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Effects of microbial phytase on apparent retention of phosphorus, calcium and zinc in broilers according to type of diet

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SUMMARY – The aim of the trial was to evaluate the effects of microbial phytase added to phosphorus-deficient diets on broiler apparent retention of phosphorus, calcium and zinc, according to type of diet (maize vs. wheat) which differ in their endogenous phytase presence. Two basal diets were used, based on maize-soybean (6 U endogenous phytase/kg) or in wheat-soybean (410 U endogenous phytase/kg). Eight experimental treatments were tested: T-1, maize control (0.45% nonphytate phosphorus, nP); T-2, maize (0.27% nP); T-3, maize (0.27% nP plus 600 FTU/kg microbial phytase); T-4, wheat control (0.45% nP); T-5, wheat (0.27% nP); T-6, wheat (0.27% nP plus 200 FTU/kg microbial phytase); T-7, wheat (0.27% nP plus 400 FTU/kg microbial phytase); and T-8, wheat (0.27% nP plus 600 FTU/kg microbial phytase). 288 one-day old male broiler chicks were distributed into 48 Petersime cages. Six replicates or cages with 6 broilers in each were used per treatment. The apparent metabolizable energy (AME) of diets was evaluated between 18 and 21 days using titanium oxide as digestibility marker. Freeze-dried excreta were analysed for energy, total and phytate phosphorus, calcium and zinc, and apparent retention of these minerals was calculated. Statistical significant differences in AME of diets between treatments were not found. Animals fed P-deficient diets showed higher apparent retention and better bioavaliability of all evaluated minerals. The addition of phytase enzyme to maize diets produced statistically significant increases in mineral bioavailability ($P<0.0001$ for total phosphorus; $P<0.0026$ for phytate phosphorus; $P<0.0151$ for calcium; and $P<0.0224$ for zinc). In wheat diets, only total and phytate phosphorus bioavailabilities were improved by phytase addition ($P<0.0010$).

Key words: Microbial phytase, phosphorus, calcium, zinc, broilers.

RESUME – *"Effets de la phytase microbienne sur la rétention apparente de phosphore, calcium et zinc chez des broilers selon le type de régime". Le but de l'essai a été d'évaluer les effets de la phytase microbienne ajoutée à des régimes ayant un déficit en phosphore chez les broilers, sur la rétention apparente de phosphore, calcium et zinc, selon le type de régime (maïs vs.blé) qui diffèrent en ce qui concerne la présence de phytase endogène chez ceux-ci. Deux principaux régimes ont été utilisés, basés sur du maïs-soja (6 U de phytase endogène/kg) ou sur du blé-soja (410 U de phytase endogène/kg). Huit traitements expérimentaux ont été testés : T-1, maïs témoin (0,45% de phosphore non-phytate, nP) ; T-2, maïs (0,27% nP) ; T-3, maïs (0,27% nP + 600 FTU/kg de phytase microbienne) ; T-4, blé témoin (0,45% nP) ; T-5, blé (0,27% nP) ; T-6, blé (0,27% nP + 200 FTU/kg phytase microbienne) ; T-7, blé (0,27% nP + 400 FTU/kg phytase microbienne) ; et T-8, blé (0,27% nP + 600 FTU/kg phytase microbienne). 288 poulets broilers mâles de 1 jour d'âge ont été distribués dans 48 cages Petersime. Six répétitions ou cages avec 6 broilers dans chacune ont été utilisées par traitement. L'énergie métabolisable apparente (EMA) des régimes a été évaluée entre 18 et 21 jours en utilisant de l'oxyde de titanium comme marqueur de digestibilité. Des excrétions séchées par procédé frigorifique ont été analysées pour l'énergie, phosphore total et phytate du phosphore, calcium et zinc, et on a calculé la rétention apparente de ces minéraux. On n'a pas trouvé de différences statistiques significatives de l'EMA des régimes entre traitements. Les animaux recevant des régimes avec déficit en P ont montré une rétention apparente plus élevée et une meilleure biodisponibilité de tous les minéraux évalués. L'addition de l'enzyme phytase aux régimes de maïs a produit des augmentations statistiquement significatives pour la biodisponibilité des minéraux ($P<0,0001$ pour le phosphore total ; $P<0,0026$ pour le phytate de phosphore ; $P<0,0151$ pour le calcium et $P<0,0224$ pour le zinc). Dans les régimes à base de blé, il n'y a eu d'amélioration, par l'addition de phytase, que pour les biodisponibilités en phosphore total et phytate de phosphore ($P<0,0010$).*

