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SUPPLEMENTATION EFFECT OF PROTEIN AND ENERGY ON NUTRIENT DIGESTION AND N METABOLISM OF BUFFALO CALVES FED WHEAT STRAW BASED DIETS

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A 4 x 4 calf metabolism trial with 2 x 2 factorial arrangement of treatments was conducted with ruminally cannulated buffalo calves to explore the effects of protein and energy supplementation of wheat straw based diets on total tract nutrient digestion and nitrogen metabolism of buffalo calves. The calves were fed experimental diets @ 2% of their body weight. They were fed either low energy low protein diets or high energy high protein diets. Total faeces were collected for OM, OM, Nand NOF digestion, whereas total urine was collected for N balance studies. Rumen samples were analyzed for rumen ammonia nitrogen and blood plasma samples for plasma urea nitrogen. Total tract OM and OM digestion was greater (P<0.05) for calves fed high energy diets, however, total tract NDF digestion was found to be the highest (P<O.O1) with low energy diets. Higher (P<O.O1) total tract N digestibility was found with high protein diets. Although high energy diets showed only a trend of greater N digestion, N retention (g/d) was 17.8,22.9, 16.4, and 24.3 for low energy, high energy, low protein and high protein diets respectively. Nitrogen retention-percent N intake and N retention-percent N digestion were greater (P<O.OI) for high energy diets. Rumen NHrN concentrations at 3, 6 and 9 hr postfeeding were higher (P<O.OI) for high protein and low energy diets. Plasma urea N concentrations at 3, 6 and 9 hr postfeeding were higher (P<O.OI) for high protein and low energy diets.

Key words: digestibility, energy, N metabolism, protein, wheat straw

INTRODUCTION

Crop residues or low quality roughages are rich in bio-cellulosic mass and represent a substantial reservoir of energy for ruminants. However, unique problems are faced by nutritionists while attempting to maximize the utilization of crop residues in ruminants, because these feeds are high in neutral detergent fibre (NOF) (>60%) and acid detergent fibre (AOF) (>40%) as well as low in available proteins «5%) and fermentable carbohydrates (NRC, 1984). This limits their ruminal fermentaion and a little, if any, feeding value is derived from these roughages. Chemical treatment and supplementation (with protein, energy, and minerals) are the two approaches which are commonly employed to improve the digestibility and utilization of low quality roughages (Males, 1987; Sultan and Loerch, 1992a). Although both the strategies to enhance the digestion and utilization of crop residues in ruminants are rational and practicable, however, the choice of application depends upon the feasibility and economics.

Crop residues contain large amounts of structural polysaccharides (cellulose and hemicellulose) but small amounts of non-structural carbohydrates (starch and sugars) as well as very low concentration of available protein. As a result, intake, digestibility and utilization of these residues by ruminants is hampered (Camp ling et al., 1962). In order to get maximum benefit from these highly cellulosic materials, supplementation with readily available energy and protein sources is needed to stimulate growth of rumen microbes and increase the rate of fermentation. Supplemental protein resulted in linear increase in digestibility of dry matter (OM), cellulose, hemicellulose and energy (Wiedmeirer et al., 1983).

In Pakistan, protein or energy supplementation compared to chemical treatment'is more practical and cost effective way of improving the nutritive value of low quality roughages because of the high prices of alkali (NaOH) and the difficulty in its handling. Little research has been done so far in Pakistan to maximize the utilization of crop residues by supplementation with protein or energy in buffaloes. Therefore the present study was designed to investigate the effects of protein or energy supplementation of wheat straw based diets on total tract nutrient digestion, nitrogen (N) status i.e. N retention and its utilization and N recycling in buffalo calves.

