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The effect of dietary concentrate level on rumen enzyme profile and ciliate protozoa population in cattle fed wheat straw diet

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ABSTRACT

An experiment was conducted to study the effect of dietary concentrate level in cattle on rumen fermentation characteristics, its enzyme profile and ciliate protozoal population. Twelve rumen fistulated adult cattle were divided into two equal groups (group C30 and C60). The concentrate fed to group C30 and C60 contained 32 and 16% crude protein (CP) while the concentrate roughage ratio in the diet was 30:70 and 60:40, respectively. Daily dry matter intake was higher (P<0.01) in group C60 than in group C30 amounting to 2.31 and 2.06% of body weight, respectively. The rumen fluid volume and its fluid flow rate were higher (P<0.01) in group C30 than in group C60. The pH of strained rumen liquor (SRL) was lower (P<0.01) in group C60 while total volatile fatty acids (TVFA) concentration was higher (P<0.01) in group C60 than in group C30. The ruminal concentration of total nitrogen, NH_3-N and trichloroacetic acid precipitable nitrogen (TCA-ppt.-N) were also higher (P<0.01) in group C60 than in group C30. The activities of carboxymethyl cellulase (CMC-ase) and xylanase in the rumen of group C30 was significantly (P<0.01) higher than in group C60 while the activities of amylase and protease enzymes were higher (P<0.01) in SRL of group C60. The activities of β-glucosidase and urease were not affected by level of concentrate feeding. The total number of rumen ciliate protozoa was higher (P<0.01) in group C60 (37.2 x 10^4/ml SRL) than in group C30 (30.1 x 10^4/ml SRL). The rumen protozoa of both groups were B-type population. The numbers of holotrich protozoa in the rumen of group C30 and group C60 were 1.4 x 10^4 and 2.1 x 10^4 whereas the numbers of entodiniomorphid protozoa were 28.7 x 10^4 and 35.2 x 10^4 per ml SRL, respectively. It was concluded that TVFA production as well as total nitrogen concentration and their fractionations were higher in the rumen of high concentrate fed animals. The total and differential protozoal

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counts were also higher in high concentrate fed groups, due to a higher availability of dietary starch. Additionally it was evident that the activities of polysaccharide-degrading enzymes depend on their respective substrate concentration in the rumen.

KEY WORDS: concentrates, wheat straw, nutrient intake, rumen fermentation, ciliate protozoa, rumen enzyme

INTRODUCTION

Livestock in developing countries, particularly in India, are reared by the farmers of low income group exclusively on grazing under extensive range management or stall feeding on crop residues like wheat straw or paddy straw with or without concentrate supplementation. Moreover the nutritional quality of cereal straws is very poor (Chaudhry, 1998). Dietary concentrate supplementation is known to have a positive effect on voluntary feed intake, feed digestibility, rumen microbial population and growth of the animals (Colucci et al., 1989; Johnson and Comb, 1992; Murphy et al., 1994). However the information on rumen ciliate protozoal population and rumen enzyme profile under such feeding regime is very scanty. Since rumen ciliate protozoa are associated with many critical functions in the rumen (Bonhomme, 1990; Santra and Jakhmola, 1998), assessment of their status under different levels of concentrate supplementation will have future application. Further, the dietary nutrients are degraded and metabolized by the rumen microbes while the end products are partly utilized by host animals for their maintenance and production (Jouany et al., 1988; Bonhomme, 1990). However little information is available in the literature regarding rumen microbial enzyme activities associated with feed degradation in the tropical environment.

This study was therefore conducted to examine the effect of dietary level of concentrate supplementation on rumen enzyme profile and the ciliate protozoa population in cattle maintained on a basal wheat straw diet.

