INFLUENCE OF EXOGENOUS ENZYMES ON NUTRIENT DIGESTIBILITY, BLOOD COMPOSITION, MILK PRODUCTION AND ITS COMPOSITION AS WELL AS MILK FATTY ACIDS PROFILE IN DAIRY BUFFALOES.

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SUMMARY

Twenty one multiparous lactating buffaloes averaging 565 ±12 kg body weight (BW) were allotted at calving to three groups, seven buffaloes per each to study the effect of feeding rations supplemented with two recently developed enzyme cocktails (Zad1 and Zad2) as exogenous fibrolytic enzymes on dry matter (DM) intake, nutrients digestibility, milk production and its composition, milk fatty acids profile, and concentration of some blood metabolites. Buffaloes within each block were assigned to one of three iso-net energy for lactation total mixed rations containing no additives (control diet), 40 g Zad1/head/day and 40 g Zad2/head/day. Diets were fed *ad libitum* from calving to week 16 of lactation. Exogenous enzymes supplementation had no effect on body weight (BW) but increased dry matter intake. Exogenous enzymes supplementation improved (P<0.05) nutrients digestibility and the nutritive value of the tested rations compared with control. Blood serum glucose was increased (P<0.05) with exogenous enzymes supplementation compared with control. Zad1 supplementation increased (P<0.05) milk production compared with control. While, Zad1 was increased (P<0.05) 4% fat corrected milk (FCM) followed by Zad2 (P<0.05) and then control. Milk components were not significantly affected by exogenous enzymes supplementation compared with control. Feed efficiency was improved with exogenous enzymes supplementation to ration. The main difference in milk fatty acid profile determined was conjugated linoleic acid (C18:2*trans*-10,*cis*12), n-3 fatty acid (C18.3N3) and total unsaturated fatty acids which were higher in milk fat of animals that fed Zad1 and Zad2 compared to control. Buffaloes fed Zad1 and Zad2 had lower proportions of total saturated fatty acids in milk fat compared with control. It's being concluded that supplementation with Zad1 is more effective than with Zad2 on nutrients digestibility, milk production and milk fatty acids profile.

Keywords: *exogenous enzymes; digestibility; milk production; milk fatty acids; blood parameters; buffaloes.*

INTRODUCTION

Ruminants depend on the indigenous bacteria, protozoa and fungi present in the rumen to digest forage fiber (White *et al.*, 1993), which occurs by numerous enzymatic activities required for hydrolysis of plant cell wall. Use of exogenous fiber degrading enzymes may be a way to increase the nutritive value of ruminant's ration (Beauchemin *et al.*, 2004). Improvement in forage fiber digestion increases energy available to ruminants (Feng *et al.*, 1996). Enzymes supplementation to ration is often accompanied by increased feed intake, which may partly be due to increased palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis. However post-ingestive enzyme effects, such as increased digestion rate and extent of digestion (Gado and Salem, 2008; Krueger *et al.*, 2008) may increase hydrolytic activity in the rumen to reduce gut fill and enhance feed intake (Adesogan, 2005). Direct-fed enzymes can also enhance microbial colonization of feed by increasing numbers of ruminal fibrolytic microbes (Morgavi *et al.*, 2000; Nsereko *et al.*, 2000) to increase rate of degradation of fiber in the rumen (Giraldo *et al.*, 2008), rumen microbial protein synthesis (Yang *et al.*, 1999; Nsereko *et al.*, 2002) and for stomach digestibility. Positive effects of adding exogenous enzymes to ruminant diets have been reported for lactating dairy cows and growing cattle. Dairy cows fed forage treated with a fibrolytic enzyme additive ate more feed

and produced 5–25% more milk (Stella *et al.*, 2007; Gado *et al.*, 2009), improved the energy balance of transition dairy cows (DeFrain *et al.*, 2005) and increased milk production in small ruminants (Stella *et al.*, 2007). A commercial exogenous enzyme mixture (Zad1), prepared from anaerobic bacterium, has been shown to improve ruminal fermentation, N balance and nutrient digestibility (Gado *et al.*, 2007; Soliman, 2006) as well as milk yield (Gado *et al.*, 2009).

The objective of this study was to determine effects of Zad1 and Zad2 (under test) as exogenous fibrolytic enzymes mixtures supplementation to diets on feed intake, nutrients digestibility, milk production and its composition, milk fatty acids profile and blood parameters in early lactation dairy buffaloes.

