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The Additivity of True or Apparent Phosphorus Digestibility Values in Some Feed Ingredients for Growing Pigs

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ABSTRACT : Two experiments were conducted to determine the additivity of apparent or true digestibility of phosphorus (P) in soybean meal (SBM), peas, faba beans, corn, oats, broken rice meal, rough rice meal, buckwheat, and sorghum for growing pigs. Chromic oxide (0.3%) was used as a digestion marker in both experiments. Each experiment lasted for 12 d, which consisted of a 7-d dietary adaptation period followed by a 5-d fecal collection period. Experiment 1 involved 6 diets: the SBM-based control diet; 4 diets with corn, oats, rough rice meal and broken rice meal substituted for SBM; and an additional diet with a representative mixture of the 5 ingredients. In Experiment 2, 6 diets were prepared similarly, except that the tested ingredients besides SBM were faba beans, peas, buckwheat, and sorghum. In each experiment, six barrows with an initial average individual BW of 20.5 kg were fed one of the six diets according to a 6×6 Latin square design. The apparent and true P digestibility values for the nine tested ingredients were determined by the substitution method. There were no differences ($p>0.05$) between the determined and the predicted true P digestibility values for the mixture of ingredients in Experiments 1 and 2. However, the determined and the predicted apparent P digestibility values for the mixture of ingredients differed ($p = 0.059$) in Experiment 1, but not in Experiment 2. These results indicate that true P digestibility values are additive in ingredients containing low levels of phytate phosphorus and anti-nutritional factors, whereas the apparent P digestibility values are not always additive in single feed ingredients for growing pigs. (**Key Words:** Additivity, Phosphorus Digestibility, Feed Ingredients, Growing Pigs)

INTRODUCTION

Excretion of phosphorus (P) in manure from intensive swine production is a major environmental issue (Jongbloed et al., 1991; 1997; Deng et al., 2007a, b; Yang et al., 2007). Accumulation of P in the soil, resulting from field application of swine manure together with runoff, potentially causes eutrophication of fresh water resources (Mallin, 2000). To minimize environmental pollution and develop more sustainable swine production systems, the excretion of P by swine should be maximally reduced (Honeyman, 1996). The majority of P in feed ingredients of plant origin is present in the form of phytate, which cannot be digested by pigs (Jongbloed et al., 1991; Cromwell, 1992). At present, dietary supplementation with various sources of exogenous microbial phytase has been shown to be effective in improving P digestion in feed ingredients of plant origin (Näsi, 1990; Simons et al., 1990; Cromwell et al., 1993; Fan et al., 2005). However, an accurate evaluation of P bioavailability in feed ingredients for the formulation of swine diets on the basis of the supply of bioavailable P is essential to ensure efficient utilization of this mineral.

Digestibility studies and the slope-ratio assay are the two major evaluation systems for assessing P bioavailability in feed ingredients for pigs (Dellaert et al., 1990; Jongbloed

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Table 1. Ingredient composition of diets (g/kg, on an as-fed basis)

	Basal	Mixture 1	Mixture 2	Corn	Oats	Rough rice	Broken rice	Faba beans	Peas	Buck-wheat	Sorghum
Maize starch	488.9	129.6	192.5	488.9	488.9	488.9	488.9	488.9	488.9	488.9	488.9
Soybean meal	491.8	106.6	106.6	137.7	245.9	142.6	142.6	250.8	162.3	186.8	162.3
Corn	-	239.0	-	354.1	-	-	-	-	-	-	-
Oats	-	145.0	-	-	245.9	-	-	-	-	-	-
Rough rice	-	179.0	-	-	-	349.2	-	-	-	-	-
Broken rice	-	179.0	-	-	-	-	349.2	-	-	-	-
Faba bean	-	-	152.0		-	-	-	241.0	-	-	-
Peas	-	-	154.0	-	-	-	-	-	329.5	-	-
Buckwheat	-	-	207.0	-	-	-	-		-	305.0	-
Sorghum	-	-	167.0	-	-	-	-	-	-	-	329.5
Calcium carbonate[a]	8.3	10.0	8.6	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
Lysine	-	0.4	0.5	-	-	-	-	-	-	-	-
Methionine	-	0.4	0.8	-	-	-	-	-	-	-	
Vitamin premix[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HCl	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix[c]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Chromic oxide	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000

[a] Limestone, with purity of 99.8%, Ninxiang County Limestone Factory, China.

[b] Supplied the following vitamins (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, 0.38; all-rac-α-tocopherol acetate, 44.0; menadione, 3.0; riboflavin, 2.2; niacin, 12.0; d-pantothenic acid, 11.0; vitamin B_{12}, 0.012; thiamine, 1.1; choline chloride, 550.0; pyridoxine, 1.1; d-biotin, 0.1; folic acid, 0.6.

