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Growth performance and fibre utilization of Murrah male buffalo calves fed wheat straw based complete feed blocks incorporated with superior anaerobic fungal zoospores (*Neocallimastix* sp. GR-1)

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ABSTRACT

In vivo study was designed to assess the influence of anaerobic fungal zoospores of *Neocallimastix* sp. GR-1, incorporated in wheat straw based complete feed blocks on growth performance, nutrient utilization, ruminal fermentation pattern and nutritive evaluation in terms of digestible crude protein (DCP) and total digestible nutrients (TDN) in male calves. Male Murrah buffalo calves (12), 5–7 months -old, weighing 82±6.60 kg, were randomly divided into 2 equal groups of 6 each. Animals in control group were fed a complete feed blocks (consisting of 50:50 wheat straw: concentrate mixture) along with 2 kg green maize fodder/animal/d. Treatment group of animals were fed with complete feed blocks as those for control group but incorporated with fungal zoospores of *Neocallimastix* sp. GR-1 to about 140×10^6 zoospores/ block of 14 kg along with 2 kg green maize/animal/d. Daily feed intake and fortnightly body weights of all the calves were recorded during the 172 d experimental period. After 90 d of experimental feeding a digestibility trial of 7 d duration was conducted and samples from feed blocks, green maize, faeces and refusals were collected. After 150 d of experimental period, samples of rumen liquor were collected through stomach tube before feeding (at 0 h) for 3 consecutive days. The results showed 16% increase in body weight gains of calves of zoospores incorporated 'Z' group of animals over the control group 'C' with similar daily feed intake. Also % feed efficiency enhanced by 28.7% over the control diet. The digestibility of DM, OM, CP, CF, NDF, ADF, ADL and cellulose of complete feed blocks increased significantly in treatment group. The digestible energy value in terms of %TDN also enhanced significantly in treatment group compared to control group. The pH, NH_3-N and protozoan count/mL were lower, whereas total-N, TCA-N, TVFAs, number of fungal zoospore and bacteria/mL in rumen liquor were significantly higher in treatment group indicating that the nutritive value of wheat straw based complete feed blocks can be enhanced with the incorporation of elite fungal zoospores. This technology developed to improve the nutritive value of wheat straw based diets, can be used at the compound feed industry level for providing a well balanced wheat straw based complete feed rations to the animals at farmers door.

Key words: Anaerobic fungi, Fibre utilization, Growth rate, Rumen, Zoospores

Most of the anaerobic fungi have active role to play in fibre degradation as evidenced by secretion of their cell wall degrading enzymes such as trans-p-feruloyl and trans-P-coumaroyl esterases, microcrystalline cellulases, hemicellulases, pectinases and protease etc. (Kopecny 1995).The enzymes p-feruloyl and p-coumroyl have got the unique ability to penetrate into the fibrous feed particles which help in access of other rumen microbes to the secondary cell wall of feed particles to make their digestible energy available to the animal. Thus rumen anaerobic fungi play an important role in the digestion of poor quality straws and their proliferation in anaerobic media to enhance their activity to breakdown bonds between the lignin and hemicellulose to improve nutritive value of cereal straw based diets (Gordon *et al.* 1995, Sehgal *et al.* 2002, Tripathi *et al.* 2007a). Efforts were made to enhance digestibility of poor quality lignocellulosic roughages by addition of microbial or chemical feed additives. Among microbial additives, there are evidences of definite positive relationships between anaerobic fungi in rumen and increased voluntary intake of low digestible fibrous feeds. Administration of elite ruminal fungi isolates from goat, cattle and buffaloes increased the digestibility and nutritive value of wheat straw based rations (Sehgal *et al.*2008, Swati *et al.* 2010).