MATERIAL AND METHODS

Experimental animals, feeding procedure and management

Twelve adult crossbred cattle (*Bos indicus* x *Bos taurus*) of about 227 kg mean body weight fitted with rumen cannulae were divided into two equal groups (group C30 and group C60) following a completely randomized design. Two concentrate mixtures were prepared which contained 32 and 16% crude protein (CP) and were isoenergetic (Table 1) in such a way that 30% concentrate (C1) in the diet of the cattle in C30 group supplied the same amount of CP as supplied by 60% concen-

trate (C2) in the diet of C60 group. Wheat straw was used as a basal roughage. Cattle in the C30 group received 30% concentrate (C1) and 70% wheat straw in their diet, while the ratio of concentrate (C2) to wheat straw received was 60:40 in the C60 group. All animals were individually fed at 9.00 am and weights of feed offered and refusals were recorded daily. Clean drinking water was offered *ad libitum* twice daily at about 08.30 and 15.00 h.

Rumen fermentation study

Strained rumen liquor (SRL) samples were collected for six consecutive days from the experimental animals from four sites of the rumen after 80 days of experimental feeding using a metallic probe at 0, 2, 4, 6 and 8 h post-feeding. After collection of rumen liquor, pH was recorded immediately and samples were preserved in plastic vials in a deep freeze until analyzed for total volatile fatty acids (TVFA), total-N and trichloroacetic acid-precipitable nitrogen (TCA-ppt –N). The rumen fluid volume and its fluid flow rate were determined by infusing polyethylene glycol (PEG, Mol.wt. 6000) solution (25 g PEG in 100 ml of distilled water) into the rumen through the fistula. The PEG estimation was carried out in SRL samples collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h post-feeding.

Staining and counting of rumen ciliate protozoa

A 5 ml portion of rumen liquor was pipetted with a wide orifice pipette into a screw-capped test tube containing 5 ml of formalinized physiological saline (0.85% sodium chloride solution containing 20% formaldehyde). Thereafter, two drops of brilliant green dye (2 g brilliant green and 2 ml glacial acetic acid diluted to 100 ml with distilled water) were added to the test tube, mixed thoroughly and allowed to stand overnight at room temperature. If necessary, further dilution was made with 30% (v/v) glycerol. Total and differential counts of protozoa were made in 30 microscopic fields at a magnification of 100 x. Ciliates were identified according to the method of Hungate (1966). Entodiniomorphs not identified to a genus level were classified into small entodiniomorphs (mainly entodinia with an average size of 42 x 23 μm) and large entodiniomorphs (largely diplodinia with an average size of 132 x 66 μm).

Processing of rumen liquor for enzyme estimation

Immediately after collection of rumen liquor at 4 h post-feeding for six consecutive days from all animals, the rumen liquor was processed to obtain a protozoa-rich fraction, bacteria-rich fraction and cell-free rumen fluid by refrigerated centrifugation to determine the rumen enzyme activities.

Protozoa-rich fraction

Thoroughly mixed 30 ml of SRL (4 h post feeding) were taken and centrifuged at 450 x g at 4°C for 5 min. The supernatant was decanted and the pellet formed in the centrifuge tube was washed by resuspending it in 0.05 M phosphate buffer (pH 6.8), the volume was adjusted to 30 ml and the supernatant was centrifuged again at 450 x g at 4°C for 5 min. The washing process was repeated twice, then the pellet was collected and resuspended in the phosphate buffer (15 ml) for further analysis.

Bacteria-rich fraction

The supernatant collected after removal of protozoa was again centrifuged at 27000 x g at 4°C for 20 min. The pellet was suspended in 15 ml phosphate buffer (0.05 M, pH 6.8). The supernatant was also collected and designated as cell-free rumen fluid, which was also used for estimation of enzyme activities.

Disruption of protozoa and bacterial fractions

The cells in the cellular fractions (protozoa and bacteria) were disrupted by ultrasonication at 10 μm for 10 min in separate containers kept in an ice bath. It was followed by centrifugation at 27000 x g for 20 min at 4°C. The supernatant was used for estimating the enzymes of protozoal and bacterial origin (cellular fraction).

