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Influence of exogenous fibrolytic enzymes on milk production efficiency and nutrient utilization in early lactating buffaloes fed diets with two proportions of oat silage to concentrate ratios

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ABSTRACT

The digestion of ruminal fibrous substrate is slow or incomplete and can hinder animal performance. Hence, the current study investigated the effect of oat-silage-to-concentrate ratios supplemented with exogenous fibrolytic enzymes (EFE) on production performance and nutrient digestibility in 32 *Nilli Ravi* early lactating buffaloes. Four diets were formulated and grossly divided across two major experimnatl groups of two proportions of silage to concentrate ratios (AS-60, silage to concentrate ratio of 60:40; and AS-70, silage to concentrate ratio of 70:30) with or without EFE supplementation. Crude protein, and neutral detergent fiber intake as well as digestible nutrient intake were significantly ($P < 0.05$) higher upon EFE supplementation than in groups without EFE. The addition of EFE improved ($P < 0.05$) all nutrient digestibility parameters. Unaltered blood glucose and urea nitrogen were found across all animal groups at 0, 3, 6, 9 h post-feeding. Animals fed on diet supplemented with EFE with 60:40 silage to concentrate ratio ($P < 0.05$) recorded the highest nitrogen balance values. Better milk production ($P < 0.001$) was observed in animals on enzyme- treated diets than in those without enzyme supplementation. Milk fat, total solids, and milk energy content were increased ($P < 0.05$) in animals on AS-60, AS-70, and NAS-70 diets. The present study demonstrated that diets containing high oat silage (60 or 70%) supplemented with EFE led to better milk production and composition in early lactating buffaloes.

1. Introduction

The constantly increasing human population is increasing pressure on infrastructural development, resulting in constant depletion of land available for fodder (Thomas and Rangnekar, 2004). In many tropical countries, the available feed resources are high in fiber, which negatively affects both feed intake and digestibility (Khan et al., 2006). According to Nisa et al. (2006) non availability of better quality fodder especially during scarcity periods are major issues in ruminant production. Many methods have been used to enhance the feeding value of forages. Fodder conservation methods like ensiling during its availability periods ensure continuous fodder supply throughout the year.

Addition of exogenous fibrolytic enzymes (EFE) is one of the methods used to improve rumen fermentation and milk production in goats (Rojo et al., 2015). The response of dairy animals to EFE supplementation in the diet has shown conflicting results. The positive impact of EFE supplementation on animals including lactating dairy cows, has been reported (Adesogan et al., 2014; Romero et al., 2016). Animals fed diets treated with EFE showed increased productive performance and better feed conversion efficiency (Iwaasa et al., 1997; McAllister et al., 1999). In contrast, some researchers reported negative or no effects of EFE on animal productive performance. Application of EFE did not improve milk production by Holstein cows (Chen et al., 1994). Similarly, milk responses to EFE were found to depend on the

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method of application (Beauchemin et al., 1999). Although EFE application could increase milk production in cows fed total mixed ration (Stokes and Zheng, 1995; Sanchez et al., 1996), improvement in milk production was dependent on the level of EFE applied (Sanchez et al., 1996; Gado et al., 2009). Moreover, the efficiency of this EFE was dependent upon the method of its application; for instance, spraying the EFE on the total mixed ration did not affect milk production, whereas applying it onto the concentrate resulted in better milk production (Beauchemin et al., 1999).

Studies focused on concentrate replacement with EFE-treated oat silage in early lactating buffaloes' rations are limited (Morsy et al., 2016). Therefore, the current research was outlined to observe the comparative effects of replacing different levels of concentrate with enzyme-treated silage on lactation performance, milk composition, dry matter intake, nutrient digestibility, urea nitrogen, and blood glucose metabolites in *Nilli-Ravi* buffaloes. It was hypothesized that oat silage fortified with EFE and reduced concentrate proportion in buffalos on total mixed ration rations would enhance nutrient digestibility, lactation performance, and may have positive economic effects on reducing the feed cost.

