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EFFECT OF FEEDING XYLANASE AND CELLULASE TREATED OAT SILAGE ON NUTRIENT DIGESTIBILITY, GROWTH PERFORMANCE AND BLOOD METABOLITES OF NILI RAVI BUFFALO CALVES

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An experiment was conducted to investigate the effect of feeding different levels of oat silage (*Avena sativa*) with and without combination of exogenous fibrolytic enzymes (xylanase and cellulase) treatment on the growth performance, nutrient digestibility and blood metabolites of *Nili-Ravi* buffalo calves. Three iso-caloric and iso-nitrogenous diets with oat silage to concentrate ratio of 50:50, 60:40 and 70:30 were formulated. Thirty six Nili-Ravi buffalo calves (9 months old) were used in 2×3 factorial arrangement under Randomized Complete Block Design. In first group, the diets were treated with enzymes (Econase Red L®) before feeding to buffalo calves. The diets with oat silage to concentrate ratio of 50:50, 60:40 and 70:30 treated with enzymes were ET50, ET60 and ET70, respectively. In second group, the diets without enzyme treatment were fed to buffalo calves. The diets with oat silage to concentrate ratio of 50:50, 60:40 and 70:30 without enzyme treatment were ET50, ET60 and ET70, respectively. Data regarding weight gain, nutrient digestibility, blood glucose and blood urea nitrogen were collected. Digestibility of nutrients was determined by total collection method. Results showed that the dry matter (DM) intake of calves fed exogenous fibrolytic enzymes treated and untreated oat silage was not significantly (P>0.05) different whereas the digestibility of DM, crude protein, neutral detergent fiber and acid detergent fiber was significantly increased (P<0.05) for all enzymes treated diets compared to calves fed oat silage without enzyme treatment. Non-significant (P>0.05) differences were observed in blood glucose and blood urea nitrogen level at 0, 3, 6 and 9 h post-prandial. Total weight gain (122.13 - 139.18 kg) and average daily weight gain (0.317-0.541 kg) of calves fed enzyme treated diets were significantly higher (P<0.05) than calves fed oat silage without enzyme treated diets. Enzyme treatment improved animal performance and nutrient digestibility. However, the performance of claves fed low concentrate and high silage diets either with or without enzyme treatment was better compared to other diets.

Keywords: Buffalo calves, xylanase and cellulase, oat silage, growth performance, nutrient digestibility, blood parameters

INTRODUCTION

Continuous supply of quality forages in sufficient quantity is essential for efficient ruminant production (Bilal, 2009). Ruminants are getting 40 and 75% of their crude protein and energy requirements, respectively from fodders (Inam-ur-Rehim *et al.,* 2008). Ensiling the fodder during fodder availability periods may bridge the gap between animal requirements and nutrients availability, as fermentation process conserves the fodder in form of silage (Colombatto *et al.,* 2004). Soluble carbohydrates concentration is low in grasses so addition of fermentable carbohydrate source is prerequisite for conservation and fermentation (Iqbal *et al.,* 2005). To enhance the nutritional quality of silage many silage additives are being used. Exogenous fibrolytic enzymes are the best approach to improve the nutritive value of poor quality feedstuffs (Colombatto *et al.,* 2004). Enzymes hydrolyze the fiber into sugars that are used by silage bacteria to produce lactic acid which ultimately reduces the silage pH (Yang *et al.,* 2000). Anaerobic condition avoids the loss of organic matter during ensiling process (Bilal, 2009). This condition decreases the pH of silage and reduces the activity of plant protease enzyme that limits the further degradation of plant protein (Xing *et al.,* 2009). Fungal attack and growth of harmful bacteria is decreased at low pH (Kung *et al.,* 2000). In ruminants, the rate and extent of digestion of cell wall components improved by exogenous fibrolytic enzymes supplementation to silage (Pinos *et al.,* 2002). Enzymes supplementation increases the digestibility of fibrous portion of forage which is usually considered indigestible (Kung *et al.,* 2002). Animals fed on concentrate have high growth rate but concentrate availability is limited and expensive (Hoffman *et al.,* 2007). However, information regarding effect of concentrate and silage total mixed ration (TMR) treated with exogenous fibrolytic enzyme is limited. Therefore, the experiment was conducted at the Livestock Experimental Station, Khushab, Pakistan, to determine the effect of feeding xylanase and cellulase (Econase Red L®) treated oat silage *(Avena sativa*) on nutrient digestibility, growth performance and blood metabolites of *Nili Ravi* buffalo calves. The present research was planned to study the response of combination of exogenous fibrolytic enzymes along with different silage to concentrate ratios on growth performance, nutrients digestibility and blood metabolites of *Nili Ravi* buffalo calves.

