Phosphorus digestibility and relative phosphorus bioavailability in two dried black soldier fly larvae meals and a defatted black soldier fly larvae meal in broiler chickens

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ABSTRACT Two chicken assays were conducted to determine P availability, using three different approaches, for 2 black soldier fly larvae meals (**BSFL** 1 and 3) and one partially-defatted BSFL. Experiment 1 was conducted to determine ileal P digestibility and total tract P retention. Three experimental diets containing a BSFL as the only source of P were fed to broiler chickens from 19 to 22 days of age. Each diet contained 0.25% total dietary P. Ileal digesta and excreta were collected on day 22. Ileal digestibility of P was 87%, 75%, and 88% for BSFL 1, BSFL 3, and partially-defatted BSFL, respectively. Total tract P retention was 87%, 73%, and 85% for BSFL 1, BSFL 3, and partially-defatted BSFL, respectively. The objective of experiment 2 was to determine the relative bioavailability of P in the 3 insect meals relative to KH_2PO_4 using a chicken bone ash bioassay. Chickens were fed one of nine dietary treatments from 8 to 22 days of age. These diets included a P-deficient cornstarch-dextrose-SBM based diet (0.14% nonphytate P) or that diet supplemented with 0.05% or 0.10% P from KH_2PO_4, 7% or 14% BSFL 1, 8%, or 16% BSFL 3, and 8% or 16% partially-defatted BSFL. The slope-ratio method using multiple regression for tibia ash regressed on supplemental P intake was used to calculate the relative bioavailability of P where values for BSFL 1, BSFL 3, and partially-defatted BSFL were 54%, 51%, and 57%, respectively, relative to KH_2PO_4. The results of this study indicated that availability of P in BSFL based on ileal P digestibility and total tract P retention values was high, whereas relative bioavailability values for P based on tibia ash were approximately 20 to 30 percentage units lower.

Key words: bioavailability, black soldier fly, digestibility, insect meals, phosphorus

INTRODUCTION

It is of importance to know the proportion of the total P in a feedstuff that is available to animals. This allows nutritionists to accurately formulate diets to closely meet the P requirement of the animal. Recent research has focused on the use of insect meals, such as black soldier fly larvae meal (**BSFL**), as a potential feed ingredient for poultry diets (Spranghers et al., 2017). Studying the P availability of insect meals is required in order to compare this ingredient with traditional plant feedstuffs which are known to have two-thirds of total P in the form of phytate, making a majority of it unavailable for utilization by poultry (Liebert et al., 2005). This information can provide insights into potential replacement of these plant feedstuffs with insect meals. To our knowledge, there has been no research published on digestibility or relative bioavailability of P in insect meals for poultry.

There are three primary methods most commonly used to determine availability of P in feed ingredients for poultry. One of these is the method which measures P digestibility by collecting samples of ileal digesta from birds to obtain ileal P digestibility values or by collecting excreta to obtain excreta or total tract P retention values (Shastak and Rodehutscord, 2013; Mutucumarana et al., 2014). An alternative method to measure P bioavailability is the relative bioavailability method which usually uses bone ash as a response parameter. In this approach, a slope-ratio method using multiple-regression can be used to calculate the bioavailability of P based on the tibia ash relative to standard P sources (Hanna et al., 2017). The objective of the current study was to determine ileal P digestibility, total tract P retention, and relative bioavailability of P in two BSFL and a partially-defatted BSFL.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois.

Diets and experimental design

The three BSFL (BSFL 1, BSFL 3, and partially-defatted BSFL) were the same ones evaluated in an earlier study by Matin et al.. The BSFL 1 and BSFL 3 and the partially-defatted BSFL had been analyzed to contain 57.5, 45.2, and 56.7% protein, 21.7, 19.0, and 12.2% fat, 1.03, 0.85, and 0.87% total P, and 1.34, 3.65, and 0.91% Ca, respectively, on a DM basis in the earlier study.

