THE ROLE OF MONENSIN IN PROTEIN METABOLISM IN STEERS

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Husbandry

1980

ABSTRACT

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Digesta Passage Studies

Four abomasally cannulated steers were used in a switch-back metabolism study to determine the effect of monensin on non-ammonia nitrogen (NAN), dry matter (DM) and fiber passage to the abomasum. The steers were fed high grain rations, with (HG-M) and without (HG) monensin or high silage rations, with (CS-M) and without (CS) monensin. Grain rations contained shelled corn, oats, alfalfa, soybean meal and molasses. Silage rations consisted of corn silage [35% dry matter (DM)] and soybean meal-mineral supplement. Monensin was added at 33 ppm ration DM and all rations contained 13% crude protein (CP). Steers were fed twice daily ad libitum. Lignin and chromic oxide were used as particulate and total digesta markers, and polyethylene glycol (PEG) used to estimate liquid digesta passage.

RNA-N:total-N ratios of mixed rumen bacteria (isolated from ruminally-fistulated steers receiving the same rations) and abomasal RNA-N:total-N ratios were used to quantitate microbial contribution to NAN flow.

From lignin passage, abomasal N and NAN passage (% of N-intake) for rations HG-M, HG, CS-M and CS were: 96.86, 90.48, 100.91, 93.24;

94.81, 88.24, 97.23 and 89.76, respectively. Abomasal NAN, microbial N and feed (bypass) N passage for the same respective rations were: 124.29, 74.85, 49.44; 119.25, 82.90, 36.35; 133.59, 76.12, 57.47; 133.64, 95.86 and 37.79 g/day. Feed N bypass (% NAN passage) for rations HG-M, HG, CS-M and CS were: 39.78, 30.48, 43.02 and 28.27, respectively. Efficiencies of microbial protein synthesis for the above respective rations were: 17.31, 20.31, 15.13 and 21.00 g CP per 100 g organic matter truly digested in the rumen. Monensin decreased microbial-N passage ($P < 0.05$), increased feed N bypass ($P < 0.01$) and decreased efficiency of ruminal microbial cell production ($P < 0.05$).

The extent of ruminal degradation of DM and fiber was not significantly affected by monensin. Passage data derived from chromium flow were very similar to the lignin-based estimates. In contrast, fractional rates of ruminal PEG outflow tended to be high, thus resulting in an overestimation of the extent of liquid digesta and N-passage to the abomasum.

Nitrogen Balance

Nitrogen status of the steers used in the preceding study was also evaluated in a balance and digestibility trial. The steers were adapted to the same experimental rations for 18 days prior to 8-day nitrogen balance collections.

Monensin increased nitrogen and DM digestibility of the grain ration without significantly affecting the silage ration. Nitrogen retention (% of N-intake) were: 37.36, 32.34, 30.90 and 27.80 for HG-M, HG, CS-M and CS rations, respectively. These differences were,

however, not significant. Fiber (ADF) digestibility was higher with the silage rations ($P < 0.01$), but there was no monensin effect.

Feeding Trial and Plasma Studies

A feeding trial was conducted to determine the influence of monensin and/or elfazepam on the plasma amino acid (PAA) status and performance of Hereford steers fed an all-silage ration. Control ration consisted of 88% corn silage (35% DM) and 12% soybean meal-mineral supplement. Monensin was fed at 33 ppm; elfazepam was added at 2 ppm ration DM.

Overall PAA, plasma urea and glucose levels were not significantly affected by the addition of monensin, elfazepam or the two chemicals combined, to the rations. The trends were for slightly reduced levels of the sulfur and branched-chain amino acids with monensin-elfazepam combination. Essential, non-essential and total amino acid levels were also slightly lower with the two drug combination; the other treatments were similar to the control.

In comparison with the control silage, monensin improved the overall performance of the steers, elfazepam only stimulated intake, while the two chemicals in combination resulted in poor performance.

In memory of

my beloved parents

ACKNOWLEDGMENTS

The author is deeply grateful to Dr. W. G. Bergen for his guidance, supervision and encouragement throughout the duration of the graduate program. His expertise and constructive advice were invaluable both in the designing of these studies and in the preparation of the ensuing manuscript. Sincere thanks are also due to Drs. M. T. Yokoyama, D. R. Hawkins and R. M. Cook for their involvement in these doctoral studies. Their scholarly comments and critical review of this dissertation were very much appreciated. The author also wishes to express a deep appreciation to Dr. John Waller for his friendship and kind indulgence.

Sincere gratitude is extended to Dr. R. H. Nelson, chairman of the Department of Animal Husbandry for providing the necessary financial support and research facilities that made my academic endeavors a reality. Dr. W. T. Magee's understanding and willingness to help, especially during the difficult periods of my graduate studies will always be remembered. I am also deeply indebted to Dr. E. R. Miller, who in his quiet exemplary ways was for me, a tremendous source of inspiration and motivation.

I also wish to thank my fellow graduate students, past and present (especially Mr. D. B. Bates and Dr. G. F. Collings), for their intellectual stimulation and comradeship. A debt of gratitude is especially due to Ms. E. S. Rimpau, not only for her unreserved technical assistance, but also for her generosity and thoughtfulness. I will always cherish my association with that special lady. Special appreciation is also extended to Mrs. Pat Cramer and Sherry Mileski for their congeniality and ready assistance.

I would like to thank my beloved grandmother, Mrs. V. A. Oputa for her unqualified love and confidence in me; my beloved brothers and sisters, Maureen, Cyril, Valentine, Evangeline, Victor and Frederick, for their love, patience and understanding. Immense gratitude is also due to Ms. J. A. Okafor for her patience, support, and love. Above all, I thank the Almighty Father for giving me such exemplary role models as my wonderful parents, whose 'untimely' departure prevented their seeing the successful conclusion of my turbulent quest for independence and knowledge. But, most importantly, I thank Him for his forgiveness, guidance and love.

For all those who, by accident or by design, did in anyway touch my life, I hope the encounters were positive; as for me, I hope the experiences would help to make me a better human being.

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CHAPTER I

INTRODUCTION

The ruminant animal is unique in its nutrient metabolism, due to its symbiotic relationship with the anaerobic microorganisms that inhabit the forepart (reticulorumen) of its digestive tract. Food consumed by the animal is initially fermented by these rumen microbes, in order to meet their own nutrient requirements for growth (Hungate, 1966). Microbial cells, and the unfermented feed residues are subsequently flushed out of the rumen to the lower gastrointestinal tract, where they become exposed to the hydrolytic actions of the digestive enzymes.

Of advantage to the ruminant, is the fact that these microorganisms can degrade B-linked polysaccharides (cellulose), which are major constituents of most ruminant feeds. Rumen microbes can therefore convert feed materials the animal cannot digest, into high quality, highly digestible microbial protein. These organisms, on the other hand, are furnished with an ecological niche conducive to their own growth requirements, while at the same time, being given a first opportunity at the food the animal consumes.

Since the advent of the modern feed-lot, the ruminant is increasingly becoming a major competitor for foods more efficiently utilized by man and other monogastrics. Feeds such as corn and soybean

are now fed to cattle in order to increase their energy and protein intake, and thus improve their rate of growth. The idea is to shorten the time the animal is on feed prior to slaughter. Unfortunately, such high quality feeds can be more efficiently converted into meat protein when they are digested in the post ruminal gastro-intestinal tract, rather than being fermented by rumen microorganisms. Several techniques have therefore, been developed to protect high quality plant proteins from extensive ruminal degradation (Chalupa, 1975), and thus increase the proportion of those feed proteins digested in the lower tract.

Over the years, the emphasis of ruminant nutrition research has often alternated between the above objectives, methods to increase energy intake of the animals, and manipulations aimed at maximizing rumen microbial production. Intake stimulants (e.g. Elfazepam [Baile et al., 1976; Baile and McLaughlin, 1979]), anabolic steroids (e.g., DES, Synovex) and antibiotics (e.g., Aureomycin, Monensin), are some of the growth stimulants and feed additives widely used to manipulate the digestive and physiological responses of the ruminant animal, in order to improve its rate of gain and efficiency of feed conversion. One of these feed additives, monensin, forms the subject matter of the following studies.

Monensin is a monocarboxylic acid-polyetherin antibiotic (Harold, 1972) produced by a strain of the actinomycete, Streptomyces cinnamonensis. This compound was reported by several workers to reduce feed intake and improve the efficiency of gains in ruminants (Potter et al., 1976a; Raun et al., 1976; Utely, 1976). Other workers

(Richardson et al., 1976) observed from in vitro studies that monensin causes a shift in the molar ratios of volatile fatty acids in favor of propionic acid. Reductions in rumen ammonia levels were also reported with monensin (Dinius et al., 1976). These observations indicate that monensin exerts some effects on rumen microbial metabolism and feed degradation. It became, therefore, necessary to determine the effect of this antibiotic on the rate of digesta passage and feed protein degradation in the rumen.

The first study was conducted to determine the effect of monensin on nitrogen passage to the abomasum of growing steers fed high grain and high silage rations. Multiple markers were used to quantitate non-ammonia-nitrogen flow, microbial protein passage and the extent of rumen feed protein and dry matter bypass. The effects of monensin on nitrogen retention, dry matter, fiber and nitrogen digestion were also examined with the same rations. In another study aimed at assessing the influence of monensin on the plasma amino acid status of growing steers, monensin was fed separately, or in combination with an intake stimulant (Elfazepam), in a high silage ration. In this trial, the rate of body weight gains and efficiency of feed conversion were also determined.

CHAPTER II

REVIEW OF LITERATURE

Rumen Microbial Growth--Substrate Utilization

Overview

Rumen microorganisms rely on the ruminant not only for the supply of the nutrients they require for their metabolism and growth, the ruminant also provides them a habitat favorable for their active growth and proliferation. The anaerobic nature of the rumen and the relative constancy of its temperature (37 to 40°C) ensures these microorganisms an ecological niche that is conducive to their metabolic needs.

Under optimal conditions, rumen fluid is richly laden with nutrients which are readily available to the microbes. These include energy and nitrogen sources, metabolic intermediates and growth substances required by these organisms for the synthesis of microbial cells.

Carbohydrate Fermentation

Carbohydrates (starches, soluble sugars, cellulose, hemicellulose and pectins) are the primary feed components used by rumen bacteria and protozoa to generate the energy (ATP) required for cell synthesis. The majority of carbohydrates are converted to pyruvate via the Embden-Meyerhoff glycolytic pathway (Hungate, 1966). Pyruvate is further

utilized to produce ATP and volatile fatty acids (VFA's), CH_4, CO_2 and H_2. The major VFA's produced (acetate, propionate, butyrate and valerate) are absorbed via the rumen wall and utilized by the host animal as energy substrates. Pathways for the fermentation of pyruvate are fairly well established (Hungate, 1966; Baldwin, 1965) and the proportion of each VFA produced depends on the type of substrate, rumen pH and the fermentation pathways utilized by the microbial species predominant in the rumen. Acetate is produced primarily via phosphoroclastic reactions which involve the decarboxylation of pyruvate. Propionate is produced via the dicarboxylic acid (randomizing) pathway, and also by the acrylate (non-randomizing or direct reductive) pathway. The former route is usually more prominent, but the latter becomes more important as the carbohydrate availability of the diet increases (Baldwin, 1965). Butyrate and other higher fatty acids are synthesized from acetyl-CoA via a reversal of B-oxidation (Baldwin, 1965).

Even though some estimates of the theoretical ATP yield from the above reactions are still in contention, the following stoichiometric relationships have been proposed (Baldwin, 1965).

$$2 \text{ Pyruvate} \rightarrow 2 \text{ Acetate} + 2 \text{ ATP} + 2 H_2 + 2CO_2$$

$$\text{Pyruvate} + 2H_2 \rightarrow \text{Propionate} + \text{ATP}$$

$$2 \text{ Pyruvate } (2 \text{ Acetate} + H_2) \rightarrow \text{Butyrate} + 2\text{ATP} + 2CO_2$$

$$2 \text{ Pyruvate} + 3H_2 \rightarrow \text{Valerate} + \text{ATP} + CO_2.$$

The presence of electron transport systems in certain rumen organisms (especially some propionate producers) could still increase the

theoretical yield of ATP per mole of VFA produced. The currently assigned ATP values per mole of end-product formed are: acetate, 2; propionate, 3; butyrate, 3; and methane, 1. The relative energetic efficiency for the conversion of one mole of glucose to propionate is 109% (based on bomb calorimetric determinations--Hungate, 1966); it is 62.2% and 77.9% for acetate and butyrate, respectively. This indicates that of the three major VFA's produced, propionate fermentation results not only in a higher ATP yield to the microorganisms, but is also energetically more useful to the host animal.

Nitrogen Metabolism

Dietary proteins and non-protein nitrogen (NPN) are degraded by rumen microbial proteolytic enzymes and deaminases to ammonia--nitrogen (NH_3-N), amino acids and peptides, which are differentially incorporated into microbial protein. Ammonia is the central intermediate in the degradation and assimilation of dietary nitrogen in the rumen, and seems to be required by several species of rumen bacteria (Hungate, 1966; Bryant and Robinson, 1962). Many microbial species can maintain their rates of growth with ammonia and other NPN as their sole nitrogen source, provided they have available a readily fermentable energy source, and other nutrient requirements such as carbon skeletons, sulfur and other co-factors required for microbial protein synthesis.

Quantitative Aspects of Microbial Protein Synthesis in the Rumen

Rumen microbial growth is dependent primarily on the availability of substrates. These include not only adequate energy and nitrogen supply, but also other co-factors such as sulfur, carbon skeleton and unidentified growth substances required for optimal cell synthesis. Any of the above requirements can limit the efficiency of microbial cell growth and organic matter degradation in the rumen.

In recent years, considerable effort has been directed to a quantitative estimation of the extent of microbial protein synthesis in the rumen. The amount of microbial cells (or microbial crude protein) produced in the rumen is usually estimated in relation to some unit of organic matter apparently digested in the rumen (DOM), or of organic matter truly fermented (FOM). The second method is more accurate and facilitates the calculation of ATP production, but the former is more precise and can be applied on a wider scale, especially when organic matter incorporation into microbial cells is not specifically determined.

Table 1 contains a summary of rumen microbial protein estimates obtained by several workers using different quantitation techniques. Estimates obtained range from 6 to 34 gm of microbial crude protein synthesized per 100 g DOM. This is equivalent to 12 to 68 g of dry microbial cells per 100 g DOM, if a 50% crude protein content for rumen bacteria is assumed (Smith, 1974). The wide variation in estimates obtained is indicative of problems encountered with the use of different markers to quantitate digesta and microbial protein flow from the rumen.

TABLE 1. RUMINAL OUTFLOW OF CRUDE PROTEIN AND MICROBIAL PROTEIN SYNTHESIS--SUMMARY OF EXPERIMENTS USING VARIOUS FLOW MARKERS

Flow marker	Microbial marker	Crude protein: Dietary intake %	Crude protein: Dietary intake g/day	Crude protein: Rumen outflow g/day	Crude protein: Rumen outflow % of ingested	Microbial yield g/100 g OM(DM)	Diet	Species	Reference
Lignin	RNA	11-12	476-512	482-658	94-124	8-15	78% corn grain (dry rolled, flaked, ensiled high moisture)	Cattle (429 kg)	Prigge et al. (1978)
Lignin	RNA	13	610-642	440-516	72-80	7-10	90% corn grain (flaked or dry rolled)	Cattle (460 kg)	Cole et al.
		12	551-734	439-829	77-118	8-12	Corn grain (whole shelled) + 0, 7, 14 or 21% cottonseed hulls		
		12-13	586-666	358-599	61-90	6-11	Corn grain (flaked or dry rolled) + 0 or 21% cottonseed hulls		
Lignin	RNA	9-10	421-466	336-381	79-82	10-12	Range grass + soybean meal - urea supplements	Cattle (275 kg)	Kropp et al. (1977a)
	RNA	8-13	386-573	541-656	105-140	18-20	Cottonseed hulls + urea or soybean meal	Cattle (350 kg)	Kropp et al. (1977b)
PEG	--	11	93	91	98	13	Semipurified diet with urea	Sheep	Hume (1970a)
PEG	--	3-21	16-100	32-48	200-48	9-13	Purified diet + urea levels	Sheep (40 kg)	Hume et al. (1970)
PEG	^{35}S	2	9	30	343	10	Barley roughage	Sheep	Liebholz & Hartman (1972), Liebholz (1972)
		14-16	101-119	65	69-52	7-12	Barley roughage + casein or gluten		
		15-26	97-188	65-63	67-35	6	Lucerne; lucerne + casein		
Cr_2O_3	--	8-21	173-637	159-506	61-97	--	Straw + flaked maize + casein	Cattle (125 kg)	Williams & Smith (1976)
Cr_2O_3	RNA DAP	15-16	464-551	369-443	77-86	10-14	Straw + flaked maize + groundnut meal, fish meal or soybean meal	Cattle (170 kg)	Smith et al. (1978)
Cr_2O_3	DAP	10-16	75-122	100-104	133-85	14-16	Rolled barley & cassava meal plus 0, 0.7, 1.4 or 2.1% urea	Sheep (22 kg)	Orskov et al. (1972)
Cr_2O_3	--	14	58	56	97	--	Hay + flaked maize (0.9 maint.)	Sheep	Nicholson & Sutton (1969)
		12	86	111	129	--	Hay + flaked maize (1.7 maint.)		
		11	123	188	153	--	Hay + flaked maize (2.3 maint.)		

TABLE 1--Continued

Flow marker	Microbial marker	Crude protein: Dietary intake %	Crude protein: Dietary intake g/day	Crude protein: Rumen outflow g/day	Crude protein: Rumen outflow % of ingested	Microbial yield g/100 g OM(DM)	Diet	Species	Reference
Cr_2O_3	Nucleic acid-N	26-29 26-29	166-180 271-281	186-213 314-324	106-118 115-116	21-23 18-22	Dried ryegrass (low intake) Dried ryegrass (high intake)	Sheep (48 kg)	Coelho da Silva et al. (1972a)
Cr_2O_3	Nucleic acid-N	16-18	146-166	189-201	130-114	21-22	Dried lucerne (chopped, wafered, pelleted)	Sheep (48 kg)	Coelho da Silva et al. (1972b)
Cr_2O_3	DAP	9-11 9-11	136-148 218-236	98-128 150-178	68-94 64-82	18-34 15-28	Fresh herbages--ryegrass & clover (low intake) Fresh herbages--ryegrass & clover (high intake)	Sheep	Ulyatt et al. (1975)
^{51}CrEDTA	--	14	111-118	90-148	76-125	17-23	Semi-purified diet with urea, casein, gelatin or zein	Sheep	Hume (1970b)
^{51}CrEDTA	--	16	128-138	109-126	83-92	18-20	Semi-purified diet with urea + gelatin + sulfur levels	Sheep (40 kg)	Hume and Bird (1970)
^{51}CrEDTA	^{35}S	--	89-98	97-119	105-122	--	Semi-purified diet + lupin peanut or soybean meal	Sheep	Hume (1974)
^{103}Ru & ^{51}CrEDTA	^{35}S	12	218	259	119	30-34	Bromegrass pellets	Sheep (51 kg) (cold vs. warm)	Kennedy et al. (1976)
PEG	^{35}S	13	125-141	---	---	19-26	Corn-soy diet	Sheep (30 kg)	Isichei & Bergen Unpublished
Lignin	--	11	506	399	79	--	Corn silage/low protein	Steers (213 kg)	Crickenberger et al. (1979)
Lignin	--	13	644	428	67	--	Corn silage/high protein	Steers (218 kg)	Crickenberger et al. (1979)
Lignin	--	13	663	588	85	--	High grain/low protein	Steers (216 kg)	Crickenberger et al. (1979)
Lignin	--	16	931	615	68	--	High grain/high protein	Steers (185 kg)	Crickenberger et al. (1979)

Also evident is the fact that both the nitrogen and energy content of the ration do affect microbial cell yield.

Hume et al. (1970) demonstrated the effects of nitrogen insufficiency in a series of experiments in which minimal protein diets containing urea were fed at 2-hour intervals. They fed diets containing 3.3%, 5.9%, 11.4% and 21.0% crude protein levels to sheep and obtained microbial protein estimates of 9.1, 10.5, 12.8 and 13.3 g per 100 g DOM, respectively. Ruminal organic matter digestion was similar in all four treatments. Subsequent experiments (Hume and Bird, 1970; Hume, 1970a, 1970b) showed that rumen microbial protein synthesis can be maximized by the inclusion of sulfur, branched-chain fatty acids and readily degradable plant proteins to the diets. These workers reported estimates as high as 22.5 g of microbial CP/100 g DOM. Another group (Bucholtz and Bergen, 1973), from in vitro studies of ^{33}P uptake by washed rumen bacteria, estimated that 22.0 g of microbial protein per 100 gm DOM were synthesized in a 4-liter sheep rumen. Subsequent in vivo studies in our laboratory based on the rate of $^{35}SO_4$ incorporation into microbial protein in sheep fed high concentrate diets (13% CP) at 2-hourly intervals, gave estimates of 19.3 to 25.7 g microbial crude protein per 100 gm DOM (Isichei and Bergen, 1975--unpublished data).

It is apparent from Table 1, that microbial crude protein yields (per 100 gm DOM) are lower for high concentrate rations and processed feeds (Prigge et al., 1978; Cole et al., 1976; Ørskov et al., 1972), than for high roughage rations, or feeds with a high roughage

content (Coelho da Silva et al., 1972a, 1972b; Ulyatt et al., 1975; Kennedy et al., 1976). This observation is related to the rate of digesta flow. It has been shown in in vitro continuous culture studies that the efficiency of microbial cell synthesis, or growth rate increases with increase in turnover or dilution rate (Isaacson et al., 1975). This is due to a decline in the energy expended by microbial cells for maintenance (Stouthammer, 1976). It is not unlikely that such situations also occur in the rumen, where it has been demonstrated that rumen liquid turnover rates are slower for concentrates than for forage diets (Hungate, 1966). It has also been reported from a few in vivo passage studies, that as the fractional rate of liquid outflow from the rumen increases, the efficiency of ruminal microbial protein synthesis (g CP/100 g DOM) also increases (Cole et al., 1976; Kropp et al., 1977a, 1977b).

Even though the amino acid composition of mixed rumen bacteria appears to be relatively constant (Purser and Buechler, 1966), their macromolecular composition on a dry matter basis do vary. Crude protein content of mixed rumen bacteria vary from 32 to 57% (Smith, 1975; Hungate, 1966). These values are lower than the 60 to 65% CP often used. There are also some variations in the carbohydrate (polysaccharide) content of rumen bacteria with time after feeding (Walker and Nader, 1970) and with the starch and soluble sugar content of the feed (McAllan and Smith, 1977). All the above variations are bound to affect the microbial dry matter values obtained from yield experiments. However, despite all these variable inputs, it is still

possible to estimate with a reasonable degree of accuracy, the efficiency of ruminal microbial crude protein synthesis.

With a knowledge of the factors regulating rumen microbial growth, the fermentation pathways predominant in the rumen, and the amount of ATP generated per unit of substrate oxidized, the actual efficiencies of microbial cell yield can be estimated.

Energetics of Rumen Microbial Growth--Y_{ATP}

It is evident from previous discussions that energy supply forms the basis for all rumen microbial activity. Synthesis of new cell material and maintenance of homeostasis both depend on the amount of energy available to the organism, and with most ruminant rations, energy availability constitutes the most common limitation to maximizing microbial output from the rumen (Owens and Isaacson, 1977).

Rumen fermentation can be likened to an open continuous flow system (Hungate, 1966), analogous to a chemostat (Bergen and Yokoyama, 1977). This fermentation model has characteristic features that include: the continual supply of substrates and end-products removal, buffering capacity, low electrical potential (relative absence of oxygen) and temperature control (Bergen and Yokoyama, 1977). Bacterial output from the rumen is dependent on two factors:

1. Bacterial concentration or population; and
2. Growth rate (or dilution rate) (Owens and Isaacson, 1977).

Some of the nutrient and ecological factors that regulate rumen bacterial numbers or population have been mentioned above, most of the data having been obtained from *in vivo* and *in vitro* experiments.

On the other hand, the bulk of our current knowledge on the factors that influence the growth rate and efficiency of microbial cellular yields were obtained primarily from *in vitro* studies.

Bauchop and Elsden (1960) first demonstrated from batch culture experiments that the amount of microbial growth was directly proportional to the amount of ATP that could be obtained from the degradation of an energy substrate, provided that energy was the rate-limiting nutrient. They introduced the Y_{ATP} concept, defining it as the dry cellular yield of microbial cells (g) per mole of ATP generated. It is clear that such a calculation is possible only when the substrate fermentation pathways for the organism in question are known. A knowledge of the catabolic pathways and steps at which ATP is synthesized by the organism, makes possible the estimation of the total amount of ATP formed for each mole of the substrate oxidized. The absence of oxidative phosphorylation in the rumen, however, impedes a complete oxidation of energy substrates, thus severely limiting the amount of ATP generated. Based on Bauchop and Elsden's data (Bauchop and Elsden, 1960), and from the work of several others (Stouthammer, 1969; Payne, 1970; Forest and Walker, 1971), it was proposed that Y_{ATP} was a biological constant for anaerobic microorganisms. Hence, there must be an upper limit for anaerobic microbial cellular growth (Hungate, 1966). A Y_{ATP} value of 10.5 was generally accepted. Subsequent studies (Stouthammer and Betterhausen, 1973; Hobson and Sommers, 1972, Isaacson *et al.*, 1975), however, showed that Y_{ATP} was variable for anaerobic organisms grown in continuous culture. So also is the

Y substrate which is defined as the molar growth microbial yield, per mole of substrate fermented.

Reasons proposed for this variation in Y_{ATP} include the following (Stouthammer, 1976; Owens and Isaacson, 1977):

1. Differences in bacterial macromolecular cellular composition (variable ash and polysaccharide content).
2. Transfer of metabolic intermediates between bacterial species in mixed cultures or in the rumen, thus enhancing the efficiency of substrate utilization and cell synthesis.
3. Specific growth rate or dilution rate influences Y_{ATP} values and consequently the molar growth yields. Increases in dilution rate in an energy-limiting chemostat (Stouthammer, 1976; Isaacson et al., 1975) have been shown to correspondingly increase Y_{ATP}. Stouthammer and Bettenhausen (1973) determined the Y_{ATP} for A. aerogenes at various growth rates (D). By plotting the relationship between Y_{ATP} and dilution rate, they obtained a maximum Y_{ATP} value (Y_{ATP}^{MAX}) of 25.5, close to the theoretical upper limit. The difference between the observed and theoretical values represents the energy expended for maintenance.
4. The availability of cell components would also influence cell yields. Synthesis of required precursors and other cellular materials involves energy expenditure. It was shown in vitro that complex media in which higher specific growth rates could be obtained, gave higher Y_{ATP} values than minimal media (Stouthammer, 1976).

5. Y_{ATP} is also related to the amount of available energy utilized by the bacteria for cell maintenance (cell turnover and maintenance of homeostasis). The amount of the energy which is used independently of growth is called the maintenance coefficient. Higher Y_{ATP} values were obtained at higher dilution rates due to a dilution of maintenance. It has been shown that slow-growing organisms have a much higher maintenance coefficient. Stouthammer (1977) suggested that the low Y_{ATP} values obtained in slow-growing cultures may be due to the uncoupling of growth, especially when bacterial cultures are grown in minimal media or in the presence of inhibitory compounds. Thus, the energy realized from substrate catabolism may not be completely used for new cell synthesis and maintenance of homeostasis. The discovery of the presence of ATPase(s) in certain anaerobes may indicate that the excess ATP produced under the above circumstances, would be dissipated (Stouthammer, 1976; 1977).

