# Casein Supplementation Does Not Affect the Estimates of True Total Tract Digestibility of Phosphorus in Soybean Meal for Growing Pigs Determined by the Regression Method

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**ABSTRACT:** Forty-eight barrows with an average initial body weight of 25.5±0.3 kg were assigned to 6 dietary treatments arranged in a 3×2 factorial of 3 graded levels of P at 1.42, 2.07, or 2.72 g/kg, and 2 levels of casein at 0 or 50 g/kg to compare the estimates of true total tract digestibility (TTTD) of P in soybean meal (SBM) for pigs fed diets with or without casein supplementation. The SBM is the only source of P in diets without casein, and in the diet with added casein, 1.0 to 2.4 g/kg of total dietary P was supplied by SBM as dietary level of SBM increased. The experiment consisted of a 5-d adjustment period and a 5-d total collection period with ferric oxide as a maker to indicate the initiation and termination of fecal collection. There were interactive effects of casein supplementation and total dietary P level on the apparent total tract digestibility (ATTD) and retention of P ($p<0.05$). Dietary P intake, fecal P output, digested P and retained P were increased linearly with graded increasing levels of SBM in diets regardless of casein addition ($p<0.01$). Compared with diets without casein, there was a reduction in fecal P in the casein-supplemented diets, which led to increases in digested P, retained P, ATTD, and retention of P ($p<0.01$). Digested N, ATTD of N, retained N, and N retention were affected by the interaction of casein supplementation and dietary P level ($p<0.05$). Fecal N output, urinary N output, digested N, and retained N increased linearly with graded increasing levels of SBM for each type of diet ($p<0.01$). The estimates of TTTD of P in SBM, derived from the regression of daily digested P against daily P intake, for pigs fed diets without casein and with casein were calculated to be 37.3% and 38.6%, respectively. Regressing daily digested N against daily N intake, the TTTD of N in SBM were determined at 94.3% and 94.4% for diets without casein and with added casein, respectively. There was no difference in determined values of TTTD of P or N in SBM for pigs fed diets with or without casein ($p>0.05$). In summary, our results demonstrate that the estimates of TTTD of P in SBM for pigs were not affected by constant casein inclusion in the basal diets. (**Key Words:** Casein, Phosphorus, Pig, Soybean Meal, True Total Tract Digestibility)

## INTRODUCTION

The majority of P in grains is bound to phytate, which leads to a poor utilization of P by swine (Cromwell, 1980; Raboy, 1997; Weremko et al., 1997). Apparent P digestibility was used to measure the P utilization in previous studies (Jongbloed et al., 1992; Fan and Sauer, 2002). However, true total tract digestibility (TTTD) of P is preferred to determine the P utilization using the regression method due to the account for the confounding effect of

endogenous P loss (EPL) in recent years (Fan et al., 2001; Ajakaiye et al., 2003; Dilger and Adeola, 2006; Akinmusire and Adeola, 2009).

Semi-purified diets, used for the determination of TTTD of P associated with feedstuffs, are different from practical diets for swine in dietary P sources and amino acid profiles. In experimental situations, the semi-purified diets were used to determine TTTD of P in feedstuffs for swine and poultry, which in general derive all the P from the test feedstuff (Fan et al., 2001; Dilger and Adeola, 2006; Akinmusire and Adeola, 2009). However, ingredients containing high P digestibility are included in practical diets (Stein et al., 2008). Furthermore, the protein quality in practical diets may be higher than that in semi-purified diets and dietary

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protein quality could be improved by the supplementation of casein (contains highly digestible amino acids). It has been reported that the estimates of metabolizable energy content of corn distillers dried grains with soluble by the regression method are not consistent between semi-purified and practical diets (Adeola and Ileleji, 2009). It is noteworthy that the estimates of TTTD of P in feedstuffs obtained from experimental semi-purified diets are used for diet formulation based on P evaluation studies for digestibility in feed ingredients and the assumption of additivity. However, there is no report on the effects of additional highly digestible P source inclusion in the basal diets and dietary protein quality of semi-purified diets on the determination of TTTD of P in soybean meal (SBM) for pigs. Therefore, the objective of the current study was to use the regression method to compare the determined values of TTTD of P and EPL associated with SBM in pigs fed diets without or with casein.

