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Effects of Cannulation, BST Administration and Protein Degradability on Rumen and Duodenal Characteristics and Milk Production Response in Holstein Dairy Cows

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EFFECTS OF CANNULATION, BST ADMINISTRATION AND PROTEIN DEGRADABILITY ON RUMEN AND DUODENAL CHARACTERISTICS AND MILK PRODUCTION RESPONSE IN HOLSTEIN DAIRY COWS

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

BOVINE SOMATOTROPIN IN LACTATING DAIRY COWS

Bovine somatotropin (bST) is a protein synthesized at the base of the brain and released by the pituitary gland into the circulatory system. BST is transported by the circulatory system and absorbed only by cells of target organs that possess cell surface receptors for the protein (11, 41). Its effect is initiated via a protein receptor initiation and cyclic AMP cascade. This effect on the cell continues well past the degradation of the bST molecule. BST is likely transported into the cell, where it is degraded. Its constituent blocks, amino acids, are used to synthesize new proteins or converted to other metabolites such as sugars (1).

The administration of bovine somatotropin to lactating animals has been known to increase milk production since the 1920s (6). During World War II, efforts to utilize bovine somatotropin to increase milk production and to minimize feed consumption failed because of difficulty in producing sufficient quantities of bST from pituitaries of slaughtered cattle.

In the 1970s, the ability to synthesize bST in microbial organisms via recombinant DNA technology was developed (43). This led to mass production of bST by many drug companies.

Multiple research experiments on dairy cattle were conducted to determine if increased milk production and feed efficiency were possible.

The first long-term experiment on injecting somatotropin was done by Bauman et al. at Cornell University (9). In the trial, 3 groups of high-producing cows were injected daily beginning 84 days after calving and continuing for 188 days. The animals received 3 doses of somatotropin (13.5, 27 and 40.5 milligrams/day (mg/d)). Cows receiving supplemental somatotropin at tested doses showed an increase in milk production (23-41%) and in feed efficiency (5-15%). No differences in incidence of mastitis, milk fever or ketosis were observed. Conception rates and services per conception during the lactation period or next lactation were not different from the controls (28, 39, 48). Research by Chalupa et al. (15, 16, 17, 18) showed that 24 cows injected daily with 12.5, 25 or 50 mg bST/day for the last 266 days of lactation produced 0.57 lb. more milk (24% increase) per pound of feed than control cows.

Baird et al. (7) found that 28 cows had average increases of 17% in milk production with increased dry matter intakes of 6.4%. There were no significant differences in milk composition, somatic cell count, health or body weight between treatment and control cows.

A long-term trial by Bauman et al. (9) saw an increase in milk production of 16%. Milk constituents did not change

over time. Feed intake increased by week 10 in the long-term study. However, in a short-term study by Peel et al. (50), feed intake decreased.

Peel et al. (47) and Bauman et al. (9) examined the effects of short- and long-term treatment with bST on high-yielding dairy cows. Doses ranged from 0-50 international units (IU) bST/day of subcutaneous and intramuscular injections. BST administered to high-yielding cows during early lactation resulted in increased milk production, milk fat percentage and lactose yield, indicating that bST mediates a major shift in the partitioning of absorbed nutrients towards milk synthesis. The dramatic increase in lactose, the major osmotic regulator of milk volume, and milk fat cannot be due merely to an alteration in nutrient supply to the mammary gland. Long-term bST injections also resulted in increased milk production. Richard et al. (51) also studied early lactational responses in dairy cattle but did not get such dramatic responses as Peel et al. (49). One explanation for this is that the response may be limited by nutrient availability during early lactation because cows are in negative energy balance.

Peel et al. (48) examined the effects of bST on early, mid- and late-lactation performances of dairy cows. During early and mid-lactation, high-yielding dairy cows divert dietary energy and mobilize body tissue to meet the high metabolic demands of lactation. Administration of bST to dairy cows has

consistently increased milk production in both short- (10, 47, 48) and long-duration studies (39, 49). Daily administration of bST in early and late lactation results in similar increases in milk yield and efficiency of milk production (33).

Annexstad et al. (5) measured circulating metabolites, hormones and physiological parameters in 28 cows. They injected 12.5, 25 or 50 mg bST/day from 5 weeks to 301 days postpartum. There was a 24% increase in 3.5% fat-corrected milk and an 11% increase in dry matter intake of treatment over control animals.

Somatotropin does not affect blood constituents, body temperatures or respiration (58, 59). There is an increase in insulin and free fatty acids with increased somatotropin dosage that appears to be related to increased milk production and feed intake.

Body composition in cows differs with treatment. In a study by Soderholm et al. (58), body fat decreased but body weights did not differ. It was felt that control cows diverted extra energy to body fat, and the treatment animals diverted the extra energy to milk production. Bauman et al. (10) suggested that increases in milk yield are caused by overall nutrient repartitioning and by enhanced capability of the mammary gland to synthesize milk.

Soderholm et al. (58) measured effects of varying doses of bST on lactational performance, body composition, blood

constituents, circulating metabolites, hormones and physiological parameters in 28 lactating cows. They injected 10.3, 20.6 and 41.2 mg bST/day from 35 days postpartum to day 266 of lactation. There was an increase of 12-25% in 3.5% fat-corrected milk (FCM) and a 4-10% increase in dry matter intake in treatment over control cows during the trial. Treated cows were 11-17% more efficient in converting feed to milk. Body weight changes were not significantly different among treatments. Estimated weights of body protein and minerals, most blood constituents, respiration rates and body temperatures were not affected by the dose of bST.

Other dose response experiments were conducted by Eppard et al. (27) to determine the effect of bST on lactation as well as on milk composition. The lactation trial was conducted on four Holstein cows by three injection methods: 1) pulse iv injection of bST at 4-h intervals, 2) single daily injection of bST and 3) continuous infusion of bST. There appeared to be no effect of pattern on bST administration which indicates flexibility in the method of administration to dairy cows. Milk production was increased with all three treatments, with a 10% decrease in milk protein percentage. Dry matter intake declined with all treatments and feed efficiency increased (5, 9, 12, 18, 58).

The effect of bST dose on milk composition (26) was determined from 6 cows receiving 0, 5, 10, 25, 50 and 100 IU/day. The concentration of alpha-lactalbumin in milk

increased linearly across the range of treatments. Short-chain and medium-chain length fatty acid concentrations were increased through the 50 IU bST/day dose, and long-chain fatty acids increased at all doses.

Fronk et al. (31) and McCutcheon and Bauman (40) studied the effects of pattern on bST administration. Both used various subcutaneous, pulse and continuous injections, with bST doses ranging from 0 to 51.5 IU/day. Results were similar in that dry matter intake was increased, and milk production increased as did feed efficiency. Both studies concluded that the amount of bST administration is the concern not the pattern.

Staples and Head (59) and Zoa-Mboe et al. (66) evaluated the effects of somatotropin and environment, specifically heat stress on milk yield, feed intake, select hormones in plasma and physiological functions. Ten Holstein cows, 196 d in lactation, received daily injections of either 0 or 50 IU somatotropin. Treatment cows under heat stress produced more milk (9.3%) with similar feed intake. The increased production was accompanied by increased respiration rates (6%), increased serum nonesterified fatty acids (150%) and insulin-like growth factor (222%).

Zoa-Mboe et al. (66) conducted a project to determine the effects of 20.6 mg somatotropin injected daily and environment (shade and no shade) on milk yield, feed intake, rectal temperatures, respiration rates and concentrations of hormones

in plasma. Milk yield and feed intake was less for animals in no shade. Heat stress had the largest effect on dry matter intake, decreased by 16% in no shade animals. Fat-corrected milk (FCM, 3.5%) and component yields were increased by bST, but response of the cows was not different in the two environments, except that treatment cows had a slight increase in body temperature and respiration rate.

Collier et al. (21) also determined environmental effects on milk production and components. They found daily injections of 25 mg somatotropin increased milk yield, and milk fat percentage when adjusted for feed intake. Milk yield was greatest for (bST) treated cows in both shade and no shade (5.7%). Feed intake was not different, but milk production was higher for animals housed in the shaded area. The increase in milk production is much lower than other experiments (28, 31, 48, 51) but was probably due to increased body temperature and decreased feed intake, which would cause a decrease in the effectiveness of somatotropin.

As can be seen, bST has been approached from all aspects of lactation and dose effect. A different approach to the effects of bST may be in relation to various feed additives and nutrient effects. Lough et al. (38) determined the effects of blends of dietary fats (5%) and bST (50 IU/d) on the performance of lactating cows. The dietary fat ration increased milk production and 4% fat-corrected milk (FCM) with bST injections. Dry matter intake decreased with the

addition of dietary fat.

DeBoer and Kennelly (23, 24) studied the affects of bST (33 mg/d) and dietary protein (11 vs. 16%) concentration on milk yield. Milk yields were increased with the higher protein concentration.

This project examines the possibility that these varying nutrient concentrations, in conjunction with bST injections, can affect ruminal and duodenal fermentation as well as other parameters.

EFFECTS OF DEGRADABLE AND UNDEGRADABLE PROTEIN SUPPLEMENTS

The large cost of protein supplements, as well as the increased nutritional stress placed on high-producing animals, has stimulated reevaluation of protein requirements for lactating dairy cows (46). New systems to express protein requirements have recently been developed that have placed emphasis not only on protein content of the diet but also rumen degradability (54, 56). The nitrogen requirement for rumen bacteria (ruminal degradable protein) and for amino acids that reach animals' small intestine (ruminal undegradable protein) are separate. The amount of nitrogen required for the host is higher than the amount supplied by ruminal microflora (14, 34, 45, 56). Therefore, interest has been stimulated to meet the animals' protein requirements while minimizing protein wastage.

Undegradable protein is protein that escapes (or

bypasses) digestion in the rumen. This protein is then digested in the small intestine of the animal and absorbed as amino acids used for production. Such protein comes from three sources: undegradable protein, microbial and endogenous protein. Endogenous protein, present at the small intestine but not in large amounts, cannot be easily separated from other proteins in the small intestine, so determination of this fraction of the contributed protein is not taken into consideration.

When microbial protein is inadequate, additional protein can only be supplied to the animal with undegradable protein. Most proteins are undegradable to some extent (19, 36), but some are more undegradable than others. Corn gluten meal (CGM) and meat and bone meal (MBM) are 55% and 60% undegradable, respectively. Soybean meal (SBM) is 28% undegradable (19), making SBM an excellent source of degradable protein (as ammonia) for microbial utilization not directly available to the host. Degradability of proteins is directly correlated to the amino-acid profile presented to the small intestine (57). The most desirable protein balance would feature combinations of slowly degraded protein sources that would reach the small intestine partially undegraded. Roth et al. (55) fed a combination of blood meal and corn gluten meal that improved performance in steers. This may be due to the improved amino-acid pattern that reached the small intestine through a complimenting effect. Methionine and

lysine, both important in milk production, have been shown to be limiting amino acids in ruminant rations (52). CGM is low in lysine and high in sulfur amino acids (44) while MBM is a good source of lysine (3). Together with sufficient energy, these two protein sources represent complimentary feeds.

Factors improving the amino acid profile presented to the small intestine have been examined. Processing and chemical treatment have been used to alter proteins by decreasing their degradability in the rumen. Annexstad et al. (4) compared effects of three combinations of extruded whole soybeans and CGM or SBM on milk production. The milk yield of cows consuming 75% of their supplemental protein from CGM was similar to all other treatments.

Ahrar and Schingoethe (2) and Mielke and Schingoethe (42) examined the effects of heat-treated soybeans and SBM in lactating cows. Dry matter intake and milk yield were similar for both trials. Ahrar and Schingoethe (2) conducted a total collection trial that showed no differences in rumen ammonia or VFAs. Heat treatment did not alter the profile of amino acids in venous or arterial samples nor affect uptake of amino acids by the mammary gland.

Feeding formaldehyde-treated proteins to increase the amount of protein that escapes ruminal degradation has proved beneficial for weight gains in young ruminants (63). However, results have not produced significant increases in milk production when fed to lactating dairy cows (20, 22, 35).

Combinations of degradable and undegradable protein sources have also been investigated to improve milk production and/or milk constituents in lactating dairy cows. Drackley and Schingoethe (25) examined the effects of an extruded blend of SBM and or sunflower seeds on milk yield and composition in cattle during early lactation. Cows fed the sunflower diet did not increase milk yield but decreased milk solids-non-fat relative to cows fed only SBM. Zerbini et al. (64) fed a highly degradable protein source (SBM) or a relatively undegradable protein source (Fish Meal, FM) to determine their effects on nitrogenous compounds entering the small intestine of dairy cows. Results showed that substituting FM for SBM resulted in less ruminal protein degradation and less microbial protein synthesis. Amino acid analysis revealed no difference in duodenal amino acid flow to the small intestine. In feeding a ration containing FM, provide sufficient degradable protein to allow for maximal microbial synthesis. Other feeds that have been analyzed for their undegradable potential are whole cottonseed, extruded whole cottonseed, directly heated whole cottonseed, soybean meal, heated SBM and CGM (62). Results have shown extruded whole cottonseed shows a lower potential for degradation and therefore can function as a bypass protein.

Corn gluten meal, a by-product of the corn wet-milling industry, is relatively resistant to microbial degradation in the rumen (61). Microbial degradation of protein in corn

gluten meal range from 38 to 54% when fed to growing cattle (65). Fed in combination with urea, slowly degraded proteins such as corn gluten meal, brewers grains, distillers grains and blood meal can provide performance superior to SBM-supplemented rations in beef cattle (53).

Another area of concern when feeding bypass protein is stage of lactation. New systems for estimating ruminant protein requirements suggest early-lactation animals need resistant dietary protein (44). Kung and Huber (37) fed early-lactating Holstein cows treatments containing various amounts of percent protein. Heat-treated soybean meal and ammonia in corn silage were fed in combination to limit rumen degradability and furnish nonprotein nitrogen. The ration calculated to present 17% crude protein (CP), was the most productive. Early-lactation dairy cows were examined through first, second and third lactation to determine the effect of dietary protein (12.2 or 16.2% CP) on dry matter intakes and milk production (54). Dry matter intakes were higher for cows fed high-protein diets (16.2%), and milk production was significantly higher in the second and third lactations.

A nylon bag study measuring protein disappearance was conducted by Barney et al. (8) in rumen-fistulated steers. They found that in the ration containing CP as 17% of the total ration, protein disappeared more rapidly, and a greater percentage of the CP was broken down in the rumen. A lactation trial was also conducted to determine if the

requirement for CP by mature, lactating animals remains high during the first 19 wk of lactation or declines as dry matter consumption in the cow increases. Results showed that feeding 17% CP past week 14 was neither productive nor economical. Forster el al. (30) and Erdman and Vandersall (29) tested the effects of protein degradability on milk production of dairy cows in early lactation. Forster used soybean meal, corn gluten meal and urea, with 3 diets of 14% CP and one of 17% CP. Erdman used soybean meal, corn gluten meal, corn, cottonseed meal and brewer' grains in various quantities to determine the effects of 18.9 and 19.2% CP on milk production. Neither dry matter intake nor milk production was affected by different amounts or sources of protein.

