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Short communication

Ruminal dynamics of ad libitum feeding in buffalo bulls receiving different level of rumen degradable protein

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ABSTRACT

Four ruminally cannulated *Nili-Ravi* buffalo bulls were used in a 4×4 Latin Square Design to determine the influence of varying level of ruminally degradable protein (RDP) on dry matter intake (DMI), ruminal characteristics, digestibility, blood pH, blood urea nitrogen (BUN) and nitrogen (N) balance. Four isonitrogenous and isocaloric diets were formulated. The C diet contained 50% RDP while medium, high and very high RDP diets had 66, 82 and 100% RDP of the total crude protein (CP) and were denoted as MRDP, HRDP and VHRDP, respectively. The bulls were fed *ad libitum*. Nutrients intake decreased linearly with increasing the RDP proportion of total dietary CP. A quadratic effect of RDP on ruminal pH was noticed with increasing level of RDP with quadratic maxima at 66% RDP diet. Increasing level of dietary RDP also had a quadratic effect on total bacterial and protozoal count with maximum microbial count at 82% RDP diet. Increasing dietary RDP resulted in linear increase in DM digestibility. However, neutral detergent fiber digestibility was decreased linearly with increasing the level of dietary RDP. A linear increase in ruminal NH_3–N and BUN was noticed due to increasing level of dietary RDP. Higher positive N balance was noticed in bulls fed C diet compared to those fed MRDP, HRDP and VHRDP diets. The findings of this study indicated that buffalo bulls can effectively utilize 13.12% RDP of DM without any adverse effect on rumen and blood parameters.

1. Introduction

Dietary crude protein (CP) is the most costly nutrient and is generally bifurcated into ruminally degradable protein (RDP) and ruminally undegradable protein (RUP). The RDP is considered essential for ruminal microbial growth. This not only improves the ruminal fermentation but also ensures an adequate supply of microbial protein to the host animal which supplies 70 to 80% of the required amino acids to ruminant animal (Chumpawadee et al., 2006).

Nonprotein nitrogen (NPN) can be used as a whole (Hannah et al., 1991) or a part of the supplemental protein to meet the ruminant requirement (Russell et al., 1992) and one of the most effective methods to enhance ruminal microbial protein supplies to the host is an efficient utilization of NPN substances. This makes the ruminant animal production cost-effective through minimizing its RUP needs (Baumann et al., 2004). The RDP causes excessive ammonia (NH_3) production in ruminants when fed in excess. Excessive ammonia absorbed from the rumen increases the synthesis of urea in the liver and increases the urinary nitrogen (N) excretion. Higher blood urea and ruminal ammonia concentrations are considered responsible for decreased feed intake (Fenderson and Bergen, 1974).

Adequate RDP to maximize microbial protein synthesis within the rumen before supplementation of RUP is, therefore, of vital consideration for ruminant nutritionist. Increasing dietary RDP from 8 to 11% of dietary DM increases feed intake, organic matter, hemicellulose digestibility (Griswold et al., 2003) and ruminal fermentation (Davidson et al., 2003). Increasing dietary RDP from 10.6 to 13.2% (on DM) has a positive linear effect on ruminal microbial protein with maximal protein production at 12.3% RDP (Reynal and

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Broderick, 2005). Increased digestibility of fibrous materials due to substantial increase in microbial activity because of RDP has also been reported by Sarwar et al. (2006). Thus, it is important to maximize ruminal microbial protein production through enhancing the utilization of RDP. However, scientific information regarding these effects in buffalo which is reared in tropical and sub-tropical countries is limited. Therefore, the present study was planned to determine the impact of varying level of RDP on DMI, ruminal characteristics, digestibility, blood pH, blood urea nitrogen (BUN) and N balance in buffalo bulls.