Experiment 1 was conducted using commercial broiler chickens (Ross 308) to determine ileal P digestibility and total tract P retention values of the 3 BSFL. The chickens were fed a standard, nutritionally complete corn-soybean meal (**SBM**) pretest diet for 18 days and kept in heated Petersime starter batteries with wire floors in an environmentally controlled room and had ad libitum access to feed and water. On Day 19, the broilers were weighed, wing banded, and allotted to one of three dietary treatments, ensuring consistency in their average body weights across treatments while using a completely randomized design. There were 8 replicate pens of 5 chickens per treatment. Experimental diets were provided for ad libitum consumption from day 19 to day 22 where diet 1 contained BSFL 1 at 27% inclusion in the diet, diet 2 contained BSFL 3 at 30% inclusion in the diet, and diet 3 contained the partially-defatted BSFL at 30% inclusion in the diet (Table 1). All diets contained 0.25% total dietary P, where the BSFL was the only source of P in the diets, and TiO_2 was added as an indigestible marker. The chickens were euthanized on Day 22 with CO_2 gas and ileal digesta (Meckel's diverticulum to the ileal-cecal junction) were collected for ileal P digestibility determination using the indigestible titanium marker method. Excreta samples were also collected for 3 days from Day 20-22 for determination of total tract P retention values using the indigestible titanium marker method. Apparent ileal P digestibility and apparent total tract P retention values were calculated as described by Mutucumarana et al. (2014).

Experiment 2 was conducted using New Hampshire x Columbian female chickens that were hatched at the University of Illinois poultry research field laboratory. The objective of this experiment was to determine the relative bioavailability of the P in three insect meals relative to the P in potassium phosphate (KH_2PO_4). The chickens were fed a standard, nutritionally complete starter pretest diet for 8 days and kept in heated Petersime starter batteries (Petersime nv, Centrumstraat 125, 9870 Zulte, Olsene, Belgium) with wire floors and had ad libitum access to feed and water. At Day 8, chickens were weighed, wing banded, and allotted to one of nine dietary treatments using a

Table 1. Ingredient composition (%) of Diets 1−3 in Experiment 1.

Ingredient	Dietary treatments		
	1	2	3
Dextrose	52.79	49.27	49.12
BSFL 1[1]	26.80	0	0
BSFL 3[1]	0	30.38	0
Partially-defatted BSFL[1]	0	0	30.30
Soybean oil	4.0	4.0	4.0
Cornstarch	10.0	10.0	10.0
Limestone	0.06	0	0.23
Solka floc[2]	5.0	5.0	5.0
Salt	0.40	0.40	0.40
Vitamin mix[3]	0.20	0.20	0.20
Mineral mix[4]	0.15	0.15	0.15
TiO_2	0.50	0.50	0.50
Choline chloride (60%)	0.10	0.10	0.10
Analyzed Ca and P:			
Ca	0.37	1.10	0.32
Total P	0.26	0.25	0.23

[1]BSFL = black soldier fly larvae.
[2]Powdered cellulose; International Fiber Corporation, Urbana, OH.
[3]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25µg; 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 bisulfate, 2.33 mg.
[4]Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \cdot H_2O$; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

completely randomized design. There were 5 replicate pens of 5 chickens each per treatment. Experimental diets were provided for ad libitum consumption from day 8 to day 22 where diet 1 was a P deficient cornstarch-dextrose-SBM diet that was formulated to contain 0.14% nonphytate P and 0.78% Ca, diets 2 to 3 contained 0.05% and 0.10% supplemental P from added KH_2PO_4, respectively, diets 4 to 5 contained added 7% and 14% BSFL 1, respectively, diets 6 to 7 contained added 8% and 16% BSFL 3, respectively, and diets 8 to 9 contained added 8% and 16% partially defatted BSFL, respectively (Table 2). The BSFL were added to the diets at the expense of cornstarch and dextrose in a 2:1 ratio. The calculated level of dietary Ca for each diet was maintained at 0.78% by adding supplemental limestone to the diets. On Day 22, all the chickens were weighed, feed intake was measured, and feed efficiency was later calculated. The chickens were then euthanized with CO_2 gas in order to collect their right tibia bone. The right tibia bone of each chicken was collected and autoclaved. Following this, tibias were cleaned, oven-dried at 100°C for 24 h, pooled within pen and ashed at 600°C in a muffle furnace for 24 h. The individual pen of birds was the experimental unit for this study.

