Asian-Aust. J. Anim. Sci.
Vol. 21, No. 1 : 51 - 58
January 2008

www.ajas.info

Ruminal Characteristics, Blood pH, Blood Urea Nitrogen and Nitrogen Balance in *Nili-ravi* Buffalo (*Bubalus bubalis*) Bulls Fed Diets Containing Various Levels of Ruminally 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 levels of ruminally degradable protein (RDP) on ruminal characteristics, digestibility, blood pH, blood urea nitrogen (BUN) and nitrogen (N) balance. Four isonitrogenous and isocaloric diets were formulated (NRC, 2001). The control diet contained 50% RDP. The medium (MRDP), high (HRDP) and very high (VHRDP) ruminally degradable protein diets had 66, 82 and 100% RDP, respectively. Increasing the level of dietary RDP resulted in a linear decrease in ruminal pH. A quadratic effect of RDP on ruminal pH was also observed with quadratic maxima at the 66% RDP diet. Dietary RDP had a quadratic effect on total bacterial and protozoal count with maximum microbial count at the 82% RDP diet. Increased microbial count was due to increasing level of ruminal ammonia nitrogen (NH_3-N). Increasing dietary RDP resulted in a linear increase in dry matter digestibility. Provision of an adequate amount of RDP caused optimum microbial activity, which resulted in improvement in DM digestibility. Increasing the level of dietary RDP resulted in a linear decrease in crude protein (CP) and neutral detergent fiber digestibility. Blood pH remained unaltered across all diets. A linear increase in ruminal NH_3-N and BUN was noted with increasing level of dietary RDP. The increase in BUN was due to increased ruminal NH_3-N concentrations. A positive N balance was noted across all diets. The results are interpreted to suggest that buffalo bulls can utilize up to 82% RDP of total CP (16%) with optimum results. (**Key Words :** Buffalo Bull, Ruminal Characteristics, BUN, RDP, Blood pH, N Balance)

INTRODUCTION

Ruminal degradable protein (RDP) is considered essential for ruminal microbial growth. This not only improves the ruminal fermentation but it also ensures an adequate supply of microbial protein to the host animal. The ultimate goal of proper rumen nutrition is to maximise the microbial growth and the amount of RDP that is converted into ruminal microbial cells. Maximising the growth of ruminal microbes not only improves the supply of amino acids to the small intestine but also improves the nitrogen (N) utilization. Microbial protein generally supplies 70 to 80% of the required amino acids to ruminants (Chumpawadee et al., 2006) and that is why ruminant nutritionists are trying to maximize ruminal microbial protein production from non-protein sources. One of the most effective methods to enhance ruminal microbial protein supply to the host is an efficient utilization of non-protein nitrogen substances (Sarwar et al., 2004). This makes the ruminant animal production cost-effective through minimizing its ruminally undegradable protein (RUP) needs (Blummel et al., 1999).

Optimum utilization of dietary crude protein (CP) requires selection of complementary feed protein sources, which provide the types and amounts of RDP that will optimize the N requirement of rumen microbes (NRC, 2001). According to NRC (1989), 9.6% RDP of dietary dry matter (DM) is sufficient to meet the needs of ruminal microbes for ruminal microbial protein synthesis. Increasing dietary RDP from 8 to 11% of dietary DM improves feed intake, organic matter (OM), hemicellulose digestibility (Griswold et al., 2003) and ruminal fermentation (Davidson et al., 2003). Increasing dietary RDP from 10.6 to 13.2% (of DM) has a positive linear effect on microbial protein with maximal protein production at 12.3% RDP (Reynal and Broderick, 2005). Another report also confirmed the linear effect of RDP on microbial growth (Hoover and Stokes, 1991). The substantial microbial activity improvement due to RDP, increases the

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Received January 14, 2007; Accepted May 13, 2007

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

Ingredients	Control	MRDP [1]	HRDP [2]	VHRDP [3]
Wheat straw	30.00	30.00	30.00	29.94
Enzose	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
Minerals	1.50	1.50	1.50	1.50
Chemical composition				
NE (M cal/kg)	1.52	1.52	1.52	1.52
OM [4]	93.80	93.46	92.22	95.05
CP [5] (%)	16.10	15.90	15.97	15.87
NDF [6]	47.94	34.0	25.0	19.0
Ash	6.20	6.52	7.78	4.95
RDP, % of CP	50	66	82	100
RDP, % of DM	8.0	10.56	13.12	16.0
RUP, % of DM	8.0	5.44	2.88	0.0
RDP:RUP ratio	50:50	66:34	82:18	100:00
NSC [7]:RDP	4	4	4	4
N:S	10	10	10	10

