



## Phytases and *myo*-inositol modulate performance, bone mineralization and alter lipid fractions in the serum of broilers

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**ABSTRACT.** Under conditions simulating intestinal digestion in broilers, phytase B was found to free *myo*-inositol from low non-phytate phosphorus maize–soyabean meal-based and wheat–soyabean meal-based diets, whereas both 3- and 6-phytases A accelerated *myo*-inositol release only in the presence of phytase B. In the feeding experiment, *myo*-inositol at 1 g · kg<sup>-1</sup> increased the feed intake and body weight gain of broilers fed both types of feeds. Phytase B at 1300 acid phosphatase units (AcPU) · kg<sup>-1</sup> enhanced feed consumption, body weight gain, and tibia mineralization, whereas phytase B mixed with 6-phytase A at 500 phytase units (FTU) · kg<sup>-1</sup> produced further enhancements in performance and bone mineralization. After 6 weeks, broilers fed the maize-based diets supplemented with both phytase B and 6-phytase A had increased serum concentrations of triglycerides and HDL-cholesterol, as well as an enhanced HDL/total cholesterol ratio. The increases in this ratio cannot be attributed to the release of phosphorus or to the generation of *myo*-inositol by the supplemented enzymes, but can only be explained as effects of intermediate products of phytate dephosphorylation.

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### Introduction

The physiological effects of exogenous commercial phytases in poultry are related directly to improved absorption and reduced endogenous losses of nutrients (Pirgozliev et al., 2012), but are also likely to extend far beyond these phenomena. In broilers, phytase A added in varying doses (500–12500 phytase units (FTU) · kg<sup>-1</sup>) to feeds alleviates the detrimental effects of phytate on performance, on phosphorus, protein and amino acid digestibility, on the activities of intestinal amylase, disaccharidases, and Na<sup>+</sup>K<sup>+</sup>-ATPase, as well as contributes to the elevation of hepatic carotenoid,  $\alpha$ -tocopherol,

retinol, and coenzyme Q<sub>10</sub> concentrations (Kardas et al., 2008; Liu et al., 2008). The enzyme also increases the sensitivity of the broiler liver insulin receptor and inhibits the expression of the insulin-like growth factor 1 gene (Józefiak et al., 2010). Moreover, in healthy broilers, phytase A increased the proliferation of blood lymphocytes and intestinal IgA levels, blood T-helper and cytotoxic T cells (Liu et al., 2008). Interestingly, most of these effects have been attributed to the biological activity of lower inositol phosphates, the concentrations of which increase in the intestine as a result of enzymatic hydrolysis of phytate. *Myo*-inositol, the final

product of phytate dephosphorylation, has been studied by our team as a feed supplement in both broilers (Żyła et al., 2004) and laying hens (Żyła et al., 2012). When added as a pure substance at  $1 \text{ g} \cdot \text{kg}^{-1}$ , this compound improved growth performance in broilers, but suppressed laying performance in hens. Due to differences in substrate specificity, commercial preparations of phytases used in poultry nutrition offer multiple pathways of phytate dephosphorylation that may generate distinct pools of lower inositol phosphates. The enzyme, 3-phytase A, such as Natuphos® (BASF, Germany), produced by an *Aspergillus niger* strain carrying *phyA* genes from *Aspergillus ficuum*, initiates phytate hydrolysis by removing the phosphate residue (iP) from position 3 of the *myo*-inositol ring, whereas 6-phytases A form another subclass of phytases. The enzymes from a recombinant *Aspergillus oryzae* strain transformed with the phytase gene of *Peniophora lycii* (Ronozyme P®, DSM-Nutritional Products, Switzerland) or enzymes from hosts carrying the *appA* gene from *Escherichia coli* (Phyzyme XP, Danisco, UK; Quantum phytase, AB Enzymes GmbH, Germany; OptiPhos, JBC, USA) start degradation of phytate by removing iP from position 6 or 4 on the phytic acid molecule (Onyango et al., 2005). In contrast to 3- and 6-phytases A, phytase B is an enzyme with broad substrate specificity, high specific activity, and low optimal pH (Vats et al., 2005). Phytase B, previously known as non-specific acid phosphatase (EC 3.1.3.2), is currently classified as a histidine acid phytase (HAP). It has been postulated previously that simultaneous application of either phytase A along with phytase B offers an effective means of enhancing phytate dephosphorylation in poultry (Żyła et al., 2000). In contrast to various phytases A, phytase B has not been investigated thoroughly and little information is available about its effects when fed as the sole supplemental enzyme. In previous studies with broilers fed maize–soyabean meal-based diets, phytase B enhanced feed intake, body weight gain (BWG), retention of toe ash and P, but not that of Ca (Żyła et al., 2004).

