In Vitro Prediction of Phosphorus Availability in Feed Ingredients for Swine

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The percentage of phosphorus (P) dialyzability was determined in 10 plant-origin and 4 animal-origin feed ingredients using an in vitro procedure that simulates the digestive system of growing swine. The test feed ingredients included alfalfa meal, barley, canola meal, corn, grain sorghum, oats, rice bran, soybean meal, wheat, wheat bran, menhaden fish meal, meat and bone meal, spray-dried blood meal, and dry whey. Repeatability of the in vitro procedure was tested for each ingredient using quadruplicate samples. The in vitro P dialyzability percentages were correlated with published in vivo P availability values for swine. Results showed that P dialyzability with the in vitro procedure was highly repeatable (>96%). For the 10 plant-origin feed ingredients, significant correlations (r = 0.72−0.88) were found among the in vitro P dialyzability percentages and the published in vivo P availability values. For the four animal feed ingredients, however, the in vitro dialyzability percentages obtained were poorly correlated (r = −0.26 to 0.70, p > 0.3−0.7) with published in vivo P availability values. In conclusion, the in vitro P dialyzability procedure presented here is a valid alternative to conducting in vivo studies for ranking plant-origin feed ingredients in P availability. Fine grinding of the ingredients and the addition of acid phosphatase increased the validity of this in vitro procedure.

Keywords: *In vitro dialyzability; phosphorus; feed ingredients; swine*

INTRODUCTION

Most feed ingredients of plant origin have 50−80% of their total phosphorus (P) as phytate (Nelson et al., 1968). Swine cannot digest phytate P because their digestive system lacks phytase, the enzyme that cleaves the phosphate groups from the phytate molecule. Therefore, swine diets are supplemented with inorganic P (National Research Council, 1988) and/or microbial phytase (Liu et al., 1997a, 1998) to meet the available P requirements based on the estimated availability of P in feed ingredients. Biological assays conducted with test pigs are the reliable means of estimating the availability of P in diets for pigs. However, such assays are impractical for routine evaluation and screening of feedstuffs or diets because they are time-consuming and expensive (Cone and Van der Poel, 1993; Waldroup, 1996). In vitro P solubilities in dilute acid solutions have been used to predict in vivo P availabilities in poultry (Day et al., 1973; Yoshida et al., 1979). However, those procedures in general lack validity for estimating P availability in swine (Dellaert et al., 1990; Waldroup, 1996).

We have described an in vitro procedure that simulates the digestive system of growing swine for the purpose of estimating the enzymatic dephosphorylation of phytate in corn−soybean meal diets for growing swine (Liu et al., 1997b). Using that procedure, we found a significant positive correlation (r = 0.999) between the in vitro P dialyzability and the in vivo P digestibility in pigs fed a corn−soybean meal diet.

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We hypothesized that our in vitro procedure could be used as an alternative method for ranking feed ingredients with respect to their P availabilities to swine. Therefore, the purpose of this experiment was to determine the validity of our in vitro procedure for predicting in vivo P availability of 14 common feed ingredients.

MATERIALS AND METHODS

Enzymes. Porcine pepsin (P-6887; EC 3.4.23.1), pancreatin (P-7545; activity = 8 × USP), and acid phosphatase (P-3627; EC 3.1.3.2) were purchased from Sigma Chemical Co., St. Louis, MO. A dry powdered phytase (EC 3.1.3.8) preparation containing 47 000 phytase units (PU)/g (declared by BASF, Mount Olive, NJ) was used as a standard reference. One PU is defined as the amount of enzyme that liberates 1.0 μmol of inorganic P/min from 5.1 mM sodium phytate from rice (P-3168, Sigma) at 37 °C and pH 5.5 (Engelen et al., 1994). One unit (U) of acid phosphatase is defined as the amount of enzyme that hydrolyzes 1.0 μmol of p-nitrophenyl phosphate/per min at 37 °C and pH 4.8.

