trapping of recycled nitrogen by the microorganisms in the rumen. If an increase in plasma urea was found, usually a high quality protein, such as soybean

TABLE 11. Pre and post feeding mean plasma levels of glucose, urea nitrogen, somatotropin, and insulin at day 60 of sampling within each treatment as affected by Iso-Plus, BST and ISO-BST.

	VARIABLE							
	GLUCOSE(mg/dl)		BUN(mg/dl)		ST(ng/ml)		INSULIN(uIU/ml)	
TRT	PRE	POST	PRE	POST	PRE	POST	PRE	POST
CONTROL	68.0	68.2	17.1	15.2d	4.41	4.96	10.8	15.1e
ISOPLUS	71.8	70.8	15.5	14.3c	4.28	4.17	10.4	14.8e
BST	71.1	71.2	16.7	14.9d	14.15	7.78a	12.01	17.6e
ISO-BST	73.6	72.2	16.2	15.7	16.7	7.33b	11.2	17.8e
SEM	1.061		0.352		2.820		0.923	

Pre and post refer to pre-feeding and post-feeding. Means between each feeding time within each treatment differ if represented by a, $p<0.10$; b, $p<0.01$; c, $p<0.005$; d, $p<0.001$; e, $p<0.00001$.

meal was fed . The enhancement for N utilization is less clear and plasma urea levels would be mainly affected by body protein turnover (Towns and Cook, 1987).

Bines et al., (1980) found non-significant decreases in plasma urea when 30mg of BST was administered to lactating Friesian and Hereford-cross cows. It was suggested that this was reflection of increased mammary protein synthesis and resultant decrease in urinary N excretion (29% decrease in the Friesians ($p<0.1$) and a 39% in the Herefords ($p<0.05$). Both isoacids (Cline et al., 1966; Umunna et al., 1975; Oltjen et al., 1971) and BST (Bines et al., 1980; Bauman and McCutcheon, 1986) stimulate N retention. There was no significant response found in plasma urea levels in this experiment when either ISO or BST was given. The differences found in the pre and post- feeding BUN levels at 30 days could be due to the source of supply of the increased post-feeding levels of glucose. For example, if the supply came from amino acids this would raise the BUN levels (Bines et al., 1980). On day 30 of sampling, BUN levles increased after feeding. The opposite was found at the 60 day sampling. Post-feeding means were significantly lower for three of the four treatments. This could be a reflection of increased mammary protein synthesis for the increased milk production. Bines et al. (1980) found a non-significant decrease in plasma urea and a reduction in urinary nitrogen excretion when cows were administered BST.

