
EFFECT OF PHYTASE SUPPLEMENTATION TO BARLEY-CANOLA MEAL AND BARLEY-SOYBEAN MEAL DIETS ON PHOSPHORUS AND CALCIUM BALANCE IN GROWING PIGS

Willem C. Sauer, Miguel Cervantes, Jinming M. He and Hagen Schulze

SUMMARY

Two metabolism experiments were carried out to determine the effect of microbial phytase addition to barley-canola meal and barley-soybean meal diets on P and Ca balance in growing pigs. In experiment 1, six barrows (29.6kg initial LW) were fed a barley-canola meal diet without or with phytase (500 units·kg⁻¹) during 24 days, according to a crossover design. The diet provided suboptimal levels of P. In experiment 2, twelve barrows (53.4kg initial LW) were fed four barley-soybean meal diets, according to a two-period changeover design. Diet 1 was supplemented with inorganic P and Ca to meet requirements; diets 2, 3 and 4 contained suboptimal levels of P, diet 3 being supplemented with phy-

tase; diet 4 was supplemented with both phytase and a mixture of xylanase and β -glucanase. The supplementation of phytase to the barley-canola meal and the barley-soybean meal diets increased ($P<0.05$) digestibility and retention of P, as well as digestibility of Ca. Supplementation of both phytase and a mixture of xylanase and β -glucanase to diet 2 gave similar ($P>0.05$) values for P retention and P and Ca digestibilities as for diets 1 and 3. In conclusion, phytase supplementation to the barley-canola meal and barley-soybean meal diets improved the utilization of P and digestibility of Ca; but no further effect was observed with the supplementation with the mixture of xylanase and β -glucanase.

RESUMEN

Se realizaron dos experimentos metabólicos para determinar el efecto de adicionar una fitasa microbiana a dietas cebada-pasta de canola y cebada-pasta de soja en el balance de P y Ca de cerdos en crecimiento. En el experimento 1, seis cerdos (29,6kg peso vivo inicial) se alimentaron con dietas elaboradas con cebada y pasta de canola, sin o con fitasa (500 unidades·kg⁻¹ dieta) durante 24 días, en un diseño de sobrecambio. Las dietas proporcionaron niveles de P inferiores al óptimo. En el experimento 2, doce cerdos (53,4kg peso vivo inicial) recibieron 4 dietas cebada-pasta de soja, en un diseño de sobrecambio con dos periodos experimentales. Las dietas 2, 3 y 4 contenían niveles de P inferiores al óptimo. La dieta 1 se adicionó con P y Ca inorgánico

para cubrir los requerimientos; las dietas 2, 3 y 4 contenían niveles de P inferiores al óptimo, adicionándose la dieta 3 con fitasa y la dieta 4 con fitasa y una mezcla de xilanasa y β -glucanasa. La adición de fitasa incrementó ($P<0,05$) la digestibilidad y la retención de P, así como la digestibilidad de Ca. La adición conjunta de fitasa y mezcla de xilanasa y β -glucanasa a la dieta 2 produjo valores de retención de P y digestibilidades de P y Ca similares ($P>0,05$) a los de las dietas 1 y 3. En conclusión, la adición de fitasa a dietas cebada-pasta de canola y cebada-pasta de soja mejoró la utilización de P y la digestibilidad de Ca, pero no se obtuvo efecto adicional con la adición de xilanasa y β -glucanasa.

Introduction

Phosphorus is an essential mineral for pigs. Adequate amounts of P are required for optimal performance and bone development in growing pigs. The content of available P in feed ingredients of plant origin is usually insufficient for growing pigs; therefore, additional inorganic P is supplemented to the diet. Because

of the low bioavailability of organic P of plant origin, a large proportion is excreted in manure, which is a concern in regions with dense animal and human populations (Jongbloed and Kemme, 1990; Kornegay, 1999).

The difference in P bioavailability among feed ingredients of plant and animal origin is related to the amount of phytate P. In most cereal

grains and oilseeds and their meals, P is found as phytate, an organic complex chemically referred to as myo-inositol hexakis-phosphate. For example, pigs are able to use only about 10 to 12% of P in corn and 25 to 35% in soybean meal (Cromwell, 1992). Both intrinsic plant and microbial phytases are involved in the release of P from phytate in feedstuffs. The supplement-

ation of microbial phytase to release P from phytate, thereby reducing the need of supplementary inorganic P, has been studied in swine production during the last decade.

