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Full Length Research Paper

Effect of isolates of fibre degrading bacteria on body weight changes, milk production and its composition, nutrient intake and nutrient utilization in lactating Murrah buffaloes

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Fibrolytic bacterial strains were isolated from the rumen liquor of permanently fistulated buffaloes kept on high fibre diet (roughage: concentrate: 60:40; w/w) on the basis of *in vitro* true dry matter digestibility study on pure neutral detergent fibre isolated from wheat straw and on high fibre based diet (wheat straw: concentrate: 80:20; w/w). Based on enzyme activity, the most potent fibre degrading bacterial isolate was selected which was further characterized on the basis of its morphology, biochemical properties and molecular properties and was found to be *Ruminococcus flavefaciens* strain FD-1 which was finally used as feed supplement for *in vivo* trial on lactating Murrah buffaloes. 12 lactating buffaloes divided into treatment and control groups of six animals each were fed with experimental diets and live and autoclaved culture of best selected fibrolytic bacterial isolate (NB-1) that is, *R. flavefaciens* strain FD-1; 300 ml orally alternate day continuously for one month period. No significant difference was observed in the mean body weight changes and daily milk yield between the treated and control groups although the live body weight and daily milk yield was increased in live culture supplemented treated group. There was no effect on milk composition of the animal. The difference in mean dry matter intake was significant (P <0.05) between control (11.11 kg/day) and treated groups (11.77 kg/day) during the experimental period. The digestibility of NDF and ADF was found to be higher in treated group by 9.66 and 19.20% over that of the control group although the effect was not significant. Thus, the bacterial culture of *R. flavefaciens* strain FD-1 showed the potential to be used as feed additive in the diet of ruminants for improving live body weight gain, daily milk yield as well as utilization of nutrients from lignocellulosic feeds.

Key words: Fibrolytic bacterial culture, lactating buffaloes, milk yield, nutrient utilization.

INTRODUCTION

Ruminants mainly depend upon fibrous lignocellulosic feeds for their energy requirement. Close association of lignin with cellulose act as the major constraint for release of energy from cellulose. The agricultural by-products which serve as a staple livestock feed are rich in lignin content. The manipulation of ruminal fermentation to maximize efficiency of feed utilization to increase ruminant productivity, that is, increase milk, meat, and wool production, continues to be of great interest to rumen microbiologists and ruminant nutritionists. In simplistic terms, the objectives of ruminal manipulation are to enhance ruminal fermentation processes that are beneficial to the host, minimize, alter, or delete inefficient or deleterious ruminal fermentation processes (Nagaraja

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et al., 1997). To extract more energy from such feeds, manipulation of rumen microbial ecosystem by the introduction of a superior fibre degrading bacterium may serve the needful purpose. The present and potential practices of rumen manipulation to increase the nutriational value of ruminant feeds in the tropics have been reviewed by many authors. Russell and Wilson (1988) suggested the potential and limitations of rumen microbial eco-system. Bello et al. (1997) suggested microorganisms from systems other than the rumen, such as digestive bacteria from wild herbivores and N-fixing bacteria from legume roots or fungi may well represent a source of enzymes to increase degradation of recalcitrant components of the diet.

Therefore, the present study was conducted to evaluate the effect of supplementation of fibrolytic bacterial isolate on body weight changes, milk production, nutrient intake and nutrient utilization in lactating Murrah buffaloes. This study was conducted to evaluate the digestibility of lignocellulosic feeds by some selected bacterial isolates isolated from domestic Murrah buffaloes under *in vitro* conditions, and a superior potent fibrolytic bacterial strain was selected to be used as microbial feed additives in *in vivo* trial on lactating Murrah buffaloes.

