

## Effect of Dietary Supplementation With Fibrolytic Enzymes on the Productive Performance of Early Lactating Dairy Cows

Dyaa El-Din A. Mohamed<sup>1</sup>, Borhami E. Borhami<sup>1</sup>, Khaled A. El-Shazly<sup>1</sup> & Sobhy M. A. Sallam<sup>1</sup>

<sup>1</sup> Animal Production Department, Faculty of Agriculture, Alexandria University, Egypt

Correspondence: Sobhy M. A. Sallam, Alexandria University, Faculty of Agriculture, Alexandria, El-Shatby, Egypt. Tel: 20-111-100-3962. E-mail: s\_sallam@yahoo.com

Received: March 28, 2013 Accepted: April 25, 2013 Online Published: May 15, 2013

doi:10.5539/jas.v5n6p146

URL: <http://dx.doi.org/10.5539/jas.v5n6p146>

### Abstract

This study was conducted to investigate the impacts of exogenous fibrolytic enzymes (Fibrozyme, *Alltech inc* company, USA) supplementation for 12 weeks on milk production and composition as well as blood metabolites in early lactating dairy cows. Total of 120 multiparous Holstein dairy cows at early lactation (57±4.2 days in milk) were randomly assigned into two groups according to lactation period "stage of lactation" and lactation season. The first group (control, n=60) were fed total mixed ration (TMR) without a supplement of exogenous fibrolytic enzymes. The second group (treatment, n=60) were fed TMR supplemented with a commercial exogenous fibrolytic enzymes at the rate of 15 g/cow/d for 12 weeks. Each group was placed in a shaded pen equipped with free stalls. An exogenous fibrolytic enzymes was added to the TMR at the time of feeding once per day at 10 am. Cows were fed as a group open feed, with free access to water.

The results of this experiment on dairy cows showed that there were not significant changes in dry matter intake of lactating dairy cows with or without a supplement of exogenous fibrolytic enzymes at early lactation. Exogenous fibrolytic enzymes supplementd to lactating dairy cows improved ( $P<0.003$ ) milk yield (41.0 vs. 39.5 kg/cow/d) compared to untreated dairy cows. Also, the fat corrected milk was increased ( $P<0.025$ ) as a response to exogenous fibrolytic enzymes supplementation to lactating dairy cows compared to un-supplemented dairy cows. In addition, the supplementation of exogenous fibrolytic enzymes enhanced ( $P<0.006$ ) the energy corrected milk (40.6 vs. 39.4 kg) and feed efficiency in early lactating dairy cows compared to the control group. The results revealed that supplementation of exogenous fibrolytic enzymes had no significant effect on milk fat, protein lactose and solid not fat (SNF) percentage compared to the control group of dairy cows. While, the quantities of milk protein (1.36 vs. 1.30kg), lactose (2.0 vs. 1.92kg) and SNF (3.47 vs. 3.31kg) in supplemented-dairy cows were improved significantly compared to the control group except quantity of milk fat ( $P<0.096$ ). Serum glucose, albumin, urea and triglycerides were not affected ( $P>0.05$ ) but total protein, globulin and cholesterol were declined ( $P<0.05$ ) due to fibrozyme inclusion compared to control group of dairy cows. The supplementation of exogenous fibrolytic enzymes to early lactating dairy cows achieved higher net profit by 0.93 US\$ per cow than control group. It is concluded that exogenous fibrolytic enzymes supplementation to early lactating dairy cows improved significantly milk production, SNF and energy corrected milk.

**Keywords:** fibrolytic enzymes, productive traits, blood metabolites, dairy cow

### 1. Introduction

The ruminant production systems are dependent worldwide on forage as the main nutritional components (Wilkins, 2000). The digestion of forage occurs through the microbial fermentation as a result of the presence of microorganisms at the reticulo-rumen and its adaptation to digest lignocellulosic components. The microbial mode of digestion allows ruminants to better unlock the unavailable energy in the plant cell wall components than other herbivores (Krause et al., 2003). This gives ruminant animals the ability to convert low nutritive and resistant lignocellulosic biomass to milk, meat, wool and hides (Weimer et al., 2009). However, most forage plants are high in cell walls and low in nitrogen (N) and energy content (Romney & Gill, 2000). Despite the importance of fibrous components in forages for salivation, rumen buffering and efficient production of ruminal end products (Mertens, 1997) only 10 to 35% of energy intake is available as net energy (Varga & Kolver, 1997). This is because the ruminal digestion of plant cell walls is not complete (Krause et al., 2003).

The use of exogenous fibrolytic enzymes (EFE) to enhance quality and digestibility of fibrous forage is on the

verge of delivering practical benefits to ruminant production systems. In this regard, 2 cellulases and xylanases are respectively amongst the two major enzyme groups that are specified to break  $\beta$ 1-4 linkages joining sugar molecules of cellulose and xylans found in plant cell wall components (Beauchemin et al., 2003). Several studies with EFE have made mention of the increase of microbial activities in the rumen, which resulted in an enhancement of animal performance traits. Despite the increase in feed digestibility and subsequent production traits, the relationship between the improvement in forage utilization and enzymatic activities is yet to be explained in ruminant systems (Eun et al., 2007). In addition, results with EFE addition in ruminant systems are variable and somewhat inconsistent (Beauchemin et al., 2003; Colombatto et al., 2003), making their biological response difficult to predict.