2. Materials and methods

Four ruminally cannulated *Nili Ravi* buffalo bulls (412 ± 15 kg) were used in a 4 × 4 Latin Square Design. Four isonitrogenous and isocaloric diets were formulated (NRC, 2001). The control diet contained 50% ruminally degradable protein (RDP). The medium ruminally degradable protein (MRDP), high ruminally degradable protein (HRDP) and very high ruminally degradable protein (VHRDP) diets had 66, 82 and 100% RDP, respectively (Table 1). The bulls were fed *ad libitum*. The bulls were individually housed on concrete floor in separate pens and fed twice a day (morning and evening). The bulls were fed for four periods and each period was of four weeks. The first three weeks of each period served as adaptation time while the fourth week was a collection period. Digestibility was determined by using total collection method (Kauffman and St-Pierre, 2001). For urine collection, special leather bag fitted with plastic pipe were made to surround the prepuce (Nisa et al., 2006). The urine excreted by each animal was acidified with 50% H_2SO_4 and 20% of it was sampled and preserved at −20 °C. In the end of each collection period, the preserved urine samples, after thawing, were composited by animal and 10% of the composited sample was used for analysis.

Table 1
Ingredients and chemical composition of diets (on DM basis).

Ingredients	Diets [1] C	MRDP	HRDP	VHRDP
Wheat straw	30.00	30.00	30.00	29.94
Enzose [a]	6.00	25.88	36.96	60.30
Corn steep liquor	1.5	4.0	15.00	–
Maize bran	45.57	25.84	8.24	–
Corn gluten meal 60%	15.0	11.2	6.76	–
Urea	0.33	1.8	1.54	5.401
Oil	0.10	0.15	–	2.859
Mineral mixture	1.50	1.50	1.50	1.50
Chemical composition				
NE, Mcal/kg	1.52	1.52	1.52	1.52
Organic matter, %	93.80	93.46	92.22	95.05
Crude protein, %	16.10	15.90	15.97	15.87
Neutral detergent fiber, %	47.94	34.0	25.0	19.0
Ash, %	6.20	6.52	7.78	4.95
RDP [2] % of CP	50	66	82	100
RDP, % of DM	8.0	10.56	13.12	16.0
RUP [3], % of DM	8.0	5.44	2.88	0.0
RDP: RUP	50:50	66:34	82:18	100:00
NSC [4]: RDP	4	4	4	4
N: S [5]	10	10	10	10

[a] Enzose, derived from the enzymatic conversion of corn starch, is a byproduct of the corn milling industry which contains 85% dextrose, with a pH of 3.5–4.5. It is a cheap rich source of fermentable carbohydrates (Sarwar et al., 2007).
[1] C, MRDP, HRDP and VHRDP stand for control, medium ruminally degradable protein, high ruminally degradable protein and very high ruminally degradable protein, respectively.
[2] Ruminally degradable protein.
[3] Ruminally undegradable protein.
[4] Nonstructural carbohydrates.
[5] Nitrogen to Sulpher ratio.

Ruminal samples were taken from four different locations in the rumen at 3, 6, 9 and 12 h after morning feeding for determination of pH, NH_3–N, bacterial and protozoal population. Ruminal pH was measured immediately after the sampling using a portable pH meter (Hanna HI 8314, Hanna industries, Romania). The sample thereafter was squeezed through 4 layers of cheesecloth and about 50 mL of it was acidified with 3 mL of 6 N HCl to terminate the fermentation and was frozen. Ruminal NH_3–N was steam distilled using kjeldahl equipment and titrated against sulphuric acid (Giri et al., 2005).

Rumen liquor was collected in sterilized plastic bottles at 3, 6, 9 and 12 h post feeding for the determination of microbial count according to the procedure as described by modified technique of Knaysi and Ford (Knaysi and Ford, 1983). Blood samples were collected from the jugular vein of each animal in heparinized syringes at 3, 6, 9 and 12 h post feeding to determine its pH (AOAC, 1990). Blood urea concentration was determined following the Berthelot method. Feed offered and fecal samples were analyzed for dry matter (DM), organic matter (OM), nitrogen (N) content (AOAC, 1990) and neutral detergent fiber (NDF; Van Soest et al., 1991).