[c] Supplied the following minerals (mg/kg diet): $FeSO_4 \cdot H_2O$, 152; $ZnCO_3$, 95.9; $MnSO_4 \cdot H_2O$, 6.2; $CuSO_4 \cdot 5H_2O$, 11.8; KI, 0.6; Na_2SeO_3, 0.3.

Table 2. Nutrient composition of the experimental diets (on an as-fed basis)

	Basal	Mixture 1	Mixture 2	Corn	Oats	Rough rice	Broken rice	Faba beans	Peas	Buckwheat	Sorghum
Dry matter (g/kg)	918.6	889.6	901.0	858.9	916.0	879.9	887.0	919.1	890.2	899.9	872.6
DE (MJ/kg)	14.9	14.21	14.21	14.17	14.21	14.17	14.50	14.21	14.21	14.21	14.21
Crude protein (g/kg)	217.8	117.7	159.4	106.5	131.2	16.71	155.8	195.4	249.4	162.2	172.8
Lysine (g/kg)	13.8	9.5	9.5	9.5	9.5	9.5	9.5	12.5	16.2	9.5	9.5
Methionine (g/kg)	2.8	2.5	2.5	2.5	2.5	2.6	2.8	2.5	2.5	2.5	2.5
Calcium (g/kg)	5.5	5.3	5.8	5.1	4.9	7.5	5.1	6.4	7.8	6.1	5.8
P (g/kg)	3.7	3.5	3.9	3.4	3.2	5.0	3.4	4.3	5.3	4.0	3.8
Apparent digestible P (g/kg)	1.1	0.9	1.2	-	-	-	-	-	-	-	-
P in ingredients/total P in diet (%)	-	77	79	76	58	66	52	70	68	62	47
Phytate-P (g/kg)	1.1	1.7	1.8	2.0	1.3	1.8	1.5	1.5	2.1	2.2	1.5
Intrinsic phytase (FTU/kg)	38	75	71	84	21	100	88	138	27	70	33

[a] Calculated values according to the Chinese Data Bank of Feedstuffs (1999).

et al., 1991; Cromwell 1992; Fan et al., 2001; Fang et al., 2007). Digestibility studies estimate P availability by measuring its digestion and absorption, whereas the slope-ratio assay provides a combined estimation of digestion and post-absorptive utilization of P at the tissue level (Weremko et al., 1997). The net absorption of P, determined as the difference between the quantities of P ingested and excreted via feces, decreases with increasing levels of dietary P. Apparent P digestibility values in feed ingredients for pigs vary greatly with P contents in assay diets and the levels of endogenous P contribution (Fan et al., 2001).

The additivity of P digestibility values is a crucial factor in the formulation of swine diets. It is usually assumed that the supply of nutrients in a complete diet is equal to the sum of its constitutive components on the basis of the digestibility values determined from single ingredients (Furuya et al., 1991). However, few studies have been undertaken to verify this assumption. Available evidence shows that apparent P digestibility values are variable and not always additive in swine diet formulation (Fan et al., 2002). However, currently, there is little information about true digestibility values of P in individual ingredients or their additivity in diets for pigs. The objective of the present study was to examine the additivity of apparent or true P digestibility values in eight common feed ingredients of plant origin when they were incorporated into swine diets.

MATERIALS AND METHODS

Experimental design and diets

Two P digestibility experiments were conducted, utilizing a 6×6 Latin square design (Byrkit, 1987; Yin et al., 2004). In Experiment 1, six test diets were formulated: the SBM-based control diet; 4 diets with 72% corn, 50% oats, 71% rough rice meal or 71% broken rice meal substituted for SBM, respectively; and a sixth diet that was a mixture of the above 5 ingredients (Table 1). In Experiment 2, six test diets were also formulated: the SBM-based control diet; 4 diets with 49% faba beans, 67% peas, 62% buckwheat or 67% sorghum substituted for SBM, respectively; and a sixth diet that was a mixture of the 5 ingredients (Table 1). The amount of SBM replaced by each testing ingredient was expressed on a w/w basis. Chromic oxide (0.3%) was used as a marker for nutrient digestibility in both experiments. The grouping of the ingredients was based on their levels of phytate phosphorus and anti-nutritional factors.