MATERIALS AND METHODS

Isolation of bacteria

Rumen liquor was collected from permanently fistulated buffaloes kept on high fibre diet (concentrate: roughage: 60:40; w/w) from the farms of National Dairy Research Institute, Karnal. Rumen liquor was collected through a stomach tube and stored in a screw cap test tube to its full capacity, avoiding air space to minimize exposure to oxygen and immediately taken to the anaerobic work station for enrichment in selective medium. Culture medium for isolation of anaerobic fibre degrading bacteria was prepared as described by Bryant and Burkey (1953). Carboxymethylcellulose (CMC) (0.5%) was used as the only carbohydrate source in the culture medium for isolation of fibre degrading bacteria. 1 ml of fresh rumen liquor was transferred into CMC medium for enrichment of bacteria able to degrade CMC and incubated for 48 h at 39°C inside the anaerobic work station. After 48 h of incubation, culture from different isolates were diluted up to 10^{-5} to 10^{-8} with anaerobic dilution fluid and streaked on the agar plate containing CMC as the only carbohydrate source. Isolated colonies from the highest dilution plate were selectively picked-up and transferred into CMC broth culture. This procedure was repeated thrice to get pure culture of bacterial isolates.

Pure cultures of the bacterial isolates were scrutinized to select only cellulolytic bacteria for further studies. Extracellular CMCase activity of the supernatant of the different cultures was tested by using Congo-red solution in the agar-plate by cup diffusion method as described by Teather and Wood (1982). On the basis of *in vitro* true dry matter digestibility study on pure neutral detergent fibre isolated from wheat straw and on high fibre based diet (Wheat straw: Concentrate: 80:20; w/w) and on study based on enzyme activity, the most potent fibre degrading bacterial isolate was selected which was further characterized on the basis of morphology, biochemical properties and molecular properties and was found to be *R. flavefaciens* strain FD-1 which was used as feed supplement for *in vivo* trial on lactating Murrah buffaloes.

Lactation trial

Selection, distribution and feeding of animals

12 lactating Murrah Buffaloes (Mid to Late lactation) were selected from the buffalo herd of National Dairy Research Institute, Karnal and randomly distributed into two groups of six each according to their milk yield, live body weights and days in lactation. Animals were fed with experimental diets and live and autoclaved culture of best selected fibrolytic bacterial isolate (*Ruminococcus flavefaciens* strain FD-1); 300 ml orally alternate day continuously for one month period after 21 days adaptation period of animals. The animals were housed in open shed having proper arrangement of individual feeding and watering.

Formulation of rations and dosing of experimental animals

Rations were made for individual animals according to their body weight and daily milk yield. The animals were given concentrate and roughage diet which comprised of concentrate mixture, maize fodder and wheat straw in the ratio of 40:30:30. Nutritional requirment was as per Paul and Lal (2010) Central Institute on Buffaloes on the basis of recommendation for a period of 100 days. Treated group of animals were fed control diet plus live bacterial culture (300 ml) orally alternate day for a period of one month whereas, the control group were fed control diet plus autoclaved bacterial culture (300 ml) alternate day for a period of one month. The bacterial culture contained 3×10^{12} number of cells /ml of diluent so total number of bacterial cells dosed in an animal was 9×10^{14} cells on alternate day basis continuously for one month period. Drinking water was offered on free choice basis thrice a day. The following parameters were recorded throughout the experimental period of 100 days.

Daily milk yield

Milking was done twice daily that is, morning at 5:30 A.M and evening at 5:00 P.M and milk yield record was maintained throughout the experimental period.

Milk composition

Milk composition; milk fat, protein, solid not fat and total solids was determined fortnightly by using automatic milk analyzer. Milk samples were collected from individual animals in 100 ml bottles and pre warmed at 40°C in water bath before analysis for all individual animals in treated and control groups.

Fortnightly body weight and dry matter intake

The animals were weighed before feeding and watering in the morning on two consecutive days at the start of the experimental feeding and thereafter at fortnightly intervals during the experimental period. Dry matter (DM) intake was recorded daily by subtracting the residual DM from the quantity of DM offered.

Nutrient utilization and balance studies (digestion trial)

A digestion trial was conducted in mid of the experimental period with seven days collection period to determine the nutrients digestibility. Animals were weighed before and after trial consecutively for two days. Allotted rations were fed to each animalb as per requirment. Fresh drinking water was provided thrice a day.

Feeding and sampling of feeds and residues during digestibility trial

During the digestibility trial weighed quantity of concentrate mixture, maize fodder and wheat straw were offered in the form of total mixed ratio (TMR) to individual animals separately twice daily in the morning and evening at the milking time. Clean and fresh drinking water was offered thrice daily *ad libitum*.

