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Effect of Exogenous Fibrolytic Enzyme Application on Productive Response of Dairy Cows at Different Lactation Stages

[1]N.E. El-Bordeny, [2]A.A. Abedo, [1]H.M. El-Sayed, [3]E.N. Daoud, [1]H.S. Soliman and [4]A.E.M. Mahmoud

[1]Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
[2]Department of Animal Production, National Research Center, Dokki, Giza, Egypt
[3]Agricultural Research Center, Regional Center for Food and Feed, Egypt
[4]Department of Animal Production, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt

Corresponding Author: N.E. El-Bordeny, Department of Animal Production, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

ABSTRACT

This study aimed to evaluate effect of using exogenous fibrolytic enzymes on productive performance of dairy cows and milk curve response at different lactation stages. One hundred and sixteen multiparous cows were randomly assigned into two groups; fifty eight cows in each. Each group was contained 12 cows in early lactation 40±6 Days In Milk (DIM), 18 cows in mid lactation (122±4 DIM) and 29 cows in late lactation (216±2 DIM). The animals were fed total mixed ration with or without 15 g fibrolytic enzymes head^{-1} day^{-1} for five weeks. Adding fibrolytic enzymes to dairy cows ration caused a significant increase in serum total protein and glucose concentration compared to control group, while Albumin, globulin, ALT, AST, alkaline phosphates activity and total bilirubin and urea concentration were not affected. Insignificant differences were observed in feed intake as dry matter, total digestible nutrient, crude protein and net energy lactation between the two groups. Enzymes supplementation to dairy cows rations increased milk yield, 4% FCM and ECM as well as milk fat contents compared to control group, while insignificant increased protein, lactose, total solid and solid not fat contents. Feed conversions as well as nitrogen efficiency utilization were significantly improved for treated group compared to control. Fibrolytic enzyme supplementation to dairy cows ration slightly increased positive slope (b-value) at early lactation compared to control group and no significant difference at mid lactation was observed. While, significant decrease in negative b-value was noticed for cows fed ration supplemented with fibrolytic enzymes compared to control group. It could be concluded that fibrolytic enzymes supplementation to dairy cows ration at early, mid and lactation has the potential to improve its productive performance as well as it is affect milk curve response.

Key words: Fibrolytic enzymes, milk yield, feed conversion, milk curve response

INTRODUCTION

Many researches has demonstrated that supplementing dairy animal and feedlot diets with fiber degrading enzymes can improve feed utilization and animal performance by enhancing fiber degradation *in vitro* (Gado *et al*., 2009; Rodrigues *et al*., 2008), *in situ* (Tricarico *et al*., 2005; Krueger *et al*., 2008) and *in vivo* (Salem *et al*., 2007; Gado and Salem, 2008; El-Bordeny *et al*., 2010). Feeding enzymes is often accompanied by increased feed intake, which may partly be due to increase palatability of the diet due to sugars released by pre-ingestive fiber hydrolysis and post-ingestive enzyme effects, such as increased digestion rate and /or 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).

The reported positive effects of adding Exogenous Fibrolytic Enzymes (EFE) to ruminant diets could be understood through, dairy cows fed forage treated with a fibrolytic enzyme additive ate more feed and produced 5-25% more milk (Lewis *et al*., 1995; Tricarico *et al*., 2005; El-Bordeny *et al*., 2010) improved the energy balance of transition dairy cows (De Frain *et al*., 2005) and increased milk production in small ruminants (Titi and Lubbadeh, 2004).

The above results cleared that exogenous enzymes can be effective for ruminants but it is important to determine the conditions that are most likely to result in positive responses (Beauchemin *et al*., 2003). Many factors, such as the specific activity of the enzymes, their mode and level of application, as well as the type of animal, its diet, energy balance and animal productivity may affect animal response to fibrolytic enzyme additive. So, the objective of this study was to evaluate effect of using exogenous fibrolytic enzymes on productive response of dairy cows and milk curve response at different lactation stages.

MATERIALS AND METHODS

Exogenous fibrolytic enzyme (EFE): Fibrozyme (commercial product), 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 at minimum 100 XU g^{-1}, Alltechinc Company, USA.

