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Effect of Probiotic and Exogenous Fibrolytic Enzyme Treated Rice Straw on the Performance of Hariana Heifers

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ABSTRACT

This study evaluated the probiotic and exogenous fibrolytic enzyme (EFE) treated rice straw on the performance of Hariana heifers. Animals were allocated into three groups having six animals and fed diet for 120 days. The control group was fed on basal diet whereas, group-1 was supplemented basal diet with 5g/kg DM probiotic and group-2 was supplemented basal diet with 7g/kg DM EFE. The nutrient requirements of heifers were met by feeding concentrate, Jowar, and paddy straw (50:30:20 ratio) on DM basis. No effect on DMI,Feed conversion ration (FCR) or Feed conversion efficiency(FCE) but body weight gain was significantly higher ($P<0.05$) in supplemented group.There was no significant effect ($P<0.05$) on blood parameters except Hb and HCT levels which was significantly higher in the treatment groups**.**Treatment groups showed significantly higher ($P<0.05$) plasma total protein, globulin, cholesterol, ALP and lower bilirubin whereas, no significant effect ($P>0.05$) was found on albumin, glucose, triglycerides, ALT, AST, creatinine and PUN levels. BHBA and IGF-1 were similar in all group but NEFA, FRAP and SOD were significantly different ($P<0.05$) in control, probiotic and EFE group, respectively. No effect on plasma minerals except P and Se.there was no effect on animal behavior parameters but rumen enzyme i.e., cellulase, avicelase, and xylanase activity in EFE group showed significantly higher ($P<0.05$). Finally, it may be concluded that probiotics (*Saccharomyces cerevisiae*) and EFE @ 5 and 7 g per kg DM, respectively improved weight gain and digestibility of nutrients without affecting blood haematology, biochemical parameters, mineral profile and ingestive behaviour adversely.

KEYWORDS: Heifers,Exogenous fibrolytic enzyme, Paddy straw,Probiotics

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INTRODUCTION

In tropical zones in the world, ruminants depend on year-round grazing on natural pastures, or the animals are fed with cut grass and crop residues. Most of these areas face seasonal dry periods in which the availability of pasture decreases and its quality by a reduction in the content of digestible energy and nitrogen (Zhuang et al., 2020). In these areas, rice straw is abundantly available from cultivating rice, and farmers offer rice straw as the main roughage source for their animals. The high level of lignification and silicification, the slow and limited ruminal degradation of the carbohydrates, and the low content of nitrogen are the main deficiencies of rice straw, affecting its value as feed for ruminants (Van Soest, 2006). Rice straw is usually fed untreated without supplements in spite of the fact that many methods for improved utilization of rice straw have been developed and recommended. Manipulation of the rumen microbial ecosystem by feed additives showed potential for manipulation of rumen fermentation (Jouany and Morgavi, 2007). The most important feed additives in this direction are "probiotics and enzymes" which have no residual effects (Zadrazil et al., 1995). Among probiotics, *Saccharomyces cerevisiae* (brewers and baker's yeast) have been reported by nutritionists to be used as additive (Guedes et al., 2018). It stabilizes the pH of rumen and therefore, favors the growth of cellulolytic bacteria sensitive to low pH. The oxygen scavenger property of yeast in the rumen helps to protect obligate anaerobes from the air ingested in the rumen along with feed intake. An increase in production of total VFA, ratio of acetate to propionate and *in vitro* dry matter digestibility was reported in cattle (Ganai et al., 2006). The usage of exogenous fibrolytic enzymes has been reported to have a positive effect on digestion. Many recent studies have reported increased digestion of dry matter and fiber measured *in situ* and *in vitro* on the usage of fibrolytic enzymes (Giraldo et al., 2014).But the extent to which level the probiotic and enzyme mix can be incorporated in livestock ration to increase nutrient intake and utilization as well as animal performance has not been well established. Hence, present study was performed to study the effect of *Saccharomyces cerevisiae* or enzyme mix supplementation on growth, haematology and biomarkers of energy, lipid and protein metabolism, rumen enzyme activity and animal behaviors of Hariana heifers fed paddy straw-based diet.