Diets in Experiment 1 and 2 were analyzed for calcium and total phosphorus and ileal digesta samples and excreta samples in Experiment 1 were analyzed for total phosphorus using inductively coupled plasma optical emission spectroscopy (Method 985.01 A, B, and D; AOAC International, 2007) and diet. Ileal and excreta samples were analyzed for titanium using the method described by Myers et al. (2004).

Table 2. Ingredient composition (%) of Diets 1–9 in Experiment 2.

Ingredient, %	Dietary treatments								
	1	2	3	4	5	6	7	8	9
Cornstarch	33.33	33.11	32.89	28.83	24.31	28.50	23.67	28.14	22.93
Dextrose	16.68	16.68	16.68	14.42	12.15	14.25	11.84	14.07	11.46
KH_2PO_4	0	0.22	0.44	0	0	0	0	0	0
BSFL 1[1]	0	0	0	7.0	14.0	0	0	0	0
BSFL 3[1]	0	0	0	0	0	8.0	16.0	0	0
Partially defatted BSFL[1]	0	0	0	0	0	0	0	8.0	16.0
Soybean meal	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0
Soybean oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Limestone	1.63	1.63	1.63	1.39	1.18	0.89	0.13	1.43	1.25
Dicalcium phosphate	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
DL-Met	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Analyzed Ca and P:									
Ca	0.78	0.75	0.81	0.73	0.80	0.78	0.75	0.79	0.83
Total P	0.28	0.31	0.36	0.35	0.42	0.33	0.42	0.36	0.45

[1]BSFL = black soldier fly larvae.

[2]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25μg; 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 bisulfate, 2.33 mg.

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

Statistical analysis

For Experiments 1 and 2, the SAS software (SAS Institute Inc., 2010) was used to statistically analyze the data obtained and the individual pens of birds were the experimental unit. For both experiments, an ANOVA was completed within the software for completely randomized designs and the least significant difference test was used to determine if differences among treatments were significant at $P < 0.05$. For Experiment 2, multiple linear regression (GLM procedure of SAS Institute, Inc. 2010) was performed with either total tibia ash (mg/tibia) or tibia ash percent being the response parameters. The slope-ratio method (Finney, 1964) was used to determine the bioavailability of P in the insect meals relative to the KH_2PO_4 using the regression coefficients obtained from multiple regression of tibia bone ash regressed on supplemental P intake from KH_2PO_4 or a BSFL. Bioavailability values were calculated relative to KH_2PO_4 with KH_2PO_4 set at 100%. Intersection and curvature validity tests were calculated using the general methods described by Finney (1964) and Littell et al. (1997) for slope-ratio assays.

RESULTS AND DISCUSSION

Experiment 1

Ileal P digestibility ranged from 75% to 88% (Table 3). There was no difference observed in ileal P digestibility between BSFL 1 and the partially-defatted BSFL. The BSFL 3, however, had a lower ileal P digestibility value compared with the other two BSFL. All three insect meals generally had greater values for ileal P digestibility compared with a number of high protein feed ingredients commonly used in poultry diets as published in the literature. For example, Munoz et al. (2020) precision-fed chickens conventional ingredients such as SBM and meat and bone meal and found the apparent ileal P digestibility to be 64% and 42%, respectively. Camden et al. (2001) determined the ileal P digestibility of a conventional corn-SBM-based diet at 54%. In terms of total tract P retention, the values ranged from 73% to 87% among the three BSFL and BSFL 3 had a lower value for total tract P retention compared with BSFL 1 and partially-defatted BSFL. All values were greater than the total tract P retention value for a conventional corn-SBM-based diet reported by Camden et al. (2001)