Animals management and experimental design: One hundred and sixteen multiparous Holstein dairy cows (body weight 525±15.5 kg) were assigned to two groups of fifty eight cows each, according to lactation stage and lactation season; control and supplemented group. Each group contain 12 cows in early lactation 40±6 Days In Milk (DIM)), 18 cows in mid lactation (122±4 DIM) and 28 cows in late lactation (216±2 DIM). The animals were fed Total Mixed Ration (TMR, Table 1) without or with 15 g fibrozyme/animal/day according to the guide of the manufacture and the experiment lasted 5 weeks. Each group was placed in a shaded pen equipped with free stalls. The diets was formulated to cover NRC requirement for dairy cattle (NRC., 2001).

Sampling and analytical methods: Samples of TMR were collected and composed weekly and dried at 55°C then ground to pass a 1 mm screen in a Wiley mill before analyzed. The TMR samples were analyzed for proximate chemical analyses according to AOAC (2000). Nitrogen free extract was calculated by difference. The NDF and ADF were determined according to Van Soest *et al.* (1991).

Cows were fed as a group with free access to water. Cows were milked 3 times daily at 4 am, 12 and 8 pm. Milk yield for all cows were determined daily using De-laval milk manager model sortie, milk samples were obtained once every weeks (for six consecutive milking) from each cow for the three consecutive milking and pooled within cow relative to production to obtain one composite milk sample per animal and stored at ±4°C until subjected to chemical analyses. Milk samples were analyzed for total solids, fat, true protein and lactose by infrared spectrophotometry (Foss 120 Milko-Scan, Foss Electric, Hillerød, Denmark) according to AOAC (2000). While, Solids-Not-Fat (SNF) was calculated.

Table 1: Formulation and chemical composition of total mixed ration

Composition	Percentage
Ingredients	
Alfalfa hay	10.27
Corn silage	18.60
Alfalfa	12.40
Sunflower meal	5.70
Linseed meal	5.66
Gluten meal 60	2.60
Soybean meal 44%	16.37
Yellow corn	23.65
Lime stone	0.74
Vitamin mixture**	0.17
Minerals mixture*	0.08
Protected fat(Magnapac)	2.81
Sodium bicarbonate	0.74
Dicalcium phosphate	0.21
Chemical composition (g kg^{-1} DM)	
Dry matter	658.80
Organic matter	896.60
Crude protein	178.00
Ether extract	37.20
Crude fiber	140.00
Nitrogen free extract	455.00
Neutral detergent fiber (NDF)	293.70
Acid detergent fiber (ADF)	205.10
NE_L (Mcal/kg DM)***	1.72

*Contained Ca: 141 g kg^{-1}, P: 27 g kg^{-1}, Mg: 65 g kg^{-1}, S: 14 g kg^{-1}, Na: 120 g kg^{-1}, K: 6 g kg^{-1}, Fe: 944 mg kg^{-1}, Zn: 1613 mg kg^{-1}, Cu: 484 mg kg^{-1}, Mn: 1748 mg, I: 58 mg kg^{-1}, Co: 51 mg kg^{-1}, Se: 13 mg kg^{-1}, **Contained vitamin A: 248,000 U, Vitamin D: 3 kg^{-1}, 74,000 UI kg^{-1} and Vitamin E: 1656 IU kg^{-1}, ***Calculated using published values of feed ingredients (NRC., 2001)

Fat corrected milk (4% fat) was calculated according to equation of Gaines (1928), while Energy Corrected Milk (ECM) was calculated according to equation of Tyrell and Reid (1965) as follow, respectively:

$$4\%\ FCM = 0.4\ \text{milk yield (gm)}+15\ \text{fat yield (g)}$$
$$ECM = 0.327^{*}\text{milk yield (kg)}+12.95^{*}\ \text{fat yield (kg)}+7.20^{*}\ \text{protein (kg)}$$

At the end of lactation trial, blood samples were taken from 10 experimental animals of each group. A sample of 10 mL of blood per animal was withdrawn from the jugular vein. The blood sample was directly collected into a clean dried glass culture tubes at 3 prior to morning feeding. The blood serum was obtained by centrifuging the blood samples 2 h after collection at 4000 (rpm) for 15 min. Blood serum was transferred into a clean dried glass vials and then stored in deep freezer at -20°C for subsequent specific chemical analysis. Blood serum samples were analyzed using commercial kits. Total serum protein concentrations was determined as described by Henry (1974), albumin concentrations was determined using methods of Doumas *et al.* (1971), blood serum glucose was determine, blood serum urea was determined according to Patton and Crouch (1977), blood serum Alkaline phosphates activity was determined according to Belfield and Goldberg (1971), total bilirubin was determined according to Burtis *et al.* (1999) and activity of serum Alanin Transaminase (ALT) and Aspartate Transaminase (AST) were determined using AST and ALT kits (Quimica Clinica Aplicada S.A., Spain) based on reaction of Young (1997). Globulin level was calculated.