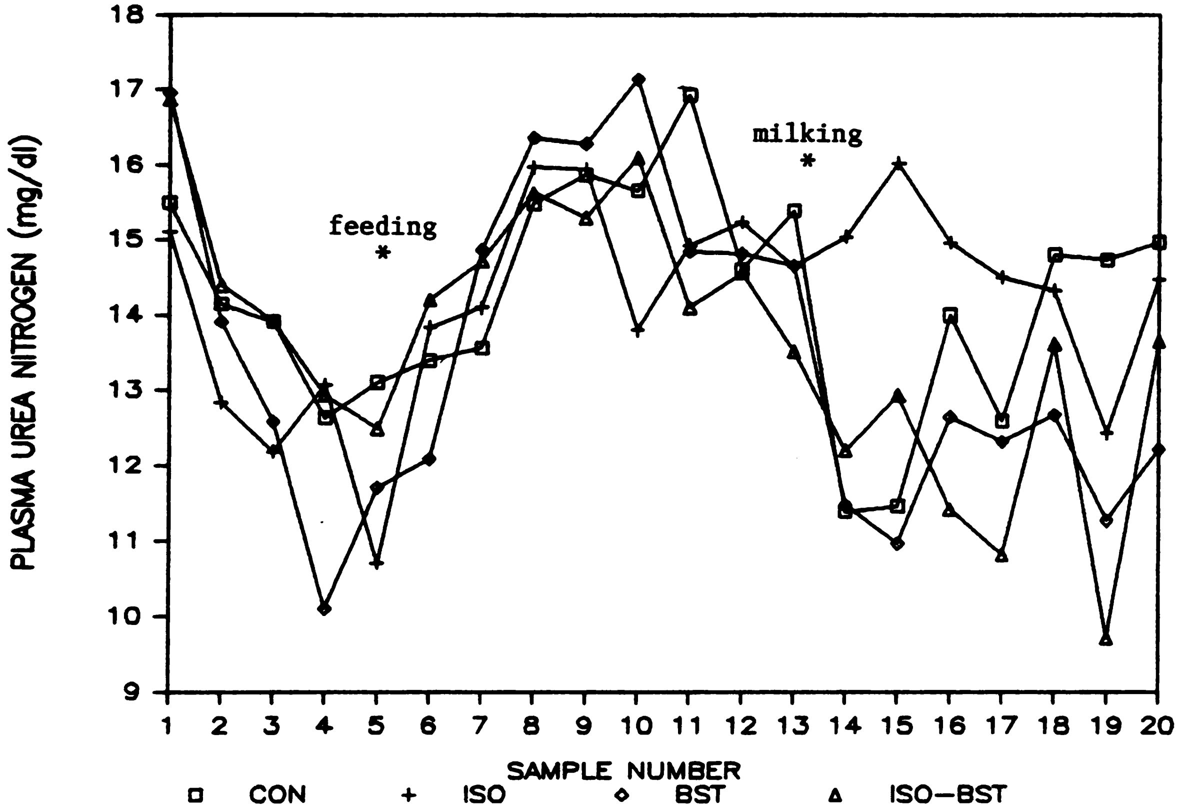

Figure 8. Effect of ISO, BST, & ISO-BST on plasma concentrations of urea nitrogen in lactating dairy cows at 30 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

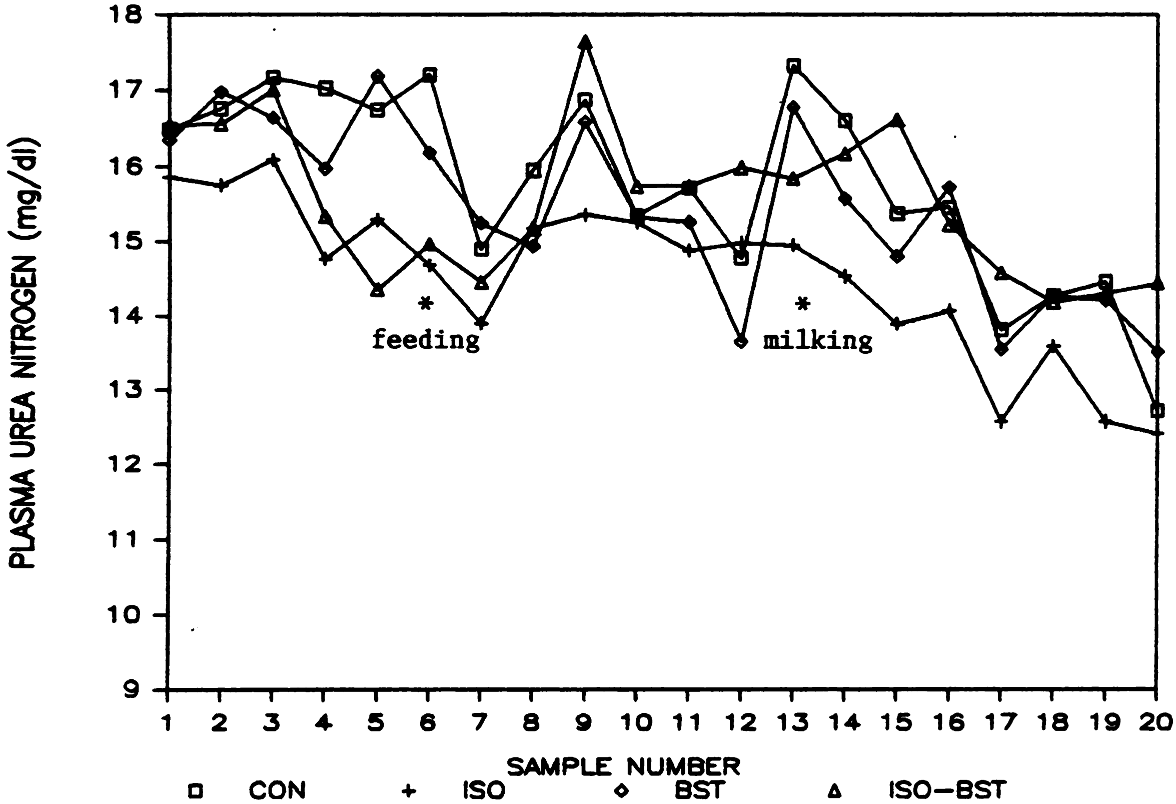

Figure 9. Effect of ISO, BST, & ISO-BST on plasma concentrations of urea nitrogen in lactating dairy cows at 60 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

The rate of milk secretion is primarily determined by glucose uptake by the bovine mammary gland (Bines and Hart, 1982). The mammary gland requires glucose for the synthesis of lactose, the glycerol moiety of milk lipids and for provision of reducing equivalents (NADPH) for lipogenesis. Little glucose is stored in the ruminant animal. The availability of glucose to the mammary gland is primarily set by gluconeogenesis from propionate, amino acids, lactate and glycerol from the liver. Bennink et al. (1972) reported that glucose entry rates increased during early lactation while intake was held constant. During BST administration, metabolic adaptations in glucose turnover and oxidation had been reported to meet the additional glucose need (Peel and Bauman, 1987). It appears that the reduction in glucose oxidation during BST treatment provides about 30% of the additional glucose required for lactose synthesis. Irreversible loss of glucose increased by 270 g/day. No significant differences were reported in this study in the BST or ISO-BST treated animals in plasma glucose levels (Tables 8 & 9). This is in agreement with others (Peel et al., 1981, 1982, 1983; Eppard et al., 1985). This does not suggest that there was no effect on glucose utilization. It is possible that the circulating concentrations do not reflect adequately the amount of glucose available for metabolic purposes. Other variables which help to evaluate the status of carbohydrate metabolism were not measured such as glucose irreversible loss rate