Apparently, milk production does not respond to some supplements of less degradable protein because 1) specific amino acids are limiting rather than the protein per se, 2) energy is limiting (34) or 3) intake is altered. Microbial protein synthesis can be limited at low intake (29) that can result in different values for degradability. Increased dry matter intake can also effect degradability of CP through retention time in the rumen (29, 60). Microbial protein could supply a much higher proportion of the total needs of a cow at higher intakes (32).

The ratio of degradable/undegradable dietary CP, amount of CP (percentage) and source of supplement have proven variable in research due to microbial and dietary influences

(8, 13, 19, 30, 53). Research conducted with undegradable/degradable protein should be continued until relationships between microbial protein and ruminal feed breakdown are more fully understood.

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CHAPTER II

DUODENAL T-CANNULATION IN DAIRY COWS: PROCEDURE AND COMPLICATIONS

ABSTRACT

Proximal duodenal T-cannulae were placed in 11 adult Holstein females. Pregnancy, parturition, lactation and future reproductive performance were considered normal in the animals following surgery. Average daily milk production (first lactation) for 6 cows was 27.9 $\pm$ 3.1 kg, while average peak milk production was 31.9 $\pm$ 3.8 kg/d. The complications following T-cannulation were related to the surgical procedure, after-care and housing environment. Six lactating animals have had functional duodenal T-cannulae in place for more than 22 months. Duodenal T-cannulation was a suitable method to collect digesta samples to study ruminant digestive functions.

INTRODUCTION

Learning how rumen fermentation modifies the nutrient composition of feeds has been a subject of investigation for some time. Digestive sample collection requires the use of surgically-placed intestinal reentrant cannulae (1, 3, 5) or intestinal T-cannulae (8). Several factors influence the type and amount of nutrients that pass through the rumen and are available for absorption in the small intestine. There are several drawbacks to reentrant cannulae that have been used to study these factors. Cannulation may affect gastrointestinal motility (15). Transection of the intestine at the cannulation site may alter nerve conduction and smooth muscle contraction. Abomasal outflow due to low pressure beyond the pylorus is altered as a result of continual sample removal (4). The reentrant cannula affects voluntary feed intake if blocked or if flow is restricted (5, 9). Finally, the external portion of the reentrant cannula is exposed and subject to damage.

The intestinal T-cannula is designed to minimize the problems associated with intestinal reentrant cannulae. Because the intestine need not be transected during placement, there is less risk of altering nerves and smooth muscles. Intestinal obstruction has not been reported with the T-cannula. A support boot and peritoneal ring protect

the T-cannula and help minimize damage.

A few reports have discussed the procedures and complications of intestinal T-cannulation in cattle (8, 14). Only one of these discusses duodenal T-cannulation in lactating dairy cows (14). This paper describes a successful procedure as well as the complications encountered. Lactation and reproductive performance are discussed, however, no true control animals were available for statistical comparisons.

MATERIALS AND METHODS

Duodenal T-cannulae (Ancom, Spencer Port, NY) (Figure 1) were placed in 9 Holstein heifers and 2 first-calf Holstein cows. The animals were 7-8 months pregnant at the time of surgery. The procedure was a modification of descriptions by Komarek (8) and Robinson et al. (14) to accommodate the location of cannulation and the use of pregnant adult Holsteins. The duration of the surgical procedure and the exposure required that animals be under general anesthesia. Food and water were withheld prior to anesthesia for 24 to 48 hours and 12 to 24 hours, respectively. Presurgical antibiotics were administered. Prior to induction, the surgical site was clipped from the 4th rib caudally to the tuber coxae and from the ventral midline to the dorsal midline. An intravenous catheter was placed in the right jugular vein.

General anesthesia was induced with a mixture of 50 g guaifenesin and 2.5 g thyamylal sodium in 500 ml sterile water administered via the jugular catheter. General anesthesia was maintained with a closed circle inhalation system with Halothane as the anesthetic agent. Pulse, respiration and mean arterial blood pressure were recorded every 5 min during surgery. Mean arterial blood pressure was monitored using percutaneous arterial catheterization in

the caudal auricular artery. Jaw tone, ocular reflexes and ocular position were also monitored (7, 12, 13).

Following induction of anesthesia, the animals were positioned in left lateral recumbency with appropriate padding under the hip, shoulder and head (7, 13). The surgical field was prepared and draped for aseptic surgery. A 25-cm incision was made over the 12th rib, extending ventrally just beyond the costochondral junction. The 12th rib was exposed by elevating the periosteum and surrounding muscles. A 20-cm portion of the 12th rib was removed from the costochondral junction dorsally with a gigli-wire saw. The incision was extended into the peritoneal cavity.

The proximal descending duodenum was identified, exposed and packed off. The easiest way to accomplish this was to pull on the greater omentum to expose the abomasum, pyloris and duodenum. Exposure can be increased with an abdominal retractor. Ingesta was manually evacuated from a 14-cm segment of the proximal duodenum and wide rubber bands held with hemostats prevented the reentry of ingesta. A 4-cm incision was made in the antimesenteric side of the duodenum approximately 10 cm distal to the pylorus. One end of the T-cannula was inserted into the lumen of the duodenum distally and the other end gently fitted through the incision. The blunt portion of a Senn retractor was used to help pull the intestine edges over the end of the cannula. The duodenal incision was then closed using 2-0 braided polyglycolic acid

with a taper point needle. The incision was closed in a simple continuous pattern with a Lembert oversew. A purse string suture was placed around the stem of the T-cannula.

The support boot was wrapped around the intestine and cannula base. The fingers of this boot were passed through the greater omentum after carefully punching a Kelly forcep through the omentum, exercising caution to avoid the wall of the duodenum. Each finger of the boot was then pulled through separate holes. The boot snugly seals the duodenal wall over the T-cannula so ingesta will not leak and bypass the cannula. The fingers were then sutured together using #2 non-absorbable suture and the excess removed. The peritoneal ring was placed over the stem of the cannula to prevent leakage around the cannula neck by promoting tissue infiltration.

Gauze sponges were placed in the stem of the cannula and the head of the insertion instrument fitted on the cannula. The obstructing rubber bands were removed, and the duodenum with cannula was rinsed with sterile saline and returned to the abdomen.

The site for exteriorization of the cannula was identified and a 2.3-cm cork bore used to cut a hole through the skin at this site. The final positioning was variable but was generally ventral to the costal cartilage and just under or slightly cranial to the 12th rib incision. The natural positioning of the duodenum in the abdomen should be maintained so that minimal tension is placed on the cannula

and intestinal tract. The T-bar was then pushed through the muscle layers into the abdomen and threaded into the head of the insertion instrument (Figure 2). The cannula was exteriorized by retraction of the T-bar and minimal dissection of the muscle layers. This assures a tight fit around the cannula, thereby minimizing potential infection. The insertion instrument was removed and a teflon outer ring placed over the neck of the cannula and held in place by a stainless steel c-clip to hold the cannula neck external. Placement of the duodenum was checked to ensure that the duodenum was not kinked and no intestines trapped beneath the cannula. The gauze was removed from the cannula and the compression plug inserted. The muscle layers and skin of the incision were finally closed using standard closure procedures.

Animals were placed in sternal recumbency while recovering from anesthesia. Topical antiseptic and fly spray were applied to the incision and around the cannula when appropriate. Following recovery, the animals were given free-choice feed and water as appropriate. Antibiotics were continued for 5 days post-surgery. Further medical care was provide as needed (Figure 3).

RESULTS AND DISCUSSION

All animals except 2 recovered from anesthesia and began eating within 3 hours. The 2 animals that did not begin eating were later euthanized due to anorexia and metabolic disturbances. The specific cause for this was not determined at necropsy. The remaining 9 animals calved within one week of their expected due dates. One animal had a dead fetus that was delivered by fetotomy.

These animals were used in nutritional research projects during their lactations. Dry matter intake during this time averaged 21.0 +_ 0.3 kg/d. Dry matter intake for other first-lactation cows at a similar stage of lactation at the USU Dairy was also 21 kg/d. Milk production data for 6 cannulated animals with complete first lactations is presented in Table 1. Average daily milk production was 27.9 $\pm$ 3.1 kg, while average peak milk production was 31.9 $\pm$ 3.8 kg/d. Average daily milk production, average peak milk production, milk protein percentage and percentage of milk fat for all heifers in the USU dairy herd at this time were 28.9 kg, 33.1 kg/d 3.16% and 3.49%, respectively.

Six of 7 animals bred back have conceived within 3 services. Due to specific management of these animals, typical reproductive indexes could not be used to compare with animals in the herd. However, that 6 of 7 animals conceived

within 3 services is good indication that reproductive performance was not adversely affected. Service per conception for this group was 2.17. The average service per conception for first-lactation cows at the USU dairy during this time was 2.29.

Of the 11 animals, 7 still had functional cannulae at 22 months, one had its cannula removed after it was broken, one was salvaged for slaughter due to a heart defect and two were euthanized due to post-surgical complications.

Numerous complications can arise following surgical placement of a duodenal T-cannula. Several complications are associated with general anesthesia and include infection, nerve paralysis, compartmentalization syndrome, abortion, bloat, regurgitation and aspiration pneumonia (7, 13). Most of these complications can be minimized by properly preparing and attending the animal before, during and after anesthesia.

Occasionally, animals would develop mild to severe anorexia. In the mild cases, rumen motility was only slightly depressed and rumen or transfaunation stimulated the appetite. Two heifers that exhibited severe anorexia with profound ruminal atony did not respond to corrective treatments and were finally euthanized. No gross abnormalities were observed at necropsy except a large atonic rumen and abomasum. The findings suggested a vagal indigestion-like syndrome (2, 11), which could have resulted from manipulation and stretching of the vagus nerve during

surgery or possibly from contamination and local peritonitis. No signs of septic peritonitis were noted at necropsy. Clinical signs of septic peritonitis were not observed in any of the other animals. Outflow problems associated with the placement of the T-cannula in relation to the pyloris could also contribute to vagal indigestion.

The most common complication observed was pressure necrosis and the development of an open sore around the cannula. Normal ventral pull of the intestines and the cannula back into the animal, along with postoperative swelling, resulted in pressure of the holding ring against the abdominal fistula. Some degree of necrosis occurred in all cases. Postoperative swelling has been reported previously (14). Adhesions of the cannula and duodenum to the abdominal wall develop rapidly, as noted also by Robinson et al. (14). By 2 months after the operation, these adhesions have developed sufficiently to allow removal of the retaining rings. This is important if animals are to be milked in a milking parlor since rings are commonly caught and torn off when moving cows to be milked. Thus, surgery should be performed at least 2 months prior to the anticipated calving date of pregnant cows or heifers.

The final positioning of the cannula through the abdominal wall is an important consideration, with the most natural positioning possible. The proximal duodenum normally runs dorsally between the 9th and 13th ribs (6, 10). This

changes somewhat with the stage of pregnancy. We recommend that the cannula not be externalized between ribs since this may increase the risk of breaking the cannula at the T-joint. Rib distortion and cannulae breakage have been reported previously when the cannula is placed in the intercostal space (14). The cannula may be externalized either caudal to the 13th rib or ventral to the costal cartilage when cannulating the proximal duodenum. If the cannula is situated caudally, pressure from the late-term fetus and uterus of a pregnant cow may cause partial obstruction of the duodenum, as occurred in 1 of the 11 animals cannulated. Therefore, externalization of the cannula ventral to the costal cartilages below the 10th to 12th ribs is preferred. Depending on individual variation, however, this positioning may not always be attainable. It is also important that the intestinal tract be placed such that there are no kinks or twists with minimal tension.

Fracture of the cannula during or after recovery from anesthesia is a potential complication, which constitutes an emergency that does not necessitate euthanasia. One of the animals in this study fractured the cannula the day after surgery. Aggressive treatment of endotoxic shock with intravenous fluids, anti-prostaglandins, heparin and antibiotics, followed by immediate surgical replacement of the cannula and peritoneal lavage, successfully saved the animal. The repair was performed in the field with local anesthesia

and physical restraint.

Most future complications with duodenal T-cannulae are related to the housing environment, use of the animal and the presence or lack of daily monitoring. Non-lactating cows that are housed in tie stalls seem to have minor problems related to the cannula. However, lactating cows that are run through the milking parlor alone or in a group can suffer several problems. Bars, walls, corners, fences and other animals tend to catch and sometimes remove or break the retaining rings or the T-cannula itself. Interaction between animals during estrus also risks damage to the cannula. Thus, it is very important that animals be confined individually, preferably in a tie-stall, for at least 2 months following surgery. This gives the cannula time to adhere to the abdominal wall so that it will not fall into the abdomen if the retaining rings are lost or the cannula breaks. After 2 months, the retaining rings are not necessary and can be removed.

We have had 2 cannulas break several months after surgery. In the first, the cannula was replaced by making a small incision to remove the base of the broken cannula and insert a new cannula. The incision was sutured as well as possible and the wound allowed to granulate in. There is continued leakage around this cannula since a tight seal could not be made; however, the amount of leakage has decreased with time and may be acceptable under some

circumstances.

In another animal, a decision was made to close the fistula. First, the remaining base of the cannula was removed. The fistula was then aggressively debrided and sutured closed. The first attempt failed, but a second attempt resulted in a successful closure.

No matter how closely the animals are watched, the potential exists for the compression plug to be lost. This can result in severe complications if not detected quickly. Initially, hypochloremic metabolic alkalosis develops, as in the case of a high intestinal obstruction or abomasal torsion (2). This in itself is relatively easy to correct with intravenous and oral fluid and electrolyte replacement. However, since large volumes of fluid are lost through the open cannula, the animal becomes dehydrated, and omasal impactions are encountered. Oral fluids and mineral oil placed in the rumen were not effective in relieving these impactions. One of the primary functions of the omasum is water absorption and secretion (4). Based on this, hyper-hydration of the animal effectively relieves the impaction. Large-volume intravenous fluid therapy is expensive so fluid was delivered with electrolytes by gravity flow through the duodenal cannula via a cuffed endotracheal tube (size 12 or 14) and funnel. Administering 3 to 5 gallons of fluids 3 times daily for 3 days is a was very effective treatment for relieving these impactions.

One final problem involves future growth of the animal, swelling of the cannula site and size of the cannula. It is advisable to keep the cannula stem long in animals that are expected to continue growing. Once the site has adhered to the abdominal wall, there is little problem with pulling the cannula stem in. If the cannula stem is cut too short, there is a tendency for the body wall to grow over it. An extension piece is available that screws into the cannula stem; however, it is advisable to keep the cannula stems long until the cannula has had time to adhere to the body wall and thus account for future growth.