MATERIALS AND METHODS

Animals and Feeding Regimen: Four male buffalo calves (Av. Wt. 175 kg) equipped with ruminal cannulae were used in a 4 x 4 Latin square design with 2 x 2 factorial arrangement of treatments. Four rations varying in protein and energy levels were formulated (Table I). High and low energy diets contained 63 and 40% wheat straw respectively, supplemented with 9.25 and 12% crude protein (addition of urea). Calculated ME was 1.87 and 2.15 Meal/kg for the low and high energy diets respectively (NRC, 1984). Ingredients and chemical composition of the experimental diets are shown in Table I. The calves were housed in metabolism pens which were designed for separate collection of faeces and urine. They were fed the experimental diets @ 2% of their body weights in two equal portions at 0800 and 2000 hr. Water was available continuously throughout the experiment. Each experiment was of 15 days duration with day 1-10 for the diet adaptation and day 11-15 for' sample collection.

Table 1. Ingredients and chemical composition of diets

Ingredients	Low energy		High energy	
	Low protein (A)	High protein (B)	Low protein (C)	High protein (D)
	Dry matter basis (%)			
Wheat straw	63.89	62.92	41.28	40.3
Liquid molasses (cane)	18	18	18	18
Urea	0.5	1,48	0,47	1,46
Rice broken	-	-	28.66	28.66
Cottonseed meal	16	16	9.99	9.98
Oicalcium phosphate	1	I	I	I
Trace mineral salt"	0.6	0.6	0.6	0.6
Vitamin A	0.001	0.001	0.001	0.001
Vitamin O	0.001	0.001	0.001	0.001
Chemical composition				
Organic matter	88,40	88,48	89.65	89.73
Crude protein	9.22	11.99	9,27	12.09
Neutral detergent fibre	55.99	55.94	41.34	40.57
Acid detergent fibre	35.24	34.76	24.68	24.2
Metabolizable energy (Meal/kg)	1,88	1,86	2.16	2.15

*See Table 2 •

Sampling Procedures: Samples of feed, orts, total faeces and urine were collected on day II through 15 of each period. Urine from each calf was collected in a separate container containing 200ml of 6N HCI to maintain pH below 2.0. Of the measured total urine from individual calves, 20ml was saved and stored at -20°C for further analysis. Ten grams of faeces from each calf/day were collected separately and dried at lO5°C for OM determination and lOOg from each calf/day was saved for future analysis. Feed, orts and faecal samples were dried at 60°C and ground through Imm screen before laboratory analysis. Ruminal and blood samples were collected from each calf at 3, 6, 9 and 12 hr after the 0800 hr feeding, on 15th day. Ten ml of ruminal fluid from each calf, after straining through 4 layers of cheese cloth, were acidified with O.3ml of 6 N HCI to terminate the microbial activity. From each calf., 10ml of blood was collected from jugular vein in heparinized tubes to avoid clotting. The tubes were centrifuged at 500rpm for 15 minutes right after collection to harvest plasma. The plasma was decanted and stored at ~20°C for further analysis of plasma urea nitrogen (PUN).

Lab Analysis: Samples of feed, orts and faeces were analyzed for organic matter (OM), OM and N (A.O.A.C.,. 1984). These were also analyzed for NOF (Robertson and Van Soest, 1977) and for AOF (Goering and Van Soest, 1970). The urine samples were analyzed for N. Rumen samples were analyzed for ammonia N (NHrN) by Nessler's reagent (Oser, 1965) and blood plasma samples for plasma urea N (PUN) by the DAM method using a kit (Merck Oiagnostica, France). Total tract digestibility of OM, OM, NOF and N was calculated from the nutrient composition of the specific nutrient in the feed and subtracting the specific nutrient composition in the total faeces. Nitrogen balance was calculated by N intake in the feed and subtracting N outgoin faeces and urine. Data were analyzed by analysis of variance procedures using MSTATC. Model sum of squares were partitioned into period, animal and diet effects. Diets sum of squares were partitioned to test for effects of energy and protein level and the energy x protein interaction.

