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Research Article

MICROBIAL PROTEIN SYNTHESIS AND URINARY ALLANTOIN EXCRETION IN BUFFALO CALVES: EFFECT OF SUPPLEMENTATION OF DIFFERENT CARBOHYDRATES IN THE DIET

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ABSTRACT

The investigations were carried out to study the effect of various carbohydrates given as supplements to a basal diet of high roughage and low protein on ruminal ammonia nitrogen, microbial protein synthesis and urinary excretion of allantoin in three healthy fistulated male Murrah buffalo calves. Results revealed that relative to basal diet, all sugars reduced the microbial protein synthesis. All supplements reduced the ruminal ammonia nitrogen concentration as compared with the basal diet. Ammonia nitrogen concentration did not differ significantly between control and treatment groups such as T_2 and T_3 however, there was significant decrease in group-T_1. The results of different carbohydrate supplementation revealed variation in urinary allantoin excretion in different groups which was found to be highest in control group fed basal diet as compared to treatment groups.

Key words: Allantoin, microbial protein, buffalo, purine derivatives

The studies on the effect of supplementation of different carbohydrates on the ruminal digestion of grass silage suggests that supplementation of sugar promote more efficient utilization of nitrogen in the rumen by microbes for synthesis of microbial proteins (Chamberlain *et al.*, 1993). Rumen microbes constitute the major source of protein supply to the ruminant's intestine. Rumen microbes are rich in nucleic acid. The purine from the rumen microbes are metabolized and excreted as their end products. The urinary excretion of purine derivatives such as allantoin, uric acid, hypoxanthine and xanthine in cattle and sheep could be used as an index of microbial protein supply to the animals provided the endogenous excretion is accounted for (Liang *et al.*,1999). This communication describes the effect of different sources of carbohydrates supplementation on ruminal ammonia nitrogen concentration and microbial protein synthesis, and its availability to the host animal by estimating allantoin excretion in the urine.

MATERIALS AND METHODS

Three apparently healthy male Murrah buffalo calves 1.5 – 2 years of age, used for this study were fed ration comprising of concentrate and wheat straw in 35:65 ratio, once daily for a period of three weeks. After the completion of three weeks adaptation period, rumen liquor samples were collected from each animal before feeding (0 h) and at 2, 4 and 6 h post feeding for two consecutive days. The samples were analyzed for ammonia nitrogen (Schwartz *et al.*,1964) and microbial protein synthesis (Cline *et al.*,1958). In the last days of experiment, experimental animals were housed in metabolism crates fitted with urinary bags. The urine collection period consisted of two days. Urine was collected over 24 h into about 200 ml of 1M sulphuric acid. Daily sub samples of urine (approximately 20 ml) were stored at -20^{0}C until analyzed for allantoin (Borchers, 1977). These animals served as control group (C). Thereafter, the animals were maintained on basal diet supplemented with (i) starch (T_1), (ii) gur (T_2), (iii) sucrose (T_3) in three separate trials. All the supplements were given @ 300 g/day/animal. Each treatment consisted of 21 days adaptation period on a particular diet. Thereafter, the rumen fluid and urine samples were collected at similar

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intervals and were analyzed in similar fashion as followed in control group.

RESULTS AND DISCUSSION

There was no significant change in overall mean ammonia nitrogen concentration in T_2 and T_3 as compared to control, whereas the values in T_1 were significantly lower as compared to other groups (Table). The decrease in T_1 may be attributed due to higher protozoal counts which has a negative correlation with rumen ammonia concentration. The overall mean microbial protein nitrogen concentrations revealed that control diet (65% roughage and 35% concentrate) had higher and other diets a lower efficiency of microbial protein synthesis in the rumen (Table). The presence of slowly degradable carbohydrates in the wheat straw (control diet) probably allowed over all utilization of the available energy by the bacteria in comparison to those grown with readily fermentable substrates. The different values obtained may not be due to the difference between carbohydrates as an energy source to support microbial protein synthesis, but could have been confounded by the influence of other factors associated with rumen environment.