## MATERIALS AND METHODS

All animal protocols were approved by the Purdue University Animal Care and Use Committee.

**Diets and experimental design**

The six dietary treatments (Table 1) consisted of 3 graded P concentrations (1.42, 2.07, or 2.72 g/kg), and 2 diet type (without or with casein supplementation) arranged in a 3×2 factorial. The SBM levels were 200, 310, or 420 g/kg and 145, 255, or 365 g/kg in the diets without and with casein, respectively. Diets were semipurified and based on SBM, cornstarch, dextrose, and sucrose. The concentrations of SBM in diets were increased at the expense of cornstarch. The only P source in diets without casein was SBM, while P in casein-supplemented diets were derived from SBM and casein. All diets were formulated to have a constant Ca:total P ratio at 1.2:1 by adjusting limestone.

**Animals, feeding and sample collection**

Forty-eight barrows, with an average initial body weight (BW) of 25.5±0.3 kg, were assigned to 6 dietary treatments according to a randomized complete block design with 8 replicates per diet. All the pigs were placed in metabolism crates (0.83 by 0.71 m) and randomly allocated to the 6 dietary treatments such that the average initial BW of pigs was similar among treatment groups. The metabolism crates were located in an environmentally controlled room with a

**Table 1.** Ingredient composition of the experimental diets (as-fed basis)

| Item (g/kg) | Without casein (0 g/kg), dietary total P level (g/kg) | | | With casein (50 g/kg), dietary total P level (g/kg) | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 1.42 | 2.07 | 2.72 | 1.42 | 2.07 | 2.72 |
| Ingredients | | | | | | |
| Soybean meal (crude protein 48%) | 200.0 | 310.0 | 420.0 | 145.0 | 255.0 | 365.0 |
| Cornstarch | 520.9 | 409.6 | 298.3 | 525.8 | 414.5 | 303.2 |
| Soybean oil | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Salt | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 | 3.3 |
| Sucrose | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 |
| Dextrose | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Limestone | 2.8 | 4.1 | 5.4 | 2.9 | 4.2 | 5.5 |
| Casein | 0.0 | 0.0 | 0.0 | 50.0 | 50.0 | 50.0 |
| Vitamin premix[1] | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Mineral premix[2] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Selenium premix[3] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 | 1,000.0 |
| Calculated nutrient composition[4] | | | | | | |
| N | 15.77 | 24.25 | 32.74 | 18.60 | 27.08 | 35.57 |
| Ca | 1.70 | 2.50 | 3.29 | 1.69 | 2.48 | 3.28 |
| Total P | 1.42 | 2.07 | 2.72 | 1.42 | 2.07 | 2.72 |
| Analyzed nutrient composition | | | | | | |
| N | 16.18 | 23.30 | 31.34 | 18.33 | 27.73 | 35.20 |
| Ca | 1.58 | 2.47 | 3.11 | 1.71 | 2.42 | 3.19 |
| Total P | 1.28 | 1.95 | 2.61 | 1.32 | 1.91 | 2.58 |

[1] Provided per kilogram of diet: 3,630 IU of vitamin A; 363 IU of vitamin D; 36.4 IU of vitamin E; 1.3 mg of vitamin K (menadione sodium bisulfite); 23.1 μg of vitamin $B_{12}$; 5.28 mg of riboflavin; 13.1 mg of D-pantothenic acid; 19.8 mg of niacin.
[2] Provided per kilogram of diet: 121 mg of Fe (as iron carbonate); 15 mg of Mn (as manganese oxide); 11.3 mg of Cu (as copper chloride); 0.46 mg of I (as ethylenediamine dihydroiodide); and 121 mg of Zn (as zinc oxide).
[3] Selenium premix provided 300 μg of Se per kilogram of diet.
[4] The nutrient composition of diets was calculated according to NRC (2012).

temperature of 21°C±2°C. Feed allowance was calculated as approximately 4% of initial BW of each pig during the experimental period. The barrows were fed one-half of the daily feed allowance at 0800 and 1800 h and the water was provided at 3 L/kg feed supply. Pigs were fed the experimental diets for 10 d with the initial 5 d being an adjustment period. Five grams of ferric oxide, indigestible marker, was included to 100 g of feed in the morning meals of d 6 and 11. Fecal collection began with the first sign of marked feces after d 5 and stopped with the appearance of the marker in the feces after d 11. Fecal samples were collected from 0800 to 1800 h during the collection period. Urine collection started at 0800 h of d 6 and ceased at 0800 h of d 11. A preservative of 50 mL of 6 *N* sulfuric acid was used for urine collection. Feces and urine were collected daily, weighed, and all the feces and a 20% subsample of the urine were stored at –20°C until further analysis. All the collected samples were pooled per pig at the end of the experiment.

