Phosphorus digestibility and bioavailability in soybean meal, spray-dried plasma protein, and meat and bone meal determined using different methods

J. A. Munoz, P. L. Utterback, and C. M. Parsons[1]

Department of Animal Sciences, University of Illinois at Urbana-Champaign, Champaign, IL, USA

ABSTRACT Three experiments were conducted to determine phosphorus (**P**) digestibility and bioavailability using different methods. The objective of the first experiment was to determine ileal P digestibility of soybean meal (**SBM**), meat and bone meal (**MBM**), and spray-dried plasma protein (**SDPP**) using a precision-fed broiler chick assay. This assay involved feeding 8 g of SBM, MBM, or SDPP to broiler chicks at 21 D of age. At 6 h after feeding, ileal digesta were collected. Ileal P digestibility of SBM, MBM, and SDPP was 64, 42, and 94%, respectively. In the second experiment, ileal P digestibility and excreta P retention of SBM, SDPP, and MBM were determined using an ad libitum fed chick assay. On day 17 of age, chicks were placed on 1 of 12 dietary treatments that consisted of diets containing increasing levels of SBM, SDPP, or MBM. On day 21, ileal digesta and excreta were collected. True ileal P digestibility and true excreta P retention estimated using regression of ileal P or excreta P output on dietary P content yielded true ileal P digestibility values for SBM, SDPP, and MBM (2 diet methods for MBM) to be 83, 98, 61, and 23%, respectively. True excreta P retention values for SBM, SDPP, and MBM (2 methods) were determined to be 51, 99, 32, and 53%, respectively. The third experiment determined bioavailability of P in SBM, SDPP, and MBM relative to KH_2PO_4 using a chick bone ash bioassay. Dietary treatments included a P-deficient cornstarch–dextrose–SBM diet supplemented with 2 increasing levels of P from KH_2PO_4, SBM, SDPP, or MBM. Bioavailability of P based on tibia ash estimated using the multiple regression slope ratio method was 36, 125, and 76% for SBM, SDPP, and MBM, respectively, relative to KH_2PO_4. The results of this study indicated the digestibility/relative bioavailability of the P in SDPP was very high for all 3 methods, but values for SBM and MBM varied greatly among different methods.

Key words: phosphorus digestibility, soybean meal, plasma protein, meat and bone meal

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INTRODUCTION

It is among debate within the scientific community as to which method is most effective and accurate for determining phosphorus (**P**) digestibility and bioavailability for poultry. Shastak and Rodehutscord (2013) evaluated several methods for determining P availability in broilers and concluded that prececal digestibility may be the most appropriate method for poultry. The prececal digestibility method, combined with regression analysis, was then evaluated in a study (Rodehutscord et al., 2017) to determine P digestibility in soybean meal (**SBM**) with 17 collaborating research stations. The results of the latter study indicated that there were significant differences among stations for P digestibility of the same SBM sample. Mutucumarana et al. (2015a,b) used a prececal or ileal digestibility method to estimate true ileal P digestibility in several ingredients using a regression method where ileal P output was regressed on dietary P content. Their experiments yielded true ileal P digestibility coefficients for 3 sources of meat and bone meal (**MBM**) that were 0.693, 0.608, and 0.420 and true ileal P digestibility coefficients for SBM that were determined to be 0.740 and 0.523. The results of the aforementioned ileal or prececal digestibility studies suggested that the digestibility of P in SBM is equal to or greater than that for MBM, which is unexpected. Dilger and Adeola (2006) also reported a high prececal P true digestibility value of 94% for SBM.

Another method that has been used often to evaluate P bioavailability in feed ingredients is the relative bioavailability method in which bioavailability is determined by comparing bone ash responses for feed

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[1] Corresponding author: poultry@Illinois.edu

ingredients with a highly available P reference standard such as potassium phosphate or dicalcium phosphate. Using this type of method, Sands et al. (2003), Karr-Lilienthal et al. (2005), and Hanna et al. (2017) all reported that the relative bioavailability of P in SBM is approximately 35%. For MBM, the bioavailability of P has been reported to be approximately 100% when using the relative bioavailability method (Waldroup et al., 1965; Sell and Jeffrey, 1996). Thus, the results that have been obtained using the relative bioavailability method for SBM and MBM seem to differ greatly from those obtained by the ileal or prececal digestibility method discussed previously. One potential reason for part of the variation among studies is that different samples of SBM and MBM were evaluated in the different studies.