2. Material and methods

Oat grass was harvested after seventy days of sowing. It was then chopped to 1–2 cm lengths and 20 g molasses was mixed with 980 g chopped oat. The blend (chopped oat and molasses) was pressed to exclude air and was ensiled and for 25 days. The blend (chopped oat and molasses) was sealed according to the procedure described by Sarwar et al. (2006) to achieve anaerobic conditions. The chemical composition, pH, and lactic acid of the silage are presented in Table (1).

Exogenous fibrolytic enzymes (Commercially available - Accellerase®XC, DuPont Co., New York, USA) used in the present study are a complex of active cellulase and xylanase, which are a dried mixture of fermentation extracts from *Penicillium funiculosum* fungi and possess endoglucanase and xylanase activity at 1000–1400 carboxymethycellulose U g^{-1} and 2500–3800 acid birchwood xylanase units g^{-1}, respectively, as specified by the manufacturer. The activity of Accellerase®XC is expressed in both carboxymethycellulose activity units and acid birchwood xylanase units. One carboxymethycelluloseof activity liberates 1 μmol of reducing sugars (expressed as glucose equivalents) in one minute under specific assay conditions of 50 °C (122°F) and pH 4.8. Xylanase activity is reported as acid birchwood xylanase units. One xylanase unit is defined as the amount of enzyme required to generate one μmol of xylose-reducing sugar equivalents per minute under the conditions of the assay.

Thirty-two early lactating *Nili Ravi* buffaloes (435 ± 5 kg) were divided into four similar groups (eight animals per group). Animals were housed on concrete floor in separate pens. Diets were offered twice a day (6 a.m. and 6 p.m.) and were treated with enzymes one hour prior to feeding throughout the experimental period. The diets were fed ad libitum. Four iso-caloric and iso-nitrogenous diets were formulated, which were divided into two groups of two proportions of silage to concentrate ratios (60:40, 70:30 silage to concentrate ratio, respectively) supplemented with (AS-60 and AS-70) or withour (NAS-60 and NAS-70) Accellerase®XC as exogenous fobrolitic enzymes. In AS-60 and AS-70 diets, which had 60:40, 70:30 silage to concentrate ratio respectively, silage was directly treated with 0.25 mL Accellerase®XC per gram neutral detergent fiber (NDF) using a sprinkler and mixed for 5 min, before at least one hour of feeding. In NAS-60 and NAS-70 diets, which had 60:40 and 70:30 silage to concentrate ratio respectively, silage was not treated with EFE, prior to feeding. The formulation and chemical composition of the experimental diets is listed in Table 2.

Table 1
Chemical composition (mean % based on dry matter ± standard error) of experimental oat silage.

	Oat silage
Dry matter	32.8 ± 0.52
Organic matter	87.6 ± 0.37
Crude protein	12.6 ± 0.77
Neutral detergent fiber	51.1 ± 0.23
Acid detergent fiber	25.1 ± 0.67
pH	4.12 ± 0.04
Lactic acid	2.57 ± 0.06

Table 2
Ingredients and chemical composition (% of dry matter) of the experimental diets[a] of two silage to concentrate ratios supplemented with or without exogenous fibrolytic enzymes.

	With enzymes		Without enzymes	
Ingredients	AS-60	AS-70	NAS-60	NAS-70
Oat silage	60	70	60	70
Cotton seed cake	4	3	4	3
Sunflower meal	6	5	6	5
Rice polish	10	5	10	5
Corn gluten 30%	9	9	9	9
Rapeseed cake	3	3	3	3
Rice Barn	1.5	0.5	1.5	0.5
Wheat Barn	4	3	4	3
Enzose	2	1	2	1
Mineral mixture	0.5	0.5	0.5	0.5
Chemical composition, g/kg of dry matter				
Crude protein	171	168	174	167
Neutral detergent fiber	443	467	436	452
Acid detergent fiber	218	237	209	221
Metabolizable energy (Mcal/ kg)[b]	2.53	2.53	2.53	2.53

[a] AS-60 and AS-70 represent silage to concentrate ratios of 60:40 and 70:30 treated with fibrolytic enzymes at the rate of 2 g kg^{-1} of neutral detergent fiber, respectively. NAS-60 and NAS-70 represent silage to concentrate ratios of 60:40 and 70:30 without fibrolytic enzymes.
[b] Calculated according to the equation of NRC (2001).