Phytase supplementation to swine diets has given positive results (Kornegay, 1999). For example, studies with low activity phytase (Cromwell *et al.*, 1993; Cromwell *et al.*, 1995a) and recombinant-derived high

KEYWORDS / Calcium / Digestibility / Phosphorus / Phytase / Pigs / Retention /

Received: 05/08/03. Modified: 07/31/03. Accepted: 07/31/03

Willem C. Sauer. Ph.D. in Animal Nutrition, University of Manitoba, Canada. Professor, Department Animal Food and Nutritional Science, University of Alberta, Canada T6G 2P5. Miguel Cervantes. Zootecnical Engineer. Ph.D in Animal Nutrition, University of Kentucky,

USA. Professor, Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Mexicali, Baja California, México (ICA-UABC). Address: Lago Reindeer N° 933, Jardines del Lago, Mexicali, B.C., México. e-mail: Miguel_Cervantes@uabc.mx.

Jinming M. He. Ph.D. in Animal Nutrition, Technischen Universität München-Weihenstephan, Germany. Research Associate, Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Canada. Hagen Schulze. Ph.D. in Animal Nutrition, Wageningen Univer-

sity, The Netherlands. DSM Food Specialties, 2600, Maastricht, The Netherlands.

Realizaram-se dois experimentos metabólicos para determinar o efeito de adicionar uma fitasa microbiana a dietas cevada-pasta de canola e cevada-pasta de soja no balance de P e Ca de porcos em crescimento. No experimento 1, seis porcos (29,6kg peso vivo inicial) se alimentaram com dietas elaboradas com cevada e pasta de canola, sem ou com fitasa (500 unidades·kg⁻¹ dieta) durante 24 dias, em um desenho de sobre câmbio. As dietas proveram níveis de P inferiores ao ótimo. No experimento 2, doze porcos (53,4kg peso vivo inicial) receberam 4 dietas cevada-pasta de soja, num desenho de sobre câmbio com dois períodos experimentais. As dietas 2, 3 e 4 continham níveis de P inferiores ao ótimo. A dieta 1 se adicionou com P e

Ca inorgânico para cobrir os requerimentos; as dietas 2, 3 e 4 continham níveis de P inferiores ao ótimo, adicionando-se a dieta 3 com fitasa e a dieta 4 com fitasa e uma mistura de xilanasa e β-glucanasa. A adição de fitasa incrementou (P<0,05) a digestibilidade e a retenção de P, assim como a digestibilidade de Ca. A adição conjunta de fitasa e mistura de xilanasa e β-glucanasa a dieta 2 produziu valores de retenção de P e digestibilidades de P e Ca similares (P>0,05) aos das dietas 1 e 3. Em conclusão, a adição de fitasa a dietas cevada-pasta de canola e cevada-pasta de soja melhorou a utilização de P e a digestibilidade de Ca, mas não se obteve efeito adicional com a adição de xilanasa e β-glucanasa.

activity phytase (Cromwell *et al.*, 1995b) with growing-finishing pigs fed soybean meal or corn-soybean meal diets showed improvements in the bioavailability of P from phytate. Studies with young pigs (e.g., Lei *et al.*, 1993a,b; Young *et al.*, 1993) fed corn-soybean meal diets also showed improvements in absorption and utilization of P, as well as in growth rate. An increase in growth rate likely only occurs when the basal diet is deficient in available P.

The first objective of these studies was to determine the effect of microbial phytase supplementation to a barley-canola meal diet upon P and Ca balance in growing pigs, as there is scarce information in the literature on the effect of phytase supplementation on canola meal-containing diets for pigs. The second objective was to determine the effect of phytase and phytase plus xylanase and β-glucanase to a barley-soybean meal diet for growing pigs on P and Ca balance.

Materials and Methods

Animal trial procedures

Experiment 1. The effect of phytase supplementation to a barley-canola meal diet (Table I) was evaluated. The diet was formulated to contain 16% CP and to provide a suboptimal level of P. Natuphos 5000 (BASF Canada Inc.) was supplemented at a rate of 500 phytase units·kg⁻¹ diet. One unit of phytase is defined as

the amount which liberates 1μmol·min⁻¹ of orthophosphate from 1.5μmol Na phytate solution at pH 5.5 and 37°C (Engelen *et al.*, 1994). Barley and canola meal were ground through a 2.8mm mesh screen prior to diet incorporation. The diets were fed in the form of mash. Canola oil was included in the diets to increase the digestible energy content to the NRC (1998) standards level. Crystalline L-lysine and DL-methionine were included to meet NRC (1998) standards. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards. Chromic oxide (0.25%) was included in the diets as an indigestibility marker. However, after the preparation of the diets it was decided to base the digestibility measurements on total collection of feces rather than on chromic oxide.

