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Exogenous Fibrolytic Enzymes Addition in Concentrate Ration of Lactating Nili Ravi Buffaloes: Effects on Milk Production and Diet Digestibility

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Authors' Contribution
BA and S conceived the idea and conducted the experiments. MNT and FS analysed the data and wrote the manuscript. AG, GA and MG helped in writing and revising the manuscript.

Key words
Concentrate ration, Feed intake, Fibrozyme™, Lactating buffaloes, Plant cell wall.

ABSTRACT

Poor availability of good quality feedstuffs for the feeding of livestock has been identified as the major hurdles in the development of livestock sector in Pakistan. To improve the poor quality roughages, exogenous fibrolytic enzyme may play important role by enhancing digestibility of fibre. This study was carried out to evaluate the effects of varying level of fibrolytic enzyme in concentrate ration on dry matter (DM) intake, milk production and composition, and diet digestibility in Nili Ravi buffaloes. Four lactating Nili-Ravi buffaloes of 3rd and 4th parity and milk production of 8±2.3 kg/day were selected and offered a basal diet based on sorghum forage and concentrate ration supplemented with fibrolytic enzyme (Fibrozyme™, Alltech Inc. Company, USA) at four levels (T1 = 0, T2 = 10, T3 = 15 and T4 = 20 g/animal/day) in a 4 × 4 Latin Square change-over design with four experimental periods. Each period consisted of a 14-days enzyme supplementation period following a 3-day transition period without enzyme supplementation between consecutive experimental periods. The results showed a significant linear ($P<0.001$) increase in the intakes of DM, CP and NDF by the animals with increasing level of enzyme in the diet. The milk yield increased until an enzyme supplementation of 15 g/day (T3) and then dropped. The yield of milk protein, fat, solids not fat and lactose were not affected ($P>0.05$). Milk fat, solids not fat and lactose concentrations differed ($P<0.001$) among the treatments but did not show ($P>0.05$) any linear or quadratic trends with increasing level of enzyme in the diet. Milk protein concentration linearly ($P<0.001$) increased with increasing level of enzyme in the diets. It is concluded that fibrolytic enzyme addition in the diets of lactating Nili Ravi buffaloes improves the dry matter intake and diet digestibility which results in improved milk production.

INTRODUCTION

Fibre degradation in ruminants depends upon the type of feed and number of microbes present in rumen. The fibre from the tropical plants is more resistant to rumen degradation by microbes present in forestomach which produce enzymes to digest cellulose, hemicelluloses and lignin (Van Soest *et al*., 1978). Exogenous fibrolytic enzymes, in the form of feed additives are included in the diets of ruminants consuming tropical region plants worldwide 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). Fibrolytic enzymes used during ensiling improve fermentation by hydrolyzing the cell wall and digestibility of corn- (Colombatto *et al*., 2003) and Bermuda grass-silage (Dean *et al*., 2005).

Fibrozyme™ which is a fibrolytic enzyme have cellulases and xylanase like activities (Alltech Inc. Company, USA) and breaks beta 1-4 glucosidic linkages present in fibre, provides readily fermentable substrate to ruminal microbes (McAllister *et al*., 2001; Bowman *et al*.,

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2002) which results in increased microbial colonization by increasing their number (Nsereko *et al*., 2000), thus increasing the fibre degradation in the rumen and improving feed efficiency (Yang *et al*., 1999; Meale *et al*., 2014). In addition, exogenous polysaccharidases stay in small intestine for long time and maintain the digesting activity in that environment too (Morgavi *et al*., 2002). The purpose of the present research was to evaluate the effect of dietary addition of fibrolytic enzyme on the performance of dairy Nili Ravi buffaloes.

Table I.- Chemical composition of forage and concentrate ration.

Ingredients	Concentrate ration	Sorghum
Sorghum	---	100.0
Maize	8.0	---
Wheat bran	32.0	---
Maize gluten	20.0	---
Cotton Seed cake	22.0	---
Rape seed cake	3.0	---
Molasses	14.0	---
Mineral mixture	1.0	---
Chemical composition		
Dry matter	88.0	36.1
Crude Protein	18.0	8.3
Ether Extract	2.9	5.6
Neutral detergent fibre	32.1	51.8
Ash	1.7	5.6

All values are presented as percentage of dry matter unless otherwise notified.