Kim et al. (2011) developed a precision-fed chick ileal digestibility broiler assay to determine amino acid digestibility in 21-day-old broiler chicks. This assay consists of precision-feeding chicks' 6 to 10 g of a test ingredient and collection of ileal digesta approximately 6 h after feeding. The results of that study indicated that this assay may be useful for determining amino acid digestibility in chicks. The precision-fed chick assay may also be accurate and useful for determining ileal digestibility of P in feed ingredients for poultry. No research has been published in scientific journals on studies to evaluate the latter.

The objective of the present study was to determine ileal P digestibility, excreta P retention, and relative P bioavailability in the same sample of SBM and MBM. In addition, spray-dried plasma protein (**SDPP**) was also evaluated as a high-protein ingredient that was expected to have high P digestibility based on research published in swine (Almeida and Stein, 2011).

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the institutional animal care and use committee.

Ingredients and Nutrient Analysis

Samples of dehulled solvent-extracted SBM, SPDD, and MBM were obtained from commercial sources. Ingredients were analyzed for DM (Method 930.15; AOAC International, 2007), CP (Method 990.03; AOAC International, 2007), crude fat (Method 920.93 (A); AOAC International, 2007), crude fiber (Method 978.10; AOAC International, 2007), and ash (Method 942.05; AOAC International, 2007). The P and Ca levels in the 3 ingredients were determined by inductively coupled plasma optical emission spectroscopy (Method 985.01 A, B, and D; AOAC International, 2007) after wet washing (Method 975.03 B[b]; AOAC International, 2007). Titanium content of experimental diets, ileal digesta, and excreta were determined by UV spectroscopy (Meyers et al., 2004). All analyses were conducted at the Columbia Experiment Station Chemical Laboratory of the University of Missouri.

Diets and Experimental Design

Chicks in all experiments were housed in stainless-steel batteries with raised wire floors in an environmentally controlled room. In Experiment 1, SBM, MBM, and SDPP were evaluated to determine ileal P digestibility using a precision-fed chick assay (Kim et al., 2011). Ross 308 broiler chicks were fed a nutritionally complete corn–SBM starter diet until 20 D of age. On day 20 of age, chicks were fasted overnight and were then precision fed 8 g of SBM, MBM, or SDPP. The chicks were then placed in a starter battery where water was available ad libitum. There were 4 pens of 4 chicks per dietary treatment. All dietary treatments contained 0.5% TiO_2 as an indigestible marker. At 6 h after precision feeding, chicks were euthanized using CO_2, and ileal digesta from Meckel's diverticulum to the ileocecal junction were collected. Apparent ileal P digestibility was then calculated.

In Experiment 2, SBM, SDPP, and MBM were evaluated to determine ileal P digestibility and excreta P retention using ad libitum fed chicks. The first 9 diets were dextrose based and contained the test ingredients as the only source of dietary P (Table 1). Diets 1 to 3 contained increasing levels of 18, 36, and 54% SBM, respectively. Diets 4 to 6 contained increasing levels of 7, 14, and 21% SDPP, respectively. Diets 7 to 9 contained increasing levels of 2, 4, and 6% MBM, respectively. Diets 10 to 12 were formulated to evaluate ileal P digestibility and excreta P retention for MBM using a different dietary approach. Increasing levels of 0, 2, and 4% MBM were added to a corn–SBM–potato protein–based diet (Table 2) as per the general method described by Rodehutscord (2013) that was recommended for testing P sources of mineral or animal origin. Calculated nonphytate P (**NPP**) levels in all diets were maintained at 0.30% or less to be well below the NPP requirement of the chicks (NRC, 1994). Ross 308 broiler chicks were fed a nutritionally complete corn–SBM starter diet until 16 D of age. On day 16 of age, chicks were fasted overnight before being placed on experiment. On day 17, chicks were weighed, wing banded, and allotted to the 12 dietary treatments, so that mean BW was similar among dietary treatments. There were 5 chicks per pen and 5 replicate pens per dietary treatment. From 17 to 21 D of age, experimental diets and water were available for ad libitum consumption. All diets contained 0.5% titanium dioxide as a digesta marker.

On day 21, feed intakes per pen and BW of each chick were recorded. The chicks were then euthanized using CO_2 gas, and ileal digesta were collected. On 20 and 21 D of age, excreta were collected. Ileal digesta and excreta were then freeze-dried and analyzed for P. Apparent ileal P digestibility and excreta P retention were then calculated.