Well-mixed representative samples of concentrate, maize fodder and wheat straw offered and residue left were taken daily in previously tarred trays and dried at 100°C overnight for dry matter estimation. The dried material obtained during trial period was pooled animal wise ground to pass through 1 mm sieve and stored for proximate and fiber analysis.

Collection and sampling of faeces

The faeces voided in 24 h by the individual animal in each group were collected quantitatively in labeled polythene bags. A representative sample from each animal was taken separately in a labeled polythene bag. From the sample, a suitable aliquot was kept (1/100 of fresh faeces) for drying at 100°C in a hot air oven for dry matter estimation. The dried materials obtained daily were pooled animal wise ground to pass through 1 mm sieve and used for proximate analysis. A suitable aliquot (1/100 of fresh faeces) was mixed with suitable quantity of 25% sulphuric acid and preserved for nitrogen estimation in previously weighed air-tight bottle.

Proximate analysis

The Association of Official Analytical Chemists (AOAC) (1995) methods of analysis were followed for estimation of proximate composition for the following components: dry matter, crude protein, ether extract, total ash, and organic matter. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined as per the methods of Van Soest et al. (1991).

RESULTS AND DISCUSSION

Effect of bacterial culture supplementation on body weight changes

The effect of supplementation of bacterial culture on body weight changes is shown in Table 1. At the start of the experiment, the body weights were 601.08 and 602.25 kg and the same at the end of 100 days of the experimental trial were 595.50 and 610.39 kg in the control and treated groups, respectively. No significant difference in body weights was recorded among the control and treated groups. Although total live weight gain was more in treated group (+8.14 kg) as compared to the control group where body weight decreased (- 5.58 kg) but the difference in total live weight gain was also not statistically significant.

Sahu (2002) also found similar results with increase in the live body weight in live cellulolytic bacterial culture supplemented treatment group of buffaloes in comparison to control group in which autoclaved culture was given.

Effect of bacterial culture supplementation on milk production performance and milk composition in experimental lactating Murrah buffaloes

Average daily milk yield during the entire experimental period varied from 5.88 to 6.36 kg/day in control group and 5.91 to 6.65 kg/day in the treated group (Table 1), respectively during different fortnights (Figure 1). Overall, average milk yield was 6.01 kg/day in control group while it was 6.20 kg/day in the treated group at the end of the experiment which was higher than initial milk yield of 5.88 kg and 5.91 kg/day in the control and treated groups, respectively. Overall average increase in daily milk yield (kg) was 0.130 kg/day in the control group and 0.290 kg/day in the treated group which was higher than control group although statistically no significant difference was found in average daily milk yield. Thus, there was increase in daily milk yield in treated group of animals due to live bacterial culture supplementation but the effect was not significant in comparison to control group.

There was no significant effect of bacterial culture supplementation on milk composition as well as on fat corrected milk yield of experimental group of lactating buffaloes (Table 1). There was no significant difference in milk protein and milk fat content over the entire experimental period between the treated and control groups of animals, the mean total solid and solid not content of the milk of two groups also remained same over the experimental period. The present findings match the findings of Chiquette et al. (2008) who used ruminal anaerobic bacteria *Prevotella bryantii* 25A as direct fed microbial (DFM) sources for dairy cows in early lactation. Six cows were given 2×10^{11} cells/dose of *P. bryantii* 25A inoculated directly with a syringe through the rumen cannula. Administration of *P. bryantii* 25A did not change milk yield, but tended to increase milk fat. In another study (Nocek and Kautz, 2006), cows supplemented with *E. faecium* and yeast produced more milk/cow per day. There were no differences in 3.5% fat-corrected milk (FCM) between cows supplemented with DFM and controls. There were no differences in milk fat yield or milk protein percentage and yield. Raeth-Knight et al. (2007) evaluated the effects of the combination of *L. acidophilus* LA747 and *P. freudenreichii* PF24 on 84 day dairy cattle performance and 28 day periods ruminal characterizations. DFM was top dressed on the TMR once daily. DFM did not affect 4% fat-corrected milk (FCM) yield, percentage or yield of milk components.