Orpinomyces sp. (C-14) isolated from cattle was found the most promising isolate for degradation of wheat and

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paddy straws. Tripathi *et al.* (2007a) isolated and identified *Piromyces* sp WNG-12 from blue bull as elite fungus that has higher CMCase, cellobiase and xylanase activities than other 11 normal fungal species isolated from the faeces of the blue bull. Further *Piromyces* sp. WNG-12 culture administration increased growth rate, feed efficiency and nutritive value of wheat straw based ration (Tripathi *et al.* 2007b) in buffalo calves. Sehgal *et al.* (2008) concluded that *Neocallimastix* sp. GR-1, isolated from goat, was a promising anaerobic fungus to act as a probiotic in the rumen of buffalo calves to improve the nutritive value of wheat straw based complete feed diets. They also studied the influence of anaerobic fungal administration on growth, rumen fermentation and nutrient digestion in female buffalo calves and reported that *Neocallimastix* sp. GR-1 gave higher growth rate and improved biochemical parameters compared to control diet. Swati *et al.* (2010) showed that *Piromyces* sp. WNG-12 could be a potential anaerobic fungi that could be exploited as probiotic in the rumen of lactating buffaloes fed on a wheat straw based total mixed ration for enhancing milk production and its composition compared to control. Elite fungi developed into zoospores that can resist adverse environmental conditions like low, normal and high temperature which incorporated in wheat straw based feed blocks to study the growth, nutrient utilization and ruminal fermentation, pattern of a wheat straw based complete feed block, supplemented with inoculums of lignin degrading superior anaerobic fungal zoospores of *Neocallimastix* sp. GR-1.

MATERIALS AND METHODS

Fungal culture and inoculum preparation: Rumen anaerobic fungal isolates of fungi *Orpinomyces* sp C-14 (isolated from domestic cattle), *Neocallimastix* sp GR-1 (from grazing and browsing goats) and *Piromyces* sp WNG-12 (from wild blue bull), preserved in the anaerobic broth medium (Hungate 1969) were revived by subculturing and tested morphologically by microscopy with cotton blue dye. These were cultured and purified in anaerobic broth (Joblin 1981) to make them free from any contamination. The isolates of *Neocallimastix* sp. GR-1 (Thareja *et al.* 2006) were chosen for *in vitro* studies because of their maximum hydrolytic enzyme activity.

These were also tested for type of colony: monocentric or polycentric and morphological characteristics with respect to feature and their enzymatic activities of zoospores including cellulase, cellobiase and xylanase to match them as per that of their parent culture. With the exception of reducing agents (sodium bicarbonate and cysteine hydrochloride), all the quantified ingredients of the medium were taken in 250 mL flask. The pH was adjusted to 6.7±0.11. The medium was boiled to free it from dissolved oxygen and cooled under the flow of carbon dioxide. Before autoclaving of media, the quantified reducing agents were added. The flask containing the media was closed by rubber cork and tied through wire and autoclaved. The colorless or slight yellowish appearance of fungal culture was indicative of perfect anaerobic condition, and was selected for the *in vitro* study while any pinkish coloration was indicative of aerobic or imperfect anaerobic condition and was discarded (Joblin 1981). 1 mL anaerobic rumen fungal culture was transferred to each test tube having anaerobic broth media filled up to brim, in the presence of recommended dose of antibiotics. By this sub culturing the culture was revived and checked first by roll tube technique and thereafter by microscopy for purity and counting. For roll tube anaerobic diluents were used. For the selected *Neocallimastix* sp GR-1 culture (0.5 mL inoculum) at desired level of dilution was added in roll tubes containing 4 mL molten media and desired quantity of antibiotic solution. The tubes were rolled over platform of crushed ice to solidify media uniformly as a thin film on the inner wall of roll tubes. The oxygen free carbon dioxide was passed through roll tubes. The tubes were kept in carbon dioxide incubator at a temperature of 39±1°C in inverted position. During incubation, roll tubes were regularly inspected for appearance of colonies visible through naked eye. The numbers of colonies were also counted.

Development and production of fungal zoospores: Sub culturing of *Neocallimastix* sp. GR-1 in anaerobic media was done for the growth of large number of zoospores by providing stress or using complex nutrients replacing simple sugars in the anaerobic broth. For providing stress, temperature was increased or decreased from 39° to 45°C or 33°C or numbers of days were increased for the production of fungal zoospores. The zoospores were also counted by direct microscopic method (Fitts *et al.* 2004).