The experiment lasted for 60 days. In first two weeks animals were adapted to the diet and samples were collected during remaining 45 days of the trial. Daily feed intake and milk production was averaged over 45 days. Milk samples were collected (5 a.m. and 5 p.m.) fortnightly to determine its composition. Digestibility was determined by the total collection methods. During the collection periods, complete collection of urine and feces was conducted according to the procedure described by Nisa et al. (2006) The feces of each animal was collected daily, weighed, mixed thoroughly and 20% of it was sampled and dried at 55 °C. At the end of each collection period, the dried fecal sample was composited and 10% of the composite sample was taken for analysis. For urine collection, special small metal buckets fitted with plastic pipes were prepared to surround the vulva. This plastic pipe was discharged into a large container (30 L). The urine excreted by each animal was acidified with 50% H_2SO_4 and a 10% volume was sampled and preserved at −20 °C. At the end of each collection period, the preserved urine sample was thawed for analysis. The feed offered and oats were sampled daily and stored for analysis. Milk samples were collected and analyzed for protein, fat, solid not-fat, and urea nitrogen content. Blood samples were collected from the jugular vein of the animal to determine the blood glucose and urea nitrogen levels.

Feed and fecal samples were subjected to analysis for dry matter (DM) and crude protein (CP) (methods 930.15 and 954.01, respectively) according to the AOAC (2006) NDF and acid detergent fiber (ADF) were determined as described by Van Soest et al. (1991). Blood glucose and blood urea concentrations were determined using the Vitalab Selectra E blood chemistry analyzer from Merck®.

Fat corrected milk (FCM, 4% fat) was calculated as described by Gains (1928) as follows:

$$FCM = 0.4 \times \text{milk yield} + 15 \times \text{fat yield}$$

Milk energy content (Mcalkg^{-1}) was calculated as: milk $NE_L = 0.0929 \times \%$ fat $+ 0.0547 \times \%$ protein $+ 0.0395 \times \%$ lactose according to the NRC (2001).

The obtained results of milk yield during adaptation period were used as covariates and the data were subjected to analysis of variance as a 2 × 2 factorial design of SAS (2002). The following statistical model was used:

$$Y_{ijkl} = \mu + E_j + R_k + ER_{jk} + \varepsilon_{ijkl}$$

where Y_{ijk} = an observation; μ = the overall mean; Ej = the fixed effect of fibrolytic enzyme (with or without supplementation); R_k = the fixed effect of oat silage to concentrate ratio (60:40 and 70:30); ER_{jk} = the interactions between the two variables;and ε_{ijkl} = residual error. Statistical analysis of blood glucose and urea nitrogen was performed via repeated measures model. Sampling time (0, 3, 6, 9 h relative to the morning feeding) and sampling time × treatment interactions were added to the previous model. The difference between means was considered significant at $P < 0.05$.

3. Results

3.1. Nutrient intake and digestibility

The obtained values of DM intake as kg day^{-1} indicated no significant difference in due to EFE supplementation. However, the calculated values of DM)as % of body weight and g kg^{-1} $W^{0.75}$), CP, NDF, and ADF (as kg day^{-1}) intake were higher ($P < 0.05$) in EFE supplemented groups. In the same trend, digestible nutrient intake as kg day^{-1} was higher ($P < 0.05$) as a result of EFE addition than in groups without EFE addition. The highest digestible nutrient intake was found in AS-60 followed by the AS-70 anmals (Table 3).

Nutrient digestibility of DM, CP, NDF and ADF with the inclusion of EFE (AS-60 and AS-70) in buffalo rations improved ($P < 0.05$) versus control animals of no EFE addition. The highest DM, CP, NDF, and ADF digestibility was found in buffaloes fed with AS-60 and the lowest digestibility values were observed in animals fed diets without EFE. Generally, no significant effect on nutrient intake and nutrient digestibility was observed between animals fed with two different ratios of oat silage to concentrate (Table 3).

3.2. Blood parameters and nitrogen balance

Blood urea nitrogen and glucose levels stayed within the normal range and no effect ($P > 0.05$) among dietary treatment groups was observed at 0, 3, 6 and 9 h post feeding (Table 4).

