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Effect of supplementation of an exogenous fibrolytic enzyme mixture on the feed intake, nutrient digestibility and nitrogen balance in crossbred dairy cattle

Veterinary Science

KEYWORDS: Exogenous fibrolytic enzyme, finger millet straw, concentrate supplement

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ABSTRACT

Nine crossbred cows (132 ± 13.1 DIM, 10.8 ± 0.70 kg of milk, 406 ± 14 kg of body weight) were divided into three groups of three animal each (based on comparable days in milk, milk yield and body weight) and were utilized in an 12 week feeding trial comprising of three periods of four weeks each in a switch over design. The cows in all three groups received finger millet straw (FMS) as the sole roughage and a concentrate supplement (CS). Dietary treatment included the following: 1) Control (T1) 2) exogenous fibrolytic enzyme supplemented in CS @ 8g/animal/day (T2) and 3) exogenous fibrolytic enzyme supplemented in CS @ 12g/animal/day (T3). There was no difference in the digestibility of DM, OM, CP, NDF and ADF between the control and the EFE supplemented groups. All the cows were in positive nitrogen balance. EFE supplementation @ 8 and 12g/animal/day failed in improving digestibility of nutrients in crossbred dairy cattle.

Introduction

Cereal and millet crop residues are the major feed sources for dairy cattle in India as well as in most of the developing countries. The high lignin and fiber content makes these crop residues less digestible thus limiting animal performance. The forage degradability can be improved by various physical, chemical and biological treatments. The use of fibrolytic enzymes to enhance the quality and digestibility of fibrous forage is one such area which can benefit to ruminant production system. The effectiveness of EFE is influenced by many factors such as type and dose of enzyme and type of diet fed to animals (Adesogan et al., 2014). Therefore enzyme should be tested on different forages at different doses to identify the optimum enzyme dose for particular forage. Enzyme application to diet in vitro improved digestion of dry matter (DM) and neutral detergent fiber (NDF) suggesting that applying fibrolytic enzymes to feed may enhance digestion of forage by cattle (Feng et al., 1996). However, results of application of fibrolytic enzymes to ruminant diets have been variable. Some studies showed that enzyme addition increased digestibility of DM, organic matter (OM), NDF and acid detergent fiber (ADF) in dairy cattle (Beauchemin et al., 1999; Yang et al., 2000; Bowman et al., 2002) whereas others did not (Lewis et al., 1999; Yang et al., 1999; Kung et al., 2002; Knowlton et al., 2002). These discrepancies may be due to differences in enzyme activity, application rate, stage of lactation of dairy cows, ruminal activity and stability of enzymes (Adesogan, 2005). Hence, the present study was carried out to evaluate the effect of adding EFE on the intake and digestibility of nutrients in lactating cows.

