Influence of rumen ammonia concentration on the rumen degradation rates of barley and maize

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1. Four rumen-cannulated steers were given barley and maize diets supplemented with graded levels of an ammonium acetate solution.

2. Animals were fed hourly from automatic feeders and water consumption was controlled to achieve steady-state conditions in the rumen.

3. Dacron bags containing rolled barley or ground barley were incubated in the rumen of barley-fed steers, while ground maize and autoclaved maize were incubated in the rumen of maize-fed steers.

4. Fractional degradation rates of dry matter were estimated for each cereal substrate incubated using a single-pool exponential decay model.

5. No differences in degradation rate due to the method of feed processing were detected; however, barley was degraded at a faster rate than maize. Furthermore, the minimum rumen ammonia-nitrogen concentration required to maximize the degradation rate of barley (125 mg/l) was greater than that required to maximize the degradation rate of maize (61 mg/l).

6. These results indicate that the optimal NH_3-N concentration required to maximize the rate of grain digestion in the rumen is influenced by the chemical or structural characteristics of the grain.

Ammonia is the main nitrogen source for rumen bacteria (Bryant & Robinson, 1962) while amino-N is the main N source for rumen protozoa (Coleman, 1979). The requirement for NH_3-N (considered to be the sum of NH_3-N and NH_4-N) by the mixed microbial population of the rumen can be described by the optimal rumen NH_3-N concentration, which can be defined as the minimum concentration of NH_3-N necessary to support maximum bacterial growth rates. This definition is consistent with the use of specific growth rate (Schaefer *et al.* 1980), rate of microbial protein synthesis (Satter & Slyter, 1974), rumen outflow rate of microbial protein (Hume *et al.* 1970; Allen & Miller, 1976), microbial protein concentration (Slyter *et al.* 1979; Kang-Meznarich & Broderick, 1981) and $^{15}NH_4$ incorporation rate (Nikolic *et al.* 1975) as dependent variables for determining an NH_3-N requirement. These variables contrast with the rate of dry matter disappearance (Mehrez *et al.* 1977; Ortega *et al.* 1979) and concentration of total volatile fatty acids (VFA) (Slyter *et al.* 1979), variables which also have been used to define optimal rumen NH_3-N concentrations but which reflect the effects of NH_3-N concentration on the degradative activities of the rumen microbial population.

The lack of agreement between estimates of 14 mg NH_3-N/l (Schaefer *et al.* 1980) and 50 mg NH_3-N/l (Satter & Slyter, 1974) for optimal rumen NH_3-N concentration based on microbial growth and an estimate of 194 mg NH_3-N/l based on barley dry matter disappearance from dacron bags (Mehrez *et al.* 1977) demands an explanation. Mehrez *et al.* (1977) suggested that the estimate of optimal rumen NH_3-N concentration was dependent on whether rate of bacterial protein synthesis or rate of dry matter degradation was the dependent variable.

We hypothesized that a portion of the variation between estimates of optimal rumen NH_3-N concentration, which were based on experiments with mixed populations of rumen microbes, could be attributed to the types of degradable substrates provided. Maize has been used in some experiments (Hume *et al.* 1970; Satter & Slyter, 1974; Ortega *et al.* 1979;

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Slyter *et al.* 1979; Kang-Meznarich & Broderick, 1981), barley in others (Mehrez *et al.* 1977; Wallace, 1979) and a combination of maize and barley in others (Allen & Miller, 1976; Pisulewski *et al.* 1981). Since barley has been associated with relatively high estimates of optimal rumen NH_3-N concentration and since barley is more extensively digested in the rumen than maize (Waldo, 1973), we considered that rumen fermentability of cereal grains could be a factor affecting estimates of optimal rumen NH_3-N concentration. This possibility was investigated in the experiment reported here using four processed grains as fermentable substrates. Rolled barley was chosen for ease of comparison with previous literature (Mehrez *et al.* 1977), ground barley and ground maize were selected to allow comparison of two grains nominally similar in physical form, and autoclaved maize was used to assess the importance of starch gelatinization.