**Chemical analyses**

Feces and urine were oven-dried at 55°C. Diets and feces were ground through a 0.75-mm sieve. Dry matter content was determined by drying samples at 105°C for 24 h in a drying oven (Precision Scientific Co., Chicago, IL, USA; method 934.01; AOAC 2006). Concentration of P in diets, feces and urine samples were determined using a colorimetric assay after a wet-ash digestion with nitric and perchloric acids (method 935.13; AOAC, 2006). The acid molybdate and Fiske-Subbarow reducer solution were added to wet-ashed samples to measure the concentrations of P through the formation of a phosphomolybdenum complex by spectrophotometry at a wavelength of 620 nm (SpectraCount, model AS1000, Packard, Meriden, CT, USA; method 946.06; AOAC, 2006). Concentration of Ca in the supernatant of nitric and perchloric acids-digested diets was determined using flame atomic absorption spectrometry (AAnalyst 300, Perkin Elmer, Norwalk, CT, USA; method 985.01; AOAC, 2006). The N contents in diets, feces and urine were analyzed by the combustion method (method 990.03; AOAC, 2006).

**Calculations and statistical analysis**

Feed refusal was used to adjust total nutrient consumption. The apparent total tract digestibility (ATTD) of P and N were calculated by the following equation:

$$ATTD\ (\%) = 100 \times (P_I - P_O)/P_I,$$

where $P_I$ represents the dietary P (mg/d) or N (g/d) intake; and $P_O$ is the fecal P (mg/d) or N (g/d) output. Digested P or N ($P_D$) is calculated by the difference between dietary P or N intake and fecal P or N output. The TTTD of

P or N in SBM was determined by regressing $P_D$ against $P_I$ for diets with or without casein supplementation using the following model:

$$P_D = (TTTD \times P_I) - EPL,$$

where TTTD (%) is the slope of the regression; and EPL (mg/d) is the negative intercept of the regression.

The MIXED procedure was used for data analysis, and pig served as the experimental unit. Casein, dietary total P level, and the interaction of casein and P level were the fixed effects, and block was considered as the random effect in the model. Linear and quadratic contrasts were used examine the effects of P level within each diet type (with or without casein supplementation). Regression coefficients were compared between diets with or without casein supplementation. The differences in TTTD of P or N and EPL between pigs fed diets with or without casein supplementation were compared according to the pooled *t*-test (Zhai and Adeola, 2013). An α level of 0.05 was used to represent significance level.

**RESULTS**

All barrows were healthy and consumed all the provided feed, except one mortality at the beginning of the study in one of the casein-supplemented diets. The analyzed concentrations of Ca, P, and N in experimental diets were close to the calculated values (Table 1).

Daily balance and ATTD of P are presented in Table 2. Dietary P intake, fecal P output, digested P, and retained P increased linearly with graded increasing inclusion of P from SBM regardless of casein supplementation (p<0.01). With increasing dietary P intake, urinary P output increased linearly for pigs fed diets with casein supplementation (p<0.05). However, there was no effect of P levels on P output in urine for diets without casein supplementation. Consumption of casein-supplemented diets decreased fecal P output, increased digested and retained P, and ATTD and retention of P compared with pigs fed diets without casein (p<0.01). There were interactive effects of casein supplementation and dietary P level on the ATTD and retention of P (p<0.05). The ATTD and retention of P tended to decrease for diets with added casein as dietary P increased (linear, p<0.10). However, retention of P tended to increase with increasing dietary P levels without casein supplementation (linear, p<0.10). The ATTD of P ranged from 31.25% to 35.84% or 44.97% to 51.15% for pigs fed diets without or with added casein, respectively.