CONCLUSION

The duodenal T-cannula is an alternative to the reentrant cannula procedure for obtaining digestive samples from the proximal duodenum. While the T-cannula design may alleviate some drawbacks and complications associated with the reentrant cannula, complications with the procedure and aftercare need to be considered. The surgical procedures necessitates general anesthesia. However, aftercare is perhaps the most critical concern. Special problems may arise later when milking cannulated cows.

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Table III. Milk production data from 6 first-lactation Holstein cows with duodenal T-cannulae

Cow	Average Daily Milk (kg/d)	Peak Milk (kg/d)	% Fat	% Protein
1	21.9	24.6	3.25	2.93
2	27.8	32.7	3.42	3.01
3	30.3	34.6	3.27	3.10
4	30.6	35.0	3.33	3.03
5	28.1	33.2	2.94	2.62
6	28.6	31.4	3.17	2.93
Average	27.9	31.9	3.22	2.94
Standard Deviation	3.1	3.8	0.16	0.17

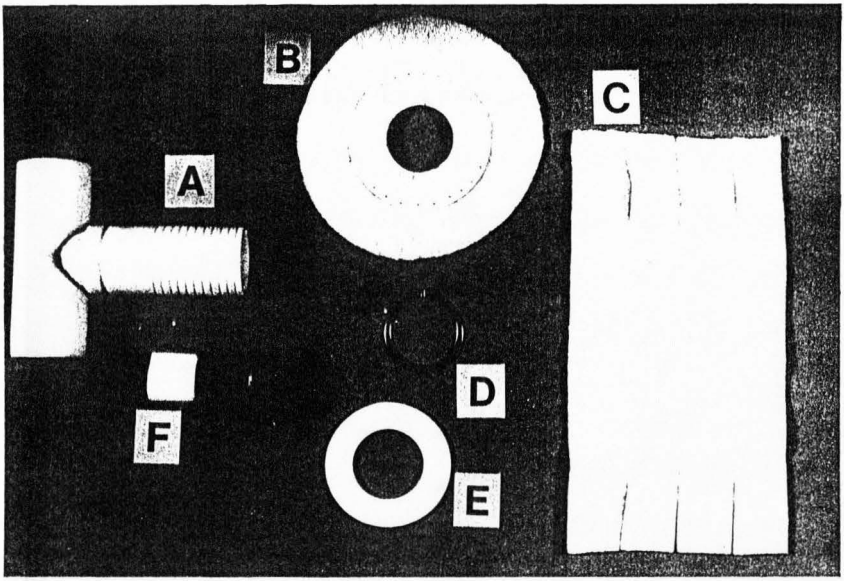

Figure II1: The parts supplied with the Ancom Intestinal T-cannula; (A) T- cannula, (B) Peritoneal ring, (C) Support boot, (D) Stainless steel c-clip, (E) Teflon outer ring, (F) Compression plug.

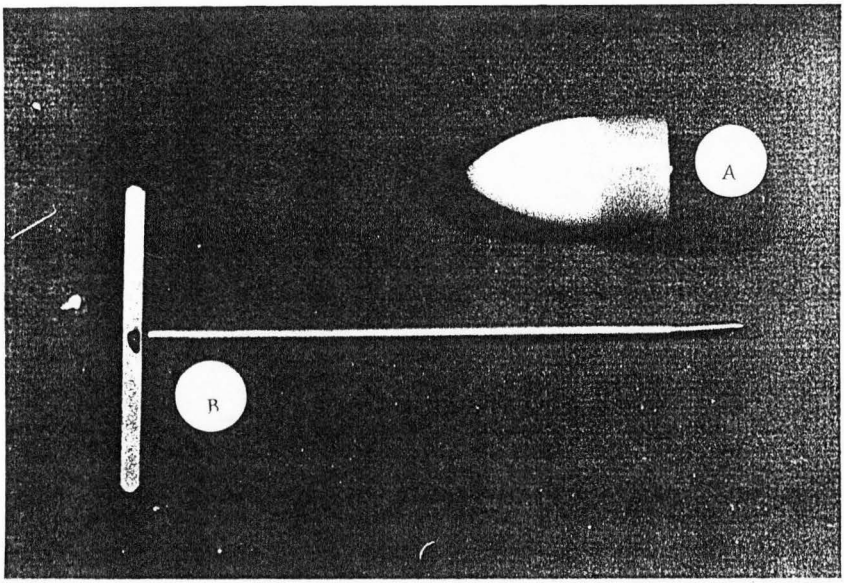

Figure II2. Instruments used to pull the stem of the T-cannula through the body wall; (A) Insertion instrument, (B) T-bar.

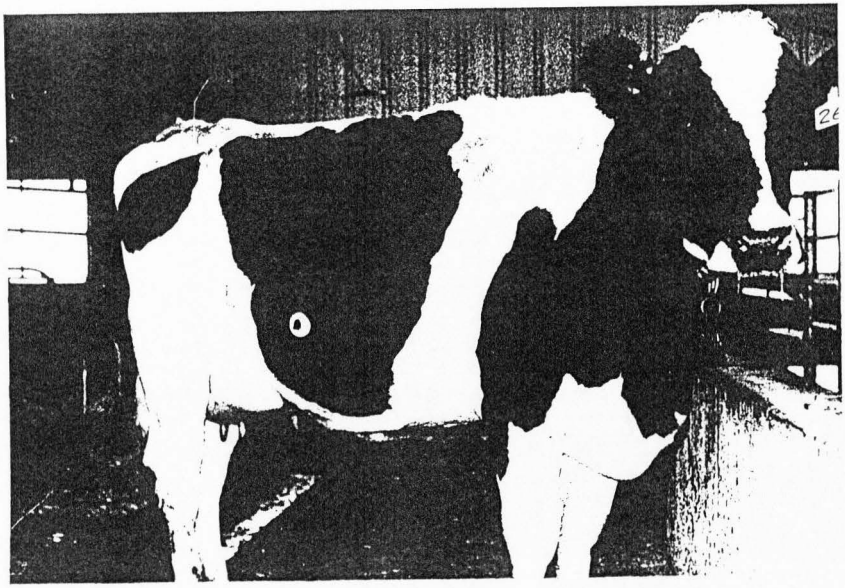

Figure II3. Holstein cow with a proximal duodenal T-cannula in place for over 22 months. This animal was housed in a tie stall during lactation to minimize potential damage to the cannula.

CHAPTER III

THE EFFECT OF SOMETRIBOVE (RECOMBINANT METHIONYL BOVINE SOMATOTROPIN) ON RUMEN FERMENTATION CHARACTERISTICS, DIGESTA RATE OF PASSAGE, NUTRIENT DIGESTIBILITY AND MILK PRODUCTION RESPONSES IN LACTATING DAIRY COWS.

ABSTRACT

Six ruminally- and duodenally-fistulated Holstein cows 60 days postpartum were randomly assigned to each of 2 treatments in a single reversal design. Treatment periods consisted of 1) placebo or 2) 25 mg Sometribove (bST) injected daily. All cows received a total mixed ration consisting of 16% crude protein and 1.67 Mcal\kg NeL. Influence of bST on rumen fermentation characteristics, digesta rate of passage, apparent nutrient digestibility and milk production were evaluated.

Percentage of rumen cellulolytic bacteria (of total viable bacteria) tended to be higher ($P<0.09$) for bST treated animals (6.39 vs. 3.40%). Total rumen protozoa numbers tended ($P<0.21$) to be higher in bST-treated animals (7.25 vs. 6.55 x 10^3/ml). Ruminal percentage of crude protein, ammonia nitrogen, alpha-amino nitrogen, ph or VFAs were unaffected by treatment.

Amino acid content of both rumen and duodenal samples was

determined. Some amino acids ran significantly higher ($P<0.05$) for bST-treated animals in rumen samples but not enough to be biologically significant. Bacterial nitrogen content of rumen samples was similar for both treatment and control. BST treatment had no significant affect on liquid dilution or solid turnover rates. Percentage of crude protein, alpha-amino nitrogen and ammonia-N content in duodenal samples were unaffected by treatment.

Arterial and venous blood samples were also analyzed for amino acid content, with arginine being significantly increased ($P<0.05$) by treatment in venous samples (3.58 vs. 1.69% of total amino acid content); however, in this case statistical difference does not correlate to biological importance.

Apparent total tract digestibility of nutrients was unaffected by treatment. Mean daily dry matter intake was unaffected by treatment. Animals receiving bST averaged 4.0 kg\d higher mean daily milk yield than controls. Fat-corrected milk (3.5% FCM) and milk-production efficiency (3.5% FCM\DMI) were significantly improved in treated animals when compared to controls (29.0 vs. 25.4 kg\d and 1.38 vs. 1.21 kg\d, respectively). Percentage of milk protein was lower in bST-treated animals when compared to controls (2.89 vs. 2.98%, respectively).

BST did increase milk production and efficiency but did not effect any other parameters tested.

INTRODUCTION

Bovine somatotropin (bST) is a protein that occurs naturally in the pituitary gland of dairy cattle. It is produced in small amounts in a pulsatile release and is a factor in the regulation of milk production (22).

The effects of bST in lactating dairy cows have been a subject of interest for many years. Asimov and Krouze (1) first demonstrated that injections of crude pituitary extracts increase milk production in dairy cows. These findings were the beginnings of research to determine whether large-scale utilization of the hormone could be possible (20, 21, 24, 31, 39, 43).

Bovine somatotropin, when injected into the animal, supplements the cows' naturally occurring somatotropin and improve milk production efficiency. Because manipulation of the somatotropic axis increases productivity in the ruminant, interest has rapidly grown in the varied effects of bST. BST can be used in various doses and given a number of ways. Research projects range from 12.5 to 60 mg/d and routes of administration include continuous infusion, daily and biweekly subcutaneous and intra muscular injections (4, 18, 19, 29, 34, 39). Responses to bST injections have also varied in a dose-response fashion from 10 to 40% increase in milk yield and from 5 to 15% increase in feed efficiency above

control animals (5, 11, 13, 14, 18, 37, 38).

Along with increased milk production, milk constituents have been affected. Increased percentage of milk fat (1 to 13%) and protein yield (1 to 10%) have been observed with increased milk yield with lactose unchanged (4, 20, 21). However, the percentage of milk constituents can change depending upon the animals' energy and protein status (5, 6, 12). Milk lactose content remains relatively constant regardless of nutritive status, but a negative energy balance in the animal can affect milk fat production by increasing the amount of milk fat relative to milk production. Percentage of milk protein tends to decrease with cows in negative energy balance due to an increase in milk protein secretion into the milk, which is proportionally less than the total milk yield increase (20, 21, 44, 47).

The response to bST is progressive and persists as long as treatment continues (20). The voluntary feed intake of treated animals increases to accommodate the increased nutrient requirements of a greater milk yield (26). But the increase in dry matter intake during early lactation can be masked by the animal's inability to rapidly increase nutrient intake to meet milk production. This results in an increase in production efficiency from a dilution of the maintenance requirement, which could represent a substantial increase in financial return (5).

The exact mechanism by which bST works to increase milk

production is not completely understood. Theoretically, growth hormone stimulates IGFs, hormones produced by body tissues such as the liver that direct nutrients through the blood to the cells in the udder and aid in milk production (3, 32). Of special interest are insulin and IGF-1 effects on lactating bovine mammary tissue. The insulin effect is unlikely through the insulin receptor because these receptors are low in number during lactation. Growth hormone (bST) injected into lactating dairy cows does increase milk production, but bST does not directly act on bovine mammary tissue. Therefore, increase in milk production may occur because of insulin resulting, in nutrients directed to the area (without the insulin receptor being primarily involved) and physiological IGF-1 levels via the somatomedin hypothesis (7, 8, 22). Administration of bST can result in a dramatic shift in the partition of available energy towards milk yield at the expense of body tissue (46). Inhibition of acetate use in body fat synthesis inhibits fatty acid synthesis in body fat depots and increases body-adipose sensitivity to lipolytic signals. Therefore, more nutrients (fatty acids) are directed towards milk component synthesis (27, 28).

Because bST affects nutrient utilization, this secondary effect must be addressed through increased dry matter intake (5, 12, 13, 36, 43). Although nutrient digestibility does not appear to be influenced by bST administration (36, 40), more nutrients directed towards milk component synthesis

under increased dry matter intake may affect the animals' feed utilization.

The objectives of this study were to compare the effect of administering bovine somatotropin (bST) on:

1) rumen fermentation characteristics.
2) passage rate of nutrients through the gastrointestinal tract.
3) apparent digestibility of nutrients.
4) milk production response.

MATERIALS AND METHODS

Six primiparous Holstein cows, surgically equipped with rumen fistulae and proximal duodenal T-cannulae, were assigned to each of 2 treatments in a switchback design. Treatments were 1) control (2.5 ml, daily subcutaneous injection of 75 mM Bicarbonate and 2) treatment (subcutaneous daily injection of 2.5 ml of 10 IU/ml bovine somatotropin (bST). Bovine somatotropin (25 mg) was solubilized in 11.9 ml of bacteriostatic water for injection. Injections (2.5 ml of excipient or bST solution) were administered in 1 of 4 alternating sites at approximately 1200 h daily. Any solubilized hormone that was not used immediately, was stored (48-h maximum) at 5 C.

Treatments were initiated at 60± 7 days postpartum and maintained for 6 weeks with a 3-week readjustment period. Cows were housed in tie stalls and had free access to water. Cows were fed ad libitum a total mixed ration (Table 1) twice daily at 0700 and 1500 h. The diet was offered in amounts calculated to provide 120% of the National Research Council recommended amount (33). Feed refusals were recorded once daily. Feed was sampled once weekly, composited monthly, dried (2) and ground through a Wiley mill (Philadelphia, PA) using a 1-mm screen. Samples were analyzed for dry matter, (DM) (2), crude protein, (CP) (23), acid detergent fiber,

(ADF) and neutral detergent fiber, (NDF) (50) and ADF-ash (49) (Table 2).

Milk yield was recorded twice daily at 300 h and 1500 h, with samples being composited once weekly (am-pm) and analyzed for percent lactose, fat, protein, SNF and somatic cell count, using a Multispec M Infrared Analyzer from Wheldrake, York, England.

Rumen Fermentation

On days 1 through 3 (the last week of the trial), rumen samples were collected to determine diaminopimelic acid (DAPA) content (15). Samples were strained through 4 layers of cheesecloth and centrifuged at 3,000 X g for 15 min. The supernatant was recentrifuged at 18,000 X g for 15 min. The resulting supernatant was poured off, producing a bacterial pellet that was resuspended in a acetate buffer and recentrifuged at 18,000 X g for 15 min. This step was repeated 3 more times to remove any additional contaminants. The bacterial pellet was lyophilized, ground in a 1-mm Udy cyclone mill (Fort Collins, Co.) and analyzed for DAPA (15), CP (23) and total amino acids (41, 42). The procedure for DAPA was modified from J.W. Czerkawski (15). The hydrolyzed samples (1 ml) were mixed with 4.0 ml of reagent (2.5 g of ninhydrin dissolved in 60 ml of glacial acetic acid and 40 ml of 6 M-phosphoric acid) and heated in boiling water for 5 min. The optical density was determined at 425 nm using cells with 2-cm light path. Bacterial nitrogen content was

calculated using the DAPA values for each animal. The calculations were made using a ratio of mg bacterial nitrogen (N) to g total N established for each animal fed (mg bacterial N\g dry marker).

