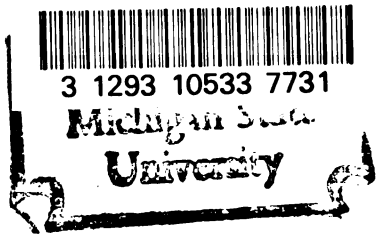

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NITROGEN PASSAGE TO THE LOWER
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STUDIES ON THE EFFECT OF DIET ON NITROGEN PASSAGE TO THE LOWER GASTROINTESTINAL TRACT IN STEERS

By

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M.S.

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ABSTRACT

STUDIES ON THE EFFECT OF DIET ON
NITROGEN PASSAGE TO THE LOWER GASTROINTESTINAL
TRACT IN STEERS

By

Stephen Scott Sachtleben, M.S.

Nitrogen Passage Studies

Three experiments were conducted to evaluate the effect of diet on nitrogen passage to the lower gastrointestinal tract in steers. In the first two experiments a total of four concentrate rations were utilized. These diets were corn based and were formulated so that the low protein diet { 7.0% crude protein (CP) } served as the basal ration and the subsequent two rations were supplemented with soybean meal to final levels of 9.9% and 12.6% CP, respectively. The fourth ration was the 12.6% CP diet supplemented with urea to a final CP level of 15.6%. Lignin and chromic oxide were used as markers to determine nitrogen (N) flow rates. Total nitrogen passage per day was 60.9, 106.8 and 145.8 grams for rations 1-3, respectively. These means were significantly different ($P < .05$) across all treatments. Non-ammonia nitrogen (NAN) passage per day for treatments 1-3 were 53.7, 94.3 and 127.3 grams, respectively. Again, all treatments were significantly different ($P < .05$) across treatments. NAN as a percent of total N intake was not significantly different ($P < .05$) for rations 1, 2 and 3 (87.3, 77.8 and 77.4%, respectively). Data from steers fed ration 4 could not be analyzed statistically since

different animals were utilized. Their total N and NAN passage/day were 112.7 and 98.5 grams, respectively. NAN as a percent of total N intake was 54.0%.

In experiment three, four Hereford steers fitted with abomasal cannulae were fed a total of six corn silage rations differing in the amount of anhydrous ammonia applied { control, 7.8 g Anhydrous-NH_3 per kilogram corn silage dry matter (AN/KGCSDM) and 15.6 g AN/KGCSDM} and the absence or presence of monensin in the supplement. Chromic oxide, polyethylene glycol and lignin were used as markers to estimate N passage to the lower gut. However, due to marker system difficulties, the estimated flow data obtained was not realistic.

A correlation study between individual abomasal samples and a composite made from samples representing a 24 hour period showed no relationship between any one or two times of the day when contrasted to a 24 hour composited sample. Thus, collections over an extended period must be made to ensure a representative sample.

Nitrogen Balance

A nitrogen balance was conducted to evaluate the N status of eight Hereford steers fed the six silage treatments from the previous experiment. Rumensin had no significant effect on nitrogen retention ($P > .10$), but the treatment of silage with anhydrous ammonia at either level resulted in significantly higher ($P < .05$) N retention in steers when compared to control animals. Nitrogen retained as a percent of total N intake was affected by the level of anhydrous ammonia ($P < .10$) and monensin addition ($P < .10$). The nitrogen retained as a percent of

N intake increased with increasing dietary protein, however, with the addition of monensin, N retained as a percent of N intake decreased with the highest protein level.

MEMORIAL

This dissertation is dedicated to and written in the memory of my brother, Peter, whose short time on God's earth will always be cherished by all those he touched. As close as we were in life, he'll always be closer in my heart and memory.

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INTRODUCTION

The optimal levels of non-protein nitrogen (NPN) addition to rations fed to high producing cattle in respect to the efficiency of microbial cell yield has been debated for a number of years. Several researchers have attempted to pinpoint at what level further increases in rumen ammonia concentration will not further enhance microbial cell protein (MCP) yields thus making further NPN additions to the diet unnecessary.

Ørskov (1972) showed that in lambs fed barley-based rations one could supplement the diet with urea up to 12% crude protein equivalent (CPE) and still enhance live weight gains.

Satter and Slyter (1974), in an *in vitro* chemostat that simulated rumen activity, determined that 5.0 mg % ammonia nitrogen (NH_3-N) was sufficient for maximal microbial growth. This is equivalent to approximately 11-14% CPE. Diets producing rumen NH_3 concentration above this point would not increase MCP synthesis. Various factors affect microbial cell yields such as energy availability, growth conditions, cofactor levels and nitrogen availability for *de novo* synthesis. Research is needed to evaluate the addition of NPN above the 11-14% CPE level and to determine the amounts of N flow to the lower gut of animals fed high concentrate rations.

Silage treatment with anhydrous ammonia (AN) at ensiling time is another method of adding NPN to low protein feed sources. There is limited research concerning nitrogen availability (i.e. N passage) to animals

fed such AN treated silage. Research is needed to evaluate the availability of this NPN (at various application levels at ensilement) to the animal in regard to microbial cell yield and actual non-ammonia nitrogen passage to the lower gastrointestinal tract and subsequent absorption.

REVIEW OF LITERATURE

RUMINAL PROTEIN DEGRADATION AND MICROBIAL NH_3-N UTILIZATION

Ruminants fed diets with varying protein sources produce microbial protein of similar amino acid profiles and of similar nutritional quality (biological value = 70-80%; Bergen et al., 1968; Ibraham and Ingalls, 1972). Since the amino acid composition of microbial protein synthesized from differing preformed protein sources is essentially the same, researchers should direct their emphasis to manipulating rumen fermentation so that proteins of poorer quality are degraded in the rumen and resynthesized into microbial protein and the higher quality proteins escape rumen degradation and pass to the lower gut for direct utilization by the host. In reviewing the available literature dealing with protein metabolism in the rumen it would be beneficial to discuss the aspects of nitrogen (N) metabolism i.e., protein degradation to amino acids and ammonia (NH_3), available NH_3-N sources for the rumen NH_3-N pool and the synthesis of microbial crude protein (MCP) and its dependency on available N and carbohydrate fermentation.

Two major considerations that influence protein degradation in the rumen are the protein solubility in the rumen fluid (Hendrick and Marten, 1963; El Shazly, 1958 and Chalupa, 1975) and digesta retention time in the rumen (Chalupa, 1975 and Ørskov and Fraser, 1973). Most highly soluble proteins are degraded extensively and rapidly regardless of the type of ration, but the less soluble proteins are not broken down as extensively with higher dietary intakes and higher dilution rates (Ørskov and Fraser, 1973; Chalupa, 1975).

Goering and Waldo (1974), through their work with feed processing and its effects on protein utilization, implied that protein utilization was a curvilinear function of solubility with optimal nitrogen retention occurring in ruminants which had been fed a diet with protein that was intermediary in regards to protein solubility. El-Shazly (1958) concluded that protein solubility was the major factor in determining ruminal ammonia production and that highly soluble protein should be considered lower in nutritive value since ruminants retain less nitrogen while on highly soluble protein diets.

Bergen, Cash and Henderson (1974) reported that the water soluble nitrogen (WSN) portion of chopped whole corn plant could not be utilized or converted to NH_3-N quickly enough to support rapid cellulose digestion. This information when coupled with an *in vitro* ammonia production study (Bergen, 1974) which showed extremely slow ammonia release by rumen microbes with WSN as a N source when compared to a urea source, suggests why overall rumen fermentation is suppressed when ruminants are fed an all silage ration that is low in protein and high in corn silage WSN.

Aitchison and coworkers (1976) conducted a metabolic trial with dairy cattle fed corn silage and grain diets, supplemented with varying amounts of urea, that ranged from 32 to 49% WSN. Using coefficients of utilization derived from a linear regression model, data clearly showed that the insoluble nitrogen was used to a greater extent than the soluble N. The utilization of the soluble N was relatively constant, but total dietary nitrogen utilization decreased with increasing levels of soluble N. This suggests that the WSN could not be utilized fast enough by

rumen microbes to support cellulose breakdown equivalent to urea hydrolysis and ammonia formation, thus the decrease in the efficiency of total N utilization as soluble N increased. The better coefficient of utilization for the insoluble N could have been the result of a rumen fermentation in which the available energy released from the fermentation more closely matched the release of NH_3-N from the hydrolysis of urea or the portion of the insoluble N which escaped rumen degradation was degraded and utilized by the host of the lower gut.

Pichard and Van Soest (1977) proposed that feed proteins are degraded in a series of steps. First, that portion which is degraded very rapidly represents the soluble dietary protein. This rapid hydrolysis of the soluble fraction is followed by a slower breakdown of less soluble nitrogen components and finally the rumen microbial degradation of a portion of the insoluble protein to NH_3-N and amino acids. That part of the insoluble protein which escapes the rumen relatively unaltered may be degraded postruminally. Bull et al. (1977) stated that although a portion of the protein is insoluble and undegraded in the rumen this does not mean that the protein is undigestable in the small intestine.

A review of the current literature concerning the effect of the level of dry matter intake has shown that depending on that level of intake certain physical and biochemical changes occur. An increase in dry matter intake will cause increased outflow (dilution) rate (Castle, 1956; Grovum and Hecker, 1973; Goshtasbpour-Parsi et al., 1977 and Tammenga et al., 1978), rumen fill (Rumsey and coworkers, 1969), rumination (Welch and Smith, 1969) and volatile fatty acid concentrations (Rumsey et al., 1970). Restricted feed intake will increase rumen pH

with corresponding increases in microbial populations (Slyter et al., 1970).

The level of intake did not affect the degradation of highly soluble proteins, but increased intake decreased the degradation of intermediary soluble proteins due to a higher digesta outflow rate from the rumen thus decreasing the rumen retention time (Miller, 1973).

Goshtasbpour-Parsi et al. (1977) fed lambs two dietary intake levels (500 or 1000 grams/head daily) which contained equal amounts of dietary nitrogen. Total N recovered in the omasum was higher than the dietary N intake with both diets. They found that significantly ($P < .05$) more total nitrogen and non-protein nitrogen (NPN) reached the omasum in sheep fed the higher level of intake. More NPN (amino acids liberated from proteins) was recovered at the abomasum when lambs ingested 1000 grams/day ($P < .05$). In other investigations greater amounts of NPN reached the abomasum than the omasum, probably as a result of abomasal hydrolysis of some preformed protein that passed from the omasum (Amos et al., 1970).

Tammenga, Van Der Koelen and Van Vuuren (1978) utilized dairy cows fitted with re-entrant cannulae in the small intestine to determine the quantity of protein entering the small intestine when the animals were fed mixed diets which varied in N content. As dry matter intake increased, the level of N reaching the small intestine as a percentage of dietary N intake also increased. The authors felt that this was a direct consequence of a decrease in digestion of dietary N with decreased rumen turnover time. Increasing amounts of N also reached the lower gut with increasing dietary protein levels particularly with higher dry matter intakes. These results support the data

obtained by Miller et al. (1973), Ørskov et al. (1974) and Crickenberger et al. (1979).

Tammenga (1978) found that when the lambs ingested more feed, that greater amounts of non-ammonia nitrogen (NAN) entered the duodenum. The author suggests that the difference may be due to an increase in microbial N flow, an increase of undegraded dietary N or a combination of both. Using a regression equation developed by Hvelplund et al. (1976), Tammenga determined that increased NAN passage to the lower gut in lambs that consumed more feed was mainly due to decreased dietary protein breakdown in the rumen. This observation was in agreement with the findings of other researchers who evaluated the effect of increased intake on intestinal N parameters (Zinn, 1978; Goshtasbpour-Parsi et al., 1977; Ørskov and Fraser, 1973).

Contradictory results were obtained by Hogan and Weston (1967a) when they fed sheep diets which ranged from 7.8 to 19.9% crude protein. The NAN recovered at the abomasum was similar regardless of the protein content of the diet consumed by the sheep. Such a low NAN recovery in the abomasum of sheep fed the 19.9% crude protein ration was probably the result of insufficient energy available to the rumen microbes for the assimilation of NH_3-N for microbial protein synthesis. This conclusion was supported by Ørskov et al. (1973) who showed that NAN passage to the lower gut increased with increasing amounts of dietary nitrogen consumed up to about 20% crude protein when adequate energy was made available to rumen microbes.

The quality of feed protein becomes much more important when ruminants are fed at high protein intake levels since more dietary protein bypass is observed.

McDonald (1948) and Mangan (1972) have shown that dietary proteins are degraded by rumen microbes to essentially amino acids and ammonia. Although NH_3-N is the predominant end product of ruminal protein breakdown, small polypeptides and nucleic acid bases are also present in much smaller concentrations (Smith, 1975). Preformed protein is not the only precursor of ammonia. Non protein nitrogen (NPN) from ingested feeds, recycled urea from saliva (McDonald, 1948; Somers, 1961) and urea diffusing through the rumen epithelium (Houpt, 1959; Dobson, 1961) may be utilized. According to Pearson and Smith (1943) in an in vitro system, rumen bacteria hydrolyzed urea to ammonia by using a microbial enzyme, urease. To substantiate this observation, the authors added toluene, a microbial inactivator to the media, and found that no hydrolysis of urea occurred, thus confirming the participation of rumen microbes in urea hydrolysis. The amount of nitrogen recycled as urea is dependent on the circulating levels of urea in the blood and the nitrogen content of the feed (Phillipson, 1962). Ammonia not assimilated by bacteria for protein synthesis will move into the vascular bed of the rumen, then to the portal vein and finally to the liver where it is synthesized to urea (McDonald, 1948; and Cocimano and Leng, 1967) recycled or excreted. Such NH_3-N movement into the rumen wall is against a concentration gradient and dependent on the ruminal NH_3-N concentration and the pH of the rumen digesta (Chalmers and White, 1969). A lower pH would increase NH_3-N passage to the lower gut where most NH_3-N absorption occurs (McDonald, 1948). Hogan (1967) and Smith (1971) have shown that extensive ammonia absorption occurs in the omasum with little or no absorption further down the gastrointestional tract.