MATERIALS AND METHODS

Animals, experimental design and diets

This research was conducted at Buffalo Research Institute (BRI), Bhunikey, Kasur, Pakistan. Four Nili-Ravi lactating buffaloes of the 3rd & 4th parity and 8±2.3 kg/day milk production were randomly selected from the herd maintained at BRI and designated to four experimental dietary treatments in a 4 × 4 Latin square change over design with four experimental periods. Each period consisted of a 14-days enzyme supplementation period following a 3-day transition period without enzyme supplementation between consecutive experimental periods. All animals were tied up and fed individually. Animals were de-wormed using Nilzan® Plus at the rate of 2.5 ml/10 kg body weight and vaccinated using Niab® HS™ oil based at the rate of 3-5 ml/animal before the initiation of the experiment. The live weight of the animals was measured fortnightly early in the morning using a digital weighing scale.

The basal diets consisted of sorghum forage which was provided ad libitum and the concentrate ration at a rate of 4 kg/animal/day. Fibrozyme™ (Alltech Inc., Nicholasville, KY, USA) was supplemented at a rate of 0 (T1), 10 (T2), 15 (T3) and 20 (T4) g/animal/day by mixing with the concentrate ration (Table I). According to the manufacturers, Fibrozyme is a fibrolytic enzyme powder preparation containing xylanase and cellulose activities (from *Aspergillus niger* and *Trichoderma viride* fermentation extract) with a cellulose and xylanase activity of 31.0 and 43.4 IU, respectively. Fresh, clean water was available all the time.

Sampling and chemical analyses

Feed, experimental diets and orts were sampled weekly, composited for each experimental treatment and analyzed for dry matter (DM), crude protein (CP), ether extract and ash according to AOAC (1999) and for neutral detergent fibre (NDF) according to Van Soest *et al*. (1991) at Nutrition Laboratory of BRI.

Forage intake was measured weekly as feed offered minus refusal collected. Milk yield was recorded twice a day at 0545 h and 1745 h. Representative milk samples were collected weekly from each animal, stored at -20°C, composted against each animal for morning and evening milkings and evaluated for milk composition (fat, protein, solids not fat and lactose) using LactiCheck™-01 RapiRead (Page and Pedesen International Ltd. USA).

Determination of digestibility of dry matter, crude protein and neutral detergent fibre

During last three days of each experimental period, total faeces were collected using polythene bags during 24 h and composited for each animal. Bags were covered from top to avoid any loss of moisture from faeces. Representative faecal samples were analyzed for DM, CP and NDF and digestibility for these nutrients, which is hereby denoted by X was calculated according to the following equation:

$$\text{Digestibility } (X) = \frac{\text{Dietary concent. of } X - \text{ Facial concent. of } X}{\text{Dietary concent. of } X} \text{ x } 100$$

Statistical analyses

Data from the feeding trial were analyzed according to Latin Square Design using GLM procedure of MINITAB® (version 16.1.1.0). Each animal on a specific diet was considered as the experimental unit. The linear and quadratic effects of increasing level of fibrolytic enzyme

in the concentrate ration were examined by replacing the qualitative variable diet in the model with the quantitative variable proportion of fibrolytic enzyme using Fitted Line Polynomial Regression Analysis in MINITAB® (version 16.1.1.0). Data are presented as mean±standard error of mean. The level of significance was set at $P<0.05$.

Following mathematical model was applied:

$$Y_{ijkl} = \mu+P_i+A_j+T_k+E_{ijk}$$

Where, Y_{ijkl} is dependent variable, μ is overall mean, P_i is effect of period i, A_j is effect of animal j, T_k is effect of treatment k and E_{ijk} is residual error.

RESULTS AND DISCUSSION

Effects on feed intake

The results showed a significant linear (P<0.001) increase in the intakes of DM, CP and NDF by the animals with increasing level of enzyme in the diet (Table II). The results of our study for increase in DM intake were similar to those of Atrian and Shahryar (2012) who observed higher DM intakes when the enzyme in liquid form was supplemented to beef cattle and also with those of Gaafar *et al*. (2010) in lactating buffaloes and Bowman *et al*. (2003) in lactating cows. However, the findings of current study are not similar with those of Dhiman *et al*. (2002) who found no effects of fibrolytic enzyme supplementation on DM intake in Holstein cows. A linear increase in DM intake of the buffaloes with the enzyme supplementation is attributed to the corresponding increases in the digestibility of all nutrients resulting in more digestible energy supply at rumen level.

