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Effect of dietary supplementation of fibrolytic enzyme on the mean body weight gain, dry matter intake, nutrient, energy intake and apparent digestibility of nutrients in lactating upgraded buffaloes

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Abstract
Eight upgraded buffaloes (371.40 ± 15.73 kg of body weight, 5.88 ± 0.22 milk yield, 67.38 ± 3.56 lactation length) in mid lactation were used in a switch over design. Three groups were made based on the milk production and stage of lactation. Buffaloes within each group were assigned to one of the three dietary treatments in each period. The total duration of the lactation trial was 12 weeks comprising three periods of four weeks each. The buffaloes in all the three groups received urea treated (@ 2 %) paddy straw (PS) as the sole roughage and compound feed mixture (CFM). Dietary treatment included as following: without enzyme mixture - control (T1), exogenous fibrinolytic enzyme supplemented in CFM @ 1.6 g/kg that was @ 15 % more than the control or normal level of xylanase enzyme activity in the rumen liquid (T2) and exogenous fibrinolytic enzyme supplemented in CFM @ 3.2 g/kg that was 30 % more than the control or normal level of xylanase enzyme activity in the rumen liquid (T3). The dry matter intake (DMI) was calculated as the difference between the DM offered and DM left over for the individual animal. Digestion trial was conducted by indicator method using ADF ash as an internal marker in a "grab collection" method. Representative samples of the feed offered and residues left were collected daily during the collection period for analysis of DM and nutrients. Statistical analysis revealed there was no significant difference in, mean body weight gain (g/d), daily dry matter intake (DMI) (kg/d), nutrient intake (kg/d), energy intake (MJ/d) and apparent digestibility of nutrients (%) in lactating buffaloes.

Keywords: exogenous fibrinolytic enzyme, xylanase, paddy straw, compound feed mixture

Introduction
In India, crop residues constitute a major portion of the diet in ruminant production system. Paddy straw is one such main agricultural byproduct which farmers usually stored for use as ruminant feed in tropical areas. Paddy straw contain low nitrogen, vitamins and minerals, which hinder the bioavailability of cellulose and hemicelluloses present in crop residue due to the presence of complex lignocellulosic bond and eventually limit the degradation by rumen microbes and necessary nutrient uptake for a satisfactory performance of ruminants. Various treatment methods have been tried to improve nutritive value of paddy straw including physical, biological and chemical treatment (Wanapat *et al*., 1996) [37]. One such biological treatment is use of Exogenous fibrinolytic enzymes (EFE) in the form of feed additives are included in the diets of ruminants in tropical region and they are reported to have exerted variable effects on the digestibility of fibre and animal performance (Beauchemin *et al*., 2000; Dhiman *et al*., 2002) [9, 38, 14]. The results of supplementation of EFEs to ruminant diets have been variable. Variable responses could be due to differences in enzyme activity, rate of enzyme application, stage of lactation of animals, ruminal activity and stability of enzymes (Adesogan, 2005) [4]. The mode of action of EFE is influenced by several factors *viz*., type and dose of enzyme and type of the diet fed to animals (Adesogan *et al*., 2014) [3]. Hence, the experiment was carried out to evaluate the impact of fibrinolytic enzymes supplementation in the paddy straw based diet in lactating buffaloes on their production performance.