The objective of this study was to determine the influence on feed intake, BWG, and bone mineralization of supplemental *myo*-inositol, phytase B alone, and phytase B combined with 6-phytase A added to NPP-deficient maize-based (M) and wheat-based (W) diets. In the second experimental period, the study was also aimed at evaluating the possible influences of dietary supplements on the blood lipid fractions of chickens fed maize-based diets. The potential effects of pure *myo*-inositol were contrasted with the effects of enzymes, the actions of which

may produce inositol in the birds' intestine. In addition to the feeding experiment, the *myo*-inositol concentrations released from diets M and W by 6-phytase A, phytase B, and both enzymes were determined using a model that simulates digestion in different parts of the bird intestine and the HPAEC-PAD method of *myo*-inositol quantification.

## Material and methods

### Enzyme activity measurements and units

Two enzyme preparations were used: phytase B (from Finase AP, with a declared activity of 225,000 units  $\cdot \text{g}^{-1}$  from AB Enzymes OY, Rajamäki, Finland) and 6-phytase A (from Ronozyme PM with a declared activity of 20 kFTU  $\cdot \text{g}^{-1}$  donated by the manufacturer, Hoffman la Roche, Switzerland). One unit of phytase B-acid phosphatase activity (AcPU) is equal to  $1 \mu\text{M} \cdot \text{min}^{-1}$  of p-nitrophenol liberated from 5.5 mM disodium p-nitrophenylphosphate at 40°C, pH 4.5. One unit of phytase A activity (FTU) is defined as the quantity of enzyme that liberates  $1 \mu\text{M}$  of inorganic P from 2 mM sodium phytate in 1 min at 40°C, pH 4.5. The activities of phytase and acid phosphatase were determined using previously described procedures (Żyła et al., 2012); the units of enzymatic activity used in the current study are the same as in the cited work.

### In vitro digestions and dialyzable inositol

The *in vitro* procedure of Żyła et al. (2000) that simulates digestion in the intestinal tract of broilers was used. Determinations of inositol in the dialysates of maize- and wheat-based starter diet samples subjected to the procedure of multiple digestions were performed using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described previously (Duliński et al., 2011).

### Experimental design, birds, diet composition and preparation

The influence of dietary additives and cereal components was determined in a 2-by-5 factorial completely randomized design from day 1 to 21. The dietary supplements included *myo*-inositol at  $1 \text{ g} \cdot \text{kg}^{-1}$ , phytase B at  $1300 \text{ AcPU} \cdot \text{kg}^{-1}$ , phytase B enriched in 6-phytase A at  $500 \text{ FTU} \cdot \text{kg}^{-1}$ , or 1.8 g of monocalcium phosphate added to low non-phytate phosphorus (NPP) diets, either maize-based or wheat-based. For the second experimental period (days 22 to 42), chickens fed the wheat-based diets were removed from the experiment so that the experiment proceeded only with broilers fed the low NPP, maize-based, grower type diets. The experi-

mental protocol was approved by the Local Bioethics Committee for Experiments with Animals in Krakow (Poland).

Four hundred male one-day-old Ross broiler chickens were purchased from a commercial hatchery and allotted randomly to stainless steel battery brooders with wire-mesh floors. The temperature was maintained at  $32^{\circ} \pm 1^{\circ}\text{C}$  in the first 3 days, and was thereafter gradually reduced according to normal management practices. Lighting was continuous and feed and water were provided *ad libitum*. The experimental design consisted of ten dietary treatments with five replicates of eight birds allotted ran-

domly to each dietary treatment from day 1 to 21.