Effects on milk production and composition

The milk yield (Table III) increased with enzyme supplementation of 15 g/day (T3) and then dropped. The yield of milk protein, fat, solids not fat and lactose were not affected (P>0.05). The results of current study are similar to the findings of Kung *et al*. (2002) who found increased milk production of cows fed on total mixed ration supplemented with fibrolytic enzymes and also with those of Shekhar *et al*. (2010) in Murrah buffaloes.

Table II.- Effects of increasing level of fibrolytic enzyme in concentrate rations on intake parameters in lactating Nili-Ravi buffaloes.

Item	Experimental diets[1]				SEM	P value (diet)	L	Q
Intake (kg/day)	T1	T2	T3	T4				
Dry matter	19.22d	19.86c	20.28b	20.79a	0.283	<0.001	0.002	0.853
Crude protein	1.89d	1.94c	1.97b	2.01a	0.023	<0.001	<0.001	0.780
Neutral detergent fibre	9.25d	9.58c	9.79b	10.06a	0.145	<0.001	0.028	0.901

[1]T1, T2, T3 and T4 represent the diets containing 0, 10, 15 and 20 g/animal/day supplementation of exogenous fibrolytic enzymes in the concentrate ration. SEM, standard error of mean; L, linear effect of increasing level of fibrolytic enzyme addition in experimental diets; Q, quadratic effect of increasing level of fibrolytic enzyme addition in experimental diets.

Table III.- Effects of increasing level of fibrolytic enzyme in concentrate rations on milk yield and composition in lactating Nili-Ravi buffaloes.

Item	Experimental diets[1]				SEM	P value (diet)	L	Q
	T1	T2	T3	T4				
Yield (kg/day)	8.49^{c}	9.53^{b}	9.98^{a}	9.40^{b}	0.968	<0.001	<0.001	<0.001
Milk protein	0.33^{c}	0.37^{b}	0.39^{a}	0.37^{b}	0.005	0.215	0.636	0.301
Milk fat	0.48^{d}	0.54^{b}	0.58^{a}	0.50^{c}	0.007	0.246	0.576	0.370
Solids not fat	0.83^{c}	0.96^{b}	1.01^{a}	0.95^{b}	0.012	0.117	0.616	0.228
Lactose	0.44^{c}	0.51^{ab}	0.53^{a}	0.50^{b}	0.006	0.149	0.663	0.237
Composition (%)								
Milk protein	3.85^{a}	3.91^{b}	3.92^{b}	3.94^{b}	0.035	0.001	0.040	0.442
Milk fat	5.68^{ab}	5.70^{ab}	5.88^{a}	5.41^{b}	0.293	0.032	0.354	0.111
Solids not fat	9.82^{b}	10.04^{a}	10.07^{a}	10.15^{a}	0.113	<0.001	0.441	0.441
Lactose	5.23^{b}	5.34^{a}	5.31^{a}	5.34^{a}	0.048	<0.001	0.074	0.282

[1]T1, T2, T3 and T4 represent the diets containing 0, 10, 15 and 20 g/animal/day supplementation of exogenous fibrolytic enzymes in the concentrate ration. For abbreviations, see Table II.

The findings of current study are also similar to those of Dean *et al*. (2007) who fed low and high energetic diets with or without fibrolytic enzyme supplementation and determined that feeding high energetic diets along with fibrolytic enzyme increased milk production. However, our results of milk production also suggest that the animals' production performance was improved to a certain level of enzyme supplementation *i.e.* T3 diet (15 g/kg diet DM) as depicted in Figure 1. Although dry matter intake kept on increasing with level of enzyme; this increased energy intake was not reflected by the milk production. It might be true that the surplus energy had been utilized to build up the body reserves.

