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EFFECTS OF TWO ENZYME FEED ADDITIVES ON DIGESTION AND MILK PRODUCTION IN LACTATING EGYPTIAN BUFFALOES*

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Abstract

The aim of this study was to evaluate the effect of two commercial enzyme products on milk production in Egyptian buffaloes. Twenty-one lactating buffaloes (570±15 kg BW) were divided into three groups (n=7) in a randomized block design for four months. Buffaloes were fed a total mixed ration containing 60% forage [rice straw and berseem hay (*Trifolium alexandrinum*)] and 40% concentrates with either no enzymes added (Control) or an addition of 40 g of Veta-Zyme Plus® (VET) or 40 g of Tomoko® (TOM) enzyme product per day for each buffalo. Enzyme addition did not affect feed intake (P>0.05), but increased the digestibility of nutrients (P<0.05) and serum glucose concentration (P=0.011). Furthermore, the addition of VET increased milk (P=0.017) and fat corrected milk (P=0.021) yields, fat content (P=0.045), total unsaturated fatty acid (P=0.045) and total conjugated linoleic acid (P=0.031) contents in milk and decreased the content of total saturated fatty acids (P=0.046), while the addition of TOM increased milk total protein (P=0.023) and true protein (P=0.031) contents. The two enzyme products both resulted in higher concentrations of lysine (P=0.045) and total essential amino acids (P=0.036) in milk. It was concluded that addition of commercial fibrolytic enzyme products (i.e. Veta-Zyme Plus® and Tomoko®) to the diet of early lactating buffaloes enhanced nutrient digestibility and milk production and quality.

Key words: buffalo, digestibility, fibrolytic enzyme, milk composition, milk production

Animal production is maximised with various approaches including manipulation of rumen fermentation (Kholif et al., 2014; Alsersy et al., 2015; Salem et al., 2015; Valdes et al., 2015). Exogenous enzyme supplementation is one of the methods used to manipulate rumen fermentation (Rojo et al., 2015). Addition of exogenous enzymes to animal diets can improve feeding values by increasing feed intake and

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improving fibre degradation (Salem et al., 2015; Valdes et al., 2015). Some studies showed that enzyme addition increased nutrient digestibility and increased milk production of dairy animals by about 5 to 25% (Khattab et al., 2011; Kholif et al., 2012; Rojo et al., 2015), but others showed only weak effects on animal performance (Sutton et al., 2003). Inconsistent results may be due to animal species, enzyme activity, application rate, enzyme composition, the stage of lactation, the mode and time of enzyme delivery, ruminal activity and stability of enzymes, enzyme-feed specificity and diet fed to the animals. Despite this, enzyme products are increasingly used in the animal industry to improve feed digestion and animal performance.

Buffalo husbandry is an emerging industry in Egypt and provides quality milk. Poor efficiency of feed utilisation is a constraint to the development of the industry. However, very little information is available about the effect of exogenous enzymes on the performance of buffaloes. Only few studies so far were reported in Egypt. Kholif et al. (2012) reported that adding two enzyme preparations to the diet of lactating buffaloes resulted in increased dry matter intake (DMI), improved nutrient digestibility and milk production and the milk produced having more healthy fatty acids [ω-3 and total conjugated linoleic acid (CLA)]. The objective of the current study was to investigate the effects of two commercial dietary enzyme supplements on feed digestion and milk production in early lactating Egyptian buffaloes.

Material and methods

Animals were cared for and handled in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Buffaloes, treatments and milk production

Twenty-one well-managed, multiparous lactating Egyptian buffaloes aged 5–6 years, having calved 3–4 times and with an average body weight of 570±15 kg were divided into three treatment groups (n=7) to study the effect of two commercial enzyme preparations on milk production and composition over four months.

Two weeks before the experiment, buffaloes were blocked for similar expected calving dates and fed on a total mixed ration (TMR; Table 1) consisting of 60% berseem hay (*Trifolium alexandrinum*) and rice straw and 40% concentrate mixture. Newborn calves were kept with their mothers to suckle colostrum, and then taken away from their mothers after 14 days. Buffaloes were housed in tie stalls and fed individually according to body weight to meet their requirements for lactation (NRC, 2001).

