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Effect of Improved Protein Nutrition on Rumen Parameters and Blood Metabolites in Water Buffalo (*Bubalus bubalis*) Heifers

T.M.I.R. Sahama, E.R.K. Perera[1] and A.N.F. Perera[1]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya.

ABSTRACT. *An experiment was conducted to examine the response of rumen parameters and blood metabolites in local buffalo heifers to provision of improved protein nutrition. Twelve buffalo heifers were allotted to two groups balanced by body weight (89 kg) and age (12 months). All the animals were offered "Guinea A" grass (Panicum maximum) ad libitum during day time. At night, the treatment group (n=6) was given 4% urea treated straw. But the control group (n=6) was given untreated straw, wetted with the same amount of water. Daily, the group feed intake of Guinea grass and straw was recorded. Representative samples of Guinea grass, straw and refusals were obtained periodically, for proximate analysis. Rumen samples were obtained every 4th week to determine rumen pH and rumen ammonia (NH_3-N). Blood samples were obtained on the same days to determine circulating Blood Urea Nitrogen (BUN), Beta Hydroxy Butyrate (BHB), Serum Albumin (Al) and Total Protein (TP) contents. Urea treated straw had a higher ($P<0.01$) Crude Protein content than untreated straw. Treatment group animals had a greater straw dry matter intake ($P<0.01$), total dry matter intake ($P<0.01$), nitrogen intake ($P<0.01$) and an energy intake ($P<0.01$). The rumen ammonia content was higher ($P<0.05$) in treatment group animals. Rumen pH, circulating BHB, BUN, Al and TP were not different ($P>0.05$) among the groups. But, trends in rumen pH and BUN in two groups suggested better utilization of available dietary protein by the treatment group animals.*

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[1] Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya.

INTRODUCTION

The majority of the 0.9 million buffalo population in Sri Lanka belongs to the indigenous type, and is maintained under natural grazing conditions with no supplementary feeding. However, many traditional farmers in Sri Lanka have recognized rice straw as a potential supplementary feed to be used for buffaloes during lean periods of feed supply. Urea treatment of rice straw has been shown to improve dry matter intake (Gadre and Jackson, 1980), dry matter digestibility (Deschard, 1983), enhance rumen function and improve protein supply to the animal (Massaki *et. al.*, 1992). Animals respond to changes in nutritional status in the diet *via* changes in metabolism, hormones and performance. Hence, significant changes in the nutritional status can cause changes in the circulating metabolites and reflect in metabolic profiles (Lee *et. al.*, 1978). Information is limited regarding blood metabolic profiles of domesticated water buffalo in Sri Lanka. This experiment was conducted to investigate the effect of improved protein nutrition on rumen parameters, and blood metabolites in water buffalo heifers.

MATERIALS AND METHODS

The study was conducted at the Department of Animal Science, Faculty of Agriculture, University of Peradeniya using 12 buffalo heifers. Animals were divided into two groups (n=6/group), balanced by age (12.0 ± 0.52 month) and body weight (89.0 ± 8.9 kg). All the animals were offered medium quality Guinea A grass (*Panicum maximum*) *ad libitum* during the day. During the night, one group of heifers (treatment) was offered urea treated straw (4% urea treated straw, ensiled for 7 days prior to feeding); while the other group of heifers (control) was offered untreated straw, but wetted with the same amount of water. Except for this treatment difference, the rest of the management practices were similar for both groups during the 45 week experimental period. Group feed intake and refusals of both grass and straw were recorded daily. Representative samples of grass, untreated straw, urea treated straw and refusals were collected weekly to determine dry matter (DM), organic matter (OM) and nitrogen content (Kejltec Autoanalyzer), using the standard methods (Anonymous, 1980). Rumen samples were obtained from individual animals 3 h post feeding, using a stomach tube at every 4th week, to determine rumen ammonia nitrogen [NH_4-N] content (Markham, 1942) and rumen pH (electrometric method). Blood samples were collected 2 h post feeding on the same days to determine blood urea nitrogen (BUN), ß-hydroxy butyrate (BHB), serum albumin (Al) and total protein (TP). These blood metabolites were quantified by standard enzymatic and colourimetric methods using the International Atomic Energy Agency (IAEA) nutritional metabolite kits. All the data were subject to analysis of variance procedures (Snedecor and Cochran, 1979).

