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EFFECT OF FIBROLYTIC ENZYME SUPPLEMENTATION AND FIBER CONTENT OF TOTAL MIXED RATION ON PRODUCTIVE PERFORMANCE OF LACTATING BUFFALOES

H.M.A. GAAFAR[1]*, E.M. ABDEL-RAOUF[2], K.F.A. EL-REIDY[1]

[1]Animal Production Research Institute, Agricultural Research Center, Nadi El-Said St., Dokki, Egypt;
[2]Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Egypt

ABSTRACT

Thirty multiparous lactating buffaloes weighing 500 to 600 kg were fed three levels of fiber with or without fibrolytic enzyme (cellulase and xylanase) treated roughage. Rations were formulated from berseem hay, corn silages and rice straw, yellow corn grain, soybean meal, wheat bran, minerals, and vitamins. The contents of crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were 21.95, 49.48 and 32.21% (high), 19.50, 45.46 and 29.20% (medium) and 16.97, 41.14 and 26.01% (low) for rations contained 75, 62.5, and 50% roughage consisted of 40% berseem hay, 40% corn silage and 20% rice straw (on DM basis), respectively. The digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) and nitrogen free extract (NFE) and nutritive values decreased significantly (P<0.05), but crude fiber (CF) increased significantly (P<0.05) with increasing fiber content. The digestibility of all nutrients and nutritive values increased significantly (P<0.05) with fibrolytic enzyme supplementation. The intake of DM, total digestible nutrients (TDN), CP and digestible crude protein (DCP) increased significantly (P<0.05) with decreasing dietary fiber content as well as with fibrolytic enzyme supplementation. The pH value increased significantly (P<0.05), however total volatile fatty acids (TVFA's) and ammonia nitrogen (NH_3-N) concentrations decreased significantly (P<0.05) with increasing dietary fiber content. The pH value and NH_3-N concentration were significantly lower (P<0.05), but TVFA's concentration was significantly higher (P<0.05) with fibrolytic enzyme supplementation. The milk yield and 7% fat corrected milk (FCM) increased significantly (P<0.05) with decreasing dietary fiber content as well as with fibrolytic enzyme supplementation. Fat and TS contents increased and lactose and SNF contents decreased significantly (P<0.05) with increasing dietary fiber content. The contents of milk constituents increased significantly (P<0.05) with fibrolytic enzyme supplementation. The DM and CP/kg FCM increased significantly (P<0.05) with increasing dietary fiber content, but TDN, CP and DCP/kg FCM did not change significantly (P>0.05) by dietary fiber content. Feed conversion improved significantly (P<0.05) with fibrolytic enzyme supplementation. Average daily feed cost, feed cost/ kg 7% FCM and output of 7% FCM decreased significantly (P<0.05), but economic efficiency increased significantly (P<0.05) with increasing dietary fiber content. Feed cost/ kg 7% FCM decreased significantly (P<0.05), but output of 7% FCM and economic efficiency increased significantly (P<0.05) with fibrolytic enzyme supplementation. It could be concluded that using high fiber total mixed ration due to increasing forage level was economically effective in buffaloes feeding and fibrolytic enzymes supplementation improved productive performance.

Key words: fibrolytic enzyme; fiber, feed intake; milk yield and composition; feed conversion and economic efficiency

***Correspondence:** E-mail: dr.gaafar@hotmail.com
H.M.A. Gaafar, Animal Production Research Institute, Agricultural Research Center, Nadi El-Said St., Dokki, Egypt.
Tel.: 0020473239156 Fax: 0020473229507

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INTRODUCTION

The goal of any feeding program is to provide the correct amount and balance of nutrients to cows at the proper time to achieve optimum production, reproductive efficiency and profitability. The nutritional advantages of a TMR over individual component feeding programs are: 1) Elimination of cow selectivity of individual feeds. 2) Capability of accurately determining dry matter (DM) intake of cows. 3) Overall better control of feeds offered and consumed by cows and, thus, better control of feed costs. 4) Ability to use a wider variety of feedstuffs including less palatable feeds and commodity feeds (Linn, 1995). He also stated that to be an efficient and effective feeding program, the TMR has to be managed correctly. Critical management factors include: 1) knowing and adjusting for changes in forage DM content; 2) good feed bunk management; 3) correct grouping of cows; 4) continuous monitoring of DM intake; 5) good mixing of feeds and correct ration formulation.