[1] MRDP = Medium ruminally degradable protein. [2] HRDP = High ruminally degradable protein.
[3] VHRDP = Very high ruminally degradable protein. [4] Organic matter.
[5] Crude protein. [6] Neutral detergent fiber. [7] Nonstructural carbohydrates.

digestibility of fibrous materials (Sarwar et al., 2006). On the other hand, feeding RDP below the requirements for efficient rumen microbial growth can compromise microbial protein production, ruminal digestion, energy and protein availability (Reynal and Broderick, 2005). Adequate RDP to maximize microbial protein synthesis within the rumen before supplementation of RUP is, therefore, of great significance.

Urea can be used as all (Hannon and Trenkle, 1990) or a part of the supplemental protein to meet the dairy cow protein requirement (Russell et al., 1992). It is documented that exotic lactating cows perform equally well when urea contributes up to 12% RDP of the ration (Gould, 1969). However, scientific information regarding this effect in buffalo is limited. Therefore, the present study was planned to determine the impact of varying level of RDP on ruminal characteristics, digestibility, blood pH, blood urea nitrogen (BUN) and N balance in buffalo bulls.

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 (C) 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. Fertilizer grade urea was used to attain this desired RDP (Table 1). The bulls were fed at the rate of 1.5% of their body weight. The bulls were individually housed on a concrete floor in separate pens and fed twice a day (morning and evening). Fresh water availability was ensured round the clock during the experimental period. The bulls were fed for four periods and each period was of five weeks. The first four weeks of each period served as adaptation time while the fifth week was a collection period. Feed offered was weighed and recorded daily during each collection period.

Digestibility was determined by using a total collection method. During collection periods, complete collections of urine and feces were made according to the procedure described by Nisa et al. (2006). The feces of each animal were collected daily, weighed, mixed thoroughly and 20% of it was sampled and dried at 55°C. At the end of each collection period, dried fecal samples were composited and 10% of the composited samples were taken for analysis. For urine collection, special leather bags fitted with plastic pipe were made to surround the prepuce (Nisa et al., 2006). The plastic pipe ended in a large container (30 L). 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.

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 populations. Ruminal pH was measured

Table 2. Nutrient intake and digestibility coefficient in buffalo bulls fed diets containing different level of RDP.

	Control	MRDP	HRDP	VHRDP	SE	L[1]	Q[2]
Intake (kg/d)							
DM	6.18	6.18	6.18	6.18	0.009	1.0	1.0
CP	0.995	0.983	0.987	0.981	0.0025	0.084	0.506
NDF	2.963[a]	2.101[b]	1.545[c]	1.174[d]	0.203	0.001	0.001
Digestibility (%)							
DM	69.80[c]	71.18[c]	75.80[b]	80.28[a]	1.29	0.001	0.098
CP	76.84[a]	75.57[a]	73.81[b]	73.65[b]	0.42	0.001	0.095
NDF	64.37[a]	51.30[b]	47.21[c]	40.24[d]	2.70	0.001	0.036

[a, b, c] Means within row with different superscripts differ ($p<0.05$).
[1] p value for linear effect. [2] p value for quadratic effect.

immediately after 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 counts. The bacterial counts were determined with modified technique of Knaysi and Ford (1938). The 5 mL of strained rumen fluid was added with 5 ml of 10% formaline to kill bacteria in a test tube and then 2 ml out of this was added with 8 ml of distilled water (10 times dilution). From the final dilution, 0.01 ml fluid was transferred to a thoroughly cleaned slide upon which an area of 1 cm^2 had been previously marked. The sample was spread evenly over the marked area and air dried and fixed by passing over the flame. It was stained with Gram's stain. The counts were made from five microscopic fields and calculations were made according to the following equation.

$$\text{TBC/ml of rumen liquor} = N \times DF \times MF \times 100$$

TBA stands for total bacterial count, N for average number of bacteria counted per field, DF for dilution factor and MF for microscopic factor. The MF of the microscope used in present study was 576.6.

The protozoal counts were determined at 3, 6, 9 and 12 h post feeding. One ml of strained rumen fluid was added with 9 ml of lugal solution (10 g KCl, 30 ml glycerol, 5 g iodine, 10 ml formalin and 60 ml distil water) to fix the protozoa. From final dilution, 0.1 ml fluid was transferred to a slide and covered with a cover slip of 24×50 cm^2. The counts were made from thirty microscopic fields and calculations were made according to following equation.

$$\text{Protozoa/ml of rumen liquor} = N \times DF \times MF \times 10$$

Where N is average number of protozoa counted per field, DF is dilution factor and MF is the microscopic factor.

