

# Phytase and Acid Phosphatase Activities in Plant Feedstuffs

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A total of 183 samples representing 24 feedstuffs were analyzed for total phosphorus, phytate phosphorus content, phytase (Phy), and acid phosphatase (AcPh) activities with the objective to predict the capacity to hydrolyze phytic acid and to contribute to formulating environmentally adequate diets for monogastric animals. Of the cereals and cereal byproducts analyzed, only rye (5147 U kg<sup>-1</sup>; 21 955 U g<sup>-1</sup>), wheat (1637 U kg<sup>-1</sup>; 10 252 U g<sup>-1</sup>), rye bran (7339 U kg<sup>-1</sup>; 56 722 U g<sup>-1</sup>), and wheat bran (4624 U kg<sup>-1</sup>; 14 106 U g<sup>-1</sup>) were rich in Phy and AcPh activities. Legume seeds and oilseeds contained negligible Phy activity and a moderate amount of AcPh activity, except for kidney bean (33 433 U g<sup>-1</sup>) and full-fat linseed meal (13 263 U g<sup>-1</sup>). On the other hand, a significant linear regression between phytate phosphorus ( $y$ ) and total phosphorus ( $x$ ) was observed in cereal byproducts ( $R^2 = 0.95$ ;  $y = 0.8458x - 0.0367$ ;  $P < 0.001$ ) and oil seeds ( $R^2 = 0.95$ ;  $y = 0.945x - 0.20$ ;  $P < 0.001$ ). Phy and AcPh were positively correlated with respect to phytate phosphorus in cereals, cereal byproducts, and other byproducts and negatively correlated in legume seeds and oilseeds. Except for cereals, the highest correlation between enzyme activities and phytate phosphorus was found for phytase. It is not possible to predict Phy and AcPh activities from phytate phosphorus content by linear and quadratic regressions. Finally, only highly significant and positive correlation was found between Phy and AcPh activities for cereals, cereal byproducts, and oilseeds.

**Keywords:** Feedstuffs; phosphorus; phytate; phytase; acid phosphatase

## INTRODUCTION

Phosphorus in plant feedstuffs is found in two separate groups: organically bound phosphorus present as salts of phytic acid (phytate phosphorus) and phosphorus present in other forms (nonphytate phosphorus) (Waldroup, 1999). Approximately two-thirds of the total phosphorus in plant materials is in the form of phytate (Nelson et al., 1968; Punna and Roland, 1999) and is either unavailable or poorly utilized by human and other monogastric animals (Reddy et al., 1982; Pointillart et al., 1984; Jongbloed, 1987; Fourdin et al., 1988). This unavailability is due to the very low phytase activity found in the digestive tract (Pallauf et al., 1994). Moreover, the phytate phosphorus not degraded is excreted and contributes to environmental pollution in areas with a high density of animal production.

Phytase has been studied intensively in the past few years because of the great interest in using such enzymes for reducing the phytate content in animal feed and food for human consumption. The phytases (*myo*-inositol hexakisphosphate 3- and 6-phosphohydrolases; EC 3.1.3.8 and 3.1.3.26) are a subfamily of high molecular weight histidine acid phosphatases that can hydrolyze phytic acid to inositol and free orthophosphate (Pointillart, 1984; Wyss et al., 1999). As a class, phytases have been rather poorly characterized biochemically (Wyss et al., 1999). Phytases are naturally found in

microorganisms, particularly fungi, and in a number of seeds including cereals, legumes, byproducts, and other feedstuffs (Eeckhout and De Paepe, 1994; Ravindran et al., 1999).

On the other hand, the positive role of acid phosphatase (pH optimum 2.5, biosynthesized by *Aspergillus ficum*; EC 3.1.3.2) in the hydrolysis of phytate and lower phosphate of *myo*-inositol has been reported by Ullah and Phillippy (1994). Acid phosphatase in *Aspergillus niger* phytase accelerated the hydrolysis of sodium phytate solution (Zyla, 1993) and complemented the breakdown of phytate by attacking the inositol phosphate intermediate (Zyla et al., 1995a). This activity had a vital role in a corn-soybean meal feed that was desphosphorylated by a commercial phytase under simulated intestinal conditions (Zyla et al., 1995b). However, Nasi et al., (1999) indicated that the synergistic interaction between microbial phytase and acid phosphatase activity levels was rather limited in the degradation of phytates in barley, maize, and soybean meal. Likewise, Gibson and Ullah (1988), Hayakawa et al. (1989, 1990), and Greiner et al. (1998) isolated nonspecific acid phosphatases in soybean, rice grains, and rye, respectively. All of these findings demonstrate the importance of acid phosphatase activity in commercial phytase preparations intended for use in the feed industry.

