

EFFECT OF PROTEIN SUPPLEMENTS OF VARYING RUMINAL DEGRADABILITY ON NUTRIENTS INTAKE, DIGESTIBILITY, NITROGEN BALANCE AND BODY CONDITION SCORE IN EARLY LACTATING NILI-RAVI BUFFALOES

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ABSTRACT

A study on the effect of protein supplements of varying ruminal degradability on nutrients intake, digestibility, nitrogen balance and body condition score was conducted in early lactating Nili-Ravi buffaloes. Twenty four multi-parous early lactating (22± 10 days) Nili-Ravi buffaloes were selected and randomly divided into four groups (six animals in each group) and fed total mixed rations A, B, C and D containing 30, 40, 50 and 60 % rumen undegradable protein (RUP), respectively in a completely randomized design. Results showed that DM, CP, NDF and ADF intakes were similar ($P>0.05$) on rations A, B, C and D. DM, CP and NDF digestibility was similar on all rations whilst NDF digestibility was highest ($P<0.01$) on ration C. Nitrogen intake and total nitrogen output (g/d or % of N intake) was similar on all rations. Of the N output fractions, there was no difference in fecal N excretion however, urinary N excretion as percent of intake was highest ($P<0.01$) on ration A and lowest on ration C. Milk N output was maximum ($P<0.05$) on diet C. Nitrogen balance and utilization were uninfluenced by the protein degradability of rations. Percent change in body condition score of the early lactating Nili-Ravi buffaloes was also unaffected ($P>0.05$) by the experimental rations.

Key words: Protein supplements, rumen degradability, nutrient digestibility, body condition score.

INTRODUCTION

In Pakistan, crop residues and grazing constitute a major feed resource for ruminant livestock which are often supplemented with energy and protein feeds to improve animal productivity. The protein supplements presently used for feeding to ruminants particularly lactating cows constitute mostly oil seed cakes obtained as a by-product of oil industry. However, the recent increasing trend in large commercial dairy and fattening farms has also encouraged the use of total mixed ration. It has facilitated the use of cakes and meals of vegetable and animal origin differ in degradability which influences feed intake (Kumar *et al.*, 2005; Flis and Wattiaux, 2005) and digestibility (Pattanaik *et al.*, 2003; Yoruk *et al.*, 2006).

The protein is the costly ingredient in total mixed ration or supplemented diets of animals. The conventional feeding system based on crude protein contents may result in overfeeding of nitrogen in the rumen resulting in low efficiency of N utilization. However, it is believed that lactating cows utilize nitrogen more efficiently than other ruminants yet, they still excrete 2-3 times more N in feces than milk (Broderick, 2006). Many studies agree with the concept that the efficiency of nitrogen utilization can be obtained when diets have proper composition of rumen degradable protein (RDP) and rumen undegradable protein (RUP) proportions. An imbalance in RDP and RUP proportions

can increase nitrogen excretion (Kauffman and Pierre, 2001). Conversely, nitrogen retention can increase with increasing level of RUP supplementation (Paengkoum *et al.*, 2004).

Reported literature gives comprehensive knowledge in dairy cattle; however, it is lacking information about new concept of protein feeding in buffaloes. Buffaloes have some difference in digestion due to large rumen volume, more cellulolytic activity and relatively less rumen passage outflow rate (Moran and Wood, 1982). Therefore, this study planned to know the effect of protein supplement of varying ruminal degradability on nutrients intake, utilization, nitrogen balance and body condition score in early lactating Nili-Ravi buffaloes.

MATERIALS AND METHODS

Twenty four multi-parous early lactating (22± 10 days) Nili-Ravi buffaloes were selected and randomly divided into four groups in a completely randomized design. Each group was consisted of six buffaloes of approximately similar milk yield (4.8±0.5 L) and lactation (2nd lactation). The experiment lasted for 105 d, with the first 15 d being allocated for adaptation to their respective diets while the remaining period (90 d) used for data collection. Initial body condition score of all buffaloes were recorded using the method described by Peters and Ball (1987). All animals of each group were

housed, tied and fed *ad libitum* in individual mangers. Rations were offered daily at 9:00 a.m. Each morning prior to feeding the previous day's feed refusals (orts) were weighed, recorded and discarded before offering the fresh rations. Representative samples of feed and orts were taken daily and pooled for individual animals of each group.