MATERIALS AND METHODS

Thirty six *Nili-Ravi* buffalo male calves (9 months old, average weight 98.6 kg) were allocated into six groups (6 calves in each group) and housed in semi opened pens where they were individually fed.

Experimental diets**:** Oat grass was sown and harvested after seventy days of sowing. Oat was chopped and 980 g chopped oat was mixed with 20 g molasses. Commercially available 4 g exotic fibrolytic enzyme (Econase Red L®, Finland) was sprinkled over 1 kg fodder molasses blend. The blend (fodder, molasses and enzyme) was pressed to exclude air and was ensiled for 21 days which was sealed according to the procedures described by Sarwar *et al.* (2006) to achieve anaerobic conditions. After the fermentation of oat silage treated with xylanase and cellulase (Econase Red L®), three enzyme treated ET50, ET60 and ET70 iso-caloric and iso-nitrogenous diets with oat silage to concentrate ratio of 50:50 60:40 70:30 were formulated respectively. Similarly, three iso-caloric and iso-nitrogenous diets without enzyme treatment EU50, EU60 and EU70, with oat silage to concentrate ratio of 50:50 60:40 70:30, respectively were formulated. Diets were offered twice daily (morning and evening). The ingredient and nutrient composition of experimental diets is presented in Table 1.

Exogenous fibrolytic enzymes**:** Econase Red L® was used as source of fibrolytic enzymes. It was composed of xylanase 350,000 BXU/gram and cellulase 10,000 ECU/gram. The BXU is the amount of enzyme that will release 0.06 micromoles of reducing sugars (xylose equivalents) from brich xylan per minute at pH 5.3 and 50°C. The ECU is the amount of enzyme that will release 0.06 micromoles of reducing sugars as glucose from hydroxyethyl cellulose per minute at pH 4.8 and 50°C. *Trichoderma reesei* was source organism, ingredients of the products also included concentrated liquid *Trichoderma reesei* fermentation extracts, glycerin, and benzoate.

Data collection**:** The growth trial was consisted of 75 days. Diets were offered twice daily. The offered and refused feed was daily recorded. The experimental calves were individually weighed weekly before feeding in the morning and offered feeds were weekly adjusted according to changes of body weight. Animals were fed ad-libitum and availability of fresh water was ensured. For nutrient digestibility, total feces were collected per selected animal manually for 3 days. Feces samples were composited and laboratory samples were taken.

Harness was attached to each selected calf and complete urine collection was made possible through the pipes directly connected to the harness in collection drums and one sample per calf per day was taken in plastic bottles and stored at -20°C for the determination of nitrogen content. At end of trial, blood samples were taken after every 3 h over a period of 0 to 9 h post prandial from jugular

Table 1. Ingredient and nutrient composition of experimental diets

Ingredients	Enzyme Treated[1]			Enzyme Untreated[2]		
	ET50	ET60	ET70	EU50	EU60	EU70
Oat silage	50	60	70	50	60	70
Cotton seed cake	17	11	3.0	17	11	3.0
Corn	8.0	4.0	9.0	8.0	4.0	9.0
Sunflower meal	12	9.0	8.0	12	9.0	8.0
Wheat bran	7.0	7.0	1.0	7.0	7.0	1.0
Corn gluten 30%	2.0	5.0	4.5	2.0	5.0	4.5
Urea	0.5	0.5	1.0	0.5	0.5	1
Di-calcium	0.5	0.5	0.5	0.5	0.5	0.5
Molasses	2.0	2.0	2.0	2.0	2.0	2.0
Min-premix	1.0	1.0	1.0	1.0	1.0	1.0
Nutrient composition (%)						
Crude protein	17.26	16.95	17.14	17.26	16.95	17.14
Neutral detergent fiber	41.18	43.93	44.35	41.18	43.93	44.35
Acid detergent fiber	21.57	22.31	22.17	21.57	22.31	22.17
Metabolizable energy (Mcal/ kg)	2.39	2.39	2.39	2.39	2.39	2.39