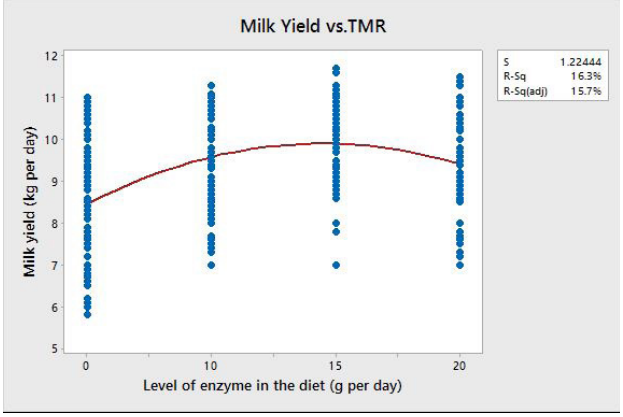

Fig. 1. Graphical representation of increasing level of fibrolytic enzyme in concentrate rations on milk yield in lactating Nili-Ravi buffaloes. The equation is milk yield = 6.52 + 2.33 level of enzyme – 0.404 level of enzyme*2.

Milk fat, solids not fat and lactose concentrations differed (P<0.001) among the treatments but did not show (P>0.05) any linear or quadratic trends with increasing level of enzyme in the diet. Milk protein concentration linearly (P<0.001) increased with increasing level of enzyme in the diets. The results of current trial agree with the findings of Dean *et al*. (2007) who examined the effects of fibrolytic enzyme on milk composition by mixing it in total mixed ration, silage, concentrate and fresh fodder. Animals fed on concentrate + enzyme produced more protein in milk while the animals fed on TMR + enzyme produced more fat and protein in milk. Also our results are in accordance with those of Miller *et al*. (2008) who determined the effects of adding different levels of liquid fibrolytic enzyme in lactating cows' diets and observed that enzyme supplementation linearly increased milk protein and quadratically increased milk fat concentrations. The lactose concentration of milk remained unaltered across many studies (e.g. Mohamed *et al*., 2013; Shadmanesh, 2014) and did the same in our study.

Effects on diet digestibility

Table IV shows that the increasing level of enzyme in the diets had a positive linear (P<0.001) effect on the digestibility of DM (2%), CP (3%) and NDF (3%) at the final supplementation level of enzyme. Performance of dairy animals is determined by amount of digestible nutrient that it consumes each day; which is the product of intake and digestibility. The digestibility of forage is related to the content and digestibility of NDF (Mertens, 2009).

The results of current study for digestibility agree with the findings of Yang *et al*. (1999) and El-Kady *et al*. (2006) who observed positive effects of fibrolytic enzyme on digestibility in the diets of cows and buffaloes, respectively. Our results are also similar to those of Arriola *et al*. (2011) where the diets of lactating cows were supplemented with fibrolytic enzyme at low and high concentrate proportions and it was observed that supplemented high concentrate groups had higher digestibility compared to non-supplemented groups. Titi and Tabbaa (2004) studied the effect of cellulase on growth performance and diet digestibility and observed that digestibility of DM and NDF significantly increased with the fibrolytic enzyme supplementation. However, our results are not in accordance with those of Sutton *et al*. (2003) and Muwalla *et al*. (2007) who reported no effects of fibrolytic enzyme supplementation in the diets on productive performance of Holstein cows and growth performance of Awassi lambs, respectively.

Table IV.- Effects of increasing level of fibrolytic enzyme in concentrate rations on diet digestibility in lactating Nili-Ravi buffaloes.

Item	Experimental diets[1]				SEM	P value (diet)	L	Q
Digestibility (%)	**T1**	**T2**	**T3**	**T4**				
Dry matter	71.31	71.43	72.98	74.23	1.145	<0.001	<0.001	0.008
Crude protein	52.91	53.03	55.96	57.67	2.232	<0.001	<0.001	0.038
Neutral detergent fibre	51.39	51.51	53.49	55.05	1.580	<0.001	<0.001	0.014

[1]T1, T2, T3 and T4 represent the diets containing 0, 10, 15 and 20 g/animal/day supplementation of exogenous fibrolytic enzymes in the concentrate ration. For abbreviations, see Table II.

CONCLUSIONS

Based on the results presented, it is concluded that increasing level of enzyme supplementation in the diets of lactating Nili Ravi buffaloes increases the amount of digestible nutrients intake; which is attributable to improved digestibility.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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