Although information on total and phytate phosphorus content and phytase activity of some foods and feed ingredients has been published, there is little information about the acid phosphatase activity for a variety of plant feedstuffs. Moreover, the possibility to establish a relationship among these parameters is also considered. Therefore, the purpose of this study was to collect

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samples from feedstuffs used in the animal feed industry and to determine the total phosphorus and phytate phosphorus contents as well as the phytase and acid phosphatase activities.

## MATERIALS AND METHODS

**Materials.** Clean and uncontaminated samples from different locations in Spain were used in the present study. Different varieties of wheat (soft, hard, spring, and fall) and barley (two and six rows) were determined. The samples were freeze-dried and stored at room temperature in airtight containers prior to chemical analysis. Samples were ground in a hammer mill and passed through a 0.5-mm sieve. All samples were analyzed in triplicate.

**Determination of Total and Phytate Phosphorus.** Total phosphorus concentration was determined spectrophotometrically by the molybdo-vanadate reagent after mineralization of the sample with HCL (AOAC, 1995). Phytate phosphorus was determined according to the indirect method of Haugh and Lantzsch (1983). In this method, the samples were extracted in 10 mL of 0.2 N HCL. To 0.5 mL of this extract, 1 mL of the ferric solution [0.2 g of ammonium iron (III) sulfate  $12\text{H}_2\text{O}$  in 100 mL of 2 N HCL and made up to 1 L] was added. The tubes were heated in the boiling water bath for 30 min. Once the tubes reached room temperature, 2 mL of 2,2'-Bipyridine solution was added. The decrease of iron determined colorimetrically (519 nm) in the supernatant is a measure of the phytic acid content.

**Enzyme Activity Measurements.** Phytase activity was analyzed according to the method described by Eeckhout and De Paepe (1994). Finely ground samples (50–100 mg) were weighed in 50-mL volumetric flasks, and the flasks were filled to the mark with sodium phytate solution [1.722 g of sodium phytate (Sigma P3618 from rice), 180 mL of  $\text{H}_2\text{O}$ , and 820 mL of 0.25 M acetate buffer, pH 5.5]. The flasks were shaken for 15 min and incubated in a water bath at 37 °C. After 10- and 70-min incubation, a 2-mL portion of the incubate was transferred to a test tube containing 2 mL of 10% trichloroacetic acid (TCA). The contents of both tubes were filtered, and the reaction was terminated by adding 1 mL of reaction mixture to 1 mL of a freshly prepared color reagent. The color reagent was a mixture of four parts of solution A (15 g of ammonium heptamolybdate  $4\text{H}_2\text{O}$  in 55 mL of 36 N  $\text{H}_2\text{SO}_4$  and made up to 1 L) and one part of solution B (27 g of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , a few drops 36 N  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{O}$  to 250 mL). The liberated phosphorus was determined spectrophotometrically (700 nm). In this method, phytase activity was calculated as follows: phytate units  $\text{kg}^{-1} = (P \times 1000)/(W \times 60)$ , where  $P$  is micromoles of phosphorus liberated by phytase in 60 min,  $W$  is sample weight (g), and 60 is the incubation time taken into account (i.e., 70 minus 10 min). From the method described, the phytase unit is defined as that amount of phytase activity that liberates inorganic phosphorus from a 0.0015 M sodium phytate solution at a rate of  $1 \mu\text{mol min}^{-1}$  at pH 5.5 and 37 °C.