MATERIALS AND METHODS

Animals, experimental design and diets:

This study was conducted at the Experimental Farm in Shalakan, Faculty of Agriculture, Ain Shams University and Dairy Science Department, National Research Center, Dokki, Cairo, Egypt during November 2010 to April 2011 using 21 lactating buffaloes (body weight (BW) was 565±12 kg). Buffaloes were blocked for similar expected calving dates. The experiment was extended from calving to 16th week of lactation. Buffaloes were housed in tie stalls and fed individually. Buffaloes within groups (seven buffaloes per each) were assigned randomly to one of three total mixed rations (TMR; Table 1). The enzyme product (Zad1) was made from natural sources of anaerobic ruminal bacteria including 7.05 unit/g of cellulase, 2.32 unit/g of xylanase, 61.5 unit/g of α-amylase and 29.2 unit/g of proteases (patent no. 22155 of Egypt, Molecular Biology Laboratory of the Ain Shams University (Cairo) according to Gado, 1997) and (Zad2 under test) including 0.892 unit/g of cellulase, 0.058 unit/g of xylanase, 3.39 unit/g of α-amylase and 1.56 unit/g of proteases. The three TMR consisted of a control TMR with no additives (control), a TMR with 40 g Zad1/head/day (Zad1), and 40g Zad2/head/day (Zad2). All diets were designed to have similar concentrations of crude protein (CP) and net energy for lactation (NE_L). Diets were formulated to meet the animal's requirements (NRC, 2001). Diets were fed twice daily at 08:00 and 16:00h ad libitum. Animals were milked twice daily at 06:00 and 15:00 h. Milk production was recorded at every milking. The BW of each buffalo was determined before feeding every month through experimental period. Dry matter intake (DMI) was recorded every month by weighing feeds offered and refused by the animals. Fresh water was available to the animals all time.

Apparent digestibility:

Three digestibility trials were applied during the last three days every month using three animals from each group. Silica was used as an internal marker for determining the digestibility (Ferret *et al.* 1999). At 4 h after the morning feeding, fecal samples (approximately 100 g) were collected from the rectum during the last three days every month and pooled by buffalo for each period, dried at 55 °C for 48 h, and then ground to pass a 1 mm sieve in a feed mill (FZ102, Shanghai Hong Ji instrument Co., Ltd., Shanghai, China) for chemical analysis. Dry matter excreted in feces was calculated by dividing silica input in the feeds (grams of silica per day) by silica output in the feces (grams of silica per day). The digestibility coefficient of certain nutrient was calculated according to the following formula (Ferret *et al.* 1999):

$$Digestibility = 100 - \left[100 \; x \; \frac{\%\ indicator\ in\ feed}{\%\ indicator\ in\ feces} \; x \; \frac{\%\ nutrient\ in\ feces}{\%\ nutrient\ in\ feed} \right]$$

Sample collection and analyzes:

Samples of TMR were collected and composed monthly, composited samples were mixed thoroughly and sub sampled for chemical analyzes. Milk samples were obtained once every two weeks from each buffalo for two consecutive milkings and pooled within buffalo relative to production to obtain one composite milk sample per buffalo for determine milk composition. Milk samples were stored at +4 °C until chemical analysis. Milk samples were pooled every month within treatment relative to production to obtain one composite milk sample per treatment and frozen at −20 °C until analyzed for milk FA profile. Blood was collected from all buffalo on week 4, 8, 12 and 16 postpartum at 4 h after the morning feeding to determine some blood parameters concentrations. Blood was withdrawn from the jugular vein into vacutainer tubes (Becton Dickinson and Cie, Rutherford, NJ, USA). Tubes were immediately placed on

ice and centrifuged within 1 h at 4 °C for 30 min at 3000×g.b. Blood serum were separated and frozen at −20 °C for subsequent analysis.