At 4 h post-feeding (1000 h) on the 4th day of collection, 300 ml of rumen digesta were collected from the mid-ventral sac and analyzed for viable cellulolytic and total bacteria using habitat-stimulating media in anaerobic roll tubes as described by Leedle and Hespell (30). Samples of whole digesta were strained through 4 layers of cheese cloth and placed in a Waring blender, flushed with carbon dioxide, agitated 1 min. and serially diluted with anaerobic dilution solution. Roll tubes for total viable bacteria consisted of a total carbohydrate-based media at dilutions of x 10^{10} and x 10^{9}, and roll tubes for cellulose bacteria consisted of a cellulose/cellobiose based media at dilutions of x 10^{9} and x 10^{8}. Roll tubes were incubated at 39 C for at least 7 days and individual colonies counted. Rumen samples were diluted 1:1 with a 50% formalin solution and placed in scintillation vials for total protozoa counts (16). A 1 ml aliquot of the preserved sample was pipetted into a test tube and mixed with 2 drops of 2% brilliant green. Samples were allowed to stand for at least 4 h; then 9 ml of a 30% glycerol solution was added. If further dilutions were necessary to facilitate counting of protozoa, the 30% glycerol solution was used. A 1 ml aliquant was pipetted into a Sedgwick-Rafter counting

chamber and total protozoa counted after 5 min.

On days 5 through 7, rumen digesta samples were collected at 0, 2, 4, 6, 8, 10 and 12 h post-feeding. Rumen samples were collected from the mid-ventral sac and separated into liquid and solid fractions by centrifuging the sample at 7,000 x g for 15 min. The solid portion was lyophilized and ground through a Wiley mill using a 1-mm mesh screen. Samples were analyzed for CP (23) and total amino acids (42).

The procedure for total amino acid content was modified from Smith and Wonnacott (42). A 0.100-g rumen and duodenal sample was placed in a round bottom 16 x 150 mm culture tube with 4 ml 6 N HCL, 0.2 ml norleucene (.125 umole/ml internal standard) and sealed. These were placed in a hot oil bath (110-120 C) and hydrolyzed for 12. When cool, a 1-ml aliquot was removed from the sample and placed in a another culture tube. The sample was dried under compressed air, and 3 mls of dicloromethane was added and repeated 3 times. Esterification reagent of 1 to 2 mls was added to samples that were heated for 1 h at 120 C. Samples were dried; again dicloromethane was added and repeated 2 times, always drying between procedures. Three mls of acetylation reagent (3/1, dicloromethane/ trifloroacetic acid) was added and allowed to stand for 20 min. (or longer). Samples were dried and 2 mls of dicloromethane added, shaken and placed in vials for analysis using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) with an Altech (Deerfield, IL) amino acid

packing 30.48 cm x 0.3175 cm ss column.

The liquid portion of the rumen sample was analyzed for alpha-amino nitrogen (35), ammonia nitrogen (23), pH and VFAs using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) with an Alltech carbowax 20 M capillary column (Deerfield, IL) with a 10 mm x 0.53 mm x 1.33 um film thickness.

Duodenal Digesta

On day 5, chromium mordanted straw was made using a modified procedure of Uden et al. (48), and added to the rumen via the fistula before feeding (0600). The modified mordanted straw, consisting of 500 g chopped straw, 40 g sodium dichromate, and 4 liters of deionized water, was used to determine particulate rate of passage. Cobalt-EDTA was made according Teeter and Owens (46) and also added to the rumen via the fistula at the 6 h post-am feeding. Duodenal digesta was collected at 0, 2, 4, 6, 8, 10, 12, 15, 18, 24, 30, 36, 42, 48, 60 and 72 h post-dosing with chromium mordanted straw. The samples were separated into liquid and solid fractions by centrifuging at 7,000 x g for 15 min. The solid portion was lyophilized and ground through a Wiley mill using a 1-mm mesh screen. Samples were analyzed for chromium (T0 to T72) using a Buck 200, Technicon Auto Analyzer (East Norwalk, Conn.) to determine particulate rate of passage through the duodenum according to Uden et al. (48). Crude protein (23) and total amino acids were also measured as previously described. The liquid portion (hours 6 through 72) was analyzed for cobalt by

a Buck 200, Technicon Auto Analyzer (East Norwalk, Conn) to determine liquid dilution rates. Ammonia-nitrogen (23) and alpha-amino nitrogen (35) were also analyzed.

Mammary Gland Amino Acid Uptake

During the collection period, blood samples were taken from cows to determine amino acid uptake (42) by the mammary gland. Blood samples were withdrawn from the internal iliac artery and sub Q abdominal vein at 2 h pre-injection of bST and 2 and at 4 h post-injection.

Nutrient Digestibility

On days 1 through 5, fecal grab samples were obtained and composited, dried in a 60-C oven for 48 h, ground through a Wiley mill using a 1-mm mesh screen and analyzed for DM (2), CP (23), ADF and NDF (50) and ADF-ash according to Undersander et al. (49). ADF-ash was used to determine total-tract apparent nutrient digestibility.

Analysis of Data

The data collected in the experiment was analyzed by analysis of variance under a model appropriate for the single-reversal experimental design. Differences due to treatment were considered.

RESULTS

Rumen Parameters

The results of daily injections of bST during early lactation on rumen total viable bacteria, cellulolytic bacteria, percentage of cellulolytic bacteria and protozoal numbers are in Table 3. Treatment had no effect on parameters tested but percentage of cellulolytic bacteria tended ($P<0.09$) to be higher for treatment groups over control.

Ruminal crude protein percentage, alpha-amino nitrogen, ammonia-nitrogen, pH and VFA concentrations are shown in Table 4; results were similar for treatment and control. Results for total amino acid content of solid phase and bacterial N are in represented in Table 5. Total amino acid concentrations were similar for both treatment and control except the significant increase of phenylalanine ($P<0.05$) for treatment (5.74 vs. 3.02%). Regardless of treatment, bacterial N content of bacterial cell mass was not different.

Duodenal Parameters

The effect of daily bST injections on total amino-acid concentrations was not different from control, as represented in Table 5. Results of treatment on liquid dilution rates, particulate rate of passage, CP, ammonia-nitrogen and alpha-amino nitrogen were similar (Table 6).

Mammary Parameters

Blood amino-acid concentrations were measured in samples from the iliac artery and mammary vein. Results were significantly higher for treatment animals (3.58 vs. 1.69%) for arginine content of venous samples (Table 7).

Digestibility Coefficients

Apparent total-tract nutrient digestibility of the ration was unaffected by daily bST injections (Table 8). Crude protein percentage, acid detergent fiber percentage and neutral detergent fiber percentage were similar for both treatment and control.

Dry Matter Intake and Milk Production

Dry matter intake, milk yield, 3.5% FCM and production efficiency are in Table 9. Injection of bST did not influence DM intake ($P>0.05$). Milk yield increased ($P<0.05$) with daily injections of bST. The mean daily average milk yield during the 42-day injection period increased by 4.0 kg/d. This response falls within the range reported for cows given bST injections (12, 18). The 3.5% FCM was significantly increased with bST treatment (29.0 vs. 25.4 kg/d). Production efficiency of bST-treated animals (1.38 kg milk/kg feed) was significantly increased ($P<0.05$) (1.21 kg milk/kg feed).

Milk components were examined and results are shown in

Table 10. Percentage of fat, lactose, solids-non-fat and somatic cell count were unaffected by treatment. Percentage of protein was significantly ($P<0.05$) lowered for bST-treated animals (2.89 vs. 2.98%).

DISCUSSION

At the beginning of lactation, cows must undergo many altered metabolic functions and partitioning of nutrients to accommodate the increased demands of the mammary gland for milk production (3). During this time, nutrient requirements of the mammary gland are greatly increased relative to the metabolism of the total animal (5). Results from studies confirm that administration of bST to lactating dairy cows does increase milk production (5, 31, 33, 36). The administration of exogenous bST increases the partitioning of nutrients to the mammary gland. These nutrients, which come from dietary intake, fat metabolism and muscle mass, are the result of ration, water and body stores. That diet and water can affect the total rumen bacterial counts in cattle accounts for much of the variation in bacterial populations seen among researchers (40).

Total bacterial concentration drops 1 to 2 h after feeding, possibly due to the dilution of rumen fluid with feed and saliva. The bacteria concentration then increases 2 to 4 h post-feeding but drops again at 5 to 7 h post-feeding so time is an important factor in determining bacterial numbers. Breakdown of particulate material is primarily dependent on the enzymatic degradation of cellulolytic materials. This is influenced by a number of factors

including 1) moisture content of the fiber; 2) the degree of crystallinity; 3) diffuse and size of the digestive enzyme; 4) the degree of polymerization; 5) the nature of the other material in the rumen; and 6) the nature, amount and variation of other bacteria (40).

Moisture plays an important role in the fermentation of cellulose. Water swells the substrate, making it more available to enzyme degradation. Water also provides a medium where nutrients are available for microbial growth and for hydrolysis (40). Variation of water intake (by ruminants) is responsible for the difference seen in percentage of cellulolytic populations. The slight increase in cellulolytic populations seen in treatment cows (6.39 vs. 3.40%) may be due to differences in water intake associated with increased milk production.

Ruminal and duodenal nutrient parameters were similar for both treatment and control animals. Analysis of ruminal and duodenal samples for crude protein, alpha-amino nitrogen and ammonia nitrogen would not be expected to be different (37).

Balancing a ruminant ration must take into consideration protein available to the microbes as well as the host. The protein available to the host is microbial protein and ruminal-escape protein, presented to the small intestine in the form of amino acids and/or peptides. Amino acids can then be beneficial to the animal but only as beneficial as the limiting amino acids they present. The limiting amino acid in

ruminants is not determined by the essential amino-acid profile content of dietary proteins but rather the essential amino-acid content of the bulk protein (composed of microbial protein and partially degraded and undegraded feed proteins). Cystine as well as most sulfur AA seem to be limiting in ruminal microbial protein (9). Other amino acids considered limiting for microbial protein and diet dependent are methionine, lysine, threonine and phenylalanine (10, 25). Free amino acids arise as intermediate products in the breakdown of proteins by rumen microorganisms (10). Low concentrations of free amino acids in the rumen suggest rapid utilization; in this study, however, variation seen in amino-acid concentrations that was significant is still not biologically significant.

DAPA has been found in various bacteria species but not in plants and yeast (40). Because DAPA is found in bacteria but not in feeds commonly given to ruminants, it is used as a marker to determine the amount of bacteria in the rumen. DAPA which may be used to predict rumen bacterial N concentration, must be constant or be corrected easily for variations that can occur due to diet fed (15). In this study, one diet was fed to all animals and DAPA did remain unchanged between treatments (17.4 vs. 18.6 ug/g organic matter). Previous studies have reported no change in DAPA among treatments (10, 40, 45), but results from the present study were lower than those reported previously (45) ranged

from 34 to 71 mg/g N. Because of the variation found in N and DAPA content of bacterial preparations across and within studies, another method of bacterial estimation may be necessary.

Venous plasma arginine content increased significantly ($P<0.05$) in bST-treated cows (3.58 vs. 1.69% of total amino acid). However, unless specific conditions are met, plasma amino acid (PAA) profiles will not necessarily reflect dietary AA patterns or represent protein (AA) status and cannot be used to represent nutritional status of the animals. The size of the free amino-acid pool is much smaller than the amount of variation of AA flowing into and out of the free tissue pools (9). Therefore, low plasma levels can represent either dietary protein deficiency or an increase in uptake of essential amino acids into tissue. High plasma levels can be a result of either increased dietary protein to excess or extensive catabolism of body protein.

Daily injection of bST for 42 d had no effect on dry matter intake but stimulated large daily increases in production of milk (4 kg/d) and a decrease in some milk constituents. Generally, this finding agrees with similar studies (21, 27). Increased feed intake was observed in cows treated with 25 mg/d bST. Also, Bauman et al. (4) found feed intake to increase after a 5-week lag period. We observed no difference in feed intakes between animals on bST and control

which was also noted in other short-term studies (24). Milk production was increased by 4.0 kg/d with bST injections, as supported by previous work (5, 37), but further research is necessary to determine whether bST injections would be more beneficial later in lactation to avoid some of the problems stated previously (i.e. increased degree and duration of negative energy balance). At this time, more beneficial results may be observed during mid to late lactation, which would increase milk production at a time of lower production, therefore increasing persistency (37).

Percentage of milk protein was decreased ($P<0.05$) with bST injections (2.98 vs. 2.89%) with all other milk constituents remaining unchanged (4, 14, 18). The observed decrease in percentage of milk protein in bST-treated cows may have been a result of limited feed intake and the greater negative energy balance stimulated by higher milk production.

The effects of bST, centering on nutrient partitioning, are specifically secondary because increase of circulating bST does not cause increased milk production directly (8). Rather, increased mobilization of nutrients to the mammary gland stimulates milk production, thus altering nutrient partitioning. Most likely, a key regulatory enzyme causes an increased synthesis rate of nutrients that does not affect the microbial environment directly or change microbial populations. BST does not directly affect other nutrient parameters of the rumen or duodenum. A possible mechanism

by which bST could indirectly affect these parameters would be when intake increases to meet increasing energy demands, which could affect the rate of passage of nutrients through the rumen to the duodenum by causing a decrease in the digestibility of nutrients and altering nutrient rate of passage. Previous short-term studies with bST have demonstrated that the digestibilities of nutrients are not altered by treatment (6, 36), but the addition of bST affects production efficiency and milk yield (4, 39, 43).

Possibly a revised study could examine these same parameters at late lactation rather than early lactation; some studies have found bST as beneficial in late lactation as in peak lactation (17). During mid- or late lactation, energy intake exceeds energy output, and excess energy is channeled towards milk production. Combining these effects with bST could have a greater effect on milk production. Peel et. al. (37) found substantially increased milk yield and efficiency in cows injected with bST during late lactation.

CONCLUSIONS

BST injected daily did increase milk production. Ruminal and duodenal parameters are not effected by bST injections during early lactation; however, later in lactation (mid to late), dry matter intake tends to increase and an affect parameters.

The use of cannulated and fistulated animals in this project has been beneficial to the research, based on a comparison of milk production between cannulated and intact animals from previous research (51). The results of milk production in this project were similar to non-cannulated animals in the same environment (27.9 vs. 31.9 kg/d); therefore, all other data is representative of intact animals.

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TABLE IIII. Composition of total mixed ration.