Some studies have shown substantial improvement of feed digestibility and animal performance traits (Cruywagen & Goosen, 2004; Bala et al., 2009; Arriola et al., 2011), while others reported either negative effects or none at all (Baloyi, 2008). If the potential intake and/or the density of available nutrients of forages can be increased with EFE as feed additives, then poor quality forages can be economically and successfully converted into meat and milk for human consumption. Moreover, an increase in the input costs in the dairy industry has demonstrated the need for methods to increase production efficiency. One way of increasing efficiency would be to increase the bioavailability of nutrients in a feedstuff, which might be accomplished through the exogenous fibrolytic enzymes supplement. Therefore, the objective of the undertaken experiment was to evaluate the effect of exogenous fibrolytic enzymes "Fibrozyme" supplementation on dry matter intake, feed efficiency, blood metabolites, milk production and milk composition in early lactating dairy cows.

## 2. Materials and Methods

### 2.1 Animals' Management and Experimental Design

This experiment was conducted at Alexandria commercial company of agriculture at Alexandria governorate during March to May, 2011 for 12 weeks. Total of 120 multiparous Holstein dairy cows at early lactation ( $57 \pm 4.2$  days in milk) were randomly assigned into two groups. The first group (control,  $n=60$ ) were fed total mixed ration (TMR) without a supplement of exogenous fibrolytic enzymes. The second group (treatment,  $n=60$ ) were fed TMR supplemented with a commercial fibrolytic enzymes (Fibrozyme is enzymes blend, which is prepared from fermentation extract and fermentation soluble of *Aspergillus Niger* and *Trichoderma longibarachiatum*, and having xylanase activity by minimum 100 XU/g, *Alltech inc* company, USA) at the rate of 15 g/cow/d according to the guide of the manufacture for 12 weeks. Fibrozyme was added and mixed to the TMR at the time of feeding once per day. Each group was placed in a shaded pen equipped with free stalls. Cows in the two groups fed a total mixed ration (TMR, Table 1), which composed of alfalfa hay, corn silage, green clover, soybean meal, yellow corn, limestone, vitamins, minerals mixture, protected fat (magnapac), sodium bicarbonate and mono calcium phosphate. The proximate analysis and calculated nutritive value of the TMR is given in Table 2.

Table 1. Ingredients of the experimental total mixed ration (TMR) in the lactation trial

Items	% of DM
Alfalfa hay	10.7
Green clover	7.3
Corn silage	26.0
Ground yellow corn	25.8
Soy bean meal	24.6
Limestone	1.2
NaCl	1.0
Minerals mixture and vitamins	0.3
Magnabac (protected fat)	2.7
Sodium bicarbonate	0.1
Mono calcium phosphate	0.30

Roughage concentrate ratio was 44:56%.

Table 2. Proximate analysis (%) and calculated nutritive value of the experimental total mixed ration (TMR) in lactation trial

Nutrients	As fed basis (%)	As dry matter basis (%)
Organic matter	51.17	94.0
Crude Protein	10.39	19.1
Neutral detergent fiber	18.51	34.0
Acid detergent fiber	8.71	16.0
Hemicellulose	9.79	18.0
Cellulose	7.35	13.5
Lignin	2.45	4.5
Ash	3.27	6.0
Non Fiber carbohydrates	19.9	36.6
Ether Extract	2.4	4.4
Nutritive value		
TDN, %	38.71	71.1
ME (M cal/kg DM)	1.71	3.13
NE <sub>L</sub> (M cal/kg DM)	0.88	1.62

TDN: Total digestible nutrients; ME: Metabolizable energy; NE<sub>L</sub>: Net energy for lactation.

Cows were fed as a group open feed, with free access to water. Amount of TMR delivered was measured with electronic scales on mixer–feeder wagon. The TMR was mixed and fed using Delaval mixer wagon. The diet was formulated using Gavish computer operated cattle feeding system 2008 to cover or exceed NRC recommendations (NRC, 2001) as in Table 2. Cows were milked three times daily at 4 am, 12 pm. and 8 pm in a Dobell 20-parallel milking parlor equipped with automatic cow identification, milk recording system, and automated detacher milker units.

### 2.2 Sampling Analysis

During the entire experiment, representative samples of TMR were collected weekly and stored at -20°C until chemical analysis for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fibers (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest, (1970); Van Soest et al., (1991); AOAC, (2006). Cellulose and hemicellulose were calculated by differences among NDF, ADF and ADL. Total digestible nutrients (TDN), metabolizable and net energy for lactation were calculated according to NRC (2001).

Milk samples were collected biweekly and analyzed immediately for fat, protein, lactose and SNF content using infrared method by Milk Analyzer (Milko tester Instruments Inc, Bulgaria). Average fat and CP yields were calculated by multiplying milk yield by fat and CP content of milk on an individual cow basis. Milk energy (MJ/kg) was calculated on an individual cow basis using the milk fat, CP and lactose content of the milk (Tyrrell & Reid, 1965).