MATERIALS AND METHODS

A total of 18 growing Hariana heifers were selected from Livestock Farm Complex (LFC), DUVASU, Mathura, India and randomly assigned into three groups (six animals in each group) on body weight and age basis. Animals in control group fed with basal diet i.e., rice straw (particle size- 1.5 to 2.0 cm), chaffed green Jowar fodder and compounded concentrate mixture as per NRC (2001) requirements. Concentrate mixture was prepared by mixing maize, barley, wheat grain, wheat bran, gram chunni, mustard oil cake and mineral mixture in 23, 10, 10, 20, 5, 30 and 2 parts, respectively. Control group were fed on basal diet whereas, animals in treatment group-1 (Prob) and treatment group-2(EFE) group were fed basal ration with 5g/kg DM probiotic and7g/Kg DM exogenous fibrolytic enzyme, respectively for 120 days. They were mixed in concentrate mixture at the time of feeding of individual animal.Probiotic (*Saccharomyces cerevisiae* 25 cfu/g) was purchased from Bioven ingredients, Gautam Buddha Nagar, Uttar Pradesh and exogenous fibrolytic enzymes mix (Amylase, Xylanase, Beta Glucanase, Cellulase, Protease, Lipase, Pectinase, Phytase with activity of 2, 15, 2.5, 4, 2, 1.5, 4 and 2 lakh IU/kg, respectively) were purchased from Mafezyme Forte, Bengaluru.Experimental heifers were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. Deworming of all the experimental animals was done before the start of the experiment by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at the dose level of 10 mg/kg bodyweight.

Body weight of the experimental heifers was recorded at the start of the experiment and subsequently at fortnightly intervals. Heifers were weighed for two consecutive days in the morning at 06:00h before offering feeds, fodders, and water. The average of two consecutive days was considered as body weight for that fortnight and considered for ADG. The samples of feeds and fodders offered and orts left were dried in a hot air oven at 60 °C and grounded in a Wiley mill to pass a 1-mm sieve for further analysis of DM (Method 973.18c), crude protein (CP; Method4.2.08), ether extract (EE; Method 920.85), and total ash (TA; Method 923.03) by following Association of Official Analytical Chemists procedures (AOAC, 2005). Neutral detergent fibre (NDF), acid detergent fibre (ADF), were determined according to the procedures described by Van Soest et al. (1991). Calcium (Ca), Phosphorus (P),Copper (Cu), Selenium (Se), Iron(Fe) and Zinc (Zn) contents in samples of feeds and fodders were analysed by inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA) facility at Animal Nutrition Department, DUVASU Mathura. The ingredient and nutrient composition of TMR fed during experimental period are presented in Table 1.

Table 1. Ingredients and chemical composition of TMR fed during the experimental period

Ingredient composition (%DM)	Control	Prob	EFE
Jowar fodder	30	30	30
Paddy straw	20	20	20
Concentrate	50	50	50
Concentrate ingredients (%DM)			
Maize grain	23	23	23
Barley grain	10	10	10
Wheat grain	10	10	10
Gram chunni	20	20	20
Wheat bran	5	5	5
Mustard cake	30	30	30
Mineral mixture	2	2	2
Chemical composition (%)			
DM	68.7	68.7	68.7
OM	91.4	91.4	91.4
EE	3.09	3.09	3.09
CP	14.0	14.0	14.0
ASH	8.59	8.59	8.59
CF	23.5	23.5	23.5
NFE	50.8	50.8	50.8
NDF	58.6	58.6	58.6
ADF	35.8	35.8	35.8
Probiotic	-	5g/kg	-
Exogenous fibrolytic enzyme		-	7g/kg

Blood samples were collected before feeding and watering of heifers at 07:00 h in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60, 90 and 120 days post supplementation. Collected blood samples were analyzed for haematological, blood biochemical and hormonal attributes. A fraction of whole blood samples was used for white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb) concentration, haematocrit values, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets and mean platelets volume was estimated with the help of Celltac-± (MEK-6500K) auto haemoanalyser made by Nihon kohden Europe.A fraction of whole blood samples were used for the analysis of superoxide dismutase (SOD; Madesh and Balasubramanian, 1998) and FRAP (Benzie and Strain, 1999) activity. Remaining amount of blood samples was centrifuged at 3000 rpm for 15 min to separate the plasma. Plasma samples were stored at -20°C until further analysis of biomarkers of liver and kidney function (glucose, triglycerides, AST, ALT, ALP, bilirubin, and creatinine), biomarkers of protein metabolism (total protein, albumin, globulin, and PUN). The plasma concentration of glucose, triglycerides, AST, ALT, ALP, bilirubin, creatinine, total protein, albumin, and PUN was determined by an automated biochemical analyser (BS-120 Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co.Ltd., China) using Span Diagnostic kits (Span Diagnostic Ltd., Surat, India). NEFA, BHBA and IGF-1 were estimated using ELISA kits supplied by Bioassay Technology Laboratory, China. Plasma globulin concentration was determined by subtracting the albumin content from total protein content. The minerals were analyzed by the inductively coupled plasma-optical emission spectroscopy (5800 ICP-OES Agilent, CA, USA). Enzyme activity is determined by the absorbance method in the spectrometer read absorbance at 575 nm against reagent blank. Ingestive behavior traits of experimental heifers were evaluated in the mid of study by camera.

