EFFECT OF BENTONITE SUPPLEMENTATION ON NITROGEN METABOLISM FROM DIETS CONTAINING UREA IN CATTLE

T.K. GHOSHAL, MADHU MOHINI AND G.P. SINGH
National Dairy Research Institute, Karnal - 132 001, India

(Received on August 6, 1997)

ABSTRACT

In an *in vitro* study three substrates viz. concentrate mixture with different levels of urea (a), synthetic diets with urea (b) and urea treated straw (c) supplemented with bentonite (0, 2, 3, 4 and 6% w/w) were evaluated. IVDMD and IVOMD did not differ irrespective of diet or level of bentonite. Ammonia -N concentration from concentrate mixture containing 1% urea decreased with the increase in bentonite level, however, at the other levels of urea it remained similar irrespective of bentonite level. The peak ammonia-N level was lower at all the levels of bentonite than those without bentonite but in synthetic diets as well as in treated straw no such variation was recorded.

In an *in vivo* experiment twelve ruminally fistulated crossbred calves, distributed in three equal groups, were fed concentrate mixture and wheat straw *ad lib*. Group II and III were supplemented with 2 and 4% bentonite, respectively in concentrate mixture. N excretion and balances were similar among groups. Lower total-N and ammonia-N ($P<0.01$) in rumen liquor in groups II and III than in group I indicated variation in N metabolism at rumen level, however, blood N parameters were similar among all the groups. Hence, this study indicated that ammonia -N was not adsorbed on bentonite and decrease in ammonia -N with protein diets might be due to the decrease in protein degradability.

Key words: Bentonite supplementation, Dietary urea, Urea treated straw, Synthetic diet.

Bentonite, a clay of montmorillonite group of minerals, possesses high ion exchange capacity which allows it to adsorb and bind a wide range of inorganic and organic cations. Its use in ruminant's diet has shown some beneficial effects[1,2]. Its supplementation increased pH of ruminal fluid in sheep fed high grain based diets[3]. Recent studies have shown its beneficial effect on the protein nutrition also[4], as it adsorbs ammonium ions. It was hypothesised[5] that it might affect nitrogen metabolism from non protein nitrogen source (urea) by adsorbing and releasing ammonia ions on dilution. Hence, this study was undertaken to see the effect of bentonite on nitrogen utilization from urea containing diets.

MATERIALS AND METHODS

Experiment-I Following substrates supplemented with different levels of bentonite (0,2,3,4 and 6% w/w) were evaluated in *in vitro* trials.

(i) Concentrate mixture (CP-20, TDN-72%) with three levels of urea (1,2 and 3%) (Table 1) and wheat straw in the ratio of 60:40.

(ii) Untreated and urea treated (4% w/w, 40% mixture, 30 d incubation following its air exposure) wheat straw.

(iii) Three synthetic diets containing urea (Table 2) as only source of nitrogen.

Each set of trials consisted estimation of IVDMD, IVOMD and NH_3-N with one substrate using single stage *in vitro* technique. One trial included incubation upto 48 h. In other trial, tubes were arranged in such a way so that one set of tubes (three tubes) was taken out at 0, 2, 4, 6, 8, 12, 24 and 48h after incubation, respectively, for the estimation of NH_3-N[7].

Experiment 2 Twelve crossbred rumen fistulated cattle were divided into 3 groups of four each. Animals were fed on wheat straw *ad lib* as basal roughage and concentrate mixture (Table 1) at maintenance level[8]. Groups II and III were supplemented with bentonite @ 2 and 4% of concentrate mixture required by each animal of the respective group. Chemical composition of the feeds is depicted in (Table 3). A 7 day metabolic trial was conducted after 27 days of preliminary feeding. Samples of feed, residue, faeces and urine were collected and analysed for proximate principles[9]. Data was analysed statistically[10] for interpretation.

Table 1 Composition of concentrate mixtures in Expt. I & II

	Expt. I.			
Ingredient	U_1	U_2	U_3	**Expt. II**
Groundnut cake	19	11.2	4	19
Maize	35	40.8	46	35
Wheat bran	42	43.0	44	42
Urea	1	2.0	3	1
Mineral mixture	2	2.0	2	2
Common salt	1	1.0	1	1

Table 2 Composition of synthetic diets

Ingredient	SU_1	SU_2	SU_3
Starch	60	60	60
Cellulose	32	32	32
Linseed oil	5	5	5
Urea	1	2	3
Mineral mixture	2	2	2
Commom salt	1	1	1

Table 3 Chemical composition of feeds (Expt-II, % DM)

	Concentrate mixture	Wheat Straw
Organic matter	92.85	90.77
Crude protein	18.88	3.59
Crude fibre	4.58	34.82
Ether extract	4.47	1.29
NFE	64.93	51.07
Total ash	7.15	9.23

