Response to Phytase Addition on Production Variables, and Excretion of Phosphorus in Faeces of Finishing Lambs

Germán Buendía Rodríguez[1*], María Denisse Montoya Flores[1], Ahmed F. Sayed[2], Nora Idolina Ortega Álvarez[3]

[1]Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal (CENID-FyMA), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Querétaro, México
[2]Dairy Science Department, National Research Centre, 33 Bohouth st. Dokki, Giza, Egypt
[3]Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana Unidad Xochimilco, México, D.F. México
*Corresponding author: buendia.german@inifap.gob.mx

Abstract: Phosphorus exists in grains mostly as phytate (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) which required sufficient digestive phytase enzyme to break the molecule and release P. The aim of the study was to evaluate the effect of phytase on some production variables of lambs fed with finishing diet with high sorghum content (70%). Thirty two Dorper × Pelibuey lambs (27.45 ± 2.46 kg BW) were completely randomly distributed in four treatments with 8 repetitions, housed in individual cages and fed *ad libitum* for 60 d were used. The treatments were 0, 150, 300 and 450 g phytase enzyme/ton of sorghum grain-based diet. The daily gain was increased ($P<0.05$) by the addition of phytase in feed. Feed intake and feed conversion were not affected ($P>0.05$) by treatments. However, greater faecal phosphorus was noted with lambs fed on diet without phytase addition. The inclusion of phytase increased weight gain and reduced the faecal excretion of P. The dose of 150 g phytase/ton had the best daily gain; however, the dose 300 g phytase/ton had the lowest P excretion in manure.
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1. Introduction

Recently, environmental regulations related to ruminants excretion of N and phosphorus (P) have increased constantly all over the world (Buendía *et al*., 2010, 2014a,b). About 60 to 80% of P of cereal grains and oilseeds is bound to phytic acid in the form of phytate; a component rarely used by non-ruminants (Lott *et al*., 2000) due to low phytase activity. Firstly, phytase additives were used in diets for non-ruminants to manipulate P availability and excretion; then, the exogenous phytase additives were used to reduce excretion and improve P digestibility in ruminants (Knowlton *et al*., 2007; Buendía *et al*., 2010, 2014a,b).

Phytase is the enzyme that can neutralize phytic acid and liberates the P. Incorporation of phytase in ruminant diets allows a significant fraction of P phytate exploited in the digestive tract by hydrolysis of the compound, for producing inorganic phosphoric esters and orthophosphates of high bioavailability (Bravo *et al*., 2002). The amount of P in manure is spread on a crop field, because the limited ability of plants to extract minerals from the terrain provided by animal excreta; an excess of the needs causes environmental pollution (Knowlton *et al*., 2004). A significant portion (about 50 to 80%) of P of the diet are ended in animal excreta (Lang *et al*., 2009). The importance of phytase in the feeding of ruminants relates to the ability for the removal of the antinutritional effects of phytic acid; by hydrolysis of the compound, with better utilization of phytate phosphorus content, reducing the incorporation of inorganic sources of the element in the diet, substantially reducing environmental pollution (Buendía *et al*., 2010, 2014a,b).

In Mexico, most of the sheep is found in backyards or grazing, but in recent years, the intensive production systems of sheep increased based on grains diets (Candanosa *et al*., 2005). Therefore, the objective of this research was to evaluate the effect of exogenous phytase, at different doses, on some production variables and faecal excretion of P in Dorper × Pelibuey lambs finished on a high sorghum grain diet.

2. Materials and methods

2.1. Study site

Productive performance testing and analysis of the samples were performed at the Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal (CENID-FyMA), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Querétaro, México.

2.2. Animals and treatments

In the current experiment, 32 Dorper × Pelibuey lambs with initial body weight of 27.45 ± 2.46 kg BW were distributed in a completely randomized design for four treatments with eight repetitions each, considering each lamb as an experimental unit. Four levels of

phytase (Natuphos 5000G, BASF Mexicana, México City, México) were used: 0, 150, 300 and 450 g of phytase produced by *Aspergillus niger* with 5000 FTU (phytase units)/g; 1 FTU is the amount of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37°C)/ ton of the experimental diet.