Acid phosphatase activity was determined according to the method of Zyla et al. (1989). In this method, the acid phosphatase activity was measured spectrophotometrically at 405 nm by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl phosphate. Finely ground samples (100–150 mg) were weighed and extracted with 5 mL of 100 mM acetate buffer, pH 4.5, for 20 min. The reaction mixture consisted of 1 mL of substrate (10 mM disodium *p*-nitrophenyl phosphate) and 0.2 mL of enzyme extracted. After 30-min incubation at 37 °C, the reaction was stopped by the addition of 5 mL of 40 mM NaOH. One unit of acid phosphatase (AcPh) was defined as  $1 \mu\text{mol}$  of *p*-nitrophenol liberated/min under the above conditions.

**Statistical Analysis.** Data were processed using SAS (1986). Analysis of variance and coefficients of correlation and linear and quadratic regressions were determined to investigate whether, within each feedstuffs classes, there was a

relationship among several components analyzed (total phosphorus, phytate phosphorus, phytase, and acid phosphatase activities).

## RESULTS AND DISCUSSION

### Total and Phytate Phosphorus Concentrations.

The total phosphorus and phytate phosphorus of cereals, cereals byproducts, legume seeds, oilseeds, and other byproducts are summarized in Table 1. The different values found in the literature on the content of phytate phosphorus of the feedstuffs must be taken into account, being that these are related to cultivars, processing conditions, and analytical methods used. Phytate phosphorus content, as a percentage of total phosphorus content, for the six cereals studied (oat, wheat, barley, rye, corn, and millet) was highest for wheat (79%) and lowest for oat and rye (59%). These values agree fairly well with those mentioned by Sauveur (1989), Ravindran et al. (1994), Eeckhout and De Paepe (1994), NRC (1994), Liu et al. (1998), and Ravindran et al. (1999). All cereal byproducts listed in Table 1 showed a higher total (0.96–1.16%) and phytate (0.29–0.88%) phosphorus content than cereal seeds. This can be explained by the abundance of phytate phosphorus (87%) in the protein-rich aleurone layer of the cereals, except in corn, where about 90% of the phytate is located in the germ portion (O'Dell et al., 1972; Sauveur, 1989). Likewise, total phosphorus and phytate phosphorus contents for hard wheat as well as fall wheat were significantly ( $P < 0.05$ ) higher as compared to soft and spring wheat, respectively (Table 2). These results are in disagreement with those reported by NRC (1994), which revealed higher values for soft wheat. These differences may be due to genetic, variety, soil, and climatic conditions and also for the analytical method used. On the other hand, the barley of six rows had higher phytate phosphorus concentration ( $P < 0.05$ ) as compared to the one of two rows (Table 2).

Various legume seeds were found to contain 0.08–0.33% phytate phosphorus, and these values are similar to those analyzed for cereal grains (Table 1). However, the percentage of phytate phosphorus, as a percentage of total phosphorus content, was lower for legume seeds. These results were in the range reported by Thompson (1986), Ravindran et al. (1994), Eeckhout and De Paepe (1994), NRC (1994), and Ravindran et al. (1999). Unlike cereals, in legume seeds, phytate is distributed throughout the entire protein complex of the seed (Swick and Ivey, 1991).

Oilseeds and cereal byproducts had the highest levels of phytate phosphorus (0.34–0.76%), being about 57–76% of the total phosphorus in the form of phytate phosphorus (Table 1). Our results are similar to those obtained by other authors (Thompson, 1986; Eeckhout and De Paepe, 1994; NRC, 1994; Ravindran et al., 1999), considering that the majority of oilseeds analyzed by those were in the form of seed meal defatted, which had a higher phytate concentration than whole seed.

Published information on the phytate content of beet pulp, grape cake, soybean hulls, and soybean pulp + molasses is limited. These byproducts contained only moderated amount of phytate phosphorus. Values obtained for beet pulp and soybean hulls compared closely to those reported by Eeckhout and De Paepe (1994).

