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RUMEN FERMENTATION, BACTERIAL GROWTH AND RUMINAL DEGRADATION OF FEEDSTUFFS AS INFLUENCED BY RUMINAL AMMONIA CONCENTRATION

by

MAN K. SONG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled RUMEN FERMENTATION, BACTERIAL GROWTH AND RUMINAL DEGRADATION OF FEEDSTUFFS AS INFLUENCED BY RUMINAL AMMONIA CONCENTRATION by Man K. Song in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION.

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ABSTRACT

Effect of ruminal ammonia concentration on microbial growth, ruminal fermentation characteristics and degradation of feed ingredients by rumen microorganisms was examined in a series of in vitro and in vivo studies. Feeding value of ammoniated barley silage was also evaluated.

Ammonia concentration did not influence the proteolytic activity of rumen microorganisms in vitro. Removal of protozoa by centrifugation reduced ($P<.05$) the extent of protein degradation. Increasing ruminal ammonia concentration by continuous infusion of NH_4Cl or NH_4HCO_3 increased ($P<.05$) bacterial numbers. However, bacterial rowth as influenced by increasing ammonia concentration did not proportionally increase ruminal degradation of feed ingredients. Ammonia concentration did not markedly influence volatile fatty acid concentrations. Ammoniation of barley silage increased ($P<.05$) pH, lactic acid, total nitrogen (N) and water insoluble N contents. Milk yield and milk composition of cows fed ammoniated barley silage were similar to cows fed untreated barley silage supplemented with canola meal or urea. Ammoniation of barley silage increased ($P<.05$) ammonia concentration and propionate proportion in rumen fluid, and supply of total N, non-ammonia N and microbial N to the small intestine compared to barley silage supplemented with canola meal or urea. It is concluded that increasing ruminal ammonia concentration increased microbial growth. But increased microbial numbers did not proportionally increase rumen microbial degradation of feed ingredients. Microbial hydrolytic activity may be primarily a function of the physio-chemical properties of feedstuffs. Feeding value of ammoniated barley silage for dairy cows was equal to

untreated silage supplemented with canola meal. Increased water insoluble nitrogen content in ammoniated silage [redacted] have contributed to enhanced utilization of ammonia nitrogen.

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TABLE OF CONTENTS

LIST OF TABLES

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Table III-1. Ammonia concentration and pH in rumen fluid, blood urea N and body weight change as influenced by NH_4Cl-N infusion.

Items	NH_4Cl-N infusion (g d^{-1})				SEM[1]	Contrast[2]
	0	32	63	102		
Body weight gain (kg)[3]	14.1	14.3	13.5	11.5	2.14	NS
Ammonia, mg 100 mL^{-1}	11.2^a	16.3^b	24.8^c	34.9^d	1.34	L/Q
pH	6.50^c	6.46^c	6.34^b	6.19^a	.08	L/C
Blood urea N, mg 100 mL^{-1}	14.8^a	18.2^b	22.2^c	24.5^c	2.02	L

[a,b,c] Means within a row having different superscipts differ ($P<.05$ for pH, $P<.01$ for ammonia and blood urea N).

[1] Standara error of means.

[2] Orthogonal contrast where L=linear; Q=quadratic; C=cubic and NS=not significant at $P<.05$.

[3] Mean body weight change during experiment.

Table III-2. Effects of ammonia concentration on volatile fatty acid (VFA) concentrations in rumen fluid.

Parameters	Ammonia (mg 100 mL^{-1})[1]				SEM[2]	Contrast[3]
	11.2	16.3	24.8	34.9		
Total VFA (mmole 100 mL^{-1})	10.3	10.3	10.9	9.9	.24	NS
Molar percent (mmole 100 $mmole^{-1}$)						
Acetate (C_2)	63.7	63.4	61.5	62.1	.59	NS
Propionate (C_3)	17.6	17.9	19.9	18.7	.71	NS
Isobutyrate	1.2	1.1	1.1	1.0	.03	NS
Butyrate	14.3	14.5	14.5	15.3	.17	NS
Isovalerate	1.7	1.6	1.7	1.5	.11	NS
Valerate	1.5	1.5	1.5	1.4	.06	NS
C_2/C_3 ratio	3.62^{b}	3.54^{b}	3.09^{a}	3.32^{ab}	.14	Q/C

[a,b] Means within a row having different superscripts differ ($P<.05$).
[1] Ruminal ammonia concentrations as influenced by NH_4Cl infusion.
[2] Standard error of means
[3] Orthogonal contrast where Q=quadratic; C=cubic and NS=not significant at $P<.05$.

Table III-3. Effect of ammonia concentration on viable counts (x 10^8 mL^{-1}) of total mixed bacteria (TMB) and mixed proteolytic bacteria (MPB) in rumen fluid.

Bacterial Population	Ammonia (mg 100 mL^{-1})[1]				SEM[2]	Contrast[3]
	11.2	16.3	24.8	34.9		
TMB	7.64	9.30	9.91	8.79	2.90	NS
MPB	.87[a]	1.04[ab]	1.29[b]	1.01[ab]	.37	L/Q

[a,b] Means within a row having different superscripts differ (P<.05).
[1] Ruminal ammonia concentration as influenced by level of NH_4Cl infusion.
[2] Standard error of means.
[3] Orthogonal contrast where L=linear; Q=quadratic and NS=not significant at P<.05.

Table III-4. In situ disappearance (%) and effective degradability (%) of dry matter (EDDM) of barley grain at various rumen outflow rates in relation to ruminal ammonia concentration.

	Incubation	Ammonia (mg 100 mL^{-1})[1]				SEM[4]	Contrast[5]
	(h)	11.2	16.3	24.8	34.9		
DM	.1	49.0	49.2	49.8	49.9	.02	NS
	2	69.7^{bc}	72.0^{c}	67.0^{ab}	65.0^{a}	1.59	L/Q
	4	77.1	77.6	75.7	75.3	.50	NS
	8	81.2	82.4	79.0	79.2	.91	NS
	12	82.9	84.1	80.5	81.5	.92	NS
	24	85.9	87.0	85.2	84.5	.80	NS
EDDM %	.04[2]	80.8	81.9	79.3	79.5	.78	NS
	.08[2]	78.3	79.4	76.6	76.5	.82	NS
	.12[2]	76.1	77.2	74.4	74.0	.79	NS

[a,b,c] Means within a row having different superscripts differ (P<.05).
[1] Ruminal ammonia concentrations as influenced by NH_4Cl infusion.
[2] Effective degradability of dry matter (EDDM) at rumen outflow rates of .04 h^{-1}, .08 h^{-1} and .12 h^{-1}.
[3] Standard error of means.
[5] Orthogonal contrast where L=linear; Q=quadratic and NS=not significant at P<.05.

Table III-5. In situ disappearance (%) and effective degradability (%) of dry matter (EDDM) and protein (EDCP) for soybean meal at various rumen outflow rates in relation to ruminal ammonia concentration.

	Incubation	Ammonia (mg 100 mL^{-1})[1]				SEM[4]	Contrast[5]
	(h)	11.2	16.3	24.8	34.9		
DM	.1	40.7	41.6	42.3	40.8	.03	NS
	2	47.4	49.5	48.5	48.8	.86	NS
	4	53.3	53.9	54.9	51.4	.88	NS
	8	68.2^{ab}	73.0^{c}	63.4^{a}	64.5^{a}	1.68	L/Q
	12	83.6^{bc}	87.9^{c}	80.4^{ab}	75.4^{a}	1.82	L/Q
	24	97.3	97.6	96.0	94.9	.52	NS
	EDDM.04[2]	85.3	86.0	85.5	84.8	1.09	NS
	.08[2]	72.6^{ab}	74.6^{b}	71.6^{ab}	70.0^{a}	.87	Q
	.12[2]	65.3^{ab}	67.6^{b}	64.3^{ab}	62.6^{a}	.91	Q
CP	.1	24.1	25.4	23.9	24.6	.07	NS
	2	37.1^{b}	38.1^{b}	35.2^{ab}	33.8^{a}	1.05	L/Q
	4	46.7^{b}	46.8^{b}	45.4^{b}	39.8^{a}	.73	Q
	8	65.5^{b}	65.9^{b}	63.2^{b}	53.6^{a}	1.90	Q
	12	78.1^{b}	87.2^{c}	76.6^{b}	71.3^{a}	2.52	Q
	24	98.4	97.6	97.2	95.0	.53	NS
	EDCP.04[3]	82.3	83.6	81.8	79.1	1.61	NS
	.08[3]	67.6^{b}	69.8^{b}	66.9^{ab}	64.0^{a}	1.47	Q
	.12[3]	58.8^{bc}	61.3^{c}	56.4^{ab}	53.6^{a}	1.33	Q

[a,b,c] Means within a row having different superscripts differ ($P<.05$).
[1] Ruminal ammonia concentrations as influenced by NH_4Cl infusion.
[2,3] Effective degradability of dry matter (EDDM) and protein (EDCP) at rumen outflow rates of .04, .08 and .12 h^{-1}.
[4] Standard error of means.
[5] Orthogonal contrast where L=linear; Q=quadratic and NS=not significant at $P<.05$.

Table III-6. In situ disappearance (%) and effective degradability (%) of dry matter (EDDM) and protein (EDCP) for corn gluten meal at various rumen outflow rates in relation to ruminal ammonia concentration.

	Incubation	Ammonia (mg 100 mL^{-1})[1]				SEM[4]	Contrast[5]
	(h)	11.2	16.3	24.8	34.9		
DM	.1	10.0	10.7	10.9	9.9	.01	NS
	2	13.9	14.3	14.2	13.9	.42	NS
	4	15.7	15.8	16.5	14.8	.83	NS
	8	19.2	18.7	19.1	16.8	.57	NS
	12	22.5	21.6	21.7	18.6	.88	NS
	24	29.1^{ab}	31.1^{b}	29.3^{ab}	25.9^{a}	.52	Q
	EDDM.04[2]	25.0	29.1	25.9	23.3	.77	NS
	.08[2]	20.9	21.8	21.1	18.8	.81	NS
	.12[2]	18.6	18.8	18.8	16.7	.76	NS
CP	.1	8.2	8.8	8.5	9.1	.03	NS
	2	10.3	11.0	9.8	10.8	.33	NS
	4	10.5	13.5	10.5	11.9	.83	NS
	8	11.4	15.2	13.6	14.1	.64	NS
	12	12.1^{a}	17.3^{b}	14.6^{ab}	15.3^{b}	.85	Q
	24	13.8^{a}	23.3^{b}	17.9^{ab}	17.0^{ab}	.87	Q
	EDCP.04[3]	12.3^{a}	20.5^{b}	14.9^{a}	15.3^{a}	.84	Q
	.08[3]	11.6^{a}	16.8^{b}	13.5^{a}	13.9^{a}	.92	Q
	.12[3]	11.1^{a}	15.0^{b}	12.6^{a}	13.1^{a}	.84	Q

[a,b] Means within a row having different superscripts differ ($P<.05$).
[1] Ruminal ammonia concentrations as influenced by NH_4Cl infusion.
[2,3] Effective degradability of dry matter (EDDM) and protein (EDCP) at rumen outflow rates of .04, .08 and .12 h^{-1}.
[4] Standard error of means.
[5] Orthogonal contrast where Q=quadratic and NS=not significant at $P<.05$.

Table III-7. In situ disappearance (%) and effective degradability (%) of dry matter (EDDM) and protein (EDCP) for fish meal at various rumen outflow rates in relation to ruminal ammonia concentration.

	Incubation	Ammonia (mg 100 mL^{-1})[1]				SEM[4]	Contrast[5]
	(h)	11.2	16.3	24.8	34.9		
DM	.1	26.1	25.8	26.3	26.1	.02	NS
	2	28.2	28.4	28.3	28.6	.34	NS
	4	29.4	29.2	29.6	29.2	.27	NS
	8	30.1	29.4	30.0	29.5	.22	NS
	12	30.9	31.0	31.4	29.8	.40	NS
	24	34.6	34.8	33.4	33.1	.65	
	EDDM .04[2]	32.7	34.0	31.8	32.2	.78	NS
	.08[2]	31.0	31.0	30.7	30.2	.59	NS
	.12[2]	30.1	29.8	30.0	29.4	.61	NS
CP	.1	20.8	19.5	21.5	21.2	.03	NS
	2	24.6	26.9	27.6	28.6	.25	NS
	4	26.6	27.8	29.4	28.9	.35	NS
	8	28.2	28.9	31.7	30.3	.55	NS
	12	29.1	30.7	34.4	31.7	.31	NS
	24	36.1	38.7	38.2	36.8	.90	NS
	EDCP .04[3]	32.1	35.0	35.4	33.9	.47	NS
	.08[3]	28.4	31.0	32.8	31.4	.40	NS
	.12[3]	26.5	28.9	31.3	30.0	.52	NS

[1] Ruminal ammonia concentrations as influenced by NH_4Cl infusion.
[2,3] Effective degradability of dry matter (EDDM) and protein (EDCP) at rumen outflow rates of .04, .08 and .12 h^{-1}.
[4] Standard error of means.
[5] Orthogonal contrast where NS=not significant at P<.05.

Table III-8. In situ disappearance (%) and effective degradability (%) of dry matter (EDDM) and cell wall (EDCW) for barley silage at various rumen outflow rates in relation to ruminal ammonia concentration.

	Incubation	Ammonia (mg 100 mL^{-1})[1]				SEM[4]	Contrast[5]
	(h)	11.2	16.3	24.8	34.9		
DM	.1	48.4	49.2	49.3	49.2	.03	NS
	4	51.1	50.7	50.4	50.4	.32	NS
	8	54.9	52.3	51.8	52.5	.89	NS
	12	57.3	56.1	55.5	53.5	1.28	L
	24	64.5^{b}	64.7^{b}	63.1^{ab}	58.7^{a}	2.06	L
	48	74.3^{b}	73.7^{b}	72.8^{ab}	68.9^{a}	1.92	L
	EDDM.04[2]	61.8^{b}	61.7^{b}	59.4^{ab}	57.4^{a}	1.32	L
	.08[2]	56.0^{b}	55.8^{b}	54.2^{ab}	53.5^{a}	1.21	L
	.12[2]	53.6	53.5	53.0	52.2	1.09	NS
CW	.1	6.6	6.5	7.1	6.7	.04	NS
	4	8.3	8.6	8.3	8.3	.88	NS
	8	12.1	12.9	10.7	11.3	2.18	NS
	12	20.5^{b}	18.6^{ab}	17.6^{ab}	16.0^{a}	2.21	L
	24	38.1^{b}	37.1^{b}	36.4^{b}	29.0^{a}	2.53	Q
	48	52.7^{c}	51.5^{bc}	47.8^{b}	43.4^{a}	2.68	Q
	EDCW.04[3]	30.2^{c}	29.3^{bc}	27.2^{ab}	25.4^{a}	2.56	L/Q
	.08[3]	18.5^{b}	18.2^{b}	17.0^{ab}	16.5^{a}	2.42	L/Q
	.12[3]	14.2	14.0	13.4	12.9	1.98	NS

[a,b,c] Means within a row having different superscripts differ ($P<.05$).

[1] Ruminal ammonia concentrations as influenced by NH_4Cl infusion.

[2,3] Effective degradability of dry matter (EDDM) and cell wall (EDCW) at rumen outflow rates of .04, .08 and .12 h^{-1}.

[4] Standard error of means.

[5] Orthogonal contrast where L=linear; Q=quadratic and NS=not significant at $P<.05$.

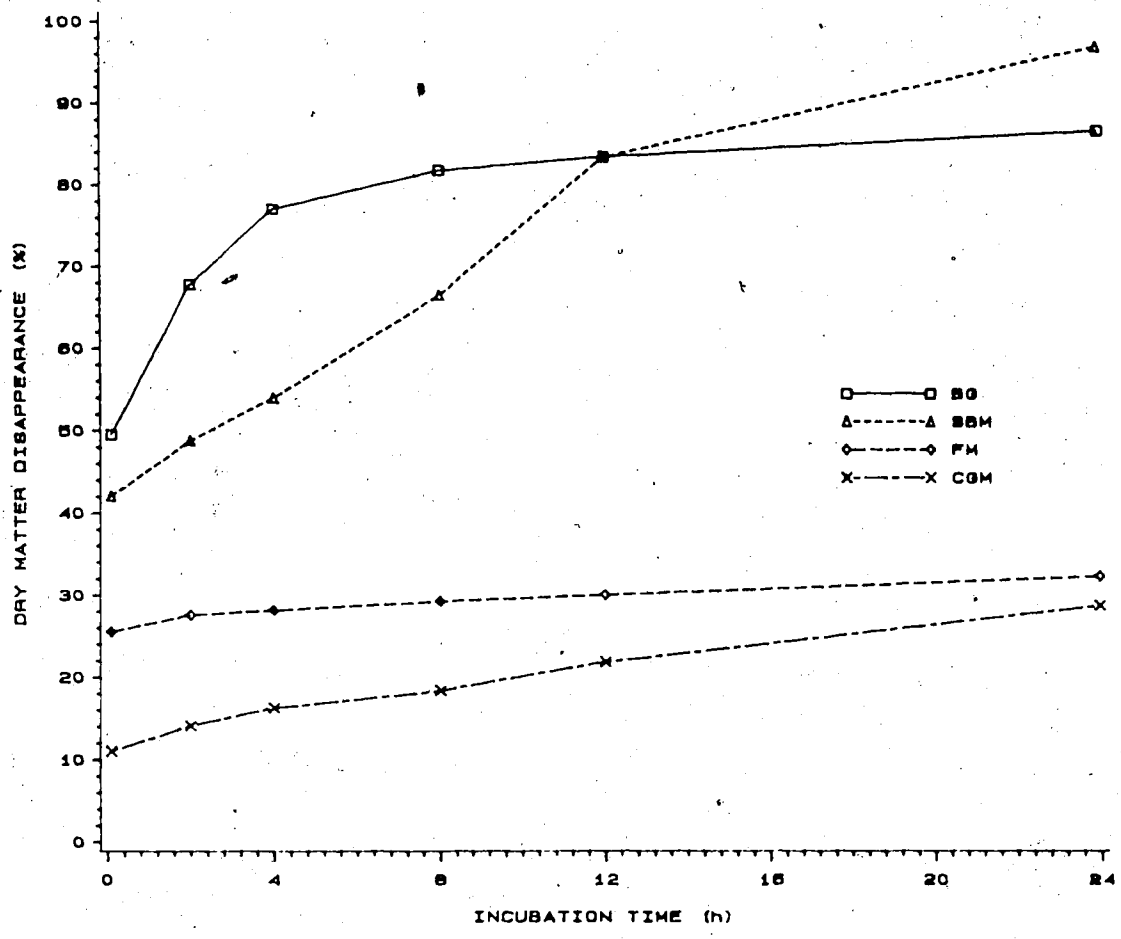

Figure III-1. Ruminal dry matter disappearance for barley grain (BG), soybean meal (SBM), fish meal (FM) and corn gluten meal (CGM).

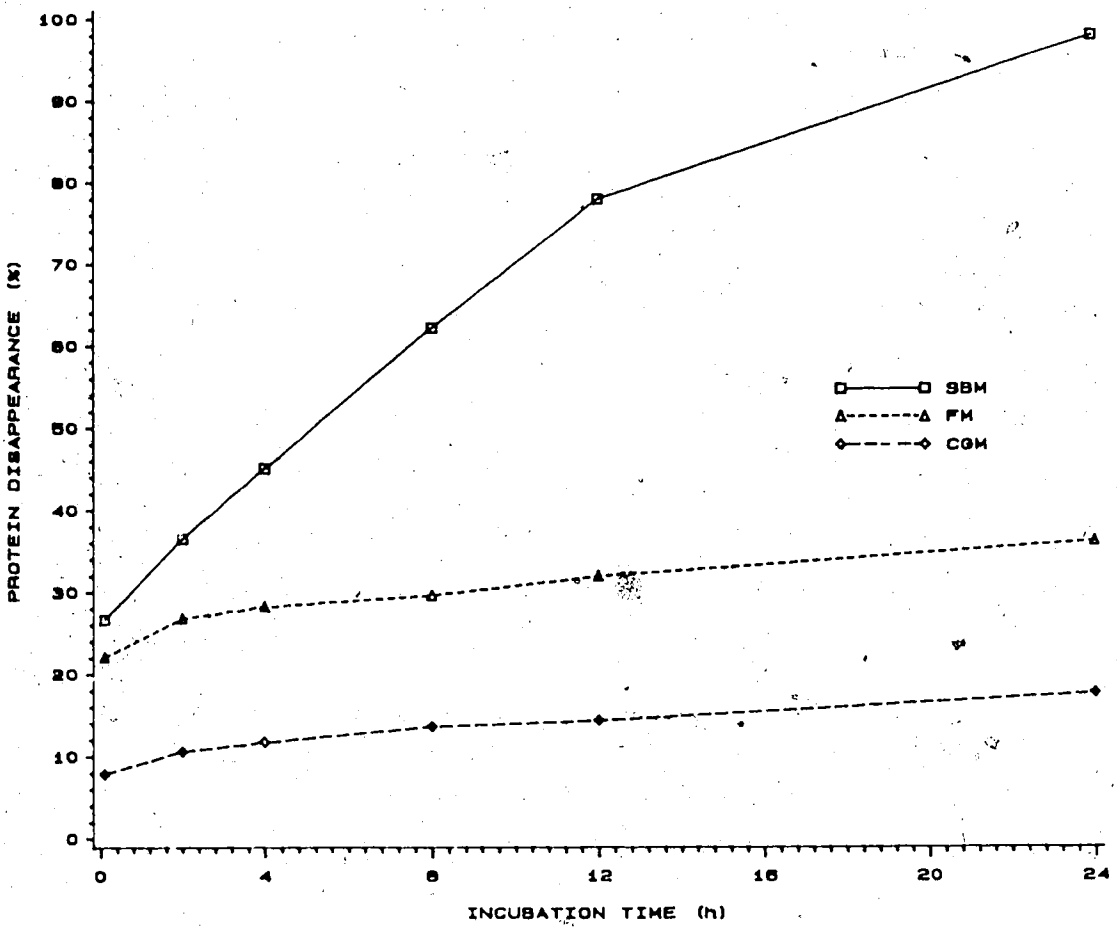

Figure III-2. Ruminal protein disappearance for soybean meal (SBM), fish meal (FM) and corn gluten meal (CGM).

