Effect of Rumen Degradable Protein (RDP) in Straw Based Ration on Purine Derivatives Excretion and Microbial Nitrogen Supply in Cattle

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ABSTRACT : Four local cattle were (145±9.7 kg) used in a 4 × 4 Latin square design to study the effect of different levels of rumen degradable protein (RDP) in straw based ration on purine derivatives excretion and microbial N supply in cattle. The four rations were formulated at the same amount of energy but varying RDP approximately 50 (U0), 75 (U1), 100 (U2) and 150 (U3) percent levels of RDP requirement for maintenance. They were fed ranged from 101 to 304 g RDP/d. Apparent digestibility of all nutrients increased significantly ($p < 0.01$) in cattle fed ration U2 than other rations. Rumen NH_3-N concentration increased from 43 to 130 mg/l in response of RDP intake. Purine derivatives excretion increased significantly ($p < 0.01$) with incremental level of 203 g RDP/d (U2) intake and positively correlated ($r = 0.69$, $p < 0.01$, $n = 16$) with amount of RDP intake. The rates of rumen microbial N supply were 16.8, 27.2, 39.1 and 32.9 g/d for rations U0, U1, U2 and U3 respectively. Efficiency of microbial N supply (EMNS) per kg of DOMR were 19.0, 25.3, 33.0 and 28.6 g and per MJ of ME. Intake were 0.62, 1.00, 1. 44 and 1.21. g for U0, U1, U2 and U3 respectively and highest results were obtained in cattle fed U2 ration. Results of this study suggest that PD excretion and EMNS were increased as incremental level of RDP intake (U2) in local cattle.

(**Key Words**: Rumen Degradable Protein, Purine Derivatives, Microbial Nitrogen, Cattle)

INTRODUCTION

The protein requirements of ruminants and protein values of their feedstuffs have for a long time been expressed as digestible crude protein (DCP). With the increasing knowledge of protein nutrition in ruminants, it is now known that these DCP values are not satisfactory. In order to overcome the limitations of DCP system, ARC (1984) has proposed that the dietary CP needs of the ruminants must be supplied in terms of rumen degradable protein (RDP) to meet the N requirement of rumen microorganisms and undegradable dietary protein (UDP) which should be made available to the animal whenever microbial protein synthesized in the rumen is insufficient to meet the N requirement of host animal tissues. For the optimum efficiency of microbial protein production, it is necessary that as much as possible of the degraded nitrogen (ammonia) is incorporated in to the microbial nitrogen. If excess rumen degradable protein is present in the rumen, this is lost to the blood stream by absorption through the rumen wall to the animal (ARC, 1984).

Purine derivatives excreted in the urine of ruminants comprise allantoin, uric acid, xanthin and hypoxanthine (Oser, 1965). They originate from degradation of purine of both endogenous and exogenous origin. In ruminants, exogenous purine are largely of rumen microbial origin (McAllan and Smith, 1973). It has been suggested that the urinary excretion of purine derivatives could be used as an estimator of the microbial protein supplied to the animal (Topps and Illiott, 1965, Rys et al., 1975 and Verbic et al., 1990).

The feed available for ruminant production in Bangladesh are largely the agro-industrial by products usually crop residues such as straws from rice and wheat production. These straws are low in nitrogen, energy and mineral matter which limits the extent of microbial fermentation in the rumen. Since nitrogen is one limiting nutrient in low quality roughages, supplementation with urea to supply ammonia to the rumen microbes is very important. As RDP is required for microbial protein synthesis and ARC (1984) recommendation is based on the research work carried out on *Bos-taurus* cattle and in temperate zones. Therefore, the present experiment was carried out to examine the effect of different levels of RDP with urea of a nitrogen deficient diets on the urinary excretion of purine derivatives and microbial nitrogen supply in cattle.

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MATERIALS AND METHODS

Animals and treatments

Four local cattle of mean initial live weight 145±9.7 kg (4-5 years old) were used in a 4 × 4 Latin square experiment. They were individually fitted with permanent ruminal cannula and trials were conduted on these cannulated animals. Each cattle was fed with one of the 4 experimental rations containing 65% straw and 35% concentrates (DM −2.5% of body weight) given in table 1. The four rations were formulated at the same amount of energy (7.6 MJ/kg DM) but varying RDP supplemented with urea for U0, U1, U2 and U3 respectively. These levels were approximately 50, 75, 100 and 150 percent levels of RDP requirement for maintenance (ARC, 1984). Animal were kept on four different rations consecutively. Rations were divided into two equal parts and were given at 08:00 and 16:00 hours daily. Rations were completely consumed by animals. Fresh water was freely available.

