Effects of dietary total phosphorus concentration and casein supplementation on the determination of true phosphorus digestibility for broiler chickens

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ABSTRACT
A study was conducted to compare the regression-driven estimates of true precaecal digestibility (TPD) of phosphorus (P) in soybean meal (SBM) for broiler chickens fed diets with or without casein supplementation at moderate or low total P concentration. A total of 768 male Ross 308 broilers were allocated to 12 diets in a 2 × 2 × 2 × 3 factorial arrangement of two total P concentration (moderate-P or low-P), two diet types (with or without casein) and three SBM levels (low, medium or high). There were 8 chicks per cage and 8 replicate cages per treatment group in a randomised complete block design. The birds were fed experimental diets from d 14 to 21 post-hatching. Chromic dioxide was used as an indigestible marker. The results showed that dietary casein supplementation improved body weight (BW) gain, feed intake and feed efficiency of broilers (p < .01). Broilers fed the moderate-P diets had greater dietary total P intake, precaecal flow and total tract output of P, precaecal digested P, and total tract retained P compared with birds fed the low-P diets (p < .01). Dietary casein supplementation increased precaecal digested P, total tract retained P, apparent precaecal digestibility (APD) and total tract retention (TTR) of P (p < .01). Broilers fed the moderate-P diets had a linear decrease in the APD and TTR of P with increasing SBM levels (p < .01). Precaecal digested P and total tract retained P increased with graded inclusion of SBM levels (linear, p < .01). Regression of precaecal digested P against dietary P intake showed that chicks fed the moderate-P diets had lower estimates of TPD of P in SBM than that for chicks fed the low-P diets (p < .05), while dietary casein supplementation had no effects on the determined values of TPD of P. In conclusion, the regression-driven true phosphorus digestibility in assay feed ingredients for broiler chickens was affected by dietary total P concentration.

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Introduction
Utilisation of phosphorus (P) in plant feedstuffs by poultry has been extensively investigated in previous experiments and the values of P digestibility vary between and within laboratories (Weremko et al. 1997; Dilger and Adeola 2006; Selle et al. 2009; Liu et al. 2013; Mutucumarana et al. 2015). This inconsistency may be attributed to differences in the methodologies used to calculate P digestibility (Rodehutscord 2009; Selle et al. 2009). To avoid the variability caused by the methodologies, the Nutrition Working Group of the European Federation of Branches of World’s Poultry Science Association recommends that the regression method determined true precaecal digestibility (TPD) should be employed as a standard criterion to determine P availability (WPSA 2013). However, the composition of test diets proposed by WPSA (2013) is different with traditional experimental diets used in the regression approach suggested by Dilger and Adeola (2006). The assay feed ingredient was the sole source of dietary protein and P in traditional experimental diets when the regression method was applied to determine true P digestibility (Fan et al. 2001; Shen et al. 2002; Dilger and Adeola 2006). In contrast, highly digestible feedstuffs, such as casein and dried egg albumen, were included in test diets proposed by WPSA (2013), Liu et al. (2014), Mutucumarana et al. (2015) and Liu and Adeola (2016).
The inclusion of casein or dried egg albumen in experimental diets was to improve the growth performance and utilisation of protein in broiler chickens, which was associated with precaecal digestion of P (Xue et al. 2016). It is noteworthy that the effects of dietary supplementation of highly digestible feedstuffs on the regression-driven TPD of P in soybean meal (SBM) were inconsistent among previous studies (Liu et al. 2014; Mutucumarana et al. 2015). In the study of Liu et al. (2014), there were no effects of dietary casein supplementation on the estimation of TPD of P in SBM for birds fed diets containing total P from 1.28 to 2.55 g/kg. However, dietary addition of dried egg albumen decreased the determined values of TPD of P in SBM for broilers fed diets at total P levels from 2.77 to 4.82 g/kg (Mutucumarana et al. 2015). Dietary concentration of P in experimental diets should be formulated below the requirement of broiler chickens when the regression approach was employed to determine true P digestibility because high levels of dietary total P depressed the digestibility of P (Selle et al. 2009; Shastak et al. 2014). The discrepancy in the effects of dietary supplementation of highly digestible feedstuffs on the determination of TPD of P in SBM might be caused by differences in dietary total P concentration between the study of Liu et al. (2014) and Mutucumarana et al. (2015).