The generated data were analysed by a mixed model for repeated measurements in Statistical Package for the Social Sciences (SPSS for windows, V21.0; SPSS Inc., Chicago, IL, USA) by using the following model:

$$Y_{ij} = \mu + H_i + T_j + e_{ij}$$

Where Y_{ij} is the dependent variable, lis the overall mean of the population, H_i is the random effect of heifers, T_j is the fixed effect of treatment (*j* = 0, 5g/kg probiotic or 7g/kg EFE),and e_{ij} is the unexplained residual element assumed to be independent and normally distributed. Turkey honestly significant test was applied to treatment means which showed a statistically significant variation in the samples. Difference was considered significant at $P<0.05$.

RESULTS AND DISCUSSION

There was no significant effect on dry matter intake (kg/day or % BW) in allgroups. Higher (P<0.05) average daily gain was observed in probiotic supplemented group.No effect of probiotic and EFE supplementation was observed on average body weight, FCR, FCE in heifer fed paddy straw-based diet (Table 2).These findings were similar to report on heifers supplemented with 5g/Kg DM probiotics (Jiang et al., 2020). A similar effect was also reported for EFE supplementation ingrowing buffaloes with no effect on the feed intake (Malik and Srinivas, 2010) and weaned dairy calves (Titi and Tabbaa, 2004). The total weight gain and ADG were improved (P<0.05) as compared to heifers receiving no treatment in between experimental periods. The significantly improved weight gain for treatment group heifers may be due to the nutritional superiority of the probiotic and EFE paddy straw-based ration over the untreated paddy straw-based ration (Dean et al., 2013, Yadav et al 2018). Besides, the possible positive effect of TMR having probiotic and EFE-treated rice straw on body weight gain could be attributed to increased microbial cellulolytic activity, leading to improved fiber fermentation and better synthesis of microbial protein, resulting in increased supply of post-ruminal amino acid (Erasmus et al., 1992).

Table 2. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on dry matter intake and growth performance in heifers

Parameters	Treatment groups			SEM	P value		
	Control	Prob	EFE		Treatment (T)	Period (P)	T×P
Initial BW (kg/day)	119	119	120	10.6	0.99	-	-
Final BW (kg/day)	169	171	172	10.2	0.99	-	-
Av. BW (kg/day)	138	139	140	3.92	0.80	0.90	1.00
DMI (kg/day)	4.15	4.18	4.22	0.12	0.80	0.90	1.00
ADG (g/day)	492^{ab}	516^{a}	512^{b}	5.58	0.001	0.001	0.001
FCR %	10.23	10.3	10.0	0.38	0.74	0.009	1.00
FCE %	0.13	0.12	0.12	0.01	0.68	0.004	0.99

P<0.05: significant. SEM, standard error of mean; DMI, dry matter intake; BW, body weight; ADG, average daily gain; FCR, feed conversion ratio; FCE, feed conversion efficiency. Control: basal diet;Prob: Basal diet containing concentrate with 5g/Kg DM probiotic (*Saccharomyces cerevisiae)*; EFE: Basal diet containing concentrate with 7 g/Kg DM exogenous fibrolytic enzymes.

Table 3.Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on nutrient digestibility (%) of heifers

Parameters	Group			SEM	P value
	Control	Prob	EFE		
Initial BW (Kg)	163	165	166	10.3	0.99
Final BW (Kg)	167	169	170	10.3	0.99
ADG (g/day)	453	511	546	40.2	0.66
Digestibility coefficients					
DM	58.5	62.6	64.3	0.33	<0.001
OM	59.9	64.5	66.2	0.50	<0.001
CP	69.4	72.2	72.0	0.32	<0.001
EE	79.2	80.0	81.5	0.24	<0.001
CF	51.3	53.5	58.6	0.75	<0.001
NFE	69.9	72.2	74.8	0.55	<0.001
NDF	52.6	54.7	59.7	0.82	<0.001
ADF	46.0	47.8	49.0	0.31	<0.001