Table 3. Ileal P digestibility and total tract P retention values for chickens in Experiment 1.[1]

Dietary treatment	Weight gain (g/chicken)	Feed intake (g/chicken)	Gain: feed (g/kg)	Ileal P digestibility (%)	Total tract P retention (%)
1. 27% BSFL 1[2]	115[a]	217[a]	530[a]	87.4[a]	87.0[a]
2. 30% BSFL 3[2]	73[b]	201[b]	365[c]	74.6[b]	73.4[b]
3. 30% partially defatted BSFL[2]	104[a]	218[a]	475[b]	87.8[a]	85.3[a]
Pooled SEM	4.7	4.8	18.2	0.70	0.94

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

[1]Values are means of eight pens of five chickens from 19 to 22 days of age for weight gain, feed intake, and gain: feed ratio. Ileal P digestibility and excreta P retention values are at 22 days of age.

[2]BSFL = black soldier fly larvae.

Table 4. Growth performance and tibia ash for chickens in Experiment 2 from 8 to 22 days of age.[1]

Dietary treatment	Weight gain (g/chicken)	Feed intake (g/chicken)	Gain: feed (g/kg)	Bone ash[2] (mg/tibia)	Bone ash[3] (%)
1. P deficient dextrose-cornstarch (basal)	223[e]	359[c]	622[c]	250[f]	33.4[e]
2. Basal + 0.05% P[4]	242[cd]	385[ab]	628[c]	312[d]	36.9[c]
3. Basal + 0.10% P[4]	252[abc]	399[a]	632[c]	371[a]	39.5[a]
4. Basal + 7% BSFL 1[5]	241[cd]	361[c]	666[b]	287[e]	35.8[d]
5. Basal + 14% BSFL 1[5]	255[ab]	361[c]	708[a]	330[c]	37.4[bc]
6. Basal + 8% BSFL 3[5]	238[d]	358[c]	665[b]	295[e]	35.7[d]
7. Basal + 16% BSFL 3[5]	249[bcd]	359[c]	694[a]	332[c]	37.2[bc]
8. Basal + 8% partially defatted BSFL[5]	246[bcd]	365[c]	675[b]	299[de]	35.4[d]
9. Basal + 16% partially defatted BSFL[5]	261[a]	371[bc]	705[a]	351[b]	38.0[b]
Pooled SEM	4.1	5.0	5.3	5.7	0.28

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

[1]Values are means of five pens of five chickens; average initial BW was 86.8 g.

[2]Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), BSFL 1 (X_2), BSFL 3 (X_3), and partially defatted BSFL (X_4) yielded the equation: $Y = 250.3 + 304.6 \pm 16.5 X_1 + 166.5 \pm 13.9\ X_2 + 177.7 \pm 13.9 X_3 + 204.4 \pm 13.4\ X_4$ ($R^2 = 0.91$). The ($\pm$) values are standard errors of the regression coefficients.

[3]Multiple regression of tibia ash (Y; %) on supplemental P intake (g) from KH_2PO_4 (X_1), BSFL 1 (X_2), BSFL 3 (X_3), and partially defatted BSFL (X_4) yielded the equation: $Y = 33.7 + 14.9 \pm 0.918\ X_1 + 8.08 \pm 0.773\ X_2 + 7.65 \pm 0.771 X_3 + 8.48 \pm 0.745 X_4$ ($R^2 = 0.88$). The ($\pm$) values are standard errors of the regression coefficients.

[4]From KH_2PO_4.