Nitrogen intake, fecal nitrogen and urinary nitrogen (as g day^{-1}) were not different ($P > 0.05$) among the experimental groups. However, the highest nitrogen absorption, retention, and milk nitrogen ($P < 0.05$) was observed in enzyme-supplemented groups, AS-60 and AS-70. The nitrogen balance in animals fed AS-60 ($P < 0.05$) was the highest among that of other groups. The lowest nitrogen balance was observed in animals fed NAS-60 and NAS-70 diets (Table 5).

3.3. Milk production and composition

Significantly better milk production was observed in animals fed enzyme-treated diets than in those without enzyme supplementation. The highest ($P < 0.001$) milk production was observed in animals fed the AS-70 diet. There was no difference ($P > 0.05$) in milk production between AS-60 and AS-70 diets. The lowest milk production was observed in animals fed the NAS-70 diet. Milk fat, total solids (%), and milk energy content were increased ($P < 0.05$) in animals fed the AS-60, AS-70, and NAS-70 diets. A non-significant difference was observed in milk protein, lactose, and solid not-fat content across all treatment groups. Concerning the feed conversion expressed as the amount of DM or CP intake to produce one kg of 4% FCM, animals fed with the AS-60 and AS-70 diets showed better feed conversion than did those on other diets (Table 6).

4. Discussion

4.1. Nutrient intake and digestibility

In the current study, no change in DM, CP, and NDF intake found in enzyme-treated groups, which is in agreement with previous studies (Reddish and Kung, 2007; Shekhar et al., 2010) showing no effect of EFE on dry matter intake in cattle. These results might be due to factors like enzyme type, particle size of forage, level of supplementation, method of enzyme application, and the level of milk production in the experimental animals (Beauchemin et al., 2003; Yu et al., 2005).

Table 3
Nutrient intake and digestibility of the experimental diets[a] of two proportions of oat silage to concentrate ratios supplemented with or without exogenous fibrolytic enzymes in *Nilli-Ravi* buffaloes.

	With enzymes		Without enzymes		SEM	P value[b]		
	AS-60	AS-70	NAS-60	NAS-70		E	R	E × R
Nutrients intake								
Dry matter, kg day^{-1}	12.23	11.65	10.55	10.62	0.280	0.053	0.604	0.501
Dry matter, % of body weight	2.76	2.69	2.51	2.39	0.051	0.003	0.208	0.714
Dry matter, g kg^{-1} metabolic body size^{-1}	126.5	122.5	113.8	109.6	2.373	0.004	0.283	0.984
Crude protein, kg day^{-1}	2.09	1.96	1.84	1.77	0.051	0.018	0.246	0.660
Neutral detergent fiber, kg day^{-1}	5.41	5.44	4.60	4.80	0.131	0.005	0.602	0.685
Acid detergent fiber, kg day^{-1}	2.67	2.76	2.21	2.35	0.072	0.001	0.285	0.823
Nutrients digestibility, %								
Dry matter	74.37	72.18	68.94	66.59	1.043	0.004	0.958	0.173
Crude protein	71.65	68.82	66.92	65.07	0.811	0.001	0.105	0.993
Neutral detergent fiber	57.41	53.39	48.93	47.55	1.222	0.001	0.190	0.497
Acid detergent fiber	40.43	38.59	34.45	33.84	0.824	<0.001	0.112	0.418
Digestible nutrient intake, kg day^{-1}								
Dry matter	9.09	8.41	7.28	7.08	0.262	0.001	0.217	0.491
Crude protein	1.50	1.37	1.23	1.15	0.044	0.001	0.089	0.614
Neutral detergent fiber	3.11	2.91	2.25	2.28	0.111	<0.001	0.453	0.318
Acid detergent fiber	1.08	1.07	0.76	0.80	0.043	<0.001	0.759	0.582

SEM indicates standard error of the mean.

[a] AS-60 and AS-70 represent silage to concentrate ratios of 60:40 and 70:30 treated with fibrolytic enzymes at the rate of 2 g kg^{-1} of neutral detergent fiber, respectively. NAS-60 and NAS-70 represent silage to concentrate ratios of 60:40 and 70:30 without fibrolytic enzymes.

[b] Probability of main effects of exogenous fibrolytic enzyme supplementation (E), proportions of oat silage to concentrate ratio (R), or the E × R interaction.