Six PIC barrows (Canabrid x Camborough) were obtained from the University of Alberta Swine Research Unit. They were housed individually in stainless steel metabolic crates in a temperature controlled barn (22 to 23°C). Water was freely available from a low-pressure drinking nipple.

A crossover design (Gill and Magee, 1976) was used and, following a 7 days adjustment period to the metabolism crates, the pigs were fed the experimental diets twice daily (08:30 and 16:00), equal amounts at each meal. The daily allowance was offered at a rate of 5% of the individual body weight of each pig, which was measured at the

start of each experimental period. The average body weights at the start of experimental periods 1 and 2, and at the end of the experiment were 29.6 ±4.4, 39.5 ±4.5, and 49.6 ±6.2kg, respectively.

Each experimental period lasted 12 days. A 5 days total collection of feces and urine was carried out from 08:30 on day 8 until 08:30 on day 12) of each experimental period. Urine was collected in a pail that contained 30ml 6N HCl. Subsamples of 2.5 or 5% of

the total volume of urine were taken at 08:30 and 16:00 during the collection period. All feces were collected. Subsamples of urine and the total collection of feces were frozen at -20°C immediately after collection.

The experimental proposal, surgical procedures, and procedures for care and treatment of the barrows were reviewed and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the Uni-

TABLE I
EXPERIMENTAL DIETS (%)^a IN EXPERIMENT 1

Ingredients	Diets	
	Barley + canola meal	Barley + canola meal + phytase
Barley	73.50	73.50
Canola meal	19.50	19.50
Canola oil	4.00	4.00
Calcium carbonate ^b	1.20	1.20
Vitamin-mineral premix ^c	1.00	1.00
Iodized salt ^d	0.30	0.30
Chromic oxide	0.25	0.25
L-lysine (78%)	0.10	0.10
DL-methionine (98%)	0.15	0.15
Natuphos 5000 ^e	-	0.01

^a As-fed basis.

^b Provided 38% Calcium. Supplied by Continental Lime, Exshaw, AB, Canada.

^c Provided the following (kg⁻¹ diet): vitamin A 7500IU, vitamin D₃ 500IU, vitamin E 50mg, vitamin K₃ 2.0mg, vitamin B₁₂ 0.03mg, riboflavin 12mg, niacin 40mg, D-panthothenic acid 25mg, choline 600mg, D-biotin 0.25mg, folic acid 1.6mg, thiamine 3.0mg, ethoxyquin 5.0mg, pyridoxine 2.25mg, Cu 125mg, Fe 150mg, I 21mg, Se 0.3mg, Mn 20mg, Zn 150mg. Supplied by Hoffmann-LaRoche, Mississauga, ON, Canada.

^d Provided (%): NaCl, 99. Supplied by Sifto Canada, Mississauga, ON, Canada.

^e BASF Canada, Toronto, ON, Canada.

TABLE II
EXPERIMENTAL DIETS (%)^a
IN EXPERIMENT 2

Ingredients	Diets			
	1	2	3	4
Barley	87.00	87.00	87.00	87.00
Soybean meal	5.60	5.60	5.60	5.60
Dextrose	-	0.30	0.30	0.30
Canola oil	4.00	4.00	4.00	4.00
Dicalcium phosphate ^b	0.80	-	-	-
Calcium carbonate ^c	0.80	1.30	1.30	1.30
Vitamin-mineral premix ^d	1.00	1.00	1.00	1.00
Iodized salt ^e	0.30	0.30	0.30	0.30
Amino acid mixture ^f	0.50	0.50	0.50	0.50
Natuphos 5000 ^g	-	-	0.01	0.01
Porzyme 9100 ^h	-	-	-	0.10

^a As-fed basis.

^b Provided 15-18% available P and 24% Ca. Supplied by Continental Lime, Exshaw, AB, Canada.

^{c,d,e} Refer to Table I.

^f Provided 0.20% lysine HCl and 0.05% L-threonine (98%). The remainder was corn starch.

^g BASF Canada, Toronto, ON, Canada. Provided 5 000 phytase units per g.

^h Finnfedds International, Wiltshire, UK. Provided 400U of each β -glucanase and xylanase per g.

