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Short communication

Effects of different protein sources in low-phosphorus diets on calculated basal endogenous loss of phosphorus by growing pigs

M.E. Nelson [a], S.A. Lee [b], H.H. Stein [a,b,*]

[a] Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801, United States
[b] Department of Animal Sciences, University of Illinois, Urbana, IL 61801, United States

A R T I C L E I N F O

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A B S T R A C T

The objective of this experiment was to test the hypothesis that the basal endogenous loss (BEL) of P from pigs fed a diet containing spray dried plasma, casein, or potato protein concentrate is not different from that of pigs fed a diet containing gelatin. Forty pigs (body weight: 19.34 kg; SD = 0.80) were housed individually in metabolism crates and allotted to four low-P diets using a completely randomized block design with two blocks of 20 pigs and five pigs per diet in each block. Diets were based on cornstarch and sucrose and contained 200 g/kg gelatin, 200 g/kg spray dried plasma, 185 g/kg casein, or 200 g/kg potato protein concentrate. With the exception of Ca and P, diets were formulated to meet requirements of all nutrients. Feces and urine samples were collected for 4 d following a 5-d adaptation period. Fecal samples were dried and ground, and fecal and urine samples were analyzed for P. Data were analyzed using a model that included diet as fixed effect and block as random effect. Results indicated that feed intake and fecal excretion of dry matter were greater ($P < 0.05$) in pigs fed diets containing spray dried plasma, casein, or potato protein concentrate compared with pigs fed the gelatin diet, with pigs fed potato protein concentrate having the greatest ($P < 0.05$) excretion. The apparent total tract digestibility (ATTD) of dry matter was least ($P < 0.001$) in pigs fed the diet containing potato protein concentrate, but there were no differences among gelatin, spray dried plasma, and casein diets. The ATTD of P was greater ($P < 0.001$) in pigs fed diets containing spray dried plasma or casein compared with pigs fed gelatin or potato protein concentrate diets. The BEL of P was not different between the gelatin and casein diets (i.e., 176 and 234 mg/kg dry matter intake), but pigs fed diets containing spray dried plasma or potato protein concentrate had greater ($P < 0.001$) BEL of P (i.e., 338 and 374 mg/kg dry matter intake) compared with pigs fed gelatin or casein diets. In conclusion, the BEL of P was greater if calculated from diets containing spray dried plasma or potato protein concentrate compared with gelatin. However, casein may be an alternative to gelatin to estimate the BEL of P because casein provides a greater amount of P compared with gelatin, which compensates for the deficient level of P in gelatin, but does not affect the BEL of P.

Abbreviations: ATTD, apparent total tract digestibility; BEL, basal endogenous loss; DM, dry matter; DMI, dry matter intake; STTD, standardized total tract digestibility.

* Correspondence to: Department of Animal Sciences, University of Illinois, Urbana, 61801, United States.
E-mail address: hstein@illinois.edu (H.H. Stein).

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1. Introduction

Phosphorus is one of the most expensive nutrients used in swine diets. Formulating diets based on values for standardized total tract digestibility (STTD) of P instead of values for apparent total tract digestibility (ATTD) may reduce the cost of diets because STTD values, unlike ATTD values, are additive in mixed diets, which may prevent an oversupply of P (Almeida and Stein, 2010). Values for STTD of P in plant ingredients, animal proteins, and feed phosphates have been determined (NRC, 2012; Stein et al., 2016) by correcting ATTD of P for the endogenous P loss. The basal endogenous loss (BEL) of P can be estimated using a P-free diet and values are relatively constant for growing-finishing pigs regardless of the body weight and dietary Ca concentration (Baker et al., 2013; Son and Kim, 2015; Kim et al., 2017). There are, however, differences in endogenous loss of P between growing pigs and gestating sows (Son and Kim, 2015; Bikker et al., 2017; Lee et al., 2018).