MATERIALS AND METHODS

Diets and animals

Four mature Holstein steers, mean weight 610 kg, were used. They were housed in an open-air barn in individual pens bedded with shredded bark. Each animal was surgically fitted with a 100 mm rumen cannula and was allowed a 4-week postoperative period to recover. During that time, the steers were accustomed to their first dietary treatments, and were conditioned to receive feed on an hourly basis from automatic feeders as described by Finner & Baumgardt (1963). Dry-matter intakes were restricted (11 g/kg body-weight) to ensure complete food consumption each hour. The diets provided sufficient energy to support small weight gains throughout the experiment.

Two basal diets were formulated (Table 1) and given either unsupplemented or supplemented with ammonium acetate (NH_4Ac). Levels of NH_4Ac given ranged from 0 to 500 g/steer per d in 100 g increments, resulting in a total of twelve experimental diets.

Diets were mixed in two stages. First, the barley or maize grain was combined with the maize cobs in 700 kg batches and mixed in a horizontal mixer. The mixture was then subdivided and stored in 20 kg bags. Later, daily rations were prepared in a small Hobart mixer. To the grain–cob mixture was added a dry vitamin–mineral premix and an aqueous supplement containing NH_4Ac and sodium sulphate (N:sulphur, 10:1) in volumes appropriate to give the desired level of NH_4Ac in the ration. The rations were mixed weekly and stored in sealed plastic bags at $-10°$ until used.

Design

The four steers were given the diets according to a cross-over, split plot design. Animals were randomly assigned a basal diet in period 1 and were switched to the other basal diet during period 2. Within each period, five NH_4Ac levels were randomly assigned after the unsupplemented basal diets were fed in the first subperiod, giving six subplots. During period 1, the same NH_4Ac randomization was applied to steer nos. 1 and 3 (in sequence of 0, 400, 100, 300, 500 and 200 g NH_4Ac/d) and to steer nos. 2 and 4 (in sequence of 0, 100, 500, 400, 200 and 300 g NH_4Ac/d), while in period 2, steer nos. 1 and 4 (in sequence of 0, 300, 200, 400, 500 and 100 g NH_4Ac/d) and steer nos. 2 and 3 (in sequence of 0, 200, 400, 300, 100 and 500 g NH_4Ac/d) received the same sequence of NH_4Ac treatments.

While only two basal diets were given, four grain treatments designated rolled barley, ground barley, ground maize and autoclaved maize were used for dacron-bag incubations in the rumen. During each subperiod, both barley treatments were incubated in the rumen of steers given the barley basal diet. Likewise, both maize treatments were incubated in the rumen of each steer fed on the maize basal diet.

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Table 1. *Composition (g/kg dry matter) of basal diets to which graded amounts of ammonium acetate were added*

Ingredient	Barley diet	Maize diet
Barley, rolled	738·0	—
Maize, cracked	—	731·0
Maize cobs, ground	250·0	250·0
Calcium carbonate	6·8	5·2
Dicalcium phosphate	—	5·2
Potassium chloride	0·9	4·3
Trace mineral salt*	2·8	2·8
Vitamin premix†	1·5	1·5

* Trace mineralized salt composition (g/kg) was a minimum of 3·5 Zn, 2·0 Mn, 2·0 Fe, 0·3 Cu, 0·05 Co, 0·07 I and NaCl between 960 and 985.

† Contained (mg/g): 1·21 vitamin A palmitate, 0·055 vitamin D_3, 0·22 vitamin E.

Experimental protocol

Animals were allowed to adapt to their basal diets for at least 3 weeks before the initiation of each period. The 42 d periods were each subdivided into six subperiods each 7 d long. During the first 6 d of each subperiod, steers were allowed to adapt to the level of NH_4Ac in the diet before experimental measurements were made.

In a preliminary experiment with one animal, the level of NH_4Ac fed was raised from 0 to 500 g/d, and rumen NH_3-N concentration was monitored for 10 d. The NH_4Ac was then removed and rumen NH_3-N concentration was monitored for another 10 d. The results indicated that 6 d were more than sufficient for rumen NH_3-N concentration to reach a new steady-state after a dietary perturbation. This preliminary experiment also indicated the importance of controlling water intake when attempting to establish a steady-state rumen NH_3-N concentration. Therefore, on days 5 and 6 of each subperiod, voluntary water consumption was determined and averaged for each steer. On the 7th day, steers were tethered and access to water was denied. Average daily water consumption of each steer was divided into nine equal portions, one of which was poured into the rumen at each sampling time.

