

**Research Article**

## **EFFECT OF DIETARY SUPPLEMENT WITH FIBROLYTIC ENZYMES ON THE PRODUCTIVE PERFORMANCE OF EARLY LACTATING DAIRY COWS**

**\*Ali Shadmanesh**

*Faculty of Veterinary Medicine Islamic Azad University – Eghlid Branch, Iran*

*\*Author for Correspondence*

### **ABSTRACT**

An experiment was conducted to study the effects of supplementing the diets of early lactating dairy cows with exogenous fibrolytic enzymes (Fibrozyme, Alltechinc Company, USA) on feed intake, feed efficiency, milk production and composition of milk for 70 days. 28 Holstein dairy cows at early lactation ( $50 \pm 6.2$  days in milk) were randomly divided into two groups according to lactation period "stage of lactation" and lactation season. The first group (control,  $n=14$ ) were fed total mixed ration (TMR) without a supplement of fibrolytic enzymes. The second group (treatment,  $n=14$ ) were fed TMR supplemented with a commercial fibrolytic enzymes at the rate of 15 g/cow/d for 70 days. Each group was placed in an open pen. Fibrolytic enzyme was added to TMR at the time of feeding twice per day at 8 am and 15 pm.

The results showed that treatments had no effect on dry matter intake. Milk yield (4%) and the fat corrected milk were increased as a response to fibrolytic enzymes supplementation to lactating dairy cows compared to un-supplemented dairy cows ( $P<0.005$ ). In addition, feed efficiency in treated was increased significantly than of the control group ( $P<0.001$ ).

The milk fat, protein, lactose and solid not fat (SNF) percentages in treated were not higher than of the control group. While, quantities of milk protein, lactose and SNF in treated were improved significantly compared to control group except quantity of milk fat ( $P<0.008$ ). It is concluded that fibrolytic enzymes supplementation to early lactating dairy cows improved significantly milk production and SNF.

**Keywords:** *Fibrolytic Enzymes, Productive Traits, Dairy Cow*

### **INTRODUCTION**

The digestion of plant cell wall in the rumen is slow and incomplete and can limit milk production and increase the cost of production. Over the years, there has been a continual search for new additives that will enhance feed utilization so that the greater nutrient demands of ever-increasing animal productivity can be met. The availability of new enzyme products, combined with the high cost of livestock production, has prompted researchers to examine the potential role of exogenous enzymes in ruminant production (Beauchemin, 1995; Chen, 1995).

The use of exogenous fibrolytic enzymes (EFE) to enhance quality and digestibility of fibrous forage is on the some of delivering practical benefits to ruminant production systems. In this matter, cellulase and xylanase are two major enzyme groups that are specified to break  $\beta$ 1-4 linkages joining sugar molecules of cellulose and xylan found in plant cell wall components (Beauchemin, 1999). 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. In addition, results with EFE addition in ruminant systems are variable and somewhat inconsistent (Beauchemin, 1999; Colombatta, 2003), making their biological response difficult to predict.

Some studies have shown substantial improvement of feed digestibility and animal performance traits (Arriola, 2011; Bala, 2009), 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 fibrolytic enzyme 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

## Research Article

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 e 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, milk production and milk composition in early lactating dairy cows.

## MATERIALS AND METHODS

### *Animals' Management and Experimental Design*

The experiment was conducted at Islamic Azad University Eghlid- Branch, from August to November, 2013 for 70 days. 28 Holstein dairy cows at early lactation ( $50 \pm 6.2$  days in milk) were randomly divided into two treatment groups. One group (N=14) was fed TMR supplemented with enzyme mixture while the other group (N=14) saved as control. The enzyme preparation Fibrozyme TM, used in the present study is a blend of active xylanase and cellulase, which are a dried mixture of fermentation extracts from *Aspergillus niger* and *Trichoderma longibrachiatum* fungi, and having xylanase activity by minimum 100 XU/g, Alltechinc Company, USA) at the rate of 15 g/cow/d according to the guide of the manufacture for 90 days. Fibrozyme was added and mixed to the TMR at the time of feeding. Cows in the two groups fed a total mixed ration (TMR, Table 1), which composed of alfalfa hay, corn silage, soybean meal, Barley grain, Blood meal, Corn gluten meal, vitamins- minerals mixture, calcium bicarbonate, dicalcium phosphate and mono sodium 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**

| Ingredients                    | % of DM |
|--------------------------------|---------|
| Alfalfa hay                    | 15      |
| Corn silage                    | 24      |
| Barley grain                   | 44      |
| Blood meal                     | 0.95    |
| Corn gluten meal               | 5.2     |
| Soybean meal, 48% CP           | 4       |
| Calcium carbonate              | .95     |
| Dicalcium phosphate            | 0.48    |
| Mono-sodium phosphate          | 0.1     |
| Mineral-vitamin mix            | 1.5     |
| Cr <sub>2</sub> O <sub>3</sub> | 0.3     |