Chemical analysis

The feed samples were analyzed for proximate composition (AOAC, 1984) and cell and cell wall constituents (Van Soest et al., 1991). The pH of SRL was estimated using a digital pH meter. Ammonia-N (Conway, 1957), total-N and TCA-precipitable nitrogen were also estimated in SRL (AOAC, 1989). Total volatile fatty acid (TVFA) analysis of rumen liquor was carried out as described by Barnett and Reid (1957) and individual VFA were fractionated by gas liquid chromatography (Erwin et al., 1961). Rumen fluid volume and its fluid flow rate were determined according to the method of Smith and McAllan (1970) using polyethylene glycol (PEG) as a soluble marker. Carboxymethyl cellulase (EC 3.2.1.4), total amylase (EC 3.2.1.1) activity, β-glucosidase (EC 3.2.1.21) and xylanase (EC 3.2.1.6) activities were measured by incubating the assay mixture (2 ml) for 60, 15, 15 and 15 min, respectively, at 39°C. Two ml of assay mixture contained 1 ml 0.1 M phosphate buffer pH 7.0, 0.5 ml test sample and 0.5 ml substrate. Substrates were carboxymethyl cellulose (1%), starch (1%), salicin (0.5%) and xylan (0.25%) (from

oat spelts) for cellulase, amylase, β-glucosidase and xylanase, respectively. The reducing sugars produced were estimated as monosaccharide by dinitrosalicyclic acid method (Miller, 1959). For protease estimation the assay mixture consisted of 1 ml of 0.1 M phosphate buffer pH 7.0, 0.25 ml of 1% casein, 0.25 ml enzyme and 0.5 ml distilled water. The mixture was incubated for 2 h at 39°C. The reaction was stopped by adding 2 ml of 10% trichloroacetic acid. The supernatant was collected by centrifugation and the hydrolyzed protein was estimated by the method of Lowry et al. (1951). Enzyme (cellulase, amylase, β-glucosidase, xylanase and protease) activity is defined as mg product released $ml^{-1}h^{-1}$ at 39°C. Urease (EC 3.5.1.5) activity was determined by estimating hydrolysis of urea to ammonia as described by Weatherburn (1967). Urease enzyme activity was defined as μg of ammonia released $ml^{-1}h^{-1}$ at 39°C. For estimation of urease enzyme, one ml of assay mixture contain 0.5 ml 0.1 M phosphate buffer pH 7.0, 0.25 ml test sample, 0.25 ml substrate (15 mg urea and 8 mg EDTA di-sodium salt in 25 ml 0.1 M phosphate buffer pH 7.0). Counting of rumen ciliate protozoa was done in 30 microscopic fields in a haemocytometer counting chamber at a magnification of 100 times and calculated as:

$$\text{Number of protozoa/ml rumen liquor} = \frac{N \times A \times D}{a \times V}$$

where:

N – average number of cells/microscopic field
A – area on slide on which the sample is spread (area of the cavity of haemocytometer)
D – dilution
a – area of microscopic field
V – volume of rumen liquor in the counting chamber.

Statistical analysis

Statistical analysis of the data was carried out following the method of Snedecor and Cochran (1989) and treatment differences were compared by Duncan's Multiple Range test (Duncan, 1955).

RESULTS

Chemical composition

The physical and chemical composition of the experimental ration is given in Table 1. The concentrate mixture C1 and C2 offered to group C30 and group C60 were contained 16.0 and 32.0% CP, respectively.

TABLE 1

Composition of experimental diets

Indices	Concentrate mixture		Wheat straw
	C_1	C_2	
Physical composition, %			
crushed maize	26	39	
wheat bran	7	40	
groundnut cake	64	18	
mineral mixture [a]	2	2	
salt	1	1	
Chemical composition, % DM			
organic matter	90.2	89.9	93.1
crude protein	31.9	16.0	3.1
ether extract	4.1	3.20	1.5
total carbohydrate[c]	54.2	70.6	88.5
neutral detergent fibre	37.9	37.4	81.7
acid detergent fibre	11.7	10.4	51.8
lignin	3.6	3.9	10.9
Gross energy, kcal/g	4.3	4.3	4.1

[a] mineral mixture contained (%): Ca, 28.0; P, 6.2; common salt, 35.8; Fe, 0.4; I, 250 ppm; Mn, 740 ppm; Cu, 280 ppm, S, 0.15