Development of zoospores of Neocallimastix sp. *GR-1 for mixing in complete feed blocks: Neocallimastix* sp. GR-1 broth culture was prepared in glass bottle (280 mL capacity) containing 250 mL of autoclaved anaerobic fungal media (Joblin 1981) along with required quantity of antibiotic solution to prevent any bacterial contamination (it was standardized by dilution and counting) in roll tube. 8 mL broth culture was to be added to 250 mL media to get a zoospores count of 10^6 zoospores/mL in culture). Oxygen-free carbon dioxide gas was also passed through the media to make the media completely anaerobic. After keeping the bottles in CO_2 jar, the jar was shifted to the CO_2 gas incubator at 39±1°C temperature for 7 d. The fungal colony count was also made from Hungate's roll tube and 140 mL of media containing *Neocallimastix* sp. GR-1 at a concentration of 10^6 zoospores/mL was mixed in a complete feed blocks of 14 kg and was fed to each animal in the treatment group for 172 d experimental period. The animals were also made to adapt to experimental feed for 5 d before the start of experiment.

Selection and distribution of experimental animals: Murrah male buffalo calves (12) of similar age (5 to 7 months) with an average initial body weight of 81±7.31 kg were selected from the herd of National Dairy Research Institute, Karnal. The initial live weights were based on 2 consecutive daily weighing after 5 days feeding of experimental complete feed blocks without fungal

zoospores for adaptation. The animals were randomly allocated into 2 groups of 6 each. Control group of 6 animals were fed individually *ad lib*. with complete feed blocks consisting of 50% wheat straw and 50% concentrate mixture (The ingredient composition of complete feed block was wheat straw 50%, maize 15.42%, GN cake (Exp.) 9.81%, mustard cake (Exp.) 5.60%, wheat bran 9.35%, deoiled rice bran 5.14%, molasses 2.80% mineral mixture 0.94% common salt 0.47% and urea 0.47% on DM basis) along with 2 kg green maize/calf/day for meeting its vitamin A requirements. A weighed quantity (14 kg complete feed block) was offered to each calf to meet the nutritional requirements of the growing buffalo calves as per Kearl (1982) for a body weight gain of 400 g/d. When this block got finished; another complete feed block of the same weight was placed before the animal and so on. No refusal was collected till the experiment was over. Treatment group of 6 animals were offered the similar complete feed blocks consisted of 50% wheat straw and 50% concentrate mixture having same ingredient composition as of control group but these blocks were also incorporated with zoospores of *Neocallimastix* sp. GR-1 broth culture (1420×10^6 zoospores/mL) in a complete feed blocks of 14 kg along with 2 kg green maize/oats/calf/d up to an experimental period of 172 d. Refusal of all the animals was weighed only at the end.

Housing and management: Throughout the experimental period, animals were maintained in an open shed with *pucca* floor, having arrangement for individual feeding. Healthy surroundings and proper sanitary conditions were maintained. During this period, mangers, floor and walls were cleaned regularly, so as to keep the buffalo calves away from any infection. The animals were provided with fresh and clean tap water free choice twice daily at 10.00 h and 17.00 h.

Growth studies: At the start of the experiment, animals were weighed for 2 consecutive days to record their average initial body weight. For the growth study, the weight of each individual calf was recorded at a fortnightly interval for 172 d. Animals were weighed in the morning before they were fed or watered and the absolute rate of growth was estimated (Brody 1945). The total and daily feed intake was calculated by subtracting a negligible quantity of feed refusal both from total feed offered for the whole experimental period. The feed conversion ratio was also calculated for both the groups.

Digestibility trial study: After 90 d of experimental feeding, a digestibility trial of 7 days was conducted on all the 12 male buffalo calves in a specially designed stall. Body weights of the animals were recorded before and after digestibility trial on 2 consecutive days. A proper record of total quantity of complete feed blocks offered, refusal, consumed and faeces voided by each buffalo calf was maintained during the period. At the end of the digestibility trial, rumen liquor was collected through a stomach tube before feeding animals (Lane *et al.* 1968).