Materials and Methods

Eight buffaloes (371.40 ± 15.73 kg of body weight, 5.88 ± 0.22 milk yield, 67.38 ± 3.56 lactation length) in mid lactation were used in a switch over design based on the milk production and stage of lactation. Buffaloes within each group were assigned to one of the three dietary treatments in each period. The total duration of the lactation trial did 12 weeks comprises three periods of four weeks each, during which time milk yield, milk composition (Total solids, Total ash, Fat, Protein, Lactose and SNF) and feed intake were recorded. The individual buffaloes in the experiment were allocated to one of the three dietary treatments. The treatments designated as T1, T2 and T3 and contained different levels of enzyme mixture as following treatments. 1) Treatment I (T1) – Adlbitum urea treated (@ 2%) paddy straw and CFM with no enzyme mixture. These groups of animals were considered to have normal level of xylanase activity in the rumen liquid and xylanase activity of the rumen liquid is 240 IU/liter (Vaithiyanathan *et al*., 2015) [36] and this group served as control. 2) Treatment II (T2) - Ad Libitum urea treated (@ 2%) paddy straw and CFM with enzyme mixture at 1.6 g per kg. The enzyme level is fixed at 15 % more than the control or normal level of xylanase enzyme activity in the rumen liquid. 3) Treatment III (T3) - Ad Libitum urea treated (@ 2%) paddy straw and CFM with enzyme mixture at 3.2 g per kg. The enzyme level is fixed at 30 % more than the control or normal level of xylanase enzyme activity in the rumen liquid. The ingredient composition (%) of CFM is presented in Table 1. The fibrinolytic enzyme mixture (Fibromas ®) used in the study was procured from All tech Inc., Nicholasville, KY, USA. The enzyme mixture was tested for the xylanase and cellulase enzymes activity at National Institute of Animal Nutrition and Physiology (NIANP), Adugodi, Bengaluru, Karnataka, India. The enzyme activity level was analyzed as per Miller (1959). The activity of xylanase and cellulase was 178 IU/g and 625.624 IU/g, respectively. The buffaloes were housed in individual stalls, in an open type protected shed in a single row and were provided with uniform management practices. All the buffaloes were individually dewormed with Fenbendazole (Panacur ® bolus) and vaccinated against HS and FMD before starting the feeding trial.

The diets for the experimental buffaloes were formulated individually to meet the energy and protein requirement as per Paul *et al*. (2002) [26]. Buffaloes were fed with the required amount of energy and protein in the diet for maintenance and milk production. The buffaloes were offered with the Ad Libitum urea treated (@ 2 %) paddy straw. The left-over were weighed on the next day morning to obtain the estimate of intake. The fibrinolytic enzyme mixture was added directly to the CFM during preparation of the diets as per the plan described above. The allowance of CFM for individual buffaloes was varied to supply the required ME. The daily allowance of CFM supplement for individual buffaloes for maintenance and milk yield was calculated based on the previous weeks' urea treated paddy straw intake, milk yield, milk fat content and body weight at initial week of respective period. The CFM was offered in three equal parts at 6.00 am, 2.00 pm and 6.00 pm. The buffaloes were provided with sufficient drinking water throughout the day. The total duration of the feeding trial was 12 weeks comprising three periods of four weeks each. Daily intake of urea treated paddy straw and CFM were recorded. Samples of paddy straw offered were collected and analyzed once in a week for the estimation of dry matter by drying samples at 70 ℃ to a constant weight but the CFM samples were dried at 100 ℃. The dry matter intake (DMI) was calculated as the difference between the DM offered and DM left over for the individual animal. Representative samples of the feed offered and residues left were collected daily during the collection period for analysis of DM and nutrients.

The buffaloes were weighed in the beginning and at the end of each period (on the same days and at same time) after morning milking before having access to feed and water. The body weights were recorded using a platform weighing scale. Digestion trials were conducted using all animals, during the last week of each period of the lactation trial/feeding trial. Digestion trial was conducted by indicator method using ADF ash as an internal marker in a "grab collection" method as described by Singh *et al*. (1994) [32].