RESULTS AND DISCUSSION

Experiment-1 Addition of bentonite decreased the NH_3-N in rumen liquor at different intervals (Fig. 1) with concentrate mixture U_1. Highest value was observed in control and lowest (P<0.01) with 6% bentonite. However, there was no significant difference among 2,3 and 4% levels of bentonite supplementation. After 24h incubation NH_3-N concentration corresponded to that of 0h in control while it remained low in bentonite supplemented groups. The NH_3-N values in concentrate mixture U_2 and U_3 remained similar (Fig. 1). With concentrate U_2 no significant difference was observed among the highest value of different treatments. It remained between 10.43 to 11.44. At 12h incubation, the values were lower in bentonite supplemented groups as compared to control. In concentrate mixture U_3 the values remained similar and no significant difference was observed. IVDMD and IVOMD of these diets also did not differ significantly (Table 4) on account of bentonite supplementation.

On urea treated wheat straw the values of NH_3-N decreased after 6h of fermentation (Table 5) as a result of microbial utilization without any significant difference at time intervals on account of bentonite supplementation. The NH_3-N value for synthetic diets containig urea as only N source with different level of bentonite supplementation did not reveal significant difference thereby indicating NH_3-N is not absorbed on the clay particles (Table 6).

These results showed that on diets with concentrate, NH_3-N values decreased with the increase in bentonite level. Though the differences in the average values at 24h and 48h were not significant yet the difference between highest and lowest value decreased with bentonite supplementation. This confirms the hypothesis that bentonite binds N when it is excess and releases when it is less in the rumen liquor to maintain the nitrogen concentration[5]. However, this hypothesis does not fit when only NPN is present in the diet as evident from urea treated straw and synthetic diet in which urea the was the only N source. It shows probably bentonite binds amino acids, amides, or protein molecules rather NH_3-N, only causing decrease in NH_3-N in liquid phase. This was also observed with casein, soy meal and urea separately[11].

Experiment II Animals were fed concentrate mixture containing 1% urea (Table 1) with 19% CP (Table 3) according to their nutritional requirement. Digestibility of the nutrients did not vary among the treatment groups[12]. N intake was similar in all the treatment groups (Table 7). Supplementation of bentonite at both the levels did not affect the N-excretion, N-digestibility and nitrogen retention[3,13].