RESUL TS AND DISCUSSION

Dry Matter and Organic Matter Digestion: Dry matter and OM intake was not affected (P>0.05) by protein or energy levels and average dry matter (OMI) and organic matter intakes (aMI) were 3.7 and 3.3kg respectively for diets varying in energy and protein concentration (Table 3). Dry matter and OM outgo differed (P<O.OI) among treatments. High energy diets had 17% less OM and 18.42% less OM outgo (P<O.O1) than the low energy diets, however, protein level had no effect on both OM and OM digestion. Due to these differences in outgo, high energy diets had 10.25 and 9.64% higher OM and OM digestibility (P<0.05) respectively than that of low energy diets. The higher digestibility (OM and OM) is due to the 28.66% broken rice contents in the high energy diets, having inherently high starch contents, being more digestible than wheat straw.

Similar results were also observed by Sultan and Loerch (1992a), when they compared the wheat straw with corn starch with or without supplementation in lambs. They found 20% greater (P<0.03) OM and OM digestion for high energy diets and found no effect of protein levels. Total tract OM and OM digestion was also found to be higher (P<O.I) for pulp and corn compared with soybean meal and barley supplements (Carey et al., 1993), due to the reason that pulp and corn contained greater proportion of fermentable carbohydrates. Increasing metabolic energy (1.5X) content of wheat straw by alkaline hydrogen peroxide significantly increased the total tract OM and OM digestion (Sultan et aI., 1992b). With adequate protein levels, energy supplementation of crop residues showed beneficial effect on OM and OM digestibility (Hartnell and Satter, 1979).

Neutral Detergent Fibre Digestion: Neutral detergent fibre (NDF) intake was lower (P<O.OI) for high energy diets (28.66% broken rice), because of its low structural carbohydrates than low energy diets. Total tract NDF digestion as percent of NDF intake was 14.8% greater (P<O.Ol) for low energy diets (Table 3) due to the high nonstructural carbohydrates availability in rumen, but protein level had no effect.. With increase in amylolytic activity, cellulolytic activity decreases (Mertons and Lofteen, 1980),- which subsequently decreased NDF digestion. Addition of 10% starch to roughage based diets did not affect fibre digestion while 30% starch resulted in a reduction in fibre digestibility (Mullholland et al., 1976), and was attributed to pH inhibition of fibre fermentation (Cremin et al., 1991). Hartnell and Satter (1979) concluded that apparent total tract NDF digestibility increased (P<0.05) as the proportion of hay increased in the diets. Addition of grain to the ration increased DM digestibility but decreased crude fibre digestibility (Joaning et al., 1981).

Nitrogen Digestion and Retention: Intake of N was not affected (P<O.O1) by energy level.. The calves fed high protein diets consumed 23% more N than those fed low protein diets. Despite more N intake with high protein diets, N excretion in faeces was almost the same for all the diets due to the reason that N digestibility differed among treatments. Apparent total tract N digestibility was affected by protein level.. However, there was an increasing trend for N digestion in diets with high energy level.. Apparent total tract N digestibility was higher (P<O.OI) for high 'protein level and increased by 10.59% than in low protein diets (Table 4). Urea or protein supplementation of low quality roughage diets led to increased N digestibility (Steen and Moore, 1989; Petit and Flipot, 1992b).

Table 2. Composition of total mineral salt used in diets

Salt	Contribution (%)
Sodium chloride	96.65
Zinc sulphate	0.87
Manganese sulphate	1.40
Ferrous sulphate	0.86
Copper sulphate	0.20
Potassium sulphate	0.03
Selenium oxide	0.07

Table 3. Effect of energy and protein supplementation on dry matter, organic matter and NDF digestion in buffalo calves