[b] vitamin supplement (Rovimixe)(R) was added 20 g per 100 kg of concentrate mixture. Rovimixe(R) contained vit. A , 40000 IU; vit. B_2, 20 mg and vit D_3, 5000 IU per g

[c] total carbohydrate: organic matter - (crude protein + ether extract)

Dry matter intake and rumen fermentation pattern

Daily dry matter intake was higher ($P<0.05$) in group C60 (diet contained 60% concentrate) than in group C30 (diet contained 30% concentrate), amounting to 2.31 and 2.06% of body weight, respectively (Table 2). The rumen fluid volume and fluid flow rate were higher ($P<0.01$) in group C30. The rumen pH decreased from 0 to 4 h post-feeding in the SRL samples followed by increase at 6 to 8 h post-feeding (Figure 1). The TVFA concentration at similar time intervals exhibited reverse trend to that of pH (Figure 2) as the TVFA and pH are inversely related. The pH of SRL was lower ($P<0.01$) in group C60 than in C30 (6.74 vs 6.48) while TVFA concentration was higher ($P<0.01$) in group C60 than in group C30. The acetate concentration was higher ($P<0.01$) in SRL of group C30 while propionate was higher in SRL of group C60. However, the butyrate concentration was similar in the two groups. The concentration of total-N, NH_3-N and TCA-ppt.-N were also higher ($P<0.01$) in SRL of group C60 than in group C30. The concentration of total N, TCA-ppt.-N and NH_3-N in the serial rumen liquor sample peaked at 4 h

TABLE 2

Nutrient intake and rumen fermentation pattern

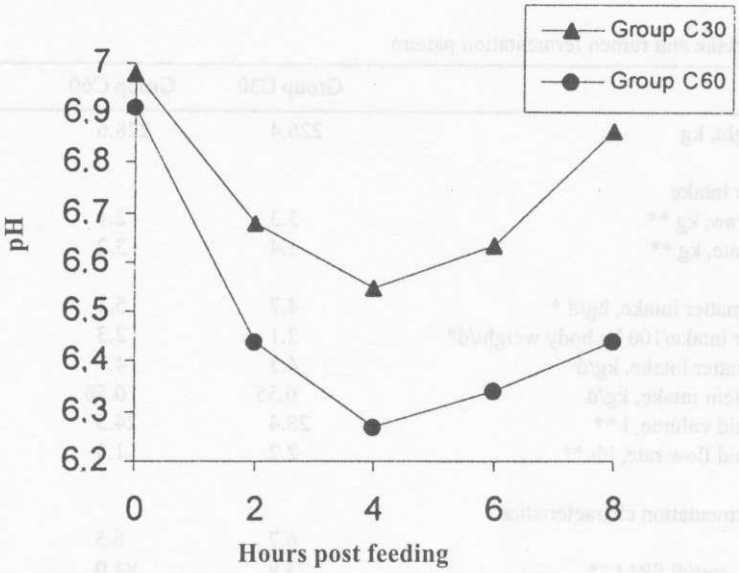
Indices	Group C30	Group C60	SEM
Body weight, kg	226.4	228.6	5.13
Dry matter intake			
wheat straw, kg **	3.3	2.1	0.11
concentrate, kg **	1.4	3.2	0.5
Total dry matter intake, kg/d *	4.7	5.3	0.07
Dry matter intake/100 kg body weight/d*	2.1	2.3	0.04
Organic matter intake, kg/d	4.3	4.7	0.21
Crude protein intake, kg/d	0.55	0.56	0.14
Rumen fluid volume, l **	28.4	24.5	1.01
Rumen fluid flow rate, l/h **	2.2	1.3	0.06
Rumen fermentation characteristics			
pH**	6.7	6.5	0.03
total – N, mg/dl SRL[a] **	73.9	84.0	2.85
NH_3 – N, mg/dl SRL**	24.3	28.0	1.03
[b]TCA – ppt. – N, mg/dl SRL**	59.9	64.2	1.79
[c]TVFA, mM/dl SRL**	8.1	9.8	0.31
acetate, %**	68.2	60.0	1.01
propionate, %**	22.5	29.9	0.63
butyrate, %	9.4	10.2	0.29

[a] strained rumen liquor
[b] trichloroacetic acid precipitable nitrogen
[c] total volatile fatty acids
* P<0.05, **P<0.01

post-feeding followed by a gradual decrease achieving a minimum at 0 h (Figures 3, 4 and 5).