Blood samples were collected from Jugular vein (10 cows per group were randomly selected) prior to morning feeding monthly. Serum were obtained by centrifugation the blood tubes for 20 min, 3000xg and stored at -20°C until blood metabolites analysis. Concentrations of serum total protein, albumen, urea, glucose, triglycerides and cholesterol were determined using commercial kits manufactured by Stanbio Diagnostic Company, Germany. The concentrations of serum total protein, albumin, urea, glucose, triglycerides and cholesterol were measured calorimetrically according to Henry (1974); Doumas et al. (1971); Patton and Crouch (1977); and Tietz et al. (1995), respectively. The concentration of globulins in each serum sample was obtained by subtracting the value of albumin from the total blood serum protein concentration. The ratio of A: G was calculated.

### 2.3 Economic Efficiency

Economic efficiency expressed as the daily feed and supplement cost and price of milk. The price of one ton of TMR was 400 US\$, while the price of fibrozyme was 12.73 US\$/kg and milk price (fat=3.5% and SNF=8.5%) was 0.524US\$ according to Egyptian prices of year 2011.

### 2.4 Statistical Analysis

Statistical analysis was completed using a completely randomized design with a 2 factorial arrangement to analyze the data. The MIXED procedure of SAS (Version 9.2 SAS. 2002) and a model containing treatment,

week (repeated measure), and all interactions of these terms and as the random effect was used to analyze the data from measurements that were repeated weekly. Contrast statements were used to determine the effects of enzymes application (control vs. enzymes) and the interaction (enzymes treatment vs. time).

### 3. Results

The mean values of the proximate analysis of TMR are shown in Table 2. The results showed that the organic matter (OM), crude protein content (CP) and ether extract (EE) were 94.0, 19.1 and 4.4%, respectively. The fiber fractions content of TMR were 34, 16, 18, 13.5, 4.5 and 36.6% for NDF, ADF, hemicellulose, cellulose, lignin and non-fiber carbohydrates, respectively. The calculated total digestible nutrients (TDN), metabolizable energy (ME) and net energy for lactation (NE<sub>L</sub>) in TMR were 71.1%, 3.13 Mcal/kg DM and 1.62 Mcal/kg DM, respectively.

The effects of fibrolytic enzymes supplementation as a nutritional manipulation to lactating dairy cows at early lactation on dry matter intake (DMI), milk yield, fat corrected milk (FCM), energy corrected milk (ECM) and feed efficiency are shown in Table 3. The response to fibrolytic enzymes supplement to dairy cows on milk yield profile within the treatment period (12 weeks) is presented in Figure 1. The results indicated that there were not significant changes in DMI of lactating dairy cows with or without a supplement of exogenous fibrolytic enzymes at early lactation. Exogenous fibrolytic enzymes supplement to the diet of lactating dairy cows improved ( $P<0.003$ ) milk yield (41.0 vs. 39.5 kg/cow/d) and feed efficiency ( $P<0.001$ ) compared to untreated dairy cows. Also, the fat corrected milk was increased ( $P<0.025$ ) as a response to exogenous fibrolytic enzymes supplement to lactating dairy cows compared to un-supplemented dairy cows.

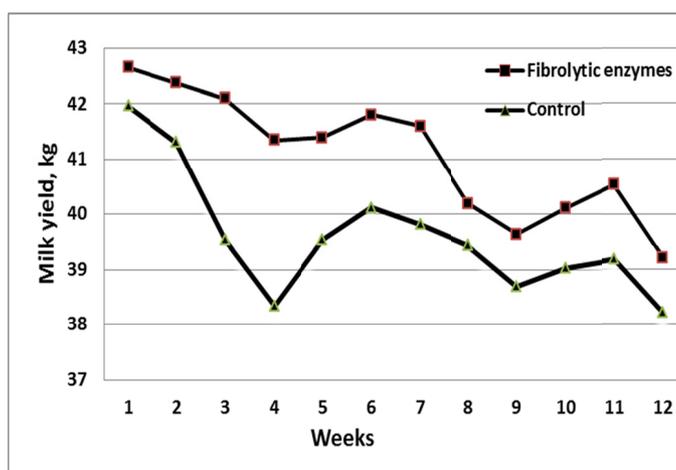


Figure 1. Milk production profile of early lactating dairy cows supplemented with exogenous fibrolytic enzymes

Table 3. Dry matter intake, milk yield, fat corrected milk, energy corrected milk and feed efficiency of lactating dairy cows at early lactation fed total mixed ration with or without exogenous fibrolytic enzymes (EFE) ( $\pm$  SE)

Items	Exogenous fibrolytic enzymes		<i>P</i> values		
	(-)	(+)	W	EFE	W*EFE
DMI, kg/d	24.78 $\pm$ 0.17	24.73 $\pm$ 0.17	0.845	0.845	0.954
Milk yield	39.5 $\pm$ 0.31 <sup>b</sup>	41.0 $\pm$ 0.31 <sup>a</sup>	0.018	0.003	0.947
FCM, kg/d	35.67 $\pm$ 0.285 <sup>b</sup>	36.58 $\pm$ 0.285 <sup>a</sup>	0.018	0.025	0.950
ECM, kg	39.4 $\pm$ 0.32 <sup>b</sup>	40.6 $\pm$ 0.32 <sup>a</sup>	0.018	0.006	0.945
Milk: Feed ratio	1.58 $\pm$ 0.01 <sup>b</sup>	1.64 $\pm$ 0.01 <sup>a</sup>	0.001	0.001	0.930

Different letters (a, b) in the same row indicate significant differences.