	Energy		Protein		SE
	Low	High	Low	High	
Dry matter					
Intake (kg/d)	3.77	3.73	3.75	3.75	0.0015
Excretion (kg/d)	1,56	1,3	1,44	1,42	0.0049[a]
Digestion, % intake	58.59	65.28	61.61	62.25	1.378°
Organic matter					
Intake (kg/d)	3.34	3.35	3.34	3.34	0.001
Excretion (kg/d)	1,35	1,14	1,25	1,24	0.037[a]
Digestion, % intake	59.57	65.93	62.52	62.99	1,238[b]
Neutral detergent fibre					
Intake (kg/d)	2.11	1,52	1,82	1,81	0.02[a]
Excretion (kg/d)	1,10	0.87	0.996	0.98	0.07[a]
Digestion, % intake	48.91	42.6	46.13	42.67	3.17[a]

a=Energy effect (P<O.O1); b=Energy effect (P<0.05).

Table 4. Effect of energy and protein on nitrogen digestion and retention in buffalo calves

	Energy		Protein		
Nutrient	Low	High	Low	High	SE
N intake (g/d)	63.95	63.73	55,46	72.23	0.395[a]
Faecal N excretion (g/d)	23.73	22.33	21.87	23.83	0.736
Urinary N excretion (g/d)	22.71	18,49	17.12	24.08	1.309[ac]
Apparent total tract N digestion, % intake	62.71	62.34	60,45	67.61	1,169[a]
N retention (g/d)	17.84	22.92	16,44	24.33	1,428[ab]
N retention, % N intake	27,43	35.90	29.60	33.74	1.583[b]
N retention, % N digestion	43.76	55.53	48.78	50.52	2.562"

a=Protein effect (P<O.OI); b=Energy effect (P<O.OI); c=Energy effect (P<0.05).

Apparent N digestibility was greater (P<0.05) for 12.5% CP diets than for 9.5% diet, i.e. 69.2 vs 62% respectively and also greater (P<0.05) for high energy than for low energy diets i.e. 67.4 vs 63.7% respectively (Sultan and Loerch, 1992a). Beneficial effects of increasing energy level on N digestion were also reported by Pritchard and Males (1982). Urinary N excretion was affected by both energy and protein levels. Low energy diets excreted 18.58% (P<0.05)

more N than high energy diets. Nitrogen excretion in urine was 28.9% more for high protein diets (P<O.OI) (Table 4). Urea supplementation of straw diets increased urinary N excretion but had little effect on faecal N excretion (Sultan and Loerch, 1992a). Nitrogen retention (g/d) was affected by both energy and protein level (P<O.OI). The calves fed high energy diets retained 22.16% more N than those fed low energy diets. Those fed high protein diets retained 32.43% more N than those on low protein diets (Table 4). Studies of Sultan and Loerch (1992a) indicated that increasing energy level of wheat straw diets improved N retention but increasing protein level through urea did not affect N retention. Nitrogen retention as a percentage of N intake and percentage of digestion was also improved by both energy and protein level (Table 4). Energy level, however, had significant effect.. High energy diets showed 23.59% more N retention as a percentage of N intake and 21.2% greater N retention as a percentage of N digestion. Although protein level had no effect (P<0.05) on N retention-percent N intake and N retention-percent N-digestion yet high protein diets resulted in 12.27% greater N retention as a percentage of N intake than low protein diets.

Table 5. Effect of energy and protein on rumen and plasma ammonia nitrogen in buffalo calves

Item	Energy		Protein		SE
	Low	High	Low	High	
Rumen ammonia N (mg/dl)					
3hr	16.68	16,39	12.74	20.33	1.58"
6hr	13.27	10.67	10.65	13.26	1.87"b
9hr	10,46	9.34	9.27	10.54	1.88^{ab}
12hr	7.61	7.67	7.68	7.59	0.54
Plasma urea N (mg/dl)					
3hr	15.75	12.71	11.57	16.84	I.73ab
6hr	13.82	11.56	10.87	14.51	1.88^{ab}
9hr	13.24	10,42	10.68	12.99	1.85^{ab}
12hr	10.73	10.65	10,44	10.95	0.06

a=Protein effect (P<O.OI); b=Energy effect (P<O.OI).