[5]BSFL = black soldier fly larvae.

at 49%. In addition, Liu et al. (2012) determined values of 51% to 53% standardized total tract P retention for SBM fed to broiler chickens where the exact value was dependent on the time of total excreta collection.

The higher Ca in BSFL 3 could be at least partially responsible for the lower ileal P digestibility and lower total tract P retention. There was substantial variation in Ca content among the BSFL, with the BSFL 3 containing a much higher Ca level than the other two BSFL. Consequently, this resulted in the BSFL 3 diet having a higher level of Ca compared with the BSFL 1 and partially-defatted BSFL diets. High dietary Ca has the potential to negatively impact P absorption and retention (Li et al., 2016). Previous studies in broilers have shown that diets containing increased dietary Ca concentrations yielded reduced true P ileal digestibility and/or true phosphorus retention compared with diets containing lower Ca levels (Liu et al., 2013; Mutucumarana et al., 2015b; Perryman et al., 2016; Hanna et al., 2020).

The weight gain, feed intake, and feed efficiency results are also displayed in Table 3. The BSFL 3 diet yielded lower values in comparison with the BSFL 1 and partially-defatted diets. These differences could be due to the higher Ca content and lower ileal P digestibility and lower total tract P retention for BSFL 3. No differences were observed between BSFL 1 and partially-defatted BSFL treatments with regards to weight gain and feed intake.

Experiment 2

Increased inclusion of the 3 BSFL in the P-deficient diet (diet 1) corresponded to increased weight gain and gain:feed ratio (Table 4). Higher inclusion of KH_2PO_4 and BSFL also led to an increase in bone ash (mg/tibia and %). Consequently, there was a strong, positive linear relationship between tibia ash (mg/tibia and %) and supplemental P intake from KH_2PO_4 and BSFL 1, BSFL 3, or partially-defatted BSFL (R^2 values of 0.91 and 0.88 for tibia ash in mg/tibia and tibia ash %, respectively, and $P < 0.0001$ for the overall regression equations/model).

Iintersection and curvature effects were not significant ($P > 0.05$) in the multiple regression slope - ratio assay. The KH2PO4 was selected as the standard for comparison because of its assumed high P bioavailability and to be consistent with many previous published studies from our laboratory on relative P bioavailability. The bioavailability values for P in the BSFL relative to the P in KH2PO4, calculated by the slope-ratio method, ranged from 55% to 67% in terms of tibia ash (mg/tibia; Table 5). There were no differences among the three insect meals. There were also no differences for relative bioavailability values based on tibia ash as a percent. The bioavailable content of the P in each sample was calculated by multiplying the

Table 5. Relative bioavailability of the P in BSFL 1,[1] BSFL 3, and partially defatted BSFL in Experiment 2.

Ingredient	Total P (%)	Bioavailability values[2] (%)		Bioavailable content[3] (%)	
		Tibia ash (mg/tibia)	Tibia ash (%)	Tibia ash (mg/tibia)	Tibia ash (%)
BSFL 1	1.03	54.7	54.2	0.56	0.56
BSFL 3	0.85	58.3	51.3	0.50	0.44
Partially defatted BSFL	0.87	67.1	56.9	0.58	0.50

[1]BSFL = black soldier fly larvae.

[2]Calculated by the slope-ratio method using the regression equations in footnotes 2 and 3 in Table 4. Values within a column are not different ($P>0.05$) as determined using the regression coefficients and standard errors in the multiple regression equations in footnotes 2 and 3 of Table 4. Bioavailability values are relative to the P in KH_2PO_4 which was set at 100%.

[3]Bioavailable content = (total P x bioavailability value)/100.

percentage of total P in that sample by the corresponding bioavailability value.