IV. RUMEN FERMENTATION, BACTERIAL GROWTH AND RUMINAL DEGRADATION OF FEED INGREDIENTS AS INFLUENCED BY RUMINAL AMMONIA CONCENTRATION

A. INTRODUCTION

Nitrogen Source for Microbial Protein Synthesis

Nitrogen (N) is a major limiting nutrient for microbial growth in the rumen (Cotta and Russell 1982; Owens and Bergen 1983;). Ammonia is the primary N source used by most rumen bacteria for protein synthesis (Hungate 1966; Owens and Bergen 1983). Some bacterial species also require peptides and amino acids (Smith 1979) which are precursors for branched chain fatty acids. Hespell and Bryant (1979) estimated 20 to 50% of microbial N synthesis was associated with preformed amino acids.

Despite the importance of ammonia for rumen bacteria, the efficiency of nonprotein N[illegible]e may depend on the rate of incorporation of ammonia into [illegible] protein. Mathison and Milligan (1971) observed that 50 to 60% of the bacterial N and 31 to 55% of protozoal N were derived from rumen ammonia in vivo. Later studies (Nolan et al. 1972; Mercer and Annison 1976) confirmed similar ranges (50 to 80%) of ammonia incorporation by rumen microbes. Ammonia is mostly preferred by fiber digesting bacteria (Hungate 1966), but the starch, sugar and secondary fermenters also require ammonia (Cotta and Russell 1982). An in vitro study with Bacteroides ruminicola (Pittman and Bryant 1964) has indicated that this microorganism utilises ammonia and oligopeptide N, but not amino acids or short chain peptide N for growth. Although a few studies (Maeng and Baldwin 1976; Allison 1982) have shown that some

LIST OF FIGURES

I. INTRODUCTION

The qualitative and quantitative importance of rumen microorganisms as a source of protein is apparent since up to 80% of the protein reaching the small intestine of ruminants is of microbial origin (Czerkawski 1978; Ørskov 1982; Owens and Bergen 1983; Tamminga 1983; Sniffen and Robinson 1987). Although protozoa and fungi are active in the rumen, microbial protein arises primarily from bacteria (Bergen and Yokoyama 1977).

Microbial protein synthesis is influenced by factors such as the quality, nature and composition of feed degraded in the rumen, proportions in which endproducts of ruminal fermentation are formed, nature of nitrogen (N) sources, and energy supply. Bergen et al. (1982) indicated that doubling time of bacteria ranged from 14 min to 14 h with rate of growth being a partial function of substrate availability. Russell and Hespel (1981) also indicated that dietary composition can alter the rumen ecology and influence microbial growth, total microbial mass, and extent of digestion. Production of bacterial protein is related to energy fermented in the rumen. Smith (1979) emphasized the importance of a controlled supply of energy in enhancing efficient synthesis of microbial protein. But, the energetic efficiency of microbial growth is variable, depending both on microbial species and ruminal environment (Stouthamer 1973; Harrison and McAllan 1980; Smith and Oldham 1982).

Nitrogen Sources for Microbial Protein Synthesis

Many researchers sugggest that protein is a major limiting nutrient for

microbial growth (Cotta and Russell 1982; Owens and Bergen 1983; Oldham 1984). Microbial yield in the rumen is proportional to dietary N (Hume et al. 1970). An insufficient supply of N substrate may decrease microbial synthesis of intracellular polysaccharide (Smith 1975; McAllan and Smith 1977). In addition, Hespell and Bryant (1979) indicated that slow growth of ruminal bacteria provided with a low protein and high energy diet may be due to energetic uncoupling.

Ammonia, peptides, and amino acids are major sources of N for rumen bacteria. A few studies (Maeng and Baldwin 1976; Allison 1982) have shown that some microbial species require preformed N for more efficient growth. But Theurer (1979) indicated that efficiency of microbial protein synthesis may not be altered by the availability of amino acid in vivo. Hespell and Bryant (1979) also suggested that changes in microbial yield with amino acid supplementation are not likely to be due to reduced ATP use for amino acid synthesis as this cost is relatively small. Certain peptides and amino acids may serve as sources of branched chain fatty acids (BCFA) which are growth factors for a number of bacterial species including cellulolytic bacteria (Bryant 1973; Russell and Hespell 1981; Russell and Sniffen 1984). Lack of BCFA has been suggested to cause energy (ATP) uncoupling, during which fermentation continues to produce ATP without concomitant use of ATP for anaerobic processes (Russell and Hespell 1981).

While ammonia is the primary N source of most ruminal bacteria for protein synthesis (Hungate 1966), some species require peptides and amino acids (Smith 1979). Rumen protozoa generally require amino acids or intact protein for growth, and do not utilize ammonia directly (Leng

1976; Leibholz and Kellaway 1979). Mathison and Milligan (1971) observed that 50 to 60% of bacterial N was derived from rumen ammonia in vivo. Later studies (Nolan et al. 1972; Mercer and Annison 1976) confirmed similar ranges (50 to 80%) of ammonia incorporation by rumen microbes. Ammonia is preferred by most fibrolytic bacteria (Hungate 1966), and starch, sugar and secondary fermenters also require ammonia (Pittman and Bryant 1964; Allison 1970; Cotta and Russell 1982).

Since precursors of amino acids are synthesized from fermentable carbohydrates, the amount of nonprotein N (NPN) incorporated into microbial protein is dependent on energy in the rumen (Satter and Roffler 1975; Chalupa 1978). The strong dependence of N utilization on dietary energy has been used for the development of models to predict protein concentrations at which NPN supplementation is beneficial (Burroughs et al. 1975; Roffler and Satter 1975; Satter and Roffler 1975). One of the major problems in using NPN for ruminants may be the rate of release of ammonia from the NPN compound. Oldham (1981a) indicated that if the rate of release does not match microbial incorporation N supplements will be utilised ineffectively and microbial growth may be limited due to inadequate or excess rumen ammonia concentration during different phases of the feeding cycle. Shiehzadeb and Harbers (1974) suggested that processed starch-urea N forms (Starea) promote greater utilization of N than simple mixture of corn and urea. Edwards et al. (1980) demonstrated that at ruminal ammonia concentrations as high as 100 mg $100mL^{-1}$ rumen fluid microbial protein synthesis was increased by changing the substrate from urea plus grain to Starea. These results suggest that synchronization between rate

of ammonia production and rate of energy availability may be important for optimal microbial growth.

Ruminal Ammonia Concentration and Microbial Growth

Many in vitro studies (Satter and Slyter 1974; Roffler and Satter 1975; Slyter et al. 1979; Schaefer et al. 1980) suggest that ammonia concentrations in excess of 5 mg 100 mL^{-1} rumen fluid has no effect on microbial growth. Kang-Meznarich and Broderick (1981), and Pisulewski et al. (1981) indicated a slightly higher ammonia concentration (8 to 10 mg 100 mL^{-1}) for maximum microbial growth. In contrast, in vivo studies (Mehrez et al. 1977; Wallace 1979; Erdman et al. 1986) have demonstrated a positive response to ammonia concentrations as high as 20 mg 100 mL^{-1} rumen fluid. It is suggested that the requirement for ammonia is directly related to substrate availability, fermentation rate and microbial mass (Hespell and Bryant 1979; Russell et al. 1983). The study of Teather et al. (1980) with lactating dairy cows has shown that supplementation of a basal diet containing 9.4% CP with various N sources increased bacterial number by up to 230%, and this increase was closely associated with both cellulose digesting bacteria (Ruminococcus spp.) and the dominant propionate producing bacteria (Selenomonas spp.).

Ruminal Ammonia Concentration and Degradation of Feedstuffs

Given that ruminal degradation of ingested feed is the function of microbes, the extent of degradation may be related to microbial numbers within a residence time of feedstuffs in the rumen. However, limited data is available to support the relationship between microbial numbers

and ruminal digestion. Wallace (1979) observed increased dry matter (DM) degradation of barley grain in association with increased bacterial number when ammonia concentration was increased from 6.1 to 13.4 mM (9.7 to 21.4 mg 100 mL^{-1}) by urea supplementation.

Despite nitrogen being a limiting nutrient for ruminal bacteria (Hespell and Bryant 1979; Cotta and Hespell 1986), with many ruminal bacteria having an absolute requirement for ammonia (Bryant and Robinson 1961; Hungate 1966; Owens and Bergen 1983), only a few studies have examined the relationship between ammonia concentration and microbial enzymatic activity. Bacterial proteolytic activity was induced by the presence of protein substrates (Mangan 1972). Oldham (1984) suggested that insufficient supply of N substrate may limit microbial activity and potentially impair digestion in the rumen. Digestibility and feed consumption have been shown to increase with increasing dietary protein content (Chalupa 1982; Oldham 1984; Oldham and Smith 1982). In contrast, Grummer and Clark (1982) observed slowest degradation of soybean meal at 1 to 4 h postfeeding when ruminal ammonia concentration was at a maximum (14 to 19 mg 100 mL^{-1}) compared to that at later times postfeeding. Blackburn (1968a, b), reported that protease production was neither induced nor repressed by vitamins, VFA, tryptose, proteose peptone, casamino acids, glutamates, aspartate, arginine, or lysine. Russell and Hespell (1981) indicated that proteolytic activity of rumen bacteria may be altered by changes in population.

There is very little evidence that ammonia concentration regulates starch and cellulose digestion in the rumen. Early studies (Belasco

1954a, b) demonstrated that addition of urea to short term (24 h) semicontinuous fermentations of rumen contents greatly improved cellulose digestion with maximul digestion occurring when ammonia concentrations reached 43 mg 100 mL^{-1}.

Ruminal pH and Microbial Hydrolytic Activity

Some enzymatic activities of ruminal bacteria have been shown to be sensitive to pH changes. Most proteolytic bacteria have a broad pH range (Hazlewood et al. 1981; Cotta and Hespell 1986), but the pH optima for maximal activity of mixed bacterial proteases are in the range of pH 6 and 7 (Kopecny and Wallace 1982). As pH decreases bacterial proteolytic activity and deaminase activity are generally inhibited, but deamination seems to be more sensitive to this inhibition than proteolysis (Erfle et al. 1982). In most in vitro and in vivo studies, reduced pH has a major impact on fiber digestion (Terry et al. 1969; Stewart 1977; Mould and Ørskov 1984). Mould et al. (1984) observed that pH reduction from 6.8 to 6.0 resulted in a moderate depression in fiber digestion, whereas decreasing pH below 6.0 caused severe inhibition. Continuous culture studies support this observation (Crawford et al. 1980; Hoover et al. 1984; Shriver et al. 1986). The depression in fiber digestion caused by pH reduction from 6.8 to 6.0 is not readily explained since the activity of isolated fibrolytic enzymes remains high in this range (Smith et al., 1973; Stanley and Kesler 1959; Groleau and Forsberg 1983). Results of some studies suggest that reduced fiber digestion associated with a moderate decrease in pH may be due to inhibition of bacterial attachment to feed

particles. Smith et al. (1973) reported an apparent interaction between pH and attachment, and Cheng et al. (1984) also indicated that low ruminal pH appeared to prevent a tight attachment of bacteria to plant cell walls. Starch digesting bacteria are usually tolerant to lower pH (Hobson and Wallace 1982). There is evidence that Bacteroides amylophilus (Abou Akkada and Blackburn 1963) and Streptococcus bovis (Russell et al. 1983), which are two of the major proteolytic and starch digesting bacteria in the rumen, can proliferate at lower pH.

Protein Solubility and Ruminal Protein Degradation

Up to 60% of the total N in common feed ingredients may be soluble in buffer (Oldham 1981b; Wholt et al. 1973; Crooker et al. 1978; Krishnamoorthy et al. 1982). Protein solubility is positively related to rate of ruminal degradation of protein (Crooker et al. 1978; Crawford et al. 1978). However, this relationship is not absolute. Buffer soluble proteins such as serum albumin (Baldwin and Denham 1979), ovalbumin (Mangan 1972), fraction 1 leaf protein (Nugent and Mangan 1981) and soluble proteins from soybean meal and rapeseed meal (Mahadevan et al. 1980) have variable resistance to ruminal degradation. Nugent and Mangan (1978) suggested that buffer solubility of protein is not a good indicator of susceptibility to hydrolysis by rumen microbial protease. Stern and Satter (1984) also indicated that solubility may be a poor predictor of protein degradation. Mahadevan et al. (1980) reported that structural characteristics of proteins and the presence of disulfide bond have a greater influence on degradation of protein by rumen microbes than protein solubility.

Microbial Growth and Volatile Fatty Acid Production

Efficiency of production of different VFA's by rumen microbes has not been established. Russell and Hespell (1981) indicated that the proportion of VFA's varies with diet and frequency of feeding due to changes in microbial metabolism and species. Pisulewski et al. (1981) monitored the efficiency of microbial growth, and observed a tendency for high microbial yield when the diet resulted in high propionate production. Teather et al. (1980) and Grummer et al (1984) found trends to increased propionate production with increasing ammonia concentration associated with urea supplementation or NH_4Cl infusion.

Utilization of Ammoniated Silage

Supplementation with NPN is generally considered useful only when the resulting ammonia is utilised by ruminal bacteria. Silage has been widely used as a carrier for NPN in ruminant diets. Studies comparing urea treated and untreated silages in isonitrogenous diets have shown slightly improved milk yields for treated silages (Huber 1975; Huber and Thomas 1971), but others have shown no difference (Van Horn et al. 1967; Polan et al. 1968;). The superiority of ammoniated over urea- treated silages was supported by studies (Huber et al. 1979; Huber et al. 1980) in which cows fed ammoniated silage had higher milk yield than those fed urea treated silage. Compared to untreated silage, ammoniation of silage increased lactic acid (Buchanan-Smith 1982; Lomas and Fox 1982; Heinrichs and Conrad 1984) and water insoluble N concentrations(Huber et al. 1980; Smith et al. 1982; Hargreaves et al. 1984). In addition silage was less susceptible to heating and spoilage when exposed to air

(Britt and Huber 1975; Glewen and Young 1982; Thoracius and Robertson 1984). This increased stability results from the antifungal action of ammonia and ammonium salts (Britt and Huber 1975). Increased insoluble N concentration in ammoniated silage results partly from binding of free ammonia to the water insoluble fraction of the forage and partly from a decrease in proteolysis of plant protein (Huber and Kung 1981). Improved feeding value of ammoniated silage is presumably associated with increased water insoluble N content and reduced proteolysis of plant protein. The effect of altered fermentation characteristics of ammoniated silage on animal performance has not been well explained except that improved net energy value of corn silage was closely related to increased lactic acid concentration arising from ammoniation (Lomas and Fox 1982).

Objectives of the studies described herein were to examine the effect of ruminal ammonia concentration on bacterial growth, degradation of feed ingredients, and fermentation patterns in the rumen. The influence of ammoniation of barley silage on fermentation characteristics in the silo, metabolic responses and silage feeding value for dairy cows were also studied.

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II. EFFECTS OF AMMONIA CONCENTRATION AND MICROBIAL POPULATION ON IN VITRO DEGRADATION OF ^{14}C-LABELLED DIETARY PROTEINS

A. INTRODUCTION

Utilization of ammonia by ruminants depends upon its conversion to microbial protein by rumen microbes and subsequent protein digestion in the intestine. Efficiency of ammonia utilization by microbes is closely related to supply of fermentable energy (Russell et al. 1983). Rate and extent of protein degradation by rumen microbes have been the subject of extensive research. However, little information is available on the relationship between rate of protein degradation and microbial growth as influenced by ammonia concentration. Indeed, recommended ammonia concentrations for maximal bacterial growth based on in vitro continuous culture studies (Roffler and Satter 1975; Slyter et al. 1979) are much less (5 mg 100 mL^{-1} rumen fluid) than levels recommended (more than 20 mg 100 mL^{-1} rumen fluid) based on in situ studies (Mehrez et al. 1977; Erdman et al. 1986). These results suggest that ammonia concentration for maximal microbial growth may not be equal to concentration required for optimal rate of ruminal protein degradation. Wallace (1979) reported that increased dry matter (DM) and protein degradation in situ was accompanied by increased bacterial growth when ammonia concentration was increased from 6.1 to 13.4 mM (9.7 to 21.4 mg mL^{-1} rumen fluid). In contrast, Nikolic and Filipovic (1981) reported that in vitro degradation of maize was not influenced by ammonia concentrations up to 25.8 mg 100 mL^{-1}. Differences in protein degradation in relation to ammonia concentration could be attributed to type of degradable

substrate. Nikolic and Filipovic (1981) used maize, while Wallace (1979) used barley grain which has a higher fermentability than maize (Waldo 1973). On the other hand, Grummer and Clark (1982) observed slower degradation of soybean meal at 1 h to 4 h postfeeding when ruminal ammonia concentrations were highest (14 to 19 mg 100 mL^{-1}). They postulated that this slow degradation could be due to either a lag time prior to microbial attachment to feed particles or suppressed bacterial protease activity via a mechanism analogous to classical feedback inhibition.

Protein solubility is positively related to rate of ruminal degradation of protein (Crooker et al. 1978; Crawford et al. 1978). However, this relationship does not always hold true. Nugent and Mangan (1978) observed differences in the rate at which casein, fraction 1 leaf protein, and bovine serum albumin were hydrolyzed in vitro. As these proteins were all buffer soluble they suggested that buffer solubility of protein was not a good indicator of susceptibility to hydrolysis by rumen bacterial proteases. Stern and Satter (1984) also postulated that solubility may be a poor predictor of ruminal protein degradation.

Bacteria are considered to be primarily responsible for protein degradation while protozoa play a minor role. Forsberg et al. (1984) observed that protozoa had 10% of hydrolytic activity of bacteria in degradation of azocasein in vitro. Wallace and Brammall (1985) confirmed that most of the proteolytic activity in the rumen is associated with bacteria.

Objectives of this study were to examine effects of ammonia concentration on degradation rate of dietary protein by total mixed

ruminal microorganisms and mixed ruminal bacteria, and to determine the relationship between protein solubility and protein degradation.

B. MATERIALS AND METHODS

Animals and Feeding

Two non-lactating Holstein cows fitted with rumen cannulae were fed 11 kg d^{-1} (DM basis) of a mixed diet consisting of 75% barley silage and 25% concentrate in two equal portions at 0800 and 1700 h. The concentrate consisted of 95% rolled barley grain, 3.33% cane molasses and 1.67% minerals on a DM basis. Crude protein (CP) content of the diet was 11.1%.

Extraction of Soluble Protein

Soluble protein was extracted from protein sources according to the method of Crooker et al. (1978), except that a borate-phosphate buffer solution consisting of 12.2 g $NaH_2PO_4.H_2O$ and 8.91 $Na_2B_4O_3.10H_2O$ per 1 L distilled water was used instead of a bicarbonate-phosphate buffer. Ground (0.5 mm mesh) soybean meal (SBM), fish meal (FM) and corn gluten meal (CGM) were weighed into 1 L flasks and buffer solution was added (1 g feed 65 mL^{-1} buffer). The flask was sealed with a rubber stopper and incubated on a shaker in a temperature controlled room (39°C) for 60 min. After incubation the protein suspension was filtered through Watman no. 54 filter paper. Buffer soluble protein in the filtrate and residual protein content were measured by the Kjeldahl method (AOAC 1980). Buffer soluble protein content (%) was estimated as the difference between total protein in filtrate and that in the protein

source prior to extraction.

^{14}C-Labelling of Proteins

Labelling of proteins with ^{14}C-HCHO was carried out by the method of Wallace (1983). Briefly, a protein suspension in distilled and deionized water (10 mg mL^{-1}) was kept on ice and 0.015 volume of sodium borohydride ($NaBH_3$) solution (0.5 mg mL^{-1}) was added. Within 10 sec, 0.05 volume of ^{14}C-HCHO solution (0.1 mg mL^{-1}) was added. The mixture was kept on ice for 30 min and then dialysed against distilled and deionized water at 4°C until the radioactivity of dialysing water was minimized to a plateau. The material was then freeze-dried. The specific radioactivity of ^{14}C-HCHO used in this experiment was 5 mCi 1 mL^{-1} and that of SM, FM, and CGM, were 1.29, 2.08, 1.92 uCi g^{-1}, respectively.

Sampling and Preparation of Rumen Fluid

Rumen fluid was taken from two cows at 2 h post-feeding, strained through 4 layers of cheese cloth, and an equal volume from each animal was mixed. Strained rumen fluid was brought to the laboratory within 15 min and was used without further treatment for measurement of protein degradation by total mixed ruminal microorganisms (TMM). For measurement of protein degradation by mixed bacteria (MB), strained rumen fluid was centrifuged at 500 x g for 10 min and the supernatant was used. Oxygen free CO_2 gas was flushed into tubes before and after centrifugation. Tubes were screw capped while being centrifuged.

Measurement of Protein Degradation by Rumen Microorganisms

Rate of protein degradation in rumen fluid was estimated with ^{14}C-labelled proteins. The reaction mixture (3 mL), in a 10 mL culture tube, contained 1.5 mL of strained or centrifuged rumen fluid and approximately 6 mg of ^{14}C-labelled protein in 25 mM potassium phosphate (KH_2PO_4), pH 7.5. Ammonium sulfate ($(NH_4)_2SO_4$, 1 M) solution was added to the mixture, prior to addition of rumen fluid, to achieve 10, 20, and 30 mg NH_3-N 100 mL^{-1}. Control tubes (4 mg NH_3-N 100 mL^{-1}) contained no added $(NH_4)_2SO_4$. Reaction mixtures were incubated in a water bath with a shaker for 0.5, 1, 2, 3, and 4 h at 39°C. The reaction was stopped by adding 25% (wt/vol) trichloroacetic acid (TCA) solution to a final concentration of 5 g 100 mL^{-1}. Trichloroacetic acid insoluble material was removed by centrifugation (26,000 x g for 10 min 4°C). For zero time incubations TCA was added immediately after addition of rumen fluid to the protein and buffer mixture. Incubation of blanks was carried out in the same way as the reaction mixture except that rumen fluid was not added. Duplicate tubes were used for each incubation time, ammonia level and microbial population. Tubes containing the reaction mixture were tightly capped with rubber stoppers during incubation. All incubation procedures were conducted anaerobically.