Table 4
Blood glucose and blood urea nitrogen concentrations (mg dl^{-1}) after 0, 3, 6 and 9 h of morning feeding of the experimental diets[a] of two proportions of oat silage to concentrate ratios supplemented with or without exogenous fibrolytic enzymes in *Nilli-Ravi* buffaloes.

	With enzymes		Without enzymes		SEM	P value[b]		
	AS-60	AS-70	NAS-60	NAS-70		E	R	E × R
Blood glucose								
0	64.00	69.50	65.25	63.75	1.991	0.298	0.081	0.245
3	64.50	65.75	64.75	66.00	2.150	0.544	0.999	0.903
6	65.75	67.75	63.50	66.75	1.682	0.125	0.701	0.327
9	62.50	65.00	63.00	64.75	2.371	0.351	0.867	0.955
Blood urea nitrogen								
0	36.25	34.50	32.50	34.50	1.240	0.943	0.297	0.297
3	32.00	34.50	29.50	33.00	1.901	0.199	0.825	0.383
6	31.75	31.25	31.00	32.50	2.532	0.847	0.701	0.923
9	34.00	34.25	30.00	33.00	1.621	0.469	0.539	0.250

SEM indicates standard error of the mean.
[a] AS-60 and AS-70 represent silage to concentrate ratios of 60:40 and 70:30 treated with fibrolytic enzymes at the rate of 2 g kg^{-1} of neutral detergent fiber, respectively. NAS-60 and NAS-70 represent silage to concentrate ratios of 60:40 and 70:30 without fibrolytic enzymes.
[b] Probability of main effects of exogenous fibrolytic enzyme supplementation (E), proportions of oat silage to concentrate ratio (R), or the E × R interaction.

Table 5
Nitrogen utilization and balance of the experimental diets[a] of two proportions of oat silage to concentrate ratios supplemented with or without exogenous fibrolytic enzymes in *Nilli-Ravi* buffaloes.

Items	With enzymes		Without enzymes		SEM	P value[b]		
	AS-60	AS-70	NAS-60	NAS-70		E	R	E × R
N intake, g/d	333	317	287	289	7. 6	0.053	0.604	0.501
Fecal N, g/d	94.3	95.6	94.9	101.0	1.87	0.458	0.363	0.562
Fecal N, % of intake	28.4	30.2	33.1	34.9	–	–	–	–
Absorption, g/d	238.3	221.3	192.0	188.1	6.68	0.001	0.260	0.477
Absorption, % of intake	71.7	69.8	66.9	65.1	–	–	–	–
Urinary N, g/d	65.1	53.3	50.1	52.5	2.42	0.088	0.293	0.120
Retention, g/d	173.2	167.9	141.9	135.5	4.93	<0.001	0.339	0.921
Retention, % of intake	52.2	53.1	49.4	46.9	–	–	–	–
Milk N, g/d	53.6	55.59	44.66	41.1	1.88	<0.001	0.730	0.259
N Balance, g/d	119.5	112.4	97.3	94.5	3.85	0.007	0.434	0.730

SEM indicates standard error of the mean.
[a] AS-60 and AS-70 represent silage to concentrate ratios of 60:40 and 70:30 treated with fibrolytic enzymes at the rate of 2 g kg^{-1} of neutral detergent fiber, respectively. NAS-60 and NAS-70 represent silage to concentrate ratios of 60:40 and 70:30 without fibrolytic enzymes.
[b] Probability of main effects of exogenous fibrolytic enzyme supplementation (E), proportions of oat silage to concentrate ratio (R), or the E × R interaction.

Table 6
Milk production and composition of the experimental diets[1] of two proportions of oat silage to concentrate ratios supplemented with or without exogenous fibrolytic enzymes in *Nilli-Ravi* buffaloes.