**Phytase and Acid Phosphatase Activities.** Because of the important role of these enzymes in the dephosphorylation process of the phytic acid, one of the

**Table 1. Total Phosphorus, Phytate Phosphorus, Phytase (Phy), and Acid Phosphatase (AcPh) Activities of Feed Ingredients<sup>a</sup>**

feed ingredients	<i>n</i> <sup>b</sup>	total P (%)	phytate P (%)	(phytate P/ total P) × 100	Phy (U kg <sup>-1</sup> )	AcPh (U g <sup>-1</sup> )	ratio AcPh/Phy
cereals							
oat	9	0.29 ± 0.02 <sup>c</sup>	0.17 ± 0.03	59 ± 0.07	84 ± 39	2 314 ± 274	27.5
wheat	30	0.29 ± 0.03	0.23 ± 0.03	79 ± 0.07	1637 ± 275	10 252 ± 3 436	6.3
barley	21	0.31 ± 0.03	0.19 ± 0.02	61 ± 0.04	1016 ± 330	3 816 ± 1 401	3.8
rye	6	0.34 ± 0.03	0.20 ± 0.01	59 ± 0.02	5147 ± 649	21 955 ± 4 017	4.3
corn	7	0.23 ± 0.01	0.18 ± 0.01	78 ± 0.01	70 ± 5	1 640 ± 12	23.4
millet	6	0.20 ± 0.01	0.15 ± 0.01	75 ± 0.01	56 ± 0.6	4 676 ± 5.8	83.5
cereal byproducts							
rye bran	10	0.96 ± 0.01	0.73 ± 0.01	76 ± 0.01	7 339 ± 11	56 722 ± 22	7.7
wheat bran	6	1.16 ± 0.01	0.88 ± 0.01	76 ± 0.01	4 624 ± 4	14 106 ± 4	3.1
oat bran	3	0.83 ± 0.01	0.68 ± 0.01	82 ± 0.05	25 ± 1	3 034 ± 4	121.4
corn gluten meal	3	0.42 ± 0.01	0.29 ± 0.01	69 ± 0.01	173 ± 3	2 189 ± 6.5	12.7
other byproducts							
soybean hull	5	0.21 ± 0.004	0.05 ± 0.01	24 ± 0.06	127 ± 3	2 283 ± 3	20.0
sunflower hull + molasses	4	0.15 ± 0.003	0.07 ± 0.01	47 ± 0.11	97 ± 3	2 697 ± 3.5	27.8
grape cake	5	0.14 ± 0.01	0.002 ± 0.01	1.4 ± 0.001	20 ± 1	2 188 ± 2	547.0
beet pulp	4	0.067 ± 0.03	0.01 ± 0.001	15 ± 0.01	20 ± 1	2 530 ± 12	633.0
legume seeds							
peas	6	0.43 ± 0.01 <sup>c</sup>	0.24 ± 0.01	56 ± 0.01	86 ± 1	5 410 ± 20	62.9
soybeans	4	0.73 ± 0.01	0.33 ± 0.01	45 ± 0.01	32 ± 2.5	1 875 ± 16.2	58.6
chickpea	6	0.31 ± 0.04	0.17 ± 0.02	55 ± 0.03	130 ± 20	5 067 ± 1 847	39.0
bean	3	0.39 ± 0.02	0.08 ± 0.01	21 ± 0.01	258 ± 1.5	4 575 ± 17	17.7
kidney bean	15	0.38 ± 0.02	0.17 ± 0.01	45 ± 0.01	144 ± 12.5	33 433 ± 6811	232.0
lupin	6	0.33 ± 0.03	0.16 ± 0.04	48 ± 0.07	219 ± 22	9 680 ± 2 787	44.2
oilseeds							
full-fat linseed	7	0.60 ± 0.01	0.34 ± 0.03	57 ± 0.01	295 ± 4.58	13263 ± 20	45.0
full-fat sesame	8	0.88 ± 0.01	0.67 ± 0.01	76 ± 0.01	175 ± 5	2661 ± 10	15.2
sunflower seeds meal (extracted)	4	0.98 ± 0.02	0.72 ± 0.01	73 ± 0.01	73 ± 2	6737 ± 8.5	92.3
rapeseed seeds meal (extracted)	5	1.05 ± 0.01	0.76 ± 0.01	72 ± 0.01	41 ± 2.5	4988 ± 12	121.7

<sup>a</sup> Analyzed based on dry matter. <sup>b</sup> Number of samples. <sup>c</sup> Each mean ± standard deviation represents three replications.