Additional factors which by influencing molar growth yields would influence the efficiency of microbial growth include, the pathway for the degradation of the energy substrate, and the possibility for anaerobic electron transport during fermentation. Cytochrome b has been found in Selenomonas ruminantium, Propionibacteria and Anaerovibrio lipolytica and functions in electron flow-linked (oxidative) phosphorylation. Some other membrane-bound primitive electron transport systems have been found in some other anaerobes, thus implying that

additional ATP could be produced in those organisms during propionate formation (Stouthammer, 1976). On the other hand, it is not impossible that ATP-conserving schemes, such as those identified in Bacteroides fragilis, a human-intestinal anaerobe (Macy et al., 1978), do occur in rumen bacteria. In B. fragilis, the PP_i-dependent 6-phosphofructokinase utilizes inorganic pyrophosphate instead of ATP, for the formation of fructose-1, 6-diphosphate during glucose catabolism. Carboxylation of PEP to oxaloacetate by PEP carboxykinase was also shown to occur in the same bacteria, with the concommitant formation of ATP (Macy et al., 1978). Further studies are needed to determine whether such in vitro pure culture findings do occur in vivo with mixed rumen bacterial populations.

Digesta and Non-Ammonia-Nitrogen Passage

The value to the animal of dietary protein conversion into microbial cells, is largely dependent on the relative biological values of the protein from both sources, although the importance of a viable microbial population to fiber digestion and NPN utilization cannot be overlooked. According to Smith (1969), 50 to 80% of dietary protein is believed to be degraded in the rumen, so that the flow of total protein should reflect microbial synthesis. Previous studies have shown that mixed rumen bacteria have a high biological value and digestibility (Purser, 1970; Bergen et al., 1968).

Generally, the rates of digesta and non-ammonia-nitrogen passage from the rumen are both a function of the rumen dilution rate and the rate of feed degradation. Increased food intake, feeding

frequency or processing of feed are some factors that have been reported to influence the rate of digesta flow (Prigge et al., 1978; Coelho da Silva et al., 1972a). Particle size and specific gravity, however, form the basis for the selective retention of feed particles in the rumen. Here, the extent of comminution and degradation of roughages determines their rates of exit. With high concentrates and more digestible feeds, the rates of microbial digestion of soluble carbohydrates and proteins vastly exceed the rate of digesta flow or dilution rate. This results in a greater microbial contribution to post-ruminal digesta. The more insoluble (less degradable) proteins such as zein or fish meal, and those high quality feed proteins protected from extensive rumen microbial attack (e.g., formaldehyde-treated casein or soybean meal), leave the rumen at the rate of digesta flow, thus increasing the extent of feed protein bypass (Sharma and Ingalls, 1974; Chalupa, 1977; Bergen and Yokoyama, 1977; Williams and Smith, 1976). Table 1 summarizes the nitrogen outflow data obtained by several workers using different marker combinations to quantitate total crude protein and microbial protein passage. It is difficult to compare all the results obtained by these workers since, in most cases, the experimental animal species, the level of intake, type of ration, energy and protein intake differ between experiments. Also, the validity of any one marker used over another is difficult to establish. However, it is still possible to make some quantitative assessments of the effects of a dietary regimen on the rate of feed protein degradation, microbial protein output and nitrogen passage to the lower gut.

Level of Feed Intake and NAN Flow

Few studies have been done with the primary objective of examining the influence of the level of dry matter and protein intake on the components of nitrogen reaching the abomasum. Using bacterial nucleic acid -N to estimate microbial protein flow, and Cr_2O_3 to quantitate particulate matter flow, Coelho da Silva et al. (1972a) (see Table 1) compared the effects of high and low levels of dried rye grass intake (19% CP) on nitrogen passage in sheep. They reported a net gain of 6 to 18% (with low intake) and 15 to 16% (with high intake) in the total nitrogen reaching the duodenum. Bacterial protein passage (as a percent of total duodenal CP) was higher for the low intake than for the high intake level (55 vs. 48%). But total bacterial protein passage was greater for the high forage intake (156 vs. 104 gm). Microbial nitrogen (Protozoa plus bacteria) contributed 86.8% and 76.3% to total duodenal nitrogen, for the low and high intake levels, respectively. NAN passage was not calculated, but the NH_3-N levels averaged 5.6% and 4.4% of total nitrogen flows for the low and high feeding levels, respectively. If the NH_3N levels are accounted for, duodenal NAN passage showed no net ruminal N losses for the low feed intake, and a 10% increase for the high intake, over their respective N-intakes. Extensive ruminal nitrogen loss would have been expected with a diet containing 19% dietary crude protein as was the case in the above experiment. The results could probably be explained by the fact that the rye grass was fed in ground or pelleted form, thus decreasing digesta transit time and consequently rumen ammonia loss. Nicholson

and Sutton (1969) (see Table 1) fed hay plus flaked maize to sheep at 0.9, 1.7 and 2.3 X maintenance and obtained a 3% net loss, 29% and 53% net gains, respectively, in total nitrogen flow (as % of N-intake). The dietary nitrogen effect was confounded in this study because the rations contained 14%, 12% and 11% CP, respectively, for the three levels of intake, due to a progressive increase in dietary starch content. No microbial protein values were reported.

The effect of dietary nitrogen and energy levels are more clearly demonstrated in two studies (Leibholz, 1972; Leibholz and Hartmann, 1972) in which sheep fitted with rumen and duodenal cannulae, were fed barley roughages supplemented with various levels of lucerne, casein and gluten. The diets are as follows:

1. Medium nitrogen, lucerne--13.2% CP;
2. Low nitrogen--1.8% CP;
3. High nitrogen, lucerne plus casein--23.8% CP;
4. Medium nitrogen, casein--12.9% CP;
5. Medium nitrogen, gluten--15.0% CP; and
6. Medium nitrogen, low energy--14.2% CP.

Diets 1 to 5 contained an estimated metabolizable energy (ME) level of 1.86 mcal/kg. Diet 6 had a reduced ME level (1.34 mcal/kg), and diet 2 was not supplemented with nitrogen. NAN passage were 64%, 339%, 31%, 66%, 60% and 50% of the dietary nitrogen intake for diets 1 to 6, respectively. All the diets (except 2) showed net losses of feed nitrogen in the rumen. Microbial nitrogen outflow were: 43%, 53%, 72%, 76% and 56% of total duodenal nitrogen for diets 1, 3, 4, 5 and 6,

respectively. All the duodenal nitrogen in those sheep fed ration 2 was of microbial origin. Their data seem to indicate that for growing ruminants fed sufficient energy in a 12-15% crude protein ration, 60 to 80% of the nitrogen that reaches the lower tract is probably microbial. However, the extent of dietary nitrogen loss in the rumen in the above studies indicate that dietary energy may have limited the efficiency of ammonia utilization with those rations. Results with diet 2 seem to indicate a high degree of urea-N recycling in the rumen. Dietary nitrogen was more efficiently utilized for microbial growth and nitrogen was probably the rate-limiting nutrient. Conclusions on the effects of protein concentration in this study are, however, limited because the effects of dietary protein source were not determined.

In a more recent passage study with abomasally cannulated steers, the rations (all silage and corn-corn silage) were variously supplemented with soybean meal (SBM) to crude protein levels of 11 to 16% (Crickenberger et al., 1979). Total nitrogen passage (as a percent of nitrogen intake) were: 79.2, 66.7, 88.4 and 67.8% for the 11.3, 12.8, 12.9 and 16.4% CP rations, respectively. NAN passage (percent of nitrogen intake) were: 70.6, 50.9, 77.5 and 57.5% for the same respective rations. There were considerable apparent ruminal nitrogen losses in all four treatments. This may be due to variations in abomasal spot sampling and poor marker (lignin) recoveries in that experiment. Microbial protein was not estimated.

Dietary Protein Source and NAN Passage

The effects of dietary nitrogen source on nitrogen passage were studied in a series of experiments with sheep fitted with rumen and omasal cannulae (Hume, 1970b). In this study, a semi-purified basal diet was supplemented with urea and higher VFA's (A), casein (B), gelatin (C) and zein (D). Urea supplied 50% of dietary nitrogen in diets B, C and D. Total crude protein averaged 14% in all rations. Total nitrogen flow to the omasum (percent of N-intake) were 89.9, 107.9, 100.0 and 139.7% for A, B, C and D, respectively. NAN passage (percent of N-intake) were 82.5, 100.6, 91.8 and 135.0 for diets A, B, C and D, respectively. Microbial nitrogen flow (percent of total omasal NAN) were 92.3, 91.0, 86.3 and 65.4 for the same respective dietary treatments. Feed nitrogen bypass and total amount of crude protein reaching the lower gut were markedly greater for zein diet than for the other nitrogen sources. There was a net gain in omasal nitrogen flow in the last three treatments. But some of the increase can be accounted for by the relatively high omasal NH_3-N levels (11 to 20 mg %) obtained. Most of the NH_3-N, however, would be absorbed before the digesta reaches the abomasum (Nolan, 1975).

In another experiment, Hume (1974), compared the effects of four protein sources (fish, lupin, peanut and soybean) on NAN and microbial protein passage in sheep. These protein supplements were added to high concentrate rations. ^{35}S label was used as the microbial marker and ^{51}Cr.EDTA for digesta flow estimation. There were no significant differences between treatments in the rates of digesta passage

to the duodenum. However, flow of NAN was greater for soybean meal (18.2 g/day) than for lupin meal and peanut meal treatments (14.5 and 16.2 g/day, respectively). NAN flow for the fish meal treatment was 20.6 g/day. There were net gains in dietary nitrogen intake at the duodenum with all four treatments. Abomasal nitrogen flow increased by 22.4% over the N-intake for peanut and SBM supplements. The lupin-meal fed sheep showed only a 5% increase in duodenal nitrogen over their N-intake levels, while the fish meal-fed animals gained 25%. Microbial contribution to duodenal NAN were the same (60%) for the lupin and peanut meals, 50% for soybean and 40% for the fish meal treatment. Dietary CP bypass was highest with the fish meal treatment (71%). Soybean meal fed animals had 61% protein bypass, lupin meal and peanut meal had only 35 and 33%, respectively.

In another passage study (Kropp et al., 1977a), steers fitted with rumen and abomasal cannulae were fed low quality roughages, supplemented with soybean meal or soybean meal plus urea (with urea replacing 0, 25, 50 and 75% of the soybean). All the rations were isocaloric and isonitrogenous. Microbial protein production was relatively constant regardless of nitrogen source. But, there were net nitrogen losses from the rumin with all the rations. Abomasal NAN decreased as urea partially replaced soybean meal. Feed-N bypass values were 35.7, 29.0, 30.0 and 24% of total nitrogen intake for 0, 25, 50 and 75% urea supplemented rations, respectively.

In a second study (Kropp et al., 1977b), the steers were fed cottonseed hulls supplemented with soybean meal or urea. Protein

levels were 11.1% (no urea), 8.5%, 10.8% and 12.6% for the four treatments, and the rations were isocaloric. There were no statistical differences in total abomasal nitrogen flow, but they reported net gains in nitrogen reaching the abomasum with all treatments. An estimated 20% of SBM-nitrogen escaped ruminal degradation. In both studies (Kropp et al., 1977a, 1977b) RNA was the microbial marker and lignin was used to determine digesta flow.

Smith et al. (1978) estimated the amount of microbial and dietary nitrogen entering the duodenum in steers fitted with rumen and duodenal cannulae. The rations consisted of: flaked maize and hay (A); flaked maize and straw--supplemented with decorticated groundnut meal (B); or fishmeal (C); or heated soybean meal (D); or raw soybean meal (E); or dried grass (F). All the rations contained 15 to 16% CP levels, except for rations A and F which had 8.0 and 18.0%, respectively. Duodenal NAN passage (percent of nitrogen intake) were 71.0, 85.5, 85.7 and 76.7 for rations B, C, D and E, respectively. Treatment A resulted in a net 5% gain in NAN output. Although there were no significant differences among the dietary treatments, decorticated groundnut meal tended to produce smaller amounts of microbial nitrogen output. Microbial protein passage was the same (54 and 56% of duodenal protein) for the groundnut meal and the fish meal, respectively. With heated soybean meal, microbial protein contributed 61% to the total duodenal protein. Raw soybean meal gave the highest level of microbial protein (70%). Feed protein bypass was similar for groundnut meal, fish meal and heated soybean meal (average 28%), and less than 18% of the raw soy protein bypassed the rumen.

All the above data suggest that dietary protein source and levels may markedly alter NAN passage and ruminal microbial protein output. The indication is that less soluble feed proteins more resistant to rumen degradation are more prone to enter the abomasum undergraded (or partially degraded). Microbial protein synthesis per unit of organic matter fermented in the rumen appears to be less affected by the protein source. Variations in this parameter probably reflect the extent of energy supply and rumen turnover (Theurer, 1979).

Forage to Concentrate Ratios and NAN Flow

Data on the effects of forage to concentrate ratio on NAN passage and microbial flow are rather limited. Forage rations are believed to have a higher fluid dilution rate than concentrates (Hungate, 1966). Addition of roughages to high concentrate ration elevates the turnover rate of the ruminal fluid and some solids, probably through increased saliva flow or reduced fluid space in the rumen (Owens and Isaacson, 1977).

The proportions of bacterial and dietary protein entering the duodenum of sheep fed a perennial ryegrass, or a short-rotation ryegrass or white clover were assessed in a study in which 2,6-diaminopimelic acid was used as the bacterial marker (Ulyatt *et al.*, 1975). About 70% of all the dietary protein in the fresh forages were degraded in the rumen. Bacterial contribution to duodenal NAN were: 43.1, 57.1 and 52.9% for the perennial rye, short-rotation ryegrass and white clover, respectively. Increased organic matter intake

increased duodenal NAN passage, even though there were extensive ruminal NH_3-N losses with all treatments.

Cole et al. (1976) fed four levels of cottonseed hulls (0, 7, 14, 21%) to abomasally cannulated steers on corn-based diets. Abomasal N passage was 80 and 77% of the dietary N-intake for the steers fed 0 and 7% cottonseed hulls, respectively. The steers that received 14 and 21% cottonseed hulls had net abomasal nitrogen gains of 18 and 7%, respectively. Microbial nitrogen flow was lowest with the all-concentrate ration, increasing with increase in the roughage content of the rations. Although an increase in the grain content of a roughage ration is known to depress the dry matter digestibility of the roughage component (associative effects of feeds), in contrast, increase in the roughage content of a concentrate ration alters rumen turnover rate, thereby increasing the rate of microbial growth (Owens and Isaacson, 1977).

Feed Processing and NAN Passage

The effect of physical and chemical alterations of forages and grains on rumen microbial protein synthesis have both been previously discussed in this review. Since both affect rumen turnover, they would also affect the composition of post-ruminal digesta. The studies of Coelho da Silva et al. (1972a) previously described, indicate that a larger percentage of the total protein reaching the duodenum was microbial in origin when the diets were given to sheep in chopped rather than in pelleted form (85% vs. 69%). Pelleting of forages seems to increase their rate of exit from the rumen, consequently increasing

the extent of feed protein bypass. Grinding and chopping of forages also increase their rates of passage (Thomson, 1972). In the latter situation, however, the rates of organic matter and feed protein degradation in the rumen would also be increased, in contrast with pelleted forages. Similar results were obtained with sheep fed chopped, wafered or pelleted lucerne by the same workers (Coelho da Silva et al., 1972b). In that study, feed N bypass (as % of duodenal N) was 68% for pelleted lucerne, compared to 54% for the chopped or wafered lucerne.

Current thinking is that grinding and pelleting both reduce apparent rumen digestion (due to increased rate of digesta passage) and metabolizable energy (ME) content of the forage, although the ME is utilized more efficiently for growth and fat deposition in pelleted feeds than in chopped ones (Thomson, 1972), when the organic matter in pelleted forages is post-ruminally digestible. As a result, the net energy (NE) of pelleted forages may be as high as that of chopped ones, or higher, as with legumes, when grinding and pelleting cause only a small reduction in digestibility (Thomson, 1972).

Concentrates and cereal grains are often processed by such methods as steam-flaking, dry-rolling, grinding and acid treatment, to enhance their utilization by the ruminant. Prigge et al. (1978) evaluated the effects of four methods of processing corn with ruminally and abomasally cannulated steers fed 78% corn rations. The processing methods included, dry-rolling (DR), steam-flaking (SF), propionic acid treatment and ensiling of high moisture whole shelled corn (AHM) and grinding and ensiling of high moisture shelled corn (GHM). The rations

contained an average of 11 to 12% CP. Microbial protein represented a greater percentage of the abomasal-N for GHM than for AHM (42% vs. 35%), and was similar for DR and SF treatments (38% vs. 40.9%). Total-N passage was greater for acid-treated whole corn than for all the others. There were net gains in abomasal-N only with acid treatment and steam flaking (24 and 14%, respectively).

The indication is that grain processing (by acid treatment and steam flaking) increase organic matter digestion in the rumen, and improve the efficiency of utilization over grinding and dry rolling of grains. The above workers (Prigge et al., 1978) showed that rumen turnover and efficiency of microbial protein synthesis were greatly improved by acid treatment of whole shelled high moisture corn.

Influence of Microbial and Digesta Markers on Passage Studies

Digesta passage studies often depend on the use of techniques or markers to monitor digesta flow and microbial contribution to post-ruminal nitrogen. Microbial markers used include such components of microbial cells as DAP (in bacteria) and nucleic acids. Digesta markers may also be a normal component of the feed (e.g., lignin, indigestible-ADF), or they may be external, relatively indigestible material incorporated into the feed. The latter group includes soluble markers such as polyethylene glycol (PEG) and Cr-ETDA, and solid-particulate markers which include such compounds as chromic oxide (Cr_2O_3), ruthenium (Ru), Lanthanium (La) and Ytterbium (Yb). The last three are some of the series of rare-earth elements that are recently being used in digesta passage studies.

Soluble and particulate markers are commonly applied simultaneously to estimate fluid and dry matter flow, respectively. Such an approach is often necessary because the liquid and particulate digesta pools exhibit differential flow kinetics (Hungate, 1966), so that markers that preferentially equilibrate with specific pools may, in combination, provide increased accuracy in the estimation of total flow from a reconstituted digesta (Faichney, 1975). Ideally, a marker should meet the following criteria in order to be useful as an accurate index of the particular component of digesta it is supposed to estimate (Faichney, 1975):

1. It must be strictly nonabsorbable;
2. It must not affect, or be affected by, the G.I. tract or its microbial population;
3. It must be physically similar to, or intimately associated with the material it is to mark; and
4. Its method of estimation in digesta samples must be specific and sensitive, and it must not interfere with other analyses.

The most commonly used non-radioactive digesta markers such as polyethylene glycol (PEG), Cr_2O_3 and lignin do not meet all the above criteria.

Fluid Markers

Water soluble markers such as PEG, Cr-EDTA and Co-EDTA are most often used to estimate fluid or water turnover in the rumen. However, neither one of them fully meets those prerequisites of an ideal marker. Hyden (1955) reported that PEG equilibrates with about 95% of rumen

water. But other workers (Czerkawski and Breckenridge, 1969; Malawer and Powell, 1967) have questioned the validity of PEG as a liquid flow marker. Even though its laboratory analysis has undergone several modifications through the years (Malawer and Powell, 1967; Ingham and Ling, 1978), the problems of PEG's association with dry matter and solid digesta flow still remain. It was reported that some PEG is absorbed from the rumen (Downes and McDonald, 1964). Dobson et al. (1976) found that PEG absorption was affected by the osmotic pressure in the rumen. Others (Czerkawski and Breckenridge, 1969) demonstrated that PEG was unevenly distributed when added to suspensions of food in aqueous buffer solutions in which PEG was reported to associate with feed residues.

CrEDTA, on the other hand, seems to occupy a larger fluid volume than PEG (99%), even though it is equally absorbed from the rumen (MacRae, 1975). This marker (in the labelled form) was also reported to associate to a variable extent with abomasal digesta. The consequence of such aberrations in the flow of these three markers is that liquid flow from the rumen is usually overestimated, resulting in considerably high rumen dilution rates. Fractional rates of rumen liquid outflow, when studied with the three markers, is highest with PEG, and lowest with CrEDTA. CoEDTA is intermediate.

Compounding the above problems are the reliability and reproducibility of Hyden's turbidimetric assay for PEG. PEG analysis, and most of its modifications, are based on the development of an oil-in-water emulsion of PEG when exposed to trichloroacetic acid, in the

presence of barium ions. Several factors such as temperature, pH, presence of interfering ions and proteins, vibration, method of mixing and other factors that affect colloidal solutions in general, determine the amount and persistence of the turbidity obtained when the final reagent is added (Ingham and Ling, 1978; Malawer and Powell, 1967). Co-EDTA, on the other hand, can be analyzed with a fairly high degree of accuracy.

Particulate Flow Markers

Lignin and chromic oxide seem to be the two particulate markers preferentially used in a lot of passage studies. This is perhaps due to the fact that lignin is a typical constituent of plant cell walls and Cr_2O_3 is cheap and readily available, even though both have questionable values as digesta flow markers.

Lignin passage is usually related to digesta components that have the slowest turnover--that is, if lignin is part of that component (Bull et al., 1979). The results of some studies indicate that lignin is up to 10% digestible, most of the degradation occurring in the rumen (MacRae, 1975). Another view is that lignin undergoes a partial modification in the rumen resulting in less post-ruminal recovery of feed lignin. There is nonetheless, a tendency for rumen outflow estimates based on lignin passage to be less than 100% of the total lignin intake, and this results in an overestimation of protein flows. A partial explanation of such discrepancies may rest with the lack of one reliable and accepted laboratory technique for lignin analysis. Acid lignin method gives lignin values often lower than those

obtained from the $KMnO_4$ procedure. There is concern that the acid detergent reagent, through its strong peptising ability, dissolves some lignin (Van Soest, 1975). The extent to which this occurs may vary between samples. NAN passage studies based on acid lignin/N ratios could therefore show an inconsistent or variable ruminal nitrogen losses (Crickenberger et al., 1979).

$KMnO_4$ lignin approach also has its problems. A comparative evaluation of lignin content of digesta samples obtained from different segments of the gastrointestinal tract (GIT), when analyzed by this technique may give impossible results. Rumen and abomasal samples have to be exposed to $MKnO_4$ for 1½ hours, while fecal samples require up to 5 hours. Fecal samples may also have to remain longer than 30 minutes in the demineralizing solution before filtration becomes possible, and this strongly acidic solution does dissolve cellulose. The point here is that $KMnO_4$ technique is also non-specific, and the above situation could overestimate the extent of lignin flow when residual digesta components such as cellulose are dissolved during $KMnO_4$ lignin determination. The net effect, in passage studies, would be an underestimation of digesta flow rate, NAN passage and ruminal dry matter digestion. Lignin would not become fully accepted as a digesta passage marker until its fate in the GIT is better understood, and a more reliable method for its laboratory estimation developed.

Chromic oxide is also regarded as a poor passage marker in some circles because its physical properties do not correspond to those of the solid-particulate fraction of digesta (Faichney, 1974). Various

alterations in the physical form of this marker reported in several studies (e.g., impregnation in paper, baking and coarse grinding of Cr_2O_3-flour mix) were simply aimed at simulating the particle size and specific gravity of the particulate digesta pool, in order to obtain a more uniform Cr_2O_3 outflow from the rumen. The independent movement of Cr_2O_3 may not present a great problem when continuous collection techniques are used, with duodenal re-entrant cannulae (MacRae, 1975). Recoveries of this marker in various studies range from 56 to 100% (MacRae, 1975; Sutton et al., 1976). This has necessitated the use of fecal Cr_2O_3 to obtain a corrected or adjusted chromium flow, especially when short-term spot sampling and 24 hr. re-entrant collections are obtained (MacRae, 1975). Depressions in dry matter flow as high as 20 to 30% due to duodenal collection procedures have been reported with fresh forages when Cr_2O_3 was used (Ulyatt and MacRae, 1974). With abomasal T-shaped cannulas, the use of Cr_2O_3 invites the problem of obtaining a sample truly representative of the digesta passing the point of cannulation. The adequacy of longer-term samplings in correcting this problem is not fully known. Rumen outflow values obtained with Cr_2O_3 data often exceed 100% and has resulted in abomasal-N outflows that are greater than the amount ingested. Lignin-based data, on the other hand, underestimates nitrogen passage (Theurer, 1979).

The current approach to passage studies is to use a dual marker (liquid and particulate types), each of which should be closely associated with the phase of digesta it is supposed to estimate. In this respect, liquid-phase markers such as CoEDTA and solid-phase ones such

as cerium or chromium-mordanted plant cell walls may show some promise. Rare-earth elements previously mentioned are excellent particulate and total digesta flow markers when applied with all the metal absorbed to feed materials. If excess metals are not washed away, these elements may show some movement between different digesta pools. Their recoveries with the particulate matter of forage-fed animals were reported to be higher than with grain fed ones (Hartnell and Satter, 1979).

Microbial Markers

Various methods for measuring the rate of ruminal microbial protein output generally depend on calculations based on values for the concentration of a particular microbial component in rumen or post-ruminal digesta, and the ratio of this component to microbial-N. In digesta passage studies, it is also essential to determine the microbial contribution to nitrogen flow, if the extent of ruminal feed protein degradation is to be assessed.

Microbial markers used have included DAP, RNA, DNA and radioisotope labelled compounds such as ^{35}S (labels sulfur amino acids in microbial cells), ^{32}P label (for RNA's and microbial phospholipids) and ^{15}N (labels nitrogen components in microbial crude protein). All the above methods depend on sampling of rumen and/or post-ruminal digesta at specified times. Microbial markers also suffer from the same limitations as digesta markers, since their effectiveness depends on obtaining digesta samples that represent the true proportions of the various components within that segment of the digestive tract. This may lead to significant errors in the quantitative estimates obtained.

It has been reported that different strains and species of bacteria show a considerable variation in their DAP content (Purser and Buechler, 1966). DAP-N/Total-N ratios in mixed rumen bacteria so far reported range from 0.5 to 1.15 (Smith, 1975). Another problem with the use of DAP is that this technique fails to account for the contribution of protozoa to NAN passage, due to the absence of that amino acid in protozoa. This would lead to an underestimation of the microbial component of ruminal outflow, regardless of the extent of protozoal passage from the rumen.

Nucleic acids, particularly RNA, is almost widely accepted as a better index of microbial passage. DNA reflects the number of microorganisms present, whereas RNA is associated more closely with protein synthesis (El-Shazly and Abou Akkada, 1972). It is therefore believed that total nucleic acid-N and the total microbial-N are proportional. However, there is a greater variability in DNA-N/Microbial-N ratios than in RNA-N/Microbial-N ratios (McAllan and Smith, 1971). Therefore, RNA is more generally used as a microbial marker in passage studies. The use of RNA is also based on the assumption that dietary nucleic acids are rapidly degraded in the rumen (McAllan and Smith, 1969; Smith and McAllan, 1970), so that all the nucleic acid present in the post-ruminal digesta should be of microbial origin. Smith et al. (1978) compared microbial protein estimates based on RNA-N of duodenal digesta in steers, some of which received doses of ^{32}P-labelled PO_4. They reported that estimates based on ^{32}P-labelled RNA were on the average 85% of those based on total RNA. They attributed these

differences to the presence of small amounts of dietary RNA in the duodenum. This questions the basic assumption that all the dietary RNA's are degraded in the rumen.