The TMR were prepared, with each treatment group having different proportions of RDP and RUP in the ration (Table 1). The rations designated A, B, C and D, contained 30, 40, 50 and 60 % RUP of total CP, respectively. Ration B served as the control group having 60:40 (RDP: RUP) ratios as recommended for large dairy cattle breeds (NRC, 2001). All TMR were iso-caloric and iso-nitrogenous (NRC, 2001). Prior to commencement of the feeding trial, all four TMRs were evaluated for RDP and RUP fractions using the *in situ* technique (Cottrill and Evans, 1984) in an adult rumen fistulated Nili-Ravi buffalo steer.

At the end of feeding trial, body condition score of animals were again recorded. Nitrogen balance was determined using total collection method during the last five days. Three cows from each group were randomly selected and total feces, urine and milk of each animal were collected and recorded daily. For urine collection, cows were kept in individual partitions that had special arrangement for automatic flow-in containers (acidified with 50% H₂SO₄) placed under pens. Each morning, the volume of urine was recorded, thoroughly mixed and 20% sub-samples taken and pooled for individual cow (Marghazani *et al.*, 1999). Similarly, feed, fecal, and milk samples (20%) were pooled separately for each cow. All collected samples were chemically analyzed for DM, Nitrogen (Kjeldhal method, AOAC, 2000), NDF and ADF (Van Soest *et al.*, 1991) to calculate nutrients intake, digestibility, N balance and N utilization.

Data thus obtained on nutrients intake and digestibility, N balance, N utilization and body condition score were statistically analyzed through analysis of variance technique under completely randomized design (Steel *et al.*, 1997) using Statistical Analysis System (SAS, 1997) software package. Means were compared for significance of difference with the Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Nutrients intake and digestibility (Mean \pm S.E) in early lactating Nili-Ravi buffaloes fed rations varying in protein degradability are given in table-2. Results showed non significant differences ($P > 0.05$) in dry matter, crude protein, neutral detergent and acid detergent fiber intakes. The experimental diets were iso-caloric and iso-nitrogenous in composition however, degradability of protein in these diets varied which didn't influence DM, CP NDF and NDF intake in early lactating buffaloes.

These results are in part agree with Santos *et al.* (1998) who in review study concluded that total DM intake in dairy animals remained same despite of increase in RUP proportion in diets. Castillo *et al.* (2001) also reported that increasing CP level or changing CP degradability had no effect on total DM intake. Lack of difference in nutrients intake in early lactating buffaloes may be due to the fact that our experimental diets were TMR (total mixed ration) based that contained mixed nutrients and hence, assured complete nutrients intake in each bolus. Samanta *et al.* (2003) also reported that this feeding system facilitate synchronized supply of nutrients to animals.

Similar nutrients intake resulted in similar ($P > 0.05$) dry matter, crude protein and acid detergent fiber digestibility except neutral detergent fiber digestibility which was significantly maximum ($P < 0.01$) on diet C (table-2). NDF digestibility increased with increase in RUP in the diet as diet A < B < C. However, diet D didn't follow this trend which may be the due to low N availability in the rumen for microbial growth and fermentation. Earlier findings (Pattanaik *et al.*, 2003; Flis and Wattiaux, 2005) also report increased NDF digestibility with increased RUP level in the diets. Our results agree with the concept that low nitrogen cause low microbial growth and hence low fiber utilization (Leng, 1990) and excess nitrogen decreased efficiency of fiber utilization due to more demand of ATP for converting extra nitrogen it to urea. Although statistically similar, dry matter and ADF digestibility were also higher on diet C. Such increased trend of nutrients utilization in early lactating experimental animals on diet C suggest proper proportion of RDP and RUP level in the diet which provide sufficient RDP for microbial and RUP level that bypassed rumen for fulfilling the requirement of host animal.