Ingredients	(% Dry Matter)
Alfalfa haylage	8.5
Corn silage	14.8
Chopped alfalfa hay	26.8
Rolled barley	18.8
Cottonseed, whole	11.4
Beet pulp	9.6
Brewers dried grains	7.8
Beet molasses	0.9
Vitamin/mineral premix[a]	1.4

[a] Consisted of sodium chloride, 99.445%; Manganese, .2%; Iron, .30%; Zinc, .01%; Iodine, .007%; Cobalt, .005%; 2000 I.U. vitamin A; 2000 I.U. vitamin D; and .2 I.U. vitamin E per gram.

TABLE III2. Nutrient content of total mixed ration.

Nutrient (DM basis)	
Crude protein, (%)	15.00
Neutral detergent fiber, (%)	49.70
Acid detergent fiber, (%)	32.50
Ne_l (Mcal/kg)	1.67
Calcium, (%)	0.70
Phosphorus, (%)	0.46
Potassium, (%)	1.99
Magnesium, (%)	0.31
Sodium, (%)	0.16
Zinc, (mg/kg)	54.50
Manganese, (mg/kg)	47.30
Copper, (mg/kg)	32.70
Iron, (mg/kg)	221.80

TABLE III3. Effect of bST treatment on ruminal microbial numbers.

Item	Treatment		SEM
	Control	bST	
Bacteria			
Total, x 10^{10} /ml	10.70	10.40	0.60
Cellulolytic, x 10^{9} /ml	3.42	4.83	2.76
Cellulolytic, (%)	3.40	6.39	2.33
Protozoa, x 10^{3} /ml	6.55	7.28	0.80

TABLE III4. Effect of bST treatment on ruminal fermentation characteristics.

Item	Treatment		SEM
	Control	bST	
Crude protein (%)	17.49	17.82	3.75
Alpha amino nitrogen (umoles/dl)	587.62	563.43	30.60
Ammonia-N (mg/dl)	4.67	6.33	0.87
pH	5.46	5.51	0.90
Total VFA (mmol/L)	155.92	154.50	3.93
(Molar %)			
Acetic	61.28	62.43	1.07
Propionic	22.91	21.99	0.82
Isobutyric	.79	.82	0.06
Butyric	12.05	11.58	0.46
Isovaleric	1.12	1.24	0.12
Valeric	1.83	2.02	0.14
Acetic/Propionic Ratio	2.67	2.84	0.82

TABLE III5. Effect of bST treatment on total amino acid and bacterial N content.

Item	Rumen		SEM	Duodenal		SEM
	Control	Treatment		Control	Treatment	
Total amino acids (umoles\g)	66.56	64.06	10.95	58.56	42.11	9.10
	--------% of total amino acids--------					
Alanine	8.70	13.87	2.92	5.68	6.78	1.91
Valine	8.71	8.32	1.23	5.90	10.78	2.25
Glycine	23.44	17.85	2.95	17.39	18.59	3.75
Isoleucine	7.74	6.26	2.95	6.58	3.93	1.37
Leucine	4.98	4.13	1.07	4.87	5.02	1.39
Proline	2.30	3.47	0.85	3.80	1.56	1.51
Serine	16.59	12.52	2.40	10.76	14.30	3.52
Methionine	5.24	6.13	2.30	3.99	7.96	2.34
Phenylalanine	3.02[a]	5.75[b]	1.13	2.74	5.26	0.87
Histidine	6.29	5.12	3.35	7.41	5.31	1.03
Tyrosine	3.96	6.20	0.84	5.05	5.04	1.05
Ornithine	1.61	1.68	0.32	12.08	3.66	4.20
Lysine	3.23	3.11	0.45	4.94	3.01	0.79
Arginine	2.36	3.50	0.46	7.26	7.19	1.01
		Control		Treatment		SEM
Bacterial N Content (mg bacterial N\g DM)		17.41		24.31		4.37

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE III6. Effect of bST treatment on duodenal digesta flow kinetics.

Item	Treatment		SEM
	Control	bST	
Liquid dilution rate, (%/h)	7.30	6.09	1.90
Particulate rate of passage, (%/h)	3.49	3.51	1.42
Crude protein (%)	15.57	16.28	1.89
Ammonia-N (mg/dl)	1.91	1.61	0.37
Alpha-amino nitrogen (umoles/dl)	471.18	442.13	30.11

TABLE III7. Effect of bST treatment on blood amino-acid content of arterial and venous samples.

Item	Vein		SEM	Artery		SEM
	Control	Treatment		Control	Treatment	
Total amino acids (umoles/100 ml)	12.27	14.70		10.26	12.97	1.61
	--------- % of total amino acids ---------					
Glycine	2.20	7.30	3.52	1.18	2.20	1.15
Isoleucine	0.11	0.09	0.12	1.17	0.77	0.40
Leucine	1.00	0.86	0.19	2.76	5.36	2.24
Proline	22.70	19.71	7.80	8.58	3.51	4.61
Histidine	3.51	9.14	3.12	5.71	4.78	1.32
Tyrosine	14.70	7.52	3.80	9.68	16.26	4.28
Ornithine	39.64	37.01	7.58	48.65	44.33	7.81
Lysine	6.44	7.27	5.90	12.90	8.67	5.97
Arginine	1.69[a]	3.58[b]	1.36	1.43	4.88	1.60

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE III8. Effect of bST treatment on total-tract apparent nutrient digestibility.

Item	Treatment		SEM
	Control	bST	
Crude protein (%)	61.72	62.18	2.91
Acid detergent fiber (%)	42.56	44.62	6.43
Neutral detergent fiber (%)	43.50	44.97	6.91

TABLE III9. Effect of bST treatment on mean daily DM intake, milk yield and production efficiency.

Item	Treatment		SEM
	Control	bST	
DM Intake, (kg/d)	21.00	21.10	0.30
Milk yield, (kg/d)	26.60[a]	30.60[b]	0.37
3.5% FCM, (kg/d)	25.40[a]	29.00[b]	0.94
Production efficiency (3.5% FCM/DMI)	1.21[a]	1.38[b]	0.02

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE III10. Effect of bST treatment on milk composition.

Item	Treatment		SEM
	Control	bST	
Fat (%)	3.23	3.25	0.05
Protein(%)	2.98[b]	2.89[a]	0.02
Lactose(%)	4.77	4.79	0.17
Solids-non-fat(%)	8.44	8.38	0.25
Somatic cell count(100/ML)	154.44	93.24	52.50

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE III10. Effect of bST treatment on milk composition.

Item	Treatment		SEM
	Control	bST	
Fat (%)	3.23	3.25	0.05
Protein(%)	2.98[b]	2.89[a]	0.02
Lactose(%)	4.77	4.79	0.17
Solids-non-fat(%)	8.44	8.38	0.25
Somatic cell count(100/ML)	154.44	93.24	52.50

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

CHAPTER IV

THE EFFECT OF DEGRADABLE AND UNDEGRADABLE PROTEIN SOURCES ON MICROBIAL ACTIVITY AND NUTRIENT DIGESTIBILITY IN A NON-LACTATING HOLSTEIN COWS.

ABSTRACT

An in situ dacron bag study was conducted to determine the rate (%/h) and extent of (%/24 h) disappearance of a rapidly-degradable rumen protein source (soybean meal, SBM) and two slowly-degradable rumen protein sources corn gluten meal (CGM) and meat and bone meal (MBM). Rate of disappearance (%/h) was significantly increased ($P<0.05$) for SBM over CGM and MBM (7.48 vs. 5.17 and 3.02%). The extent of degradation (%/24 h) was significantly different ($P<0.05$) among all 3 protein sources (SBM, CGM and MBM were 98.8, 45.5 and 37.6 %/24 h, respectively).

Six Holstein cows surgically equipped with rumen fistula and proximal duodenal T-cannulae were assigned to each of 2 treatments in a single reversal design. Treatment period consisted of a 14-day adaptation period, 21 days of collection and a 7-day washout period. Treatments consisted of 1) a basal ration containing SBM (control) and 2) a basal ration containing 34% of the dietary crude protein requirement from CGM and MBM (treatment). The influence of treatment on rumen fermentation characteristics, digesta rate of passage, total

amino acid content and apparent nutrient digestibility was calculated. Total bacteria, cellulolytic bacteria, percentage of cellulolytic bacteria and protozoal numbers were not different ($P>0.05$) between treatment and control. Treatment had no effect on ruminal or duodenal crude protein percentage or alpha-amino nitrogen. Ammonia nitrogen in rumen samples was significantly higher ($P<0.05$) in control animals (9.56 vs. 7.88 mg/dl). Treatment had no effect on liquid dilution rates, rate of passage or pH of rumen samples. The molar percentage of propionic was significantly increased ($P<0.05$), and acetic\propionic ratio decreased in rumen fluid of treatment- fed animals compared to control (25.16 vs. 19.36, 2.63 vs. 3.07%, respectively).

Dry matter intake was unaffected by treatment ($P>0.05$). Apparent total-tract digestibility of nutrients was unaffected by treatment.

Results from the in situ trial conclude that SBM is a rapid, ruminally-degradable protein source. Results from both trials suggest that an increase in the amount of the less ruminally degradable protein sources (CGM and MBM) passed through to the small intestine. This could be of benefit to the host animal because protein available to the host without microbial synthesis is more efficient.

INTRODUCTION

Protein, necessary for animal tissue synthesis and milk production comes from three sources: dietary protein, microbial protein and endogenous protein. Degradable dietary protein entering the rumen is degraded to ammonia, which is necessary for microbial growth through protein synthesis, and to volatile fatty acids (VFA) necessary for animal production. Microbial protein then passes to the abomasum and small intestine where it is digested to amino acids and absorbed for utilization by the host (4). The microbes themselves can provide sufficient protein for maintenance, slow growth and early pregnancy (27) but cannot maintain the increased demands of high milk production or rapid growth in young animals (17, 26). Therefore, dietary protein sources must be maintained to acquire the exact ratio of degradable to undegradable and thus accommodate the demands of increased milk production.

Protein degradation is influenced by a number of rumen functions. Rate of passage of the digesta, feed intake, density of the ration, particle size and the degradability of the protein source (3, 27) all affect the amount of amino acids that will reach the small intestine. If the rate of passage of nutrients is increased, digestibility can decrease. When feed intake increases above the maintenance tolerance of

the animal, the rate of passage of nutrients increases, as explained above, and digestibility of the ration can decrease. Specific gravity can affect rate of passage. If the diet is dense (i.e., increased concentrates in the diet), rumen Ph will decrease and liquid dilution rates decrease (6). A smaller particle size of ration can have an increased rate of passage through the rumen, thereby decreasing the amount digested. The degree to which proteins are broken down also depends on the rate of degradation. Slowly degraded proteins, such as meat and bone meal (MBM) and corn gluten meal (CGM), can bypass degradation (bypass protein) in the rumen by the microorganisms and be utilized more efficiently in the gastrointestinal tract (29). Protein that escapes rumen fermentation could be used to the animals advantage, either to improve milk production or to reduce the amount of a conventional protein source required (9, 23, 32). Also, degradable protein is not used as efficiently as undegradable protein due to losses of ammonia from the rumen, that result in less protein available to the host (20, 24). Soybean meal (SBM) is highly degradable and more than sufficient in supplying the microbes with nitrogen to be used as ammonia. However, its easy degradability makes it less efficient, loosing ammonia across the rumen wall. The excess ammonia is removed in saliva for recirculation or lost in urine.

The ideal situation would be to create a ration adequate

both in degradable and undegradable protein to meet the requirements of microbes and host. Rumen microbes would be supplied with enough nitrogen to fill ammonia requirements and sufficient amounts of protein to pass out of the rumen intact and directly to the host. A ration consisting of only undegradable protein would be unable to meet nitrogen requirements of the microorganisms, and fiber digestion and performance would decrease in the animal (29), biasing results and underestimating the apparent value of the protein. If the ration consisted of only degradable protein, the microbes would be satisfied as long as energy is sufficient (31), but the requirements of the host could be lacking.

The objective of the in situ dacron bag study was to test the rate and extent of protein disappearance of individual protein supplements in the rumen of Holstein cows. Coupled with bag study, a 6-animal study was conducted to determine the influence of altering quantity and source of degradable/undegradable dietary protein on microbial activity requirements and the requirements for metabolizable protein in mature, non-lactating Holstein cows.

MATERIALS AND METHODS

In Situ Disappearance

An in situ, protein-disappearance dacron bag study was conducted to determine the rate of disappearance (%/h) and the extent of disappearance (%/24 h) of separate protein sources (SBM, CGM and MBM). The actual CP percentage was also determined in the supplemental protein sources (SBM, CGM and MBM) (16) so a ration could be balanced more precisely. Two rumen fistulated and duodenally cannulated cows were fed a straw diet for 10 days. Dacron-polyester bags (6 x 10 cm, with a pore size of 50 microns) with 5.0-g samples of separate protein sources (SBM, CGM, MBM) were placed in duplicate in the rumen of experimental animals. Samples were ground through a 1-mm screen, tied with a silk thread and fastened to a 50-cm nylon cord weighted at the end. Bags were placed mid-ventrally in the rumen 0.5 h post-am feeding and removed at 2, 4, 6, 9, 15 and 24 h along with subsequent rumen and duodenal samples. The time zero bag was not placed in the rumen. Bags were removed from the rumen at assigned intervals, washed with deionized water and freeze dried. Samples were analyzed for rate of disappearance and CP percentage (16).

Rumen and duodenal samples were analyzed for CP percentage (16), ammonia nitrogen (16) and VFAs using a

Hewlett-Packard 5890 gas chromatograph (Avondale, PA) with an Alltech carbowax 20 M capillary column with a 10 mm x .53 mm x 1.33 um film thickness (Deerfield, IL), alpha amino nitrogen (28), liquid dilution rates (38), rate of passage (39) and total amino acids (34).

Total amino-acid content of duodenal and rumen samples was analyzed according to a modified version of Smith & Wonnacott, 1980 (34). A 0.1-g sample was placed in a round bottom 16 x 150 mm culture tube with 4 ml 6 N HCL, 0.2 ml norleucene (.125 umoles/ml internal standard) and sealed. These were placed in a hot oil bath (110-120 C) and hydrolyzed for 12 hours. When cool, a 1-ml aliquot was removed from the sample and placed in a culture tube. The sample was dried under compressed air, and 3 mls of dicloromethane was added. Samples were heated for 1 h at 120 C and dried. Addition of dicloromethane followed by drying was repeated 2 times. Three mls of acelytation reagent (3/1, dicloromethane/trifloroacetic acid) was added to the dried sample and allowed to stand for 20 min. These samples were dried, and 2 mls of dicloromethane was added. The samples were allowed to sit and placed in vials for amino acid analysis using a Hewlett-Packard 5890 gas chromatograph (Avondale, Pa.) with an Altech (Deerfield, IL) amino acid packing 60.96 cm. x .306 cm. ss column.

Six-Cow Nonlactating Trial

Six Holstein, second-lactation cows (approximately 610

kg), surgically equipped with rumen fistula and proximal duodenal T-cannulae, were assigned to each of 2 treatments in a switchback design. Treatments consisted of 1) a basal ration (control) with soybean meal (SBM) as its primary degradable protein source and 2) a basal ration (treatment) consisting of a 66/34 degradable/undegradable protein with corn gluten meal (CGM) and meat and bone meal (MBM) representing the undegradable portion. Other degradable protein sources in the ration consisted of alfalfa haylage, corn silage and barley (Table 4).