Another major problem with the use of RNA as the microbial marker in passage studies lies in the laboratory methods for its estimation. Alkali digestion of post-ruminal digesta as outlined by McAllan and Smith (1969) is nonspecific for RNA and there is evidence of contamination of separated ribonucleotides (in solution) by plant lipids, pentose polymers and pigments most of which are not removed by extraction in organic solvents prior to alkali hydrolysis. Further purification of the RNA extracts by ion-exchange could still leave remnants of peptides, sugars, proteins and other interfering materials which absorb in the U.V. range of 260 nm, and also react with orcinol reagent (Munro and Fleck, 1966).

The use of labelled markers such as ^{35}S and ^{15}N (urea) have their own problems. Besides the common problem of obtaining samples representative of the microbial population in that part of the GIT, other concerns include, the ability to establish a steady state of the labelled compounds within the rumen and corrections for extensive urea, and some sulfur recycling occurring within the rumen (Nolan, 1975; Hume and Bird, 1970; Bird, 1974). If pool sizes are changing, the appearance of labelled metabolites may show a particular metabolic pathway but may not necessarily allow it to be quantified, especially when recycling process is involved (Bray and Till, 1975). Other considerations with all microbial markers are the effects of diurnal variations on the composition of rumen microbes, since marker

concentration is usually determined in relation to the microbial cell mass (McAllan and Smith, 1977).

Monensin

Monensin is a polyether antibiotic produced in fermentation broths by a strain of Streptomyces cinnamonensis. It is known to inhibit gram-positive microorganisms (Haney and Hoehn, 1967), and since its discovery, has been used as an antibacterial agent effective in controlling coccidiosis in poultry (Shumard and Callender, 1967).

This compound was discovered to cause a shift in vitro in the molar proportions of VFA's in favor of propionate, without affecting total VFA production (Richardson et al., 1974; Richardson et al., 1976). Subsequent in vivo studies with rumen-fistulated steers confirmed the above monensin effect. In feeding trials, it was reported that monensin also improved the performance of beef cattle fed different rations (Raun et al., 1976; Potter et al., 1976a, 1976b).

After considerable investigations into the safety and efficacy of this antibiotic, it was approved by the Food and Drug Administration in 1975 for use as a feed additive in feedlot rations.

Monensin and VFA Production

Richardson et al. (1974) conducted in vitro experiments to characterize the effects of monensin on ruminal fermentation. They reported that addition of 1.0 ppm of monensin increased propionate production by 45%, without concomitant changes in total VFA levels. This response was obtained with rumen fluid from high grain-fed

cattle or sheep when incubated with high grain feed as the substrate. A similar response was also obtained by the same workers using rumen fluid from pasture fed cattle, and high roughage feed as substrate.

The same workers, designed another series of *in vitro* studies (Richardson *et al.*, 1976) to determine the optimum dose--response level, of monensin. Monensin was added at 0.1, 0.25, 0.5, 1.0, 5.0 and 25.0 ppm to concentrate--or high roughage--incubation systems, containing rumen innocula from cattle fed the same respective rations. With concentrates, monensin reduced acetic, isovaleric and valeric acid production at $\geq$ 1 ppm. They reported an average of 50% increase in propionic acid production at 1.0 ppm monensin. With high roughage substrates, acetate production was reduced at 5 ppm monensin, butyrate at 1 and 5 ppm, while propionate levels increased at 1 and 5 ppm. Total VFA (TVFA) production remained unchanged, indicating that equal quantities of substrates were fermented in comparison with the controls.

A preliminary *in vivo* study was also conducted by the above workers (Richardson *et al.*, 1974) in which monensin was fed at 25 to 500 mg per day for 3 weeks to rumen fistulated cattle. They reported a 52% increase in ruminal propionic acid when 200 mg/day monensin was fed. This increase persisted throughout the trial. In five other *in vivo* dose-response studies (Richardson *et al.*, 1976), ruminally-cannulated steers were fed concentrate rations with monensin given at 25, 50, 100, 200 and 500 mg/head/day. The results obtained were similar to those found *in vitro*, but the effects on TVFA were not consistent. Acetate decreased at dosages $\geq$ 100 mg/head/day, while

propionate increased , and butyrate decreased at each dosage. These shifts in VFA patterns were shown to persist throughout the 148-day trial. Other studies (Dinius et al., 1976; Perry et al., 1976) have confirmed this shift in the molar proportions of VFA towards propionic acid in monensin-fed ruminants.

On the basis of this redistribution of VFA's associated with the addition of monensin to the ration, Richardson et al. (1976) calculated a theoretical energy savings to the animal of 5.6% (assuming a 10 moles per 100 moles increase in ruminal propionate). They stated that fermentation efficiency was improved by monensin treatment as a result of increased recovery of metabolic hydrogen in the rumen, in the form of VFA's. Raun et al. (1976), however, observed that even though propionate fermentation is energetically more efficient than acetic and butyric acid fermentations (Hungate, 1966; Wolin, 1960), it did not account for all the improvement in performance they obtained in feed lot studies. They, therefore, suggested that increased propionate may also:

1. lower heat increment (Smith, 1971);
2. spare amino acids normally used in gluconeogenesis (Reilly and Ford, 1971; Leng et al., 1967); and
3. stimulate body protein synthesis (Eskeland et al., 1974).

Feedlot Studies

Since the recognition of the effects of monensin on beef cattle performance, several other studies have been conducted to determine its optimum dose-response level and the performance of animals fed this antibiotic, under practical feedlot conditions.

Raun et al. (1976) fed monensin at levels ranging from 0 to 88 ppm to steers on high energy rations and reported average daily gain (ADG) equal to or greater than the controls (except for the 88 ppm level). Feed intake progressively declined with increase of monensin in the rations, while the efficiencies of feed conversion improved. The result of these studies (Raun et al., 1976) indicate that 33 ppm was the optimum level for feeding monensin to cattle. At this level, intake declined by 13.1%, while feed efficiency (F/G) improved by 17%.

In another study, Potter et al. (1976a) fed monensin at 0 to 400 mg/head/day to beef cattle on pasture and green chop. They reported a 17% improvement in gain and a 20% improved feed efficiency when monensin was fed at 200 mg/head/day. Improvements in performance were obtained at 100 to 300 mg monensin per animal per day. Further studies in which carcass composition was examined, Potter et al. (1976b) showed that monensin had minimal effects on dressing percent and other carcass characteristics.

The results of 29 university feedlot studies (growing, finishing and growing-finishing cattle performance studies) summarized by Goodrich et al. (1976) showed that monensin at 50 and 100 mg/head/day improved ADG; the other levels (200 to 400 mg/head/day) were not different from the controls. Dry matter intake and F/G also declined with increasing levels of monensin. Carcass composition was not significantly altered by feeding monensin at 200 or 300 mg/head/day.

The reduction in dry matter intake observed with monensin in high energy rations prompted speculations that dietary protein intake may need to be adjusted when this additive is fed to growing ruminants. Several studies were therefore conducted to determine the effect of ration protein levels and sources on the performance of cattle fed monensin (Dartt et al., 1976; Davis and Erhart, 1976; Hanson and Klopfenstein, 1979; Gates and Embry, 1977). Dartt et al. (1976) reported that withdrawal of supplemental protein from the rations of growing steers resulted in a significant reduction in ADG (17.1%) and a 12.9% poorer feed efficiency for those steers not given monensin. In comparison, monensin feeding during protein withdrawal improved F/G by 11.9% and reduced ADG by only 6.1%.

In a study with urea-supplemented rations, Davis and Erhart (1976) obtained improvements in F/G when monensin was fed to growing steers. In contrast, Hanson and Klopfenstein (1979) reported a reduction in the rate of gain and poorer feed efficiency with the addition of monensin to urea-supplemented corn and corn-silage rations. Monensin and brewers grain supplementation to the same basal rations resulted in significant improvements in feed efficiency (Hanson and Klopfenstein, 1979). The latter studies also showed that the largest responses to monensin occurred at lower dietary crude protein levels (10.5 vs. 12.5% CP), thus indicating that monensin may spare protein. The above protein studies (Dartt et al., 1976; Davis and Erhart, 1976; Hanson and Klopfenstein, 1979) all indicate that monensin is effective with different dietary protein sources and that adjustments in protein levels may not be necessary when monensin is fed.

Monensin and Rumen Microbial Growth

Effects on Microbial Population and Growth

Initial characterization studies with monensin revealed that this drug inhibits the growth of several gram-positive micro-organisms (Haney and Hoehn, 1967). Subsequent experiments were therefore conducted by various workers to determine the effect of the antibiotic on rumen microbial population and growth (Dinius et al., 1976; Van Nevel and Demeyer, 1977; Chen and Wolin, 1979).

Dinius et al. (1976) in continuous culture fermentation studies observed small decreases in methane production with monensin in the media. This decline was transient, however, methane production returning to normal levels with prolonged monensin treatment. A similar temporary decline was also observed in vivo by Thornton et al. (1976).

Other workers (Van Nevel and Demeyer, 1977) also showed in vitro that monensin has no direct toxic effects on methanogenic rumen bacteria. Monensin did not inhibit methane formation from H_2 and CO_2 by washed-cell suspensions of mixed rumen bacteria, but did inhibit formate decomposition during carbohydrate fermentation. These workers (Van Nevel and Demeyer, 1977) reasoned that since only about 18% of rumen methane is derived from formate (Hungate et al., 1970), this may explain the less dramatic inhibition of methane production found in their studies.

In contrast, Chen and Wolin (1979) found that the sensitivity of methanogenic bacteria to monensin varies with the specific organism. However, none of the methanogens they examined was completely inhibited.

In most cases, the antibiotic appeared to cause only a delayed growth response. The studies of Chen and Wolin (1979) were designed to examine the effects of monensin on the growth of methanogenic and rumen saccharolytic bacteria in a complex medium containing rumen fluid. Bacteroides succinogenes and B. ruminicola, both important succinate producers, were found to be less sensitive to monensin. Selenomonas ruminantium was also resistant to the drug. B. succinogenes and S. ruminantium are both cellulolytics, while B. ruminicola uses starch and other carbohydrates. Other important cellulolytics (Ruminococci and B. fibrisolvens) were found to be highly sensitive to monensin.

This inhibitory effect on cellulolytic rumen bacteria was confirmed by Simpson (1978) who found that monensin strongly inhibited cellulolytic activity in vitro, when inoculum from animals not previously exposed to the antibiotic was used. He obtained this response after a 48-hour incubation, and the effects of a long-term exposure to monensin were not examined in the study. On the other hand, Dinius et al. (1976) reported from a series of in vitro and in vivo experiments that there were no reductions in cellulose, hemicellulose and dry matter digestibilities when the animals (or inocula from adapted animals) were exposed to monensin for 21 days. Neither the numbers of protozoa, total bacteria nor cellulolytic bacterial numbers were affected by up to 33 ppm dietary monensin.

Chen and Wolin (1979) hypothesized from their studies, and from others (Dinius et al., 1976) that monensin acts by selecting a rumen microbial community that produces proportionally more propionate.

Monensin and Efficiency of Microbial Growth

Based on the results of *in vitro* studies conducted by Richardson *et al.* (1976), it was demonstrated that monensin increased propionate production without influencing either the amount of hexose fermented, microbial cell yield and efficiency, or the production of metabolic hydrogen (Chalupa, 1977). Other workers, however, reported significant reductions in microbial efficiency by monensin *in vitro* (Van Nevel and Demeyer, 1977). In the latter study, both the total and net microbial growth (expressed as milligrams of N incorporated per 100 μ mole of hexose fermented) were severely inhibited by monensin. Other workers, as previously discussed (Chen and Wolin, 1979) have reported varying degrees of selective inhibition of different rumen microbial species by monensin *in vitro*.

In vivo data on the efficiency of ruminal microbial growth are, however, scarce. Poos *et al.* (1979), in protein passage studies, reported significant decreases in bacterial-N flow to the abomasum with monensin in the diet. Ruminal organic matter digestion was, however, not determined in that study, so that microbial efficiencies could not be accurately estimated. More *in vivo* studies are needed to determine the effect of this compound on the efficiency of rumen microbial protein synthesis.

Monensin and NAN Passage

In addition to the transient reductions in cellulose and D.M. digestibilities observed when monensin is added to ruminant diets, the results of some studies indicate that rumen proteolysis and deamination may also be inhibited. Reductions in rumen ammonia levels have frequently been reported with monensin (Dinius et al., 1976; Van Nevel and Demeyer, 1977; Hanson and Klopfenstein, 1979), an observation that led to earlier suggestions that the antibiotic may act in the rumen as a deaminase inhibitor.

Van Nevel and Demeyer (1977) observed a considerable decrease in casein degradation when monensin was added to incubations of mixed rumen bacteria. Addition of energy substrates (cellebiose and maltose) to the media failed to elevate the rate of casein protein breakdown. They also reported a lowered NH_3-N level and increased accumulation of α-amino nitrogen, which indicate that deaminase activity, rather than proteolysis may be more inhibited by monensin.

The effect of monensin on in vivo protein degradation has also been examined in a few studies (Poos et al., 1979; Lemenager et al., 1979). Poos et al. (1979) reported 37% and 55% increases in plant-N bypass when monensin was added to steer rations supplemented with brewers' dried grains (BDG) and urea, respectively. Owens et al. (1979) reported a 22% feed protein bypass when steers fed a rolled corn--16% CP ration were given monensin. The two studies thus indicate that monensin reduces the extent of feed protein degradation in the rumen.

Increases in the amount of NAN reaching the abomasum were also reported in the same two studies. Monensin-fed steers showed increases in total-N and NAN flow to the lower gut over their dietary nitrogen intakes. Poos et al. (1979), however, reported increases in abomasal amino acid flow only with natural protein supplements. Both the essential and non-essential amino acids reaching the small intestines were increased by the combination of BDG and monensin over the controls (164 vs. 150 gm and 194 vs. 174 g, respectively). Abomasal amino acid patterns and concentrations should reflect the level of NAN passage to the lower gastro-intestinal tract (GIT).

Poos et al. (1979) also reported significant decreases in bacterial-N flow to the abomasum by the addition of monensin to steer rations. In comparison with the control treatments, monensin decreased bacterial-N passage by 32% for the natural protein, and 33% for the urea supplement. Other workers (Dinius et al., 1976; Owens et al., 1979) found no decreases in bacterial numbers or in the amount of bacterial-N reaching the abomasum of monensin-fed steers.

It seems well-established that monensin reduces the extent of feed protein degradation in the rumen. The effect of monensin on rumen turnover, however, still remain to be conclusively determined. Even though Owens et al. (1979) reported a 10% reduction in ruminal DM digestion with monensin in concentrate rations, they also obtained a reduced (24%) rumen liquid turnover rate with monensin treatment. Another group (Lemenager et al., 1978) reported a 30.8% slower rumen liquid turnover rate and a 43.6% slower turnover of rumen solids in

steers fed low quality dry winter range grass, supplemented with 30% SBM and monensin. They had, however, a 19.6% reduction in feed intake and also a large decrease in estimated rumen liquid volume for the monensin treatment. In a second trial in which a high concentrate ration was limit-fed to steers (Lemenager et al., 1978) monensin still decreased rumen liquid dilution rate.

In contrast, Poos et al. (1979) obtained significant increases in both the DM and liquid flow to the abomasum per day, with monensin in both the natural protein--and urea-supplemented rations. This indicates a faster rumen turnover, unless there were differences in rumen volumes between treatments.

Monensin--Mode of Action

Despite the preponderance of information so far collected on the effects of monensin in the metabolism of the ruminant, its mechanism of action still remains to be elucidated. The energetic benefits of a shift in the molar ratios of VFA's towards propionate and, therefore, the increased recovery of metabolic hydrogen in propionate coupled with subsequent improvement in the animal's amino acid utilization, offer only partial explanations for the drug's efficacy. The advantages of monensin's reduction of ruminal protein degradation rate can be more fully exploited if the ruminant is given more high quality plant proteins better utilized in the post-ruminal tract. However, decreases in the efficiency of rumen microbial protein synthesis and Y_{ATP} (Bergen, 1979) could be a disadvantage to the animal, depending on the nature of the diet and the amount of protein escaping rumen degradation (Van Nevel and Demeyer, 1977).

Reductions in rumen ammonia levels and the overall rate of protein degradation with monensin, suggest that rumen deaminase activity, proteolysis and perhaps, peptide transport into bacterial cells may be some physiological functions inhibited. And since the inhibition of microbial growth by monensin does not affect the rate of energy substrate catabolism (Van Nevel and Demeyer, 1977), it seems plausible to suggest that monensin may uncouple growth from fermentation. Being an ionophore, monensin could cause an alteration in cell membrane permeability, thus affecting the rate of substrate transport into microbial cells.

Monensin is a strong lipophilic cation-binding ionophore of the acid polyetherin family (Anteunis and Rodios, 1978). Other polyether antibiotics or ionophores such as lasalocid exert similar effects on rumen microbial fermentation as monensin (Bartley et al., 1979; Chen and Wolin, 1979).

CHAPTER III

MATERIALS AND METHODS

Nitrogen Metabolism Studies

General Design

Four Hereford steers fitted with abomasal cannulae and weighing approximately 287.5 kg each, were used in a switch-back metabolism study to determine the effects of monensin in high concentrate and high silage rations on digesta and nitrogen passage to the lower gastro-intestinal tract. During each period, two steers were fed one type of ration, the other pair receiving a similar ration with or without monensin (see Table 2). The rations were mixed and fed twice a day ad libitum. Feed intake and unconsumed portions were recorded, subsamples frozen for subsequent analyses. The animals were individually housed in 91 cm x 244 cm collection pens in an environmentally controlled metabolism room. They had free access to water.

Chromic oxide and feed lignin were used as particulate flow markers, and polyethylene glycol (PEG; MW 4000) used to quantitate the liquid digesta flow. Chromic oxide was mixed with wheat flour (1:4 ratio) and water added to form a paste which was baked at 100°C until dry. This baked mixture was then ground coarse in a Wiley mill and added to each ration at a level of 1% of ration dry matter (Ørskov

TABLE 2. COMPOSITION OF DIETS USED IN DIGESTA PASSAGE AND NITROGEN METABOLISM STUDIES

Ingredient	International reference no.	1	2	3	4
		% of DM			
Corn, aerial pt, w. ears, ensiled, mature, well-eared, mx 50% mn, 30% dry matter	3-08-153	--	--	85.00	85.00
Corn, dent, yellow, grain gr 2 US	4-02-931	58.00	58.00	--	--
Soybean, seeds, meal solv-extd	5-04-604	4.00	4.00	--	--
Soybean meal-mineral supplement[a]		--	--	13.50	13.50
Alfalfa, aerial part dehy meal, mn 17% protein	1-00-023	20.00	20.00	--	--
Oats, grain	4-03-309	10.00	10.00	--	--
Sugarcane, molasses	4-04-696	4.00	4.00	--	--
Phosphate defluorinated, grnd	6-01-780	0.48	0.48	--	--
Trace mineral salt		2.00	2.00	--	--
Chromic oxide-four mix		1.00	1.00	1.00	1.00
Polyethylene glycol (MW4000)		0.50	0.50	0.50	0.50
Rumensin premix[b]		--	--	--	--
Vitamin A premix[c]		0.01	0.01	--	--
Vitamin D premix[d]		0.01	0.01	--	--

[a]Composition listed in Table 3 supplements 1 and 2 used for diets 3 and 4, respectively.

[b]Diets 2 and 4 contained 30 g Rumensin per 907 kg air-dry feed.

[c]30,000 IU vitamin A per g.

[d]3,000 IU vitamin D_3 per g.

et al., 1971). PEG was mixed with the rations at 0.5% of ration dry matter.

Total digesta flow to the abomasum and nitrogen balance were estimated during each period. The steers were allowed an 18-day period to adapt to each ration, followed by nitrogen balance collections for 8 days. After a two-day rest, abomasal samples were collected three times a day (at six-hour intervals) for a period of four days.

Abomasal Cannula Design

A brass mold obtained from Dr. L. D. Satter of the University of Wisconsin was used to make the cannulae. Clear plastisol (manufactured by Norton's Plastic and Synthetics Division, Akron, Ohio) was placed under vacuum for at least one hour to remove air bubbles. It was then poured into the preheated mold and allowed to solidify in the oven at 190°C for about 20 minutes. The mold was then cooled by immersing it in cold water for several minutes, after which the cast was removed. Properly prepared cannulae should be pliable, transparent, amber-colored and free of air bubbles. In a one-stage surgical procedure, the cannulae were inserted through an abomasal incision, and retained by purse-string sutures.

Rumen Microbial Studies

Two Hereford steers fitted with rumen fistulae were adapted for 14 days in sequential order to each of the four rations used in the digesta passage studies (Table 2). The steers were also housed in individual stalls in the same metabolism room. They were fed twice

TABLE 3. COMPOSITION OF SOYBEAN MEAL-MINERAL SUPPLEMENTS USED IN DIGESTA PASSAGE STUDIES AND FEEDING TRIAL

Ingredient	Supplement No. 1	2	3	4
	% of DM			
Soybean meal (48% CP)	92.70	92.70	92.70	92.70
Defluorinated phosphate	3.00	3.00	3.00	3.00
Ground limestone	2.00	2.00	2.00	2.00
Trace mineral salt	2.00	2.00	2.00	2.00
Vitamin A premix[a]	0.15	0.15	0.15	0.15
Vitamin D premix[b]	0.15	0.15	0.15	0.15
Monensin (mg/454 g)[c]	--	125.00	--	125.00
Elfazepam (mg/454 gm)[d]	--	--	8.35	8.35
Analysis of Supplement:				
Crude protein (%)	49.40	49.40	49.40	49.40
Calcium (%)	2.00	2.00	2.00	2.00
Phosphorus (%)	1.20	1.20	1.20	1.20
Trace mineral salt	2.00	2.00	2.00	2.00

[a]30,000 IU vitamin A per g.

[b]3,000 IU vitamin D_3 per g.

[c]Added to provide 33 ppm monensin in total diet.

[d]Added to provide 2 ppm elfazepam in total diet.

TABLE 4. CHEMICAL COMPOSITION OF RATIONS[a] USED IN DIGESTA PASSAGE STUDIES

Ingredient	Ration no. 1	2	3	4
	% of DM			
Dry matter	93.10	93.15	35.53	35.73
Crude protein	13.10	13.25	13.26	13.20
Neutral detergent fiber	22.50	21.82	44.21	44.15
Acid detergent fiber	10.32	10.04	24.62	24.86
Permanganate lignin	3.09	3.09	3.93	3.93
Organic matter	93.31	93.04	94.38	94.39
Ash	6.70	6.97	5.62	5.62
Polyethylene glycol	0.488	0.544	0.443	0.487
Chromium (mg/g DM)	1.981	2.025	1.781	1.726

[a]Actual laboratory determinations of feed samples, each from two abomasal collection periods.

a day ad libitum and had free access to water. Following each adaptation period, rumen digesta samples were collected from each steer at specified times and quickly transported to the laboratory for processing.

Collection and Preparation of Samples

Nitrogen Balance Study

Following adaptation to each ration, total urine was collected from each steer into large plastic carboys containing 200 ml of 18N sulfuric acid. The acid was added to prevent the liberation of ammonia and other nitrogenous compounds. Carboys were emptied at least every two days, the urine volume measured and diluted to 10 liters with water. After mixing, a 10% aliquot was stored in the cooler. At the end of each collection period, the urine samples were pooled, thoroughly mixed and a 500 ml subsample frozen for later analysis.

Feces were collected in a plastic-lined steel trough placed behind each steer. The feces were weighed, properly mixed and several subsamples taken from different points in the mixture. Ten percent of each collection was saved. At the end of the period, subsamples from each steer were composited and a 10% aliquot was kept frozen for subsequent analysis.

Several grab samples were taken from each ration as the mixed feed was being discharged from the mixer. Samples for each steer were kept frozen until the end of the collection period. They were then thawed, composited and chopped in a Hobart feed grinder. Sufficient subsamples (approximately 1 kg) were frozen to be analyzed later.

Digesta Passage Studies

After each nitrogen balance, abomasal samples were collected from each of the four steers. Samples were collected three times a day at six hour intervals for four consecutive days. The sampling frequency was designed to give a net effect of a three-hour collection interval, if the four days were condensed into one (see Figure 1). Abomasal contents were agitated with air from an air-pump and approximately 150 ml digesta were collected into a plastic beaker. Attempts were made to keep in sequence with the wave of contraction during abomasal emptying. Individual samples were kept frozen in plastic bottles until the end of each period of collection. The samples were subsequently thawed, mixed and 50 ml aliquot from each steer homogenized and composited. Approximately 200 ml from each composite were freeze-dried and stored in a dessicator. Both the dried and the wet abomasal samples were later analyzed.

Rumen Microbial Studies

Rumen contents were collected from the two ruminally-cannulated steers which were fed the same rations used in the passage studies. The contents of the rumen were mixed by inserting an arm through the cannula prior to collection. Four hundred milliliter rumen contents were taken at 2, 4, 6 and 8 hours after the morning feeding. The samples were collected in vacuum bottles and quickly taken to the laboratory for processing. Rumen liquor was obtained by squeezing the samples through two layers of cheese cloth. The solid residues were resuspended in 0.9% NaCl, and strained again. The liquors were

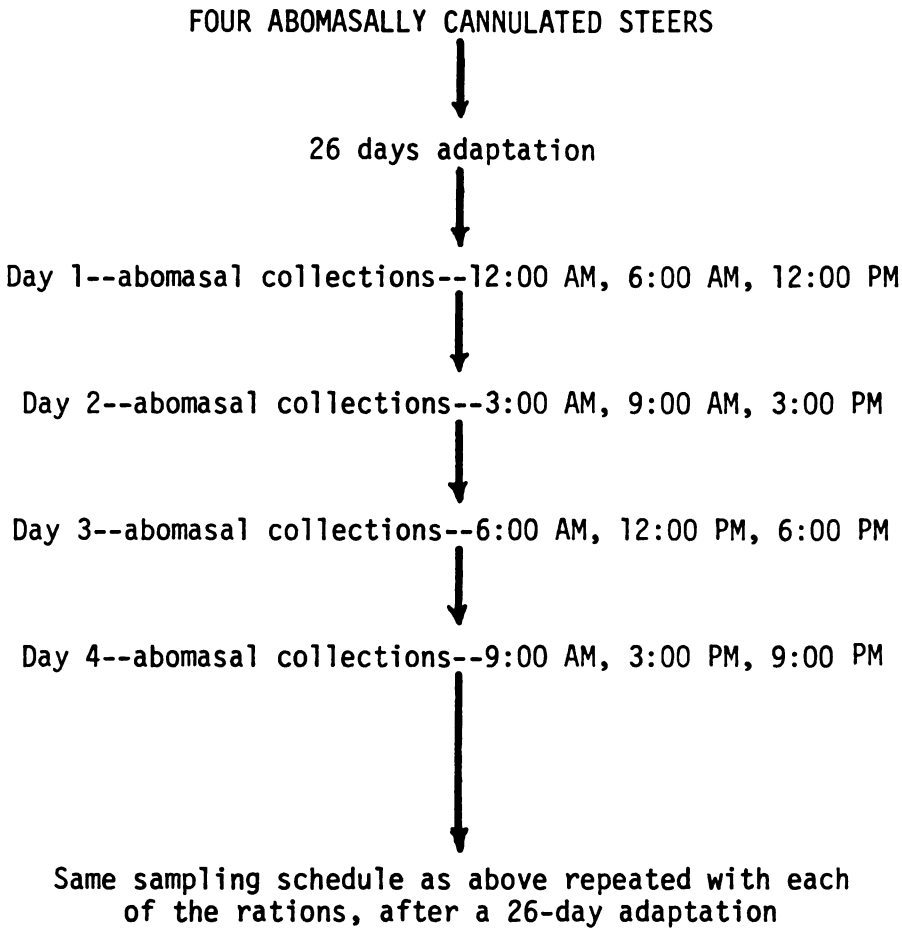

Figure 1. Flow Chart of Sampling Protocol* (Digesta Passage Studies).