Neitz and Dugmore (2008) found that total mixed ration (TMR) can be described as a mixture of both the roughage and the processed ingredients, formulated and mixed to supply the cow's requirements in a form that precludes selection. It is designed to be the sole feed source given over a 24 hour period and fed *ad libitum* for optimum results. This cannot be accomplished without the use of accurate weighing equipment and adequate and proper mixing equipment. Increasing dietary forage and NDF decreased yield of milk and all milk components; these responses were linear. Moreover, milk concentrations of protein, true protein, lactose, and SNF also were decreased linearly with greater dietary NDF (Broderick, 2003).

The beneficial effects of fibrolytic enzymes in ruminant diets appear to be a result of, in part, improvements in feed digestibility (Beauchemin *et al.*, 1995; Beauchemin *et al.*, 1999; Feng *et al.*, 1996; Lewis *et al.*, 1995 and Yang *et al.*, 1999). Dairy cows fed forage treated with a fibrolytic enzyme additive ate more feed and produced 5 to 25% more milk (Lewis *et al.*, 1995 and Stokes and Zheng, 1995). Supplementing dairy cow diets with a fibrolytic enzyme mixture has the potential to enhance milk yield and nutrient digestibility of cows in early lactation without changing feed intake (Rode *et al.*, 1999). Fibrolytic enzymes applied to the forage portion of the rations prior to feeding improved lactational performance of early and midlactation cows (Lewis *et al.*, 1999).

Spraying enzymes directly onto forages prior to feeding can improve milk yields (Kung *et al.*, 2000). Enzyme supplementation increases total tract digestibility of organic matter and fiber and cows receiving the enzyme product added to the concentrate had a numerically higher FCM compared to the control cows (Bowman *et al.*, 2002). Fibrolytic enzymes can be used to improve milk production in lactating cows, which cows fed forages treated with cellulase D and xylanase B tended to produce more 3.5% FCM than did cows fed the untreated forages (Kung *et al.*, 2002). It is well recognized that fibrolytic enzymes (FE), such as cellulases and hemicellulases, assist with the digestion of structural carbohydrates. Recent research has shown increased milk yield (3–10%) when FEs have been fed with grain based concentrates in dairy feedlot systems (Beauchemin *et al.* 1999; Rode *et al.* 1999; Yang *et al.* 1999, 2000). Responses in milk yield in these reports were attributed to increased NDF digestibility (15–85 g/kg), in conjunction with small, but consistent increase in intake (1–2%).

The aim of the present study was to investigate the effect of fibrolytic enzyme and fiber content of total mixed ration (TMR) on digestibility, feed intake, milk yield and composition, feed conversion and economic efficiency of lactating buffaloes.

MATERIAL AND METHODS

Experimental animals and rations

Thirty multiparous lactating buffaloes weighing 500 to 600 kg were fed three levels of fiber with or without fibrolytic enzyme treated roughage. The rations were fed as TMR formulated from berseem hay, corn silages and rice straw, yellow corn grain, soybean meal, wheat bran, minerals, and vitamins (Table 1).

Table 1: Formulation of experimental rations (% on DM basis)

Feedstuffs	Fiber content		
	High	Medium	Low
Berseem hay (BH)	30.0	25.0	20.0
Corn silage (CS)	30.0	25.0	20.0
Rice straw (RS)	15.0	12.5	10.0
Corn grain (CG)	8.0	13.5	20.0
Soybean meal (SBM)	10.0	10.0	10.0
Wheat bran (WB)	6.6	13.35	19.1
Common salt	0.3	0.3	0.3
Premix*	0.1	0.1	0.1
Sodium bicarbonate	0	0.25	0.50
Total	100	100	100

* Premix contained 50 g Ca, 40 g P, 50 g Mg, 10 g K, 22 g Na, 12 g S, 2000 mg Cu, 4500 mg Zn, 4500 mg Mn, 7000 mg Fe, 3000 mg I, 50 mg Co, 12 mg Se, 200000 IU V_A, 20000 V_{D3} and 20000 V_E.