Counting of Radioactivity and Estimation of Protein Degradation

After designated incubation times, reaction mixtures were centrifuged and 1 mL of supernatant was added to liquid scintillation cocktail (Beckman Ready Gel, pseudocumine/xylene surfactant), and radioactivity (dpm) was determined by liquid scintillation spectroscopy (Beckman LS

5801). Radioactivity of ^{14}C-labelled proteins were determined by oxidizing in a biological material oxidizer (Harvey Ins. Co; Model, OX-100, N.J.) and measuring radioactivity of CO_2 trapped in the coctail (mixture of toluene, methyl ethylene glycol monomethyl ether, and monothanolamine) by liquid scintillation counter.

Percent degradation of protein was estimated for each time of incubation as the difference between dpm of ^{14}C-labelled test protein before incubation and that from the supernatant of reaction mixture after incubation corrected for the blank. The degradation rate was fitted to the equation $P=a+b(1-e^{-kt})$ (Ørskov and McDonald 1979), where P is the percent degraded at time t, a is an intercept representing the soluble portion of protein at time zero, b is the potentially degradable fraction and k is the rate of degradation of fraction b. Because b values were considerably higher than 100% rate of degradation of SBM was estimated by simple regression from the linearly increased portion of degradation which is shown between 0 to 3 h incubation, and expressed as mg SBM degraded per mL rumen fluid per h. Values of a, b and k were compared for FM and CGM.

Statistical Analysis

Data obtained were subjected to analysis of variance. Where significant, treatment means were compared at probability levels of .01 or .05 using Student-Newman-Keul's test (Steel and Torrie 1980).

C. RESULTS AND DISCUSSION

Solubility on Protein Degradation

Nitrogen contents of SBM, FM and CGM used in this experiment were 8.3, 10.2 and 11.2% on a DM basis, respectively. Soluble N concentrations were 17.3, 11.7 and 4.6% of total N for SBM, FM and CGM, respectively. This presumably reflects the physical and chemical properties of these protein sources (Mahadevan et al. 1980).

Degradation of protein in this experiment was not corrected for changes in microbial biomass associated with test proteins and ammonia concentrations since microbial growth during in vitro incubation would be expected to be small (Wallace et al. 1987).

As expected, differences were observed between protein sources with the extent of degradation of protein being in the order SBM > FM > CGM. Extent of degradation of protein sources followed a similar order to that observed for protein solubilities. This data is supported by the observations of Crooker et al. (1978), Crawford et al. (1978) and Stern and Satter (1984) which indicate that solubility of dietary protein is positively correlated to ruminal degradation.

Protein Degradation -Total Mixed Microorganisms vs Mixed Bacteria

Removal of protozoa and feed particles reduced protein degradation by 20% (Table 1), but the three protein sources responded differently. Ratios of MB to TMM degradation were in the range of .80 to .87, .85 to .91 and .90 to .96 for SBM, FM and CGM, respectively. These results are in agreement with observations of Nugent and Mangan (1978), Forsberg et al. (1984) and Wallace and Brammall (1985) who all found that most rumen

proteolytic activity is associated with bacteria. Craig and Broderick (1981) emphasized that the physical form of protein was one of the most important factors in the relative contribution of bacteria and protozoa to proteolysis. Hino and Russell (1987) found a synergistic increase in ammonia and a decrease in nonammonia-, nonprotein-N when protein was degraded by combinations of bacteria and protozoa compared to bacteria or protozoa only. They concluded that soluble proteins are primarily degraded by bacteria while protozoa could contribute to the degradation of insoluble, particulate proteins. However, care must be taken in interpretating these data because feed particles collected with protozoa could be heavily colonized by bacteria (Minato et al. 1966; Forsberg and Lam 1977).

Ammonia Concentration and Protein Degradation

Patterns of protein degradation differed between protein sources. While degradation of SBM was almost linear up to 3 h incubation those of FM and CGM were curvilinear (Figure 1). Barrio et al. (1985) suggested that protein disappearance might not be expected to be strictly first order since most feedstuffs contain several protein types, and each type has a digestion rate which may or may not be first order.

Percent degradation of protein sources after 4 h incubation by ruminal microorganisms was not influenced by ammonia concentrations except for SBM which the highest ammonia concentration (30 mg 100 mL^{-1}) decreased ($P<.05$) protein degradation by TMM (Table 1).

Rate of degradation of SBM by TMM (mg protein degraded mL^{-1} rumen fluid h^{-1}), as estimated by simple linear regression ($r^2=.99$), tended

to decrease with increased ammonia concentration, but the differences were not significant (Table 2). Similarly, no effect of ammonia concentration was observed on rate of degradation of SBM by MB. However, significant ($P<.01$) differences between TMM and MB in rates of degradation of SBM were observed at all ammonia concentrations. The average rate of degradation of SBM by MB was 78% of that achieved with TMM.

Non-linear parameters (a, b and k) of FM were not influenced by ammonia concentration (Table 3). But decreased ($P<.05$) potentially degradable portion (b) and increased ($P<.05$) rate of degradation (k) of b, by both microbial populations were observed for CGM at higher ammonia levels (10 to 30 mg 100 mL^{-1}). Removal of protozoa and feed particles by low speed centrifugation did not change the values of the soluble fraction (a), b or k fractions for FM, but appeared to decrease a and k values for CGM. These results may indicate that degradation patterns of protein by rumen microorganisms varies with protein sources, presumably due to their different physical and chemical characteristics.

Little is known about the effect of ammonia concentration on proteolysis or deamination by rumen bacteria. Mehrez et al. (1977) found increased in situ DM disappearance of barley grain with increased ammonia concentration in the rumen. Increasing ammonia concentration from 6.1 to 13.4 mM (9.7 to 21.4 mg 100 mL^{-1}) by supplementing the diet with urea caused a 90% increase in the rate of degradation of DM from rolled barley in situ, but smaller increases were observed for protein (Wallace 1979). This increase in DM degradation was accompanied by increased bacterial numbers. Nikolic and Filipovic (1981) were not able

to demonstrate an effect of ammonia concentration (up to 25.8 mg 100 mL^{-1}) on rate of degradation of maize protein. Differences in protein degradation in relation to ammonia concentration could be attributed to type of degradable substrate used. Nikolic and Filipovic (1981) used maize while Mehrez et al. (1977) and Wallace (1979) used barley grain. Waldo (1973) indicated that barley grain has a higher fermentability than maize. deFaria and Huber (1985) suggested that higher ammonia N is required for feeds of high fermentability. Erdman et al. (1986) also reported that ruminal ammonia concentration required for maximal digestion is not constant but rather a function of diet fermentability.

Results of this study (Tables 2 and 3) suggest that ammonia concentration in the range of 4 to 30 mg mL^{-1} is not likely to affect rate or extent of protein degradation. These results differ from those of Grummer and Clark (1982) who observed reduced SBM degradation at ammonia concentrations of 14 to 19 mg 100 mL^{-1}. It is not clear whether this depressed degradation is due to a microbial population or response of proteolytic enzymes to increased ammonia concentrations. Russell et al. (1981) indicated that the greatest increases in ammonia concentration in ruminants occurs 1 to 3 h after ingestion of a meal. During this period soluble carbohydrates are present, and thus microbial growth is at its maximum. They found little effect of ammonia level (6 to 45 mg 100 mL^{-1}) on protein degradation unless adequate readily available energy is supplied for bacterial growth. This may indicate that protein degradation in the rumen is at least in part a function of the number of the microbial population. In this study energy supply was fixed for all treatments, and microbial growth was not estimated. Care must be

taken when comparing the result of in vitro studies to in vivo or in situ studies since method of incubation has been demonstrated to influence results obtained. For in situ degradation studies soluble portion (a) of a protein source is estimated by washing in water, but in vitro incubations are of relatively short duration and soluble protein is not removed.

Results of this study indicate that rumen microbial protein degradation in vitro was not influenced by ammonia concentration in the range of 4 to 30 mg 100 mL^{-1}. Dietary protein degradation by rumen microorganisms tended to follow solubility of protein source. Proteolytic activity was primarily associated with bacteria while protozoa played a minor role.

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Table II-1. Effect of ammonia concentration on percent degradation of protein in vitro after 4 h incubation with total mixed ruminal microorganisms (TMM) or mixed ruminal bacteria (MB).

Protein[1]		Ammonia (mg 100 mL^{-1})[2]				SEM[3]
		4	10	20	30	
SBM	TMM	58.5[ab]	59.5[b]	55.8[ab]	54.1[a]	.42
	MB	47.4	47.5	48.4	46.1	.38
	MB/TMM[4]	.81	.80	.87	.85	
FM	TMM	33.9	34.3	34.2	32.4	.14
	MB	28.7	31.0	31.1	27.8	.20
	MB/TMM	.85	.90	.91	.86	
CGM	TMM	29.9	27.3	25.1	25.6	.33
	MB	26.8	25.1	24.0	24.2	.29
	MB/TMM	.90	.92	.96	.95	

[a,b] Means (percent degradation) in the same row with different superscripts differ ($P<.05$).

[1] Protein - SBM, soybean meal; FM, fish meal; CGM, corn gluten meal.

[2] Ammonia concentration was adjusted with 1 M $(NH_4)_2SO_4$.

[3] Standard error of means.

[4] MB to TMM ratio in percent degradation of protein.

Table II-2. Effect of ammonia concentration on the rate of degradation (mg mL^{-1} h^{-1})[1] of soybean meal (SBM) by total mixed ruminal microorganisms (TMM) or mixed ruminal bacteria (MB).

Ammonia-N[2]	Microbial population[3]		SIG[4]	TMM/MB[5]
(mg 100 mL^{-1})	TMM	MB		
	- (mg mL^{-1} h^{-1}) -			
4.0	1.45	1.09	*	.75
10.0	1.46	1.06	*	.73
20.0	1.32	1.08	*	.82
30.0	1.20	1.02	*	.85
Mean	1.37	1.06		.78

[1] Rate of degradation was expressed as mg protein degraded per mL rumen fluid per hour.
[2] Ammonia concentration was adjusted with 1 M $(NH_4)_2SO_4$.
[3] TMM, total mixed ruminal microorganisms (bacteria plus protozoa); MB, mixed ruminal bacteria.
[4] Significant difference at the probability of .01.
[5] TMM to MB ratio.

Table II-3. Effect of ammonia concentration on non-linear parameters (a, b, k) for fish meal (FM) and corn gluten meal (CGM) after incubation with total mixed ruminal microorganisms (TMM) or mixed ruminal bacteria (MB).

Parameters[1]		Microbes[2]	NH_3-N (mg 100 mL^{-1})[3]				SEM[4]
			4	10	20	30	
FM	a	TMM	3.9	4.2	4.7	4.1	.26
		MB	3.9	3.4	3.4	3.8	.43
	b	TMM	28.9	27.0	26.7	26.1	.61
		MB	25.9	28.5	27.5	25.2	.58
	k	TMM	.94	.97	1.06	.89	.91
		MB	1.01	.97	1.17	1.09	.88
CGM	a	TMM	1.4	1.7	1.9	1.5	.45
		MB	.7	.8	.8	1.0	.30
	b	TMM	35.6^{b}	27.6^{a}	26.9^{a}	29.7^{ab}	.24
		MB	34.5^{b}	28.9^{a}	31.0^{ab}	29.2^{a}	.25
	k	TMM	$.41^{a}$	$.57^{b}$	$.55^{b}$	$.53^{b}$	.42
		MB	$.38^{a}$	$.52^{c}$	$.44^{b}$	$.43^{b}$	.37

[a,b,c] Means in the same row with different letters differ ($P<.05$).
[1] Parameters a, b, k were estimated according to the equation $p=a+b(1-e^{-kt})$ (Ørskov and McDonald 1979).
[2] TMM, total mixed ruminal microorganisms (bacteria plus protozoa); MB, mixed ruminal bacteria.
[3] Ammonia concentration was adjusted with 1 M $(NH_4)_2SO_4$.
[4] Standard error of means.

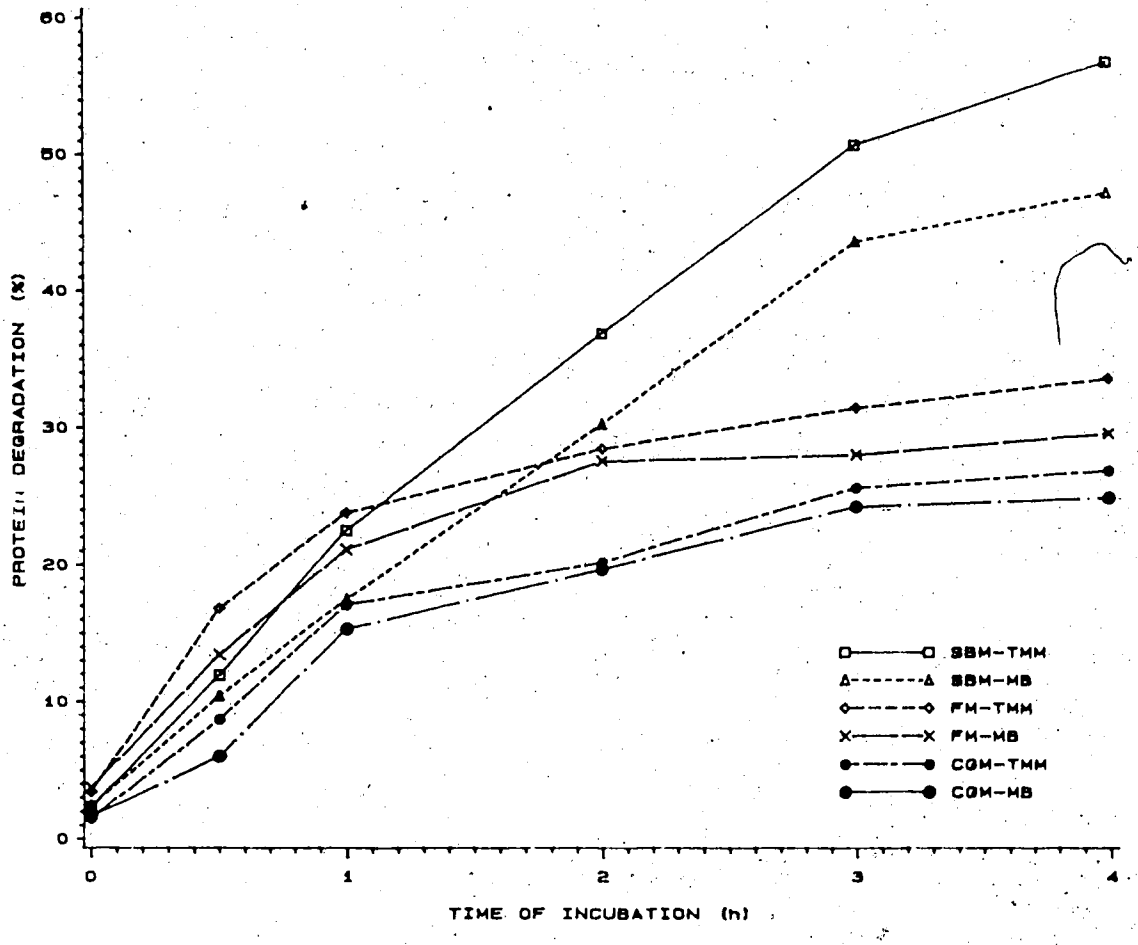

Figure II-1. In vitro degradation of ^{14}C-labelled proteins by total mixed ruminal microorganisms (TMM) or mixed ruminal bacteria (MB).

III. IN SITU DEGRADATION OF FEED INGREDIENTS, FERMENTATION PATTERN AND MICROBIAL POPULATION AS INFLUENCED BY RUMINAL AMMONIA CONCENTRATION

A. INTRODUCTION

Benefits from dietary non-protein nitrogen (NPN) supplementation is primarily derived from incorporation of ammonia into ruminal microbes. Many fiber digesting bacteria in the rumen prefer ammonia as a nitrogen source (Hungate 1966) and starch, sugar and secondary feremters also require ammonia (Cotta and Russell 1982). However, efficiency of ammonia utilization for microbial protein synthesis depends on substrate availability and fermentation rate (Russell et al. 1983).

Attempts to establish optimum ruminal ammonia concentration have focused on maximal microbial growth and ruminal degradation of feedstuffs. However, in vitro and in situ studies have tended to yield conflicting results. In vitro continuous culture studies (Satter and Slyter 1974; Satter and Roffler 1977; Slyter et al. 1979) recommend ammonia concentration of less than 5 mg 100 mL^{-1} for maximal microbial growth, while in situ studies (Mehrez et al. 1977; Wallace 1979; Erdman et al. 1986) have suggested ammonia concentrations in excess of 20 mg 100 mL^{1}. Discrepencies also exist within in situ studies (Wallace 1979; Nikolic and Filipovic 1981; Grummer and Clark 1982) suggesting that ammonia concentration required for maximal microbial growth is not equal to that required for optimal rate of digestion. However, Wallace (1979) observed both increased microbial numbers and increased in sacco dry matter (DM) disappearance with urea supplementation.

Little information is available on the regulation of proteolytic enzyme activity. Grummer and Clark (1982) observed slower degradation of soybean meal protein at 1 to 4 h postfeeding, when ruminal ammonia concentrations were 14 to 19 mg 100 mL^{-1}, than later times postfeeding when ruminal ammonia concentrations were lower. They postulated that the proteolytic activity of rumen microbes might be suppressed by higher ammonia concentration via a mechanism analogous to classical feedback inhibition. However, in a previous experiment (Chapter II of this thesis), in vitro degradation rate of various dietary protein sources was not affected by ammonia concentrations ranging from 4 to 30 mg 100 mL^{1}. Nikolic and Filipovic (1981) also were unable to find any influence of ammonia concentration, up to 25.8 mg 100 mL^{-1}, on disappearance of maize protein. Poos et al. (1979) suggested that very low ammonia concentration could affect proteolytic activity to the extent that ammonia might limit microbial growth.

Since microbial populations in the rumen are influenced by the amount and type of substrate provided (Hungate 1966) it might be expected that the microbial population may differ depending on the structural characteristics of feedstuffs. Wallace et al. (1987) observed altered proteolytic flora when albumin replaced casein in the diet although they found little change in total proteolytic activity.

Objectives of this study were to examine the influence of ammonia concentration on ruminal fermentation patterns, in situ disappearance of selected feed ingredients, and on bacterial populations in rumen fluid.

B. MATERIALS AND METHODS

Animals and Feeding

Four non-lactating Holstein cows fitted with rumen cannulae were assigned in a 4 x 4 Latin square design to four treatments which differed in ruminal ammonia concentration.

Cows were fed 11 kg d^{-1} (dry matter (DM) basis) of a complete mixed diet by automatic feeders in 12 equal portions at 2 h intervals. The diet consisted of 70 % barley silage, 28 % rolled barley grain, 0.5 % fish meal (FM), 1.0 % cane molasses, 0.14 % dicalcium phosphate, 0.16 % limestone, 0.15% trace mineral salts, and 0.05% vit A,D,E mixture on a DM basis. The diet contained 11.5 % crude protein (CP).

Infusion of NH_4Cl

In period one cows were allowed 2 wk to adapt to the basal diet prior to infusion of ammonium chloride (NH_4Cl) solution. Between periods 7 d were allowed for adaptation to the basal diet prior to commencement of NH_4Cl infusion. Infusion of NH_4Cl solution was initiated on d 15 of the first period, and on d 8 of periods two to four. Cows were gradually adapted to NH_4Cl during the first 3 d of infusion. Ammonia concentrations in rumen fluid and rate of NH_4Cl infusion were monitored from d 4 of infusion to achieve the desired ammonia concentration for each treatment.

Three levels of ammonium chloride (NH_4Cl) were dissolved in distilled water to a total volume of 1.8 L. Solutions were continuously infused through a rumen cannulae using a Technicon Autoanalyzer Proportioning pump with tubing (Gradco Analytical Ins. Sci. BD 1.42) to achieve

ammonia concentrations of 15, 25 and 35 mg/100 mL rumen fluid. In control animals ammonia concentration was 11.2 mg/100 mL. Five to 7 d were required to stabilise ammonia concentrations. Average NH_4Cl infusions were 122.3, 240.7 and 389.8 g d^{-1} to achieve ruminal ammonia concentrations of 16.3, 24.8 and 34.9 mg 100 mL^{-1}, respectively. Infusions of NH_4Cl were equivalent to 32, 63 and 102 g N d^{-1}. Infusion rates were 5.10, 10.03 and 16.24 g NH_4Cl h^{-1}, respectively.

Sampling and Analysis

Samples of rumen fluid were obtained using a 120 mL syringe attached to a plastic tube. Samples were collected 1.5 h postfeeding (0930 h and 1330 h) for 3 consecutive d each period, and pH was measured immediately. Rumen fluid was prepared for volatile fatty acid (VFA) analysis by adding 1 mL of 25% orthophosphoric acid to 5 mL rumen fluid. Samples of blood were taken from the tail vein at 1.5 h postfeeding (1130 h and 1530 h) for 3 consecutive d during each period, and plasma was separated by centrifuging whole blood at 2,800 x g for 10 min. Samples of barley silage and concentrate were taken twice during each period. All samples were kept frozen at -20°C until analyzed.

Crude protein (CP) contents of test ingredients and total diet were analyzed by Kjeldahl method (AOAC 1980). Cell wall (CW) in barley silage was determined using a neutral detergent solution as described by Goering and Van Soest (1970). Ammonia in rumen fluid was determined by the method of Fawcett and Scott (1960). Ruminal VFA was determined by gas chromatography (Varian Model 3700, OV-351 capillary column, 0.25 mm ID x 30 m). Blood urea N was analyzed by the method of Croker (1967).

In Situ Incubation of Feed Ingredients

Samples of soybean meal (SBM), fish meal (FM), corn gluten meal (CGM), barley grain (BG), and barley silage (BS) were subjected to ruminal degradation. Samples of BG, FM and BS were taken from the same batches used to feed the animals. Barley silage was dried in a forced air oven at 50°C for 3 d and ground through a 2 mm screen (Wiley mill) prior to in situ incubation. Barley grain, SBM and CGM were ground through a 1 mm screen, while commercial FM was used without further processing. Composition of ingredients is given in Table 1.