The major source of nitrogen for microbial protein synthesis is ammonia. Bryant and Robinson (1962) concluded from their work that 82% of rumen bacteria were capable of growing with ammonia nitrogen (NH_3-N) as their principal N source. Researchers using labelled N^{15} ammonia or N^{15} ammonium nitrate showed that rumen bacteria employed labelled N for their own microbial protein synthesis (Warner, 1965; Phillipson, 1962) and was further found as milk protein in dairy cows (Land and Virtanen, 1959). Although NH_3-N is the predominant source of N for microbial protein, amino acids and small polypeptide are assimilated by various bacterial strains but the mechanism is not known (Bryant and Robinson, 1963; Pitman and Bryant, 1964; Hungate, 1966; Wright, 1967 and Chalupa, 1974). Nolan and Leng (1972) concluded that in sheep fed lucerne (alfalfa) chaff, 29% of the nitrogen digested was incorporated into microbial protein as amino acid nitrogen. Similar data from Pilgram et al. (1970) and Mathison et al. (1971) have been published.

Salter et al. (1979) have shown in rumen fistulated steers fed diets which consisted mainly of straw and tapioca and supplemented with decorticated groundnut meal (DSGM), DCGM plus urea or entirely urea, that when ^{15}N urea was infused into the rumen, the extent at which the labelled N was assimilated into microbial amino acids was dependent on the preformed protein content of the diet. When sufficient preformed units (amino acids and peptides) were available, proline (pro), arginine (arg), histidine (his), methionine (met) and phenylalanine (phe) were used directly by the bacteria. However, in a diet that was inadequate in preformed units such as the diet supplemented solely with urea, synthesis of pro, arg and his increased; met and phe synthesis rates remained constant. Salter et al. (1979) suggest that methionine and

phenylalanine supplies from preformed units may limit bacterial growth on low preformed protein diets that is high in NPN.

Rumen ciliate protozoa utilize bacterial amino acids and possibly other growth factors through bacterial engulfment and digestion (Allison, 1969 and Coleman, 1975). The value of this protozoal protein to the host is not certain, but Oxford (1955) believed that such microbial protein would provide protein similar to natural animal protein. According to Bergen et al. (1968a, b) the protein quality, protein digestibility and lysine concentration of protozoa were superior to that of rumen bacteria. A review of current literature has shown that little is known about the quantitative contribution of protozoal protein to the host animal. However, Hungate et al. (1971) has shown that sheep fed alfalfa pellets produced rumen microbes of which more than 50% were protozoa. Of this large ciliate protozoa population only a small portion was passed to the duodenum, however, Hungate (1966) indicated that perhaps the protozoa were retained in the rumen or omasum thus implying that the microfauna had a slower dilution rate than rumen liquid and small particle pool. Weller and Pilgrim (1974) compared actual appearance of protozoa to that population expected from dilution rate calculations and found only 6-29% of the theoretical yield actually appeared in the omasum. These findings confirmed what Hungate (1966) had speculated in regard to protozoal retardation within the rumen and they moved independently of the liquid pool. The data of Weller and Pilgrim (1974) and Hungate et al. (1971) could imply that a recycling of protozoa occurred with relatively little passage of rumen ciliate protozoa to the lower gastrointestional tract. If these protozoa are indeed retained within the rumen, they

would actually limit total ruminal protein production and decrease the nitrogen available to the host for its own tissue synthesis (Bergen and Yokoyama, 1977).

Ration type (natural versus purified diets) will influence the quantity of total microbial protein which reaches the duodenum. According to Bergen, Purser and Cline (1968), protozoa will represent a higher percentage of total microbial protein in animals fed natural diets when compared to those fed a purified or semi-purified diet. Increasing concentrations of protozoa within the rumen with any given ration will decrease the amount of microbial protein available to the host. Utilizing roughage rations, Hungate et al. (1971), Pilgrim et al. (1970) and Bucholtz and Bergen (1973) found that as a ratio of bacterial: protozoal protein synthesized, considerable variation could have been the result of the experimental approach used to quantitate microbial protein.

FACTORS WHICH AFFECT MICROBIAL SYNTHESIS IN THE RUMEN

The amount of microbial cells synthesized in the rumen is dependent on two major factors: first, the presence of an energy supply adenosine triphosphate (ATP) derived from the fermentation of a substrate and second, the precursors (intermediates from rumen fermentation or its end products) of microbial cell synthesis must be present in adequate concentrations and in a form utilizable by the microbes (Hungate, 1966). Under ideal conditions, rumen fermentation is coupled to cell growth due to carbohydrate degradation, VFA production and ATP generation and their interrelationships with the process of microbial cell synthesis (Walker and Forrest, 1965) . The major end products of this coupled rumen fermentation process are VFA, gas and microbial cells (Hungate, 1966).

Anaerobic microorganisms utilize various limiting metabolites as electron acceptors in the place of oxygen, thus having a lower potential of ATP generation from a particular substrate (Gunsalus and Shuster, 1961). Under such a system, the first limiting factor for microbial growth is energy availability (Gunsalus and Shuster, 1961). According to Hungate (1966) a molecule of hexose fermented in the rumen yields only 10-12% of its aerobic ATP potential.

During ruminal CHO degradation by microbes, the amount of ATP generated is dependent on the type of CHO, the fermentation pathways and final end products (eg. e^- acceptors). The end products of the ruminal fermentation are primarily acetic, propionic and butyric acids and methane (CH_4). Their associated ATP yields are (as moles of ATP/mole VFA or CH_4 formed): acetate, 2; propionate, 3; butyrate, 3; CH_4, 1 (Isaacson et al.,

1975). Portions of the substrate are only broken down to intermediary compounds and these are used as precursors of microbial cell synthesis. Little, if any, ATP is generated by such partial degradation (Allison, 1969), but the energy loss is minute compared to the potential energy cost of de novo microbial macromolecular synthesis from fermentation end products (Stouthamer, 1973).

Although the basic rumen fermentation pathways have been elucidated (Hungate, 1966; Baldwin, 1965) there remains an uncertainty in the theoretical ATP yields associated with the production of propionate and CH_4. According to Baldwin et al. (1963), propionate is produced via the dicarboxylic acid pathway or the direct reductive pathway depending on the CHO source (eg. diet). The pathway utilized to synthesize propionate is dependent on the concentration of dietary carbohydrate ingested. The dicarboxylic pathway is the predominant route, but with increasing amounts of readily fermentable CHO in the diet, the role of the direct reductive pathway increases (Baldwin et al., 1963). The theoretical ATP yield of the dicarboxylic pathway and the direct reduction pathway differ by a single ATP, the former producing 3 ATP (Hobson and Summers, 1972) and the latter 2 ATP (de Vries et al., 1973). According to Wolin (1975), most, if not all, of the propionate in the rumen is produced via succinate (eg. dicarboxylic pathway).

Hydrogen is rapidly utilized in the rumen through its reaction with CO_2 to form CH_4 or acetate (Hungate, 1966). In vitro work conducted by Carroll and Hungate (1955) has shown that CH_4 is the predominant end product in regard to H_2 utilization in an anaerobic system. Methanogenesis has been evaluated in regard to its possible effect on energy yields

within the rumen (Scheifinger et al., 1975; Hungate, 1966). Methane production represents a loss of H_2 that could be transmitted to propionate in the absence of methanogenic bacteria (Scheifinger et al., 1975). There is no net change in ATP synthesis when shifting from CH_4 production to propionate synthesis, yet metabolic H_2 is conserved and propionate production is enhanced (Demeyer et al., 1975).

Nevertheless, in curbing CH_4 synthesis rumen function and feed utilization are disrupted (Garton et al., 1972; Demeyer and Van Nevel, 1975). According to Demeyer and Van Nevel (1975) inhibiting methanogenesis may result in depressed cellulolysis and fermentation rates, protozoa production may be decreased, proteolysis is depressed and H_2 easily accumulates. As a result of the above, microbial growth may decrease and alter the site and extent of protein and CHO degradation. Even though the propionic levels will increase with the prevention of methane production, the use of methanogenic bacteria inhibitors may supress rumen function and microbial synthesis to a point where the beneficial energy derived from increased propionate levels will not be realized to the fullest extent.

Bauchop and Elsden (1960) concluded that the amount of microbial growth was directly proportional to the amount of ATP that could be obtained from the fermentation of the energy source present in the medium. This relationship between cell growth and ATP was defined by Bauchop and Elsden (1960) as Y_{ATP} (grams dry weight synthesized/mole ATP). Initially, the Y_{ATP} was thought to be a constant factor of 10.5 for all microbes (Forrest and Walker, 1971 and Stouthamer, 1969). Based on a Y_{ATP} of 10.5 and assumed stoichimetry of ATP/mole VFA, Hungate (1966) calculated that

the rumen fermentation process can produce approximately 10.0 grams of microbial protein/100 grams of organic matter (OM) digested in the rumen. This 10 grams/100 grams OM fermented would represent the "upper limit" of microbial protein synthesis due to the available energy in 100 grams of fermented carbohydrate. Studies by Stouthamer and Bettenhaussen (1973) indicated, however, that Y_{ATP} was not constant for all microbes and that the efficiency depended on the microbes' growth rate and maintenance requirements. These researchers studied the growth rate of A. aerogenes in an energy limiting chemostat. Stouthamer and Bettenhaussen (1973) showed that as growth rate increased, a concomitant increase in Y_{ATP} was evident. The Y_{ATP}^{MAX} (molar growth yield for ATP corrected for energy of maintenance) was determined to be about 25. They showed that slower growing organisms had a much higher maintenance requirement (biosynthetic reactions). Therefore, it was suggested that Y_{ATP} was a function of the dilution rate (growth rate) and maintenance requirements of that microorganism (Stouthamer and Bettenhaussen, 1973; Bergen and Yokoyama, 1977).

Maintenance energy for bacteria is necessary for the turnover of cell constituents, motility, the preservation of right ionic composition and intracellular pH of the cell and replacement of lysed cells (Marr et al., 1963; Stouthamer, 1977 and Owens and Isaacson, 1977). The amount of energy used for the maintenance is termed m_e or maintenance coefficient and the yield in grams dry cells/mole ATP above maintenance, Y_{ATP}^{MAX} (Stouthamer and Bettenhaussen, 1973).

Isaacson et al., (1975) and Van Nevel and Demeyer (1979) supported the observations of Stouthamer and Bettenhaussen (1973) with mixed rumen

cultures grown at various dilution rates in a continuous culture system with glucose as substrate. They found that Y_{ATP} in rumen bacteria was not constant but varied with dilution rate. At 2% turnover per hour ATP energy used for maintenance as a percent of the total available ATP was 60%, but when the dilution rate increased to 12% per hour, only 15-20% of the total energy was utilized for maintenance. In a continuous culture system maintained at a constant dilution rate, Isaacson et al. (1975) showed bacterial growth efficiency did not change with varied substrate levels. However, when dilution rate was increased, a corresponding increase in microbial growth efficiency was observed. This increase in cellular growth rate efficiency was due to a decreased microbial population and residence time within the fermentor.

Cole et al. (1976a), Kropp et al. (1977) and Harrison and coworkers (1975) observed with ruminants fed various rations of roughages, grains and protein supplements that as dilution rate increased (.02 to .06 h^{-1}), microbial synthesis also increased. The above in vivo data exemplify the conclusions made by Stouthamer and Bettenhaussen (1973) and Isaacson et al. (1975) that the efficiency of microbial growth is dependent on maintenance expenditures which, in turn, is a function of rumen turnover.

Rumen turnover will effect the molar proportion of propionic acid (Hodgson et al., 1975). With every 1% increase in rumen turnover, a decrease of 1.5 to 8% propionate is observed concomitant with increases in acetate and butyrate. Isaacson et al. (1975) concluded that fermentation balance equations indicated that such a shift in VFA was accompanied by increased levels of CH_4 and heat losses and decreased ATP synthesis.

Contrary results to those of Isaacson et al. (1975) and Hodgson et al. (1975) regarding the molar proportions of VFA produced during the fermentation were reported by Van Nevel and Demeyer (1979). In a chemostat with glucose as the growth limiting energy and carbon source, mixed rumen bacteria were maintained at four dilution rates. Increased dilution rates shifted the proportions of the end products: methane decreased and propionate increased. The reasons why propionate increased with increased dilution rates are unclear. However, Van Nevel and Demeyer (1979) suggest several possibilities for such a shift. First, with increased dilution rate, more glucose can be fermented per unit of time resulting in a shift of metabolism toward lactate production and ultimately propionate synthesis. A change in microbial populations (eg. decreased methanogenic bacteria) due to faster rumen turnover may also stimulate alternate electron sink acceptors (eg. propionate).

From fermentation balance equations, metabolic H_2 recoveries were calculated and found to be similar to those of batch cultures (Demeyer and Van Nevel, 1975). Such data contradict the metabolic H_2 recoveries calculated by Isaacson et al. (1975). The difference was suggested by Isaacson and coworkers (1975) to be a result of a bacterium that could utilize acetate or longer chained fatty acids for methanogenesis. If this was the case, their Y_{ATP} values were erroneous since a portion of their maintenance requirements were met by energy obtained from a source other than glucose. Van Nevel and Demeyer (1979) found no such bacterium within their culture system and attributed the presence of the microbe to a difference in inoculum donors and/or the rations they were consuming.

The capacity for microbial cell synthesis is dependent on the ATP availability mainly derived from rapidly fermented carbohydrates and the

efficiency at which the ATP is utilized. From a general viewpoint, higher cereal grain diets encourage lower dilution rates (at least less than full ad lib. intakes) with higher ATP production but lower Y_{ATP} yields. Conversely, those diets that encourage high dilution rates will produce less total ATP but a higher Y_{ATP}. From this example one can see how under two different flow rates the amount of microbial cells produced could be similar.

In order to obtain maximal microbial synthesis, energy availability and CHO source are not the only factors to be considered. Adequate supplies of NH_3-N, carbon skeletons, sulfur, free amino acids and other cofactors are necessary. The whole scheme of VFA production from energy sources (eg. carbohydrates) and microbial synthesis is a coupled process and a deficiency of any one of the metabolites will create limitations on catabolic and anabolic processes which will ultimately lead to decreased VFA and microbial production (Bergen and Yokoyama, 1977).

MONENSIN EFFECTS ON PERFORMANCE

Monensin, a biologically active compound (Haney and Hoehn, 1967) synthesized by Streptomyces cinnamonensis has improved cattle performance by altering rumen fermentation patterns thereby enhancing feed efficiency (Potter et al., 1976a; Utley et al., 1976; Mowat et al., 1977; Perry et al., 1979; Hanson and Klopfenstein, 1979). Data from many research stations have consistently shown that feeding monensin to sheep and cattle has resulted in a decrease in rumen acetate production with a concommitant increase in propionate synthesis of 35-40%; total VFA production changed little, if any (Richardson et al., 1976; Prange et al., 1978). It has been suggested that since the propionic acid is used more efficiently in terms of a metabolizable energy source than acetate (Raun et al., 1976; Van Nevel et al., 1969; Hungate, 1966; Wolin, 1960), cattle fed rations plus monensin should gain more efficiently than those fed rations without the benefit of the additive. However, Ørskov et al. (1979) has recently shown that in sheep infused with various volatile fatty acid (VFA) mixtures (ie. acetic, propionic and butyric) there were no significant differences in the efficiency of utilization of VFA mixtures for maintenance or energy retention. This agrees with the observations of Armstrong and Blaxter (1957), Bull et al. (1970) and Ørskov and Allen (1966).