TABLE III
CHEMICAL ANALYSES^a OF THE EXPERIMENTAL
DIETS (EXPERIMENT 1)

Items	Diets	
	Barley + canola meal	Barley + canola meal + phytase
Dry matter, %	89.93	89.87
Organic matter, %	85.15	85.37
Crude protein, %	16.53	16.23
Gross energy, Mcal·kg ⁻¹	4.27	4.29
Ash, %	4.78	4.51
Phosphorus, %	0.43	0.43
Calcium, %	0.71	0.69

^a As-fed basis.

versity of Alberta. The barrows used in this experiment, as well as in experiment 2, were cared for in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC, 1993).

Experiment 2. Twelve PIC barrows were used in this study to determine the effect of phytase and phytase plus xylanase and β -glucanase to a barley-soybean meal diet (Table II). Diet 1 was supplemented with inorganic P and

Ca to meet NRC (1998) standards. Diets 2, 3, and 4 were formulated to contain suboptimal levels of P (NRC, 1998). Diet 3 was supplemented with phytase at a rate of 500 phytase units·kg⁻¹ diet. Diet 4 was supplemented with phytase at a rate of 500 phytase units kg⁻¹ diet and Porzyme 9100 (Danisco Animal Nutrition, Wiltshire, UK) at a rate of 0.1g·kg⁻¹ diet to supply 400 units of xylanase and 400 units of β -glucanase·kg⁻¹ diet. The enzyme mixture was de-

TABLE IV
CHEMICAL ANALYSES^a OF THE EXPERIMENTAL
DIETS (EXPERIMENT 2)

Items	Diets ^b			
	1	2	3	4
Dry matter, %	88.89	88.55	88.28	88.72
Organic matter, %	84.29	84.34	83.96	84.73
Crude protein, %	12.29	12.26	12.11	12.66
Gross energy, Mcal·kg ⁻¹	4.11	4.11	4.16	4.16
Ash, %	4.61	4.21	4.32	4.05
Phosphorus, %	0.45	0.35	0.35	0.35
Calcium, %	0.71	0.61	0.63	0.59

^a As-fed basis.

^b Refer to Table II.

rived from *Trichoderma longibrachiatum*. Barley and soybean meal were ground through a 2.8mm mesh screen prior to diet incorporation. The diets were fed in the form of mash. Canola oil was included in the diets to increase the digestible energy

content to that recommended by NRC (1998). Crystalline L-lysine and L-threonine were included to meet NRC (1998) standards. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards.

Following a 7 days adjust-

TABLE V
EFFECT OF PHYTASE SUPPLEMENTATION
TO THE BARLEY-CANOLA MEAL DIET ON P
AND Ca BALANCE AND DIGESTIBILITY
IN GROWING PIGS (EXPERIMENT 1)

Items	Diets		SEM ^a
	Barley + canola meal	Barley + canola meal + phytase	
Balance of phosphorus			
Intake (g·d ⁻¹)	7.3	7.7	
Fecal output (g·d ⁻¹)	3.3	3.2	0.18
Urinary output (mg·d ⁻¹)	87.7	100.0	14.20
Absorbed (g·d ⁻¹)	4.0 ^c	4.5 ^b	0.10
Retained (g·d ⁻¹)	3.9 ^c	4.4 ^b	0.09
Balance of calcium			
Intake (g·d ⁻¹)	12.1	12.3	
Fecal output (g·d ⁻¹)	3.9	3.9	0.32
Urinary output (mg·d ⁻¹)	51.8	50.0	6.02
Absorbed (g·d ⁻¹)	8.1	8.4	0.33
Retained (g·d ⁻¹)	8.1	8.3	0.33
Digestibility (%)			
Dry matter	84.3	83.7	0.87
Organic matter	86.0	85.2	0.80
Ash	62.6	63.6	1.97
Energy	84.1	83.6	0.89
Crude protein	79.0	78.0	1.34
Phosphorus	52.5 ^c	61.0 ^b	1.60
Calcium	60.6 ^c	68.7 ^b	2.06

^a Standard error of the mean.