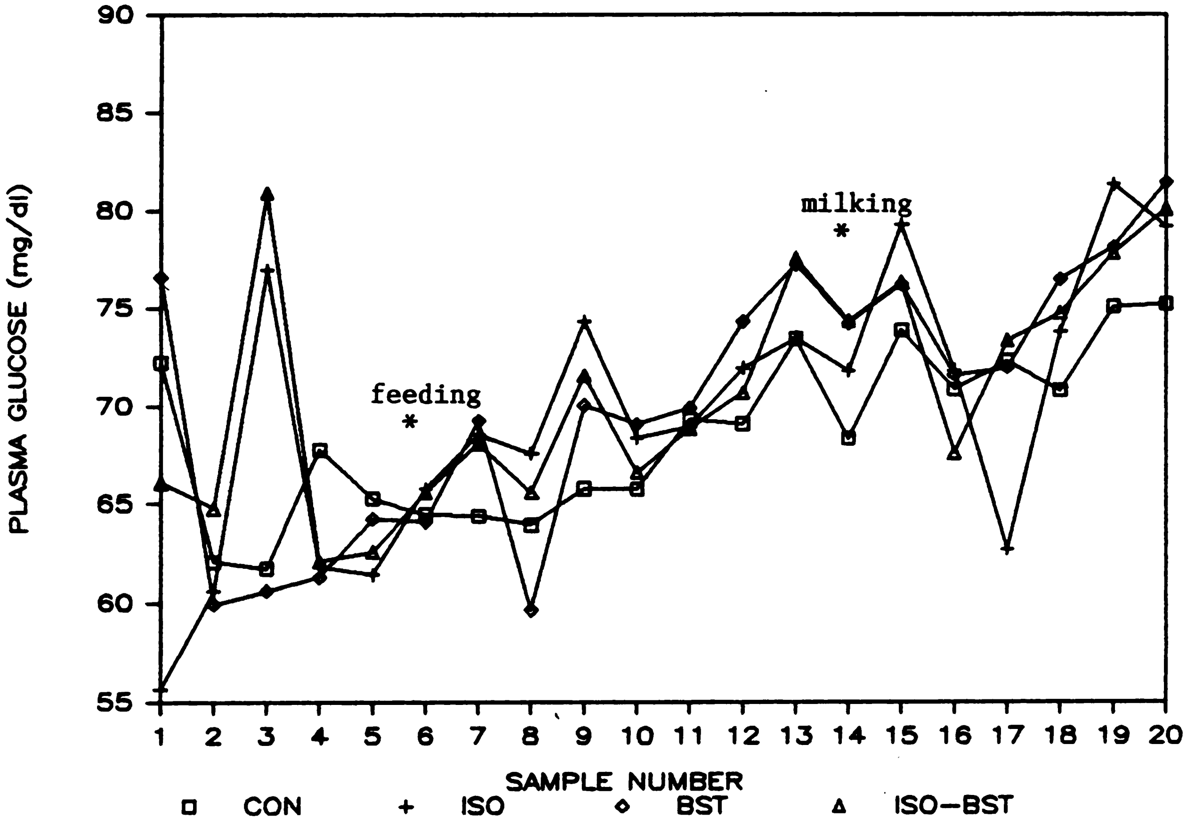

Figure 10. Effect of ISO, BST, & ISO-BST on plasma concentrations of glucose in lactating dairy cows at 30 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