Table 3 shows role of probiotics and exogenous fibrolytic enzyme treated rice straw in digestibility of nutrients. Mean digestibility of nutrients was significantly higher (P<0.001) in treatment groups than control. Probiotic and enzyme treatment improved utilization of carbohydrate, protein, and lipid as nutrient digestibility was observed better in treatment group heifers (P<0.001). The increasing trend of digestibility might be because probiotics likely improved gut health, enhancing nutrient absorption while enzyme-mediated digestibility increases might be due to increased microbial colonization of feed particles or by direct cell wall hydrolysis and degraded the lignocellulosic contents of rice straw.Similarly, Kholif et al. (2022) found that paddy straw yeast treatments increased nutrient digestibilityand thus their feeding values as TDN and digestible crude protein (DCP) increased compared with the untreated paddy straw. Generally, probiotics may increase enzyme activity in the gastrointestinal tract and improve digestibility (Sharma et al., 2018).Similarly, Gado et al. (2013) also reportedthe potential of enzymes in improving digestibility and degradation of NDF and ADF of rice straw pre-treated with exogenous enzymes.

Table 4. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on haematological parameters

Parameters	Treatment groups			SEM	P value		
	Control	Prob	EFE		Treatment (T)	Period (P)	T×P
RBC (x 10^6/µL)	8.27	8.55	8.35	0.10	0.72	0.008	0.329
Hb (g/dl)	10.0^{ab}	10.3^{b}	10.6^{a}	0.11	0.042	0.57	0.997
platelets (x10^9 / L)	285	292	297	9.32	0.44	0.002	0.397
WBC (x10^9 / L)	11.1	10.8	11.8	0.20	0.20	0.53	0.444
HCT (%)	28.5	30.10	27.9	0.36	0.54	0.72	0.848
MCV (fL)	36.6	35.90	35.7	0.38	0.33	0.53	0.705
MCH (pg)	12.6	12.19	11.9	0.15	0.05	0.91	0.987
MCHC (g/dL)	34.4	34.41	34.1	0.12	0.07	0.001	0.213
MPV (fL)	6.71	6.73	7.02	0.13	0.26	0.27	0.075

The concentration for hematological indices was well within the normal range reported for heifer after treatment of probiotic and exogenous fibrolytic enzyme, indicating that TMRs had no adverse effect on the physiological status of the heifers, which is also a sign of good health. Hemoglobin concentration was statistically ($P<0.05$) greater in the treatment group than control group. The observed elevated effects might be due to dietary supplementation of probiotics and EFE triggered blood-cell formation processes as a result of better iron salt absorption from the small intestine and vitamin B production (Kander, 2004). Mean values for RBC, Platelets, WBC, HCT, MCV, MCH, MCHC, and MPV did not show any significant effect ($P>0.05$) in the supplemental group. Findings were similar to reported by Milewski and Sobiech (2009) in heifers fed probiotics and EFE supplemented TMR.

Table 5. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on blood biochemical parameters

Parameters	Treatment groups			SEM	P value		
	Control	Prob	EFE		Treatment (T)	Period (P)	T×P
Glucose (mg/dl)	49.5	50.9	50.0	0.32	0.48	0.14	0.298
Triglyceride (mg/dl)	29.4	30.8	31.0	0.41	0.02	0.75	0.374
Cholesterol (mg/dl)	90.1^{b}	94.8^{a}	96.7^{a}	0.48	0.00	0.69	0.997
Total protein (g/L)	6.10^{b}	7.38^{a}	6.26^{b}	0.08	0.43	0.53	0.695
Albumin (g/L)	2.91^{c}	4.08^{a}	3.27^{b}	0.07	0.02	0.94	0.445
Globulin (g/L)	3.20	3.30	2.98	0.06	0.11	0.04	0.014

Plasma levels of glucose, cholesterol and triglycerides were used as biomarkers of energy and lipid metabolism while, plasma total protein, plasma albumin and globulin were used as biomarkers of protein metabolism (Table 5). The plasma cholesterol level was significantly higher ($P<0.05$) due to mobilization of fat supplemented animals (Liu et al., 2922) while no difference was observed in triglyceride and glucose level. All biomarkers of protein metabolism are within normal physiological limit and no significant ($P>0.05$) difference were observed in supplemented as well as unsupplemented heifers. In heifers, EFE supplementation up to 6 g/Kg DM had no effect on mean plasma glucose, cholesterol, or triglyceride concentrations (Singh et al., 2018). Both the probiotic and enzyme treatments increased the concentrations of serum total protein and albumin, which are important indicators for the improved nutritional and physiological status of the heifers due to increased nutrient intake and digestibility. Similar findings were observed in *Corriedale* sheep on feeding paddy straw treated with probiotic and enzyme @ 9 g/kg DM to the complete feed (Sheikh et al.,2017).The reasons for the lower content of globulin in the present study may be due to individual variations of animal, breed, or seasonal effects.