Gelatin has been widely used in P-free diets because it does not contain any P and has high levels of digestible amino acids (Petersen et al., 2005; Petersen and Stein, 2006). However, most gelatin products are in a powder form that may make diets dusty and sticky, which can make it difficult to work with such diets and this may also result in reduced palatability of diets. In addition, feeding pigs a diet containing no P may result in physiological issues in pigs (Fan et al., 2001; She et al., 2017). Therefore, spray dried plasma, casein, or potato protein concentrate are possible protein alternatives to gelatin because P in spray dried plasma and casein is close to 100% digestible and potato protein concentrate has a low concentrations of P (NRC, 2012). Indeed, blood plasma is sometimes used to determine the BEL of P (Bünzen et al., 2012) because it is assumed that the P contributed by plasma is completely absorbed (Almeida and Stein, 2011). However, to our knowledge, it has never been confirmed that the BEL of P is the same if estimated from diets containing gelatin, spray dried plasma, casein, or potato protein concentrate. Therefore, the objective of this experiment was to test the hypothesis that the BEL of P calculated from pigs fed a diet containing spray dried plasma, casein, or potato protein concentrate are not different from that of pigs fed a diet containing gelatin.

2. Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment. Pigs used in the experiment were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

2.1. Diets and feeding

Four semi-purified diets with low concentrations of P were formulated based on cornstarch and sucrose (Tables 1 and 2). These

Table 1
Ingredient composition of experimental diets.

Item, g/kg	Protein Source			
	Gelatin	Spray dried plasma	Casein	Potato protein concentrate
Cornstarch	468.1	493.8	513.6	495.0
Sucrose	200.0	200.0	200.0	200.0
Gelatin	200.0	-	-	-
Spray dried plasma	-	200.0	-	-
Casein	-	-	185.0	-
Potato protein concentrate	-	-	-	200.0
Soybean oil	40.00	40.00	40.00	40.00
Cellulose	40.00	40.00	40.00	40.00
L-Arg	-	-	-	-
L-His	3.00	-	-	-
L-Ile	3.90	1.80	-	-
L-Leu	7.50	-	-	-
L-Lys·HCl	6.60	0.40	-	2.00
DL-Met	2.00	2.40	-	0.50
L-Thr	3.80	0.20	0.80	-
L-Trp	1.80	-	-	0.50
L-Val	3.80	-	-	-
Limestone, ground	5.50	7.40	6.60	8.00
Potassium carbonate	4.00	4.00	4.00	4.00
Magnesium oxide	1.00	1.00	1.00	1.00
Sodium chloride	4.00	4.00	4.00	4.00
Vitamin-mineral premix[a]	5.00	5.00	5.00	5.00

[a]The vitamin-mineral premix provided the followign quantities of vitamins and minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1660 IU; vitamin E as DL-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as odium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Table 2
Analyzed nutrient composition of experimental diets (as-fed basis).

Item, g/kg	Protein source			
	Gelatin	Spray dried plasma	Casein	Potato protein concentrate
Dry matter	916.2	917.6	920.1	929.5
Crude protein	211.2	179.1	163.0	158.7
Gross energy, MJ/kg	17.19	16.94	17.22	17.63
Ash	14.4	33.6	17.6	20.7
Ca	3.00	3.70	2.80	3.80
P	0.10	3.10	1.40	0.30
Calculated				
Ca	3.00	3.00	3.00	3.00
P	-	2.60	1.30	0.30

diets also contained 200 g/kg gelatin, 200 g/kg spray dried plasma, 185 g/kg casein, or 200 g/kg potato protein concentrate, which were the only sources of P in the diets (Table 3). The inclusion rate of the protein sources was determined to meet the amino acid requirement of pigs. Crystalline amino acids, vitamins, and minerals with the exception of Ca and P were included in the diets to meet the current requirements for 11- to 25 kg pigs (NRC, 2012). Limestone was included in all diets to reach a level of 3.00 g/kg of total Ca. Daily feed allotments were divided into two equal meals that were provided at 0800 and 1600 h and pigs were provided feed at 2.5 times the daily maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy per kg body weight$^{0.60}$; NRC, 2012). Experimental diets were fed for 12 days, with the initial 5 days considered the adaptation period.