The data collected on ruminal pH, ruminal ammonia N, bacterial count, protozoal count, digestibility, blood pH, BUN and N balance was subjected to ANOVA using general linear model procedure of SPSS (SPSS, 1999).

3. Results

A linear decrease in nutrient intake by increasing the RDP level of diet was observed in bulls. Maximum and minimum DMI was noticed in bulls fed C and VHRDP diets, respectively (Table 2). Other nutrients also followed the similar trend. The DM digestibility increased with increasing the RDP level of diet. Maximum and minimum DM digestibility was observed in bulls fed VHRDP and C diets, respectively. However, DM digestibility in bulls fed HRDP and VHRDP diets remained non-significant (Table 2). Increasing the dietary RDP level resulted in linear decrease ($P<0.01$) in N balance (Table 2). Increasing dietary RDP level resulted in linear decrease ($P<0.01$) in fecal and urinary N.

Ruminal pH remained highest ($P<0.05$) in bulls fed MRDP diet and it was lowest in bulls fed VHRDP diet (Table 3). At 12 h post feeding, the ruminal pH was higher ($P<0.05$) in bulls fed MRDP and HRDP diets than those fed C and VHRDP diets. Increasing dietary RDP level resulted in quadratic decrease ($P<0.01$) in ruminal pH with quadratic maxima at 66% RDP across all time periods. A linear decrease ($P<0.05$) in ruminal pH was also noticed at 3, 6 and 9 h post feeding. Ruminal NH_3–N concentration in bulls fed HRDP and VHRDP diets was higher ($P<0.05$) than those fed C and MRDP diets

Table 2
Nutrient intake, digestibility and N balance in buffalo bulls fed diets containing different level of rumen degradable protein.

Parameters	Diets [1] C	MRDP	HRDP	VHRDP	SE	L [2]	Q [3]
Intake, kg/d							
Dry matter	8.16[a]	8.0[a]	7.60[a]	6.61 [b]	0.18	0.001	0.076
Crude protein	1.313[a]	1.272[a]	1.214[a]	1.049 [b]	0.030	0.001	0.096
Neutral detergent fiber	3.91[a]	2.72[b]	1.90[c]	1.26 [d]	0.26	0.001	0.012
Digestibility, %							
Dry matter	67.10[c]	70.09[bc]	75.63[ab]	78.66 [a]	1.5	0.001	0.992
Crude protein	73.32	73.17	74.75	75.85	0.43	0.015	0.409
Neutral detergent fiber	59.0[a]	52.92[b]	47.9 [b]	41.22 [c]	1.84	0.001	0.859
Nitrogen balance							
N Intake, g/d	210.07[a]	203.46[a]	194.2 [a]	167.84[b]	4.81	0.001	0.096
Fecal N , g/d	55.72 [a]	54.53 [b]	48.96 [c]	40.52 [d]	1.81	0.001	0.001
Urinary N, g/d	119.53 [a]	116.19 [b]	115.03 [b]	111.16 [c]	0.92	0.001	0.579
N Balance, g/d	34.82 [a]	32.74 [b]	30.21 [c]	16.16 [d]	2.21	0.001	0.001

Means within row with different superscripts differ ($P<0.05$).
[1] C, MRDP, HRDP and VHRDP stand for control, medium ruminally degradable protein, high ruminally degradable protein and very high ruminally degradable protein, respectively. The RDP:RUP in C, MRDP, HRDP and VHRDP diets was 50:50, 66:34, 82:18 and 100:00, respectively.
[2] p value for linear effect.
[3] p value for quadratic effect.