Material and methods

Nine crossbred cows (132 ± 13.1 DIM, 10.8 ± 0.70 kg of milk, 406 ± 14 kg of body weight) were divided into three groups of three animal each (based on comparable days in milk, milk yield and body weight) and were utilized in an 12 week feeding trial comprising of three periods of four weeks each in a switch over design. The cows in all three groups received finger millet straw (FMS) as the sole roughage and a concentrate supplement (CS). Dietary treatment included the following: 1) Control (T1) 2) exogenous fibrolytic enzyme supplemented in CS @ 8g/animal/day (T2) and 3) exogenous fibrolytic enzyme supplemented in CS @ 12g/animal/day (T3). The ingredient composition of CS is presented in Table 1. The exogenous fibrolytic enzyme (Fibromase™) used in the study was procured from Alltech Inc., Nicholasville, KY, USA and contained fermentation extracts and solubles from *Aspergillus niger* and *Trichoderma longibrachiatum* and xylanolytic activity of 100 IU of xylanase/g of product, as indicated by the manufacturer. The cows were housed in individual stalls, in an open type protected shed in a single row and were provided with uniform management care. The cows were offered adequate FMS (7 kg /cow/day). The left over were weighed on the next day morning to obtain the estimate of intake. The fibrolytic enzyme was added directly to the concentrate supplement (8g/cow/day-T2 and 12g/cow/day-T3) daily just before feeding as per the plan described above. The daily allowance of concentrate supplement for individual cows for maintenance and milk yield was calculated based on the previous weeks' milk yield, milk fat content, body weight change and FMS intake. The CS was fed in three equal parts at 5.30 a.m., 2.00 p.m. and 5.30 p.m. The cows were allowed to have free access to water at 8.30 a.m. and 3.00 p.m. The feeding trial lasted for a total period of 12 weeks. A metabolism study was conducted for 5 days at the end of each period during which daily intake of feed and output of dung and urine were recorded. The dung and urine from each cow was collected manually as and when voided. One hundredth of the total weight of dung voided by each animal every day was weighed on a tray and dried at 60 °C to a constant weight (72 h) for DM estimation. The dried samples were pooled and ground through 1mm sieve and preserved for analysis except for nitrogen. For nitrogen estimation, one 1000^{th} of weight of dung voided by individual animal was weighed into 500 ml wide mouth polypropylene bottles. Five days aliquot was added to the same bottles so as to make one pooled sample for each animal and stored at -20 °C in a deep freezer for further analysis. One hundred milliliters of urine was taken to the laboratory from total voided urine of individual animal every day for sub-sampling to determine nitrogen. One 200^{th} of the total urine (undiluted) voided each day was measured separately into 250 ml polypropylene bottle for the determination of nitrogen content. The samples were preserved at -20 °C. Five days aliquots of urine sample for nitrogen estimation was added to the respective bottle to make one pooled sample for each animal. Urine samples stored at -20° C for N-determination were thawed to room temperature and 5 ml was taken to determine nitrogen by using Kjeldhal method.

The pooled and ground dung samples of individual animals were subjected to proximate analysis (AOAC, 1995) except for crude fiber and ether extract. NDF and ADF were determined according to Goering and Van Soest (1970). Nitrogen in the dung slurry was determined by Kjeldhal method. Milk sample was analysed for nitrogen (Kjeldhal method). The digestibility of nutrients was calculated as the difference between nutrient intake and nutrient outgo.

The samples of FMS and CS were analyzed for proximate composition (AOAC, 1995) except for crude fiber. Fiber fractions (NDF, ADF and ADL) were determined as per the method described by Goering and Van Soest (1970). The NDF in concentrate supplements was estimated by the method of Van Soest and Robertson (1991) using amylase enzyme.

The energy content (ME) of FMS and CS was estimated by rumen *in vitro* gas production technique (RIVIGP-24) test according to Menke and Steingass (1988). Data were analyzed using the mixed model procedure of SAS software (SAS Institute Inc., Version 9.1., 2004, Cary, NY, USA) and the treatment means were compared using the Tukey's HSD.

Results and discussion

Chemical composition of FMS and CS is presented in Table 2. The crude protein (%) and metabolizable energy (MJ/kg DM) content of the FMS and CS were 3.44, 7.37 and 17.9, 12.25 respectively. The chemical composition of FMS and CS were similar to the values reported in earlier studies (Kiran and Krishnamoorthy, 2007., Tang et al 2008).