2.2. Animals and housing

Forty growing barrows (body weight: 19.34 kg; SD = 0.80) were allotted to the four diets using a completely randomized block design with two blocks of 20 pigs with 5 pigs per diet in each block for a total of 10 replicate pigs per diet. Pigs in the two groups were born and weaned two weeks apart and the weaning group, therefore, was the blocking factor. Pigs were individually housed in metabolism crates that were equipped with a nipple waterer, a feeder, and fully slated floors. A mesh screen and pan were installed under the slatted floor during the collection period allowing for total, but separate, feces and urine collection.

2.3. Method of collection

Samples of the diets and main ingredients were collected at the time of diet mixing. Feces were collected for 4 days following the adaptation period using the marker-to-marker procedure (Adeola, 2001). Fecal collection began when the first marker (i.e., chromic oxide) fed in the morning of day 6 appeared in the feces and ceased when the second marker (i.e., ferric oxide), which was fed in the morning of day 10, appeared in the feces. Urine was collected from day 6 to day 10. Feed consumption was recorded daily, and orts were collected to determine feed intake from day 6 to day 10. Pigs had free access to water throughout the experiment.

Fecal collection occurred twice daily, and samples were stored at −20 °C immediately following collection. Buckets containing a preservative of 50 mL of 6 *N* HCl were placed under each crate for urine collection. Urine buckets were weighed and emptied once per day and one-tenth of the collected urine was stored at −20 °C. At the conclusion of the experiment, urine samples were thawed at room temperature and subsamples were collected for analysis.

2.4. Chemical analyses

Fecal samples were thawed, dried in a 50 °C forced air drying oven, and finely ground using a 500 G swing type grain mill (RRH, Zhejiang, China) prior to analysis. The dry matter (DM) in diets and ingredients was determined in duplicate by oven drying at 135 °C for 2 h (method 930.15; AOAC Int., 2019). Diets were also analyzed in duplicate for ash at 600 °C for 2 h (method 942.05; AOAC Int.,

Table 3
Analyzed nutrient composition of ingredients (as-fed basis).

Item, g/kg	Protein source			
	Gelatin[a]	Spray dried plasma[b]	Casein[c]	Potato protein concentrate[d]
Dry matter	874.90	877.0	887.1	918.4
Crude protein	1 010.0	759.6	878.3	823.1
Ca	0.4	1.2	0.3	0.2
P	0.5	16.2	7.1	1.0

[a] Gelatin obtained from Gelita USA Inc. (Sioux City, IA, USA).
[b] Spray dried plasma was obtained from APC Inc. (Ankeny, IA, USA).
[c] Casein was obtained from NZMP (Auckland, New Zealand).
[d] Potato protein concentrate was obtained from Royal Avebe (Veendam, Netherlands).

2019). Diet and ingredient samples were analyzed for Ca and P and feces and urine samples were analyzed for P (method 985.01 A, B and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). Diets were also analyzed for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA) and diets and ingredients were analyzed for N using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as N × 6.25.

2.5. Calculations

The BEL of P was calculated using the fecal flow of P and feed intake of pigs and was expressed as mg/kg of DM intake (DMI) as previously outlined (Petersen and Stein, 2006; Almeida and Stein, 2010; NRC, 2012):

$$\text{BEL of P (mg/kg DMI)} = [(P_{\text{fecal output}}/\text{DMI}) \times 1000 \times 1000]$$

The ATTD of P in each experimental diet was calculated by modifying the equation by Almeida and Stein (2010):

$$\text{ATTD of P (g/g)} = [(P_{\text{intake}} - P_{\text{fecal output}})/\ P_{\text{intake}}]$$

Retention of P was calculated by modifying the equation by Petersen and Stein (2006):

$$\text{P retention (g/day)} = [P_{\text{intake}} - (P_{\text{feces}} + P_{\text{urine}})]$$

where P_{intake}, P_{feces}, and P_{urine} are in g/day.

2.6. Statistical analysis

Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures (SAS Institute Inc., 2016) and outliers were identified using Internally Studentized Residuals (Tukey, 1977). Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., 2016). The model included diet as fixed effect and block and pig within block as random effects. Pig was the experimental unit for all analyses and results were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$. Treatment mean values were calculated using the LSMeans statement in SAS and, if significant, were separated using PDIFF option with Tukey's adjustment. An alpha value of 0.05 was used to assess significance among means.

