ABOMASAL NUTRIENT INFUSIONS FOR STEERS FED LOW QUALITY ROUGHAGE OR HIGH CONCENTRATE RATIONS

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CHAPTER I

INTRODUCTION

The ruminant has a digestive tract which allows a wide variety of feedstuffs to be digested. As an energy source, the ruminant can use poor quality roughages or highly digestible concentrates. As a protein source, the ruminant can use non-protein nitrogen or pre-formed protein. These various feedstuffs are usable due to microbial digestion in the rumen. Ruminal microbial protein forms a large portion of the protein supply for the ruminant animal post-ruminally.

This complex digestive tract raises several questions not answerable through study of non-ruminants. First, what principles govern feed intake of different diets? Is the digestibility of one feedstuff or nutrient influenced by feeding another feedstuff or nutrient? Is digestibility constant within a feedstuff at different levels of feed intake? Is efficiency of metabolism in the rumen or in tissues independent of nutrient supply?

This thesis examined the influence of post-ruminal nutrient supply on feed intake and nitrogen retention of cattle fed low quality roughage or highly digestible concentrate rations.

Low quality roughages are a major energy source for maintenance of ruminant animals. Intake of roughage is limited due to the bulkiness of the ration and the physical capacity of the digestive tract.

Digestion of roughages relies upon the cellulolytic bacteria to degrade the fiber and utilize the released energy together with available nitrogen sources to multiply and replace bacteria continually flushed to the small intestine.

Energy and nitrogen requirements are interrelated. At some point, each will limit microbial growth. These interrelated requirements are difficult to quantitate. Furthermore, animal performance may or may not be limited by microbial protein production.

Winter roughages are considered to be of low quality since much of the soluble nutrients have been leached away. This leaves a cellulose skeleton which is partially lignified. Since both protein and carbohydrates have been depleted, either available energy or nitrogen could limit animal performance. When ruminal ammonia is deficient, adding non-protein nitrogen (NPN) for the fuminal microbes will increase digestibility and possibly forage intake. But even when ammonia is adequate, the amount of microbial protein reaching the small intestine may be inadequate to meet the ruminant animal's protein need for maintenance. As compared with addition of NPN, adding intact protein to a range grass ration usually increases weight gain or reduces weight loss. Part of the intact protein will escape ruminal digestion to reach the small intestine and supplement microbial protein. This would suggest that microbial protein supply may be inadequate to meet the animal's need for maintenance although direct measurements have not been made to date.

Microbes in the rumen degrade much of the dietary protein fed. Such degradation is detrimental when the dietary protein has a good amino acid balance since the catabolized protein serves merely as a source of

ammonia for the microbes. Some researchers have treated proteins or amino acids in attempts to more extensively bypass destruction in the rumen and thereby supplement the diet more efficiently. But the circumstances under which increased protein bypass will increase animal performance remain unknown. If the post-ruminal protein supply is already adequate, increased bypass is useless.

The first part of this dissertation concerns post-ruminal nutrient supplementation of steers fed a roughage ration. Research was conducted to determine if supplemental energy or protein provided post-ruminally would improve feed utilization and intake of a low quality roughage ration. This ration is similar to that consumed by cattle grazing range grass during the winter.

The second part of this dissertation concerns post-ruminal supplementation of growing steers. Rations for vast numbers of cattle in feedlots contain large amounts of digestible energy. With readily available energy in the rumen, microbial growth and protein production are high, but so is the animal's energy supply. Post-ruminal protein has been hypothesized by many workers to limit feedlot performance since cattle performance is often lower with NPN supplements than other protein supplements. This effect has not been verified experimentally with growing steers consuming large amounts of typical feedlot rations, however.

With a high intake of a typical feedlot ration, the post-ruminal supply of protein comes from ruminal microbes and non-degraded feed protein. Is the amount adequate for feedlot rates of gain? Past trials to examine post-ruminal protein benefits generally have fed low protein rations at low intakes. Can one extrapolate findings to feedlot

conditions? The final trials of this dissertation were designed to test if an increased supply of protein would increase nitrogen retention of rapadily growing cattle.

CHAPTER II

REVIEW OF LITERATURE

This review concerns only those areas directly examined in the dissertation. It discusses, (1) the role and control of feed intake in the ruminant, (2) effects of post-ruminal nitrogen infusion, and (3) factors that control microbial protein production and efficiency.

Role and Control of Feed Intake

Environmental Control

Environment plays an important role in the daily behavior pattern of the ruminant animal. Changes in environmental temperature can stimulate the ruminant animal to eat. Ruminant animals will usually consume larger quantities of feed during cold stress and reduced quantities during warm periods (Baile, 1968). Though feed intake and temperature are correlated, no cause and effect relationship has been demonstrated (Johnson et al., 1958; Baile & Bayer, 1968; Spector et al., 1968; Dinius et al., 1970). Recent work by Gonyou et al (1977) suggests that increased intake during cold stress is associated with a higher rate of passage of digesta and increased thyroxin secretion. Other weather factors such as humidity and rain, age, sex and pecking order also may prove important.

Neural Control

The central nervous system plays a dominant role in regulation of feed intake. Stellar (1954) suggested that hypothalamic centers regulate meal size as well as total feed intake.

Eating behavior in satiated ruminants was induced by electrical or chemical stimulation of the lateral hypothalamus in sheep and goats (Larson, 1954). Stimulation of another area, the ventromedial hypothalamus, decreased eating behavior of goats (Wyricka, 1960). This led Arees and Mayer (1967) to suggest a direct neural fiber connection between the ventromedial neurons and the lateral hypothalamus. But neurophysiological or neuroanatomical evidence for such a connector has not been found to date (Murphy & Renaud, 1969).

Baile and Mayer (1970) reviewed research that indicated that lesions in the hypothalamic area of goats resulted in a slow, sluggish eating behavior and that electrolytic lesions in the ventromedial hypothalamus led to obesity. These effects could not be duplicated in sheep (Holmes & Fraser, 1965; Tarttelin & Bell, 1968).

Dorsal medial hypothalamic lesions in the rat (Bernardis et al., 1978) disrupted spontaneous food intake but did not impair the rat's ability to choose a nutritionally complete diet. It was suggested that the dorsal medial hypothalamus controls food intake and meters energy intake. But because of the variability in response to hypothalamic lesions, Grossman (1968) suggested that there may be extrahypothalamic mechanisms involving other brain regions.

Injection of high levels of gold thioglucose (Liebelt & Perry, 1957; Wagner & DeGroot, 1963) produced ventromedial lesions in the

rat and mouse and altered feeding pattern. But lesions could not be produced by this manner in the ruminant (Baile & Mayer, 1970). They postulated that the ruminant animal does not have the appropriate ventromedial receptors necessary for a gold thioglucose response.

Challenging this concept, Peterson (1972) injected gold thioglucose into calves. Injection into the lateral hypothalamus increased intake. Injection of a neural depressent, sodium pentobarbital, increased eating behavior in cattle and sheep. Rezek and Novin (1976) vagotomized rabbits and duodenally infused glycerol, casein or glucose. They noted that vagal mediation of information from peripheral metabolic organs is vital to the short-term glucostatic regulation of food intake.

Another hypothalamic control theory involves volatile fatty acids. Many researchers (Manning et al., 1959; Simkins et al., 1965a,b; and Conrad, 1966) have suggested that in the ruminant, the hypothalamus has receptor sites for volatile fatty acids as the non-ruminant has receptor sites for glucose. These could depress eating behavior. However, little experimental evidence to date supports this theory. There is a hypothesis that a blood plasma propionate concentrations increase, the liver allows more to pass through which may reduce feed intake (Theurer, 1975).

Dinius and Baile (1977) fed an intake stimulant, elphazepam, to steers. Intake was increased by 13%. Because it is chemically similar to cannibitols, elphazepam most likely acts on the central nervous system.

Digestible Energy

In an excellent review, Baumgardt (1970) concluded that there must

be two mechanisms for feed intake control in the ruminants. The first is the "bulk fill" or ruminal distention theory that controls intake of high fiber diets. The second is some form of chemical control of intake (chemostatic control) active with ruminants fed highly digestible, high energy diets.

"Bulk fill" intake control was first visualized when Blaxter and Wilson (1962) noted a close relationship between feed intake and ration digestibility. Capacity (a function of body weight), passage rate and dry matter digestibility were considered to be the factors limiting feed intake of long forage diets with digestibilities ranging from 52% to 66% (Conrad et al., 1964). With higher energy diets, from 67% to 80% digestibility, metabolic size, production status (gain, milk) and ration digestibility were considered to be the limiting factors.

Since digestible energy and dry matter are closely related, Baumgardt (1970) suggested that digestible energy density should be included with all descriptions of rations. He felt this was necessary since high density feeds generally had increased rates of passage and digestion. Baumgardt and Peterson (1971) suggested that caloric density be used based upon results of adding up to 50% sawdust to rations. Digestible energy intake decreased at higher levels of sawdust addition, but at the lower levels, digestible energy intake remained remarkably constant while feed intake increased.

Nutrient deficiencies or excesses can influence intake as well. Fat and protein are the two nutrients which have received most attention. Kowalczyk et al. (1977) administered a high fat supplement either in the dry form or as a liquid. Feed intake and digestibility of the roughage diet decreased when the dry form of fat was fed but dosing

with liquid fat had no effect on feed intake or digestibility of their dried grass ration.

Papas et al. (1974) infused corn oil postruminally into lambs fed a highly digestible semi-purified diet. They observed a decrease in feed intake when 28 grams of oil was infused. Digestibility of the diet was not affected.

The postruminal protein to total calorie ratio also may be important. Egan (1977) noted intake responses of up to 15% to duodenally infused casein with sheep. He suggested that feed intake will increase six units for every unit increase in protein digested in the intestine.

Acetic acid infusion depressed intake of sheep in a study by Egan (1977). This prompted him to conclude that energy and protein infusion have qualitatively opposite effects on feed intake. Consequently, factors for both postruminal energy and protein should be included in any equation to predict feed intake.

Physical or Capacity Factors

Many researchers had suggested that bulk fill limits intake of forage rations. To test this, Weston (1966) added ruminal digesta from donor animals to the rumen of test cattle. He observed that the addition of digesta reduced hay intake. Purser and Moir (1966) approached this same theory from the opposite direction. They removed ruminal digesta from cattle and observed increases in feed consumption.

Most intake studies have used mature or older growing animals for

such studies. In contrast, Hodgson (1971a,b,c,d) studied solid food intake by the pre-ruminant calf. He found that the concentration of energy in a milk or milk substitute ration did not effect intake, but ration volume did. Also "palatability" of the diet had a large effect on intake. Physical and metabolic factors became more important as regulators of intake as solid food became a larger percentage of the diet.

Processing of hay into wafers or grinding of hay generally increases dry matter consumption by sheep and cattle (Hogan & Weston, 1967). When processing increases intake, digestibility may decline, supposedly due to a decreased ruminal residence time for dry matter. The relationship between digestibility and rate of passage and their effect on feed intake was addressed by Weston (1966). By adding polyvinyl chloride chips and sawdust to a concentrate diet fed to sheep, he observed that feed intake decreased. However, he cautioned readers about extrapolation of the data since the chips and sawdust may not behave as more typical roughage. Differences in roughage digestibility and rates of digestion and passage might alter results.

