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Short Communication

Effects of Exogenous Phytase on Growth Performance of Weaned Dorper x Pelibuey Lambs

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ABSTRACT

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The objective of this study was to evaluate the effect of an exogenous phytase on *in vitro* residual phosphorus (P) concentration and performance of 30 weaned ¾ Dorper *vs* ¼ Pelibuey lambs (12.12±1.46 kg BW). *In vitro* treatments were: 0 and 0.12 mg phytase per g of sorghum, corn gluten meal (CGM), alfalfa hay and experimental diet (70% ground sorghum grain, 16.9% CGM, 12% alfalfa hay, 1.1% calcium carbonate). There were significant ($P<0.05$) differences in the residual P concentration (%) between 0 and 0.12 mg phytase at 24h of incubation, the values being 0.086 and 0.050 for ground sorghum grain, 0.259 and 0.119 for CGM, 0.365 and 0.240 for alfalfa hay, and 0.276 and 0.240 for the mixed diet with the corresponding 48h values of 0.054 and 0.048 for ground sorghum grain, 0.178 and 0.161 for CGM, 0.198 and 0.131 for alfalfa hay, and 0.237 and 0.211 for the mixed diet. For the performance trial, 30 lambs were allotted to three different groups using complete randomised design, and were fed the experimental diet supplemented with 0, 6 or 12 g t^{-1} phytase. Parameters recorded were ADG, DM intake, feed conversion (FC), apparent DM digestibility and faecal P excretion (FPE). Phytase supplementation did not change ($P>0.05$) ADG (251, 294 and 266 g/d), DMI (905, 1119 and 975 g/d), FC (4.06, 4.37 and 3.94). However, phytase addition increased DMD (72.34, 82.54 and 82.57%) and FPE (1.01, 1.09 and 1.26 g/d). It may be concluded that apparent DM digestibility as well as faecal excretion of phosphorus were affected when an exogenous phytase was added to a 70% sorghum grain diet, fed to weaned Dorper x Pelibuey lambs.

Key words: Faecal excretion, *In vitro*, Phosphorus, Phytase, Sheep.

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INTRODUCTION

About 60 to 80% of phosphorus (P) in cereal grains and oil seeds is bound in the form of phytate, a component of little use for non-ruminants (Lott *et al.,* 2000), due to their low intestinal phytase activity. The addition of exogenous phytase in the diets would led a significant fraction of phytic P to be absorbed from the gastrointestinal tract by hydrolysis of the compound, producing inorganic orthophosphates and phosphoric esters of high bioavailability (Bravo *et al.,* 2002). The effect of phytase on the availability of P of the diet in lambs has been assayed in other studies, and the analysis of the results suggests the opportunity of reducing excretion and improving the availability of P in goats, dry and lactating cows (Kincaid *et al.,* 2005, Knowlton *et al.,* 2005; Knowlton *et al.,* 2007). This reduces the use of inorganic phosphates, and thereby environmental pollution caused by the excess excretion of P. Until the mid-1980s the excretions had a residual value as fertilizer of crop land because of their high input of N, P and other nutrients, but the intensification of production and concentration of livestock in specific areas, along with new standards of environmental conservation, has limited this pathway of excreta disposal. The amount of manure to be spread in a crop field is limited by the capability of the plants to extract minerals from the ground supplied by excreta; an excess over the needs causes environmental pollution (Knowlton *et al.,* 2004). An important proportion (50 to 80%) of N, P and K of the diet of an animal ends up excreted (Lang *et al.,* 2009). In Mexico, the majority of sheep is found in backyard or grazing, but in recent years intensive systems of production have increased wherein specie are fed with diets based on grains (Candanosa *et al.,* 2005). Therefore, the objective of this research was to evaluate the effect of exogenous phytase on *in vitro* digestibility and productive performance of early weaned sheep.

MATERIALS AND METHODS

Chemical analyses were performed in the Animal Nutrition Laboratory of the National Disciplinary Research in Physiology and Animal Breeding Center, of the National Institute of Forestry Investigations, Agriculture and Livestock.