In Experiment 3, SBM, MBM, and SDPP were evaluated for relative P bioavailability using a chick bone ash

Table 1. Ingredient composition of diets 1 to 6 in Experiment 2.

	Dietary treatments					
	Soybean meal			Spray-dried plasma protein		
Ingredient,%	1	2	3	4	5	6
Dextrose	63.575	45.50	27.43	74.31	66.96	59.62
Cornstarch	10.00	10.00	10.00	10.00	10.00	10.00
Meat and bone meal	–	–	–	–	–	–
Corn	–	–	–	–	–	–
Soybean meal	18.00	36.00	54.00	–	–	–
Spray-dried plasma protein	–	–	–	7.00	14.00	21.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.075	0.15	0.22	0.34	0.69	1.03
Solka floc	5.00	5.00	5.00	5.00	5.00	5.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Analysis:						
Ca, calculated	0.08	0.16	0.24	0.14	0.28	0.41
Nonphytate P, calculated	0.04	0.08	0.12	0.07	0.14	0.21
Total P, analyzed	0.11	0.20	0.34	0.07	0.12	0.23

[1] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4$ H_2O; iron, 75 from $FeSO_4$ H_2O; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4$ $5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

assay. A P-deficient cornstarch–dextrose–SBM diet (0.11% NPP) was fed as diet 1 (Table 3). Diets 2 and 3 contained 0.05 and 0.10% added P from potassium phosphate, respectively. Diets 4 and 5 contained 0.05 and 0.10% added P from potassium phosphate but also contained 0.10 and 0.20% added Ca from limestone, respectively, to provide Ca and P in a 2:1 ratio. The latter 2 diets were included to provide a reference standard for MBM because this ingredient contains a large amount of Ca in an approximate 2:1 ratio to P. Diets 6 and 7 contained 12.5 and 25.0% SBM, respectively (Table 4). Diets 8 and 9 contained 5.0 and 10.0%

Table 2. Ingredient composition of diets 7 to 12 in Experiment 2.

	Dietary treatments					
	Meat and bone meal			Meat and bone meal		
Ingredient,%	7	8	9	10	11	12
Dextrose	79.34	77.39	75.45	–	–	–
Cornstarch	10.00	10.00	10.00	15.37	13.39	11.39
Meat and bone meal	2.00	4.00	6.00	–	2.00	4.00
Corn	–	–	–	51.00	51.00	51.00
Soybean meal	–	–	–	20.00	20.00	20.00
Spray-dried plasma protein	–	–	–	–	–	–
Potato protein				10.00	10.00	10.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	–	–	–	0.28	0.26	0.26
Solka floc	5.00	5.00	5.00	–	–	–
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
KH_2PO_4	0.31	0.26	0.20	–	–	–
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Analysis:						
Ca, calculated	0.20	0.40	0.60	0.17	0.36	0.56
Nonphytate P, calculated	0.10	0.20	0.30	0.085	0.18	0.28
Total P, analyzed	0.10	0.22	0.33	0.27	0.35	0.48

[1] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4$ H_2O; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4$ $5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

Table 3. Ingredient composition of diets 1 to 5 in Experiment 3.

	Dietary treatments				
	P-deficient control	KH_2PO_4		KH_2PO_4 + limestone	
Ingredient,%	1	2	3	4	5
Dextrose	16.68	16.61	16.54	16.48	16.28
Cornstarch	33.38	33.22	33.07	33.09	32.95
Soybean meal	42.00	42.00	42.00	42.00	42.00
Spray-dried plasma protein	–	–	–	–	–
Meat and bone meal	–	–	–	–	–
Dicalcium phosphate	0.10	0.10	0.10	0.10	0.10
Soybean oil	5.00	5.00	5.00	5.00	5.00
Limestone	1.65	1.65	1.65	1.91	2.18
DL-Methionine	0.30	0.30	0.30	0.30	0.30
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10
KH_2PO_4	–	0.23	0.45	0.23	0.45
Bacitracin-BMD premix[3]	0.04	0.04	0.04	0.04	0.04
Analysis:					
Ca, calculated	0.77	0.77	0.77	0.87	0.97
Nonphytate P, calculated	0.11	0.16	0.21	0.16	0.21
Total P, analyzed	0.28	0.33	0.35	0.32	0.37