**Table 2. Influence of Hardness and Season of Wheat and Rows of Barley on Total Phosphorus, Phytate Phosphorus, Phytase (Phy), and Acid Phosphatase (AcPh) Activities<sup>a</sup>**

cereals	total P (%)	phytate P (%)	Phy (U kg <sup>-1</sup> )	AcPh (U g <sup>-1</sup> )
wheat				
hardness				
soft	0.28 <sup>b</sup>	0.22 <sup>b</sup>	1605	12 104 <sup>a</sup>
hard	0.31 <sup>a</sup>	0.25 <sup>a</sup>	1714	5931 <sup>b</sup>
<i>P</i> value	0.0026	0.0138	NS	0.0001
season				
spring	0.26 <sup>b</sup>	0.20 <sup>b</sup>	1791	13 179 <sup>a</sup>
fall	0.29 <sup>a</sup>	0.24 <sup>a</sup>	1590	10 251 <sup>b</sup>
<i>P</i> value	0.0021	0.0149	NS	0.0129
SEM <sup>b</sup>	0.005	0.006	50	627
barley				
files				
2 rows	0.28 <sup>b</sup>	0.18	1265 <sup>a</sup>	4427 <sup>a</sup>
6 rows	0.34 <sup>a</sup>	0.19	834 <sup>b</sup>	3053 <sup>b</sup>
<i>P</i> value	0.0001	NS	0.0043	0.0491
SEM	0.007	0.004	72	305

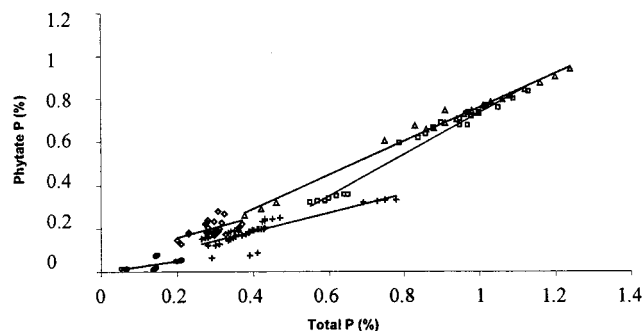
<sup>a</sup> Means within a column not sharing common letter are significantly different (*P* < 0.05). <sup>b</sup> Standard error means.

main objectives of the current study was to investigate, for each feedstuff class, the Phy and AcPh activities and to determine which of them was more related to phytate phosphorus content. Despite numerous attempts to purify plant phytases, only a few have been purified to homogeneity or near homogeneity (Greiner et al., 1998). In our study, both enzymes have not been separated. The physicochemical and catalytic properties of Phy and AcPh of microbial origin have been studied (Wodzinski and Ullah, 1996); however, from plant sources there are few data. Hayakawa et al. (1989, 1990) isolated non-

specific acid phosphatases with and without phytase activity in rice bran. Gibson and Ullah (1988), Konietzny et al. (1995), and Greiner et al. (1998) have also purified phytases and acid phosphatases from germinated soybean cotyledons, spelt, and rye, respectively.

The phytase and AcPh activities of the different feedstuffs analyzed are presented in Table 1. Of the cereals, rye had the highest Phy and AcPh activities (5147 U kg<sup>-1</sup> and 21 955 U g<sup>-1</sup>), followed by wheat (1637 U kg<sup>-1</sup> and 10 252 U g<sup>-1</sup>). Barley had a relatively high level of phytase activity (1016 U kg<sup>-1</sup>), but a moderate amount of AcPh activity (3816 U g<sup>-1</sup>), whereas oat, corn, and millet contained negligible phytase activities and low amounts of AcPh activities. In cereal byproducts, rye bran had evidently the highest Phy and AcPh activities (7339 U kg<sup>-1</sup> and 56 722 U g<sup>-1</sup>), followed by wheat bran (4624 U kg<sup>-1</sup> and 14 106 U g<sup>-1</sup>). This could be explained by the great amount of phytates found in the outer layers (Sauveur, 1989). However, oat bran and corn gluten meal had lower activities of both enzymes. As other authors reported, in our data, rye bran and rye exhibit higher Phy (Eeckhout and De Paepe, 1994; Greiner et al., 1998) and AcPh activities (Greiner et al., 1998), in comparison with other cereals (Han et al., 1997; Nasi et al., 1999; Ravindran et al., 1999).