Estimation of proximate constituents and fibre fractions: Ground samples of concentrate mixture, wheat straw, complete feed blocks, green maize fodder and faeces were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), nitrogen free extracts (NFE) and total ash (TA) according to AOAC (1992). The fibre fractions NDF, ADF, ADL, hemicellulose and cellulose were estimated as per Van Soest *et al.* (1991).

Analysis of rumen liquor: Immediately after collection of rumen liquor samples, the pH was recorded and total VFA were estimated (Barnett and Reid 1957). Total nitrogen and tricarboxylic acid precipitable nitrogen were estimated by Kjeldahl's method (AOAC 1992). Micro- diffusion technique of Conway (1962) was used to estimate NH_3-N. After appropriate dilution of samples, direct microscopic counts of fungal zoospores were made and expressed per mL of rumen fluid. All data were subjected to statistical analyses using the randomized block design by Snedecor and Cochran (1980). Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Per cent proximate principals and cell wall contents of complete feed blocks and green fodder maize: The per cent proximate composition of complete feed blocks and green maize fodder is given in Table 1. Experimental complete feed blocks were also incorporated with 140×10^6 zoospores/mL of *Neocallimastix* sp. GR-1 per block of 14 kg. Because of 50% of wheat straw in the blocks the NDF, ADF and acid detergent lignin contents were higher; and hemicellulose was low, however the chemical composition of the block can meet the nutritional requirements for growth about 400g/d of calves (Kearl 1982). Maize fodder being offered to animals about 2 kg/d was also a good source of CP 10.25%, EE 3.35% and hemicellulose 22.89% (Table 1). However, this was given only to meet the vitamin A requirements of the animals. In this study the dry matter of maize given was 21.0% and the analysed values were commensurating with that of Tripathi *et al.* (2007b). The

Table 1. Proximate analysis (%) and cell wall contents (%) of concentrate mixture, wheat straw, green maize and complete feed block (CFB) fed to experimental animals (on DM basis)

Principles	Concentrate mixture	Wheat straw	Maize green	Complete feed block
Dry matter	89.55	89.61	21.00	91.41
Organic matter	92.50	89.62	91.25	90.16
Crude protein	20.74	2.88	10.25	12.41
Ether extract	4.47	1.23	3.35	3.33
Crude fibre	9.67	42.44	31.30	29.77
Nitrogen free extracts	57.62	43.07	46.35	44.65
Total ash	7.50	10.38	8.75	9.84
Neutral detergent fibre**	25.17	74.49	59.05	51.85
Acid detergent fibre**	15.25	56.35	36.16	38.37
Acid detergent lignin	4.48	8.66	5.23	7.6
Hemicellulose	9.92	18.14	22.89	13.48
Cellulose	10.77	38.21	30.93	30.77

** Determined on ash-free basis.

Table 2. Growth performance of male Murrah buffalo calves fed wheat straw based complete feed blocks without or with fungal zoospores of *Neocallimastix* sp., GR-1

	Groups		't' values
	Control	Treatment	
Initial body wt. (kg)	81.25±7.30	82.33±6.60	0.19^{NS}
Final body wt. (kg)	156.37±13.06	171.00±14.33	8.32*
Total body wt. gain (kg)	75.12±4.38	88.38±3.34	4.89*
Daily Gain (g)	436.74±35.18	515.52±20.11	3.87*
Dry matter intake (DMI) (kg)			
a) From complete feed blocks	525.91±30.53	512.47±16.76	0.22^{NS}
b) From green maize	72.78±0.39	72.75±0.49	0.22^{NS}
Total DMI (kg)	598.50±30.53	584.70±16.76	0.22^{NS}
Average DMI /day (kg)	3.47±0.17	3.39±0.09	0.22^{NS}
Feed conversion ratio (kg DM/kg gain)	8.00±0.06	6.53±0.07	3.87**
% Feed efficiency (kg gain/ 100 kg DMI)	12.48±0.12	16.08±0.08	3.96*

[a], After 172 days; * P≤0.05; NS, nonsignificant ; **P≤0.01.