[1]Enzyme treated ET50, ET60 and ET70 contained oat silage to concentrate ratio of 50:50 60:40 70:30 were formulated, respectively. [2]Enzyme untreated EU50, EU60 and EU70 contained oat silage to concentrate ratio of 50:50, 60:40 and 70:30, respectively.

vein to determine blood glucose and blood urea nitrogen (mg/dL) concentrations of each animal. The samples were placed immediately into tubes containing heparin as anticoagulant and pH was determined. The samples were stored at -20°C for further analysis. Blood glucose and blood urea nitrogen concentration was determined through the Clinilab200 semi auto chemistry analyzer delivered from New Jersey, United States.

***Chemical analysis*:** The DM content was determined by oven drying the sample at 60°C for 48 h and ash content was determined by incineration at 550°C (AOAC, 2000) for 4 h. The nitrogen (N) was determined by Kjeldhal method and crude protein (CP) was calculated as N × 6.25 (AOAC, 2000). Neutral detergent fiber (NDF) was determined by method of Van Soest *et al*. (1991) with the use of sodium sulfite. Acid detergent fiber (ADF) was determined by boiling NDF residue with ADF reagent and expressed with residual ash.

***Statistical analysis*:** The data recorded were subjected to the Analysis of Variance using 2×3 factorial arrangement under Randomized Complete Block Design and means were compared by least significance difference (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

***Nutrient intake*:** Table 2 shows data on nutrient intake and digestibility of exogenous fibrolytic enzyme treated and untreated diets fed to *Nili Ravi* buffalo calves. Non-significant differences (P>0.05) for DM, CP, NDF and ADF intake were observed between animals fed enzymes treated and without enzyme treated oat silage. Exogenous fibrolytic enzymes showed no effect on total intakes of DM, CP, NDF and ADF content. However, the high level of concentrate in both enzymes treated and untreated diets showed higher nutrient intake compared to diets contain low levels of concentrate. The results of present study are in accordance with the previous findings which indicate no effect of different fibrolytic enzymes supplementation to feed on nutrient intake of lactating sheep (Flores, 2004) and lactating goats (Titi and Lubbadeh, 2004a). Sutton *et al.* (2003) also reported no difference in nutrient intake when fibrolytic enzymes were supplemented in TMR or forage. In contrast, increase in feed intake has been reported in dairy cows and feedlot cattle supplemented with cellulase enzymes (Beauchemin *et al.,* 2000).

***Nutrient digestibility*:** Supplementation with exogenous fibrolytic enzymes increased (P<0.05) digestibility of CP, NDF and ADF for all enzymes treated groups compared to the control one. The DM digestibility of calves fed ET50 diet (enzyme treated) was 3.4, 7.7 and 12.3% higher than those calves fed EU50, EU60 and EU70 diets (enzyme untreated), respectively. Lewis *et al*. (1999) also reported similar trend in the DM digestibility. The CP digestibility was 14.2% higher (P <0.05) in animals fed diet ET50 (enzymes treated) compared to EU70 (enzymes untreated). Findings regarding higher CP digestibility in response to exogenous enzyme supplementation was supported by Beauchemin *et al.* (2000). The NDF digestibility of calves fed ET50 diet (enzyme treated) was 4.7, 11.1 and 17.5% higher than those calves fed EU50, EU60 and EU70 diets (enzyme untreated), respectively. Likewise, the ADF digestibility of calves fed ET50 diet (enzyme treated) was 3.7, 10.19 and 17.5% higher than those calves fed EU50, EU60 and EU70 diets (enzyme untreated), respectively. Animals fed ET60 diet showed statistically non-significant difference in NDF and ADF digestibility compared to the animals fed enzyme untreated EU50 diet. Previous studies showed that dietary enzymes are more effective if directly applied to the diets and enhance NDF digestibility (Pinos-Rodriguez *et al.,* 2002). Higher nutrient digestibility in response to enzyme treatment might be due to additive effect of enzymatic action between ruminal micro-flora and externally added enzyme (Morgavi *et al.,* 2000).