The detailed composition of the basal experimental diets is given in Table 1. The five experimental starter diets based on the NCM formula were: 1) a negative control diet (NC); 2) an internal control diet, NC +  $1.8 \text{ g} \cdot \text{kg}^{-1}$  of monocalcium phosphate (IC); 3) NC +  $1 \text{ g} \cdot \text{kg}^{-1}$  of myo-inositol (Inos); 4) NC + phytase B at  $1300 \text{ AcPU} \cdot \text{kg}^{-1}$  (Phy B); 5) NC + phytase B at  $1300 \text{ AcPU} \cdot \text{kg}^{-1}$  + 6-phytase A at  $500 \text{ FTU} \cdot \text{kg}^{-1}$  (Phy A+B). Five (6–10) starter wheat-based diets were formulated according to the same design but the wheat-based IC diet was supplemented with  $1.57 \text{ g} \cdot \text{kg}^{-1}$  of monocalcium phosphate. The NPP levels of the IC diets were established experimentally and were equivalent to the NPP concentrations in the Phy A+B diets. The quantity of NPP released from the diets by phytases was determined *in vitro* by the procedure described previously (Żyła et al., 2000). All starter diets were isonitrogenous and isocaloric (Table 1). The grower-type, low NPP maize-based experimental diets were formulated using the same design. The diets were prepared in mash form. Powdered enzymes were premixed with a small quantity of feed and added to the remaining part of a diet during final mixing. The protein and energy contribution from enzyme addition was considered insignificant. During the course of the experiment all of the diets were stored in a cooler at  $4^{\circ}\text{C}$ .

**Table 1.** Composition and nutrient content of the negative control (NC), maize-based (M) and wheat-based (W) diets fed to growing broilers<sup>1</sup>,  $\text{g} \cdot \text{kg}^{-1}$

Indices	Basal diets		
	NCM -starter	NCW -starter	NCM -grower
Ingredient			
maize	60.03	–	63.77
wheat	–	62.07	–
soyabean meal	36.00	31.90	31.00
limestone	1.00	1.00	1.00
rapeseed oil	1.80	3.80	3.00
NaCl	0.30	0.30	0.30
DL-methionine	0.22	0.24	0.22
Lys-HCl	0.15	0.19	0.21
vitamin and mineral premix <sup>2</sup>	0.50	0.50	0.50
Calculated composition, per kg of feed; on an as-is basis			
metabolizable energy, MJ	12.75	12.75	13.10
crude protein, g	225	225	205
NPP, g	1.45	1.50	0.95
lysine, g	12.0	12.0	11.50
methionine + cystine, g	9.10	9.10	8.20
Ca analysed, g	5.16	5.53	5.27
P total analysed, g	3.56	4.06	3.38

<sup>1</sup> Four experimental diets were prepared based on each NCM and NCW formulae: internal control diets (IC): NC +  $1.80 \text{ g}$  of monocalcium phosphate per kg; myo-inositol supplemented diets (Inos): NC +  $1 \text{ g}$  of myo-inositol per kg; phytase B supplemented diets (Phy B): NC + phytase B  $1300 \text{ AcPU}$ ; (from Finase AP); phytase B ( $1300 \text{ AcPU} \cdot \text{kg}^{-1}$ ) and 6-phytase A ( $300 \text{ FTU} \cdot \text{kg}^{-1}$ ) supplemented diets (from Ronozyme PM). For definitions of an enzymatic activity units (AcPU, FTU) see Material and methods. Dietary ingredients were added at the expense of maize and the metabolizable energy was adjusted by increasing amounts of the vegetable oil

<sup>2</sup> The premix provided, per 1 kg of starter diet: IU: retinyl acetate 13500, cholecalciferol 3500; mg: tocopheryl acetate 45, menadione sodium bisulfite 3, thiamin mononitrate 3.25, riboflavin 7.5, pyridoxine 5, cyanocobalamin 0.0325, biotin 0.15, Ca-pantothenate 15, niacin 45, folic acid 1.5, choline chloride 600, manganese 100, zinc 75, iron 67.5, copper 17.5, iodine 1, selenium 0.275, cobalt 0.4; per 1 kg of grower diet; IU: retinyl acetate 12000, cholecalciferol 3250, tocopheryl acetate; mg: 40 menadione sodium bisulfite 2.25, thiamin mononitrate 2, riboflavin 7.25, pyridoxine 4.25, cyanocobalamin 0.03, biotin 0.1, Ca-pantothenate 12, niacin 40, folic acid 1.0, choline chloride 450, manganese 100, zinc 65, iron 65, copper 15, iodine 0.8, selenium 0.25, cobalt 0.4