Table 1. Composition of rations (g/kg DM)

Ingredients	Rations			
	U0	U1	U2	U3
Rice straw	650	650	650	650
Wheat bran	150	150	150	150
Rice polish	100	94.4	88.8	77.6
Molasses	88	88	88	88
Urea	−	5.6	11.2	22.4
Mineral mixture*	8	8	8	8
Common salt	4	4	4	4

* The mineral mixture contained (g/100 g): $CaCo_3$ − 44, $MgSO_4$ −7, K_2PO_4 −45, Trace mineral mixture ** −4.

** Composition of trace mineral mixture (g/100 g): $FeSo_4$ $7H_2O$ −48, $CuSo_4$ $7H_2O$ −9, $MnSo_4$ H_2O −41, KI −0.4, $ZnSO_4$ $7H_2O$ −0.5, $CoCl_2$ $6H_2O$ −1 and Na_2SeO_4 −0.1.

Experimental procedures

Each experiment was maintained for twenty three consecutive days.

In situ ruminal effective degradability of CP of rations

From day 13 to day 15, the CP disappearances(%) were estimated 4 g of each ration in nylon bags (pore size 0.42mm) suspended in the rumen for 2, 6, 12, 24 and 48 hours. After withdrawal, the bags were thoroughly rinsed in a tap water and dried in an oven at 60℃. Crude protein disappearance was estimated from the CP loss during the incubation period. From degradability data obtained at different intervals, the constant a, b and c from the expression $P = a + b(1-e^{-ct})$ were obtained proposed by Orskov and McDonald (1979), where P is the degradability at time t. The degradation kinetics constants (a, b and c) were combined with those of rate of passage (5%) to calculate effective degradability of CP (EDCP) as proposed by McDonald, 1981.

$EDCP = a + b*c/(c + k)*EXP\{-(c + k)*T\}$

where, the constants 'a' is intercept, 'b' is the potential degradable fraction, 'c' is the degradation rate, T = lag time and k is the fractional outflow rate from the rumen.

Faeces and urine collection

Nutrients digestibility was determined for each animal in each period, for which total faeces and urine excretion were collected for 7 days. Faeces were collected manually by floor scraping immediately after voiding and weighed every 24 hours. A 200 g of sample was then taken and dried at 60℃ in the conventional oven overnight to determine DM content and daily dry faecal output. The dried samples for each 7 days collection period were pooled, ground through a 1mm sieve and stored for later chemical analysis.

Daily urine output was collected in about 100ml of 1 M N−free sulfuric acid to prevent ammonia−N loss (final pH of the urine < 3) and at each 24 hours urine collection was diluted to 5 litre (to prevent precipitation of uric acid during storage), filtered and sampled. The samples were stored at −20℃ until analysis for purine derivatives.

Sampling of rumen liquor

On the last three days of each experimental period (day 21−23) rumen liquor samples were taken by means of manual pump at 0, 1, 3, 5 and 7 hours after feeding. The samples were acidfied with HCl (0.2M) and stored at −20℃ for NH_3-N analysis.

Estimation of microbial N supply

Purine absorption and PD excretion: The PD were determined according to the procedure of Chen et al. (1992). The amount of microbial purine absorbed (X mmol/d) corresponding to PD excreted (Y mmol/d) was calculated based on the relationship derived by chen et al. (1990) namely:

$Y = 0.85X + (0.385W^{0.75})$

were

$W^{0.75}$ = the metabolic body weight (kg) of the animal

0.85 = represents the recovery of absorbed purines as PD to total excretion after correction for the utilization of microbial purines by the animals.

0.385 = the endogenous contribution is taken as a constant at 0.385 mmol/kg $W^{0.75}$ per day.