Treatments lasted for 3 weeks with a 1 week adjustment period. Cows were housed in tie stalls and had free access to water. Cows were fed ad libitum a total mixed ration twice daily at 0700 and 1500 h. The diet was offered in amounts calculated to provide 120% of their nutrient requirements (26). Feed refusals were recorded once daily. Feed was sampled once weekly, composited monthly, dried (2) and ground through a Wiley mill (Philadelphia, PA) using a 1-mm screen. Samples were analyzed for dry matter (DM) (2), crude protein (CP) (16), acid detergent fiber (ADF), neutral detergent fiber (NDF) (41) and ADF-ash (40).

Rumen Fermentation

At 4 h post-feeding (1000 h) on the first day of collection, 300 ml of rumen fluid was collected from the mid-ventral sac and analyzed for viable cellulolytic and total bacteria using habitat-stimulating media in anaerobic roll

tubes as described by Leedle and Hespell (19). Samples of whole digesta were strained through 4 layers of cheesecloth and placed in a Waring blender, flushed with carbon dioxide, agitated 1 min and serially diluted with anaerobic dilution solution. Roll tubes for total viable bacteria consisted of a total carbohydrate-based media at dilutions of x 10^{10} and x 10^{9}. Roll tubes for cellulose bacteria consisted of a cellulose/cellobiose-based media at dilutions of x 10^{9} and x 10^{8}. Roll tubes were incubated at 39 C for at least 7 days, and individual colonies were counted. Rumen samples were diluted 1:1 with a 50% formalin solution and placed in scintillation vials for total protozoa counts (11). A 1-ml aliquot of the preserved samples was pipetted into a test tube and mixed with two drops of 2% brilliant green. Samples were allowed to stand for at least 4 h; then 9 ml of 30% glycerol solution was added. A 1 ml aliquot was pipetted into a Sedgwick-Rafter counting chamber, and total protozoa were counted after 5 min.

On day 5, chromium mordanted straw was made using a modified procedure of Uden et al. (39) and added to the rumen via the fistula before feeding (0600) to determine particulate rate of passage. The modified mordanted straw consisted of 500 g chopped straw, 40 g sodium dichromate and 4 liters deionized water. Cobalt-EDTA was made according to Teeter and Owens (38) and added to the rumen via the rumen fistula at 6 h post-am feeding. Cobalt-EDTA was used to

determine liquid dilution rates. On days 5 through 7, rumen digesta samples were collected pre-feeding (0600) and at 2, 4, 6, 9, 15, 24, 36, 48, 60 and 72 h post-feeding. Rumen samples were collected from the mid-ventral sac and separated into liquid and solid fractions by centrifuging the samples at 7,000 x g for 15 min. The solid portion was lyophilized and ground through a Wiley mill using a 1-mm mesh screen. Solid samples were analyzed for CP (16) and chromium (T0 through T72) (39), using a Buck 200, Technicon Auto Analyzer (East Norwalk, Conn) to determine particulate rate of passage. Total amino acid content (34) was determined using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) with an Altech (Deerfield, IL) amino acid packing, 60.96 cm. x .306 cm. SS column. The liquid portion of the samples was analyzed for alpha-amino nitrogen (28), ammonia nitrogen (16), cobalt (38) by a Technicon Auto Analyzer (East Norwalk, Conn) to determine dilution rates, pH and VFAs using a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) with an Alltech (Deerfield, Il) carbowax 20 M capillary column with a 10 mm x 0.53 mm x 1.33 um film thickness.

Duodenal Digesta

Duodenal digesta was collected at 0, 4, 12, 24, 48 and 72 h post-feeding. The samples were separated into liquid and solid fractions by centrifuging at 7,000 x g for 15 min. The solid portion was lyophilized, ground through a Wiley mill using a 1-mm mesh screen and analyzed for total amino-acid

content (33, 34) and CP (16). Liquid samples were analyzed for ammonia nitrogen (16) and alpha-amino nitrogen (28).

Nutrient Digestibility

On day 1 through 5, fecal grab samples were obtained, composited, dried in a 50-C oven for 48 h, ground through a Wiley mill using a 2-mm mesh screen and analyzed for DM (2), CP (16), ADF and NDF (41) and ADF-ash according to Undersander et al. (40). ADF-ash was used as an internal marker to determine total-tract apparent nutrient digestibility.

Analysis of Data

The data collected in the experiment was analyzed by analysis of variance under a model appropriate for the single-reversal experimental design. Differences due to treatment were considered.

RESULTS

In Situ Disappearance

Protein disappearance rate (%/h), extent of degradation (%/24 h) and CP percentage from the in situ protein study are represented in Table 1. Disappearance rate (%/h) of SBM was significantly increased ($P<0.05$) (7.48 vs. 5.17 and 3.02 %/h). The percentage degradation rates over 24 h (Figure 1) were significantly different ($P<0.05$) for all protein sources, SBM, CGM and MBM (98.8, 45.5, 37.6%/24 h, respectively). Percentage of CP of the protein sources was significantly higher ($P<0.05$) in MBM over SBM or CGM (58.36 vs. 48.28, 50.70%). Ruminal CP percentage was significantly increased ($P<0.04$) in samples of control animals (24.20 vs. 18.05%). Alpha-amino nitrogen was also significantly increased ($P<0.05$) in duodenal samples of control animals (574.11 vs. 492.00 umoles/dl). Liquid dilution rates and rate of passage of particulate matter were unaffected by treatment, as were ammonia nitrogen and total VFAs. Molar percentage of butyric acid was increased ($P<0.05$) with control animals (19.39 vs. 15.83%) and is presented in Table 2. Percentage of total amino-acid content is presented in Table 3, and specific amino acids were increased ($P<0.05$). Percentage of serine was increased in treatment animals in rumen samples (2.40 vs. 1.92% of total amino acids).

Percentage of leucine and histidine was increased in duodenal samples of treatment animals (7.57 vs. 5.22 and 1.82 vs. 1.51% of total amino acid, respectively.)

Six-Cow Nonlactating Trial

The ingredient and chemical composition of SBM and CGM/MBM rations are shown in Table 4 and Table 5.

The results of treatment on rumen total viable bacteria, cellulolytic bacteria, percentage of cellulolytic bacteria and protozoal numbers are shown in Table 6. Treatments had no effect ($P>0.05$) on the parameters tested.

Ruminal crude protein percentage, alpha-amino nitrogen, ammonia-N, liquid dilution rate, rate of passage, pH and VFA concentrations are shown in Table 7. Ammonia-N was significantly increased ($P<0.05$) for control animals (9.56 vs. 7.88 mg/dl). Molar percentage of propionic acid was increased and the acetic/propionic ratio significantly decreased for treatment over control (25.16 vs. 19.36%, 2.63 versus 3.07%, respectively).

Results for dry matter intake were similar for both treatment and control (28.90 vs. 29.34 kg/d). Total amino acid contents of rumen and duodenal samples are represented in Table 8. Regardless of treatment, dry matter intake and amino-acid concentrations in rumen and duodenal samples were similar for both treatment and control, except for significant increases of ornithine in duodenal samples ($P<0.05$) in treatment over control (5.44 vs. 3.78% of total

amino acid).

The effects of degradable/undegradable protein ratios on CP percentage, ammonia-nitrogen and alpha-amino nitrogen in duodenal samples were similar (Table 9).

Apparent total-tract nutrient digestibility of the ration was unaffected by treatment. Crude protein percentage, acid detergent fiber percentage and neutral detergent fiber percentage were similar for both treatment and control (Table 10).

DISCUSSION

The in situ method for protein disappearance determination is descriptive of protein sources because it provides both rate and extent of disappearance (37). The rate of disappearance of protein DM %/h and extent of disappearance over 24 h was highest for SBM, CGM next and MBM last. A significant difference ($P<0.05$) was observed with SBM in %/h (7.48 vs. 5.17, 3.02 %/h). Over 24 h significant difference was observed among all 3 protein sources (98.8, 45.5, 37.6 %/24 h, respectively). Similar results for protein disappearance (%/24 h) of SBM and CGM were found in studies by Annexstad et al (1), Lu et al. (22) and Stern et al (35). The CGM DM disappearance (%/h) is much higher than previous studies but was grossly underestimated in pervious work (35, 36, 37). SBM is a more degradable protein source (72%) than CGM and MBM, which tend to be less degradable (45%, 40%, respectively) and represent by-pass protein (1, 6, 36). Degradability is important to the animal for two reasons 1) availability to the rumen microbes that require protein in the form of ammonia, amino acids and peptides and 2) availability of protein to the animal (6). The protein available to the animal is presented as dietary (by-pass protein), microbial protein. By-pass protein escapes digestion in the rumen, is digested in the lower tract of

the animal and is absorbed as amino acids to be used for production functions (18).

Percentage of CP in rumen samples from both the in situ study and the 6-cow non-lactating trial were higher for the SBM diet with a significant increase ($P<0.05$) in the in situ study (24.20 vs. 18.05%). In duodenal samples from both studies, CP percentage was higher for treatment animals fed a CGM/MBM-based diet. This represents a shift in protein availability, increasing the amount of amino acids presented to the small intestine from the combination of dietary, microbial and endogenous protein. Ammonia nitrogen present in both studies was increased in rumen samples with the addition of SBM (1, 20, 22). This increase was significant ($P<0.05$) in the 6-cow non-lactating trial (9.56 vs. 7.88 mg/dl). This is consistent with the increased percentage of CP seen in rumen samples (15) of animals fed SBM. Also, ammonia nitrogen (mg/dl) in duodenal samples was higher for both studies when CGM/MBM was fed. This was reported by Loerch et al. (21) and Santos et al. (30) where diets containing slowly-degraded protein sources supply more nitrogen to the small intestine than diets containing SBM. Alpha-amino nitrogen was higher in rumen samples of control animals and was significantly increased ($P<0.03$) in duodenal samples (574.11 vs. 492.00 umoles/ml) in the in situ study.

VFAs were similar between treatments for both studies except butyric acid and propionic acid. Butyric acid was

significantly higher ($P<0.05$) in the in situ study for SBM-fed animals (19.39 vs. 15.83 molar %) and also higher in the 6-cow non-lactational trial, as noted Mielke and Schingoethe (25). In the 6-cow non-lactating trial, molar percentage of propionic acid was increased and the acetic/propionic acid ratio was decreased ($P<0.05$) with treatment fed animals (25.16 vs. 19.36 molar %, 2.63 vs. 3.07%, respectively). VFAs were not expected to be different since varying a protein source should not affect rumen VFAs greatly unless such a change causes a great deficiency in nitrogen available to the rumen microorganisms (5). The fact that butyric acid was increased in the in situ trial and propionic acid increased in the 6-cow non-lactating trial is probably not due to a protein deficiency but a digestibility difference.

Calculation of estimated amino-acid supplies as a percentage of requirements suggests that methionine, lysine, histidine and arginine are the amino acids in shortest supply in growing cattle (4). Derrig et al. (12) have suggested that the order of limiting amino acids for milk protein synthesis in a lactating cow is phenylalanine, methionine, lysine and threonine. Others have cited histidine, lysine, tyrosine and methionine as limiting milk production (8, 13). Research shows consistency as to the importance of amino acids to milk production, but the means of producing results for specific amino acids supplied to the animal is still under investigation. Even though there significant difference

($P<0.05$) was observed in this study regarding specific amino acids from rumen and duodenal samples, it is not biologically significant. However, the trend for total amino-acid content to increase in duodenal samples of treatment animals could possibly be an increase in amino acids available to the host. A concern in feeding the less degradable ration could be that an increase in the amount of protein escaping ruminal degradation in cows fed CGM/MBM diet compared with SBM could be counterbalanced by less microbial synthesis in the rumen.

Bacterial and protozoal populations analyzed in the non-lactation trial were not influenced by degradable/undegradable concentrations or source. This agrees with results by Stern et al. in 1983 (36) where no effect was found on bacterial populations from animals fed CGM. However cellulolytic and percentage of cellulolytic populations tended ($P<.11$) to be higher for control. This could be a response of bacterial populations to ration containing a very degradable protein source, SBM, as its major degradable protein (5) and a lack of response to CGM/MBM, a less degradable protein. If the microbial yield from the CGM/MBM diet was lower due to the lower degradability, there would be less microbial synthesis in the rumen (42). Proteolytic enzymes must contact proteins through some interaction involving water so that soluble proteins are more quickly degraded and insoluble proteins are often degraded slowly (7). SBM is approximately 72% degradable (6); therefore, if energy is sufficient, CP

can be maximally utilized (6, 18), or if CP is in excess of the microbes' capability, an increase in ruminal ammonia nitrogen can occur as seen in both studies, significantly ($P<0.05$) in the non-lactating study (9.56 vs. 7.88 mg/dl).

Rumen liquids and solids move at different rates. The turnover rates of liquids influence rumen microbial populations and outflow of nutrients. Soluble substances moving with the liquid phase are more apt to pass through the rumen at higher liquid turnover rates (4). Therefore, faster liquid dilution rates can change the microbial population, increasing the number of young rumen microbes that are important as energy. Liquid dilution rates of SBM-fed animals were increased but not significantly ($P>0.05$). The rate of passage between diets was not different.

Dry matter intake and apparent digestibilities of crude protein, acid detergent fiber and neutral detergent fiber were not affected by dietary treatment. This agrees with Crooker el al. (10) who compared the effects of SBM and formaldehyde-treated SBM on DM intake and apparent digestibilities. Also, Forster et al. (14) fed various CGM diets, and digestibilities were not influenced by the source of CP.

CONCLUSIONS

Results from the in situ trial concluded that soybean meal is a rapid ruminally-degradable protein source. This was also observed in the non-lactating trial with the increased ammonia-N content of rumen samples of animals fed the control ration (SBM). This degradability of SBM could work to the animal's disadvantage. If a protein source is rapidly degraded in the rumen, breakdown byproducts could be lost across the rumen wall and decrease microbial efficiency, which would be a waste of protein. Corn gluten meal and MBM ruminally degrade at slower rates based on DM disappearance. The non-lactating trial confirmed the ruminal bypass of CGM and MBM by the increase seen in the percentage of crude protein, total amino acid content and ammonia-N content of duodenal samples of animals fed treatment ration. This could benefit the host animal because protein available to the host without microbial synthesis is more efficient.

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TABLE IV1. Dry matter disappearance (%/h), DM percentage degraded over 24 h and CP percentage of SBM, CGM and MBM from dacron bags suspended in the rumen of non-lactating cows.

Item	Treatment			SEM
	SBM	CGM	MBM	
Dry matter disappearance (%/h)	7.48^{b}	5.17^{a}	3.02^{a}	1.10
DM degraded over 24 h (%)	98.80^{c}	45.50^{b}	37.60^{a}	1.88
Crude protein (%)	48.28^{a}	50.70^{a}	58.38^{b}	2.71

a, b, c
Means in the same row with different superscripts differ ($P<0.05$).