Therefore, it is hypothesised that the effects of dietary casein supplementation on the estimation of TPD of P in assay feed ingredients would be affected by dietary total P concentration. The objective of the present study was to compare the estimates of TPD of P in SBM for broiler chickens fed diets with or without casein supplementation at moderate or low total P concentration.

Materials and methods

This experiment was conducted in accordance with the Chinese guidelines for animal welfare and all experimental procedures were approved by the Southwest University of Science and Technology Ethics Committee.

Birds, diets and sample collection

A total of 768 male broiler chicks (Ross 308) were fed a commercial broiler starter diet (CP: 23%, Ca: 1.0%, non-phytate P: 0.5%, without coccidiostat and enzyme supplementation) in cages in an environmentally controlled room from d 1 to 14 post-hatching. Birds had free access to feed and water during the experimental period. On d 14 post-hatching, all chicks were weighed individually, grouped into 8 blocks by BW and randomly assigned to one of the 12 diets in each block with 8 chicks per cage in a randomised complete block design. All chicks received one of the 12 experimental diets from d 14 to 21 post-hatching. The room temperatures were maintained at 35, 32 and 27°C on d 1 to 7, d 8 to 14 and d 15 to 21 post-hatching, respectively. There were 12 diets in a $2 \times 2 \times 3$ factorial arrangement, which included 2 total P levels (low or moderate), 2 diet types (with or without casein) and 3 levels of SBM (low, medium or high). The low-P diets and moderate-P diets were formulated to contain dietary total P levels from 1.3 to 3.0 g/kg and 2.3 to 4.0 g/kg, respectively. Casein was included into the casein-supplemented diets at 50.0 g/kg on an as-fed basis. Limestone was included in the diets to keep a constant dietary Ca to P ratio at 1.2:1. Chromic dioxide was incorporated into experimental diets as an indigestible marker at 5.0 g/kg to calculate the digestibility of P. The composition and nutrients levels of experimental diets are shown in Table 1 and Table 2, respectively. The individual BW of birds was recorded on d 21 post-hatching to calculate BW gain, and feed consumption of each cage was recorded from d 14 to 21 post-hatching.

Excreta samples of each cage were collected from d 19 to 20 post-hatching from pans placed underneath the cages to calculate apparent total tract retention of P. On d 21 post-hatching, all birds were euthanised by carbon dioxide asphyxiation and precaecal digesta was collected from the distal two-thirds of the ileum by flushing the ileal content with deionised water. Digesta samples from 8 chicks in each cage were pooled into a plastic container. Excreta and precaecal digesta samples were dried in a forced-draft oven at 55°C for 5 d.

Chemical analyses

Diets, precaecal digesta and excreta samples were ground through a 0.75-mm sieve before analyses. Dry matter was measured by drying the samples at 105°C for 24 h in a drying oven. Nitrogen content in diets was determined by the combustion method (AOAC 2006; 990.03) using the Leco CHNS-932 Analyzer (Leco Corp., St. Joseph, MI). Diets, precaecal digesta and excreta samples were digested in concentrated nitric acid and 70% perchloric acid (AOAC 2006; 935.13) before the determination of chromium, Ca and P concentrations. The concentration of chromium was estimated by spectrophotometric reading of absorption at
440 nm (AOAC 2006; 946.06; Pharmacia LKB Ultraspec III, Cambridge, UK). Fiske-Subbarow reducer solution and acid molybdate were incorporated into the digested samples to determine the concentration of P by spectrophotometric reading of absorption at 620 nm (AOAC 2006; 946.06; Pharmacia LKB Ultraspec III, Cambridge, UK). Calcium content was determined by flame atomic absorption spectrometry (AAnalyst 300, PerkinElmer, Norwalk, CT).