With the assumption that the purine:protein ratio of mixed ruminal microbes remained constant, the amout of microbial nitrogen supplied to the animal was calculated as follows:

Microbial N (g/d) = 70X / (0.83 × .116 × 1,000)
= 0.727X

where

0.83 = digestibility co-efficient for microbial purines (Chen, 1989)

70 = N content of purines (mg−N/mmol) and

0.116 = ratio of purine N to total N in mixed microbial biomass measured (Chen, 1989).

The microbial N estimated from PD excretion corresponds to the quantity of microbial biomass reaching the duodenum rather than the synthesized within the rumen. Therefore, the term "efficiency of microbial nitrogen supply"(EMNS) is used. It is expressed as grams of microbial N per kilogram of digestible OM apparently.

Chemical analysis

Nutrient contents of feed and faeces samples were determined according to AOAC (1984) procedures and N content of feed, faeces and urine by Kjeldhal method. Analysis of NDF and ADF were done by the method of Goering and Van Soest (1970). Ammonia concentration of rumen liquor samples was determined by direct distillation with $Na_2B_4O_7$.

Statistical analysis

Data were examined by analysis of variance as a 4 × 4 Latin square design with degree of freedom partitioned to animal, period and treatments assuming no carryover effect. The least significant difference ($p < 0.05$ and $p < 0.01$) was used to compare treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The mean chemical composition of the rations and the intake of various nutrients are presented in table 2. Rations were formulated to maintain all dietary constituents except nitrogen for maintenance. Energy intake (ARC, 1984) was achieved by restricting dietary intake (DM −2.5% body weight).

Table 2. Chemical composition of rations, intake and digestibility of nutrients

Constituents	Rations				SEM
	U0	U1	U2	U3	
Composition (g/kg DM)					
OM	866±28	867±21	868±26	869±25	
CP	63±4	78±7	91±6	123±8	
RDP	28±3	43±5	57±2	85±6	
CF	270±21	269±12	268±17	267±18	
NFF	511±35	496±19	482±31	451±39	
NDF	554±38	548±42	543±28	532±19	
ADF	411±12	407±24	403±30	394±19	
P/ME (g/MJ)	8.3	10.3	11.9	16.2	
Intake (g/d)					
DM	3,580±371	3,577±395	3,571±361	3,566±384	
OM	3,099±337	3,100±308	3,099±279	3,099±314	
CP	225±18	279±17	323±24	440±29	
RDP	101±7	152±12	203±19	304±23	
ME (MJ/d)	27.6	27.2	27.1	27.1	
Apparent digestibility (%)					
DM*	50.0^a	56.5^b	58.3^b	53.5^a	1.6
OM*	46.4^a	53.9^a	58.8^b	56.2^b	1.3
CP**	46.9^a	57.6^b	66.9^c	75.7^d	1.3
NDF*	49.2^a	52.3^b	54.9^b	51.3^b	1.2
ADF*	41.3^a	47.3^b	48.7^b	49.2^b	2.0

^{a,b,c,d} Values in the same row with different superscripts differ significantly; * $p < 0.05$, **$p < 0.01$.
SEM Standard error of means.

The digestibility of DM, OM, CP, NDF and ADF are given in table 2. The digestibility of DM and OM increased significantly ($p < 0.01$) in response of the rations U1 and U2, but no further increases were achieved by increasing the RDP supply (U3).

Raleigh and Wallace (1963) found that total DM and OM digestibilities by steers fed low quality roughage diet increased from 9 to 12% with supplemental protein. Some previous research has reported no difference in apparent DM digestion due to level and sources of nitrogen. (Hume et al., 1970 and Hume, 1970). In the present experiment, the OM intake was not different ($p > 0.05$), but the proportion of DOM apparently digested in the rumen (based on the model assumes DOMR to be 65% of digestible OM intake ARC, 1984) increased from 935g/d on the ration U0 to 1,086, 1,184 and 1,132 g/d with successive level of RDP supplementation. Neutze et al. (1986) observed that an increase proportion of DOM apparently digested in the rumen as a result of urea supplementation.

The digestibility of NDF and ADF were significantly ($p < 0.01$) higher in U1, U2 and U3 than U0. This increase could be attributed to the availability of extra N to rumen microbes, which improved the rumen fermentation. Decreased digestibility in diet U0 might be due to shortage of RDP for proper microbial fermentation in the rumen. Similar reports regarding the increase in degestibility of cell wall components due to supplementation of urea were noted (Singh et al., 1990).