2.3. Nutritional management

Lambs were housed in individual cages and fed with the experimental diets for 60 days plus15 days of adaptation to the diet (Table 1). The diet was formulated to meet animal requirements according to NRC (1994). Lambs had free access to diet (fed at 07:00 and 16:00 h) ensuring orts of 1% of the amount fed daily, as well as to water.

2.4. Sample collection

The daily feed intake was measured by subtracting the daily refused feed of the daily offered feed. Weight gain for lambs were weighed at before morning feeding after 0, 15, 30, 45 and 60 days using a digital scale (300 kg capacity, accuracy 0.05 kg). Feed conversion was calculated with the previous two variables.

2.5. Laboratory analysis

Samples of diet were analyzed for dry matter (DM), organic matter (OM), and N according to AOAC (1995). Neutral detergent fibers (NDF) and acid detergent fibers (ADF) were determined by Van Soest *et al*. (1991) using ANKOM200 Fiber Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed with the use of an alpha amylase with sodium sulfite. Both NDF and ADF are expressed without residual ash. The concentration of P in faeces was determined as described by Fiske and Subbarow (1925).

2.6. Statistical analysis

Data of the experiment was analyzed in a completely randomized design with four treatments with evaluating the effect of time using the MIXED procedure of SAS (SAS 2002, North Carolina, USA). Orthogonal polynomials were used to test effects of phytase supplementation levels on lambs. The following model was used:

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

Where,

Y_{ijk} = response variable

μ = overall mean

δ_i = fixed effect of the *i*th treatment

d_{ij} = random effect associated with the *j*th lamb in the *i*th treatment

t_k = fixed effect of the *k*th period

$(\delta t)_{ik}$ = fixed effect of the interaction of the *i*th treatment with the *k*th period

ε_{ij} = random error associated with the *j*th lamb in the *i*th treatment and *k*th sampling time.

3. Results and discussion

The daily weight gain was greater for lambs fed diet with 150 and 450 g phytase/ton. However, the control lambs fed on 0 g phytase/ton had the lowered daily gain. The use of exogenous phytase improved lambs' weight gain and decreased the concentration of P in feces. However, feed intake and feed conversion of finishing lambs were not affected. This may be probably due to saturation of its ability to hydrolyze the substrate (Godoy and Meschy, 2000). Moreover, phytase helped phytic P release and increased weight gain and decreased phosphorus excretion in faeces. Moreover, the improved daily gain may be related with improved nutrients digestibility as reported by Buendía *et al*. (2010) who found that total tract digestibility of DM, NDF and P were linearly ($P<0.01$) increased as phytase supplementation level increased in the diet of Suffolk × Creole crossbreed male lambs fed sorghum-based diets. However, they stated unaffected body weight, average daily gain, feed intake and feed conversion by phytase supplementation. Phosphorus availability may be improved as a result of phytase supplementation, result in enhanced enzyme systems of ruminal microorganisms and fermentation kinetics of structural carbohydrates (Durand and Komisarczuk, 1988). Breves and Schroder (1991) noted that reduced P in the diet reduced cellulose digestion, microbial protein synthesis and protein degradation in the rumen. In a study on exogenous phytase on phosphorus digestibility in dairy cows, Kincaid *et al*. (2005) found that P digestibility of diets with high grain contents was improved due to phytase supplementation. The results may be explained based on the synergism occurred with phytase-producing bacteria, which are the main starch-fermenting organisms (Knowlton *et al*., 2007). Moreover, Gartner *et al*. (1982) stated that body weight gain and feed efficiency were improved by P supplementation to beef heifers diets. Shanklin (2001) stated increased nutrients digestibility in lambs fed diets supplemented with phytase exogenous enzyme, which indicates increased ruminal fluid P concentration which may have enhanced ruminal microorganism activity for these lambs.

No effects were observed for phytase supplementation in the diets of lambs on feed intake and feed conversion. Dilip (2004) noted no significant effect on dry matter intake of lactating cows fed diets containing barley and corn and supplemented with exogenous phytase.