Fig. 1 Ammonia-N concentration in concentrate diets *(in vitro)*

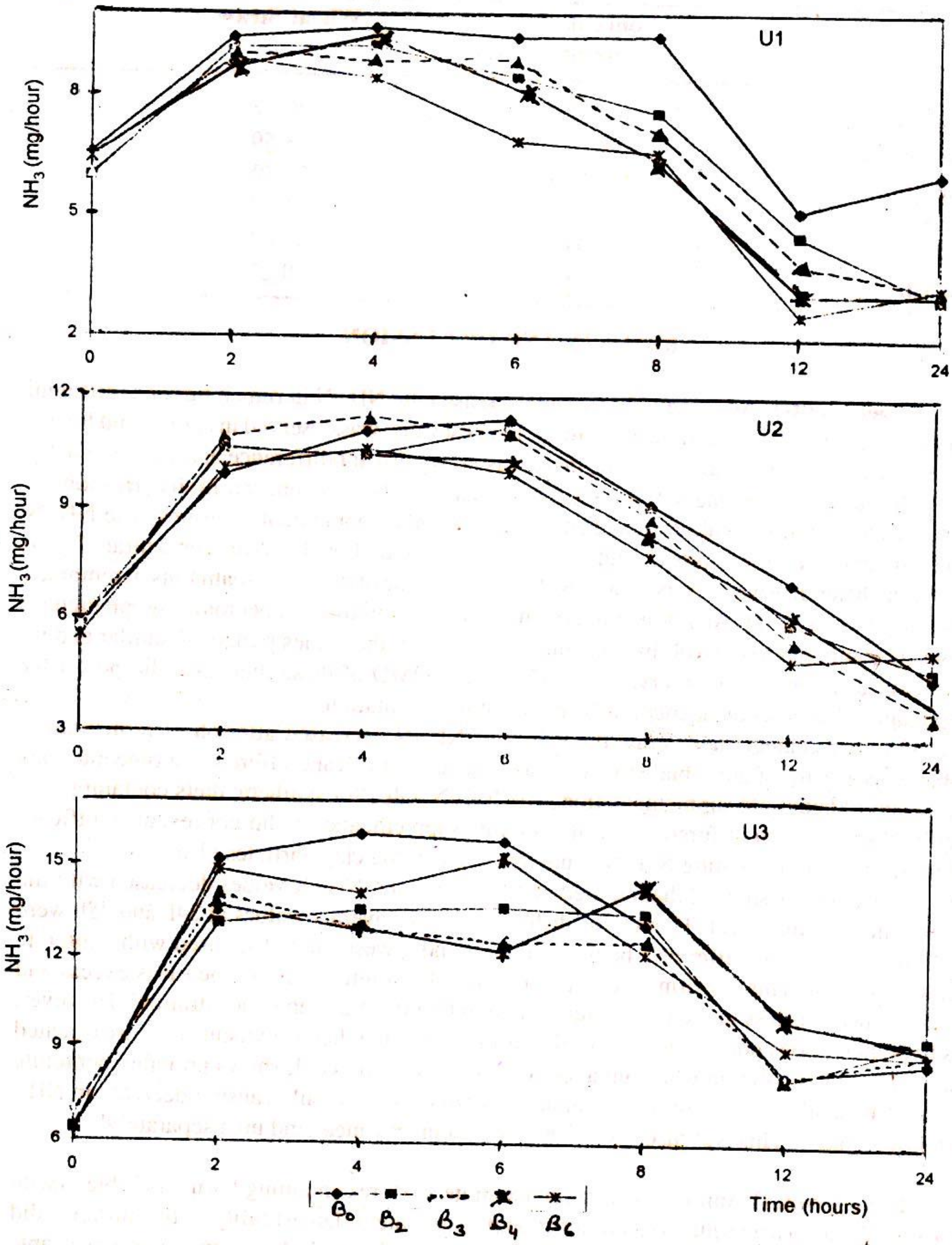

Table 4 IVDMD and IVOMD with different types of substrates at different levels of bentonite

Bentonite Level	Substrate				
	Wheat straw + Concentrate mixture U_1	Wheat straw + concentrate mixture U_2	Wheat straw + concentrate mixture U_3	Untreated wheat straw	Urea treated wheat straw
			IVDMD		
B_0	72.86	72.19	71.24	44.90	54.51
B_2	71.82	73.39	69.96	41.75	51.59
B_3	73.08	71.58	72.32	45.66	53.39
B_4	72.34	72.85	71.63	38.81	50.44
B_6	71.51	73.12	71.88	40.76	51.94
Mean ± S.E.	72.32 ±0.30	72.63 ±0.33	71.41 ±0.40	42.38[a] ±1.28	52.37[b] ±0.71
			IVOMD		
B_0	74.71	74.51	73.69	52.60	62.03
B_2	74.08	75.68	72.40	51.14	60.60
B_3	75.03	74.11	74.88	55.01	62.30
B_4	74.39	75.07	74.37	50.16	61.25
B_6	74.60	75.42	74.56	51.77	62.96
Mean ± S.E.	74.56 ±0.16	74.96 ±0.29	73.98 ±0.44	52.14[a] ±0.82	61.83[b] ±0.41

Figures bearing different superscripts in a row differ significantly (P<0.01)

Table 5 ***In vitro*** **NH_3-N concentration (mg/100ml) on urea treated wheat straw with different levels of bentonite**

Treatment	Time (h)								Mean ± S.E.
	0	2	4	6	8	12	24	48	
UB_0	11.08	12.07	12.44	11.48	10.34	6.62	2.32	11.20	9.69 ± 0.69
UB_2	10.98	12.06	12.58	11.52	10.38	7.58	1.17	7.80	9.26 ± 0.79
UB_3	11.40	12.30	12.64	11.32	11.13	6.38	1.26	10.78	9.65 ± 0.77
UB_4	11.50	12.25	12.31	11.20	10.06	6.47	2.58	7.36	9.22 ± 0.74
UB_6	11.55	12.41	12.39	11.79	9.72	5.67	2.13	8.38	9.25 ± 0.83

Table 6 *In vitro* NH_3-N concentration (mg/100 ml) on synthetic diets (U_1, U_2, U_3) with different levels of bentonite

Treatment	Time (h) 0	2	4	6	8	12	24	48	Mean ± S.E.
U_1B_0	4.63	12.02	12.22	10.11	9.46	8.92	9.05	8.59	9.37 ± 0.50
U_1B_2	4.94	11.41	12.01	11.22	9.56	9.53	8.95	7.02	9.33 ± 0.51
U_1B_3	4.94	10.80	12.11	10.30	10.52	8.76	8.10	7.84	9.17 ± 0.47
U_1B_4	4.73	11.80	11.20	10.91	10.29	7.28	8.72	6.89	8.98 ± 0.51
U_1B_6	4.93	11.26	12.16	11.49	9.38	9.22	7.84	7.32	9.20 ± 0.52
U_2B_0	5.99	20.49	19.97	18.58	19.26	16.82	15.01	14.53	16.23 ± 0.95
U_2B_2	5.89	19.33	18.63	19.82	15.46	15.65	15.02	13.70	15.44 ± 0.94
U_2B_3	6.05	19.70	21.14	17.39	16.93	14.57	15.26	14.87	15.74 ± 0.91
U_2B_4	5.78	19.44	20.26	16.95	17.83	14.71	15.76	14.13	15.61 ± 0.91
U_2B_6	6.13	18.84	18.81	20.34	17.07	15.86	15.32	14.13	15.81 ± 0.92
U_3B_0	8.10	22.13	29.31	32.30	26.02	26.54	24.61	25.45	24.31 ± 1.49
U_3B_2	8.42	21.95	29.65	28.14	27.36	29.30	25.99	25.18	24.50 ± 1.38
U_3B_3	8.27	22.49	30.95	29.27	26.70	26.67	29.63	25.77	24.97 ± 1.44
U_3B_4	7.71	22.3	31.09	28.95	21.73	25.57	26.76	25.30	23.68 ± 1.41
U_3B_6	8.38	22.92	30.86	30.66	25.70	25.08	25.50	24.54	24.18 ± 1.37