Buffaloes in each block were assigned randomly to one of three treatments: to be fed on TMR with no enzyme additives (Control), TMR with 40 g of Tomoko®/head/day (TOM), or TMR with 40 g of Veta-Zyme Plus®/head/day (VET). Tomoko® is a commercial enzyme mixture sourced from Biogenkoji Research Institute, Kirishima, Japan. Veta-Zyme Plus® is a product of Vetagri Consulting Inc., Bramp-

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ton, Canada. The full characteristics of the two enzyme products are shown in Table 2. Buffaloes were fed twice daily at 08:00 and 16:00 h in equal portions. A daily dose of enzymes was mixed individually for each buffalo with the feed portion for the morning feeding at 08:00 h. The mixing process was conducted just prior to feeding.

Table 1. Ingredients and chemical composition of total mixed ration fed to dairy buffaloes

Total mixed ration	% DM
Ingredients (%)	
Berseem hay	33.0
Rice straw	27.0
Maize corn	6.6
Soybean meal	8.3
Wheat bran	20.0
Sunflower meal	3.0
Urea	0.5
Calcium carbonate	0.3
Mineral and vitamin mix*	1.3
Chemical composition (%)	
Dry matter	90.1
Organic matter	89.7
Crude protein	16.2
Ether extract	3.7
Non-structural carbohydrates	31.1
Neutral detergent fibre	38.7
Acid detergent fibre	23.1
Net energy of lactation (NEL, MJ/kg)**	4.89

*Contained: Ca (141 g/kg), P (87 g/kg), Mg (45 g/kg), S (14 g/kg), Na (120 g/kg), K (6 g/kg), Fe (944 mg/kg), Zn (1613 mg/kg), Cu (484 mg/kg), Mn (1748 mg), I (58 mg/kg), Co (51 mg/kg), Se (13 mg/kg), vitamin A (248,000 U/kg), vitamin D_3 (74,000 UI/kg), vitamin E (1656 IU/kg).

**Calculated according to NRC (2001).

Body weight was recorded biweekly before feeding throughout the experimental period. Dry matter intake was recorded daily at the last two weeks of every month by weighing feeds offered and refused. Samples of feeds and orts were collected daily and composited monthly, and then composited samples were mixed thoroughly and subsampled. The subsamples were dried at 55°C and ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) before analysis. Fresh drinking water was available at all times.

Buffaloes were milked twice daily at 06:00 and 15:00 h. Milk production was recorded daily for four months. Milk samples were obtained biweekly from morning and afternoon milking and pooled for each buffalo. The samples were preserved with potassium dichromate and stored at 4°C until analysis. Milk samples for the analysis of fatty acids and amino acids were pooled every month for each treatment and frozen at −20°C until analysis.

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Table 2. Composition and full characteristics of commercial enzyme products Tomoko® and Veta-Zyme® fed to dairy buffaloes

Item	Tomoko®	Veta-Zyme®
Microbes included	*Aspergillus awamori*	*Lactobacillus acidophilus*
Colony-forming units	3×10^6	2×10^8
Protease (units/g)	1000	2000
Pectinase (units/g)	30	ND
Xylanase (units/g)	25	ND
α-Amylase (units/g)	20	550
Phytase (units/g)	10	ND
Glucoamylase (units/g)	5	ND
Cellulase (units/g)	4	400

ND – not detected.

Nutrient digestibility and blood chemistry

Apparent nutrient digestibilities were determined during the last seven days of each month (d 23–30, d 53–60, d 83–90 and d 113–120) following the method of Ferret et al. (1999) where acid insoluble ash was used as an internal marker. Faeces were sampled from the rectum of each buffalo at 4, 8, 12, 16 and 20 h post the first feeding each day. Faecal samples were composited over all collection days for each buffalo, dried at 55°C for 48 h, ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and stored for further chemical analysis.

At 4 h after morning feeding on d 30, 60, 90 and 120, about 10 ml of blood was collected from each buffalo by jugular venipuncture using a vacutainer (Becton Dickinson and Cie, Rutherford, NJ, USA), immediately placed on ice and centrifuged at 4,000 × *g* at 4°C for 20 min. Serum was separated into a clean dried glass vial and frozen at –20°C until analysis.