Phytase activity was measured using *in vitro* technique. Ruminal fluid extracted from two Suffolk sheep (54.5 kg BW) fitted with ruminal cannula, receiving the control diet for 15d. Ruminal fluid was taken and placed in a thermal container, then it was filtered and mixed with McDougall's artificial saliva (pH 6.8), in a proportion of 4:1; 50 ml of this mixture was placed in polypropylene tubes (100 ml) with caps and Bunsen Tilley and Terry (1963) valves. The treatments were 0 and 0.12 mg of enzyme per g of sorghum grain, corn gluten meal, alfalfa hay and the mixed (experimental) diet; CO_2 was added to each tube, which was capped to mix their content, placed in a double boiler at 38°C, and incubated in triplicate for 24 and 48h.

Thirty lambs (¾ Dorper *vs* ¼ Pelibuey) of initial LW of 12.12±1.46 kg were fed on a diet containing 70.0% of sorghum grain, 16.9% corn gluten, 12.0% alfalfa

Table 1. Chemical composition (%) of the experimental diet

Phytase‡ (g/t diet)	Dietary groups†		
	0 (T_1)	6 (T_2)	12 (T_3)
Dry matter	88.73	88.50	88.67
Organic matter	94.45	94.19	94.43
Crude protein	18.75	18.83	18.43
NDF	19.85	19.72	19.90
ADF	8.89	8.73	8.21
Calcium	0.68	0.70	0.68
Phosphorus	0.32	0.31	0.32

‡FINASE®, AB Enzymes, from *Trichoderma reesei*; 40,000 FTU g^{-1}

†Phytase was added at 0, 6 and 12 g/ton for the diet T_1, T_2 and T_3, respectively.

hey and 1.1% of Ca carbonate. The lambs were housed in individual cages and received food and water *ad libitum*. Animals were distributed according to a completely randomized design into three treatments i.e. supplementation of 0 (T_1), 6 and 12 (T_2) g phytase (FINASE®, AB Enzymes, from *Trichoderma reesei*; 40,000 FTU g^{-1}) per ton of the experimental diet. Each treatment had ten replicates considering each lambs as experimental unit. Lambs were adapted to the experimental diet for 15d, and the test lasted 60d. The lambs were weighed at 7:00h in the morning of 0, 15, 30, 45 and 60d of experimented feeding on a digital scale (300 kg capacity; 0.05 kg precision). Productive performance variables studied were: average daily gain (ADG), DM intake (DMI), and feed conversion (FC).

Faecal samples were collected at 72h on the days 15, 30, 45 and 60d from each lamb, and 10% of the samples were mixed and dried in an oven at 55°C and ground, to calculate apparent digestibility (Merchen, 1988). Samples of the experimental diets were analysed for DM, OM, N and total ash (AOAC, 1990), NDF and ADF (Van Soest *et al.*, 1991), Ca (ISO 6490-1, 1985) and P (AOAC 1984).

The data were analyzed adopting the MIXED procedure (SAS, 2002) to evaluate the effect of time using the following model:

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

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 lambs within each treatment (J = 1-8); t_k = fixed effect of the experimental period (k = 1-5). $(\delta t)_{ik}$ = is interaction effect of treatment with experimental period; ε_{ij} = is the random error.

RESULTS AND DISCUSSION

Residual P in the incubating medium decreased ($P < 0.05$) after the *in vitro* test at 24h with the addition of exogenous phytase, for sorghum grain, corn gluten, alfalfa hay and the experimental diet. However, at 48h of incubation, the reduction in

residual P was apparent only with the alfalfa hay and the mixed diet as substrates (Table 2). The importance of exogenous phytases in feed of ruminants is related to the decrease of the anti-nutritional effects of phytic acid and the best use of phosphorus as phytates which reduces the incorporation of inorganic sources of the element in the diets, decreasing the cost of the diet and substantially diminishing environmental pollution. Reducing residual P concentration in feed samples after *in vitro* fermentation of feeds sample may be due to the increased efficiency of phytic acid hydrolysis with the addition of exogenous phytase. This suggests that, P was liberated and could be used by ruminal microorganisms. Phosphorous is essential for the development of ruminal microorganisms; it is involved in different enzymatic pathways and is particularly relevant in carbohydrate metabolism, mainly structural cellulose fermentation (Durand *et al.*, 1983).