Five grab samples of feces were collected from each buffalo in four days period by rectal sampling in a design that equally represents the 78 hours collection period as well as 24 hours of the day (10.30 hours on day 1st, 05.45 hours on day 2nd, 01.00 and 20.15 hours on day 3rd and 15.25 hours on day 4th). Four hundred grams of the faecal sample collected each time was composited and frozen (-20 ℃) until further analysis. Composited samples of feces were thawed to room temperature, mixed thoroughly and dried at 55 ℃ for analysis. The digestibility of DM, OM, N, NDF and ADF was calculated by using the following equations.

$$\text{Digestibility of DM (\%)} = 100 - 100 \times \frac{\text{(Per cent indictor in DM of feed)}}{\text{(Per cent indictor in DM of faeces)}}$$

$$\text{Digestibility of Nutrients (\%)} = 100 - 100 \times \frac{\text{(Per cent indictor in feed} \times \text{per cent nutrient in faeces)}}{\text{(Per cent indictor in faeces} \times \text{per cent nutrient in feed)}}$$

Data on mean body weight gain (g/d), dry matter intake (DMI kg/d), nutrient (kg/d) and energy intake (MJ/d) and apparent digestibility of nutrients (%) were analysed by ANOVA using Graph Pad Prism software (version 5.00) as per Snedecor and Cochran (1994) [34].

Table 1: Ingredients Composition (%) of compound feed mixture

Ingredients	Parts
Maize	50
Wheat bran	45
SRNP	2
Mineral mixture	2
Salt	1
Total	100

Results and Discussion

Chemical composition

The chemical composition of PS and CFM was analysed as per method described by AOAC (2016) is presented in Table 2. The crude protein content (%) and metabolizable energy (MJ/kg DM) values of PS and CFM were 9.02, and 5.62; 18.26 and 11.03, respectively. The chemical composition of both PS and CFM was similar to the values reported in earlier studies (Reddy *et al.*, 2016 and Anupkumar *et al*., 2017) [5].

Table 2: Chemical composition[1] (% DM), and predicted metabolizable energy (ME, MJ/kg DM) of roughages and compounded feed mixture used in *in vitro* and *in vivo* studies

Parameter	Paddy straw	Urea treated (@2%) Paddy Straw	CFM
DM	95.69	94.48	91.74
OM	87.28	85.54	92.36
CP	4.48	9.02	18.26
EE	1.01	1.00	2.75
TA	12.72	14.46	7.65
NDF	74.08	64.29	16.77
ADF	52.12	52.30	6.26
ADL	4.58	4.61	1.44
ME[2]	4.32	5.62	11.03

[1]Mean of two replicates. Variations in duplicate measurements were within ± 3% of the mean
[2] Determined by RIVGPT (Menke *et al*., 1979) [29]

Body weight gain

The body weight changes in experimental animals in T1, T2 and T3 were presented in Table 3. There was no significant difference in the body weight changes (467 g in T1, 427 g in T2 and 373 g in T3) between treatment groups indicating that supplementation of fibrinolytic enzymes in the diet did not bring any significant difference in weight gain of animals. However, at higher enzyme level (3.2 g/kg DM) numerically decreased body weight changes were observed when compared to control and T3 (lower enzyme level). The present study results were in agreement with the findings of Knowlton *et al*. (2002) [19], Elwakeel *et al*. (2007) [16] and Anupkumar (2016) [6] in lactating cows. The body weight changes in all the groups indicated that the buffaloes were on positive energy balance.