Statistical analysis: Data were analyzed according to statistical analysis system User's Guide, (SAS., 1999). Separation among means was carried out by using Duncan's multiple range test

(Duncan, 1955). The model used for Statistical analysis of blood bio-chemical parameters and productive performance data was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = Observation on the ith treatment
μ = Overall mean
T_i = Effect of the ith treatment
e_{ij} = Random experimental error

But the model used for statistical analysis of animal productivity at different lactation stages was:

$$Y_{ijk} = \mu + T_i + L_j + L^*T + e_{ijk}$$

Where:

Y_{ij} = Observation of the ith treatment and jth lactation stage
μ = Overall mean
T_i = Effect of the ith treatment
L_j = Effect of lactation stage
e_{ijk} = Random experimental error

The relationship between actual milk production and day in milk (milk curve) was therefore realized by regression analysis within each treatment for each lactation stage (early, mid and late lactation stage. The lactation stage defined as early lactation stage "1-90 DIM", mid lactation stage "91-180 DIM" and late lactation stage "181-305 DIM"). T-paired test was performed to compare between regression coefficients for the two treatments within each lactation stage.

RESULTS AND DISCUSSION

Effect of exogenous fibrolytic enzyme application on blood metabolites: Adding EFE to dairy cows ration showed significant increase ($p<0.05$) in serum total proteins and glucose concentration compared to cows fed ration not supplemented (Table 2). This may be attributed that EFE supplementation improve metabolic process as a response to increase apparent nutrients digestibility specially, protein and organic matter in the rumen and flow of microbial protein from the rumen. In this connection, Kumar *et al.* (1980) and Bush (1991) reported that serum total proteins concentration reflects the nutritional status of the animal and it has a positive correlation with dietary protein level. Moreover, Holtshausen *et al.* (2011) found that adding exogenous enzymes to dairy cattle ration improve its energy availability.

On the other hand albumin, globulin, total bilirubin and urea concentration and ALT, AST, alkaline phosphates activity were not significantly ($p>0.05$) affected by EFE supplementation to dairy cows ration. The present values of AST and ALT activity indicated normal activity of the animal hepatic tissues, consequently, EFE application in the present study had no an adverse effect on the liver function. Furthermore, El-Bordeny *et al.* (2010) found that adding EFE to dairy buffaloes rations had not any significant effect on buffalo's blood bio-chemical parameters.

Table 2: Effect of EFE supplementation on some blood bio-chemical parameters of dairy cows

Items	Control	EFE	p-value
Total protein (g dL^{-1})	7.870±0.26[b]	8.830±0.30[a]	0.048
Albumin (g dL^{-1})	3.390±0.29	4.220±0.32	0.096
Globulin (g dL^{-1})	4.480±0.10	4.610±0.11	0.415
A/G ration	0.760±0.07	0.920±0.08	0.203
Glucose (mg dL^{-1})	42.420±1.79[b]	49.890±1.96[a]	0.009
ALT (IU L^{-1})	52.750±0.62	54.130±0.62	0.136
AST (IU L^{-1})	42.750±3.42	40.630±3.42	0.666
Alkaline phosphatase (IU L^{-1})	16.800±1.70	17.280±2.08	0.864
Total bilirubin (mg L^{-1})	0.230±0.40	0.319±0.45	0.182
Urea (mg dL^{-1})	45.370±3.25	44.130±3.25	0.7950

[a] and [b] means with different superscripts are significant (p<0.01) difference

Table 3: Effect of EFE supplementation on milk yield and composition, milk content yield and feed conversion