Two different sizes of nylon (Nytex, B and SH Thompson and Co. Ltd., Montreal, pore size 50 um) bags were prepared. Small bags (3.5 x 5.5 cm) were used for incubation of SBM, FM and CGM; while large bags (7 x 11 cm) were used for BG and BS. Approximately 1 g (air dry basis) of SBM, FM and CGM were placed in small bags and approximately 5 g of BG (air dry basis) and BS (oven dried) were placed in large bags. Bags were incubated in the rumen as described by deBoer et al. (1987). Small bags were duplicated for each incubation time. Large bags were incubated in duplicate for 24 and 48 h incubations only. Bags containing SBM, FM, CGM and BG were suspended in the rumen for 0.1, 2, 4, 8, 12 and 24 h, and bags for BS were suspended for 0.1, 4, 8, 12, 24 and 48 h. Upon removal from the rumen, bags were mechanically washed as described by deBoer et al. (1987). Washed bags were dried in a forced air oven at 60°C for 2 d.

Estimation of Effective Degradability in The Rumen

Percent disappearance of DM, CP and CW at each incubation time was calculated from the portion remaining after incubation in the rumen.

Disappearance rate was fitted to the equation (Ørskov and McDonald 1979): $P=a+b(1-e^{-kt})$, where P is disappearance at time t, a is an intercept representing the portion of DM, CP and CW solubilized, b is the fraction that is degraded at time infinity, k is the rate constant of disappearance of fraction b, and t is incubation time. Nonlinear parameters a, b and k were estimated by an iterative least-square procedure to calculate effective degradability of DM (EDDM), CP (EDCP) and CW (EDCW) according to the equation (Ørskov and McDonald 1979): effective degradability = $a+(bxk)/(k+r)$, where r is the fractional ruminal outflow rate, and a, b and k are as defined above. Three hypothetical fractional ruminal outflow rates (.04, .08 and .12 h^{-1}) were used for estimation of effective degradability.

Enumeration of Viable Count in Rumen Fluid

Approximately 500 g of rumen digesta was taken from the middle part of the rumen solid mat and mixed with rumen liquid. The digesta was strained through 4 layers of cheese cloth into a 200 mL flask. Sampling of rumen fluid was carried out at 1130 h for total viable counts, and at 1530 h for proteolytic bacterial counts on 2 consecutive d during each period.

Nine fold dilutions were prepared in anaerobic diluting fluid (Bryant and Robinson 1961). Total viable bacteria were determined by inoculating dilutions of 10^{-7}, 10^{-8} and 10^{-9} in triplicate into roll tubes containing non-selective artificial medium with rumen fluid (Scott and Dehority 1965). Similar inoculations were made into roll tubes containing casein medium 7 of Hobson (1969) as modified by Wallace

(1979) for proteolytic bacteria. Anaerobic culture techniques as described by Hungate (1966) were employed throughout the study.

Statistical Analysis of Data

Data obtained were subjected to analysis of variance with treatment (ammonia concentration), animal and period as factors. When treatment effects were significant, treatment means were compared at probability levels of .05 and .01 using Student-Newman-Keuls' test (Steel and Torrie 1980). Linear, quadratic and cubic contrasts in response to ammonia concentration were tested using the Statistical Analysis System (SAS 1982).

C. RESULTS AND DISCUSSION

All cows were in positive energy balance with no significant difference between treatments (Table 1). Body weight gain for all cows indicates over-supply of nutrients. The National Research Council (1978) suggests 19.95 Mcal of digestible energy (DE) and 515 g of CP per day for the maintenance requirements of mature cows. In the present study, 31.1 Mcal (DE) and 1.25 kg (CP) as calculated values were provided. A slight depression in DM intake
was observed for cows at the highest level of NH_4Cl infusion.

Ruminal Characteristics and Blood Urea Nitrogen

While ammonia concentration increased ($P<.01$) linearly, pH decreased ($P<.05$) from 6.50 to 6.19 with NH_4Cl infusions (Table 1). Decreased pH in rumen fluid was presumably due to the acidic characteristics of

NH_4Cl. Blood urea N also linearly increased ($P<.05$) with increasing NH_4Cl infusion. Blood urea N content tended to be closely correlated ($r=.74$) to ruminal ammonia concentration up to 24.8 mg 100 mL^{-1} but the correlation was lower at the higher NH_4Cl infusion level. Ha and Kennelly (1984) also observed a relatively high correlation between ruminal ammonia concentration and blood urea N.

Total VFA concentration was not influenced by ammonia concentration (Table 2). But there was a trend for decreased molar percent of acetic acid and increased propionic acid in rumen fluid with increasing ammonia concentration. No differences due to ammonia concentrations were observed for other VFAs. This is in agreement with Teather et al. (1980) and Grummer et al. (1984) who found trends to increased propionate production with increasing ammonia concentration due to urea supplementation or NH_4Cl infusion. Ha and Kennelly (1984), and deFaria and Huber (1984) did not observe differences in VFA proportions with increasing protein content in the diet from 13 to 19% and from 8.1 to 13.3%, respectively. However total VFA concentration increased with protein supplementation.

Bacterial Counts

Viable counts of total mixed bacteria (TBM) and mixed proteolytic bacteria (MPB) in rumen fluid tended to increase with ruminal ammonia concentration up to 24.8 mg 100 mL^{-1} (Table 3). Wallace (1979) olso observed that urea supplementation increased bacterial numbers in rumen fluid. Teather et al. (1980) reported increased microbial growth by increasing N content of diet with supplementations of urea, urea+silage

or SBM. They also found that increased dietary N resulted in large changes in numbers of some bacterial species.

Ruminal Degradation of Feedstuffs

Ruminal disappearances of DM, CP or CW of feed ingredients increased with incubation time in the rumen. Ruminal DM disappearance for BG was not influenced by ammonia concentration with the exception of 2 h incubation where disappearance was lowest ($P<.05$) at highest ammonia concentration (Table 4). No effect of ammonia concentration on EDDM was observed. However, both ruminal disappearance and effective degradability of DM tended to be highest at ammonia level of 16.3 mg 100 mL^{-1}. Ruminal disappearances of SBM decreased ($P<.05$) with increasing ammonia concentration (8 and 12 h for DM, and 2 to 12 h for CP) with highest values again being observed at 16.3 mg 100 mL^{-1} (Table 5). Similar trends ($P<.05$) were observed in EDDM and EDCP at simulated fractional outflow rates of .08 and .12 h^{-1}. Ruminal ammonia concentration was without effect on DM disappearance from CGM except at the 24 h incubation ($P<.05$, Table 6) where DM disappearance was lowest at highest ammonia concentration. Highest DM disappearance was again obtained at an ammonia concentration of 16.3 mg 100 mL^{-1}. Despite the difference in DM disappearance for the 12 h incubation there were no differences in EDDM due to ammonia concentrations. Disappearance of CGM CP for 12 and 24 h incubation was greatest at 16.3 mg ammonia per 100 mL rumen fluid. Differences in EDCP were also observed ($P<.05$) between the first two levels of ammonia for all outflow rates. As observed for BG and SBM, ammonia concentration of 16.3 mg 100 mL^{-1} again appeared to be optimal

for maximal ruminal degradation of CGM. In contrast, ruminal disappearance and effective degradability of DM and CP of FM were not influenced by ruminal ammonia concentration (Table 7). Ruminal disappearance of BS DM and CW tended to decrease with ruminal ammonia concentration with the differences being significant ($P<.05$) during later incubations (24 and 48 h for DM and 12 to 48 h for CW, Table 8). Differences ($P<.05$) in EDDM and EDCW were also observed at simulated outflow rates of .04 and .08 h^{-1}. Highest ruminal degradation values were obtained at the lowest ammonia level (11.2 mg 100 mL^{-1}) while the lowest values were observed at 34.9 mg 100 mL^{-1}.

The extent of ruminal disappearance of feedstuffs appears to be dependent on their fermentability which may in turn be related to solubility. Higher mean disappearance of DM from BG, SBM and BS than those from CGM and FM are closely related to their soluble portions which were estimated by washing after 0.1 h incubation in the rumen (Figure 1). Dry matter of BG and SBM disappeared quickly during earlier incubations (up to 4 h) while that of FM and CGM tended to disappear more slowly over time. Similar trends in rate and extent of protein disappearance were observed (Figure 2). Ørskov et al. (1983) indicated that protein supplements of animal origin are digested more rapidly but incompletely, whereas plant protein are degraded more slowly, but potentially to a greater extent. Ammonia concentration which maximized ruminal degradation was 16.3 mg 100 mL^{1} for most feed ingredients examined in this study. A few authors (Mehrez et al. 1977; Wallace 1979, Erdman et al. 1986) suggest that ammonia concentrations greater than 20 mg 100 mL^{-1} are required for maximal degradation of feedstuffs in

situ. Wallace (1979) reported that increased DM degradation in situ, associated with urea supplementation, was accompanied by increased bacterial growth. In the present study increased bacterial numbers (Table 3) as influenced by NH_4Cl infusion partly supports observed increases in degradation. Degradation of DM and CW of BS, however, decreased with ammonia concentration. The decline in DM degradation at higher ammonia level might be due to reduced CW digestion since most of the rapidly degradable portion disappeared by washing. Reduced CW digestion could be related to lower pH associated with NH_4Cl infusion. Growth of fiber digesting bacteria is inhibited by lower pH, resulting in reduced fiber digestion (Russell and Dombrowski 1980). Steward (1977) reported that reducing ruminal pH from 7.0 to 6.0 almost completely eliminated cellulolytic activity. Mould et al. (1984) observed a moderate depression in fiber digestion when pH was reduced from 6.8 to 6.0. Low ruminal pH appears to prevent a tight attachment of bacteria to plant cell walls (Cheng et al. 1984). But the depression in fiber digestion caused by pH reduction from 6.8 to 6.0 is not readily explained since the activity of isolated fibrolytic enzymes remains high in this range (Smith et al. 1973; Groleau and Forsberg 1983). Change in bacterial flora would also be expected as a result of altered pH or ammonia concentration. Starch digesting (amylolytic) bacteria are generally tolerant to lower pH, while pH optima of proteolytic bacteria is between 6 and 7 although activity is maximized at 7.5 (Hobson and Wallace 1982). Bacteroides amylophilus (Abou Akkada and Blackburn 1963) and Streptococcus bovis (Russell et al. 1983) have been known to proliferate at lower pH. Erfle et al. (1982) indicated that deamination

seems to be more sensitive to lower pH than proteolysis. The increase in total mixed bacterial numbers but decreased CW digestion might indicate altered microbial flora arising from infusion of NH_4Cl. In the present study cows were fed 11 kg d^{-1} (DM basis) at 2 h intervals. Under this feeding regime depletion of readily available nutrients would not be expected. Bacterial population preferring readily available nutrients thus are likely to proliferate.

Little information is available on the mechanism of regulation of proteolytic activity in rumen bacteria. Grummer and Clark (1982) observed slower degradation of SBM at higher ammonia concentrations (14 to 19 mg 100 mL^{-1}), and they postulated that proteolytic activity of rumen microbes might be suppressed at a higher ammonia concentrations. In a previous in vitro study (Chapter II) degradation of various ^{14}C-labelled dietary protein sources was not affected by ammonia concentration. Nikolic and Filipovic (1981) also were not able to find an effect of ammonia concentration at levels up to 25.8 mg 100 mL^{-1} on disappearance of maize protein.

Supplementation of N by NH_4Cl infusion increased microbial growth but did not proportionally increased ruminal degradation of concentrate ingredients. Decreased ruminal CW degradation of barley silage with increasing NH_4Cl infusion may indicate a confounding effect of pH on influence of ammonia concentration due to the acidic characteristics of NH_4Cl.

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microbial species require preformed N (amono acids) for more efficient growth, Theurer (1979) did not observe improved in vitro microbial protein synthesis with amino acid supplementation.

Microbial Growth and Degradation of Feedstuffs as Influenced by Ammonia Concentration

In vitro studies (Satter and Slyter 1974; Slyter et al. 1979; Schaefer et al. 1980) have reported that no more than 5 mg 100 mL^{-1} of ammonia concentration is required for maximum microbial growth. Kang-Meznarich and Broderick (1981), and Pisulewski et al. (1981) suggested ammonia concentrations of 8 to 10 mg 100 mL^{-1} for maximum microbial growth. Allison (1970) observed that the growth of Bacteroides amylophilus, which is one of the major starch and protein fermenting bacteria in the rumen, was restricted when ammonia concentration fell below 7.4 mg 100 mL^{-1} in vitro. Schaefer et al. (1980) determined that ammonia saturation constants in vitro for the predominant species of rumen bateria were less than 50 uM (0.8 mg 100 mL^{-1}). They stated that microorganisms growing in a medium containing 1 mM (1.6 mg 100 mL^{-1}) ammonia should achieve 95% of their maximum specific growth rate, but acknowledged that this concentration would not necessarily provide for maximal microbial growth. On the other hand, in situ degradation rates have been shown to plateau at greater than 20 mg 100 mL^{-1} rumen fluid (Mehrez et al. 1977; Wallace 1979; Erdman et al. 1986). The study of Teather et al. (1980) with lactating dairy cows has shown that supplementations of various N sources (urea, urea-treated maize silage and soybean meal) to basal diet (9.4% CP) increased bacterial numbers by up to 230%, and this increase

was closely associated with cellulose digesting bacteria (Ruminococcus spp.) and the dominant propionate producing bacteria (Selenomonas spp.).

Since ruminal degradation of ingested feed is a function of microbes, the extent of degradation might be expected to be related to microbial numbers within a residence time. However, limited data is available on this relationship and the extent to which it is influenced by ruminal ammonia concentration. Wallace (1979) observed that increased dry matter (DM) and crude protein (CP) degradation of barley grain was accompanied by increased bacterial growth when ammonia concentration was increased from 6.1 to 13.4 mM (9.7 to 21.4 mg 100 mL^{-1} by urea supplemention. However, in our studies (Chapter II) protein degradation in vitro was not influenced by ammonia concentration. Increasing ruminal ammonia concentration up to 16.3 mg 100 mL^{-1} by NH_4Cl infusion had limited influence on ruminal degradation of concentrates ingredients despite significantly ($P<.05$) increased bacterial number in response to increased ammonia concentration (Chapter III). Comparisons of data from in vitro and in situ studies suggest that ammonia concentration for maximal microbial growth is not equal to that required for maximal degradation of feedstuffs.

Little information is available on ruminal cellulose digestion in relation to ammonia concentration. Early studies (Bélasco 1954a, 1954b) demonstrated that addition of NPN to short term (24 h) semicontinuous fermentations of rumen contents greatly improved cellulose digestion, and maximum cellulose digestion occurred when ammonia concentrations reached 43 mg 100 mL^{-1}.

Relationship between Microbial Growth and Volatile Fatty Acid Production in the Rumen

Volatile fatty acid (VFA) are end products of ruminal fermentation. Proportion of VFA varies with diet and frequency of feeding, and are caused by changes in microbial metabolism and species (Russell and Hespell 1981). Efficiency of individual VFA production has been partially examined. Pisulewski et al. (1981) monitored the efficiency of microbial growth, and there was a tendency for high microbial yield when diets which enhanced propionate production were fed. Chalupa (1977) indicated that the efficiencies of fermenting hexose to acetate, propionate and butyrate are 62, 109 and 78%, respectively. Thus, the metabolically useful energy recovered in fermentation end products may be increased by enhancing the production of propionate.

Objectives of this study were to determine the influence of ruminal ammonia concentration on rumen fermentation pattern, bacterial growth and ruminal degradation of feed ingredients.

B. MATERIALS AND METHODS

Animals and Feeding

Three non-lactating Holstein cows fitted with rumen cannulae were maintained in individual pens and fed 5 kg d^{-1} of a complete mixed diet (90% oatlage and 10% concentrate mixture, dry matter (DM) basis) in two equal portions at 0900 and 2100 h. The concentrate mixture consisted of 71.6% rolled barley, 6.0% canola meal, 4.3% fish meal, 5.6% wheat shorts, 8.8% ground shell corn, 2.9% molasses, 0.11% monocalcium phosphate, 0.56% trace mineral salts, and 0.13% Vit.A,D,E mixture (DM

basis). The diet contained 11.1% crude protein (CP).

Cows were assigned to four treatments in a 4 x 3 Youden square design as described by Pearce (1952), consisting of four experimental periods and three cows. Cows were allowed 7 d to adapt to the basal diet.

Infusion of NH_4HCO_3

Four different amounts of NH_4HCO_3 (0, 95, 190 and 280 g d^{-1}) were dissolved in distilled water to a total volume of 1.8 L, and were continuously infused through the rumen cannulae using a Technicon Autoanalyzer Proportioning pump with tubing (Gradco Analytical Inc. Sci. BD 1.42).

Between periods infusion level of NH_4HCO_3 was changed abruptly to new treatment level. Infusion rate of NH_4HCO_3 solution and ammonia concentration in rumen fluid were monitored from d 4 of each period. Four to 8 days required to achieve stable ruminal ammonia concentration.

In Situ Ruminal Effective Degradability of Feed Ingredients

Samples of feed ingredients used for ruminal incubation were soybean meal (SBM) and fish meal (FM) as protein sources, barley grain (BG) as an energy source, and oatlage as a fiber source. Air dried SBM and BG were ground through a 1 mm screen (Wiley mill) while commercial FM was not further processed prior to ruminal incubation. However, large particles, such as bone, were separated from FM by sieving (1 mm mesh). Oatlage was dried in a forced air oven at 45°C for 3 d. Visible oat grain was removed and oatlage was ground through a 2 mm screen.

Nylon bags (3.5 x 5.5 cm, Nytex, B and SH Thompson and Co. Ltd., Montreal, pore size 50 um) were used for ruminal incubation of feed ingredients. Approximately 1 g (air dry basis) of SBM, FM and BG, and 0.5 g (oven dried) of oatlage were placed in nylon bags which were then heat sealed. Duplicate bags for each incubation time were placed in a polyester mesh bag (25 x 30 cm, mesh size 3 mm) which was equipped with a draw string. The draw string was extended to approx. 70 cm, and a 250 mL plastic jar filled with pebbles was attached to the middle of the draw string using nylon string.

Incubation of feed ingredients in the rumen was carried out during sampling days for rumen fluid and blood. Bags containing SBM, FM and BG were suspended in the rumen for 6, 12, 24, 36 and 48 h while bags containing oatlage were incubated for 6, 12, 24, 48 and 72 h. Upon removal from the rumen, bags were washed as described by deBoer et al. (1987). Disappearance for t=0 incubation was estimated by washing with cold water only. Washed bags were dried in a forced oven at 60°C for 2 d.

Percent disappearance of DM, CP and cell wall (CW) at each incubation time was calculated from the portion remaining after incubation in the rumen. The disappearance rate was fitted to the equation (Ørskov and McDonald 1979): $P=a+b(1-e^{-kt})$, where P is percent disappearance at time t, a is an intercept representing the portion of DM, CP or CW solubilized, b is the fraction which is degraded at time infinity, k is rate constant of disappearance of fraction b, and t is incubation time. Nonlinear parameters (a, b and k) were estimated by an iterative least-square procedure to calculate effective degradability of DM (EDDM), CP (EDCP) and CW (EDCW) according to the equation (Ørskov and

McDonald 1979): effective degradability = a+(bxk)/(k+r), where r is the fractional rate of outflow from the rumen. Effective degradability was calculated at three hypothetical fractional outflow rates (.03, .05 and .08 h^{-1}).

Sampling and Analysis

Samples of rumen fluid were taken through the rumen cannulae using a 120 mL syringe connected to plastic tube, at 0.5, 1.5, 3.0, 5.0, 7.0, 9.0 and 11.0 h after the 0900 h feeding for 2 consecutive d during each period. pH was measured immediately after rumen fluid sampling. One mL of 25% orthophosphoric acid was added to 4 mL of rumen fluid for volatile fatty acid (VFA) analysis. Samples of blood were taken from the tail vein at 1, 5, 6 and 11 h after the 0900 h feeding for 2 consecutive d during each period. Plasma was separated by centrifuging whole blood at 2800 x g for 15 min. Oatlage and concentrate mixture were sampled twice during each period. All samples were kept frozen at -20°C until analyzed.

Crude protein was determined by Kjeldahl method (AOAC 1980). Cell wall residue in oatlage was estimated with neutral detergent solution as described by Goering and Van Soest (1970), but CW residues in nylon bags were estimated by washing in commercial detergent solution using a washing machine for one complete cycle. Ammonia concentration in rumen fluid was determined by the method of Fawcett and Scott (1960) using colorimeter (Brinkman PC 800). Volatile fatty acid in rumen fluid was determined by gas chromatography (Varian Model 3700, OV-351 capillary column, 0.25 mm ID x 30 m). Blood urea N was determined by the method of

Croker (1967).

Enumeration of Total Viable Bacteria in Rumen Fluid

Rumen digesta was grab sampled and mixed with rumen liquid. Rumen liquid was separated using four layers of cheese cloth and brought to the laboratory within 15 min.

Eight-fold dilutions were prepared using an anaerobic diluting fluid (Bryant and Robinson 1961). Total viable counts of bacteria were determined by incubating dilutions of 10^{-6}, 10^{-7} and 10^{-8}, in triplicate, in roll tubes containing non-selective artificial medium with rumen fluid (Scott and Dehority 1965). The anaerobic culture techniques of Hungate (1966) were employed throughout the study. Sampling of rumen fluid and incubation of bacteria were conducted twice (1030 and 1900 h) on the final day of each period.

Statistical Analysis

All data were subjected to analysis of variance with treatment (infusion level of NH_4HCO_3), animal and period as factors. When treatment effects were significant treatment means were compared at probability level of .05 using the Statistical Analysis System (SAS 1982). Linear, quadratic and cubic contrasts in response to level of NH_4HCO_3 infusion were tested as described by Steel and Torrie (1980) for factorial experiments.

C. RESULTS AND DISCUSSION

Dietary N intake was 88.8 g d^{-1} for all cows. Continuous infusion of 95, 190 and 280 g d^{-1} NH_4HCO_3 was equivalent to 16.8, 33.7 and 46.9 g N d^{-1}, respectively (Table 1). Rates of NH_4HCO_3 N infusion were 0.7, 1.4 and 2.07 g h^{-1}. Total N supplied to cows ranged from 88.8 to 138.4 g d^{-1}. Daily N supply exceeded requirements (82.4 g) for the maintenance of mature nonlactating cows (NRC 1978), but calculated daily digestible energy (DE) supply (13.3 Mcal) was considerably less than the requirements of 31.1 Mcal.

Ruminal pH and Ammonia Concentration, and Blood Urea Nitrogen

Ammonia concentration in rumen fluid clearly reflected infusion levels of NH_4HCO_3 (Figure 1). Ruminal ammonia concentration peaked at 1.5 h postfeeding for all infusion levels. Thereafter, ammonia concentration declined until 7 h postfeeding after which it remained relatively constant.