Goodrich and coworkers at Minnesota (1976) summarized data from 28 trials with steers and heifers which were fed various types of rations with and without monensin. A range of monensin concentrations (5.5, 11.0, 22.0, 27.5, 33.0 and 44.0 ppm of ration DM) were provided in the various rations. The results showed that all cattle with the

exception of those fed 44.0 ppm monensin, had gains equal to or greater than the controls. All cattle fed the additive decreased their feed intakes with increasing monensin levels. Feed efficiency was improved across all treatment levels with the maximum improvement (eg. feed/gain decreased but improved) noted with cattle fed 27.5 ppm monensin.

Two separate trials were conducted by Boling et al. (1977) to study: (1) the influence of monensin level on gain and VFA production in Angus steers grazing on a Kentucky Bluegrass - clover mixture supplemented with either 0, 25, 50 or 100 milligrams monensin per head per day and; (2) the influence of monensin (0, 100, 200 or 300 mg + 4.54 kg corn gain/head/day) on finishing steers fed corn silage ad libitum. In the first trial, average daily gains were greatest for those Angus fed the 50 or 100 mg monensin per day ($P < .01$) when contrasted to steers fed either 0 or 25 mg monensin. Ruminal propionate increased ($P < .01$) in all monensin fed groups. Carcass data were similar although those cattle fed 300 mg monensin per day tended to have lower marbling scores, smaller ribeye areas and less fat over the rib. Data from Brown et al. (1974), Potter et al. (1976) and Thonmey (1977) support the findings of Boling in that monensin had no consistent effect on carcass quality or cutability.

Heifers fed monensin (33 ppm) and implanted with Synovex-H gained 11.5% faster and 6.5% more efficiently than heifers fed monensin without the benefit of a hormonal implant (Woody and Fox, 1977). Similar results were obtained by Burroughs et al. (1976) in feedlot steers fed monensin.

According to Nissen and Trenkle (1976), the addition of monensin to feedlot rations initially reduced dry matter intake by as much as 15-30%. However, intake returns to 90% of the intake recorded for steers fed the control ration by the end of 30 days. The adaptation of both the animal and rumen ecosystem to the monensin may have made an adaptation period a necessity (Poos et al., 1979). In vitro studies by Simpson et al. (1976) and Simpson (1978) found monensin to inhibit cellulolytic activity in an inoculum obtained from an animal not previously exposed to the additive. However, if the animals are adapted to monensin for three weeks prior to inoculum removal from the rumen, no inhibition of cellulolytic activity could be observed (Dinius et al., 1976). In cattle that had been adapted to monensin and then fed a diet supplemented with the additive (33 ppm), Dinius et al. (1976) reported that the additive had no major effect on dry matter, protein, hemicellulose or cellulose digestibilities nor did it effect the total microbial population. This is in conflict with the report by Simpson et al. (1976). Simpson (1978) and Poos et al. (1979) found that monensin was a potent inhibitor of protozoa and cellulolytic bacteria.

The relationship among fermentation rate, dilution rate and the extent of digestion in the rumen is paramount in the determination of protein and AA nitrogen (NAN) reaching the duodenum (Bergen and Yokoyama, 1977). The conversion of dietary N to microbial protein is less efficient in terms of energy utilized with slower rumen turnover rates. When rumen turnover increased, efficiency is significantly increased since microbial populations and residence time in the rumen decline (Owens and Isaacson, 1977).

Lemenager, Owens and coworkers (1978) in Oklahoma conducted a series of *in vivo* studies to evaluate the effect of monensin on rumen turnover, cellulose disappearance and nitrogen components of rumen fluid. Rumen cannulated steers fed low quality winter grass, supplemented with soybean meal and 200 mg monensin per day had a 15.6% ($P < .02$) lower daily feed intake than steers fed grass and soy without monensin. The authors suggested that in steers fed monensin supplemented roughage rations, the lower feed intake was due to a decreased rumen digestion rate resulting in a 44% slower solid and 31% slower liquid turnover rate. Hence the limit of feed intake in steers fed monensin roughage ration is rumen capacity. In the second trial, steers fed a high concentrate ration with or without monensin maintained constant intake levels, however, rumen turnover rate decreased.

Lemenager *et al.* (1978) suggests several reasons why there seems to be a relationship between forage intake and rumen dilution rate. With roughage rations, rumen turnover may simply be depressed due to a decrease in intake (Balch and Campling, 1965). However, with high concentrate rations, intake usually remains constant, yet turnover decreases thus indicating the monensin's effect of depressed dilution rate is independent of intake. Therefore, decreased rumen turnover in ruminants fed monensin may cause a decrease in intake dependent on the type of ration fed, ie. concentrate or roughage.

The authors further speculated that a decreased rate of digestion possibly was responsible for reduced intake. If the digestion of feed particles is retarded, the small size needed to pass into the omasum will not have been reached for a longer period of time resulting in a prolonged rumen retention time. The decrease in dry matter intake

(energy) does not necessarily indicate a detriment to animal performance since increased propionate synthesis, decreased methane (CH_4) production, heat loss and gross fecal energy compensate for that decrease in intake (Lemenager and coworkers, 1978).

With the shift of molar percentages of VFA's towards more propionic and less acetic and butyric acids, the question arose as to how this increase in propionic acid caused improved efficiencies within the ruminant's metabolic system. Hungate (1966) concludes that propionic fermentations are more efficient due to the reduction in CH_4 losses which occur with the production of acetate and butyric. Using the equations for the calculation of theoretical fermentation balances of Wolin (1960), it could be seen that the reduction in CH_4 production accounted for some increase in propionate efficiency. Demeyer and Van Nevel (1975) stated in a review on methanogenesis and its control, that a decline in CH_4 production as a result of high rates of rumen fermentation or turnover would increase the molar percentage of propionate and decrease that of acetate. A shift from a 60% acetate: 30% propionate: 10% butyrate to a 52:40:8 ratio would reflect a 5.6% increase in gross energy savings to the animal (Hungate, 1966).

Eskeland et al. (1974) reported that propionic acid infusion increased N retention in ruminants when contrasted to infusions of acetic and butyric acids. The increase in retention may have been mediated through a protein sparing effect. Since both propionate and amino acids can serve a precursors of gluconeogenesis (Leng et al., 1977), a greater efficiency of propionate utilization may allow more amino acids to be used for anabolism rather than gluconeogenesis (Reilly and Ford, 1971).

Potter et al. (1976) showed increased blood glucose concentrations in cattle pastured on orchard grass, alfalfa, brome and ladino clover mixtures and supplemented with soybean meal and monensin when compared to those fed only the forage.

Blaxter (1962) suggested that the efficiency of propionate utilization in ruminant tissue is greater than for either acetate or butyrate. Smith (1971), in a general review of the subject has found that researchers are not in agreement as to propionate's efficiency at the tissue level. However, if the propionate is used more efficiently at this point, the benefit would be additive to that obtained during rumen fermentation by lowering CH_4 losses.

Poos et al. (1979) conducted a metabolic study with steers fitted with abomasal cannulae to determine any differences in digestibility, ruminal protein degradation (bypass) and microbial protein synthesis of ground corn-milo based ration supplemented with Brewer's dried grain or urea; with and without monensin. Monensin decreased bacterial nitrogen flow ($P < .05$) while increasing plant protein passage to the abomasum regardless of protein supplement. As previously discussed, monensin tends to reduce the cellulolytic bacteria population. These bacteria are required to have NH_3-N as an N source irregardless of the presence of preformed amino acids (Blackburn, 1964). A decrease in the total N and NAN flow might be indicative of this decrease in cellulolytic bacteria in ruminants fed diets supplemented with urea and monensin.

Further work at Nebraska by Hanson and Klopfenstein (1979) with similar rations indicated that when a preformed protein source was used as the supplement, that the addition of monensin did not result in an

increased dietary protein need as dry matter intake was depressed. An increase in dietary levels of preformed protein (10.5% to 12.5%) caused significant ($P < .05$) increases in gain. Such an increase in protein equivalent from urea did not improve performance, however. Hanson and Klopfenstein (1979) suggested that the protein reaching the duodenum was the limiting factor for improved animal performance. Gain differences were not apparent at different urea levels thus indicating that ammonia nitrogen was not the limiting factor but rather microbial protein synthesis. If monensin caused increased microbial protein synthesis, one would expect increased average daily gain in those steers fed the higher urea levels, however, the opposite occurred. The work of Poos et al. (1979), Richardson et al. (1978), and Hanson and Klopfenstein (1979) support the idea that microbial protein synthesis is inhibited (or at least not changed) with the addition of monensin, but that total ruminal degradation of preformed protein is markedly lower. Producers would benefit to the greatest extent if they utilized monensin when feeding a natural protein supplement. Although monensin depresses protein degradation at any dietary protein level, once the animal's requirement has been exceeded with a large supply of protein escaping rumen degradation any further increase in ruminal bypass of protein would not stimulate production any further.

MARKERS SYSTEMS USED IN DIGESTA PASSAGE STUDIES

Various types and combinations of marker systems have been employed to assess digesta passage to the abomasum or duodenum of ruminants in an effort to estimate rumen liquid and dry matter turnover rates, non-ammonia nitrogen passage, extent of ruminal organic matter digestion, microbial protein yields and protein which escapes ruminal degradation. An ideal marker must satisfy the following criteria in order to be considered for use in passage studies (Engelhardt, 1974 and Kotb et al., 1972): (1) the marker must be non-absorbable, (2) it must not affect or be affected by the animal's gastrointestinal tract, its environment or the microbes, (3) the substance should be physically similar to or closely associated with the fraction of digesta under study, and (4) the method of analysis for the marker must be sensitive and specific for that marker and not interfere with any other chemical analyses. As researchers can attest, there is not one marker available for animal research today that will qualify as an ideal marker and satisfy all the criteria previously described. It next becomes the main objective of the researcher to obtain a marker that will satisfy as many criteria as possible. Any great deviation from the criteria aforementioned will result in serious miscalculations in regards to the extent of digestion, time of rumen retention (i.e. dilution rate) and digesta passage.

Markers may be administered by many techniques such as continuous infusion with time-sequenced sampling, continuous infusion with total collection or single dosage with time sequence sampling. Liquid passage may be measured through employing polyethylene glycol (PEG) (Sperber et al., 1953), the ^{51}chromium complex of ethylenediaminetetra-acetic acid

(^{51}Cr-EDTA) (Downes and McDonald, 1964), Cr-EDTA (Downes and McDonald, 1964) or phenol red (Hecker et al., 1964) as inert markers either singularly, in combination with each other and/or in a mixture with particulate markers chromic oxide (Purser and Moir, 1966), ruthenuim phenathroline (Tan et al., 1971), other rare-earth elements (Hartnell and Salter, 1979) and the internal marker already present in the ration, lignin (this by no means is the total number of markers available to researchers). Since this author dealt only with Cr_2O_3, PEG and lignin as indigestible markers, the review of literature will pertain only to these entities.

Faichney (1975), in a review article on the use of markers in digesta studies, pointed out that Cr_2O_3 moves independently of the liquid and particulate phases of the digesta thus making any accurate estimate of flow rates rather dubious. Drennan, Holmes and Garrett (1970) concluded that in trials with sheep and cattle fed high concentrate diets, that chromic oxide, fed as a powder mixed into the ration, flowed much more rapidly out of the rumen than did the digesta. Their calculations of ruminal digestion using Cr_2O_3 as the marker gave digestion coefficients which were not possible, i.e. too high.

The amount of the starch digested in the rumen of these sheep when lignin was used as a marker was less than the organic matter (OM) digested, but when chromic oxide was used as the marker, more starch was digested than total OM by about 500 grams. Likewise, Cr_2O_3 data depicted that twice as much protein left the abomasum than was ingested, indicating a rather absurd value for endogenous nitrogen secretion. Lignin based data for nitrogen passage was credible.

The data of Drennan et al. (1970) was in agreement with that provided by other workers that used chromic oxide powder and similar

sampling techniques. The general conclusion was that passage data based on Cr_2O_3 powder was unusable. The values recorded by using lignin as the marker were reasonable and consistent when contrasted to investigators (Topps et al., 1968; Nicholson and Sutton, 1969; Ørskov and Fraser, 1968) using PEG or Cr_2O_3 impregnated paper as their reference sources. It would seem from this study that lignin represented a better estimate of digestion and passage than Cr_2O_3 powder.

Chromic oxide, whether administered as a powder, impregnated into paper or baked with wheat flour and ground into a powder, shows a pattern of diurnal variation (Faichney, 1975; Wilkinson, 1970, Kane et al., 1952 and Davis et al., 1958) which may correspond to cyclic changes in gastrointestinal physiology with the lowest point in the cycle occuring at night. It would therefore become apparent that when Cr_2O_3 is utilized, regardless of form, that long sampling periods be employed at various times, day or night. These individual samples in order to be representative should be pooled and subsampled for analysis since the concentration of Cr_2O_3 will vary (MacRae and Armstrong, 1969; Harris and Phillipson, 1962).

Incomplete marker recoveries are quite common in the majority of experiments reviewed. According to Sutton et al. (1976), incomplete recoveries could be the result of a depression in flow due to sampling interference with the animal. Sutton et al. (1976) claimed marker collection for recovery purposes beyond day three is useless and an adjustment to 100% recovery should be made at this point. Sutton et al. (1976) further pointed out that adjusted recoveries are often undesirable due to higher standard errors associated with such an adjustment.

Pitzen (1974) obtained recoveries of Cr_2O_3 as low as 59% and when adjusted to 100% recovery, the flow data was much more variable and inconsistent. Some of the adjusted microbial crude protein values were higher than theoretically possible under normal ruminal conditions (Walker, 1975). Pitzen concluded that Cr_2O_3 paper was unsuitable for determining digesta flow rates in the ruminant.