	With enzymes		Without enzymes		SEM	P value[4]		
	AS-60	AS-70	NAS-60	NAS-70		E	R	E × R
Milk yield, kg day^{-1}	9.63	9.80	7.70	7.13	0.341	<0.001	0.573	0.305
4% FCM[2], kg day^{-1}	12.14	12.52	8.80	8.94	0.490	<0.001	0.564	0.780
Milk composition								
Protein yield, kg day^{-1}	335.2	347.4	279.1	256.6	11.76	<0.001	0.730	0.259
Fat yield, kg day^{-1}	552.4	573.6	381.4	405.6	24.05	<0.001	0.308	0.944
Protein, %	3.48	3.55	3.62	3.63	0.071	0.471	0.783	0.833
Fat, %	$5.75^{a,b,c}$	$5.85^{a,b,c}$	$4.95^{a,b,c}$	$5.70^{a,b,c}$	0.122	0.010	0.017	0.046
Lactose, %	4.84	4.98	5.02	5.02	0.044	0.246	0.475	0.443
Solid not fat, %	8.30	8.35	8.50	8.60	0.062	0.091	0.552	0.842
Total solids, %	$14.61^{a,b,c}$	$14.78^{a,b,c}$	$14.03^{a,b,c}$	$14.85^{a,b,c}$	0.101	0.030	<0.001	0.009
Milk energy content[3], Mcal kg^{-1}	0.92	0.93	0.86	0.93	0.011	0.063	0.020	0.147
Feed conversion								
Dry matter, kg^{-1} FCM milk	1.01	0.93	1.20	1.19	0.041	<0.001	0.377	0.496
Crude protein, g kg^{-1} FCM milk	171.5	158.6	204.6	202.8	6.234	<0.001	0.370	0.499

SEM indicates standard error of the mean.
[1]AS-60 and AS-70 represent silage to concentrate ratios of 60:40 and 70:30 treated with fibrolytic enzymes at the rate of 2 g kg^{-1} of neutral detergent fiber, respectively. NAS-60 and NAS-70 represent silage to concentrate ratios of 60:40 and 70:30 without fibrolytic enzymes.
[2]Fat corrected milk (FCM, 4% fat) was calculated as described by Gains (1928) as follows: FCM = 0.4 × milk yield + 15 × fat yield.
[3]Milk energy content (Mcalkg^{-1}) was calculated as milk NE_L = 0.0929 × % fat + 0.0547 × % protein + 0.0395 × % lactose according to the NRC (2001).
[4]Probability of main effects of exogenous fibrolytic enzyme supplementation (E), proportions of oat silage to concentrate ratio (R), or the E × R interaction.
[a,b,c]Means in a row with different superscripts differ significantly ($P < 0.05$).

The CP, NDF, and ADF intake besides DM, CP, NDF and ADF digestible intake found in this study is in accordance with that reported by Shekhar et al. (2010) and Miachieo and Thakur (2007) who recorded higher total digestible nutrient intake in cows on rations supplemented with EFE. Salem et al. (2013) reported increased CP and NDF intake in animals fed EFE supplemented dies. The increased digestible nutrient intake might possibly be due to the increased hydrolytic capacity of the rumen with enzyme-treated diets fed to animals which indirectly decreased the gut fill (Adesogan et al., 2014). It could also be due to increased palatability, resulting in pre-ingestive release of sugar and partial solubilization of fiber by the enzyme (Dado and Allen, 1995).

Better nutrients digestibility in animals fed enzyme-supplemented diets is similar to previous studies (Gado et al., 2009; Shekhar et al., 2010; Salem et al., 2013) that recorded improved nutrient digestibility with EFE supplementation. The enhanced nutrient digestibility in enzyme-treated diets could be ascribed to the additive effects of enzymatic action and ruminal micro-flora (Morgavi et al., 2001). According to Beauchemin et al. (2003) synergism with ruminal microbes, stimulation of bacterial colonization, stimulation of ruminal microbial populations, stimulation of bacterial attachment, and improvement in ruminal hydrolytic capacity were some of the main factors in improving feed efficiency and digestion in response to EFE supplementation.

In contrast, Sutton et al. (2001) and Dean et al. (2005) observed no effect of EFE on DM, CP, NDF, and ADF digestibility. Treacher and Hunt (1996) and Kung Jr et al. (2000) reported that excessive use of EFE in diets results in binding of EFE to substrates and secretion of antinutritional factors such as phenolic compounds that might affect microbial growth in the rumen and decrease fiber digestion. Furthermore, the use of higher doses of EFE could cause reduced forages chewing and saliva production and subsequently resulting in lower rumen pH and fiber degradation. The present results differ from their results because they used higher doses of enzymes (8800 units carboxyl cellulase and 40,000 xylanase per kg of forage on DM basis) and their method of application was also different.