Chemical analysis

Feed, ort and faecal samples were analysed for dry matter (DM, method 930.15), nitrogen (N, method 954.01), ether extract (method 920.39) and ash (method 942.05) according to AOAC (1997). Neutral detergent fibre (NDF, Van Soest et al., 1991), acid detergent fibre (ADF) and lignin (AOAC, 1997; method 973.18) analyses were conducted using an ANKOM200 Fibre Analyser unit (ANKOM Technology Corporation, Macedon, NY, USA). For NDF assays, samples were pre-treated with an α-amylase and sodium sulfite. Both NDF and ADF are expressed without residual ash. Organic matter (OM) and non-structural carbohydrates (NSC) were calculated.

Serum metabolites were analysed colorimetrically using specific kits from Stanbio Laboratory (Boerne, Texas, USA) following manufacturer instructions. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using AST and ALT kits (Quimica Clinica Aplicada SA., Spain) following manufacturer instructions.

Milk samples were analysed for total solids, fat, true protein, urea-N and lactose by infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Den-

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mark), and non-fat solids content was calculated. Fat corrected milk (4% FCM) was calculated according to the equation of Gaines presented in NRC (2001).

Milk fatty acids were determined using methyl esters prepared by base-catalysed methanolysis of the glycerides (KOH in methanol) according to International Standards (ISO-IDF 2002) using a GC system. Fatty acid methyl esters were separated using a Cp-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Chrompack, Middelburg, Netherlands) on a Perkin-Elmer chromatograph (model 8420, Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization detector.

Milk amino acid profiles were determined as described by Spackman et al. (1958). The analysis was performed using an LC 3000 amino acid analyser (Eppendorf-Biotronik, Germany) at the Central Service Unit, National Research Centre, Egypt. The analysis was based on the separation of amino acids using strong cation exchange chromatography followed by the ninhydrin colour reaction and photometric detection at 570 nm.

Non-casein-N (NCN) content was determined according to Aschaffenburg and Drewry (1959). Casein content was calculated by subtracting the NCN value from the corresponding total N value and multiplying by 6.38 for each sample. Non-protein-N (NPN) content was determined from the N content of the 12% tricholoroacetic acid supernatant and multiplied by 3.60 according to AOAC (1997). Whey protein content was calculated by subtracting the NPN value from the corresponding NCN value and multiplying by 6.38.

Statistical analysis

All data were analysed using the MIXED procedure of SAS (2004). Data on feed intake, nutrient digestibility, blood chemistry, milk production, milk composition and milk efficiency were analysed as a randomized block design with the model:

$$Y_{ijk} = \mu + T_i + P_j + A_k + E_{ijk}$$

where:

Y represented every observation of the kth buffalo in the ith treatment in the jth period,

μ expressed the overall mean,

T expressed the dietary treatment with a fixed effect,

P expressed period with a fixed effect as repeated measurements,

A expressed animal with a random effect,

E expressed the residual, assumed to be normally distributed.

When a significant F-test was detected (i.e., $P<0.05$), treatment means were separated using Duncan's multiple range test (Duncan, 1955). Differences were considered significant at $P<0.05$ unless otherwise noted and trends were discussed at $P<0.10$.

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Results

Feed intake, digestibility and blood chemistry

No differences in the feed intakes of various nutrients were observed (P>0.05) between treatments (Table 3). The addition of either VET or TOM to the diets increased the digestibilities of DM (P=0.023), CP (P=0.006), NDF (P=0.041), ADF (P=0.012) and NSC (P=0.018).

Table 3. Feed intake, apparent nutrient digestibility and nutritive value of diets supplemented with enzymes in dairy buffaloes (n=7 each treatment)

	Diets*			SEM	P-value
	Control	VET	TOM		
Intake (kg/d)					
Dry matter	13.4	14.0	14.0	1.56	0.310
Organic matter	12.0	12.6	12.6	1.42	0.141
Crude protein	2.17	2.27	2.27	0.296	0.062
Ether extract	0.50	0.52	0.52	0.111	0.180
Non-structural carbohydrates	4.17	4.35	4.35	0.533	0.209
Neutral detergent fibre	5.19	5.42	5.42	0.447	0.144
Acid detergent fibre	3.10	3.23	3.23	0.586	0.102
Digestibility (%)					
Dry matter	61.4 b	68.0 a	65.1 a	1.15	0.023
Organic matter	60.7 b	68.2 a	65.8 ab	1.33	0.011
Crude protein	61.7 b	66.8 a	66.3 a	1.67	0.006
Ether extract	61.2	63.1	62.6	2.38	0.588
Non-structural carbohydrates	51.6 b	56.0 a	55.9 a	0.61	0.018
Neutral detergent fibre	54.3 b	61.0 a	58.8 a	2.56	0.041
Acid detergent fibre	56.4 b	61.6 a	61.3 a	2.75	0.012

a, b – means with different letters differ significantly (P<0.05).