The use of exogenous phytase did not alter ($P>0.05$) the average daily gain, DM intake or feed efficiency, while it increased ($P<0.05$) faecal P excretion and apparent digestibility of DM (Table 3).

There was no effect of phytase supplementation on DM intake by lambs. However, phytase might have facilitated release of more P and thereby increased the efficiency of DM degradation in the rumen. This was reflected in the apparent DM digestibility (Table 3) of the experimental diets, which was increased ($P\leq0.05$) in lambs fed diets supplemented with phytase. High concentrate diets reduce retention

Table 2. *In vitro* residual P concentration (%) in sorghum grain, corn gluten meal, alfalfa hay and mixed diet following 24 and 48h of incubation

Incubation period	24h		SEM	48h		SEM
Phytase (mg)	0	12		0	12	
Sorghum grain	0.086[a]	0.050[b]	0.017	0.054	0.048	0.002
Corn gluten meal	0.259[a]	0.119[b]	0.035	0.178	0.161	0.017
Alfalfa hay	0.365[a]	0.240[b]	0.016	0.198[a]	0.131[b]	0.005
Experimental diet	0.276[a]	0.240[b]	0.008	0.237[a]	0.211[b]	0.005

[ab]Means with different superscript differ ($P<0.05$). SEM: standard error of the mean.

Table 3. Average daily gain, DM intake, feed efficiency, faecal phosphorus excretion and apparent DM digestibility of lambs fed a sorghum grain based diet with phytase

	g phytase t^{-1}			SEM
	0	6	12	
Avarage daily gain (g/d)	251	294	266	0.017
DM intake (g/d)	905	1119	975	0.151
Feed effeciency	4.06	4.37	3.94	0.272
Faecal P excretion (g/d)	1.01[b]	1.09[ab]	1.26[a]	0.033
DM digestibility (%)	72.34[b]	82.54[a]	82.57[a]	1.338

[ab]Means with different superscript differ ($P\leq0.05$). SEM: standard error of the mean.

time of digesta, which could also decrease hydrolysis of phytates in the rumen (Sauvant *et al.*, 1999). But addition of phytase in the present case appeared to have negate this trend.

The use of exogenous phytase did not alter the BW gain, feed intake and feed conversion of lambs (Table 3), probably due to a saturation of its capability to hydrolyze the substrate (Godoy and Meschy, 2000). Godoy and Chicco (2004) reported ADG of 112 and 108 g, which suggests that the enzyme hydrolyzed phytate and P was available for its absorption and maintain growth similar to those obtained in lambs fed with a greater concentration of P in the diet. In calves from 1 to 6 weeks of age that consumed 0.83 kg of concentrate and 4.1 L of milk daily, it was found that phytic acid was degraded in the rumen, but phytate was not completely hydrolyzed, which may be because of the fact that the rumen was not well developed (Duskova *et al.*, 2001), and therefore, did not have enough microorganisms in the rumen to hydrolyze phytic P. The present results suggested that the same could also occur in young lambs, and giving the possibility of the use of phytase in lambs in early growth stages, but more research is needed on the subject.

Wu *et al*. (2000) indicated that faecal excretion of P is related to its intake, while Weiss and Wyatt (2004) reported that faecal excretion of P linearly increases as intake of these mineral raises. In the present study, the addition of phytase to the diet reduced the amount of P in faeces and therefore may help reducing soil and water pollution by this element. The results obtained herein suggest the possibility of adding phytase to the diet, knowing the importance of the production of protein of animal origin, but conscious of environmental protection.

It is concluded that the growth performance of lambs was not affected by phytase supplementation, however, the use of exogenous phytase improved the digestibility and decreased excretion of P which may contribute to reduced environmental pollution.

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