3. Results

Feed intake and fecal excretion were greater ($P < 0.05$) for pigs fed diets containing spray dried plasma, casein, or potato protein concentrate compared with pigs fed the gelatin diet, but pigs fed the diet containing potato protein concentrate had the greatest ($P < 0.05$) fecal excretion compared with pigs fed the other protein sources (Table 4). The ATTD of DM was less ($P < 0.05$) for the diet containing potato protein concentrate compared with diets containing gelatin, spray dried plasma, or casein. Urine excretion from pigs fed the gelatin diet was not different from that of pigs fed the other diets, but pigs fed the spray dried plasma diet had greater ($P < 0.05$) urine excretion compared with pigs fed diets containing casein or potato protein concentrate.

Table 4
Basal endogenous loss and retention of P by pigs fed low-P diets[1,2].

Item	Protein source				SEM	*P*-value
	Gelatin	Spray dried plasma	Casein	Potato Protein Concentrate		
Feed intake, kg/day	0.59[b]	0.72[a]	0.73[a]	0.73[a]	0.02	< 0.001
Fecal excretion, g DM/day[1]	21.13[c]	31.12[b]	29.07[b]	43.16[a]	0.002	< 0.001
ATTD of DM[1]	0.96[a]	0.95[a]	0.96[a]	0.94[b]	0.003	< 0.001
Urine excretion, kg/day	3.93[ab]	4.93[a]	2.93[b]	2.59[b]	0.57	0.004
P balance						
P intake, g/day	0.09[d]	2.23[a]	1.12[b]	0.23[c]	0.02	< 0.001
P in feces, g/kg	4.50[c]	7.10[a]	5.30[bc]	5.70[b]	0.04	< 0.001
Fecal P output, g/day	0.10[c]	0.22[a]	0.16[b]	0.25[a]	0.01	< 0.001
Absorbed P, g/day	-0.01[c]	2.01[a]	0.96[b]	-0.03[c]	0.02	< 0.001
ATTD of P	-0.07[b]	0.92[a]	0.85[a]	-0.12[b]	0.05	< 0.001
BEL of P, mg/kg DMI[1]	176[b]	338[a]	234[b]	374[a]	16.8	< 0.001
P in urine, g/kg	0.003[b]	0.144[a]	0.005[b]	0.005[b]	0.001	< 0.001
Urine P output, g/day	0.01[b]	0.68[a]	0.01[b]	0.01[b]	0.04	< 0.001
P retention, g/day	-0.02[c]	1.33[a]	0.96[b]	-0.04[c]	0.04	< 0.001

[a,b,c,d]Means within a row without a common superscript letter are different ($P < 0.05$).
[1]DM = dry matter; ATTD = apparent total tract digestibility; BEL = basal endogenous loss; DMI = dry matter intake.
[2]Least squares means represent 10 replicate animals per diet.

Phosphorus intake was different ($P < 0.05$) among all diets, with pigs fed the diet containing gelatin having the least and pigs fed the diet containing spray dried plasma having the greatest P intake. There was no difference in concentration of fecal P (g/kg) between pigs fed the gelatin diet and pigs fed the casein diet, but pigs fed the gelatin diet had less ($P < 0.05$) fecal P output (g/day) compared with pigs fed diets containing the other protein sources. Likewise, pigs fed the casein diet had less ($P < 0.05$) fecal P output (g/day) than pigs fed diets containing potato protein concentrate or spray dried plasma. Absorbed P was greatest ($P < 0.05$) by pigs fed the diet containing spray dried plasma, and pigs fed the casein diet had greater ($P < 0.05$) daily absorption of P than pigs fed the gelatin diet or the potato protein diet. Pigs fed diets containing spray dried plasma or casein had greater ($P < 0.05$) ATTD of P compared with pigs fed diets containing gelatin or potato protein concentrate. The BEL of P was not different between the gelatin and casein diets, but pigs fed diets containing spray dried plasma or potato protein concentrate had greater ($P < 0.05$) BEL of P than pigs fed the other diets. Phosphorus excreted in urine, expressed as g/kg or g/day, was not different among diets containing gelatin, casein, or potato protein concentrate, but pigs fed diets containing spray dried plasma had greater ($P < 0.05$) urine P excretion compared with pigs fed the other protein sources. Retention of P was lowest ($P < 0.05$) for pigs fed diets containing gelatin or potato protein concentrate and was greatest ($P < 0.05$) for pigs fed the spray dried plasma diet.