*Diets supplemented with no enzyme products (Control; 0 g/head/day), Veta-Zyme Plus® (VET; 40 g/head/day) and Tomoko® (TOM; 40 g/head/day).

SEM – standard error of the mean.

Feeding buffaloes on VET and TOM diets increased blood serum glucose concentration compared to the Control diet (P=0.011; Table 4). Also, VET and TOM treatments tended to increase total protein (P=0.091), albumin (P=0.082) and globulin (P=0.052) concentrations relative to the Control treatment.

Milk yield, composition and milk efficiency

Buffaloes fed VET diet had increased milk (P=0.017) and FCM (P=0.021) yields, and had higher fat contents in milk (P=0.045) compared to the Control diet, while buffaloes fed TOM diet increased milk total protein (P=0.023) and true protein (P=0.031) concentrations (Table 5). Both TOM and VET diets increased milk casein content (P=0.010), but decreased milk urea-N content (P=0.010).

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Milk efficiency expressed as milk yield/DMI (P=0.013) and FMC yield/DMI (P=0.024) were higher for VET buffaloes than for TOM and Control buffaloes (Table 5).

Table 4. Serum parameters of dairy buffaloes fed diets supplemented with enzymes (n=7 each treatment)

	Diets*			SEM	P-value
	Control	VET	TOM		
Total protein (g/dl)	6.99	8.31	8.11	1.130	0.091
Albumin (g/dl)	3.89	4.18	4.71	1.242	0.082
Globulin (g/dl)	3.10	4.13	3.40	1.100	0.052
Albumin/globulin ratio	1.25	1.01	1.39	0.149	0.100
Urea (mg/dl)	38.9	39.4	39.2	4.38	0.554
Total lipids (mg/dl)	267	273	276	24.6	0.677
Cholesterol (mg/dl)	143	136	131	15.8	0.612
Glucose (mg/dl)	66.4 b	74.0 a	73.0 a	5.83	0.011
ALT (units/l)	15.1	15.6	15.1	1.89	0.175
AST (units/l)	30.4	30.1	30.4	4.42	0.638

a, b – means with different letters differ significantly (P<0.05) different.

*Diets supplemented with no enzyme products (Control; 0 g/head/day), Veta-Zyme Plus® (VET; 40 g/head/day) and Tomoko® (TOM; 40 g/head/day).

ALT – alanine aminotransferase; AST – serum aspartate aminotransferase; SEM – standard error of the mean.

Table 5. Milk yield and composition of dairy buffaloes fed diets supplemented with enzymes (n=7 each treatment)

	Diets*			SEM	P-value
	Control	VET	TOM		
Milk production (kg/day)					
Yield	7.26 b	7.91 a	7.59 ab	0.195	0.017
4% fat corrected milk (FCM)	10.36 b	11.56 a	10.93 b	0.171	0.021
Milk composition (%)					
Fat	6.86 b	7.10 a	6.96 ab	0.440	0.045
Total protein	3.88 b	4.02 ab	4.20 a	0.510	0.023
Casein	2.87 b	3.14 a	3.30 a	0.581	0.010
Whey	0.83	0.80	0.85	0.014	0.495
Non-protein N	0.04 a	0.04 a	0.03 b	0.001	0.033
True protein	3.84 b	3.99 ab	4.17 a	0.652	0.031
Urea-N	2.81 a	2.08 b	2.10 b	0.165	0.010
Lactose	4.66	4.73	4.68	0.297	0.871
Ash	0.80	0.80	0.78	0.010	0.776
Total solids	16.20	16.65	16.62	1.511	0.336
Solids not fat	9.34	9.55	9.66	1.383	0.405
Milk efficiency					
Milk yield/DMI	0.54 b	0.57 a	0.54 b	0.023	0.013
ECM/DMI	0.77 b	0.83 a	0.78 b	0.011	0.024

a, b – means with different letters differ significantly (P<0.05).