Table 2: Chemical composition of ingredients

Item	BH	CS	RS	CG	SBM	WB
DM %	90.25	28.67	89.63	90.58	93.46	90.26
Composition of DM %						
OM	88.57	93.17	82.21	97.68	93.62	94.08
CP	13.16	8.22	3.36	9.50	43.18	12.20
CF	29.01	22.05	33.98	2.74	5.24	11.97
EE	2.83	2.31	1.27	3.23	1.41	2.96
NFE	43.57	60.41	43.60	82.21	43.79	66.95
Ash	11.43	6.83	17.79	2.32	6.38	5.92
Fiber fractions %						
NDF	52.30	58.65	73.55	11.20	15.82	40.72
ADF	38.80	32.78	51.45	5.36	10.60	23.15
ADL	5.94	5.35	6.74	0.85	1.15	3.67
Hemicllulose	13.50	25.87	22.10	5.84	5.22	17.57
Cellulose	32.86	27.43	44.71	4.51	9.45	19.48

The contents of CF, NDF and ADF were 21.95, 49.48 and 32.21% (high), 19.50, 45.46 and 29.20% (medium) and 16.97, 41.14 and 26.01% (low) for rations contained 75, 62.5, and 50% roughage consisted of 40% berseem hay, 40% corn silage and 20% rice straw (on DM basis), respectively. Chemical composition of ingredients used in buffaloes feeding is shown in Table 2.

Commercial fibrolytic enzyme contained 4000 units of cellulase and 10000 units of xylanase per gram. Concentrated enzyme mixture was dissolved in water (100 g/liter) and then added to roughage (20 liter/ton DM) to give 8000 units cellulase and 20000 units xylanase / kg roughage and leaving it overnight.

Management procedure

Lactating buffaloes were individually fed according to Animal Production Research Institute (1997) to cover the recommended requirements. Rations were recalculated every two weeks based on milk yield and body weight of animals and offered in three equal meals daily at 9 and 12 a.m. and 3 p.m. buffaloes were allowed to drink water three times at 7 and 12 a.m. and 6 p.m.

Digestibility trials

Digestibility trials were conducted on the experimental animals to determine nutrient digestibility coefficients and nutritive values of the experimental rations using acid insoluble ash (AIA) as a natural marker (Van Keulen and Young, 1977). Feces samples were taken from the rectum of each animal twice a day with 12 hour interval during the collection period. Samples of tested feedstuffs were taken at the beginning, middle and the end of collection period. The samples of feedstuffs and feces were composted and representative samples were analyzed according to AOAC (1995).

Rumen liquor samples

Rumen liquor samples were collected at 3 hours after the morning feeding from buffaloes using a stomach tube and filtered through double layers of cheese cloth and pH value was determined directly using Orian 680 digital pH meter. The concentration of total VFA's was determined in rumen liquor samples by the steam distillation method (Warner, 1964) using markham micro-distillation apparatus. The concentration of NH_3-N was determined using saturated solution of magnesium oxide distillation according to the method of AOAC (1995).

Milk yield and samples

Individual morning and evening milk yields of lactating buffaloes were recorded daily and corrected for 7% fat content (FCM) using the formula of 7% FCM = 0.265 x milk yield (kg) + 10.5 x fat yield (kg) as stated by Raafat and Saleh (1962). Milk samples from consecutive evening and morning milking were taken at the 4^{th} week of each period and mixed in proportion to yield. Milk fat, protein, lactose and total solids were determined using Milko-Scan (133B Foss Electric).

Feed conversion

Feed conversion was calculated as the amounts of DM, TDN (kg), CP and DCP (g) required to produce 1 kg of 7% FCM.