Likewise, no differences were found in phytase activity between soft and hard wheat nor between spring and fall wheat. However, AcPh activity was significantly higher (*P* < 0.05) in soft and spring wheat than in hard and fall wheat (Table 2). Maga (1982) found higher phytase activity in hard wheat as compared with soft wheat; however, we did not observe this result. Finally, Phy and AcPh activities of other byproducts analyzed



**Figure 1.** Linear relationship between total phosphorus and phytate phosphorus of cereals (diamonds), cereal byproducts (triangles), legume seeds (+), oilseeds (squares), and other byproducts (circles). Increasing total phosphorus ( $x$ ) in the feed ingredients, there was a linear response in phytate phosphorus ( $y$ ) of cereals ( $r = 0.44$ ;  $R^2 = 0.20$ ;  $y = 0.416x + 0.079$ ;  $P < 0.001$ ), cereals byproducts ( $r = 0.97$ ;  $R^2 = 0.95$ ;  $y = 0.8458x - 0.0367$ ;  $P < 0.001$ ), other byproducts ( $r = 0.55$ ;  $R^2 = 0.30$ ;  $y = 0.33x - 0.01$ ; NS), legume seeds ( $r = 0.79$ ;  $R^2 = 0.62$ ;  $y = 0.426x + 0.015$ ;  $P < 0.001$ ), and oilseeds ( $r = 0.98$ ;  $R^2 = 0.95$ ;  $y = 0.945x - 0.20$ ;  $P < 0.001$ ).

(beet pulp, grape cake, soybean hulls, and sunflower hull + molasses) were not detected with the method used.

Legume seeds and oilseeds contained negligible phytase activity, according to the observations reported by Thompson (1986), Eeckhout and De Paepe (1994), Liu et al. (1998), Ravindran et al. (1999), and Nasi et al. (1999). However, for each feedstuff class studied, we obtained moderate amounts of AcPh activity (1875–9680 and 2661–6737 U g<sup>-1</sup>, respectively), which were, in the case of chickpea, lupin, sunflower, and rapeseed, higher than those found in some cereals and cereal byproducts (Table 1). It is necessary to mention the great amount of AcPh activity found in kidney bean (33 433 U g<sup>-1</sup>) and full-fat linseed (13 263 U g<sup>-1</sup>). In the literature reviewed, we have not found any mention of AcPh activity in legume seeds and oilseeds.

**Correlation Coefficients and Linear and Quadratic Regressions between the Several Components Analyzed.** Correlation coefficients and linear and quadratic regressions among total phosphorus, phytate phosphorus, phytase, and acid phosphatase activities for the several feedstuff classes were analyzed. The results showed that total phosphorus and phytate phosphorus were positively correlated in cereals ( $r = 0.44$ ,  $P < 0.001$ ), cereal byproducts ( $r = 0.97$ ,  $P < 0.001$ ), other byproducts ( $r = 0.55$ , NS), legume seeds ( $r = 0.79$ ,  $P < 0.001$ ), and oilseeds ( $r = 0.98$ ,  $P < 0.001$ ) (Figure 1). However, when the linear and quadratic regressions were determined, the predictability of phytate phosphorus ( $y$ ) content from total phosphorus ( $x$ ) content was only justified for cereal byproducts ( $R^2 = 0.95$ ;  $y = 0.8458x - 0.0367$ ;  $P < 0.001$ ) and oilseeds ( $R^2 = 0.95$ ;  $y = 0.945x - 0.20$ ;  $P < 0.001$ ) (Figure 1). Eeckhout and De Paepe (1994) also found a high correlation between total and phytate phosphorus for wheat and corn as well as its byproducts but not for oil meals.