Animals and housing

The use of animals in this experiment was approved by the Animal Care Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences. Twelve Landrace×Yorkshire×Duroc barrows with an initial body weight of 20.5±2.0 kg were obtained from the Swine Research Farm of the Hunan Animal Science Research Institute (Yang et al., 2005; Li et al., 2007a, b). Pigs were housed in large adjustable metabolic crates in a temperature-controlled room at 20-22°C (Yin et al., 2002a). During a one-week adaptation period, the pigs were fed a grower diet twice daily (0830 and 1630) and were given unlimited access to drinking water. Thereafter, individual pigs were randomly allocated to one of the six experimental diets according to the two 6×6 Latin Square designs. Each trial lasted for 12 d, with a 7-d adaptation period followed by a 5-d fecal collection period. The pigs were offered their assigned diets in the mash form (a water to feed ratio of 2:1) at 0830 and 1630 (Yin et al., 2002b; Tang et al., 2005). Additional drinking water was provided from low-pressure drinking nipples. The feeding level, based on 2.6 times the maintenance energy requirement (NRC, 1998), was adjusted for every experimental period according to the BW of pigs. During each experimental period, all of the feces was collected continuously for 24 h and immediately frozen at -20°C for subsequent processing.

Sample preparation and chemical analyses

Fecal samples were pooled for each pig during the 5-d collection period. They were oven-dried at 105°C and, along with diet samples, were ground using a Wiley mill through a 0.5-mm screen and thoroughly mixed before chemical analyses (Yin et al., 2001a,b; Huang et al., 2003). The DM content in the diets and in the feces was determined according to AOAC procedures (AOAC, 1993). Chromic oxide was determined according to Saha and Gilbreath (1981) by using an atomic absorption spectrometer (SpectrAA-10/20, Varian, and Mulgrave, Australia). Approximately 1.0 g diet or 0.5 g fecal sample was added into 60-ml Prex beakers and baked overnight at 550°C. Chromic oxide was oxidized to dichromate by digestion in 6 ml of a phosphoric acid (16.7 M)-manganese sulphate (13.5 mM) solution mixed with 8 ml of a potassium bromate (0.27 M) solution on a hot plate with a surface temperature of 350°C. Potassium dichromate was used as a standard. Absorbance of dichromate was read at 375 nm with a slit width of 0.5 nm on the atomic absorption spectrometer. Total phosphorus (TP) content was determined spectrophotometerically using the molybdo-vanadate reagent after mineralization of the sample with HCl (AOAC, 1995). Phytate phosphorus (PP) was determined using trichloroacetic acid according to the procedure of Yang (1993). In this method, Na-phytate (Sigma, St. Louis, USA) and a spectrophotometer at 415 nm were utilized. Potassium monobasic phosphate was used as a standard inorganic phosphate compound for establishing standard curves. The intrinsic phytase activity (IPHYE) of the ingredients was measured using the method of Engelen et al. (2001).

Calculations and statistical analyses

The apparent digestible P content in diets, expressed as g/kg dry matter intake (DMI), was calculated from Equation (1) according to previous studies (Fang et al., 2007).

$$P_{Ai} = P_{Di} \times D_A \quad (1)$$

Where P_{Ai} represents the apparent fecal digestible P content in the ith diet (g/kg DMI), P_{Di} is the total P content in the ith diet (g/kg DMI), whereas D_A is the apparent fecal P absorption (%) in the ith diet (%).

The true P digestibility was determined from equation (2) according to the two basic assumptions.

$$D_T(\%) = [(TPI_2\text{-}FPD_2)\text{-}(TPI_1\text{-}FPD_1)]/(TPI_2\text{-}TPI_1) \quad (2)$$

Where TPIi is the total P input from diet at ith intake (I = 1, 2) and FPDi is the fecal P output for the ith diet (I = 1, 2). D_T represents true P digestibility that was expressed as

Table 3. Analyzed chemical composition of dry matter (DM), neutral detergent fiber (NDF), crude protein (CP), fat, total phosphorus (TP), phytate phosphorus (PP) and activity of intrinsic phytase activity (IPHYE) in the tested ingredients and mixture diets 1 and 2.

Tested ingredients	DM (g/kg)	NDF	CP	Fat	TP (g/kg)	PP (g/kg)	IPHYE (FTU/kg)
Soybean meal	880	154	412	210	760	2.3	77
Maize	848	186	105	44	3.6	2.4	105.5
Rough rice	864	150	115	14	4.7	1.8	118.3
Broken rice	833	124	110	12	2.5	1.4	100.8
Oats	869	302	133	53	3.7	1.9	15.2
Faba beans	854	250	272	139	6.2	2.3	255.7
Peas	858	200	238	145	5.4	2.3	14.3
Buckwheat	853	218	130	31	4.0	2.8	88.1
Sorghum	855	240	139	35	2.7	1.4	19.7
Mixture 1[a]	85.5	223	120	48	0.34 (0.35)	0.12 (0.17)	58.8 (70.8)
Mixture 2	85.6	256	149	45	0.36 (0.39)	0.21 (0.18)	133.2 (74.8)

[a] Data (expressed on DM basis) in brackets are calculated values.

percent (%). The endogenous P output was calculated from equation (3) for the ith diet if the true P digestibility was determined from equation (2).

$$P_E \text{ (g/kg DMI)} = D_T \times P_{Di} - D_{Ai} \times P_{Di} \quad (3)$$

Where P_{Di} is the total P level of the ith diet and D_{Ai} is the apparent digestibility of TP for the ith diet.