Feeding three different diets containing either chopped or pelleted hay or sawdust, Egan (1972) noted a close relationship between nutritive value (quality) of the diet and the capacity of the gut to accomodate indigestible materials. He concluded that gut capacity for indigestible material in the rumen can limit intake of roughage.

When low protein roughage rations are fed, urea addition can increase rate of digestion, rate of passage and intake feed (Balch & Campling, 1962; Freer & Balch, 1962; Moir & Harris, 1962; Egan, 1965; Campling, 1966; Kropp et al., 1977; Mizwicki et al., 1978). The

increase in feed intake has been attributed to an increased rate of passage from the rumen. This may be a result of increased cellulolytic activity of the rumen microorganisms. Urea addition often improved the cellulolytic activity in the rumen while energy addition did not in the trials where both were monitored. An increased rate of passage permits feed intake to increase while the addition of energy will not effect or will decrease roughage intake.

Physical limitation may play an important role in feed intake of pregnant or obese ruminants. Obese cows would not consume enough hay to maintain their weight, while thin or normal weight cows had little problem consuming enough feed to maintain their weight (Bines, 1971). Pregnancy compounded this effect. This was attributed to a reduction in rumen or gut capacity due to accumulation of fat or fetal tissues (Bines, 1971; Forbes, 1971).

Energy

Plasma glucose concentration and glucose utilization are accepted as short term feed intake regulators for the non-ruminant animal. In the ruminant animal, however, injections of glucose into either the rumen or blood stream (arterial or venous) have failed to decrease grain intake (Baile & Mayer, 1970). They had noticed previously (Baile & Mayer, 1967) that infusion of glucose into the abomasum could actually increase total energy intake while ruminal infusion had no effect. Papas (1974) abomasally infused a starch solution and observed increased energy intake by lambs. Effects of oil infusion (Papas, 1974) into the abomasum of sheep varied depending upon the quantity infused. A high level of oil decreased feed intake, apparently due to

a post-ruminal effect. He postulated that saturated long chain fatty acids may increase release of cholecystokinin and thereby depress feed intake.

The effects of volatile fatty acids (VFA) on feed intake have varied. Dowden and Jacobsen (1960) infused acetic acid, proprionic acid and butyric acid intravenously into dairy cows at a rate of 12.5% of the metabolizable energy requirement. Daily energy intake was unchanged. However, they did observe a slight decrease in meal size during the infusion period. At an infusion rate of 15% of the digestible energy requirement, only acetate decreased total energy intake (Simkins et al., 1965b). The other two acids decreased meal size but did not change 24 hr. feed consumption. They also measured volatile fatty acid concentrations in the rumen and blood plasma. Blood plasma VFA concentrations peaked approximately two hours after feeding. They stated that ruminal and blood plasma concentrations of volatile fatty acids peaked when satiety occurred. However, Thye et al. (1970) discounted this relationship showing that blood plasma metabolites continued to change after satiety occurred in lactating ewes.

Although the ruminant animal is believed to be more sensitive to changes in the intravenous concentration of acetate than other short chain fatty acid (Baile & Mayer, 1970), Weston (1966) found infusion of solutions low in pH decreased intake more than for solutions of neutral pH, but that reduced energy intake was proportional to the calories infused. With his abomasal infusions of volatile fatty acids, Papas (1970) noticed that caloric content was less important than molar amount infused. As more acid was infused, intake decreased. Eskeland

et al. (1974) intravenously infused either saline or short-chain fatty acids. With acid infusions, they observed an increase in nitrogen balance of sheep fed a concentrate diet. They concluded that calorie input may limit protein retention.

Monensin, an antibiotic feed additive, alters the acetate to propionate ratio in the rumen. Emby and Swan (1974), Raun et al. (1976), Burroughs (1975), Farlin et al. (1975), Sherrod et al. (1975), Gill et al. (1977), and Rust (1979) have all observed decreased feed intakes with monensin feeding. In an effort to determine the reason for this intake effect, Baile et al. (1979) either ruminally infused or feed monensin to cattle offered roughage or concentrate rations. With infusion at a rate equivalent to 60 g per ton of feed, they noted no depression in feed intake of the roughage diet when monensin was infused but found intake depressed when monensin was fed. The authors hypothesized that monensin causes a malaise. Some sensory cue, such as flavor or smell of Rumensin was sensed as being related. Hence intake was reduced to avoid the malaise.

Protein and Amino Acids

Aminostatic control theories have been suggested as intake regulators for many years. In 1916, Osborn and Mendel, as cited by Rodgers and Laung (1973) stated that animals do not eat satisfactorily when protein concentration of the food becomes very low. Limitation of a single amino acid depresses growth and feed intake as well. For the ruminant animal, nutritionists must consider both dietary and microbial sources of protein from qualitative and quantitative standpoints.

In the ruminant animal, blood plasma amino acid patterns remain

relatively constant following a meal and are a function of both bypass of dietary amino acids and microbial protein composition (Liebholz, 1967; Ely et al., 1969; Purser, 1969; Potter et al., 1972). Most work with protein levels and imbalances with ruminant animals has been approached by dietary modification.

Considering first the microbial protein, Bergen et al. (1968) identified cystine and histidine as limiting amino acids in microbial protein and protozoa for growing rats. But application to ruminants was only by extrapolation. Methionine, lysine, and threonine were suggested by Nimrick et al. (1970a,b,c; 1971) to be limiting amino acids in microbial protein for nitrogen balance of slowly growing sheep. Richardson and Hatfield (1978) identified methionine and lysine to be the first limiting amino acids in microbial protein of cattle fed a semi-purified diet.

Papas et al. (1974) abomasally infused an amino acid mixture patterned after casein and observed increased feed intake by growing lambs. Abomasal infusion of casein also increased feed intake when either a semi-purified or an intact protein diet was fed. When a disproportionate amino acid mixture, lacking methionine, lysine and threonine was infused abomasally, feed intake dropped. This depression indicated that sheep can sense an amino acid imbalance.

Amino acid imbalance, toxicity, and antagonism, as well as blood ammonia level, appear to act as aminostatic control mechanisms for intake control by non-ruminants. Harper et al. (1970) and Rodgers and Leung (1973) have found that amino acid imbalances decrease food intake, but little research has examined meal size or meal number. After ingestion of an imbalanced diet containing excessive isoleucine, more

efficient use of the limiting amino acids was suggested since limiting amino acids in the blood plasma decreased. As levels fell, the remaining pattern resembled the blood plasma pattern seen when high amounts of indispensable amino acids are fed. This signal they theorized, curtailed feed intake.

Rats fed amino acid deficient diets exhibit depressed intake by decreased meal size with no change in meal frequency (Rodgers & Leung, 1973). Switched back to a normal diet, rats readjusted to a normal meal size within 24 hours. Harper et al. (1970) and Rodgers and Leung (1973) also reported that high protein diets also decreased meal size for a few days but then intake returned to normal. Feeding excesses of methionine, tryptophan and leucine all depressed intake. Rodgers and Leung stated that since these effects are short-term, control in these cases is attributable to some rapidly varying factor when an imbalance is not present.

Harper et al. (1970) found a close correlation between metabolic adaption to a high protein diet, decreased blood plasma amino acids and restoration of food intake. This suggested that aminostatic control may be due not to absolute concentration of plasma free amino acids but rather to the pattern or ratios of amino acids.

With sheep, Hogan et al. (1968) found that plasma essential amino acids changed to reflect the amino acid composition of duodenal infusate solutions. Nimrick et al. (1970), Tao et al. (1974), Fenderson and Bergen (1975), and Richardson and Hatfield (1978) all have shown that infusion of methionine increases plasma methionine concentration when the supply of methionine is in excess. Below the methionine requirement, plasma concentration remains low. This break

point matches the point at which both nitrogen balance and growth rate reach plateaus. Excesses of amino acids may prove slightly detrimental. Infusing an excess of methionine (9 g daily) abomasally into sheep fed a high protein diet depressed feed intake by 4% (Schelling, 1975). Since no single amino acid infusion has markedly altered intake of ruminants, the practical importance of amino acid imbalance, toxicity, and antagonism, as well as marked deficiencies of a single amino acid for ruminant animals remains questionable.

The effect of a postruminal protein deficiency on feed intakes remains unclear. Weston (1971) concluded that a post-ruminal level of about 18 grams of digestible protein per 100 grams of digestible organic matter maximized feed intake. Excess protein concentrations had no effect on production for sheep with ad libitum consumption.

Protein concentration in the ration may influence feed intake. Goshtasbpour Parsi et al. (1977) fed a 7% crude protein semi-purified diet or a 14% crude protein hay diet to lambs. Intakes were 500 g and 1000 g daily for the semi-purified and roughage diet, respectively. The higher energy semi-purified diet produced a greater flow of protein to the abomasum of lambs.

Protein excesses do not appear to be harmful to ruminants. Fenderson and Bergen (1977) fed dietary protein levels of 10 to 40% to steers and found no adverse effects of high levels. Initially when steers were switched from the 10% to the 40% protein ration, feed intake decreased, but intake returned to normal after 5 to 10 days on the high protein diet. No effects on plasma amino acid concentrations were detected.

Post-Ruminal Infusion of Protein Into Ruminants

Infusion of Intact Protein

Extensive protein degradation by microbes of the rumen complicates study of post-ruminal protein requirements. Requirements must be studied through physically or surgically bypassing the rumen. Post-ruminally fistulated animals are commonly used. Nutrients can be infused post-ruminally and effects measured.

This technique was first used by Cuthbertson and Chalmers (1950). For sheep fed a roughage ration they observed that casein was much more beneficial when it was infused duodenally rather than ruminally. Chalmers et al. (1954) later fed or ruminally or post-ruminally infused casein. They again observed that post-ruminal infusion was far superior to either oral or fuminal administration. Feeding wheaten hay, Egan (1965a,b,c,d) and Egan and Moir (1965) observed that intake increased with duodenal infusion of either urea or casein. This was attributed to a reduction in the retention time of the residues in the digestive tract. When he fed urea as a supplement and infused casein duodenally, dry matter intake increased 11% but retention time of feed in the digestive tract was not effected. Hogan et al. (1968) suggested that the intake increase was mediated by plasma amino acid concentration since essential amino acid concentrations in plasma increased as casein was administered.

Feeding or abomasally infusing either casein or soybean oil meal into sheep, Little and Mitchell (1967) observed a greater increase in nitrogen retention with abomasal infusion of casein over feeding casein.

They also observed that infusion of a low quality protein (gelatin) did not increase nitrogen retention over feeding of that same low quality protein. This suggested that one or a series of specific amino acids must be deficient in the combination of microbial protein plus bypassed feed protein.

Schelling and Hatfield (1967a) increased nitrogen retention of lambs fed a semi-purified diet containing urea by abomasally infusion of casein plus methionine. They noted a similar increase in nitrogen retention when they infused ten essential amino acids combined in the same ratio as that found in casein. Dove et al. (1977) also increased utilization of dietary nitrogen in the pre-ruminant lamb by abomasally infusing essential and non-essential amino acids simultaneously in equal amounts. Schelling and Hstfield (1968) felt their results may have been confounded with intake. Their lambs were ad lib fed and casein infusion increased feed intake and nitrogen retention. They also found an increase in nitrogen retention with casein infusion when the lambs were limit fed, however.