A greater P excretion in faeces was observed with the control lambs and lambs fed diet with 450 and 150 g phytase/ton. Phosphorus is an important mineral element needed for numerous functions in the body including, bone formation, cell membrane structure, energy transfer, and structure of nucleic acids. The main source is animal diets and therefore it is presented

in manure (Satter *et al*., 2002). Eeckhout and De Paepe (1994) stated that more than 60% of the P in sorghum is found in a form of phytic acid. Dietary supplementation of animal feed with exogenous phytase can increase the availability of P from grain resulted in reduced amount of inorganic P of feeds and consequently reduces manure P concentration. The enzyme phytase releases phosphate groups from phytin result in more P available to the animal, thereby reducing P excreted from poultry and swine by 15 to 30%. However, the ruminal microbial phytase helps to increase the P availability in the rumen and increase P excretion in urine and faeces of finishing lambs when fed on a high grains in their diet (Harmon and Britton,1983). In our study, phytase reduced the P excretion in manure, which reflect better utilization of P and also not high P content in sorghum grains. However, Buendía *et al*. (2010) noted unaffected P intake and P urine and faeces excretion by phytase addition to lambs diets.

Table 1. Composition (%) of experimental diets on a dry basis.

	Phytase g/ton			
Ingredients (%)	0	150	300	450
Oat Straw	18	18	18	18
Rolled grain sorghum	70	70	70	70
Molasses	10	10	10	10
Urea	2	2	2	2
Chemical composition (%)				
Dry matter	93.72	93.75	93.76	93.71
Organic matter	96.25	96.24	96.27	96.25
Crude protein	18.28	18.28	18.27	18.27
Neutral detergent fibers	19.84	19.83	19.84	19.85
Acid detergent fibers	8.78	8.78	8.79	8.80
Ash	3.75	3.76	3.73	3.75
Calcium	0.64	0.64	0.64	0.64
Phosphorus	0.31	0.31	0.31	0.31
Phytic phosphorus	0.21	0.21	0.21	0.21

Table 2. Effect of phytase in productive variables in lambs Dorper × Pelibuey lambs in finishing stage.

Variables	Phytase (g/ton)				SEM	*P*-value	
	0	150	300	450		Linear	Quadratic
Daily weight gain							
0 day	191	222	192	192			
15 day	177	245	185	198			
30 day	165	210	188	210			
45 day	192	205	172	213			
60 day	190	198	198	192			
Average	183[b]	216[a]	187[b]	201[ab]	12.2	0.042	0.032
Dry matter intake							
0 day	1002	1250	1010	985			
15 day	1270	1080	1205	1110			
30 day	1210	987	920	1125			
45 day	1172	1292	1025	980			
60 day	901	1351	1105	1105			
Average	1111	1192	1053	1061	123.6	0.634	0.851
Feed conversion							
0 day	5.25	5.63	5.26	5.13			
15 day	7.18	4.41	6.51	5.61			
30 day	7.33	4.70	4.89	5.36			
45 day	6.10	6.30	5.96	4.60			
60 day	4.74	6.82	5.58	5.76			
Average	6.12	5.57	5.64	5.29	0.884	0.500	0.732
Fecal P (g/d)							
0 day	1.62	1.38	1.24	1.37			
15 day	1.62	1.37	1.21	1.35			
30 day	1.66	1.37	1.18	1.34			
45 day	1.64	1.37	1.20	1.37			
60 day	1.66	1.36	1.17	1.32			
Average	1.64[a]	1.37[b]	1.20[c]	1.35[b]	0.071	0.014	0.023

[a, b, c] Means in a row with different literal, are different (P < 0.05).

Conclusions

Feed intake and feed conversion of lambs were not affected by phytase; however, exogenous phytase increased daily gain and decreased excretion of faecal P, contributing the reduction of environmental pollution caused by phosphorus. The best daily gain was obtained with the dose of 150 g phytase/ton;

however, the dose 300 g phytase/ton had the lowest P excretion in manure.