The relative bioavailability values in Experiment 2 were lower than the values from Experiment 1 for ileal P digestibility and total tract P retention. A possible explanation for this for BSFL 1 and partially defatted BSFL could be that there are higher amounts of Ca present in the diets of Experiment 2 compared with those of Experiment 1. The higher Ca and Ca:P ratio in all of the diets for Experiment 2 may also be at least part of the reason for the lack of significant differences among the relative P bioavailability values for the 3 BSFL since, as discussed earlier, it is hypothesized that the main reason for the lower ileal digestibility and lower total tract retention of P in BSFL3 in Experiment 1 was due to its high Ca content, resulting in a much higher dietary Ca:P ratio for just that one diet. Higher dietary Ca concentrations can reduce P digestibility, particularly due to reduced hydrolysis of phytate in the intestinal tract, thus decreasing P availability (Selle and Ravindran, 2007). Higher ileal digestibility and/or total tract P retention values compared with relative P bioavailability values based on bone ash have been reported previously for other feed ingredients such as SBM and canola meal. For example for SBM, values for ileal P digestibility and total tract P retention have often been reported to be in the range of 70% to 85% or higher (Nwokolo et al., 1976; Dilger and Adeola, 2006; Liu et al., 2013; Mutucumarana et al., 2014; Munoz et al., 2020) compared with relative P bioavailability values mostly in the range of 30% to 40% (Sands et al., 2003; Karr-Lilienthal et al., 2005; Hanna et al., 2017). Similar types of results have been reported for meat and bone meal (Waldroup et al., 1965; Sell and Jeffrey, 1996; Mutucumarana et al., 2015a; Munoz et al., 2020) where relative bioavailability values in experiments based on bone ash have generally indicated that the P in animal products such as meat and bone meal is highly or totally bioavailable for poultry in contrast to digestibility experiments that have often indicated that the digestibility of P is only 50% or lower. It might be expected that the relative bioavailability of P in BSFL would be higher than in plant feedstuffs such as corn and soybean meal and would be high and similar to animal meals because of a lack of phytate in BSFL. The reason for relative bioavailability of the P in BSFL being much less than 100% is unknown and may warrant further study. Finally, the overall results indicated that the BSFL contain approximately 0.8% digestible P or approximately 0.5% relative bioavailable P, indicating that BSFL is a source of substantial dietary available P for poultry.

In summary, the results of this study indicated that the availability of P in BSFL based on P digestibility and total tract P retention was high, with values ranging from 73 to 88%. However, relative bioavailability values based on bone ash were approximately 20 to 30 percentage units lower than the values based on ileal digestibility and total tract retention

DISCLOSURES

There is no conflict of interest for this article.

REFERENCES

AOAC International. 2007. Official Methods of Analysis. 18th ed. rev. 2. AOAC Int., Gaithersburg, MD.

Camden, B. J, P. C. Morel, D. V. Thomas, V. Ravindran, and M. R. Bedford. 2001. Effectiveness of exogenous microbial phytase in improving the bioavailabilities of phosphorus and other nutrients in maize-soya-bean meal diets for broilers. Anim. Sci. 73:289–297.

Dilger, R. N., and O. Adeola. 2006. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing chicks fed conventional and low-phytate soybean meals. Poult. Sci. 85:661–668.

Finney, D. J. 1964. Statistical Method in Biological Assay. 2nd ed. Charles Griffin & Co., London, UK.

Hanna, C. D., C. K. Foran, P. L. Utterback, H. H. Stein, and C. M. Parsons. 2017. Phosphorus bioavailability in increased-protein, reduced-fiber canola meal, conventional canola meal, and soybean meal fed to crossbred chicks. Poult. Sci. 97:188–195.

Hanna, C., J Munoz, P. Utterback, and C. M. Parsons. 2020. Research note: phosphorus digestibility in conventional canola meal determined using different balance assays. Poult. Sci. 99:2650–2654.

Karr-Lilienthal, L. K., P. L. Utterback, C. M. Amezcua, C. M. Parsons, N. R. Merchen, and G. C. Fahey Jr. 2005. Relative bioavailability of phosphorus and true amino acid digestibility by poultry as affected by soybean extraction time and of low-phytate soybeans. Poult. Sci. 84:1555–1561.