Monro (1964) reviewed the literature on the influence of dietary energy on nitrogen balance. He summarized that under normal conditions nitrogen retention increases with energy intake as long as protein intake is adequate. Conversely, an increase in protein percentage will not increase nitrogen retention if energy intake is inadequate. Since post-ruminally infused protein supplies both energy and protein, it is difficult to interpret results of trials without isocaloricisonitrogenous controls.

Black and Tribe (1973) infused both energy and protein post-ruminally. They increased average daily gain, wool growth and nitrogen

retention when they duodenally infused both energy and protein above that achieved with ruminal infusions. Black (1973) designed an experiment with casein infusion to determine the post-ruminal digestible protein requirement of growing lambs to help facilitate ration formulation. He found the requirement to be: $Y = 13.4 - .242X$ when Y = grams of reference protein per MJ of metabolizable energy and X = live weight in kilograms. He expressed the tissue protein requirements of lambs in terms of grams of reference protein per MJ of metabolizable energy. With knowledge of the efficiency with which dietary protein and energy are used to supply the tissue needs, Black felt it was possible to formulate diets for lambs given protein from different sources more accurately.

Papas et al. (1974) fed a semi-purified 9% crude protein diet and infused either water or casein at one of two levels into growing lambs. He noted that at an infusion rate of 30 grams per day, no increase in nitrogen retention occurred. At a rate of 60 grams per day, nitrogen retention increased. They felt that this may be due to an increased amount or an improved balance of amino acids available for absorption.

Colebrook and Reis (1969) and Bird and Moir (1972) noted that when either casein supplemented with methionine or sulfur amino acids alone were infused abomasally, wool production increased. Colebrook and Reis (1968) abomasally administered whole egg, egg albumin, or corn gluten. Whole egg and egg albumin gave a response equal to casein, but corn gluten gave a response only half this magnitude. Probably differences in wool growth and nitrogen retention were due to differences in amino acid composition of the proteins infused and hence, differences in the amount and proportions of individual amino

acids absorbed.

Not all scientists have used the infusion technique to bypass the degradative action of ruminal microorganisms. Orskov et al. (1970) bottle fed lambs to close the esophageal groove hoping to achieve rumen bypass. They administered protein supplements via bottle to weaned lambs. An additional 5 grams of nitrogen from the supplement to increase nitrogen intake from 12 to 16 grams daily increased nitrogen retention 31% with casein, 27% with fishmeal and a 24% with soybean meal.

Another popular technique to prevent rumen degradition of protein is chemical treatment of the protein. Formaldehyde treatment is the most common chemical treatment for protein bypass. Reis and Tunk (1969), Wright (1971), Barry (1972), Faichney (1971), Peters et al. (1971) and Johnson and Hatfield (1976) found increases in nitrogen retention, wool growth or body weight gain when soybean meal was treated with formaldehyde prior to feeding to ruminants. Faichney (1971) noted an increase in average daily gain and blood plasma amino acid concentrations when casein was treated with formaldehyde prior to feeding to growing lambs.

Casein Infusion for Lactating Ruminants

Ranawana and Kellaway (1977) studied the effect of post-ruminal infusion of casein for lactating goats. Feeding a diet at 85% of ad libitum intake and infusing graded levels of casein abomasally, they noted that casein infusion (0, 15, 30 and 45 g daily) increased milk production from 2.41 kg to 2.52, 2.80, and 2.94 kg per day. Composition of the milk remained unchanged. As casein infusion increased,

nitrogen retention also increased.

Derrig et al. (1974) ruminally or abomasally infused 400 grams of casein per day into lactating dairy cows consuming alfalfa hay ad libitum plus a 14% crude protein concentrate mix. No effect on dry matter intake, milk fat, or percentage lactose in the milk was noted between infusion sites. Increases of 4 to 5% were seen in dry matter digestibility, milk protein and grams milk nitrogen when casein was abomasally administered. They hypothesized (1) that the casein increased the supply of one or more specific amino acids that are deficient in the digesta passing from the rumen, (2) infusion increased the supply of glucogenic amino acids to the liver thereby increasing glucose availability to the mammory gland, or (3) infusion had some direct or indirect hormonal effect on milk production.

Broderick et al. (1974) abomasally infused either 800 grams of casein or an iso-caloric, iso-nitrogenous glucose-urea mixture into the lactating dairy cow. He observed a 10% decrease in grain dry matter consumption but an 11.6% increase in milk protein production and 6.2% increase in total milk production.

Spires et al. (1975) abomasally infused 450 g casein per day or an iso-caloric, iso-nitrogenous glucose-urea solution. They noted a 5% increase in milk production and a 14.2% increase in milk nitrogen. Increased efficiency of conversion of absorbed nitrogen into milk nitrogen accounted for 80% of the increase in efficiency of milk production with the casein infusion. Clark et al. (1977) then abomasally infused casein or a glucose-urea solution. They again observed an increase in milk yield and milk protein production. Abomasal infusion of a glucose-urea mixture again gave no response.

Clark measured arterial blood plasma amino acids in the mammory artery and vein and found that methionine, lysine, and phenylalanine uptake paralleled milk output most closely. He concluded that if milk production is limited by protein synthesis, these three amino acids would be the most limiting amino acids for milk production.

Schwab et al. (1978) fed lactating dairy cows a 10 to 11.5% crude protein diet that met requirements for net energy for lactation but only 75% of the requirement for crude protein. They then abomasally infused 11 grams of melthionine, 27 grams lysine, methionine plus lysine or 425 grams casein. Casein was the positive control while dibasic ammonium citrate served as the negative control. Milk yield increased only slightly (1.2 kg daily) with infusion of methionine and lysine. This response was less than half that seen from infused casein. They concluded that lysine and methionine are first and second limiting or co-limiting amino acids for secretion of milk protein for cows fed a corn, corn-silage and alfalfa hay protein deficient diet.

Amino Acid Infusion

Post-ruminal or tissue amino acid requirements of the ruminant animal have not been studied extensively due to the excessive ruminal protein degradation that occurs in the rumen. Work that has been conducted suggests that supplementing the microbial digesta with methionine, lysine, and threonine increases the nitrogen status of the ruminant animal. However, the response varies greatly with experimental conditions and type and amount of ration fed.

Devlin and Woods (1965) abomasally infused lysine into eight growing steers limit fed a 65% corn, 24% corn cob, 5% corn gluten meal

ration. They found that with infusion of 9 grams of lysine per day, nitrogen retention increased by 5 grams per day. In a similar study, Boila and Devlin (1972) fed a similar corn-corn gluten meal diet. They abomasally administered graded levels of lysine or a water control into four growing Holestein steers and noted that 3 grams of lysine daily gave the largest increase on nitrogen retention. Thinking that nitrogen retention might be limited by energy intake, Boila and Devlin (1975) repeated these graded levels of lysine infusion or a water control with steers fed a corn based diet at the rate of 7.27 kg/day in many small meals or twice daily at the rate of 8.18 kg/day. They observed that nitrogen balance response to lysine infusion was lower with continuous feeding than with feeding twice daily. They concluded that continuous feeding as an experimental tool may reduce the interactions encountered between lysine infusion and the concentration of blood plasma amino acids. Steers eating continuously had variable results while animals allowed to eat only twice daily gave a more consistent response to infusion.

Burris et al. (1974) fed a concentrate ration at a restricted level of intake (1.5% of body weight) to growing steers and abomasally infused lysine at 0, 15, 30 and 45 grams per day. Nitrogen retention increased most at the highest infusion level. Burris et al. (1976) repeated their experiment and infused 0, 12, 24, and 35 grams per day of lysine with steers fed at a rate of 2% of body weight. The infusion of 24 g daily gave the greatest nitrogen retention response. At this level, the equivalent of 165% of the infused nitrogen was retained. In these trials, nitrogen retention seldom increased by an amount equal to the amount of nitrogen infused. Since control

treatments did not receive NPN infusion, interpretation is difficult. Infusing methionine abomasally into lambs fed a diet of 50% lucerne chaff and 50% wheaten chaff Reis and Schinckel (1963) observed an increase in average daily gain and a 2.5 gram daily increase in wool growth above that obtained by gelatin infusion. Lambs are known to have a high sulfur amino acid requirement for wool growth.

To see if sulfur amino acids were limiting in post-ruminal microbial digesta for growth, Bergen et al. (1968) fed microbial protein to rats. They found that the concentrations of sulfur amino acids, as well as histidine, in microbial protein limited growth rate of rats. By comparing blood plasma amino acids to the amino acids infused into the duodenum of the sheep, Wakeling and Lewis (1970) examined amino acid limitations. They interpreted a decrease in blood plasma concentration of other essential amino acids as indicative that the limiting amino acid is being supplied. In contrast, when the blood plasma concentration of the infused amino acid increased, the requirement had been met. Using this procedure, they reported that methionine was the limiting amino acid in post-ruminal digesta of their low protein diet for lambs.

Feeding a high quality natural diet to lambs, Schelling et al. (1973) also infused methionine. The increase in nitrogen retention and wool growth with infused methionine supported the theory that methionine was the first limiting amino acid for tissue and wool growth. From this experiment with high quality diets, they concluded that post-ruminally, methionine plus one or more other amino acids may be deficient for lambs fed a high energy, low protein diet. This conclusion was based upon responses they noted to abomasally infused casein.

Chalupa et al. (1972), 1973, 1975) conducted several experiments with steers. They abomasally infused water, methionine, lysine or several combinations of amino acids. Their 65% concentrate diet ranged from 10.5 to 11.8% crude protein. They observed that 3 to 9 grams per day of methionine and 5 to 15 grams of lysine per day would increase nitrogen retention by 5 to 10 grams daily with limit-fed steers. These trials as well as those of Hill et al. (1980) used restricted feeding without isonitrogenous controls. In both studies, nitrogen balance paralleled nitrogen intake very closely. Nitrogen balance responses may have been due to non-specific nitrogen and not to amino acids per se.

Fenderson and Bergen (1975) infused incremental levels of methionine, lysine, methionine and lysine, and threonine, and tryptophan into the abomasum of growing steers. Their steers were fed a 9.5% crude protein 65% TDN ration at 3.0% of body weight. Using blood plasma amino acid response curves as an evaluation criterion, only methionine produced the two phase curve indicative of response to a limiting amino acid.

Feeding 165 kg steers a semi-purified diet containing 14.2% protein at a rate of 80% of ad libitum intake, Richardson and Hatfield (1978) noted responses in both nitrogen balance and blood plasma to methionine infusion over lysine and threonine. However the combination of all three amino acids increased nitrogen balance response most. Continuing their trials, they infused methionine at a constant rate and added either lysine, threonine, a histidine. They found methionine plus lysine gave a favorable response.

Based upon the theory that methionine was the first limiting amino acid for ruminant rations in general, Hill et al. (1980) infused a

constant level of methionine with either graded levels of lysine or 140 grams of casein. Feeding 4.4 kg per day of a 10.2% protein corn, cottonseed hull diet to 230 kg steers, they noted the greatest nitrogen balance response to the added casein infusion. Nitrogen balance also increased with lysine infusion. One must point out again that nitrogen retentions were closely correlated with the added nitrogen infused suggesting that non-specific nitrogen, not amino acids, may have been the beneficial factor.