^{b,c} Means in the same row with different superscript letters differ (P<0.05).

ment period to the metabolism crates, the pigs were allotted to four dietary treatments according to a two-period changeover design (Gill and Magee, 1976). The pigs were fed twice daily at 08:30 and 16:00, equal amounts at each meal. The daily allowance was offered at a rate of 5% of the individual body weight of each pig, which was measured at the start of each experimental period. The average body weights were 53.4 ±5.4 and 74.5 ±6.0kg, at the start of experimental periods 1 and 2, and 84.9 ±7.2kg at the end of the experiment. The pigs were fed a 16% CP grower diet, which supplied all nutrients and energy according to NRC (1998), for 7 days between the experimental periods. The length of the experimental period, the collection period for urine and feces, and collection procedures were similar to those described under experiment 1.

Chemical analyses

Samples of diets, collected each time the meal allowances were weighed, were pooled for each dietary treatment. Samples of diets and feces were freeze-dried, and thereafter ground in a Wiley Mill through a 0.8mm mesh screen. Dry (DM) and organic (OM) matter, and N₂ were determined according to AOAC (1990) procedures N° 7003 and 2057. Gross energy in diets and feces was determined with an AC-300 Leco Automatic Calorimeter (Leco Corporation, St. Joseph, MI). The P content in the diets, feces and urine were determined spectrophotometrically according to AOAC (1990) procedure N°965.17. Atomic absorption spectrophotometry was used to determine Ca according to AOAC (1990) procedure N°977.29; lanthanum oxide (0.4%) was included at the final dilution step to minimize interference from other minerals. Urine samples for analyses of P and Ca were first filtered with Whatman #2 filter

TABLE VI
EFFECT OF PHYTASE AND PHYTASE PLUS XYLANASE AND β-GLUCANASE TO THE BARLEY-SOYBEAN MEAL DIET ON PHOSPHORUS AND CALCIUM BALANCE AND DIGESTIBILITY IN GROWING PIGS (EXPERIMENT 2)

Items	Diets ^a				SEM ^b
	1	2	3	4	
Balance of phosphorus					
Intake (g·d ⁻¹)	9.8	8.8	9.0	8.9	
Fecal output (g·d ⁻¹)	4.5 ^c	4.6 ^c	3.6 ^d	3.3 ^d	0.27
Urinary output (mg·d ⁻¹)	74.3	94.5	84.0	104.2	15.00
Absorbed (g·d ⁻¹)	5.3 ^c	4.3 ^d	5.5 ^c	5.7 ^c	0.30
Retained (g·d ⁻¹)	5.3 ^c	4.2 ^d	5.4 ^c	5.6 ^c	0.29
Balance of calcium					
Intake (g·d ⁻¹)	18.2	15.4	16.3	15.0	
Fecal output (g·d ⁻¹)	6.7 ^c	7.0 ^c	4.5 ^d	4.5 ^d	0.45
Urinary output (mg·d ⁻¹)	62.8	48.0	51.8	49.3	8.50
Absorbed (g·d ⁻¹)	11.6	8.5	11.8	10.5	0.60
Retained (g·d ⁻¹)	11.6	8.4	11.8	10.5	0.59
Digestibility (%)					
Dry matter	85.3 ^c	83.5 ^c	85.2 ^c	86.9 ^d	0.74
Organic matter	87.0	85.4	86.8	88.3	0.65
Ash	55.4 ^c	50.4 ^d	61.0 ^c	62.9 ^c	2.26
Energy	84.9	83.2	84.8	86.5	0.75
Crude protein	79.2	77.9	79.5	81.3	0.97
Phosphorus	53.8 ^{c,d}	47.9 ^d	60.1 ^c	63.1 ^c	2.58
Calcium	63.7 ^c	54.9 ^d	72.1 ^c	71.0 ^c	2.61

^a Refer to Table IV.

^b Standard error of the mean.

^{c,d} Means in the same row with different superscript letters differ (P<0.05).

paper, oven-dried and then ashed overnight at 450°C, followed by hydrolysis with 3N HCl. The chemical composition of the diets used in experiments 1 and 2 is presented in Tables III and IV.

Statistical analyses

Data from each experiment were first subjected to ANOVA according to a cross-over design (Gill and Magee, 1976). Where appropriate, the means of dietary treatments and experimental periods were compared with the Student-Newman-Keuls multiple range test (Steel and Torrie, 1980). The ANOVA and treatment comparisons were carried out using SAS (1988).

Results

Experiment 1. The effect of phytase supplementation to the barley-canola meal diet is presented in Table V. Phytase supplementation increased (P<0.05) absorption and re-

tention of P (3.9 to 4.4g·d⁻¹ retained); however, it did not affect (P>0.05) absorption and retention of Ca, neither digestibilities of DM, energy and CP. The digestibilities of both P (52.5 to 61.0%) and Ca (60.6 to 68.7%) were increased (P<0.05) upon phytase supplementation.