Feed Ingredients and Sample Preparation. The feed ingredients tested in the present study were 10 plant-origin feed ingredients (alfalfa meal, barley, canola meal, corn, grain sorghum, oats, rice bran, 49% CP soybean meal, wheat, and wheat bran) and 4 animal-origin ingredients (menhaden fish meal, meat and bone meal, spray-dried blood meal, and dry whey) that were obtained from local feedmills. All 14 air-dried ingredient samples (~1 kg) were ground in a hammermill with screen openings of 1.0 mm (Arthur H. Thomas, Philadelphia, PA). A ground sample was removed and hand-mixed in a plastic cup. After mixing, ~100 g of sample was further ground in a Tecator 1093 sample mill with screen openings of 0.5 mm (Fisher Scientific, Pittsburgh, PA). All ingredient samples were kept in sealed cups at 0 °C until analyzed. Three sample preparation treatments were (1) ingredient samples ground through a 1.0-mm screen, (2) ingredient samples ground through a 0.5-mm screen, and (3) ingredient samples

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ground through a 0.5-mm screen with the addition of acid phosphatase at 0.5 U/g of feed.

Assays. Indigenous phytase activity in the feed ingredients was determined according to the procedure described by Engelen et al. (1994). In brief, duplicate samples (5 g) of each ingredient were ground through a 1.0-mm screen and placed in 250-mL Erlenmeyer flasks containing 50 mL of 0.25 M acetate buffer (pH 5.5). A suspension was prepared by stirring on a magnetic stirrer for 60 min. After the suspension was filtered through a Q-5 filter paper (Fisher Scientific), the clear solution was incubated with substrate (5.1 mM sodium phytate) at 37 °C for 60 min. Phytase activity of ingredient samples was determined by fitting the absorbance into the standard curve established with the standard phytase preparation. Dietary P was determined colorimetrically on a spectrophotometer (Beckman DU-65, Beckman Instruments, Fullerton, CA) at 415 nm as described by Engelen et al. (1994) in triplicate samples, which were digested by a wet-ash procedure (Association of Official Analytical Chemists, 1990).

Determination of in Vitro Phosphorus Dialyzability. The in vitro procedure was previously described (Liu et al., 1997b). In brief, a 1-g sample of test feed ingredients was mixed with 2 mL of a 0.18 N HCl solution containing a total of 3000 units of pepsin with or without 0.5 U of acid phosphatase. The sample was then incubated in a water bath at 39 °C for 75 min. The peptic digesta were mixed with 0.65 mL of a 1 M $NaHCO_3$ solution containing 3.7 mg of pancreatin/mL and transferred into a segment of dialyzing tubing (molecular weight cutoff 12 000−14 000, diameter 1.6 cm; Sigma Chemical). The segment was then placed in 100 mL of a 0.05 M succinate buffer (pH 6.0) containing 0.1 M NaCl and dialyzed in a shaking water bath at 39 °C for 4 h. Released P was determined colorimetrically as described previously. The P dialyzability was determined in quadruplicate samples of each ingredient ground through a 1.0- or 0.5-mm screen and in samples ground through a 0.5-mm screen with acid phosphatase at 0.5 U/g of feed. The P dialyzability was calculated according to the equation

$$\text{dialyzability (\%)} = D/W \times A \times 100$$

where D is the amount of dialyzed P (mg), W is the air-dry weight of the feed ingredient sample (g), and A is the P concentration in the air-dry sample (mg/g).

Statistical Analysis. Data (percent P dialyzability on an air-dry basis) were analyzed as a one-way analysis of variance (Snedecor and Cochran, 1989) using SAS (1989). The three sample preparation treatments and the 14 feed ingredients were tested as a 3 × 14 factorial arrangement. Main effect and interaction effect means were tested using the F-protected least significant difference (LSD).

The sample preparation treatments were also tested for repeatability using another analysis of variance. The error mean square for each sample preparation treatment provides an estimate of within-ingredient variability pooled over all feed ingredients. F values were obtained by comparing the error mean squares between any two sample preparation treatments. The sample preparation treatment that has the largest F value is more repeatable and is superior to the other processing treatments. Coefficients of correlation and regression were analyzed among the three sample preparation treatments and the three sets of published in vivo P availability values of the 14 ingredients.