Items	Control (Mean value)	EFE (Mean value)	±SE	p-value
Feed intake				
Dry matter (kg day^{-1})	24.16	24.09	0.300	0.870
TDN (kg day^{-1})	14.90	14.86	0.185	0.869
CP (kg day^{-1})	4.601	4.587	0.057	0.870
NEL(Mcal day^{-1})	38.42	38.31	0.478	0.870
Milk yield (kg day^{-1})				
Actual milk yield (kg day^{-1})	25.22	28.07	0.556	0.0005
4% FCM (kg day^{-1})	22.64	27.39	0.523	<0.0001
ECM (kg day^{-1})	24.61	29.44	0.565	<0.0001
Milk composition (%)				
Fat	3.32[b]	3.84[a]	0.23	0.01
Protein	3.04	3.12	0.20	0.24
Lactose	4.95	5.01	0.32	0.24
TS	11.87	12.48	0.56	0.21
SNF	8.55	8.64	0.48	0.21
Milk content yield (g day^{-1})				
Fat	837.16	1077.71	20.052	<0.001
Protein	766.56	875.63	17.15	<0.001
Lactose	1248.18	1406.06	27.72	0.001
SNF	2155.96	2424.84	47.83	0.001
TS	2993.13	3502.54	67.85	<0.001
Feed conversion				
Dry matter (kg kg^{-1} FCM milk)	1.092	0.896	0.0214	<0.001
TDN (kg kg^{-1} FC M milk)	0.672	0.553	0.0131	<0.001
CP (g kg^{-1} FC M milk)	208.57	170.610	4.11	<0.001
NEL	1.736	1.424	0.034	<0.001
Nitrogen efficiency of utilization*	16.32	18.700	0.365	<0.001

*Nitrogen efficiency of utilization: Nitrogen in feeds (g)/nitrogen in milk (g) 100

Effect of adding EFE on feed intake, milk yield, milk composition and feed conversion: Data in Table 3 indicated insignificant differences in feed intake as DM, TDN, CP and NEL between the two experimental groups. Similar trends were observed by Lewis *et al.* (1999), Ahn *et al.* (2003) and Arriola *et al.* (2011), who reported that supplementing dairy cow diets with fibrolytic enzyme not enhance DMI and no significant difference was found between cows fed supplemented diet or un-supplemented diet. 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 (Ware and Zinn, 2005; El-Bordeny *et al.*, 2010).

Adding EFE to lactating cows ration increased (p>0.01) actual milk yield, 4% FCM and ECM by 11.30, 20.89 and 19.63%, respectively compared to the cows fed not supplemented rations (Table 3). The increase in blood glucose and total protein concentration for group supplemented with EFE compared to control group (Table 2) suggest that increased milk yield was due to EFE application in dairy cows rations. Blume *et al.* (1983) reported that total protein, globulin, cholesterol and phospholipids were positively, correlated with actual or corrected milk yield during several periods of lactation.

Significant increase ($p<0.05$) was noticed in milk fat content for group supplemented with EFE compared to the control group (Table 3). The increase in milk fat of cows fed ration supplemented with EFE was expected, which the main effect of the exogenous fibrolytic enzyme is increase fiber digestibility which led to increase acetic proportion (Rode *et al.*, 1999; Giraldo *et al.*, 2008), consequently increase milk fat synthesis. Moreover, Stokes (1992) postulated that the increase in fat percentage may be due to the increase in available energy and fatty acids for fat synthesis, when adding fibrolytic enzymes. The present results agree with findings of El-Bordeny *et al.* (2010) found that adding EFE to lactating buffalo's rations resulted in increase milk fat, protein and solid not fat contents compared to control.

On the other hand, insignificant effects were noticed in protein, lactose, total solid and solid not fat contents due to EFE supplementation. Also, Knowlton *et al.* (2002), Elwakeel *et al.* (2007) and Reddish and Kung Jr. (2007) noticed that there was no significant effect for fibrolytic enzymes (xylanase and cellulase) supplementation on milk composition of lactating cow.

Furthermore significant increase ($p<0.05$) in total fat yield, total protein yield, total lactose yield, total solid yield and solid not fat yield at rate of 28.73, 14.23, 12.65, 12.47 and 17.02, respectively for supplemented group compared to control group (Table 3). These may be due to the significant increase in actual milk yield and milk fat contents, parallel to the insignificant differences between the two experimental groups in protein, lactose, total solid and solid not fat contents.

The present results showed that feed conversion as DM, CP, TDN and NEL per kg 4% FCM as well as nitrogen efficiency utilization were significantly improved ($p<0.01$) for cows fed ration supplemented with EFE compared with those fed rations not supplemented (Table 3). These may be attributed to the higher actual milk yield and FCM yield recorded for supplemented group compared to not supplement one. In addition, the improvement in feed conversion efficiency observed for the cows fed ration supplemented with EFE might be attributable to greater NDF digestibility in the rumen (Holtshausen *et al.*, 2011).