Rumen Ammonia Nitrogen: Ruminal concentrations of NHrN for all diets showed a decreasing trend after 0800 feeding. At 3, 6 and 9 hr after 0800 hr feeding high protein diets represented 37, 19 and 12% greater R-NH_3-N concentration (mg/dl) compared to low protein diets (Table 5). However, at 12 hr there was increasing trend of R-NHr N concentration for high protein diets. The high levels of R-NHrN on high protein diets may be due to rapid degradability of urea. Greater ruminal NHrN concentrations (P<O.OI) were also observed in steers fed fish meal-urea supplemented diet (Petit and Flipot, 1992b). Rapid degradation of urea into ammonia increased R-NHr N to a greater extent than an iso-nitrogenous supplement of fish meal, even though total N intakes were similar on the two diets. Similar observations were reported by Titgemeyer et al. (1989).

At 3 hr postfeeding the R-NHrN was not affected (P<0.05) by energy levels, whereas at 6 and 9 hr, high energy diets showed 24.37 and 11,99% low (P<O.OI) ruminal NHrN compared to low energy diets (Table 5). This might be due to increased microbial efficiency to capture more NH_3-N on high energy diets. At 12 hr postfeeding, there was no difference (P>0.05) among the high and low energy diets. Sultan et al. (1992b) also reported lower ruminal N pool for high energy diets.

Plasma Urea Nitrogen: Plasma urea nitrogen (PUN) concentration (mg/dl) at 3, 6 and 9 hr was affected (P<O.OI) by both energy and protein levels. No differences existed among diets at 12 hr after 0800 feeding. There was 19, 16 and 21% greater PUN for low energy diets than with high energy diets at 3, 6 and 9 hr postfeeding respectively' (Table 5). The low PUN in high energy diets may be due to increased N recycling to meet the N requirements for microbial protein synthesis in the rumen as was observed by Sultan et al. (1992c) that high metabolic energy contents of wheat straw based diets increased N recycling fro,m blood to rumen.

High protein diets always showed greater values of PUN (P<O.OI) but at a decreasing rate with non-significant effect at 12 hr postfeeding. At 3, 6 and 9 hr high protein diets showed 31, 25 and 18% more PUN (P<O.OI) respectively than low rotein diets. With high protein diets there was always higher PUN concentration because increasing the concentration of N in the diet increased PUN concentration (Kennedy, 1980). The reason for no difference in PUN at 12 hr postfeeding is that excessive N on low energy diets could have been recycled to rumen, because R-NHrN for low energy diets was low and research has shown that the relationship for clearance of plasma urea to the rumen is inversely proportional to the concentration of rumen NH} (Kennedy, 1980; Bunting et al., 1989).

When dietary N supply is inadequate, recycling of endogenous urea N provides a substantial amount of N for microbial protein synthesis in the rumen (Kennedy, 1980). It has also been reported that increasing ruminal OM digestion by the introduction of readily fermentable energy source, increased N recycling (Kennedy, 1980). In the present study, broken rice was used as a readily fermentable energy source in high energy diets and results showed

increased OM digestion (P~.05), which may be the reason for increased N recycling.

Conclusions: It may be stated that DM and OM digestion was increased by increasing energy level of diet, due to availability of readily fermentable carbohydrates. Neutral detergent fibre digestion reduced with high energy diets. Nitrogen digestion and retention increased by increasing protein and energy level in diet. It might be due to increased efficiency of rumen microbes to capture more N. Rumen NH_3-N concentration increased on high protein diets due to degradation of dietary protein and urea, while its concentration decreased on high energy diets due to capture of N by rumen microbes resulting into enhanced microbial protein synthesis. Plasma urea nitrogen increased on high protein diets, while it decreased on high energy diets.

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