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 samples were also collected from each bull without anticoagulant to harvest blood serum which was stored in aliquots at -20°C awaiting analysis for biochemical parameters. Blood urea concentration was determined photometrically using the analytical kit (Cat # CS 612, Crescent Diagnostics, Saudi Arabia) 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).

Statistical analysis

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 10.0.1., 1999).

RESULTS AND DISCUSSION

Dry matter and CP intakes remained unaltered because bulls were fed restricted diets. However, NDF intake was highest (2.963 kg) in bulls fed C diet and was lowest (1.174 kg) in bulls fed VHRDP diet (Table 2). A linear decrease ($p<0.001$) in NDF intake was due to its reduced dietary contents (Table 1). Ruminal pH remained unaltered in bulls fed C and MRDP diets but it was lower ($p<0.05$) in animals fed VHRDP diets than those fed HRDP diets across all time periods (Table 3). Increasing dietary RDP level resulted in linear ($p<0.01$) decrease in ruminal pH. A quadratic ($p<0.01$) effect of RDP level on ruminal pH was also observed with quadratic maxima at 66% RDP diet. The reduced ruminal pH in bulls fed HRDP and VHRDP diets might be attributed to their higher enzose (Sarwar et al., 2005), a readily fermentable carbohydrate source. Similar results were reported by Baumann et al. (2004) who explained that the ruminal pH was decreased ($p<0.01$) when RDP was added to corn grain based diets in steers. Similar findings were reported by Nisa et al. (2005).

Ruminal NH_3-N concentrations were different ($p<0.05$) across all treatments at 3 h post feeding (Table 3). However,

Table 3. Ruminal pH and ruminal NH_3-N in buffalo bulls fed diets containing different level of RDP

	Control	MRDP	HRDP	VHRDP	SE	L[1]	Q[2]
3 h							
pH	6.81[a]	6.82[a]	6.64[b]	6.43[c]	0.049	0.001	0.003
NH_3-N	22.73[d]	29.33[c]	37.18[b]	46.30[a]	2.39	0.001	0.719
6 h							
pH	6.80[a]	6.85[a]	6.73[b]	6.49[c]	0.041	0.001	0.001
NH_3-N	23.8[c]	27.67[b]	29.07[b]	37.67[a]	1.57	0.001	0.021
9 h							
pH	6.94[a]	6.97[a]	6.90[a]	6.46[b]	0.064	0.001	0.001
NH_3-N	18.00[c]	21.16[bc]	25.90[b]	44.00[a]	3.21	0.001	0.011
12 h							
pH	7.03[a]	7.06[a]	7.00[a]	6.64[b]	0.054	0.001	0.001
NH_3-N	16.67[b]	18.11[b]	18.67[b]	35.92[a]	2.42	0.001	0.001

[a, b, c, d] Means within row with different superscripts differ ($p<0.05$).
[1] p value for linear effect. [2] p value for quadratic effect.

Table 4. Total bacterial count ($\times 10^{10}$cells/ml) and Protozoal count ($\times 10^{6}$ cells/ml) in buffalo bulls fed diets containing different level of RDP

	Control	MRDP	HRDP	VHRDP	SE	L[1]	Q[2]
3 h							
Bacteria	6.53[bc]	10.12[b]	40[a]	1.11[c]	4.61	0.107	0.001
Protozoa	1.38[b]	2.58[a]	3.05[a]	0.75[b]	0.26	0.20	0.001
6 h							
Bacteria	42.23[c]	52.77[b]	153.41[a]	6.5[d]	16.46	0.326	0.001
Protozoa	0.75[b]	2.10[a]	1.88[a]	0.47[b]	0.20	0.254	0.001
9 h							
Bacterial	91.74[c]	150.0[b]	183.45[a]	60.49[c]	14.54	0.001	0.001
Protozoa	1.24[b]	1.38[b]	3.01[a]	0.31[c]	0.28	0.275	0.001
12 h							
Bacterial	4.48[b]	5.81[b]	7.87[a]	0.74[c]	0.80	0.001	0.001
Protozoa	0.93[b]	1.29[a]	1.56[a]	0.44[c]	0.12	0.032	0.001

[a, b, c, d] Means within row with different superscripts differ ($p<0.05$).
[1] p value for linear effect. [2] p value for quadratic effect.

it remained highest in bull fed VHRDP diets at 6, 9 and 12 h post feeding. A linear ($p<0.01$) increase in ruminal NH_3-N concentrations was noticed due to increasing dietary RDP level. Increased ruminal NH_3-N concentration with increased dietary RDP has also been reported by Roffler and Satter (1975). 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. Fu et al. (2001) also reported increased ruminal NH_3-N concentrations with increasing the dietary RDP level. Similar findings were reported by Lee et al. (2001) in goats.