Table 6. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on antioxidant status, liver and kidney function parameters

Parameters	Treatment groups			SEM	P value		
	Control	Prob	EFE		Treatment (T)	Period (P)	T×P
Liver and kidney function parameters							
ALT (IU/L)	5.76	6.15	6.28	0.09	0.002	0.134	0.063
AST (IU/L)	25.4	24.5	25.7	0.20	0.875	0.613	0.663
ALP (IU/L)	57.8[a]	58.6[ab]	58.6[b]	0.18	0.004	0.271	0.126
Bilirubin (mg/dl)	0.99[b]	0.88[a]	0.89[a]	0.02	<0.001	0.359	0.866
Creatinine (mg/dl)	0.92	0.90	0.90	0.00	0.058	0.051	0.063
PUN (mg/dl)	7.15	7.05	6.96	0.05	0.2294	0.061	0.058
BHBA (pmol/ml)	0.16	0.16	0.17	0.002	0.098	0.064	0.075
NEFA (µmol/L)	0.018[a]	0.018[a]	0.019[b]	0.0001	0.002	0.076	0.743
IGF (ng/ml)	0.32	0.32	0.32	0.001	0.876	0.067	0.089
Antioxidant activity parameters							
SOD (µmol MTT formazan/mg Hb)	0.50[b]	0.52[b]	0.33[a]	0.03	<0.001	<0.001	0.010
FRAP (µmol/L)	1133[a]	1177[b]	1176[b]	12.6	0.001	<0.001	<0.001

Studied biomarkers of liver function test in the present study were ALT, AST, ALP, and bilirubin whereas, plasma creatinine and PUN is used as biomarker of kidney function test. In the present study, significantly decreased plasma bilirubin and creatinine levels in supplemented groups, and values were found highest in control groups were in line with findings of Singh et al. (2018). Similarly, Magaye et al. (2014) found a substantial reduction in total bilirubin levels in the serum after using probiotics, however, the values were found to be normal physiological levels in this study. No adverse effect of supplementation on liver function was evident from similar plasma activity of ALT and AST in the control, probiotic, and EFE groups. The ALP level was higher in both supplemented groups but it was under normal physiological range. It might be due to the supplementation of EFE. Moreover, treatments did not affect the concentrations of serum ALT or AST, suggesting minimal effects of treatments on liver health (Pettersson et al.,2008*)*. Although ruminal NH_3-N concentration elevated with the additive use, this was not reflected in blood urea-N concentration. In agreement with the present findings, no effect of yeast and EFE feeding treatments on the concentrations of NEFA in plasma was also observed in Holstein steers (Hosoda et al., 2006) and early and mid-lactation Holstein cows (Peters et al., 2015). Additionally, the unchanged or minor changes in concentrations of serum NEFA, BHBA and IGF-1 indicate that body-fat breakdown was not changed and the animals were not in a negative energy balance in the EFE or probiotic treatments (Ye et al*.,* 2009). In the present study, FRAP and SOD were estimated to access antioxidant status of animals. SOD were significantly lower (P<0.05) in heifers receiving EFE-supplemented diets. FRAP were significantly higher (P<0.05) in heifers receiving probiotic and EFE-supplemented dietsItmight be due to improvement in nutritive digestibilityand related to physical conditioning causing improvement of feed palatability (Arjomand et al., 2022).

Table 7. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on plasma mineral profile

Parameters	Treatment groups			SEM	P value		
	Control	prob	EFE		Treatment (T)	Period (P)	T×P
Ca (mg/dl)	10.5	10.4	10.5	0.14	0.988	0.737	0.786
P (mg/dl)	5.00[c]	6.08[b]	6.62[a]	0.09	0.001	0.360	1.000
Se (mg/dl)	0.05[a]	0.06[b]	0.06[b]	0.02	0.004	0.000	0.000
Fe (mg/L)	2.60	2.64	2.57	0.04	0.793	0.888	0.612
Cu (mg/L)	1.19	1.20	1.27	0.03	0.207	0.055	0.601
Zn (mg/L)	1.41	1.50	1.48	0.03	0.334	0.098	0.212

