EFFECT OF DIETARY PHOSPHORUS REDUCTION AND PHYTASE SUPPLEMENTATION ON GROWTH OF RABBITS

Eiben Cs.[1]*, Gippert T.[2], Gódor-Surmann K.[1], Podmaniczky B.[1], Kustos K.[3]

[1]Research Institute for Animal Breeding and Nutrition (ÁTK), Isaszegi út 200, H-2100 Gödöllő, Hungary
[2]GBT PRESS Ltd., Sportföld u. 21/F, H-2112 Veresegyház, Hungary
[3]Lab-Nyúl Ltd., Malomtó u. 8, H-2100 Gödöllő, Hungary
*Corresponding author: eiben@katki.hu

ABSTRACT

New Zealand White rabbits were used to study the influence of exogenous phytase enzyme additions at the expense of supplemental inorganic phytate on growth performance. A total of 300 rabbits aged 35 d were weaned and with respect to body weight half were housed individually or in pairs up to 77 d of age. Within each housing system, rabbits were assigned randomly to three dietary groups in a balanced manner according to body weight at weaning. A basal diet with 0.24% added inorganic monocalcium phosphate (MCP) was used in the control group (C). This feed was supplemented with 1000 FTU/kg of *Aspergillus niger* phytase (Natuphos®5000) at the expense of MCP that was reduced by 50% of the control level (i.e. from 0.24 to 0.10%) in the Phytase group (P) or not used (0% MCP) in the Phytase-MCP group (P-MCP). Total phosphorus contents of the diets were 0.58, 0.45 and 0.35%, respectively. The 35-77 d feed intake was 136, 138 and 131 g/d in the C, P and P-MCP groups, respectively (P=0.112). Feed conversion was not affected (3.30, 3.32, 3.27; P=0.592). Differences in 35-77 d weight gain (40.3, 40.2, 39.6 g/d; P=0.556) and 77-d body weight (2706, 2699, 2677 g; P=0.651) were not significant. Health risk was comparable during the fattening period (30, 26, 28%; P>0.05). Supposing lower faecal phosphorus excretions with Phytase or Phytase-MCP feeds than with the control diet (under evaluation), both enzyme supplemented diets can be promising since using them resulted in production similar to that of conventional rabbit feed.

Key words: Phosphorus, Phytase, Growth.

INTRODUCTION

Rabbit manure has been a precious by-product but recently it is considered as an environmental polluter, at least in Europe, due to its nitrogen and phosphorus content (Maertens, 1999; Maertens *et al*., 2005). The solution can be the reduction of output and/or the N and P content of rabbit faeces. Dietary N and P are not totally utilized in the rabbit but that can be improved via nutritional strategies.

A major part of the phosphorus in cereals and seeds is stored as phytate (Poulsen *et al*., 2007) that can interact with proteins and starch as well as binding elemental P and minerals, thereby reducing digestibility of these nutrients (Huang *et al*., 2006; McClung *et al*., 2006; Kim *et al*., 2007). The enzyme phytase that is present to some extent in cereals but may partly be inactivated during feed processing (Simon and Igbasan, 2002; Boyce and Walsh, 2006), catalyses the hydrolysis of phytate rendering P available for absorption (Poulsen *et al*., 2007). Ruminants effectively utilize phytate P due to rumen bacterial fermentation whilst it is difficult to digest for poultry and particularly for pigs. Rabbits are between ruminants and pigs (Gutiérrez *et al*., 2000; Falcão-e-Cunha *et al*., 2007) since phytase has been measured in the small intestine (Cooper and Gowing, 1983) and, thanks to gut bacteria, phytic acid is hydrolysed efficiently in the caecum but the absorbtion of soluble inorganic phosphates is incomplete (Marounek *et al*., 2003). It is well known that phytase addition to pig diets improves P digestibility, and thus reduces faecal P excretion (Gundel *et al*., 2004; Nitrayova *et al*., 2006; Guggenbuhl *et al*., 2007). Growth was not affected at minimum levels of recommended dietary P (Lebas *et al*., 1998; Ritskes-Hoitinga *et al*., 2004) but supplemental inorganic phosphates are generally used in rabbit diets. This is costly, in excess it can affect the bioavailability of other minerals and unutilized P in rabbit faeces increases the phosphate load of the environment. In rabbits, there are few studies about the performance and digestibility responses to dietary phytase addition compared to other livestock (Ayers *et al*., 1996; Gutiérrez *et al*., 2000; El-Adawy, 2004; Guo-Xian *et al*., 2004; Ritskes-Hoitinga *et al*., 2004).