Table 3. Digestibility coefficient (%) of various nutrients of wheat straw based complete feed block without or with fungal (*Neocallimastix* sp., GR-1) zoospores in growing male Murrah buffalo calves

	Groups		't' values
	Control	Treatment	
Dry matter	53.04±0.49	61.70±1.24	4.72*
Organic matter	53.37±1.51	62.43±1.05	5.15*
Crude protein	57.55±1.03	65.17±0.46	6.19**
Ether extract	76.00±1.00	78.82±1.27	1.35^{NS}
Crude fibre	48.88±0.96	58.98±1.18	6.60**
Nitrogen free extracts	53.88±0.69	62.25±1.67	3.69*
Neutral detergent fibre	47.50±1.39	57.22±0.61	5.57**
Acid detergent fibre	44.26±0.62	54.29±0.72	7.23**
Cellulose	57.39±0.63	68.19±0.85	7.13**

** P≤0.01 ;* P≤0.05.

proximate principals of wheat straw (Table 1) viz. CP 2.88, CF 42.44, NFE 43.07 and total ash 10.38% were in close agreement with the values reported in the literature i.e. 3.0 to 4.0, 30.5 to 48.9; 37.5 to 57.4 and 7.2 to 10.9% respectively (Sehgal and Makkar 1994, Givens and Moss 1995, Sehgal *et al.* 1999, Dey *et al.* 2004 and Tripathi *et al.* 2007b).

Animal growth performance: The average daily gain was highest in treatment group followed by control group (Table 2). Between treatment group and control group, the differences were statistically significant (P<0.01). Body weight gain of buffalo calves was improved by 16% fed with wheat straw based complete feed blocks with fungal zoospores of *Neocallimastix* sp.GR-1 over the control group.

The reason for getting higher body weight gains in treatment group (515.52 g/d) compared to control (436.74 g/d) group with almost at similar level of DM intake in both the groups and was due to the incorporation of elite fungal zoospores of *Neocallimastix* sp. GR-1 in treatment group which in turn, have increased the digestibility of nutrients particularly that of CF, NDF and ADF (Table 2). Initial differences were narrow, but as buffalo calves grew older the differences widened, indicating the establishment of zoospores in rumen of treatment group (Table 2). Dey *et al.* (2004) also observed an improved daily gain with administration of fungi into the diet of cow calves. Compared to the control group, they observed a 15.4% higher body weight of crossbred cow calves fed a wheat straw based complete feed mixture supplemented with *Orpinomyces* sp. C-14. The present study showed that the DMI per day remained nearly the same in 2 groups. However, due to variation in body weight gain, the feed conversion ratios were found to be significantly improved in wheat straw based complete feed blocks with zoospores of *Neocallimastix* sp. GR-1 over the control group (Table 2). Tripathi *et al.* (2007b) reported that compared to the control group, body weight gain of buffalo calves improved by 20.6 and 29.7% after feeding diets supplemented with *Orpinomyces* sp. C-14 and *Piromyces* sp. WNG-12, respectively. Gordon *et al.* (2000) reported that supplementation of sheep with a non-indigenous rumen anaerobic fungi, having highest cellulolytic activities, increased voluntary DMI by 12%. The present study is in a close agreement with results of Dey *et al*. (2004) and Tripathi *et al.* (2007b). This indicated that the feed efficiency of complete feed blocks based on wheat straw incorporated with zoospores of *Neocallimastix* sp. GR-1 can be enhanced in growing buffalo calves.