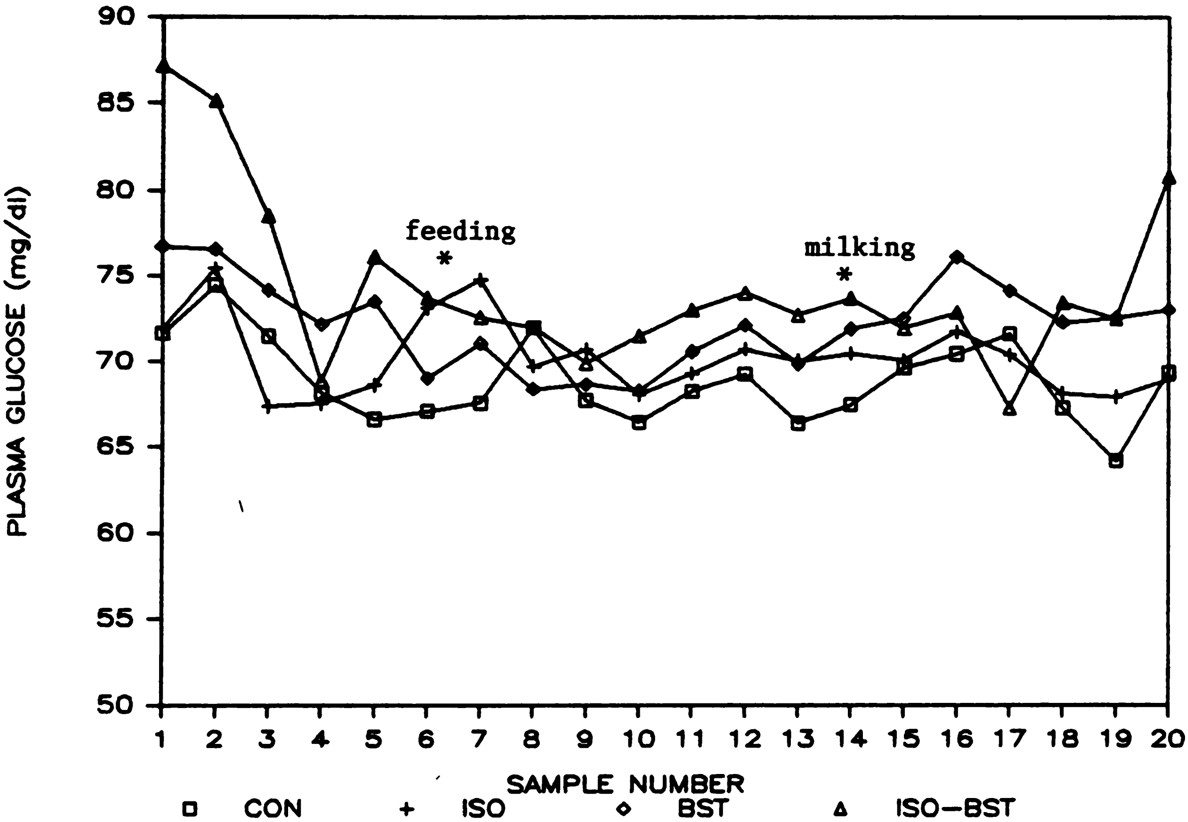

Figure 11. Effect of ISO, BST, & ISO-BST on plasma concentrations of glucose in lactating dairy cows at 60 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

glucose and oxidized to CO_2. Glucose production was most likely increased in the animals administered BST. Precursors for the increase in glucose production are not readily apparent especially since the animals did not differ in feed intake (Table 4). It may be that increased rates of gluconeogenesis from propionate can account for the increase in glucose. Pocius and Herbein (1986) found an increased capacity for glucose production from propionate in liver biopsies from cows treated with BST. Amino acids also could contribute to the glucose supply. Most likely this involves the process of gluconeogenesis from body reserves of protein (Collier et al., 1984). A final source includes glycerol produced by the hydrolysis of adipose triglycerides. It has been reported that up to 27% of the additional glucose supply was received from glycerol (Peel and Bauman, 1987).

In cattle fed isoacids lower glucose levels have been reported (Towns and Cook, 1984). However, Brondani (1986) reported no difference in plasma glucose levels in sheep fed isoacids. The results in this experiment are in agreement with Brondani (1986). Since there was a small response in milk production in the cattle fed isoacids a lowering of glucose levels was not expected.

Although not all treatments showed a glucose response to feeding at 30 days, the trend was still present. Most likely this could be contributed to wide animal variation. It appears that the utilization of glucose by the dairy animals at 60 days was not as great as it was at 30 days. There were no significant differences between pre and post-feeding levels for all treatments. This may reflect a change in metabolic demand of the animal as lactation progresses.

The hormone insulin has a primary role in carbohydrate and protein metabolism. Insulin appears to be a major factor in understanding nutrient utilization as a means of manipulating tissue growth to optimize the type and quality of product produced. Insulin's actions involve the inhibition of lipolysis, gluconeogenesis, glucose release from the liver and proteolysis. It also acts by stimulating lipogenesis, the uptake and utilization of glucose by many peripheral tissues and the uptake and incorporation of amino acids into protein.