Rumen enzyme activities

The activities of total carboxymethylcellulase and xylanase were higher (P<0.01) in SRL of group C30 while the activities of amylase and protease were higher in group C60 (Table 3). The activities of β-glucosidase and urease were not affected by level of concentrate feeding. CMC-ase and xylanase activities were higher (P<0.01) in the cellular fraction whereas the activities of amylase was higher in extracellular fraction. The activities of β-glucosidase, protease and urease were detected only in the cellular fraction. The trends in rumen enzyme activities for both cellular and extracellular fraction were however similar in the two treatment groups.



Figre 1. Effect of dietary concentrate supplementation on rumen pH in crossbred cattle

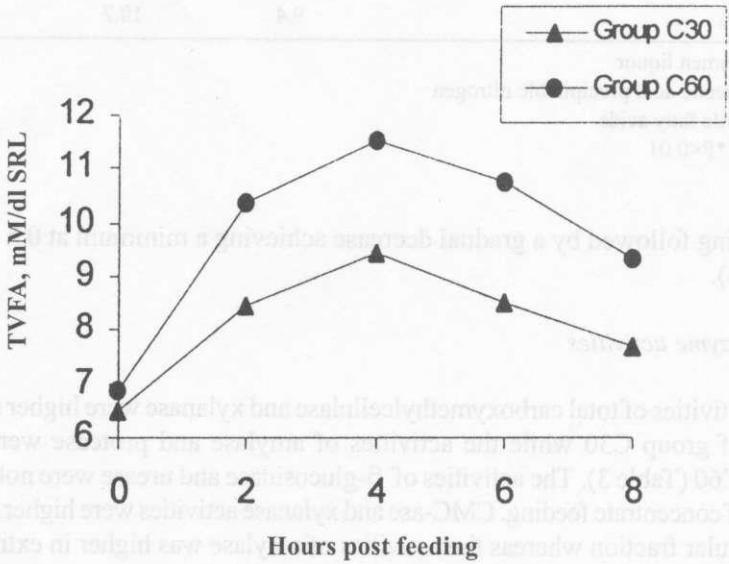


Figure 2. Effect of dietary concentrate supplementation on total volatile fatty acids concentration (TVFA) in the rumen of crossbred cattle

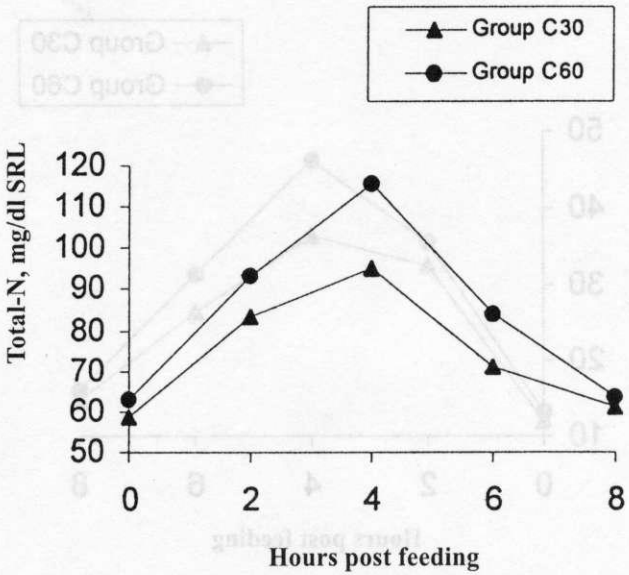


Figure 3. Effect of dietary concentrate supplementation on total nitrogen concentration in the rumen of crossbred cattle