Corresponding Author:
Dr. Germán Buendía Rodríguez
Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal (CENID-FyMA), Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Querétaro, México
E-mail: buendia.german@inifap.gob.mx

References

1. AOAC, (Association of Official Analytical Chemists). Official Methods of Analysis of AOAC International. 16th ed. AOAC International, Gaithersburg, MD; 1995.
2. Bravo D, Meschy F, Bogaert C, Sauvant D. Effects of fungal phytase addition, formaldehyde treatment and dietary concentrate on ruminal phosphorus availability. Anim Feed Sci Technol 2002; 99: 73–95.
3. Breves G, Schroder B. Comparative aspects of gastrointestinal phosphorus metabolism. Nutr Res Rev 1991; 4: 125-140.
4. Buendía RG, González MS, Pérez SM, Ortega NI, Aceves HC, Montoya FMD, Almaraz BI, Partida PJE, Salem AZM. Effect of an Exogenous Phytase on Growth Performance in Growing Holstein Calves. Anim Nutr Feed Technol 2014a; 14: 169-174.
5. Buendía RG, González MS, Mendoza MGD, Ortega ANI, Aceves HC, Crosby GMM, Meneses TAP, Salem AZM. Exogenous Phytase Effects on Growth Performance of Weaned Dorper X Pelibuey Lambs. Anim Nutr Feed Technol 2014b; 14: 183-188.
6. Buendía G, Mendoza G, Pinos-Rodríguez JM, González-Muñoz S, Aranda E, Miranda L, Melgoza L. Influence of supplemental phytase on growth performance, digestion and phosphorus balance of lambs fed sorghum-based diets. Ital J Anim Sci 2010; 9: 187-189.
7. Candanosa E, Mendoza M, Salcedo R. Efecto de bicarbonato de sodio y glucosa sobre la fermentación ruminal, equilibrio ácido-base y química sanguínea en borregos. Rev Científ FCV-LUZ. XV 2005; 1: 41-48.
8. Dilip KG. Effect of exogenous phytase addition to diets on phytate phosphorus digestibility in dairy cows. MSc thesis. Washington State University, USA; 2004.
9. Durand M, Komisarczuk S. Influence of major minerals on rumen microbiota. J Nutr 1988; 118:249-260.
10. Eeckhout W, De Paepe M. Total phosphorus, phytate phosphorus and phytase activity in plant feedstuffs. Anim Feed Sci Technol 1994; 47:19-29.
11. Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem 1925; 66:375-400.
12. Gartner RJW, Murphy GM, Hoey WA. Effects of induced, sub clinical phosphorus deficiency on feed intake and growth of beef heifers. J Agr Sci 1982; 98: 23-29.
13. Godoy S, Meschy F. Utilization of phytate phosphorus by rumen bacteria in a semi-continuous culture system (Rusitec) in lactating goats fed on different forage to concentrate ratios. Reprod Nutr Dev 2000; 41:259-265.
14. Harmon DL, Britton RA. Balance and urinary excretion of calcium, magnesium and phosphorus in response to high concentrate feeding and lactate infusion in lambs. J Anim Sci 1983; 57:1306-1315.
15. Kincaid RL, Garikipati DK, Nennich TD, Harrison JH. Effect of grain source and exogenous phytase on phosphorus digestibility in dairy cows. J Dairy Sci 2005; 88:2893-2902.
16. Knowlton F, Radcliffe S, Novak L, Emmerson A. Animal management to reduce phosphorus losses to the environment. J Anim Sci ; 82: E173–E195.
17. Knowlton KF, Taylor MS, Hill SR, Cobb C, Wilson KF. Manure nutrient excretion by lactating cows fed exogenous phytase and cellulose. J Dairy Sci2007; 90:4356-4360.
18. Lang D, Burcham T, Elmore R, Moore R. Sampling and application of dairy lagoon effluent. Mississippi, EEUU, Extension Service of Mississippi State University; 2009.
19. Lott I, Ockenden V, Raboy G, Batten D. Phytic acid and phosphorus in crop seeds and fruits: A global estimate. Seed Sci Res 2000; 10:11–33.
20. NRC. Nutrient Requirements of Sheep. 6th Revised edn. National Academy Press, Washington, DC, USA; 1994.
21. SAS. System for Windows. SAS User's Guide Statistics. SAS Inst. Inc. Cary. North Carolina. USA; 2002.
22. Satter LD, Klopfenstein TJ, Erickson GE. The role of nutrition in reducing nutrient output from ruminants. J Anim Sci 2002; 80: E143.
23. Shanklin RK. Effect of form and amount of phosphorus and phytase supplementation on phosphorus utilization by ruminants. MSc Thesis. Faculty of the Virginia Polytechnic Institute. State University. Blacksburg, Virginia; 2001.
24. Van Soest P, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991; 74:3583–3597.

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