SE: standard error; SNF: solid not fat; W: weeks.

Fat Corrected milk (FCM) = milk yield\*0.4+ fat yield\*15

Energy corrected milk (ECM) = 0.327\*milk yield (kg)+12.95 \*fat (kg)+7.20\*protein (kg) (Tyrrell & Reid, 1965).

In addition, the supplementation of exogenous fibrolytic enzymes enhanced ( $P < 0.006$ ) the ECM (39.4 vs. 40.6 kg) in dairy cows compared to un-treated group. The significance contrasts in Table 3 showed that there were no interaction between treatment and time, while time had significant effects on milk yield, FCM, ECM and feed efficiency in treated group compared to the control group.

Effects of exogenous fibrolytic enzymes supplement to the diet of lactating dairy cow at early lactation on milk composition are given in Table 4. The results denoted that supplementation of exogenous fibrolytic enzymes had no significant effect on milk fat, protein lactose and solid not fat (SNF) percentage compared to the control group of dairy cows. While, the quantities of milk protein (1.36 vs. 1.30 kg), lactose (2.0 vs. 1.92 kg) and SNF (3.47 vs. 3.31 kg) in supplemented-dairy cows were improved significantly compared to the control group of dairy cows except quantity of milk fat ( $P < 0.096$ ).

Table 4. Milk composition of lactating dairy cows at early lactation fed total mixed ration with or without exogenous fibrolytic enzymes (EFE) ( $\pm$  SE)

Items	Exogenous fibrolytic enzymes		<i>P</i> values		
	(-)	(+)	W	EFE	W*EFE
Fat, %	3.43 $\pm$ 0.16	3.38 $\pm$ 0.16	0.520	0.384	0.858
Fat, kg	1.34 $\pm$ 0.01	1.36 $\pm$ 0.01	0.017	0.096	0.954
Protein, %	3.32 $\pm$ 0.02	3.37 $\pm$ 0.02	0.001	0.277	0.809
Protein, kg	1.30 $\pm$ 0.01 <sup>b</sup>	1.36 $\pm$ 0.01 <sup>a</sup>	0.018	0.001	0.939
Lactose, %	4.92 $\pm$ 0.20	4.95 $\pm$ 0.190	0.897	0.897	0.978
Lactose, kg	1.92 $\pm$ 0.015 <sup>b</sup>	2.00 $\pm$ 0.015 <sup>a</sup>	0.018	0.004	0.984
SNF, %	8.49 $\pm$ 0.09	8.59 $\pm$ 0.09	0.132	0.864	0.648
SNF, kg	3.31 $\pm$ 0.027 <sup>b</sup>	3.47 $\pm$ 0.027 <sup>a</sup>	0.018	0.001	0.945

Different letters (a, b) in the same row indicate significant differences.

SE: standard error; SNF: solid not fat; W: weeks.

The significance contrasts in Table 4 showed that there were no interaction between treatment and time, while time had significant effects on yield of milk fat, protein, lactose and SNF in treated group compared to the control group. Effects of exogenous fibrolytic enzymes inclusion to lactating dairy cows at early lactation on blood metabolites are presented in Table 5.

Table 5. Least square means ( $\pm$  SE) of blood metabolites of lactating dairy cows at early lactation fed total mixed ration with or without exogenous fibrolytic enzymes (EFE)

Items	Exogenous fibrolytic enzymes		<i>P</i> values		
	(-)	(+)	M	EFE	M*EFE
Glucose (mg/dL)	52.0 $\pm$ 2.68	51.3 $\pm$ 2.71	0.532	0.802	0.197
Total protein (g/dL)	12.8 $\pm$ 0.49 <sup>a</sup>	10.4 $\pm$ 0.47 <sup>b</sup>	0.016	0.001	0.263
Albumin (g/dL)	4.14 $\pm$ 0.25	4.04 $\pm$ 0.25	0.089	0.758	0.364
Globulin (g/dL)	8.9 $\pm$ 0.59 <sup>a</sup>	6.3 $\pm$ 0.59 <sup>b</sup>	0.212	0.002	0.143
A/G ratio	0.50 $\pm$ 0.29	1.2 $\pm$ 0.28	0.543	0.085	0.416
Urea (mg/dL)	34.4 $\pm$ 1.01	35.0 $\pm$ 1.01	0.007	0.678	0.707
Triglycerides (mg/dL)	28.8 $\pm$ 2.98	23.4 $\pm$ 2.98	0.888	0.195	0.710
Cholesterol (mg/dL)	242.0 $\pm$ 15.2 <sup>a</sup>	193.7 $\pm$ 15.2 <sup>b</sup>	0.956	0.002	0.224

Different letters (a, b) in the same row indicate significant differences.

SE: standard error; M: months.