TABLE IV2. Effect of treatment on ruminal fermentation characteristics determined from rumen and duodenal samples in an in situ trial involving non-lactating animals.

Item	Ruminal			Duodenal		
	Treatment	Control	SEM	Treatment	Control	SEM
Crude protein, (%)	18.05[a]	24.20[b]		21.35	16.31	1.18
Ammonia nitrogen (mg/dl)	4.14	5.46		2.44	1.97	0.36
Alpha-amino nitrogen (umoles/dl)	638.11	684.01		492.00[a]	574.11[b]	1.11
Liquid Dilution Rate (%h)	6.03	7.25				0.45
Rate of passage, (%h)	3.55	3.76				0.61
Total VFA (mmol/L)	114.05	107.06	2.94			
VFA (molar %)						
Acetic	64.02	62.22	0.84			
Propionic	27.68	19.88	1.88			
Isobutyric	1.21	1.09	0.06			
Butyric	15.83[a]	19.39[b]	0.75			
Isovaleric	2.25	2.16	0.18			
Valeric	3.13	2.29	0.22			
Acetic/Propionic Ratio	2.39	3.21	0.21			

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE IV3. Effect of treatment on total amino-acid content from rumen and duodenal samples in an in situ trial involving non-lactating animals.

Item	Ruminal			Duodenal		
	Control	Treatment	SEM	Control	Treatment	SEM
Total amino acids (umoles/g)	65.04	54.24		59.03	69.38	16.22
	% of total amino acids					
Alanine	9.82	10.57	2.03	10.73	12.03	1.39
Valine	4.77	3.48	0.08	4.03	6.08	1.91
Glycine	34.52	22.48	7.61	27.39	15.87	2.19
Isoleucine	6.61	8.09	0.83	4.44	6.94	1.87
Leucine	6.63	7.74	0.57	5.22[a]	7.57[b]	0.19
Proline	9.20	5.20	1.88	12.92	8.31	5.65
Serine	1.92[a]	2.40[b]	0.18	13.97	2.07	4.96
Methionine	1.86	2.15	2.14	0.93	0.04	0.98
Phenylalanine	9.01	10.67	1.78	12.07	8.07	5.25
Histidine	1.01	1.31	0.51	0.51[a]	1.82[b]	0.01
Tyrosine	1.78	3.11	1.24	1.20	4.37	0.92
Ornithine	8.34	8.56	1.98	6.20	13.04	3.00
Lysine	1.33	1.83	0.30	0.88	6.01	0.85
Arginine	2.76	2.81	0.82	3.71	8.52	2.30

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE IV4. Composition of total mixed ration.

Ingredients	(% Dry Matter)	
	Treatment	Control
Alfalfa haylage	10.03	8.15
Corn silage	50.16	50.13
Rolled barley/corn	27.59	26.69
Beet pulp	6.27	6.27
Meat and bone meal	2.51	---
Corn gluten meal	3.13	---
Soybean meal	---	7.77
Dicalcium phosphate	0.06	0.37
Limestone	0.25	0.69

TABLE IV5. Nutrient content of total mixed ration.

Nutrient (DM basis)	Treatment	Control
	- - - - % - - - -	
Dry Matter	64.10	67.90
Crude protein	14.95	15.00
Acid detergent fiber	24.50	23.60
Neutral detergent fiber	42.68	41.40
Ne_l (mcal/kg)	1.65	1.64
ASH	1.55	1.45

TABLE IV6. Effect of treatment on ruminal microbial numbers.

Item	Treatment	Control	SEM
Bacteria			
Total, x 10^{10}	13.14	12.33	1.48
Cellulolytic, x 10^{9} /ml	49.30	52.46	7.60
Cellulolytic, (%)	5.83	8.46	1.21
Protozoa, x 10^{3} /ml	6.85	5.03	0.67

TABLE IV7. Effect of treatment on ruminal fermentation characteristics.

Item	Treatment	Control	SEM
Crude protein (%)	16.07	17.63	0.88
Alpha amino nitrogen (umoles/dl)	642.80	672.80	23.50
Ammonia-N (mg/dl)	7.88[a]	9.56[b]	0.51
Liquid dilution rate (%\h)	6.57	7.73	0.47
Rate of passage (%\h)	2.65	2.49	0.53
pH	6.06	6.18	0.08
Total VFA (mmol/L)	90.82	105.99	4.53
VFA (molar %)			
Acetic	64.42	57.50	2.40
Propionic	25.16[b]	19.36[a]	1.44
Isobutyric	0.89	0.99	0.05
Butyric	10.52	12.51	0.66
Isovaleric	1.34	1.52	0.08
Valeric	1.25	1.37	0.08
Acetic/Propionic Ratio	2.63[a]	3.07[b]	0.10

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE IV8. Effect of treatment on total amino-acid content.

	Ruminal		SEM	Duodenal		SEM
	Control	Treatment	SEM	Control	Treatment	SEM
Total amino acids (umoles/g)	66.53	46.71		35.78	44.87	9.60
	---------- % of total amino acids ----------					
Alanine	9.81	9.38	1.16	4.88	6.21	1.71
Valine	4.18	5.16	0.78	4.22	4.82	0.75
Glycine	27.51	28.09	3.22	26.16	34.46	4.89
Isoleucine	5.76	6.75	0.99	10.35	6.26	1.60
Leucine	7.07	11.09	1.89	17.42	12.55	1.88
Proline	12.14	11.68	1.47	13.77	15.28	1.43
Serine	3.03	2.47	1.70	3.73	2.65	0.58
Methionine	5.42	2.59	0.70	2.46	2.43	0.75
Phenylalanine	11.42	7.11	4.43	3.84	5.50	0.75
Histidine	1.20	1.19	0.10	1.11	0.64	0.16
Tyrosine	1.86	3.05	0.54	0.94	1.16	0.27
Ornithine	5.68	5.96	0.53	3.78[b]	5.44[a]	0.72
Lysine	1.22	1.55	0.28	1.91	1.40	0.36
Arginine	2.06	2.37	0.20	2.16	1.91	0.53

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE IV9. Effect of treatment on duodenal digesta nitrogenous components.

Item	Treatment	Control	SEM
Crude protein (%)	18.24	17.43	0.80
Ammonia-N (mg/dl)	3.66	3.06	0.39
Alpha-amino nitrogen (umoles/dl)	546.10	523.80	25.50

TABLE IV10. Effect of treatment on total-tract apparent nutrient digestibility.

Item	Treatment	Control	SEM
Crude protein (%)	68.73	66.63	3.20
Acid detergent fiber (%)	49.32	41.93	3.42
Neutral detergent fiber (%)	50.66	50.70	2.31

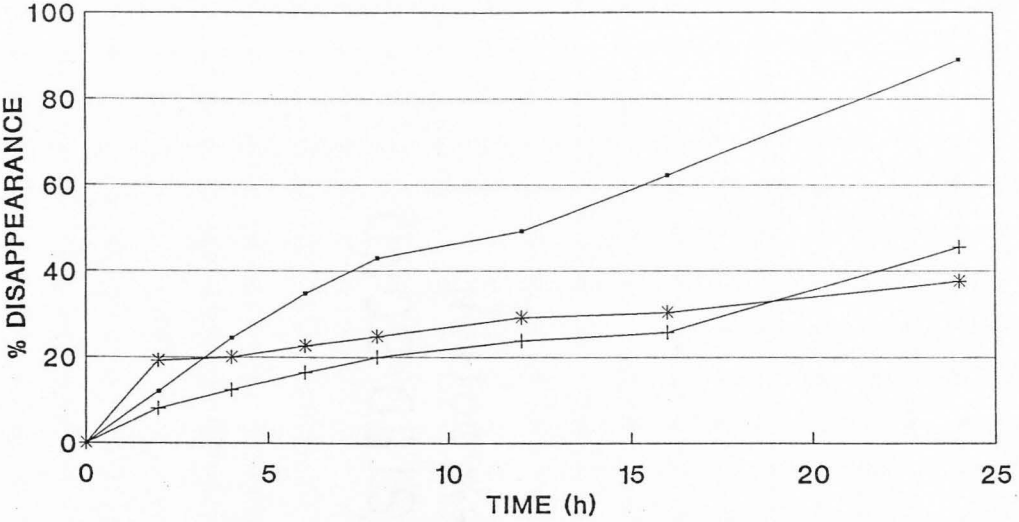

Figure IV1. Rate of in situ DM disappearance (%/h) of protein sources. Protein sources consisted of SBM (·), CGM (+), MSM (*).

CHAPTER V

THE EFFECT OF DEGRADABLE AND UNDEGRADABLE PROTEIN SOURCES ON MILK COMPOSITION AND PRODUCTION OF EARLY-LACTATION DAIRY COWS INJECTED WITH BOVINE SOMATOTROPIN

ABSTRACT

Twenty multiparous Holstein cows in early lactation were assigned to 1 of 2 treatments to examine the influence of varying dietary crude protein sources (degradable versus degradable/undegradable) while receiving 500 mg somatotropin (bST) injected every 2 weeks. The effects of degradable/undegradable protein sources were determined on milk production, milk components and nutrient digestibility. Treatments consisted of 1) a basal ration containing soybean meal (SBM, control) as its primary degradable protein source and 2) a ration containing corn gluten meal and meat and bone meal (CGM/MBM, treatment) as its primary undegradable protein source.

For animals receiving somatotropin, the undegradability of protein sources did not influence dry matter intake and body weights. Milk yield, 3.5% fat-corrected milk (FCM) and production efficiency were not affected by somatotropin administration and increased percent undegradable protein. Milk fat and solids-non-fat were not significantly increased ($P>0.05$) with addition of an undegradable protein source.

Lactose was significantly increased ($P<0.05$) as the percentage of degradability of the diet increased (5.00 vs. 4.9%) but not of any biological significance. Total protein (g/l) and casein percentage were significantly increased ($P<0.05$) with treatment (31.49, 28.69 g/l and 62.11, 58.24%, respectively). Apparent total-tract digestibility of nutrients was unaffected by treatment; however, crude protein percentage digestibility tended ($P<0.08$) to be higher as undegradability increased (67.85 vs. 62.83%).

The combination of degradable and undegradable protein sources, together with sufficient energy, should have resulted in a more efficient utilization of these sources for milk production, but this was not the case.

INTRODUCTION

High producing cows have a high requirement for protein in the form of amino acids, a requirement that is fulfilled by dietary protein, microbial protein and endogenous protein (2, 40) and provides amino acids for absorption by the small intestine. New systems for determining requirements for dietary protein sources have emphasized the need for protein to be resistant to rumen proteolysis (10, 36), and degree of resistance depends on degradability. Dietary protein sources are either degradable, such as soybean meal (SBM) (10, 13) and therefore susceptible to proteolysis, or undegradable, such as corn gluten meal (CGM) and meat and bone meal (MBM) (2) and less susceptible to proteolysis. The percentage of degradability affects the amount that reaches the small intestine to be utilized by the animal. Degradable dietary protein entering the rumen is broken down to ammonia, which is necessary for microbial growth through protein synthesis or to volatile fatty acids (VFA) necessary for animal production (8, 11). The microbes can provide sufficient protein for maintenance, slow growth and early pregnancy (32, 40) but cannot maintain the increased demands for high milk production or rapid growth in young animals (30). Therefore, manipulating dietary protein sources is necessary to acquire the exact ratio of degradable/undegradable protein

supplements to accommodate the demand for increased milk production. Because of today's increased demands put on high producing animals, that amount of dietary protein has increased (38).

Demands put on high-producing cows are even greater when animals are injected with bovine somatotropin (bST). Bovine somatotropin is a naturally-occurring protein in the pituitary gland of cattle. BST, when injected into the animal, is intended to supply cows with naturally-occurring somatotropin to increase milk production efficiency (42). Much research has been conducted on the effects of bST in lactating dairy cows (7, 12, 33, 34, 42). Administration of bST results in a dramatic shift in the partitioning of available energy towards milk yield at the expense of body tissue (41). This partitioning of nutrients is mediated by the endocrine system with insulin, glucagon and bST most likely responsible for energy and protein partitioning (14). Because of the effect bST has on nutrient utilization, an essential area of investigation is exactly how dietary protein is being manipulated (14, 15).

Protein degradation is influenced by a number of functions in the rumen besides percent degradability without taking into consideration the influence of bST. Rate of passage of the digesta, feed intake, density of the ration, particle size and degradability of the protein source (41) all affect the amount of amino acids that will reach the

small intestine. When feed intake increases above the maintenance requirement of the animal, rate of passage of nutrients increases, as explained above, and nutrient digestibility of the ration can decrease. Specific gravity can affect rate of passage in that if the diet is more energy dense (i.e., increased concentrates in the diet), pH will decrease and liquid dilution rates decrease (7). A smaller particle size of the ration can result in an increased particulate rate of passage through the rumen, therefore decreasing the amount of nutrients digested. The degree to which proteins are broken down also depends on the rate of degradation. Slowly-degraded proteins, such as meat meal (MM) and corn gluten meal (CGM), can bypass ruminal degradation (bypass protein) in the rumen by the microorganisms and be utilized more efficiently in the gastrointestinal tract (37). This could be used to the animals' advantage because protein that escapes rumen fermentation could be used either to improve milk production or to reduce the amount of conventional protein source required (22, 40). Degradable protein is not used as efficiently as undegradable protein due to the increased loss of ammonia from the rumen; thus, less protein is utilized by the host (23, 27, 29). Soybean meal is highly degradable and more than sufficient in supplying microbes with nitrogen to be used as ammonia. However, its easy degradation is less efficient due to ammonia loss across the rumen wall, which

will be cleared from circulation either by recycling ammonia in saliva or in urine as waste.

Some studies have reported an increase in milk production when diets were formulated to have low ruminal protein degradabilities (19, 24) while others have not (1, 17). New systems (30) are under investigation to determine the exact requirements for dietary protein in high-producing dairy cows. Some feel the new requirements for rumen undegradable protein are too high (36), and the effects of bST have not yet been considered. In vivo and in situ studies have estimated rumen protein degradation (18, 31). However, studies where diets are formulated on estimated rumen degradability are limited, and the number of studies including bST and rumen protein degradability are nil (14, 15).

The objective of this study was to test effects of feeding diets formulated to contain a high or low degradable protein source, in combination with 500 mg bST injected every 2 weeks, on milk production and milk components response in early-lactating dairy cows.

MATERIALS AND METHODS

Twenty multiparous Holstein cows, selected based on milk production and days postpartum (60+/- 7 days), were allotted to 1 of 2 treatments following a 2-week adjustment period. A 10-week lactation trial was conducted to evaluate in combination slowly rumen degradable protein source (CGM/MBM) as partial replacement for a rapidly degradable protein source (SBM) in a complete mixed ration (Table 1). The 2 treatments consisted of 1) a basal ration in which 34% of the total protein was supplied by CGM and MBM, representing the undegradable fraction (treatment) and 2) a basal mixed ration with SBM the single degradable protein source (control). All animals received subcutaneous injections of 500 mg of bST every 2 weeks, administered in 1 of 4 alternating sites at approximately 1200 h.