*Total of four collection periods. During each period, two steers received one type of ration; the other two received the alternate ration (with or without monensin). The rations were switched at the end of each collection period.

combined and centrifuged at 150 xg for 5 minutes to remove the protozoa. The supernatant fraction was next centrifuged at 2000 rpm (500 xg) for 10 minutes, to remove the feed residues. The supernatant was then centrifuged at 18,000 xg for 15 minutes. The supernatant was discarded and the residue (bacterial cells) was resuspended in 0.9% saline solution and centrifuged again. The supernatant was discarded. This procedure was repeated three times. The resulting pellet was finally washed in a small amount of deionized-distilled water to remove the excess NaCl. After the final centrifugation, the bacterial pellet was frozen and lyophilized. The protozoal fractions were subjected to a similar series of washings and centrifugation and the pellets also freeze-dried.

Further Sample Preparations

Portions of the feed samples and weighbacks (from the nitrogen balance and abomasal collections) and fecal samples, were dried at 60°C to a constant weight (see Figures 2a and 2b). Each was then ground through a Wiley mill and retained for fiber and chromium analyses. The freeze-dried abomasal, protozoal and bacterial samples were individually finely ground with mortar and pestel.

Nitrogen Determination

Total nitrogen content of feed, feces, urine, abomasal and bacterial samples was determined with the Technicon Auto Kjeldahl System. Undried feed and fecal samples were used for the determination. Abomasal fluid composites were separated (by high speed centrifugation)

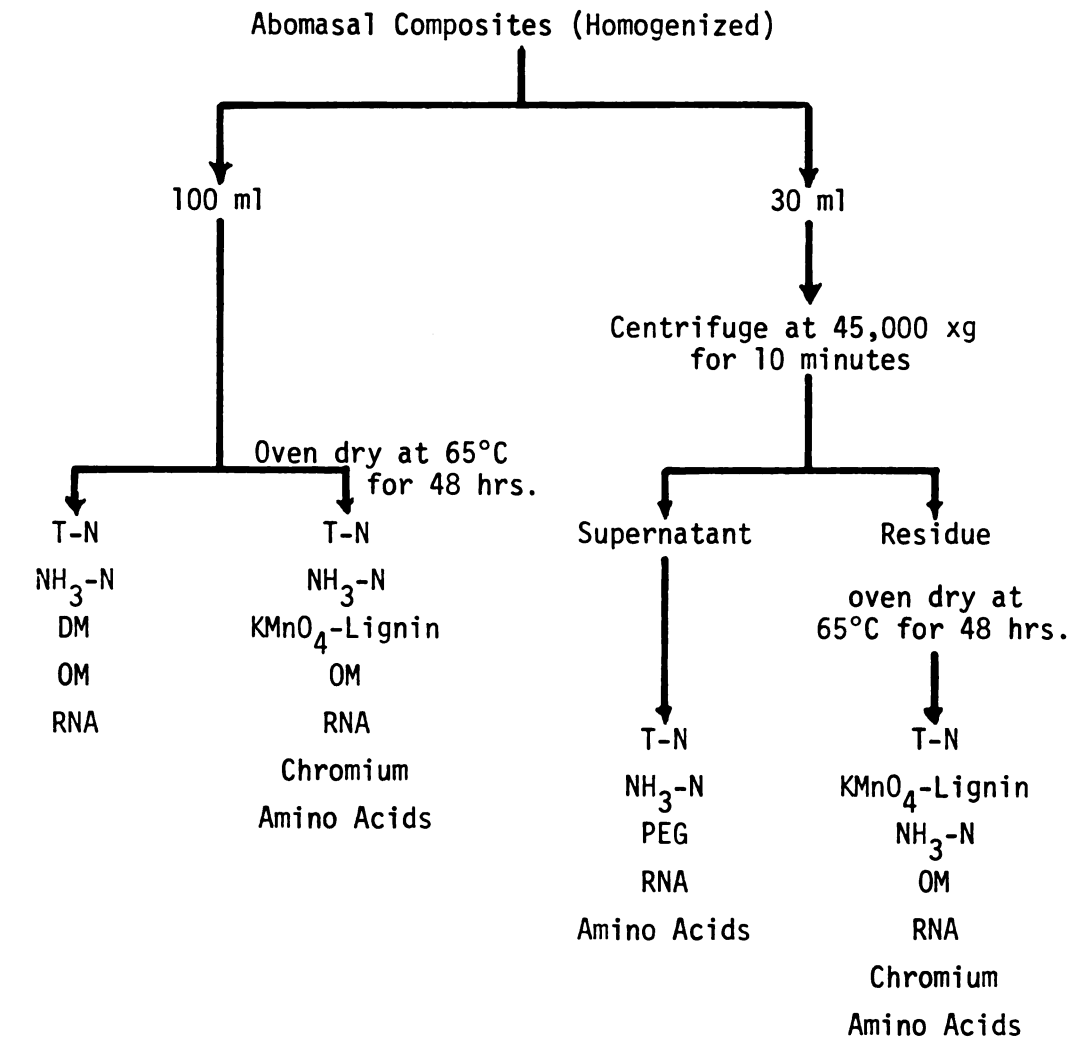

Figure 2a. Scheme of Laboratory Analysis for Composited Abomasal Samples (Digesta Passage Studies).

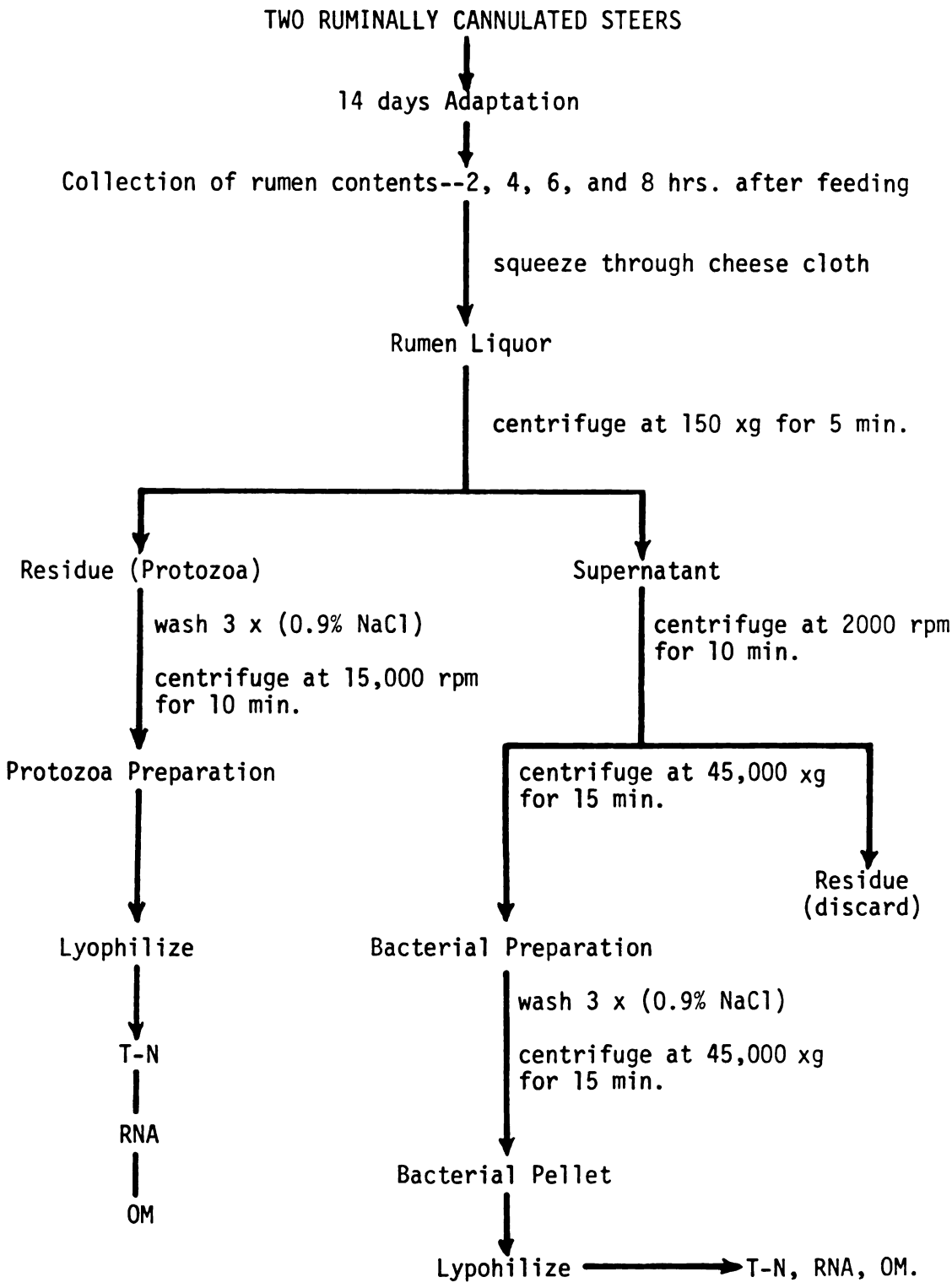

Figure 2b. Flow Chart of Sampling Protocol and Laboratory Analyses (Rumen Microbial Studies).

into the supernatant and residue. The solids were oven dried to a constant weight at 60°C and then finely ground. Five milliliter of the supernatant, 2 gm dried residues and 5 gm portions of wet abomasal composites were also analyzed for total nitrogen using the same procedure. Ammonia nitrogen was determined on the wet abomasal composites by Conway Microdiffusion technique (1960).

Chromium Analysis

Dried feed, feces, and abomasal (wet, dried and residues) composites were analyzed for chromium by nitric-perchloric acid digestion followed by atomic emission spectrophotometry using the I.L. 453 Atomic Absorption/Emission Spectrophotometer.

Approximately 300 mg of the dried samples (3 gm wet samples) were digested in 30 ml of concentrated nitric acid in a 250 ml Phillips beaker on a hot plate, until the volume was just sufficient to cover the bottom of the beaker. The beaker was cooled to room temperature, and the digest oxidized by adding 7 ml of 72% perchloric acid, followed by heating again until 2 to 4 ml of the digest remained (or the digest turned orange). The beaker was cooled and the contents diluted to 100 ml with deionized-distilled water. The oxidized and diluted samples were then analyzed for chromium by atomic emission spectrophotometer at a wavelength of 425.4 nm, scale of 2.5, slit width of 80, photomultiplier voltage at 700 volts, acetylene flame, hollow-cathode chromium lamp and nitric oxide solid burner. A standard curve was prepared by serial dilutions of stock ammonium chromate solution. Subjected to the same treatment as the samples were 1, 2, 5, 10, 15 and 20 ppm chromium from the solution.

Dry and Organic Matter Determination

Dry matter of the feed, feces, abomasal composites and bacterial cells were determined by oven-drying the samples at 60°C to constant weights. Corrections for moisture absorbed in storage were made by second dry matter determination during other analyses. Organic matter of the moisture-free samples was obtained by ashing in a muffle furnace at 660°C.

Polyethylene Glycol Determination (PEG)

PEG in the feed, weighbacks, and abomasal supernatant were determined by the method of Hyden (1955) as modified by Smith (1959). Five grams of the ground feed were rehydrated in 25 gm of water, homogenized and centrifuged to provide the supernatant used for PEG determinations.

Fiber Determination

Neutral detergent fiber, acid detergent fiber and potassium permanganate lignin contents of the dried feed, weighbacks, fecal and abomasal composites and residues were determined by the standard Van Soest procedures (Van Soest, 1963; Van Soest and Wine, 1967; Robertson and Van Soest, 1977).

Nucleic Acid Analysis

Ribonucleic acid content of rumen bacteria, protozoa, abomasal fluid composites, abomasal supernatant and residues were determined by a modification of the methods of McAllan and Smith (1969) and Munro and Fleck (1966).

One hundred to one hundred-fifty milligrams of dried (and 2 to 4 gm of wet) samples were homogenized under ice in 5 ml cold 0.2 N perchloric acid (PCA) for 15 minutes. Five milliliters of 1.2 N PCA were used for the wet samples. Each was transferred into Corex tubes in ice; the homogenizer was washed twice with 2 ml of cold 0.2 N PCA. The washings were added to the samples and the tubes centrifuged at 18,000 rpm for 15 minutes. The residues were separated, washed twice with cold 0.1 N PCA and centrifuged each time, and the supernatants discarded. The pellets were next dispersed in 10 ml buffered ethanol (1% potassium acetate in 95% ethanol W/V), centrifuged at 18,000 rpm for 15 minutes and the supernatants discarded. The above lipid/pigment extraction procedure was repeated with chloroform:methanol (1:3), methanol:ether (3:1) and ether, after which the samples were dried in a vacuum-dessicator. All the above extractions were performed at < 4°C.

The samples were next hydrolyzed in 10 ml 1.0 N KOH at 37°C for one hour. They were then cooled in ice and centrifuged at 35,000 xg for 15 minutes. The supernatant was separated and the residue washed twice in 2 ml 1.0 N KOH, centrifuged, and the liquid portions combined with the above supernatant. The latter was acidified with 9 ml of 2.0 N PCA and kept in ice (with occasional shaking) for 30 minutes. The sample was next centrifuged for 15 minutes at the above speed. The supernatant was saved. The residue was washed in 2 ml 0.2 N PCA, centrifuged, and the supernatant therefrom combined with the former. The solution was then adjusted to pH 7 (with

approximately 2 ml of 3 N KOH), filtered into 100 ml volumetric flasks and the filter paper washed down with some deionized water. Five milliliters 2 N PCA were added to the flask and the volume made up to mark with deionized water. The acid-soluble RNA, now in 0.1 N PCA, was analyzed by ultra-violet absorption at 260 nm. Sample DNA content could be determined on the combined residues. Corrections for acid-soluble peptides could be made on the RNA values by performing a Lowry-protein analysis on the RNA solutions, and then subtracting 0.001 OD units for each mg peptide found, per ml of the solution.

A standard curve was prepared from serial dilutions in 0.1 N PCA (0, 12.5, 25.0, 37.5 and 50.0 μg/ml) of purified highly polymerized yeast RNA.

Statistical Analyses

Analysis of variance (Snedecor and Cochran, 1967) was used to examine the effects of treatments on the digesta passage data, nitrogen balance and microbial growth studies. Orthogonal contrasts (Snedecor and Cochran, 1967) were designed to compare the means of treatment combinations of primary interest. All statistical analyses were performed on Hewlett Packard Model 9825A Calculator.

Feeding Trial and Blood Metabolite Studies

General Design

Twenty-four Hereford steer calves weighing approximately 277 kg each, were used in a feeding trial employing all-corn silage diets to study the effects of monensin and elfazepam on plasma amino acid status, blood glucose levels and performance of the animals.

The steers were wormed, treated for lice, vaccinated for IBR, BVD and PI_3 and then adapted to a ration containing 88% corn silage and 12% soybean meal-mineral supplement, prior to the study. All the steers were implanted with Synovex S.

Six steers, ranging from small to average frames, were randomly allotted to each of the four experimental rations listed in Table 5. The composition of the supplements are listed in Table 3. The steers were individually fed once a day and had free access to water. Rations were mixed immediately before feeding in a horizontal batch mixer. Daily feed records were maintained for each steer and unconsumed feed was removed, weighed and recorded. Feed was provided in such a manner that the bunks were nearly clean at the next feeding period. Several grab samples of the rations were taken as the mixed feed was being discharged from the mixer. These were composited, comminuted, subsampled and kept frozen for subsequent dry matter analysis. The trial lasted for 118 days and each ration contained 13% crude protein.

The steers were individually weighed on days 0, 28, 63, 90 and 118 of the trial. Initial and final weights were taken 16 hours after feed and water removal. During the intermediate weighing periods, only water was removed.

Jugular blood samples were taken from each steer by venipuncture prior to weighing on days 28, 63, 90 and 118. Efforts were made to keep the animals calm and restrained during blood withdrawal.

The steers were confined to individual pens (91 x 244 cm) with completely slotted floors in an environmentally controlled metabolism room, throughout the trial.

TABLE 5. COMPOSITION OF RATIONS USED IN FEEDING TRIAL AND PLASMA STUDIES

Ingredient	Percent--DM basis
Corn silage (35% DM)	88
Soybean meal-mineral supplement 1,[a] 2,[b] 3,[c] 4,[de]	12
Analysis of Supplement:	%
NX6.25	49.4
Calcium	2.0
PO_4	1.2
TM salt	2.0

[a]Contained vitamin A and vitamin D premix.

[b]Contained 1 plus 125 mg minensin per 454 gm.

[c]Contained 1 plus 8.35 mg elfazepam per 454 gm.

[d]Contained 1 plus 125 mg monensin and 8.35 mg elfazepam per 454 gm.

[e]Supplements 1, 2, 3 and 4 used in Rations 1, 2, 3, and 4, respectively; ingredients listed in Table 3.

Sample Preparation and Analyses

Blood Samples

Blood samples drawn from the right jugular vein of each steer were collected into 100 x 16 mm tubes (vacutainers) containing 2 to 3 drops of heparin. The tubes were kept under ice, deproteinized and used for analyses of the plasma metabolites. Samples were kept frozen until analyzed.

Plasma was prepared for amino acid analyses by the standard procedure used in our laboratory (Bergen et al., 1973). Plasma free amino acid concentrations were determined by ion-exchange chromatography on a Technicon Amino Acid Analyzer TSM-1 as described by Bergen and Potter (1971).

Plasma urea nitrogen levels were determined by Conway Micro-diffusion method (1960) as outlined by Fenderson (1972).

Plasma glucose levels were determined by a modification of the colorimetric method in which glucose is oxidized in a glucose oxidase-peroxidase reaction (Boehringer Mannheim Corporation Cat. No. 15754, 1974).

Feed Samples

Dry matter of the feed and weighback were determined by drying the samples in an oven at 65°C for 48 hours.

Statistical Analysis

Least square analyses (Snedecor and Cochran, 1967) were used to examine the main effects and interactions for average daily gain

(ADG), average daily dry matter intake (ADDMI) and feed efficiency (F/G). Differences between least square means were determined using Duncan's New Multiple Range Test.

The effects of treatment on plasma glucose, urea and amino acid levels were examined by analysis of variance (Snedecor and Cochran, 1967).

Calculations

Estimates obtained were based on the following calculations:

A. Crude protein (CP) = N x 6.25;

B. Organic matter (OM) = dry matter (DM) - ash;

C. Organic matter digested in the rumen (OMD) = OM intake - (OM reaching the abomasum - microbial OM);

D. Non-ammonia nitrogen (NAN) = total nitrogen (TN) - ammonia-nitrogen (NH_3-N).

1. DM flow (g/day)

$$= \frac{\text{Marker}^1 \text{ Intake (g)}}{\text{Marker}^1\text{/g Abomasal DM}}$$

2. Passage of digesta components[2] (g/day): (A)

$$= \frac{\text{DM Intake x Component (g/g DMI)}}{\text{DM Flow x Component (g/g Abomasal DM)}}$$

3. TN (NAN) passage (g/day)

   a. From chromium or lignin outflow

$$= \frac{\text{Marker}^1 \text{ Intake (g)}}{\text{g Marker}^1\text{/ml Abomasal Fluid}} \times \text{TN(NAN)/ml Abomasal Fluid}$$

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[1]Chromium or lignin.

[2]Digesta component: DM, OM, NDF, ADF.

b. From N:Marker[1] ratio

$$= \frac{\text{N:Marker}^1\text{ Ratio in Abomasal DM}}{\text{N:Marker}^1\text{ Ratio in Feed DM}} \times \text{N-Intake (g)}$$

c. From Reconstituted Digesta: (B) + (C)

$$(B) = \frac{\text{PEG Intake (g)}}{\text{PEG g/ml Abomasal Fluid}} \times \text{N g/ml Liquid Digesta}$$

$$(C) = \frac{\text{Chromium Intake (g)}}{\text{Chromium g/g DM Abomasal Residue}} \times \text{N (g/g Abomasal Residue)}$$

4. Proportion microbial N in abomasal N: (D)

$$= \frac{\text{Total N/g Bacterial DM}}{\text{RNA-N}^4\text{/g Bacterial DM}} \times \frac{\text{RNA-N}^{3,4}\text{/g Abomasal DM}}{\text{NAN/g Abomasal DM}}$$

5. Microbial N flow (g/day)

= (D) x NAN Passage

6. Microbial DM flow

$$= \frac{\text{Microbial N Flow}}{\text{N/g Bacterial DM}}$$

7. True ruminal digestion[5] of component[2] (g/day)

$$= \frac{\text{(A) - (Microbial DM Flow x Component/g Bacterial DM)}}{\text{Component in Feed (g/day)}}$$

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[1]Chromium or lignin.

[2]Digesta component: DM, OM, NDF, ADF.

[3]From 'adjusted RNA' per gm abomasal DM (i.e., RNA x 0.85, see discussion: Nucleic Acid Methodology.

[4]Microbial RNA assumed to contain 14.8% N (Ling and Buttery, 1978).

[5]Digestion in the rumen corrected for microbial, but not for endogenous contribution.

8. True efficiency of microbial protein synthesis (CP/100 g OMD)

$$= \frac{\text{Microbial N Flow} \times 6.25}{\text{True OMD (g)}} \times 100 \text{ g OM}$$

9. Digesta passage rate (ruminal outflow rate)

   a. Liquid Digesta (liters/day)

$$= \frac{\text{PEG Intake (g)}}{\text{PEG/ml Abomasal Supernate}}$$

   b. Particulate Digesta (liters/day)

$$= \frac{\text{Chromium Intake (g)}}{\text{Chromium/g DM Abomasal Residue}}$$

   c. Total Digesta (liters/day)

$$= \frac{\text{Marker}^1 \text{ Intake (g)}}{\text{Marker}^1 \text{ g/ml Abomasal Fluid}}$$

10. Rumen dilution rate (% per day)

$$= \frac{\text{Digesta Passage Rate (liters/day)}}{\text{Rumen Volume}^6 \text{ (liters)}}$$

11. Rumen turnover time (days)

$$= (\text{Rumen Dilution Rate})^{-1}$$

12. Post-ruminal ADF digestion (% abomasal)

$$= \frac{\text{Abomasal ADF Passage (g/day)} - \text{Fecal ADF (g/day)}}{\text{Abomasal ADF Passage (g/day)}} \times 100$$

13. Fecal chromium recovery (% of intake)

$$= \frac{\text{Fecal DM (g/day)} \times \text{Chromium/g Fecal DM}}{\text{Chromium Intake (g/day)}}$$

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[1]Chromium or lignin.

[6]Rumen volume separately determined. In this study, 50 liters assumed for Hereford steers (277 kg BWT).

CHAPTER IV

RESULTS

Digesta Passage Studies

Total digesta outflow from the rumen was estimated from the rates of particulate markers (lignin and chromium) appearance in abomasal digesta. These estimates were compared with abomasal digesta flow rates determined from the partitioning of liquid (PEG) and particulate (chromium) fractions of abomasal digesta (Faichney, 1975). Total digesta flow rates based on the three above markers are shown in Table 6. Estimates from the reconstituted digesta (PEG + chromium passage) were consistently higher than the corresponding values determined from lignin and chromium flows. Lignin and chromium-estimated digesta flow rates were 32 to 41% lower than PEG chromium flow rates. But, laboratory values of chromium in particulate digesta (dry abomasal residues) when reconstituted, were similar to the chromium values obtained from the corresponding total digesta composites. Since chromium was not detected in the liquid fraction of the abomasal composites, it was assumed that the particulate chromium values obtained did not, within the limits of our abomasal sampling accuracy and sampling technique used, overestimate solid digesta flows. PEG-estimated liquid digesta flow rates were 27 to 38% higher than total digesta flow rates determined from abomasal lignin and chromium passage, while total digesta flows based on the passage of the

TABLE 6. COMPARISON OF TOTAL DIGESTA FLOW RATES[a] ESTIMATED BY REFERENCE TO PEG,[b] LIGNIN AND CHROMIUM

Ration	Flow rate from PEG (liters/day)	Flow rate from lignin (liters/day)	Flow rate from chromium (liters/day)	Ratio (%) of lignin/PEG estimates	Ratio (%) of chromium/PEG estimates	Ratio (%) of lignin/chromium estimates
High grain w/o monensin	62.26	42.73	42.10	68.63	67.62	101.50
High grain w. monensin	68.51	42.50	44.05	62.03	64.30	96.48
High silage w/o monensin	89.39	60.21	58.83	67.36	65.81	102.35
High silage w. monensin	104.34	61.67	62.95	59.11	60.33	97.97

[a]Overall treatment means of 4 steers per treatment; composite sample for each steer consisted of 16 individual abomasal spot-samples collected over a 4-day period.

[b]Consists of reconstituted digesta flow determined from the partitioned liquid (PEG) and solid (chromium) digesta (Faichney, 1975).

latter two markers were very similar within treatments (Table 6). This implied that there could be a problem with abomasal PEG passage in the present experiment.

However, abomasal flow of the various components of digest was estimated from lignin, chromium, N:lignin, N:chromium and PEG + chromium passage, using the different methods of calculation previously discussed (see section: Calculations). Here again, abomasal N recoveries estimated from the reconstituted digesta were higher than the corresponding lignin and chromium-based N recoveries. Recoveries obtained by the former method seemed physiologically impossible for steers on the dietary regimen used in this study (see Table 13). Based on those observations and other reasons to be discussed later, it was decided to calculate abomasal passage of the various components of digesta from lignin and chromium passage rates only.

Ruminal Dry Matter and Fiber Digestion

Abomasal passage of dry matter (DM), fiber (NDF and ADF) and the extent of ruminal digestion of those feed components are shown in Tables 7 and 8. Variations in intake and the basic differences in fiber content of the grain and silage rations make a direct statistical comparison of the absolute estimates untenable.

There was a trend toward a decrease in abomasal DM passage with monensin rations, which was more evident in the silage rations. But these differences were not significant. NDF and ADF passage to the abomasum were similar in the high grain rations, and slightly lower

TABLE 7. DRY MATTER AND FIBER FLOW FROM THE RUMEN: ESTIMATED FROM LIGNIN-BASED DRY MATTER FLOW

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	
Intake (g/day):					
Dry matter	6468.35	6187.44	7184.21	6503.56	474.35
NDF	1455.83	1389.65	3226.75	2753.04	195.45
ADF	672.34	655.66	1785.02	1584.83	81.01
Abomasal Flow (g/day):					
Dry matter	4598.19	4164.01	5668.74	4460.19	514.51
NDF	1002.10	1035.10	2276.59	1900.60	170.13
ADF	512.16	551.26	1276.18	1140.91	82.65
Microbial DM[a]	996.16	894.43	1148.00	909.70	78.07
Feed DM	3602.03	3269.58	4520.74	3550.49	462.67
Apparent Rumen Digestion:					
Dry matter	1870.16	2023.43	1515.47	2043.37	236.23
NDF	453.72	354.55	950.16	852.45	65.92
ADF	160.18	104.40	508.85	443.91	38.75
Rumen DMD[b]	2866.32	2917.86	2663.47	2953.07	265.51
% Rumen Digestion					
Apparent DMD[c]	28.91	32.70	21.10	31.42	3.95
Apparent NDF	31.17	25.52	29.45	30.96	2.27
Apparent ADF	23.82	15.92	28.51	28.01	2.90
Rumen DMD[b]	44.31	47.16	37.07	45.41	3.99

[a]Significant monensin effect ($P < 0.05$)

[b]Rumen dry matter corrected for microbial flow.