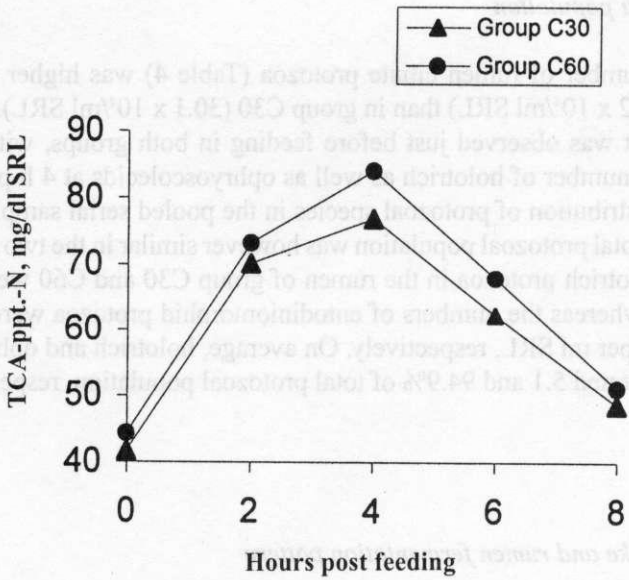


Figure 4. Effect of dietary concentrate supplementation on TCA precipitable nitrogen concentration in the rumen of crossbred cattle

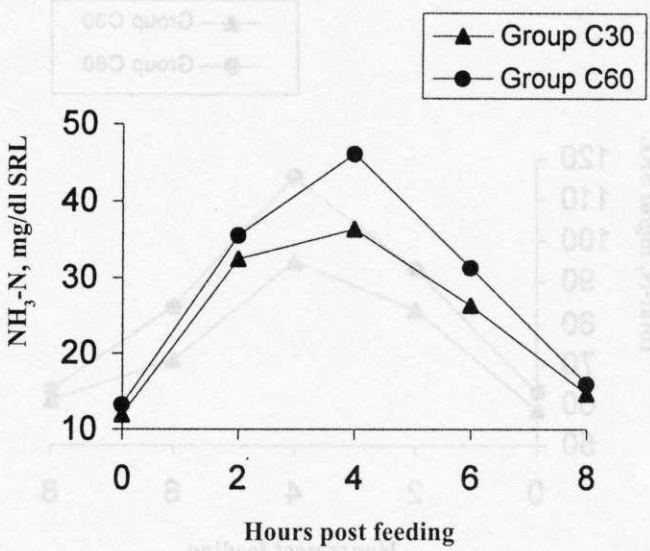


Figure 5. Effect of dietary concentrate supplementation on NH_3-N concentration in the rumen of crossbred cattle

Rumen protozoa population

The total number of rumen ciliate protozoa (Table 4) was higher ($P<0.01$) in group C60 (37.2×10^4/ml SRL) than in group C30 (30.1×10^4/ml SRL). The lowest protozoal count was observed just before feeding in both groups, with an abrupt increase in the number of holotrich as well as ophryoscolecids at 4 h post feeding. The relative distribution of protozoal species in the pooled serial samples as a percentage of the total protozoal population was however similar in the two groups. The numbers of holotrich protozoa in the rumen of group C30 and C60 were 1.4×10^4 and 2.1×10^4 whereas the numbers of entodiniomorphid protozoa were 28.7×10^4 and 35.2×10^4 per ml SRL, respectively. On average, holotrich and ophryoscolecid protozoa constituted 5.1 and 94.9% of total protozoal population, respectively.

DISCUSSION

Dry matter intake and rumen fermentation pattern

The daily intake and digestibility of nutrients by ruminant animals depend on the nature and proportion of roughage in the animal diet (Slabbert et al., 1992;

TABLE 3

Rumen enzyme activities in cattle

Enzymes	Enzyme activity						SEM
	cellular		extra cellular		total		
	Group		Group		Group		
	C30	C60	C30	C60	C30	C60	
Cm-cellulase**	87.3	71.2	26.4	23.4	113.7	94.6	4.93
α-Amylase**	71.8	85.5	179.0	207	250.8	292.5	10.11
Xylanase**	131.9	115.5	68.9	59.3	200.7	174.8	7.03
β-Glucosidase	10.9	10.8	00	00	10.9	10.8	0.98
Protease**	25.3	40.0	00	00	25.3	40.0	5.97
Urease	2.6	2.5	00	00	2.6	2.5	0.81