At 08·00 hours on day 7 of each subperiod, eight polyethylene bottles with bags attached were soaked in water for 1 min and subsequently placed in the ventral rumen of each steer. One bottle was removed from each steer after 2, 4, 7, 10, 13, 16, 19 and 22 h of rumen incubation. At each sampling time, approximately 400 ml ventral rumen fluid were obtained and immediately placed on ice. One portion of the steer's water allowance was then poured into the rumen.

Incubated grains

The barley grain (Glenn variety) used for the incubation measurements was harvested from the agronomic plots of the University of Wisconsin-Madison and held in dry storage for 1 year before being used in this experiment. A portion of the barley was processed through a small roller mill. Kernels that were not crushed were sorted out, and the remaining rolled grain comprised the rolled barley treatment. A second portion of the whole grain was ground through a 6 mm screen and mechanically sieved for 3 min. Of the ground grain 4% passed through a 40-mesh screen and was discarded. Grain fragments larger than 40 mesh were recombined and mixed in a Hobart mixer for 2 min, resulting in the ground barley treatment.

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The maize grain used was of the Trelay 8000 variety (Trelay Farms, Livingston, Wisconsin). For the ground-maize treatment, the grain was processed in exactly the same manner as the ground barley. Of the weight before sieving, 9% passed through the 40-mesh screen and was discarded as fines. The autoclaved maize was prepared by submerging whole maize in water and autoclaving at 121° for 30 min. Excess water was drained off and the wet maize remaining was spread out in broad-based pans and dried at 65° in a forced-air oven for 24 h. This material was then ground and sieved through a 40-mesh screen as described earlier; fines comprised 5% of presieved weight.

Dacron-bag technique

Dacron cloth (Erlanger Baumgardt & Co., New York), with a pore size of 4000 (SD 1450) μm^2, was cut and sewn into bags with internal dimensions of 80 × 55 mm. Seams were double stitched and the stitch holes were filled by applying glue (Duco cement; Devcon Corp., Danvers, Maine) to the seams. The mouth of each bag was singed with a flame to prevent fraying. Bags were inverted and dried at 65° in a forced-air oven for 24 h. After standing in a desiccator for at least 3 h, they were weighed to the nearest 0·0002 g and were filled with approximately 2 g air-dry grain sample with equal precision. This resulted in a value for the ratio, substrate:bag surface area, of 0·227 mg/mm².

The mouths of the bags were tied shut with non-sterile surgical suture (Ethicon no. 0; Ethicon Inc., Somerville, New Jersey). Polyethylene bottles (125 ml) filled with 0·5 kg lead shot were used to weight the bags. A total of six bags, either three rolled barley plus three ground barley, or three ground maize plus three autoclaved maize, were attached to each bottle.

Wohlt *et al.* (1976) have shown that rumen NH_3-N concentration is higher in the dorsal portion than in the ventral portion of the rumen. Thus, weighting the bags was designed to ensure that they were confined to the ventral portion of the rumen so that the NH_3-N concentration determined in that region would represent the concentration in the fluid surrounding the bags.

After bottles were removed from the rumen, bags were submerged in ice-cold water for 2 h, after which they were cut from the bottles. Any feed residue on the exterior of the bags was removed, and the excess water in the bags was gently expressed. Bags were subsequently dried at 65°, placed in a desiccator for 3 h and weighed.

Sample preparation and chemical analysis

The pH of the chilled rumen fluid samples was determined with a combination glass electrode, and the samples were strained through one layer of cheese-cloth. Approximately 30 ml were centrifuged at 43 500 ***g*** for 10 min at 4° to remove feed particles, protozoa and bacteria. Microscopic examination of the supernatant fraction revealed that only a few very small bacteria remained. Of the supernatant fraction, 10 ml from each sample were acidified with 2 ml metaphosphoric acid (250 g/l) for NH_3-N determination. In addition, 5 ml of the supernatant fraction from each steer were saved at each sampling time and mixed to form a composite sample for each steer on each collection day. Of each composite, 10 ml were acidified with 2 ml metaphosphoric acid (250 g/l) for VFA analysis. All samples were stored at −10° until analysed.