A decrease in digesta flow is not the only possible theory for incomplete marker recoveries. Curran, Leaver and Weston (1967) suggested that losses due to regurgitation, fecal loss, loss while grinding samples, analytical losses and the absorption of soluble chromates will decrease marker recovery. Deinium et al. (1962) found traces of chromate in the liver, lymph, kidneys and lungs of cows fed chromic oxide impregnated paper. The data of Deinium et al. (1962) clearly shows a violation of one of the paramount criteria of a marker; that it be non-absorbable.

In more recent data, Poos et al. (1979) using a dual marker system (Cr_2O_3 and PEG) in steers, calculated that total N reaching the abomasum exceeded the N intake. The researchers suggest that an underestimation of ruminal degradation and an over-estimation of particulate matter passage to the abomasum was the reason for the increased total N reaching the abomasum when compared to the N intake. Such problems with Cr_2O_3 may be found without difficulty throughout current literature.

Polyethylene glycol was first introduced as a soluble marker to study the movement of water in the rumen by Sperber, Hyden and Ekman (1953). Their method of administration (injection into the rumen) resulted in a "hit and miss" type of approach which ultimately led to misconceptions and misleading data (Termouth, 1967). Under some conditions

approximately 5% of the PEG (molecular weight = 4000) was found to be associated with the particulate phase of the digesta, a violation of Engelhardt's (1974) and Kobt et al.'s (1972) criteria for a reference marker. Another serious drawback to use of PEG as a marker is the lack of a specific, sensitive and accurate method of analysis (Downes et al., 1964). All known methods are based on the precipitation of PEG from an aqueous solution. With this type of procedure, incomplete recoveries of the marker were obtained due to analytical losses (Smith, 1955; Corbett, 1958). This underestimation of PEG concentration will ultimately result in an overestimate of total liquid flow from the gastrointestinal site (Bergen, 1979; Lemenager et al., 1978).

Lignin is often employed as an internal marker to check the validity of the other liquid and particulate markers calculated digesta flows. Lignin, although there is no question as to its association with the particulate phase of the digesta, does have problems associated with its usage as a reference marker. In high grain rations the concentration of lignin is rather small, thus sampling and analytical procedures become limiting. Acceptable methods for lignin analysis (Van Soest, 1963; Collings, 1979; Johnson et al., 1961) are highly variable among laboratories thus limiting the usefulness of lignin as a marker since comparisons of flow data would become futile. According to Drennan et al. (1970), data based on lignin calculations may not be correct, but in comparison to data based on Cr_2O_3 as a marker, it is at least feasible.

The section of the gastrointestinal (GI) tract choosen for cannulation, the location of cannulation within the choice of organ and the type of cannual system utilized (eg. "T" type or re-entrant) may influence passage data. Sampling digesta through an abomasal "T" type

cannula will not result in an increase of digesta flow from the upper GI tract to compensate for that lost during sample collection. However, obtaining a representative sample of abomasal digesta is difficult because of the stratification of feed within the organ resulting in channels for liquid flow. Thus, a dual marker system (one each for the liquid and particulate phase) should be used since the liquid and solid portions do not necessarily move together. The cannula itself should be placed in the abomasum just anterior to the pyloric junction in order to obtain the best possible representative sample of digesta that would be available for absorption in the duodenum.

A duodenual "T" type cannual has been acceptable for spot sampling of digesta flow from the abomasum. A cannula inserted anterior to the common bile duct represents an excellent site for the collection of samples that are adequately mixed and highly representative of chyme available for absorption. However, the surgical procedure proves more difficult compared to the abomasal insertion with more complications occurring post-operatively.

A duodenal re-entrant cannula system is capable of collecting a good representative sample and excellent flow data can be obtained, but one major drawback is the necessity to replace the volume of digesta sampled with donor digesta. If the digesta is not replaced, digesta flow rates from the upper GI tract will increase to compensate for the losses to the jejenum (MacRae, 1975) thus giving erroneous rumen outflow data. Other drawbacks which cause some researchers to choose "T" type cannulae in preference to the re-entrant type are the increased labor involved with sampling, more complicated surgical procedures with increased post-operative problems and a shorter life expectancy of the animal after surgery.

OBJECTIVES

The optimal level of non-protein nitrogen addition to rations fed to high producing cattle in respect to the efficiency of microbial cell yield has been debated for a number of years. Several researchers have attempted to pinpoint at what level further increases in rumen NH_3-N concentrations will not enhance microbial cell protein yield thus making NPN additions to the diet unnecessary. Satter and Slyter (1974) in an *in vitro* chemostat experiment determined that 5.0 mg % NH_3-N was sufficient for maximal microbial growth. This was equivalent to approximately 11-14% crude protein equivalent (CPE) in the ration. Diets that produce rumen NH_3-N concentrations above this level should not increase microbial cell protein synthesis and the added CPE would be wasted. Therefore, it was the objective of Experiments One and Two to:

(1) determine the effect of protein level on total nitrogen and NAN passage to the abomasum in steers.

(2) determine if supplemental NPN above 13% CP will result in increased non-ammonia nitrogen passage to the lower gut in steers.

Corn silage may be supplemented with either preformed protein or NPN in order to provide adequate nitrogen to the rumen microbes for microbial protein synthesis. Corn silage is well suited for NPN supplementation due to its high energy and relatively low protein content. The addition of NPN at ensiling has decreased the amount of feed steers consumed per unit gained when compared to untreated silage supplemented with NPN at feeding (Ely, 1967). Data concerning N passage to the abomasum is limited in steers fed anhydrous ammonia treated silage supplemented

with monensin. Therefore, it was the objective of Experiment Three to:

(1) determine the effect of silage treatment (protein level) on nitrogen parameters that pass to the abomasum in steers,

(2) determine if monensin affects nitrogen flow to the abomasum in steers,

(3) determine if there is an anhydrous ammonia-monensin interaction on nitrogen passage to the abomasum in steers,

(4) determine the validity of using a two phase marker system in determining N passage from the rumen and ruminal liquid and particulate outflow rates,

(5) determine if monensin effects dilution rates in the ruminant and

(6) compare two systems of abomasal sample analyses (individual time analysis vs composite analysis) and to see if there is a correlation between any one or two times and the composite.

There has been little work done on the subject of nitrogen retention in steers fed corn silage that had been treated with various levels of anhydrous ammonia and supplemented with monensin. It was the purpose of Experiment Four to:

(1) determine the effect of silage treatment (CP level) on nitrogen retention and

(2) determine if monensin had any effect on nitrogen retention in steers fed the various anhydrous ammonia treated rations.

MATERIALS AND METHODS

A. EXPERIMENT ONE

1. Design of the Experiment

A total of five Holstein steers with a body weight of approximately 300 kg were fitted with an abomasal "T" type cannulae and housed in 91 x 244 cm metabolic stalls. The animals were fed one of three experimental rations twice daily (Table 1) at 90 percent voluntary intake and had free access to water. Each steer was fed at least two of the three rations in this completely randomized trial.

The concentrate diets fed were corn based with relatively constant percentages of oats and corn cobs present across treatments (see Table 1). They were formulated in order that the low protein diet{ 7% crude protein (CP)} served as the basal ration and the subsequent two rations were formulated by adding soybean meal to final levels of 9.9 and 12.6% CP respectively. A chromic oxide-wheat flour mixture (1:4) was added at one percent of the ration dry matter (Ørskov et al., 1971). The Cr_2O_3 was mixed with the flour to form a paste, oven dried and then ground to pass through a 1 mm wire mesh screen in a Wiley mill. All diets were similar in digestible energy values (mcal/kg).

2. Cannula Design and Insertion

The abomasal "T" type cannulae were manufactured from viscous plastisol (Norton's Plastic and Synthetics Div., Akron, Ohio) and Mystaflex (M & R Plastics and Coatings, Maryland Heights, Missouri). The liquid was placed under a strong vacuum for at least one hour to alleviate

TABLE 1. Rations fed to steers in Experiment One

Ingredient*	Int. Ref. No.	1	2	3
			%	
Corn	5-02-932	41.0	58.0	51.0
Soy-49	5-04-604	-	3.0	10.0
Corn cobs	1-02-782	35.0	20.0	20.0
Oats	4-03-309	10.0	10.0	10.0
Cane molasses	4-04-696	5.0	5.0	5.0
Corn starch	4-02-889	5.0	-	-
Deflourinated rock phosphate	6-01-780	1.0	1.0	1.0
Trace minerals		2.0	2.0	2.0
Cr_2O_3- wheat flour		1.0	1.0	1.0
DE (Mcal/kg, calculated)		3.08	3.33	3.31
N X 6.25 (analysis)		7.0	9.9	12.6

* Vitamins A, D, E were added as 1,000,000; 125,000 and 22,500 International Units (I.U.), respectively.

air bubbles after which it was poured into a preheated brass mold (duplicated from one obtained from Dr. L. D. Satter, University of Wisconsin) and baked at 190°C for about 25 minutes. The mold and baked plastic were then immersed in water and allowed to cool and harden. A properly constructed cannula was pliable but strong, transparent and free of air bubbles.

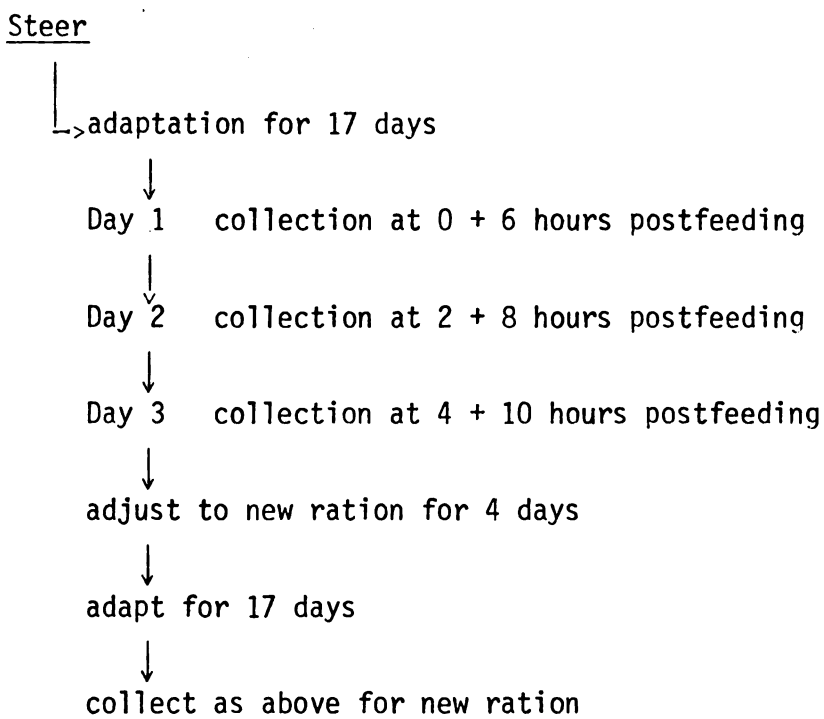
All feed and water were withheld from the steers 16 hours prior to surgery to reduce gastrointestinal fill. The cannulae were placed in the posterior end of the abomasum proximal to the pylorus. This position in the true stomach would enable us to obtain the best representative sample of digesta entering the duodenum without employing a duodenal re-entrant system. The animals were allowed to recover for at least three weeks prior to being placed on experiment. Steers were fed a predominantly hay ration to stimulate feed intake and rumination during recovery.

3. Sample Collection and Preparation

Abomasal samples were collected twice daily and obtained over a three day period. (see Figure 1). A reversal pressure pump was used to bubble air into the abomasum for at least five minutes prior to collection of abomasal fluid. This aeration was intended to agitate the abomasal contents thus delivering a representative sample of the abomasal digesta. The first twenty to thirty milliliters of sample were discarded because particulate matter tended to settle in the cannula itself and could not be mixed with the abomasal contents. Approximately 250 ml of abomasal digesta were collected each time in a plastic beaker and after the last sampling, all samples per steer, per ration, were composited, frozen and

FIGURE 1

Flow chart for abomasal sampling



stored. Daily feed samples were also retained and composited for subsequent analysis.

Prior to analyses for total nitrogen, ammonia nitrogen, chromium and acid lignin, the abomasal composites were thawed, acidified with 6N hydrochloric acid to a pH of 2 and then oven dried at 55°C. Subsequent to drying, the samples were ground through a .5 mm mesh screen in a Wiley mill.

4. Nitrogen Determinations

Total nitrogen was determined on all feed and abomasal composites with a Technicon Auto Kjeldahl system. Non-ammonia nitrogen levels were calculated by subtracting ammonia nitrogen values obtained by steam distillation over alkali from corresponding total nitrogen figures for that sample.

5. Chromium Analysis

Chromium concentrations in feed and abomasal samples were determined by a nitric-perchloric acid digestion followed by atomic emission spectrophotometry utilizing an I.L. 453 Atomic Absorption-Emmission Spectrophotometer.

Approximately 200-300 mg of sample was placed in a 250 ml Phillips beaker followed by the addition of 25 ml of nitric and 4 ml of perchloric acid. The samples were digested under a hood at a slow boil until only 1-2 ml of liquid remained. At this point the sample should have been yellow to orange in color. After cooling the sample was diluted to 100 ml (grams). The oxidized samples were then read on the Atomic Emmission spectophotometer at a wavelength of 424.8 nm, scale of 2.5, slit width

of 80 and using the nitric oxide burner head. A chromium standard curve was obtained by preparing solutions with 0, 2, 5, 10, 15 and 20 ppm chromium and subjecting them to the nitric-perchloric digestion and dilution.

6. Lignin Determinations

Dried feed and abomasal samples were subject to the permanganate lignin procedure of Van Soest (1963).

7. Determination of Daily Flow Rates From the Rumen

Daily N flow was determined by a marker ratio technique (Fenderson and Bergen, 1975) utilizing the following relationship:

$$\text{Total N flow (g/day)} = \frac{\text{N: marker ratio in abomasal digesta}}{\text{N: marker ratio in feed ingested}} \times \text{g N consumed per day}$$

Non-ammonia nitrogen passage was calculated by subtracting the NH_3-N from the total N flow. The NAN data were not adjusted for endogenous protein secretions.

The above nitrogen: marker relationship may be used when chromium or lignin are used as reference markers.

8. Statistical Analysis

Data were statistically analyzed by analyses of variance on a Hewelett Packard 9825 A computer. The difference among means were determined by the Studentized Range Test utilizing the table of Rohlf and Sokal (1969).

B. EXPERIMENT TWO

1. General Design

Three Hereford steers averaging 325 kg and housed under the identical environmental conditions as in Experiment One were fed the 12.6% CP ration used in the first trial but supplemented with urea to 15.7% CP (Table 2). The feeding regime, abomasal collection schedule, and laboratory analyses performed were similar to that described for Experiment One.