The effects of either ISO or BST in this study on plasma insulin levels appear to be inconclusive. There were no differences in plasma levels detected across treatments at either 30 or 60 days of sampling (Tables 8 & 9). Brondani (1986) reported lower levels of plasma insulin when sheep were fed 0.2 g of isoacids/kg bw/day. It was proposed that the lower insulin levels were due to a decrease in propionate production in the rumen. End products of rumen fermentation include the VFA acetate, propionate and butyrate. Propionate is a major metabolite thought to increase insulin secretion (Horino et al., 1968; Bines and Hart, 1984; Emmanuel and Kennelly, 1984; Istasse and

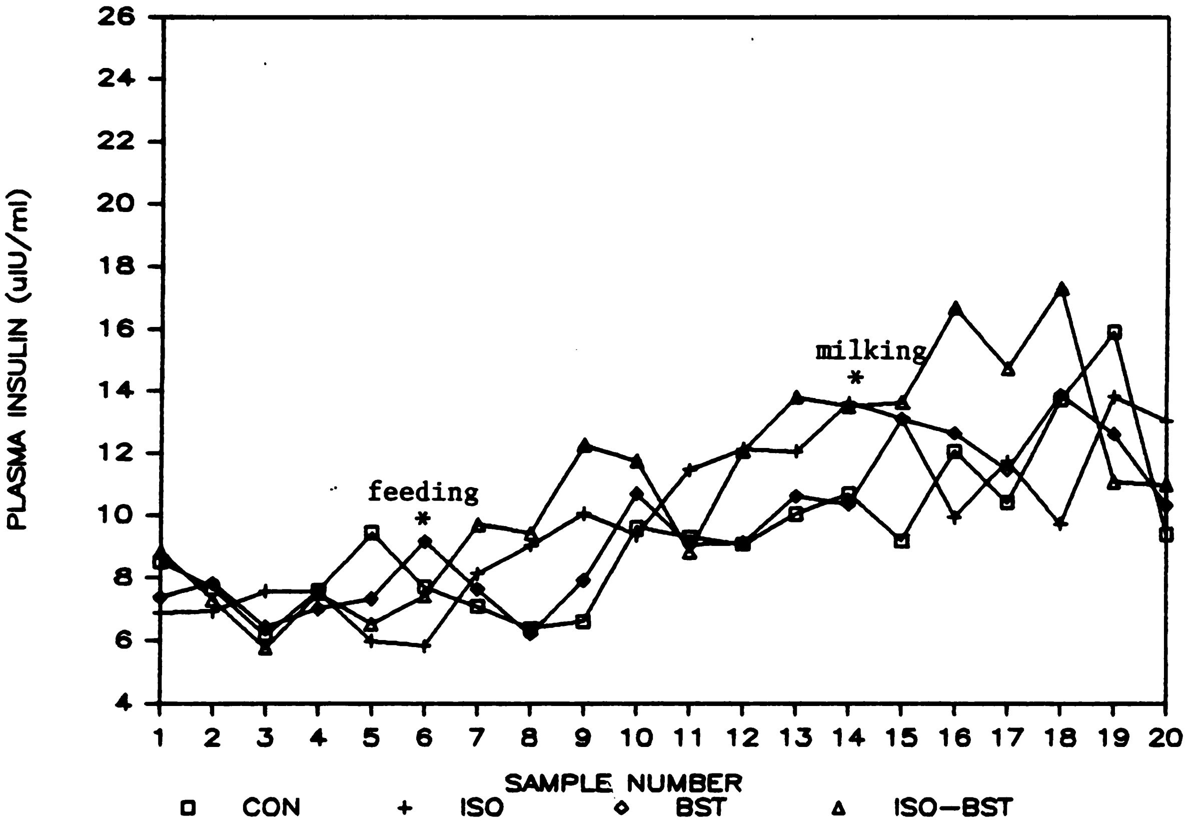

Figure 12. Effect of ISO, BST, & ISO-BST on plasma concentrations of insulin in lactating dairy cows at 30 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