The four grains to be incubated in dacron bags were analysed for crude protein (N × 6.25) using a modified micro-Kjeldahl procedure (Bradstreet, 1965; Brotz & Schaefer, 1984). Neutral- and acid-detergent fibre contents were determined according to the procedures of Goering & Van Soest (1970), and α-linked glucose polymers by the method of MacRae & Armstrong (1968) using glucan 1, 4-α-glucosidase (amyloglucosidase; *EC* 3.2.1.3) from *Rhizopus* fungi.

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Frozen samples of rumen fluid were thawed at room temperature and blown through a sintered-glass filter to remove precipitated protein. NH_3-N concentrations were determined in the samples using the Berthelot colorimetric reaction described by Chaney & Marbach (1962) and the phenol and hypochlorite reagents reported by Brotz & Schaefer (1984). VFA were separated and quantified by gas–liquid chromatography (Supelco, 1975).

Statistical methods

Analysis of rumen variables. Basal diet and NH_4Ac effects on rumen fluid pH, NH_3-N concentrations and molar proportions of VFA were analysed according to a cross-over, split plot analysis of variance (Cochran & Cox, 1957). All main effect means were separated with Duncan's new multiple-range test (Steel & Torrie, 1980).

Fractional degradation rate computation. The disappearance of dry matter from the bags over time was fitted to a single-pool exponential decay model described as follows:

$$\text{DMR} = Ae^{-K_d t}$$

where DMR is dry matter remaining in the bag (proportion of initial), A is dry matter remaining at time zero, K_d is fractional rate of dry matter disappearance (/h), and t is period of rumen incubation (h).

The equation was linearized by natural log transformation ($\ln \text{DMR} = \ln A - K_d t$) and each K_d was estimated by regression. Residual analysis revealed that the error variance was heterogeneous, increasing with length of incubation. Therefore, weighted least squares regression (Neter & Wasserman, 1974) was employed to stabilize the error variance. Each of the ninety-six disappearance curves was linearized as described and individually examined for remaining curvature by testing for the significance of a t^2 term; 24% contained significant curvature ($P < 0{\cdot}05$). Further examination of the residuals showed that the results obtained at 2 h of incubation were consistently adding curvature to the relation for each grain. For this reason, these values were discarded, and the extent of curvature was reassessed. Only 10% of the relations then contained significant t^2 effects, and this could not be improved.

Analysis of fractional degradation rates. In a preliminary analysis, K_d estimates were subjected to an analysis of variance, removing effects due to animal, period, grain and the animal × period × grain interaction. Residuals from this analysis were plotted against rumen NH_3-N concentration for each substrate separately. The plateau and exponential models described by Mehrez *et al.* (1977) were then fitted to each of the four scatter plots by the method of least squares (SAS Institute, 1982). A general extra sum of squares test (Neter & Wasserman, 1974) indicated that the rolled and ground barley models did not differ ($P > 0{\cdot}10$). A similar conclusion was reached for the two maize substrates. Hartley's test (Neter & Wasserman, 1974) indicated that the error variance was greater for the combined barley regression than for the combined maize regression so that for the final analysis the two grain types were analysed separately.

In the final K_d analysis each data set was submitted to the non-linear procedure of the Statistical Analysis System (SAS Institute, 1982). The chosen model included terms for animal effects and rumen NH_3-N concentration as either a plateau or exponential effect. The fitted models were then adjusted to the average animal and the resultant plateau and exponential equations were plotted. The residual for each K_d observation was added to the value predicted by the plateau model to obtain the scatter plots. A t test was then used to examine the difference between x coordinates of the plateau breakpoints (optimal rumen NH_3-N concentrations) and all other parameter estimates for barley and maize, considering the error variances of the estimates from the two grains to be unequal.