The Holstein steers from Experiment One could not be used in this trial because they developed severe feet and leg problems prior to the collection period of abomasal samples for the urea supplemental ration. Due to this problem, a switch in steers to Herefords was necessitated and, hence, the data obtained from the Hereford steers could not be statistically compared with data accumulated from the abomasal samples obtained from steers in the first trial.

2. Determination of Daily Flow Rates from the Rumen

Daily N flow was determined by a marker ratio technique (Fenderson and Bergen, 1975) utilizing the following relationship:

$$\text{Total N flow (g/day)} = \frac{\text{N: marker ratio in abomasal digesta}}{\text{N: marker ratio in feed ingested}} \times \text{g N intake/day}$$

Non-ammonia nitrogen passage was calculated by subtracting the NH_3-N from the total N flow. The NAN data were not adjusted for endogenous protein secretions.

The above nitrogen: marker relationship may be used when chromium or lignin are used as reference markers.

TABLE 2. Ration fed to steers in Experiment Two

Ingredient*	Int. Ref. No.	1 %
Corn	4-02-932	50.0
Soy 49	5-04-604	10.3
Corn Cobs	1-02-782	20.0
Oats	4-03-309	10.0
Cane molasses	4-04-696	5.0
Deflourinated rock phosphate	6-01-780	1.0
Trace minerals		2.0
Urea		.7
Cr_2O_3 - wheat		1.0
DE (Mcal/kg, calculated)		3.28
N X 6.25 (analyzed)		15.7

* Vitamins A, D, E were added as 10,000,000; 125,000 and 22,500 I.U., respectively.

C. EXPERIMENT THREE

1. Design of the Experiment

Four Hereford steers with an average body weight of approximately 380 kg and fitted with abomasal "T" type cannulas were fed a total of six corn silage rations differing in the level of anhydrous ammonia applied at the blower with or without monensin (Rumensin^{R} - Elanco Products Co.) in the supplement. Every steer received each ration during the duration of this switchback designed trial. The animals were fed twice daily (8 A.M. and 8 P.M.) at 90% of voluntary intake. Voluntary intake levels were dropped to 90% at least one week prior to the collection of abomasal digesta. Water was available free choice.

Exogenous chromic oxide and lignin were used as insoluble markers. The former was added to the vitamin-mineral supplement as a chromic sesquoxide:wheat flour mixture (1:4). As a soluble marker, polyethylene glycol (PEG - molecular weight 4000) was also added to the vitamin mineral premix. These markers were utilized to quantitate total nitrogen and NAN passage to the abomasum as well as ruminal liquid turnover rates for the various rations studied.

All corn silage used was obtained from the same source on the Michigan State University farm system and differed only in the level of anhydrous ammonia applied upon ensiling. Untreated corn silage served as the control and silage treated with either five (7.8 g anhydrous ammonia/kilogram corn silage dry matter AN/KGCSDM) or ten (15.6 g AN/KGCSDM) pounds of anhydrous ammonia per ton of harvested silage comprised the other two protein treatments. The control silage contained 7.4% crude

protein on a dry matter basis and the 5 lb. anhydrous and 10 lb. anhydrous ammonia treated rations contained 9.8 and 12.4% crude protein, respectively.

All three silage treatments were supplemented with a vitamin and mineral premix with or without Rumensin (Table 3 and 4), thus bringing the total number of treatments tested to six.

3. Sample Collection and Preparation

Abomasal samples were collected three times daily (around the clock) over a four day period. The designated times for sampling (Figure 2) varied in order of sequence from day to day in an attempt to obtain a representative sample from the abomasum, thus alleviating any error due to diurnal variation of chromic oxide flow.

The abomasal digesta were aerated *in situ* to promote mixing as outlined in the first experiment and approximately 125 ml of abomasal digesta were collected and frozen for analyses. It was an objective of this study to perform all analyses (i.e., dry matter, total nitrogen, ammonia nitrogen, chromium, ADF, lignin and PEG) on one silage treatment (15.6 g AN/KGCSDM with and without Rumensin) on an individual sample basis and compare that data to data obtained when abomasal samples were pooled for that particular treatment and steer. One or two time periods that abomasal samples were obtained could conceivably be highly correlated with the total sample composite thus possibly alleviating the need for around the clock or multi-collection sampling. The other silage treatments were analyzed on a composite basis, i.e., all samples per steer per treatment were pooled and analyzed.

The individual 125 ml samples were divided into three portions; 30, 70 and 25 gm. The 30 gm fraction was centrifuged at 15,000 rpm for

TABLE 3. Vitamin and Mineral Premix - MSU 376

Item	International Reference Number	Percent of Total Dry Matter
Ground corn	4-02-932	47.4
Calcium sulfate		13.1
Trace mineral salt		9.2
Rumensin - 30[a]		1.9
Vitamin A[b]		.4
Vitamin D[b]		.4
Deflourinated rock phosphate		26.1
Chromic oxide mix[c]		1.0
Polyethylene glycol[d]		.5

[a]Rumensin added at a rate of 33.0 ppm of ration DM

[b]Vitamins A & D equal to 30,000 and 3,000 International Units per gram, respectively.

[c]Chromium sesquoxide added (1 part Cr_2O_3 to 4 parts wheat flour) as 1% total ration DM.

[d]PEG - carbowax 4,000 added as .5% of total ration DM.

TABLE 4. Vitamin and Mineral Premix - MSU 377

Item	International Reference Number	Percent of Total Dry Matter
Ground corn	4-02-932	49.3
Calcium sulfate		13.1
Trace mineral salt		9.2
Vitamin A[a]		.4
Vitamin D[a]		.4
Deflourinated rock phosphate		26.1
Chromic oxide mix[b]		1.0
Polyethylene glycol[c]		.5

[a]Vitamins A & D equal to 30,000 and 3,000 International Units per gram, respectively.

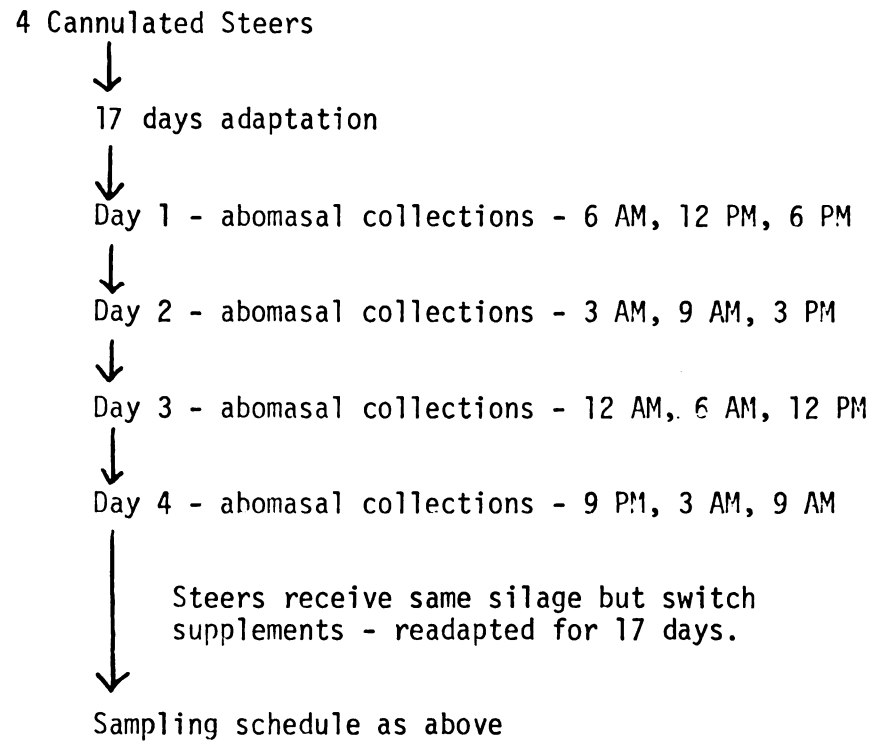
[b]Chromic sesquoxide added as 1% total ration (1 part Cr_2O_3 to 4 parts flour)

[c]PEG - carbowax 4,000 added as 0.5% of total ration DM.

FIGURE 2

Flow chart of sampling protocol

Experiment 3



Switch steers to different silage and again switch supplements so that steers do not receive same vitamin-mineral premix (i.e. 376 or 377) two collection periods in a row.

10 minutes, the supernatant pipetted off and saved for PEG, total nitrogen (N_T) and ammonia nitrogen (NH_3-N) determinations. The pellet was oven dried at 55° C overnight and stored for chromium, acid detergent fiber (ADF), lignin, N_T and possible ammonia nitrogen analysis. The 70 gm fraction was oven dried at 55° C and analyzed for dry matter, chromium, N_T, NH_3-N and PEG. The remaining 25 gm were composited (per steer/treatment) and frozen for an overall analysis for chromium, PEG, ADF, lignin, N_T, NH_3-N and dry matter. A total overview of the analysis scheme for individual samples can be seen in Figure 3 and for composited samples in Figure 4.

Prior to analysis, all samples which needed to be analyzed on a dry matter basis were adjusted to approximately a pH of 2 by adding 6 N HCl and then dried in a forced air oven at 55° C. Subsequent to drying, each sample was hand ground by mortar and pestle.

4. Nitrogen Determinations

Total nitrogen was determined on all feed and abomasal samples using the Technicon Auto Kjeldahl Micro System. Ammonia nitrogen levels in the supernatant and pellet obtained by centrifugation were determined with an ammonia electrode (model 95-10; Orion Research Incorporated, Cambridge, Massachusetts). Exactly 1.0 ml of centrifuged abomasal fluid was diluted to 50 ml with deionized water (to get an ammonia reading within a range of 10^{-6} to 10^{0}M) and then 2.0 ml of 10 M NaOH was added. The NaOH brought the pH of the solution up to at least 11.0 thus converting the $NH_4^{\pm}$ ion to the free NH_3-N form. A magnetic stirring bar was added, the solution stirred for about 30 seconds, and an electronic potential reading was taken on the millivolt (MV) scale of an expanded

FIGURE 3

Scheme of laboratory analysis for individual abomasal samples - 10# anhydrous-NH_3/ton treatment

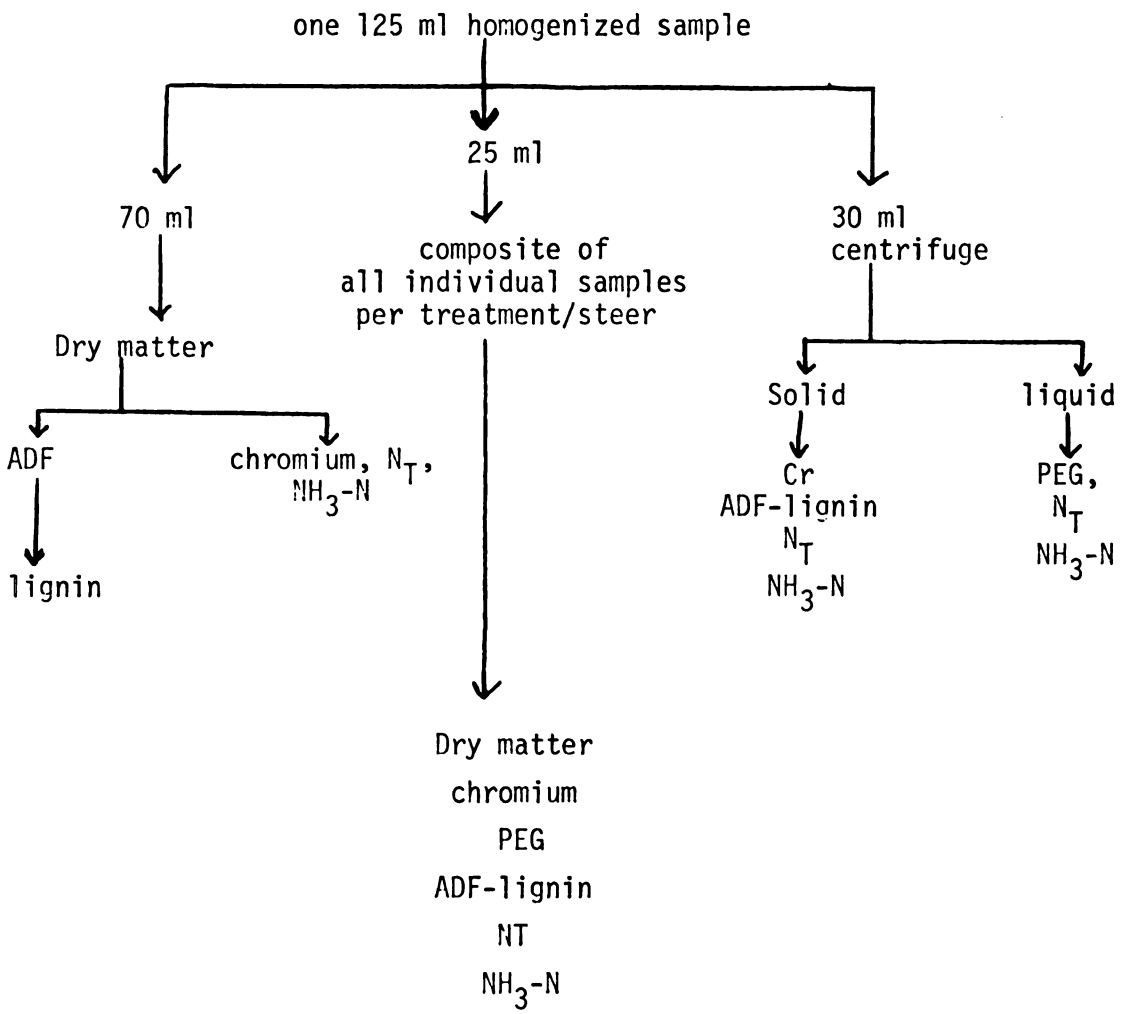
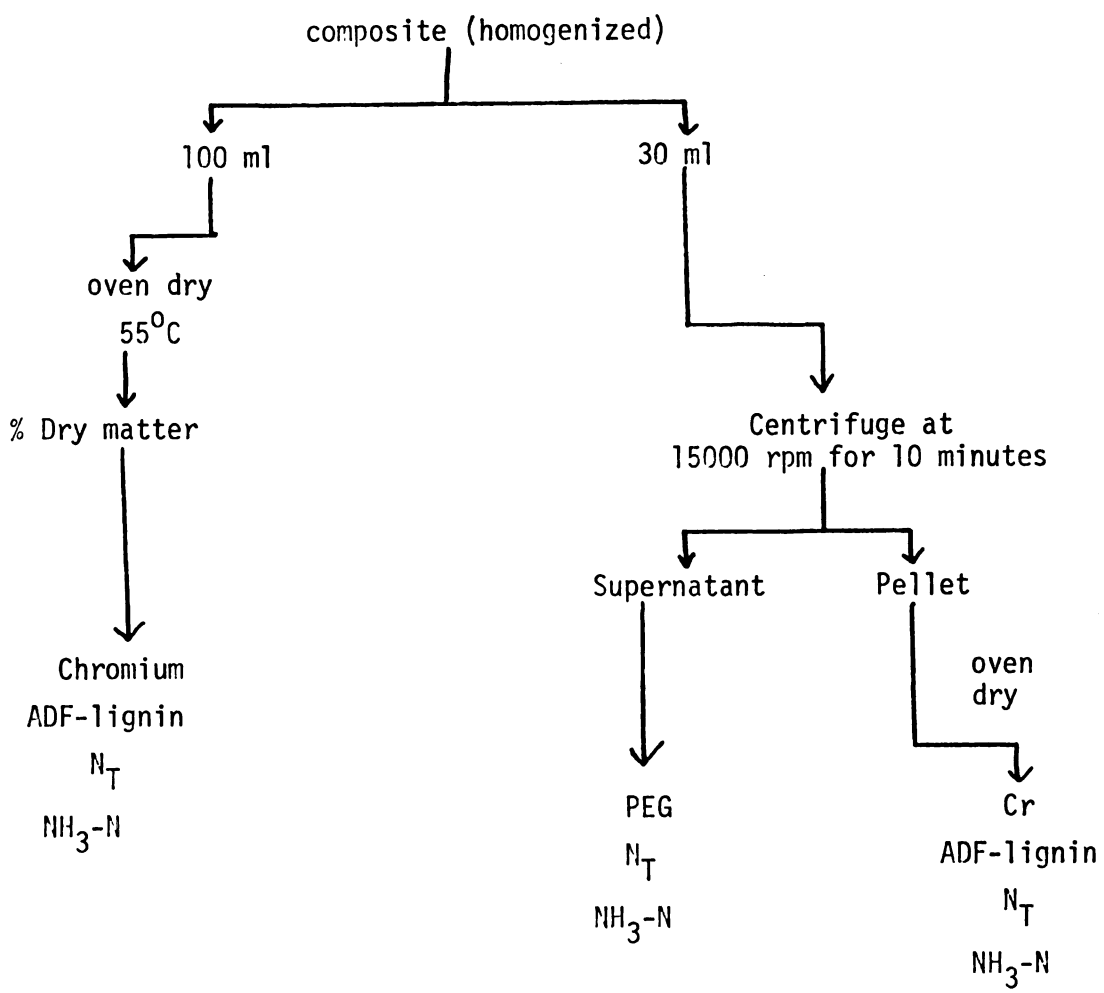