[1] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4$ H_2O; iron, 75 from $FeSO_4$ H_2O; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4$ $5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

[3] Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

SDPP, respectively. Diets 10 and 11 contained 1 and 2% MBM, respectively. All ingredients were substituted in place of equal amounts of dextrose and cornstarch. New Hampshire x Columbian male chicks were fed a nutritionally complete starter diet for 7 D. On day 7 of age, chicks were fasted overnight before being placed on experiment. On day 8 of age, chicks were weighed, wing banded, and allotted to the 11 dietary treatments in a completely randomized design so that mean BW was similar across treatments. There were 5 chicks per pen and 5 pens per dietary treatment. Chicks were on trial from 8 to 22 D of age, and experimental diets and water were available ad libitum for consumption. On day 22 of age, feed intake per pen and final BW of each chick was recorded. Then, using CO_2, chicks were euthanized, and the right tibia bone was collected, autoclaved, cleaned, and dry-ashed at 600°C in a muffle furnace.

Statistical Analysis

For Experiments 1, 2, and 3, data for ileal P digestibility, excreta P retention, weight gain, feed intake, gain-to-feed ratio and bone ash were analyzed using the PROC ANOVA procedure of SAS with pen as the experimental unit (SAS Institute, 2010). Differences among treatment means were assessed using the least significant difference test. For Experiment 2, simple linear regression analyses were used to regress ileal digesta or excreta P output (g/kg DM intake) on dietary P content (g/kg) for SBM, SDPP, and MBM treatments. Thus, the slope value from the regression equation represents indigestible P. This slope value was subtracted from 1 to obtain ileal P digestibility and excreta P retention coefficients. For Experiment 3, multiple linear regression was conducted by regressing tibia ash (mg/tibia) on supplemental P intake (g/chick) from potassium phosphate, SBM, SDPP, or MBM using the GLM procedure of SAS Institute (2010). The slope ratio method (Finney, 1964) was then used to estimate the bioavailability of P in SBM and SDPP relative to potassium phosphate alone (diets 1–3). The bioavailability of P in MBM was calculated relative to the potassium phosphate plus Ca treatments (diets 1, 4, and 5). Statistical significance of differences among treatments was assessed at $P < 0.05$.

RESULTS AND DISCUSSION

Nutrient Composition

The analyzed nutrient composition of SBM, SDDP, and MBM is shown in Table 5. The CP in SBM was higher than the value of 48.5% reported in the NRC (1994), whereas the MBM was lower than the reported value of 50.4% in the NRC (1994). The highest analyzed value of CP was for SDPP and was similar to the reported value in the NRC (2012) of 77.84%.

The analyzed Ca and total P for SBM were 0.28 and 0.59%, respectively, which are in agreement with the Ca and total P values for SBM reported in the NRC (1994). The MBM used in this study was derived from pork sources, and this may be the primary reason for the 10.94% for Ca and 5.26% for P from the NRC

Table 4. Ingredient composition of diets 6 to 11 in Experiment 3.

	Dietary treatments					
	Soybean meal		Spray-dried plasma protein		Meat and bone meal	
Ingredient,%	6	7	8	9	10	11
Dextrose	12.52	8.35	14.18	11.68	16.18	15.68
Cornstarch	25.04	16.71	30.88	28.38	32.88	32.38
Soybean meal	42.00	42.00	42.00	42.00	42.00	42.00
Test soybean meal	12.50	25.00	–	–	–	–
Spray-dried plasma protein	–	–	5.00	10.00	–	–
Meat and bone meal	–	–	–	–	1.00	2.00
Dicalcium phosphate	0.10	0.10	0.10	0.10	0.10	0.10
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.65	1.65	1.65	1.65	1.65	1.65
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
KH_2PO_4	–	–	–	–	–	–
Bacitracin-BMD premix[3]	0.04	0.04	0.04	0.04	0.04	0.04
Analysis:						
Ca, calculated	0.80	0.84	0.77	0.78	0.87	0.97
Nonphytate P, calculated	0.18	0.26	0.16	0.21	0.16	0.21
Total P, analyzed	0.35	0.44	0.32	0.38	0.33	0.36

[1] Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2] Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \, H_2O$; iron, 75 from $FeSO_4 \, H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \, 5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

[3] Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

(2012) being higher than the analyzed values for the MBM evaluated herein. The analyzed Ca and *P* values for SDPP were 0.10 and 0.98%, respectively, which are slightly lower than those reported in the NRC (2012).