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Table 2. *Chemical composition (g/kg) of grains incubated in the ventral rumen of steers fed on barley or maize basal diets supplemented with graded amounts of ammonium acetate*

Component	Rolled barley	Ground barley	Ground maize	Autoclaved maize
Dry matter	924	914	891	875
Crude protein (nitrogen × 6·25)*	120	118	89	90
Starch*	520	550	740	760
Neutral-detergent fibre*	223	244	108	105
Acid-detergent fibre*	83	69	24	29

* Dry matter basis.

Table 3. *Volatile fatty acid (VFA) concentration and composition, pH and ammonia-nitrogen in ventral rumen fluid of steers fed on maize or barley diets supplemented with ammonium acetate (NH_4Ac)*

	VFA concentration (mM)	VFA composition (mmol/mol VFA)				pH	NH_3-N (mg/l)
		Acetic	Propionic	Butyric	Other*		
Maize	102·3	673[a]	183	101	44	6·46	92·8[a]
Barley	104·7	655[b]	172	129	44	6·41	159·7[b]
SE	1·5	1	2	2	1	0·04	2·0
NH_4Ac (g/d)							
000	88·6[a]	642[a]	182	132	44	6·49	41·0[a]
100	100·8[b]	657[a,b]	181	114	48	6·40	79·6[b]
200	107·6[b]	678[b]	166	112	44	6·46	110·6[b]
300	109·7[b]	662[a,b]	180	114	45	6·41	145·7[c]
400	108·2[b]	675[b]	174	111	40	6·42	173·4[c,d]
500	106·1[b]	668[b]	182	107	43	6·43	207·3[d]
SE	3·6	2	2	2	1	0·04	3·5

[a, b, c, d] Values with different superscript letters differed significantly (Duncan's new multiple-range test): $P < 0·05$. The error term for diet effects was the animal × period × diet interaction (2 df) and the error term for the NH_4Ac main effect was the residual error (10 df).

* Sum of iso-butyric, valeric and iso-valeric acids.

RESULTS

The chemical composition of the incubated grains is shown in Table 2. The composition of the barley samples differed markedly from the maize samples, being higher in crude protein and fibre but lower in starch content. Processing of grains had no effect on their chemical composition.

The effects of basal diet and NH_4Ac on rumen VFA concentrations, pH and NH_3-N concentration are summarized in Table 3. The major difference detected between the basal diets was with respect to rumen NH_3-N concentration which was higher ($P < 0·05$) for the barley diet (159·7 mg/l) than for the maize diet (92·8 mg/l). This difference was expected since the barley basal diet contained more crude protein than the maize diet. There was also a slightly lower proportion of acetate associated with the barley diet. Total VFA

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Table 4. *Rumen ammonia-nitrogen concentrations (mg/l) in steers fed on barley or maize diets supplemented with graded amounts of ammonium acetate*

(Mean values and standard deviations for eight sampling times)

Animal no.	Ammonium acetate (g/d)	Barley diet		Maize diet	
		Mean	SD	Mean	SD
1	000	52	17	6	2
	100	114	17	43	7
	200	151	32	73	34
	300	165	36	136	63
	400	231	47	141	41
	500	209	34	160	32
2	000	68	16	45	12
	100	97	15	39	20
	200	146	27	58	21
	300	228	28	93	25
	400	208	31	154	31
	500	275	33	155	39
3	000	87	16	13	4
	100	146	17	47	9
	200	125	16	107	20
	300	152	26	135	34
	400	170	37	134	30
	500	204	36	158	28
4	000	39	10	18	3
	100	128	38	23	21
	200	142	33	83	5
	300	124	25	133	47
	400	253	82	96	20
	500	320	38	177	50

concentration and the molar proportion of acetic acid were higher ($P < 0{\cdot}05$) in the second experimental period (109·5 mM and 668 mmol/mol VFA, respectively) than for the first (97·5 mM and 659 mmol/mol VFA). This increase may have been due to the increased feeding levels required in the second period to maintain the level of dry-matter consumption at 11 g/kg body-weight.