The results also showed that Phy and AcPh activities were positively correlated with phytate phosphorus in cereals ( $r = 0.28$ ;  $P < 0.01$  and  $0.28$ ;  $P < 0.01$ ), cereal byproducts ( $r = 0.75$ ;  $P < 0.01$  and  $0.58$ ;  $P < 0.05$ ), and other byproducts ( $r = 0.86$ ;  $P < 0.001$  and  $0.51$ ; NS), and negatively in legume seeds ( $r = -0.80$ ;  $P < 0.001$  and  $-0.17$ ;  $P < 0.05$ ) and oilseeds ( $r = -0.94$ ;  $P < 0.001$  and  $-0.87$ ;  $P < 0.01$ ). Except for cereals, the highest correlation between enzyme activities and phytate P was found for phytase.

The significantly different correlation found between enzyme activities and phytate phosphorus content in legume seeds with respect to cereals could be explained by the different location and distribution of phytate phosphorus in the seeds. In dicotyledonous seeds, including legumes and oilseeds, the phytate is distributed throughout the kernel in subcellular inclusions, known as globoids (Cosgrove, 1980). The phytic acid in cereals is not uniformly distributed within the kernel but associated with specific morphological components of the seed. In wheat, the endosperm is almost devoid of phytate, but the aleurone layers of the kernel and the bran contain substantial amounts (De Boland et al., 1975).

The association between phytic acid and protein that begins in the seed during ripening and the structural and chemical properties of this protein association in cereals and legume seeds could determine the degree of phytate–protein binding, which in turn, may influence the response to Phy and AcPh. Likewise, the ability of phytic acid to complex to protein is determined largely by its solubility. De Boland et al. (1975) have reported differences in the solubility of phytate from different sources. They suggest that the variation in phytate solubility may be responsible for the differences in the extent of hydrolysis of phytate from different feedstuffs.

On the other hand, although the probability of regression coefficients was significant in many feedstuff classes, the very low values of  $R^2$  obtained in the statistical analysis suggest that it was not possible to predict Phy and AcPh activities of cereals, cereal byproducts, legume seeds, oilseeds, and other byproducts from phytate phosphorus content by linear and quadratic regression analysis. Only in the case of oilseeds, the linear regression coefficient showed an increase in phytate phosphorus ( $x_1$ ) or total phosphorus ( $x_2$ ), which results in a significant increase in phytase activity ( $y$ ) ( $R^2 = 0.89$ ;  $y = -565x_1 + 497$ ;  $P < 0.001$ ) ( $R^2 = 0.98$ ;  $y = -575x_2 + 648$ ;  $P < 0.001$ ). When both Phy and AcPh activities were separately studied, we obtained a higher  $r$  and  $R^2$  than when those enzymes were considered as a whole (results not shown). Sauveur (1989) did not find a clear correlation between phytate phosphorus content and phytase levels. Likewise, Eeckhout and De Paepe (1994) reported that no relationship could be established between phytase activity and total phosphorus or phytate phosphorus contents for barley, wheat, and wheat byproducts.

Likewise, a highly significant and positive correlation between Phy and AcPh activities for cereals ( $r = 0.82$ ;  $P < 0.001$ ), cereal byproducts ( $r = 0.91$ ;  $P < 0.001$ ), and oilseeds ( $r = 0.66$ ;  $P < 0.01$ ) was found. However, no significant correlation was found in legume seeds ( $r = -0.09$ ; NS) and other byproducts ( $r = 0.18$ ). When AcPh activity ( $x$ ) was increased, there was a significant linear increment in Phy activity ( $y$ ) in cereals ( $R^2 = 0.68$ ;  $y = 0.179x - 35.78$ ;  $P < 0.001$ ) and cereal byproducts ( $R^2 = 0.83$ ;  $y = 0.126x + 637$ ;  $P < 0.001$ ), and a quadratic increment in oilseeds ( $R^2 = 0.52$ ;  $y = -0.000062x^2 - 0.087x + 350.6$ ;  $P < 0.001$ ).

In conclusion, in this study we have reported data of acid phosphatase activity in different feed ingredients used in animal nutrition and their relationship to total phosphorus, phytate phosphorus, and phytase activity. Since the utilization of phytate phosphorus by monogastric animals can be improved by dietary incorporation of plant-derived ingredients with known phytase

and acid phosphatase activities, the results of this investigation could help to predict the capacity to dephosphorylate phytic acid of different common feedstuffs, within the digestive tract, when used in animal nutrition.

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