Total bacterial count (TBC) remained highest in bulls fed HRDP diet compared to those fed C, MRDP and VHRDP diets at all time periods (Table 4). Level of dietary RDP had a quadratic effect ($p<0.01$) on TBC with quadratic maxima at 82% RDP. Linear increase ($p<0.01$) in TBC was noticed in bulls fed C, MRDP and HRDP at 9 and 12 h post feeding. In present study, ruminal ammonia level ranged from 16.67 to 46.30 mg/dl. Maximum microbial count was reported when ruminal ammonia level ranged from 10 to 25 mg/100 ml (Leng, 1990; Orskov, 1992). Similarly, Wanapat and Pimpa (1999) reported that a ruminal NH_3-N concentration higher than 13.6 mg/dl in swamp buffaloes was considered optimum. Similar findings have been reported by Fu et al. (2001) who reported increased bacterial mass when dietary RDP was increased from 38 to 58% in crossbred steers. Hoover and Stokes (1991) also reported increased microbial growth with increased dietary RDP level in an *in vitro* experiment. The increased microbial population in bulls fed diets with increased dietary RDP may be due to increased ruminal ammonia level in present study (Table 2). Suwanlee and Wanapat (1994) reported increased ruminal bacterial count as ruminal NH_3-N increased from 1.7 to 5.6 mg/dl. In present study, maximum microbial count was observed with increasing the concentration of ruminal NH_3-N, however, microbial count decreased when ruminal NH_3-N level exceeded beyond 37.18 mg/dl. The findings of the present study are more consistent with those of Wanapat and Pimpa (1999) who reported 1.4×10^8, 1.7×10^8, 2.6×10^8, 3.7×10^8 and

1.5×10^8 cells/ml when ruminal NH_3-N concentrations were 7.1, 8.8, 13.6, 17.6 and 34.4 mg/dl, respectively, in dairy cows. Thus, the bacterial count increased with increasing NH_3-N concentration up to 17.6 mg/dl, however, it reduced when ruminal ammonia concentration was increased to 34.4 mg/dl. Increased bacterial population with increased NH_3-N concentration has also been reported in swamp buffaloes by Pimpa et al. (1996). Lowest TBC was noticed in bulls fed VHRDP diet which had about 100% N from urea and thus it was deficient in peptides and amino acids. Non-structural carbohydrates fermenting bacteria require adequate supply of peptide and amino acid N in addition to ammonia N for their multiplication (Russell et al., 1992). It is also documented that some of the amino acids may also limit ruminal bacterial growth (Atasoglu et al., 2004). 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). Likewise, Bach et al. (2005) also indicated that low concentration of peptides and amino acids could potentially limit microbial growth when feeding rations 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 (Griswold et al., 1996; Meng et al., 2000). Meng et al. (2000) stated that TBC was lower (7.5×10^9 cells/ml) in 100 % urea base diet than those in which urea was replaced with 30 or 70% soybean RDP (10.4×10^9 or 11.2×10^9 cells/ml) in a continuous culture fermenters.

On dry matter basis, C, MRDP, HRDP and VHRDP diets had 8.0, 10.56, 13.12 and 16.0% RDP, respectively, (Table 1). According to NRC (1989) 9.6% RDP of dietary DM is sufficient to meet the needs of ruminal microbes for protein synthesis. Dhiman and Satter (1997) and Fu et al. (1999) also stated that diet containing 9.3 and 9.4% RDP (DM basis) supplied sufficient N precursors to support maximum microbial growth in cows. In the present study, the C diet had 8% RDP and was insufficient to fulfill the microbial need for microbial protein synthesis. However, bulls fed diets containing 10.56 (MRDP) or 13.12% (HRDP) of total DM supported greater microbial count than that of C diet (Kalscheur et al., 2006). These results of the present study support the findings of Stokes et al. (1991) who reported that cows fed diets containing 11.8 and 13.7% RDP of DM supported greater microbial protein synthesis than those fed diets containing 9% RDP of DM.