Economic efficiency

Economic efficiency expressed as the daily feed cost, price of 7% FCM, feed cost per kg 7% FCM and the ratio between daily feed cost and price of 7% FCM. The price of one ton was 800 LE for berseem hay, 150 LE for corn silage, 90 LE for rice straw, 2200 LE for soybean meal, 1400 LE for corn grain, 1600 LE for wheat bran,

Table 3: Chemical composition of experimental rations used in feeding of lactating buffaloes

Item	Fiber content			Fibrolytic enzyme	
	High	Medium	Low	Without	With
DM	72.06	75.19	78.33	75.23	75.15
Composition of DM %					
OM	90.24	90.82	91.44	90.79	90.88
CP	12.80	12.99	13.16	13.02	12.95
CF	21.95	19.50	16.97	19.61	19.34
EE	2.33	2.42	2.51	2.42	2.42
NFE	53.16	55.91	58.80	55.74	56.18
Ash	9.76	9.18	8.56	9.21	9.12
Fiber fractions %					
NDF	49.48	45.46	41.14	45.42	45.30
ADF	32.21	29.20	26.01	29.23	29.05
ADL	4.82	4.38	3.92	4.41	4.33
Hemicllulose	17.27	16.26	15.13	16.19	16.25
Cellulose	27.38	24.82	22.10	24.82	24.72

250 LE for common salt, 2000 LE for premix and 300 LE for sodium bicarbonate. While, the price of one kg fibrolytic enzyme was 10 LE and 7% FCM was 3 LE according to prices of year 2009.

Statistical analysis

The data were subjected to statistical analysis using general linear models procedure adapted by SPSS (2008). Duncan test within program SPSS was done to determine the difference between the means.

RESULTS AND DISCUSSION

Chemical composition of experimental rations

Chemical composition of experimental rations used in feeding of lactating buffaloes is presented in Table 3. The results showed that the increase in fiber and fiber fraction contents was accompanied by the decrease in OM, EE and NFE contents and the increase in ash content. These results attributed to increasing forage level and decreasing concentrate level which led to increasing fiber content. Determining the nutritional value of feeds that have high amounts of structural carbohydrates (dietary fiber) is of great significance as it affects both the digestibility and intake of diet (Church, 1988). The plant cell wall, which is measured as a fiber is a complex structure composed of lignin, cellulose, hemicellulose, pectin, some protein, lignified nitrogenous substances, waxes, cutin and mineral components. Fiber composition is important from a nutritional point of view and varies with the type of cell wall **(**Van Soest, 1994**).**

Digestibility coefficients and nutritive values

The nutrient digestibility coefficients and nutritive values of experimental rations are presented in Table 4. The digestibility of DM, OM, CP, EE and NFE and the TDN and DCP values decreased significantly ($P<0.05$), however CF digestibility increased significantly ($P<0.05$) with increasing fiber content. Moreover, the digestibility of all nutrients and nutritive values increased significantly ($P<0.05$) with fibrolytic enzyme supplementation. These results agreed with those obtained by Rode *et al*. (1999), who found that supplementing dairy cow diets with a fibrolytic enzyme mixture has the potential to enhance nutrient digestibility of cows. Bowman *et al*. (2000) reported that the using enzyme supplementation increased total tract digestibility. Bowman *et al*. (2002) stated that enzyme supplementation increases total tract digestibility of organic matter and fiber. Broderick (2003) found that energy content of rations decreased with increasing NDF content.

Table 4: Nutrient digestibility coefficients and nutritive values by lactating buffaloes

Item	Fiber content			Fibrolytic enzyme	
	High	Medium	Low	Without	With
Digestibility coefficients %					
DM	66.15^{c}	68.68^{b}	70.68^{a}	66.77^{b}	70.24^{a}
OM	67.47^{c}	69.61^{b}	71.97^{a}	67.73^{b}	71.64^{a}
CP	65.31^{c}	67.74^{b}	69.80^{a}	66.44^{b}	68.80^{a}
CF	66.60^{a}	64.47^{b}	62.79^{c}	62.41^{b}	66.82^{a}
EE	70.86^{c}	72.41^{b}	74.73^{a}	71.61^{b}	73.73^{a}
NFE	68.97^{c}	71.34^{b}	74.22^{a}	69.15^{b}	73.86^{a}
Nutritive values %					
TDN	63.35^{c}	65.20^{b}	67.70^{a}	63.61^{b}	67.23^{a}
DCP	8.36^{c}	8.80^{b}	9.19^{a}	8.65^{b}	8.91^{a}

a, b, c: Means in the same row for each item with different superscripts differ significantly at 5% level