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

| Nutrients               | As dry matter basis (%) |
|-------------------------|-------------------------|
| DM                      | 60.5                    |
| OM                      | 92.6                    |
| Crude Protein           | 16.7                    |
| Neutral detergent fiber | 31                      |
| Acid detergent fiber    | 17.8                    |
| Ash                     | 5.8                     |
| Non Fiber carbohydrates | 36.5                    |
| Ether Extract           | 4.6                     |
| ME (M cal/kg DM)        | 3.3                     |
| NEL (M cal/kg DM)       | 1.75                    |

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.

## Research Article

The diet was formulated using of recommendations NRC (2001), (Table2). Cows were milked twice times daily at 5 am and 5 pm in a Dobell 10-parallel milking parlor, milk recording system, and automated detacher milker units.

### Sampling Analysis

During the experiment, samples of TMR were collected weekly and stored at -18°C until chemical analysis for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fibers (NDF) and acid detergent fiber (ADF). Analysis chemical of samples were determined according to Goering and Van Soest (1970), Van Soest (1991) and AOAC, (2006). Metabolizable and net energy for lactation were calculated according to NRC (2001). Milk samples were collected weekly and analyzed immediately for fat, protein, lactose and SNF content using infrared method by Milk Analyzer. Average fat and CP yields were calculated by multiplying milk yield by fat and CP content of milk on an individual cow basis.

### Statistical Analysis

Data using of software SAS (2002), version9), in a completely randomized design with a 2 factorial (enzyme and week) arrangement were analyzed. A model containing treatment, week (repeated measure), and all interactions.

## RESULTS AND DISCUSSION

The results of the proximal analysis of TMR are shown in table 2. The results showed that the organic matter (OM), crude protein (CP), ether extract (EE), NDF, ADF and non- fiber carbohydrate were 92.6, 16.7, 4.6, 31, 17.8 and 36.5 respectively. Metabolizable energy (ME) and net energy for lactation (NE<sub>L</sub>) in TMR were calculated 3.3 M cal/kg DM and 1.75 M cal/kg DM respectively.

Effect of enzyme supplement of the diet of lactating dairy cow at early lactation on dry matter intake, milk production, fat corrected milk and feed efficiency are shown in table 3. Intake of DM was not affected by fibrolytic enzymes supplementation. This is in agreement with our previous studies (Ahn, 2003; Arriola, 2011; Dyaa, 2013; Lewis, 1999; Rode, 1999; Yang, 1999), in which a high xylanase, high cellulase enzyme product was added to either the TMR, forage, or concentrate. 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, 1999) or applied to TMR or concentrate portion of the diet (Ware, 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).

**Table 3: Effect of fibrolytic enzymes supplementation on dry matter intake, milk production, fat corrected milk and feed efficiency at lactating dairy cows**

| Items            | Control                 | E-TMR                   | P values |       |       |
|------------------|-------------------------|-------------------------|----------|-------|-------|
|                  |                         |                         | W        | EF    | W*FE  |
| DMI              | 19.5±0.15               | 19.60.15                | 0.875    | 0.875 | 0.955 |
| Milk yield(kg/d) | 31.2±0.3 <sup>b</sup>   | 32.5±0.3 <sup>a</sup>   | 0.017    | 0.003 | 0.943 |
| FCM(Kg/d)        | 29.04±0.22 <sup>b</sup> | 30.05±0.22 <sup>a</sup> | 0.017    | 0.025 | 0.950 |
| milk: feed ratio | 1.48±0.01 <sup>b</sup>  | 1.66±0.01 <sup>a</sup>  | 0.001    | 0.001 | 0.937 |

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

\*W: weeks and FE: Fibrolytic enzyme

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

Milk yield (p<0.003), fat corrected milk (p<0.025) and feed efficiency (p<0.001) was significant higher for cows fed the E-TMR diet than for cows fed the control.