Dry matter of TMR, ether extract (EE), crude fiber (CF), crude protein (CP) and ash were determined according to AOAC (2007). Organic matter was determined by difference between dry matter and ash content. The concentration of NDF, ADF in TMR was determined with sodium sulfite and heat stable α-amylase and expressed exclusive of residual ash (Van Soest *et al.*, 1991). Blood serum samples were analyzed for concentrations of serum total protein (Armstrong and Carr, 1964), albumin (Doumas *et al.*, 1971), total lipids (Postma and Stress, 1968) cholesterol (Raltiff and Hall, 1973), glucose (Siest *et al.*, 1981) and serum GOT and GPT (Reitman and Frankel, 1957). Globulin was calculated by deference between total protein and albumin. pH value of milk was determined using a digital pH-meter. Milk samples were analyzed for total solids, fat, total protein and lactose by infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark). Solids-not-fat (SNF) was calculated by difference. Fat corrected milk (4% fat) was calculated by using the following equation according to Gaines (1928):

$$FCM = 0.4 \text{ milk yield (gm)} + 15 \text{ fat yield (gm)}$$

Fatty acids in milk and TMR were extracted and methylated according to method of AOAC (1998) using HPLC system.

Statistical analysis:

All results were analyzed using the MIXED procedure of SAS (2004). Data of the present study were analyzed as a randomized block design. Data were expressed as mean values when there was no interaction between week and treatment (i.e., $P>0.10$). When a significant F-test was detected (i.e., $P<0.05$), treatment means were separated using Duncan's multiple range test (Duncan, 1955).

Table (1): Ingredient and chemical composition of total mixed diets of lactating buffaloes fed no additives (control), Zad1 or Zad2.

Ingredient (g/kg)	Control	Zad1	Zad2
Berseem hay	330	330	330
Rice straw	275	275	275
Yellow corn	61	60	60
Soybean meal	83	81	81
Wheat bran	200	200	200
Sunflower meal	20	20	20
Urea	5	5	5
Zad1	0	3	0
Zad2	0	0	3
Calcium carbonate	3	3	3
Minerals and Vitamins[a]	23	23	23
Chemical composition (g/kg DM)			
Dry matter	901.1	901.5	901.6
Organic matter	896.6	896.9	897.1
Crude protein	162	161	162
Ether extract	37.2	37.1	37.2
Crude fiber	235.9	235.8	235.7
Nitrogen free extract (NFE)	461.5	463.0	462.2
Neutral detergent fiber (NDF)	387	386	384
Acid detergent fiber (ADF)	231	230	229
NE_L (Mj/kgDM)[b]	6.3	6.3	6.3

Zad1 7.05 unit/g of cellulase, 2.32 unit/g of xylanase, 61.5 unit/g of α-amylase and 29.2 unit/g of proteases, Zad2 0.892 unit/g of cellulase, 0.058 unit/g of xylanase, 3.39 unit/g of α-amylase and 1.56 unit/g of proteases

[a] Contained 141 g/kg of Ca, 27 g/kg of P, 65 g/kg of Mg, 14 g/kg of S, 120 g/kg of Na, 6 g/kg of K, 944 mg/kg of Fe, 1613 mg/kg of Zn, 484 mg/kg of Cu,1748mg of Mn, 58mg/kg of I, 51 mg/kg of Co, 13 mg/kg of Se, 248,000 U/kg of vitamin A, 74.000 UI/kg of vitamin D3 and 1656 IU/kg of vitamin E.

[b] Calculated using published values of feed ingredients (NRC, 2001).

RESULTS AND DISCUSSION

Feed intake and body weight:

Dry matter intake, nutrients digestibility and feeding value are presented in Table 2. Exogenous enzymes supplementation to buffaloes rations were increased (P<0.05) dry matter intake (DMI), by 1.98% for Zad2 and 2.13% for Zad1 compared with control while, live body weight was not affected by treatments. The improvement of DM intake by addition of enzymes may be partly due to improvement of nutrient digestibility, which is consistent with previous results that obtained by Soliman, 2006; Gado *et al.*, 2007; El-Adawy *et al.*, 2008; Gado and Salem, 2008 and Gado *et al.*, 2009. In the current study, DM intake and digestibility were improved by about 2.1 and 1.98, and 8.61and 6.84 % respectively with Zad1 and Zad2 addition. Moreover, our results are consistent with Beauchemin et al. (2001), who reported that the average increase in DM intake due to enzyme supplementation was 1.6 kg/d in dairy cows. In contrast, Schingoethe *et al.* (1999) treated forage with 0.7–1.5 l/t forage DM with concentrated cellulase and xylanase enzymes and reported no significant effect of exogenous enzyme on DM intake.