Infusion of NH_4HCO_3 slightly increased pH in rumen fluid, but the differences were small among infusion levels (Figure 2). Overall ruminal pH also increased with time after feeding.

Accumulation of ammonia in rumen fluid indicates either increased production, reduced absorption through the rumen wall or less utilization by microbes for protein synthesis. Recycling of N across the rumen wall is negatively related to ammonia concentration and positively related to blood urea N concentration (Owens and Bergen 1983). Thus, increased recycling of blood urea N is expected for relatively low ruminal ammonia concentration (up to 95 g of NH_4HCO_3 infusion),

particularly at later times postfeeding. But it is not known whether the recycled N is utilized by ruminal bacteria since ammonia concentration after 7 h postfeeding remained low (less than 5 mg 100 mL^{-1}).

Blood urea N concentration increased ($P<.05$) linearly with level of NH_4HCO_3 infusion, and overall concentrations declined with time after feeding (Table 2).

Ruminal Volatile Fatty Acid Concentrations

Total VFA concentration in rumen fluid increased ($P<.05$) with increased level of NH_4HCO_3 infusion (Figure 3). Highest VFA concentration was obtained at 1.5 h postfeeding for all infusion levels. While molar proportion (mmole 100 mmole^{-1}) of acetate increased with time (Figure 4) propionate proportion declined (Figure 5). Unlike acetate and propionate, molar proportions of butyrate (Figure 7), isovalerate (Figure 8) and valerate (Figure 9) increased at earlier times (up to 3 h) postfeeding, and then rapidly declined. For isobutyrate (Figure 6) two peaks were observed, one at 1.5 to 3 h and a second at 11 h postfeeding. Molar proportions of acetate, propionate, isovalerate and valerate were not ($P>.05$) influenced by NH_4HCO_3 infusion. But higher ($P<.05$) butyrate and lower isobutyrate proportions were obtained by infusion of NH_4HCO_3. Butyrate proportion was higher ($P<.05$) for 95 g of NH_4HCO_3 infusion than for all other infusion levels.

Effect of production of different VFA on microbial growth has not been established. Pisulewski et al. (1981) observed a tendency for high microbial yield when higher concentration of propionate was produced. Infusion of NH_4HCO_3 slightly increased bacterial counts compared

to no infusion. Optimum ammonia concentration for butyrate producing bacteria may differ from acetate producing bacteria because, while molar proportion of acetate was lowest (Figure 4) butyrate proportion was highest (Figure 7) at 95 g of NH_4HCO_3 infusion. Hungate (1966) indicated that in general, butyrate production is closely associated with acetate production the latter being primarily derived from fermentation of fibrous material. Proportional patterns of isobutyrate and isovalerate over time postfeeding are interesting. Their proportions were relatively increased by NH_4HCO_3 infusion at early fermentation stage (between 1.5 and 3 h for isobutyrate and 1.5 h for isovalerate postfeeding), but decreased after indicated times. Both VFA are known to be derived from the fermentation of protein (amino acid), and are growth factors for a number of bacterial species including cellulolytic bacteria (Bryant 1973; Prince 1977; Russell and Hespell 1981). However, it is not known whether growth and enzymatic activity of these bacteria are depressed at higher ammonia concentration or whether the associated effect of ammonia and other nutrients promotes optimal growth. Hungate (1966) indicated that substrate specificity was one of the main characteristics of rumen bacteria. Russell and Baldwin (1979) observed that affinity for the same substrate differs greatly among species. Scheifinger et al. (1976) also observed that different strains of bacteria differ in their preference to degrade different amino acids. On the other hand, Hespell and Bryant (1979) and Russell et al. (1983) suggested that the requirement for ammonia is directly related to substrate availability, fermentation rate and microbial mass. Based on data for bacterial counts and VFA production it might be postulated that

proliferation of a particular bacterial population is dependent on availability of preferred substrates. Stage of proliferation of particular bacterial population may also differ among species, and the effect of ammonia N supplementation on bacterial growth may be influenced by substrate.

Bacterial Growth

Infusion of NH_4HCO_3 increased ($P<.05$) viable counts of total mixed bacteria in rumen fluid at both 1.5 and 10 h postfeeding with bacterial numbers being relatively higher at 10 h compared to 1.5 h postfeeding (Table 3).

It has been suggested that N is a major limiting nutrient for microbial growth (Cotta and Russell 1982; Owens and Bergen 1983), and that ammonia is the primary N source of most rumen bacteria for protein synthesis (Hungate 1966; Mathison and Milligan 1971). Increased bacterial counts and total VFA production associated with by NH_4HCO_3 infusion clearly indicate the positive effect of additional N, in the form of ammonia, on bacterial growth and fermentation in the rumen. This effect appears to be proportional to levels of NH_4HCO_3 infusion. Studies on the effect of ammonia on bacterial growth and fermentation activity are limited although many researches have confirmed that ammonia N is utilized by most ruminal bacteria. Pittman and Bryant (1964) indicated that Bacteroides ruminicola utilises ammonia and oligopeptide N, but not amino acid or short chain peptide N for growth. Allison (1970) reported that the growth of Bacteroides amylophilus, which is one of the major starch and protein fermenting bacteria in the

rumen, was restricted when ammonia concentration fell below 4.6 mM (7.4 mg 100 mL^{-1}) in vitro. In a study with lactating dairy cows Teather et al. (1980) determined that supplementation of a basal diet containing 9.4% CP (DM basis) with various N sources (urea, urea-treated maize silage or soybean meal) increased bacterial numbers by up to 230%. This increase was closely associated with fiber digesting bacteria (Ruminococcus spp.) and the dominant propionate producing bacteria (Selenomonas spp.). Increased bacterial numbers and total VFA concentration with increased NH_4HCO_3 infusion during the early stage of fermentation postfeeding may be associated with increased numbers of non-fibrolytic bacteria, and at later stages (after 5 h postfeeding) of fermentation with increased fiber digesting bacteria. Depletion of readily degradable substrates may result in higher ammonia N supply, but increased ammonia N may enhance growth of fibrolytic bacteria. Earlier studies by Belasco (1954a, b) suggest a stimulating effect of ammonia, up to 43 mg 100 mL^{-1}, on in vitro growth of cellulolytic bacteria. Patterns of VFA concentration support the above postulation as molar proportion of acetate was low, but propionate proportion was high at earlier stage of fermentation while the opposite was true at later stage of fermentations. The relatively higher bacterial counts at 10 h postfeeding compared to those at 1.5 h are probably due to reduced pool size of rumen fluid. Proliferation of fiber digesting bacteria at 10 h postfeeding may also have contributed to the higher counts. Many studies (Hungate 1966; Forsberg and Lam 1977; Craig et al. 1987) have indicated that a larger bacterial population (60 to 75%) is associated with solid feed particles. Hungate (1966) suggested that since initial steps of

noncellulosic material digestion in the rumen are accomplished rapidly and fiber digestion is relatively slow, ammonia N is likely to be the primary N source available to fiber digesting bacteria.

Ruminal Degradation of Feedstuffs

For almost all incubation times infusion of NH_4HCO_3 did not influence DM disappearance or effective degradabilities of DM (EDDM) and CP (EDCP) for SBM (Table 4). An exception was increased ($P<.05$) DM disappearance at 12 h incubation and a faster ($P<.05$) rate of degradation for the degradable DM fraction. Ruminal disappearances and effective degradabilities of DM and CP of fish meal (Table 5) and DM of barley grain (Table 6) were not influenced by NH_4HCO_3 infusion. Degradation of oatlage DM was also not affected by NH_4HCO_3 infusion, but linear increases ($P<.05$) in ruminal disappearance of the soluble CW fraction were observed (Table 7). Despite higher CW digestion at 12 h incubation no significant differences were observed in effective degradability of CW (EDCW).

The degree and extent of ruminal degradation of feedstuffs would be expected to be correlated to microbial growth. In the present study, NH_4HCO_3 infusion proportionally increased mixed bacterial counts and total VFA concentration, but had little or no influence on ruminal degradation. In contrast Wallace (1979) observed that increased degradation of DM and CP of barley grain was accompanied by increased bacterial growth when ammonia concentration was increased from 6.1 to 13.4 mM (9.7 to 21.4 mg 100 mL^{-1}). Grummer and Clark (1982) observed a slower degradation of SBM at 1 to 4 h postfeeding when ruminal ammonia

concentration was relatively high (14 to 19 mg 100 mL^{-1}). Proteolytic activity was not depressed by increased ammonia concentration in this study. Similar results were observed in our in vitro study (Chapter II). Early studies (Belasco 1954a,b) indicated that addition of urea to short term (24 h) semicontinuous fermentations of rumen contents greatly improved cellulose digestion, and maximum digestion occurred when ammonia concentrations approached 43 mg 100 mL^{-1}. But ammonia concentration did not affect CW degradation of oatlage in the present study. Except for a few isolated instances, ruminal degradation of feed ingredients was not influenced by ammonia concentration although bacterial numbers increased with NH_4Cl infusion (Chapter III).

It is concluded that increasing ammonia concentration increased bacterial numbers in rumen fluid. But increased bacterial numbers did not proportionally influence ruminal degradation of feedstuffs.

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Table IV-1. Influence of NH_4HCO_3 on nitrogen (N) supply.

Items	Infusion of NH_4HCO_3 (g d^{-1})			
	0	95	190	280
Dietary N intake, g d^{-1} [1]	88.8	88.8	88.8	88.8
NH_4HCO_3 N infused, g d^{-1} [2]	0.0	16.8	33.7	49.6
Ratio[3]	.00	.19	.38	.56
Total N, g d^{-1}	88.8	105.6	122.5	138.4

[1] Dietary N supply with a complete mixed diet (5 kg, 11.1% CP, DM basis).
[2] Solution of NH_4HCO_3 were continuously infused into the rumen.
[3] Ratio of NH_4HCO_3 N to dietary N intake.

Table IV-2. Blood urea N concentration (mg 100 mL^{-1}) at various sampling times as influenced by NH_4HCO_3 infusion.

Time (h)[1]	NH_4HCO_3 infusion (g d^{-1})[2]				SEM[3]	Contrast[4]
	0	95	190	280		
1.5	7.9[a]	9.9[b]	10.6[b]	13.5[c]	.51	L
6	7.4[a]	9.2[b]	9.9[c]	11.9[d]	.36	L
11	6.7[a]	8.3[b]	9.2[c]	11.2[d]	.49	L

[a,b,c,d] Means within a row having different superscripts differ ($P<.05$).
[1] Sampling times after 0900 h feeding
[2] Solutions of NH_4HCO_3 were continuously infused into the rumen.
[3] Standard error of means.
[4] Orthogonal contrast where L=linear ($P<.05$).

Table IV-3. Numbers ($x10^8$ mL^{-1}) of total mixed bacteria in rumen fluid as influenced by NH_4HCO_3 infusion.

Time (h)[1]	NH_4HCO_3 infusion (g d^{-1})[2]				SEM[3]	Contrast[4]
	0	95	190	280		
1.5	7.40[a]	8.49[b]	8.73[b]	10.51[c]	.30	L
10	8.02[a]	10.80[b]	10.61[b]	9.99[ab]	.46	Q
Mean	7.71	9.65	9.67	10.25		

[a,b,c] Means within a row having different superscripts differ ($P<.05$).
[1] Sampling times after 0900 h feeding.
[2] Solutions of NH_4HCO_3 were continuously infused into the rumen.
[3] Standard error of means.
[4] Orthogonal contrast where L=linear; Q=quadratic ($P<.05$).

Table IV-4. In situ disappearances (%) of dry matter (DM) and crude protein (CP), non-linear parameters (a, b and k), and effective degradabilities (%) of DM (EDDM) and CP (EDCP) of soybean meal at various outflow rates as influenced by NH_4HCO_3 infusion.

	Incubation Time (h)	NH_4HCO_3 infusion (g d^{-1})[1] 0	95	190	280	SEM[2]	Contrast[3]
DM:	0	39.4	39.1	39.0	39.3	.18	NS
	6	56.9	55.5	55.5	60.0	.39	NS
	12	78.3[a]	79.6[a]	79.8[a]	85.9[b]	.38	Q
	24	94.8	97.1	96.0	97.1	.77	NS
	36	97.9	98.3	98.4	98.5	.11	NS
	48	98.5	98.7	98.6	98.5	.10	NS
Param:[4]	a	37.8	37.5	36.8	36.5	.30	NS
	b	64.1	64.1	66.2	63.6	.99	NS
	k	.078[a]	.082[ab]	.075[a]	.098[b]	.01	Q/C
EDDM:[5]	.03	84.0	84.4	84.0	86.1	.52	NS
	.05	76.7	77.3	76.4	79.6	.72	NS
	.08	69.3	70.0	68.8	72.5	.84	NS
CP:	0	23.2	23.5	23.9	24.0	.35	NS
	6	46.9	48.1	47.1	49.7	1.03	NS
	12	75.9	77.7	76.9	77.9	1.18	NS
	24	96.0	98.6	96.6	98.3	.69	NS
	36	99.2	99.8	99.4	99.6	.23	NS
	48	99.8	99.9	99.9	99.9	.04	NS
Param:[4]	a	20.9	22.0	21.5	21.9	.69	NS
	b	83.4	82.1	82.4	82.1	1.37	NS
	k	.083	.085	.083	.089	.03	NS
EDCP:[5]	.03	81.7	82.8	82.1	83.0	.76	NS
	.05	72.5	73.9	73.0	74.2	1.05	NS
	.08	62.9	64.5	63.5	64.9	1.24	NS

[a,b] Means within a row having different superscripts differ ($P<.05$).

[1] Solutions of NH_4HCO_3 were continuously infused into the rumen.

[2] Standard error of means.

[3] Orthogonal contrast where L=linear; Q=quadratic; C=cubic, and NS=not significant at $P<.05$.

[4] See text for details.

[5] EDDM and EDCP calculated at ruminal outflow rates of .03, .05 and .08 h^{-1}.

Table IV-5. In situ disappearances (%) of dry matter (DM) and crude protein (CP), non-linear parameters (a, b and k), and effective degradabilities (%) of DM (EDDM) and CP (EDCP) of fish meal at various outflow rates as influenced by NH_4HCO_3 infusion.

	Incubation Time (h)	NH_4HCO_3 infusion (g d^{-1})[1] 0	95	190	280	SEM[2]	Contrast[3]
DM:	0	27.0	26.8	27.0	27.1	.23	NS
	6	30.6	30.4	30.8	30.3	.30	NS
	12	31.5	31.1	31.6	33.2	.83	NS
	24	40.5	40.5	39.6	39.7	.91	NS
	36	48.9	49.9	48.5	47.1	.44	NS
	48	56.6	60.2	58.5	56.0	.30	NS
Param:[4]	a	10.9	10.3	9.7	11.2	1.18	NS
	b	367.3	249.2	185.3	249.9	.43	NS
	k	.001	.002	.002	.001	.01	NS
EDDM:[5]	.03	22.1	21.4	21.4	21.0	.43	NS
	.05	18.5	17.1	16.5	17.1	.59	NS
	.08	15.5	14.6	13.7	14.9	.78	NS
CP:	0	20.7	20.7	20.4	20.9	.19	NS
	6	27.6	28.6	27.8	27.8	.45	NS
	12	31.4	31.2	31.2	32.7	.82	NS
	24	43.2	42.8	41.9	42.4	.66	NS
	36	54.5	55.6	54.0	53.1	.48	NS
	48	65.1	66.7	66.4	64.8	.35	NS
Param:[4]	a	14.2	13.8	13.8	14.3	1.45	NS
	b	246.1	282.2	265.2	242.5	1.71	NS
	k	.002	.002	.002	.002	.01	NS
EDCP:[5]	.03	29.8	31.4	30.5	29.7	.43	NS
	.05	23.8	24.6	23.6	23.4	.59	NS
	.08	20.3	20.5	20.2	20.2	.78	NS

[a,b] Means within a row having different superscripts differ ($P<.05$).

[1] Solutions of NH_4HCO_3 were continuously infused into the rumen.

[2] Standard error of means.

[3] Orthogonal contrast where NS=not significant at $P<.05$.

[4] See text for details.

[5] EDDM and EDCP calculated at ruminal outflow rates of .03, .05 and .08 h^{-1}.

Table IV-6. In situ disappearance (%) of dry matter (DM), non-linear parameters (a, b and k) and effective degradability (%) of DM (EDDM) of barley grain at various outflow rates as influenced by NH_4HCO_3 infusion.

	Incubation Time (h)	NH_4HCO_3 infusion (g d^{-1})[1]				SEM[2]	Contrast[3]
		0	95	190	280		
DM:	0	53.2	52.9	52.6	52.6	.25	NS
	6	78.4	79.6	78.3	80.9	.50	NS
	12	82.5	83.3	82.0	84.1	.52	NS
	24	86.2	86.1	85.4	86.5	.75	NS
	36	88.8	88.5	87.5	88.8	.44	NS
	48	90.6	90.3	90.6	91.1	.33	NS
Param:[4]	a	53.5	53.1	52.8	52.9	.27	NS
	b	35.1	34.9	34.8	35.5	.74	NS
	k	.185	.182	.177	.235	1.02	NS
EDDM:[5]	.03	83.7	82.7	82.6	84.6	.91	NS
	.05	81.1	80.0	79.9	82.5	1.05	NS
	.08	78.0	76.8	76.8	79.8	1.15	NS

[a,b] Means within a row having different superscripts differ (P<.05).
[1] Solutions of NH_4HCO_3 were continuously infused into the rumen.
[2] Standard error of means.
[3] Orthogonal contrast where NS=not significant at P<.05.
[4] See text for details.
[5] EDDM calculated at ruminal outflow rates of .03, .05 and .08 h^{-1}.

Table IV-7. In situ disappearances (%) of dry matter (DM) and cell wall (CW), non-linear parameters (a, b and k), and effective degradabilities (%) of DM (EDDM) and CW (EDCW) of oatlage at various outflow rates as influenced by NH_4HCO_3 infusion.

	Incubation	NH_4HCO_3 infusion (g d^{-1})[1]				SEM[2]	Contrast[3]
	Time (h)	0	95	190	280		
DM:	0	38.7	38.2	38.8	38.9	.12	NS
	12	50.6	50.0	50.4	53.4	1.08	NS
	24	59.5	60.6	60.2	61.6	.67	NS
	48	68.6	68.5	68.1	70.0	.78	NS
	72	72.5	73.5	73.7	74.1	.40	NS
Param:[4]	a	38.6	37.9	38.7	39.0	.16	NS
	b	38.1	39.7	39.1	36.0	1.36	NS
	k	.034	.034	.032	.041	.01	NS
EDDM:[5]	.03	58.3	58.5	58.6	59.9	.68	NS
	.05	53.6	53.6	53.7	55.4	.77	NS
	.08	49.7	49.5	49.7	51.3	.74	NS
CW:	0	.61	.58	.64	.66	.03	NS
	12	16.4^{a}	19.0^{ab}	19.2^{ab}	22.8^{b}	.34	L
	24	32.2	35.6	35.9	36.0	.89	NS
	48	47.1	47.9	48.4	50.1	.53	NS
	72	53.7	56.3	56.5	56.1	.40	NS
Param:[4]	a	$.00^{a}$	$.11^{a}$	$.49^{b}$	$.54^{b}$	.16	L
	b	62.8	62.6	62.3	59.6	1.23	NS
	k	.030	.031	.032	.037	.01	NS
EDCW:[5]	.03	30.5	32.2	32.4	33.6	1.03	NS
	.05	22.8	24.3	24.5	26.0	1.00	NS
	.08	16.5	17.9	18.0	19.5	.94	NS

[a,b] Means within a row having different superscripts differ ($P<.05$).

[1] Solutions of NH_4HCO_3 were continuously infused into the rumen.

[2] Standard error of means.

[3] Orthogonal contrast where L=linear and NS=not significant at $P<.05$.

[4] See text for details.

[5] EDDM and EDCW calculated at ruminal outflow rates of .03, .05 and .08 h^{-1}.