RESULTS

Total P Concentrations and Indigenous Phytase Activities of Feed Ingredients. The P concentrations in feed ingredients were in the range reported by the National Research Council (1988). Alfalfa meal, canola meal, corn, grain sorghum, oats, and soybean meal contained no or negligible phytase activities, whereas wheat and wheat bran contained significant amounts of phytase activities (Table 1).

Table 1. Phosphorus Concentrations of Feed Ingredients, Air-Dry Basis[a]

feed ingredient	total P (%)	indigenous phytase (PU/kg)
Plant-Origin Ingredients		
alfalfa meal	0.24	0
barley	0.35	88
canola meal	0.93	24
corn	0.26	0
grain sorghum	0.29	0
oats	0.34	0
rice bran	1.51	0
soybean meal, 49% CP	0.69	0
wheat	0.34	420
wheat bran	1.17	1665
Animal-Origin Ingredients		
menhaden fish meal	3.46	
meat and bone meal	4.44	
spray-dry blood meal	0.39	
whey	0.68	

[a] Dietary P concentrations and phytase activities were determined in replicate and triplicate samples, respectively. Indigenous phytase activities were not determined in animal-origin ingredients.

In Vitro P Dialyzability. The mean P dialyzability percentages from our in vitro procedure and the available P values published by the National Research Council (1988) and Cromwell (1989, 1992) for the 14 feed ingredients are presented in Table 2. A large interaction ($p < 0.0001$) was obtained between sample preparation treatment and plant-origin feed source. The primary contributors to this interaction were alfalfa meal, barley, oats, soybean meal, and wheat. Generally, with the exception of wheat bran, in vitro P dialyzability percentages of the 14 feed ingredients were lower than corresponding published in vivo availabilities.

The percentages of P dialyzability were increased ($p < 0.05$) by grinding samples of alfalfa meal, barley, oats, and soybean meal through a 0.5-mm screen compared to a 1.0-mm screen. Addition of acid phosphatase further increased ($p < 0.05$) the percentages of P dialyzability in alfalfa meal and wheat (Table 2). The remaining five plant-origin feed ingredients (canola meal, corn, grain sorghum, rice bran, and wheat bran) and all four of the animal-origin ingredients were not affected ($p > 0.05$) by sample preparation treatments.

For all 10 plant-origin ingredients, the samples ground through a 0.5-mm screen with or without addition of acid phosphatase had higher ($p < 0.0001$) mean percentages of P dialyzability than the samples ground through a 1.0-mm screen. Addition of acid phosphatase to the ground feed ingredients (screen size = 0.5 mm) increased ($p < 0.05$) P dialyzability only for alfalfa meal and wheat, with no effect on the other 12 ingredients.

Repeatability Test. There were relatively high repeatabilities (>96%) in the determination of the percentage of P dialyzability in feed ingredients with the in vitro procedure (Table 3). Grinding samples through a 0.5-mm screen increased the repeatability of the in vitro test only in the animal-origin feed ingredients. The addition of acid phosphatase decreased ($p < 0.05$) the repeatability for predicting the percentage of P dialyzability in plant-origin feed ingredients but had no effect on the repeatability of predicting P dialyzability in animal-origin feed ingredients.

Correlations between in Vitro P Dialyzability and Reported in Vivo P Availability Values. Significant correlations were found between the percent-

Table 2. In Vitro P Dialyzability and Reported in Vivo P Availabilities in Feed Ingredients