Rumen NH_3-N concentration steadily increased with increased NH_4Ac supplementation. However, only minor differences in total VFA concentration and molar proportion of acetic acid were observed. Interactions between NH_4Ac and the whole plot factors were not significant ($P > 0{\cdot}05$).

Average rumen NH_3-N concentrations measured at each level of NH_4Ac supplementation for each animal given each basal diet are shown in Table 4. The relatively low standard deviations (range of 2–82 mg NH_3-N/l) indicated that reasonable steady-state conditions were maintained during sample collection. However, a plot of rumen NH_3-N concentration, adjusted to remove whole and subplot effects, *v.* sampling time showed a significant ($P < 0{\cdot}05$) diurnal pattern (Fig. 1).

The relation between rumen NH_3-N concentration and the adjusted fractional degradation rates of barley and maize is shown in Fig. 2. Parameter estimates for the plateau and exponential models displayed in Fig. 2 are given in Table 5. Differences in the mean degradation rates for rolled and ground barley treatments were not detected ($P > 0{\cdot}10$) and since the extra sum of squares test indicated that the effect of rumen NH_3-N concentration

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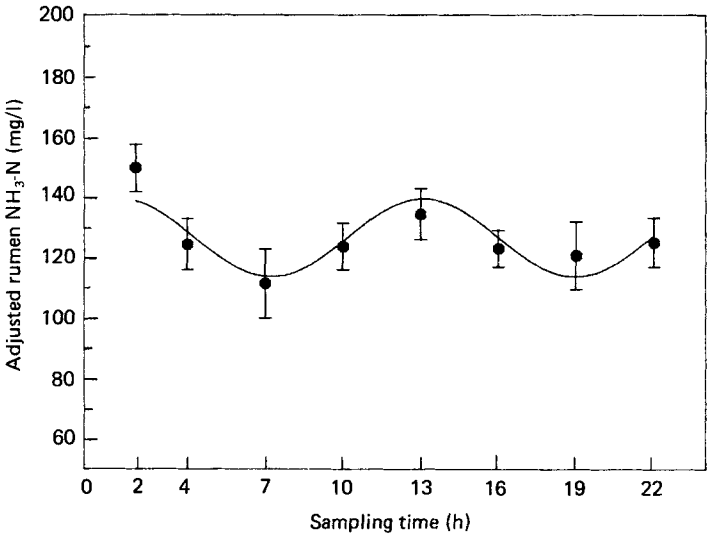

Fig. 1. Diurnal pattern of rumen ammonia-nitrogen (adjusted to remove whole and subplot effects) concentration in steers fed hourly. Zero-hour sampling time was 08·00 hours. $y = 12{\cdot}9 \sin (0{\cdot}54(x - 10{\cdot}15)) + 126{\cdot}4$. Each point is the mean, with 2 SE represented by vertical bars, for forty-eight observations.