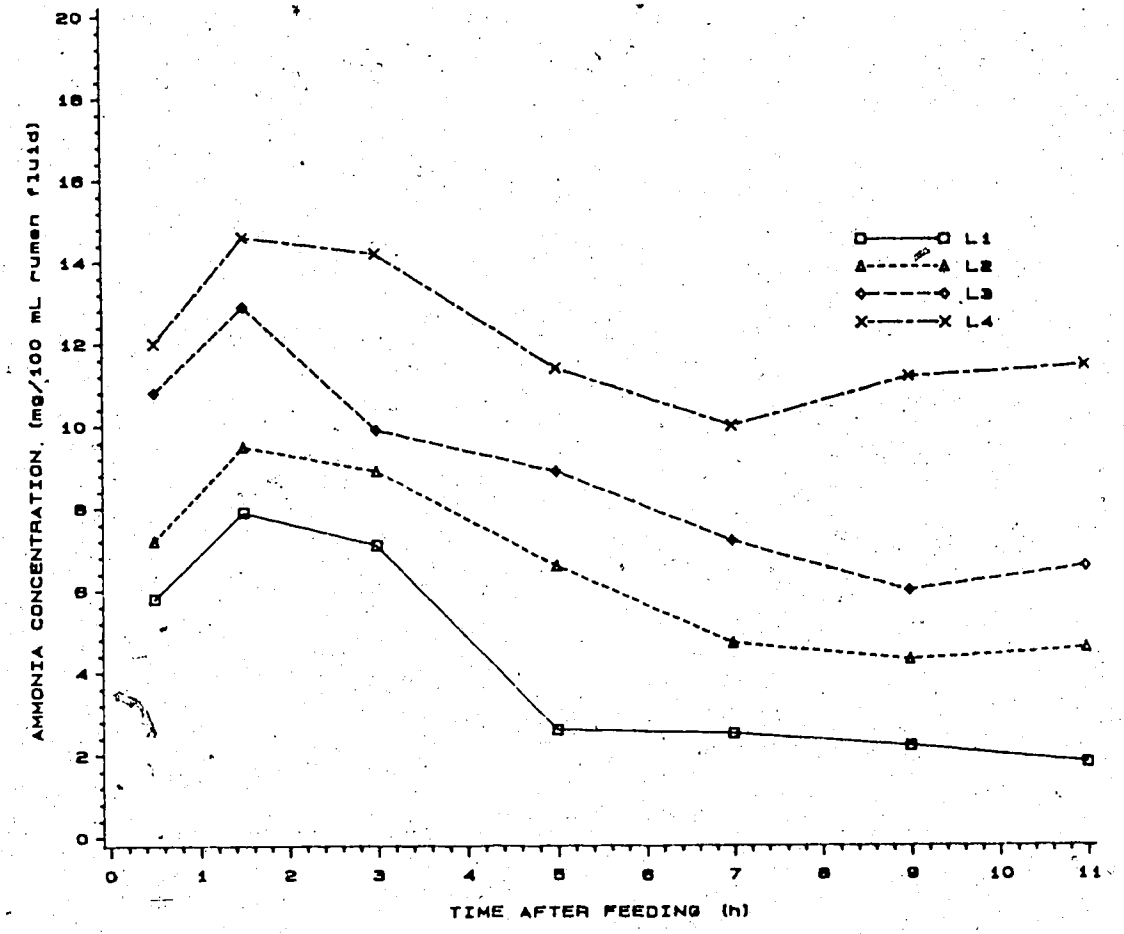
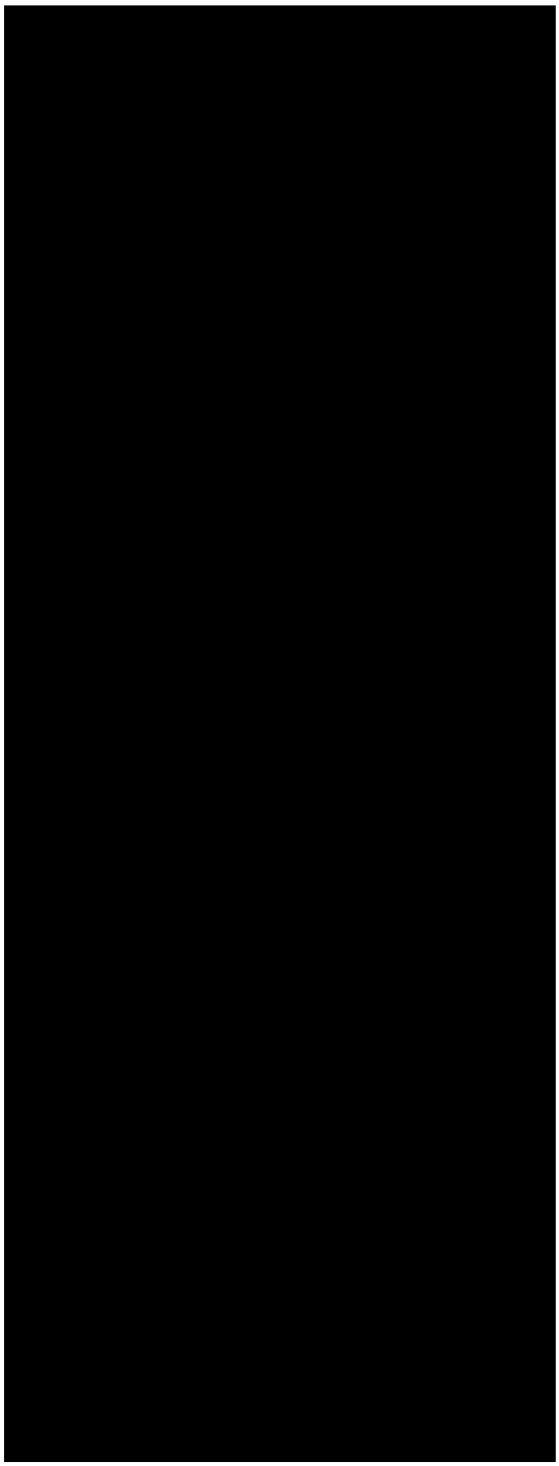


Figure IV-1. Influence of level (L1, 0.0; L2, 95; L3, 190, and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal ammonia concentration. Feeding was at 0 h and 12 h.



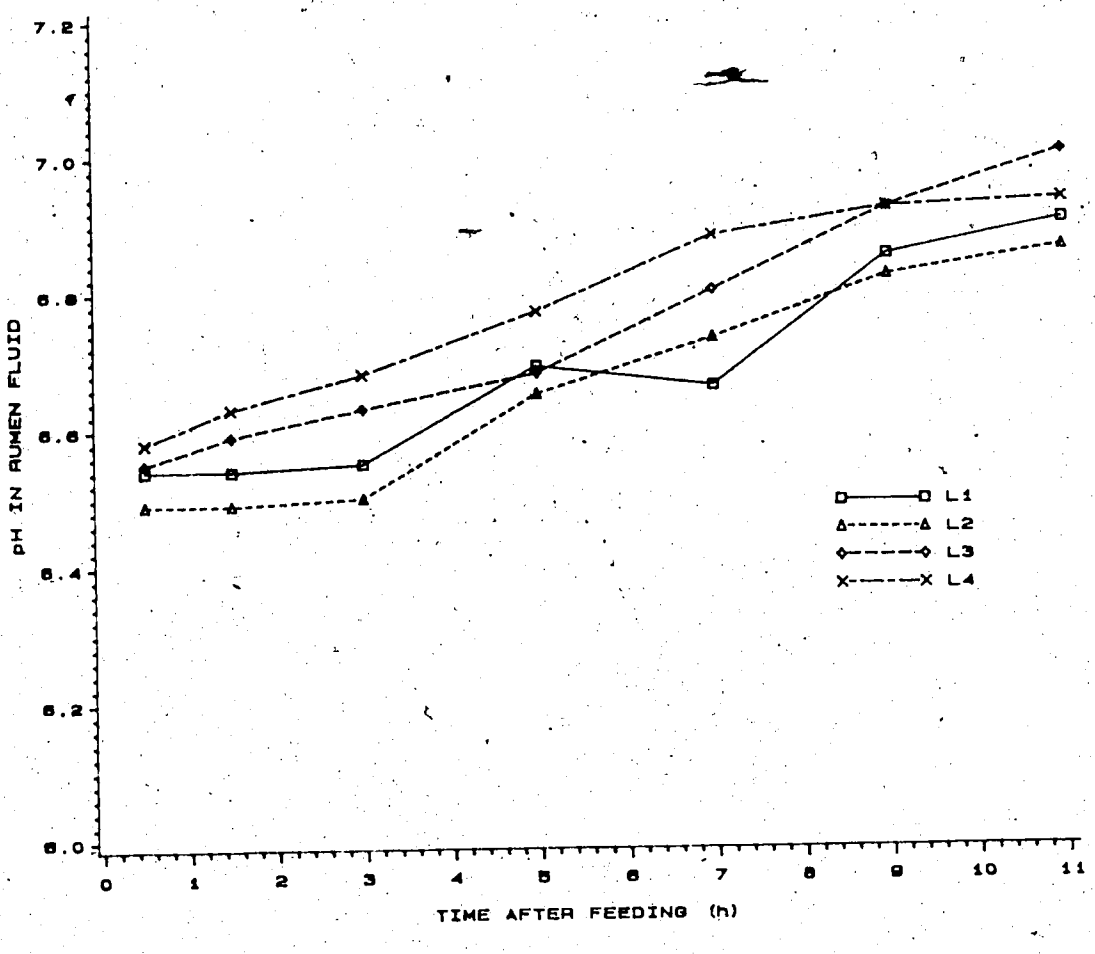


Figure IV-2. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal pH. Feeding was at 0 h and 12 h.

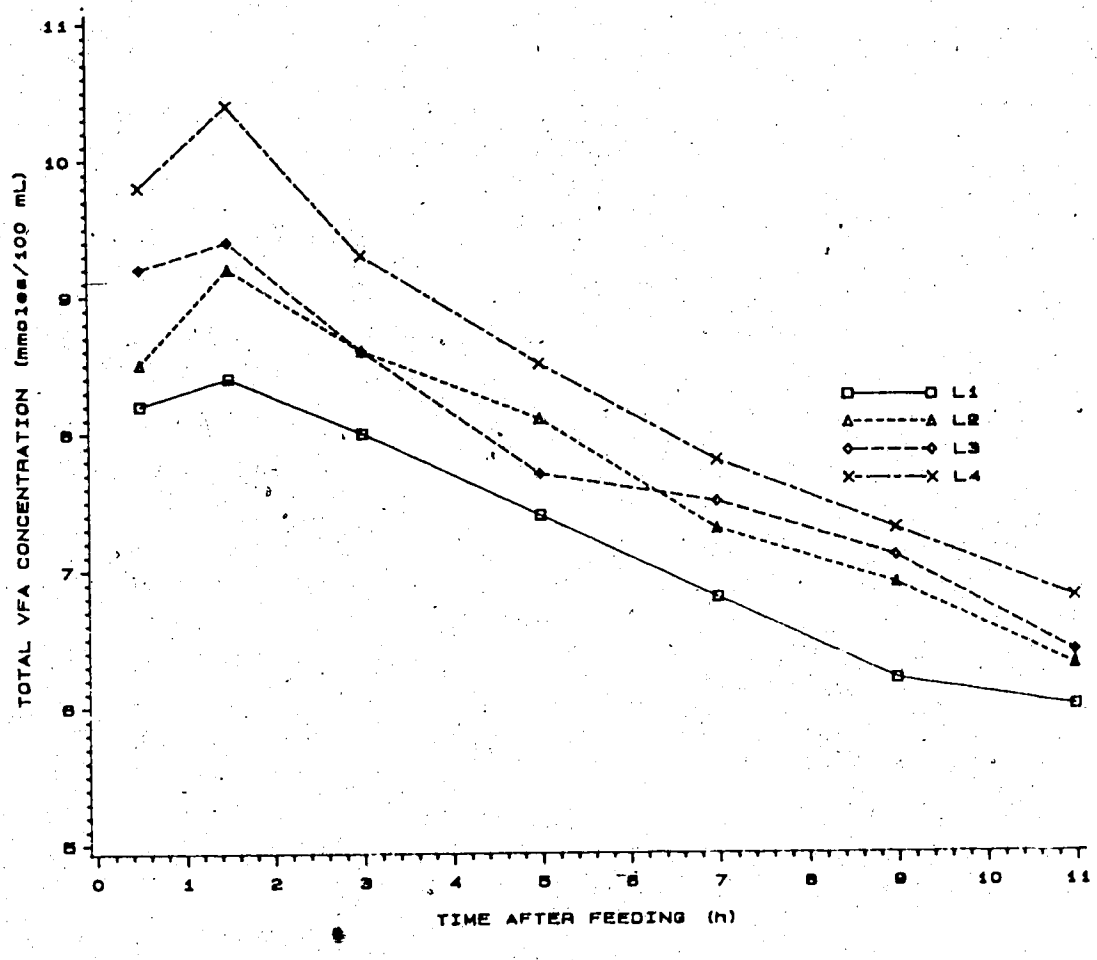


Figure IV-3. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal total VFA concentration. Feeding was at 0 h and 12 h.

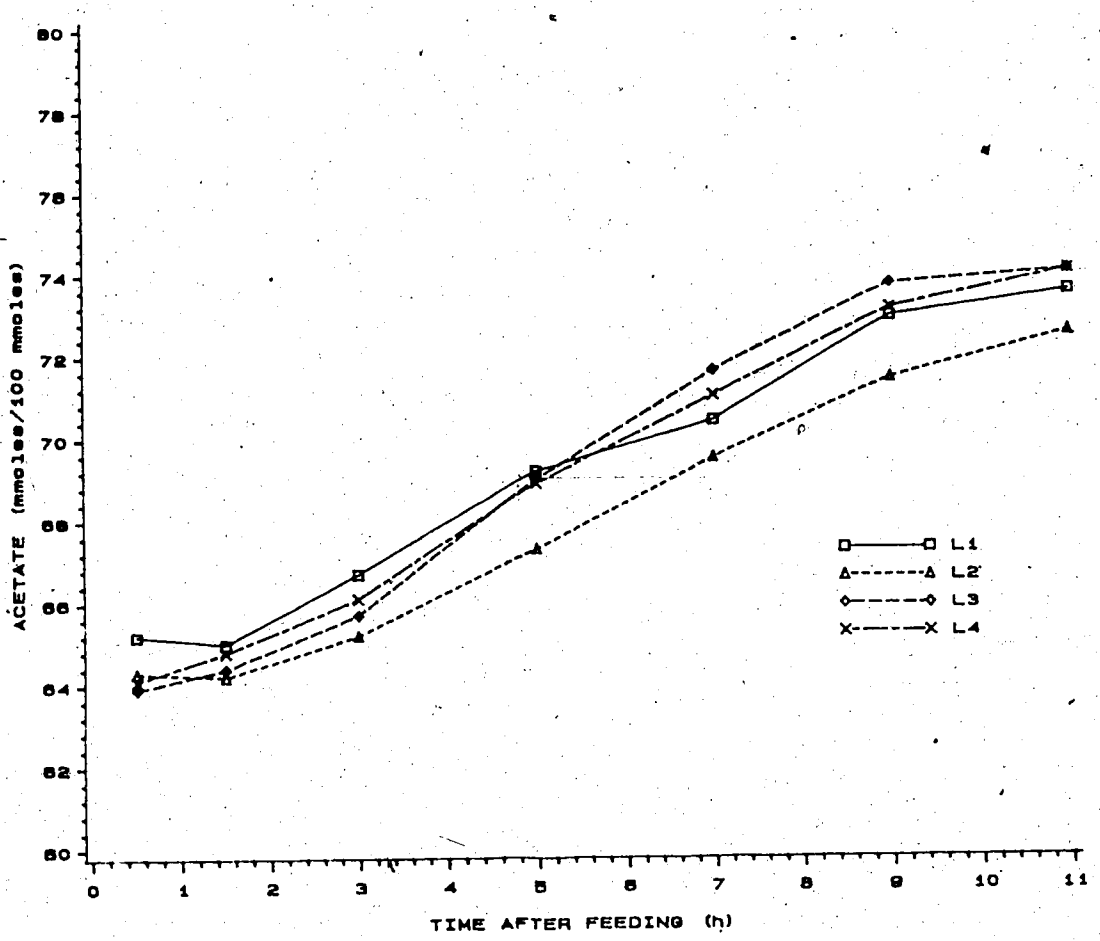


Figure IV-4. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal acetate proportion. Feeding was at 0 h and 12 h.

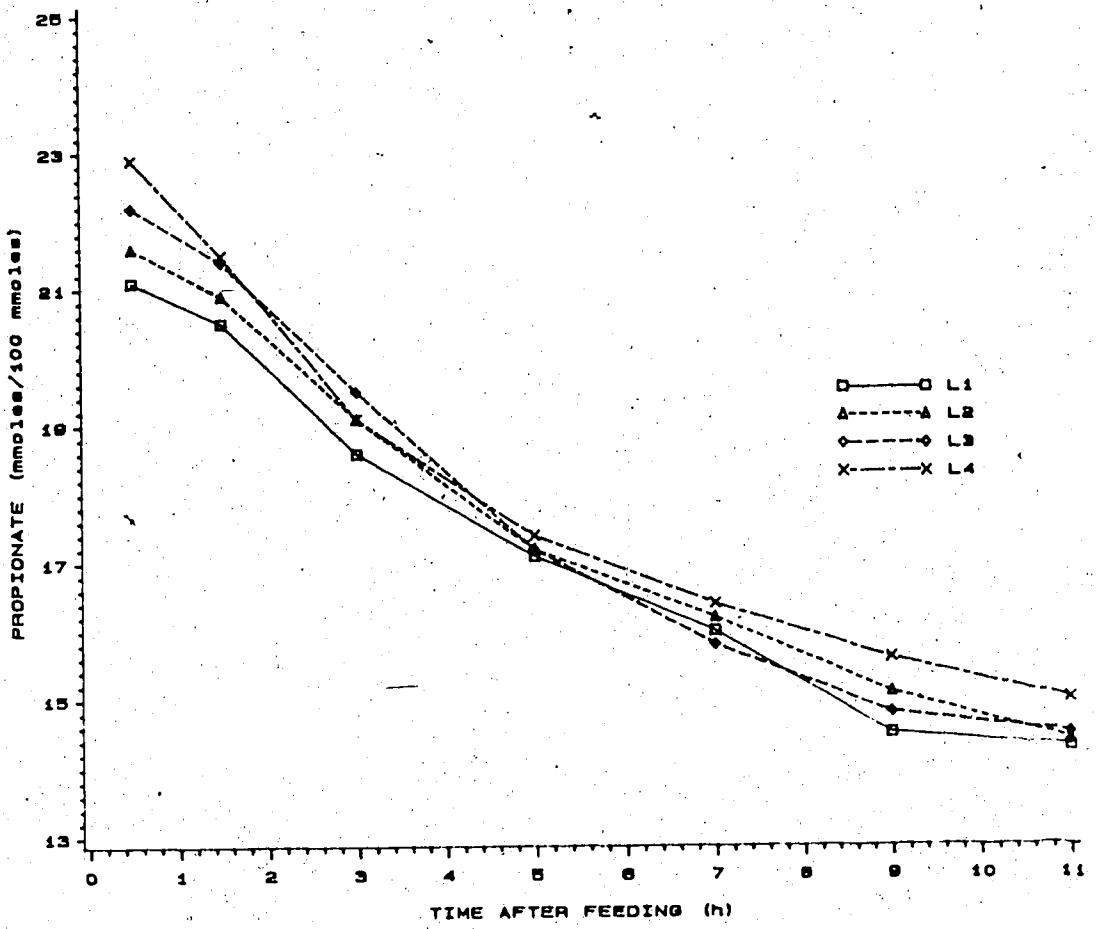


Figure IV-5. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal propionate proportion. Feeding was at 0 h and 12 h.

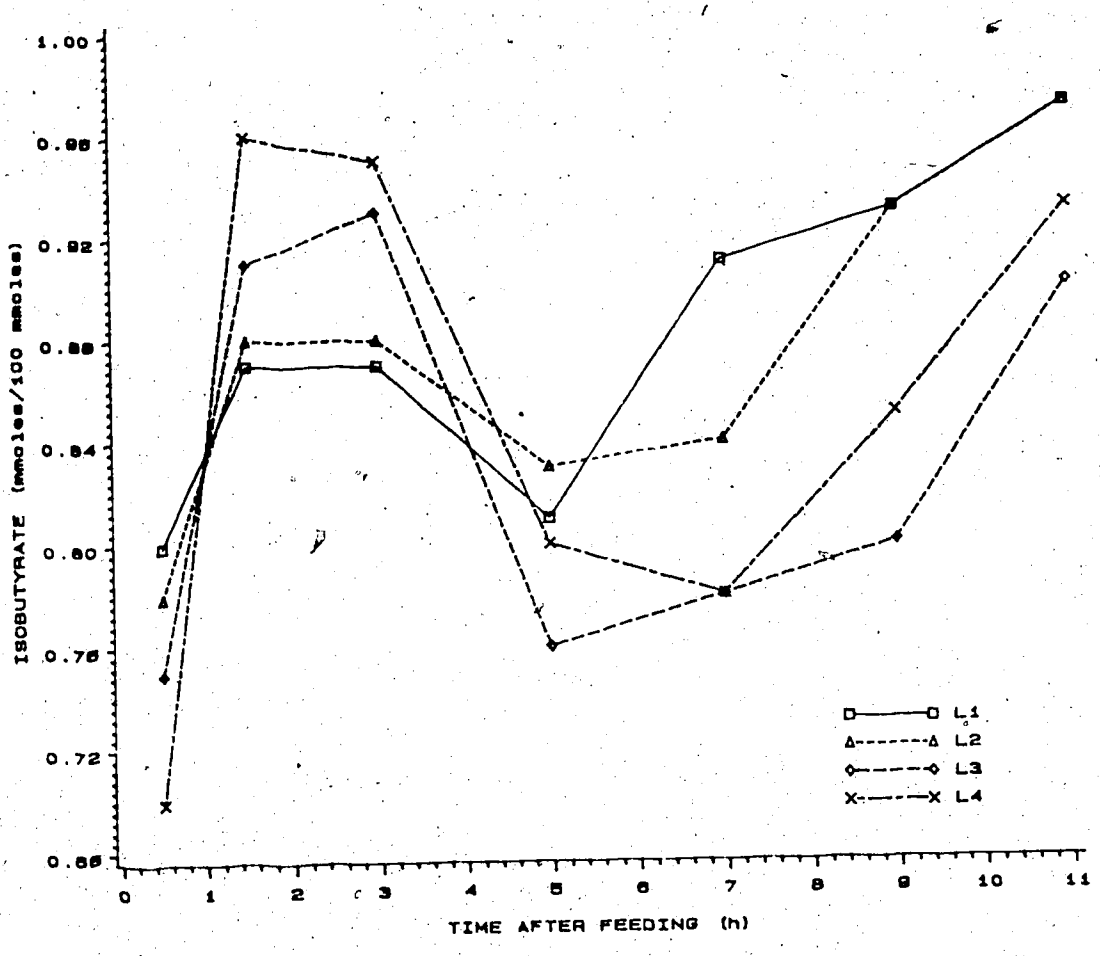


Figure IV-6. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal isobutyrate proportion. Feeding was at 0 h and 12 h.