This study compared the growth performance of rabbits fed diets supplemented with phytase at the expense of inorganic phosphate. Analyses of faecal P content are in process and not presented here.

MATERIALS AND METHODS

Dietary groups and animals

A basal diet with 0.24% added inorganic monocalcium phosphate (MCP) was used for the control group (Table 1). This feed was supplemented with 1000 FTU/kg *Aspergillus niger* phytase at the expense of MCP that was reduced by 50% of control level (i.e. from 0.24 to 0.10%) in Phytase group (P) or not used in Phytase-MCP group (P-MCP).

Table 1: Ingredients, chemical composition and nutritive value of diets

	Control (C)	P	P-MCP
Monocalcium phosphate (MCP) (%)	0.24	0.10	-
Phytase[1] (FTU/kg)	-	1000	1000
Ingredients (%):			
Alfalfa meal	34.0	34.0	34.0
Dried sugar beet pulp	12.5	12.5	12.5
Wheat grain	10.0	10.0	10.0
Full fat soybean meal	10.0	10.0	10.0
Extr. sunflower meal	8.0	8.0	8.0
Dried apple pulp	8.0	8.0	8.0
FaserMix (NaOH-treated wheat straw pellet)	6.0	6.0	6.0
Whole corn meal	5.0	5.0	5.0
Wheat bran	1.5	1.5	1.5
KNP-843 premix[2]	4.0	4.0	4.0
Chemical composition:			
Dry matter (%)	90.08	90.06	90.05
Digestible energy (MJ/kg dry matter)	10.10	10.10	10.10
Crude protein (%)	16.79	16.86	16.90
Ether extract (%)	3.94	3.94	3.94
Crude fibre (%)	16.85	16.85	16.85
NDF (%)	33.99	33.99	33.99
ADF (%)	22.52	22.52	22.52
ADL (lignin) (%)	4.24	4.24	4.24
Calcium (%)	1.22	1.00	0.90
Total phosphorus (%)	0.58	0.45	0.35
Ash (%)	8.36	7.46	6.96
Lysine (%)	0.82	0.83	0.83
Methionine (%)	0.34	0.34	0.34
Met+Cis (%)	0.58	0.58	0.59
Threonine (%)	0.68	0.69	0.69
Vitamin A (NE/kg)	10000	10000	10000
Vitamin D_3 (NE/kg)	1000	1000	1000
Vitamin E (mg/kg)	60	60	60

[1]Natuphos®5000 (BASF Aktiengesellschaft, Germany): 3-phytase (EC 3.1.3.8) produced by *Aspergillus niger*
[2]Supplemental medication: 50 mg/kg robenidine

A growth trial was carried out at Alsótold rabbit farm in a climatized building (16L:8D, 18-23°C) from July to August 2007. New Zealand White rabbits aged 35 days were weighed, ear-tagged and weaned and assigned to one of the three dietary groups according to body weight and litter. One-hundred-fifty rabbits were housed singly (50 rabbits/group) and 150 in pairs (50 rabbits/group) in all-wire cages (40x30x30 cm, i.e. 1200 and 600 cm^2 individual area) up to 77 d of age. Rabbits received *ad libitum* antibiotic-free but anticoccidial diets up to 63 d of age and medication free diets thereafter. Feed consumption was only estimated for pair housing (total intake of group/alive animals).