The mean total DMI (kg/day) for T1, T2 and T3 groups was 12.02, 11.98 and 12.19, respectively (Table 3). The finger millet straw was offered *ad libitum* while CS was offered in calculated quantities to meet the total requirement of nutrients to the individual cows. Enzyme supplementation did not influence significantly the DMI both from roughage and CS which clearly indicated that 8 and 12 g level of enzyme supplementation failed to improve DMI. Similarly, intake of nutrient- OM, CP, NDF and ADF was also statistically not significant among groups and fibrolytic enzymes could not increase intake of any nutrient. The results are in agreement with those of Elwakeel *et al.* (2007); Arriola *et al.* (2011); Bernard *et al.* (2010) and Dean *et al.* (2013) who reported no improvement in DM and nutrient intake on supplementation of enzyme. On the contrary Beauchemin *et al.* (2000) observed that adding a low or high amount of an enzyme supplement to the diet increased ($P < 0.01$) DMI and also tended to increase intake of NDF ($P = 0.17$) and ADF ($P = 0.14$). These increases were relatively small (20 and 8% for NDF and ADF, respectively) and they may be attributable to increased palatability or rate of passage.

The mean digestibility (g/kg) in T1, T2 and T3 groups is presented in Table 4. There was no significant difference among treatments in DM, OM, CP, NDF and ADF digestibility. The results are in agreement with those obtained by Hristov *et al.* (1998), Lewis *et al.* (1999), Knowlton *et al.* (2002), Elwakeel *et al.* (2007) and Arriola *et al.* (2011) who also found no improvement in nutrient digestibility when EFE supplemented diets were fed to cows in early or late lactation. However, Rode *et al.* (1999) observed significant improvement in NDF digestibility of a total mixed ration (TMR) consisting of corn silage (24%), alfalfa hay (15%) and concentrate (47%) when supplemented with an EFE. Similarly, Beauchemin *et al.* (1999) reported that applying EFE to the TMR before feeding increased digestibility in the total tract due to greater post-ruminal digestion. In the present study marginal improvement in NDF and ADF digestibility was noticed for T2 and T3 groups when compared to control group which indicated that the level of enzyme might not be sufficient to bring any improvement. Other possible reasons might be lower activity of enzyme preparation used in this study when compared to other studies.

The nitrogen intake, nitrogen out go in dung, urine, milk and nitrogen retained (g/day) in T1, T2 and T3 groups are presented in Table 4. All animals were on positive nitrogen balance with no significant difference with respect to nitrogen intake, outgo and balance among three groups. The results are in agreement with Knowlton *et al.* (2002) who reported no significant difference in nitrogen out go in dung, urine, milk and nitrogen retained in cows fed with EFE.

The percent digestible crude protein (DCP) and digestible organic matter in dry matter (DOMD) of the experimental diets in T1, T2 and T3 groups were not statistically significant. However, the level was sufficient enough to meet the requirement for maintenance and milk production.

In the present study 8 and 12g EFE/cow /day failed to bring any improvement in the performance of lactating cows in terms of DMI and nutrient digestibility. Substrate degradability is a function of rate of passage and retention time of the diet in the rumen. Unlike in *in vitro* studies the forage/diet fed to the ruminant passes through the different compartments of the digestive tract during the process of digestion. Retention time is a crucial factor for the effective degradation of forage/diet. The level of enzyme used in the present *in vivo* study (8 and 12 g/cow/day) with respect to rumen retention time might not have been sufficient enough in bringing about the desirable effect in improving the digestibility of nutrients.

Conclusion

Exogenous fibrolytic enzyme supplementation @ 8 and 12g/animal/day failed to bring improvement in digestibility of nutrients in crossbred dairy cattle.

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Table 1 Ingredient composition (per cent) of concentrate supplement used during experimental trial

Ingredients	Parts
Maize	50
Wheat bran	45
Mineral mixture	2
Salt	1
Urea	2

Table 2. Chemical composition (% on DMB), *in vitro* gas production at 24 h (GP-24, ml/g DM) and predicted metabolizable energy (ME, MJ/kg DM) of finger millet straw (FMS) and concentrate supplement (CS) used in the study.