4. Discussion

Concentrations of DM and crude protein in gelatin, spray dried plasma, casein, and potato protein concentrate and analyzed concentrations of Ca and P in spray dried plasma and casein were in agreement with expected values (NRC, 2012). Likewise, Ca and P concentrations in potato protein concentrate were consistent with reported values (Nelson et al., 2022).

The observation that pigs fed the gelatin diet had the least feed intake is in agreement with previous observations (Stein et al., 2006; Alves et al., 2016) and may be due to the reduced palatability caused by gelatin. Differences observed in P intake, fecal P (expressed as g/kg and g/day), and absorbed P were expected due to the differences in P provided by the diets. However, although no differences in feed intake were observed among pigs fed spray dried plasma, casein, or potato protein concentrate diets, pigs fed the diets containing spray dried plasma or potato protein concentrate had greater fecal P output (g/day) than pigs fed the casein diet. The greater total fecal excretion by pigs fed the potato protein concentrate diet than pigs fed the other diets may have been a result of the increased fiber and phytate in the potato protein concentrate (NRC, 2012; Nelson et al., 2022) compared with the other ingredients. The greater total urine output from pigs fed the diet containing spray dried plasma compared with pigs fed diets containing casein or potato protein concentrate may be due to the greater concentration of Na in spray dried plasma compared with casein or potato protein concentrate (NRC, 2012), which may have increased water intake and subsequently urine output. The observation that P concentration in the urine, expressed as g/kg or g/day, was greatest from pigs fed spray dried plasma diet was expected due to greater dietary concentration of P.

The observation that ATTD of P was negative in pigs fed diets containing gelatin and potato protein concentrate is a result of the low concentration of P in these diets and pigs were, therefore, excreting more P than they were ingesting from the diets. In contrast, the greater ATTD of P in the spray dried plasma and casein diets was a result of the greater concentration of P in these diets and reflects that endogenous P, as a percent of the total P excreted, decreases proportionately as P intake increases (Alves et al., 2016). Values for ATTD of P in spray dried plasma and casein are consistent with reported values (NRC, 2012).

The BEL of P observed in pigs fed the gelatin diet (176 mg/kg of DMI) is close to the 190 mg/kg of DMI, which the NRC (2012) designated as a representative value for BEL of P. Observed BEL of P from pigs fed the diet containing spray dried plasma (338 mg/kg of DMI) is consistent with the value of 370 mg/kg of DMI that was recently observed by Bailey et al. (2023), where spray dried plasma was the only source of P in the diet. In Brazil, blood plasma is sometimes utilized as a protein source in low-P diets to determine the BEL of P (Bünzen et al., 2012). Due to the high digestibility of P in spray dried plasma, it is assumed that P provided by spray dried plasma is completely absorbed, and therefore, any P present in the feces is of endogenous origin (Almeida and Stein, 2011). However, BEL of P measured from the diet containing spray dried plasma was greater than that measured from pigs fed the gelatin diet, indicating that not all P provided by spray dried plasma was absorbed by the pig. Therefore, using spray dried plasma may result in an overestimation of BEL of P. The BEL of P observed in pigs fed potato protein concentrate (374 mg/kg of DMI) is slightly greater than results observed by Lopez et al. (2022) who reported a value of 308.5 mg/kg of DMI when utilizing potato protein concentrate as the only source of P in the diet. The BEL of P observed from pigs fed the potato protein concentrate diet was greater than that observed in pigs fed the gelatin diet, indicating that the P provided from potato protein concentrate is not completely absorbed by pigs. Therefore, potato protein concentrate may also result in an overestimation of BEL of P. The observed BEL of P in pigs fed the casein diet (234 mg/kg DMI) is close to the range (110–226 mg/kg DMI) reported by Pettey et al. (2006). Because this value was not different from the value obtained for the gelatin diet it is concluded that casein, unlike spray dried protein plasma or potato protein concentrate, may be used as an alternative to gelatin in P-free diets used to estimate the BEL of P. The estimated basal endogenous loss of P from pigs fed both the gelatin and the casein diets are also within the range of values for basal endogenous loss of P (i.e., 111–325 mg/kg DMI) that has been reported in the literature (Lee and Stein, 2023). However, the result obtained in this experiment with growing pigs are different from results obtained with broiler chickens where the basal endogenous loss of P was greater from birds fed a diet based on casein than from birds fed a gelatin diet (Mutucumarana and Ravindran, 2021). It is not clear why broiler chickens appear to react different than pigs to casein and gelatin, but regardless of the diet, values for the basal endogenous loss of P in broiler chickens were much lower than what is usually obtained in pigs (i.e., 25 and 104 mg/kg dry matter intake for gelatin and casein diets, respectively).