Other rabbits that were fed the same experimental diets from 35 d of age were transferred to Gödöllő for a digestibility trial. The trial consisted of a 4-day adaptation followed by a 4-day digestibility period using 56-day-old rabbits weighing 2017±20 g and kept individually in metabolic cages (4 rabbits/group). Apparent digestibility coefficients of nutrients and total phosphorus were calculated according to the Hungarian National Standard Methods (MSZ ISO 6496, 5984, 6830-6/7 and 6491).

Statistical Analysis

The effects of three dietary treatments (Control, P, P-MCP) and two housing systems (one or two rabbits/cage) on growth parameters were assessed by two-way ANOVA with interaction (not detailed here). Contrasts were analysed by Student's *t*-test. Morbidity, mortality and health risk (morbidity plus mortality) rates were subjected to Chi-square tests with Yates correction. All analyses were performed with Statgraphics 6.0 (1992) statistical software.

RESULTS AND DISCUSSION

The reduction of supplemental inorganic phosphate of rabbit feed to half level (from 0.24 to 0.10%) or its omission combined with *Aspergillus niger* phytase enzyme additions (1000 FTU/kg Natuphos) did not significantly affect body weights of rabbits compared to animals fed a control diet with 0.24% added MCP and without phytase inclusion during the fattening period (Table 2).

Table 2: Effect of dietary inorganic phosphorus (MCP) reduction and phytase enzyme (Natuphos) supplementation on production traits

	day	Group				Housing (rabbits/cage)			MSE
		Control n=100	P n=100	P-MCP n=100	Prob.	One n=150	Two n=150	Prob.	
Body weight (g)	35	1009	1009	1009	0.999	1009	1010	0.880	4
	49	1584	1619	1606	0.328	1642[a]	1564[b]	0.001	10
	63	2178	2200	2159	0.382	2225[a]	2134[b]	0.001	12
	77	2706	2699	2677	0.651	2735[a]	2652[b]	0.002	13
Weight gain (g/d)	35-49	41.0	43.3	42.3	0.301	44.7[a]	39.7[b]	0.001	0.6
	49-63	42.3	41.4	40.1	0.332	42.0	40.6	0.243	0.6
	63-77	37.9	35.7	36.8	0.258	36.6	37.0	0.686	0.5
	35-77	40.3	40.2	39.6	0.556	41.0[a]	39.1[b]	0.001	0.3
Feed intake (g/d)	35-49	108	111	103	0.118	107	109	-	2
	49-63	138	141	134	0.181	137	141	-	2
	63-77	161	163	157	0.517	161	178	-	2
	35-77	136	138	131	0.112	135	143	-	1
Feed conversion	35-49	2.50	2.39	2.43	0.344	2.44	-	-	0.03
	49-63	3.36	3.45	3.27	0.491	3.36	-	-	0.06
	63-77	4.50	4.68	4.44	0.515	4.54	-	-	0.09
	35-77	3.30	3.32	3.27	0.592	3.30	-	-	0.02
Morbidity (%)	35-77	11.0	6.00	6.00	>0.05	8.00	7.33	>0.05	-
Mortality (%)	35-77	19.0	20.0	22.0	>0.05	20.0	20.7	>0.05	-
Health risk (%)	35-77	30.0	26.0	28.0	>0.05	28.0	28.0	>0.05	-

Values in the same row with unlike superscripts differ

Feed intake was lower in the P-MCP group than in the other two groups but the differences were not significant (Table 2).

The slightly better 35-77 d feed conversion found in the P-MCP group coincides with data obtained in the metabolic trial since nutrient digestibilities seemed to improve in this group (Table 3). Phytase supplementation in low phosphorus diets slightly improved (P=0.06) the digestibility of N-free extract (carbohydrate) and the digestible energy content (P=0.142). These findings agree with other reports (Ayers *et al*., 1996; Gutiérrez *et al*., 2000; Veum *et al*., 2006; Sands and Kay, 2007).