In some studies, the response to enzymes has been substantial. For example, Lewis (1999) treated for age with a cellulase/xylanase mixture (FinnFeedsInt.; supplying 1 mL/kg of total mixed ration [TMR], DM basis) and observed that cows in early lactation produced 6.3 kg/d (16%) more milk. However, higher and lower levels of the same enzyme product were less effective. Rode (1999) applied an enzyme product

## Research Article

(Promote, Biovance Technologies Inc., Omaha, NE) to the concentrate portion of a diet (supplying 1.3 g/kg of TMR on a DM basis) and observed a 3.6 kg/d (10%;  $P = 0.11$ ) increase in milk production for cows in early lactation. Yang (2000) added an enzyme mixture (Biovance Technol, Omaha, NE) to the concentrate, and cows in early lactation produced 2 kg/d (5.9%) more milk. However, there was no response when the same enzyme was added to the TMR. But no milk response was reported in others (Bernard, 2010; Elwakeel, 2007). 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.

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 (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.

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 and feed efficiency in treated group compared to the control group.

Effect of enzymes supplement to the diet of lactating dairy cow at early lactation on milk composition are shown in table 4. The results denoted that 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. 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.

**Table 4: Milk composition of lactating dairy cows at early lactation fed TMR with or without fibrolytic enzymes (FE)**

| Items       | control                 | E-TMR                   | P values |       |       |
|-------------|-------------------------|-------------------------|----------|-------|-------|
|             |                         |                         | W        | EF    | W*FE  |
| Fat, %      | 3.54±0.15               | 3.5 ±0.15               | 0.485    | 0.482 | 0.922 |
| Fat, kg     | 1.104±0.02              | 1.137±0.02              | 0.016    | 0.1   | 0.954 |
| Protein, %  | 3.15±0.02               | 3.25±0.18               | 0.007    | 0.25  | 0.80  |
| Protein, kg | 0.98±0.01 <sup>b</sup>  | 1.06±0.01 <sup>b</sup>  | 0.018    | 0.008 | 0.937 |
| Lactose, %  | 4.7±0.15                | 4.8 ±0.16               | 0.88     | 0.787 | 0.982 |
| Lactose, kg | 1.47±0.014 <sup>b</sup> | 1.56±0.014 <sup>a</sup> | 0.017    | 0.008 | 0.924 |
| NSF, %      | 8.4±0.07                | 8.6±0.07                | 0.12     | 0.896 | 0.732 |
| NSF, kg     | 2.62±0.03 <sup>b</sup>  | 2.79±0.03 <sup>a</sup>  | 0.017    | 0.005 | 0.958 |

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

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

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 (Bernard, 2010; Dyaa, 2013; Granzin, 2005; Lewis, 1999). On the other hand, Yang (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 (2009) concluded that milk protein yield for Brown Swiss cows was ( $P < 0.05$ ) increased (0.57 %) for cows fed ZADO® 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.

## Conclusion

Applying enzymes to the TMR before feeding dairy cows in early lactation improved milk production and SNF.