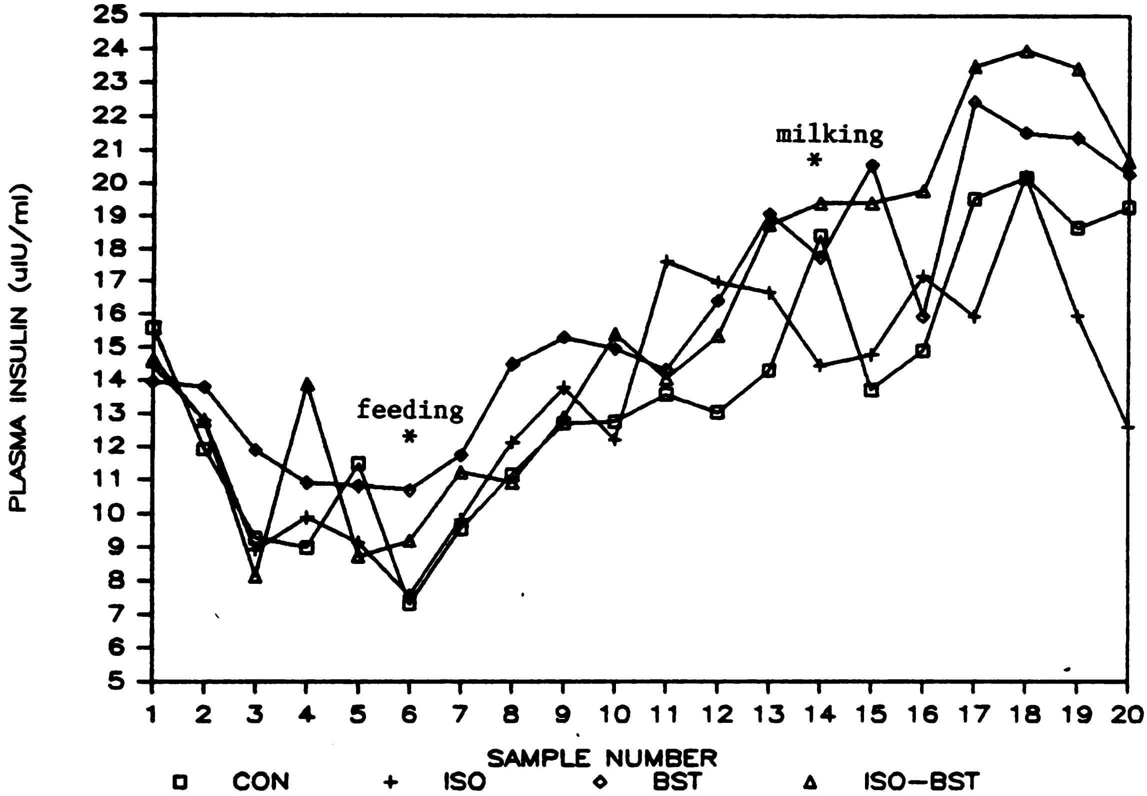

Figure 13. Effect of ISO, BST, & ISO-BST on plasma concentrations of insulin in lactating dairy cows at 60 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

Orskov, 1984). Other results suggesting little or no increases in propionate levels are in contrast to the lower propionate production reported by Brondani (1986) . Felix (1976) fed isoacids to lactating dairy cows and found a slight increase in propionate production. When isoacids were fed to calves there was no difference in propionate production (Miron et al., 1968). While differences in results have been reported, investigators have consistently found increases in acetate production when isoacids are given (Felix, 1976; Quispe, 1982; Brondani, 1986). Ruminants utilize acetate instead of glucose as a major substrate for energy storage and oxidation in the fed state. Therefore, these animals are almost totally dependent on gluconeogenic pathways for provision of glucose in the fed and fasting state. The exact sources of glucose availability have been previously discussed. In ruminants the hormonal responses induced by nutrient intake should favor acetate oxidation or incorporation into fat. At the same time glucose is synthesized at high rates to preserve metabolic homeostasis. However, in the ruminant, insulin plays a small role in the removal of large glucose loads. Still a possible action of insulin in ruminant metabolism may exist when isoacids are fed. Towns and Cook (1987) have proposed feeding isoacids may produce a non-insulin-stimulating energy. Since propionate appears to be the only VFA to stimulate insulin secretion and there tends to be no effect of isoacids on propionate production the stimulus for insulin secretion is reduced. The reduction in secretion would be most favorable as investigators have associated high levels of insulin with depressed levels of milk yield (Kronfeld et al., 1963; Schmidt, 1966; Lomax et al., 1979). Furthermore,isoacids increase acetate production resulting in the improvement of dietary energy release. Improvement in dietary energy release demonstrates a significant importance especially during early lactation. During this time period the animal usually does not take in the amount needed for full performance.

Just as there was no response on insulin level in the isoacid fed animal, no effect was detected when BST was administered. This is in agreement with other studies conducted during early lactation (Peel et al., 1981, 1983 and Eppard et al., 1985).

In regard to plasma ST levels of ISO animals, no effect of treatment was seen at 30 or 60 days (Tables 8 & 9). In fact, plasma profiles of the ISO animals were practically identical to that of the controls (Figures 14 & 15) . These results are in contrast to what has been previously reported by Towns and Cook (1984) and Fieo et al. (1984). The response found by Fieo was even without a response in milk production. Both of these studies found higher plasma ST levels when dairy cows were fed isoacids. However, the results reported in this study are in agreement with what was reported by Brondani (1986). In those experiments no