Dry matter intake

The DMI (kg per day) was 10.32, 10.49 and 10.20, respectively for groups T1, T2 and T3 (Table 3). The urea treated (@ 2%) paddy straw was offered *adlbitum* while CFM was offered in calculated quantities to meet the total requirement of nutrients to the individual buffaloes. The DMI (total, as % body weight and per kg B. wt) for all three diets were similar. While some of the previous studies reported no effect of supplementation of fibrinolytic enzyme on DMI (Rode *et al*., 1999 [29] in Holstein cows; Schingoethe *et al*., 1999 [30] in Holstein cows; Bowman *et al*., 2002 [10] in lactating Holstein cows; Granzin, 2005 [18] in mid lactating Friesian cows; Adesogan *et al*., 2007 [2] in lactating Holstein cows; Elwakeel *et al*., 2007 [16] in Holstein cows; Singh and Das, 2009 [33] in buffalo calves; Bassiouni *et al*., 2010 [8] in lactating Friesian cows; Shekhar *et al*., 2010 [31] in lactating Murrah buffaloes; Lopuszanska-Rusek and Bilik., 2011 [20]; Rajamma *et al*., 2014 [27] in Buffalo calves; El-Bordeny *et al*., 2015 [15] in Holstein cows; Morsey *et al*., 2015 [24] in lactating Egyptian buffaloes and Anupkumar *et al*., 2017 [5] in dairy cows). On contrary, few others reported increased DMI with the supplementation of fibrinolytic enzyme in the diets (Beauchemin *et al*., 2000 [9, 38] in multiparous dairy cows; Knowlton *et al*., 2002 [19] in Holstein cows; Miachieo and Thakur, 2007 [22] in lactating Sahiwal crossbred cows; Gaafar *et al*., 2010 [8, 17] in lactating buffaloes; Das and singh., 2011 [12] in crossbred cows: Dehghani *et al*., 2011 [13] in Holstein cows and Da Silva *et al*., 2015 [11] in Holstien cows). They stated that the DMI of animals fed diet supplemented with fibrinolytic enzyme was more because of palatability (Rajamma *et al* 2014) [27] and combined effect of increased intake as well as digestion (Beauchemin *et al*., 2000; Gaafar *et al*., 2010) [9, 38, 8, 17]. The result of the present study corroborated with the recent findings of Anupkumar *et al*. (2017) [5], who observed no differences in the roughages, CFM and total DM intake of cows fed fibrinolytic enzyme in dairy cows fed straw based diets.

Nutrient intake

Intake of nutrients *viz*., OM, CP, NDF and ADF (Table 3) were also statistically not affected significantly among groups and fibrinolytic enzymes could not increase intake of any nutrients. The results were in agreement with findings of Rode *et al*. (1999) [29] in Holstein cows; Yang *et al*. (2002) [39] in lactating Holstein cows; Shekhar *et al*. (2010) [31] in lactating Murrah buffaloes; El-Bordeny *et al*. (2015) [15] in Holstein cows; Morsey *et al*. (2015) [24] in lactating Egyptian buffaloes and Anupkumar *et al*. (2017) [5] in dairy cows. Contrary to this some observed increased intake of OM (Beauchemin *et al*., 2000 in cows; Gaafar *et al*., 2010 in buffaloes) [9, 38, 8, 17], CP (Gaafar *et al*., 2010 in buffaloes) [8, 17], NDF intake (Beauchemin *et al*., 2000 in cows; Knowlton *et al*., 2002 in cows and Da Silva *et al*., 2015 in Holstein cows) [9, 38, 19, 11] and ADF intake (Beauchemin *et al*., 2000 in cows) [9, 38].

Table 3: Mean body weight gain, dry matter intake and nutrient and energy intake of lactating buffaloes during feeding trial

Parameter	T 1	T 2	T 3	SEM	P
Body weight (kg)					
Initial	371.40	372.60	371.40	8.765	0.9979
Final	388.80	389.00	380.10	9.418	0.9158
Gain, g /d	467	427	373	0.039	0.534
DMI					
PS, kg/d	5.55	5.61	5.40	0.145	0.850
% b. wt.	1.42	1.44	1.35	0.021	0.203
CFM, kg/d	4.78	4.88	4.80	0.097	0.915
% b. wt.	1.23	1.26	1.21	0.018	0.547
Total DMI (kg/d)	10.32	10.49	10.20	0.226	0.884
% b. wt.	2.65	2.70	2.56	0.029	0.147
CPI					