feed ingredient	in vitro P dialyzability[a] (%)			in vivo P availability (%)		
	1.0 mm	0.5 mm	0.5 mm + AP[b]	NRC[c] (1988)	Cromwell (1992)	Cromwell (1989)
Plant-Origin Ingredients						
alfalfa meal	56.84[d] ± 1.04	71.23[e] ± 5.96	77.58[f] ± 10.79	100	100	100
barley	16.03[d] ± 1.29	27.20[e] ± 2.65	28.90[e] ± 1.59	31	30	31
canola meal	16.41 ± 0.77	20.10 ± 2.11	19.21 ± 1.07		21	21
corn	13.11 ± 2.53	16.55 ± 1.24	14.40 ± 1.55	15	14	14
grain sorghum	13.85 ± 0.35	17.73 ± 0.98	14.83 ± 1.91	22	20	19
oats	20.69[d] ± 0.79	25.48[e] ± 1.82	25.21[e] ± 1.35	30	22	30
rice bran	13.06 ± 1.60	14.35 ± 1.43	13.84 ± 0.74	25	25	25
soybean meal, 49%	10.48[d] ± 0.53	19.02[e] ± 3.30	19.73[e] ± 3.25	25	23	25
wheat	11.97[d] ± 0.36	14.30[d] ± 1.32	22.54[e] ± 4.72	50	49	50
wheat bran	45.24 ± 3.63	45.86 ± 0.57	47.98 ± 0.57	35	29	35
Animal-Origin Ingredients						
menhaden fish meal	16.18 ± 2.47	16.24 ± 0.39	15.83 ± 0.59	100	94	102
meat and bone meal	6.97 ± 1.04	5.70 ± 0.32	5.75 ± 0.27	93	67	76
spray-dry blood meal	55.16 ± 6.89	50.04 ± 4.64	51.83 ± 5.26		92	92
whey	77.02 ± 5.14	79.29 ± 0.79	77.44 ± 1.38		97	76

[a] Each mean ± standard deviation represents four replications. Means within rows with no common letters (d−f) differ ($p < 0.05$). [b] AP, acid phosphatase. [c] National Research Council.

Table 3. Repeatability (Percent) of the Three Sample Preparation Treatments

feed ingredient	1.0-mm screen[a]	0.5-mm screen[a]	0.5-mm screen[a] + AP
plant-origin ingredient	97.19[b]	97.96[b]	96.07[c]
animal-origin ingredient	98.16[b]	99.50[c]	99.32[bc]

[a] Samples were ground in a mill with screen openings of 1.0 or 0.5 mm. Means within rows with no common letters (b, c) differ ($p < 0.05$).

Table 4. Relationship between the in Vitro P Dialyzability and Reported in Vivo P Availabilities for All 10 Plant-Origin Feed Ingredients

treatment	in vivo data	regression eq[a]	correl coeff (r)	signif-icance[b]
1.0-mm screen[c]	NRC (1988)	$Y = 1.14x + 11.50$	0.76	***
	Cromwell (1992)	$Y = 1.13x + 8.67$	0.72	***
	Cromwell (1989)	$Y = 1.18x + 9.42$	0.76	***
0.5-mm screen[c]	NRC (1988)	$Y = 1.09x + 6.40$	0.82	***
	Cromwell (1992)	$Y = 1.10x + 3.48$	0.79	***
	Cromwell (1989)	$Y = 1.12x + 4.47$	0.82	***
0.5-mm screen[c] + AP	NRC (1988)	$Y = 1.05x + 6.19$	0.87	***
	Cromwell (1992)	$Y = 1.05x + 3.41$	0.85	***
	Cromwell (1989)	$Y = 1.07x + 4.48$	0.88	***

[a] Y = in vivo P digestibility (%); x = in vitro P dialyzability (%). [b] *** $p < 0.0001$. [c] Samples were ground in a mill with screen openings of 1.0 or 0.5 mm.

ages of in vitro P dialyzability and published in vivo P availability values for plant-origin type feed ingredients (Table 4). In plant-origin feed ingredients, samples ground through a 0.5-mm screen with acid phosphatase had the highest correlation coefficients ($r = 0.85-0.88$, $p < 0.0001$) with published P availability values, samples ground through a 0.5-mm screen without acid phosphatase were intermediate ($r = 0.79-0.82$, $p < 0.0001$), and samples ground through a 1.0-mm screen had the lowest values ($r = 0.72-0.76$, $p < 0.0001$).

However, no significant correlations ($r = -0.26$ to 0.70, $p > 0.3-0.7$) were found between our in vitro P dialyzability values and published in vivo P availability values (National Research Council, 1988; Cromwell, 1989, 1992) for the four animal feed ingredients (data not shown).

DISCUSSION

The P availability of a P supplement, a feed ingredient, or a diet is usually obtained by conducting a bioassay using animals (Cromwell, 1992), which is time-consuming and expensive. An alternative to the in vivo approach is to use an in vitro procedure because of the relatively low cost and the short time required for the assay (Waldroup, 1996). An in vitro method may be very useful for ranking purposes even when the prediction of bioavailability is relative rather than absolute (Wolters et al., 1993).