Experiment 2. As it was expected, P retained (Table VI) was higher (P<0.05) when the pigs were fed diet 1 (5.3g·d⁻¹) as compared to diet 2 (4.2g·d⁻¹). These results show that diet 2 supplied suboptimal levels of P, as it was intended. Digestibility of ash and Ca were higher (P<0.05) for diet 1, as compared to diet 2; however, no further differences (P>0.05) were found between diet 1 and 2 for digestibility of any other variable. Phytase supplementation (diet 3) improved (P<0.05) the absorption and retention (4.2 to 5.4g·d⁻¹) of P; besides, it also increased (P<0.05) digestibility of P (47.9 to 60.1%) and Ca (54.9 to 72.1%), and ash

(50.4 to 61.0%). Supplementation of a mixture of xylanase and β-glucanase (diet 4) did not improve (P>0.05) P and Ca absorption and retention (Table VI), and only DM digestibility was increased (85.2 to 86.9%; P<0.05).

Discussion

For experiment 1 no inorganic P was included in the diet; thus, any improvement in P digestibility and retention would be caused by the phytase effect on P of plant origin. The barley-canola meal diet provided a lower total P (0.43%; Table III) than NRC (1998) standards, which is 0.50% for pigs 20 to 50kg body weight. P bioavailability, for pigs, in canola meal and barley were 21 and 31% (Cromwell, 1992). Therefore, the barley-canola meal diet would provide 0.13% available P, which is 0.10% lower than NRC (1998) requirement (0.23%). According to Parr (1996), 500 units of phytase

activity·kg⁻¹ diet are equivalent to 1.0g of P from monocalcium phosphate, assuming that P from this source is 100% available. Then, 500 units of phytase (0.1g·kg⁻¹ diet) should replace 0.10% available P in the barley-canola meal diet and make up the difference.

Phytase supplementation increased (P<0.05) the apparent fecal digestibility of P in the barley-canola meal diet (52.5 to 61.0%; Table V). An improvement in P digestibility upon phytase supplementation to diets for pigs has been reported (Lei *et al.*, 1993a,b; Mroz *et al.*, 1994; Pallauf *et al.*, 1994; Kemme *et al.*, 1999 b). Ca digestibility was improved (60.0 to 68.7%; P<0.05) by phytase supplementation (Table V). An improvement in Ca digestibility upon phytase supplementation, in pigs, was also reported by Lei *et al.* (1993a,b), Pallauf *et al.* (1994), Liu *et al.* (1997) and Li *et al.* (1998).

Digestibility of other variables, including CP, was not changed (P>0.05) by phytase supplementation. These results were not unexpected, because as it has been pointed out (Sauer and Ozimek, 1986), amino acid digestibility measured with the ileal analysis method give a more sensitive prediction of the potential for protein utilization than apparent fecal CP digestibility. Small but significant improvements of ileal indispensable amino acids digestibility have been shown by Mroz *et al.* (1994; 1%) and Kemme *et al.* (1999a; 2.5%), when supplementing diets with phytase.

Since P digestibility was improved, the retention of P increased from 3.9 to 4.4g·d⁻¹ (Table V). These results agree with Nasi and Helander (1994) who reported retentions of 3.7 and 4.9g·d⁻¹ without or with supplementation of phytase, in studies with growing pigs (35 to 88kg body weight) fed a barley-soybean meal diet. The retention of Ca in this study was not affected (P>0.05) by

phytase supplementation, because the Ca requirement (11.4g·d⁻¹; NRC 1998) was closely met.

The objective of experiment 2 was to determine the effect of adding phytase and phytase plus xylanase and β-glucanase to a barley-soybean meal diet. As previously discussed, a positive effect of phytase supplementation on P retention can only be detected with diets deficient in available P. Diet 1 was formulated to meet or exceed total and available NRC (1998) standards for P (0.40 and 0.15%, for 50 to 110kg pigs), and inorganic P (dicalcium phosphate) was used in this diet. Based on Cromwell (1992), available P supply in diet 1 was 0.21%, as well as 30, 31, and 100% in barley, soybean meal, and dicalcium phosphate, respectively. Diets 2, 3, and 4 were formulated to contain suboptimal levels of total and available P. Based on P content (NRC, 1998) and availability (Cromwell, 1992), the total and available P contents in diets 2, 3, and 4 were 0.29 and 0.09%, and the analyzed total P content was 0.35% (Table IV).