Digestibility of nutrients: The digestibility of DM, CP and cell wall contents was significantly higher in treatment group compared to control (Table 3).These results may be attributed to the better utilization of fibrous material of the complete feed blocks after incorporation of zoospores. Kostyukovsky *et al.* (1990) isolated 2 anaerobic fungal strains from the rumen of cattle and reported that these fungi can utilize a wide spectrum of mono-, oligo- and polysaccharides. Elliot *et al.* (1987) and Gordon and Phillips (1993) reported that the *in vivo* DM digestibility was increased by 3–8% points in the presence of rumen fungi compared to control. Orpin (1989) reported that the *Neocallimastix* sp. and *Piromyces* sp. efficiently degraded plant tissues. Calderon-Cortes *et al.* (1989) found that elimination of fungi from the rumen of sheep decreased straw digestion from 53.8–44.6% which was reversed when fungi were reintroduced. Similar to the present study, Akin *et al.* (1990) observed that polycentric *Orpinomyces* (fungi) increased the dry matter digestibility of Bermuda grass stems. Manikumar *et al.* (2004) reported increased *in vitro* DM, NDF, and ADF digestibility and decreased ADL contents of wheat straw after 48 h incubation with strained rumen liquor and double log dose (10^6 cfu/mL) of *Orpinomyces* sp. (C-14) culture. Contrary to this, Samanta *et al.* (2001) reported that the addition of fungal culture

Table 4. Nutritive evaluation and nutrient utilization of wheat straw based complete feed blocks fed zoospores of *Neocallimastix* sp. GR-1

	Groups		't' values
	Control	Treatment	
Nutritive evaluation			
% DCP	6.41±0.09	7.02±0.07	3.90*
% TDN	52.89±1.89	60.55±0.58	6.38**
Nutrient intake and utilization			
Total DCP intake (kg)	24.69±0.89	29.32±1.85	2.02^{NS}
DCP intake/day (g)	251.43±14.71	277.64±17.76	2.02^{NS}
DCP intake/kg gain (g)	532.99±34.92	485.88±11.82	1.68^{NS}
Total TDN intake (kg)	191.67±8.34	214.07±10.66	2.95*
TDN intake/day (kg)	2.14±1.55	2.25±1.43	2.88*
TDN intake/kg gain	4.21±1.22	4.01±1.88	1.32^{NS}

NS, Nonsignificant.** P≤0.01;* P≤0.05.

Table 5. Influence of anaerobic fungal (*Neocallimastix* sp. GR1) zoospores on rumen fermentation pattern in growing male Murrah buffalo calves

	Groups		't' values
	Control	Treatment	
pH	7.15±0.02	6.98±0.03	4.30**
Total VFA (mM/100 mL)	10.23±0.06	13.45±0.05	5.60**
Total nitrogen (mg/100 mL)	78.18±0.63	106.54±0.58	7.55**
Ammonia nitrogen (mg/100 mL)	13.48±0.23	9.07±0.08	19.63**
TCA precipitable nitrogen (mg/100 mL)	52.53±0.68	71.46±0.57	20.24**
Average number of fungal zoospores/mL	1.60×10^5 $\pm0.57\times10^4$	4.33×10^5 $\pm1.23\times10^4$	10.36**
Average number of bacteria/mL	1.94×10^{10} $\pm2.24\times10^8$	2.77×10^{10} $\pm3.23\times10^8$	6.52**
Average number of protozoa/mL	2.05×10^6 $\pm2.43\times10^4$	1.55×10^6 $\pm3.15\times10^4$	11.45**