Daily balance and ATTD of N are presented in Table 3. Fecal N output and urinary N output increased linearly (p<0.01) with graded levels of SBM in each type of diet. There were interactive effects of casein inclusion and

**Table 2.** Daily balance and apparent total tract digestibility (ATTD) of P in pigs fed experimental diets (as-fed basis)

| Item | Without casein, dietary total P level (g/kg) | | | With casein, dietary total P level (g/kg) | | | Standard deviation | Casein | P level | Interaction | p-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Without casein | | With casein | |
| | 1.42 | 2.07 | 2.72 | 1.42 | 2.07 | 2.72 | | | | | Linear | Quadratic | Linear | Quadratic |
| No. of pigs | 8 | 8 | 8 | 7 | 8 | 8 | | | | | | | | |
| Initial body weight (kg) | 25.5 | 25.5 | 25.5 | 25.4 | 25.5 | 25.4 | 0.33 | 0.812 | 0.754 | 0.895 | 0.936 | 0.777 | 0.518 | 0.420 |
| P intake (mg/d) | 1,305 | 1,988 | 2,661 | 1,343 | 1,947 | 2,630 | 31.6 | 0.028 | <0.001 | 0.003 | <0.001 | 0.711 | <0.001 | 0.008 |
| Fecal P output (mg/d) | 899 | 1,317 | 1,713 | 652 | 1,047 | 1,448 | 111.1 | <0.001 | <0.001 | 0.957 | <0.001 | 0.824 | <0.001 | 0.946 |
| Urinary P output (mg/d) | 19 | 18 | 21 | 19 | 21 | 28 | 7.9 | 0.159 | 0.137 | 0.410 | 0.581 | 0.699 | 0.028 | 0.579 |
| Digested P (mg/d) | 406 | 671 | 948 | 691 | 900 | 1,182 | 102.9 | <0.001 | <0.001 | 0.713 | <0.001 | 0.905 | <0.001 | 0.432 |
| ATTD of P (%) | 31.25 | 33.92 | 35.84 | 51.15 | 46.33 | 44.97 | 4.75 | <0.001 | 0.812 | 0.012 | 0.124 | 0.860 | 0.081 | 0.414 |
| Retained P (mg/d) | 387 | 653 | 927 | 672 | 879 | 1,154 | 102.7 | <0.001 | <0.001 | 0.666 | <0.001 | 0.926 | <0.001 | 0.452 |
| P retention (%) | 29.81 | 32.99 | 35.07 | 49.81 | 45.23 | 43.91 | 4.72 | <0.001 | 0.961 | 0.008 | 0.082 | 0.789 | 0.091 | 0.439 |

dietary P level on digested N, ATTD of N, retained N, and N retention (p<0.05). Digested N increased (linear, p<0.01; quadratic, p<0.01) with graded levels of SBM regardless of diet type. The ATTD of N increased linearly (p<0.01) for pigs fed diets without casein and increased quadratically (p<0.05) for pigs fed diets with casein. There were linear and quadratic decreases (p<0.01) in N retention for pigs fed casein-supplemented diets. With increasing SBM inclusion, pigs fed casein-supplemented diets had increases (linear, p<0.01; quadratic, p<0.01) in retained N, and pigs fed diets without casein had a linear increase (p<0.01) in retained N.

There was significant linear relationship between dietary P intake and digested P for pigs fed each type of diet in the current study. This allowed for the estimates of EPL and TTTD of P associated with SBM for growing pigs by the regression method. According to the linear regression

equations shown in Table 4, the estimated values of TTTD of P in SBM were 37.34% or 38.57% for pigs fed diets without or with added casein, respectively. There was no significant difference in determined values of TTTD of P in SBM between pigs fed diets without and with casein inclusion. Furthermore, the determined intercepts representing for EPL were 66 or −161 mg/d in pigs fed diets without or with casein supplementation, respectively. Theses determined values of EPL were not different from zero, which might be caused by the relative large variance with the estimates.