The variation in CP intake as well as RDP to UDP ratio did influence the CP digestibility (table 2). Crude protein digestibility in cattle fed ration U0 was significantly ($p < 0.01$) lower when compared with cattle fed rations U1, U2 and U3. The significant improvement of CP digestibility by rations U1 to U3 might be due to the fact that a part of ammonia presumably released in the rumen and a good part of it absorbed through the rumen wall. Improvement of digestibility may be associated with increase in rumen microbial activity and thus microbial protein synthesis as has been suggested by Warly et al. (1992). Overall digestibility of nutrients was highest in cattle fed ration U2. This may be confirmed by CP/ME ratio which 12.0 g/MJ and said to be minimum for optimal microbial fermentation (Menke and Huss, 1987).

Potential CP degradability (a+b) of the rations was increased by the experimental treatment from 60.4 to 82.2 (table 3). However, fractional degradation was unaffected in response to the level of RDP intake. This effect was reflected in a gradual increase in effective degradation of CP that elicited the maximum response in NH_3-N concentration in rumen liquor. The supplementation of straw based diet with urea as RDP into the rumen elicited a steady NH_3-N concentration which ensured a constant N availability at each level of RDP intake.

Table 3. The constants a and b, the potential degradability (a+b), the degradation rate 'c' EDCP, RDP and UDP (%) for four rations

Rations	a	b	(a+b)	c	RSD	EDCP	RDP	UDP
U0	3.8	56.6	60.4	.093	.18	44.9	2.8	3.5
U1	26.2	42.8	69.0	.096	.67	54.4	4.3	4.5
U2	39.5	35.7	75.2	.095	.20	62.8	5.7	3.4
U3	56.2	26.0	82.2	.090	.32	69.1	8.5	3.8

Each value is the mean of four observations.
RSD Residual standard deviation.

It is well known that ammonia is an essential nutrient for proper growth of many bacteria (Hungate, 1966). Rumen ammonia nitrogen (table 4) was highest (130 mg/l) with feeding of U3 ration. In the present experiment, the low level of NH_3-N concentration (43 mg/l) recorded with the ration U0, was insufficient to meet microbial requirements as the critical levels of ammonia nitrogen has been variably reported as being between 50 and 250 mg of NH_3-N/l of rumen liquor (Preston and Leng, 1986). The level of NH_3-N concentration obtained in cattle fed the rations U1 (71 ± 12), U2 (96 ± 17) and U3 (130 ± 31) in this study (table 4) suggests that the rations achieved a level of NH_3-N necessary for optimum microbial protein synthesis.

The purine derivatives (PD) excreted in the urine originated from absorbed microbial purines and purines from the animal's own tissues. The contribution from the endogenous source is small compared with total excretion. In this experment, total PD excretion was calculated based on the relationship described by Chen et al. (1990) as the endogenous contribution is taken as constant at 0.385 mmol/kg $W^{.75}$ per day and this contribution to total

excretion declined as the exogenous supply was increased.

The daily excretion of PD is shown in table 4. The highest rate (61.8±4.2 mmol/d) of urinary PD excretion was noted in the cattle receiving the U2 ration. The response of urinary PD in the present study was due to increased RDP intake up to 203 g/d (table 4). There was a positive correlation ($r=0.69$, $p < 0.01$) between daily RDP (g) intake and total PD excretion (mmol) in the urine. Liang et al. (1995) found PD (allantion) excretion was dependent quantitably upon digestible dry matter intake (DDMI) in cattle. Chen et al. (1992) detected a positive relationship ($R^2=0.50$) between purine excretion and DMI : BW ratio for sheep, and suggested that purine excretion was related to ruminal kinetics.