Infusion of a Non-Specific Nitrogen Source

Deficiency of non-specific nitrogen in the lower tract was stated previously as a possible limiting nutrient. Egan (1965) duodenally infused urea into his lambs fed a 3.3% protein ration and noted an increase in nitrogen balance and decreased rumen retention time of wheaten hay. He felt this response may have been due to recycling of nitrogen to the rumen or an increased rate of ruminal digestion.

Feeding a semi-purified diet to growing lambs, Schelling and Hatfield (1968) observed an increase in nitrogen retention with the abomasal infusion of monosodium glutamate. Since their diet contained 12% crude protein which should have been adequate for bacterial growth in the rumen, a post-ruminal non-specific nitrogen deficiency was suggested. Nimrick (1971), using a similar diet, found that nitrogen retention increased with glutamic acid infusion. Nitrogen retention was maximized when glutamic acid was infused at a rate of 0.6% of the daily energy intake but a 0.4% infusion rate increased nitrogen retention as well. He concluded that for lambs fed this purified diet with urea as the sole nitrogen source, a non-specific nitrogen source

was limiting post-ruminally.

Ruminal infusion of urea (Slyter et al., 1979) increased dry matter digestibility as well as nitrogen retention in growing steers fed a 10.9% crude protein diet consisting of 70% concentrate and 30% roughage at a rate of 1.2% of body weight. All treatments received 900 g of cerelose infusion in addition to the various urea concentrations. Nitrogen retention was greatest when the urea infusion was equivalent to 13.3% crude protein. The authors concluded that the increase in nitrogen retention to a level of 11.1% crude protein could be attributed to an increase in microbial growth. But since nitrogen balance increased above this point, this also suggested that the increased ammonia was increasing nitrogen retention. Alternatively, the nitrogen balance increase could be attributed to errors inherent in the nitrogen balance technique.

Factors Affecting Microbial Protein Production and Efficiency

The microbial population in the rumen is extremely diverse. A wide variety of nutritional and environmental factors can influence this population. Hespell (1979) grouped those factors which affect microbial growth into two general categories: physical-chemical and nutritional. He concluded temperature, pH, oxidation-reduction potential, osmotic pressure, surface tension and viscosity in his list of the physical-chemical factors. Since these factors are known to be relatively constant, they cannot explain the wide differences in bacterial efficiency observed within the rumen. Nevertheless, manipulation of these factors could alter efficiency and production. Hespell's list

of nutritional factors included nutrient solubility and particle size, chemical form, and the various ratios and concentrations of specific nutrients.

Solubility of both protein and carbohydrate appear to regulate microbial growth in the rumen. Protein solubility (Hume, 1974; Wohlt, 1974; Owens, 1978) affects the rumen bacterial supply of nitrogen. Decreasing solubility to bypass the rumen, though beneficial to the host animal, may prove detrimental to the rumen microbial population unless a soluble non-protein nitrogen source is substituted (Owens & Issacson, 1977).

With a poor quality, low protein, lowly digestible roughage, microbial synthesis appears more responsive to added nitrogen (protein) than energy (Pilgrim et al., 1970; Kropp et al., 1973; Mizwicki et al., 1979). Utilization of high quality, highly digestible concentrate diets also can be affected by NPN as NPN influences the amount of microbial protein synthesis in the rumen (Burroughs et al., 1974; Prigge et al., 1978). Prigge et al. also suggested that where dietary protein is of poor quality, ruminal degradation and subsequent resynthesis into microbial protein could prove beneficial.

An adequate ruminal ammonia concentration is necessary to maximize microbial production. Slyter and Satter (1974) concluded that 2-5 milligrams of ammonia-N per deciliter of ruminal fluid are required for adequate microbial synthesis. Mizwicki et al. (1978) fed a low quality roughage to steers with various amounts of urea supplement. He noted no increase in microbial protein production above a ruminal ammonia concentration of 3 mg/dl, although nitrogen retention increased above this point.

With high concentrate diets, soluble carbohydrates are the major energy source utilized by ruminal microbes. Soluble carbohydrates are derived primarily from non-structural starch or intra-cellular constituents (McDonald, 1952; Phillipson et al., 1962; Hespell, 1979). Availability of soluble carbohydrates can alter cell yield as well as cell lysin and viability (Hespell, 1979).

Dilution rate or ruminal retention time also may be an important factor regulating efficiency of microbial protein production (Prigge et al., 1978; Chalupa, 1972, 1973; Issacson et al., 1975). Issacson et al. noted that as dilution rate increased, out flow and consequently total yield of microbial protein increased in his system.

Metabolizable Protein

The metabolizable protein (MP) concept is an amino acid feeding standard for the ruminant animal (Burroughs et al., 1975). Although it is based on amino acids, it was not developed by measuring individual amino acid requirements. It is one method to balance a ration to meet the requirements of cattle.

Each feedstuff has assigned a value representing the amount of MP (bypass plus bacterial protein) corrected for metabolic fecal nitrogen at the rate of 12 g/kg dry matter intake.

Microbial protein synthesis was estimated at 10.4% of TDN. This value is based upon: (1) 52% of the TDN is digested in the rumen; (2) 25% of the digested TDN is synthesized into microbial protein, and (3) 80% of the microbial protein nitrogen is alpha-amino nitrogen.

The concept also allows for an estimation of the ability for urea

utilization in the diet called the urea fermentation potential (UFP). The calculation for UFP and MP of a feedstuff and the animal's requirement for MP is presented in Appendix A.

CHAPTER III

ABOMASAL NUTRIENT INFUSIONS OF STEERS FED WEATHERED PRAIRIE HAY

Summary

Dextrose, casein, corn oil, methionine or water was infused into the abomasum of five 605 kg steers. Winter harvested prairie hay (2.3% protein) was available ad libitum to the steers and .9 kg of a 15% protein supplement was fed daily. All infusions were isonitrogenous to the control which provided 92 g of urea daily and all infusions except water provided equal amounts of metabolizable energy. The casein and methionine infusions provided equal quantities of sulfur amino acids. Dry matter intake and digestibility were not statistically altered by infusions. Infusates containing dextrose increased ($P<.1$) blood plasma glucose levels. Only the casein infusion increased ($P<.025$) nitrogen retention and blood insulin concentrations. Results may be interpreted to mean that post-ruminal protein will improve the nitrogen status of steers fed low-protein prairie hay but methionine is not the first limiting amino acid. (Key words: abomasal infusion, nutrient, protein, steers, prairie hay.)

Introduction

Weight loss is common among cattle grazing low quality roughages.

Low feed intake of low quality forages generally has been attributed to rumen distention (Balch & Campling, 1962). With certain low protein forages, feed intake may be enhanced by increasing the level of nitrogen fed (Moir & Harris, 1962). This change generally has been attributed to an increased rate of ruminal digestion of cellulose. But post-ruminal protein supply may be important as well. Egan (1965), using sheep, found wheaten hay intake was increased more with abomasal infusion of casein than with abomasal infusion of urea. This suggests that post-ruminal protein status of the animal might alter feed intake of a low quality forage.

The post-ruminal supply of other nutrients has received little attention with high forage rations. With high concentrate rations, abomasally infused casein, starch and amino acid mixtures all enhanced feed intake and performance of lambs (Papas et al., 1974). Knowledge of limiting nutrients should be useful for formulating supplements for ruminant animals grazing winter range forage.

The objective of this experiment was to determine the influence of various abomasally infused nutrients on voluntary feed intake, dry matter and protein digestibility, and nitrogen balance of mature steers fed weathered prairie hay.

Experimental Procedure

Five 605 kg Hereford steers, fitted with permanent rumen and abomasal cannulas, were housed in stanchion-type metabolism stalls. Winter-harvested prairie hay containing 2.3% crude protein was available ad libitum and .91 kg of a 15% crude protein supplement (Table I) was fed once daily.

TABLE I. RATION SUPPLEMENT[a]

Ingredient	IRN[b]	Percentage
Corn, dent yellow, grain, rolled	4-02-931	52.5
Soybean seeds, meal solv-extd	5-04-604	16.6
Alfalfa, aerial part, dehy meal	1-00-023	14.7
Cotton hulls	1-01-599	9.8
Monosodium phosphate	6-04-288	2.6
Potassium chloride	--	2.0
Phosphate, deflourinated, grnd.	6-01-780	.87
Sodium sulfate	--	.74
Trace mineralized salt	--	.05
Vit. A (30,000 IU/g)	--	.12

[a]Analyzed 14.3% crude protein.

[b]International reference number.

Five abomasal infusion treatments (Table II) were imposed. These included water, dextrose, oil, casein and methionine. Urea and dextrose addition to the infusate solutions made them isonitrogenous except for water infusion, isocaloric. The level of methionine employed in this study was equal to that infused by Richardson and Hatfield (1978). L-methionine and casein infusions provided equal quantities of sulfur containing amino acids. Water soluble materials were infused with a roller pump in 5.2 liters of water daily while the corn oil was added to the water infusion tube with a syringe pump. The infusion treatments were continuous. The tubing entered the abomasal cannula through a neoprene stopper and extended a minimum of 23 cm into the abomasum to prevent localized accumulation of nutrients.

Five nitrogen balance periods included 9 days for adjustment and 5 days for excreta collection. Venous blood and rumen samples were obtained 6 hr post-supplement feeding on the last day of each collection period. The blood was immediately centrifuged and the blood plasma frozen for later analysis. Fecal, urinary and ruminal samples were stored frozen for later chemical analysis. Dietary, fecal and urinary nitrogen were determined by the macro-Kjeldahl method. Ruminal ammonia concentration was analyzed colorimetrically (Chaney & Marbach, 1962). Blood plasma glucose was determined by the Glucostat enzyme assay and plasma insulin by radio-immunoassay using a bovine insulin standard. The insulin assay procedure was validated for specificity, accuracy and precision as described by Hafs (1977). Treatment means were compared by least significant difference testing for a 5 x 5 latin square according to Steel and Torrie (1966).

TABLE II. ABOMASAL NUTRIENT INFUSATES[a]

Infusate Treatment	Infusate Composition		
	Dry Matter	Urea	Energy[b]
	g	g	kcal
Water	--	91.4	--
Dextrose	286.2	91.4	1144.8
Oil	127.0	91.4	1143.0
Casein	286.2	--	1144.8
Methionine	10.0		
+	+	91.4	1144.8
Dextrose	286.2		

[a]All infusates were held to a constant volume of 5.2 liter/day.

[b]Calculated based on 4 kcal/g DM for carbohydrate and protein and 9 kcal/g DM for fat.

Results and Discussion

Voluntary Feed Intake

Nutrient infusions had no significant effect (P>.5) on feed intake (Table III). This supports the theory that feed intake of high forage rations is limited by ruminal fill (Campling, 1970). The slight decrease in feed intake with corn oil infusion (11%) is less than that reported by Papas et al. (1974) with high concentrate rations. Response to abomasal nutrients that they observed with their ration may be attributed to some type of chemostatic intake regulation. Forage intake by cows grazing native forage from the range where this grass was harvested was previously estimated by the chromic oxide dilution technique to be 9.6 kg per day (Lemenager, et al. 1978). The higher intake of grazing cows than of steers in metabolism stalls during this study may be due to differences in exercise, pregnancy, body condition, lactation, selective grazing or general environmental conditions.