Table 6. Economic evaluation of the exogenous fibrolytic enzymes supplementation at early lactation of dairy cows

Items	Control	Treatment
DMI, kg/d	24.78	24.73
Feed Cost, US\$	9.912	9.892
Supplement cost, US\$	0	0.191
Milk yield, kg/d	39.5	41.0
Milk Price, US\$/kg	0.524	0.527*
Return, US\$/cow/d	20.70	21.61
Profit, US\$/cow	10.79	11.72
Net profit, US\$/cow	0	0.93
Economic efficiency	2.09	2.19

Price of TMR = 0.4 US\$/kg.

Supplement cost (Exogenous fibrolytic enzymes, Fibrozyme) = 12.73 US\$ /kg.

\* The difference in milk price between two groups was due to higher 0.1% in SNF.

The results revealed that exogenous fibrolytic enzymes supplementation caused significant decline in serum total protein (12.8 vs. 10.4 g/dL), globulin (8.9 vs. 6.3 g/dL), A/G ratio content (0.81 vs. 0.54) and cholesterol (242.0 vs. 193.7 mg/dL) compared to control group of dairy cows. While, there were no significant changes regarding glucose, albumin, urea and triglycerides when exogenous fibrolytic enzymes was supplemented to the diet of lactating dairy cows at early lactation compared to control group. The results of statistical analysis showed no interaction was detected between treatments and months in the blood metabolites, while only values of total protein and blood urea N were highly affected by months.

Economic evaluation of the exogenous fibrozyme enzymes supplementation at early lactation of dairy cows is given in Table 5. The results showed the inclusion of 15 g of exogenous fibrolytic enzymes will cost 0.191 US\$ /cow/day, which will increase both milk production and SNF by 1.5 kg and 0.1% per cow/day. The daily profit of individual cow was 10.79 and 11.72 US\$ in control and treated group of lactating dairy cow at early lactation, respectively excluding the labor, veterinary medicines and other management's costs. Moreover, the net profit for exogenous fibrolytic enzymes inclusion in diet of early lactating dairy cows was higher 0.93 US\$ than untreated cows and economic efficiency increased from 2.09 to 2.19 by the treatment.

#### 4. Discussion

Feeding high-producing cows continues to challenge dairy farmers and nutritionists. Also, dairy profit margins vary as milk prices and feed costs shift yearly. Feed additives are a group of feed ingredients that can cause a desired animal response in a non-nutrient role, such as pH shift, metabolic modifier, or performance (Hutjens, 1991). The use of exogenous fiber-degrading enzyme additives for ruminants was first examined in the 1960s, as reviewed by Beauchemin and Rode (1996). Enzyme products for ruminant diets are of fungal (mostly *Trichoderma longibrachiatum*, *Aspergillus niger* and *A. oryzae*) like our tested product (Fibrozyme); bacterial (*Bacillus* spp., Pendleton, 2000) or rumen bacterial (Gado et al., 2009) origin. According to Sheppy (2001), there are four main reasons for using enzymes in animal feed: 1) to break down anti-nutritional factors; 2) to increase the availability of starches, proteins and minerals enclosed within fiber-rich cell walls; 3) to break down specific chemical bounds in raw materials which are not usually broken down by the animals' own enzymes, thus releasing more nutrients, and 4) to supplement the enzymes produced young animals.

Beauchemin et al. (1999) used lactating and cannulated Holstein cows to investigate the effects of grain source and fibrolytic enzymes supplementation on ruminal fermentation, nutrients digestion in the rumen and in intestine, and milk production. Two grains (barley and hull-less barley) were combined with and without enzymes. They proposed three ways for the arrival and action of the enzymes into the intestine: enzymes applied to dry feed may enhance the binding of the enzyme to the substrate, which may increase the resistance of the enzymes to proteolysis and prolong their residence time within the rumen; enzymes applied to silage or TMR immediately prior to feeding, may be released into the ruminal fluid and may pass through the rumen quickly before they can be effective, which would provide larger intestinal effects and exogenous enzymes may alter

digestion and nutrient absorption in the small intestine. Digestion of plant cell walls, which is carried out by ruminal microorganisms, provides a large amount of energy for ruminants. Fibrolytic enzymes isolated from fungi fermentation cultures have been utilized to improve DMI and forage digestibility; particulate passage rate; and digestibility of DM, NDF, and ADF in beef steers (McAllister et al., 1999).

In consistence with our finding on DMI, Ahn et al. (2003); Bernard et al. (2010) and Arriola et al. (2011) reported that adding fibrolytic enzymes supplementation to dairy cow diet did not enhance DMI and no difference was found between cows fed supplemented diet or un-supplemented diet with fibrolytic enzymes. On the other hand, several researchers recorded an increase in DMI of dairy cows when fibrolytic enzymes was applied to forage before mixing with other ingredients (Lewis et al., 1999) or applied to TMR or concentrate portion of the diet (Bowman et al., 2002; Ware & Zinn, 2005). However, the effects of fibrolytic enzymes on DMI appear to be vary among enzymes products and the method of applying of enzymes (Bowman et al., 2002)

A number of studies have examined the effects of fibrolytic exogenous enzymes on digestibility and milk production in dairy cows. In some studies, dietary addition of fibrolytic enzymes either to forages or concentrate portion increased milk production from 5- 16% (Lewis et al., 1999; Gado et al., 2009; Holtshausen et al., 2011) as noticed at the current study but no milk response was reported in others (Elwakeel et al., 2007; Bernard et al., 2010). Furthermore, these enhancement in milk yield at the current study are in line with those found by Guerra et al. (2007) who used Fibrozyme in diet containing alfalfa hay and they reported that Fibrozyme supplementation increased milk yield, which may be due to improved utilization of nutrients in digestive tract and in rumen and increased gain of net energy. Differences in enzyme activity, form and application rates, and diets across these studies complicate elucidation of the reasons for these discrepancies and highlight the need for caution when comparing studies involving different enzyme preparations.