**Calculations and statistical analyses**

Apparent precaecal digestibility of DM and P was calculated via the following equation:

$$\text{APD, } \% = 100 - \left(\frac{\text{Cr}\text{I}}{\text{CrO}}\right) \times \left(\frac{\text{NO}}{\text{NI}}\right) \times 100$$

where APD is the apparent precaecal digestibility of DM or P; CrI is the chromium concentration in diets; CrO is the chromium concentration in precaecal digesta; NO is the DM or P concentration in precaecal digesta; and NI is the concentration of DM or P in diets. All analysed values were expressed as milligrams per kilogram of DM.

Total tract retention of P was calculated by the following equation:

$$\text{TTR, } \% = 100 - \left(\frac{\text{CrI}}{\text{CrO}}\right) \times \left(\frac{\text{PO}}{\text{PI}}\right) \times 100$$

where TTR is the total tract retention of P; CrI is the chromium concentration in diets; CrO is the chromium concentration in excreta; PO is the P concentration in excreta output; and PI is the concentration of P in diets. All analysed values were expressed as milligrams per kilogram of DM.

Total P flow in precaecal digesta and total P output in excreta, expressed as milligrams per kilogram of DM intake (DMI), were calculated according to the following equation:

$$\text{PO-DMI, } \text{mg/kg} = \text{PO-DMO} \times \left(\frac{\text{CrI}}{\text{CrO}}\right)$$

where PO-DMI and PO-DMO represent P flow or output concentrations (in precaecal digesta or excreta) on a DMI and DM output (DMO) basis, respectively; CrI is the concentration of chromium in diets (mg/kg of DM); and CrO is the chromium concentration in precaecal digesta or excreta (mg/kg of DM).
Precaecal digested P or total tract retained P, expressed as milligrams per kilogram of DMI, was calculated via the following equation:

\[ P_{D-DMI} \text{ mg/kg} = P_{I-DM} - P_{O-DMI} \]

where \( P_{D-DMI} \) represents precaecal digested P or total tract retained P on a DMI basis; \( P_{I-DM} \) is the concentration of P in diets on a DM basis; and \( P_{O-DMI} \) is P flow or output concentrations (in precaecal digesta or excreta) on a DMI basis.

In the present study, precaecal digested P was regressed against dietary P intakes for birds fed diets with or without casein supplementation at moderate or low total P concentrations using the following equation (Iyayi et al. 2013):

\[ P_{D-DMI} \text{ mg/kg} = (TPD \times P_{I-DM}) - EPL \]

where \( P_{D-DMI} \) represents precaecal digested P on a DMI basis; \( TPD \) (%) represents true precaecal digestibility of P; \( P_{I-DM} \) is the concentration of P in diets on a DM basis; and \( EPL \) represents endogenous P loss (EPL) in precaecal digesta on a DMI basis.

The data were analysed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and cage served as the experimental unit for statistical analyses. The fixed effects included dietary total P level (moderate or low total P), diet type (with or without casein supplementation), SBM level (low, medium, or high), dietary total P level \( \times \) diet type, dietary total P level \( \times \) SBM level, diet type \( \times \) SBM level and dietary total P level \( \times \) diet type \( \times \) SBM level. The block was considered as a random effect in this model. Orthogonal polynomial contrasts were used to determine the effects of graded dietary SBM levels within each diet type at moderate or low dietary total P concentration. Statistical significance was considered at \( p \leq .05 \). The regression method was used to determine the TPD of P in SBM for diets with or without casein supplementation at moderate or low dietary total P concentration. The slope of the regression equation represents the TPD of P. Regression coefficients were compared among birds fed diets with or without casein supplementation at moderate or low total P concentration using 95% confidence intervals derived from the standard errors of respective regression coefficients.