Dry Matter and Protein Digestibility

Dry matter digestibility (Table III) for the supplemented winter range grass was near the 49% cited by Williams et al. (1969). Digestibility of the range grass alone, calculated by difference using NRC (1976) values for supplement ingredients, was 42% and equal to the NRC (1976) value for post-ripe, sun cured, native grass hay (IRN 1-03-188).

Nitrogen digestibility of the total ration, including the infusate was 67.7% (Table III). Calculating by difference, the prairie hay provided only .6% digestible protein, slightly above the .5% listed by the NRC (1976). As a percentage of the total protein, some 25% was

TABLE III. INTAKE, DIGESTIBILITY AND RUMINAL AMMONIA OF INFUSED STEERS

Item	Infusate					S.E.
	Water	Dextrose	Oil	Casein	Methionine	
Intake, g/day						
Dry matter consumed[a] g/day	5008	5182	4660	5097	5301	260
Digestible dry matter[b] g/day	2489	2467	2148	2487	2391	
Indigestible dry matter[b] g/day	2519	2715	2512	2610	2910	
Digestibility, %						
Dry matter[a]	49.7	47.6	46.1	48.8	45.1	2.29
Nitrogen[a]	66.9	65.9	65.5	66.4	64.4	1.16
Nitrogen intake[b] g/day	85.1	85.9	83.8	85.5	86.2	.92

TABLE III (Continued)

Item	Infusate					S.E.
	Water	Dextrose	Oil	Casein	Methionine	
Nitrogen retention[b]						
g/day	17.3[c]	19.5[c]	16.6[c]	29.9[d]	16.3[c]	2.38
% of intake	19.1[e]	22.3[e]	20.6[e]	34.8[f]	18.8[e]	2.80
% of nitrogen digested	28.9[e]	33.6[e]	31.2[e]	53.0[f]	29.5[e]	3.85
Ruminal ammonia, mg/dl	2.99[gh]	2.00[g]	3.70[h]	2.85[gh]	2.82[gh]	.32

[a]Not including infused materials.

[b]Including infused dry matter.

[c,d]Means in a row with different superscripts differ statistically (P<.025).

[e,f]Means in a row with different superscripts differ statistically (P<.01).

[gh]Means in a row with different superscripts differ statistically (P<.05).

digestible compared with 12% for the NRC (1976) value for this hay. Total digestible N supplied per steer daily from feed plus infusate was 57 g compared to an estimated requirement of 54 g for maintenance of a 500 kg steer (NRC, 1976).

Ruminal ammonia concentrations were low for steers on all treatments (Table III). The slightly greater ruminal ammonia concentration with the oil infusion may be associated with the slightly reduced hay intake coupled with a constant amount of protein supplement. Ruminal ammonia concentrations were all below the 2 to 5 mg/dl considered necessary for maximum microbial protein synthesis (Slyter et al., 1979). Since infusates provided equal amounts of nitrogen, equal amounts should have been available for recycling to the rumen. Nevertheless, higher ruminal ammonia concentrations might have increased bacterial protein synthesis and flow of protein to the abomasum. This might have altered response to the nutrient infusions.

Nitrogen Retention

Casein infusion enhanced nitrogen retention ($P<.025$; Table III). An equal supply of sulfur-containing amino acids from L-methionine (10 g) failed to increase nitrogen balance. This suggests an amino acid other than or in addition to methionine was limiting nitrogen balance. This conflicts with results from experimental steers with limit fed higher quality, highly digestible rations in which methionine infusions have proven beneficial (Fenderson & Bergen, 1975; Richardson & Hatfield, 1978).

Blood Plasma Glucose and Insulin

Infusion of 286 g glucose per day in the form of dextrose plus urea, with or without added methionine, tended to increase the concentration of blood plasma glucose (Table IV) while equal energy from corn oil or casein produced no increase. Since 26% of nitrogen infused as casein was retained and only about half of the amino acids are glucogenic upon catabolism, increased blood plasma glucose from casein infusion would not be expected.

Insuline concentrations increased ($P<.025$) with casein infusion despite unaltered blood glucose concentartions. Blood plasma insulin concentrations were low compared with the 25 to 40 μ U/ml reported for growing steers or the 3 to 6 μ U/ml for fasting heifers (Trenkle, 1970; 1972). These low levels may reflect an energy deficiency. Whether insulin concentrations might index protein-calorie status remains to be determined. Metabolic responses to casein infusion may have been mediated through hormonal changes.

Direct bypass of the supplement to the abomasum of steers was noted in this trial. Within seconds of supplement feeding, supplement appeared at the abomasal cannula. Reduced mixing of supplement with ruminal contents may be attributed ruminal compaction with forage. This phenomenon could alter current concepts of ruminal digestion and outflow. Direct bypass could help explain why intact protein as a winter range supplement enhances performance more than non-protein nitrogen.

The quantity of supplemental post-ruminal protein needed to improve nitrogen retention in this study may be below the quantity

TABLE IV. BLOOD PLASMA GLUCOSE AND INSULIN LEVELS OF INFUSED STEERS

Item	Infusate					S.E.
	Water	Dextrose	Oil	Casein	Methionine	
Plasma						
Glucose, mg/dl	58.1[a]	69.4[b]	57.4[a]	56.4[a]	62.4[a,b]	3.62
Insulin μU/ml	1.43[c]	1.28[c]	1.41[c]	2.20[d]	1.51[c]	.16

[a,b]Means in a row with different superscripts differ statistically ($P<.1$).

[c,d]Means in a row with different superscripts differ statistically ($P<.025$).

supplied since only 26% of the infused nitrogen from casein was retained. Qualitative and quantitative assessment of amino acid requirements must await further research. Results from this trial may be interpreted to mean that cattle fed low protein forage rations should benefit from increased protein bypass. This as well as direct bypass might help to explain the superiority of intact protein over urea supplements for gestating or lactating cows grazing a low-protein winter forage.

CHAPTER IV

ABOMASAL PROTEIN INFUSIONS FOR GROWING STEERS FED CORN GRAIN RATIONS

Summary

Casein was abomasally infused into five growing 226 kg steers consuming a high-concentrate, urea-supplemented corn grain ration ad libitum. Infusate solutions in the 5 x 5 latin square contained 0, 20, 40, 80 and 120 g casein made isocaloric and isonitrogenous by dextrose and urea addition. Feed intake averaged 5.4 kg dry matter daily and was not altered significantly by abomasal infusion treatment. Digestibility of nitrogen decreased from 71.7% to 66.8% as casein was substituted for urea in the infusate. Nitrogen retentions (NR) were 40.5, 35.5, 42.8, 35.1 and 32.7 grams per day for the five levels of infusion, respectively. A second study was conducted to determine if level of feed intake influenced the benefit seen from post-ruminal protein supplementation. Four steers (306 kg) ate either ad libitum or were limit fed (2.5 kg/day) a 1% urea-supplemented, high-concentrate corn grain ration in a 4 x 4 latin square design. These steers were abomasally infused with 120 g casein or an isocaloric, isonitrogenous dextrose-urea mixture. Daily ad libitum feed intake averaged 4.7 kg and was not altered significantly by infusion composition. NR tended to increase with casein infusion at both levels of intake, but as a

percentage, the increase tended to be greater with limit feeding (42 vs 24%). To determine if supplemental abomasal urea might prove beneficial, a third trial was conducted with four 313 kg steers consuming the same ration ad libitum in a crossover design. Infusates consisted of 120 g dextrose or 120 g dextrose plus 42.6 g urea per day. Daily feed intakes were 4.5 and 4.8 kg/day for the dextrose and dextrose plus urea infusion, respectively. NR tended to increase with urea infusion (29.9 vs 29.7 g/day). Results suggest that energy total nitrogen or other nutrients, not post-ruminal amino acids, limited nitrogen balance of young growing steers gaining weight at a rate of 0.9 kg daily fed a urea-supplemented, cracked corn ration.

Key words: Abomasal infusion, casein, protein, steers, high concentrate rations.

Introduction

Post-ruminal protein infusions have generally increased nitrogen balance or wool growth of sheep (Egan, 1965; 1965a; 1965b; Egan & Moir, 1965) and nitrogen balance of steers (Johnson, 1980) fed rations low in energy. With higher concentrate rations, infusion of supplemental amino acids has increased nitrogen retention in several experiments (Hill et al., 1980; Chalupa, 1972).

In most experiments with post-ruminal protein infusion, intake of feed, energy or nitrogen has been restricted. Results from experiments in low energy rations, purified diets or limited feed intakes may not be applicable to conditions of ad libitum consumption of high concentrate diets.

The objectives of these trials were to determine for growing steers fed a high concentrate ration, (1) the feed intake and nitrogen balance response to post-ruminal protein infusion, (2) if level of feed intake influences response to post-ruminal protein supplementation, and (3) if post-ruminal urea alters nitrogen balance.

Experimental Procedure

Experiment One

Five steers with a mean weight of 226 kg were fitted with permanent T-type ruminal and abomasal cannulas and used in a 5 x 5 latin square experiment. Each period consisted of 9 days for adaptation and 5 days of excreta collection. The infusate solutions (Table V) provided 0, 20, 40, 80 or 120 g casein daily. Casein was solubilized by the addition of 1.2 g sodium hydroxide per liter which produces a solution with a pH of 6.8. Dextrose and urea were added to make all infusion solutions isocaloric and isonitrogenous. The solution was pumped through a roller pump[1] at a continuous rate of 2.2 liters per day. The tubing entered the abomasal cannula through a neoprene stopper and extended a minimum of 23 cm into the abomosum to avoid localized accumulation of infused nutrients.

The corn-urea diet (Table VI) contained 11% crude protein with 8.9% from natural feed ingredients and the remainder from urea. Calculated from feed intake plus infusions of nitrogen from urea or casein, total protein intake for steers was equivalent to 13.2% of the

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[1]Brinkman Pump, Model MP-GE, Brinkman Mfg. Co., Des Plaines, IL 60016.

TABLE V. EXPERIMENT ONE. ABOMASAL INFUSION TREATMENTS OF STEERS

Treatments	Abomasal Infusion (G of DM)		
	Casein	Dextrose	Urea
		g daily	
0	--	120	42.6
20	20	100	35.5
40	40	80	28.4
80	80	40	14.2
120	120	--	--

TABLE VI. COMPOSITION OF DIET FED TO GROWING STEERS

Ingredient	International Reference No.	Percentage[a]
Corn, ground	4-02-931	77.80
Alfalfa, Dehy. pellet	1-00-023	5.98
Cottonseed Hulls	1-00-599	13.96
Trace mineral salt		.49
Calcium Carbonate	6-02-632	.49
Dicalcium Phosphate	6-01-080	.49
Urea		.75

[a]Dry matter basis. Analyzed 11.0% crude protein of which 2.1% came from urea.

ration with the non-urea crude protein providing 8.9 to 11.4% of total ration dry matter. Steers were allowed to consume feed ad libitum with fresh feed added twice daily.