Effect of bacterial culture supplementation on nutrient intakes in experimental lactating Murrah buffaloes during lactation trial

There was no significant difference between the control group and treated group in all the nutrient intakes except in mean DMI/day and mean TDNI/day over the entire experimental period. The difference in mean dry matter

Table 1. The effect of bacterial culture supplementation on nutrient intakes, changes in mean body weight, average daily milk yield, fat corrected milk yield and milk composition in experimental Murrah buffaloes during lactation trial.

Particular	Control	Treated	P value
Body weight/Initial (kg)	601.08 ± 48.32	602.25 ± 55.91	NS
Body weight/Final (kg)	595.5 ± 43.28	610.39 ± 54.10	NS
Daily milk yield/Initial (kg)	5.88 ± 0.31	5.91 ± 0.64	NS
Daily milk yield/Final (kg)	6.01 ± 0.09	6.20 ± 0.11	NS
FCM yield/Initial (kg)	7.98 ± 0.44	8.31 ± 0.53	NS
FCM yield/Final (kg)	8.33 ± 0.53	9.45 ± 0.36	0.019
Milk protein (%)	4.26 ± 0.04	4.21 ± 0.09	NS
Milk fat (%)	7.21 ± 0.15	7.43 ± 0.22	NS
Milk total solids (%)	14.62 ± 0.24	15.05 ± 0.20	NS
Milk solid not fat (%)	7.41 ± 0.12	7.63 ± 0.12	NS
DM Intake (kg/day)	11.11^{a} ± 1.10	11.77^{b} ± 1.00	0.033
DMI (kg/day/100 kg B.W)	1.87 ± 0.11	1.93 ± 0.05	NS
CP Intake (kg/day)	1.35 ± 0.15	1.43 ± 0.12	NS
CP Intake (kg/100 kg B.W)	0.23 ± 0.01	0.23 ± 0.01	NS
DCP Intake kg/day)	1.17 ± 0.12	1.23 ± 0.10	NS
DCP Intake (kg/100 kg B.W)	0.20 ± 0.01	0.20 ± 0.01	NS
TDN Intake (kg/day)	6.45^{a} ± 0.64	6.91^{b} ± 0.59	0.021
TDN Intake (kg/100 kg B.W)	1.08 ± 0.06	1.14 ± 0.03	NS

Values with different superscripts within a row differ significantly, $P < 0.05$.

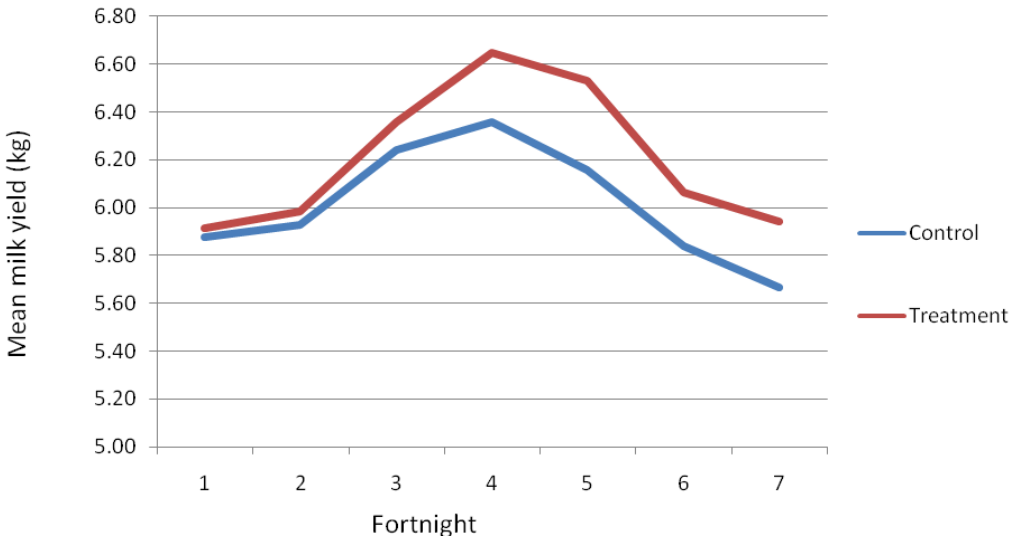

Figure 1. Fortnightly mean milk yield (Kg/day) change in control and treatment groups.

intake and total digestible nutrient intake was found to be significant ($P < 0.05$) between control group (11.11 and 6.45 kg/day) and treated group (11.77 and 6.91 kg/day), respectively over the entire experimental period which resulted in increased mean body weight changes and daily milk yield in treated group compare to control group although the two changes were not significantly different.