The contributed P rate listed in Table 4 and 5 was calculated as the total P in a single ingredient divided by the total P in the diet. The predicted value was calculated as the sum of the digestibility value of single ingredients×the rate contributed by a single ingredient in the mixture diet. Data (mean±SEM) presented in Table 4 and 5 were subjected to analyses of variance for the 6×6 Latin Square design, with pigs and feeding periods as blocking factors. Differences between the determined and the predicted P digestibility values were determined by unpaired t-test (SAS Institute, Cary, NC). A probability value ≤0.05 was taken to indicate statistical significance.

RESULTS AND DISCUSSION

The analyzed DM, TP, PP and IPHYE of the nine ingredients and two mixed diets are presented in Table 3. The TP and PP contents of the legume ingredients were higher than those of the gramineae ingredients, which agreed well with the early reports of Viveros et al. (2000), Eeckhout and De Paepe (1994), and the Chinese Data Bank of Feedstuffs (1999). The analyzed and calculated TP or PP contents in the two mixed diets (Tables 1 and 2) were also similar ($p>0.05$) but the analyzed IPHYE content differed ($p<0.05$) from the calculated value (Table 3). This finding could be explained by the differences in the cultivars, processing conditions, and/or analytical methods used, as previously reported by other investigators (Eeckhout et al., 1994; Viveros et al., 2000). The TP contents for the ingredients used in the present study were similar to those reported by Viveros et al. (2000), NRC (1998), Eeckhout and De Paepe (1994), Ravindran et al. (1994), and the Chinese Data Bank of Feedstuffs (1999).

In determining true P digestibility values, a key issue is an estimation of the gastrointestinal endogenous P output. On the basis of the endogenous outputs of other nutrients (e.g., amino acids), two major approaches are potentially useful, including the use of P-free diets and the regression analysis technique (Fan et al., 2001). In our previous studies, we found that under feeding conditions with P-free diets, pigs were unable to maintain normal nutritional or physiologic status, as evidenced by symptoms of diarrhea and shivering. Therefore, P-free feeding assays were not appropriate for the determination of true P digestibility and gastrointestinal endogenous P loss in the present study.

Apparent digestibility values for P in the basal diet, the mixture diet, and individual ingredients were similar to those in the literature (Jongbloed and Kemme, 1990a,b; Jongbloed et al., 1991; Düngelhoef et al., 1994). True digestibility values of P in the diets were also in agreement with those reported (Lei et al., 1993; Düngelhoef et al., 1994; Rodehutscord and Lorenz 1996). Using SBM as a test ingredient, Fan et al. (2001) and Shen et al. (2002) demonstrated that true P digestibility and gastrointestinal endogenous P outputs in pigs fed diets containing test ingredients could be determined using the regression analysis technique. The present study is the first to determine whether the true P digestibility values of ingredients in compound feeds were additive for growing pigs. There were no differences ($p>0.05$) between the determined and the predicted true P digestibility values for the ingredient mixture in Experiments 1 and 2 (Table 4). However, the determined and the predicted apparent P digestibility values differed for the ingredient mixture used in Experiment 1 ($p = 0.059$), but not for the ingredient mixture used in Experiment 2 ($p = 0.179$) (Table 5). There

Table 4. Determined true P digestibility values (% DM) in different ingredients and comparison of the determined and the predicted true P digestibility values in mixtures 1 and 2 (% DM) in different ingredients [1]

Item	Basal	Oat	Rough rice	Broken rice	Corn	Mixture 1	Predicted value [4]	SEM
Experiment 1								
Determined value [2]	50.3±3.7	27.5±3.8	41.9±5.8	44.6±3.2	40.3±5.2	40.4±2.8	42.0±2.6	3.9
Contributed rate [3]	21.4	16.1	25.3	13.4	25.8			

Item	Basal	Buckwheat	Pea	Faba bean	Sorghum	Mixture 2	Predicted value [4]	SEM
Experiment 2								
Determined value [2]	51.2±2.9	49.6±5.4	36.6±5.1	50.8±5.9	42.3±5.4	41.0±4.9^{b}	46.3±5.2^{a}	3.0
Contributed rate [3]	18.9	22.0	21.8	25.3	12.0			

[1] Refer to Table 1 and 2. [2] Digestibility value determined in the present study.