*Diets supplemented with no enzyme products (Control; 0 g/head/day), Veta-Zyme Plus® (VET; 40 g/head/day) and Tomoko® (TOM; 40 g/head/day).

SEM – standard error of the mean.

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Milk amino acids and fatty acids profiles

Compared to the Control group, buffaloes fed diets supplemented with either TOM or VET increased lysine (P=0.045) and total essential amino acid (P=0.036) concentrations in milk. No significant effects (P>0.05) were observed for other determined amino acids (Table 6).

Table 6. Milk amino acid contents of dairy buffaloes fed diets supplemented with enzymes (n=7 each treatment)

	Diets*			SEM	P-value
	Control	VET	TOM		
Essential AA (EAA) (% of milk protein)					
Arginine	2.71	2.44	2.17	0.062	0.729
Histidine	2.53	2.56	2.64	0.096	0.902
Isoleucine	5.44	5.62	5.50	0.056	0.813
Leucine	9.77	11.32	11.55	0.060	0.255
Lysine	7.76 b	8.11 b	10.02 a	0.068	0.045
Methionine	8.69	9.90	10.76	0.062	0.125
Phenylalanine	4.25	4.25	4.74	0.105	0.762
Threonine	4.79	5.25	6.26	0.080	0.162
Valine	6.21	6.67	6.60	0.092	0.855
Branched chain AA**	21.42	23.61	23.64	0.159	0.105
Total EAA	52.14 c	56.12 b	60.24 a	0.982	0.036
Non-EAA (NEAA) (% of milk protein)					
Alanine	3.94	3.71	4.26	0.132	0.857
Aspartic acid	7.89	8.08	9.48	0.089	0.485
Glutamic acid	24.30	23.76	23.50	0.038	0.905
Serine	4.92	5.37	5.81	0.159	0.720
Tyrosine	2.96	3.33	4.02	0.131	0.456
Total NEAA	44.02	44.25	47.07	4.620	0.805

a, b – means with different superscripts differ significantly (P<0.05).

*Diets supplemented with no enzyme products (Control; 0 g/head/day), Veta-Zyme Plus® (VET; 40 g/head/day) and Tomoko® (TOM; 40 g/head/day).

**Branched chain AA is the sum of isoleucine, leucine and valine.

AA – amino acids, EAA – essential amino acids, NEAA – non-essential amino acids, SEM – standard error of the mean.

Table 7. Milk fatty acid profile (g/100 g total fatty acids) of dairy buffaloes fed diets supplemented with enzymes (n=7 each treatment)

	Diets*			SEM	P-value
	Control	VET	TOM		
1	2	3	4	5	6
C4:0	1.70	1.50	1.60	0.099	0.768
C6:0	1.20	1.10	0.97	0.052	0.211
C8:0	0.84	0.80	0.74	0.053	0.797
C10:0	1.64	1.60	1.51	0.091	0.873
C12:0	2.12	2.02	1.89	0.074	0.510
C14:0	12.10	11.10	12.10	0.684	0.832
C14:1	0.75	0.77	0.77	0.017	0.896

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Table 7 – contd.

1	2	3	4	5	6
C15:0	0.29	0.28	0.30	0.026	0.964
C16:0	34.60	31.60	34.17	1.010	0.484
C16:1	0.37 c	1.43 a	1.12 b	0.159	0.001
C18:0	18.54	17.80	17.10	0.586	0.668
C18:1^{n9T}	23.15	24.70	26.90	1.172	0.484
C18:1^{n9C}	1.20	1.50	1.33	0.089	0.444
C18:2 *trans-10, cis-12*	0.11	0.15	0.12	0.011	0.307
C18:2 *cis-9, trans-11*	0.12 b	0.17 a	0.13 b	0.009	0.048
C18:3^{n-3}	0.06	0.10	0.08	0.008	0.125
C18:3^{n-6}	0.27	0.34	0.30	0.020	0.413
C20:0	0.77 b	0.58 c	0.84 a	0.050	0.044
C20:1	0.17	0.27	0.23	0.027	0.367
TSFA	73.80 a	68.38 b	71.22 ab	5.045	0.046
TUFA	26.20 b	31.26 a	28.78 ab	1.085	0.045
MUFA	25.64 b	30.87 a	28.15 a	1.308	0.046
PUFA	0.56 c	0.76 a	0.63 b	0.043	0.041
Total CLA	0.23 b	0.32 a	0.25 b	0.053	0.031
ω-6/ω-3	4.50	3.60	3.70	0.206	0.145

a, b, c – means with different letters differ significantly (P<0.05).