The improvement in feed efficiency observed in the current lactation study might be attributable to greater NDF digestibility in the rumen and the similar trend was concluded by Holtshausen et al. (2011). Improvements in feed conversion efficiency were due to lower DMI rather than a change in milk yield. Improved feed efficiency indicates better utilization of nutrients when TMR was treated with enzymes, with the magnitude of improvement being a linear function of enzymes dosage.

There were no responses in the percentages of milk fat, protein, lactose and SNF for supplementing exogenous fibrolytic enzymes to early lactating dairy cows under the conditions of the current study, but the yield of milk protein, lactose and SNF was enhanced significantly by fibrozymes inclusion in the early lactating dairy cows. In agreement with our finding, several studies has been reported that fibrolytic enzymes supplementation to Holstein dairy cows did not affect ( $P>0.05$ ) on milk composition (Lewis et al., 1999; Knowlton et al., 2002; Reddish & Kung, 2007; Elwakeel et al., 2007; Bernard et al., 2010; Arriola et al., 2011). On the other hand, Yang et al. (1999) and Mansour (2009) found that milk fat increased when adding fibrolytic enzymes. The increase in fat percentage may be due to the increase in available energy and fatty acids for fat synthesis. Gado et al. (2009) concluded that milk protein yield for Brown Swiss cows was ( $P<0.05$ ) increased (0.57 %) for cows fed ZADO<sup>®</sup> supplemented diet compared with 0.45 kg/h/day for cows fed control diet. The variability in responses among studies may be attributed to variety of enzyme products and experimental conditions.

Contents of albumin, glucose, triglycerides and urea were not differing significantly due to fibrozymes supplementation to early lactating dairy cows (Table 5). Broderick et al. (1997) treated alfalfa silage with four levels of fibrolytic enzymes mixture (xylanase and cellulase) and fed dairy cows, they found that the adding of fibrolytic enzymes did not influence blood glucose and urea in all levels. Also Viktor (2006) concluded that there was no significant difference in blood plasma glucose concentrations for Holstein dairy cows fed diet supplemented with fibrolytic enzyme (xylanase). On the other hand, total protein and globulin and cholesterol were declined significantly by fibrozymes supplementation, which is similar to that found by Abd El-Kareim (2004). A/G ratio indicated that the treated animals were in good health since A/G ration was more than 1.00. Exogenous fibrolytic enzymes inclusion in TMR fed to the early lactating dairy cows improved economic efficiency and achieved daily net profit 0.93 US\$ per cow over control cows. 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.

## 5. Conclusion

The results of the current study concluded that feeding a fibrozyme applied to the total mixed ration of dairy cows in early lactation has the potential to increase milk production, SNF and economic efficiency.