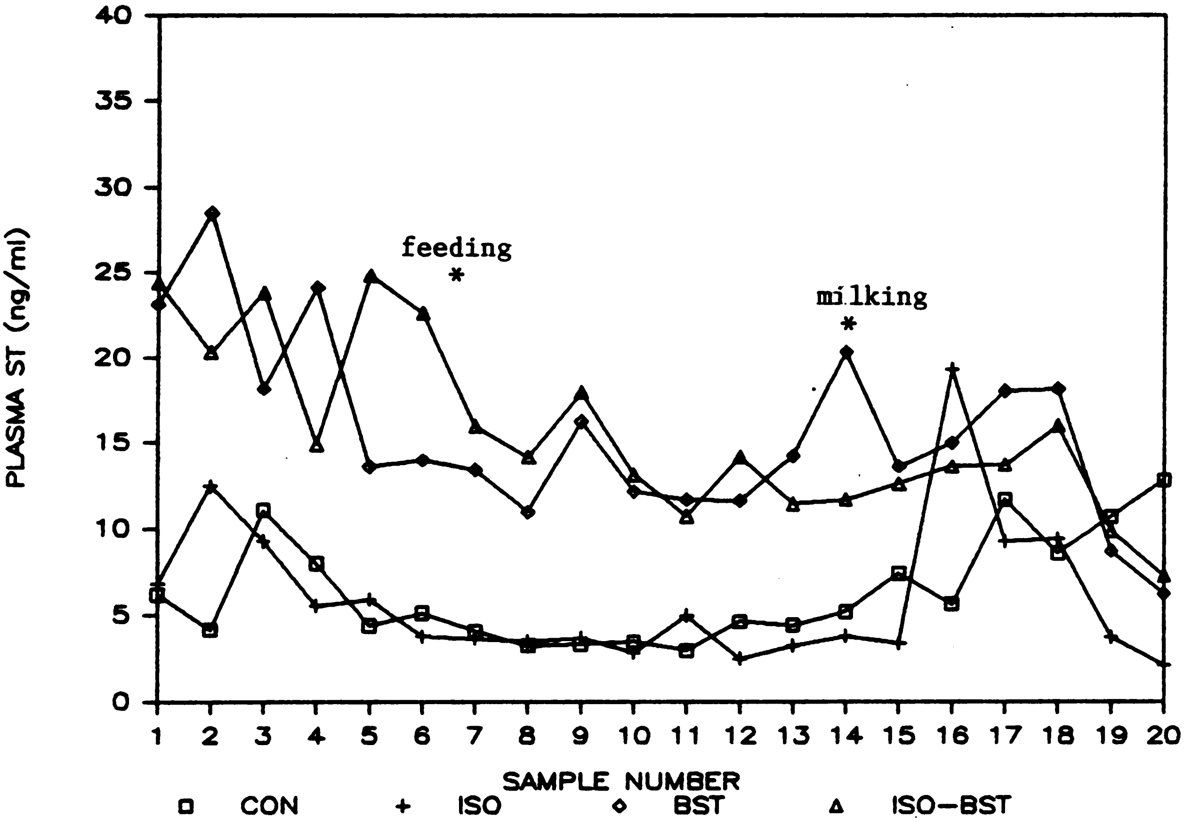

Figure 14. Effect of ISO, BST, & ISO-BST on plasma concentrations of somatotropin in lactating dairy cows at 30 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