FIGURE 4

Scheme of laboratory analysis for composited abomasal samples (5# anhydrous NH_3/ton silage and control treatments)



scale (pH/mV) meter (Orion Research Incorporated, model 801). An ammonia standard curve was obtained by preparing a 1.0 N ammonium chloride solution and through serial dilution, preparing other standards of .1, .01, .001, .0001 and .00001 normal. These standards were then subjected to similar NH_3-N liberation with the alkali addition and subsequently read on the pH/mV meter.

The electrical potential values for the standards and samples were fed into a Cannon Computer (Model Canola SX 310) using the Statistics II - SX 311 linear regression program and the output gave the molarity of each individual sample as obtained from the standard curve.

Non-ammonia nitrogen concentrations were calculated by difference $\{N_T-(NH_3-N)\}$.

5. Chromium Determinations

Chromium concentrations in feed and abomasal fractions were analyzed as outlined in Section 6 of Experiment 1.

6. Lignin Determinations

All lignin values were obtained through analysis according to the acid-lignin procedure as outlined by Van Soest (1963).

7. Polyethylene Glycol Determination

Polyethylene glycol content of the abomasal and feed samples were determined by spectrophotometry using a Gilford Spectrophotometer, model Stasar II. A 3.0 ml sample of the supernatant from the centrifuged abomasal digesta was diluted with 2.5 ml of deionized water and the following reagents were added in succession: 2.0 ml 0.3 N $Ba(OH)_2$, 2.0 ml 5% $ZnSO_4$ and .5 ml 10% $BaCl_2$. The resulting solution was centrifuged at

10,000 rpm for ten minutes. Four milliliters of the supernatant plus 1.0 ml of additional deionized water were vortexed and while being mixed, 5.0 ml of TCA-$BaCl_2$ was quickly added. The samples were read on the spectrophotometer exactly twenty minutes after the addition of TCA-$BaCl_2$ at a wavelength of 500 nm.

A PEG standard curve was obtained by subjecting PEG solutions of .50, 1.0, 1.5 and 2.0 ml of standard solution (.75 mg PEG/ml; MW = 4000) to the same treatment as the samples.

8. Determination of Daily Flow Rates from the Rumen

Daily total N flow to the abomasum was determined by various marker: N ratios as shown below:

(1) $$\text{Total N flow/day} = \frac{\text{N: } Cr_2O_3 \text{ ratio in abomasum}}{\text{N: } Cr_2O_3 \text{ ration in feed}} \times \text{N intake (g) per day}$$

(2) $$\text{Total N flow/day} = \frac{\text{N: lignin ration in abomasum}}{\text{N: lignin ratio in the feed}} \times \text{N intake (g) per day}$$

(3) $$\text{Total N flow/day} = \frac{\text{N: PEG ratio in abomasum}}{\text{N: PEG ratio in feed}} \times \text{N intake per day}$$

Non-ammonia nitrogen flow data were obtained by subtracting abomasal NH_3-N {NH_3-N flow = Total N (% NH_3-N) flow/day} from total N flow/day. NAN flows were not corrected for endogenous N secretion.

Liquid flow from the rumen (liters per day) was determined from the following calculation:

(4) $$\frac{\text{PEG intake/day}}{\text{PEG concentration/ml abomasal liquid}} = \text{liquid flow to the abomasum/day}$$

Particulate passage to the abomasum was determined by:

$$(5)\ \frac{Cr_2O_3 \text{ intake/day}}{Cr_2O_3 \text{ concentration/gm dry abomasal particulate matter}} = \text{grams DM to abomasum/day}$$

or

$$(6)\ \frac{\text{Lignin intake/day}}{\text{Lignin concentration/gm abomasal DM}} = \text{grams abomasal DM flow/day}$$

NAN flow to the abomasum for each phase of flow, i.e., liquid and solid, was calculated as follows:

(7) Liquid NAN flow/day = (liquid flow per day x total N concentration) - NH_3-N flow/day to abomasum in liquid

(8) Solid NAN flow/day = (solid flow per day x total N concentration) - NH_3-N DM flow/day to abomasum

The total NAN passage to the abomasum based on a two phase marker system was then determined by the addition of equations 7 and 8:

(9) Liquid NAN flow/day + particulate NAN flow/day = total NAN passage/day

8. Statistical Analysis

Least square analysis (Snedecor and Cochran, 1967) was used to examine main effects and interactions of protein level and rumensin on nitrogen passage and liquid and particulate outflow rates. When appropriate, orthogonal contrasts (Snedecor and Cochran, 1967) were designed for a comparison of selected treatment means.

D. EXPERIMENT FOUR

1. General Design of the Experiment

A nitrogen balance study was conducted to evaluate the N status of eight Hereford steers (270 kg) fed the six corn silage treatments described in Experiment Three (control silage, 5# anhydrous ammonia treated silage and 10# anhydrous ammonia treated silage, all silage with and without 33.0 ppm monensin).

Cattle were elevated on wooden boxes about 30 cm above the floor within individual metabolic stalls (91 x 244 cm). The elevated box made fecal collection possible without animal interference. A coarse mesh area in the center of the box facilitated urine collection in a pan placed beneath. The steers were fed twice daily at twelve hour intervals and given free access to water.

The cattle were adjusted to each ration for 21 days prior to the collection period. Each collection period lasted eight days with fecal and urinary output measured, recorded and sampled daily. Subsequent to each collection cycle the animals were allowed a day's rest and then placed on a 21 day adjustment schedule for the next treatment. Nitrogen balance was expressed as total nitrogen intake - (fecal N + urinary N).

2. Sample Collection and Preparation

a. Fecal samples

Total feces were collected daily in large plastic bags located directly behind each steer. Feces were removed each day, their weights recorded and a representative sample (10%) secured. At the end of each period all samples per steer were thoroughly mixed and grab samples equivalent to 10% of the composite were taken and frozen until analyzed

for nitrogen.

b. Urine samples

Urine was collected in the bottom section of a 55 gallon (208 liters) drum which had been cut off at approximately the 8 gallon mark (30 liters). This container, with 400 ml of 6 N HCl which had been added to prevent the liberation of ammonia or other nitrogenous compounds from the urine, was covered with ordinary house window screening and placed underneath the box. The screening prevented any extraneous material from falling into the urine. The urine was collected daily and the volume per steer was recorded. Urine output less than 10 liters was diluted to that volume. A ten percent subsample was retained each day and at the end of the 8 day collection subsamples per steer were thoroughly mixed and a 10% aliquot taken for nitrogen analysis.

Ration grab samples were obtained at each feeding as the feed was discharged from the mixer. The samples were composited for each steer at the end of the period, chopped in a Hobart food chopper and approximately 1 kilogram saved for dry matter and nitrogen determinations.

3. Nitrogen and Dry Matter Determinations

Total nitrogen content of feed, feces and urine samples were determined through utilizing the Technicon Auto Kjeldahl System. Dry matters were estimated by oven drying at 55^{o} C for 48 hours.

4. Statistical Analysis

Analysis of variance was used to determine any differences in nitrogen balance and N retained/N intake due to treatment effects (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Experiments One and Two

Nitrogen/permanganate lignin and nitrogen/chromium ratios were used to estimate nitrogen flow to the abomasum. However, due to inconsistent nitrogen passage data when the calculations were based on chromium ratios the data from that marker system were not analyzed. Therefore, all reported nitrogen passage data in these two trials are based on nitrogen/lignin ratios.

The original idea was to feed the three different crude protein rations (7.0, 9.9 and 12.6% CP) plus the 12.6% CP ration supplemented to 15.7% CP with urea to one set of steers. However, animal illness prevented the author from following that protocol and, unfortunately, the N data obtained from the last group of animals fed the 15.7% CP diet could not be compared statistically to the data obtained from steers fed the other three rations.

Nitrogen passage to the abomasum is summarized in Table 5. When nitrogen/lignin ratios for each ration were contrasted to their corresponding abomasal nitrogen/lignin ratios it was observed that some nitrogen losses from the rumen occurred in steers fed all rations except the low protein diet. The 7% CP diet produced nitrogen/lignin ratios that were similar in both the ration and abomasum. This indicates that there is no net loss of NH_3-N from the rumen and that the NH_3-N energy availability to the microbes for microbial growth correspond closely. As the percent protein in the diet increased, the amount of NH_3-N not incorporated into microbial also increased (0% to 35% losses). This supports

TABLE 5. Effect of ration crude protein level on N passage to the abomasum of steers.

Ration No. Crude Protein, %	1 7.00	2 9.90	3 12.60	SE	4 15.70
Ration:					
Daily DM intake, kg	5.53	7.65	8.16		7.26
Daily N intake, gms	62.0	121.2	164.6		182.3
Nitrogen/lignin	.30	.63	.73		.96
Abomasal Contents:					
Total N, %	1.60	2.07	2.52	.28	2.70
NAN, %	1.41	1.83	2.20	.23	2.36
NH_3-N, %	.19	.24	.32	.06	.34
Nitrogen/lignin	.31	.56	.62		.60
Nitrogen Passage:					
Total N, g/day	60.9[a]	106.8[b]	145.8[c]	13.0	112.7
NAN, g/day	53.7[a]	94.3[b]	127.3[c]	11.9	98.5
NAN Abo/total N intake, %	87.3	77.8	77.4	16.4	54.0

[a,b,c] Means on the same row with different superscripts are significantly different (P<.05)

earlier similar observations by Gray et al. (1956) and Crickenberger (1977). This observation does not suggest that protein supplementation above 7% does not increase microbial cell yield, but rather at the same energy level (Mcal/kg DM consumed), NH_3-N losses from the rumen increase with increases in protein levels and the net efficiency of NH_3-N utilization (expressed as percent of N intake) for microbial synthesis declined. In devising their systems for predicting ruminal microbial cell yield, Satter and Roffler (1977) and Roy et al. (1977) assumed a net efficiency of NH_3-N utilization of 90% and 100%, respectively, over the range of dietary protein levels utilized in this experiment. It cannot be speculated that there was insufficient NH_3-N available to the microorganisms since soybean meal is degraded at least 50-60% in the rumen (Hume, 1974). Further, a considerable portion of the increased NAN passage to the abomasum must have been due to some soybean protein escaping ruminal degradation. The bacterial contribution to the total N passage was not evaluated in this study.

Total nitrogen passage to the lower gut was 60.90, 87.59, 145.83 and 112.70 grams/day for the 7.0, 9.9, 12.6 and 15.7% rations, respectively. All treatment means were significantly different from each other ($P < .05$) excluding that obtained from the 15.7% rations. As dietary protein levels increased, greater amounts of total N reached the abomasum. These observations support similar findings by Miller et al. (1973), Ørskov et al. (1974) and Crickenberger et al. (1979).

Non-ammonia nitrogen that passed out of the rumen was significantly different ($P < .05$) across treatment (7.0, 9.9 and 12.6% CP) with the greatest amount of NAN passage in steers fed the 12.6% diet. Similar

NAN passage patterns were observed by Crickenberger et al. (1979) and Ørskov et al. (1971) who reported that NAN passage in steers was greater when diets of higher crude protein levels were consumed as long as dietary energy intake was adequate. According to the National Research Council's (1970) recommendations, all test rations contained adequate levels of digestible energy so energy did not limit NAN passage to the abomasum in this study.