Li, X., D. Zhang, T. Yang, and W. Bryden. 2016. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. Agric. 6:25.

Liebert, F., J. K. Htoo, and A. Sünder. 2005. Performance and nutrient utilization of laying hens fed low-phosphorus corn-soybean and wheat-soybean diets supplemented with microbial phytase. Poult. Sci. 84:1576–1583.

Littell, R. C., P. R. Henry, A. J. Lewis, and C. B. Ammerman. 1997. Estimation of relative bioavailability of nutrients using SAS procedures. J. Anim. Sci. 75:2672–2683.

Liu, S. B., S. F. Li, L. Lu, J. J. Xie, L. Y. Zhang, and X. G. Luo. 2012. Estimation of standardized phosphorus retention for corn, soybean meal, and corn-soybean meal diet in broilers. Poult. Sci. 91:1879–1885.

Liu, J. B., D. W. Chen, and O. Adeola. 2013. Phosphorus digestibility response of broiler chickens to dietary calcium-to-phosphorus ratios. Poult. Sci. 92:1572–1578.

Matin, N., P. Utterback, and C. M. Parsons. 2021. True metabolizable energy and amino acid digestibility in black soldier fly larvae meals, cricket meal, and mealworms using a precision-fed rooster assay. Poult. Sci. 100:101146.

Munoz, J. A., P. L. Utterback, and C. M. Parsons. 2020. Phosphorus digestibility and bioavailability in soybean meal, spray-dried plasma protein, and meat and bone meal determined using different methods. Poult Sci 99:4998–5006.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2014. Measurement of true ileal digestibility and total tract retention of phosphorus in corn and canola meal for broiler chickens. Poult. Sci. 93:412–419.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015a. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens. Poult. Sci. 94:1611–1618.

Mutucumarana, R. K., V. Ravindran, G. Ravindran, and A. J. Cowieson. 2015b. Measurement of true ileal phosphorus digestibility in maize and soybean meal for broiler chickens: comparison of two methodologies. Anim. Feed Sci. Tech. 206:76–86.

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: A procedure for preparation and quantitative analyses of samples for titanium dioxide. J. Anim. Sci. 82:179–183.

Nwokolo, E. N., D. B. Bragg, and W. D. Kitts. 1976. A method for estimating the mineral availability in feedstuffs. Poult.Sci 55:2217–2221.

Perryman, K. R., H. V. Masey O'Neill, M. R. Bedford, and W. A. Dozier. 2016. Effects of calcium feeding strategy on true ileal phosphorus digestibility and true phosphorus retention determined with growing broilers. Poult. Sci. 95:1077–1087.

Sands, J. S., D. Ragland, J. R. Wilcox, and O. Adeola. 2003. Relative bioavailability of phosphorus in low-phytate soybean meal for broiler chicks. Can. J. Anim. Sci. 83:95–100.

SAS Institute Inc. 2010. Users Guide Statistics. Version 9. 2nd ed. SAS, Inc., Cary, NC.

Sell, J. S., and M. Jeffrey. 1996. Availability for poults and phosphorus from meat and bone meals different particle sizes. Poult. Sci. 75:232–239.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Tech. 135:1–41.

Shastak, Y., and M. Rodehutscord. 2013. Determination and estimation of phosphorus availability in growing poultry and their historical development. World's Poult. Sci. J. 69:569–586.

Spranghers, T., M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, J. Michiels, M. Eeckhout, P. De Clercq, and S. De Smet. 2017. Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. J. Sci. Food Agric. 97:2594–2600.

Waldroup, P. W., C. B. Ammerman, and R. H. Harms. 1965. The utilization of phosphorus from animal protein sources for chicks. Poult. Sci. 44:1302–1306.