Experiment 1

Apparent ileal P digestibility values for P in SBM, MBM, and SDPP determined using the precision-fed chick assay are shown in Table 6. There were significant differences among ingredients with values varying from 42 to 94%. The high ileal P digestibility of 94% for SDPP is in agreement with a previous study from Almeida and Stein (2011) who reported that SDPP is a highly digestible source of P for swine. In the latter study, standardized total tract digestibility of P was 102.8%. Soybean meal had the second highest ileal P digestibility at 64% in the present study. The value of 64% is somewhat lower than the values of 78 to 81% reported by Dilger and Adeola (2006) for SBM, whereas the value of 64% is in general agreement with Mutucumarana et al. (2015b) who reported apparent ileal P digestibility of SBM to be 61 to 68% when the dietary Ca–to–total P ratio was 1.3:1. The MBM herein yielded the lowest apparent ileal P digestibility value at 42%. Mutucumarana et al. (2015a) reported apparent ileal P digestibility of 3 MBM samples to vary from 49 to 69% among diets. The results of the latter study and the present study are in contrast to several earlier growth/bone ash studies that concluded that the P in animal products are highly or totally available for poultry (Waldroup et al., 1965; NRC, 1994; Sell and Jeffrey, 1996). Thus, the results of Experiment 1 of the present study, Dilger and Adeola (2006), and Mutucumarana et al. (2015a) suggest that SBM, a plant-based feed ingredient, has apparent P digestibility equal to or higher than that of MBM, an animal by-product, when using a prececal or ileal P digestibility assay.

Table 5. Analyzed nutrient composition (%, as fed basis) of ingredients used in Experiments 1, 2, and 3.

Item	Soybean meal	Spray-dried plasma Protein	Meat and bone Meal
DM	90.10	91.52	93.11
CP	50.26	79.00	46.63
Crude fat	2.00	0.03	11.98
Crude fiber	2.70	0.28	2.19
Ash	6.21	6.33	30.70
Ca	0.28	0.10	9.93
Total P	0.59	0.98	4.89

Table 6. Apparent ileal P digestibility values determined in precision-fed chicks in Experiment 1[1].

Dietary treatment	Ileal P digestibility[1] (%)
1. 8 g soybean meal	64^{b}
2. 8 g meat and bone meal	42^{c}
3. 8 g spray-dried plasma protein	94^{a}
Pooled SEM	3.5

[a-c] Means within a column with no common superscript differ significantly ($P < 0.05$).

[1] Values are means of 4 pens of 4 chicks at 21 D of age.

Experiment 2

The ileal P digestibility and excreta P retention values for the 12 dietary treatments are shown in Table 7. For diets 1 to 9 in which the test ingredients were the only source of dietary P, apparent ileal P digestibility for SDPP was high, ranging from 80 to 94%, and apparent excreta P retention was also high, ranging from 74 to 93%. These results are in general agreement with Experiment 1, indicating that SDPP is a highly digestible ingredient for P. In agreement with Experiment 1, apparent ileal P digestibility values for SBM in diets 1 to 3 were often higher (74–85%) than those for the MBM in diets 7 to 9 (66–79%). The SBM diets 1 to 3 also yielded higher excreta P retention values of 49 to 63% than the MBM diets 7 to 9 (32–39%). The apparent ileal P digestibility values for MBM of 66 to 79% are slightly higher than values from the study by Mutucumarana et al. (2015a) who reported apparent ileal P digestibility values for 3 samples of MBM ranging from 49 to 69%. For the corn–SBM–potato protein diets (10–12), apparent ileal digestibility of P decreased when the diet contained 2 or 4% MBM ($P < 0.05$), whereas there were no significant differences among the 3 diets for apparent excreta P retention.