**Results**

**Growth performance**

Growth performance and DM digestibility of birds are presented in Table 3. Dietary casein supplementation increased BW gain, feed intake and feed efficiency (\( p < .01 \)). Chicks fed the moderate-P diets had greater BW gain, feed intake and feed efficiency, but lower precaecal DM digestibility than birds fed the low-P diets (\( p < .01 \)). There were linear increases in BW gain, feed intake and feed efficiency with increasing SBM level for birds fed diets with or without casein addition at moderate or low total P concentration as dietary SBM level increased (\( p < .01 \)). The precaecal DM digestibility was affected by the interactive effects of dietary total P \( \times \) SBM levels (\( p < .01 \)). The precaecal digestibility of DM decreased (linear, \( p < .01 \)) for birds fed the moderate-P diets with increasing levels of SBM, while there were no linear or quadratic effects of SBM levels on precaecal DM digestibility for chicks fed the low-P diets.

**P digestibility and retention**

Dietary total P intake and utilisation of P are presented in Table 4 and Table 5. The precaecal flow of P, total tract P output, precaecal digested P and total tract retained P were affected by the interactive effects of dietary total P \( \times \) SBM levels (\( p < .01 \)). Furthermore, the precaecal digested P and total tract retained P were affected by the interactive effects of dietary total P \( \times \) casein addition, and SBM level \( \times \) casein addition (\( p < .05 \)). The precaecal flow of P, total tract P output, precaecal digested P and total tract retained P increased (linear, \( p < .01 \)) for chicks fed the low-P diets with graded levels of SBM. There were linear (\( p < .01 \)) and quadratic (\( p < .05 \)) effects of increasing SBM levels on precaecal flow and total tract output of P for birds fed the moderate-P diets. Chickens fed the moderate-P diets had greater precaecal flow of P, total tract P output, precaecal digested P and total tract retained P than that for birds fed the low-P diets (\( p < .01 \)). Dietary casein supplementation decreased precaecal flow of P and total tract P output, and increased precaecal digested P and total tract retained P (\( p < .01 \)).

The APD and TTR of P are presented in Table 5. The APD of P was affected by the interactive effects of dietary total P \( \times \) SBM level (\( p < .01 \)), and casein addition \( \times \) SBM level (\( p < .05 \)). With increasing inclusion of SBM in diets, the APD of P decreased (linear, \( p < .01 \)) for birds fed the moderate-P diets without casein supplementation. Linear (\( p < .01 \)) and quadratic (\( p < .05 \)) responses to graded dietary SBM level in APD of P were observed in chicks fed the moderate-P diets with casein supplementation. There were no linear or quadratic effects of SBM level on the APD of P for birds fed the low-P diets. The TTR of P was affected by the interactive effects of dietary total P \( \times \) SBM level (\( p < .05 \)). The TTR of P had a linear decrease for...
| Casein meal levels, g/kg | Without casein | With casein | Without casein | With casein | Pooled SEM |
|-------------------------|----------------|-------------|----------------|-------------|------------|
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 |
| BW gain, g/chick | 186 | 284 | 327 | 279 | 353 | 372 |
| Feed intake, g/chick | 455 | 530 | 553 | 502 | 538 | 558 |
| Gain: feed, g/kg | 409 | 535 | 592 | 557 | 655 | 667 |
| Precaecal DM digestibility, % | 80.56 | 76.29 | 72.96 | 81.08 | 77.00 | 73.21 |

**Table 3. Growth performance and DM digestibility of chicks from d 14 to 21 post-hatching.**

| Casein | Without casein | With casein | Without casein | With casein | Pooled |
|--------|----------------|-------------|----------------|-------------|--------|
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 |
| BW gain, g/chick | 186 | 284 | 327 | 279 | 353 | 372 |
| Feed intake, g/chick | 455 | 530 | 553 | 502 | 538 | 558 |
| Gain: feed, g/kg | 409 | 535 | 592 | 557 | 655 | 667 |
| Precaecal DM digestibility, % | 80.56 | 76.29 | 72.96 | 81.08 | 77.00 | 73.21 |

**p-Value**

| Casein | Without casein | With casein | Without casein | With casein | Pooled |
|--------|----------------|-------------|----------------|-------------|--------|
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 |
| BW gain, g/chick | 186 | 284 | 327 | 279 | 353 | 372 |
| Feed intake, g/chick | 455 | 530 | 553 | 502 | 538 | 558 |
| Gain: feed, g/kg | 409 | 535 | 592 | 557 | 655 | 667 |
| Precaecal DM digestibility, % | 80.56 | 76.29 | 72.96 | 81.08 | 77.00 | 73.21 |