*Diets supplemented with no enzyme products (Control; 0 g/head/day), Veta-Zyme Plus® (VET; 40 g/head/day) and Tomoko® (TOM; 40 g/head/day).

CLA – conjugated linoleic acid; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; SEM – standard error of the mean; TSFA – total saturated fatty acids, TUFA – total unsaturated fatty acids.

The VET treatment increased the concentrations of C16:1 (P=0.001), C18:2$^{cis-9,\ trans-11}$ (P=0.048), total unsaturated fatty acids (TUFA, P=0.045), monounsaturated fatty acids (MUFA, P=0.046), polyunsaturated fatty acids (PUFA, P=0.041) and total CLA (P=0.031) and decreased (P=0.046) the concentration of total saturated fatty acids (TSFA) in milk compared to the Control treatment (Table 7).

Discussion

Feed intake, digestibility and blood chemistry

In the present study, lack of enzyme supplementation effect on DMI is inconsistent with previous studies by Khattab et al. (2011), Alsersy et al. (2015) and Salem et al. (2015) using other enzyme preparations in diets of goats and sheep. Kholif et al. (2012) noted that exogenous enzyme cocktails supplemented to the diets for Egyptian buffaloes in the early lactation stage increased DMI. The absence of an effect on DMI in this study is possibly because the enzyme products we added accelerated the availability of nutrients, but did not alter rumen fill. Different responses between some previous studies (e.g. Kholif et al., 2012) and the present study might result from the method used for the application of enzymes, and the activity and nature of the enzymes.

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The improved nutrient digestibility with exogenous enzyme supplementation was consistent with other studies (Khattab et al., 2011; Salem et al., 2013, 2015). Salem et al. (2013) reported that the addition of exogenous enzyme to the diet of Baladi × Friesian steers increased DM, OM, CP, NDF and ADF digestibilities by 12, 12, 5, 22 and 27%, respectively. Moreover, Kholif et al. (2012) added two exogenous enzyme mixtures to the diet of lactating buffaloes, leading to a significant improvement in nutrient digestibility compared to the unsupplemented diet. The main purpose of the addition of exogenous enzymes to the diet of ruminants is to improve fibre digestion. Digestion can be affected by the chemical composition of the diet (Elghandour et al., 2015 a, b), the size of the indigestible fibre fraction (Varga, 2006), the degradation rate of potentially digestible fibre fractions, and rumen outflow rate, as well as the use of feed additives (Elghandour et al., 2014; Salem et al., 2014). Increased degradation rate of the potentially digestible fibre fractions (Yang et al., 1999), altered ruminal fermentation kinetics (Khattab et al., 2011), and enhanced ruminal microorganism attachment to feed particles and colonization of the plant cell wall (Wang et al., 2001) are possible modes of action for improved nutrients digestibility as a result of fibrolytic enzyme administration. Moreover, improved synergism between ruminal endogenous and exogenous enzymes (Morgavi et al., 2000), and increased numbers of fibrolytic and non-fibrolytic bacteria in the rumen (Wang et al., 2001) are other possible mechanisms. However, the tested products differ in enzyme concentrations and activities, which both have effects on nutrient digestibility. Veta-Zyme Plus® contains greater concentrations of cellulases and *Lactobacillus acidophilus* compared to Tomoko®. As a result, Veta-Zyme Plus® is better suited for use in diets with high fibre content. On the other hand, Tomoko® had a higher protease content than Veta-Zyme Plus®. However, the two enzyme products resulted in the same CP digestibility. This might result from the degradation of proteolytic enzymes in the rumen by added exogenous protease from Tomoko®. Another possibility is that the ruminal proteolytic activity is naturally high and thus there is no need to add exogenous protease.