Effect of stage of lactation on productive response of dairy cows to EFE supplementation: The productive response of dairy cows to EFE supplementation was greatly affected by stage of lactation. The cows fed rations supplemented with EFE were significantly higher ($p<0.05$) actual milk yield than the control group during early and mid lactation by 5.37 and 12.68%, respectively, while the cows fed supplemented rations during late lactation produced 3.84% more ($p>0.05$) actual milk yield than those fed control ration (Table 4 and Fig. 1). Also the same trends were observed for 4% FCM and ECM yield. These results may be due to animal energy balance status, which it is negative at early lactation and balanced at mid lactation and EFE application improved the available energy (Lewis *et al.*, 1999), consequently, the extra energy supply led to improve milk production, while, the energy balance is positive at late lactation and any extra of energy supply get benefit for body reserves, especially during transitional period (De Frain *et al.*, 2005).

The main effect of enzyme addition was affect milk yield and the interaction between stage of lactation and enzyme treatment was not significant ($p<0.25$). This means that the direction of the

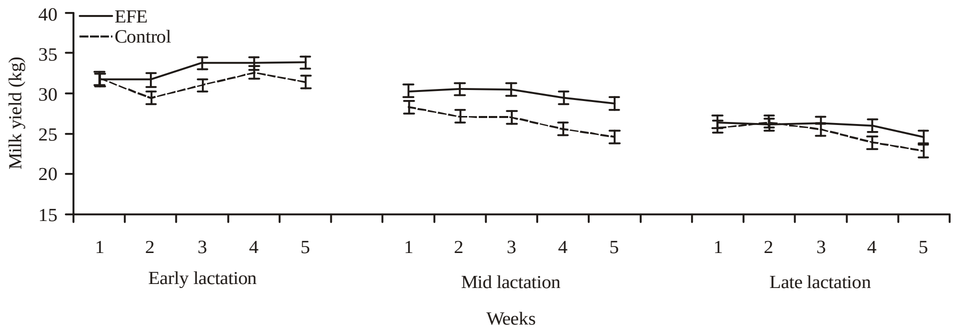

Fig. 1: Effect of EFE supplementation to dairy cows ration on milk production at different stage of lactation

Table 4: Effect of lactation stage on productive response of dairy cows fed rations supplemented with EFE

	Early lactation		Mid lactation		Late lactation			p-value		
Parameters	Control	EFE	Control	EFE	Control	EFE	SEm[1]	Trt	P	Trt*P
Milk yield (kg day^{-1})	31.25	32.92	26.52	29.89	24.92	25.88	1.55	0.039	0.004	0.249
4% FCM (kg day^{-1})	28.06	32.13	23.82	29.17	22.38	25.26	1.51	0.001	0.004	0.214
ECM	30.49	34.53	25.88	31.35	24.32	27.15	1.62	0.001	0.004	0.218

[1]Due to unequal n, largest SEm (n = 12) reported

Table 5: Effect of EFE supplementation on milk curve slope (B-value) at different lactation stages

	B-value (curve slope)		Significantly	
Stage	Control	EFE	T value	T table at 0.05
Early lactation	0.033±0.026	0.106±0.035	0.34	1.68
Mid lactation	0.030±0.015	-0.017±0.057	0.27	1.68
Late lactation	-0.094±0.007[b]	-0.059±0.005[a]	3.75	1.65

response to enzyme treatment was different in the cows at different stage of lactation (Knowlton *et al.*, 2002). The present results agreed with Beauchemin *et al.* (1996) who reported that the greatest responses will be for ruminants fed for maximal productivity and the energy balance greatly affect dairy cow response to EFE application. Similarly, the response to exogenous enzymes was higher for dairy cattle in early lactation than for those in later lactation (Nussio *et al.*, 1997; Schingoethe *et al.*, 1999; Knowlton *et al.*, 2002).

As expected, milk yield was gradually decreased ($p<0.01$), which the higher production was noticed in cows at early lactation followed by those at mid lactation while the lowest ($p<0.01$) production was recorded in cows at late lactation (Table 4).