RESULTS AND DISCUSSION

Proximate composition of the diets

The mean proximate composition of grass, untreated straw and urea treated straw is given in Table 1. Urea treated straw had a higher ($P<0.01$) crude protein content than untreated straw. These values are comparable to the values reported by Ravindran *et. al.*,(1987) and Massaki *et.al.*, 1992. Since the CP contents of all the base feeds were less than 12%, a higher dry matter intake by the animals can be expected.

Table 1. Proximate composition of the grass, untreated straw and urea treated straw fed to heifers.

	Grass	Untreated* Straw	Urea treated* Straw
Dry matter(g/kg)	286±5.2	678±4.6	671±6.2
Other Components: (g/kg DM)			
Organic Matter	885±4.0	900±1.8	893±2.6
Crude Protein	78.9±0.4	55.8±0.3	91.0±0.2
Phosphorus	3.3±0.2	1.5±0.1	2.8±0.2
Potassium	6.1±0.2	1.2±0.1	2.6±0.2

* Straw was wetted with the same amount of water

Dry matter intake (DMI)

The mean daily DMI of grass and straw are given in Table 2. The grass dry matter intake (DMI) was similar in both groups. But, the total DMI of the treatment group (3.85 kg/d/100 kg Body Weight) was greater

(P<0.01) than that of the control group, due to the greater (P<0.01) straw DMI by this group. Such increases in straw DMI following urea treatment have been reported by Wongsrikeao and Wanapat (1984). The increase in straw DMI following urea treatment has been attributed to enhanced microbial activity on straw dry matter facilitated by breaking down of ligno-cellulose complex by urea ammonia (Saadullah *et. al.*, 1981). Greater availability of nitrogen resulted from urea treatment also would have enhanced microbial activity. The results of this experiment clearly indicate significant positive effects of protein content in the diet on DMI of roughage feeds.

Energy intake

The estimated daily metabolizable energy (ME) intake of the two groups, based on the DMI and ME content of the feed (Ibrahim *et. al.*, 1987 and Ravindran *et.al.*, 1987) are given in Table 2. Grass ME intake was not different between the groups. But, the treatment group had a greater (P<0.01) total ME intake, due to greater (P<0.01) straw ME intake (Table 2). This suggests that improvement of protein nutrition in the diet exerts beneficial effects on energy intake, also *via* its effects on DMI and digestibility. According to the observed body weight gain in the two groups of heifers of this experiment (Sahama *et.al.*, 1993) and the established nutritional requirement values for buffaloes (Kearl, 1982), the daily ME requirements per 100 kg body weight of the two groups amount to 5.46 Mcal (control) and 6.28 Mcal (treatment). The estimated energy intake values of the two groups (Table 2) exceed these requirements. This implies that the heifers of both groups received sufficient energy and were not subject to any energy stress. Circulating BHB serves as an indicator of energy stress in ruminants (Lee *et.al.*, 1978). The above implication could be further confirmed by examining the circulating BHB values of the two groups.

Nitrogen Intake (NI)

The estimated NI of the two groups, based on the DMI and the N content of feed are given in Table 2. Grass NI was not different between the groups. The treatment group had a greater (P<0.01) total NI (65 *vs* 51 g/d/100 kg BW), primarily due to a greater straw NI (21 *vs* 5 g/d/100 kg BW) (Table 2). This greater NI may be of no use to the animals, if NH_3-N is eliminated from the rumen in an attempt to avoid rumen NH_3 toxicity.