Despite lowering the phosphorus contents in P and P-MCP diets, no significant decline was detected for 35-77 d gain, for 77 d body weight or for health risk (Table 2). Similarly, growth was unaffected in response to added phytase in other works (Ayers *et al*., 1996; Guo-Xian *et al*., 2004) but it reduced total phosphorus excretion (Guo-Xian *et al*., 2004). Supplemental 500 and 1000 FTU/kg phytase (Natuphos®2500) in diets differing in protein sources reduced the 7-14 wk feed intake by 2.4 and 1.7% but increased the body weight gain by 5.5 and 7.6% according to El-Adawy (2004).

Rabbits housed in pairs gained 4.6% less between 35-77 d of age and had 3.0% lower 77 d body weight than those caged singly. The reason could be the less time spent for feeding and more for moving with group vs. individual housing (Podberscek *et al*., 1991). Health risk was not affected by housing (P>0.05).

Table 3: Mean apparent faecal digestibilities (%) of nutrients (n=12)

	Control	P	P-MCP	Prob.	M.S.E
Dry matter	56.5	57.6	60.7	0.274	1.03
Digestible energy	50.9	53.1	55.8	0.297	1.21
Crude protein	75.4	74.8	75.3	0.938	0.72
Ether extract	77.6	81.5	85.6	0.247	1.80
Crude fibre	45.4	46.1	40.8	0.376	1.58
N-free extract	60.6	65.2	68.7	0.060	1.19
Total phosphorus	87.5	88.8	89.0	0.470	0.51
Digestible energy (MJ/kg)	8.50	9.04	9.60	0.142	0.20

CONCLUSIONS

Assuming lower faecal phosphorus excretion with Phytase or Phytase-MCP feeds than with the control diet (under evaluation), both enzyme supplemented diets are promising since using them resulted in similar production (growth and health status) to that of traditional rabbit feed.

ACKNOWLEDGEMENTS

This work was supported by GAK-OMFB-01336/ALAP1-00121/2004. The authors thank Farkas Zs., Horváth I. and Juhász Zs. for technical assistance and Dr. James I. McNitt for checking the manuscript. Appreciation is expressed to TENDRE Feed Industry Ltd. for producing the diets.

REFERENCES

Ayers A.C., Barrett R.P., Cheeke P.R. 1996. Feeding value of tree leaves (hybrid poplar and black locust) evaluated with sheep, goats and rabbits. *Anim. Feed Sci. Technol., 57, 51-62.*

Boyce A., Walsh G. 2006. Comparison of selected physicochemical characteristics of commercial phytases relevant to their application in phosphate pollution abatement. *J. Environ. Sci. Health A Tox Hazard Subst. Environ. Eng., 41 (5) 789-98.*

Cooper J.R., Gowing H.S. 1983. Mammalian small intestinal phytase (*EC* 3.1.3.8). *British J. of Nutrition, 50 (3), 673-678.*

El-Adawy M.M. 2004. Dietary protein sources and microbial phytase supplementation in growing rabbits. 1-Growth performance and economical evaluation. *Egyptian Journal of Rabbit Science, 14 (1), 81-99.*

Falcão-e-Cunha L., Castro-Solla L., Maertens L., Marounek M., Pinheiro V., Freire J., Mourão J.L. 2007. Alternatives to antibiotic growth promoters in rabbit feeding: a review. *World Rabbit Sci*., *15, 127-140.*

Guo-Xian Z., Zhi-Hua F., Yu-Ding W., Yun-Qi L., Guan-Zhong L. 2004. The effects of supplemental microbial phytase in diets on the growth performance and mineral excretion of rex rabbits. *In: Proc. 8th World Rabbit Congress, 2004 September, Puebla, Mexico, 1114-1120*.