Parameter	FMS	CS
Dry matter	90.0	89.6
Organic matter	91.8	93.3
Crude protein	3.44	17.9
Ether extract	1.12	2.45
Total ash	8.20	6.70
Neutral detergent fiber	65.67	34.57
Acid detergent fiber	36.69	11.17
Acid detergent lignin	5.20	4.60
Gas production-24 h	182.0	309.0
Metabolizable energy	7.37	12.25

Table 3 Effect of exogenous fibrolytic enzyme on daily intake of dry matter and nutrients in lactating cows

Parameter	T 1	T 2	T 3	P
FMS (kg/d)	5.55 ± 0.14	5.51 ± 0.17	5.68 ± 0.10	0.69
% of body weight	1.34 ± 0.04	1.34 ± 0.06	1.37 ± 0.04	0.90
CS (kg/d)	6.47 ± 0.08	6.47 ± 0.08	6.52 ± 0.07	0.86
% of body weight	1.56 ± 0.03	1.57 ± 0.03	1.57 ± 0.03	0.97
Total DMI (kg/d)	12.02 ± 0.12	11.98 ± 0.10	12.19 ± 0.11	0.39
% Body weight	2.92 ± 0.06	2.93 ± 0.08	2.96 ± 0.07	0.92
OM (kg/d)	11.13 ± 0.11	11.09 ± 0.09	11.29 ± 0.11	0.39
% of body weight	2.70 ± 0.06	2.70 ± 0.08	2.73 ± 0.06	0.92
CP (kg/d)	1.35 ± 0.01	1.35 ± 0.01	1.36 ± 0.01	0.63
% of body weight	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.96
NDF (kg/d)	5.88 ± 0.08	5.85 ± 0.08	5.92 ± 0.10	0.87

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% of body weight	1.42 ± 0.03	1.42 ± 0.05	1.43 ± 0.03	0.99
ADF (kg/d)	2.76 ± 0.04	2.74 ± 0.05	2.81 ± 0.04	0.59
% of body weight	0.67 ± 0.02	0.67 ± 0.02	0.68 ± 0.02	0.90

Mean values between the different treatment groups for all parameters do not differ significantly.

T1- Control
T2- 8g enzyme/cow/day
T3- 12g enzyme/cow/day

Table 4 Effect of exogenous fibrolytic enzyme on digestibility of nutrients (g/kg), nitrogen balance and nutrient density of diet during metabolism trial

Parameter	T 1	T 2	T 3	P
Digestibility (g/kg)				
DM	595.4 ± 9.6	589.4 ± 14.4	601.8 ± 9.9	0.75
OM	619.6 ± 8.8	612.7 ± 13.5	624.8 ± 9.1	0.72
CP	618.6 ± 14.3	621.1 ± 16.1	637.3 ± 11.6	0.60
NDF	499.8 ± 11.8	512.6 ± 8.1	523.8 ± 11.4	0.29
ADF	415.5 ± 11.7	425.8 ± 20.0	428.9 ± 15.4	0.82
N balance (g/d)				
In take	218.2 ± 1.8	219.7 ± 1.9	222.7 ± 2.3	0.31
In dung	83.2 ± 3.2	82.7 ± 3.4	79.7 ± 2.7	0.70
In urine	63.6 ± 4.8	63.9 ± 3.4	68.1 ± 3.5	0.68
In milk	47.6 ± 2.7	49.2 ± 2.9	49.5 ± 1.7	0.85
N Retained	23.8 ± 6.7	23.9 ± 7.8	25.3 ± 3.9	0.98
DCP	6.95 ± 0.17	7.01 ± 0.21	7.13 ± 0.16	0.78
DOMD	58.7 ± 0.28	58.8 ± 0.63	59.2 ± 0.33	0.68

Mean values between the different treatment groups for all parameters do not differ significantly.