***Blood glucose, blood urea nitrogen and nitrogen balance*:** Data on blood glucose, blood urea nitrogen and nitrogen balance of experimental diets are presented in Table 3. Blood glucose concentration was within the

Table 2. Nutrient intake and digestibility of exogenous fibrolytic enzyme treated and untreated diets fed to *Nili Ravi* buffalo calves.

Diets1	Nutrient intake (kg)				Nutrient Digestibility (%)			
	DM	CP	NDF	ADF	DM	CP	NDF	ADF
ET50	4.31	0.75	1.77	0.93	76.31^{a}	89.84^{a}	68.43^{a}	48.74^{a}
ET60	4.51	0.77	1.98	1.01	71.80^{b}	84.20^{bc}	62.98^{b}	43.81^{b}
ET70	4.41	0.76	1.96	0.98	65.35^{d}	79.44^{d}	53.75^{d}	32.10^{d}
EU50	4.43	0.77	1.82	0.95	72.95^{b}	64.99^{b}	63.78^{b}	45.10^{ab}
EU60	4.68	0.77	2.06	1.04	68.64^{c}	82.69^{c}	57.34^{c}	38.55^{c}
EU70	4.36	0.75	1.94	0.97	64.08^{d}	75.64^{e}	51.28^{d}	31.24^{d}
SEM	0.14	0.023	0.06	0.03	0.48	0.39	0.82	1.09

Enzyme treated ET50, ET60 and ET70 contained oat silage to concentrate ratio of 50:50 60:40 70:30 were formulated, respectively. Enzyme untreated EU50, EU60 and EU70 contained oat silage to concentrate ratio of 50:50, 60:40 and 70:30, respectively.

Table 3. Blood glucose, blood urea nitrogen and nitrogen balance of experimental diets at end of experiment.

Time	ET50	ET60	ET70	EU50	EU60	EU70	SEM
			Blood glucose (mg/dl)				
0 h	44.00	48.00	48.50	46.00	51.00	49.50	3.67
3 h	59.50	61.50	58.50	69.50	59.50	60.00	4.01
6 h	55.00	56.50	54.00	63.50	52.00	55.50	4.72
9 h	50.50	51.50	51.50	49.00	49.50	52.00	4.05
			Blood urea nitrogen (mg/dl)				
0 h	10.50	9.50	9.00	11.00	9.50	9.00	0.83
3 h	9.50	11.00	10.00	10.00	9.50	9.50	1.94
6 h	15.00	16.50	13.00	15.00	12.50	14.00	2.63
9 h	14.50	11.50	13.50	15.50	16.00	13.50	1.70
			Nitrogen balance (g/d)				
Nitrogen intake	118.80	120.95	120.95	122.25	126.75	119.60	3.71
Fecal nitrogen	12.05[e]	19.30[d]	24.85[b]	18.35[d]	21.95[c]	29.10[a]	0.52
Urinary nitrogen	40.05[b]	44.05[b]	54.65[a]	43.65[b]	51.40[a]	54.05[a]	1.22
Nitrogen balance	66.20[a]	58.85[a]	41.40b[c]	60.20[a]	53.40[ab]	36.35[c]	3.68

Table 4. Effect of exogenous fibrolytic enzyme treated and untreated diets on growth performance of *Nili Ravi* buffalo calves.