Nitrogen intake, excretion, balance and utilization (Mean \pm S.E) in early lactating Nili-Ravi buffaloes fed rations varying in protein degradability are given in table-3. Nitrogen intake was similar among experimental animals on different diets varying in degradability. This is due to the fact that all experimental diets were iso-nitrogenous in composition and were TMR based that prevented selection in experimental animals. Non significant ($P > 0.05$) differences observed in fecal N excretion as g/d or percent of intake, however, an increasing trend observed with increase in RUP level up to 50%. Likewise, Lines and Weiss (1996) also observed progressive increase in fecal N excretion with increased RUP supply. In urinary N excretion, non significant differences observed as g/d, however as percent of intake it was maximum ($P < 0.01$) on diet A and minimum on diet C. These results suggest that as RUP level increased in diet, N excretion through urine also decreased as A > B > C. However, diet D was different in trend which may be due to improper RDP and RUP levels for rumen

microbial growth and animal performance, respectively. Castillo *et al.* (2001) also reported increase in urinary N excretion with increase in degradability of experimental diets. N excretion through milk as g/d ($P < 0.01$) or percent of N intake ($P < 0.05$) was significantly maximum on diet C, while other diets were similar ($P > 0.05$) among each other. These results confirms the highest protein percent ($P < 0.001$) obtained on diet C as more N in milk among experimental rations was recorded on this diet. Nitrogen balance as g/d or percent of N intake was similar on all

diets. Although, there was non significant ($P > 0.05$) difference, the increase in nitrogen balance (% of N intake) and nitrogen utilization followed the trend of increase in RUP level in the diet, except diet D. These results are inline with the findings of Paengkoum *et al.* (2004) who reported that nitrogen balance can increase with increasing level of RUP supplementation. Similarly, Pattnaik *et al.* (2003) found that animals on low RDP diets retained more nitrogen than those given high RDP.

Table-1: Ingredients and chemical composition of experimental total mixed rations fed to early lactating Nili-Ravi buffaloes

Ingredients	Ration-1	Ration-2	Ration-3	Ration-4
Wheat straw	29	30	31	32
Wheat bran	22.4	16	20	15
Molasses	07	05	08	11
Rice Polishing	09	05	01	06
Broken maize	-	06	05	04
CGM 60 %	02	07	05	-
CGM 60% (H.T)	-	-	-	07
Rapeseed meal	12	07	-	-
Rapeseed meal (F.T)	-	-	07	06
Sunflower meal	02	09	07	03
Soybean meal	06	04	01	02
Cotton seed cake	08	09	09	08
Blood meal	-	-	04	04
Urea	0.6	-	-	-
*Mineral mixture	02	02	02	02
Total	100	100	100	100
M.E Mcal/kg	2.35	2.35	2.36	2.36
C.P %	17.01	17.29	17.24	16.99
NDF %	38.2	38.1	38.5	39.9
ADF %	22.3	21.8	22.5	22.9
RUP (% CP)	5.26	6.80	8.75	10.39
RDP: RUP	69:31	61:39	50:50	39:61

ME Mcal/kg= Metabolizable energy mega calories per kilogram; C.P= crude protein; NDF= neutral detergent fiber; ADF=acid detergent fiber; RUP= rumen degradable protein; RDP= rumen degradable protein; CGM= corn gluten meal; H.T= heat treated; F.T= formaldehyde treated. * Mineral mixture composition (per kilogram): Dicalcium phosphate 708g; Sodium chloride 189g; Magnassium sulphate 86.0g; Ferrous sulphate 8.9g; Manganese sulphate 4.9g; Zinc sulphate 3.2g; Copper sulphate 0.3g; Potassium iodide 0.087mg and Cobalt chloride 0.0089mg; Sodium selenate 0.015mg.