As it was intended, the retention of P in pigs fed diet 2 was lower (P<0.05) than for diet 1 (Table VI). The supplementation of phytase (diet 3) improved (P<0.05) the retention of P (5.4g·d⁻¹), as compared to diet 2 (4.2g·d⁻¹), due to an increase in absorption of P (Table VI). Supplementation of phytase did not change (P>0.05) retention of Ca (Table VI). As it was the case for experiment 1, phytase supplementation improved (P<0.05) P digestibility in experiment 2 from 47.9 (diet 2) to 60.1% (diet 3). Ca digestibility was also improved (P<0.05), from 54.9 to 72.1%. The improvement in P digestibility upon phytase supplementation agrees with other results (Lei *et al.*, 1993a,b; Mroz *et al.*, 1994; Pallauf *et al.*, 1994; Kemme *et al.*, 1999 b). As for the non significant improvement in Ca digestibility and retention, Yi

et al. (1996) showed only small and variable changes in Ca absorption. Since P digestibility was improved by phytase supplementation, there was an increase (P<0.05) in ash digestibility (Table VI). However, digestibilities of the other variables were not affected (P>0.05) by phytase supplementation.

Supplementation of xylanase and β-glucanase (diet 4) had no further effect (P>0.05) on P and Ca retention and digestibility (Table VI); however, there was a small but significant increase (P<0.05) in DM digestibility. Attempts to improve feed utilization through supplementation with cellulolytic and hemicellulolytic enzymes have met with mixed results (Dierick and Decuyper, 1993). The increase in DM digestibility in this study likely resulted from the action of β-glucanase on the β-glucans in barley, which contains variable levels of β-glucans (Newman *et al.*, 1989). Li *et al.* (1996) reported linear increases (P<0.05) in the DM digestibility (also of CP and energy) with increasing levels of supplementation of β-glucanase to barley-based diets for young pigs.

The results of these studies showed that the supplementation of phytase to barley-canola meal and barley-soybean meal diets improved the utilization of P and sometimes, of Ca, thereby reducing the need for supplementation of inorganic P and Ca sources.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support provided by the Alberta Agricultural Research Institute and Danisco Animal Nutrition.

REFERENCES

AOAC (1990) *Official Methods of Analysis* (15th ed.). Association of Official Analytical Chemists. Washington, DC, USA. 1298 pp.
CCAC (1993) *Guide to the care and use of experimental ani-*

mals. Vol. 1 (with addendum). Canadian Council on Animal Care, Ottawa, ON, Canada.

Cromwell GL (1992) The biological availability of phosphorous in feedstuffs for pigs. *Pig News and Information* 13: 75N-78N.
Cromwell GL, Stahly TS, Coffey RD, Monegue HJ, Randolph JH (1993) Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. *J. Anim. Sci.* 71: 1831-1840.
Cromwell GL, Coffey RD, Monegue HJ, Randolph JH (1995a) Efficacy of low activity microbial phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. *J. Anim. Sci.* 73: 449-456.
Cromwell GL, Coffey RD, Parker GR, Monegue HJ, Randolph JH (1995b) Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn-soybean meal diets for pigs. *J. Anim. Sci.* 73: 2000-2008.
Dierick NA, Decuyper JA (1993) Enzymes and growth in pigs. In Cole D, Wiseman J, Varley M (Eds.) *Principles of Pig Science*. Nottingham University Press, Nottingham, UK. pp. 169-195.
Engelen AJ, van der Heeft FC, Randsdorp HG, Smit ELC (1994) Simple and rapid determination of phytase activity. *J. Assoc. Off. Anal. Chem.* 77: 760-764.
Gill JL, Magee WT (1976) Balanced two-period changeover design for several treatments. *J. Anim. Sci.* 42: 775-777.
Jongbloed AW, Kemme PA (1990) Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. *Anim. Feed Sci. Technol.* 28: 233-242.
Kemme PA, Jongbloed AW, Mroz Z, Kogut J, Beynen AC (1999a) Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. I. Apparent ileal digestibility of amino acids. *Livest. Prod. Sci.* 58: 107-117.
Kemme PA, Jongbloed AW, Mroz Z, Kogut J, Beynen AC (1999b) Digestibility of nutrients in growing-finishing pigs is affected by *Aspergillus niger* phytase, phytate and lactic acid levels. II. Apparent total tract digestibility of phosphorus, calcium, and magnesium and ileal degradation of phytic acid. *Livest. Prod. Sci.* 58: 119-127.