## References

- Abd El-Kariem, M. A., Deyab, H. E., Ahmadi, E. A. A., & Abdel-Samee, A. M. (2004). *Heat stress of hot summer and its amelioration techniques effects on post-partum Friesian cows under Egyptian conditions*. 3<sup>rd</sup> Inter.Conf.Anim. poultry, Fish prod. and Health in Semi-Aired Areas, 7-9 sep, 2004. El-Arish-North- Sinai, Egypt. pp.172-183.
- Ahn, J. J., Seo, C., Kim, I. Y. K., Kim, D., Hong, H. N., & Lee, H. (2003). Characteristics of apoptotic cell death induced by coxsackievirus B in Vero cells. *Intervirology*, *46*, 245-251. <http://dx.doi.org/10.1159/000072435>
- Arriola, K. G., Kim, S. C., Staples, C. R., & Adesogan, A. T. (2011). Effect of fibrolytic enzyme application to low and high concentrate diets on the performance of lactating dairy cattle. *J Dairy sci.*, *94*, 832-841. <http://dx.doi.org/10.3168/jds.2010-3424>
- Bala, P., Malik, R., & Srinivas, B. (2009). Effect of fortifying concentrate supplement with fibrolytic enzymes on nutrient utilization, milk yield and composition in lactating goats. *J. Anim. Sci.*, *80*, 265-272. <http://dx.doi.org/10.1111/j.1740-0929.2009.00634.x>
- Baloyi, T. F. (2008). Effects of exogenous fibrolytic enzymes on *in vitro* fermentation kinetics of forage and mixed feed substrates. MSc (Agric.) thesis, Stellenbosch University, Stellenbosh, South Africa.
- Beauchemin, K. A., & Rode, L. M. (1996). Use of feed enzymes in ruminant nutrition. In L. M. Rode (Ed.), *Animal Science Research and Development-Meeting Future Challenges* (pp. 103-131). Minister of Supply and Services Canada, Ottawa, ON. [http://dx.doi.org/10.3168/jds.S0022-0302\(99\)75244-6](http://dx.doi.org/10.3168/jds.S0022-0302(99)75244-6)
- Beauchemin, K. A., Yang, W. Z., & Rode, L. M. (1999). Effects of grain Source and Extent of Nutrient Digestion in Dairy Cows. *J. Dairy Sci.*, *82*, 378-390.
- Beauchemin, K. A., Colombatto, D., Morgavi, D. P., & Yang, W. Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *J. Anim. Sci.*, *81*(E.Suppl. 2), E37-E47.
- Bernard, J. K., Castro, J. J., Mullis, N. A., Adesogan, A. T., West, J. W., & Morantes, G. (2010). Effect of feeding alfalfa hay or Tifton 85 bermudagrass haylage with or without a cellulase enzyme on performance of Holstein cows. *J. Dairy Sci.*, *93*, 5280-5285. <http://dx.doi.org/10.3168/jds.2010-3111>
- Bowman, G. R., Beauchemin, K. A. & Shelford, J. A. (2002). The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. *J. Dairy Sci.*, *85*, 3420-3429. [http://dx.doi.org/10.3168/jds.S0022-0302\(02\)74430-5](http://dx.doi.org/10.3168/jds.S0022-0302(02)74430-5)
- Broderick, G. A., Derosa, R., & Renal, S. (1997). *Value of Treating Alfalfa Silage with Fibrolytic Enzymes prior to Feeding the silage to Lactating Dairy Cows* (pp. 74-76). U. S Dairy Forage Research Center.
- Colombatto, D., Mould, F. L., M. Bhat, K., & Owen, E. (2003). Use of fibrolytic enzymes to improve the nutritive value of ruminant diets: A biochemical and *in vitro* rumen degradation assessment. *Anim. Feed Sci. Technol.*, *107*, 201-209. [http://dx.doi.org/10.1016/S0377-8401\(03\)00126-3](http://dx.doi.org/10.1016/S0377-8401(03)00126-3)
- Cruywagen, C. W., & Goosen, L. (2004). Effect of an exogenous fibrolytic enzyme on growth rate, feed intake and feed conversion ratio in growing lambs. *S. Afr. J. Anim. Sci.*, *34*(Suppl. 2), 71-73.
- Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clin. Chim. Acta.*, *31*, 87-96. [http://dx.doi.org/10.1016/0009-8981\(71\)90365-2](http://dx.doi.org/10.1016/0009-8981(71)90365-2)
- Elwakeel, E. A., Titgemeyer, E. C., Johnson, B. J., Armendariz, C. K., & Shirley, J. E. (2007). Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. *J. Dairy Sci.*, *90*, 5226-5236. <http://dx.doi.org/10.3168/jds.2007-0305>
- Eun, J. S., Beauchemin, K. A., & Schulze, H. (2007). Use of exogenous fibrolytic enzymes to enhance *in vitro* fermentation of Alfalfa hay and Corn silage. *J. Dairy Sci.*, *90*, 1440-1451. [http://dx.doi.org/10.3168/jds.S0022-0302\(07\)71629-6](http://dx.doi.org/10.3168/jds.S0022-0302(07)71629-6)
- Gado, H. M., Salem, A. Z. M., Robinson, P. H., & Hassan, M. (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Anim. Feed Sci. Technol.*, *154*, 36-46. <http://dx.doi.org/10.1016/j.anifeedsci.2009.07.006>
- Goering, H. K., & Van Soest, P. J. (1970). *Forage fiber analyses (apparatus, reagents, procedures and some applications)*. Agric. Handbook 379 ARS, USDA, Washington DC, USA.