Protozoal count in bulls fed C and VHRDP diets was lower ($p<0.05$) than those fed MRDP and HRDP diets at 3 and 6 h post feeding (Table 4). However, there was no difference between MRDP and HRDP diets. The protozoal count in bulls fed HRDP diet remained highest across all time periods, whereas it was lowest in bulls fed VHRDP diet. Level of RDP had a quadratic ($p<0.01$) effect on protozoal count with quadratic maxima at 82% RDP (HRDP) across all time. A linear increase ($p<0.05$) in protozoal count was also observed when dietary RDP was increased from 50 to 82% at 12 h post feeding. The increased protozoal count in bulls fed HRDP diet was due to higher level of dietary RDP than those fed C diet. Hoover and Stokes (1991) also reported increased microbial growth with increased dietary RDP level in *in vitro*. Meng et al. (2000) reported that protozoal count was lower (0.4×10^3 cells/ml) when total RDP was supplied from urea compared 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. Lower protozoal count in bulls fed C diet might be attributed to its lower level of dietary RDP and enzose than those fed MRDP diet. Moreover, higher microbial count in bulls fed HRDP diet may also be attributed to ready availability of carbon and nitrogen skeletons from enzose and RDP, respectively (Hoover and Stokes, 1991). Decreased ruminal microbial count with slowly degradable carbohydrate compared to readily degradable carbohydrates source with same RDP level is also well documented (Sarwar et al., 1991; 1992; Fu et al., 2001).

Dry matter digestibility was lower ($p<0.05$) in bulls fed C and MRDP diets than those fed HRDP and VHRDP diets. However, it was higher ($p<0.05$) in bulls fed VHRDP than those fed HRDP diet. There was no difference in DM digestibility between C and MRDP diets (Table 2). Increased DM digestibility in bulls fed HRDP and VHRDP diets might be attributed to the fact that high DM proportion of these diets was composed of readily fermentable substrates (enzose and urea) which might have increased the fermentation rate and thus digestibility. Davidson et al. (2003) reported higher rumen fermentation rate in cows fed high RDP than those fed low RDP level of diet. Increase in total microbial count associated with increasing RDP contributed to increased DM digestibility. Griswold et al. (2003) also reported that increasing dietary RDP improved DM digestibility in cows. In the present study, increasing trend in DM digestibility with increased RDP level might be because of increased fermentation rate as indicated by high ruminal NH_3-N (Table 3) and microbial count (Table 4).

Crude protein digestibility was higher ($p<0.05$) in bulls fed C and MRDP diets than those fed HRDP and VHRDP diets (Table 2). Increasing the level of dietary RDP resulted in a linear ($p<0.01$) decrease in CP digestibility. Kumar et al. (2006) also reported that increasing dietary RDP level from 52 to 59%, decreased the CP digestibility from 58.23 to 57.34%. Similarly Wright et al. (1998) and Pangkoum et al. (2004) reported reduced CP digestibility when dietary RDP increased.

The NDF digestibility was significantly different across

Table 5. Blood pH and blood urea nitrogen (BUN) in buffalo bulls fed diets containing different level of RDP

	Control	MRDP	HRDP	VHRDP	SE	L[1]	Q[2]
3 h							
pH	7.62	7.65	7.65	7.67	0.008	0.042	0.582
BUN	19.42 [c]	22.77 [bc]	25.97 [b]	35.43 [a]	1.63	0.001	0.024
6 h							
pH	7.58	7.59	7.60	7.63	0.009	0.05	0.538
BUN	24.73 [b]	26.37 [b]	28.59 [b]	33.07 [a]	1.02	0.001	0.327
9 h							
pH	7.58	7.59	7.60	7.60	0.009	0.266	1.0
BUN	23.01 [b]	25.66 [b]	25.69 [b]	31.29 [a]	0.89	0.001	0.151
12 h							
pH	7.61	7.63	7.66	7.71	0.018	0.047	0.621
BUN	21.82 [b]	23.78 [b]	24.03 [b]	32.58 [a]	1.31	0.001	0.076

[a, b, c, d] Means within row with different superscripts differ ($p<0.05$).

[1] p value for linear effect. [2] p value for quadratic effect.