True ileal digestibility values determined by linear regression of ileal or excreta P outputs on dietary P content are presented in Table 8. These results yielded true ileal digestibility and true excreta retention coefficients that were lower for MBM than for SBM, again, contradicting the earlier relative bioavailability bone ash studies that indicated P from animal sources are highly or totally available (Waldroup et al., 1965; Sell and Jeffrey, 1996). For SBM, true ileal P digestibility was 83%, which is not greatly different than the value of 80% (Mutucumarana et al., 2014) and 74% (Mutucumarana et al., 2015b) reported earlier when using the same regression procedure and similar diets to those used in the present study. True excreta P retention was lower than true ileal P digestibility for SBM in the present study. For SDPP, true ileal P digestibility and true excreta P retention coefficients were very high at 98 and 99%, respectively. These values are in agreement with the study of Almeida and Stein (2011), which indicated that the P in SDPP is 100% digestible. The low R^2 values for the SDPP regression were because of the very low amount of P output in the ileal digesta and excreta and because of the very high P digestibility, which resulted in slope values that were not significantly different from zero ($P > 0.10$). For MBM, true ileal P digestibility was 61%, which was within the range of values of 42 to 69% for MBM reported by Mutucumarana et al. (2015a) when using the same regression procedure and diets similar to those used herein.

As mentioned previously, dietary treatments 10 to 12 were based on the protocol suggested by Rodehutscord (2013) for testing P sources of mineral or animal origin where the ileal digestibility of P is recommended to be determined when adding increasing levels of the test ingredient to a corn–SBM–egg albumen diet in place of cornstarch. A similar type of corn–SBM–potato protein diet was used in the present study rather than a corn–SBM–egg albumen diet. When P output in ileal digesta and excreta were regressed on dietary P content, true ileal P digestibility and true excreta P retention values were 23 and 53%, respectively (Table 8). As discussed previously, these values are much lower than the relative bioavailability values of approximately 100% that have been reported in several earlier studies or publications (Waldroup et al., 1965; NRC, 1994; Sell and Jeffrey, 1996). The true ileal digestibility of P in MBM estimated from diets 10 to 12 was particularly low and lower than that obtained from the regression analyses of diets 7 to 9 and was also lower than the true ileal P digestibility values reported from regression analyses by Mutucumarana et al. (2015b).

The low P digestibility for MBM in diets 8 to 9 and 11 to 12 compared with diets 7 and 10 may have been largely because of the higher Ca content of the former diets. At low dietary Ca levels, such as in diets 7 and 10, the digestibility of P will usually be higher (Perryman et al., 2016). The dietary Ca level increased in diets 8 to 9 and 11 to 12, and the higher levels of dietary Ca may be largely responsible for the decreased ileal P digestibility.

Table 7. Ileal P digestibility and excreta P retention values for chicks in Experiment 2[1].

Dietary treatment	Ileal P digestibility (%)	Excreta P retention (%)
1. 18% soybean meal	$74^{d,e}$	49^{d}
2. 36% soybean meal	$85^{b,c}$	63^{c}
3. 54% soybean meal	84^{c}	61^{c}
4. 7% spray dried plasma protein	$80^{c,d}$	74^{b}
5. 14% spray dried plasma protein	$94^{a,b}$	93^{a}
6. 21% spray dried plasma protein	94^{a}	90^{a}
7. 2% meat and bone meal	$79^{c,d}$	35^{e}
8. 4% meat and bone meal	$66^{e,f}$	39^{e}
9. 6% meat and bone meal	66^{e}	32^{e}
10. 0% meat and bone meal	$83^{c,d}$	64^{c}
11. 2% meat and bone meal	65^{f}	64^{c}
12. 4% meat and bone meal	60^{f}	59^{c}
Pooled SEM	3.2	2.6

[a-f] Means within a column with no common superscript differ significantly ($P < 0.05$).

[1] Values are means of 5 pens of 5 chicks at 21 D of age.

Table 8. Results of linear regressions of ileal or excreta P outputs on total dietary P content in Experiment 2.

Item	Regression equation[1]	SE[2] of the slope	SE[2] of the intercept	R^2	Digestibility/retention coefficient
Soybean meal					
True ileal P digestibility	Y = 0.167X + 0.086	0.03	0.07	0.69	0.833
True excreta P retention	Y = 0.486X + 0.024	0.05	0.10	0.89	0.514
Spray-dried plasma protein					
True ileal P digestibility	Y = 0.024X + 0.077	0.02	0.04	0.08	0.976
True excreta P retention	Y = 0.013X + 0.173	0.03	0.05	0.02	0.987
Meat and bone meal[3]					
True ileal P digestibility	Y = 0.388X – 0.140	0.06	0.13	0.77	0.612
True excreta P retention	Y = 0.675X – 0.018	0.05	0.12	0.93	0.325
Meat and bone meal[4]					
True ileal P digestibility	Y = 0.767X + 1.778	0.05	0.22	0.94	0.233
True excreta P retention	Y = 0.473X – 0.378	0.05	0.20	0.88	0.527

[1] Regression of ileal digesta or excreta P output (g/kg dry matter intake) on dietary P content (g/kg) determined by feeding diets containing graded levels of either soybean meal, spray-dried plasma protein, or meat and bone meal. The slope represents true P indigestibility. The digestibility and excreta retention coefficients were calculated by subtracting the slope values from one.