Venous blood samples were obtained by jugular puncture on the last day of each collection period. The blood was immediately centrifuged and the plasma frozen for later analysis. Rumen samples were collected and acidified with HCl on the last day of each period. Fecal, urinary, and ruminal samples were stored frozen prior to chemical analysis. Nitrogen was determined by the macro-kjeldahl method. Ruminal ammonia nitrogen concentration was analyzed colormetrically (Chaney & Marbach, 1962). Plasma glucose was determined by the Glucostat enzyme assay.[2] Treatment means were compred by least significant difference testing for a 5 x 5 latin square according to Steel and Torrie (1966).

Experiment Two

Four steers averaging 290 kg were surgically prepared with permanent T-type ruminal and abomasal cannulas. The design was a 4 x 4 latin square with 9 day adaptation and 5 day collection periods. In the 2 x 2 factorial treatment arrangement (Table VII) feed intake was limited or ad libitum and abomasal infusion provided 120 g casein or an isonitrogenous urea-dextrose mixture. The diet (Table VIII) was a high concentrate corn based ration containing 1% urea. Infusate administration and sampling was as described for Experiment 1.

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[2]Glucostat Reagent Set, Worthington Brochemical Corp., Freehold, N.J. 07728.

TABLE VII. EXPERIMENT TWO. INTAKE AND INFUSION TREATMENTS OF GROWING STEERS

Treatments	
Intake	Abomasal Infusion
Limited	120 g Dextrose + 42.6 g Urea
Limited	120 g Casein
Ad Libitum	120 g Dextrose + 42.6 g Urea
Ad Libitum	120 g Casein

TABLE VIII. COMPOSITION OF DIET FED TO GROWING STEERS IN EXPERIMENTS 2 AND 3

Ingredient	International Reference No.	Percentage[a]
Corn, rolled	4-02-931	77.23
Alfalfa, dehy. pellet	1-00-023	5.98
Cottonseed hulls	1-01-599	13.96
Sodium sulfate		.11
Salt		.49
Trace mineral mix		.25
Calcium carbonate	6-02-632	.49
Dicalcium phosphate	6-01-080	.49
Urea		1.00
Vitamin A (30,000 IU/g)		.001
Vitamin D (15,000 IU/g)		.001

[a]Dry matter basis; analyzed 12.3% crude protein, with 2.8% coming from urea.

Abomasal samples were drawn at two and six hours post-feeding for the limit fed animals. Microbial nitrogen was estimated from RNA concentration. The orcinol procedure (Appendix B) was employed after removal of interfering undigested feed starch in the sample. This modification involved incubation with amylase prior to the RNA extraction and purification. Chromium sequioxide (Cr_2O_3) was fed as a marker the last three days of each collection period. This marker was to be used as an external marker to quantitate flow of nitrogenous components to the abomasum. Orthogonal contrasts of feed intake, infusate composition and their interaction were determined as described by Steel and Torrie (1966).

Experiment Three

Using the same steers and diets as in Experiment 2, a crossover experiment was designed. Steers consumed the diet ad libitum and received abomasal infusions of either 120 g dextrose with or without addition of 42 g of urea. Administration of the infusate solution and all sampling was the same as in Experiment 1. Treatment differences in the crossover design were compared according to procedures of Cochran and Cox (1957).

Results

Experiment One

Dry matter intake (Table IX) was not altered by casein infusion. Mean feed intake of steers in this trial was 5.3 kg DM/day or 2.4% of body weight. According to the net energy equations (Lofgreen &

TABLE IX. EXPERIMENT ONE: INTAKE AND DIGESTIBILITY OF A CORN UREA DIET BY GROWING STEERS WITH ABOMASAL PROTEIN INFUSIONS

Casien Infusion (g/day)	0	20	40	80	120	S.E.
Dry matter intake (kg/day)	5.61	5.31	5.65	5.26	4.99	.37
Total nitrogen intake (g/day)	117.8	112.6	112.5	111.1	107.1	20.7
Dry matter digestibility, %	71.5^{a}	75.2^{b}	72.6^{a}	71.1^{a}	70.4^{a}	.74
Nitrogen digestibility, %	69.5^{cd}	71.7^{c}	69.7^{cd}	67.6^{de}	66.8^{e}	.84

^{a,b}Means within a row with different superscripts differ significantly ($P<.10$).

^{c,d,e}Means within a row with different superscripts differ significantly ($P<.025$).

Garrett, 1968) this level of intake of this ration should provide sufficient energy to achieve .75 kg/day gain. Weights at 2 week intervals revealed that gains averaged .90 kg/day. Dry matter and nitrogen digestibility were increased slightly at the 20 g infusion level. No explanation for this increase is apprent. Nitrogen retention was not altered significantly by casein infusion. Nitrogen retention averaged 37 g per steer daily (Table X) or 33% of nitrogen intake. Ruminal ammonia nitrogen (Table XI) averaged 7.5 mg/dl and was not altered significantly with casein infusion. Blood plasma glucose values trended to decline from 83 to 74 mg/dl as infused casein increased.

Experiment Two

Dry matter and nitrogen intake (Table XII) differed with the feeding regime. Dry matter digestibility averaged 81% for the limit-fed steers and 77% for the steers consuming feed ad libitum ($P<.10$). Nitrogen digestibility tended to be slightly lower with the higher feed intake.

Casein infusion increased nitrogen retention with limited or ad libitum feed intake (43 and 20%; Table XIII), but the differences were nonsignificant. As a percentage of total nitrogen provided, casein infusion increased nitrogen retention by 40% with limit feeding but only 9% with consumption ad libitum.

Results of analysis of the nitrogen components of abomasal samples are presented in Table XIV. Total nitrogen as a percentage of dry matter was 23% greater with steers consuming feed ad libitum.

TABLE X. EXPERIMENT ONE - NITROGEN RETENTION OF STEERS RECEIVING CASEIN INFUSION

Abomasal Casein Infusion	0	20	40	80	120	S.E.
Fecal N, g/day	180.0	160.3	180.3	181.2	172.7	16.8
Urinary N, g/day	206.4	225.7	198.4	198.7	195.7	17.1
Nitrogen Retention grams/day	40.5	35.5	42.8	35.1	32.7	± 5.5
as % intake	34.4	31.4	36.3	31.1	32.4	± 5.5

TABLE XI. EXPERIMENT ONE - RUMINAL AMMONIA NITROGEN AND BLOOD PLASMA GLUCOSE OF STEERS RECEIVING CASEIN INFUSION

Casein Infusion	0	20	40	80	120	S.E.
Ruminal NH_3-N, mg/dl	15.5	7.3	4.0	7.1	3.5	4.6
Blood plasma glucose, mg/dl	83.3	83.0	75.4	72.8	74.2	5.4

TABLE XII. EXPERIMENT TWO - INTAKE AND DIGESTIBILITY OF A CORN-UREA DIET BY GROWING STEERS RECEIVING ABOMASAL INFUSIONS

Treatment	Limit Fed	Limit Fed 120 g Casein Infused	Ad Lib Fed	Ad Lib Fed 120 g Casein Infused	S.E.
Dry matter intake[a] (kg/day)	2.52	2.57	4.56	4.75	.3
Total nitrogen intake[a] (g/day)	65.5	66.2	105.6	109.3	6.4
Dry matter digestibility	82.3	79.6	77.1	76.3	2.5
Nitrogen digestibility	78.0	75.4	73.4	69.3	3.9

TABLE XIII. EXPERIMENT TWO - NITROGEN RETENTION OF STEERS FED A CORN-UREA DIET AND RECEIVING ABOMASAL INFUSIONS

Treatment	Limit Fed	Limit Fed 120 g Casein Infused	Ad Lib Fed	Ad Lib Fed 120 g Casein Infused	S.E.
Nitrogen retention[a] g/day	9.89	14.18	20.63	24.82	8.3
Nitrogen retention as % nitrogen intake	15.09	21.16	19.43	21.19	7.8
Nitrogen retention as % nitrogen digested	19.3	27.7	26.7	30.7	9.8

[a]Significant intake effect ($P<.1$).

TABLE XIV. EXPERIMENT TWO - ABOMASAL NITROGEN FRACTIONS OF STEERS FED A CORN-UREA DIET AND RECEIVING ABOMASAL INFUSIONS

Item	Limit-Fed		Ad Libitum Fed		+ S.E.$_m$
	0 Casein	120 Casein	0 Casein	120 Casein	
Abomasal N, mg/g DM[a]	25.7	25.0	33.8	32.0	2.14
mg/dl	266.4	339.1	394.1	329.4	50.0
NH_3-N, mg/dl	15.4	13.8	13.7	11.5	1.05
Non-Ammonia N, mg/dl	251.1	326.8	380.4	317.9	50.6
% of total abomasal N	90.4	87.8	95.1	96.0	2.2
Microbial N, mg/g DM	3.34	6.57	2.21	2.93	1.19
% of total[b] abomasal N	14.1	28.4	6.51	9.58	3.64
By-passed feed N, % of total[b] abomasal N	76.4	59.4	89.4	86.4	4.7

[a]Feed intake effect ($P<.05$).

[b]Feed intake effect ($P<.025$).

Ammonia nitrogen differences were small with a mean value of 13.6 mg/dl. Non-ammonia nitrogen as a percentage of total abomasal nitrogen was 89 and 96% for the two treatments, respectively.

Microbial nitrogen expressed as a percentage of total abomasal nitrogen decreased ($P<.0.1$) with increased feed intake. Bypassed feed nitrogen represented 68% and 88% of abomasal N for the limit and ad libitum fed steers respectively. Ruminal ammonia nitrogen was not significantly effected by intake regime or infusion level (Table XV). Ruminal ammonia-nitrogen should have been adequate to prevent an ammonia deficiency. Blood plasma glucose values were not significantly altered with intake regime or infusion level (Table XV).

Experiment Three

Daily dry matter intake averaged 4.5 and 4.8 kg DM for the dextrose and dextrose plus urea infusion respectively (Table XVI). Urea infusion provided an additional 21.5 g of nitrogen. No differences in dry matter digestibility or nitrogen digestibility were noted with infusion. Nitrogen retention (Table XVII) in grams per day was increased slightly (8.8 g/d or 42.5%) with urea infusion.

Discussion

Feed Intake

Dry matter intake and nitrogen retention were not increased by infusion of casein in the first experiment. This disagrees with results with lambs by Papas et al. (1974) who found an increase in intake of a semi-purified 9% crude protein diet with abomasal casein infusion. Higher intakes (2.4% of body weight) were obtained in this

TABLE XV. EXPERIMENT TWO - RUMINAL AMMONIA NITROGEN AND BLOOD PLASMA GLUCOSE OF STEERS FED A CORN-UREA DIET AND RECEIVING ABOMASAL INFUSION

Treatment	Limit Fed	Limit Fed 120 g Casein Infused	Ad Lib Fed	Ad Lib Fed 120 g Casein Infused	S.E.
Ruminal NH_3-N (mg/dl)	13.85	13.71	13.32	10.89	2.7
Blood plasma glucose (mg/dl)	77.07	72.46	79.73	78.99	3.9

TABLE XVI. EXPERIMENT THREE - INTAKE AND DIGESTABILITY OF A CORN-UREA DIET BY STEERS ABOMASALLY INFUSED WITH NPN

Abomasal Infusate	120 g Dextrose	120 g Dextrose + 42 g Urea	S.E.
Dry matter intake (kg/day)	4.52	4.80	.24
Total nitrogen intake (g/day)	89.18[a]	110.68[b]	10.8
Dry matter digestibility %	74.1	75.6	1.8
N Digestibility %[c]	67.7	71.8	3.8

[a,b]Means within a row differ significantly (P<.1).