### Sample collection and assays

At the end of week 3 of the experiment, chickens were deprived of feed for 6 hours, weighed individually and feed consumption was determined for each pen. Mortality was recorded in each treatment during the first experimental period. On completion of the experiment the chickens were weighed, killed by cervical dislocation and decapitated. Samples of blood from 12 birds from each treatment group were collected into serum tubes (Greiner Bio-One GmbH, Austria). Analysis of serum lipid profiles was performed using an automatic biochemical analyzer Metrolab 2300 (Argentina). Total cholesterol, triglycerides, and lipoprotein HDL and LDL fractions were assessed quantitatively by an enzymatic method using reagent sets from

Pointe Scientific, Inc. (MI, USA). Samples of toes were obtained from twelve birds in each treatment by carefully and uniformly severing the middle toe from each foot between the second and third tarsal bones. The samples were dried at  $100^{\circ}\text{C}$  for 24 h, weighed and dry-ashed at  $600^{\circ}\text{C}$  overnight for determination of toe ash. The phosphorus concentration in feed samples was determined colorimetrically by the molybdo-vanadate method (AOAC, 1995). Calcium concentrations in feed samples were determined by flame atomic absorption spectrophotometry (Spectrometer AA 240FS, Varian).

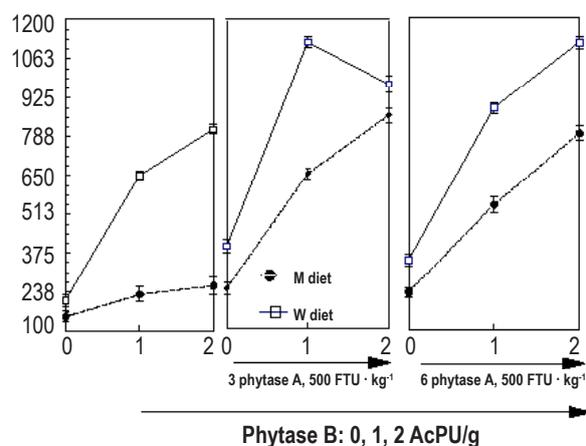
## Statistical analyses

The experimental data were analyzed by the General Linear Models Procedure of Statgraphics Plus for Windows (1996) using the one-way and two-way ANOVA models. Mean differences were determined using Fisher's least significant difference test. Mortality in each treatment was expressed in percentages and subjected to *arcsin* transformation to ensure homogeneity of variations. Statistical significance was accepted at  $P < 0.05$ .

## Results

The concentrations of *myo*-inositol released from maize- and wheat-based starter diets supplemented with phytase B and 3- or 6-phytase A and subjected to the *in vitro* procedure of multiple digestions, analyzed by the HPAEC-PAD technique, are presented in Figure 1. The type of cereal was a decisive factor affecting the efficacy of phytases in the release of *myo*-inositol. The quantities of *myo*-inositol found in the digesta of wheat-based diets were significantly higher than those found in maize-based feeds. Furthermore, phytase B tested as a single feed supplement was highly effective at  $1 \text{ ACPU} \cdot \text{g}^{-1}$  in wheat-based diets, but was ineffective in the maize-based ones. Both 3- and 6-phytases A tested at a constant level of  $500 \text{ FTU} \cdot \text{kg}^{-1}$  enhanced *myo*-inositol release from both types of diets. The highest quantities of *myo*-inositol (around  $1100 \mu\text{g} \cdot \text{g}^{-1}$ ) were released from wheat-based diets by either  $1 \text{ AcPU}$  of phytase B  $\cdot \text{g}^{-1}$  and  $500 \text{ FTU}$  of 3-phytase A  $\cdot \text{kg}^{-1}$  or by  $2 \text{ AcPU}$  of phytase B  $\cdot \text{g}^{-1}$  accompanied by  $500 \text{ FTU} \cdot \text{kg}^{-1}$  of 6-phytase A.