[2] Abbreviation: SE = Standard error.

[3] Determined using diets 7 to 9 in Table 1 where the MBM was the only source of dietary P.

[4] Determined using diets 10 to 12 in Table 1 where the MBM was added to a corn–soybean meal–potato protein diet.

When comparing ileal P digestibility and excreta P retention in this experiment, excreta P retention values were numerically lower, sometimes substantially lower, than ileal P digestibility values for all diets. These results suggest that some of the absorbed dietary P was not used or retained in the body. Part of the reason for this may be because of the low Ca level in some diets, which limited the amount of P that could be deposited in bone. Another reason, possibly indirectly associated with the former, is that the excreta P retention values are "apparent" values and are not corrected for endogenous P losses. Thus, any P excreted in the urine is not corrected for, which could also contribute to lower P retention values.

Experiment 3

In the relative P bioavailability chick assay (Experiment 3), weight gain was significantly increased ($P < 0.05$) by the highest level of supplemental P from potassium phosphate and SDPP (Table 9). Feed intake and gain:feed responses to supplemental potassium phosphate, SBM, SDPP, and MBM were not consistent. A linear response in bone ash was observed among treatments as diets increased in supplemental P, with the largest response being observed for SDPP. In addition, as Ca from limestone was supplemented along with potassium phosphate (diets 4 and 5), bone ash decreased numerically or significantly ($P < 0.05$) compared with potassium phosphate supplemented alone (diets 2 and 3), which indicated that the added Ca negatively affected the utilization of P. The reduction in P utilization by the added Ca was confirmed by the multiple regression analysis of tibia ash on supplemental P intake (R2 = 0.89), which yielded a significantly lower regression coefficient (234.5) for potassium phosphate plus Ca than for potassium phosphate alone (285.5), and the ratio of the 2 coefficients was 82%.

Calculated relative P bioavailability values from the multiple regression analysis are presented in Table 10. The relative bioavailability value was highest for SDPP and was lowest for SBM, with MBM being

Table 9. Growth performance and tibia ash content for chicks in Experiment 3[1].

Dietary treatment	Weight gain (g/chick)	Feed intake (g/chick)	Gain:feed (g/kg)	Bone ash[3] (mg/tibia)
1. P deficient diet	$263^{f,g}$	409^{e}	$644^{c,d,e}$	296^{g}
2. As 1 + 0.05% KH_2PO_4	$290^{d,e}$	$447^{b,c,d}$	$651^{b,c,d}$	$360^{d,e}$
3. As 1 + 0.10% KH_2PO_4	$313^{b,c}$	486^{a}	$644^{c,d,e}$	440^{b}
4. As 2 + 0.10% Ca from limestone	$279^{e,f,g}$	$443^{b,c,d,e}$	$630^{c,d,e}$	$356^{d,e,f}$
5. As 3 + 0.20% Ca from limestone	$299^{c,d}$	$459^{a,b,c}$	$650^{b,c,d}$	405^{c}
6. As 1 + 12.5% SBM[2]	$265^{e,f,g}$	$430^{d,e}$	$616^{d,e}$	$343^{e,f}$
7. As 1 + 25% SBM[2]	$278^{f,g}$	$419^{b,c,d,e}$	$663^{b,c}$	$360^{d,e}$
8. As 1 + 5.0% SDPP[2]	318b	$463^{a,b}$	686^{b}	406^{c}
9. As 1 + 10.0% SDPP[2]	353a	486^{a}	726^{a}	507^{a}
10. As 1 + 1.0% MBM[2]	261g	$428^{c,d,e}$	612^{e}	331^{f}
11. As 1 + 2.0% MBM[2]	$281^{d,e,f}$	$428^{b,c,d,e}$	$656^{b,c}$	377^{d}
Pooled SEM	6.7	12.3	13.7	9.7

[a-g] Means within a column with no common superscript differ significantly ($P < 0.05$).

