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Short Communication

Effect of an Exogenous Phytase on Growth Performance in Growing Holstein Calves

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ABSTRACT

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The aim of this study was to evaluate the effect on the productive performance, apparent digestibility of DM, serum phosphorus and phosphorus balance in Holstein calves which received a high concentrate diet with different doses of exogenous phytase for a period of 60d. The experiment was conducted with a completely randomized design with three treatments of eight calves each, the treatment included dietary supplementation of exogenous phytase at 0, 12 and 24 g/ton. Phytase inclusion increased ($P<0.05$) average daily gain when phytase was supplemented at 24 (850g) as compared to 0 (816g) or 12 (809g) g/ton. However, it had no effect ($P>0.05$) on DM intake, feed conversion and DM digestibility. Adding phytase in the diet decreased phosphorus excretion in faeces ($P<0.05$) showing a significant linear increase with increasing level of supplementation (the values being 9.96, 9.14 and 8.13 g/d) which, in turn, increased the P retention (4.69, 5.37 and 6.45 g/d, resectively, for the three groups). In conclusion, supplementation of phytase could improve the growth performance of calves without any discernible effects on feed intake or digestibility of nutrients.

Key words: Calves, Growth, Holstein, Phytase.

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INTRODUCTION

Since the late 1980s exogenous enzyme supplementation of animal feed has been practiced successfully. Initial investigations showed that enzymes improve nutritional value of several ingredients such as barley, wheat, rye and triticale. Recent studies have associated enzymes for animal feed with environmental and welfare benefits. By 2000 about 60 enzyme products had been authorized in the European Union (EU) as food additives, and around 40 of them have reached absolute EU approval (Brufau *et al.*, 2006).

Phosphorus pollution due to intensive livestock production has become a growing concern in recent years. Much of this pollution is caused by the inefficiency of animals to metabolize phytate. Phytic acid salts or phytate are the main storage form of phosphorus in plants seeds and the main source of phosphorus in grain based diets used in intensive livestock production. Ruminants can use phytate as phosphorus source which is undigestible for monogastric animals and phosphorus excess is excreted in the faeces. Phosphorus rich manure application to arable lands has contributed to eutrophication of surface water in areas with high density of livestock production. On the other hand, phytate has anti-nutritional properties and forms complexes with divalent cations and proteins that may interfere with digestion (Nakashima *et al.*, 2007). Rumen habitat represents a source of highly active phytases which has not been extensively studied as yet. Ruminants capability to metabolize phytate has been attributed to the microbes that live in the rumen. It has been known that rumen microbial communities produce a variety of hydrolytic enzymes including celluloses, xylenes, proteases and amylases. However, phytases diversity and phytase producing bacteria in the rumen has been examined only recently (Nakashima *et al.*, 2007).

Limited information is available on the effect of phytase supplementation on ruminant performance, especially during young age. The aim of this study was to evaluate the effect of exogenous phytase on growth performance of growing Holstein calves.

MATERIALS AND METHODS

Twenty intact Holstein calves, initial body weight of 145±7.39 kg were used according to a completely randomized design with three treatments and eight replications, considering each calf as experimental unit. Phytase was supplemented to the diet at 0, 12 and 24g of phytase (FINASE®, AB Enzymes, from *Trichoderma reesei*; 40,000 FTU g^{-1}) per ton of feed (Table 1). Calves were housed in individual cages. Feed and water was provided *ad libitum* for 60d after 15d of adaptation to a diet (16% of CP and 3.3 Mcal of ME/kg) composed of corn stover (9%), oat straw (9%), sorghum grain (70%), molasses (10%) and rea (2%).

Samples of diet and faeces were analysed for DM, OM, N and ash as per AOAC (1990) NDF, ADF as per Van Soest *et al.* (1991) and P according to Fiske and Subbarow (1925).

Dry matter intake (DMI) was measured by recording the feed offered and residues left. Body weight of individual calves was recorded at fortnightly intervals before feeding and watering in the morning. Average daily gain and feed conversion was calculated. Apparent digestibility of dry matter was calculated using the formula proposed by Merchen (1988).

Faecal samples collected at 72h on the day 15, 30, 45 and 60 of the experiment were analysed for phosphorus (faecal P) by collecting 10% of faecal void which were further mixed, dried and ground. Urine samples were taken by direct collection on the day 15, 30, 45 and 60 of the experiment for P determination according to Fiske and Subbarow (1925).