In the feeding trial, significant main effects of dietary additions and the type of cereal fed, with no



**Figure 1.** The influence of the type of phytase on the release of *myo*-inositol from samples of the maize-based (M,  $\bullet$ ) and wheat-based (W,  $\square$ ) starter diets. Samples of diets were digested by the three-step *in vitro* method (Żyła et al., 2000) and dialyzed digesta were analysed for the *myo*-inositol by the HPAEC-PAD technique as described previously (Duliński et al., 2011)

**Table 2.** Main effects of dietary additives and type of diet (maize-based or wheat-based) on performance of broilers, 1–21 day of age<sup>1,2</sup>

Main effect	Feed intake, g	Body weight gain, g	Feed conversion ratio, g	Mortality, arcsin, %
Dietary additives				
NC	565 <sup>c</sup>	263.4 <sup>c</sup>	2.195 <sup>a</sup>	2.244
IC	633 <sup>b</sup>	321.0 <sup>b</sup>	2.005 <sup>b</sup>	3.142
Inos	617 <sup>b</sup>	320.9 <sup>b</sup>	1.977 <sup>b</sup>	3.598
Phy B	652 <sup>ab</sup>	345.1 <sup>ab</sup>	1.918 <sup>b</sup>	2.244
Phy A+B	692 <sup>a</sup>	371.1 <sup>a</sup>	1.908 <sup>b</sup>	1.795
Type of diet				
maize	621	295 <sup>B</sup>	2.156 <sup>A</sup>	3.773 <sup>A</sup>
wheat	643	354 <sup>A</sup>	1.842 <sup>B</sup>	1.436 <sup>B</sup>
Pooled SEM	12	15.6	0.059	0.554
Probability				
dietary additives	0.001	0.001	0.007	NS
cereal	NS	0.001	0.001	0.004
interaction	NS	NS	NS	NS

NC – negative control diet, IC – internal control diet (NC +  $1.8 \text{ g} \cdot \text{kg}^{-1}$  monocalcium phosphate. <sup>a,b</sup> means within a column with no common superscript differ significantly ( $P < 0.05$ ); <sup>1</sup>for experimental diets formulation see Table 1; <sup>2</sup>the values are the means of 5 replicates of 8 birds each

interactions between experimental factors, were observed for body weight gain and FCR in the first experimental period. Feed intake did not depend on the cereal component of the diet, whereas mortality was significantly higher in broilers fed diets based on maize but was not altered by the tested dietary additions (Table 2). The highest feed intakes were recorded in broilers fed the diets supplemented with both phytases, while the lowest feed consumption was recorded in birds fed the NC diet. Feed intakes were significantly improved in broilers fed diets supplemented with either *myo*-inositol or monocalcium phosphate. The addition of phytase B brought FI to values that did not significantly differ from those observed in birds fed diets supplemented with both phytases. Generally, the variations in BW paralleled the changes in feed intake, but the gains were clearly higher in broilers fed the wheat-based diets. With the exception of the NC diet, FCR did not differ among dietary treatments but, again, in comparison with the maize-based diets, the FCR values for wheat-based diets were significantly improved.

At week 6, broilers fed the maize-based diets had final body weights that significantly depended on the dietary additives tested (Table 3). The highest body weights were recorded in chickens fed diets supplemented with phytase B and phytase A, whereas the lowest body weights were observed in their counterparts fed the NC diet. Similarly to the first experimental period, the addition of either phytase B or monocalcium phosphate improved body weight as compared with the NC treatment. Supplemen-

tary *myo*-inositol only numerically enhanced BWG above the level that was characteristic for birds fed the NC diet, but the value did not differ significantly from the values observed in the IC and Phy B treatments. Broilers that were fed the NC diet had poor bone mineralization, as evidenced by the low percentage of ash in the toes. The highest percentages of ash were found in the toes collected from broilers fed the diets supplemented with phytases A and B. Bone mineralization was also positively altered by phytase B used as a sole supplemented enzyme. In broilers fed the diets supplemented with monocalcium phosphate or *myo*-inositol, the percentage of toe ash was only numerically increased and did not differ significantly from that determined for birds fed the NC diet.