Nutrients digestibility and feeding values:

Zad1 and Zad2 supplemented rations were significantly improved nutrients digestibility as organic matter, crude protein, crude fiber, nitrogen free extract, neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Table 2). Zad1 and Zad2 improved NDF digestibility by about 8.67 and 6.37 % compared to control. Nutritive value in term of total digestible nutrients (TDN), digestible crude protein (DCP), digestible energy (DE), metabolizible energy (ME) and net energy for lactation (NE_L) were significantly improved with rations supplemented by Zad1 followed by Zad2 and then control ration. Other reports have also shown increases in digestibility of dry matter particularly fiber with fibrolytic enzyme addition (Gado and Salem, 2008; Hristov *et al.*, 2008). Bowman *et al.* (2002) reported a 25% increase in total tract NDF digestibility with a fibrolytic enzyme product. Exogenous fibrolytic enzymes would be expected to increase fiber digestion by increasing the rate of ruminal digestion of the potentially digestible NDF fraction (Yang *et al.*, 1999), alterations in ruminal fermentation (Nsereko *et al.*, 2002) and/or enhanced attachment and colonization to the plant cell wall by ruminal microorganisms (Nsereko *et al.*, 2000; Wang *et al.*, 2001) and/or by synergism with enzymes in rumen fluid (Morgavi *et al.*, 2000). However, increased fiber digestion is unlikely the result of supplemental enzyme activity alone because the contribution of added exogenous enzymes to total ruminal activity is relatively small (Beauchemin *et al.*, 2001). Wang *et al.* (2001) reported that enzyme supplementation increased numbers of non-fibrolytic and fibrolytic bacteria in a batch culture system with rumen fluid. Stimulation of rumen microbial numbers by the use of enzymes could result in higher microbial biomass, which would provide more total polysaccharidase activity to digest feedstuffs. Consistent with this hypothesis, Yang *et al.* (1999) reported that enzyme supplementation to dairy cow diets increased feed digestion in the rumen and flow of microbial protein from the rumen. The beneficial effects on animal performance are likely to be highest for ruminants in negative energy balance, such as cows in early lactation (Rode *et al.*, 1999), which is consistent with results of our study. The improvement of nutritive value of rations supplemented with Zad1 and Zad2 may be due to the improvement of nutrients digestibility as shown in Table 2.

Blood serum parameters:

Data in Table 3 showed that exogenous enzymes supplemented rations were not significantly affected serum total protein, albumin, globulin, albumin/globulin ratio and urea nitrogen. Also, Serum total lipid and cholesterol were not significantly affected by treatments. Serum glucose concentration tended to higher (P<0.05) with animals fed Zad1 and Zad2 compared to control. The higher concentration of serum glucose with Zad1 and Zad2 reflect the improvement of energy utilization and soluble carbohydrate in the rumen absorbed through ruminal wall to blood which resulted higher glucose available. These results are consistent with the improvement of crude fiber, NDF, ADF and NFE digestibility of Zad1 and Zad2 supplementation to buffalo's diets. Serum glucose had the same trend of milk yield (Table 4). Blood serum glutamic-oxaloacetate-transaminase (GOT) and glutamic-pyruvate-transaminase (GPT) values were not affected by treatments. The concentrations of serum proteins, total lipids, cholesterol, GOT and GPT were not significantly affected by treatments and in the normal range for healthy animals. These results indicated that tested additives to lactating buffalo's rations were not negatively affected liver activity or general animal's health.

Table (2): Apparent nutrients digestibility and nutritive value of rations supplemented with ZAD1 and Zad2.

Item	Control	Zad 1	Zad 2	±SE
Live body weight (kg)	565.6	566.1	564.5	2.152
Dry matter intake (kg/head/d)	13.14[b]	13.42[a]	13.40[a]	2.315
Nutrients digestibility (g/100g DM)				
Dry matter	61.10	66.36	65.28	1.157
Organic matter	63.10[b]	68.04[a]	66.75[a]	1.331
Crude protein	60.60[b]	69.79[a]	67.37[a]	1.372
Ether extract	61.05	62.08	61.54	0.337
Crude fiber	54.00[b]	63.56[a]	62.55[a]	2.836
Nitrogen free extract	55.10[b]	59.88[a]	58.61[a]	0.807
NDF	55.20[b]	60.32[a]	59.36[a]	2.366
ADF	56.36[b]	61.00[a]	60.25[a]	2.115
Nutritive value:				
Total digestible nutrients (g/kg)	531.40[c]	596.62[a]	582.16[b]	1.025
Digestible crude protein (g/kg)	98.17[c]	113.05[a]	108.47[b]	1.124
DE (MCal/kg)	2.343[c]	2.631[a]	2.567[b]	0.984
ME (MCal/kg)	2.321[c]	2.611[a]	2.547[b]	0.885
NE_L (MCal/kg)	1.302[c]	1.461[a]	1.426[b]	0.878

Each value represents an average of twelve samples.