There is a higher requirement for preformed amino acids/peptides for microbes utilizing sugar and starch as compared to cellulolytic organisms which grow on ammonia (Maeng *et al.*, 1976). This might have been one of the reasons of lower microbial protein synthesis observed in animals fed supplemented diets. The low efficiency of microbial protein synthesis in animals when given a diet with a higher proportion of readily fermentable carbohydrates has been attributed to higher production of propionate via acrylate pathway with a resultant lower ATP supply (Tamminga, 1979). Since both the ruminal ammonia nitrogen concentration as well as microbial protein synthesis was low on starch supplementation, it seems that low level of ruminal ammonia nitrogen was not due to its higher incorporation into the synthesis of microbial cells. It seems that average value of ammonia nitrogen (4.43mg/dl) as observed in animal fed starch supplemented diet may have been less than the optimum value (5 mg/dl) required for maximum growth of microbial cells.

The variation in urinary allantoin excretion in different groups was found to be the highest in control group (2.33 g/day) as compared to supplemented groups (1.49, 1.18 and 2.06 g/day in T_1, T_2 and T_3, respectively). Rumen microbes in general, rapidly degrade purine in diets. Purines from diet are therefore likely to be present in only negligible amounts in digesta leaving the rumen therefore, the purines present in digesta entering the small intestine are almost totally of microbial origin. Purine component are

Table
Effect of supplementation of different carbohydrates on rumen ammonia nitrogen and microbial protein nitrogen

Parameter	Group of animals	Period of sample collection: Pre-feeding hours (mean ± S.E.)	Post-feeding hours (mean ± S.E.)			Overall mean concentration (mean ± S.E.)
		0 h	2 h	4 h	6 h	
Ammonia nitrogen (mg/dl)	Control	06.83 ^{a}±0.64	09.16 ^{a}±0.84	05.83 ^{a}±0.56	04.31 ^{a}±0.49	06.54 ^{a}±0.48
	T_1	05.33 ^{b}±0.44	05.48 ^{b}±0.11	03.73 ^{b}±0.39	03.15 ^{b}±0.43	04.43 ^{b}±0.27
	T_2	05.63 ^{b}±0.46	08.40 ^{c}±0.51	06.21 ^{a}±0.48	03.45 ^{b}±0.56	05.93 ^{a}±0.44
	T_3	06.76 ^{a}±0.61	08.05 ^{c}±0.73	06.18 ^{a}±0.21	03.61 ^{a}±0.42	06.15 ^{a}±0.42
Microbial protein nitrogen (mg/dl)	Control	14.63 ^{a}±0.59	17.73 ^{a}±1.18	15.75 ^{a}±1.51	15.51 ^{a}±1.83	15.90 ^{a}±0.68
	T_1	12.98 ^{b}±1.16	13.15 ^{b}±1.45	11.05 ^{b}±1.21	13.23 ^{b}±1.24	12.60 ^{b}±0.62
	T_2	11.85 ^{b}±0.28	14.53 ^{c}±0.52	12.41 ^{c}±0.54	10.56 ^{c}±0.42	12.34 ^{b}±0.37
	T_3	10.6 $^{b\ c}$±0.22	12.10 ^{bd}±0.40	11.21 ^{b}±0.25	10.18 ^{c}±0.39	11.04 ^{b}±0.21

Values with common superscript do not differ significantly between treatments

then metabolized in ruminants to form purine derivatives (PD) that are excreted mainly in the urine. All the four components are found in urine of sheep and goat but only allantoin and uric acid are present in cattle and buffalo urine (Chen *et al.,*1996). Despite the high profile activity of xanthine oxidase, the endogenous purine excretion is lower in buffalo than in cattle (Chen *et al.,*1996, Kawashima *et al.,* 2006) suggesting a major difference between both the species. On comparing the data of present studies on rumen microbial protein synthesis with allantoin excretion, revealed that animals in control group having higher ruminal ammonia nitrogen concentration and microbial protein synthesis in the rumen also had higher allantoin excretion in the urine as compared to treatments groups. Thus we may assume that urinary allantoin excretion in buffalo may reflect microbial protein synthesis in the rumen.

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