Theoretically, the NAN flow in steers fed the 15.7% ration should equal the NAN flow from the 12.6% CP ration unless a response is observed and more microbial protein is produced. An increase in NAN passage was not seen in the steers fed the 15.7% ration. If the urea supplemented ration was corrected to the NAN passage observed for the 12.6% diet, then the expected NAN/N intake ratio would be 77.4. However, a much lower value was observed in steers fed the urea supplemented ration (54.0 vs 77.4). This lower than expected microbial growth yield in the urea supplemented steers was most likely in part due to an inability of the microorganisms to trap the NH_3-N at a rapid enough rate to match the rate of NH_3-N release during urea hydrolysis (Chalupa, 1967). This does not, however, explain a cell yield below that observed for the 12.6% CP ration except the possibility that the different group of steers employed somehow influenced the ruminal cell yield. Bloomfield et al. (1960) indicated that urea hydrolysis in the rumen occurred at a rate four times faster than the uptake of NH_3-N by the rumen bacteria.

Urea Fermentation Potentials (UFP) according to Burroughs' (1972) formula were calculated for each of the four rations. The 7.0 and 9.9% CP ration had positive values and thus could benefit from urea addition but, conversely, the two higher protein rations were negative in terms

of UFP values. The UFP values for the 7.0, 9.9, 12.6 and 15.7% CP rations were 9.56, 6.15, -2.21 and -2.65, respectively. The NAN passage data along with their corresponding UFP values indicate that NPN supplementation above 12-13% CP would not enhance microbial cell protein synthesis. However, because of the change in steers between Experiments One and Two and the variations in N data between steers within a treatment group, it cannot be said that the urea caused significantly decreased amounts of NAN flows to the abomasum as the data might indicate.

Rumen liquor outflow rates (Table 6) ranged from 27.8 to 51.87 liters per day based on the lignin ratio equation. The extreme variation in flow rates resulted in a rather large standard error ($\pm$19.5) thus no significant difference in liquid outflow rates could be delineated. Assuming a 50 liter rumen volume for the steers used in these two experiments, the rumen turnover varied from .56 to 1.04 per day.

Experiment Three

A summary of individual sample versus composite sample analysis is shown in Table 7. Only correlations that were equal to or greater than .60 are summarized. The correlations indicate that there is no one time that abomasal collections could be made and be representative of a time sequenced composite for all the parameters measured. There are, however, trends that suggest individual samples would suffice if one or two parameters were to be evaluated instead of seven or eight.

With feeding time at 8 AM and 8 PM, PEG, NH_3-N (supernatant) and total N (supernatant) were highest correlated (r=.75) with the composited sample. Each of these three parameters flow with the liquid pool and this indicates that the liquid flow may be more uniform across time

TABLE 6. Liquid flow rates from the rumen of steers fed varying levels of crude protein.

Ration CP	Flow Rate[a] (l/day)	Turnover/Day[b]
7.00	33.95	.68
9.90	42.06	.84
12.60	51.87	1.04
15.70	27.80	.56

[a] Flow rate = $\frac{\%\ \text{lignin (feed)} \times \text{DM intake/day}}{\%\ \text{lignin (abom)} \times \text{abom DM/l}}$

[b] Assuming a 50 liter rumen $\frac{\text{Flow rate}}{50}$ = turnover per day

TABLE 7 Correlations for individual samples vs. composite (10# anhydrous treatment)

Item:	Time*	Correlation to composite
Dry matter	6 AM	.62
	3 PM	.80
Total N	6 AM	.68
	3 PM	.60
Total N (pellet)**	6 PM	.85
	12 PM	.74
Total N (Supernatant)**	3 PM	.91
	12 PM	.86
	3 AM	.85
Chromium	6 PM	.79
	3 PM	.79
	9 AM	.67
PEG	6 PM	.93
	3 AM	.89
	6 AM	.82
Lignin	--	--
NH_3-N Supernatant**	12 AM	.89
	3 AM	.79
	3 PM	.77

* Only times with correlations $\geq$ .60 were summarized in this table.

** Pellet and supernatant are representative of the two phases present in a centrifuged abomasal sample.

than the particulate matter. The optimal time for collection of abomasal samples in order to obtain liquid phase data would be 3-5 hours prior to feeding (in this study, 3 AM or 3 PM).

Data obtained from the analyses of particulate matter correlated poorly with the composite, with the best correlations occuring 2-5 hours prior to feeding. The low correlations associated with dry matter analysis data are indicative of the type of passage that occurs in that pool. Feeding patterns will influence the digesta passage to the abomasum and its uniformity between meals. Lignin from individual samples was very poorly correlated with that percent obtained from the composite (the best correlation was r=.39 at 3 PM).

It becomes apparent from this study that in ruminants fed twice daily corn silage based rations, no particular sampling time, day or night, highly correlates with data obtained from a composited sample collected over a 4 day period. Liquid phase parameters may be measured with some confidence (.75 - .93), but such data alone would be of limited value since a large proportion of the microbial cells pass from the rumen in the particulate phase (Czerkawski, 1978).

The nitrogen flow data estimated by the marker ratio technique in this trial (Appendix Tables A.7-A.18) were not feasible due to certain marker difficulties. The discussion which follows is an attempt to reason why the marker system failed.

Nitrogen flow equations (Fenderson and Bergen, 1975) and the resultant daily nitrogen flows calculated from them are based on marker-nutrient ratios both in the feed and abomasal digesta. If the markers themselves are absorbed, altered or flow independently of that phase they are specifically to mark, serious deviations from actual nitrogen

flows will be seen in the calculated N and NAN values. To validate whether the marker systems utilized did, in essence, succeed or fail in regards to marker criteria (Engelhardt, 1974), a comparison of a marker: marker ratio in the feed to that ratio calculated in the abomasal digesta would indicate the stability of the marker system. Theoretically, these two ratios should not differ since the marker itself should not be absorbed or structurally changed in any way if the marker system is to work properly. If the system works properly, such a ratio comparison would be $1.0 \pm .05$.

Table 8 shows the mean marker concentrations in all three silage rations and their corresponding concentrations in the abomasal digesta obtained from steers fed that diet. The ratios of marker combinations in the feed and abomasal digesta, and the marker: marker (in feed) to marker: marker (in abomasal digesta) ratio are depicted in Table 9. From this data, it becomes apparent that lignin and chromium are the most suitable markers since their ratios in the feed and the abomasal digesta are closest to 1.0. Even though these two markers work best in this trial, the lignin and chromium ratios differ from the feed to the abomasum by 13.0% in the control, 16.0% in the intermediate protein ration and a high of 20% in the 15.6 g AN/KGCSDM ration. Such marker shifts, whether attributable to their non-association with the phase they are to mark, absorption by the animal or an analytical error, will lead to gross miscalculations of nitrogen and NAN passage data. Such data were obtained in this experiment as can be observed in Appendix Tables A.7-A.18.

As a marker, polyethylene glycol did not perform as well as lignin or chromium as can be deducted from its marker ratios when paired

TABLE 8. Marker Concentrations in Feed and Abomasal Samples for Steers Fed Experiment 3 Rations

Item	Feed	Abomasal Digesta[a]
Control		
Lignin, %	2.17	5.01
Cr_2O_3, %	.2000	.4031
PEG, %	.5000	.220
7.80 g AN/KGCSDM		
Lignin, %	2.67	5.23
Cr_2O_3, %	.2000	.4564
PEG, %	.5000	.216
15.60 g AN/KGCSDM		
Lignin, %	2.61	6.44
Cr_2O_3, %	.2000	.3939
PEG, %	.5000	.240

[a]Average of 8 composited samples per ration

TABLE 9. Marker: Marker Ratios in Feed and Abomasal Samples

Item	Control			7.8 g AN/KGCSDM			15.6 g AN/KGCSDM		
	Feed	Abomasum	Feed / Abom	Feed	Abomasum	Feed / Abom	Feed	Abomasum	Feed / Abom
Lignin: Cr	10.85	12.43	.87	13.35	11.46	1.16	13.05	16.35	.80
Lignin: PEG	4.34	22.77	.19	5.34	24.21	.22	5.22	26.84	.19
Cr: PEG	.40	1.83	.22	.40	2.11	.19	.40	1.64	.24

with chromium or lignin. In control animals, PEG: lignin ratios in the feed differed from that ratio in the abomasal digesta by 81%; in steers fed the 7.80 g AN/KGCSDM diet by 78% and again by 81% in animals fed the highest protein ration. When PEG: chromium ratios in feed and abomasal digesta were contrasted, similar patterns were observed. In inert marker systems where marker passage and recovery are so critical in estimating total N passage and subsequent microbial cell protein production, variations in marker ratios from feed to abomasal digesta cannot be tolerated and still expected to obtain feasible data.

Once a marker system has been termed a failure due to variations in the feed marker to abomasal marker ratios, the question as to why the system failed needs to be investigated. PEG, the only liquid phase marker utilized, has been used by other researchers (Termouth, 1967; Lemenager et al., 1978) with limited success. This marker, due to its association with the liquid pool, may not have been ingested at the expected .5% of daily dry matter intake. Marker losses could have occurred at the feed bunk as the animal consumed its feed due to excess salivation and water transfer from the waterer. The analytical procedure for PEG is non-specific and interferences in turbidity development has been well documented (Smith, 1955 and Corbett, 1958).

Particulate marker difficulties are found throughout the literature. Crickenberger (1977) found chromium based data to be too variable and thus based his N passage solely on lignin ratio equations since that system had less variability. Poos et al. (1979) found Cr_2O_3 was underestimated in abomasal samples, thus resulting in over-estimated particulate passage. Drennan et al. (1970) reported similar data to this

author's trial in that Cr_2O_3 based N flows resulted in NAN estimated passage that was greater than N intake.

Lignin ratios employed to evaluate N passage to the lower gut seem to be more consistent than Cr_2O_3 (Faichney, 1975; Drennan et al., 1970), however, the low lignin content in most typical feedlot rations limits the usage of lignin as a suitable marker due to the high variability observed in lignin determinations. There seems to be no ideal procedure for lignin analysis at present. Reports from various laboratories (Collings, 1979; Johnson et al., 1961 and Van Soest, 1963) indicated that different procedures in analyzing for lignin resulted in different lignin values for a specific feed. This is especially true for low lignin materials. The length of time between mixing the reagents and their usage may affect the final lignin yield as will the time that the reagents are in contact with the sample. The chemicals used for the different procedures will degrade different portions of the plant cell, thus resulting in variable lignin values.

It is the choice of a marker system that ultimately leads to whether the experimental data obtained from a N passage trial is theoretically acceptable. In this case, the markers used and the choice of analytical procedures to determine their concentrations in the feed and abomasal samples were the origin of this trial's unfeasible data.

Experiment Four

The nitrogen balance data from the six ration treatments are shown in Table 10. Rumensin had no significant effect on nitrogen retained per day ($P < .10$), but treatment of silage with anhydrous ammonia

TABLE 10. Effect of Monensin and Anhydrous Ammonia Treatment on N Metabolism in Steers[a]

Item	Level of monensin (ppm)	Anhydrous ammonia levels (g AN/KGCSDM) 0	7.80	15.60
N intake, g/day	0	39.1	67.5	84.3
	33	38.9	67.3	84.0
Fecal N excreted, g/day	0	19.2	27.9	29.5
	33	14.9	27.8	25.7
Urine N excreted, g/day	0	14.1	25.1	26.7
	33	10.1	24.8	35.0
N retained, g/day[b]	0	5.8	14.5	28.1
	33	13.9	14.7	23.3
N retained, as % N intake[c]	0	15.0	21.6	33.4
	33	35.8	22.5	28.2

[a]Eight steers per silage treatment: 4 steers per monensin treatment.

[b]EMS = 32.5 anhydrous treatment level had significant effect on N balance ($P < .05$). An AN x monensin interaction was observed ($P = .087$).

[c]EMS = 65.3 significant difference in N ret/N intake due to monensin ($P=.104$) and ammonia treatment ($P=.107$). AN x monensin interaction ($P=.01$).

at both levels resulted in significantly higher ($P < .05$) N retention when contrasted to the control silage. As the level of crude protein in the ration increased, so did the nitrogen retained per day. A slight anhydrous ammonia x monensin interaction was evident ($P = .087$) pertaining to nitrogen retention.

Nitrogen retained as a percent of total nitrogen intake was affected by the level of anhydrous ammonia ($P < .10$) and the addition of monensin ($P < .10$). Monensin supplementation resulted in 20.8 and .9% increases in N retention in the 0 and 7.80 g AN/KGCSDM (anhydrous ammonia/kilogram corn silage dry matter) treated rations respectively, but decreased the N retention by 5.2% in steers fed the 15.6 g AN/KGCSDM supplemented ration. A NH_3-N x monensin interaction was also observed concerning the nitrogen retained as a percent of intake. This data supports earlier similar results obtained by Van Nevel and Demeyer (1977), Hanson and Klopfenstein (1977) and Poos et al. (1979) which suggests that steers fed rations supplemented with monensin retained more nitrogen when the CP level is borderline to deficient in regards to their requirement and when the rations were supplemented with preformed protein rather than NPN. The decrease in N retention/N intake in steers fed the 15.6 g AN/KGCSDM ration may be the result of less efficient NH_3-N use by ruminal bacteria at the higher NH_3-N uptake from the rumen and omasum thus resulting in greater N losses in the urine (Poos et al., 1979).

CONCLUSIONS

In the first two experiments the data indicate that the following conclusions may be made:

(a) increasing CP levels in rations fed steers resulted in increased ($P < .05$) total N and NAN flows to the abomasum across all treatments, and;

(b) supplementation with urea above 13.0% crude protein did not result in any increase in abomasal total N or NAN flow although data from the two sets of steers could not be compared statistically.

The data from the third experiment suggest that:

(a) rumensin supplementation did not affect N intake.

(b) the effect of increasing dietary crude protein levels on nitrogen passage to the abomasum could not be evaluated due to poor marker flows as indicated by marker: marker contrasts between feed and abomasal samples.

(c) PEG overestimated the flow of liquid pools from the rumen thus making the validity of a dual marker system in this trial rather dubious,

(d) composites of abomasal samples collected over a 3-4 day period should be made since individual sample analysis correlated poorly with composited values.

The last experiment regarding rumensin effects on nitrogen retention showed:

(a) rumensin had no effect on N retention per day,

(b) treatment of silage at both levels resulted in significantly higher ($P < .05$) N retention when contrasted to the control,

(c) a slight anhydrous ammonia x rumensin interaction ($P = .087$) was evident in regards to N retention,

(d) the addition of monensin and treatment of silage with anhydrous ammonia resulted in an increase of N retained/N intake ($P < .10$), and

(e) an NH_3-N x rumensin interaction ($P = .01$).