Concerning milk curve response at different lactation stages, data of Table 5 and Fig. 2 and 3 showed that, EFE application in dairy cows ration slightly ($p>0.05$) improve the natural increase in milk production (b value) at early lactation compared to control group group, the corresponding values of b value was b = 0.106 vs. 0.033 for EFE and control, respectively. This mean that the group fed ration supplemented with EFE tend to increase their productivity faster than the control group during early lactation consequently, increase their productivity during the whole lactation season. In dairy cows there is highly correlation between peak yield and total lactation yield.

Moreover, the data clearly indicated that no significant differences in milk curve response during mid lactation (Table 5 and Fig. 3a, b), which mean each group maintains their productivity level during this period.

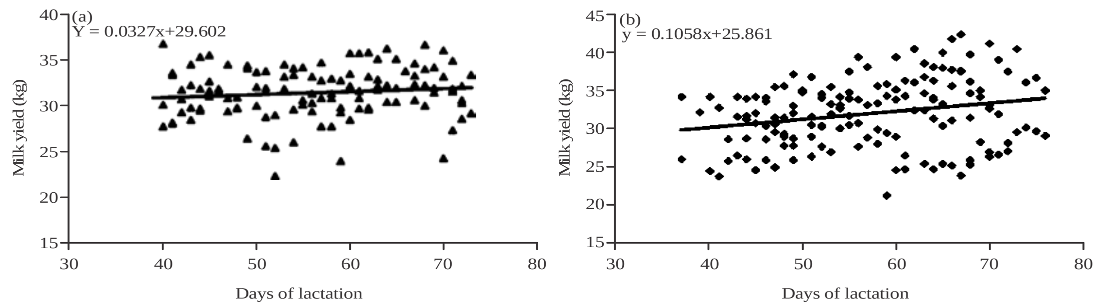

Fig. 2(a-b): Regression analysis for (a) Control group and (b) EFE group milk yield and DIM during early lactation

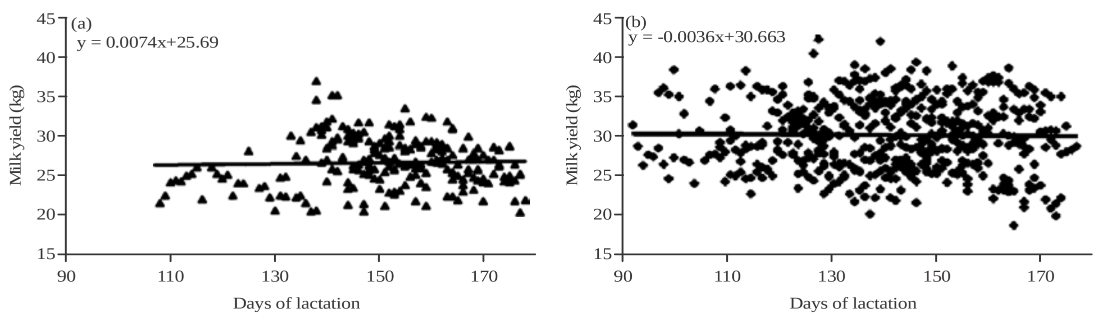

Fig. 3(a-b): Regression analysis for (a) Control group and (b) EFE group milk yield and DIM during mid lactation

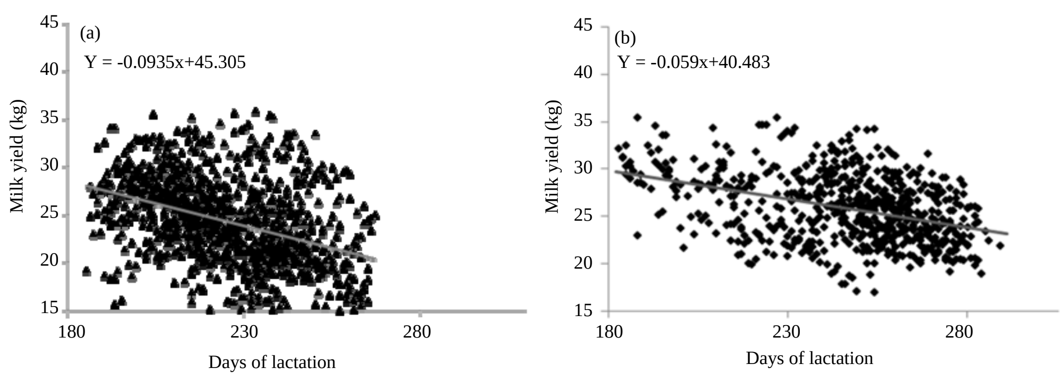

Fig. 4(a-b): Regression analysis for (a) Control group and (b) EFE group milk yield and DIM during late lactation

The greatest positive effect of EFE application in dairy cows rations was noticed at late lactation stage (Table 5 and Fig. 4a, b) where, EFE was found to play an important role in delaying the natural deterioration in milk production curve (b = -0.059 for EFE group vs -0.094 for control). Which mean that the group fed ration supplemented with EFE tend to slowly decrease their productivity than the control group.