[3] Total P in a single ingredient/Total P in the diet (%).

[4] Predicted value is the sum of the true digestibility value of a single ingredient as determined by the substitution assay times the rate contributed by the single ingredient in Mixture 1 or Mixture 2.

[a, b] Means in the same row with different superscript differs ($p<0.05$).

Table 5. Determined apparent P digestibility values (% DM) in different ingredients and comparison of the determined and the predicted apparent P digestibility values in mixtures 1 and 2 (% DM) in different ingredients [1]

Item	Basal	Oat	Rough rice	Broken rice	Corn	Mixture 1	Predicted value [4]	SEM
Experiment 1								
Determined value [2]	30.7±3.7	29.8±3.8	28.6±5.8	31.9±3.22	17.2±5.2	15.5±5.1^{b}	27.3±2.63^{a}	5.3
Contributed rate [3]	21.4	16.1	25.3	13.4	25.8			

Item	Basal	Buckwheat	Pea	Faba bean	Sorghum	Mixture 2	Predicted value [4]	SEM
Experiment 2								
Determined value [2]	31.9±4.7	30.3±5.4	26.3±8.7	31.6±5.9	31.0±5.4	21.2±2.9^{b}	29.9±5.4^{a}	4.9
Contributed rate [3]	18.9	22.0	21.8	25.3	12.0			

[1] Refer to Table 1 and 2. [2] Digestibility value determined in the present study.

[3] Total P in a single ingredient/Total P in the diet (%).

[4] Predicted value is the sum of the apparent digestibility value of a single ingredient as determined by the substitution assay times the rate contributed by the single ingredient in Mixture 1 or Mixture 2.

[a, b] Means in the same row with different superscripts differ ($p<0.05$).

was a very poor additivity of apparent P digestibility values for all the test ingredients (Table 5). Notably, the predicted values in Experiments 1 and 2 overestimated the apparent P digestibility by 53% and 42%, respectively.

An interesting finding from our present study is that the additivity of apparent P digestibility values depended on the sources of the ingredients used in the diet. The determined and the predicted apparent P digestibility values differed when the complete diet (Exp. 1) consisted of the ingredients (SBM, corn, oat, rough rice and broken rice) containing low levels of PP and anti-nutritional factors (e.g., tannin, hemagglutinin and trypsin). However, there was no additivity of apparent P digestibility values when the complete diet (Exp. 2) consisted of the ingredients (faba bean, pea, buckwheat and sorghum) with high levels of PP, tannin, hemagglutinin and trypsin (Kadirvel and Clandinin, 1974; Chubb, 1982; Yin et al., 1993; Fan and Sauer, 1999; Yin et al., 2002a,b). Thus, the difference in results between Experiments 1 and 2 can be explained by the different levels of PP and tannin, hemagglutinin and trypsin in the diets, therefore resulting in different amounts of endogenous P secretion (Leiner et al., 1974; Griffiths et al., 1983; Begbie et al., 1989). The anti-nutritional factors may regulate the total endogenous secretion of P and its recycling within the gastrointestinal tract, thereby affecting total P content in the feces (Barrier-Guillot et al., 1996; Fan and Sauer, 2002). The amount of fecal endogenous P output for calculating the true P digestibility in the present study was estimated from that of Fang et al. (2007) for growing pigs fed a SBM- and wheat bran-based diet. Clearly, the amount of fecal endogenous P used for calculating true P digestibility for all the diets used in Exp. 1 was accurate, but was not suitable for the diets used in Experiment 2. Collectively, these findings support the conclusion that true P digestibility values are additive in ingredients containing low levels of PP and anti-nutritional factors whereas the apparent P digestibility values are not always additive in single feed ingredients for growing pigs. Dietary PP and anti-nutritional factors likely influence the digestion and absorption of total P in the diets for growing pigs (Bhanja et al., 2005; Sacakli et al., 2006). Knowledge about these factors is of enormous nutritional significance because P plays an important role in intestinal function and nutrient metabolism (Wu, 1998; Jobgen et al., 2006).

In conclusion, the results of the present study indicate that true P digestibility values, rather than apparent P digestibility values, are additive in SBM-based swine diets containing low levels of PP and anti-nutritional factors. True P digestibility values should be used in formulating diets for growing pigs to meet optimal P requirement and minimize its fecal and urinary excretion.

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