5. Conclusion

The basal endogenous loss of phosphorus was greater if calculated from pigs fed diets containing spray dried plasma or potato protein concentrate compared with gelatin. However, no difference between the casein and gelatin diet was observed and casein may, therefore, be used as an alternative to gelatin to estimate the basal endogenous loss of phosphorus. Casein provides a greater amount of phosphorus compared with gelatin, which compensates for the deficient level of phosphorus in gelatin as well as the poor palatability of gelatin, but using casein does not impact values for the basal endogenous loss of phosphorus.

Declaration of Competing Interest

The authors have no conflicts of interest.

References

Adeola, O., 2001. Digestion and balance techniques in pigs. In: Lewis, A.J., Southern, L.L. (Eds.), Swine nutrition. CRC Press, Washington, DC, USA, pp. 903–916.

Almeida, F.N., Stein, H.H., 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88, 2968–2977. https://doi.org/10.2527/jas.2009-2285.

Almeida, F.N., Stein, H.H., 2011. Standardized total tract digestibility of phosphorus in blood products fed to weanling pigs. Rev. Colomb. Cienc. Pecu. 24, 617–622.

Alves, D.A., Rocha, L.Td, Camargo, C.Ad.S., Figueiredo, A.M., Ceron, M.S., Lucca, W., Zanella, I., Oliveira, Vd, 2016. Methodologies for the determination of endogenous phosphorus losses in growing pigs. Asian-Australas. J. Anim. Sci. 29, 1632–1638. https://doi.org/10.5713/ajas.15.0540.

AOAC Int, 2019. Official methods of analysis of AOAC Int, 21th ed. AOAC Int., Rockville, MD, USA.

Bailey, H.M., Campbell, J.M., Torres-Mendoza, L.J., Fanelli, N.S., Stein, H.H., 2023. Inclusion of spray dried plasma in diets based on different ingredient combinations increases the digestibility of energy, fiber, Ca, and P by young pigs. Transl. Anim. Sci. https://doi.org/10.1093/tas/txad031.

Baker, S.R., Kim, B.G., Stein, H.H., 2013. Comparison of values for standardized total tract digestibility and relative bioavailability of phosphorus in dicalcium phosphate and distillers dried grains with solubles fed to growing pigs. J. Anim. Sci. 91, 203–210. https://doi.org/10.2527/jas.2010-3776.

Bikker, P., van der Peet-Schwering, C.M.C., Gerrits, W.J.J., Sips, V., Walvoort, C., van Laar, H., 2017. Endogenous phosphorus losses in growing-finishing pigs and gestating sows. J. Anim. Sci. 95, 1637–1643. https://doi.org/10.2527/jas.2016.1041.

Bünzen, S., Rostagno, H.S., Kiefer, C., Oliveira, A.T., Ribeiro Jr., V., 2012. Digestible phosphorus levels for growing swine. Rev. Bras. Zootec. 41, 320–325 doi.10.1590/S1516-35982012000200013.

Fan, M.Z., Archbold, T., Lackeyram, D., Rideout, T., Gao, Y., de Lange, C.F.M., Hacker, R.R., Sauer, W.C., 2001. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous outputs in studies with pigs. J. Nutr. 131, 2388–2396. https://doi.org/10.1093/jn/131.9.2388.