[c]Significant monensin effect ($P < 0.01$)

TABLE 8. DRY MATTER AND FIBER FLOW FROM THE RUMEN: ESTIMATED FROM CHROMIUM-BASED DRY MATTER FLOW

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	
Intake (g/day):					
Dry matter	6468.35	6187.44	7184.21	6503.56	474.35
NDF	1455.83	1389.65	3226.75	2753.04	195.45
Abomasal Flow (g/day):					
Dry matter	4469.88	4335.75	5584.00	4314.25	556.62
NDF	972.88	1077.54	2364.62	1889.82	207.07
Microbial DM[a]	980.29	926.28	1124.08	929.31	81.71
Feed DM	3489.58	3409.48	4459.93	3384.95	490.93
Apparent Rumen Digestion:					
Dry matter	1998.48	1851.69	1600.21	2189.31	286.23
NDF	482.95	312.12	862.13	863.23	87.15
Rumen digestion[b]	2978.77	2777.96	2724.28	3118.61	275.61
% Rumen Digestion:					
Apparent DMD[c]	30.90	29.93	22.27	33.66	4.64
Apparent NDF	33.17	22.46	26.72	31.36	3.64
Rumen DMD[bc]	46.05	44.90	37.92	47.95	4.43

[a]Significant monensin effect with silage ration ($P < 0.05$).

[b]Rumen digestion corrected for microbial flow.

[c]Monensin effect with silage ration ($P < 0.10$).

in monensin-supplemented silage ration. These trends were also not statistically significant. But, microbial DM passage to the abomasum was significantly reduced by monensin ($P < 0.05$, Table 7). Monensin also increased apparent DM digestibility (%) of the silage ration ($P < 0.05$), without significantly affecting the high grain ration. Apparent NDF digestibility was also slightly increased by monensin in silage rations (Tables 7 and 8) while apparent ADF digestibility was slightly reduced by monensin in both types of ration. These trends were, however, not significant. True (or actual) ruminal DM digestibility (i.e., corrected for microbial DM passage) was not affected by monensin in high grain ration, but was increased in silage ration supplemented with monensin ($P < 0.10$). Mean percent DM truly digested in the rumen (based on combined estimates determined from lignin and chromium abomasal flows) were: 45.18, 46.03, 37.50 and 46.68 for high grain ration, without (HG) or with (HG-M) monensin, and, high silage ration, without (CS) or with (CS-M) monensin, respectively.

Total Nitrogen and NAN Passage to the Abomasum

Passage of the nitrogen constituents to the abomasum was estimated using lignin, chromium and reconstituted PEG and chromium as markers. These were also compared to abomasal N passage calculated by the marker:nutrient ratio (see section: Calculations). N passage estimates obtained by the above methods are shown in Tables 9 to 13. Abomasal N flow estimates from lignin, chromium and marker:nutrient ratio methods showed some net ruminal nitrogen losses with HG and CS

TABLE 9. EFFECTS OF MONENSIN AND TYPE OF RATION ON THE NITROGEN CONSTITUENTS OF ABOMASAL DIGESTA: ESTIMATED FROM LIGNIN FLOW

	Treatment				
	High grain		High silage		
Item	Without monensin	With monensin	Without monensin	With monensin	SEM
Intake (g/day):					
Dry matter	6468.35	6187.44	7184.21	6503.56	474.35
Nitrogen	135.15	131.10	148.89	137.40	8.59
Abomasal Flow (g/day):					
Total N	122.28	126.98	138.82	138.65	10.07
Ammonia-N[a]	3.03	2.69	5.18	5.06	0.42
Non-NH_3-N[b]	119.25	124.29	133.64	133.59	9.77
Microbial N[c]	82.90	74.85	95.86	76.12	6.51
Plant N[d]	36.35	49.44	37.79	57.47	5.08
Abomasal Flow (%):					
Plant N (% NAN)[d]	30.48	39.78	28.27	43.02	2.42
NAN (% N intake)[bc]	88.24	94.81	89.76	97.23	2.72
Digesta flow rate[e]	42.73	42.50	60.21	61.67	3.38

[a]Significant differences due to type of ration ($P < 0.05$).

[b]Not corrected for endogenous N secretion.

[c]Significant differences due to monensin ($P < 0.05$).

[d]Significant differences due to monensin ($P < 0.01$).

[e]Liters per day; significant differences due to ration ($P < 0.01$).

TABLE 10. EFFECTS OF MONENSIN AND TYPE OF RATION ON THE NITROGEN CONSTITUENTS OF ABOMASAL DIGESTA: ESTIMATED FROM N: LIGNIN RATIOS

	Treatment				
	High grain		High silage		
Item	Without monensin	With monensin	Without monensin	With monensin	SEM
Intake (g/day):					
Dry matter	6468.35	6187.44	7184.21	6503.56	474.35
Nitrogen	135.15	131.10	148.89	137.40	8.59
Abomasal Flow (g/day):					
Total N	124.04	127.46	139.38	139.66	10.01
Ammonia-N[a]	3.03	3.17	5.74	6.07	0.42
Non-NH_3-N[b]	121.01	124.29	133.64	133.59	9.68
Microbial N[c]	84.01	74.85	95.85	76.12	6.37
Plant N[d]	36.99	49.44	37.79	57.47	5.11
Abomasal Flow (%):					
Plant N (% NAN)[de]	30.57	39.78	28.28	43.02	2.42
NAN (% N intake)[be]	89.54	94.81	89.76	97.23	2.74

[a]Significant ration effect ($P < 0.05$).

[b]Not corrected for endogenous-N secretion.

[c]Significant monensin effect ($P < 0.05$).

[d]Significant monensin effect ($P < 0.01$).

[e]Significant monensin effect ($P < 0.05$).

TABLE 11. EFFECTS OF MONENSIN AND TYPE OF RATION ON THE NITROGEN CONSTITUENTS OF ABOMASAL DIGESTA: ESTIMATED FROM CHROMIUM FLOW

	Treatment				
	High grain		High silage		
Item	Without monensin	With monensin	Without monensin	With monensin	SEM
Intake (g/day):					
Dry matter	6468.35	6187.44	7184.21	6503.56	474.35
Nitrogen	135.15	131.10	148.89	137.40	8.59
Abomasal Flow (g/day):					
Total N	120.39	131.80	135.75	141.55	10.81
Ammonia-N[a]	2.98	2.78	5.04	5.17	0.40
Non-NH_3-N[b]	117.41	129.02	130.71	136.38	10.53
Microbial N	81.58	77.52	93.86	77.76	6.82
Plant N[c]	35.83	51.50	36.85	58.63	5.50
Abomasal Flows (%):					
Plant N (% NAN)[c]	30.52	39.92	28.19	42.98	2.40
NAN (% N intake)[bc]	86.87	98.41	87.79	99.26	3.12
Digesta flow rate[ad]	42.10	44.05	58.83	62.95	3.52

[a]Significant differences due to ration type ($P < 0.05$).

[b]Not corrected for endogenous N secretion.

[c]Significant differences due to monensin ($P < 0.01$).

[d]Liters per day.

TABLE 12. EFFECTS OF MONENSIN AND TYPE OF RATION ON THE NITROGEN CONSTITUENTS OF ABOMASAL DIGESTA: ESTIMATED FROM N: CHROMIUM RATIO

	Treatment				
	High grain		High silage		
Item	Without monensin	With monensin	Without monensin	With monensin	SEM
Intake (g/day):					
Dry matter	6468.35	6187.44	7184.21	6503.56	474.35
Nitrogen	135.15	131.10	148.89	137.40	8.59
Abomasal Flow (g/day):					
Total N	120.44	136.00	136.56	142.44	10.80
Ammonia-N[a]	3.03	3.17	5.74	6.07	0.42
Non-NH_3-N[b]	117.41	132.83	130.82	136.37	11.81
Microbial N	81.57	79.61	93.94	77.79	7.36
Plant N[c]	35.84	53.22	36.88	58.58	5.99
Abomasal Flow (%):					
Plant N (% NAN)[bc]	30.53	40.07	28.19	42.96	2.42
NAN (% N intake)[bc]	86.87	101.32	87.86	99.25	4.01

[a]Significant ration effect ($P < 0.05$).

[b]Not corrected for endogenous N secretion.

[c]Significant effects due to monensin ($P < 0.01$).

rations. Monensin addition to both rations resulted in virtually no net ruminal nitrogen losses. Abomasal NH_3-N recoveries were higher when the steers received the silage rations ($P < 0.05$), but showed no significant monensin effect. NAN passage (as % of N-intake) from combined lignin and chromium passage estimates, were higher when the rations contained monensin ($P < 0.01$). These, for lignin-based estimates, were 88.24, 94.81, 89.76 and 97.23% for HG, HG-M, CS and CS-M rations, respectively ($P < 0.05$). The same estimates from abomasal chromium passage were 86.87, 98.41, 87.79 and 99.26% for the above respective rations ($P < 0.01$).

But, microbial N passage was reduced ($P < 0.05$) and feed N bypass increased upon addition of monensin to HG and CS rations. Feed N contributed about 30% to abomasal NAN passage with the control rations (HG and CS rations); with monensin added to the rations, feed N bypass comprised 40% of abomasal NAN. Total digesta flow rates were higher when silage rations were fed (Table 9, $P < 0.01$; Table 11, $P < 0.05$), but monensin did not affect the rate of digesta passage. Abomasal passage of the various nitrogen constituents of digesta calculated by N:lignin ratios were virtually the same as those determined from lignin alone, while estimates from N:chromium ratios also closely paralleled the corresponding estimates from chromium passage.

Abomasal N Passage from Reconstituted Digesta

Abomasal N passage data calculated by reconstitution of the partitioned liquid (PEG) and solid (chromium) fractions of digesta are shown in Table 13. The data presented in the table show marked increases

TABLE 13. EFFECTS OF MONENSIN AND RATION TYPE ON NITROGEN CONSTITUENTS OF ABOMASAL DIGESTA IN GROWING STEERS--DETERMINED FROM RECONSTITUTED LIQUID (FROM PEG) AND SOLID (CHROMIC OXIDE) FLOWS

	Treatment				
	High grain		High silage		
Item	Without monensin	With monensin	Without monensin	With monensin	SEM
N intake (g)	135.15	131.10	148.89	137.40	8.59
Total abomasal N (g)	153.32	161.43	162.93	162.02	
Abomasal NAN (g)[a]	151.08	159.19	158.98	157.61	10.27
(1) Solids (g)[b]	61.22	60.39	84.62	79.52	4.92
(2) Liquids (g)	89.86	98.80	74.37	78.09	6.83
NH_3-N flow (g)[b]	2.24	2.24	3.94	4.41	0.22
NAN (% N intake)[a]	111.79	121.43	106.78	114.71	
	Digesta Flow Rates				
	ml/day				
Solid flow[c]	4,469.88	4,335.75	5,584.00	4,550.55	564.30
Liquid flow[d]	57,784.58	64,173.34	83,807.80	99,790.97	6995.28
Total flow[d]	62,254.46	68,509.09	89,391.80	104,341.52	

[a]Not corrected for endogenous N secretion.

[b]Significant difference due to type of ration ($P < 0.05$).

[c]Significant monensin vs. silage interaction ($P < 0.10$).

[d]Significant monensin effect on flow rate ($P < 0.05$).

in most parameters estimated, over the corresponding estimates from lignin and chromium passage. Total N and NAN passage to the abomasum were significantly higher than the dietary N intakes with all the rations fed, and there were virtually no monensin effects. Abomasal NAN from particulate digesta (solids) and NH_3-N passage were both higher for CS and CS-M than for HG and HG-M rations ($P < 0.05$). Particulate digesta flow rates were similar for the high grain rations, and were reduced by monensin in silage rations ($P < 0.10$). Liquid and total digesta flow rates were higher for monensin supplemented rations ($P < 0.05$). Table 14 represents a direct comparison of the marker ratios in ration and abomasal DM. Monensin increased % NAN and lignin in abomasal DM.

Rumen Microbial Studies

In order to accurately determine the effects of monensin on feed protein and dry matter degradation in the rumen, microbial contribution to abomasal N and DM flow also have to be quantitatively estimated. Since it is rather difficult to physically separate intact microbial cells from abomasal digesta, a microbial marker (e.g., RNA) is usually used to estimate microbial passage. Variability in the macromolecular composition of microbial cells with diets and time after feeding (McAllan and Smith, 1977) make it necessary to determine the chemical composition of the microorganisms isolated from animals under the same experimental regimen.

Table 15 presents the total N, RNA and organic matter estimates of mixed bacterial preparations isolated from the rumen of steers fed

TABLE 14. RATIOS OF MARKERS[a] IN RATION AND ABOMASAL DIGESTA

Item	Treatment				
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	SEM
% Nitrogen:					
Ration	2.10	2.10	2.10	2.10	0.10
Abomasal digesta[bc]	2.66	3.03	2.43	3.04	0.23
% Chromium:					
Ration	0.21	0.20	0.18	0.17	0.01
Abomasal digesta	0.30	0.29	0.23	0.25	0.02
% Lignin:[d]					
Ration	3.09	3.09	3.93	3.93	0.01
Abomasal digesta[e]	4.40	4.70	5.10	5.80	0.30
Nitrogen:Chromium:					
Ration	10.33	10.48	11.79	12.29	0.63
Abomasal digesta	8.97	10.54	10.32	12.22	0.73
Nitrogen:Lignin:					
Ration	0.677	0.686	0.533	0.539	0.022
Abomasal digesta	0.605	0.649	0.478	0.525	0.026

[a] mg marker/g dry matter.

[b] Abomasal NAN.

[c] Significant monensin effect ($P < 0.05$).

[d] Lignin determination by the $KMnO_4$ procedure.

[e] Significant monensin effect ($P < 0.10$).

TABLE 15. COMPOSITION OF MIXED RUMEN BACTERIAL PREPARATIONS FROM STEERS FED MONENSIN[a]

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	
Total N	83.22	83.69	83.50	83.67	2.29
RNA	87.64	87.80	87.65	87.83	3.22
RNA-N	12.97	12.99	12.97	13.00	0.46
RNA-N:TN ratio	0.156	0.155	0.156	0.155	0.14
Ash (%)	15.05	15.33	15.00	15.30	
Organic matter (%)	84.95	84.67	85.00	84.70	

[a]Rumen samples collected at 2, 4, 6, and 8 hours after feeding, and pooled.

[b]mg/g bacterial dry matter.

the same rations used in the passage study. Protozoa composition was also studied, but not used in subsequent calculations since it is believed that protozoa contribute very little to abomasal NAN (Weller and Pilgrim, 1974).

There were no differences in total N, RNA, ash and organic matter content between the bacterial preparations from steers fed the four experimental rations. However, bacterial preparations isolated two hours after feeding contained less nitrogen (DM basis) than those obtained 4, 6 and 8 hours after the steers were fed. This response was more evident with the HG ration and may account for the slightly lower N content of the bacteria isolated from the steers receiving that ration. Bacterial preparations from the steers fed monensin also contained a slightly higher amount of ash. These differences were not significant, however.

Microbial Contribution to Abomasal NAN Passage

Total N:RNA-N ratios of the rumen bacterial preparations and abomasal digesta were used to determine the microbial proportion of abomasal NAN passage. Bacterial RNA was assumed to contain 14.8% nitrogen (Ling and Buttery, 1978) and abomasal RNA estimates were multiplied by 0.85 to obtain the adjusted RNA (Smith et al., 1978; see discussion). Without this adjustment in digesta RNA, RNA-N values derived were found to be exceedingly high, and in a few cases resulted in estimates of microbial NAN passage equal to (or greater) than total abomasal NAN passage, an unlikely occurrence with the rations used in this experiment.

To assess the reliability of the nucleic acid procedure used, RNA analyses were performed on oven-dried (previously lyophilized) abomasal composites and compared to abomasal fluid composites. The results obtained with the dried samples were so inflated that it was decided to use the RNA values determined from the abomasal fluid samples for subsequent calculations. The latter were consistently lower and less variable between samples.

Microbial contributions to abomasal NAN passage are presented in Table 16. Based on these calculations, monensin reduced microbial nitrogen passage to the abomasum ($P < 0.01$). There were no significant effects of ration on microbial N flow. Microbial N:abomasal NAN ratios averaged about 0.70 and 0.60 for the control (HG plus CS) and monensin rations, respectively. Abomasal NAN per gram digesta DM were higher with monensin rations ($P < 0.05$). Microbial-N passage to the abomasum are presented in Tables 17 and 18. Abomasal passage of microbial N (in g/day) determined from lignin-estimated digesta flows were lower in monensin-fed steers (75.49 vs. 89.38; $P < 0.05$). The corresponding estimates from chromium passage were 77.64 and 87.72 for ration with and without monensin, respectively.

Efficiency of Microbial Protein Synthesis

Ruminal organic matter digestion and efficiency of microbial protein synthesis were determined from lignin and chromium-based digesta passage only (see Tables 17 and 18). There were no significant

TABLE 16. MICROBIAL CONTRIBUTION TO ABOMASAL NAN FLOW IN STEERS FED MONENSIN: DIGESTA PASSAGE STUDIES

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	
Bacterial RNA (mg/g DM)	87.64	87.80	87.65	87.33	3.22
Bacterial RNA-N[a]	13.03	12.99	12.97	13.00	0.46
Bacterial-N (mg/g DM)	83.22	83.69	83.50	83.67	2.29
Bacterial TN/RNA-N	6.39	6.44	6.44	6.43	0.16
Abomasal RNA (mg/g DM)	22.64	21.81	21.27	21.31	1.73
Abomasal RNA-N[a]	3.35	3.23	3.19	3.15	0.26
Abomasal RNA-N[b] (adjusted)	2.85	2.74	2.21	2.68	0.22
Abomasal NAN[c] (mg/g DM)	26.60	30.28	24.33	30.35	2.26
Microbial-N/Ab. NAN[d]	0.70	0.60	0.72	0.57	0.02

[a]Bacterial RNA assumed to contain 14.8% N (Ling and Buttery, 1978).

[b]Abomasal RNA concentration x 0.85 (Smith et al., 1978).

[c]Significant monensin effect ($P < 0.05$).

[d]Significant monensin effect ($P < 0.01$).

TABLE 17. EFFECTS OF MONENSIN AND TYPE OF RATION ON EFFICIENCY OF MICROBIAL PROTEIN SYNTHESIS: ESTIMATES FROM LIGNIN FLOW

	Treatment				
	High grain		High silage		
Item[a]	Without monensin	With monensin	Without monensin	With monensin	SEM
Organic matter intake	6033.38	5757.53	6784.31	6139.76	448.33
Abomasal OM flow	4218.72	3768.58	4863.78	3750.21	469.01
Microbial OM flow[b]	846.73	757.59	975.80	770.52	66.28
Feed OM flow[c]	3371.99	3010.99	3887.98	2979.69	422.40
Rumen OMD	2661.39	2746.54	2896.33	3160.88	235.07
Rumen OMD (%)	43.99	47.93	43.56	51.71	4.66
Microbial Efficiency:					
Microbial NAN (g/day)[d]	82.90	74.86	95.86	76.12	6.51
Microbial CP synthesis[d] (g/100 g OMD)	20.31	17.31	21.00	15.13	1.94
Microbial CP synthesis[de] (g CP/100 g OMD)	20.31	17.31	21.00	15.13	1.94

[a] g/day.

[b] Significant monensin effect ($P < 0.05$).

[c] Significant monensin vs. silage ($P < 0.10$).

[d] Significant monensin effect ($P < 0.05$).

[e] Estimated from N:lignin ratios.

TABLE 18. EFFECTS OF MONENSIN AND TYPE OF RATION ON EFFICIENCY OF MICROBIAL PROTEIN SYNTHESIS: ESTIMATES FROM CHROMIUM FLOW

	Treatment				
	High grain		High silage		
Item[a]	Without monensin	With monensin	Without monensin	With monensin	SEM
Organic matter intake	6033.38	5757.53	6784.31	6139.76	448.33
Abomasal OM flow	4101.53	3919.37	4788.76	3623.68	469.51
Microbial OM flow[b]	833.25	784.56	955.47	787.12	69.34
Feed OM flow	3268.28	3134.82	3833.29	2836.56	439.12
Rumen OMD	2765.10	2622.71	2951.02	3303.20	244.52
Rumen OMD (%)	45.83	45.55	43.50	53.80	4.23
Microbial Efficiency:					
Microbial NAN (g/day)	81.58	77.52	93.86	77.76	6.82
Microbial CP synthesis[c] (g/100 g OMD)	19.38	18.75	20.25	15.00	2.19
Microbial CP synthesis[cd] (g/100 g OMD)	19.38	19.25	20.25	15.00	2.31

[a] g/day.

[b] Significant monensin effect ($P < 0.10$).

[c] Significant differences, monensin vs. silage ration ($P < 0.10$).

[d] Estimated from N:chromium ratios.

differences due to monensin or the type of ration in the extent of organic matter degradation in the rumen. The trends were, however, for a slight increase with monensin-feeding. About 50% of the organic matter fed with monensin was digested in the rumen compared to 44% for rations without monensin. These differences were, however, not statistically significant.

Based on lignin flow, true efficiency of ruminal microbial protein (N x 6.25) synthesis were higher without monensin than with monensin in the rations ($P < 0.05$). Microbial efficiencies estimated from abomasal chromium passage showed the same trend, but reached a level of significance at α 0.10, with silage rations only. Microbial crude protein synthesized per 100 gm of organic matter actually degraded in the rumen were approximately 20.7 g for rations without monensin and 16.20 gm for rations supplemented with monensin. Combination of silage and monensin consistently produced the lowest microbial efficiencies (15 gm CP per 100 g OMD).

Microbial Efficiency and Ruminal Dilution Rates

Rumen dilution rates and turnover times were calculated from lignin and chromium flows, and also from PEG flows. Since rumen liquid pool size was not determined with each experimental animal for each ration fed, a 50-liter rumen liquid volume was assumed for all the steers in each treatment. There were variations in animal size and level of feed intake, and this could have affected the dilution rates obtained. The relationship between microbial efficiencies and rumen

dilution rates are shown in Table 19. There were no significant differences in dilution rates and rumen turnover time which can be attributed to monensin in the rations. However, rumen turnover time (in days) was shorter and fractional digesta outflow rates (% per hour) higher, with silage rations than with high grain rations ($P < 0.01$). Trends toward increases in rumen dilution rates, did not seem to increase the efficiency of microbial protein synthesis when monensin was fed.

In contrast, fractional liquid outflow rates derived from PEG were much higher, and consequently rumen turnover times, much faster than those estimated from either lignin or chromium outflows. Based on analytical reasons to be discussed later, it was decided that PEG outflow was too fast and would not be useful in this study as an index of digesta flow. Microbial efficiencies were therefore not determined from PEG data.

Also, the variations in animal size and level of intake, coupled with an across-the-board assumption of a 50-liter rumen volume made the establishment of a direct relationship between microbial efficiency and rumen fractional dilution rate difficult. A direct linear trend was more evident with monensin treatment than without.

TABLE 19. EFFECTS OF MONENSIN AND TYPE OF RATION ON RUMEN DILUTION RATES[a] AND EFFICIENCY OF MICROBIAL PROTEIN SYNTHESIS

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With momensin	
From Lignin Flow:					
Microbial CP synthesis (g CP/100 g OMD)	20.31	17.31	21.00	15.13	1.94
Dilution rate (%/hour)[b]	3.56	3.54	5.02	5.14	0.28
Rumen turnover time (days)[bc]	1.19	1.22	0.84	0.81	0.09
From Chromium Flow:					
Microbial CP synthesis (g/100 g OMD)	19.38	18.75	20.25	15.00	2.19
Dilution rate (%/hr)[b]	3.51	3.67	4.91	5.25	0.29
Turnover time (days)[b]	1.20	1.18	0.86	0.80	0.08
From PEG Flow:					
Liquid dilution (PEG)[b]	4.82	5.35	6.99	8.32	0.58
Turnover time (day)[bc]	0.90	0.80	0.60	0.52	0.07

[a]Fifty liter rumen volume assumed.

[b]Significant differences due to ration type ($P < 0.01$).

[c]Reciprocal of the dilution rates.

Nitrogen Balance and Digestibility Studies

Nitrogen Balance

Table 20 summarizes the data obtained from nitrogen balance studies. Variations in nitrogen intake between treatments may account for the absence of significant differences between the mean estimates of some parameters measured. There were, however, significant monensin effects on fecal nitrogen excretion ($P < 0.025$). Fecal N (gm/day) were 44.69, 37.01, 54.65 and 48.30 for HG, HG-M, CS and CS-M rations, respectively. Although monensin had no significant effect on urinary nitrogen excretion, more nitrogen (in gms) were excreted in the urine by steers on silage in comparison with steers on grain rations (44.44 and 44.00 g vs. 59.40 and 52.36 g). Daily nitrogen retentions were similar between rations HG, CS and CS-M (43 to 44 gm); monensin in high grain rations resulted in a slightly higher amount of nitrogen retained (48.32 g for ration 2), but this was not statistically significant. Apparent nitrogen digestion (%) followed a similar trend (66.07, 65.46, 66.60% for rations HG, CS and CS-M, respectively) with a significantly higher percent digestion for monensin-high grain rations ($P < 0.01$). More nitrogen (as % of N-intake) was also retained by steers given monensin in high grain rations (37.36%), in comparison with the other three rations (32.34, 27.80 and 30.90 for rations HG, CS and CS-M, respectively). Even though the above differences were not statistically significant, the trends were for higher percent of dietary nitrogen retention with monensin in the silage and grain rations.

TABLE 20. EFFECTS OF MONENSIN AND TYPE OF RATION ON NITROGEN BALANCE AND DIGESTIBILITY IN GROWING STEERS

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	
Nitrogen Balance (g/day):					
N intake	131.73	129.32	158.21	144.40	9.33
Fecal N[a]	44.69	37.01	54.65	48.30	3.24
Urinary N	44.44	44.00	59.40	52.36	7.27
N retained	42.61	48.32	44.16	43.75	4.73
% N digestion[b]	66.07	71.38	65.46	66.60	1.45
% N retention	32.34	37.36	27.80	30.90	3.12
DM Digestion (g/day):					
DM intake	6438.97	6217.25	6812.18	6493.89	388.09
DM excreted[c]	1586.90	1141.86	2022.24	2007.17	126.54
DM digestion	4852.07	5075.39	4789.94	4486.73	298.86
% DM digestion[d]	75.36	81.63	70.31	69.08	1.18
ADF Digestion (g/day):					
ADF intake	670.58	631.74	1680.36	1565.65	62.46
Fecal ADF	359.25	338.43	667.30	636.00	33.74
ADF digestion	311.33	293.32	1013.06	929.65	43.10
% ADF digestion[e]	46.43	46.40	60.29	59.38	3.32

[a]Significant monensin effect ($P < 0.025$).

[b]Significant monensin effect in grain rations ($P < 0.01$).

[c]Significant monensin effect in grain rations ($P < 0.025$); overall ($P < 0.05$).

[d]Significant ration effect ($P < 0.01$); monensin effect in grain rations ($P < 0.05$).

[e]Significant ration effect ($P < 0.01$).