Data were analysed according to a completely randomized with three treatments in order to assess the effect of time. The MIXED procedure of SAS v 9.2 (1998) was used according to Littell *et al.* (1998) and Wang and Goonewardene (2004) and treatment means were compared using Tukey's test. The statistical model was as follows:

$$Y_{ijk} = \mu + \delta_i + d_{ij} + t_k + (\delta t)_{ij} + \varepsilon_{ijk}$$

Where, Y_{ijk} = is the value measured; μ = general mean; δ_i = fixed effect of the treatment (i = 1-3); d_{ij} = associated effect (random effect) of Calves within each treatment (J = 1-8); t_k = fixed effect of the experimental period (k = 1-5). $(\delta t)_{ik}$ = interaction effect of treatment with experimental period; ε_{ij} = is the random error.

RESULTS AND DISCUSSION

Phytase inclusion increased ($P < 0.05$) ADG; however, there were no differences ($P > 0.05$) in DM intake, feed conversion and DM digestibility (Table 1). This might be presumably due to saturation in capability to hydrolyze the substrate as reported by Godoy and Meschy (2001). Besides the biochemical characteristics (pH, thermostability and capability to release P) of phytase (Yin *et al.*, 2007), they exert a direct or indirect effect in the relation to composition of the diet, intake, viscosity, intestinal transit time and intestinal microclimate, including interactions with enteropathogenic and ruminal microflora (Brufau *et al.*, 2006). In addition, recent reports demonstrate the importance of hydrogen bonding and ionic interactions of phytases for thermo stability (Vogt *et al.*, 1997; Lebbink *et al.*, 1999; Leggio *et al.*, 1999), although they were not evaluated in this study.

Phytase activity might be affected by genetic and nutritional factors (Murry *et al.*, 1997), as well as the proportion of inorganic phosphorus, Ca:P ratio and fiber (Salle and Ravindran, 2007), so that there are differences in the ability to use phytate phosphorus between species and breeds (Pizzani *et al.*, 2008). Phytates are hydrolyzed in the intestinal tract by mucosal cell phytases but also by enzymes present in the microflora (Wise and Gilburt, 1982), which determines that differences between species and ages are due to bacterial phytase activity (Pizzani *et al.*, 2008).

Table 1. Effect of phytase supplementation on growth performance in growing calves

	Phytase (g/ton)			SEM
	0	12	24	
Average daily gain, g	816[b]	809[b]	850[a]	0.197
DM intake, g/d	4442	4370	4428	0.210
Feed conversion	5.44	5.40	5.21	0.087
DM digestibility, %	69.30	68.52	70.64	1.682
Serum P	8.34	7.79	8.09	0.026

[a,b]Means in a row with different literal differ (P≤0.05); SEM=Standard error of the mean.

Table 2. Effect of phytase supplementation on P balance in growing calves

	Phytase (g/ton)			SEM
	0	12	24	
P inptake	14.65	14.97	15.05	0.063
Faecal P, g/d	9.96[a]	9.14[b]	8.13[c]	0.056
Urinary P, g/d	0.46	0.46	0.47	0.075
P retained, g/d	4.69[c]	5.37[b]	6.45[a]	0.099

[a,b]Means in a row with different literal differ (P≤0.05); SEM=Standard error of the mean

The P balance data revealed a reduction in faecal P ($P<0.05$) with increasing phytase level which, in turn was reflected in higher P retention with phytase addition. Further, both the faecal excretion as well as retention of phosphorus showed a linear relationship with increasing level of phytase. Differences in faecal P (Table 3), may be due to the fact that ruminants excrete more than 95% of P through faeces with 25 to 50% coming from microbial wastes originated from digestive fermentation. Brufau *et al*. (2006) found that faecal P was reduced (up to 60% less) upon phytase addition compared to the control diet with normal availability of P.

When releasing more P, microbial activity increased and thus apparent digestibility of DM improved. This increase in phytase activity may be due in response to higher levels of phytate in cereal grains compared with fodder (Reddy *et al.*, 1982), although DM digestibility was not modified in present study. It has been emphasized that research is needed to predict enzyme supplementation efficacy in the diet (Brufau *et al.*, 2006). Phytate degradation possibly increases absorbable P amount in small intestine. However, further studies are needed to investigate inorganic P absorption which is released from phytate in the large intestine, considering all biochemical characteristics and practical applications (Yin *et al.*, 2007).

It is concluded that the growth performance of calves could be improved by phytase supplementation. Further, exogenous phytase decreased P excretion which can contribute to the reduction in environmental pollution.