U_1 = Synthetic diet (Urea 1%)
U_2 = Synthetic diet (Urea 2%)
U_3 = Synthetic diet (Urea 3%)

Table 7 N-Metabolism as influenced by different treatments

Paremeter	Group		
	T_1	T_2	T_3
Intake (g/d)	74.11±10.35	73.08±4.68	71.02±3.46
Outgo in faeces (g/d)	35.60±6.12	32.68±2.19	32.88±3.58
Outgo in urine (g/d)	34.94±4.58	36.58±4.69	33.66±0.52
Balance (g/d)	3.58±1.13	3.82±1.48	4.48±0.51
Faecal N as % of intake (g/d)	47.98±3.70	44.72±2.14	46.03±2.73
Urinary N as % of intake (g/d)	47.30±2.44	50.05±2.47	47.58±2.07
Total N in rumen	77.65±4.47[a]	66.04±4.91[b]	55.34±3.87[c]
NH_3-N	26.21±1.79[a]	20.27±1.28[b]	17.49±1.49[b]
TCA-ppt-N	31.29±3.69[a]	28.35±2.18[b]	25.29±2.88[b]
Blood NH_3-N	1.52±0.07	1.58±0.12	1.76±0.26
Blood urea-N	16.33±1.73	12.74±1.19	11.14±0.83

It is concluded from *in vivo* as well as *in vitro* studies that bentonite supplementation did not affect the nitrogen metabolism of the diets containing urea at maintenance level of feeding.

REFERENCES

1 JORDAN, R.M. (1953) Bentonite in lamb rations. *Feedstuffs*. May 16, p. 53

2 ERWIN, E.S., ELAM, C.J. and DYER, I.A. (1957) The influence of sodium bentonite *in vitro* and in the ration of steers. *J. Anim. Sci.* 16 : 858

3 FISHER, L.J. and MACKAY, V.G. (1983) The investigation of sodium bicarbonate or bentonite as supplements in silages fed to lactating cows. *Cand. J. Anim. Sci.* 63 (4) : 939

4 FENN, P.D. and LENG, R.A. (1989) Wool growth and sulfur amino acid entry rate in sheep fed roughage based diets supplemented with bentonite and sulfur amino acids. *Aust. J. Agric. Res.*, 40:889

5 MARTIN, L.C., CLIFFORD, A.J. and TILIMAN, A.D. (1969) Studies on sodium bentonite in ruminant diets containing urea *J. Anim. Sci.*, 29:777

6 TILLEY, J.M. and TERRY, R.A. (1963) A two stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassland Soc.* 18(2):104

7 CONWAY, W.J. (1962) *Microdiffusion Analysis and Volumetric Error* (5th edn). Crossby Lockwood and Sons Ltd., London

8 SEN, K.C. and RAY, S.N. (1978) *Nutrtive Values of Indian Feeds and the Feeding of Animals*. ICAR Publ, New Delhi

9 AOAC (1984) *Official Methods of Analysis* (14th edn). Association of Official Analytical Chemist, Washington, D.C.

10 SNEDECOR, G.W. and COCHRAN, W.G. (1968) *Statistical Methods*, Oxford and IBH Publishing Company, Calcutta, India

11 BRITTON, R.A. COLLING, D.P. and KLOPFENSTEIN, T.J. (1978) Effect of complexing sod. bentonite with soybean meal or urea *in vitro* ruminal ammonia release and nitrogen utilisation in ruminants. *J. Anim. Sci.* 46:1738

12 GHOSHAL, T.K. and MADHU MOHINI (1998) Nutrient utilisation and VFA production on bentonite supplementation to the diet containing urea in cattle. *Indian J. Dairy Sci.* 51:44

13 RINDSING, R.B and SCHULTZ, L.H. (1970) Effect of bentonite on nitrogen and mineral balances and ration digestibility of high grain rations fed to lactating dairy cows. *J. Dairy Sci.*, 53:888