The estimates of TTTD of N in SBM were 94.26% or 94.43% for pigs fed diets without or with added casein, respectively, with their corresponding determined daily fecal endogenous N loss being 666 or 242 mg/d (Table 5). Dietary casein supplementation had no effect on the

**Table 3.** Daily balance and apparent total tract digestibility (ATTD) of N in pigs fed experimental diets (as-fed basis)

| Item | Without casein, dietary total P level (g/kg) | | | With casein, dietary total P level (g/kg) | | | Standard deviation | Casein | P level | Interaction | p-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Without casein | | With casein | |
| | 1.42 | 2.07 | 2.72 | 1.42 | 2.07 | 2.72 | | | | | Linear | Quadratic | Linear | Quadratic |
| No. of pigs | 8 | 8 | 8 | 7 | 8 | 8 | | | | | | | | |
| N intake (g/d) | 16.49 | 23.75 | 31.94 | 18.65 | 28.26 | 35.88 | 0.407 | <0.001 | <0.001 | <0.001 | <0.001 | 0.013 | <0.001 | <0.001 |
| Fecal N output (g/d) | 1.60 | 2.08 | 2.46 | 1.34 | 1.71 | 2.29 | 0.205 | <0.001 | <0.001 | 0.418 | <0.001 | 0.629 | <0.001 | 0.268 |
| Urinary N output (g/d) | 4.81 | 7.43 | 11.20 | 4.47 | 6.81 | 12.55 | 1.534 | 0.492 | <0.001 | 0.162 | <0.001 | 0.395 | <0.001 | 0.017 |
| Digested N (g/d) | 14.89 | 21.67 | 29.48 | 17.32 | 26.55 | 33.59 | 0.427 | <0.001 | <0.001 | <0.001 | <0.001 | 0.009 | <0.001 | <0.001 |
| ATTD of N (%) | 90.29 | 91.26 | 92.30 | 92.82 | 93.95 | 93.61 | 0.738 | <0.001 | <0.001 | 0.025 | <0.001 | 0.903 | 0.485 | 0.031 |
| Retained N (g/d) | 10.08 | 14.24 | 18.28 | 12.85 | 19.73 | 21.03 | 1.541 | <0.001 | <0.001 | 0.025 | <0.001 | 0.924 | <0.001 | <0.001 |
| N retention (%) | 61.26 | 59.93 | 57.26 | 68.82 | 69.86 | 58.58 | 5.024 | <0.001 | <0.001 | 0.043 | 0.142 | 0.766 | <0.001 | 0.009 |

**Table 4.** Linear relationship between digestible P and dietary P intake (mg/d) of pigs fed diets supplemented without or with casein

| Item | Without casein | With casein | p-value[1] |
|---|---|---|---|
| Regression equation[2] | Y = 0.3734X−65.9579 | Y = 0.3857X+160.6458 | |
| Standard error of the slope | 0.0460 | 0.0440 | |
| Standard error of the intercept | 94.9160 | 91.1898 | |
| $r^2$ | 0.7495 | 0.7851 | |
| Estimate of endogenous P loss (mg/d) | 66 | −161 | 0.095 |
| Estimate of true total tract P digestibility (%) | 37.34 | 38.57 | 0.849 |
| No. of pigs | 24 | 23 | |

[1] The p-values are resulted from *t*-test.

[2] Digested P was regressed against dietary P intake for pigs fed diets supplemented without or with casein, the slope represents an estimate of the true total tract P digestibility (%) and the intercept represents an estimate of endogenous P loss (mg/d).

**Table 5.** Linear relationship between digestible N and dietary N intake (mg/d) of pigs fed diets supplemented without or with casein

| Item | Without casein | With casein | p-value[1] |
|---|---|---|---|
| Regression equation[2] | Y = 0.9426X–0.6658 | Y = 0.9443X–0.2424 | |
| Standard error of the slope | 0.0065 | 0.0060 | |
| Standard error of the intercept | 0.1625 | 0.1740 | |
| $r^2$ | 0.9989 | 0.9991 | |
| Estimate of endogenous N loss (mg/d) | 666 | 242 | 0.083 |
| Estimate of true total tract N digestibility (%) | 94.26 | 94.43 | 0.852 |
| No. of pigs | 24 | 23 | |

[1] The p-values are resulted from *t*-test.