Dry Matter and ADF Digestion

Dry matter and ADF digestibility data are also presented in Table 20. Fecal DM excretion were similar for the high silage rations (2022.24 vs. 2007.17 g/day), but monensin decreased the amount of daily fecal dry matter output with high grain rations (1586.90 vs. 1141.86 gm; $P < 0.01$). Consequently, the trend was for a greater amount feed DM apparently digested in the total tract with monensin in the high grain rations (5075.39 g), in comparison with the other three treatments (4852.07, 4789.94 and 4486.73 gm for HG, CS and CS-M rations, respectively). Apparent dry matter digestion (as % of DM intake) were higher on concentrate rations than on silage (75.36 and 81.63 vs. 70.31, 69.08; $P < 0.01$) and monensin increased DM digestion with steers on HG-M ration (75.36 vs. 81.63%; $P < 0.05$).

Because of the differences in ADF content between the grain and silage rations which resulted in significantly different dietary ADF intakes between the two rations, a direct comparison of the extent of ADF digestion could not be made. It is evident from fecal ADF excretion, and ADF digestion data obtained, that monensin had no effect on both parameters. There were, however, greater proportions of dietary ADF digested in the animals on silage than in those receiving concentrate rations. ADF digestion (as % of intakes) were 46.43 and 46.40 for HG and HG-M rations compared to 60.29 and 59.38 for CS and CS-M rations, respectively ($P < 0.01$).

Fecal Marker and ADF Recoveries

The proportions of feed chromium intake and abomasal ADF recovered in the feces are shown in Table 21. Chromium recovery was higher for the silage than for the grain rations. There were also smaller variations between individual recoveries when the steers received silage. Fecal chromium recovered (as % of intakes) were 74.53, 70.42, 83.31 and 92.31 for HG, HG-M, CS and CS-M, respectively.

In contrast, fecal recoveries of abomasal ADF were generally lower on silage than on grain rations. Percent ADF recoveries were also low in comparison with the values obtained for chromium. This implies either a high degree of post-ruminal ADF digestion or problems with fecal ADF analysis. ADF recoveries (% of abomasal) were 69.60, 63.72, 55.80 and 56.49 for HG, HG-M, CS and CS-M rations, respectively. This means that 30.31, 36.28, 44.20 and 43.51% of ruminal ADF output were digested in the lower gastro-intestinal tract for HG, HG-M, CS and CS-M rations, respectively.

Feeding Trial and Plasma Metabolite Studies

Feeding Trial

As previously reported, the steers were fed basal silage rations consisting of 88% corn silage (35% DM) and 12% soybean meal-mineral supplement during the feeding trial and plasma studies. The composition of the rations are listed in Tables 3 and 5. Control ration consisted of the basal corn silage; monensin ration contained 33 ppm monensin; elfazepam contained 2 ppm elfazepam, and monensin-elfazepam contained 33 ppm monensin and 2 ppm elfazepam.

TABLE 21. FECAL MARKER (CHROMIUM)[a] RECOVERY AND POST-RUMINAL ACID DETERGENT FIBER DIGESTION

Item	Treatment				SEM
	High grain		High silage		
	Without monensin	With monensin	Without monensin	With monensin	
Chromic oxide recovery (%)[b]	74.33 ±8.29	70.42 ±10.45	83.31 ±2.19	92.31 ±1.91	3.41
ADF Recovery:					
Abomasal ADF[c]	515.51	531.16	1195.90	1125.91	60.42
Fecal ADF	359.25	338.43	667.30	636.00	33.74
Fecal ADF recovery (%)[d]	69.69 ±7.41	63.72 ±10.60	55.80 ±5.45	56.49 ±2.85	3.57
Post-ruminal ADF digestion (%)	30.31 ±7.41	36.28 ±10.60	44.20 ±5.45	43.51 ±2.85	3.57

[a]From nitrogen balance trial.

[b]Percent of Cr_2O_3 intake.

[c]Adjusted for ADF intake at abomasal collection.

[d]Percent of abomasal ADF flow.

The overall performance results are presented in Table 22. Initial and final weights were similar across treatments, but monensin-fed steers had the highest average final weight. Dry matter intake (DMI) varied between treatments, but these were not statistically different from control. In comparison with control, DMI was 7.62 kg per day, highest for the elfazepam, while monensin treatment resulted in slightly less dry matter consumed (6.85 kg). Monensin-elfazepam combination resulted in a significantly reduced intake, when compared with elfazepam alone (6.60 kg; $P < 0.05$). Monensin-fed steers had the highest average daily gains (ADG), while the combination of both drugs resulted in the lowest rate of gains. ADG (in kg) were: 0.98, 1.08, 0.99, and 0.86 kg for the control, monensin, elfazepam and monensin-elfazepam supplemented rations, respectively. Monensin treatment also produced the best efficiency of feed conversion, while elfazepam alone produced the poorest. F/G ratios were: 7.20, 6.34, 7.74 and 7.69 for the control monensin, elfazepam and monensin-elfazepam rations, respectively. These differences in ADG and feed efficiency were, however, not statistically significant.

To further evaluate the performance of the steers, ADG and DMI for the different treatment groups were compared through the monthly weighing periods. The trends are graphically illustrated in Figure 3. Some differences in DMI were observed during the first and third months of the experiment. During these periods, monensin-fed steers consumed slightly less feed than the control steers, but gained more. Elfazepam treatment enhanced feed intake most effectively during the first three

TABLE 22. OVERALL DRY MATTER INTAKE, ADG AND FEED EFFICIENCY FOR 118 DAY FEEDING TRIAL

	Treatment			
	Control	Monensin	Elfazepam	Monensin and elfazepam
Initial wt. (kg)	280	278	278	282
Final wt. (kg)	400	409.5	392	385
ADG[a] (kg)	0.98	1.08	0.99	0.86
DMI[ab] (kg/day)	7.02	6.85	7.62	6.60
DM/kg gain	7.20	6.34	7.74	7.69

[a]Least square means.

[b]Elfazepam different from monensin-elfazepam ($P < 0.05$).

months, but produced gains better than controls only during the first two months. Gains in the last half of the experiment were lower. Steers on the two-drug combination showed similar, but much lower intake patterns as the control and monensin groups. However, these animals gained slightly less than the control groups during the first half of the trial and subsequent gains were poor.

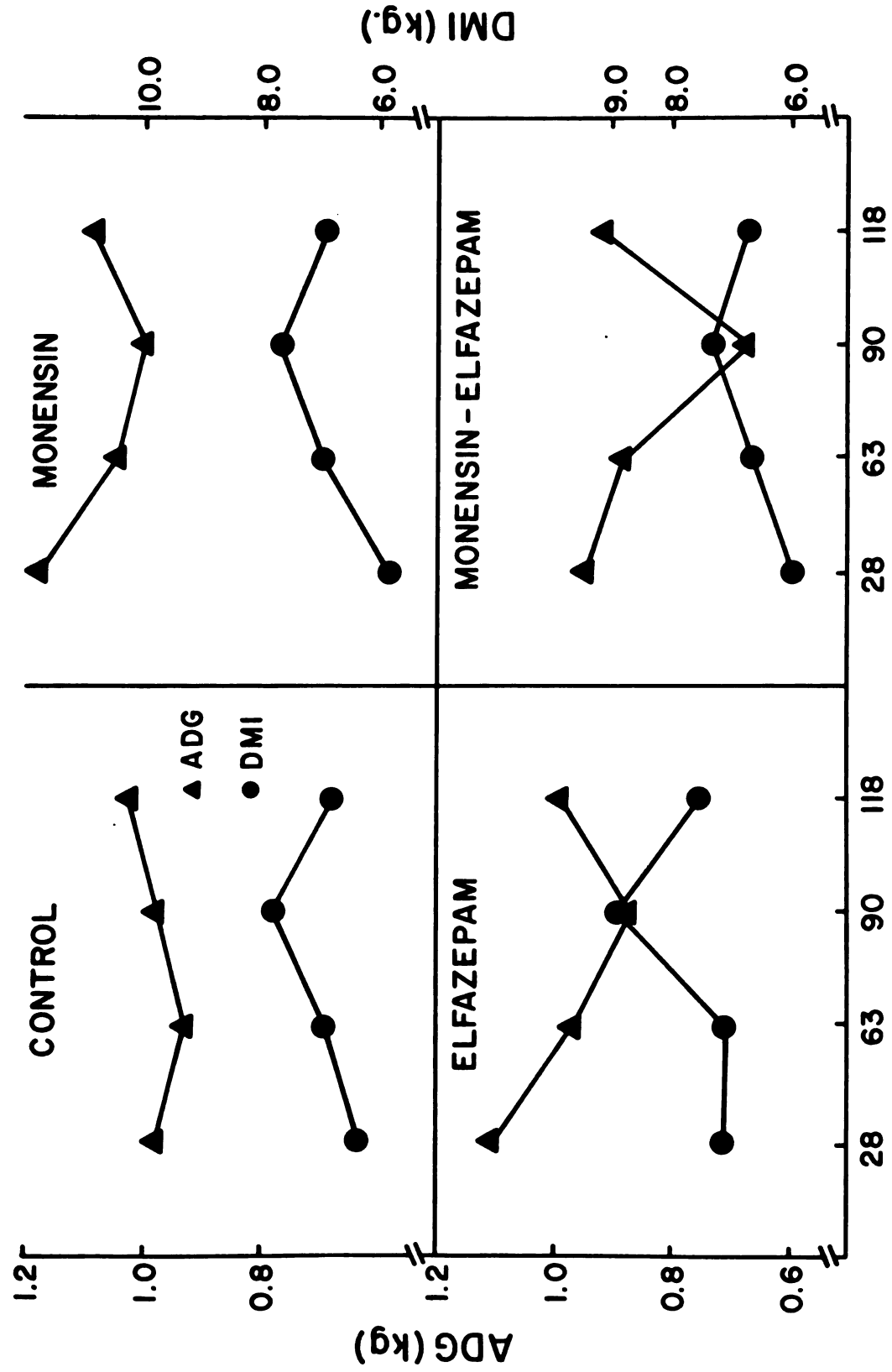

FIGURE 3. ADG, DMI at Day 28, 63, 90 and 118

Plasma Metabolite Studies

The effects of monensin and elfazepam on the overall plasma free amino acid profiles are presented in Table 23. Several of the amino acids most commonly involved in amino-group transfer were similar across treatments, but the trends were for slightly higher levels with monensin in the rations. Proline concentration was also highest with monensin treatment (8.00 μ moles per dl), elfazepam treatment and the two-drug combination also resulted in proline levels slightly higher than controls (7.20 and 7.10 vs. 6.70 μ moles per dl). Glycine levels were highest in elfazepam fed steers; the other treatment groups showed levels similar to control. Alanine levels were highest in the control steers and similar among the three other treatments. Monensin and elfazepam treatments resulted in methionine concentrations similar to control, but slightly less for the two-drug combination; but cystine levels were virtually the same across treatments. Levels of the branched-chain amino acids were similar between rations, with monensin-elfazepam combination producing slightly lower values for valine and isoleucine. Despite the above trends in amino acid concentrations, none of the observed treatment differences were significant ($P < 0.05$).

A summary of the plasma amino acid levels is presented in Table 24. Plasma urea-nitrogen and glucose levels are also reported in the same table. There were no significant differences in either the total essential amino acid (TEAA), non-essential amino acids (NEAA), or total amino acid (TAA) levels between the treatments. The trends were for slightly lower levels in the three parameters, when the

TABLE 23. INFLUENCE OF MONENSIN AND ELFAZEPAM ON PLASMA FREE AMINO ACID CONCENTRATIONS OF STEERS FED SILAGE RATIONS[a]

	Treatment				
	Control	Monensin	Elfazepam	Monensin and elfazepam	SEM
	---------- μ moles/dl ----------				
Aspartate + Asp-NH_2	3.20	3.50	3.10	3.20	0.20
Threonine	6.00	6.40	6.30	6.00	0.50
Serine	5.90	6.50	5.90	5.80	0.40
Glutamate + Glu-NH_2	26.30	26.90	26.60	25.80	1.20
Proline	6.70	8.00	7.20	7.10	0.50
Glycine	24.00	24.30	26.00	24.30	1.40
Alanine	25.10	24.30	23.70	22.80	1.40
Valine	21.50	21.10	21.70	20.50	0.90
Methionine	2.20	2.10	2.10	1.80	0.10
1/2 Cystine	1.60	1.60	1.54	1.60	0.10
Isoleucine	10.30	10.10	10.00	9.20	0.50
Leucine	11.40	11.70	11.50	11.50	0.50
Tyrosine	4.66	4.58	4.50	4.26	0.20
Phenylalanine	4.47	4.65	4.61	4.41	0.20
Lysine	9.90	9.70	9.60	8.30	0.60
Histidine	6.00	5.90	6.40	6.30	0.30
Arginine	10.40	9.70	9.60	8.60	0.50
Total amino acids	178.90	180.90	180.30	170.70	6.20

[a]All treatment comparisons N.S. at $P < 0.05$.

Table 24. PLASMA METABOLITE LEVELS IN STEERS FED MONENSIN AND ELFAZEPAM[a]

	Treatment				
	Control	Monensin	Elfazepam	Monensin and elfazepam	SEM
	---------- μ moles/dl plasma ----------				
Essential amino acid	82.10	81.30	81.80	76.60	3.00
Non-essential amino acid	96.80	99.50	98.50	94.10	3.80
Total amino acid	178.90	180.90	180.30	170.70	6.20
	---------- mg/dl plasma ----------				
Urea-nitrogen	12.10	13.50	12.20	13.60	0.46
Glucose	98.80	95.70	93.50	93.40	3.80

[a]All comparisons N.S. at $P < 0.05$.

two drugs were combined. These treatment comparisons were also not significant ($P < 0.05$).

With plasma urea levels, elfazepam group had levels similar to control (12.20 vs. 12.10 mg/dl), while the monensin and monensin-elfazepam groups gave similar, but slightly higher levels (13.50 vs. 13.60 mg/dl). These differences were also not significant ($P < 0.05$).

Plasma glucose levels were relatively high in all treatments. Levels were highest in the control steers and similar among the three treatment groups. Glucose levels (in mg per dl plasma) were: 98.80, 95.70, 93.50 and 93.40 for the controls, monensin, elfazepam and monensin-elfazepam treatments, respectively.

Figure 4 represents a graphic illustration of the changes in the levels of plasma valine, leucine and isoleucine with time through the monthly sampling periods. There was an observable decline, from days 28 to 63, in plasma valine and isoleucine of those animals on the two monensin-supplemented rations. Steers on elfazepam alone, showed the same patterns in the time trends for the three amino acids as the controls.

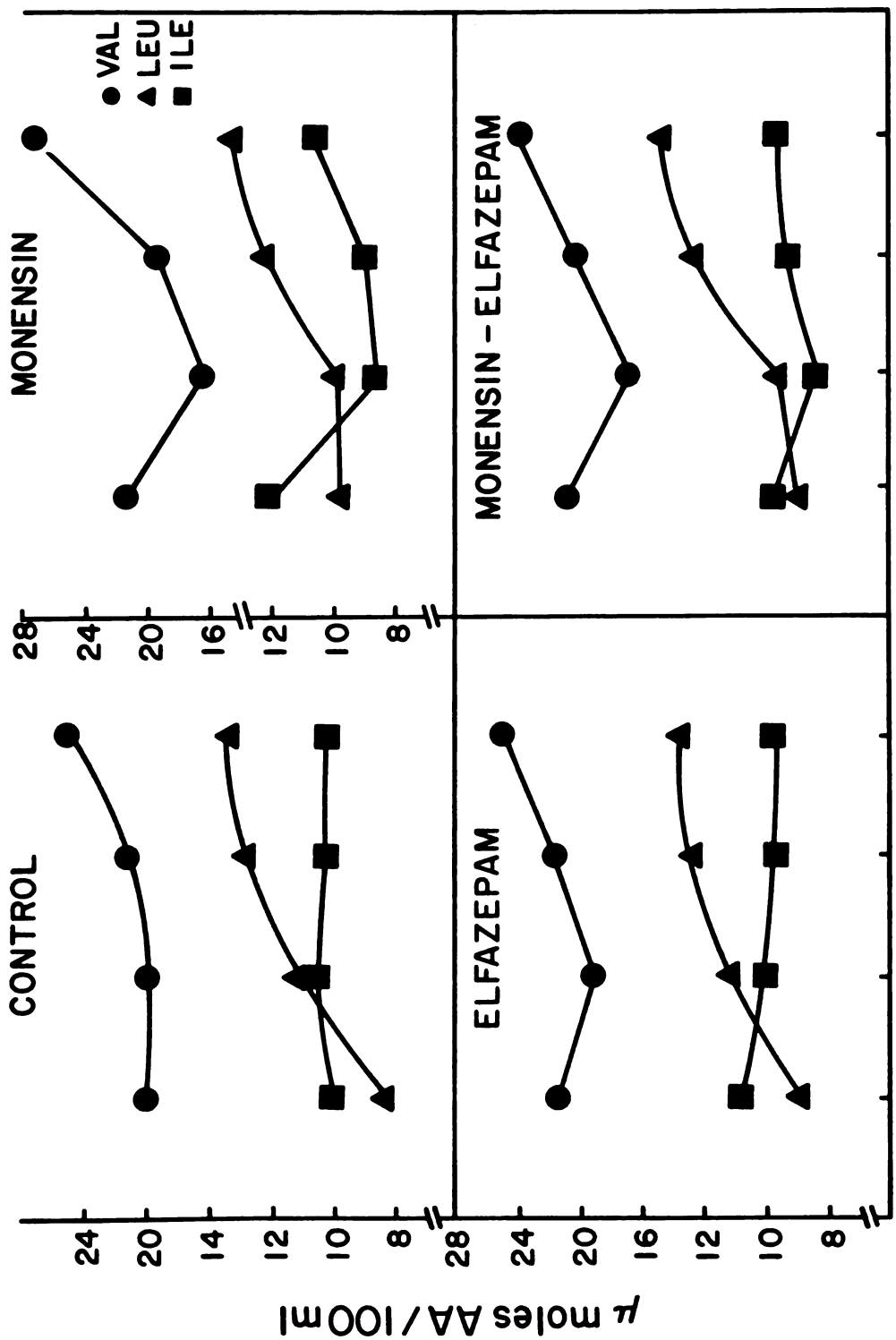

FIGURE 4. Time Trends for Valine, Leucine and Isoleucine

CHAPTER V

DISCUSSION

Digesta Passage Studies

The digesta passage data indicate that, with the grain ration, monensin did not significantly affect the amount of dry matter apparently (and truly) degraded in the rumen. With the silage ration monensin increased the extent of ruminal degradation of the dry matter consumed by the steers. Based on lignin passage to the abomasum, % DMI truly digested in the rumen was similar for the grain rations (HG, 44.31 vs. HG-M, 47.16%), and increased with monensin added to the silage (37.07 vs. 45.41%). True DMD estimates from chromium passage followed the same pattern. These were: 46.05, 44.90, 37.92 and 47.95% for HG, HG-M, CS and CS-M rations, respectively. Apparent ruminal fiber (ADF and NDF) digestibility also followed the above trends. The trends were for lower apparent ADF digestion with monesin rations.

Both the apparent and true rumen DM and fiber digestion appear to be somewhat low in this study. Such values have, however, been reported for concentrate rations (Orskov et al., 1971; Nicholson and Sutton, 1969; Kennedy et al., 1976), and for rations containing high amounts of roughages (Cole et al., 1976, Kropp et al., 1977a, 1977b). These workers (Cole et al., 1976) progressively increased the roughage content of corn rations from 0 to 21% by adding cottonseed hulls, and obtained an almost linear decrease in rumen DM digestion, ranging from

58.9 to 43.4%. Some other workers (Drennan et al., 1970) have even obtained negative net ruminal DM digestion using duodenal spot sampling techniques, with chromic oxide (corrected for 100% chromium recoveries) as digesta marker, while at the same time reporting plausible rumen digestibility estimates when lignin was used. Kropp et al. (1977a) reported a 34% apparent ruminal DM digestion of roughage rations supplemented with soybean meal.

Despite the relatively low ruminal dry matter and fiber digestion obtained in the present study, a comparative assessment of abomasal passage of the various components of digesta determined from both lignin and chromium flows through the abomasum, indicate a close agreement between the two digesta markers. It is not unlikely that variations between and within the experimental animals in the rates of digesta flow, coupled with the spot-sampling method used to obtain abomasal digesta samples may have been responsible for the low rumen digestibility estimates obtained.

Fluctuations in abomasal digesta flow rates were commonly observed throughout the experiment. Despite the attempts to simulate a three hourly abomasal collection interval (extended over a four-day period), there were periods during the trial when digesta flows were so minimal that sufficient digesta samples could not be obtained to make up the digesta composite. This situation was also more prevalent with specific animals during the trial. Abomasal samples taken during periods of reduced digesta passage would disproportionately contribute to a composite sample, while samples obtained during periods of elevated digesta flow, would be underrepresented in the composite. However,

attempts were made during the trial to keep digesta sampling in sequence with the waves of omaso-abomasal contractions.

Other problems which may contribute to low ruminal digestibility estimates may arise from the laboratory analytical procedures employed. KM_nO_4-lignin technique was used to determine the lignin content of abomasal digesta, and this method, rather than the acid-lignin method is more likely to result in an overestimation of digesta lignin, due to the fact that some of the reagents utilized can also degrade the expected residual fiber component (cellulose) after the delignification process. (For details, see Literature Review: Influence of Markers on Digesta Passage Studies.) In contrast, several investigators have suggested that chromic oxide flows independently of both the liquid and solid portions of digesta, with a tendency toward a faster ruminal outflow rate than both digesta pools (Faichney, 1975; MacRae, 1975; Drennan et al., 1970). This would also result in high post-ruminal chromium recoveries and consequently, decreased rumen digestion estimates.

In spite of all the above marker problems, it was evident from the results obtained in the present study, that monensin did not affect rumen DMD of the grain ration. This is contrary to results reported by other workers (Owens et al., 1979). In another study (Lemenager et al., 1978), no significant differences in rumen DM digestion were reported in steers fed high energy rations with and without monensin, although estimates of non-microbial-ruminal dry matter were not reported. Owens et al. (1979) reported a 10% decrease in rumen DM digestion for steers

fed monensin and high energy rations. Feed DM intake was kept constant during the trial, and that may explain the relative reduction in rumen DM digestion. In contrast, the animals were fed ad libitum throughout the present study and consumed 4 to 9.5% less feed DM when monensin was included in the rations. Reductions in feed intake could increase rumen DM retention time, thus increasing ruminal digestion (Owens and Isaacson, 1977). However, the more probable explanation was that in this experiment, the animals were adapted to each ration for 28 days prior to abomasal digesta collection. By that time, feed intake on monensin rations had gradually increased again, after the initial 12 to 18% reduction observed when the animals were first placed on monensin-supplemented rations. Other workers (Dinius et al., 1976; Van Nevel and Demeyer, 1977; Chen and Wolin, 1979) have reported that monensin does not inhibit microbial fermentation of energy substrates when incubated with cultures from animals previously adapted to monensin. But the significant increase in ruminal DM digestion of the monensin-silage rations found in this study, may also be related to a longer rumen solid retention time of roughages (Bergen and Yokoyama, 1977), together with a slower rumen solid turnover reported to occur when monensin is added to rations (Lemenager et al., 1978).

Digesta Flow Rates

Total digesta flows were quite variable between steers for each ration fed, but there were no significant interaction of monensin with ration. Monensin did not affect total digesta flow rates, even though ruminal digesta outflows were higher for silage than for the high grain

rations (42.42 to 43.28 vs. 59.52 to 62.31 liters per day--mean of combined lignin and chromium abomasal flows). Total digesta passage estimates from ruminal chromium outflows closely agree with those determined from lignin passage (Table 6). Mean digesta flow rates obtained for high grain rations (HG, 42.42 vs. HG-M, 43.28) were 23 to 30% lower than digesta flow rates reported by other workers (Poos et al., 1979) for a concentrate ration with or without monensin. A direct comparison is, however, not realistic because Poos et al. (1979) maintained the animals on a constant level of feed intake, using a continuous feeding system, and fed supplemental protein sources which were slowly degraded in the rumen (Brewers dried grains). Increased feeding frequency may therefore have increased the rates of digesta passage from the rumen in that study. However, those workers (Poos et al., 1979) also observed no monensin effect on the rates of digesta passage; and that agrees with the result obtained in this study. Some other investigators (Lemenager et al., 1978; Owens et al., 1979) reported that monensin significantly depressed ruminal fluid turnover rates in steers fed high roughage and concentrate rations. Lemenager et al. (1979) also found that feed intake, and rumen liquid volume (even though unusually high with the forage rations) were reduced by monensin. They, however, attributed the excessive rumen liquid volume to incomplete rumen PEG equilibration. With concentrate rations, Lemenager et al. (1979) related the reduced rumen liquid outflow rates by monensin, to other factors besides the observed depressions in feed intake. It is, however, interesting that rumen liquid volumes for

monensin-fed steers were 9 to 28% higher than in the control animals in that experiment. But those workers did not indicate any possible relationship between those results to the slower rumen fluid turnover rates they obtained when monensin was fed.

Liquid digesta passage estimated from abomasal PEG flows are presented in Table 6. These estimates are 30 to 40% higher than total digesta flows obtained from abomasal lignin and chromium passage. Hume et al. (1970) reported a 10% faster mean ruminal PEG dilution rate relative to lignin. Their data also showed wide variations between individual estimates of digesta flows determined from ruminal PEG disappearance, with some estimates 20 to 60% higher than their corresponding lignin-based estimates. Harrison et al. (1975) showed that rumen dilution rate could be increased by intraruminal infusion of artificial saliva. They also reported further increases by addition of PEG to the saliva.

Such results question the validity of PEG as a fluid marker in digesta passage studies. Excessively high PEG flows may, however, indicate an underestimation of PEG in rumen or abomasal digesta. This is a likely possibility, because it has been reported that several digesta components such as proteins and by-products of enzyme degradation in the GI tract do interfere in laboratory analysis of PEG in digesta fluids (Smith, 1959; Ingham and Ling, 1978).

In the present study, abomasal passage of the various components of digesta was estimated using chromium, lignin and PEG as digesta markers. Estimates of nitrogen passage from lignin and

chromium flows were very similar (Tables 9 to 12), and generally agree with results obtained by other workers from studies in which similar levels of dietary crude protein (12 to 13%) were fed to growing ruminants (Ørskov et al., 1971; Leibholz and Hartmann, 1972; Hume et al., 1970). In contrast, nitrogen passage data estimated from PEG flows were much higher, and showed excessive net abomasal gains relative to N intake (Table 13). Such ruminal nitrogen outputs are expected and have been reported with diets containing low levels of nitrogen (Hume et al., 1970; Ørskov et al., 1971). The suspicion, therefore, was that ruminal PEG outflow was excessively high in this study and resulted in an overestimation of the amounts of various components of digesta leaving the rumen. Since PEG estimates obtained by analysis of PEG in the experimental rations used were in close agreement with the calculated amounts added (Table 4), it was assumed that abomasal PEG recoveries were relatively low. PEG analysis of abomasal digesta samples may have been hindered by the presence of the interfering materials discussed above. It was therefore decided to use only lignin and chromium flow data for the calculation of abomasal passage of the various components of digesta.

Nitrogen Metabolism Studies

Ruminal Nitrogen Losses

Results of the present studies indicate that net ruminal losses of dietary nitrogen occurred to a varying extent with each ration fed (Tables 9 to 12). Based on the combined estimates from lignin and chromium flows, 8.0 to 8.9% of feed nitrogen consumed

was lost during rumen digestion in the animals consuming rations not supplemented with monensin. Steers on monensin-supplemented rations showed only a ±1.80% net change in abomasal nitrogen flow over their dietary nitrogen intakes. Net feed nitrogen losses in the rumen were also slightly higher for concentrates than for silage rations.