Table 5: Feed intake and rumen fermentation activity of lactating buffaloes

Item	Fiber content			Fibrolytic enzyme	
	High	Medium	Low	Without	With
Feed intake (kg/head/day)					
DM	16.27[c]	16.74[b]	17.15[a]	16.39[b]	17.05[a]
TDN	10.31[c]	10.92[b]	11.62[a]	10.43[b]	11.47[a]
CP	2.08[c]	2.17[b]	2.26[a]	2.13[b]	2.21[a]
DCP	1.36[c]	1.47[b]	1.58[a]	1.42[b]	1.52[a]
Rumen fermentation activity					
pH	6.26[a]	6.13[b]	6.05[b]	6.23[a]	6.06[b]
TVFA's (meq/dl)	16.94[b]	17.54[ab]	18.23[a]	16.71[b]	18.43[a]
NH_3-N (mg/dl)	22.61[c]	23.35[b]	24.05[a]	24.01[a]	22.66[b]

a, b, c: Means in the same row for each item with different superscripts differ significantly at 5% level

Feed intake

Average daily feed intake by lactating buffaloes is shown in Table 5. The intake of DM, TDN, CP and DCP increased significantly (P<0.05) with decreasing dietary fiber content as well as with fibrolytic enzyme supplementation. These results are in accordance with those obtained by Martinez *et al*. (2009), who found that increasing dietary forage level from 50 to 60% of ration DM decreased DM intake. Beauchemin *et al*. (2000) reported that the using of enzyme supplementation increased feed intake and the intake of digestible nutrients was increased to a greater extent for cows fed enzyme supplementation.

Rumen fermentation activity

Rumen liquor parameters as affected by dietary fiber content and fibrolytic enzyme supplementation are presented in Table 5. The pH value increased significantly (P<0.05), however TVFA's and NH_3-N concentrations decreased significantly (P<0.05) with increasing dietary fiber content. The pH value and NH_3-N concentration were significantly lower (P<0.05), but TVFA's concentration was significantly higher (P<0.05) with fibrolytic enzyme supplementation. The pH values obtained here are within the range (6.2-7.2) reported by Van Soest (1994) as being optimal for fiber digestion. Oosting (1993) found that increased VFA concentration increased microbial activity, which is associated with utilization of ammonia.

Milk yield

Average daily milk yield and 7% FCM are shown in Table 6. The actual milk and 7% FCM yield increased significantly (P<0.05) with decreasing dietary fiber content as well as with fibrolytic enzyme supplementation. These results agreed with those obtained by Lewis *et al*. (1999) who found that fibrolytic enzymes applied to the forage portion of the rations prior to feeding improved lactational performance of early and midlactation cows. Granzin (2005) reported positive effect of fibrolytic enzyme on milk yield of Holstein Friesian cows.

Table 6: Average daily actual milk and 7% FCM yield and milk composition of lactating buffaloes

Item	Fiber content			Fibrolytic enzyme	
	High	Medium	Low	Without	With
Milk yield (kg/head/day)					
Actual	9.93[c]	11.44[b]	12.57[a]	10.49[b]	12.13[a]
7% FCM	9.58[b]	10.54[ab]	11.07[a]	9.44[b]	11.35[a]
Milk composition %					
Fat	6.66[a]	6.24[b]	5.85[c]	6.08[b]	6.41[a]
Protein	4.20	4.29	4.30	4.16[b]	4.37[a]
lactose	5.36[b]	5.55[a]	5.71[a]	5.37[b]	5.71[a]
SNF	10.27[b]	10.54[a]	10.70[a]	10.23[b]	10.77[a]
TS	16.93[a]	16.78[ab]	16.54[b]	16.31[b]	17.19[a]
Ash	0.71[a]	0.70[ab]	0.69[b]	0.70	0.70
Milk components yield (kg/head/day)					
Fat	0.66[b]	0.71[ab]	0.74[a]	0.64[b]	0.78[a]
Protein	0.42[b]	0.49[ab]	0.54[a]	0.44[b]	0.53[a]
lactose	0.53[c]	0.63[b]	0.72[a]	0.56[b]	0.69[a]
SNF	1.02[c]	1.21[b]	1.34[a]	1.07[b]	1.31[a]
TS	1.68[c]	1.92[b]	2.08[a]	1.71[b]	2.09[a]
Ash	0.07[b]	0.08[ab]	0.09[a]	0.07	0.08

a, b, c: Means in the same row for each item with different superscripts differ significantly at 5% level.