Mots-clés : *Phytase microbienne, phosphore, calcium, zinc, broilers.*

Introduction

The majority of P in plants is contained in chemical structures called phytates. Phytate P is relatively unavailable to monogastric animals. In addition to reducing the P availability, phytates are

associated with a number of antinutritional effects, largely because they can chelate divalent cations such as Ca and Zn, and can reduce availability of nutrient such as protein and amino acids, starch and other carbohydrates (Ravindran *et al.*, 1995).

In order to increase phytate phosphorus availability present in vegetal ingredients and to reduce the quantity of inorganic phosphorus added to diets, phytase, an enzyme that hydrolyses phytic acid to inositol and phosphoric acid, has been used in monogastric diets, obtaining positive effects on mineral availability (Broz *et al.*, 1994) and on protein and amino acid digestibilities (Yi *et al.*, 1996).

Nevertheless, most of studies have been performed using maize-soybean diets, both ingredients characterised by very low endogenous phytase activity. Little is known about the effects of exogenous phytase on phosphorus and other minerals bioavailability, and on energy and nutritive value of diets based on cereals rich in endogenous phytase, such as wheat.

The aim of the trial was to evaluate the effects of microbial phytase added to phosphorus-deficient diets on broiler apparent retention of total phosphorus, phytate phosphorus, calcium and zinc, according to type of diet (maize vs. wheat) which differ in their endogenous phytase presence.

Material and methods

Eight experimental treatments were tested (Table 1).

Table 1. Experimental treatments tested

Treatment	Diet	Non phytate phosphorus (%)	Microbial phytase (U/kg)
T-1	Maize	0.45	–
T-2	Maize	0.27	–
T-3	Maize	0.27	600
T-4	Wheat	0.45	–
T-5	Wheat	0.27	–
T-6	Wheat	0.27	200
T-7	Wheat	0.27	400
T-8	Wheat	0.27	600

288 one-day old male broiler chicks were distributed into 48 Petersime cages (0.376 m^2 each). Six replicates or cages with 6 broilers in each were used per treatment.

The experiment lasted 21 days. Water and feed in mash form were supplied *ad libitum.*

The composition of basal diets are presented in Table 2. Animals and feed were wheighed at the start of the experiment and at 21 days, and productive parameters (average daily consumption, average daily weight gain and feed efficiency) were evaluated.

The apparent metabolizable energy (AME) of diets were evaluated between 18 and 21 days using titanium oxide (0.5%) as digestibility marker. Freeze-dried excreta were analysed for energy, titanium oxide, total and phytate phosphorus, calcium and zinc, and apparent retention of these minerals was calculated.

The statistical analysis was performed separately for each type of diet, using ANOVA procedure of statistical package SAS.

Results and discussion

Energy and mineral retention results (expressed as percentage according to mineral consumed) are presented in Tables 3 and 4 for maize and wheat diets, respectively.

In both types of diets, no statistical significant differences in metabolizable energy of diets were observed due to reduction of available phosphorus or phytase addition; maize diets presented higher energy values than wheat ones.