**Moderate-P diets**

| Casein | Without casein | With casein | Without casein | With casein | Pooled |
|--------|----------------|-------------|----------------|-------------|--------|
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 |
| BW gain, g/chick | 186 | 284 | 327 | 279 | 353 | 372 |
| Feed intake, g/chick | 455 | 530 | 553 | 502 | 538 | 558 |
| Gain: feed, g/kg | 409 | 535 | 592 | 557 | 655 | 667 |
| Precaecal DM digestibility, % | 80.56 | 76.29 | 72.96 | 81.08 | 77.00 | 73.21 |

**Low-P diets**

| Casein | Without casein | With casein | Without casein | With casein | Pooled |
|--------|----------------|-------------|----------------|-------------|--------|
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 |
| BW gain, g/chick | 186 | 284 | 327 | 279 | 353 | 372 |
| Feed intake, g/chick | 455 | 530 | 553 | 502 | 538 | 558 |
| Gain: feed, g/kg | 409 | 535 | 592 | 557 | 655 | 667 |
| Precaecal DM digestibility, % | 80.56 | 76.29 | 72.96 | 81.08 | 77.00 | 73.21 |
Table 4. Dietary P intake, P output and digested P of chicks.

| Soybean meal levels, g/kg | 340 | 480 | 620 | 180 | 320 | 460 | 130 | 270 | 410 | SEM |
|---------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Dietary P intake, mg/kg   | 2026| 2742| 3756| 1098| 2004| 2941| 1105| 1940| 2751| –   |
| Precaecal P flow, mg/kg   | 490 | 680 | 1132| 313 | 499 | 758 | 206 | 442 | 645 | 33.6|
| Total tract P output, mg/kg| 667 | 948 | 1493| 360 | 675 | 998 | 259 | 599 | 853 | 38.5|
| Precaecal digested P, mg/kg| 1536| 2062| 2624| 786 | 1505| 2183| 899 | 1499| 2106| 33.6|
| Total tract retained P, mg/kg| 1359| 1794| 2263| 738 | 1329| 1949| 846 | 1342| 1898| 38.5|

| p-Value                   |       |     |     |     |     |     |     |     |     |     |
|---------------------------|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Dietary P intake, mg/kg   | .007 | .007| .007| .002| .007| .001| <.001| .007| .007| .007|
| Precaecal P flow, mg/kg   | .382 | .382| .382| .382| .382| .382| .382| .382| .382| .382|
| Total tract P output, mg/kg| .007 | .007| .007| .007| .007| .007| .007| .007| .007| .007|
| Precaecal digested P, mg/kg| .164 | .164| .164| .164| .164| .164| .164| .164| .164| .164|
| Total tract retained P, mg/kg| .365 | .365| .365| .365| .365| .365| .365| .365| .365| .365|

Moderate-P diets

| Dietary P intake, mg/kg   | –     | –     | –     | –     | –     | –     | –     | –     | –     | –     |
| Precaecal P flow, mg/kg   | .001  | .002  | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Total tract P output, mg/kg| <.001 | .006  | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Precaecal digested P, mg/kg| <.001 | .662  | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |
| Total tract retained P, mg/kg| <.001 | .717  | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |

Low-P diets

| Dietary P intake, mg/kg   | –     | –     | –     | –     | –     | –     | –     | –     | –     | –     |
| Precaecal P flow, mg/kg   | –     | –     | –     | –     | –     | –     | –     | –     | –     | –     |
| Total tract P output, mg/kg| –     | –     | –     | –     | –     | –     | –     | –     | –     | –     |
| Precaecal digested P, mg/kg| –     | –     | –     | –     | –     | –     | –     | –     | –     | –     |
| Total tract retained P, mg/kg| –     | –     | –     | –     | –     | –     | –     | –     | –     | –     |
chickens fed the moderate-P diets with graded inclusion of SBM (p < .01). For chicks fed the low-P diets with casein supplementation, there were linear and quadratic decreases in the TTR of P with graded levels of SBM (p < .05).