Kim, J.W., Ndou, S.P., Mejicanos, G.A., Nyachoti, C.M., 2017. Standardized total tract digestibility of phosphorus in flaxseed meal fed to growing and finishing pigs without or with phytase supplementation. J. Anim. Sci. 95, 799–805. https://doi.org/10.2527/jas.2016.1045.

Lee, S.A., Stein, H.H., 2023. Digestibility and availability of nutrients in feed ingredients. In: L. I. Chiba, editor, Sustainable Swine Nutrition. p. 493-545. doi:10.1002/9781119583998.ch19.

Lee, S.A., Walk, C.L., Stein, H.H., 2018. Comparative digestibility and retention of calcium and phosphorus by gestating sows and growing pigs fed low- and high-phytate diets without or with microbial phytase, 83-83 J. Anim. Sci. 96. https://doi.org/10.1093/jas/sky073.154.

Lopez, D.A., Lee, S.A., Stein, H.H., 2022. Effects of microbial phytase on standardized total tract digestibility of phosphorus in feed phosphates fed to growing pigs. J. Anim. Sci. 100, 1–7. https://doi.org/10.1093/jas/skac350.

Mutucumarana, R., Ravindran, V., 2021. Measurement of endogenous phosphorus losses in broiler chickens. J. Poult. Sci. 58, 58–63. https://doi.org/10.2141/jpsa.0190118.

Nelson, M.E., Lee, S.A., Dersjant-Li, Y., Remus, J., Stein, H.H., 2022. Microbial phytase reduces basal endogenous loss of calcium in pigs fed diets containing phytate phosphorus at commercial levels. J. Anim. Sci. 100, 1–7 doi.org/10.1093/jas/skac280.

NRC, 2012. Nutrient requirements of swine, 11th rev. ed Natl. Acad. Press, Washington, DC, USA.

Petersen, G.I., Smiricky-Tjardes, M.R., Stein, H.H., 2005. Apparent and standardized ileal digestibility of amino acids in gelatin-based diets by growing pigs. Anim. Feed Sci. Technol. 119, 107–115. https://doi.org/10.1016/j.anifeedsci.2004.11.006.

Petersen, G.I., Stein, H.H., 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84, 2126–2132. https://doi.org/10.2527/jas.2005-479.

Pettey, L.A., Cromwell, G.L., Lindemann, M.D., 2006. Estimation of endogenous phosphorus loss in growing and finishing pigs fed semi-purified diets. J. Anim. Sci. 84, 618–626. https://doi.org/10.2527/2006.843618x.

SAS Institute Inc., 2016. SAS® 9.4 SQL Procedure User's Guide, 4th ed. SAS Institute Inc, Cary, NC, USA.

She, Y., Li, D., Zhang, S., 2017. Methodological aspects of determining phosphorus digestibility in swine: A review. J. Anim. Nutr. 3, 97–102. https://doi.org/10.1016/j.aninu.2017.02.003.

Son, A.R., Kim, B.G., 2015. Effects of dietary cellulose on the basal endogenous loss of phosphorus in growing pigs. Asian-Australas. J. Anim. Sci. 28, 369–373. https://doi.org/10.5713/ajas.14.0539.

Stein, H.H., Lagos, L.V., Casas, G.A., 2016. Nutritional value of feed ingredients of plant origin fed to pigs. Anim. Feed Sci. Technol. 218, 33–69. https://doi.org/10.1016/j.anifeedsci.2016.05.003.

Stein, H.H., Boersma, M.G., Pedersen, C., 2006. Apparent and true total tract digestibility of phosphorus in field peas (Pisum sativum L.) by growing pigs. Can. J. Anim. Sci. 86, 523–525. https://doi.org/10.4141/A05-091.

U.S. Environmental Protection Agency, 2000. Acid digestion of sediments, sludges, and soils, U.S. EPA, Washington, DC, USA. ⟨https://www.epa.gov/sites/production/files/2015-12/documents/3050b.pdf⟩ (accessed March, 2023).

Tukey, J.W., 1977. Exploratory data analysis. Addison-Wesley Pub, Co., Boston, MA, USA.