Digestibility of nutrients

Proximate composition of feedstuffs

The chemical composition of feedstuffs (Concentrate, Green maize fodder and Wheat straw) used during digestibility trial has been shown in Table 2. Dry matter

Table 2. Proximate composition (%) of different feed ingredients offered to the experimental animals as well as chemical composition (%) of TMR fed to the experimental animals on DM basis.

Particular	Concentrate	Green maize	Wheat straw	% DMB
DM	89.08	19	90	66.03
OM	91.74	89.27	90	90.44
CP	21.55	9.15	3.21	12.14
CF	9.80	25.55	40.10	23.85
EE	3.41	2.75	1.27	2.56
TA	8.26	10.73	10	9.56
NDF	37.60	53.33	81.13	55.61
ADF	16.13	32.46	50.33	31.53

Table 3. Nutrient digestibility (%) of the ration fed to the experimental animals during digestibility trial.

Particular	Control	Treated
DM	57.59 ± 3.77	58.10 ± 1.78
OM	61.35 ± 3.68	62.09 ± 1.49
EE	74.17 ± 2.13	75.23 ± 2.83
CP	87.12 ± 1.08	86.02 ± 1.06
NDF	44.72 ± 4.16	49.02 ± 2.66
ADF	28.85 ± 6.67	34.39 ± 3.42
HC	60.99 ± 2.95	60.23 ± 2.48
TDN	58.06 ± 3.40	58.67 ± 1.38
NFE	56.73 ± 4.27	57.92 ± 1.66

content of concentrate was 89.08% and it contained contained 91.73, 21.55, 3.41, 9.8, 8.26, 37.6, 16.13 and 56.98% OM, CP, EE, TA, NDF, ADF and NFE on DM basis respectively. The corresponding value for green maize fodder was 89.26, 9.15, 2.75, 25.55, 10.73, 53.33, 32.46, and 51.82 and for wheat straw, these values were 90, 3.21, 1.27, 40.10, 10.00, 81.13, 50.33 and 5.42%, respectively.

Effect of bacterial culture supplementation on nutrient digestibility of the ration fed to the experimental animals

In the mid of the experiment, digestibility trial was conducted for a period of seven days to see the effect of bacterial culture supplementation on nutrient digestibility. The detail of the requirement and actual intake of the nutrients is given in Table 4. No significant difference in the nutrient intake was found between the two groups during digestibility trial period. Digestibility coefficients of the different nutrients of the rations with autoclaved bacterial culture supplementation and live bacterial culture supplementation in control and treatment group are given in Table 3. The digestibility coefficients of DM, OM, EE, CP, HC, TDN and NFE were 57.59, 61.33, 74.17, 87.12, 60.99, 58.06, and 56.73% in the control in the control and treated groups, respectively. The digestibility of NDF and ADF was found to be higher in treated group by 9.66 and 19.20% over that of control group although the effect was not found to be significant. The above results indicate that an increase in digestibility of fibre components was due to the newly introduced bacterium into the rumen of buffaloes. Being cellulolytic in nature, the newly introduced bacterium might have attacked the fibre components and resulted in higher fibre digestibility. Tarakanov (1993) successfully introduced tsellobakterin (a mixture of three cellulolytic bacterial isolates from the rumen) which increased the intake and efficiency of utilization of roughage and increased 12 to 16% of crude fibre digestibility. Similarly, Laptev et al. (1994) introduced cellulolytic bacteria *Ruminococcus albus* into the rumen of cattle to enhance fibre digestibility. He suggested that the biological effects of introduced bacterial strains depend not only on their quantity and quality, but also on the state of eco-systems in the rumen. The result of the present finding matches group and 58.10, 62.09, 75.23, 86.02, 60.23, 58.67, and 57.92% in treated group respectively. The digestibility of NDF and ADF was 44.72, 28.85 and 49.02 and 34.39% with the findings of Laptev et al. (1994) and Sahu (2002) in the case of buffalo heifers dosed with 200 ml medium containing culture of cellulolytic bacterial strain; daily

Table 4. The requirement (as per Paul and Lal, 2010) and actual intakes of nutrients in experimental animals during digestibility trial.