Table 2. **Dry matter intake, energy intake and nitrogen intake of buffalo heifers fed with untreated straw (control) or urea treated straw (treatment) as a supplement.**

	Untreated Straw	Urea treated Straw	
Dry matter intake (g/d/100 kg BW)			
Grass	2.74	2.66	NS
Straw	0.53	1.19	**
Total	3.27	3.85	**
Energy intake (Mcal/d/100 kg BW)			
Grass	4.66	4.52	NS
Straw	0.83	2.09	**
Total	5.49	6.61	**
Nitrogen intake (g/d/100 kg BW)			
Grass	45.61	44.05	NS
Straw	5.13	20.49	**
Total	50.74	64.54	**

** ($P \leq 0.01$) NS = Not Significant BW=Body Weight

Rumen parameters

The mean values of Rumen pH and rumen ammonia are given in Table 3. Rumen pH of the two groups was not significantly different ($P>0.05$), although the treatment group had a slightly higher rumen pH (Table 3). Generally, an elevated pH results when urea treated straw is fed to sheep (Massaki *et.al.*, 1992). The results of this experiment suggest the existence of a more efficient buffering capacity in the rumen of buffalo compared to sheep.

Table 3. Mean values of rumen parameters and blood metabolites of Buffalo Heifers Fed with Untreated Straw (Control) or Urea Treated Straw (Treatment) as a Supplement.

	Untreated Straw	Urea treated Straw	
Rumen Parameters			
pH	7.30	8.02	NS
NH_3-N (mgN/L)	20.05	36.49	*
Blood Metabolites			
BUN (mmol/l)	7.22	6.50	NS
BHB (mmol/l)	0.23	0.21	NS
Albumin (g/l)	33.03	33.08	NS
Total Protein (g/l)	79.05	80.03	NS

* ($P<0.05$) NS = Not Significant

Rumen NH_3-N content was greater ($P<0.05$) in heifers supplemented with urea treated straw, compared to the control group (Table 3). However, the rumen NH_3-N contents of both the groups (36.49 and 20.05 mg N/L) were lower, than that required by the rumen microbes (80 mg N/L) for an optimal microbial protein synthesis (Hoover, 1986). This fact has been reported and discussed elsewhere by the authors (Sahama *et.al.*, 1993). Rapid removal of ammonia nitrogen from the rumen has been suggested as a possible reason for the existence of a low concentration of NH_3-N in the rumen (Masaaki *et.al.*, 1992).

Blood metabolites

The mean values of Beta Hydroxy Butyrate (BHB), Blood Urea Nitrogen (BUN), Serum Albumin (Al) and Total Protein (TP) are given in Table 3. Circulating BHB of the treatment group was not different from that of the control group. These values were comparable with the reference values established for well-fed non lactating cattle and sheep (Anonymous, 1993). The reference BHB values are not established for buffaloes yet. However, the results of another study (unpublished) by the authors reveal that the BHB values of well-fed buffaloes range between 0.19 and 0.28

nmol/l. The BHB values of the heifers of this experiment were within that range. Thus based on the values of ME intake (Table 2) and BHB (Table 3), it can be suggested that none of the heifers were suffering from energy stress.

Mean Blood Urea Nitrogen of the control animals was slightly greater than that of the treatment group. Although the difference between the two groups was not significant ($P > 0.05$), a tendency to have greater BUN values in control animals was apparent in more than 80% of the collected samples. As evidenced by the values of rumen parameters (Table 3), the proximate composition (Table 1) of the diet and nitrogen intake (Table 2), the control animals did not receive excess protein in the diet. As suggested by the circulatory BHB values (Table 3) and estimated ME intake values (Table 2), they were not suffering from any form of energy stress. Hence, this tendency of the control animals to have higher BUN in circulation, implies, poor utilization of available nitrogen in the rumen by the control animals, compared to the treatment animals.

Neither serum albumin (Al) nor total protein (TP) values were different between the two groups (Table 3). The values of Al and TP of these heifers were comparable to the reference values reported for cattle (Anonymous, 1993). Serum albumin and total protein change dramatically under conditions of starvation, dehydration and/or infections. The Al and TP values of these experimental animals suggest that none of the animals were suffering from starvation, dehydration or infections during the experimental period.

CONCLUSIONS

The results suggest that the urea treatment effectively improved the CP content of the diet. Supplementary feeding of urea treated straw increased straw dry matter intake, total dry matter intake, energy intake, protein intake and rumen ammonia of the heifers, compared to the supplementary feeding of untreated straw. The trends in rumen parameters and blood metabolites suggested better utilization of available nitrogen by the treatment group, compared to the control group heifers.

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