Table 2. Composition of experimental basal diets

	Maize		Wheat	
	P Normal	P low	P Normal	P low
Ingredients				
Maize	54.276	56.554	–	–
Wheat	–	–	61.977	64.587
Soybean meal 48%	37.015	36.683	29.375	28.635
Lard	4.315	3.537	4.123	3.405
Amino acids and choline chloride	0.261	0.263	0.467	0.486
Calcium carbonate	1.553	1.418	1.598	1.464
Dicalcium phosphate	1.710	0.676	1.624	0.589
Salt	0.470	0.470	0.437	0.435
Minerals and vitamins	0.400	0.400	0.400	0.400
Calculated nutritive value				
Metabolizable energy (kcal/kg)	3100	3100	3000	3000
Crude protein (%)	21.97	22.00	22.00	22.00
Crude fibre (%)	2.75	2.78	3.24	3.29
Ether extract (%)	6.95	6.25	5.88	5.20
Ash (%)	6.54	5.40	6.30	5.14
Lysine (%)	1.20	1.20	1.20	1.20
Methionine + Cystine (%)	0.92	0.92	0.92	0.92
Calcium (%)	1.10	0.81	1.10	0.81
Total phosphorus (%)	0.64	0.46	0.68	0.50
Available phosphorus (%)	0.45	0.27	0.45	0.27
Phytate phosphorus (%)	0.29	0.29	0.28	0.29
Endogenous phytase (U/kg)	5.68	5.76	410	428

Broilers fed P-deficient diets presented higher apparent retention of all minerals evaluated than control diets, formulated to satisfy NRC requirements, showing better efficiency of utilisation when mineral levels were low. The addition of phytase enzyme to maize diets produced statistically significant increases in mineral bioavailability ($P<0.0001$ for total phosphorus; $P<0.0026$ for phytate phosphorus; $P<0.0151$ for calcium; and $P<0.0224$ for zinc). In wheat diets, only total and phytate phosphorus bioavailabilities were improved by phytase addition ($P<0.0010$), but numerically improvements were observed for calcium and zinc bioavailabilities.

In conclusion, the addition of phytase enzyme to P- and Ca-deficient diets produced an increase in phosphorus, calcium and zinc bioavailabilities, decreasing the excretion of these minerals to the environment.

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Table 3. Results of AME and apparent retention of phosphorus, calcium and zinc of maize diets

Treatment	Diet	Available phosphorus (%)	Microbial phytase (U/kg)	AME (kcal/kg)	AMEn (kcal/kg)	Total phosphorus retention (%)	Phytate phosphorus retention (%)	Calcium retention (%)	Zinc retention (%)
T-1	Maize	0.45	–	3116	2960	62.72 b	46.88 c	28.57 b	–4.31 b
T-2	Maize	0.27	–	3078	2940	77.90 a	55.10 b	37.28 ab	4.35 ab
T-3	Maize	0.27	600	3063	2908	80.38 a	63.06 a	46.91 a	15.08 a
Pr>F				0.5488	0.5965	*0.0001*	*0.0026*	*0.0151*	*0.0224*
St. err.				34.57	35.69	1.74	2.39	3.58	4.07

Table 4. Results of AME and apparent retention of phosphorus, calcium and zinc of wheat diets

Treatment	Diet	Available phosphorus (%)	Microbial phytase (U/kg)	AME (kcal/kg)	AMEn (kcal/kg)	Total phosphorus retention (%)	Phytate phosphorus retention (%)	Calcium retention (%)	Zinc retention (%)
T-4	Wheat	0.45	–	2884	2744	53.33 b	44.83 c	18.92	17.90
T-5	Wheat	0.27	–	2913	2757	68.46 a	54.09 b	18.35	8.38
T-6	Wheat	0.27	200	2904	2765	72.93 a	42.28 c	25.42	8.36
T-7	Wheat	0.27	400	3002	2857	71.56 a	63.81 a	31.81	12.23
T-8	Wheat	0.27	600	2892	2757	70.82 a	54.48 b	24.71	13.43
Pr>F				0.1391	0.2025	*0.0001*	*0.0007*	0.3316	0.6343
St. err.				31.63	32.41	2.00	3.11	4.97	4.94

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