## Research Article

### REFERENCES

- Ahn JJ, Seo C, Kim IYK, Kim D, Hong HN and Lee H (2003).** Characteristics of apoptotic cell death induced by coxsackievirus B in permissive Vero cells. *Intervirolgy* **46**(4) 245-51. Available: <http://dx.doi.org/10.1159/000072435>.
- Arriola KG, Kim SC, Staples CR and Adesogan AT (2011).** Effect of fibrolytic enzyme application to low and high concentrate diets on the performance of lactating dairy cattle. *Journal of Dairy Science* **94** 832-841. Available: <http://dx.doi.org/10.3168/jds.2010-3424>
- Bala P, Malik R and Srinivas B (2009).** Effect of fortifying concentrate supplement with fibrolytic enzymes on nutrient utilization, milk yield and composition in lactating goats. *Journal of Animal Science* **80** 265-272. Available: <http://dx.doi.org/10.1111/j.1740-0929.2009.00634.x>
- Baloyi TF (2008).** Effects of exogenous fibrolytic enzymes on *in vitro* fermentation kinetics of forage and mixed feed substrates. MSc (Agric.) thesis, Stellenbosch University, Stellenbosch, South Africa.
- Beauchemin KA, Rode LM and Sewalt VJH (1995).** Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. *Canadian Journal of Animal Science* **75** 641-644.
- Beauchemin KA, Yang WZ and Rode LM (1999).** Effect of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. *Journal of Dairy Science* **82** 378-390.
- Bernard JK, Castro JJ, Mullis NA, Adesogan AT, West JW and Morantes G (2010).** Effect of feeding alfalfa hay or Tifton 85 bermudagrass haylage with or without a cellulase enzyme on performance of Holstein cows. *Journal of Dairy Science* **93** 5280-5285. Available: <http://dx.doi.org/10.3168/jds.2010-3111>
- Chen KH, Huber JT, Simas J, Theurer CB, Yu P, Chan SC, Santos F, Wu Z and Swingle RS (1995).** Effect of enzyme treatment or steam-flaking of sorghum grain on lactation and digestion in dairy cows. *Journal of Dairy Science* **78** 1721-1727.
- Colombatto D, Mould FL, Bhat MK and Owen E (2003).** Use of fibrolytic enzymes to improve the nutritive value of ruminant diets: A biochemical and *in vitro* rumen degradation assessment. *Animal Feed Science and Technology* **107** 201-209. Available: [http://dx.doi.org/10.1016/S0377-8401\(03\)00126-3](http://dx.doi.org/10.1016/S0377-8401(03)00126-3)
- Dyaa BC, El-Din A and Sallam MA (2013).** Effect of Dietary Supplementation with Fibrolytic Enzymes on the Productive Performance of Early Lactating Dairy Cows. *Journal of Agricultural Science* **5**(6). Available: [www.ccsenet.org/jas](http://www.ccsenet.org/jas)
- Elwakeel EA, Titgemeyer EC, Johnson BJ, Armendariz CK and Shirley JE (2007).** Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. *Journal of Dairy Science* **90** 5226-5236. Available: <http://dx.doi.org/10.3168/jds.2007-0305>
- Gado HM, Salem AZM, Robinson PH and Hassan M (2009).** Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Animal Feed Science and Technology* **154** 36-46. Available: <http://dx.doi.org/10.1016/j.anifeedsci.2009.07.006>
- Goering HK and Van Soest PJ (1970).** *Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications)* Agric. Handbook 379 (ARS, USDA, Washington DC, USA).
- Granzin BC (2005).** Effects of a fibrolytic enzyme supplement on the performance of Holstein Friesian cows grazing kikuyu. *Tropical Grasslands* **39** 112-116.
- Guerra NP, Bernárdez PF, Méndez J, Cachaldora P and Castro LP (2007).** Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *Animal Feed Science and Technology* **134** 89-107. <http://dx.doi.org/10.1016/j.anifeedsci.2006.05.010>
- Lewis GE, Sanchez WK, Hunt CW, Guy MA, Pritchard GT, Swanson BI and Treacher RJ (1999).** Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. *Journal of Dairy Science* **82** 611-617. Available: [http://dx.doi.org/10.3168/jds.S0022-0302\(99\)75274-4](http://dx.doi.org/10.3168/jds.S0022-0302(99)75274-4)
- Holtshausen L, Chung YH, Gerardo-Cuervo H, Oba M, and Beauchemin KA (2011).** Improved milk production efficiency in early lactation dairy cattle with dietary addition of a developmental fibrolytic enzyme additive. *Journal of Dairy Science* **94** 899-907. Available: <http://dx.doi.org/10.3168/jds.2010-3573>



**Research Article**

**Mansour MA (2009).** Alleviation of stress for promoting physiological and productive performance of Friesian cattle. MSc., Faculty of Agriculture, Cairo University, Giza, Egypt.

**NRC (2001).** *Nutrient Requirements of Dairy Cattle* 7th edition (Washington, DC: Natl. Acad. Press).

**Rode LM, Yang WZ and Beauchemin KA (1999).** Fibrolytic enzyme supplements for dairy cows in early lactation. *Journal of Dairy Science* **82** 2121–2126.

**Van Soest PJ, Robertson JB and Lewis BA (1991).** Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74** 3583-3597. Available: [http://dx.doi.org/10.3168/jds.S0022-0302\(91\)78551-2](http://dx.doi.org/10.3168/jds.S0022-0302(91)78551-2)

**Ware RA and Zinn RA (2005).** Influence of Maceration and fibrolytic enzyme on the feeding value of rice straw. *Journal of Animal and Veterinary Advances* **4** 387-392.

**Yang WZ, Beauchemin KA and Rode LM (1999).** Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *Journal of Dairy Science* **82** 391–403.