TABLE XVII. EXPERIMENT THREE - NITROGEN RETENTION OF STEERS ABOMASALLY INFUSED WITH NPN.

Abomasal Infusate	120 g Dextrose	120 g Dextrose + 42 g Urea	S.E.
Nitrogen Retention g/day	20.86	29.73	4.0
% N intake	21.67	26.73	2.4
% N digested	34.95	37.29	3.0

experiment than in their trial and our basal protein level was from intact protein, not NPN. Consequently, bypass of feed protein would be considerably greater in the present study. In the latter two experiments, protein infusion and non-specific nitrogen infusions tended to increase feed intake. In these experiments, nitrogen balance also increased with infusion. In these experiments however, feed intakes were lower than in the first experiment. These results support the concept that meeting a post-ruminal nitrogen deficiency might increase energy intake.

Digestibility

In experiment 1, a slight increase in dry matter digestibility and nitrogen digestibility was observed at the 20 g per day casein infusion level. Digestibility tended to respond quadratically to casein infusion. No explanation is apparent. In experiment 2, increasing dry matter intake tended to depress dry matter digestibility. Higher feed intake often decreases digestibility of energy (Tyrrell & Moe, 1972). Furthermore, site of digestion may be shifted downstream in the tract by higher feed intakes (Galyean et al., 1979). Such a shift might increase microbial growth in the large intestine and decrease apparent nitrogen digestibility.

Nitrogen Retention

Supplemental abomasal casein did not increase nitrogen retention in experiment 1. Since nitrogen retention did not increase with post-ruminal protein supply, this suggests that the post-ruminal supply of protein (from microbial protein produced in the rumen plus by-passed

feed protein) must have met the post-ruminal requirements of these steers and some other nutrient limited nitrogen balance. Expected daily gain calculated from nitrogen retention, assuming that deposited tissue is 15% protein, would be 1.2 kg. Average daily gain during this experiment was .92 kg.

In experiment 2, casein was abomasally infused into steers which had limited or unlimited feed available. Feed intake in this trial was less than in experiment 1, even with unlimited feed available. Most previous trials which have shown benefit from abomasal infusion of protein have limit fed their animals (Schelling & Hatfield, 1968; Chalupa, 1972; Ranawana & Kellaway, 1977). Nitrogen retention data support the hypothesis that response to post-ruminal protein is greater with limit feeding. Nitrogen retention increased 40% with infused casein for limit-fed steers contrasted with a 9% increase for steers consuming feed ad libitum. Results agree with those of Schelling and Hatfield (1968) who found a more pronounced increase in nitrogen retention with casein infusion with limit feeding than ad libitum consumption. Since most research trials use confined, limit-fed animals, caution must be exercised when extrapolating results to field conditions.

Limiting energy intake can reduce the post-ruminal protein supply in three ways. First, microbial protein supply is reduced as digestible organic matter intake declines (Isaacson et al., 1975). Furthermore, longer ruminal retention time increases degradation of protein from some feedstuffs (Galyean et al., 1979). Finally, the reduced ruminal turnover rate with lower feed intake will reduce efficiency of bacterial growth (Cole et al., 1976). Consequently, the

post-ruminal protein supply may decrease substantially with limit-feeding of ruminants. Direct measurements in another trial support this suggestion (Zinn & Owens, 1980).

For the non-ruminant animal, the protein to calorie ratio needed for maintenance is less than that needed for growth. However, for the ruminant, the post-ruminal protein calorie ratio required could decrease with increased intake and growth since metabolic fecal protein increases with feed intake. This would agree with calculations for energy and protein requirements based on NRC (1981) formulas.

In experiment 1, the diet provided 421 g MP/day, 335 from rational feedstuffs and 86 from urea addition to the feed, while the MP requirement is estimated as 430 g/day. The casein infusion raised the MP of the total diet incrementally to 541 g MP/day. If we compare the metabolizable protein (MP) (Burroughs et al., 1975) of these two diets against the MP requirements we note no increase in NR as we surpass the MP requirement with casein infusion.

In the second experiment the limit-fed animals had a MP intake (195 g/day) slightly above their requirement (169 g MP/day). Nevertheless, abomasally infused casein to increase MP supply to 299 g MP/day tended to increase nitrogen retention (14 vs 10 g). Steers consuming their feed ad libitum had MP intakes below their requirement (363 vs 445 g MP.day). Abomasally infused casein to increase the MP to 483 g/day increased NR only slightly (25 vs 21 g). This suggests that the MP value of a feedstuff may change with feed intake. If lower intakes allow for slower ruminal turnover for more complete feedstuff digestion, the assumption of 52% TDN digestion in the rumen may be incorrect.

Results may be interpreted to question whether dietary protein

requirements should be expressed on a basis of percentage or as grams per day. Based on the responses to infusion, one could speculate that at lower feed intakes, the percentage protein in the ration needs to be increased to maintain a post-ruminal protein calorie ratio.

The effect of abomasal urea infusion on nitrogen retention was examined in experiment 3. Nitrogen retention tended to increase with urea infusion. This suggests a post-ruminal non-specific nitrogen deficiency or some other benefit from urea supplementation. Several previous experiments have noted nitrogen balance increases with post-ruminal infusion of glutamic acid, urea, or other non-specific sources of nitrogen (Egan, 1965; Schelling & Hatfield, 1968; Nimrick et al., 1971). Several experiments (Hill et al., 1980; Chalupa, 1972) which have not used isonitrogenous control infusions have shown benefit from post-ruminal protein supplementation. Increased nitrogen retention with urea infusion makes one question the validity of experiments which have not used isonitorgenous infusions. In some experiments, total nitrogen intake, fed plus infused, correlates well with nitrogen balance suggesting that non-specific nitrogen may have limited nitrogen retention.

Infusion of casein in these trials did not significantly alter the concentration of nitrogen in the dry matter reaching the abomasum. As compared with limit feeding, ad libitum consumption increased the concentration of nitrogen in abomasal dry matter by 237%. To estimate total protein flow to the abomasum, samples of abomasal contents were analyzed for chromium and calcium content and related to daily intake of these minerals. Based upon chromium concentration, total N passage with limited intake calculated to be 127.3 g daily compared with 307.8 g

daily with ad libitum consumption. Using calcium as a marker, values were 103.8 g versus 244.0 g of abomasal nitrogen passage. These values all appear erroneously high since daily N intakes were only 66 and 107 g. Possible explanations for such high passage values are, (1) inadequate time for chromium to equilibrate (3 days) and (2) non-representative sampling of the abomasal contents. Relative increases in protein flow with ad lib consumption (238%) exceed the difference in protein intake (162%).

Across these experiments using 230 to 290 kg steers gaining weight at a rate of 1 kg/d and ad libitum fed a high-energy, corn based ration, an increased post-ruminal protein supply did not increase nitrogen balance or feed intake relative to equal amounts of added energy and nitrogen from glucose and urea. Failure of supplemental post-ruminal amino acids to increase nitrogen balance conflicts with most literature results. The greater feed intake, higher basal protein concentrations, and isonitrogenous controls are obvious differences between this experiment and most of those conducted previously. Yet in feedlots, cattle generally are fed protein levels greater than that used in this trial. Benefit from such protein supplementation may be attributable to factors other than post-ruminal amino acid supply from the added protein. The added protein may alter time for and extent of ruminal digestion and microbial protein synthesis by (1) relieving a ruminal ammonia deficiency, or (2) stimulating bacterial growth by providing additional amounts of certain nutrients (potassium, sulfur or phosphorus), or buffering action. Another possibility is an increased intestinal digestibility of starch or energy achieved by reducing intestinal passage rate.

To explore these hypotheses, further research is required specifically examining the influence of protein level and feed intake on (1) protein bypass, (2) microbial protein synthesis, and (3) site and extent of energy and protein digestion.

CHAPTER V

SUMMARY

Supplemental post-ruminal nutrients were provided in four experiments with steers fed rations of low or high digestibility to measure the effects on feed intake, digestibility and nitrogen retention. In the first experiment dextrose, casein, corn oil or methionine was infused into the abomasum of five 605 kg steers. Steers consumed winter harvested prairie hay ad libitum plus .9 kg daily of a 15% crude protein supplement. Feed intake was not affected by any infused nutrient suggesting that intake was limited by physical capacity or bulk fill. As casein infusion increased nitrogen retention (NR) and plasma insulin increased, suggesting that post-ruminal protein will improve the nitrogen status of steers fed low protein prairie hay. Methionine alone did not improve NR. Richardson and Hatfield (1978) had shown methionine to be one of the first limiting amino acids for growing steers and it was hypothesized that methionine may also limit NR of mature steers fed low quality roughage. Our results and a trial by Leme (1978) refute the hypothesis that methionine is deficient for cattle consuming Oklahoma low quality forages. Blood plasma insulin levels paralled NR values in this trial. Possibly insulin could be used as a tool to monitor nitrogen status of cattle fed low quality, low-protein diets.

In a second experiment, five levels of casein were abomasally infused into five growing 226 kg steers consuming a high concentrate-urea supplemented corn grain ration ad libitum. NR was not altered by supplemental casein infusion. This conflicts with past trials with limit fed steers and suggests that feed intake alters post-ruminal protein supply.

Consequently, a third experiment examined whether feed intake influenced the benefit from post-ruminal protein supplementation. The consumption of feed by four (306 kg) steers was either limit or ad libitum. The diet was a 1% urea supplemented corn grain diet and steers received a urea-dextrose or casein infusion abomasally. NR was not affected significantly by casein infusion on either intake level. However on a percentage basis, casein infusion with limited intake increased NR twice as much as with ad libitum intake.

A fourth experiment was conducted to determine if supplemental abomasal urea nitrogen might prove beneficial. Four steers (313 kg) consumed the ration ad libitum and abomasally infused with either dextrose or dextrose plus urea. A slight NR response to supplemental urea was observed. Results suggest that energy, total nitrogen or some other nutrient, other than amino acids limit NR and feed intake of young rapidly growing steers.

The last three trials conducted with growing steers, conflict with many concepts presented in the literature. In all of these trials, abomasal infusions of protein were compared with a urea-dextrose control. Thereby rations were isocaloric and isonitrogenous. Such has not been true with many trials from the literature. In past trials, NR has often paralleled total nitrogen intake (consumed plus infused)

suggesting that total nitrogen, not amino acids, may be responsible for the effects observed. Furthermore, in our experiments feed intakes and basal protein levels more similar to commercial practice were achieved.

For all rations nitrogen intake including the infusates met or exceeded the urea fermentation potential of the diet and ranged from a deficit to an excess of the calculated metabolizable protein requirements of steers. Increased protein bypass with increased feed intake failure to improve NR as MP was added suggests that MP values may change as feed intakes change. This suggests that feed intake needs to be included as a dynamic part of an ideal protein evaluation system. The concept of a linear relationship between MP protein intake and MP supply to the intestines needs re-evaluation. The MP system assumes that values for percentage protein bypass and percentage of TDN digested in the rumen are constant, neither of which match results from this thesis. Reasonable success of the MP system to predict response to additional post-ruminal protein in these trials may be due to counterbalancing errors in the MP in underestimating the additional protein requirement for growth. In addition to feed intake, type and form of diet and degree of processing probably alter rumen parameters and site and extent of digestion and need to be considered in any protein evaluation scheme. Future systems for protein and other nutrients must consider such factors.