Table-2: Mean (\pm S.E) nutrients intake and digestibility in early lactating Nili-Ravi buffaloes fed rations varying in protein degradability

Description	Groups				Sig.
	A	B	C	D	
Intake (kg/d)					
DM	14.14 \pm 0.41	14.77 \pm 0.66	14.80 \pm 0.24	14.67 \pm 0.27	NS
CP	2.41 \pm 0.07	2.55 \pm 0.11	2.55 \pm 0.04	2.49 \pm 0.18	NS
NDF	5.40 \pm 0.16	5.63 \pm 0.25	5.70 \pm 0.09	5.85 \pm 0.11	NS
ADF	3.15 \pm 0.09	3.22 \pm 0.14	3.33 \pm 0.05	3.36 \pm 0.06	NS
Digestibility (%)					
DM	60.21 \pm 1.38	62.30 \pm 1.71	64.60 \pm 1.75	60.23 \pm 0.66	NS
CP	75.61 \pm 1.38	74.30 \pm 1.47	72.97 \pm 1.65	73.43 \pm 0.94	NS
NDF	55.35 \pm 1.88 ^b	60.54 \pm 2.05 ^{ab}	65.22 \pm 1.91 ^a	57.97 \pm 1.09 ^b	**
ADF	41.98 \pm 2.48	44.95 \pm 3.10	47.11 \pm 2.71	43.60 \pm 1.96	NS

Means with different superscripts within same row are significantly different ($P < 0.05$); Sig.= significance level; **= ($P < 0.01$); NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber; DM= Dry matter; CP= Crude protein

Table-3: Mean (\pm S.E) nitrogen intake, excretion, balance and utilization in early lactating Nili-Ravi buffaloes fed rations varying in ruminal protein degradability

Description	Groups				Sig.
	A	B	C	D	
Initial	3.13 \pm 0.13	2.92 \pm 0.12	2.96 \pm 0.08	3.08 \pm 0.08	NS
Final	3.58 \pm 0.14	3.29 \pm 0.18	3.25 \pm 0.11	3.42 \pm 0.05	NS
% Change	0.46 \pm 0.08	0.21 \pm 0.08	0.29 \pm 0.08	0.33 \pm 0.08	NS

Table-4: Mean (\pm S.E) body condition score in early lactating Nili-Ravi buffaloes fed rations varying in ruminal protein degradability

Description	Groups				Sig.
	A	B	C	D	
Nitrogen intake (g/d)	385.95 \pm 11.26	407.90 \pm 18.23	406.98 \pm 6.51	399.10 \pm 7.45	NS
Nitrogen output (g/d)					
Faecal	93.62 \pm 5.12	102.16 \pm 4.32	109.45 \pm 6.02	105.72 \pm 3.70	NS
Urinary	227.65 \pm 8.73	218.63 \pm 12.55	205.54 \pm 9.32	223.76 \pm 6.74	NS
Milk	42.49 \pm 1.04 ^b	44.33 \pm 1.43 ^b	49.75 \pm 1.06 ^a	44.24 \pm 1.39 ^b	***
Nitrogen balance (g/d)	22.18 \pm 3.44	42.78 \pm 11.50	42.24 \pm 8.16	25.39 \pm 5.78	NS
Nitrogen output (% of intake)					
Faecal	24.39 \pm 1.38	25.10 \pm 1.47	27.03 \pm 1.65	26.57 \pm 0.94	NS
Urinary	58.91 \pm 1.1 ^a	53.63 \pm 1.87 ^{bc}	50.47 \pm 2.12 ^c	56.01 \pm 1.11 ^{ab}	**
Milk	11.14 \pm 0.44 ^b	11.01 \pm 0.33 ^b	12.24 \pm 0.24 ^a	11.10 \pm 0.34 ^b	*
Nitrogen balance (% of intake)	5.55 \pm 0.77	9.66 \pm 2.66	10.25 \pm 1.92	6.32 \pm 1.43	NS
Nitrogen Utilization	16.69 \pm 0.58	20.68 \pm 2.63	22.49 \pm 1.81	17.42 \pm 1.43	NS

Means with different superscripts within same row are significantly different (P<0.05)

Sig= significance level; * = (P<0.05), ** = (P<0.01); *** (P<0.001)

N.S= non significant

In BCS parameters, non significant differences (P>0.05) was observed among all groups (table-4). Early lactating experimental animals in each group showed a positive change and improved their BCS with maximum (P>0.05) in group A followed by group D. Likewise, higher increase in BCS obtained on control diet as compared to high fish meal diets (source of RUP %) in the findings of Malleeson *et al.* (2007). It is argued that increased RUP feeding causes mobilization of body fats in animals (Schroeder and Gagliostro; 2000) that ultimately results in low body condition score (BCS).

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