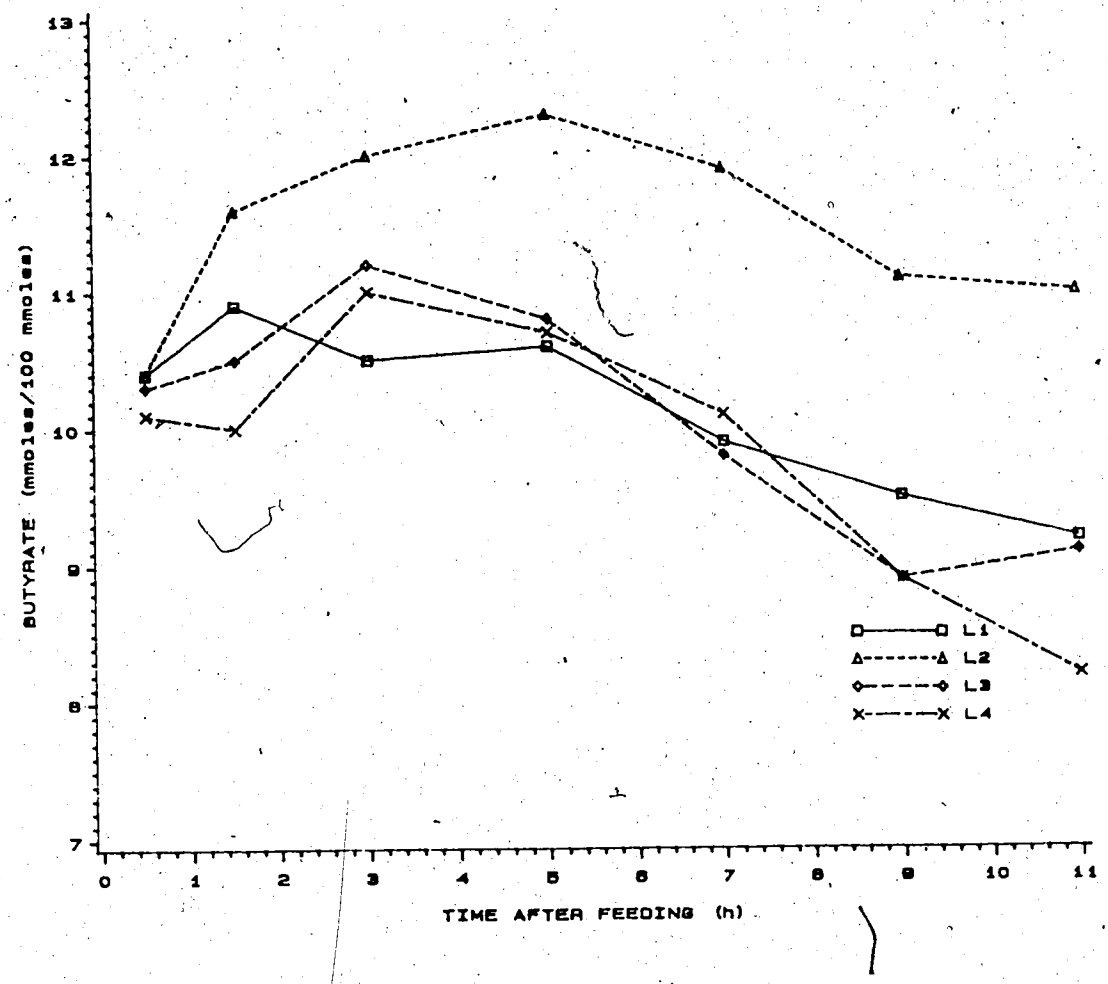


Figure IV-7. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal butyrate proportion. Feeding was at 0 h and 12 h.

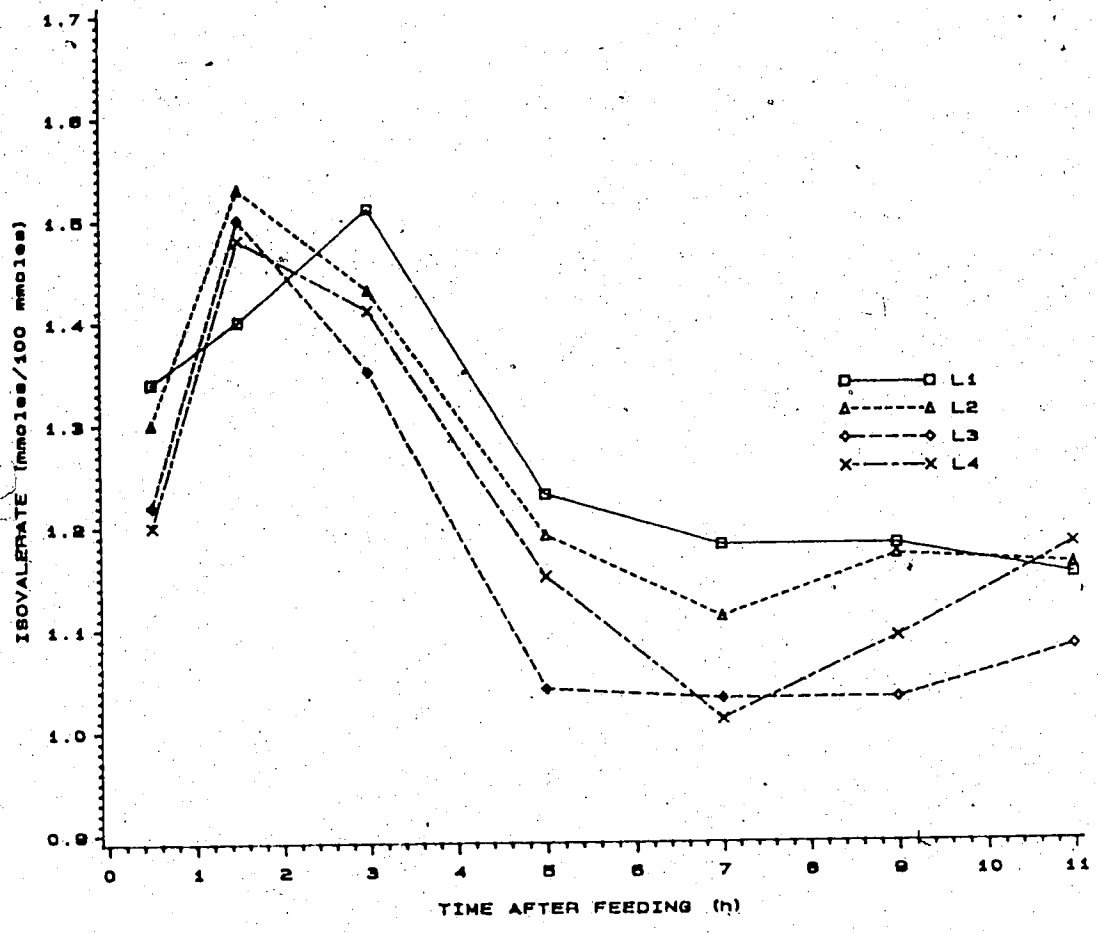


Figure IV-8. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal isovalerate proportion. Feeding was at 0 h and 12 h.

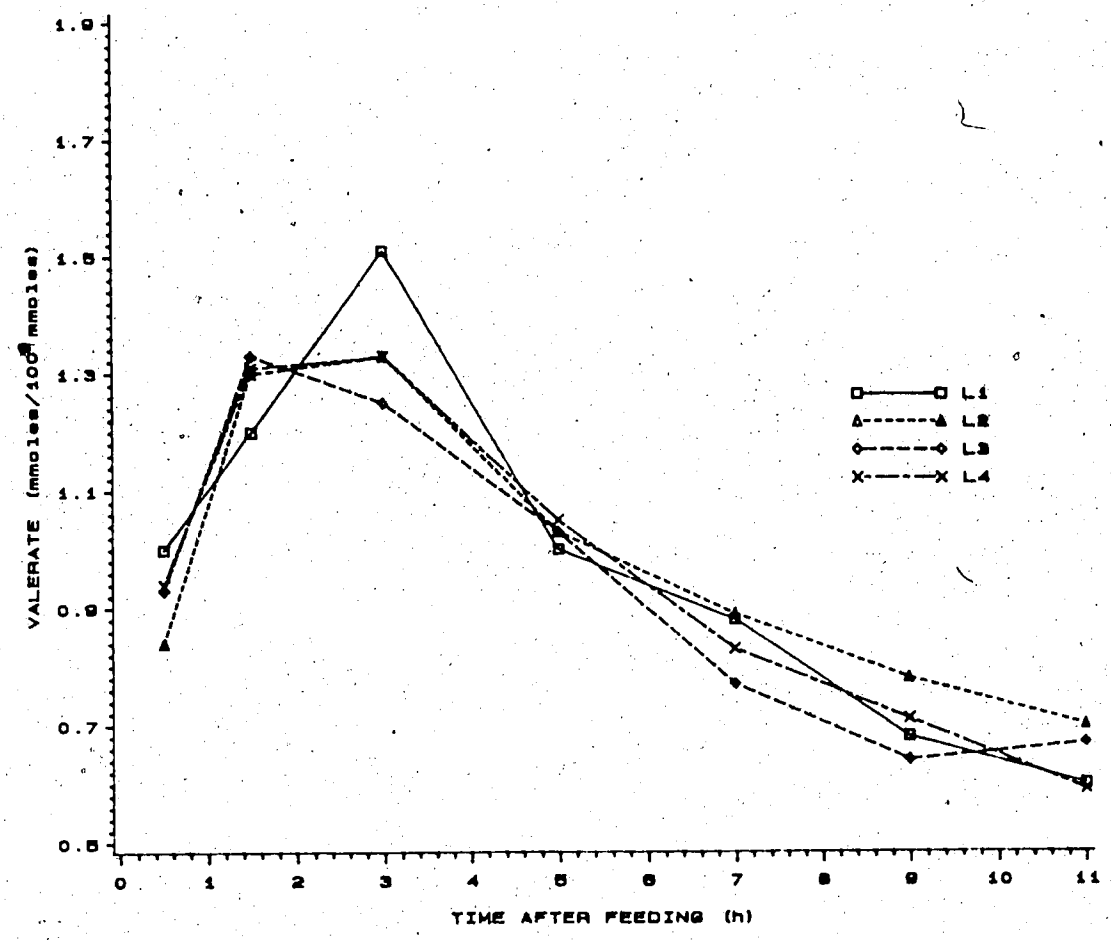


Figure IV-9. Influence of level (L1, 0.0; L2, 95; L3, 190 and L4, 280 g d^{-1}) of NH_4HCO_3 infusion on ruminal valerate proportion. Feeding was at 0 h and 12 h.

V. EFFECT OF AMMONIATION OF BARLEY SILAGE ON SILAGE AND RUMINAL FERMENTATION, NITROGEN SUPPLY TO THE SMALL INTESTINE, DIGESTIBILITY AND PERFORMANCE OF LACTATING DAIRY COWS

A. INTRODUCTION

Ammoniation has gained commercial acceptance as a means of improving the nutritive value of of whole plant cereal silage. Primary benefits of ammoniation are increased nitrogen (N) content of silage (Lomas and Fox 1982; Smith et al. 1982; Phillip et al. 1985) and greater stability of silage as a result of redused fungal growth (Britt and Huber 1975; Glewen and Young 1982; Thoracius and Robertson 1984). In adition, ammoniation at ensiling increases the water insoluble (WIS) N content of silage (Huber et al. 1980; Smith et al. 1982; Hargreaves et al. 1984), and apparently reduces loss of plant protein by proteolysis and deamination (Huber et al. 1980; Jhonson et al. 1982; Smith et al. 1982). Other effects of ammoniation include higher pH and increased lactic acid concentration (Heinrichs and Conrad 1984; Lomas and Fox 1982). Recovery of ammonia N depends upon level of added ammonia and moisture content of silage.

Several studies have demonstrated that milk production of dairy cows fed ammoniated corn silage is equal or superior to those fed untreated or urea treated silage (Huber et al. 1979; Huber et al. 1980). Improved feeding value of ammoniated silage may be associated with increased WIS-N content and redused proteolysis of plant protein, thereby, providing a more slowly degradable nitrogen source for rumen microorganisms. In a study where dairy cows were fed ^{15}N-ammonia

supplemented corn silage a considerable portion of the added ammonia N was utilized for milk protein synthesis (Smith et al. 1982). In addition the soluble and insoluble N compounds from the added ammonia were utilized with the same efficiency as other sources of dietary CP with similar solubilities.

Although digestibility of silage protein tended to be slightly improved by ammoniation (Carr et al. 1984), digestibility of cell wall component may not be influenced due to the relatively low ammonia application rates.

Information on the effect of ammoniation of cereal silage on fermentation patterns and microbial growth in the rumen is limited. Morever, unlike corn silage, very limited information is available on the effect of ammoniation of whole plant barley silage on its digestion and utilization by dairy cows.

Two experiments were conducted to determine the influence of ammoniation of barley silage on fermentation characteristics in the silo and in the rumen, nitrogen supply to the small intestine, digestibility and performance of lactating dairy cows.

B. MATERIALS AND METHODS

Ammoniation and Diet Formulation

Whole plant barley was cut at the mid-dough stage, chopped with a forage harvester and treated with 1% anhydrous ammonia (dry matter (DM) basis, BS-A) as it was packed into polyethylene silage bags (Alberta Ag Industries, Westlock, Alberta). Alternate loads of chopped whole plant barley was ensiled in silage bags without addition of ammonia.

Three isonitrogenous (15% crude protein (CP) in DM) diets were formulated (Table 1). Untreated barley silage was supplemented with either canola meal (BS-CM) or urea (BS-U) at a level equivalent to the added N arising from ammoniation. Canola meal and urea were added at the expense of barley grain. Diets consisted of 50% barley silage and 50% concentrate mixture (DM basis), and were prepared fresh each morning.

Animals and Feeding

Experiment 1: Twenty seven Holstein cows in mid-lactation were assigned on the basis of milk yield and lactation number to the three diets in a completely randomized design. Cows were fed complete mixed diets, ad libitum twice daily. Seven days were allowed for cows to adapt to diets prior to a 6 wk experimental period.

Experiment 2: Three Holstein cows, in mid to late lactation, fitted with permanent rumen and T-type duodenal cannulae were assigned in a 3 x 3 Latin square design to the same three diets as for experiment 1. Cows were fed 16 kg d^{-1} in 12 equal portions at 2 h intervals by automatic feeders. Each period consisted of 2 wk for adaptation to diets and 1 wk for sample collection

In both experiments cows were maintained in tie stalls with free access to block salt and water.

Sampling and Measurements

In both experiments samples of silage and concentrate mixture were taken twice daily. Cows were milked twice daily with milk weights recorded at each milking. Milk samples were collected for 2 consecutive

d each week. Quantities of mixed diets fed and refusals were recorded daily. Body weights were measured on a weekly basis. All samples were stored at -20°C until analyzed.

Experiment 1: Blood samples were taken from the tail vein immediately prior to, and 2 h after the morning feeding, and plasma was collected by centrifuging at 2800 x g for 10 min.

Experiment 2: Samples of rumen fluid were taken for 3 consecutive d, using a syringe attached to a rumen bullet, at 30, 60 and 90 min after feeding, and pH was measured immediately. Rumen fluid was acidified by adding 1 mL of 25% orthophosphoric acid for volatile fatty acid (VFA) analysis. Samples of duodenal digesta were collected at the same time as rumen fluid samples. Total fecal collections were on the last 4 d of each period. After throughly mixing, a portion of the total fecal collection (0.5% of fresh weight) was subsampled each day for estimation of whole tract digestibility.

Sample Analysis

For measurement of pH, ammonia concentration and water soluble (WS) N, 40 mL of distilled and deionized water was added to approx. 5 g of fresh silage in a 50 mL tube, agitated for 4 h and pH was determined immediately. The silage suspension was centrifuged at 20,000 x g for 10 min. Ammonia concentrations of silage in the supernatant and in rumen fluid were determined by the method of Fawcett and Scott (1960) using colorimeter (Brinkman PC 800). Crude protein (CP) contents in silage and diets, and WS-N content in silage were determined by the Kjeldahl method (AOAC 1980). Forty mL of ethanol was added to approx. 5 g of fresh

silage in a 50 mL tube and agitated for 4 h. After silage suspension was centrifuged at 20,000 x g for 10 min, lactic acid in the supernatant was analyzed by gas chromatography (Varian Model m3700, OV-351 capillary column, 0.25 mm ID x 30 m) as described by Mamer and Gibbs (1973). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) in silage were determined by the method of Goering and Van Soest (1970). Hemicellulose content was estimated as the difference between NDF and ADF. Water in silage was extracted with ethanol and was separated with a Porapak QS column in a gas chromatograph (Aerograph 660) as described by Fenton et al. (1981). Samples of concentrate mixture were dried in a forced oven at 60°C for 24 h for estimation of DM content. Contents of fat, protein and lactose in milk were assayed by the Alberta Central Milk Testing Laboratory using infrared procedures (AOAC 1980). Blood urea N was determined by the method of Croker (1967).

Volatile fatty acid (VFA) in rumen fluid was determined by gas chromatography (Varian Model 3700, OV-351 capillary column, 0.25 mm ID x 30 m). Microbial N in duodenal digesta was determined by estimating RNA content using 0.15 of RNA-N to Microbial-N ratio as described by Zinn and Owens (1982). An instrumental neutron activation analysis/ (INAA) procedure was used to determine marker concentrations in duodenal digesta (Kennelly et al. 1982). Non-ammonia N (NAN) in duodenal digesta was estimated by substracting ammonia content from total N content, and dietary N by substracting ammonia and microbial N from total N content. Quantitative flow of N fractions to the small intestine was determined by applying outflow rate of rumen digesta obtained from markers. Two markers (Co-EDTA as a liquid marker and Dy_2Cl_3 as a particulate marker)

were prepared in a mixture (50% to 50%) of ground barley silage (2 mm mesh) and barley grain (1 mm mesh). Sixty grams of marker mixture, containing 0.003 g of Co and 0.[illegible]53 g of Dy was added directly to the feed container containing the feed allotment for each of the 12 times daily feeding. Markers were fed for 7 d prior to commencement of sampling. Outflow rates of rumen digesta were determined as described by Faichney (1975).

In Situ Degradation of Silages and Complete Mixed Diets (Experiment 2)

Silages were dried at 50°C for 3 d and ground through a 2 mm screen (Wiley mill). Samples of concentrate mixtures were ground through a 1 mm screen. Ground silages and concentrate mixtures were mixed in similar proportions as for complete mixed diets. Approximately 5 g samples of silages and complete mixed diets were placed in nylon bags (7x15 cm). Duplicate bags for each incubation time were suspended in the rumen for 2, 4, 8, 12, 24 and 48 h, using a 70 cm line connected to a 250 mL plastic jar filled with pebbles. Upon removal, the bags were washed manually under running cold tap water until the water was clear, followed by drying in a forced air oven at 60°C for 2 days. Disappearance at t=0 was calculated on the basis of DM and CP loss on washing.

Percent disappearance of DM and CP at each incubation time was calculated from the portion remaining after incubation in the rumen. The disappearance rate was fitted to the equation (Ørskov and McDonald 1979): $P = a+b(1-e^{-kt})$, where P is a disappearance at time t, a is an intercept representing the soluble component, b is the fraction that

is degraded at time infinity, k is the rate constant for disappearance of fraction b, and t is incubation time. Non-linear parameters (a, b and k) were estimated by an iterative least-square procedure and effective dagradability of DM (EDDM) and CP (EDCP) was calculated according to Ørskov and McDonald (1979) where EDDM or EDCP = a+(bxk)/(k+r) and where r is the fractional ruminal outflow rate. Effective degradability was calculated using two hypothetical fractional outflow rates (.05 and .08).

Statistical Analysis

Data obtained were subjected to analysis of variance with treatments (diets) in experiment 1, and with treatments (diets), animals and periods as factors in experiment 2. When treatment effects were significant, treatment means were compared at probability level of .01 or .05 using Student-Newman-Keuls' test (Steel and Torrie 1980).

C. RESULTS AND DISCUSSION

Silage Characteristics -Experiment 1

Addition of ammonia increased (P<.01) contents of total N, WIS-N and WS-N by factors of 1.36, 1.31 and 1.51, respectively, over those of untreated barley silage (Table 2). Ammonia N concentration in silage was increased (P<.01) by ammonia treatment. Based on application rate, 77.7% of added ammonia was recovered in silage, and based on the assumption that WS-N arising from fermentation was similar for treated and untreated silage, 59.4% of the added ammonia N was recovered in the WIS fraction. Efficiency of N recovery from ammoniation has been reported to vary from 0.4 to 0.6. Nitrogen retention may be related to application

rate and moisture content of silage. Johnson et al. (1982) observed increased WIS-N at higher ammoniation levels (0.25 vs 1.08%, DM), and Buchanan-Smith (1982) reported that when 1% anhydrous ammonia was added to corn silage (DM basis), 61% of the added N was recovered at 28% silage DM while 54% was recovered at 50% silage DM. Increased WIS-N arising from ammoniation may be due to direct binding of ammonia to insoluble organic compounds in plants (Huber et al. 1980; Hargreaves et al. 1984) and reduced degradation of plant protein (Johnson et al. 1982; Smith et al. 1982; Phillip et al. 1985). Johnson et al. (1982) found that concentrations of free amino acids in the water phase of corn silage decreased when ammonia addition was increased from 0.25 to 1.08% of DM. Benefits from binding of ammonia as WIS-N might include improved recovery in silage and greater supply of slow release N for rumen microbes.

Addition of ammonia increased ($P<.05$) pH from 4.1 to 5.5 and lactic acid concentration from 7.2 to 9.0% (Table 3). Increased lactic acid production arising from ammoniation is in agreement with other results (Glewen and Young 1982; Johnson et al. 1982). However, Britt and Huber (1975) and Heinrichs and Conrad (1984) observed decreased lactic acid production in corn silage at higher application rates (2 to 3% of DM) of ammonia. This suggests that lactic acid production may be dependent upon application rate of ammonia and that at lower application rate, added ammonia may stimulate the growth of some lactic acid producing bacteria.

There was a trend to slightly lower cell wall components (NDF, ADF) in ammoniated silage (Table 3). In contrast to ammoniation of low quality roughage, or cleavage of ester bonds (Buettner 1978) would not

be expected in the present study due to the relatively low application rate (1%,DM) of ammonia. No difference in hemicellulose content was observed between treated and untreated barley silage.

Performance of Cows -Experiment 1

Ammoniation of barley silage depressed ($P<.05$) voluntary DM intake (17.6 kg d^{-1}) compared to BS-CM (20.5 kg d^{-1}) or BS-U (19.7 kg d^{-1}) (Table 4). Depression in DM intake associated with ammoniation was most pronounced during the first 2-3 week of experiment (Figure 1). Lower DM intake for BS-A may have been due to the strong odor of free ammonia. Diets were prepared with fresh silage each morning, thus the exposure time to air might not be long enough to evaporate the free ammonia in silage. Few studies have indicated negative effect of ammoniation on silage intake of dairy cows. Huber et al. (1979) and Henderson and Bergen (1972) observed slightly depressed DM intake of corn silage by ammoniation compared with either urea added- or untreated silage. Moore et al. (1986) observed increased DM intake when grass-legume silage was treated with ammonia, but this increased DM intake tended to be accompanied by improved digestibility associated with application rates of ammonia at 1.5 and 3% of DM. A few studies (Huber et al. 1975; Huber et al. 1979) have suggested that up to 1.4% anhydrous ammonia could be added to corn silage without depressing intake.