PS, kg/d	0.43	0.43	0.44	0.019	0.993
% b. wt.	0.110	0.114	0.110	0.004	0.911
CFM, kg/d	0.75	0.76	0.76	0.015	0.970
% b. wt.	0.191	0.195	0.191	0.003	0.794
Total CP (kg/d)	1.18	1.19	1.20	0.030	0.986
% b. wt.	0.30	0.31	0.30	0.005	0.873
OM (kg/d)	9.99	10.10	9.98	0.225	0.969
% b. wt.	2.56	2.60	2.51	0.032	0.495
NDF (kg/d)	4.33	4.40	4.16	0.105	0.653
% b. wt.	1.11	1.13	1.04	0.015	0.035
ADF (kg/d)	3.19	3.23	3.12	0.080	0.859
% b. wt.	0.82	0.83	0.78	0.011	0.217
ME (MJ/d)	86.28	87.16	86.17	1.783	0.972

Mean values between the different treatments groups do not differ significantly for all parameters
T1- Control, T2- 1.6 g enzyme /kg CFM, T3- 3.2 g enzyme/kg CFM

Apparent digestibility of nutrients

Intake (kg/d) and apparent digestibility (%) of the nutrients *viz*., OM, CP, NDF and ADF were presented in table 4 and were also statistically not affected significantly between groups and fibrinolytic enzymes could not increase digestibility of any nutrients. The previous reports were also showed the similar results in digestibility of DM (Knowlton *et al*., 2002; Adesogan *et al*., 2007) [19, 2], OM (Shekhar *et al*., 2010; Rajamma *et a.*, 2014) [31, 27], CP (Sutton *et al*., 2003; Adesogan *et al*., 2007; Shekhar *et al.*, 2010) [35, 2, 31], NDF and ADF (Yang *et al*., 2000; Sutton *et al*., 2003; Naik., 2004; Knowlton *et al*., 2002; Adesogan *et al*., 2007; Rajamma *et al*., 2014) [38, 35, 25, 19, 2, 27] in lactating animals. However, many researcher found increase in digestibility of DM (Gaafar *et al*., 2010 and Morsey *et al*., 2015) [8, 17, 24], OM (Yang *et al*., 2000 and Gaafar *et al*., 2010) [38, 8, 17], CP (Yang *et al*., 2000; Gaafar *et al*., 2010 and Morsey *et al*., 2015) [38, 8, 17, 24], NDF and ADF (Beauchemin *et al*., 2000; Shekhar *et al.*, 2010; Das and Singh, 2011; Azzaz *et al*., 2013 and Morsey *et al*., 2015) [9, 38, 31, 12, 7, 24] in lactating animals which was influenced by the enzyme supplementation.

Table 4: Nutrient intake and apparent digestibility of in experimental buffaloes during digestion trial period

Parameter	Treatment			SEM	P
	T1	T2	T3		
DM					
Intake(kg/d)	10.33	10.24	10.08	0.252	0.924
Digestibility (%)	65.83	64.94	67.19	0.619	0.339
OM					
Intake(kg/d)	9.28	9.21	9.05	0.224	0.920
Digestibility (%)	68.95	67.87	69.60	0.656	0.575
CP					
Intake(kg/d)	1.24	1.23	1.22	0.028	0.966
Digestibility (%)	71.35	68.90	74.22	0.953	0.068
NDF					
Intake(kg/d)	4.41	4.34	4.27	0.124	0.897
Digestibility (%)	50.22	49.64	53.86	1.163	0.287
ADF					
Intake(kg/d)	3.24	3.18	3.14	0.096	0.922
Digestibility (%)	52.44	52.12	54.84	0.651	0.177

Mean values between the different treatment groups s do not differ significantly for all parameter
T1- Control T2- 1.6 g enzyme/kg CFM T3- 3.2 genzyme/kg CFM

Conclusion

Fibrinolytic enzymes supplementation at 15 and 30 % more than normal level of xylanase activity in the rumen, failed to bring any improvement in performance of lactating buffaloes in terms of body weight changes, DMI, nutrient intake and apparent digestibility of nutrients.

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