across all time periods (Table 3). Total bacterial count (TBC) remained highest (P<0.05) in bulls fed HRDP diet compared to those fed C, MRDP and VHRDP diets at 3, 6 and 9 h post feeding. There was non-significant difference in TBC in bulls fed MRDP and HRDP. However, at 12 h post feeding, it was higher (P<0.05) than those fed C and VHRDP diets (Table 3). Level of dietary RDP had a quadratic increase (P<0.01) in TBC with quadratic maxima at 82% RDP across all time periods. Linear increase (P<0.05) in TBC was also noticed at 3, 6 and 9 h post feeding. Protozoal count in bulls fed C diet was higher (P<0.05) than those fed VHRDP diet. However, it was not significantly different from bulls fed MRDP and HRDP diets at 3 and 12 h post feeding. A linear increase (P<0.05) in protozoal count was also noticed with increasing dietary RDP level at 3 and 12 h post feeding (Table 3). Level of RDP had a quadratic (P<0.05) effect on protozoal count at 9 and 12 h post feeding.

Increasing level of dietary RDP had no effect on blood pH at 3 and 6 h post feeding. Blood pH was lower in bulls fed C diet than those fed MRDP, HRDP and VHRDP diets at 9 h post feeding. However, it was lower than those fed HRDP and VHRDP diets and was similar to those fed MRDP diet at 12 h post feeding (Table 4). Linear increase (P<0.05) in blood pH was noticed with increasing the dietary RDP level. Increasing dietary RDP level resulted in linear increase (P<0.05) in BUN concentrations (Table 4).

Table 3
Ruminal pH, NH_3–N, bacterial ($\times 10^{10}$cells/ml) and protozoal ($\times 10^{6}$cells/ml) counts in buffalo bulls fed diets containing different level of rumen degradable protein.

	Diets [1] C	MRDP	HRDP	VHRDP	SE	L [2]	Q [3]
3 h							
pH	6.52 [b]	6.60 [a]	6.51 [b]	6.47 [c]	0.015	0.001	0.001
NH_3–N	26.07 [b]	27.77 [b]	37.4 [a]	39.1 [a]	2.02	0.002	1.0
Bacterial	17.0 [c]	31.35 [b]	40.0 [a]	13.24 [c]	4.73	0.001	0.001
Protozoal	6.1 [a]	6.3 [a]	6.9 [a]	4.5 [b]	0.41	0.011	0.664
6 h							
pH	6.55 [b]	6.68 [a]	6.52 [b]	6.04 [c]	0.074	0.001	0.001
NH_3–N	24.37 [b]	25.27 [b]	36.17 [a]	42.5 [a]	2.75	0.004	0.47
Bacterial	12.1 [c]	71.67 [b]	77.55 [a]	6.48 [c]	10.73	0.001	0.001
Protozoal	5.93 [b]	5.67 [b]	6.7 [a]	5.5 [b]	0.18	0.219	0.31
9 h							
pH	6.63 [b]	6.74 [a]	6.67 [b]	6.55 [c]	0.023	0.013	0.001
NH_3–N	19.83 [b]	21.5 [b]	30.6 [a]	36.5 [a]	2.3	0.001	0.414
Bacterial	43.0 [c]	91.8 [b]	130.67[a]	10.5 [c]	17.21	0.015	0.001
Protozoal	6.63 [b]	6.3 [b]	8.8 [a]	6.0 [b]	0.37	0.742	0.014
12 h							
pH	6.72 [b]	6.86 [a]	6.85 [a]	6.69 [b]	0.024	0.301	0.001
NH_3–N	18.37 [b]	19.67 [b]	27.77 [a]	30.6 [a]	2.02	0.01	0.803
Bacterial	14.0 [b]	58.6 [a]	58.88 [a]	2.1 [b]	8.7	0.188	0.001
Protozoal	7.7 [a]	7.1 [a]	6.8 [a]	4.1 [b]	0.43	0.001	0.005

Means within row with different superscripts differ ($P<0.05$).
[1] C, MRDP, HRDP and VHRDP stand for control, medium ruminally degradable protein, high ruminally degradable protein and very high ruminally degradable protein, respectively. The RDP:RUP in C, MRDP, HRDP and VHRDP diets was 50:50, 66:34, 82:18 and 100:00, respectively.
[2] p value for linear effect.
[3] p value for quadratic effect.