CONCLUSION

It could be concluded that exogenous fibrolytic enzymes supplementation to dairy cows rations during early, mid and lactation has the potential to increase milk production as well as it affects milk curve response during the different stages of lactation. Using exogenous fibrolytic enzymes in dairy cattle ration has no adverse effect on cow's health and improves feed conversion as DM, CP, TDN and NEL.

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REFERENCES

AOAC., 2000. Official Methods of Analysis. 17th Edn., Association of Official Analytical Chemist, Washington, DC., USA.

Adesogan, A.T., 2005. Improving forage quality and animal performance with fibrolytic enzymes. Proceedings of the 16th Florida Ruminant Nutrition Symposium, February 1-2, 2005, Gainesville, Florida, USA., pp: 91-109.

Ahn, J.H., Y.J. Kim and H.J. Kim, 2003. Effects of fibrolytic enzyme addition on ruminal fermentation, milk yield and milk composition of dairy cows. J. Anim. Sci. Technol., 45: 131-142.

Arriola, K.G., S.C. Kim, C.R. Staples and A.T. Adesogan, 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.

Beauchemin, K.A., L.M. Rode, W.Z. Yang and T.A. McAllister, 1996. Use of Feed Enzymes in Ruminant Nutrition. In: Animal Science Research and Development-Meeting Future Challenges, Rode, L.M. (Ed.). Lethbridge Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada, ISBN-13: 9780660164991, pp: 103-131.

Beauchemin, K.A., D. Colombatto, D.P. Morgavi and W.Z. Yang, 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci., 81: E37-E47.

Belfield, A. and D.M. Goldberg, 1971. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme, 12: 561-573.

Blum, J.W., P.L. Kunz, H. Leuenberger, K. Gautschi and M. Keller, 1983. Thyroid hormones, blood plasma metabolites and haematological parameters in relationship to milk yield in dairy cows. Anim. Prod., 36: 93-104.

Burtis, A., P. Trinder and D.S. Young, 1999. Tietz Text Book of Clinical Guide to Laboratory Tests. 3rd Edn., AACC. Org., America.

Bush, B.M., 1991. Interpretation of Laboratory Results for Small Animal Clinicians. Oxford Blackwell Scientific Publications, London.

De Frain, J.M., A.R. Hippen, K.F. Kalscheur and J.M. Tricarico, 2005. Effects of dietary α-amylase on metabolism and performance of transition dairy cows. J. Dairy Sci., 88: 4405-4413.

Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta, 31: 87-96.

Duncan, D.B., 1955. Multiple range and multiple *F* tests. Biometrics, 11: 1-42.

El-Bordeny, N.E., H.M. Gado, S.M. Kholif, A.A. Abedo and T.A. Morsy, 2010. Influence of exogenous enzyme on dairy buffaloes performance. Proceedings of the 61th Annual Meeting on EAAP, August 23-27, 2010, Herklion, Greece, pp: 1-228.

Elwakeel, E.A., E.C. Titgemeyer, B.J. Johnson, C.K. Armendariz and J.E. Shirley, 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J. Dairy Sci., 90: 5226-5236.

Gado, H.M. and A.Z.M. Salem, 2008. Influence of exogenous enzymes from anaerobic source on growth performance, digestibility, ruminal fermentation and blood metabolites in lambs fed of orange pulp silage in total mixed ration. Proceedings of the 59th Annual Meeting of the European Association for Animal Production, August 24-27, 2008, Vilnius, Lithuania, pp: 228-230.

Gado, H.M., A.Z.M. Salem, P.H. Robinson and M. Hassan, 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.

Gaines, W.L., 1928. The energy basis of measuring milk yield in dairy cows. Report No. 308, University of Illinois Agricultural Experiment Station, Urbana, IL., USA., pp: 436-438.

Giraldo, L.A., M.L. Tejido, M.J. Ranilla and M.D. Carro, 2008. Effects of exogenous fibrolytic enzymes on *in vitro* ruminal fermentation of substrates with different forage: Concentrate ratios. Anim. Feed Sci. Technol., 141: 306-325.