The dietary supplements tested in the current study significantly modulated the serum lipid profiles in experimental chickens (Table 3). Serum triglyceride concentrations were in the range from 22.1 mg · dl<sup>-1</sup> to 67.3 mg · dl<sup>-1</sup> for broilers fed the negative control diet and the diet containing two phytases, respectively. The variations observed among mean values of this parameter were somehow connected to the changes in the final body weight and showed a similar pattern of variability. The serum HDL cholesterol concentrations were similar in chickens fed the different diets, with the exception of broilers fed the diets supplemented with both phytases, who had significantly higher HDL levels. Furthermore, broilers that received both phytases had the highest HDL-to-total cholesterol ratio as opposed to birds fed the IC diet, whose serum HDL-to-total cholesterol ratios were the lowest among all of the experimental birds. *Myo*-inositol and phytase B applied as a single feed supplement affected the HDL-to-total cholesterol ratio similarly and led to intermediate values of this index.

**Table 3.** Effects of dietary additives on the final body weight (FBW), toe ash, and concentration of triglycerides and HDL cholesterol in 42-day old broilers fed the maize-based starter and grower diets<sup>1</sup>

Treatment	FBW n=80, g	Toe ash n=12, %	Serum, n=12		
			triglycer- lides, mg · dl <sup>-1</sup>	HDL	HDL/total cholesterol
NC	1366 <sup>c</sup>	10.7 <sup>c</sup>	22.1 <sup>c</sup>	111.0 <sup>b</sup>	0.647 <sup>bc</sup>
IC	1611 <sup>b</sup>	11.2 <sup>c</sup>	33.2 <sup>b</sup>	109.2 <sup>b</sup>	0.585 <sup>c</sup>
Inos	1495 <sup>bc</sup>	11.7 <sup>bc</sup>	23.8 <sup>bc</sup>	115.5 <sup>b</sup>	0.679 <sup>b</sup>
Phy B	1622 <sup>b</sup>	12.7 <sup>b</sup>	26.4 <sup>b</sup>	117.5 <sup>b</sup>	0.706 <sup>b</sup>
PhyA+B	1968 <sup>a</sup>	14.2 <sup>a</sup>	67.3 <sup>a</sup>	156.5 <sup>a</sup>	0.826 <sup>a</sup>
SEM	82	0.45	4.85	7.13	0.0279
P	0.001	0.001	0.001	0.001	0.001

<sup>1</sup> for experimental diets formulation see Table 1; a-c; A-B means within a column with no common superscript differ significantly (P < 0.05). NC, IC – see table 2

## Discussion

In this study, phytase B proved again to be the key catalyst that is necessary to generate *myo*-inositol from phytates. In contrast to the experimental evidence provided in a previous study that showed the ability of the enzyme to generate inositol from a sodium phytate solution (Żyła et al., 2012), here we document its ability to free substantial quantities of this substance from fully formulated maize-based and wheat-based diets under conditions that mimic digestion in the intestine of birds. Furthermore, the much higher efficacy of phytase B in wheat-based than in maize-based diets points to possible cooperation of the supplemental microbial enzyme with endogenous phytate-degrading enzymes from wheat. This can be attributed to the much higher content of endogenous phytate-degrading enzymes in wheat than in maize (Eeckhout and De Paepe, 1994; Viveros et al., 2000).

The much higher activity of endogenous phytate-degrading enzymes in wheat also explains the generally better performance of broilers fed the wheat-based diets as compared with birds fed the maize-based diets. This well-known phenomenon that is responsible for better phosphorus availability from wheat than from maize resulted in a higher BWG, better FCR, and lower mortality rate in chickens fed the starter diets based on wheat. It seems interesting to note that, in contrast to the *in vitro* part of this study, there were no data suggesting cooperation between endogenous and supplemental phytases in the broiler feeding experiment, since the interactions between dietary additives and the cereal components of the diets were not significant for any of the parameters studied.

In contrast to phytase B, which affected broiler performance consistently throughout both experimental periods, supplemental *myo*-inositol stimulated the growth of chickens more efficiently in the starter than in the grower period. *Myo*-inositol has already been reported to enhance body weight gains in growing broilers (Żyła et al., 2004), thus the current study provides yet more evidence of the growth-stimulating effect of this compound in broilers. Furthermore, the study broadens the previous findings and extends them to wheat-based diets. It also suggests that the biological activity of *myo*-inositol may be restricted to the early stages of bird development, or that the supplementation level of 1 g · kg<sup>-1</sup> diet may be too low.