4. Discussion

Increased nutrients intake by buffaloes fed C, MRDP and HRDP diets compared to those fed on VHRDP diet might be attributed to the adequate RDP supply of this diet. This might have optimized rumen microbial proliferation by ensuring the optimal ruminal ammonia nitrogen rather than excessive ammonia (Table 3). Optimum rumen ammonia nitrogen has also been reported to increase feed intake by producing more enzymes per unit of time in ruminant animals. Rumen microbe synthesis or multiplication is very much dependent on adequate supply of nitrogen and carbon skeleton at ruminal level. The C, MRDP and HRDP diets might have favored optimum rumen microbial growth by providing corn

Table 4
Blood pH and blood urea nitrogen (mg/dL) in buffalo bulls fed diets containing different level of rumen degradable protein.

	Diets [1]						
	C	MRDP	HRDP	VHRDP	SE	L [2]	Q [3]
3 h							
pH	7.49	7.55	7.56	7.57	0.013	0.048	0.405
BUN [4]	21.68 ^{bc}	20.45 ^{c}	24.48 ^{b}	33.28 ^{a}	1.58	0.001	0.002
6 h							
pH	7.54	7.57	7.60	7.63	0.019	0.03	1.0
BUN	26.91 ^{b}	25.81 ^{b}	27.99 ^{b}	35.14 ^{a}	1.32	0.008	0.042
9 h							
pH	7.56 ^{b}	7.62 ^{a}	7.63 ^{a}	7.65 ^{a}	0.012	0.01	0.14
BUN	27.3 ^{b}	26.67 ^{b}	28.37 ^{b}	33.55 ^{a}	1.06	0.007	0.04
12 h							
pH	7.59 ^{b}	7.63 ^{ab}	7.66 ^{a}	7.69 ^{a}	0.013	0.001	0.59
BUN	22.82 ^{b}	25.96 ^{b}	26.09 ^{b}	33.29 ^{a}	1.3	0.001	0.176

Means within row with different superscripts differ ($P<0.05$).
[1] C, MRDP, HRDP and VHRDP stand for control, medium ruminally degradable protein, high ruminally degradable protein and very high ruminally degradable protein, respectively. The RDP:RUP in C, MRDP, HRDP and VHRDP diet was 50:50, 66:34, 82:18 and 100:00, respectively.
[2] p value for linear effect.
[3] p value for quadratic effect.
[4] Blood urea nitrogen.

steep liquor and enzose as precursors of nitrogen and carbon skeleton, respectively (Table 3). This might have increased feed intake. Haddad et al. (2005) also reported increased feed intake due to increased population of rumen microbes. Decreased DMI by bulls fed VHRDP diet was due to very high degradable protein portion of this diet. Rapid degradation of this protein portion might have overcome the capacity of rumen microbes to efficiently capture all the ruminal ammonia nitrogen for their growth and thus sufficient ammonia nitrogen might have escaped from the rumen as indicated by increased serum BUN concentration (Table 4). Reduced feed consumption by cows fed diet containing high degradable N has also been reported by Wilson et al. (1975). Likewise, Chaturvedi and Walli (2001) also reported linear decrease in DMI in early lactating crossbred cows with increased RDP level. Moreover, protein supplements especially those with high in RUP stimulate DMI to greater extent than diets containing highly soluble N in the form of urea (Kalbande and Chainpure, 2001). Fenderson and Bergen (1974) also reported decreased DMI with increased ruminal ammonia and plasma urea concentrations in steers fed excessive dietary N either natural protein or nonprotein nitrogen.