Losses of dietary nitrogen during ruminal digestion have generally been reported for rations containing more than 10 to 11% crude protein, even though these rations may contain adequate amounts of digestible energy to meet the energy needs of the rumen microorganisms (Hume et al., 1970; Leibholz and Hartmann, 1972). With such rations, post-ruminal N and NAN flows are often lower than the amounts of feed nitrogen consumed by the experimental animals.

The extent of ruminal nitrogen loss is controlled by factors which regulate the efficiency of rumen microbial protein synthesis. The relationship between rumen nitrogen utilization and dietary protein, energy and co-factors, have already been reviewed. Other equally important considerations include the degradability (or solubility) of the feed protein source and its retention time in the rumen (Bergen and Yokoyama, 1977). Ørskov et al. (1971) reported a 7 to 10% dietary nitrogen loss when sheep were fed maize and barley rations containing up to 15% crude protein. As the soy protein of the ration was increased, the extent of ruminal nitrogen loss was reported to also increase, especially with rations containing high levels of crude protein (Ørskov et al., 1971; Hume et al., 1970). Soybean meal and similar protein sources such as peanut meal are extensively degraded

in the rumen (Mathers et al., 1976; Sniffen, 1978). In contrast, less degradable proteins such as fish meal or zein are less prone to extensive ruminal nitrogen losses (Ørskov et al., 1974; Mathers et al., 1976; Hume, 1974). It is also unlikely that ruminal nitrogen losses from corn silage rations would be as high as losses from corn-soy rations. Fermented feeds such as corn silage have been shown to contain 40 to 50% water soluble nitrogen (Bergen et al., 1974; Wilkinson et al., 1976), which are not readily degraded to ammonia in the rumen (Bergen et al., 1974). This is consistent with results obtained from this study. Overall, slightly less ruminal nitrogen losses were found to occur on corn silage than on the high grain rations.

With rations containing crude protein levels higher than 10 to 11% rumen microbes would produce ammonia in excess of their needs, and it has been shown that the rate of rumen ammonia release is proportional to the dietary protein content, rather than to microbial requirements for nitrogen (Laughren and Young, 1979). Extensive ruminal nitrogen losses with high quality feed proteins such as soybean may limit the amount of total protein intake that becomes available to the animal, thus limiting the efficiency of feed protein utilization and consequently, the performance of the animal.

This may be of significance only in very high producing (dairy) cows since ordinarily, the beef animal would obtain sufficient nitrogen from such a ration to maintain its optimal rate of growth.

It is evident from this study that monensin reduced the extent of dietary nitrogen loss in the rumen (Tables 9 to 12). This agrees with the results obtained in other nitrogen passage studies (Poos et al., 1979; Hanson and Klopfenstein, 1979). Monensin has been reported to cause a decrease in the rates of rumen microbial proteolysis and deamination of feed protein (Van Nevel and Demeyer, 1977). Rumen ammonia levels were reported to decrease with addition of monensin to ruminant rations (Dinius et al., 1976; Thornton et al., 1976; Poos et al., 1979). Rumen ammonia levels were not determined in this experiment, but abomasal ammonia levels were slightly lower in monensin-fed steers. However, these may not accurately reflect the relative ruminal ammonia concentrations, since ruminal and omasal ammonia absorption rates may vary (Nolan, 1975). Reductions in dietary nitrogen losses in the rumen by monensin would enhance the efficiency of feed protein utilization in the ruminant given rations containing high quality and more degradable preformed plant proteins, especially when dietary nitrogen levels are low.

NAN Passage to the Abomasum

Abomasal NAN passage data are presented in Tables 9 to 13. As previously noted, estimates of NAN passage calculated from abomasal lignin and chromium flows (Tables 9 vs. 11), were in close agreement, and those calculated from N:marker ratios were also similar (Tables 10 vs. 11). Based on the combined average of the four, NAN passage (as % of N intake) were: 87.88, 97.34, 88.79 and 98.24 for HG, HG-M, CS and CS-M, respectively. These estimates for the control grain and

silage rations (HG and CS) are higher than those obtained by Crickenberger et al. (1979) for steers fed similar rations although the high grain rations used in their study contained about 42% corn silage DM (HG, 87.88 vs. 50.90%; CS, 88.79 vs. 77.50%). Crickenberger et al. (1979) also encountered digesta sampling problems and varying abomasal lignin recoveries.

In contrast, others have reported abomasal NAN recoveries within the range of estimates obtained in the present study, using rations containing similar crude protein concentrations (Hume, 1970d; Nicholson and Sutton, 1969; Ørskov et al., 1971; Prigge et al., 1978; Kropp et al., 1976b). Hume (1970d) fed urea, casein, gelatin and zein as alternative proteins to sheep on rations containing about 14% CP. He recovered 82.5, 100.6, 91.8 and 135.2% of the dietary N from the four respective rations as abomasal NAN. Ørskov et al. (1971) recovered even higher amounts with barley-soybean rations. In steer nitrogen passage studies, Kropp et al. (1977b) recovered 99.7 to 136.0% of the feed N as abomasal NAN using roughage rations supplemented with soybean meal or urea (8.55 to 12.76% CP).

The increased recovery of NAN with monensin-supplemented ration found in the present study, is consistent with previous findings that monensin reduces the rate of protein degradation in the rumen. Poos et al. (1979) also reported increases in abomasal NAN passage in steers fed concentrate rations containing monensin. Daily abomasal NAN passage (as gm crude protein) from this study were: 742.3, 797.6, 826.3 and 843.6 for HG, HG-M, CS and CS-M rations, respectively. These amounts

of total daily protein are sufficient to meet the maintenance and growth protein needs of steer calves, estimated to gain between 0.9 to 1.3 kg per day, based on NRC estimates. The extra protein passage obtained in monensin-fed steers may be realized in terms of additional growth, only if the net efficiencies of N utilization are generally low, due to high maintenance costs (e.g., stresses due to confinement). Otherwise, the expected optimal growth rates would be ≤1.1 kg per day, and the NAN passage estimates obtained from all the rations would seem adequate for optimal growth.

Based on the combined estimates from lignin and chromium flow data, feed N contributed 30.53 and 28.23% to abomasal NAN passage in the steers fed the control rations (HG or CS). The corresponding values for monensin rations were 39.89 and 43.00% (HG-M or CS-M). Therefore, 26.82 and 25.07% of feed N passed the rumen undegraded in steers receiving HG and CS, respectively. Feed N bypass values for HG-M and CS-M were 38.83 and 42.24%, respectively. These estimates are comparable to those reported from digesta passage studies in which grain and roughage rations, supplemented with soybean meal (Kropp et al., 1977a, 1977b; Cole et al., 1976) or rations containing monensin (Poos et al., 1979) were fed to growing steers. Soy and ground-corn proteins have been estimated to be 60 to 90% degraded in the rumen (Mathers et al., 1976; Ørskov et al., 1971) while ruminal degradation of corn silage was estimated to be lower--50 to 60% (Ørskov and Mehrez, 1977). These estimates do not differ between the two ration-types used in this study, but monensin increased feed protein bypass by 12 and 17% with the high grain and silage rations, respectively.

In contrast to this increase in feed N passage observed, monensin reduced the amount of microbial nitrogen leaving the rumen. Microbial N as a percent of abomasal NAN were: 69.48, 60.1, 71.77 and 57.70 for the combined mean estimates from steers fed HG, HG-M, CS and CS-M, respectively. This observation agrees with the results reported by others from in vivo nitrogen passage studies (Poos et al., 1979). Similar findings have also been reported from in vitro studies with monensin (Bartley et al., 1979; Chen and Wolin, 1979). In the present study, monensin decreased microbial N passage by 9.48 and 14.07% for the grain and all silage rations, respectively. This reduction in microbial N flow should be offset by the increases in feed N bypass observed in this experiment, if the nutritional quality of the bypassed feed N is similar to that of microbial N. The nutritional quality of soybean meal is only slightly lower than that of microbial protein (Bergen et al., 1978). However, some corn protein and water soluble nitrogen would also be expected to pass the rumen undegraded, or partially degraded. The nutritional value to the animal of the amino acids and aromatic nitrogen constituents of water soluble nitrogen fraction of silages has not been clearly determined.

But the added advantage of monensin in this experiment is that these nitrogen passage estimates were obtained, despite reductions in nitrogen and DM intake observed when the steers received monensin rations. The net gains of monensin supplementation in this study should therefore be realized primarily in terms of reduced feed costs and enhanced efficiency of feed N utilization.

Efficiency of Rumen Microbial Protein Synthesis

As indicated earlier, there was no significant monensin effect on the amount of organic matter digested in the rumen. However, since there were differences in daily microbial nitrogen passage to the abomasum, lower efficiencies of ruminal microbial protein production were obtained with monensin supplemented rations.

Estimates of percent apparent rumen organic matter digestion in the present study are similar to those reported in other studies for steers fed roughage (Kennedy, 1980; Thomson et al., 1972), and concentrate rations (Kropp et al., 1977a, 1977b), but lower than values obtained from studies in which sheep were given concentrate or semi-purified diets (Orskov et al., 1971; Hume, 1970a). Percent organic matter actually digested in the rumen was slightly higher for rations containing monensin, although these estimates were not statistically different (avg. 50 vs. 44%). As was previously explained for ruminal DM digestion estimates, the steers were allowed a 28 day adaptation to the rations, before digesta samples were collected. The results obtained from the present study do not contradict the conclusion reached by other investigators that monensin decreases the rate of rumen microbial protein breakdown, without inhibiting microbial fermentation rate (Dinius et al., 1976; Van Nevel and Demeyer, 1977). Since monensin also decreased ruminal microbial protein output in this study, the net effect was a decrease in the efficiency of microbial synthesis.

Microbial crude protein synthesized per 100 gm of organic matter actually digested in the rumen, were lower for rations containing monensin than for the controls (Tables 17 and 18). The mean combined estimates from the four quantitation methods used were 16.76 g for monensin-supplemented rations and 20.24 g for the controls. True microbial efficiencies were, however, only slightly reduced by monensin in the grain ration (19.85 vs. 18.44 g per 100 g OMD), and more drastically reduced in monensin-silage ration (20.63 vs. 15.07 g/100 g OMD). Perhaps rumen ammonia levels were less than optimum in the steers fed the CS-M rations. Since a major proportion of silage nitrogen consists of the ruminally less degradable water soluble nitrogen (Bergen et al., 1974), this, in addition to the reduced rate of feed protein degradation by monensin, may have created suboptimal rumen ammonia levels which limited microbial growth.

Van Nevel and Demeyer (1977) had previously reported that monensin may inhibit ruminal protein deamination, rather than proteolysis. They further suggested that peptide uptake by bacteria may be another step in bacterial protein metabolism that is also inhibited by monensin. Bartely et al. (1979) observed decreases in the rate of urea hydrolysis by monensin in vitro. Such findings, together with previous reports of reduced rumen ammonia levels by this antibiotic (Dinius et al., 1976; Thorton et al., 1976), suggest that the generation of ammonia in the rumen may be one step at which monensin interferes with microbial growth. Since the results presented here and those from other studies (Van Nevel and Demeyer,

1977) indicate that monensin does not impede microbial fermentation of organic matter, ammonia supply may be limiting the growth of many rumen bacteria to such an extent that energetic uncoupling occurs. This would inhibit bacterial growth since many rumen bacteria use ammonia as their preferred nitrogen source, even in the presence of amino acids (Bryant and Robinson, 1962).

Energetic uncoupling occurs with most bacteria when their potential to produce energy from substrates exceeds their ability to utilize that energy for biosynthetic purposes (Hespell and Bryant, 1979). As a result of such an ammonia insufficiency as proposed above, microbial cell yield would decrease without a concomitant decrease in fermentation rate (Hespell and Bryant, 1979). Alternatively (or in addition to the above), monensin may also affect the rate of amino acid and peptide (as well as other nutrient) uptake by rumen microbes and cause a growth rate depression.

Other investigators have offered various suggestions as to the mode of action of this antibiotic. Chen and Wolin (1979) concluded from their in vitro studies that monensin may act by selectively inhibiting, to a variable extent, the growth of certain bacteria species. They further hypothesized that monensin may act in the rumen by selecting for a microbial population that produces more propionate.

Nucleic Acid Methodology

The use of bacterial RNA-N:total N ratios to estimate the proportion of microbial N passage from the rumen, hinges on the assumption that this ratio is similar to that of the microorganism

passing through the abomasum. The validity of that assumption depends on the similarity of this ratio between rumen protozoa and bacteria and also on the contribution of protozoa to post-ruminal digesta flow. Contrary to the findings of Smith et al. (1978), RNA estimates of protozoal preparations isolated from rumen contents obtained in this study were much lower than the corresponding estimates from bacterial preparations. A similar trend was indicated in the data presented by Ling and Buttery (1978), if it is assumed that total N content of the bacteria and protozoa they isolated in that study were similar. The use of bacterial RNA-N:total N ratio alone to estimate microbial N passage to the abomasum in this study would therefore underestimate microbial passage to a varying extent, depending on the rate of protozoal N exit from the rumen of each experimental animal with each of the rations fed.

The assumption that all the nucleic acid passing out of the rumen is microbial in origin has also been disputed by other investigators (Ling and Buttery, 1978; Smith et al., 1978). These workers compared duodenal microbial-N passage data estimated by the RNA approach with the same estimates obtained using ^{35}S or ^{32}P labels incorporated into microbial cells and observed that RNA-based estimates were consistently higher. Smith et al. (1978) found that RNA-based estimates of duodenal microbial N flow were consistently about 15% higher than those determined by the radioisotope incorporation method and hence corrected duodenal RNA values by multiplying by 0.85 (adjusted RNA). Smith et al. (1978) reasoned that this correction approach would equate

RNA-based microbial N flow with the more quantitatively accurate ^{35}S incorporation method (Ling and Buttery, 1978). They (Smith et al., 1978) also concluded that some dietary RNA may have escaped degradation in the rumen. Based on the above considerations and also on the fact that RNA estimates from abomasal digesta in the present study appeared exceedingly high and, in a few cases, resulted in calculated microbial NAN passage equal to (or greater than) total abomasal NAN passage, the adjusted RNA values were used to determine microbial contribution to abomasal NAN flow. Estimates of RNA in bacterial preparations in this study gave RNA-N:total N ratios within the range of those obtained by Smith et al. (1976; 1978) from animals fed similar rations (0.156 vs. 0.12 to 0.19), even though the extensive ion-exchange procedure was eliminated from our analysis.

Wide variations in RNA values between and within digesta samples obtained from individual steers were observed. Such problems were also encountered by others (Ling and Buttery, 1978; Smith et al., 1978). RNA concentrations determined from oven-dried abomasal digesta samples were 20 to 30% higher than the corresponding values from undried abomasal digesta samples. The reason for such a discrepancy is not clear. It is possible that more of the lipid/pigment and other materials which absorb light at 260 nm were not removed to the same extent from both samples during the initial extraction with organic solvents. RNA values from undried abomasal digesta were also less variable within animals and were therefore used for subsequent calculations.

Nitrogen Balance and Digestibility Studies

The nitrogen balance trials were conducted to examine the effects of monensin on overall dry matter digestibility and efficiency of nitrogen utilization. Monensin reduced the amount of nitrogen excreted in the feces without increasing urinary nitrogen excretion. Nitrogen digestion as a percent of N-intake was higher for HG-M than for HG rations (71.38 vs. 66.07), and similar for CS and CS-M rations (65.46 vs. 66.60, respectively). This increase in N digestion by monensin in the grain ration also resulted in an increase in the percent of N-intake retained, although these were not significant. Poos et al. (1979) reported increases in apparent N digestibility and retention in lambs fed monensin in concentrate rations in one trial, although these authors observed the opposite effect in another balance study. In the present study, the trend was for an increase in the amounts of nitrogen retained with HG-M and CS-M rations, which may be attributed primarily to increases in total N digestion. There is no ready explanation for these results. Monensin-fed steers consumed less amount of feed DM and protein since the rations were offered ad libitum. It may be that the feed protein that bypassed ruminal degradation with monensin rations was high in nutritional quality. This protein, together with microbial protein, may have been more digestible in the gastrointestines. As indicated above, the slightly higher (though not statistically different) percent N digestibility and retention with CS-M ration (in comparison with control silage) may simply be due to a lower N intake by the steers when fed the

CS-M ration (see Table 20). The data were not adjusted to equal intakes. However, the above speculation may be valid for HG-M versus HG rations, although N retention for the grain rations were also not statistically different.

The estimates of DM digestion followed the same pattern as that observed in N digestibility. Monensin increased DM digestibility of the high grain rations (75.36 vs. 81.63%), but did not affect corn silage dry matter digestion. There was also no monensin influence on ADF digestibility. ADF digestibility values were: 46.43, 46.40, 60.29 and 59.38 for HG, HG-M, CS and CS-M rations, respectively. The higher amounts of ADF digested in the silage rations may indicate a longer rumen retention time of forage particulate matter (size factor), and perhaps a more extensive silage fiber digestion in the lower G.I. tract. The latter observation appears to be a credible explanation, in view of the percent fecal ADF recoveries obtained (Table 21). Fecal recoveries of abomasal ADF were about 10% higher for CS and CS-M rations, than for HG and HG-M rations. This implies that about 10% more ADF was digested in the lower G.I. tract of the steers when they received the silage rations.

Nitrogen retention and DM digestibility estimates obtained in the present study are about 5% higher than those reported by Poos et al. (1979) for lambs fed monensin and concentrate rations, but are similar to values obtained by Crickenberger et al. (1979) for growing steers receiving similar grain and silage rations (without monensin). Poos et al. (1979), however, reported reductions in DM and ADF

digestibility in lambs fed monensin and concentrate rations. The latter investigators also observed no monensin effect on both parameters, when the lambs were fed for a longer period. This latter observation may explain the absence of a significant monensin effect on DM and fiber digestion in this study since, as previously reported, the animals were allowed a relatively long period to adapt to each of the rations. Dinius et al. (1976) also reported that monensin did not affect DM and fiber digestion with steers adapted to monensin rations for 21 days.

As was previously stated, microbial protein passage to the abomasum was reduced in the steers that received monensin (especially in the silage rations). Yet those steers had a slightly greater amount of nitrogen retained. A possible explanation is that the increased abomasal NAN passage with HG-M and CS-M must have compensated for reduced microbial-N passage in steers given those rations. Since microbial protein is high in nutritional quality, the protein that bypassed ruminal degradation with monensin rations must have had a quality comparable to that of microbial protein. Perhaps more soybean, which has a slightly lower nutritional quality than microbial protein (Bergen et al., 1978) escaped ruminal degradation in monensin rations. On the other hand, maybe the bypassed protein and the microbial protein blended better in terms of the total amino acid profile.

The amino acid profile of bypass protein and the relative proportion of that protein that is unavailable to the animal (bound

protein) should both be considered when assessing the nutritional value of that protein to the animal. In this regard, soy protein contains only about 2.5% bound protein, and the amino acid profile of its total and insoluble protein (75% of the total) are quite similar (Sniffen and Hoover, 1978). The animal would therefore benefit from an increase in soy protein bypass.

The amount of nitrogen retained by the animal is an approximate indicator of the efficiency with which a dietary nitrogen source is utilized. It is possible to estimate from the amounts of nitrogen retained (NR), the expected daily weight gains (DWG) of the steers for each of the rations fed during the nitrogen metabolism studies. This was calculated from the following equation, which converts nitrogen retention into muscle protein accretion (muscle protein was assumed to contain 80% water, although this should be lower in the yearling steers):

$$DWG\ (kg) = NR\ (kg) \times 6.25 \times 5.$$

Based on such calculations, the expected daily weight gains on the HG, HG-M, CS and CS-M rations were 1.33, 1.51, 1.38 and 1.37 kg, respectively. The values are slightly higher than the previous estimates from abomasal crude protein passage (see discussion: NAN Passage). The NAN passage data are closer to the actual daily weight gains obtained during the study. The steers gained about 0.97 kg per day on HG and HG-M rations combined. The effect of monensin on ADG during the periods the steers received the grain rations cannot be

determined because the animals were not weighed at the end of period 1, when the grain rations were switched. But ADG on the CS and CS-M rations were 0.87 and 0.92 kg, respectively, and these also fall within the range of daily gains estimated from the abomasal crude protein flows (and NRC tables). In addition to the fact that nitrogen retention tends to overestimate weight gains, such deviations from the expected growth rates may also relate to the stresses of confinement to which the steers were subjected during the entire study. The slightly better performance observed with the high grain rations may reflect a relatively higher efficiency of corn nitrogen and energy utilization for rumen microbial synthesis.

Feeding Trial and Plasma Metabolite Studies

Feeding Trial

The indication from the results presented in Table 22 is that the combination of monensin and elfazepam resulted in the highest reduction in feed intake. In comparison with controls, monensin-elfazepam treatment reduced intake by 8.0%, while monensin alone resulted in a 3% reduction in feed consumption. In contrast, elfazepam alone increased feed intake by 8.5%. Despite this stimulation of intake, elfazepam did not significantly affect ADG (0.99 vs. 0.98 kg for the control). Monensin improved ADG by 10%, while monensin-elfazepam depressed ADG by 12%. Monensin treatment also resulted in the best efficiency of feed conversion (12.5% improvement over the control treatment), while elfazepam and the two-drug combination each produced F/G ratios that were 8% poorer than the controls.

Elfazepam has long been recognized as a potent feed intake stimulant when given to various animals (Baile et al., 1976; Wise and Dawson, 1974; Dinius and Baile, 1977). Dinius and Baile (1977) fed elfazepam at increasing dosage levels to steers on a ground hay ration and reported 26.8, 7.3 and 16.4% improvement in ADG, feed intake and feed efficiency, respectively. They found no differences in response between the different levels (up to 2 ppm) of elfazepam fed. The only major similarity between their findings and the results obtained in the present study is that elfazepam stimulated intake in this study. However, differences in both performance results may be due to differences in the type of rations used in the two studies.

Prior et al. (1978) did not even ellicit an intake response in steers fed elfazepam and corn silage rations. In the latter study, the steers were fed to a constant weight, and elfazepam reduced ADG by 9%. Some other workers (Gonzalez et al., 1977; Wangsness et al., 1977) have, however, obtained increases in intake with elfazepam and roughage rations, along with increased DM and protein digestibility.

It seems that positive responses to elfazepam were often obtained with dry roughage and concentrate rations (Gonzalez et al., 1977; Farlin and Baile, 1977; Dinius and Baile, 1977). With fermented feeds such as corn silage rations, elfazepam may stimulate intake, but has not improved performance (Prior et al, 1978). Prior et al. (1978) speculated that the lack of growth response they observed with corn silage may be related to the ration moisture content and perhaps to the stability of elfazepam in fermented feeds.

The results obtained in the present study for monensin-elfazepam treatment combination also contradicts those reported by Farlin and Baile (1977). These workers obtained improvements in ADG and F/G ratios in heifers fed concentrate rations with monensin and elfazepam included in the same diets. Dinius and Baile (1977) also reported that elfazepam tended to alleviate the depression in feed intake induced by monensin in steers given both chemicals. In contrast, the results here indicate that the two drugs when combined further inhibited feed intake (Table 22). It is interesting to note that the former investigators (Dinius and Baile, 1977) also reported that one of the steers became hypophagic when placed on monensin-elfazepam rations, and therefore had to be removed from the experiment. Baile and McLaughlin (1979) nonetheless concluded that elfazepam may still be helpful, in some rations, in overcoming the initial reduction in feed intake caused by monensin, but that it may not be effective with all rations.

The results obtained in this study with monensin treatment are consistent with the performance data reported by several other investigators (Potter et al., 1976a; Raun et al., 1976; Hanson and Klopfenstein, 1979). Monensin appears to increase the efficiency of protein and feed ME utilization. This would result in decreased feed costs and increased net returns to the ruminant livestock producer. On the other hand, elfazepam may be beneficial in situations in which feed consumption by the ruminant is restricted or regulated by factors other than energy balance. This may apply to high roughage rations and fermented feeds such as corn silage, where intake may be

limited by factors such as bulk (rumen fill) and ruminal solid digesta flow rates.

Plasma Metabolite Studies

As previously stated, there were no significant effects of treatments on overall plasma amino acid (PAA) profiles, blood glucose and blood urea levels ($P < 0.05$). The overall patterns were for slightly elevated PAA levels in steers fed the two monensin rations. These trends were more apparent for glutamate, aspartate, threonine and serine, the typical amino acids involved in transamination and gluconeogenesis. Although there were no ration differences in the overall levels of the branched-chain amino acids (Table 24), it is evident from Figure 4 that monensin caused some depression in the plasma levels of valine and isoleucine during the first half of the experiment. Plasma urea-nitrogen (PUN) was also slightly elevated with both monensin-supplemented rations, in comparison to the responses obtained with elfazepam and control rations. As previously reported, plasma glucose levels seemed relatively high with all treatments, but were highest in the control steers.

The relative usefulness of PAA profile as an indicator of the nutritional status of the ruminant has been extensively reviewed by some investigators (Bergman and Heitmann, 1978; Bergen, 1979b). Plasma or tissue free amino acids represent only a small proportion of total body amino acids and may not accurately reflect the magnitude of AA fluxes between the different tissue pools. Changes in PAA patterns in the ruminant also do not usually reflect the dietary AA profile,

since microbial protein generally constitutes a major proportion of post-ruminal nitrogen flow. The exception to this are proteins that are relatively more resistant to ruminal degradation, but digestible in the lower tract. Large excesses or deficiencies of AA in proteins that escape ruminal degradation will be reflected to some extent, in the PAA profile (Bergen et al., 1973). Also, PAA levels of the branched-chain amino acids can often indicate the animal's nutritional state. Under starvation or protein deprivation, there is an elevation in plasma levels of those amino acids, and this indicates muscle protein catabolism (Leibholz, 1970).

In contrast, intravenous administration of glucose, propionate or other VFA's results in an insulin-mediated depression of PAA levels (Call et al., 1972), with subsequent increase in nitrogen retention (Eskeland et al., 1974). Glucose and propionate were shown to produce greater decline in PAA levels, with the branched-chain AA's most rapidly declining (Potter et al., 1968). The above response was interpreted to mean an increase or induction of tissue protein synthesis (Eskeland et al., 1974). In the ruminant, 50 to 60% of blood glucose may be synthesized from propionate (Young, 1977). Since dietary carbohydrates are usually fermented in the rumen to VFA's, often less than 10% of the animal's glucose needs is obtained from glucose absorption in the gastro intestines. This implies that gluconeogenesis is of tremendous importance to the ruminant. It also means that other glucogenic substrates have to provide up to 40% of the animal's glucose needs, and these are usually the glucogenic amino acids, which could be more

efficiently utilized for tissue synthesis. Since it has been definitely shown that monensin increases rumen propionate levels, while simultaneously decreasing the rate of feed protein degradation in the rumen, it is apparent why the efficiency of nitrogen utilization would be improved in ruminants fed monensin-supplemented good quality plant proteins.

The trends in PAA, PUN and plasma glucose found in the present study are consistent with those reported by Pendlum et al. (1980). The decline in plasma levels of valine and isoleucine in the steers receiving monensin rations during the first half of this study, may indicate an increase in the rate of protein synthesis and growth. Since no adjustments in the dietary crude protein level were made as the trial progressed, the subsequent rise in PAA and PUN levels may be due to protein reaching the lower gut in excess of the animal's protein needs, during the latter phase of growth.