Henry, R.J., 1974. Clinical Chemistry, Principles and Techniques. 2nd Edn., Harper and Row, Hagerstown, MD, USA., Pages: 525.

Holtshausen, L., Y.H. Chung, H. Gerardo-Cuervo, M. Oba and K.A. Beauchemin, 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.

Knowlton, K.F., J.M. McKinney and C. Cobb, 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.

Krueger, N.A., A.T. Adesogan, C.R. Staples, W.K. Krueger, S.C. Kim, R.C. Littell and L.E. Sollenberger, 2008. Effect of method of applying fibrolytic enzymes or ammonia to Bermuda grass hay on feed intake, digestion and growth of beef steers. J. Anim. Sci., 86: 882-889.

Kumar, N., U.B. Singh and D.N. Verma, 1980. Effect of different levels of dietry protein and energy on growth of male buffalo calves. Ind. J. Anim. Sci., 15: 513-517.

Lewis, G.E., W.K. Sanchez, R.C. Treacher, W. Hunt and G.T. Pritchard, 1995. Effect of direct-fed fibrolytic enzymes on lactational performance of midlactation Holstein cows. Proc. West. Sect. Am. Soc. Anim. Sci. Can. Soc. Anim. Sci., 46: 310-313.

Lewis, G.E., W.K. Sanchez, C.W. Hunt, M.A. Guy, G.T. Pritchard, B.I. Swanson and R.J. Treacher, 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. J. Dairy Sci., 82: 611-617.

NRC., 2001. Nutrient Requirements of Dairy Cattle. 7th Edn., National Academies Press, Washington, DC., USA., ISBN: 0309069971, Pages: 381.

Nussio, L.G., J.T. Huber, C.B. Theurer, C.B. Nussio and J. Santos *et al*., 1997. Influence of a cellulase/xylanase complex (C/ X) on lactational performance of dairy cows fed Alfalfa Hay (AH) based diets. J. Dairy Sci., 80: 220-220.

Patton, C.J. and S.R. Crouch, 1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464-469.

Reddish, M.A. and L. Kung Jr., 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.

Rode, L.M., W.Z. Yang and K.A. Beauchemin, 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci., 82: 2121-2126.

Rodrigues, M.A.M., P. Pinto, R.M.F. Bezerra, A.A. Dias and C.V.M. Guedes *et al*., 2008. Effect of enzyme extracts isolated from white-rot fungi on chemical composition and *in vitro* digestibility of wheat straw. Anim. Feed Sci. Technol., 141: 326-338.

SAS., 1999. SAS User's Guide. SAS Institute Inc., Cary, NC., USA.

Salem, A.Z.M., M.M. El-Adawy, H. Gado and M.S.M. Khalil, 2007. Feed intake, nutrient digestibility and animal growth performance in sheep and goats fed wheat straw. J. Anim. Sci., 8: 107-107.

Schingoethe, D.J., G.A. Stegeman and R.J. Treacher, 1999. Response of lactating dairy cows to a cellulase and xylanase enzyme mixture applied to forages at the time of feeding. J. Dairy Sci., 82: 996-1003.

Stokes, M.R., 1992. Effects of an enzyme mixture, an inoculant and their interaction on silage fermentation and dairy production. J. Dairy Sci., 75: 764-773.

Titi, H.H. and W.F. Lubbadeh, 2004. Effect of feeding cellulase enzyme on productive responses of pregnant and lactating ewes and goats. Small Rumin. Res., 52: 137-143.

Tricarico, J.M., J.D. Johnston, K.A. Dawson, K.C. Hanson, K.R. McLeod and D.L. Harmon, 2005. The effects of an *Aspergillus oryzae* extract containing alpha-amylase activity on ruminal fermentation and milk production in lactating Holstein cows. Anim. Sci., 81: 365-374.

Tyrell, H.F. and J.T. Reid, 1965. Prediction of the energy value of cows milk. J. Dairy Sci., 48: 1215-1223.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Ware, R.A. and R.A. Zinn, 2005. Influence of Maceration and fibrolytic enzyme on the feeding value of rice straw. J. Anim. Vet. Adv., 4: 387-392.

Young, D.S., 1997. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edn., AACC Press, Washington, DC., USA., ISBN-13: 9780915274888, Pages: 1285.