Table 4. Purine derivatives (PD) excretion, calculated microbial supply as affected by the level of RDP intake

Particulars	Rations					Stat. Sig. of treatment effects
	U0	U1	U2	U3	SEM[p]	
DOMI (g/d)	1,438	1,671	1,822	1,742		
DOMR[q] (g/d)	935	1,086	1,184	1,132		
Ammonia Conc. (mg/l)	43[a]	71[b]	96[c]	130[d]	9	**
PD excretion (mmol/d)						
Allantoin	30.9[a]	40.9[b]	53.2[c]	46.8[d]	.76	**
Uric acid	4.8[a]	7.0[ab]	8.3[b]	7.7[b]	.66	**
Total	35.7[a]	47.9[b]	61.8[c]	54.5[d]	1.03	**
Efficiency of microbial−N supply (EMMS)						
g/d	16.8[a]	27.2[b]	39.1[c]	32.9[d]	1.18	**
g/kg DOMR[q]	19.0[a]	25.3[b]	33.0[c]	28.6[bc]	1.61	**
g/kg DOMR[r]	29.9[a]	34.8[b]	37.9[b]	36.3[b]	1.10	**
g/kg $W^{.75}$/d	0.40[a]	0.65[b]	0.94[c]	0.79[b]	0.05	**
g/kg DDMI	9.40[a]	13.50[b]	18.80[c]	17.30[b]	0.58	**
g/MJ of ME intake	0.62[a]	1.00[b]	1.44[c]	1.21[d]	0.02	**

[p] Residual d. f. 6.
[q] DOMR calculated as 0.65 XDOMI.
[r] calculated as 32 g/kg DOMR (ARC, 1984).
[a,b,c,d] Values in the same row with different superscripts differ significantly; ** $p < 0.01$.
SEM Standard error of means.

Microbial nitrogen flow was expressed per unit OM apparently digested in the rumen. Rations fed different in the RDP concentration and caused differences in the rumen microbial N supply estimated using the microbial nucleic acid flow method (table 4). The calculated microbial N supply ranged from 16.8 to 39.1 g/d and EMNS from 19.0 to 33.0 g/kg of DOMR (table 4). The lowest rate of microbial−N supply was observed in cattle fed ration U0 and highest rate in cattle fed U2 ration. There was a positive correlation ($r=0.76$; $p < 0.05$) between EMNS and RDP intake. Robinsons et al. (1985) found the increased bacterial N yield with increased N intake. There was a significant relationship ($p < 0.01$) between EMNS (y: g/kg $W^{0.75}$ per day) and rumen ammonia concentration (x: mg/l) described by the equation: $y = 0.40 + 0.003$ (SE 0.0008)X ($r = 0.69$, RSD = 0.15). This equation shows that the ammonia concentration required to attained the maximum response (96 mg/l) seems to be higher than the critical concentration necessary to maximize the EMNS. Hume et al. (1970) also found the requirement of higher ammonia concentration (139 mg/l) to increase duodenal flow of micronbial nitrogen. Balcells et al. (1992) also found linearly increased microbial N flow to the duodenum with rumen ammonia concentration. The reported value in literature for the efficiency of microbial nitrogen synthesis in the rumen (20-50 g N/kg of OM apparently digested in the rumen) of steers and sheep have been shown considerably large variations (Smith et al., 1978 and McAllan and Smith, 1984).

Rumen microbial protein synthesis is depend upon the amount of energy, usually calculated as moles of ATP released during fermentation in the rumen, but for practical purposes, microbial protein synthesis can be

predicted from metabolizable energy (ME) intake (ARC, 1984). The preferred value of 1.4 g microbial N/MJ of ME intake was adopted by ARC (1984). Calculation of microbial N yield as per Chen et al. (1992), gave the values 9.4, 13.5, 18.8 and 17.3 g microbial N/kg of DDM intake (table 4). This values when converted into per MJ of ME intake gave the values of 0.62, 1.00, 1.44 and 1.21 g microbial N of which the value obtained in ration U2 in present experiment is similar to the preferred value of the ARC (1984) and Liang et al. (1994). The estimated mean values of EMNS in cattle fed ration U2 either expressed as microbial N supply g per kg of DOMR or microbial N supply g per MJ of ME intake were in the range those reported by Agriculture Research Council for a variety of diets (ARC, 1984).

The efficiency of microbial N supply was higher in cattle fed the U2 ration in which the protein : energy ratio was at the level recommended by the protein digested in the intestine (PDI) system (INRA, 1989) than in those fed rations in which the ratios were not balanced (table 2). Whatever the basis for the calculations of EMNS in the present experiment, a significant ($p < 0.01$) increases was always evident in cattle fed ration U2. It is suggested that more work is reguired to determine the influence of the level and sources of RDP on the PD excretion and microbial N supply in local cattle under different feeding regime and physiological condition with available feed resources in Bangladesh.

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