Table 6. Nitrogen balance in buffalo bulls fed diets containing different level of RDP

	Control	MRDP	HRDP	VHRDP	SE	L[1]	Q[2]
N intake (g/d)	159.22	157.22	157.91	156.92	0.50	0.206	0.634
Fecal N (g/d)	36.87 [b]	38.42 [ab]	41.36 [a]	41.36 [a]	0.70	0.004	0.416
Urinary N (g/d)	98.95 [c]	99.68 [bc]	101.51 [b]	103.73 [a]	0.62	0.001	0.282
N balance	23.38 [a]	19.12 [b]	15.02 [c]	11.83 [d]	1.33	0.001	0.323

[a, b, c, d] Means within row with different superscripts differ ($p<0.05$).

[1] p value for linear effect. [2] p value for quadratic effect.

all treatment (Table 2). Increased NDF digestibility with decreased RDP level might be attributed to low rumen pH (Table 3) which might have increased lag time and thus NDF digestibility (Mould et al., 1983; Sarwar et al., 1991, 1992). Flis and Wattiaux (2005) reported increased apparent NDF digestibility because of lower RDP in Holstein cows. Pattanaik et al. (2003) also reported that NDF digestibility was increased with low RDP as compared to high RDP (49 vs. 55).

Increasing the level of dietary RDP had no effect on blood pH (Table 5). Elrod et al. (1993) also reported that dietary RDP level had no effect on blood pH. Linear increase ($p<0.05$) in blood pH was noticed at 3, 6 and 12 h post feeding. This increased blood pH might be attributed to increased concentration of ruminal ammonia.

Blood urea nitrogen concentrations were lowest ($p<0.05$) in bulls fed C diet compared with those fed HRDP and VHRDP diets at 3 h post feeding. However, there was no difference between C and MRDP diets. The BUN concentrations were lower ($p<0.05$) in bulls fed C diet than those fed VHRDP, however, it was not different from bulls fed MRDP and HRDP diets at 6, 9 and 12 h post feeding (Table 5). Increasing level of dietary RDP from 50 to 100% resulted in a linear increase ($p<0.01$) in BUN. The linear increase in BUN with increasing the level of dietary RDP was due to increased ruminal NH_3-N concentration. Blood urea nitrogen concentrations showed almost a similar trend of the ruminal NH_3-N. Similarly, Wanapat and Pimpa (1999) reported linear increase in BUN (13.0, 17.8, 23.4, 29.3 and 39.3 mg/dl) with increasing (7.1, 8.8, 13.6, 17.6 and 34.4 mg/dl) ruminal NH_3-N concentrations. Chumpawadee et al. (2006) reported that an increase in ruminal NH_3-N concentrations increased BUN concentration. Vertanen (1966) also reported higher BUN in cattle fed purified diets containing urea than those fed natural diets. Aziz et al. (1983) also reported increased BUN in buffalo bulls fed diet containing urea.

Nitrogen balance was positive for all bulls fed different diets. Nitrogen balance was highest ($p<0.05$) in bulls fed C diet compared with those fed other diets (Table 6). Increasing the dietary RDP level resulted in linear decrease ($p<0.01$) in N balance. Similar findings were reported by Paengkoum et al. (2004) who described that N retention decreased linearly (28.03, 26.59, 23.69 and 20.36%) as the level of RDP increased (94, 96, 98 and 100% of CP) in goats. Highest N balance in bulls fed C diet was due to its low RDP level (50%). Pattanaik et al. (2003) also reported higher N retention in low RDP diet as compared to high degradable protein diet in crossbred calves. Fecal N in bulls fed C diet was lowest ($p<0.05$) than those fed HRDP and VHRDP diets. However, it was not significantly different from those fed MRDP diet. Increasing dietary RDP resulted in linear increase ($p<0.01$) in fecal N. Similarly, Wright et al. (1998) reported that increasing the dietary RDP level in dairy cows increased fecal N. Urinary N in bulls fed C diet was lowest ($p<0.05$) compared with those fed HRDP and VHRDP diets, however, it was significantly different from those fed MRDP. There was a linear increase ($p<0.01$) in urinary N by increasing the level of RDP from 50 to 100% (Table 6). These findings were consistent with those of

Kalscheur et al. (2006) who reported that there was a linear increase in urinary N with increasing the dietary RDP (6.8, 8.2, 9.6 and 11% of DM) in dairy cows. Similar results were reported by Castillo et al. (2001) and Davidson et al. (2003).

In conclusion, buffalo bulls can effectively use diets containing 82% RDP without any adverse effects. However, some more trials involving larger number of animals at *ad libitum* feeding are suggested prior to making any recommendations for the farming community. The data strongly suggest that the level and type of carbohydrates (structural vs. non-structural and relative fermentation rates) is very important to the effective utilization of the RDP and would need to be defined in future recommendations.

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