Monensin-Na: Mechanism of Action

Monensin belongs to the class of carboxylic ionophores that catalyzes the electro-neutral exchange of cations for protons. It is similar in structure and function to nigericin, but mediates the exchange of Na^+ for H^+ across cell membranes (Harold, 1972). These compounds have strong affinity for specific monovalent metal cations, which they capture by ligand-binding to several oxygen atoms within their framework (Pressman, 1973). The terminal carboxylic acid must be deprotonated in order to form the complex (Pressman, 1973). Consequently, an ionophore such as monensin forms a charged-neutral

isostoichiometric complex (zwitterion) with Na^+. Monensic acid would most likely adopt a similar conformation when in solution abundant with monovalent cations, such as rumen fluid. Monensic acid has a pK_a of 6.65 (Haney and Hoehn, 1968), so that it would be easily deprotonated in rumen fluid.

It has been demonstrated in many facultative and anaerobic bacteria (e.g., E. coli, S. faecalis) that, as in mitochondria and aerobic microorganisms, nutrient uptake depends on the maintenance of a proton-motive force (comprising of a pH and an electro-chemical gradient) between the cell and the surrounding media. According to the chemi-osmotic hypothesis, protons produced as a result of substrate oxidation are extruded from the cell, thereby generating a pH gradient (interior alkaline) and an electro-chemical gradient, to which nutrient transport is coupled (Mitchell, 1972). Addition of monensin to the media may initially reverse the flow of protons, thereby collapsing the pH gradient (Harold, 1972). Since the ionophore facilitates antiport exchange of Na^+ for H^+, the electrochemical gradient would remain intact. This reduction in total proton-motive force may perhaps reduce the rate of uptake of nutrients required for microbial synthesis.

Rumen fluid usually has a high concentration of Na^+ and K^+, both of which are obligate growth requirements for rumen bacteria, although K^+ requirement is less stringent than that of Na^+ (Caldwell and Anderson, 1974). Both cations are required for many cellular functions which include nutrient transport and activation of many enzymes (e.g., ATPases; Harold, 1977). Rumen bacteria would therefore

tend to have a high intracellular concentration of those cations under normal feed lot conditions. Addition of monensin to ruminant rations may initially result in the rapid extrusion of those intracellular cations coupled to an influx of protons into rumen microbial cells, thereby inhibiting nutrient uptake. It has been shown that amino acid and PO_4 transport in S. faecalis, and in many other bacteria is inhibited by monensin, indicating that the pH gradient may play a greater role in the transport of those nutrients (Harold, 1972).

Other investigators (Estrada-O and Calderon, 1970) reported that monensin inhibited the energy-dependent PO_4 uptake, and its incorporation into ADP in mitochondrial preparations, a situation they described as analogous to the inhibition of mitochondrial oxidation of TCA intermediates under conditions of cellular hyperosmolarity. Estrada-O and Calderon (1970) further reported that the above inhibition was reversed by the addition of succinate, B-hydroxybutyrate, glutamate + malate, or by increasing the PO_4 concentration of the media. The preferential accumulation and oxidation of succinate and B-hydroxybutyrate thus, provided the energy required for the uptake of PO_4's. It is not unlikely that the initial inhibition of energy substrate degradation and the increased production of propionate, both of which occur when ruminants are initially exposed to monensin, are based on similar physiological responses. In such a situation, bacterial species that can metabolize those metabolic intermediates (e.g., succinate and propionate producers with membrane-bound cytochromes) would have a selective advantage for growth in the rumen.

Adaptation to monensin by rumen microbial species probably occurs as a result of the cyclical conductance of ions, since that seems to be the basic mode of action of most carboxylic ionophores (Pressman, 1973). A gradient of monovalent cations has to exist for the ionophore to functionally exchange cations for protons. This exchange, which is facilitated by the ionophore moving in an alternating fashion to either surface of the lipid membrane, continues until equilibrium is established (Mitchell, 1976). In this situation, rumen bacteria may probably have sufficient intracellular cations (Na^+ and K^+) for adequate growth. The specific mechanism by which monensin alters or influences nutrient metabolism in rumen microbial cells cannot be conclusively determined until adequate in vitro and in vivo studies with rumen microorganisms are conducted. However, it seems likely that alterations in microbial membrane permeability caused by monensin, may also result in tremendous expenditure of ATP in order to maintain the transmembrane potential necessary for nutrient uptake.

CHAPTER V

CONCLUSIONS

Based on the data obtained in these studies, the following conclusions are made:

1. Monensin increased ruminal dry matter digestion of the corn silage rations, but did not significantly affect DM digestion of the high grain rations in the rumen.
2. Apparent ADF and NDF digestion in the rumen were not affected by monensin.
3. Monensin increased NAN (% of N-intake) passage to the abomasum in silage and high grain rations.
4. Monensin increased the proportion of dietary nitrogen that escaped degradation in the rumen, but did not affect abomasal digesta flow rates.
5. Ruminal organic matter digestion was increased by monensin in steers fed silage, but not in steers that received grain rations.
6. Monensin reduced the amount of microbial N leaving the rumen.
7. Efficiency of ruminal microbial protein synthesis was reduced by monensin in the rations.
8. Abomasal appearance of the various components of digesta based on lignin and chromium passage were similar between the two digesta markers.

9. PEG tended to overestimate liquid digesta passage to the abomasum.
10. Monensin did not influence nitrogen retention or fiber digestion in the steers, but increased apparent N digestibility of grain rations.
11. From the results of the feeding trial, it is concluded that plasma amino acid, plasma urea and plasma glucose levels of growing steers were not affected by addition of monensin, elfazepam and the two chemicals combined, to all-silage rations.
12. Monensin improved the overall performance of steers fed all-silage rations; elfazepam only stimulated silage intake, while the combination of elfazepam and monensin in the silage resulted in poor performance.

APPENDIX

TABLE A.1 INDIVIDUAL STEER APPARENT FIBER DIGESTION (FROM LIGNIN-BASED DM FLOW) IN THE RUMEN: DIGESTA PASSAGE STUDIES

Steer[a]	Feed NDF-intake (gm)	Abomasal NDF (gm)	App. rumen NDF (gm)	Feed ADF-intake (gm)	Abomasal ADF (gm)	App. rumen ADFD (gm)
992-1[b]	1439.02	895.79	543.24	663.87	537.16	126.61
528-1	1343.09	980.67	362.42	619.61	525.42	94.19
994-2	1617.01	1174.59	442.42	745.98	640.40	105.57
799-2	1271.22	1035.22	236.00	586.46	511.16	75.30
992-2	1430.86	1009.71	421.15	691.18	549.12	142.06
528-2	1239.52	920.88	318.64	599.02	504.36	94.66
994-1	1808.06	1155.57	652.49	824.11	566.24	257.87
799-1	1216.33	870.07	346.26	573.31	444.82	128.49
992-3	2722.73	1905.69	817.04	1607.69	1152.51	455.18
528-3	2683.76	1728.30	955.46	1584.76	974.25	610.52
994-4	2976.64	1968.87	1007.79	1766.75	1186.44	580.31
799-4	2422.78	1702.21	720.57	1431.73	1058.26	373.47
992-4	2877.01	1990.52	886.49	1595.78	1161.05	434.73
528-4	2735.74	1940.80	794.95	1545.04	1157.89	387.14
994-3	4000.27	3113.43	886.84	2105.41	1682.09	423.32
799-3	3500.24	2358.93	1141.31	1842.23	1295.87	546.36

[a]Steer notations represent rations: HG, HG-M, CS and CS-M, respectively, for Tables A.1 through A.10.

[b]Not used in calculations; steer developed abomasal lacerations.

TABLE A.2 INDIVIDUAL STEER: TRUE RUMEN DRY MATTER DIGESTION. PASSAGE STUDIES--LIGNIN BASED ESTIMATES

Steer	Abom. DM flow (gm)	g Microb. N/g DM	Microb. NAN flow (g/day)	Microb. DM flow (g/day)	Feed DM flow (g)	Rumen DMD (g/day)
992-1[a]	5160.05	.08322	72.20	867.58	4292.47	2103.18
528-1	3674.30	.08322	69.85	839.34	2834.96	3134.32
994-2	5058.54	.08369	83.88	1002.27	4056.27	3130.45
799-2	4227.12	.08369	66.70	796.99	3430.13	2219.73
992-2	4382.44	.08369	85.54	1022.11	3360.33	3021.72
528-2	2987.94	.08369	63.30	756.37	2231.58	3299.53
994-1	5772.09	.08322	106.29	1277.22	4494.87	3537.40
799-1	4348.18	.08322	72.56	871.91	3476.27	1927.24
992-3	4501.98	.08350	89.73	1074.61	3427.37	2995.68
528-3	4745.47	.08350	95.95	1149.10	3596.37	2735.07
994-4	4575.54	.08367	81.72	976.69	3598.85	3423.19
799-4	3656.74	.08367	68.38	817.26	2839.48	2875.99
992-4	5101.29	.08367	67.85	810.924	4290.37	2674.08
528-4	4507.19	.08367	86.51	1033.94	3473.25	2839.02
994-3	7621.22	.08350	111.60	1336.53	6284.69	2239.22
799-3	5805.88	.08350	86.15	1031.74	4774.14	2684.28

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.3 INDIVIDUAL STEER: TRUE RUMEN DMD. PASSAGE STUDIES: CHROMIUM-BASED ESTIMATES

Steer	DM flow (gm)	g Microb. N/g DM	Microb. NAN flow (g/day)	Microb. DM flow (g/day)	Feed DM flow (g)	Rumen DMD (g/day)
992-1[a]	4975.00	.08322	69.62	836.58	4138.42	2257.23
528-1	3515.00	.08322	70.00	841.14	2673.86	3295.42
994-2	5788.00	.08369	94.98	1134.90	4653.10	2533.62
799-2	4175.00	.08369	65.86	786.95	3388.05	2261.81
992-2	4252.00	.08369	83.00	991.76	3260.24	3121.81
528-2	3128.00	.08369	66.24	791.49	2336.51	3194.69
994-1	5586.00	.08322	102.85	1235.88	4350.12	3682.15
799-1	4308.63	.08322	71.89	863.86	3444.77	1958.74
992-3	4444.00	.08350	88.26	1057.01	3386.99	3036.06
528-3	4325.00	.08350	87.43	1047.07	3277.93	3053.51
994-4	4631.00	.08367	82.55	986.61	3644.39	3377.65
799-4	3767.00	.08367	70.38	841.16	2925.84	2789.63
992-4	4161.00	.08367	67.92	811.76	3349.24	3615.21
528-4	4698.00	.08367	90.17	1077.69	3620.31	2691.96
994-3	7654.00	.08350	112.04	1341.80	6312.20	2211.71
799-3	5913.00	.08350	87.71	1050.42	4862.58	2595.84

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.4 INDIVIDUAL STEER NAN PASSAGE--CALCULATED FROM LIGNIN OUTFLOW FROM THE RUMEN

Steer	DMI (gm)	Lignin intake (g/day)	mg Lignin/ml ab. fluid	Lignin outflow liters	mg NAN/ml ab. fluid	g NAN flow day
992-1[a]	6395.65	197.63	2.0840	94.830	2.2394	212.36
528-1	5969.28	176.02	4.4879	39.220	2.800	109.82
994-2	7186.72	222.07	4.4300	50.129	2.920	146.38
799-2	5649.86	174.58	3.890	44.880	2.549	114.40
992-2	6382.05	197.21	4.4910	43.911	2.9923	131.40
528-2	5531.11	170.91	5.5027	31.060	3.380	104.98
994-1	8032.21	248.20	4.9622	50.017	3.010	150.55
799-1	5403.51	166.97	4.2860	38.957	2.500	97.39
992-3	6423.05	249.86	4.0515	61.670	2.0464	126.20
528-3	6331.44	246.29	3.8670	63.691	2.180	138.85
994-4	7022.04	273.16	4.1432	65.929	2.3610	155.66
799-4	5715.47	222.33	4.0067	55.490	2.2819	126.62
992-4	6964.45	276.49	4.401	62.824	1.960	123.14
528-4	6312.27	250.60	4.0143	62.427	2.0652	128.92
994-3	8523.91	338.40	5.11932	66.102	2.3579	155.86
799-3	7458.42	296.10	5.9976	49.370	2.3021	113.66

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.5 INDIVIDUAL STEER NAN PASSAGE: FROM N:LIGNIN RATIOS

Steer	Abom. lignin (g/g DM)	Feed lignin (g/g DM)	Abom. N/lignin	Feed N/lignin	NAN passage (g/day)
992-1[a]	.0383	.0309	1.07490	.68350	212.46
528-1	.0502	.0309	.62390	.68366	115.07
994-2	.0439	.0309	.65923	.68350	146.41
799-2	.0413	.0309	.65521	.6822	114.38
992-2	.0450	.0309	.66629	.68867	131.40
528-2	.0572	.0309	.61425	.68867	104.98
994-1	.0430	.0309	.60658	.67327	150.55
799-1	.0384	.0309	.58333	.67259	97.40
992-3	.0555	.0389	.50504	.58393	126.19
528-3	.0519	.0389	.56377	.57979	138.85
994-4	.0597	.0389	.56985	.59229	155.66
799-4	.0608	.0389	.56952	.59596	126.63
992-4	.0542	.0397	.4454	.47786	123.16
528-4	.0556	.0397	.51446	.49159	128.92
994-3	.0444	.0397	.46058	.48363	155.86
799-3	.0510	.0397	.3838	.48363	113.64

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.6 INDIVIDUAL STEER NAN PASSAGE: CALCULATED FROM CHROMIUM OUTFLOW

Steer	feed DM	Chromium intake (g/day)	mg chr./ml ab. fluid	Chromium outflow (liters)	mg NAN/ml ab. fluid	g NAN per day
Collection 1:						
992-1[a]	1.8042	11.54	0.1262	91.44	2.2394	204.76
528-1	1.8042	10.77	0.2740	39.31	2.800	110.06
994-2	1.9406	13.95	0.2457	56.76	2.920	165.75
799-2	1.9406	10.964	0.2474	44.32	2.549	112.97
Collection 2:						
992-2	2.1095	13.463	0.3160	42.60	2.9923	127.59
528-2	2.1095	11.67	0.359	32.50	3.380	109.85
994-1	2.1572	17.33	0.358	48.40	3.010	145.68
799-1	2.1572	11.66	0.302	38.60	2.50	96.494
Collection 3:						
992-3	1.7188	11.04	0.18134	60.66	2.0464	124.13
528-3	1.7188	10.88	0.18754	58.040	2.180	126.53
994-4	1.7020	11.95	0.1791	66.731	2.3610	157.55
799-4	1.7020	9.73	0.17022	57.121	2.2819	130.34
Collection 4:						
992-4	1.7490	12.18	0.19360	62.885	1.960	123.26
528-4	1.7490	11.04	0.16967	65.068	2.0652	134.38
994-3	1.8429	15.71	0.2367	66.366	2.3579	156.48
799-3	1.8429	13.75	0.2735	50.263	2.3021	115.71

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.7 INDIVIDUAL STEER NAN PASSAGE: FROM N:CHROMIUM RATIOS

Steer	Abom. N_2 (g/g DM)	g Chr./g abom. DM	Feed nitrogen (g/g DM)	Feed chromium (g/g DM)	Abom. N: chromium	Feed N: chromium	NAN passage (g/day)
Collection 1:							
992-1[a]	.04117	.0023195	.02112	.0018042	17.750	11.706	204.85
528-1	.03132	.0030643	.021125	.0018042	10.221	11.709	110.07
994-2	.02894	.0022373	.02112	.0019406	12.935	10.8626	180.76
799-2	.02706	.0026264	.02108	.0019406	10.3031	10.8626	112.97
Collection 2:							
992-2	.02998	.0031600	.02128	.0021095	9.4873	10.088	127.72
528-2	.035135	.0037312	.02128	.0021095	9.4170	10.088	109.87
994-1	.026083	.0031026	.020804	.0021572	8.4068	9.644	145.66
799-1	.022400	.0027062	.020783	.0021572	8.2773	9.634	96.49
Collection 3:							
992-3	.02803	.0024841	.022715	.0017188	11.284	13.216	124.57
528-3	.02926	.0025173	.022554	.0017188	11.6236	13.123	126.48
994-4	.03402	.0025805	.023040	.0017020	13.1707	13.537	157.41
799-4	.03463	.0025830	.023183	.0017020	13.407	13.621	130.42
Collection 4:							
992-4	.02414	.0023853	.018971	.001749	10.1203	10.847	123.27
528-4	.028604	.002350	.019516	.001749	12.172	11.1584	134.38
994-3	.02045	.0020526	.019200	.0018429	9.963	10.4184	156.51
799-3	.019576	.0023254	.019200	.0018429	8.41834	10.4184	115.71

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.8 INDIVIDUAL NAN--LIQUID OUTFLOW: FROM PARTITIONED LIQUID (PEG) DIGESTA PASSAGE

Steer	PEG (gm) intake	gm PEG/ml abomasal supernate	PEG outflow ml	NAN/ml supernate	NAN/day supernate (gm)
992-1[a]	32.60	0.3136	103,954.10	1.19975	124.72
528-1	30.41	0.6882	44,187.70	1.60560	70.95
994-2	37.54	0.5459	68,767.20	1.68990	116.21
799-2	28.13	0.3870	72,687.34	1.03881	75.51
992-2	36.17	0.5351	67,594.80	1.66340	112.44
528-2	31.35	0.6580	47,644.00	1.91070	91.03
994-1	34.535	0.6285	59,721.60	1.72780	103.19
799-1	24.50	0.3528	69,444.44	1.37430	95.44
992-3	33.81	0.3696	91.477.27	0.86670	79.28
528-3	33.15	0.4513	74,008.42	0.91560	67.76
994-4	37.342	0.3936	94,872.97	0.89412	84.83
799-4	30.513	0.2818	108,278.92	0.7707	83.45
992-4	29.82	0.2439	122,267.32	0.65712	80.34
528-4	27.61	0.3744	73,744.66	0.86404	63.72
994-3	30.83	0.3744	82,318.40	0.9675	79.64
799-3	26.98	0.3086	87,427.10	0.8097	79.79

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.9 INDIVIDUAL STEER NAN--SOLID DIGESTA OUTFLOW: FROM PARTITIONED SOLID (CHROMIUM) PASSAGE

Steer	Chromium intake (g/day)	mg Chromium per gm. abom. residue	Residue outflow (gm)	NAN/gm residue	g NAN solid flow/day
992-1[a]	11.54	2.3195	4,975.00	.02007	99.85
528-1	10.77	3.0643	3,515.00	.01638	57.57
994-2	13.95	2.2373	5,788.00	.01273	73.684
799-2	10.964	2.6264	4,175.00	.012604	52.62
992-2	13.463	3.1666	4,252.00	.01448	61.56
528-2	11.67	3.7312	3,128.00	.01717	53.70
994-1	17.33	3.1026	5,586.00	.01364	76.19
799-1	11.66	2.7062	4,308.63	.01158	49.89
992-3	11.04	2.4841	4,444.30	.01764	78.40
528-3	10.88	2.5173	4,325.00	.01849	79.97
994-4	11.95	2.5805	4,630.90	.018404	85.23
799-4	9.73	2.5830	3,767.00	.02070	77.98
992-4	12.18	2.3853	5,106.30	.01423	72.66
528-4	11.04	2.3500	4,698.00	.01750	82.21
994-3	15.71	2.0526	7,653.70	.01310	100.26
799-3	13.75	2.3254	5,913.00	.01350	79.83

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.10 INDIVIDUAL STEER--NH_3-N AND NAN PASSAGE TO THE ABOMASUM

Steer	mg NAN/ml abomasal fluid	mg NH_3/ml abomasal fluid	mg TN/ml abomasal fluid	% DM abomasal fluid	mg NH_3-N/ abomasal DM	mg TN/g abomasal DM
992-1[a]	2.2394	0.056015	2.29542	5.44	1.0297	42.20
528-1	2.800	0.07163	2.87163	8.94	0.80123	32.12
994-2	2.920	0.06157	2.98157	10.09	0.61021	29.60
799-2	2.549	0.062115	2.611115	9.42	0.6594	27.72
992-2	2.9923	0.06490	3.0572	9.98	0.6503	30.63
528-2	3.380	0.06491	3.4449	9.62	0.6747	35.81
994-1	3.010	0.07446	3.08446	11.54	0.64523	26.73
799-1	2.5000	0.06548	2.5655	11.16	0.58674	22.99
992-3	2.0464	0.08396	2.13036	7.30	1.15014	29.18
528-3	2.180	0.10024	2.28024	7.45	1.3455	30.61
994-4	2.3610	0.09574	2.45674	6.94	1.3795	35.40
799-4	2.2819	0.07948	2.3614	6.59	1.20607	35.83
992-4	1.960	0.06272	2.02272	8.12	0.7724	24.91
528-4	2.0652	0.08955	2.15475	7.22	1.2403	29.84
994-3	2.3579	0.07840	2.4363	11.53	0.67996	21.13
799-3	2.3021	0.08008	2.3822	11.76	0.6810	20.26

[a]Not used in calculations; steer developed abomasal lacerations.

TABLE A.11 RNA AND N CONSTITUENTS OF RUMINALLY ISOLATED BACTERIAL PREPARATIONS

	mg RNA/g sample DM	mg RNA - N/g bact. DM	mg N/g bacteria	Bacteria TN:RNA-N	$\bar{x}$	$\bar{x}$ Bacteria N (mg) per g DM
High Grain without Monensin:						
A2 Bact.	72.20	10.867	76.19	7.0113		
A4 Bact.	--	--	--	--		
A5 Bact.	94.22	13.945	87.18	6.252	6.44	83.22
A8 Bact.	96.49	14.281	86.29	6.0423		
High Grain with Monensin:						
B2 Bact.	86.54	12.808	85.94	6.7098		
B4 Bact.	86.68	12.829	83.22	6.4869	6.44	83.69
B6 Bact.	91.17	13.49	84.82	6.2876		
B8 Bact.	86.82	12.85	80.76	6.2848		
High Silage without Monensin:						
C2 Bact.	82.40	12.20	75.23	6.1701		
C4 Bact.	87.65	12.97	85.79	6.6211	6.43	83.50
C6 Bact.	94.04	13.92	90.06	6.470		
C8 Bact.	86.52	12.81	82.92	6.4802		
High Silage with Monensin:						
D2 Bact.	85.21	12.61	82.94	6.58		
D4 Bact.	87.98	13.02	79.90	6.14	6.44	83.67
D6 Bact.	90.23	13.35	86.10	6.45		
D8 Bact.	87.89	13.01	85.74	6.59		

TABLE A.12 MICROBIAL CONTRIBUTION TO NAN PASSAGE TO THE ABOMASUM: INDIVIDUAL STEERS

Steer	mg NAN/g abomasal DM	mg RNA/g abomasal DM	Ab. RNA-N[b] adjusted (mg)	$\bar{x}$ T-N bacteria: RNA-N	Microbial-N: g abomasal-NAN
992-1[a]	41.17	17.19	2.1624	6.435	0.340
528-1	31.32	24.59	3.0934	6.435	0.636
994-2	28.94	20.46	2.5739	6.44	0.573
799-2	27.06	16.55	2.0820	6.44	0.583
992-2	29.983	24.10	3.0318	6.44	0.651
528-2	35.14	26.14	3.2884	6.44	0.603
994-1	26.08	22.73	2.8594	6.435	0.706
799-1	22.40	20.61	2.5928	6.435	0.745
992-3	28.03	24.64	3.0997	6.432	0.711
528-3	29.26	25.01	3.1463	6.432	0.691
994-4	34.02	22.06	2.7752	6.438	0.525
799-4	34.63	23.07	2.9022	6.438	0.540
992-4	24.14	16.41	2.0644	6.438	0.551
528-4	28.604	23.68	2.9789	6.438	0.671
994-3	20.45	18.09	2.2757	6.432	0.716
799-3	19.58	18.33	2.3059	6.432	0.758

[a]Not used in calculations; steer developed abomasal lacerations.

[b]mg RNA x 14.8% x 0.85, see discussions; nucleic acid methodology.

TABLE A.13 INDIVIDUAL STEER: NITROGEN BALANCE STUDIES

Steer	DMI (kg)	N-intake (gm)	Fecal-N (gm)	Urin-N (gm)	N-retained (gm)	% N-digested	% N-retained
992-1	6.36	132.18	45.62	35.00	51.57	65.49	39.01
528-1	5.93	123.37	47.75	30.00	45.62	61.30	36.98
994-2	7.24	150.61	49.78	40.87	59.96	66.95	39.81
799-2	5.89	122.57	32.21	43.18	47.18	73.72	38.50
992-2	6.29	130.77	35.91	46.80	48.06	72.54	36.75
528-2	5.49	113.33	30.12	45.13	38.09	73.42	33.61
994-1	7.998	166.35	47.96	79.76	38.63	71.17	23.22
799-1	5.47	105.02	37.42	33.00	34.60	64.37	32.95
992-3	6.563	150.63	57.60	63.97	29.06	61.76	19.29
528-3	6.57	149.89	50.84	59.29	39.76	66.08	26.52
994-4	7.35	169.81	58.42	72.47	38.91	65.59	22.91
799-4	6.01	138.93	45.77	53.33	39.84	67.06	28.68
992-4	6.55	139.88	45.55	51.33	43.00	67.44	30.74
528-4	6.08	128.97	43.45	32.29	53.23	66.31	41.28
994-3	7.54	177.44	59.04	57.57	60.84	66.73	34.29
799-3	6.57	154.87	51.12	56.77	46.96	66.99	30.33

TABLE A.14 INDIVIDUAL STEER DRY MATTER DIGESTION (N_2 BALANCE CONTINUED)

Steer	DMI/day (kg)	DM feces (kg)	DMD (kg)	% Digested
992-1	6.35483	1.56645	4.78838	75.35
528-1	5.931174	1.75540	4.17577	70.40
994-2	7.2407	1.51755	5.72315	79.04
799-2	5.8929	0.95401	4.93891	83.81
992-2	6.28679	0.98856	5.29823	84.28
528-2	5.4486	1.107303	4.34125	79.68
994-1	7.99774	1.79481	6.20293	77.56
799-1	5.47214	1.23094	4.2412	77.51
992-3	6.56291	1.97798	4.5849	69.86
528-3	6.5673	1.89153	4.6758	71.20
994-4	7.34457	2.43266	4.91191	66.88
799-4	6.00919	1.84735	4.16184	69.26
992-4	6.547305	1.96195	4.58536	70.03
528-4	6.07451	1.78671	4.2878	70.59
994-3	7.54415	2.30614	5.2301	69.43
799-3	6.574368	1.91332	4.66105	70.90

TABLE A.15 INDIVIDUAL STEER FECAL CHROMIUM RECOVERY--NITROGEN BALANCE STUDIES

Steer	mg Cr/g DM feces	Fecal DM per day	Chromium excretion per day	Chromium intake (g/day)	% Recovery chromium
992-1	5.166	1566.45	8.0923	11.089	72.98
528-1	5.074	1755.40	8.9070	10.350	86.06
994-2	6.619	1517.55	10.0450	12.264	81.91
799-2	6.990	954.01	6.6690	9.08	73.45
992-2	6.698	988.56	6.6214	11.66	56.79
528-2	6.346	1107.30	7.0270	10.11	69.51
994-1	5.483	1794.81	9.8410	14.84	66.31
799-1	5.327	1230.94	6.5572	9.01	72.78
992-3	4.450	1977.98	8.8020	10.54	83.51
528-3	4.5186	1891.53	8.5471	10.65	80.25
994-4	4.823	2311.03	11.1460	11.79	94.54
799-4	5.125	1754.99	8.9943	9.65	93.21
992-4	4.801	1961.95	9.4193	10.33	91.12
528-4	4.845	1786.71	8.657	9.58	90.37
994-3	4.598	2306.14	10.604	12.614	84.07
799-3	4.905	1913.32	9.385	10.99	85.40

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