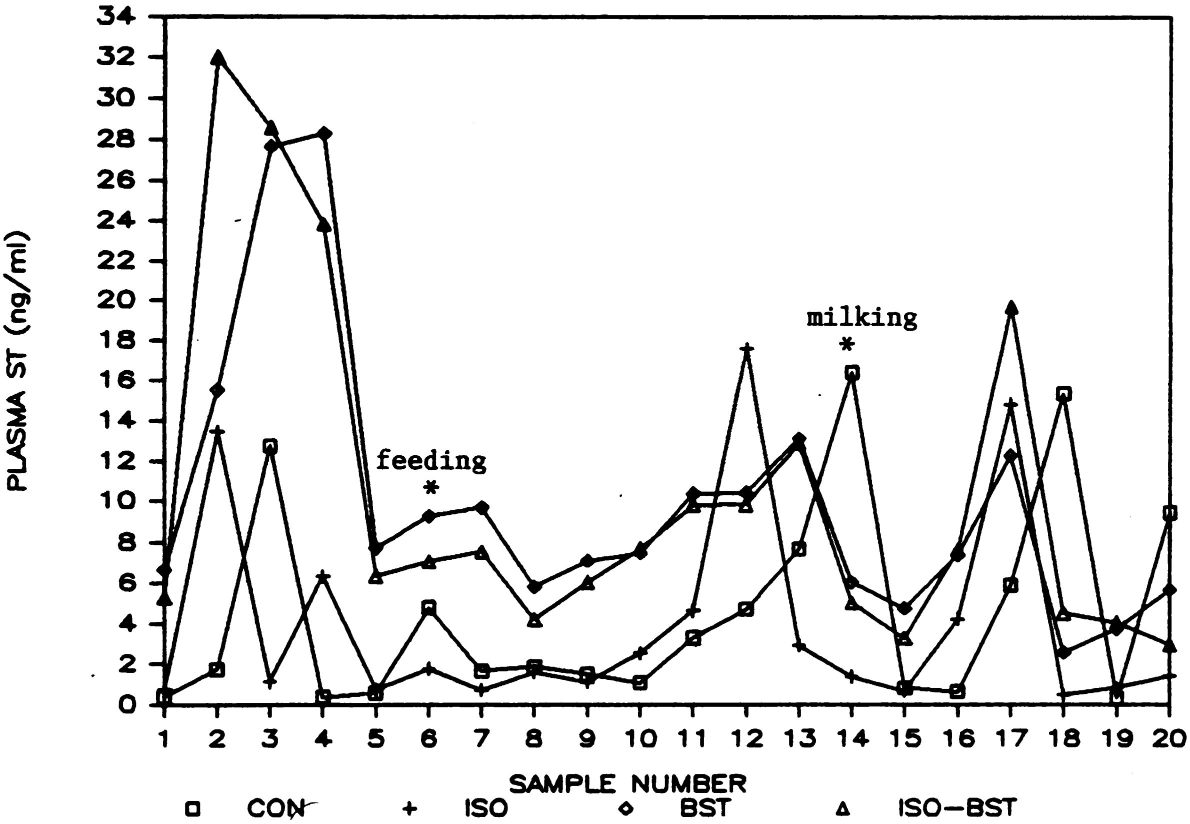

Figure 15. Effect of ISO, BST, & ISO-BST on plasma concentrations of somatotropin in lactating dairy cows at 60 days of treatment. Blood samples were taken every 20 minutes between 1020 hours and 1840 hours.

differences in plasma ST were found in sheep or dairy cows fed isoacids. From this particular study it appears that Iso-Plus does not affect plasma ST levels. The differences reported form other studies can not be fully explained.

Plasma levels of ST in the BST and ISO-BST treated animals were elevated at both sampling periods (Tables 8 & 9), showing highest response post injection. Post-feeding values were significantly lower than pre-feeding values (Table 10 & 11). Plasma profiles for these two treatments were very similar (Figures 14 & 15). The question of how this increase in ST acts within the animal to enhance production performance is continually pursued. The physiological mechanism involved in the stimulus of milk production may include a combination of events. One mechanism involves the nutrient partitioning theory. The exogenous hormone somehow acts directly or indirectly on other tissues such that nutrients are preferentially channeled to the mammary gland. Hart et al. (1980a) found a positive correlation between changes in milk yield and changes in the ratio of plasma ST to insulin. The high plasma ST relative to insulin may result in fat mobilization. This appears to be especially expressed in early lactation as the BST treated cows lost weight and took longer to rebuild their body stores when compared to the controls. When voluntary food intake increases, allowing the animal to maintain energy homeostasis, the daily energy intake matches daily energy output. Eventually the rate of live-weight loss is first stabilized and then reversed (Peel et al., 1985). Mobilization can then increase the availability of energy-precursors for milk production. Positive correlation between changes in ratios of plasma ST to glucose have also been cited (Hart et al., 1980a). Since the ruminant so highly depends on gluconeogenesis, low levels of insulin would be very compatible for high yields of milk production. Insulin inhibits gluconeogenesis. When exogenous somatotropin is presented to the animal and there is an increase in the plasma ST:INS ratio, exogenous BST may be acting by reducing the effects of insulin. Herbein et al. (1985) reported an increase in ST:INS by increasing plasma ST with no change in plasma insulin. The effect may be at the pancreas. The data presented by Lomax et. al. (1979) suggest that during early lactation, when insulin concentrations are decreased, there appears to be a decrease in pancreatic output of insulin rather than any increase in hepatic uptake. The increased ST levels from the exogenous administration may be acting similarly. The data presented support the role of BST as a nutrient partitioning agent most likely acting on the ST:INS ratio to physiologically benefit the animal for higher production performance. Still, many questions remain unresolved, requiring further research.

CONCLUSIONS

The results from this study indicate a positive response from feeding Iso-Plus to increase milk production in early lactation. However, the effect was minimal. Although there was no significant difference between the control and treated animals by the sixth week of treatment the ISO animals did begin to exhibit a positive trend of higher milk production. It is proposed that if the animals were fed longer a greater response would have been detected. There may be an additional benefit to feeding isoacids. That is, a carryover effect may exist. When treatments were withdrawn the ISO animals continued to have higher levels of milk production than the controls. This is in contrast to what is seen when BST is withdrawn. Milk production for the BST treated animals dramatically declined when treatment was withdrawn. In addition the ISO-BST animals consistently had higher milk production levels than the BST animals during the withdrawal period but not during the treatment period.

When 25 mg of BST was administered milk production was increased dramatically. A similar response was seen with the ISO-BST treatment. There was no significant difference between the BST and ISO-BST treatments. Yet the ISO-BST animals were numerically lower in production levels when compared against the BST animals.

None of the treatments had any significant effects on milk composition, feed intake, body weight changes or net energy balance. Both BST treatments did show a trend for higher dry matter intake. If the trial would have continued for a longer period of time most likely a significant difference would have been detected. This would be in agreement with what has been reported in the literature. No adverse effects on health were reported in this study.

Effects of treatments on plasma hormones and metabolites showed no significant differences for plasma insulin, urea nitrogen and glucose. BST and ISO-BST treated animals had significantly higher plasma somatotropin levels. This was to be expected.

Finally there was no additive effect when Iso-Plus and BST were given together.

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