Future trials measuring the effect of intake and processing on the metabolizable protein value of a feedstuff are needed. Interactions between feedstuffs also need to be tested. For example, the MP value of a corn grain soybean meal mixture may have one value when fed as a feedlot ration and a different value when fed as a supplement

for a low quality forage. Consequently, type of diet may prove important when evaluating a feedstuff as a supplement.

Finally, level of feed intake in all trials needs to be reported. If practical application is intended, feed intake should be as close to that attained under field conditions as possible. As exemplified by historical starch digestion trials reporting 100% digestibility, subnormal intake levels in research trials may prove useless and misleading for researchers and livestock producers.

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APPENDIXES

APPENDIX A

UREA FERMENTATION POTENTIAL EQUATIONS

Following are equations for determining Urea Fermentation Potential (UFP) of a diet and the Metabolizable Protein Values and Requirement. Also included is a calculator program for the TI-59 programmable calculator for these determinations.

1. $$UFP = \frac{1.044\ (\%\ TDN) - \frac{10\ (\%\ CP)\ (\%\ \text{degraded in rumen})}{100}}{2.8}$$

2. Metabolizable protein in a feed if UFP is ≥ 0.

$$MP = .8\ (\%\ \text{degraded in rumen})\ (\%\ CP)\ (.1) - 12 + .9\ 10\ (\%\ CP) - \frac{10\ (\%\ CP)\ (\%\ \text{degraded in rumen})}{100}$$

if UFP is < 0

$$MP = .8\ [1.044\ (\%\ TDN] - 12 + .9\ \ 10\ (\%\ CP) - \frac{10\ (\%\ CP)\ (\%\ \text{degraded in rumen})}{100}$$

3. Metabolizable protein of an animal is:

$$(\text{wt in lbs})^{.734} \times 24) + [\text{ADG in lbs})\ (3527 - \text{wt in lbs})] \times .0526$$

Title: MP and UFP Determinations and Requirement

Part 1 - calculates the MP and UFP of individual feedstuff and multiply by % of diet to calculate MP and UFP of a total diet.

Part 2 - calculates MP and UFP of supplement.

Part 3 - calculate the MP requirement of the animal.

Step	Procedure		Enter	Press	Display
Part 1 - Determination of MP and UFP of a Feedstuff and a Ration.					
	ALWAYS Clear Memory after reading.				
1	Example corn	% CP	10	A	2.0
2		% TDN	91	R/S	3.0
3	Det. MP	% Protein degraded	62	R/S	32.6 MP g/lb feedstuff
4		% Feedstuff in ration	.7	R/S	22.8
5	Det. UFP			R/S	5.3 UFP (feedstuff)
6				R/S	3.7
	- repeat steps 1, 2, 3, and 4 for each feedstuff -				
	Hay	% CP	18	A	
		% TDN	53	R/S	
		% Protein degraded	68	R/S	38.1 MP g/lb feed-stuff
		% in ration	.3	R/S	11.4
				R/S	-10.9 UFP of feed-stuff
				R/S	-3.3
	MP of total ration			Rcl 20	34.2 MP g/lb of ration
	UFP of total ration			Rcl 21	.5 UFP of ration

Step	Procedure	Enter	Press	Display
Part 2 - Determine the MP and UFP of a commercial supplement.				
1	% CP	36	B	36.0
2	% CP NPN	0	R/S	54.4 Mp g/lb
3			R/S	-34.1 UFP
Part 3 - Determination of MP requirement.				
1	weight of animal (lbs)	500	D	500.0
2	ADG (lbs)	2.42	R/S	506.2 MP total reqt.

```
000  76 LBL
001  11  A
002  18 C'
003  65  ×
004  10 E'
005  95  =
006  42 STO
007  17  17
008  99 PRT
009  91 R/S
010  42 STO
011  18  18
012  43 RCL
013  18  18
014  65  ×
015  43 RCL
016  17  17
017  95  =
018  44 SUM
019  20  20
020  99 PRT
021  91 R/S
022  43 RCL
023  05  05
024  65  ×
025  10 E'
026  95  =
027  42 STO
028  16  16
029  99 PRT
030  91 R/S
031  43 RCL
032  18  18
033  65  ×
034  43 RCL
035  16  16
036  95  =
037  44 SUM
038  21  21
039  99 PRT
040  98 ADV
041  91 R/S
042  76 LBL
043  16 A'
044  18 C'
045  91 R/S
046  43 RCL
047  05  05
048  91 R/S
049  76 LBL
050  18 C'
051  58 FIX
052  01  01
053  65  ×
054  01  1
055  00  0
056  95  =
057  42 STO
058  01  01
059  02  2
060  95  =
061  91 R/S
062  65  ×
063  01  1
064  93  .
065  00  0
066  04  4
067  04  4
068  95  =
069  42 STO
070  03  03
071  03  3
072  95  =
073  91 R/S
074  65  ×
075  43 RCL
076  01  01
077  55  ÷
078  01  1
079  00  0
080  00  0
081  95  =
082  42 STO
083  02  02
084  94 +/-
085  85  +
086  43 RCL
087  03  03
088  95  =
089  55  ÷
090  02  2
091  93  .
092  08  8
093  95  =
094  42 STO
095  05  05
096  77  GE
097  38 SIN
098  43 RCL
099  03  03
100  61 GTO
101  39 COS
102  76 LBL
103  38 SIN
104  43 RCL
105  02  02
106  76 LBL
107  39 COS
108  65  ×
109  93  .
110  08  8
111  75  -
112  01  1
113  02  2
114  85  +
115  93  .
116  09  9
117  65  ×
118  53  (
119  43 RCL
120  01  01
121  75  -
122  43 RCL
123  02  02
124  95  =
125  42 STO
126  04  04
127  92 RTN
128  76 LBL
129  10 E'
130  93  .
131  04  4
132  05  5
133  03  3
134  05  5
135  09  9
136  02  2
137  03  3
138  07  7
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```
139  92 RTN
140  76 LBL
141  12  B
142  47 CMS
143  58 FIX
144  01  01
145  42 STO
146  10  10
147  91 R/S
148  42 STO
149  11  11
150  94 +/-
151  85  +
152  43 RCL
153  10  10
154  95  =
155  42 STO
156  12  12
157  65  X
158  01  1
159  93  .
160  05  5
161  01  1
162  01  1
163  95  =
164  92 RTN
165  76 LBL
166  19 D'
167  43 RCL
168  12  12
169  65  X
170  93  .
171  09  9
172  04  4
173  08  8
174  06  6
175  85  +
176  43 RCL
177  11  11
178  65  X
179  01  1
180  93  .
181  06  6
182  01  1
183  04  4
184  95  =
185  94 +/-
186  92 RTN
```

```
187  76 LBL
188  17 B'
189  12  B
190  55  ÷
191  10 E'
192  95  =
193  91 R/S
194  19 D'
195  55  ÷
196  10 E'
197  95  =
198  91 R/S
199  76 LBL
200  14  D
201  47 CMS
202  42 STO
203  00  00
204  45 Y^x
205  93  .
206  07  7
207  03  3
208  04  4
209  65  X
210  02  2
211  04  4
212  95  =
213  42 STO
214  19  19
215  43 RCL
216  00  00
217  91 R/S
218  65  X
219  53  (
220  03  3
221  05  5
222  02  2
223  07  7
224  75  -
225  43 RCL
226  00  00
227  95  =
228  44 SUM
229  19  19
230  93  .
231  00  0
232  05  5
233  02  2
234  06  6
235  49 PRD
236  19  19
237  43 RCL
238  19  19
239  91 R/S
240  00  0
241  00  0
242  00  0.
243  00  0
```

APPENDIX B

RNA ANALYSIS

RNA Analysis

Sample Preparation:

A. Homogenize rumen samples (strained through double layer of cheesecloth).

B. Weigh out .4-.6 g of very finely ground solid (or 25 ml. of the liquid portion of homogenized sample).

C. Incubate in glucosidase (reference origin) for 12 hrs @ 60°C to eliminate carbon sugar interference.

Purification Procedure:

A. Extract 10 min. @ 25°C in 20 mls ethanol-NaCl
Spin (15 min. 12,000 x g) and decant

B. Extract 10 min. @ 70°C in 20 mls ethanol
Spin (15 min., 12,000 x g) and decant.

C. Extract 10 min. @ 70°C in 20 mls ethanol-NaCl
Spin (15 min, 12,000 x g) and decant (repeat until the supernatant solution remains colorless.

Extraction:

A. Extract nucleic acid from moist residue with 10 ml. of 10% NcCl for 30-60 min. @ 100°C in plastic stoppered centrifuge tubes.

B. Centrifuge 5 min. @ 12,000 x g (decant supernatant and save).

C. Extract residue a second time for 30-60 min. at 100°C and filter the suspension while not through 4.25 cm discs of Whatman No. 1 filter paper with suction.

D. Wash centrifuge tubes with 5 ml of water then pour through the residue on filter paper. Add filtrate to supernatant from first extraction and chill in ice water.

Precipitate:

A. Precipitate nucleic acids by adding 5 mls. of cold 10% trichloroacetic acid.

B. Stir suspension and keep 1 hr. @ 0°C.

C. Centrifuge 10 min. @ 27,000 x g @ 0°C.

D. Discard supernatant

E. Wash pellet with .2 N PCA and centrifuge (10 ml (PCA).

F. Solubilize in 7 mls .5 N perchloric acid for 46 min. in a 90°C water bath and mix periodically.

G. The supernatant solution resulting after centrifugation is decanted through glass wool into a 10 ml volumetric.

H. The ppt. in the tube is washed in 1 ml. .5 N PCA and filtered into the 10 ml vol.

I. Glass wool is rinsed and the flask made to volume with .5 N PCA.

J. This solution is used for orcinol procedure.

Solutions:

ETOH-NaCl - 800 ml, 95% ETOH and 200 ml 10% NaCl

10% NaCl - 100 g NaCl per 1000 ml

10% TCA - 100 g TCA per 1000 ml

.5 N PCA - 4.25 ml PCA per 100 ml

Orcinol - .6 g orcinol per 10 ml 95% ETOH

HCl - .5 ml of 10% $FeCl_3$ .6 H_2O in 100 ml HCl

.1 N KOH - 5.6 g KOH per 100 ml

RNA - .0025 g per 25 ml of .1 N KOH

VITA

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Education: Graduated from Fairfield Community High School, Fairfield, Illinois in May, 1971; received a Bachelor or Science in Agriculture from the University of Illinois at Urbana-Champaign, Illinois, in January, 1975; received a Master of Science from the University of Illinois at Urbana-Champaign, Illinois in May, 1976; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in December, 1980.

Professional Experience: Undergraduate research assistant at University of Illinois Metabolism Unit, 1973-1975; graduate research assistant at University of Illinois, 1975-1976; research associate, Dixon Springs Agricultural Center, 1976; graduate research and teaching assistant at Oklahoma State University, 1976-1979.

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