The values of TPD of P are shown in Table 6. The TPD of P in SBM was determined to be 62.38 and 60.95% for chicks fed the moderate-P diets without casein and with casein, respectively. For chicks fed the low-P diets, the TPD of P in SBM was estimated to be 75.78 and 73.32% for diets without casein and with casein, respectively. Broilers fed the moderate-P diets had lower estimates of TPD of P than that for birds fed the low-P diets (p < .05). There were no effects of dietary casein supplementation on the determination of TPD of P in SBM.

Discussion

The regression method has been proposed as a reliable approach to determine the true digestibility of P in assay feed ingredients (Fan et al. 2001; Shen et al. 2002; Dilger and Adeola, 2006; Liu et al. 2014). It is suggested that highly digestible feedstuffs should be included in experimental diets to improve the growth performance of chickens when the regression method was used to estimate the true P digestibility in assay feedstuffs (WPSA 2013). Therefore, the effects of dietary supplementation of highly digestible feedstuffs, such as casein and dried egg albumen, on the determination of TPD of P in test ingredients for birds should be further investigated. The present study was conducted to compare the regression-driven estimates of true P digestibility in SBM for broiler chickens fed diets with or without casein supplementation at moderate or low concentration of total P. The results demonstrated that increasing dietary P level decreased the estimates of TPD of P in SBM, while the inclusion of casein in diets had no effects on the determination of TPD of P.

In the current study, birds fed the moderate-P diets had greater feed intake, BW gain and feed efficiency than chickens fed the low-P diets during the experimental period. The increased growth performance can be attributed to the increasing consumption of protein, energy and P from SBM. In addition, an improvement in growth performance of chickens fed diets with casein supplementation compared with birds fed diets without casein addition was found. These observations agree with the results of Dilger and Adeola (2006) and Liu et al. (2014), who reported that increasing dietary levels of SBM and casein improved the growth performance of broiler chickens.
Furthermore, the precaecal digestibility of DM decreased with graded inclusion of SBM for birds fed the moderate-P diets, regardless of casein addition. This may be attributed to the replacement of highly digestible corn starch with assay feed ingredients, which has been reported in previous studies (Dilger and Adeola 2006; Iyayi et al. 2013; Liu et al. 2013). However, there were no linear or quadratic effects of SBM levels on the precaecal digestibility of DM for broilers fed the low-P diets, which is in agreement with the study of Liu et al. (2014) using experimental diets at low levels of SBM.

The moderate-P diets and low-P diets had dietary total P levels from 2.3 to 4.0 g/kg and 1.3 to 3.0 g/kg, respectively. The concentration of P in experimental diets was lower than the requirements of P for broiler chickens suggested by NRC (1994), which meet the requirement for the use of the regression method to calculate TPD of P in assay feed ingredients. With increasing dietary SBM levels, the determined values of APD of P in SBM decreased from 75.83 to 69.87% and 80.05 to 72.31% for chicks fed the moderate-P diets without and with casein inclusion, respectively. The estimates of APD of P ranged from 71.54 to 74.21% and 81.36 to 76.56% for broilers fed the low-P diets without and with casein inclusion as dietary SBM level increased, respectively. The determined values of APD of P in SBM for broilers fed diets without casein were consistent with the results of Liu et al. (2014), who reported that the values of APD of P in diets varied from 69.4 and 81.0% for birds fed casein-supplemented diets at dietary Ca:P ratio of 1.2. The estimated values of APD of P in diets were increased by dietary casein addition which is in agreement with the study of Liu et al. (2014). This observation is expected because the digestibility of P in casein for broilers is greater than that in SBM (NRC 1994).