Highly significant correlations were found between the in vitro P dialyzability and reported in vivo P availability in plant-type ingredients in the present study, confirming our hypothesis. Our in vitro procedure has a high repeatability of 96−99.5% in predicting P dialyzability. This high degree of validity may be related to the design of our in vitro procedure that simulated the digestive system (stomach and small intestines) of growing swine (Liu et al., 1997b). Another in vitro method (Yoshida et al., 1979) that was not based on a digestive physiology approach lacked adequate sensitivity for use with swine (Dellaert et al., 1990).

The availability of P in feed ingredients and diets depends primarily on the amount of dietary phytate P present and the concentration of phytase in the plant sources. We found that alfalfa meal, canola meal, corn, grain sorghum, oats, and soybean meal were essentially devoid of phytase activity, which is in agreement with previous results (Pointillart et al., 1985; Oksbjerg, 1988; Pointillart, 1988; Bosi et al., 1997). Both wheat and wheat bran contained significant measurable phytase activities, which were in the range reported by Pointillart (1988) and Bosi et al. (1997). The high dialyzability of P in wheat bran may be due to the high indigenous phytase activity of 1665 PU/kg measured in the present experiment. Alfalfa meal does not contain phytate (Nelson et al., 1968) and should have a P availability of 100% (National Research Council, 1988; Cromwell, 1989, 1992). However, in the present experiment only 57−78% of the P in alfalfa meal was dialyzable by our in vitro procedure. The in vitro P dialyzability of the 14 feed ingredients tested in the present experiment were generally below the corresponding published in vivo P availability values with the exception of wheat bran. Therefore, we are in agreement with Wolters et al. (1993) that these in vitro results provide a relative rank order of P availability and do not provide absolute P availabilities.

We hypothesized that grinding samples through a 0.5-mm screen would increase in vitro P dialyzability

compared to a 1.0-mm screen. The grinding through a 0.5-mm screen significantly increased P dialyzability in 4 of the 14 feed ingredients. We used a water to feed ratio of 2 to 1 during the peptic digestion. The finely ground sample generally absorbed more water, making the mixture less fluid compared to the samples ground through a 1.0-mm screen. The effect of the water to feed ratio on enzyme function in an in vitro P dialyzability procedure deserves further evaluation.

The addition of acid phosphatase to the in vitro procedure in the present experiment did not increase P dialyzability in any of the feed ingredients except alfalfa meal and wheat. Zyla et al. (1995a,b), using an in vitro procedure that simulates the digestive system of turkeys, found that the addition of acid phosphatase increased P dialyzability in corn−soybean meal diets when microbial phytase was present. However, when microbial phytase was not present, the addition of acid phosphatase was without effect. Therefore, we attribute the general lack of response to acid phosphatase in the present experiment to the fact that microbial phytase was not present in our in vitro procedure.

Correlation analysis indicated that all three sample preparation treatments were significantly correlated with the in vivo P availabilities in plant-origin feed ingredients. However, grinding samples through a 0.5-mm screen improved the correlation coefficients compared to those obtained when a 1.0-mm screen was used. Supplementation of acid phosphatase further increased the correlation coefficients because the addition of acid phosphatase increased the in vitro P dialyzability in alfalfa meal and wheat. Therefore, the addition of acid phosphatase increased the overall validity of our in vitro procedure for predicting in vivo P availability for swine.

In summary, we obtained significant positive correlations between our in vitro P dialyzability values and the published in vivo P availabilities for plant-type ingredients. This suggests that our in vitro procedure is a valid alternative to conducting in vivo studies for ranking plant-origin feed ingredients with respect to P availability. When plant-type ingredient samples were ground through a 0.5-mm screen and supplemented with acid phosphatase, the equations of $Y = 1.05x + 6.19$ and $Y = 1.05x + 4.48$ could be used to predict the in vivo P availabilities published by the National Research Council (1988) and Cromwell (1992), respectively. However, on the basis of P dialyzability and correlation analysis, the in vitro procedure in the present experiment is not valid for predicting in vitro P availability in animal-origin feed ingredients.

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