With the exception of blood glucose, blood metabolites were not affected by enzyme addition and lay within the reference ranges for normal animals (Boyd, 2011). Serum total protein, albumin and blood urea lay within the reference range, reflecting a good nutritional status in the buffaloes (Kumar et al., 1980) and absence of muscle protein catabolism, and normal kidney function. Normal concentrations of both ALT and AST enzymes, which are the most important indicators for liver activity, demonstrated that there are no pathological lesions in the liver.

The higher serum glucose concentration with enzyme addition may have resulted from the improved OM, NDF, ADF and NSC digestibilities, as these improvements enhance energy utilization and increase propionate absorption through the ruminal wall, leading to a high rate of glucose synthesis.

Milk production, composition and milk efficiency

Improved milk and FCM yields are consistent with those obtained with different enzymatic products in dairy cattle, goats and buffaloes (Gado et al., 2009; Khattab et al., 2011; Kholif et al., 2012; Rojo et al., 2015). The enhancement of milk and FCM

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production occurred without increasing feed intake, reflecting an improvement in feed utilization and nutrient digestibility providing additional energy to the enzyme-fed buffaloes. Increased milk production in the VET treatment can be explained by increased nutrient digestibility (Gado et al., 2009; Khattab et al., 2011) which increased energy available for milk production.

Increased milk efficiency with VET enzyme reflects better feed utilization than TOM enzyme and the control diet, better feed utilization because of the improved feed digestibility with VET enzyme addition.

The increase in milk fat content of buffaloes supplemented with exogenous enzyme may be attributed to the larger amount of fibre digested in the rumen, which might provide more acetate for fatty acid synthesis. In the present study, increased casein and total amino acid concentrations and decreased non-protein nitrogen and urea nitrogen concentrations in milk might suggest microbial protein synthesis increased in the rumen of buffaloes supplemented with enzymes. These results agreed with those obtained by Khattab et al. (2011) who found increased milk production, milk protein and fat contents with a mixture of exogenous enzymes supplied to Baladi goats.

Results of improved amino acid concentration in the milk might be related to the high protease concentration in Tomoko® which also increased milk casein and true protein contents. Increased concentration of essential amino acids in milk is important for human growth and health (Reeds, 2000).

Enzymes supplemented in the buffalo diet improved the milk fatty acid profile, increased PUFA and MUFA contents and decreased TSFA content. Improved fibre digestion with enzyme supplementation, in the present study, is suspected to be associated with altered milk fatty acid profiles via a change in the ratio of acetate to propionate in the rumen. This change might increase precursor availability for fatty acid synthesis, particularly for lactating ruminants in early lactation when nutrient intake lags nutrient demand (Eun et al., 2007). Gado et al. (2009) suggested that enzyme supplemented diets alter ruminal acetate, propionate and butyrate proportions. In the present study, the increase in CLA and the decrease in *ω-6/ω-3* milk fat caused by enzyme additives have a great benefit for human consumer's health. Conjugated linoleic acid has been shown to have antiadipogenic (Pariza et al., 2001), anticarcinogenic (Ip et al., 1999), antiatherogenic (Koba et al., 2002), antidiabetogenic (Ryder et al., 2001) and anti-inflammatory properties (Yang and Cook, 2003).

Veta-Zyme Plus® improved milk production and composition more than Tomoko®. Veta-Zyme® product contained more cellulase than Tomoko®, however, this did not affect fibre intake but did affect digestibility. This may be a reason for the altered TUFA, MUFA and PUFA concentrations in milk. Our results are in agreement with Kholif et al. (2012) who found that the improvement in the milk fatty acid profile was correlated to increased TUFA and decreased TSFA contents.

Conclusions

Addition of fibrolytic enzymes in high fibre diets of dairy buffaloes is beneficial for milk production and composition. Increased milk production and fat content from the addition of enzymes to the diet may be a direct result of improved feed digest-

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ibility in the rumen rather than a change in feed intake. The commercial enzyme products Veta-Zyme Plus® and Tomoko® at 40 g/buffalo/day can be used to improve nutrient digestibility, milk production and composition of Egyptian buffaloes without negative effects on blood chemistry. Further study using a larger number of buffaloes fed for a longer duration is needed to confirm the effects of the addition of enzyme to high fibre diets reported in the current study. More attention is required to be paid to the Egyptian buffaloes to improve their ability for milk production with better milk composition.

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