*DE (digestible energy) = 0.04409 * TDN, ME (metabolizible energy) = 1.01 * (DE – 0.45),*

*NE_L (net energy for lactation) = 0.0245 * (TDN – 0.12) (NRC, 2001).*

[a, b, c] means with different superscripts are significant (P<0.05) difference.

Table (3): Blood serum parameters of buffaloes fed ZAD1 and Zad2 supplemented diets.

Item	Control	Zad 1	Zad 2	±SE
Total protein (g/dl)	6.59	7.34	6.92	0.130
Albumin (g/dl)	3.41	3.78	3.72	0.242
Globulin (g/dl)	3.19	3.55	3.19	0.100
A/G ratio	1.08	1.08	1.22	0.049
Urea (mg/dl)	38.85	38.56	40.71	0.380
Total lipids (mg/dl)	267.8	274.8	277.7	1.613
Cholesterol (mg/dl)	145.8	138.5	132.8	2.781
Glucose (mg/dl)	69.20[b]	73.66[a]	72.02[a]	3.83
GPT (Units/ml)	15.42	15.68	15.39	0.187
GOT (Units/ml)	34.13	35.09	34.48	0.422

Each value represents an average of twenty eight samples.

[a, b] means at the same row with different superscript are significantly (P< 0.05) different

Milk yield and its composition:

Milk production and milk analysis data are shown in Table 4. Milk production was significantly higher (P<0.05) with buffaloes fed Zad1 and insignificantly higher with Zad2 compared with control animals. Also, 4% fat corrected milk yield was higher (P<0.05) with buffaloes fed Zad1 followed by Zad2 and then control. Milk production and fat corrected milk (4%FCM) were higher in Zad1 and Zad2 supplemented diets by about 9.12 and 15.99% and 3.78 and 8.30%, respectively. The increase in DM intake and digestibility suggest the increased milk yield which refered to feeding enzymes. Soliman (2006) reported 23% higher milk production of dairy cows fed peanut hay ensiled with enzymes for 45 day, and explained the improvement as being due to increased nutrient digestibility. Studies on enzyme supplementation to dairy cow diets have shown increased milk yields of 7–15% (Yang *et al.*, 2000; Gado *et al.*, 2009), probably due to increased digestibility (Tricarico *et al.*, 2005; Gado *et al.*, 2009), as well as alteration of acetic/propionic acid ratio in the rumen (Rode *et al.*, 1999; Giraldo *et al.*, 2008), which increased energy available for milk production (Lewis *et al.*, 1999; Yang *et al.*, 1999). Beauchemin *et al.* (2003) suggested that animal responses to exogenous enzyme are likely greatest when net energy intake is first limiting, such as in early lactation dairy cows (Schingoethe *et al.*, 1999) and that exogenous enzyme may help to overcome feed digestion restrictions that separate actual from potential animal performance. In contrast, Elwakeel *et al.* (2007) evaluated 7 different enzymes under *in-vitro* and *in-vivo* conditions at increasing levels and did not observe any effect on the performance of lactating dairy cows fed diets based on corn silage, corn gluten feed, and concentrate.

Milk components except SNF were not significantly affected by exogenous enzymes supplementation (Table 4). Previous studies have reported that exogenous enzyme supplementation has had little effect on milk components when cows have been in positive energy balance (Dhiman *et al.*, 2002) which consistent with our results. Other studies, Reddish and Kung (2007) did not observe any effect on milk composition for lactating cows fed a supplemental cellulase-xylanase enzyme mixture top dressed on a diet based on corn silage, alfalfa silage and concentrate. Feed efficiency calculated as milk yield/DMI and FCM/DMI were significantly improved by Zad1 followed by Zad2 supplementation and then control (Table 4).

Holtshausen *et al.* (2011) noted that adding a high level (1.0 mL/ kg of TMR DM) of fibrolytic enzyme to the diet of cows in early lactation increased milk production efficiency (kg of milk/kg of DMI) by 10.7% and FCM production efficiency (kg of FCM/kg of DMI) by 11.3%. Improved feed efficiency indicates better utilization of nutrients when TMR was treated with enzymes. The improvement in feed conversion efficiency observed in the lactation study might be attributable to greater NDF digestibility in the rumen. The exogenous enzyme Zad1 is more effect on lactating buffalo's productivity than Zad2 may be due to the higher concentrations of fibrolytic enzymes in Zad1 than Zad2.