T1- Control
T2- 8 g enzyme/cow/day
T3- 12 g enzyme/cow/day

References

1. A.O.A.C., 1995. Official Methods of Analysis, Association of Official Analytical Chemists, 15th Edition, Washington D.C.
2. Adesogan, A.T., 2005. Improving forage quality and animal performance with fibrolytic enzymes. In: Proceedings of 16th Annual Florida Ruminant Nutrition Symposium, Gainesville, pp. 91-109.
3. Adesogan, A.T., Ma, Z.X., Romero, J.J. and Arriola, K.G., 2014. Improving cell wall digestion and animal performance with fibrolytic enzymes. J. Anim. Sci, 92: 1317–1330.
4. Arriola, K.G., Kim, S.C., Staples, C.R.. and 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.
5. Beauchemin, K.A., Yang, W.Z. and Rode, L.M., 1999. Effect of grain source and enzyme additive on site and extent of nutrient digestion in dairy cows. J. Dairy Sci., 82:378–390
6. Beauchemin, K.A., Rode, L.M., Maekawa, M., Morgavi, D.P. and Kampen, R., 2000. Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. J. Dairy Sci., 83:543–553.
7. Bernard, J.K., Castro, J.J., Mullis, N.A., Adesogan, A.T., West, J.W. 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. J. Dairy Sci., 93:5280–5285.
8. Dean, D.B., Staples, C.R., Littell, R.C., Kim, S.C. and Adesogan, A.T., 2013. Effect of method of adding a fibrolytic enzyme to dairy cow diets on feed intake digestibility, milk production, ruminal fermentation, and blood metabolites. Anim. Nutr. Feed Techn., 13:287-302.
9. Elwakeel, E. A., Titgemeyer, E. C., Johnson, B. J., Armendariz, C. K. and Shirley, J. E., 2007. Fibrolytic Enzymes to increase the nutritive value of dairy feedstuffs J. Dairy Sci. 90 (11):5226-5236.
10. Feng, P.C., Hunt, W., Pitchard, G.T. and Julien, D.W.E., 1996. Effect of enzyme preparations on in situ and in vitro digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci., 74:1349-1357.
11. Goering, H. K. and Van Soest, P. J., 1970. Forage Fiber Analysis. Agricultural Handbook. Agricultural Research Services, US Dept. of Agriculture. Washington, D.C.
12. Hristov, A.N., MCallister, T.A. and Cheng, K.J., 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide- degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci.,76:3146–3156
13. Kiran , D. and Krishnamoorthy, U., 2007. Rumen fermentation and microbial biomass synthesis indices of tropical feedstuffs determined by the in vitro gas production technique. Ani. Feed Sci and Tech. 134 :170–179.
14. Knowlton, K.F., McKinney, J.M. and 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
15. Kung Jr, L., Cohen, M. A., Rode, L. M. and Treacher, R. J., 2002. The effect of fibrolytic enzymes sprayed onto forages and fed in a total mixed ratio to lactating dairy cows. J. Dairy Sci., 85:2396-2402.
16. Lewis, G. E., Sanchez, W.K., Hunt, C.W., Guy, M.A., Pritchard, G.T., Swanson, B.I. and Treacher, R.J., 1999. Effect of direct-fed fibrolytic enzymes on the lactational performance of dairy cows. J. Dairy Sci., 82:611–617.
17. Menke, K. H. and Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Devpt., 28: 7-55.
18. Rode, L.M., Yang, W.Z. and Beauchemin, K.A., 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci., 82:2121-2126.
SAS Institute, 2004. The SAS system for Microsoft windows, release 9.1.2, SAS institute Inc., Cary, NC.
19. Tang, S.X., Tayo, G. O., Tan, Z. L., Sun, Z. H., Shen, L. X., Zhou, C. S., Xiao, W. J., Ren, G. P., Han, X. F. and Shen, S. B., 2008. Effects of yeast culture and fibrolytic enzyme supplementation on in vitro fermentation characteristics of low-quality cereal straws. J. Anim. Sci. 86:1164–1172.
20. Van Soest, P.J. and Robertson, J.B., 1991. Methods of dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.
21. Yang, W.Z., Beauchemin, K.A. and Rode, L.M., 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci., 82:391–403.