Increased DM digestibility with increasing the RDP level might be attributed to readily RDP degradation which not only increased rumen ammonia concentration but also facilitated the proliferation of ruminal bacterial population (Table 3). Higher ruminal bacterial population might have increased enzymes production per unit of time which might have increased DM digestibility. Decreased NDF digestibility in bulls fed VHRDP diet might be attributed to low dietary intake of NDF which might have increased its rumen retention time and thus digestibility. Highest N balance in bulls fed C diet was due to its low RDP level (50%). Higher N retention in crossbred calves fed low RDP diet than those fed high RDP diet was reported by Pattanaik et al. (2003). Linear decrease in fecal and urinary N was due to linear decrease in N intake. Similarly, Paengkoum et al. (2004) also reported linear decrease in fecal N (g/d) when dietary RDP increased from 94 to 100%.

The lowest ruminal pH in bulls fed VHRDP diet might be attributed to its higher enzose content, a readily fermentable carbohydrate source. Moreover, enzose is acidic in nature and its pH ranges from 3.5–4.0. Similar results were reported by Baumann et al. (2004) who explained that the ruminal pH was decreased in steers fed corn grain based diets containing high level of RDP. A linear increase ($P<0.05$) in ruminal NH_3–N concentration was due to increasing dietary RDP level. Increased ruminal ammonia N was due to readily degradation of RDP in the rumen. A positive co-relation between ruminal NH_3–N and RDP has been documented by Kung et al. (1983) who reported 12.7, 19.0, 25.1 and 18.7 mg/dL ammonia concentration at 2 h post feeding in lactating cows fed 55.0, 58.8, 63.3 and 66.0% RDP, respectively.

Increased microbial population in bulls fed C, MRDP & HRDP diets with increased dietary RDP may be due to increased ruminal ammonia (Table 3). In the present study, ruminal NH_3–N level ranged from 18.37 to 42.50 mg/dL. Orskov (1992) reported maximum microbial count when ruminal NH_3–N level ranged from 10 to 25 mg/dL. Increased bacterial mass with increasing dietary RDP (38 to 58%) has been reported by Fu et al. (2001) in crossbred steers. Lowest TBC in bulls fed VHRDP diet might be attributed to inadequate supply of peptide and amino acid for their multiplication as about 100% dietary N of this diet was coming from urea (Russell et al., 1992). It is also documented that some of the amino acids may also limit the ruminal bacterial growth. Moreover, in the present study, VHRDP diet had about 60% non-structural carbohydrates and ruminal bacteria responsible to ferment this type of diet need 66% N from peptides and amino acids and 34% N from ammonia for their multiplication (Russell et al., 1983). Low concentration of peptides and amino acids could potentially limit microbial growth when feed was rich in non-structural carbohydrates. Microbial growth was improved when urea or ammonia as the sole or major source of N was replaced with peptide or amino acids. Meng et al. (2000) stated that TBC was lower in 100% urea base diet than those in which urea base RDP was replaced with 30 or 70% soybean in continuous culture fermenters. Lowest protozoal count in bulls fed VHRDP diet than those fed C, MRDP and HRDP diets was due to urea base RDP which was deficient in natural protein. Meng et al. (2000) reported that protozoal count was lower (0.4×10^3 cells/mL) when total RDP was supplied from urea compared with when urea base RDP was replaced with 30 or 70% soybean (3.0×10^3 or 4.8×10^3 cells/mL) in a continuous culture fermenters.

The plausible reason for linear increase in blood pH with increasing level of dietary RDP might be attributed to increasing concentration of absorbed ruminal ammonia and BUN. The linear increase in BUN with increasing the level of dietary RDP was due to increased ruminal NH_3–N concentration because BUN concentrations were highly correlated ($r=94$) with ruminal NH_3–N. Similarly, Chumpawadee et al. (2006) reported that an increase in ruminal NH_3–N concentrations increased BUN concentration. Highest BUN concentration (35.14 mg/dL) was recorded in bulls fed VHRDP diet which was purely urea base. Slightly higher BUN in animals fed C diet than those fed MRDP diet was due to higher RUP intake. This might have resulted by supplying more quantity of amino acids, which might be used as energy and ammonia from deamination of these amino acids and it might had resulted in higher BUN.

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