[2] Digested N was regressed against dietary N intake for pigs fed diets supplemented without or with casein, the slope represents an estimate of true total tract digestibility of N (%) and the intercept represents an estimate of endogenous N loss (mg/d).

estimates of TTTD of N in SBM and daily endogenous N loss for growing pigs.

## DISCUSSION

To improve P utilization by pigs, it is important to estimate the digestibility of P in feedstuff for accurate diet formulation. Compared with apparent digestibility, the true digestibility of P preferred because of the correction for the endogenous loss (Fan et al., 2001; Petersen and Stein, 2006). The P-free diet, $^{32}$P-labeled phosphates and the regression method have been used to measure the tandrue digestibility of P in assay ingredients and EPL for animals (Al-Masri and Günther, 1988; Al-Masri, 1995; Fan et al., 2001; Dilger and Adeola, 2006; Petersen and Stein, 2006; Akinmusire and Adeola, 2009). Because of disturbances in normal physiologic status of animals from the P-free diets and the rapid recycling of $^{32}$P-labeled phosphates in the gut (Al-Masri and Günther, 1988; Al-Masri, 1995; Petersen and Stein, 2006), the regression method is widely used to estimate the TTTD of P in test ingredients and EPL for pigs. However, there were little information about dietary factors affecting the use of the regression mathematical model and limitations of this approach have been published previously. To our knowledge, the concentrations of P in experimental diets are lower than the requirements of pigs and dietary P levels being within the linear response range are the only reported conditions influencing the use of regression analysis (Poulsen et al., 1999; Fan et al., 2001; Dilger and Adeola, 2006). This is because of the digestibility of P in diets is depressed by excessive dietary phytate-P intake (Selle et al., 2009).

Dietary supplementation of casein, which is high in protein quality and digestibility of P, did not affect regression-derived estimates of TTTD of P in SBM for pigs in the current study. However, added casein resulted in the determined value of EPL being negative even though the estimate was not different from zero. The estimation of EPL was considered as a key issue to measure TTTD of P because the values of EPL are needed for correcting ATTD

of P. However, the novel finding of the current study suggests that the estimates of TTTD of P in assay ingredients are not dependent on the accurate determination of EPL by regressing daily digested P against daily P intake. In agreement with previous publications using SBM as the test feedstuff, a strong linear relationship between digested P and dietary total P intake was observed in this study, which meet the requirement for the use of the regression method (Fan et al., 2001; Dilger and Adeola, 2006; Akinmusire and Adeola, 2009). As expected, diets supplemented with casein increased the ATTD of P due to the higher digestibility of P in casein than that in SBM (NRC, 2012). This is reflected in the ATTD of P ranging from 31.25% to 35.84% in diets without casein and 44.97% to 51.15% in diets with casein supplementation. The estimates of TTTD of P in SBM are 37.3% and 38.6% in diets without and with casein supplementation, respectively, in the current study. Our determined values of TTTD of P were lower than the estimates of 48.5% and 44.5% for SBM reported by Fan et al. (2001) and Dilger and Adeola (2006), whereas consistent with the estimates of 40.9% and 36.0% reported by Akinmusire and Adeola (2009) and Zhai and Adeola (2013). The current regression-derived estimates of TTTD of P in SBM are in agreement with Liu et al. (2014b), who reported that the determined values of TTTD of P in SBM are 41.0% and 37.5% for pigs fed a cornstarch-based semi-purified diet and a corn-based practical diet, respectively. The differences in the TTTD of P in SBM among previous studies and the current experiment may be due to dietary Ca:total P ratio, which was shown in broiler by Liu et al. (2013). There was no dietary limestone inclusion in the study of Fan et al. (2001) and Dilger and Adeola (2006), whereas dietary Ca:total P ratio was maintained at 1.2 by limestone supplementation in the current work and the study of Liu et al. (2014b). Furthermore, P digestibility in the basal diets does not affect the determination of TTTD of P in SBM in the current study, which is consistent with the study of Liu et al. (2014b). However, effects of P digestibility and levels in basal diets on the determination of true ileal digestibility of P in SBM

for broiler chickens were not consistent between the studies of Liu et al. (2014a) and Mutucumarana et al. (2015). There was no effect of casein supplementation (50.0 g/kg) on the estimates of true ileal digestibility of P in SBM for chickens fed diets at total P from 1.02 to 2.55 g/kg (Liu et al., 2014a), whereas dietary dried egg albumen addition decreased the determined values of true ileal P digestibility in SBM for chickens fed diets at total P from 2.77 to 4.82 g/kg (Mutucumarana et al., 2015). The differences in the range of total P levels of experimental diets are probably responsible, in part, for the differences between the study of Liu et al. (2014a) and Mutucumarana et al. (2015).