APPENDIX

TABLE A1. Individual Steer Data: Intake Components - Control - Experiment 3

Animal No.	Rumensin (ppm)	DM Intake (kg)	% N, Feed	N Intake g/day	% Lignin, Feed	% Cr_2O_3 Feed	% PEG, Feed
528	0	4.45	1.24	55.23	2.17	.200	.500
528	33	5.81	1.24	72.17	2.17	.200	.500
799	0	5.81	1.24	72.17	2.17	.200	.500
799	33	4.45	1.24	55.23	2.17	.200	.500
992	0	4.45	1.24	55.23	2.17	.200	.500
992	33	5.81	1.24	72.23	2.17	.200	.500
994	0	5.81	1.24	72.23	2.17	.200	.500
994	33	4.45	1.24	55.23	2.17	.200	.500

TABLE A2. Individual Steer Data: Intake Components - 7.80 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	DM Intake (kg)	% N, Feed	N Intake g/day	% Lignin, Feed	% Cr_2O_3 Feed	% PEG, Feed
528	0	4.45	1.56	69.48	2.67	.200	.500
528	33	4.45	1.56	69.48	2.67	.200	.500
799	0	4.45	1.56	69.48	2.67	.200	.500
799	33	4.45	1.56	69.48	2.67	.200	.500
992	0	4.45	1.56	69.48	2.67	.200	.500
992	33	5.81	1.56	90.80	2.67	.200	.500
994	0	4.45	1.56	69.48	2.67	.200	.500
994	33	4.96	1.56	77.48	2.67	.200	.500

TABLE A3. Individual Steer Data: Intake Components - 15.60 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	DM Intake (kg)	% N, Feed	N Intake g/day	% Lignin, Feed	% Cr_2O_3 Feed	% PEG, Feed
528	0	4.45	1.99	87.63	2.61	.200	.500
528	33	4.45	1.99	88.63	2.61	.200	.500
799	0	3.45	1.99	68.75	2.61	.200	.500
799	33	4.45	1.99	88.63	2.61	.200	.500
992	0	3.75	1.99	74.63	2.61	.200	.500
992	33	3.18	1.99	63.42	2.61	.200	.500
994	0	4.45	1.99	88.63	2.61	.200	.500
994	33	4.45	1.99	88.63	2.61	.200	.500

TABLE A4. Individual Steer Data: Abomasal Components - Control - Experiment 3

Animal No.	Rumensin (ppm)	Total N, (%)	NH_3-N, (%)	Cr (ppm)	PEG mg/ml	% Lignin	% DM
528	0	3.11	.21	2472	.25	4.13	4.08
528	33	3.89	.38	4923	.22	4.53	3.51
799	0	3.51	.28	5238	.21	4.09	3.80
799	33	4.92	.21	4300	.19	6.64	4.84
992	0	3.04	.25	3771	.24	4.97	5.23
992	33	3.55	.36	3187	.23	5.55	4.84
994	0	3.91	.39	4262	.22	5.06	3.39
994	33	3.45	.38	4108	.20	5.13	3.79

TABLE A5. Individual Steer Data: Abomasal Components - 7.80 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	Total N, (%)	NH_3-N, (%)	Cr (ppm)	PEG mg/ml	% Lignin	% DM
528	0	3.59	.48	3745	.32	5.29	4.08
528	33	3.68	.34	5595	.28	5.24	3.83
799	0	3.34	.28	3992	.18	5.43	4.41
799	33	3.51	.32	3936	.15	4.42	3.48
992	0	4.31	.44	4822	.19	5.98	2.57
992	33	3.92	.39	5066	.13	4.32	2.68
994	0	4.24	.37	4693	.22	5.52	3.60
994	33	3.49	.42	4851	.26	5.67	3.96

TABLE A6. Individual Steer Data: Abomasal Components - 15.60 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	Total N, (%)	NH_3-N, (%)	Cr (ppm)	PEG mg/ml	% Lignin	% DM
528	0	3.55	.220	4078	.41	6.250	4.40
528	33	3.13	.183	3642	.45	6.285	4.96
799	0	3.43	.190	3808	.23	7.05	3.96
799	33	3.48	.190	4166	.20	6.89	3.74
992	0	3.38	.24	3322	.25	6.01	4.75
992	33	4.05	.21	3941	.20	5.07	4.55
994	0	3.48	.25	4106	.33	7.45	3.18
994	33	3.65	.26	4461	.30	6.56	3.70

TABLE A7. Individual Steer Data: Estimate of N Passage - Control - Experiment 3[a]

Animal No.	Rumensin (ppm)	N Passage (Lignin)	NAN Passage (Lignin)	N Passage (Cr)	NAN Passage (Cr)	N Passage (PEG)	NAN Passage (PEG)
528	0	72.87	71.59	112.06	110.10	115.70	113.68
528	33	108.58	104.80	91.98	88.78	190.91	184.27
799	0	108.39	105.64	78.00	76.03	192.23	187.36
799	33	71.67	70.63	101.92	100.45	299.76	295.44
992	0	59.08	57.50	71.81	69.88	155.66	151.49
992	33	80.79	78.35	129.66	125.76	230.46	223.51
994	0	97.69	93.03	106.79	101.69	178.49	169.96
994	33	65.00	62.07	74.81	71.44	153.39	146.48

[a]All N and NAN passage (pass) estimates are grams per day.

TABLE A8. Individual Steer Data: Estimate of N Passage - 7.80 g AN/KGCSDM - Experiment 3[a]

Animal No.	Rumensin (ppm)	N Passage (Lignin)	NAN Passage (Lignin)	N Passage (Cr)	NAN Passage (Cr)	N Passage (PEG)	NAN Passage (PEG)
528	0	80.70	77.36	85.39	81.86	106.27	101.87
528	33	83.59	80.67	58.59	56.54	114.71	110.70
799	0	73.14	70.59	74.53	71.93	185.68	179.20
799	33	94.54	91.93	79.43	77.24	186.02	180.88
992	0	85.75	82.33	79.62	76.44	133.25	127.93
992	33	140.98	135.29	90.07	86.44	238.17	228.56
994	0	91.42	87.94	80.48	77.41	163.02	156.80
994	33	81.70	77.84	71.47	68.09	137.40	130.91

[a]All N and NAN passage (pass) estimates are grams/day.

TABLE A9. Individual Steer Data: Estimates of N Passage - 15.60 g AN/KGCSDM - Experiment 3[a]

Animal No.	Rumensin (ppm)	N Passage (Lignin)	NAN Passage (Lignin)	N Passage (Cr)	NAN Passage (Cr)	N Passage (PEG)	NAN Passage (PEG)
528	0	66.08	63.46	77.54	74.47	87.95	84.47
528	33	57.89	55.98	76.55	74.03	80.26	77.62
799	0	56.60	54.56	80.23	77.35	137.29	132.36
799	33	45.55	43.94	57.72	55.68	118.26	114.09
992	0	55.09	53.36	64.32	62.31	154.85	150.00
992	33	66.51	64.75	77.71	75.65	121.03	117.82
994	0	54.34	50.31	75.49	69.91	76.02	70.40
994	33	64.68	61.76	72.88	69.59	102.93	98.28

[a]All N or NAN passage estimates are grams per day.

TABLE A10. Individual Steer Data: Particulate and Liquid Flow Rates - Control - Experiment 3

Animal No.	Rumensin (ppm)	Liquid Passage l/day (PEG)	Particulate Passage g/day (Cr)	Particulate Passage g/day (lignin)
528	0	87.47	3603	2343
528	33	134.92	2365	2791
799	0	138.64	2222	3088
799	33	119.79	2072	1457
992	0	92.77	2362	1943
992	33	127.64	3653	2276
994	0	130.10	2731	2499
994	33	112.87	2168	1884

TABLE A11. Individual Steer Data: Particulate and Flow Rates - 7.80 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	Liquid Passage l/day (PEG)	Particulate Passage g/day (Cr)	Particulate Passage g/day (lignin)
528	0	69.59	2379	2248
528	33	78.27	1592	2272
799	0	120.50	2231	2190
799	33	146.99	2263	2693
992	0	117.20	1847	1990
992	33	220.63	2298	3596
994	0	102.95	1898	2156
994	33	95.48	2048	2341

TABLE A12. Individual Steer Data: Particulate and Liquid Flow Rates - 15.60 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	Liquid Passage l/day (PEG)	Particulate Passage g/day (Cr)	Particulate Passage g/day (lignin)
528	0	53.83	2184	1861
528	33	49.14	2446	1850
799	0	97.07	1659	1650
799	33	87.47	2339	1309
992	0	91.87	1903	1630
992	33	62.69	1919	1642
994	0	66.52	2169	1561
994	33	73.40	1997	1772

TABLE A13. Individual Steer Data: NAN Passage Based on Dual Marker System[a] - Control - Experiment 3

Animal No.	Rumensin (ppm)	NAN-Liquid g/day (PEG)	NAN-Solid g/day (lignin)	NAN-Solid g/day (Cr)	Total NAN g/day (PEG & lignin)	Total NAN g/day (PEG & Cr)
528	0	49.23	59.84	92.03	109.06	141.26
528	33	64.42	94.62	80.16	159.04	144.58
799	0	57.57	123.06	88.56	180.62	146.12
799	33	41.53	35.13	49.96	76.66	91.50
992	0	54.32	43.79	53.22	98.10	107.54
992	33	64.23	63.72	102.27	127.95	166.50
994	0	63.45	88.30	96.52	151.74	159.97
994	33	63.22	64.13	73.81	127.35	137.03

[a]Dual marker system = In abomasum, one marker to associate with liquid phase, one marker to associate with solid phase, and together they represent the total abomasal digesta pool.

TABLE A14. Individual Steer Data: NAN Passage Based on Dual Marker System[a] - 7.8 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	NAN-Liquid g/day (PEG)	NAN-Solid g/day (lignin)	NAN-Solid g/day (Cr)	Total NAN g/day (PEG & lignin)	Total NAN g/day (PEG & Cr)
528	0	33.64	67.08	70.97	100.72	104.61
528	33	34.96	83.50	58.52	118.47	93.49
799	0	49.12	70.71	72.05	119.83	121.17
799	33	52.67	98.63	82.87	151.30	135.54
992	0	43.58	56.56	52.52	100.14	96.09
992	33	65.22	133.82	85.50	199.04	150.72
994	0	46.82	87.99	77.46	134.82	124.28
994	33	51.06	77.91	68.15	128.97	119.21

[a]Dual marker system = In abomasum, one marker to associate with liquid phase, one marker to associate with solid phase, and together they represent the total abomasal digesta pool.

TABLE A15. Individual Steer Data: NAN Passage Based on Dual Marker System[a] - 15.60 g AN/KGCSDM - Experiment 3

Animal No.	Rumensin (ppm)	NAN-Liquid g/day (PEG)	NAN-Solid g/day (lignin)	NAN-Solid g/day (Cr)	Total NAN g/day (PEG & lignin)	Total NAN g/day (PEG & Cr)
528	0	26.44	87.95	72.47	88.20	98.91
528	33	24.85	65.99	87.26	90.84	112.12
799	0	34.67	57.73	81.85	92.41	116.52
799	33	31.15	50.77	64.34	81.92	95.49
992	0	41.40	52.96	61.83	94.36	103.24
992	33	30.98	45.96	53.55	76.82	84.53
994	0	36.59	53.57	74.43	90.16	111.02
994	33	41.84	62.99	70.98	104.83	112.82

[a]Dual marker system = In abomasum, one marker to associate with liquid phase, one marker to associate with solid phase, and together they represent the total abomasal digesta pool.

TABLE A16. Effect of anhyrous ammonia treatment level on N passage to the abomasum of steers

Item:	Level of anhydrous ammonia (g AN/KGCSDM) 0	7.80	15.60
Ration:			
Daily DM intake, kg	5.13	4.68	4.08
Daily N intake, g	63.70	73.14	81.24
Nitrogen/lignin	.48	.58	.76
Nitrogen /Cr_2O_3	6.20	7.80	9.95
Lignin / Cr_2O_3	10.85	13.35	13.05
Abomasal Contents:			
Total N, %	3.61	3.78	3.52
NH_3 %	.31	.38	.23
NAN, %	3.30	3.40	3.29
Nitrogen/lignin	.72	.72	.55
Nitrogen/Cr_2O_3	9.03	8.24	8.93
Lignin/Cr_2O_3	12.43	11.46	16.35
Nitrogen Passage:			
Total N, g/day[a]	95.88	77.45	72.80
NAN, g/day[a]	93.01	74.49	69.87
Total N / N intake[a]	1.52	1.06	.91
Total N, g/day[b]	83.01	91.48	58.34
NAN, g/day[b]	80.45	87.99	56.02
Total N / N intake[b]	1.29	1.24	.73

a = chromium based b = lignin based

TABLE A17. Effect of monensin on abomasal nitrogen passage parameters.

Item:	Monensin Levels[a] 0	33	EMS
		g/day	
N intake	72.7	72.6	85.96
Total abomasal N[b]	75.1	80.1	304.58
Total abomasal N[c]	82.2	81.9	210.95
Total abomasal N[d]	140.5	164.4	1839.23
Abomasal NAN[b]	72.3	77.3	280.49
Abomasal NAN[c]	79.1	79.1	200.81
Abomasal NAN[d]	135.5	159.0	1792.19
Total abo. N/N intake[b]	1.06	1.11	.03
Total abo. N/N intake[c]	1.17	1.16	.05
Total abo. N/N intake[d]	2.00	2.37	.48

[a] No significant differences due to monensin level.

[b] Based on lignin as references marker.

[c] Based on chromium as reference marker.

[d] Based on PEG as reference marker.

TABLE A18. Effect of monensin on daily ruminal flows to the abomasum

Item	monensin levels[a] 0	33
Liquid flow (l)[b]	97.4	109.1
NAN (liquor)[b], g	44.7	47.2
Particulate flow[c], g	2322.5	2206.5
NAN (particulate)[c], g	74.5	73.1
Particulate flow[d], g	2096.6	2156.9
NAN (particulate)[d], g	68.6	73.1
NAN (liquor)[b] + NAN (part.)[c]	119.2	120.3
NAN (liquor)[b] + NAN (part.)[d]	113.3	120.3
NAN[b] (total sample)	135.5	159.0
NAN[c] (total sample)	79.1	79.1
NAN[d] (total sample)	72.3	77.3

* No significant differences due to monensin.

[a] Based on PEG as reference marker.

[b] Based on chromium as reference marker.

[c] Based on lignin as reference marker.

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