- Guerra, N. P., Bernárdez, P. F., Méndez, J., Cachaldora, P., & Castro, L. P. (2007). Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *Anim. Feed Sci. Technol*, *134*, 89-107. <http://dx.doi.org/10.1016/j.anifeedsci.2006.05.010>
- Henry, J. B. (1974). Todd, Sanford, Davidsohn: "Clinical Diagnosis and Measurement by Laboratory Methods" (16th edn, pp. 260-264). W.B. Saunders and Co, Philadelphia P.A.P.
- Holtshausen, L., Chung, Y. H., Gerardo-Cuervo, H., Oba, M., & Beauchemin, K. A. (2011). Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. *J. Dairy Sci.*, *94*, 899-907. <http://dx.doi.org/10.3168/jds.2010-3573>
- Hutjens, M. F. (1991). Feed additives. *Vet Clinics North Am.: Food Animal Practice*, *7*(2), 525.
- Knowlton, K. F., McKinney, J. M., & Cobb, C. (2002). Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows. *J. Dairy Sci.*, *85*, 3328-3335. [http://dx.doi.org/10.3168/jds.S0022-0302\(02\)74421-4](http://dx.doi.org/10.3168/jds.S0022-0302(02)74421-4)
- Krause, D. O., Denman, S. T., Mackie, R. I., Morrison, M., Rae, A. L., Attwood, G. T., & McSweeney, C. S. (2003). Opportunities to improve fibre degradation in the rumen: Microbiology, ecology and genomics. *FEMS Microbiol. Rev.*, *27*, 663-693. [http://dx.doi.org/10.1016/S0168-6445\(03\)00072-X](http://dx.doi.org/10.1016/S0168-6445(03)00072-X)
- Lewis, G. E., Sanchez, W. K., Hunt, C. W., Guy, M. A., Pritchard, G. T., Swanson, B. I., & Treacher, R. J. (1999). Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. *J. Dairy Sci.*, *82*, 611-617. [http://dx.doi.org/10.3168/jds.S0022-0302\(99\)75274-4](http://dx.doi.org/10.3168/jds.S0022-0302(99)75274-4)
- Mansour, M. A. (2009). *Alleviation of stress for promoting physiological and productive performance of Friesian cattle*. MSc., Fac. Agric., Cairo Univ. Giza, Egypt.
- McAllister, T. A., Oosting, S. J., Popp, J. D., Mir, Z., Yanke, L. J., Hristov, A. N., ... Cheng, K. J. (1999). Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. *Can. J. Anim. Sci.*, *79*, 353-360. <http://dx.doi.org/10.4141/A98-099>
- Mertens, D. R. (1997). Creating a system for meeting the fibre requirements of dairy cows. *J. Dairy Sci.*, *80*, 1463-1481. [http://dx.doi.org/10.3168/jds.S0022-0302\(97\)76075-2](http://dx.doi.org/10.3168/jds.S0022-0302(97)76075-2)
- NRC. (2001). *Nutrient Requirements of Dairy Cattle* (7th edn). Washington, DC.: Natl. Acad. Press.
- Patton, C. J., & Crouck, S. R. (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. *Anal. Chem.*, *49*, 464-469. <http://dx.doi.org/10.1021/ac50011a034>
- Pendleton, B. (1996). The regulatory environment. In S. Muirhead (Ed.), *Direct-Fed Microbial, Enzyme and Forage Additive Compendium* (pp. 47-52). Minnetonka, Minnesota: The Miller Publishing Company.
- Reddish, M. A., & Kung, L. (2007). The effect of feeding a dry enzyme mixture with fibrolytic activity on the performance of lactating cows and digestibility of a diet for sheep. *J. Dairy Sci.*, *90*, 4724-4729. <http://dx.doi.org/10.3168/jds.2007-0269>
- Romney, D. L., & Gill, M. (2000). Forage evaluation for efficient ruminant livestock production. In D. L. Givens, E. Owen, R. F. E. Axford, & H. M. Omed (Eds.), *Forage evaluation in ruminant nutrition* (pp. 43-62). CAB inter.
- SAS. (2002). *SAS user's guide: statistics (Version 9)*. Cary, NC: SAS Inst. Inc.
- Sheppy, C. (2001). The current feed enzymes market and likely trends. In M. R. Bedford, & G. G. Partridge (Eds.), *Enzymes in farm animal nutrition* (pp. 1-10). CAB inter.
- Tietz, P. S., Holman, R. T., Miller, L. J., & LaRusso, N. F. (1995). Isolation and characterization of rat cholangiocyte vesicles enriched in apical or basolateral plasma membrane domains. *Biochemistry*, *34*, 15436-15443. <http://dx.doi.org/10.1021/bi00047a007>
- Tozer, P. R., Bargo, F., & Muller, D. L. (2003). Economic analyses of feeding systems combining pasture and total mixed ration. *J. Dairy Sci.*, *86*, 808-818. [http://dx.doi.org/10.3168/jds.S0022-0302\(03\)73663-7](http://dx.doi.org/10.3168/jds.S0022-0302(03)73663-7)
- Tyrrell, H. F., & Reid, J. T. (1965). Prediction of the energy value of cow's milk. *J. Dairy Sc.*, *48*, 1215-1223. [http://dx.doi.org/10.3168/jds.S0022-0302\(65\)88430-2](http://dx.doi.org/10.3168/jds.S0022-0302(65)88430-2)
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, *74*, 3583-3597. [http://dx.doi.org/10.3168/jds.S0022-0302\(91\)78551-2](http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2)

- Varga, G. A., & Kolver, E. S. (1997). Microbial and animal limitations to fibre digestion and utilization. *J. Nutr.*, *127*, 819S-823S.
- Viktor, J. D. V. (2006). *Use of Fibrolytic Enzymes produced by fungus Thermomyces lanuginosus in ruminant nutrition*. PhD Thesis, Szentistvan University, Hungry.
- Ware, R. A., & Zinn, R. A. (2005). Influence of Maceration and fibrolytic enzyme on the feeding value of rice straw. *J. Anim. Vet. Adv.*, *4*, 387-392.
- Weimer, P. J., Russell, J. B., & Muck, R. E. (2009). Lessons from the cow: What the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. *Bioresour. Technol.*, *100*, 5323-5331. <http://dx.doi.org/10.1016/j.biortech.2009.04.075>
- Wilkins, R. J. (2000). Forages and their role in animal systems. In D. L. Givens, E. Owen, & R. F. E. Forage (Eds.), *Evaluation In Ruminant Nutrition* (pp. 1-12). Axford & H.M. Omed. CAB inter.