Parameter	Control	Treated
Average Body Wt. (kg)	587.17 ± 40.73	615.67 ± 55.23
DM offered (kg/day/animal)		
Concentrate	4.90	5.17
Maize fodder	3.71	3.94
Wheat straw	3.68	3.90
Total DM Offered	12.29	13.01
Concentration : GF : WS	39.86:30.18:29.94	39.75:30.28:29.97
DM required as per CIRB (Kg)	12.29	13.01
Actual DMI	11.33 ± 1.01	11.75 ± 0.74
DMI/100 kg of body weight	1.93 ± 0.10	1.94 ± 0.07
DM consumed as % of CIRB requirement	92.18	90.31
CP required as per CIRB (Kg)	1.50	1.57
Actual CPI	1.37 ± 0.12	1.37 ± 0.08
CPI/100 kg of body weight	0.23 ± 0.01	0.23 ± 0.01
CP consumed as % of CIRB requirement	91.33	87.26
TDN Required as per CIRB (Kg)	7.26	7.67
Actual TDNI	6.69 ± 0.91	6.93 ± 0.55
TDNI/100 kg of body weight	1.12 ± 0.08	1.13 ± 0.04
TDNI consumed as % of CIRB requirement	92.14	90.35

showed that there was increase in fibre digestibility in treated group of animals in comparison to control group, respectively. The result of the present finding also matches with the result of Krause et al. (2001) in the case of sheep dosed with 500 ml medium containing culture of Ruminococcus strain for two weeks; continuously showed that *in situ* nylon bag digestibility and whole tract dry matter digestibility showed no differences between control and dosed groups. These results demonstrate that increasing the numbers of cellulolytic bacteria in the rumen to the extent that fibre digestion gets enhanced is very difficult. In the dosing protocol used, we hoped that the microbial population would be perturbed to a sufficient extent to allow the introduced bacteria to establish and multiply but this did not happen in this case and this was also confirmed by Dehority and Tirabasso (1998) who could not demonstrate any improvement in the proportion of cellulose digested with a 10-fold increase in the number of cellulolytic bacteria in the rumen. It is also known that fibrolytic strains can undergo subtle changes in phenotype because of repeated transfer under laboratory conditions. It is likely that key elements are lost from the strains and many of these could be critical for the ability of strains to colonize and persist *in vivo*.

Conclusions

It appears from the results that there is a potential in the bacterial isolates isolated from buffalo rumen liquor to be used for the manipulation of rumen fermentation as well as production performance in other domestic ruminants and for enhancement of fibre digestion. Supplemented bacterial culture showed improved results in terms of live body weight gain, daily milk yield, and fibre digestion whereas there was significant ($P < 0.05$) improvement in terms of dry matter intake in lactating buffaloes. More studies are needed to find its potential as a microbial feed additive in an *in vivo* experiment.

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Abbreviations: ADF, Acid detergent fibre; **AOAC,** Association of Official Analytical Chemists; **CIRB,** Central Institute for Research on Buffaloes; **CMC,** carboxy methyl cellulose; **CP,** crude protein; **CPI,** crude protein intake; **DCP,** digestible crude protein; **DFM,** direct fed microbials; **DM,** dry matter; **DMI,** dry matter intake; **DMI/day**, dry matter intake per day; **EE,** ether extract; **FCM,** fat corrected milk; **GF,** green fodder; **HC,** hemicellulose; **NDF,** neutral detergent fibre; **NB,** NDRI buffaloes; **NFE,** nitrogen free extract; **OM,** organic matter; **TA,** total ash;

TDN, total digestible nutrient; **TDNI/day,** total digestible nutrient intake per day; **TMR,** total mixed ration; **WS,** wheat straw.

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