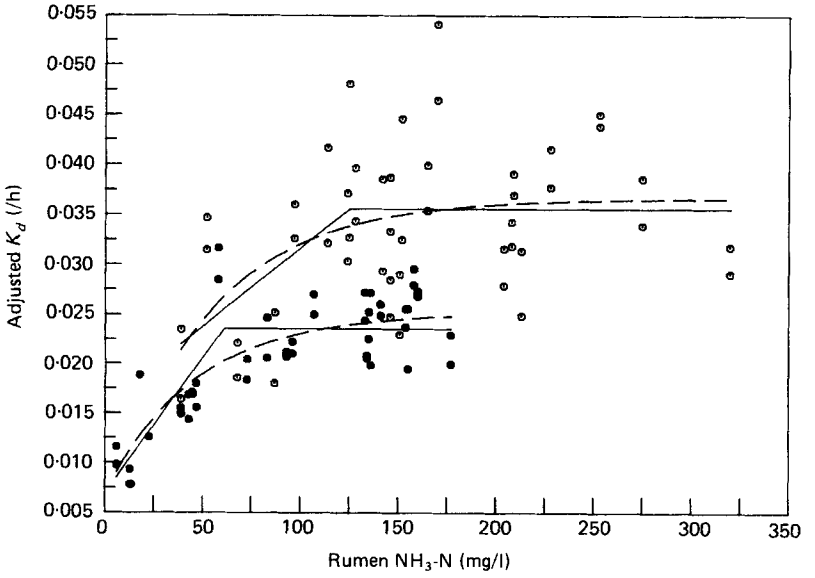

Fig. 2. Relation between rumen ammonia-nitrogen concentration and fractional degradation rates (K_d; adjusted to the average animal effect) for barley (⊙) and maize (●) dry matter in dacron bags suspended in the ventral rumen of steers. See Table 5 for parameter estimates of the plateau and exponential models which have been plotted. The x coordinates of the plateau breakpoint for barley and maize models are 125 and 61 mg NH_3-N/l respectively ($P < 0{\cdot}05$).

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Table 5. *Parameter estimates of the plateau and exponential models which describe the effect of rumen ammonia-nitrogen concentration on adjusted* K_d *for barley and maize*

Model	Grain	Parameter					
		A	SE	*B*	SE	*C*	SE
Plateau*	Barley	0·00016	0·00007	125[a]	23	0·036[a]	0·0013
	Maize	0·00028	0·00006	61[b]	7	0·024[b]	0·0007
Exponential†	Barley	0·037[a]	0·0023	−0·034	0·023	0·021	0·014
	Maize	0·024[b]	0·0017	−0·018	0·002	0·020	0·007

[a,b] For the model given, parameter estimates with different superscript letters differed significantly ($P < 0{\cdot}05$) for barley and maize.

K_d, fractional rate of dry-matter disappearance (/h).

* The plateau model has the form: $y = \gamma + Ax$ for $x < B$, and $y = C$ for $x \geqslant B$, where $\gamma = (C - AB)$.

† The exponential model has the form: $y = A + Be^{-Cx}$.

was similar for both, the rolled and ground barley treatments were pooled. The same was true for the ground- and autoclaved-maize treatments. Plateau models fitted the values better than exponential models as indicated by smaller residual sums of squares. Rumen NH_3-N concentration for the barley diets ranged from 39 to 320 mg/l, while the maize diets ranged from 6 to 177 mg/l. A difference between slopes of the ascending portions of the barley and maize plateau models was not detected ($P > 0{\cdot}10$). The maximum degradation rate for barley grain was greater than that for maize (0·036 *v.* 0·024/h respectively; $P < 0{\cdot}01$). Furthermore, the minimum rumen NH_3-N concentration required to maximize the degradation rate of maize was 61 (SE 7·3) mg/l which was lower ($P < 0.05$) than the 125 (SE 23·6) mg/l concentration required to maximize the rate for barley.

DISCUSSION

It has been suggested (Van Soest, 1982) that the single-pool exponential decay model used to describe forage dry matter remaining in dacron bags over time may fit better if the undegradable dry matter fraction is estimated and subtracted from each observation. This correction would help approach the single-pool assumption. However, in a preliminary investigation with barley and maize samples, we determined that this correction did not noticeably improve the fit of the model to our findings and therefore it was not utilized in this experiment. Quadratic effects present after the natural log transformation suggested that the correction may have been necessary; however, curvature introduced by the values for the 2 h period of incubation contributed substantially. After removal of the 2 h values, many of the plots of ln DMR *v.* time with significant t^2 effects showed negative quadratic relations. This remaining curvature was considered to result from lack of precision in the intrarumen incubation technique rather than effects due to a second pool.

Precision of the dacron-bag technique is limited by the large number of factors which can influence dry-matter disappearance (Figroid *et al.* 1972; Meyer & Mackie, 1986). A specific problem encountered with the high-grain diets used in this experiment was accumulation of gas in the bags. Gas accumulation has been reported by others (Lowrey, 1970; Uden & Van Soest, 1984). Increased rumen fluid viscosity in grain-fed animals may provoke occlusion of pores in the dacron material. Likewise, incubation in the highly fluid ventral rumen may prevent the gas from being expressed.