Despite lower feed intake for cows fed BS-A, milk yield and milk composition (fat, protein and lactose) were similar among diets, and this resulted in slightly improved milk efficiency (milk yield/DM intake) for cows fed BS-A. Results of this study are consistent with

previous studies (Huber et al. 1979; Colenbrander et al. 1983; Heinrichs and Conrad 1984) where cows fed ammoniated corn silage had similar milk yield to cows fed untreated silage in isonitrogenous diets. Higher levels of N addition in the form of ammonia, than as urea, can be achieved without depressing feed intake (Huber et al. 1975). This may be associated with the higher concentration of WIS-N in ammoniated silage (Huber et al. 1979; Smith et al. 1982). Milk composition were not different among diets. Heinrichs and Conrad (1984) obtained a higher milk fat content (3.29%) for cows fed corn silage treated with 0.45% of anhydrous ammonia (fresh weight basis) compared to those fed untreated silage (3.04%) or silage treated with 0.32% ammonia, suggesting that increased WIS-N in silage at higher application rates of ammonia may result in increased fiber digestion, thereby increasing milk fat content as a result of elevated acetate productions.

Ruminal Fermentation Characteristics and Digestibility -Experiment 2

Addition of anhydrous ammonia at ensiling increased ($P<.05$) ruminal ammonia concentration compared to cows fed BS-CM or BS-U (Table 5). Molar percent of propionate was higher ($P<.05$) for cows fed ammoniated silage than those fed BS-U. Increased propionate and somewhat lower acetate concentration for cows fed BS-A resulted in a lower ($P<.05$) acetate to propionate ratio. Ammoniation did not influenced total VFA concentratiosn. Higher ruminal ammonia concentration in cows fed BS-A is surprising since NPN addition was similar to the BS-U diet. A probable reason for higher ammonia concentration in animals fed BS-U diet may be due to the associated effect of increased WS-N and WIS-N contents in

ammoniated silage resulting in higher ammonia concentration at the 3 sampling times (30, 60 and 90 min after feeding). Increased WS-N and WIS-N in corn silage arising from ammoniation has been reported (Huber et al. 1980; Smith et al. 1982; Hargreaves et al. 1984). Moore et al. (1986) observed elevated ruminal ammonia concentration up to 8 h after feeding ammoniated (1 and 3% of DM) grass-legume silage. Lower ruminal ammonia from urea supplementation may be due to rapid ruminal hydrolysis and absorption prior to sampling at 30 min. Little information is available on the effect of NPN supplementation on ruminal VFA production. Ammoniation has resulted in increased total VFA, acetate and propionate concentrations (Moore et al. 1986). These increases were associated with improved silage digestion as a result of higher application of ammonia (3%, DM) to grass-legume silage. Other studies (Teather et al. 1980; Grummer et al. 1984) found some trends for increased propionate production under situations of higher ammonia concentration arising from NH_4Cl infusion or urea supplementation.

Compared to BS-CM, ammonia addition increased ($P<.05$) total-N and non-ammonia N (NAN) concentrations in duodenal digesta. Increased NAN was due to greater ($P<.05$) microbial N concentration (Table 6). Dietary treatment did not influence concentration of dietary protein in duodenal digesta. Ammoniation increased ($P<.05$) supply of total N and NAN to the small intestine (533 and 521 g d^{-1}, respectively) compared with BS-C (490 and 480 g d^{-1}) and BS-U (486 and 477 g d^{-1}) (Table 7). Increased NAN supply was mainly due to greater ($P<.05$) microbial N since no significant dietary differences were observed in dietary N escaping ruminal degradation. Microbial N supply (238 g d^{-1}) to the small

intestine was least for cows fed BS-U. Increased microbial protein yield arising from ammoniation may be due to the growth of non-fibrolytic bacteria being favored by an adequate and steady supply of ammonia.

Ammoniation slightly increased EDDM of barley silage, but there were no differences in EDCP (Table 8). Effective degradability of DM and CP in the rumen, and whole tract digestibility of DM and CP of complete mixed diets were not affected by supplemental N source (Table 9). Results indicate that low application rate (1%, DM) of ammonia may not influence ruminal degradation characteristics of barley silage. These results are in agreement with other studies (Hargreaves et al. 1984; Moore et al. 1986) who found no improvement in silage digestibility at low application rates up to 1.5% of ammonia.

Performance of Cows -Experiment 2

Intake was maintained constant (16 kg d^{-1}, DM) across treatments to avoid effects of feed intake on parameters being measured. No differences in milk yield or milk composition were observed between cows fed BS-A, BS-CM and BS-U (Table 10). A few studies (Huber et al. 1979; Henderson and Bergen 1972) have reported slightly depressed intake of ammoniated corn silage compared with either urea added- or untreated silage supplemented with natural protein. However, Huber et al. (1975) suggested that anhydrous ammonia could be added at 1.4% (DM) without depressing feed intake. Phillip et al. (1985) observed greater organic matter intake of steers fed ammoniated high moisture ear corn than steers fed corn with added urea. Result of the present study are in agreement with previous studies (Huber et al. 1979; Colenbrander et al.

1983; Heinrichs and Conrad 1984) which indicated that cows fed ammoniated corn silage based diet had similar milk production to cows fed untreated silage in isonitrogenous diets. Colenbrander et al. (1983) also found no difference in milk yield among diets supplemented with either soybean meal or NPN (ammonia and urea). Other studies have indicated no significant effect of ammonia addition to corn silage on milk composition, although slight increases in protein (Huber et al. 1979; Colenbrander et al. 1983) and fat content (Heinrichs and Conrad 1984) were reported. Milk components in this study were in the normal range for cows in mid to late-lactation.

Based on the results of the present studies, the feeding value of ammoniated barley silage for dairy cows is similar to that of untreated silage supplemented with canola meal. Thus, under these circumstances ammonia would be an economical source of N for dairy cows. Comparable performance for ammonia N and canola meal protein is presumably associated with increased microbial protein yield in the rumen, and this increased microbial protein yield is, in turn, favored by higher and more stable ammonia concentration in the rumen associated with increased water insoluble N content in ammoniated barley silage.

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Table V-1. Composition (%) of complete mixed diets (dry matter basis).

Ingredients	Diets[1]		
	BS-A	BS-CM	BS-U
Ammoniated barley silage	50.00	-	-
Untreated barley silage	-	50.00	50.00
Barley grain	44.32	37.47	43.58
Canola meal	3.30	10.15	3.30
Urea	-	-	.74
Cane molasses	1.13	1.13	1.13
Dicalcium phosphate	.50	.50	.50
Limestone	.50	.50	.50
Trace mineral salts[2]	.25	.25	.25

[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal ; BS-U, untreated barley silage based diet supplemented with urea.

[2] Commercial product: contained 94% salt, 0.25% Cu, 0.004% Co, 0.01% I, 0.35% Mn, 0.75% Zn and 25 ppm Se. All diets were fortified with vitamins A, D and E (500 g/ton), and vitamin D_3 (300 g/ton).

Table V-2. Total nitrogen (N), water insoluble (WIS) N, water soluble (WS) N and ammonia (NH_3) N in ammoniated (ABS) and untreated (BS) barley silage (dry matter basis).

Fractions	ABS	BS	SEM[1]	SIG[2]	Ratio[3]
Total-N, %	2.42	1.78	.98	**	1.36
WIS-N[4], %	1.74	1.33	.09	**	1.31
WS-N, %	.68	.45	.08	**	1.51
NH_3-N, mg g^{-1}	1.73	.44	.15	**	3.91

[1] Standard error of means.
[2] Significant difference at the level of .01
[3] Ratios of ABS to BS.
[4] WIS-N is equal to total-N minus WS-N.

Table V-3. Chemical constituents of ammoniated (ABS) and untreated (BS) barley silage (dry matter basis).

Items	ABS	BS	SEM[1]	SIG[2]
Dry matter, %	38.0	36.7	.36	
pH	5.5	4.1	.35	**
Lactic acid, %	9.0	7.2	.33	**
NDF[3], %	49.8	51.7	.79	
ADF[4], %	28.6	30.2	1.11	
Hemicellulose, %	21.2	21.5	.52	

[1] Standard error of means.
[2] Significant difference at probability level of .01
[3] Neutral detergent fiber.
[4] Acid detergent fiber.

Table V-4. Effect of ammoniation of barley silage on dry matter intake, milk yield and milk composotion, body weight gain and blood urea nitrogen.

Items	Diets[1]			SEM[2]
	BS-A	BS-CM	BS-U	
Dry matter intake, kg d^{-1}	17.6[a]	20.5[b]	19.7[b]	.73
Milk yield, kg d^{-1}	22.3	22.9	22.4	1.71
Milk composition, %				
Fat	3.42	3.40	3.50	.20
Protein	3.35	3.39	3.35	.07
Lactose	4.78	4.88	4.89	.09
Milk efficiency	1.25	1.13	1.14	.68
Body weight gain, kg d^{-1}	.34	.42	.38	.91
Blood urea nitrogen, mg 100 mL^{-1}				
prior to feeding	15.8	15.8	15.9	.24
2 h after feeding	20.2	18.7	19.4	.41

[a,b] Means in the same row with different letters differ ($P<.05$).
[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal; BS-U, untreated barley silage based diet supplemented with urea.
[2] Standard error of means.

Table V-5. Effect of supplemental nitrogen source on ruminal fermentation.

Parameters	Diets[1]			SEM[2]
	BS-A	BS-CM	BS-U	
pH	6.4	6.2	6.3	.09
Ammonia-N, mg 100 mL^{-1}	8.8[b]	7.4[a]	7.6[a]	.26
Total VFA, mmole 100 mL^{-1}	12.1	11.1	11.0	.18
Molar percent, mmole 100 $mmole^{-1}$				
Acetate (C_2)	57.7	59.2	59.9	.41
Propionate (C_3)	25.3[b]	22.1[ab]	21.4[a]	.29
Isobutyrate	.8	1.1	.9	.05
Butyrate	12.8	13.7	14.5	.75
Isovalerate	1.9	2.3	2.4	.12
Valerate	1.8	1.7	1.6	.07
C_2/C_3	2.36[a]	2.73[b]	2.79[b]	.18

[a,b] Means in the same row with different letters differ ($P<.05$).
[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal; BS-U, untreated barley silage based diet supplemented with urea.
[2] Standard error of means.

Table V-6. Effect of supplemental nitrogen source on nitrogen fractions in duodenal digesta (mg g^{-1}, dry matter basis).

Parameters	Diets[1]			SEM[2]
	BS-A	BS-CM	BS-U	
Total-N	39.5^{b}	35.0^{a}	35.6^{a}	.49
Ammonia-N	$.87^{b}$	$.74^{ab}$	$.65^{a}$	.04
Nonammonia-N (NAN)[3]	38.6^{b}	34.2^{a}	35.0^{ab}	.45
Microbial-N	20.9^{b}	17.0^{a}	18.6^{ab}	.31
Dietary-N[4]	17.7	17.3	16.4	.34
Micobial-N/NAN	.54	.51	.53	.21

[a,b] Means in the same row with different letters differ (P<.05).
[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal; BS-U, untreated barley silage based diet supplemented with urea.
[2] Standard error of means.
[3] Total-N minus ammonia-N.
[4] Nonammonia-N minus Microbial-N.

Table V-7. Effect of supplemental nitrogen source on nitrogen supply to the small intestine (g d^{-1}).

Parameters	Diets[1]			SEM[2]
	BS-A	BS-CM	BS-U	
Total-N	533[b]	490[a]	486[a]	.39
Ammonia-N	11.7[b]	10.4[ab]	8.9[a]	.18
Nonammonia-N[3]	521[b]	480[a]	477[a]	.21
Microbial-N	282[b]	238[a]	256[a]	.19
Dietary-N[4]	239[b]	242[b]	225[a]	.37

[a,b] Means in the same row with different letters differ ($P<.05$).
[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal; BS-U, untreated barley silage based diet supplemented with urea.
[2] Standard error of means.
[3] Total-N minus ammonia-N.*
[4] Nonammonia-N minus microbial-N.
* Outflow rates of total digesta were .56 (BS-A), .58 (BS-CM) and .57 (BS-U) kg h^{-1}.

Table V-8. In situ effective degradability (%) of dry matter (EDDM) and crude protein (EDCP) of ammoniated barley silage (ABS) and untreated barley silage (BS).

Items	Silage[1]		SEM[2]
	ABS	BS	
EDDM:			
.05[3]	54.5	52.8	.31
.08[4]	51.0	50.6	.26
EDCP:			
.05[3]	86.9	87.6	.47
.08[4]	86.3	86.4	.30

[1] ABS - ammoniated barley silage; NBS - untreated barley silage.
[2] Standard error of means.
[3,4] Fractional outflow rates of .05 and .08 h^{-1}.

Table V-9. Effect of supplemental nitrogen source on ruminal effective degradabilities of dry matter (EDDM) and crude protein (EDCP), and whole tract digestibility of DM and CP of complete mixed diets (%).

Items	Diets[1]			SEM[2]
	BS-A	BS-CM	BS-U	
EDDM:				
.05[3]	70.5	69.4	70.8	.33
.08[4]	67.4	65.7	67.2	.29
EDCP:				
.05[3]	80.4	78.7	81.5	1.10
.08[4]	77.7	75.3	79.0	.74
Whole tract digestibility, %				
DM	68.9	67.4	68.2	.63
CP	72.7	72.6	71.0	.45

[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal; BS-U, untreated barley silage based diet supplemented with urea.
[2] Standard error of means.
[3,4] Fractional outflow rates of .05 and .08 h^{-1}.

Table V-10. Effect of supplemental nitrogen source on dry matter intake, milk yield and milk composition.

Parameters	Diets[1]			SEM[2]
	BS-A	BS-CM	BS-U	
Dry matter intake, kg d^{-1}	15.3	15.8	15.3	.65
Milk yield, kg d^{-1}	17.5	16.9	17.4	.74
Milk composition (%)				
Fat	4.01	3.92	4.00	.94
Protein	3.53	3.63	3.52	.47
Milk efficiency	1.14	1.07	1.13	.11

[1] BS-A, ammoniated barley silage based diet; BS-CM, untreated barley silage based diet supplemented with canola meal; BS-U, untreated barley silage based diet supplemented with urea.
[2] Standard error of means.

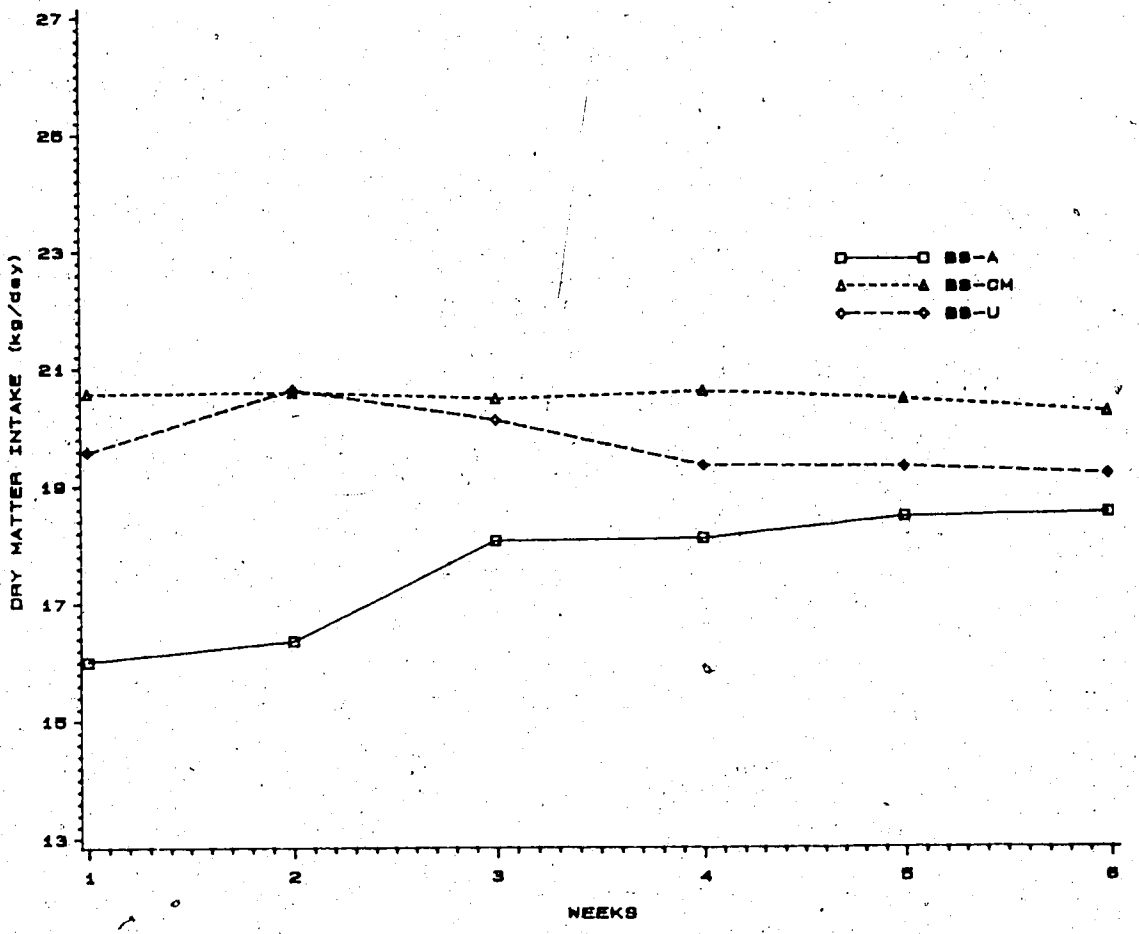

Figure V-1. Patterns in dry matter intake of ammoniated barley silage based diet (BS-A), untreated barley silage diet supplemented with canola meal (BS-CM) and untreated barley silage diet supplemented with urea (BS-U).

VI. GENERAL DISCUSSION

Ruminant animals receive up to 80% of their amino acid requirements from ruminal microorganisms (Sniffen and Robinson 1987). Ammonia has been accepted as the major nitrogen (N) source for microbial growth in the rumen (Hungate 1966; Mathison and Milligan 1971; Cotta and Russell 1982). However, the relationship between ammonia concentration and rumen microbial growth has been the subject of controversity. In vivo studies generally suggest higher ammonia concentrations than in vitro studies for maximal microbial growth. This may indicate that ammonia concentration for maximal microbial growth is not constant. It is also considered that ammonia concentration for maximal microbial growth is not equal to concentration required for optimal rate of diet degradation. A positive relationship exists between N supplementation and microbial growth (Wallace 1979; Teather et al. 1980; Erdman et al. 1986), and between ammonia concentration and ruminal degradation of feedstuffs. Results of our in vitro study (Chapter II) indicated no influence of ammonia concentration, in the ranges from 4 to 30 mg 100 mL^{-1}, on the rate of protein degradation. Blackburn (1968a,b) reported that protease production of ruminal microorganisms was neither induced nor depressed by various N sources. Russell and Hespell (1981) indicated that proteolytic activity of rumen bacteria may be altered by changes in population. Rate and extent of protein degradation by ruminal microorganisms, thus, may mainly depend upon size of microbial population. Increasing ruminal ammonia concentration by infusion of NH_4Cl (Chapter III) or NH_4HCO_3 (Chapter IV) increased bacterial numbers. However, increased bacterial growth, as influenced by ammonia

concentration, did not positively influence ruminal degradation of feed ingredients except for a few isolated instances where linear and/or quadratic effects were observed. Based on the ruminal degradation of concentrate ingredients, optimal ammonia concentration was 16.3 mg 100 mL^{-1} rumen fluid. The hydrolytic activity of ruminal microorganisms tended to be depressed with increased NH_4Cl infusion. Depressed hydrolytic activity of ruminal microorganisms at higher NH_4Cl infusion may be related to reduced pH, due to the acidic characteristics of NH_4Cl. Proteolytic activity of ruminal microorganisms are sensitive to lower pH (Erfle et al. 1982), but cellulolytic activity of ruminal microorganisms is most sensitive to pH (Cheng et al. 1984; Hoover et al. 1984; Shriever et al. 1986). In a second in vivo study (Chapter IV) only small increases in ruminal degradation of feed ingredients were observed despite increased bacterial numbers as result of NH_4HCO_3 infusion.

Results of these studies (Chapters II to IV) indicate that ruminal degradation of protein is positively related to its solubility since rate and extent of protein degradation was in the same order as solubility in a borate-phosphate buffer (pH 7.8). A positive relationship between protein solubility and its degradation by ruminal microorganisms has been indicated (Crooker et al. 1978; Crawford et al. 1978), but other factors are also involved (Nugent and Mangan 1981; Mahadevan et al. 1980).

Several studies (Forsberg et al. 1984; Wallace and Brammall 1985) reported that most ruminal proteolytic activity is associated with bacteria. Removal of protozoa reduced protein degradation (Chapter II). This suggests that ruminal protozoa play a minor role in protein

degradation compared to bacteria. However, bacteria attached to feed particles as well as large bacteria may also have been removed by centrifuging at 500 x g for 10 min.

Supplementation with non-protein nitrogen (NPN) is generally considered useful only when it provides ammonia for use by ruminal microorganisms. Silage has been used as a carrier for NPN in ruminant diets. Ammoniation (1%, dry matter basis) of whole plant barley silage increased concentrations of lactic acid, total N and WIS-N in silage (Chapter V). These results are in agreement with other studies (Huber et al. 1980; Heinrichs and Conrad 1984; Johnson et al. 1982; Lomas and Fox 1982; Smith et al. 1982). Equal feeding value of ammoniated barley silage based diet (BS-A) to untreated silage supplemented with canola meal (BS-CM) was observed using dairy cows (Chapter V). Ammoniation also increased supplies of total N and microbial N to the small intestine (Chapter V). Increased ruminal ammonia concentration from cows fed BS-A may be related to increased WIS-N content arising from ammoniation. Increased microbial protein supply to the small intestine by ammoniation may be in turn related to increased WIS-N content in barley silage.

Based on results of the present studies (Chapters II to IV), it is suggested that increasing ammonia concentration positively stimulates rumen microbial growth, but increased microbial numbers as influenced by increased ammonia concentration may not affect ruminal degradation of feedstuffs within the range of ammonia concentration used in this study. It is also suggested that ruminal ammonia concentration is not the primary factor regulating proteolytic activity of ruminal microorganisms. Ruminal degradation of feed ingredients may primarily

depend upon their physical and chemical properties rather than on ruminal conditions. Ammonia N is an economical alternative to plant protein under conditions of these studies.

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