- Kornegay ET (1999) Feeding to reduce nutrient excretion: effects of phytase on phosphorus and other nutrients. In Lyons TP, Jacques KA (Eds.) *Biotechnology in the Feed Industry*. Proc. Alltech's Annual Symp. Nottingham University Press, Nottingham, UK. pp. 461-490.
- Lei XG, Ku PK, Miller ER, Yokoyama MT (1993a) Supplementing corn-soybean meal diets with microbial phytase linearly improved phytate phosphorus utilization by weaning pigs. *J. Anim. Sci.* 71: 3359-3367.
- Lei XG, Ku PK, Miller ER, Yokoyama MT (1993b) Supplementing corn-soybean meal diets with microbial phytase maximizes phytate phosphorus utilization by weaning pigs. *J. Anim. Sci.* 71: 3368-3375.
- Li S, Sauer WC, Mosenthin R, Kerr B (1996) Effect of β -glucanase supplementation of cereal-based diets for starter pigs on the apparent digestibilities of dry matter, crude protein and energy. *Anim. Feed Sci. Technol.* 59: 223-231.
- Li D, Che X, Wang Y, Cao H, Thacker PA (1998) Effect of microbial phytase, Vitamin D₃, and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. *Anim. Feed Sci. Technol.* 73: 173-176.
- Liu J, Bollinger DW, Ledoux DR, Ellersieck MR, Veum TL (1997) Soaking increases the efficacy of supplemental microbial phytase in a low-phosphorus corn-soybean meal diet for growing pigs. *J. Anim. Sci.* 75: 1292-1298.
- Mroz Z, Jongbloed AW, Kemme PA (1994) Apparent digestibility and retention of nutrients bound to phytate complex as influenced by microbial phytase and feeding regimen in pigs. *J. Anim. Sci.* 72: 126-132.
- Nasi M (1990) Microbial phytase supplementation for improving availability of plant phosphorus in the diet of growing pigs. *J. Agric. Sci. Finl.* 62: 435-442.
- Nasi M, Helander E (1994) Effects of microbial phytase supplementation and soaking of barley soybean meal on availability of plant phosphorus in the diet of growing pigs. *Acta Agric. Scand. Sect. A. Anim. Sci.* 44: 79-86.
- Newman PK, Lewis SE, Newman CW, Boik RJ, Ramage RE (1989) Hypocholesterolemic effect of barley foods on healthy men. *Nutr. Rep. Int.* 39: 749-760.
- NRC (1998) *Nutrient Requirements of Swine* (10th ed.). National Research Council. National Academy Press. Washington, DC, USA. 183 pp.
- Pallauf J, Rimbach G, Pippig S, Schindler B, Most E (1994) Effect of phytase supplementation to a phytate-rich diet based on wheat, barley and soya on the availability of dietary phosphorus, calcium, magnesium, zinc and protein in piglets. *Agribiol. Res.* 47: 39-48.
- Parr J (1996) Formulating swine diets with Natuphos phytase. *BASF Technical Symposium*. Des Moines, IO, USA. pp.94-121.
- SAS (1988) *SAS/STAT user's guide*: Statistics. Release 6.03. SAS Institute, Inc., Cary, NC. USA. 956 pp.
- Sauer WC, Ozimek L (1986) Digestibility of amino acids in swine: Results and their practical application. A review. *Livest. Prod. Sci.* 15: 367-388.
- Simons PCM, Versteegh HAJ, Jongbloed AW, Kemme PA, Slump P, Bos KD, Wolters MGE, Beudeker RF, Verschoor GJ (1990) Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Brit. J. Nutr.* 64: 525-540.
- Steel RGD, Torrie JH (1980) *Principles and Procedures of Statistics: A Biological Approach* (2nd ed.). McGraw-Hill. New York, NY, USA. 622 pp.
- Yi Z, Kornegay ET, Ravindran V, Lindemann MD, Wilson JH (1996) Effectiveness of Natuphos phytase in improving the bioavailabilities of phosphorus and other nutrients in soybean meal-based semi-purified diets for young pigs. *J. Anim. Sci.* 74: 1601-1611.
- Young LG, Leunissen M, Atkinson JL (1993) Addition of microbial phytase to diets for young pigs. *J. Anim. Sci.* 71: 2147-2150.