Table (4): Milk yield and its composition of buffaloes fed ZAD1 and Zad2 supplemented diets.

Item	Control	Zad 1	Zad 2	±SE
Milk yield (kg/d)	7.13^{b}	7.78^{a}	7.40^{ab}	0.119
4% FCM (kg/d)	9.88^{c}	11.46^{a}	10.70^{b}	0.635
Milk composition %				
Fat	6.57	7.15	6.97	0.062
Protein	4.19	4.10	4.18	0.018
Lactose	4.71	4.79	4.96	0.081
TS	16.78	16.89	16.85	0.048
SNF	10.21^{a}	9.75^{b}	9.88^{b}	0.076
Ash	0.782	0.782	0.816	0.008
pH	6.35	6.22	6.40	0.265
Feed efficiency (kg/kg):				
Milk yield/DMI	0.543^{b}	0.581^{a}	0.551^{ab}	1.145
FCM yield/DMI	0.752^{c}	0.855^{a}	0.797^{b}	1.201

Each value represents an average of fifty six samples.

$^{a, b, c}$ means with different superscripts are significant (P<0.05) difference.

Milk fatty acid profile:

Milk fatty acids profile is presented in table 5; proportions of fatty acids (6:0, 8:0, 10:0, 12:0, 14:0, 14:1 and 16:.0) in milk fat were not significantly affected by treatments. On the other ward, 16:1 proportion was increased (P<0.05) with exogenous enzymes supplementation to the diet (Table 5). C17:0 and C17:1 were only appeared with Zad1 supplemented diet. C18:0 was decreased with Zad2 and C18:1n9c was decreased with Zad1 supplemented diets compared with other treatments. Feeding Zad1 and Zad2 enzymes increased ($P < 0.05$) conjugated linolenic acid (CLA) C18:2 *trans*-10, *cis*-12 and decreased CLA, C18:2 *cis*-9, *trans*-11. Total CLA was not affected by Zad1 and decreased with Zad2 supplementation. Zad1 and Zad2 supplementation significantly increased the proportion of n-3 (C18:3 n-3) and n-6 FAs (C18:3 n-6). The n-6: n-3 ratio was significantly decreased in milk fat from 28.0 in control milk fat to 3.0 and 2.21 with Zad1 and Zad2 supplementation, respectively. The C20:0 and C20:1 were not significantly affected by Zad1 and Zad2 supplementation while, C20:4 was appeared with Zad1 and Zad1 supplemented diets.

Milk from buffaloes receiving exogenous enzymes supplementation displayed an improvement in the FA profile, with an increase in the poly- and mono-unsaturated FA content and a decrease in saturated FA content. These results may be due to the improvements of NDF and organic matter digestibility which produce more soluble carbohydrates for fatty acids synthesis. Gado *et al.* (2009) suggest that enzyme supplemented diet increased ruminal acetate, propionate and butyrate proportions. Also, enzyme supplemented diet stimulated lactic acid-utilizing bacteria which also produce propionate. Beauchemin *et al.* (2003) reported that supplementing the diet of feedlot cattle with lactic acid bacteria increased the proportion of propionate and, consequently, decreased the proportion of butyrate in rumen fluid compared with the control. This shift in short chain fatty acids proportions could increase precursor availability for fatty acids synthesis, particularly for dairy cows in early lactation when nutrient intake lags nutrient demand (Eun *et al.*, 2007). In particular, in the present study the decrease in n-6/n-3 from 28.0 in control milk to 3.0 and 2.21 in Zad1 and Zad2 milks may be a positive goal from a consumer's health.

Table (5): Milk fatty acids composition of lactating buffaloes fed ZAD1 and Zad2 supplemented diets.