Plasma concentrations of Ca, Fe, Cu and Zn indicated that dietary supplementation in heifers did not show any significant effect. Heifers were in positive balance for all minerals in this experiment. Other researchers also showed that EFE supplementation had no significant effect on Ca and P levels (Bhasker et al., 2013), Fe and Cu levels (Avellaneda et al., 2009). Similarly, supplementation of yeast culture in the TMR group had no effect on Ca, Fe, Cu and Zn retention. This result is in agreement with the findings of other researchers (Chandrasekhar et al., 2009). Serum P and selenium concentrations were significantly increased ($P<0.05$) in this experiment after supplementation compared control group. This may be due to the increasing absorption of nutrients in the supplemented group than control group.

Table 8. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on rumen enzyme activity

Enzyme	Group			SEM	P value
	Control	Prob	EFE		
CMC (µmole/h/ml)	45.5[a]	58.6[ab]	76.8[b]	4.94	0.010
Avicelase (µmole/h/mL)	32.3[a]	35.4[a]	39.1[b]	1.02	<0.001
Xylanase (µmole/h/ml)	10.9[a]	10.5[a]	1200[b]	0.76	0.019
Glucosidase (µmole/h/ml)	3.42	3.73	4.32	0.51	0.060
Proteases (µg/h/ml)	3.04	3.68	3.95	0.60	0.062
Amylase (mg/min/ml)	1.04	1.11	1.14	0.13	0.079
Urease (µg/min/ml)	2.53	2.83	3.37	0.16	0.43

The activity of cellulase, avicelase, and xylanase in the EFE group were statistically significantly ($P<0.05$) higher than probiotic and control group. There was no significant difference between the control, probiotic, and EFE groups in the case of glucosidase, protease, amylase, and urease activity ($P>0.05$). Many authors suggested that concentrations of EFE below 9 g/kg of DM in the diet, as that observed in the present EFE feeding groups, do not have adverse effects on ruminal fermentation (Salawu et al., 1999). Forsberg et al. (1981) demonstrated that the cellulase breaks to the outer membrane after coming into physical contact with its substrate in order to hydrolyze it. For these reasons, enzymes from bacteria were more active compared to paddy straw. Considering the nature of the urease enzyme that is produced in bacterial cells and its extracellular action, no clear difference was found among all three groups.

Table 9. Effect of probiotics and exogenous fibrolytic enzyme treated rice straw supplementation on animal behaviors

Animal Behaviour	Group			SEM	P value
	Control	Prob	EFE		
Daily time spend on feeding (min/day)	300	270	250	13.2	0.319
Feeding time DM (min/Kg DM)	29.3	27.1	23.1	1.90	0.429
RTDM (min/kg DM)	36.8	41.9	42.8	2.05	0.460
Chewing time (min/day)	236	233.0	234	3.81	0.955
Chewing time DM (min/Kg DM)	24.2	23.6	22.9	2.00	0.971
Time spent on idleness (min/day)	710	730	750	37.1	0.918
BCS (body condition score)	2.83	3.00	3.17	0.18	0.775
Urination time /day	6.33	5.83	7.17	0.49	0.578

Note : RTDM : rumination time /kg DM; BCS : body condition score

There was no significant difference (P>0.05) between the control and the supplemented group for most of the ingestive behavior variables studied. The feeding behavior of animals may be altered by physical activity which thus influences total energy expenditure and feed efficiency. Similarly, Refat (2018) found that low RFI animals spent less time and energy in this activity and more time in sedentary activities, possibly saving energy that is directed towards weight gain. In theory, pretreating fibrolytic enzymes to feed may decrease chewing time and saliva output which may increase the risk of acidosis (Arriola et al., 2011). Bowman et al. (2002) noted that adding enzymes before feeding may induce a modification in plant cell wall structure which could cause a reduction in the physical effects of the fiber. Earlier study found that fibrolytic enzymes showed no effect on total chewing activity, urination activity, and body condition score (Refat, 2018).

CONCLUSION

It may be concluded that probiotics (*Saccharomyces cerevisiae*) and exogenous fibrolytic enzyme @ 5 and 7 g per kg DM, respectively treated paddy straw based diet improved weight gain and digestibility of nutrients without affecting blood haematology, biochemical parameters, mineral profile and ingestive behaviour adversely. Thus, rice straw may be included in ration of ruminants up to 20% of DM with probiotics (*Saccharomyces cerevisiae*) and exogenous fibrolytic enzyme treatments.

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