In agreement with the study of Liu et al. (2014), dietary casein inclusion had no effects on the determined values of TPD of P in SBM for broilers. However, dietary dried egg albumen supplementation caused lower estimates of TPD of P in SBM was reported by Mutucumarana et al. (2015). The main reason for that discrepancy might be the differences in dietary Ca: P ratios between studies. In the study of Mutucumarana et al. (2015), the dried egg albumen-supplemented diets had greater dietary Ca:P ratio of 1.2 than the ratio of 0.7 in diets without dried egg albumen. In contrast, a constant dietary Ca:P ratio of 1.2 was applied in the study of Liu et al. (2014) and in the present study. Chicks fed the moderate-P diets had lower estimates of TPD of P in SBM compared with birds fed the low-P diets and were observed in the current study. The slope derived from regressing digested P and dietary P intake can be applied for the determination of TPD of P and is based on the assumption that the digestibility of P in assay feed ingredients was not affected by increasing consumption of the assay feed ingredients (Liu et al. 2014). However, the APD of P decreased with graded inclusion of assay feed ingredients was observed for broiler chickens fed experimental diets at moderate total P levels, even if there is a strong linear relationship between digested P and dietary P intake (Iyayi et al. 2013; Liu et al. 2013; Mutucumarana et al. 2014, 2015). The reduced P digestibility may be caused by excessive intake of

### Table 6. Linear relationships between precaecal digested P (mg/kg DMI) and dietary P intake (mg/kg DMI) in broilers fed diets with or without casein at different concentration of total P.

| Dietary total P and casein addition | Regression equation | SE of the slope | SE of the intercept | r² | True precaecal digestibility of P, % |
|-----------------------------------|---------------------|----------------|-------------------|----|-----------------------------------|
| **Moderate-P diets**              |                     |                |                   |    |                                   |
| Diets without casein              | Y = 0.6238X + 301.57| 0.0312         | 92.01             | 0.93| 62.38a                            |
| Diets with casein                 | Y = 0.6095X + 449.09| 0.0422         | 123.21            | 0.90| 60.95a                            |
| **Low-P diets**                   |                     |                |                   |    |                                   |
| Diets without casein              | Y = 0.7578X – 35.50 | 0.0305         | 64.10             | 0.91| 75.78b                            |
| Diets with casein                 | Y = 0.7332X + 84.79 | 0.0293         | 51.64             | 0.92| 73.32b                            |

a,bMeans not sharing a common superscript differed (p < .05).

bRegression of precaecal digested P (mg/kg of DMI) against dietary P intake (mg/kg of DMI) as determined from feeding broilers with or without casein at different concentration of total P. The linear term represents true precaecal digestibility of P.

dStandard errors of regression components (n = 24 observations).
dietary phytate P as the level of assay feed ingredients increased (Leytem et al. 2008; Selle et al. 2009; Shastak et al. 2014). Therefore, the depressed P digestibility in treatment groups containing high levels of assay feed ingredients leads to a decreased slope, which results in lower estimates of TPD of P for broilers. In the present study, the values of TPD of P in SBM were determined to be 62.38 and 60.95% for moderate-P diets without casein and with casein supplementation, which are consistent with the estimates of 55.3 and 52.3% for broiler chickens fed the moderate-P diets at a Ca:P ratio of 1.2 reported by Liu et al. (2013) and Mutucumarana et al. (2015). By contrast, these estimates of TPD of P in SBM for broilers fed the moderate-P diets were lower than the determined values of 74.4, 77.8 and 81.3% for broilers fed the low-P diets (P levels from 1.02 to 2.55 g/kg) reported by Liu et al. (2014). Based on previous data and the results of the present study, it indicates that the regression-driven estimates of TPD of P in SBM were lower for broiler chickens fed the moderate-P diets than that for birds fed the low-P diets.

Conclusions

In summary, dietary total P concentration affects the estimates of TPD of P in assay feed ingredients determined by the regression approach. The levels of total P in test diets should be maintained at a reasonable range, which allowed the APD of P was not affected by increasing levels of assay feedstuffs. The supplementation of casein at 50.0 g/kg in basal diets has no effects on the estimation of TPD of P for broiler chickens. Therefore, constant supplementation of highly digestible feedstuffs in test diets, which was proposed by WPSA (2013), should be applied to determine the TPD of P in plant feed ingredients for broiler chickens.

Disclosure statement

The authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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