REFERENCES

AOAC, 1995. *Official Methods of Analysis*, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Brufau, J., Francesch, M. and Pérez-Vendrell, A. 2006. The use of enzymes for improve cereal diets for animal feeding. *Journal of the Science of Food and Agriculture,* 86: 1705-1713.

Duffield, T., Plaizier, J., Fairfield, A., Bagg, R., Vessie, G. and Dick, P. 2004. Comparison of techniques for measurement of rume pH in lactating dairy cows. *Journal Dairy Science,* 87: 59-66.

Fiske, C. and Subbarow Y. 1925. The colorimetric determination of phosphorus. *Journal of Biological Chemistry,* 66: 375-380.

García, E. 1981. Modificación al Sistema de Clasificación Climática de Koppen. Instituto de Geografía, Universidad Autónoma de México, México, D.F. 251 p.

Godoy, S. and Meschy F. 2001. Utilization of phytate phosphorus by rumen bacteria in a semi-continuous cultural system (Rusitec) in lactating goats feed on different forage to concentrate ratios. *Reproduction Nutrition, Development,* 41: 259-265.

INEGI. 2000. Cartas topográficas de climas de temperaturas y precipitación. Instituto Nacional de Geografía Estadística e Informática en México.

Lebbink, J., Knapp, S., Oost, J., Rice, D., Ladenstein, R. and de Vos W. 1999. Engineering the activity and stability of Thermotoga maritime glutamate dehydrogenase, II: Construction of a 16-residue ion-pair network at the subunit interface. *Journal of Molecular Biology,* 289: 357-369.

Leggio, L., Kalogiannis, S., Bhat, M. and Pickersgill, R. 1999. High resolution structure and sequence of *T. aurantiacusxylanasa* I: Implications for the evolution of thermostability in family 10 xylanases and enzymes with-barrel architecture. *Proteins,* 36: 295-306.

Littell, R., Henry, P. and Ammerman, C. 1998. Statistical analysis of repeated measures data using SAS procedures. *Journal Animal Science*, 76: 1216-1231.

Merchen, N.R. 1988. Digestión, absorción y excreción en los rumiantes. El rumiante, fisiología digestiva y nutrición (Ed. D.C. Church), *Acribia, Zaragoza, España*, pp. 191-223.

Murry, A., Lewis, R. and Amos, H. 1997. The effect of microbial phytase in a pearl millet-soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration, and bone mineral density of nursery pigs. *Journal Animal Science,* 75: 1284-1291.

Nakashima, B., McAllister, T., Sharma, R. and Selinger, L. 2007. Diversity of phytases in the rumen. *Microbial Ecology,* 53: 82-88.

Pizzani, P., Godoy, S., León, M., Rueda, E., Castañeda, M.Y. and Arias, A. 2008. Efecto de concentraciones crecientes de fósforo fítico sobre la actividad de las enzimas fitasa y fosfatasa alcalina en el epitelio intestinal de ovinos jóvenes. *Revista Científica, Facultad de Ciencias Veterinarias-LUZ,* 18: 59-64.

Reddy, N.R., Sathe, S.K. and Salunkh, D.K. 1982. Phytases in legumes and cereals. In: *Advances in Food Chemistry* (Eds. C.O. Chichester, E.M. Mrak and F. Stewart), Academic Press, New York, pp. 1-92.

Salle, P. and Ravindran, V. 2007. Microbial phytase in poultry nutrition. Review. *Animal Feed Science and Technology,* 135: 1-41.

SAS. 1998. System for Windows. SAS *User's Guide Statistics*, SAS Institute Incorporate, Cary. USA. 558 p.

Van Soest P., Robertson J. and Lewis B. 1991. Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. *Journal of Dairy Science,* 74: 3533-3597.

Vogt, G., Woell, S. and Argos, P. 1997. Protein thermal stability hydrogen bonds, and ion pairs. *Journal of Molecular Biology,* 269: 631-643.

Wang, Z. and Goonewardene, L. 2004. The use of MIXED models in the analysis of animal experiments with repeated measures data. *Canadian Journal of Animal Science,* 84: 1-11.

Wise, A. and Gilburt, D. 1982. Phytate hydrolysis by germfree and conventional rats. *Applied and Environmental Microbiology*, 43: 753-756.

Yin, Q., Zheng, Q. and Kang, X. 2007. Biochemical characteristics of phytases from fungi and the transformed micro-organism. *Animal Feed Science and Technology,* 132: 341-350.