4.2. Blood parameters and nitrogen balance

No change was observed in blood urea nitrogen and glucose similar to the results of previous studies (Varlyakov et al., 2010; Wahyuni et al., 2012). The use of iso-nitrogenous diets in animals feeding together with the efficient utilization of ruminal ammonia nitrogen by microraganisms could be responsible for the non-significant alterations in blood urea nitrogen values (Dehghani et al., 2012). The non-significant change in blood glucose level could be linked to the high metabolic rate of glucose consumption and homeostatic mechanism of animal body does not permit appreciable variation in glucose concentration (Dehghani et al., 2012).

Increased the levels of blood glucose (post-feeding) in response to enzyme-supplemented diets possibly due to release of soluble sugars by the action of EFE. Highly fermentable carbohydrates yield increased proportions of propionate, which may be transformed to glucose, thus increasing the blood glucose level in animals (Newbold, 1997; Wang et al., 2001).

The nitrogen balance results are in a harmony with previous studies (Miller et al., 2008; Gado et al., 2009) reporting better nitrogen retention due EFE supplementation in the ruminant diet. Enzyme supplementation in the ruminant diet might result in the release of soluble sugars, which helps improve microbial growth (Beauchemin et al., 2003). According to Wang et al., (2001) addition of EFE to the ruminant diet improved attachment and the number of cellulolytic bacteria in the rumen by up to 10 times. Increased microbial protein synthesis due to EFE supplementation was also supported by some studies (Beauchemin et al., 1999; Rode et al., 1999) reporting increased nitrogen fixation, digestion, and retention due to enzyme supplementation in the ruminant diet.

4.3. Milk production and composition

Higher milk production in animals fed enzyme-supplemented diets than in those without added enzymes in the diet are supported by previous reports (Ahn et al., 2003; Titi, 2003) showing increased milk production in dairy animals fed with EFE-supplemented diets. Beauchemin et al., (2003) reported 3–4% increased milk production by EFE supplementation in the ruminant diet. This improvement in milk production might be due to the increased absorption of nutrients in the gastrointestinal tract or rumen, thus resulting in gain of more net energy (Kung Jr, 2001). Despite this, Reddish and Kung (2007) and Dean et al. (2013) reported no effect of EFE on milk production. No change in milk production might be a result of similar DM, CP, NDF, and ADF digestibility (Dean et al., 2013). The difference in experimental results, might be attributed to different enzyme products resulting in high variability in animal responses (Beauchemin et al., 2003).

The present results regarding milk fat are in accordance with previous reports (Yang et al., 1999; Bowman, 2001) showing improvement in milk fat when forages or silages were supplemented with enzymes. Fiber digestion is directly proportional to *de novo* fatty acid synthesis of milk through the process of rumen fermentation where acetate is produced as a precursor of milk fat synthesis (Ma, 2012). Acetate produced in the rumen is transformed into acetyl CoA by the enzyme acyl-CoA synthetase. Acetyl-CoA is then converted to malonyl-CoA by the enzyme acetyl-CoA carboxylase (Zhang and Kim, 1998). Fatty acid synthase finally catalyzes *de novo* fat synthesis from malonyl-CoA(Ma, 2012). No change in milk protein, milk lactose, and solid not-fat content is in accordance with previous studies (Arriola et al., 2011; Shadmanesh, 2014) that reported no effect of EFE supplementation on milk protein, lactose, and SNF.

Improvement in the feed conversion ratio of DM and CP (kg^{-1} FCM milk) could be attributed to the higher FCM yield recorded in EFE-supplemented groups (AS-60 and AS-70) than that with the non-supplemented groups. In addition, the feed conversion enhancement might be attributable to superior NDF digestibility in the rumen (Holtshausen et al., 2011).

4. Conclusion

Supplementation with exogenous fibrolytic enzymes in early lactating buffalo diets containing high oat silage (60 or 70%) resulted in higher milk production and fat content. The improved milk yield and composition may be a direct result of enhanced digestible nutrient intake, digestion coefficients, nitrogen balance, and feed conversion.

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