** P≤0.01.

(*Piromyces* sp.) to a mixed rumen inoculum did not increase *in vitro* dry matter digestibility (IVDMD) significantly may be due to a lesser dose of added fungi. Lee *et al.* (2000) administered *Orpinomyces* strain KNGF–2 isolated from Korean native goat, to the rumen of sheep and observed an increase in the nutrient digestibility resulting from an increase in the number of bacteria and fungi in the rumen by altering the pattern of VFA production. Dey *et al.* (2004) reported that the digestibility coefficients (%) of DM (59.95 vs. 53.94), OM (62.31 vs. 56.21), CP (65.82 vs. 59.76) and ADF (51.98 vs. 42.94) were significantly higher (P≤0.05) in treatment group compared to control group. Also, the CF and NDF digestibilities were significantly (P≤0.01) higher in treatment group than control group of crossbred cow calves. This may be attributed to the better utilization of fibrous material of the wheat straw with the dosing of *Orpinomyces* sp. in the treatment group. Paul *et al.* (2004) also evaluated the effect of administration of an elite strain of anaerobic fungi (*Piromyces* strain, isolated from the faeces of wild herbivore) for a very short period to buffalo calves by mixing this in the feed and showed that the fungal strain resulted in an increase in total tract digestibility of DM, OM, NDF and ADF but no effect on the digestibility of CP and EE was observed. Effect of zoospores of *Neocallimastix* sp. GR-1 incorporated in the wheat straw based complete feed blocks fed to buffalo calves was studied. This helped in higher digestibility of various nutrients as fungal zoospores get developed in fungi and has ability to breakdown the lignocellulose and ligno-hemicellulose bonds of the cell wall contents of wheat straw (Akin *et al.* 1983, Ho *et al.* 1996, Ushida *et al.* 1997, Manikumar *et al*. 2004, Paul *et al.* 2004, Dey *et al.* 2004, Tripathi *et al.* 2007b, Sehgal *et al.* 2008 and Prashant *et al.* 2009) and amylolytic bonds of the starch contents of maize grains (Pearce and Bauchop (1985); Yanke *et al.* (1993), Kopecny and Hodrova 1995) constituting the concentrate mixture part of the wheat straw based complete feed mixture.

Nutritive evaluation and efficiency: In treatment group, the content of DCP and of TDN was significantly higher than control group (Table 4). The higher availability of energy may be also due to the higher digestibility of nutrients in zoospores incorporated fungal group. The CP intake in treatment group was higher than the required intake reported by Kearl (1982) for buffalo calves at a growth rate of 500 g/d. The lower TDN intake in the control group can be attributed to the higher proportion of wheat straw in the complete feed block due to lower digestibility.

Rumen fermentation parameters: The pH of the rumen liquor varied significantly between control and treatment group (Table 5). Compared to the control group, the content of total VFAs, total nitrogen and TCA precipitable nitrogen increased, while the content of NH_3 – nitrogen decreased significantly.The increase of zoospores counts per mL in treated group might be responsible for lowering the pH and increasing the total VFAs due to increase in nutrient digestibility (Table 5). Thus, the additional availability of digestible energy in zoospores incorporated group might be also responsible for higher growth rate in buffalo calves.

The amounts of total-N and NH_3-N in rumen liquor of buffalo calves fed on the control diet in present experiment are in close agreement with results of Bhatia *et al.* (1982). The optimal NH_3-N for maximal rumen fermentation rate was very high in comparison with most published values for optimum NH_3-N for maximal microbial protein synthesis per unit of OM fermented. Studies *in vivo* have shown the corresponding NH_3-N to be 8.8–13.3 mg/100 mL (Hume *et al.* 1970) or 28.9 mg/100 mL rumen fluid (Miller 1973). In the treated group, the pH was lower and a significant change in total VFA content was noticed, which indicated a changed fermentation pattern (Lee *et al.* 2000). Dey *et al.* (2004) also observed a 2.4- fold increase in zoospore count after supplement of *Orpinomyces* sp. C-14 in the rumen of crossbred calves. Similarly Paul *et al.* (2004) observed an increased concentration of TVFA, and total N

but NH_3-N was lower in treated group. Tripathi *et al.* (2007b) also reported that the pH was significantly lesser in the rumen liquor ($P \leq 0.05$) in treated groups (6.92 and 6.88) than that in control group (7.11). The total VFAs (mM/100 mL) were 7.35±0.38, 10.55±0.65 and 11.58±0.35 in the SRL of control group, *Orpinomyces* and *Piromyces* treated groups, respectively, also indicated an increased in the TVFA concentration of the treated group and statistically the differences were significant ($P \leq 0.05$) in these 3 groups.

Elite rumen anaerobic fungi, *Neocallimastix* sp.GR-1 isolated from grazing and browsing goat is a promising fungi which can form fungal zoospores, which can be incorporated in wheat straw based complete feed blocks to improve the nutritive value of cereal straws and improvement in growth performance, nutrient digestion, ruminal fermentation pattern and nutritive evaluation in terms of % DCP and % TDN in male Murrah buffalo calves.

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