* P<0.05, ** P<0.01

TABLE 4

Total and differential counts of ciliate protozoa population (x 10^4/ml) in rumen liquor of cattle

Protozoa	Group		SEM
	C30	C60	
Isotricha	0.4	0.6	0.39
Dasytricha	1.1	1.4	0.51
Total holotrich*	1.5	2.0	0.04
Large entodiniomorphs**	11.4	13.5	0.15
Small entodiniomorphs**	17.3	21.7	0.32
Total entodiniomorphs**	28.7	35.2	0.04
Total rumen protozoa**	30.2	37.2	1.73

* P<0.05, ** P<0.01

Murphy et al., 1994). This is also evident from the results of the present experiment, in that increasing the level of wheat straw in the diet of experimental cattle (group C30) decreased daily dry matter intake. In the present study, the rumen fluid volume and its fluid flow rate were higher in low concentrate fed animals as dietary concentrate level influence the rumen fluid volume and its outflow (Sutton, 1980). Such a response was expected due to lower bulk density of high roughage fed animals (Goetsch and Galyean, 1982). Moreover, high-roughage and low-concentrate diets also stimulate saliva secretion, which leads to higher rumen fluid volume and its flow rate (Church, 1988). The observed lower pH and higher TVFA in animals of group C60 were due to their higher starch intake which will favour higher production of TVFA leading to significant decrease in pH of rumen liquor

(Eun, 1990). The higher concentration of acetate in group C30 and propionate in group C60 resulted from different proportion of roughage and concentrate in experimental diets. The higher concentration of total nitrogen, TCA precipitable nitrogen and ammonia nitrogen in high concentrate fed group (C60) in comparison to low concentrate fed group (C30) was due to higher microbial activity and higher microbial biomass production (Punia and Sharma, 1980; Mallikarjunappa et al., 1983). It is also a fact that the relative dominance of microbial population varies according to the nature of the diet.

Rumen enzyme profile

The higher contents of dietary cellulose, hemicellulose and starch stimulated the production of respective enzymes, i.e. cellulase, amylase and xylanase responsible for their hydrolysis. The higher proportion of wheat straw, as a source of ligno-cellulose substrate in the diet of group C30 would have stimulated the microbial population to synthesize the enzymes responsible for its hydrolysis leading to higher digestibility of cell wall constituents in high roughage fed animals (Poore et al., 1990; Kennedy and Bunting, 1992). This finding is in agreement with earlier report of Agarwal et al. (1993) that green oats, containing higher concentrations of cellulose and hemicellulose in comparison to berseem, stimulated higher production of CMC-ase and xylanase in the rumen. Despite similar crude protein intake in the two groups, the significantly higher production of protease in high concentrate fed animals might be due to higher rumen microbial activity and microbial biomass production contributing to observed higher rumen concentration of total nitrogen and ammonia nitrogen. Further, the majority of rumen bacteria are capable of producing proteolytic enzymes and the production of protease might be a direct function of quantity of microbial biomass in the rumen.

The values of fibre degrading enzymes (cellulase and xylanase) were lower in extracellular fraction because these enzymes are bound with cellular coat of the rumen microbes and only a little amount is released in the rumen medium due to mechanical injury or disintegration of the fibre degrading microbes. The observation is supported by an earlier finding by Agarwal et al. (2000). However, higher amylase enzyme activity was observed in extracellular than in cellular fraction. Higher amylase enzyme activity in extracellular fraction might be due to mechanical injury or disintegration or lysis of amylolytic microorganisms before separation of bacterial and protozoal fraction. Similar type of finding also observed by Santra (1992) in buffaloes. β-glucosidase and protease enzymes are cell bound and that's why no activity of these enzymes observed in extracellular fraction. Similarly, no urease enzyme activity was observed in extracellular fraction. Urease is associated with the particulate microbial fraction of rumen fluid and mainly of bacterial origin which is cell bound (Wallace and Cotta, 1988).