In the current study, dietary casein inclusion did not affect the estimate of EPL associated with SBM in pigs using the regression technique. This indicates that the negative intercepts derived from regressing digested P against dietary total P intake cannot be used for EPL estimation while additional ingredients containing high digestible P are included in the basal diets. This notion has been reported previously (Liu et al., 2014a). The slope and the negative intercepts represent the TTTD of P and EPL by the regression method, respectively. The following equations may responsible for dietary casein supplementation affects the negative intercepts while the estimates of TTTD of P in SBM was not altered:

$$P_D = TTTD_{SBM} \times P_{SBM} + TTTD_{casein} \times P_{casein} - EPL$$

$$P_D = TTTD_{SBM} \times (P_I - P_{casein}) + TTTD_{casein} \times P_{casein} - EPL$$

$$P_D = TTTD_{SBM} \times P_I + P_{casein} \times (TTTD_{casein} - TTTD_{SBM}) - EPL$$

where $TTTD_{SBM}$ and $TTTD_{casein}$ represent the TTTD of P (%) in SBM and casein, respectively; $P_{SBM}$ and $P_{casein}$ is dietary P (mg/d) originate from SBM and casein, respectively; $P_D$ is digested P (mg/d); $P_I$ is dietary total P intake (mg/d), and EPL is EPL (mg/d). These equations indicate that the negative intercept is $EPL + P_{casein} \times (TTTD_{SBM} - TTTD_{casein})$ using a linear regression of $P_D$ on $P_I$. However, the $TTTD_{SBM}$ is lower than $TTTD_{casein}$. Therefore, the determined negative intercept representing for EPL is negative when adequate amounts of P derived from casein was supplemented in the basal diet. These equations suggested by Liu et al. (2014b), indicated that the $P_D$ is increased by graded $P_I$ derived from SBM because the increased dietary P intake is due to increasing inclusion of SBM. Therefore, it is reasonable that the estimates of slope representing for TTTD of P in SBM were not affected by constant casein addition in the basal diet. In the current study, the estimate of EPL associated with SBM in pigs is 66 mg/d, which is consistent with the 48 mg/d reported by Akinmusire and Adeola (2009). The values of EPL reported in previous studies ranged from 70 mg/kg of dry matter

intake (DMI) (Dilger and Adeola, 2006; Pettey et al., 2006) to 670 mg/kg of DMI (Shen et al., 2002) by regression method. Using the P-free diet, an estimate of EPL at 139 mg/kg of DMI was also reported by Petersen and Stein (2006). Jongbloed et al. (1991) indicated that EPL is decreased when P intake is lower than the requirements for P. However, dietary concentration of P being lower than the requirements of the animal is a fundamental condition needed for using regression approach (Poulsen et al., 1999; Fan et al., 2001; Dilger and Adeola, 2006). Taken together, accurate EPL is not an essential requirement for reliable estimate of regression-derived TTTD of P in test feedstuffs.

In the current study, the ATTD of N increased with increasing levels of SBM. This may be due to decreased ratio of endogenous N loss to fecal N output derived from indigested N from SBM as dietary SBM level increased. As expected, pigs fed casein-supplemented diets had lower fecal N output than pigs fed diets without casein, thus leading to greater ATTD and retention of N. Consistent with the effects of casein supplementation on the estimate of TTTD of P, the determined values of TTTD of N in SBM were not influenced by dietary casein inclusion.

In conclusion, the regression-derived estimates of TTTD of P in SBM were not influenced by supplementing casein at 50 g/kg basal diet. The negative EPL for pigs fed diets with casein supplementation resulted from greater P digestibility in casein than that in SBM. Therefore, feedstuffs containing highly digestible protein and P can be included in cornstarch-based diets to determine the TTTD of P in the assay ingredients using the regression method.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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