An absorption method similar to that described by Mehrez *et al.* (1977) was used to

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supplement the basal diets with an NH_4Ac–Na_2SO_4 solution, thereby avoiding technical difficulties associated with continuous rumen infusions. Hourly feeding of the supplemented diets assisted in achieving steady-state rumen NH_3-N concentrations. However, additional control over water consumption improved the steady-state approximation markedly. Although these efforts were reasonably effective in stabilizing rumen NH_3-N concentration (Table 4), a diurnal fluctuation was still detectable (Fig. 1).

Wohlt *et al.* (1976) observed an increase in rumen NH_3-N concentration beginning at 01·00 hours which was 9·5 h after the previous feeding. They attributed this, in part, to previously reported post-prandial patterns of saliva secretion (Bailey & Balch, 1961; Meyer *et al.* 1964) and reasoned that influx of salivary urea into the rumen accounted for increased NH_3-N concentrations. Post-prandial patterns of saliva secretion are not sufficient to explain the present results since the data were collected from steers fed hourly and given their daily water allowance in nine equal intrarumen doses. These results suggest that there was an underlying diurnal pattern to rumen NH_3-N concentration which was not suppressed by hourly meal consumption. This resilient characteristic could be caused by diurnal patterns of rumination, salivation independent of rumination, or transrumen flux of urea-N.

The use of NH_4Ac as the supplemental non-protein-N source had the desired effect of increasing rumen NH_3-N concentration without adversely affecting pH, which could be a confounding factor when urea supplements are given. Furthermore, acetate was selected as the associated anion based on the consideration that it would have little influence on the large rumen acetate pool.

No differences in mean dry-matter degradation rates were detected between processing methods for each grain type. Similarly, the relation between rumen NH_3-N concentration and degradation rate (Fig. 2) was not significantly affected by grain-processing method. Chemical analyses revealed that processing did not alter composition, but autoclaving should have increased the fermentability of the maize starch. In subsequent experimentation, 100 mg ground and autoclaved maize were exposed for 1–5 h to *Rhizopus* glucan 1, 4-α-glucosidase. Rates of glucose appearance were not different ($P > 0{\cdot}10$) for the two maize treatments. This implies that the gelatinized starch content of the autoclaved maize used for intrarumen incubations may not have been as high as intended. Exposure of maize to dry heat after autoclaving may have offset any increase in starch degradability due to autoclaving.

The minimum rumen NH_3-N concentration required to maximize the fractional degradation rate of barley exceeded that for maize (Fig. 2). This suggests that the optimal rumen NH_3-N concentration is higher for barley than for maize diets. Caution should be exercised, however, since the barley values refer to a higher rumen NH_3-N concentration range than the maize values. Nevertheless, chemical or structural characteristics of the degradable substrate may influence estimates of optimal rumen NH_3-N concentration, and this may in part explain the divergent estimates occurring in the literature.

The NH_3 saturation constants estimated for predominant species of rumen bacteria (Schaefer *et al.* 1980) indicate that lower concentrations than those reported here should support maximum bacterial growth rates. However, those results were obtained with bacterial suspensions grown in media without substrates for attachment. It is tempting to speculate that the relatively high estimates of optimal rumen NH_3-N concentration obtained in vivo are due to the physical association of bacterial cells with fermentable substrates. This speculation invokes the earlier suggestion (Allison, 1969; Ørskov, 1982; Owens & Bergen, 1983) that localized concentrations of nutrients or metabolites in micro-habitats could influence microbial growth and metabolism.

The extent of bacterial attachment may vary in direct proportion with the fermentability

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of grains and this could enhance differences in concentrations of soluble nutrients or metabolites between the microenvironments within attached bacterial strata and the suspending milieu. Maize is less extensively digested in the rumen and is associated with relatively low estimates of optimal rumen NH_3-N concentration (Satter & Slyter, 1974; Slyter *et al.* 1979; Kang-Meznarich & Broderick, 1981) while barley is extensively degraded in the rumen and was a diet component or an incubated feed in reports of relatively high optimal rumen NH_3-N concentration (Allen & Miller, 1976; Mehrez *et al.* 1977; Wallace, 1979).

Another possible explanation is that specific dietary components give rise to antimetabolites of NH_3-N. Methylamine has been reported to inhibit the extent of NH_3-N-limited growth of *Bacteroides amylophilus* (Ricke & Schaefer, 1984).

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