Rumen protozoal population

The rumen protozoa of both groups belonged to B-type population, due to the presence of *Polyplastron multivesiculatum* (Coleman, 1980). The total number of protozoa, holotrichs and spirotrichs (large and small) were higher ($P<0.01$) in the high concentrate fed group due to the availability of more dietary starch, which is one of the stimulating factors for protozoal growth (Bonhomme, 1990). After the feed was ingested, protozoal numbers in the rumen liquor increased within the first 4 h of post feeding due to the migration of ciliate protozoa from the rumino-reticular wall to the rumen in response to chemical stimuli originating from the diet (Abe et al., 1981; Dehority and Tirabasso, 1989; Ankrah et al., 1990; Kamra et al., 1991). Progressive increase in total number of ciliate protozoa in the rumen liquor of the animals of both the groups up to 4 h post feeding, followed by a gradual decline in their number, could be ascribed to sequestration of rumen protozoa (Kamra et al., 1991). These finding are in agreement with the observation of Soni and Sharma (1982) in goats, Prasad and Pradhan (1990) in cattle and sheep and Kamra et al. (1991) in black bucks.

CONCLUSIONS

It is concluded from this study that the TVFA concentration, total nitrogen concentration and their fractions were higher in the rumen of high concentrate fed animals. The total and differential protozoal count were also higher in high concentrate fed groups, due to the higher availability of dietary starch. Additionally, the activities of polysaccharide-degrading enzymes depend on their respective substrate concentration in the rumen.

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STRESZCZENIE

Wpływ udziału paszy treściwej w dawce ze słomy pszennej na skład enzymów i produkcję orzęsków w żwaczu bydła

Celem pracy było zbadanie wpływu udziału paszy treściwej w dawce dla bydła na przebieg fermentacji w żwaczu, skład enzymów oraz produkcję orzęsków w żwaczu. Doświadczenie przeprowadzono na 12 dorosłych zwierzętach, podzielonych na dwie grupy. Stosunek paszy treściwej do objętościowej wynosił 30:70 (grupa C30) i 60:40 (grupa C60), a zawartość białka ogólnego w paszy treściwej 32 i 16%, odpowiednio. Zwierzęta grupy C60 spożywały więcej ($P<0,01$) suchej masy niż

grupy C30, odpowiednio 2,31 i 2,06% masy ciała. Objętość płynnej treści żwacza i jej tempo wypływu były większe ($P<0,01$) w grupie C30 niż C60. U zwierząt grupy C60 pH płynu żwaczowego było niższe ($P<0,01$), podczas gdy stężenie sumy LKT, azotu całkowitego, $N\text{-}NH_3$ oraz związków azotu wytrąconych kwasem trójchlorooctowym było większe ($P<0,01$) niż u zwierząt grupy C30.

Aktywność celulazy karboksylmetylowej oraz ksylanazy w żwaczu zwierząt C30 była większa ($P<0,01$) niż w grupie C60, natomiast aktywność amylazy i proteazy była większa ($P<0,01$) w płynie żwaczowym zwierząt grupy C60. Aktywność β-glukozydazy i ureazy nie zależała od ilości paszy treściwej w dawce. Całkowita liczba orzęsków żwaczowych, należących do typu B, była większa ($P<0,01$) w grupie C60 ($37,2 \times 10^4$/ml) niż w grupie C30 ($30,1 \times 10^4$/ml). Liczebność holotricha w płynie żwacza wynosiła $1,4 \times 10^4$/ml i $2,1 \times 10^4$, liczebność *entodiniomorpha* $28,7 \times 10^4$ i $35,2 \times 10^4$/ml, odpowiednio u zwierząt z grup C30 i C60.

Stwierdzono, że produkcja LKT oraz stężenie azotu i jego frakcji w żwaczu, jak też liczba pierwotniaków były większe w żwaczu zwierząt otrzymujących w dawce więcej paszy treściwej.