Each cow was assigned to an individual Calan gate and fed twice daily (0630 and 1830 h). Refusals were measured and adjustments made in daily offerings so that each cow was fed ad libitum (30). Feed was sampled once weekly, composited monthly, dried (3) and ground through a Wiley mill (Philadelphia, PA) using a 1-mm screen. Samples were analyzed for dry matter, (DM) (3), crude protein (CP) (21), acid detergent fiber, (ADF), and neutral detergent fiber, (NDF) (45) and ADF-ash (44) (Table 2).

Milk yield was recorded twice daily (0500 and 1700 h) with weekly milk samples being composited from the last 4 consecutive milkings for each cow and analyzed for percentage of lactose, fat, solids-non-fat (SNF) and somatic cell count (SCC) using a (Multispec M Infrared Analyzer from Wheldrake, York, England). Total milk protein (casein, whey and non-protein-nitrogen) was analyzed. Determination of total milk protein was accomplished according to A.O.A.C. methods (3). The milk components, total protein (g/l), whey, casein and percentage of non protein-nitrogen (NPN) were calculated from 1 initial milk sample. Percentage of total protein was analyzed using 0.2 mls of milk followed by digestion with sulfuric acid through the Kjeldahl procedure (3) and titration with boric acid as an indicator. Whey was determined by first adjusting a 10-g milk sample to Ph 4.6 with 0.1 N HCL, followed by filtering sample through Whatman #5 paper. One gram of filtrate was used for Kjeldahl digestion. Non-protein-nitrogen (NPN) was analyzed using 10 g milk plus 5 g of 6% trichloroacetic acid (TCA) to make a 2% solution of TCA which was filtered through Whatman # 42 paper. Five mls of filtrate was used for digestion. Casein was calculated by difference.

Body weights were recorded every 2 weeks.

Nutrient Digestibility

Fecal samples were collected twice daily (0630 and 1800 h) on the last 5 consecutive days of the experiment and were

composited for each cow. Following drying at <60 C, fecal samples were ground through a Wiley mill using a 1-mm mesh screen and analyzed for DM (3), CP (21), ADF and NDF (45) and ADF-ash according to Undersander et al. (44). ADF-ash was used as an indicator to determine total-tract apparent nutrient digestibility.

Analysis of Data

All data were subjected to analysis of variance procedures, and the means for the treatments were compared for significant difference.

RESULTS

Dry Matter Intake and Milk Production

Performance and body weights of lactating cows fed the degradable or undegradable protein sources did not differ ($P>0.05$). Dry matter intake, milk yield, 3.5% FCM, production efficiency and the change in body weights are shown in Table 3. Protein source or degradability did not influence mean daily milk yield. Production efficiency (3.5% fat-corrected milk/DM intake) was not significant ($P>0.05$) between treatments. However, the ration containing the degradable protein source (SBM) tended ($P<0.10$) to be higher (2.56 vs. 2.39 fat-corrected milk/DM intake). Milk components examined are presented in Table 4. Percentage milk fat, somatic cell count and SNF were not significantly ($P>0.05$) different between treatments. Percentage of lactose was significantly ($P<0.05$) decreased for treated (SBM) animals over controls (CGM/MBM), (4.91 vs. 5.00%). Total milk protein and percentage of casein were significantly increased ($P<0.05$) by protein undegradability (31.49, 28.69 g/l and 62.11, 58.24% of total protein, respectively).

Digestibility Coefficients

Apparent total-tract nutrient digestibility of the ration was unaffected by treatment. Crude protein percentage , acid

detergent fiber percentage and neutral detergent fiber percentage were similar for both treatment and control. However, CP digestibility of animals fed the treatment diet tended ($P<0.08$) to be higher than control-fed cattle (67.85 vs. 62.83%) Table 5.

DISCUSSION

At the beginning of lactation, cows must undergo many altered metabolic functions and partitioning of nutrients to accommodate the increased demands of the mammary gland for milk production (5). During this time, nutrient requirements of the mammary gland are greatly increased relative to the metabolism of the total animal (7). Results from bST administration confirm that bST does increase milk production in lactating dairy cows (6, 26, 33). Administration of exogenous bST increases the partitioning of nutrients to the mammary gland. Changes that occur during lactogenesis in ruminants are 1) a change in fatty acid synthesis (i.e., increased lipolysis and decreased lipogenesis), 2) increased gluconeogenesis and increased use of fats as an energy source and 3) increased use of nutrients, specifically an increase in carbohydrate metabolism (glucose) and an increase in insulin responsiveness of various tissues (20).

The nutrients available to the mammary gland come from dietary intake, fat metabolism and muscle mass. BST influences the partitioning of energy and protein to the mammary gland as well as glucagon and insulin (14).

Balancing a ruminant ration must take into consideration protein available to microbes as well as host. The protein available to the host is microbial protein and

ruminal escape protein amino acids and/or peptides that are presented to the small intestine where they can benefit the animal.

Animals injected with bST, when fed either of the protein sources (SBM or CGM/MBM) for 70 d, showed no effect on feed intake or body weight gain. In this respect, the results of the study are consistent with results of similar short-term studies with animals receiving bST (28) at varying dietary CP intakes (4, 19). Increase in feed intake has been well documented with bST administration (12), but results vary as to amount of bST administered (20) and stage of lactation of the animal (35). Previous studies have shown feed intake to be lower at peak lactation than mid-lactation, with cows in negative energy balance at peak lactation and positive energy balance by mid-lactation (9, 28). In the present study, animals were 60 days postpartum at the initiation of the project. At peak lactation, intake appears depressed relative to increased milk production due to administration of bST. High milk yields require high intakes, but the animal cannot physically consume enough food because of rumen limitations to meet energy demands of peak lactation (7). Crude protein percentage in both diets was low (14.5%); therefore, a decrease in dry matter intake could result that would decrease ruminal microbial synthesis. Research has shown that cows fed rations deficient in protein do not respond as favorably to bST injections as cows fed adequate in protein (14), but

low CP percentage was necessary to ensure accurate comparisons. If the percentage of crude protein in the diet was not kept below the animals' requirements, protein would not be the first limiting nutrient, and a valid comparison could not be made (23).

Production of milk and production efficiency were not significantly ($P>0.05$) affected by the degradability of the protein source in animals receiving bST. De Boer and Kennelly (15) and others reported that as percentage of CP increased in the diet and animals were injected daily with 33 mg of bST, milk production increased (15, 22, 27). Forster et al. (19) found milk production to increase as degradability decreased in early-lactation cows fed 14% dietary CP. Milk production increases with bST injection alone have been reported (6, 34).

Lactose was the only milk component for control animals that was significantly ($P<0.05$) increased in cows fed the more degradable ration (SBM) (5.00 vs. 4.91%) but not enough to be of any biological value. In previous studies, percentage of milk lactose did not alter greatly during lactation regardless of bST injections or percentage of CP or degradability in the ration (4, 19, 25, 28). Changes in milk protein components were significantly increased in animals fed the less degradable protein source. Total protein and percentage of casein increased by feeding the lower-ruminally degradable protein source.

Milk protein content is affected by nutritional factors (39, 43). Parameters specifically compared in this project were the response of various milk protein components to rumen degradability of protein in the ration. Milk protein content has been altered in response to the cow's diet in various experiments (39, 43). DePeters et al. (16) reported increases in total milk protein (2.1 vs. 2.4 lb/d) with the addition of 3.5% fat to the ration. Also, NPN increased with the addition of 3.5% fat (from 5.98 to 6.68%) but the increase reported in milk protein tends to be small (43).

The mechanism that regulates the synthesis of milk protein remains to be clearly understood; however, through infusion experiments utilizing casein, results have consistently improved milk protein concentration by 5% (43). The response to milk protein content is most likely due to the change in dietary protein supply during the passage of amino acids to the small intestine (32). Previously in this study, the total amino acid supplied to the small intestine was increased by the less degradable protein source in the ration fed in this trial (46), which suggests that the effect on milk protein content is observed when dietary protein sources are less degradable (16, 43).

Based on research over the last 20 years, the assumption was that casein proteins accounted for 78% of the total nitrogen in cow's milk, whey proteins for 17% and NPN for 5% (16). In results from animals fed the less degradable

protein source in our trial, casein was lower (62.11%), while whey and NPN were higher than reported values for treatment-fed animals (23.27 and 14.80%, respectively). Therefore, milk protein components can be manipulated by the degradability of the protein source. However, the effects of amount of protein undegradability and/or type of undegradable protein in the diet produce results with variability and inconsistency (43).

Apparent digestibilities of ADF, NDF or CP were not influenced by the source of dietary CP. However, the CP percentage digestibility tended ($P<0.08$) to be increased with decreased degradability (67.85 vs. 62.83%). Previous short-term studies with bST have demonstrated that digestibilities of nutrients are not altered (33).

CONCLUSIONS

BST when injected daily into lactating dairy cows was determined to increase milk production (46). The combination of corn gluten meal and meat and bone meal was low in undegradability, representing a bypass protein. Together with sufficient energy, the results should have channeled towards a more efficient utilization of degradable and undegradable protein sources for milk production. Animals receiving bST and various percentage of protein degradabilities should have had a greater effect on milk production (18, 33), but this was not the case. However, milk proteins were effected by protein degradability. Possibly some effect on milk production might have been accomplished if the CP percentage in the diet were increased (16-17%), increasing DM intake (13, 24).

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TABLE V1. Composition of experimental diet.

Ingredient (DM %)	Control	Treatment
Corn silage	50.13	50.16
Alfalfa Hay	8.15	10.03
Rolled barley/corn	26.69	27.59
Beet pulp	6.27	6.27
Corn gluten meal	---	2.51
Meat and bone meal	---	3.13
Soybean meal	7.77	---
Dicalcium phosphate	0.37	0.06
Limestone	0.69	0.25

TABLE V2. Chemical analysis of the treatment diet.

Nutrient (DM basis)	Control	Treatment
Dry Matter, (%)	69.75	71.30
Crude protein, (%)	14.50	14.30
Acid detergent fiber, (%)	22.00	20.40
Neutral detergent fiber, (%)	41.90	43.20
Ne_l (mcal/kg)	1.67	1.67
Ash, (%)	1.57	1.50

TABLE V3. Effect of treatment on mean daily dry matter intake, milk yield, production efficiency and body weight.

Parameter	Control	Treatment	SEM
DM Intake (kg/d)	22.37	22.12	0.90
Milk yield (kg/d)	35.72	34.08	1.90
3.5% Fat corrected milk (FCM) (kg/d)	35.15	35.19	2.10
Production efficiency (3.5% FCM/DMI)	2.56	2.39	0.07
Body weight change (kg/10 weeks)	13.20	32.30	10.45

TABLE V4. Effect of treatment on milk composition.

Item	Control	Treatment	SEM
Fat, (%)	3.42	3.71	0.17
Lactose, (%)	5.00[b]	4.91[a]	0.03
Solids non-fat, (%)	8.89	8.78	0.08
Somatic cell count (100/ml)	101.72	120.36	27.20
Total protein, (g/l)	28.69[a]	31.49[b]	0.07
% Total Protein:			
Whey, (%)	26.07	23.27	1.85
Casein, (%)	58.24[a]	62.11[b]	0.67
NPN (%)	16.72	14.80	0.97

[a,b]Means in the same row with different superscripts differ ($P<0.05$).

TABLE V5. Treatment effects on apparent total-tract nutrient digestibilities.

Item	Control	Treatment	SEM
Crude protein, (%)	62.83	67.85	1.94
Acid detergent, (%)	56.45	53.72	2.14
Neutral detergent, (%)	67.56	68.62	1.38

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EDUCATION

Doctor of Philosophy (June, 1990), Utah State University, Department of Animal Science, Effect of Bovine Somatotropin on Rumen Fermentation, Digesta, Rate of Passage and Digestibility of Nutrients in Lactating Dairy Cows.

Master of Science, University of Arizona, Tucson, Arizona. May 1987, Department of Animal Science, Utilization of Chopped Wheat in Complete Rations for Lactating Dairy Cows.

Bachelor of Science, University of Arizona, Tucson. May 1984, Department of Animal Science, Animal Health Science.

PROFESSIONAL EXPERIENCE

July 1987 to present: Graduate Research Assistantship, Department of Animal Science, Utah State University, Logan.

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Fall 1985 to May 1987: Graduate Research Lab Technician, Department of Animal Science, University of Arizona, Tucson.

Jan 1987 to May 1987: Teaching Assistant, Department of Animal Science, University of Arizona, Tucson. #430 Dairy Nutrition Lab.

AWARDS

- Research Assistantship, Utah State University, Logan. July 1987 to July 1990.
- Deans List, Utah State University, Logan. January 1988 to June 1988.

PROFESSIONAL ACTIVITIES

University of Arizona, Tucson, Block and Bridle Club member, 1982 to 1984.

University of Arizona, Tucson, Animal Science Graduate Student Association member, 1985 to 1987.
- Organize meetings
- Planned fund raisers, sold cheese weekly
- Traveling arrangements

Representative for ADVS Department as a Graduate Student Senator, Utah State University, Logan, Utah, 1989 to 1990.
- Executive chairperson GSS
- Chairperson in charge of activity committee
- Library committee member
  - Responsible for informing the ADVS graduate students of rights, responsibilities and funds available

PROFESSIONAL ORGANIZATIONS

American Dairy Science Association, (1987 to present)
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RESEARCH EXPERIENCE

Surgical procedures - duodenal T cannulation and rumen fistulation.

Wet Chemistry - proximate analysis, Van Soest Forage Analysis, ammonia nitrogen, alpha amino nitrogen determinations, amino acid analysis (including diaminopimelic acid), blood amino acids, protein determinations (Kjeldahl, Hach), volatile fatty acids, chromium and cobalt analysis, milk protein (Udy), fat (Babcock) and solids-non-fat (Watson) determinations, simple sugar analysis.

Equipment use - spectrophotometer, Hach, high-speed centrifuge, amino acid analyzer, atomic absorption spectrophotometer, bomb calorimeter, distillation apparatus, lyophilizer, aerobic and anaerobic fermentation systems.

Microbial population determinations using roll tube and anaerobic glove-box techniques. Also protozoa determinations.

Digesta passage collection procedures.

Computers - Working knowledge of Harvard Graphics, Word Star, Word Perfect and other software packages.

ARTICLES IN PRESS

Kent B. A., M. Olson, M. J. Arambel, M. D. Winsryg, and J. L. Walters. 1988. Effect of live bacterial inoculant on ensiling characteristics of corn silage. J. Dairy Sci. 71 (supplement 1) Abstract.

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PRESENTATIONS

1989 American Dairy Science Association/ American Animal Science Association Meeting.

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