Fatty acids	Control	ZAD1	ZAD2	±SE
C6	1.10	1.12	0.68	0.041
C8	0.87	0.9	0.78	0.122
C10	1.71	1.69	1.55	0.063
C12	2.18	2.03	1.92	0.235
C14.0	11.26	10.39	10.19	0.027
C14.1	0.67	0.67	0.77	0.321
C16.0	33.67	32.33	33.43	0.745
C16.1	0.45^{b}	1.62^{a}	1.65^{a}	0.302
C18.0	17.82^{a}	18.57^{a}	15.78^{b}	0.547
C18.1N9T	27.02	26.57	25.46	0.125
C18.1N9C	1.78^{a}	0.27^{b}	1.68^{a}	0.085
C18:2*trans*-10,*cis*12	0.10^{b}	0.13^{a}	0.13^{a}	0.096
C18:2*cis*-9, *trans*-11	0.18^{a}	0.13^{b}	0.01^{c}	0.035
Total CLA	0.28^{a}	0.26^{a}	0.14^{b}	0.055
C18.3n-3	0.01^{b}	0.11^{a}	0.14^{a}	0.188
C18.3n-6	0.28^{b}	0.33^{a}	0.31^{a}	0.065
N-6/N-3	28.00^{a}	3.00^{b}	2.21^{b}	0.199
C20.0	0.71	0.24	0.73	0.056
C20.1	0.19	0.22	0.26	0.784
C20.4	0.0	2.68	4.53	0.622

Each value represents an average of four samples.

$^{a, b, c}$ means with different superscripts are significant ($P<0.05$) difference.

CONCLUSIONS

The exogenous enzymes product Zad1 sourced from anaerobic bacterium and added to the TMR of buffaloes in early lactation, improved 4%FCM production and feed efficiency. The exogenous enzymes product Zad1 is more effect on lactating buffalo's productivity than Zad2. This improvement in production efficiency might be due in part to the increase in dry matter intake and NDF digestibility of TMR with addition of the enzyme. The stepwise approach taken in this study provided an appropriate characterization of enzyme activity of the developmental enzyme product for use in ruminant diets. Further work is necessary to determine if the enzyme additive would be effective for other types of feedstuffs and what the optimal dosage for different enzyme–substrate combinations would be.

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تأثير إضافة الإنزيمات المحلله للألياف على معاملات الهضم و مكونات الدم و إنتاج اللبن وتركيبة ومحتواه من الأحماض الدهنية فى الجاموس الحلاب

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تم استخدام 21 جاموسة حلابة فى بداية موسم الحليب بمتوسط وزن 565 +12 كجم قسمت إلى ثلاثة مجموعات فى كل منها سبع حيوانات تغذت على العلائق التالية:

1- العليقة المقارنة :عبارة عن مخلوط العلف المركز والخشن بدون إضافات.
2- المعاملة الأولى: العليقة المقارنة مضاف إليها 40 جم Zad1/رأس/يوم.
3- المعاملة الثانية: العليقة المقارنة مضاف إليها 40 جم Zad2/رأس/يوم.

وكانت أهم النتائج المتحصل عليها كما يلى:

1- لم تتأثر أوزان الحيوانات بينما ارتفع المأكول من المادة الجافة بإضافة كلا الإنزيمين.
2- إرتفعت معاملات الهضم والقيمة الغذائية بإضافة كلا الإنزيمين بينما لم تتأثرمكونات الدم بإضافة كلا الإنزيمين ما عدا جلوكوز الدم الذي ارتفع بإضافة كلا الإنزيمين.
3- ارتفع إنتاج اللبن مع المعاملة الأولى (Zad1) كما ارتفع اللبن المعدل نسبة الدهن بإضافة Zad1 تبعه Zad2 ثم المجموعة المقارنة. بينما لم تتأثر مكونات اللبن معنويا بإضافة كلا الإنزيمين. كما تحسنت الكفاءة الغذائية بإضافة بإضافة كلا الإنزيمين مقارنة بالعليقة المقارنة.
4- ارتفعت نسبة حمض اللينولينيك المرتبط CLA والأحماض الدهنية أوميجا 3 والأحماض الدهنية غير المشبعة بينما انخفضت نسبة الأحماض الدهنية المشبعة بإضافة كلا الإنزيمين.

ويستخلص من هذة النتائج أنه يمكن استخدام إضافة 40 جم Zad1/ رأس/يوم كسياسة غذائية لحيوانات اللبن لتحسين هضم الألياف وشقوقها وتحسين القيمة الغذائية وانتاج اللبن. كما أن ارتفاع محتوى دهن اللبن من الأحماض الدهنية غير المشبعة خاصة CLA و أوميجا 3 وخفض نسبة الأحماض الدهنية المشبعة فى دهن اللبن الدم له فائدة صحية للمستهلك.