Guggenbuhl P., Piñón Quintana A., Simões Nunes C. 2007. Comparative effects of three phytases on phosphorus and calcium digestibility in the growing pig. *Livest. Sci., 109, 258-260.*

Gundel J., Regius Möcsényi Á., Hermán A., Fébel H., Huszár Sz., Szelényi M., Szabó A. 2004. Change of the apparent digestibility of nutrients and phosphorus as a function of phosphorus source and phytase supplementation in pigs. *J. Anim. Feed Sci., 13, 133-141.*

Gutiérrez I., García J., Carabaño R., Mateos G.G., De Blas J.C. 2000. Effects of exogenous phytase on phosphorus and nitrogen digestibility in growing-finishing rabbits. *In: Proc. 7th World Rabbit Congress, 2000 July, Valencia, Spain, Vol. C, 277-281.*

Huang H., Luo H., Yang P., Meng K., Wang Y., Yuan T., Bai Y., Yao B. 2006. A novel phytase with preferable characteristics from *Yersinia intermedia. Biochem. Biophys. Res. Comm., 350, 884-889.*

Kim J.C., Sands J.S., Mullan B.P., Pluske J.R. 2007. Performance and total-tract digestibility responses to exogenous xylanase and phytase in diets for growing pigs. *Anim. Feed Sci. Technol., doi:10.1016/j.anifeedsci.2007.07.004*

Lebas F., Lamboley-Gaüzere B., Delmas D., Auvergne A. 1998. Effect of dietary phosphorus level on growth performance, slaughter rate and bones mechanical resistance of fattening rabbits. *In: Proc. 7th Journée Recherche Cunicole, 1998 May, Lyon, France, 141-146.*

Maertens L. 1999. Towards reduced feeding costs, dietary safety and minimal mineral excretion in rabbits: a review and opinion article. *World Rabbit Sci., 7, 65-74.*

Maertens L., Cavani C., Petracci M. 2005. Nitrogen and phosphorus excretion on commercial rabbit farms: calculations based on the input-output balance. *World Rabbit Sci., 13, 3-16.*

Marounek M., Dušková D., Skřivanová V. 2003. Hydrolysis of phytic acid and its availability in rabbits. *British Journal of Nutrition, 89 (3), 287-294.*

McClung J.P., Stahl C.H., Marchitelli L.J., Morales-Martinez N., Mackin K.M., Young A.J., Scrimgeour A.G. 2006. Effects of dietary phytase on body weight gain, body composition and bone strength in growing rats fed a low-zinc diet. *J. Nutr. Biochem., 17 (3), 190-6.*

Nitrayova S., Patras P., Sommer A., Heger J. 2006. Effect of microbial phytase on apparent ileal amino acid digestibility of phosphorus-adequate diets in growing pigs. *Arch. Anim. Nutr., 60 (2), 131-40.*

Podberscek A.L., Blackshaw J.K., Beattie A.W. 1991. The behaviour of group penned and individually caged laboratory rabbits. *Appl. Anim. Behav. Sci., 28 (4), 353-363.*

Poulsen H.D., Blaabjerg K., Feuerstein D. 2007. Comparison of different levels and sources of microbial phytases. *Livest. Sci., 109, 255-257.*

Ritskes-Hoitinga J., Grooten H.N.A., Wienk K.J.H., Peters M., Lemmens A.G., Beynen A.C. 2004. Lowering dietary phosphorus concentrations reduces kidney calcification, but does not adversely affect growth, mineral metabolism, and bone development in growing rabbits. *British Journal of Nutrition, 91 (3), 367-376.*

Sands J.S., Kay R.M. 2007. Phyzyme XP phytase improves growth performance and nutrient utilization in wheat-based diets fed to weaned pigs. *Livest. Sci., 109, 264-267.*

Simon O., Igbasan F. 2002. *In vitro* properties of phytases from various microbial origins. *Intern. J. Food Sci. Technol., 37 (7), 813-822.*

Statgraphics ® 1992. Reference Manual, Version 6.0, *Manugistics Inc., Rockville, MD, USA*

Veum T.L., Bollinger D.W., Buff C.E., Bedford M.R. 2006. A genetically engineered *Escherichia coli* phytase improves nutrient utilization, growth performance, and bone strength of young swine fed diets deficient in available phosphorus. *J. Anim. Sci., 84, 1147-1158.*