The experimental design employed in this study included the IC diet formulated to contain amounts of available phosphorus similar to those for the Phy

A + B diet. The significant differences found between these two experimental groups could, therefore, be attributed to factors other than the increase in the concentration of available phosphorus resulting from enzymatic dephosphorylation. Further contrasting of the effects against the diet supplemented with *myo*-inositol may clearly point to the involvement of lower *myo*-inositol phosphates, the generation of which is accelerated by the joint action of phytases A and B on feed phytates (Żyła et al., 2000). Furthermore, in the diet containing both phytases, *myo*-inositol, which is also freed from phytate as evidenced by the *in vitro* part of the study, may interact with its lower phosphate esters creating a unique environment of 'enzymatically generated inositol'. In this study, broilers that were fed the diet supplemented with phytase A and B not only consumed more feed, had higher weight gains at days 21 and 42, but also had higher percentages of ash in the toes than chickens fed the IC diet. This suggests the interaction of compounds other than phosphorus or *myo*-inositol in bone mineralization and may reflect the well-known involvement of *myo*-inositol triphosphates in the calcium signaling pathway (Suzuki et al., 2010). Indeed, phytase B alone and, to a much higher extent, in combination with 6-phytase A not only enhanced bone mineralization, but also increased body weight gains in broilers fed the NPP- and Ca-deficient diets, emphasizing the important role that phytases play in Ca metabolism.

The possible impact of different feed supplements on the serum lipid profile of broilers has not attracted much attention in recent research. Moreover, some of the findings about the impact of dietary  $\omega$ -3 unsaturated fatty acids on the serum lipid profiles of birds are inconsistent (Szymczyk et al., 2001; Atakisi et al., 2009). Liu et al. (2010) have documented the effects of phytate and phytase on intestinal lipase activity and lipid metabolism in broiler chickens. The addition of sodium phytate (Liu et al., 2010) or chitosan (Razdan and Pettersson, 1994) to diets for broiler chickens produced significant decreases in the plasma concentration of total cholesterol and a concomitant increase in the HDL-to-total cholesterol ratio. This was probably caused by enhanced reversed cholesterol transport in response to the intestinal losses of the dietary lipids that interacted with phytate or chitosan during digestion. This explanation, however, cannot be offered for the significant reduction in total plasma cholesterol of broilers consuming diets enriched in a microbial culture of mixed *Lactobacillus* strains as reported by Kalavathy et al. (2003). From among all of the experimental birds tested in our study, broil-

ers fed the diets supplemented with phytase A and B had the highest HDL cholesterol contents and the highest HDL-to-total cholesterol ratios. Zamudio et al. (2001) reported not only high levels of phytate-degrading enzymes in six *Lactobacillus* strains, but also pointed out that the enzymes were a type of non-specific acid phosphatase, whose catalytic properties resemble the phytase B tested in our study. It seems, therefore, that among the products generated by phytase from phytate there must have been compounds with the ability to modulate lipid metabolism in broilers. Taking into account the results of the study presented here, neither *myo*-inositol nor free phosphorus can be candidates for this role, thus the single remaining possibility is the *myo*-inositol phosphate intermediates generated by the concerted action of 6-phytase A and phytase B.

## Conclusions

*Myo*-inositol fed at  $1 \text{ g} \cdot \text{kg}^{-1}$  was found to stimulate the growth of broilers fed both maize-based and wheat-based diets at week 3. At week 6, however, it did not significantly affect the toe ash content in birds fed the maize-based diets. At week 3, phytase B fed at  $1300 \text{ AcPU} \cdot \text{kg}^{-1}$  enhanced performance parameters in broilers fed maize- and wheat-based diets, whereas 6-phytase A added to feeds ( $500 \text{ FTU} \cdot \text{kg}^{-1}$ ) on top of phytase B produced further enhancements in these parameters. At week 6, both phytases increased the toe ash of chickens fed the maize-based diets. Broilers fed the maize-based diets supplemented with both phytase B and 6-phytase A for 6 weeks had increased concentrations of serum triglycerides and HDL-cholesterol, as well as an enhanced HDL-to-total cholesterol ratio.

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