[1] Values are means of 5 pens consisting of 5 chicks.

[2] Abbreviations: SBM = soybean meal, SDPP = spray-dried plasma protein, MBM = meat and bone meal.

[3] Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), KH_2PO_4 + Ca (X_2), SBM (X_3), SDPP (X_4), and MBM (X_5) yielded the equation: $Y = 299.9 + 285.5 \pm 22.1X_1 + 234.5 + 23.2X_2 + 103.9 \pm 16.8X_3 + 357.4 \pm 18.2X_4 + 177.1 \pm 25.3X_5$ ($R^2 = 0.89$). The (±) values are SE of the regression coefficients.

Table 10. Relative bioavailability of the P in soybean meal, spray-dried plasma protein, and meat and bone meal in Experiment 3.

		Bioavailability values[1] (%)	Bioavailable content[2] (%)
Sample	Total P (%)	Tibia ash (mg)	Tibia ash (mg)
Soybean meal	0.59	36.4^{c}	0.21
Spray-dried plasma protein	0.98	125.0^{a}	1.22
Meat and bone meal	4.89	75.5^{b}	3.69

$^{a\text{-}c}$Values within columns containing no common superscripts are significantly different ($P < 0.05$) as determined using the regression coefficients and standard errors in the multiple regression equation in footnote 3 of Table 9.

[1] Calculated by the slope ratio method using the multiple regression equation in footnote 3 of Table 9. Bioavailability values for soybean meal and spray-dried plasma protein are relative to the P in KH_2PO_4 alone(X_1) which was set at 100%. The value for meat and bone meal is relative to KH_2PO_4 + Ca (X_2) in the multiple regression equation.

[2] Bioavailable content = (total P × bioavailability value)/100.

intermediate. The high relative bioavailability value for P in SDPP (the calculated value exceeded 100%) is in agreement with previous Experiments 1 and 2 and further indicated that it is a highly digestible or available source of P. The reason why the value for SDPP exceeded 100% is unknown and may warrant further study. One possible reason for the high value for SDPP is that the SDPP beneficially affected intestinal structure and possibly reduced endogenous P losses. The relative bioavailability value of 36% for SBM is in excellent agreement with the values of 39% reported by Hanna et al. (2017) and 36% reported by Sands et al. (2003) when using the same type of chick bone ash method as that used herein.

One of the primary objectives of the present study was to evaluate and compare ileal P digestibility and/or excreta P retention values from balance assays with relative bioavailability values from a chick growth bone ash assay for the same samples of SBM and MBM in the same study. Indeed, relative bioavailability values for P in SBM and MBM from Experiment 3 did not agree with ileal P digestibility and excreta P retention values from Experiments 1 and 2, with the results of Experiment 3 indicating the P in MBM is more highly available than the P in SBM. The results that the P in MBM was more highly available than the P in SBM seem reasonable considering previous research has indicated that MBM is a highly available source of P (Waldroup et al., 1965; Sell and Jeffrey, 1996), and the NRC (1994) states that the P in animal products is generally considered to be well used. Considering that SBM is a plant-based source of P and a large amount of the P is present as phytate P, which is poorly digested (NRC, 1994), it is expected that animal-based P such as MBM is more highly available than that of a plant-based source of P such as SBM. In contrast, the results of the ileal digestibility and excreta P retention values in Experiments 1 and 2 in the present study and previous such balance studies by Nwokolo et al. (1976), Dilger and Adeola (2006), Mutucumarana et al. (2014), Mutucumarana et al. (2015a), and Mutucumarana et al. (2015b) indicated that the ileal digestibility and/or excreta P retention values for the P in SBM were equal to or greater than MBM. The reason for discrepancy between results obtained using a relative bioavailability chick assay and the results obtained with ileal digestibility or excreta retention balance assays are unknown. It is also unknown as to why many balance-type assays are yielding values that are seemingly too high for SBM and too low for MBM. A major reason for the differences may be due to dietary calcium levels. Often, the balance assays use diets that contain low levels of calcium, and it has been shown that dietary calcium level can greatly affect P digestibility in balance studies (Liu et al., 2013; Mutucumarana et al., 2015b; Perryman et al., 2016). Thus, the overall results of this study indicate that the P digestibility and retention values obtained for some ingredients using diets that are semisynthetic or semipurified, particularly when they contain low Ca levels, may be misleading and not representative of diets used in commercial practice.

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