Variable	Diets[1]						SEM
	ET50	ET60	ET70	EU50	EU60	EU70	
Dry matter intake (kg)	4.37	4.51	4.57	4.44	4.56	4.52	0.11
Initial weight (kg)	98.58	99.17	98.33	99.66	99.33	98.50	3.63
Final body weight (kg)	139.18[a]	133.58[ab]	122.13[b]	135.60[a]	129.08[ab]	122.42[b]	3.92
Weight gain (kg)	40.60[a]	34.42[b]	23.80[d]	35.93[b]	29.75[c]	23.92[d]	0.63
Daily weight gain (kg)	0.541[a]	0.459[b]	0.317[d]	0.479[b]	0.397[c]	0.319[d]	8.42
Feed conversion	8.09[d]	9.82[c]	14.43[a]	9.28[c]	11.50[b]	14.19[a]	0.37

[1] Enzyme treated ET50, ET60 and ET70 contained oat silage to concentrate ratio of 50:50 60:40 70:30 were formulated, respectively. Enzyme untreated EU50, EU60 and EU70 contained oat silage to concentrate ratio of 50:50, 60:40 and 70:30, respectively.

normal range and non-significant differences (P>0.05) were observed among all the enzymes treated and untreated groups. The reason may be a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body does not allow appreciable change in glucose level. The blood glucose and urea levels recorded in the present study are in agreement with the results reported by Shekhar *et al.* (2010) and Varlyakov *et al.* (2010) in buffaloes. There was no difference in blood urea nitrogen level in control and treatment groups. Similarly, non-significant differences in blood glucose and urea levels were reported by Shekhar *et al.* (2010) and Varlyakov *et al.* (2010) in buffaloes.

Buffalo calves were in positive nitrogen balance across all diets. Significant differences (P<0.05) in nitrogen balance were observed in calves fed diets supplemented with the enzymes versus those fed the control diets. The calves fed enzyme treated diets retained more (P<0.05) nitrogen (g/day) in their bodies than those fed untreated diets. These findings are in agreement with Gado *et al.* (2009) who observed a significant increase in nitrogen retention in animals fed enzyme treated diets. Nitrogen retention was 6, 12.8, and 29.9 % higher in calves fed ET50 (enzyme treated) compared to calves fed EU50, EU60 and EU70 diets (enzyme untreated), respectively. Similarly, nitrogen retention was 29.85 and 22.5% higher in calves fed ET50 and ET60 diets, respectively compared to calves fed EU70 diet (enzyme untreated). The results of present study are also supported by other researchers (Rodriguez *et al.*, 2002; Rode *et al.*, 1999) who reported improved nitrogen balance in response to exogenous fibrolytic enzymes.

***Growth performance*:** Gains in body weight (BW) were 0.54, 0.46, 0.32, 0.48, 0.39 and 0.31 kg/day on diets ET50, ET60, ET70, EU50, EU60 and EU70, respectively. Gains in BW were significantly (P<0.05) higher in buffalo calves fed enzymes treated diets versus control diets. Body weight increased linearly with increasing levels of concentrate in the diets. The results revealed that the inclusion of enzymes in the diets improved the BW gain. Initial body weight of the calves groups was almost same. Total final weight gain and average daily gain of calves fed enzyme treated rations were significantly higher (P<0.05) than those untreated diets. These results showed that enzyme supplementation in concentrate feed mixtures had a positive response to average daily gain. The results of present study showed that enzyme supplementation in concentrate feed mixtures had a positive response to average daily gain (ADG) which are in accordance with El-Kholy *et al.* (2003). Supplementation of exogenous fibrolytic enzymes showed a significant ($P<0.05$) change in feed conversion. Titi and Tabbaa (2004a) reported that enzymes increased the weight gain and lower the feed costs due to improved digestibility in Awassi lambs. However, Titi and Lubbadeh (2004b) also reported that fibrolytic enzyme improved ($P<0.05$) feed conversion ratio of fattened sheep with no effect on feed intake. Results indicated that fibrolytic and enzymes could enhance the growth of lambs and improve their conversion ratios mainly through improving digestibility.

***Conclusion*:** The comparative study concluded that animals fed enzyme treated diets furnished better results than those fed enzymes untreated diets. The calves fed enzymes treated diets showed improved nutrient digestibility and growth performance compared to untreated diets. High concentrate levels in diets showed better growth performance. Animals fed ET60 diet showed statistically similar results with the animals fed enzyme untreated EU50 diet so ET60 diet can replace the EU50 diet. Enzyme treatment improved animal performance despite of low concentrate and high silage proportion in ET60 diet than EU50 diet.

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