Milk composition

As shown in Table 6, fat and TS contents increased and lactose and SNF contents decreased significantly (P<0.05) with increasing dietary fiber content. However, protein content did not changed significantly (P>0.05) by dietary fiber content. The contents of fat, protein, lactose, SNF and TS increased significantly (P<0.05) with fibrolytic enzyme supplementation. The yield of all milk components increased significantly (P<0.05) with decreasing dietary fiber content as well as with fibrolytic enzyme supplementation. These results agreed with those obtained by Martinez *et al*. (2009), who found that increasing dietary forage level from 50 to 60% of ration DM increased milk fat percentage due to increasing dietary fiber content.

Feed conversion

Feed conversion expressed as the amounts of DM, TDN and DCP per kg 7% FCM are shown in Table 7. The amounts of DM and CP/kg FCM increased significantly (P<0.05) with increasing of dietary fiber content, but TDN and DCP/kg FCM did not changed significantly (P>0.05) by dietary fiber content. Feed conversion has been improved significantly (P<0.05) with fibrolytic enzyme supplementation. These results agreed with those obtained by Martinez *et al*. (2009) who found that decreasing dietary forage level of ration DM increased the efficiency of converting feed to milk. Sarwar *et al.* (1999) reported that high fiber diets had lower efficiency of metabolizable energy for maintenance or production than low fiber diets.

Economic efficiency

Economic efficiency presented in Table 7, revealed that average daily feed cost, feed cost/ kg 7% FCM and output of 7% FCM decreased significantly (P<0.05), but economic efficiency increased significantly (P<0.05) with increasing dietary fiber content. Average daily feed cost did not significantly (P>0.05) affected by fibrolytic enzyme supplementation, while feed cost/ kg 7% FCM decreased significantly (P<0.05), but output of 7% FCM and economic efficiency increased significantly (P<0.05) with fibrolytic enzyme supplementation. These results agreed with those obtained by Tozer *et al*. (2003) who found that although costs per kilogram of milk produced were lowest for pasture-concentrate cows, cows on TMR had the highest net income per cow per day because of higher yields of milk and milk components, but cows on the pasture-concentrate had lower daily net income due to lower yields of milk and milk components. Martinez *et al*. (2009) reported that increasing forage level in diets decreased feed cost.

CONCLUSION

It could be concluded that using of high fiber total mixed ration (high roughage) was economically effective in feeding buffaloes and fibrolytic enzyme supplementation improved productive performance.

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Table 7: Feed conversion and economic efficiency of lactating buffaloe fed experimental rations

Item	Fiber content			Fibrolytic enzyme	
	High	Medium	Low	Without	With
Feed conversion					
DM (kg/kg FCM)	1.70[a]	1.59[b]	1.55[b]	1.74[a]	1.50[b]
TDN (kg/kg FCM)	1.08	1.04	1.05	1.11[a]	1.01[b]
CP (g/kg FCM)	217.12[a]	205.88[b]	204.16[b]	225.64[a]	194.71[b]
DCP (g/kg FCM)	143.15	140.67	142.98	150.59[a]	133.94[b]
Economic efficiency					
Daily feed cost LE	15.01[c]	17.75[b]	20.53[a]	17.40	18.13
Feed cost LE/kg FCM	1.57[c]	1.68[b]	1.85[a]	1.84[a]	1.60[b]
Output of FCM LE	28.74[c]	31.62[b]	33.21[a]	28.32[b]	34.05[a]
Economic efficiency	1.91[a]	1.78[b]	1.62[c]	1.63[b]	1.88[a]

a, b, c: Means in the same row for each item with different superscripts differ significantly at 5% level

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