Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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

Farzana Abbasi, Jingbo Liu, Hongfu Zhang, Xiaoyun Shen & Xuegang Luo

**To cite this article:** Farzana Abbasi, Jingbo Liu, Hongfu Zhang, Xiaoyun Shen & Xuegang Luo (2018) Effects of dietary total phosphorus concentration and casein supplementation on the determination of true phosphorus digestibility for broiler chickens, Italian Journal of Animal Science, 17:1, 135-144, DOI: 10.1080/1828051X.2017.1346489

**To link to this article:** https://doi.org/10.1080/1828051X.2017.1346489

ITALIAN JOURNAL OF ANIMAL SCIENCE, 2018
VOL. 17, NO. 1, 135–144
https://doi.org/10.1080/1828051X.2017.1346489

Taylor & Francis
Taylor & Francis Group

PAPER

OPEN ACCESS Check for updates

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

Farzana Abbasi[a], Jingbo Liu[a,b], Hongfu Zhang[b], Xiaoyun Shen[a,c] and Xuegang Luo[a]

[a]School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang, Sichuan, China; [b]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, Hebei, China; [c]State Engineering Technology Institute for Karst Desertification Control, Guizhou Normal University, Guiyang, Guizhou, China

**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 $2 \times 2 \times 3$ 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 form 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.

**ARTICLE HISTORY**
Received 19 April 2017
Revised 19 June 2017
Accepted 21 June 2017

**KEYWORDS**
Broiler; casein; phosphorus; soybean meal; true precaecal digestibility

## 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).

**CONTACT** Prof. Xuegang Luo luoxgswust@163.com School of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, China

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

**Table 1.** Diet formulation of experimental diets (as-fed basis, g/kg).

| Dietary total P | Moderate-P diets | | | | | | Low-P diets | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Casein | Without casein | | | With casein | | | Without casein | | | With casein | | |
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 | 180 | 320 | 460 | 130 | 270 | 410 |
| Soybean meal (44% CP) | 340.0 | 480.0 | 620.0 | 290.0 | 430.0 | 570.0 | 180.0 | 320.0 | 460.0 | 130.0 | 270.0 | 410.0 |
| Corn starch | 354.6 | 213.2 | 71.9 | 354.4 | 213.0 | 71.6 | 516.2 | 374.8 | 233.4 | 516.0 | 374.6 | 233.2 |
| Casein | 0.0 | 0.0 | 0.0 | 50.0 | 50.0 | 50.0 | 0.0 | 0.0 | 0.0 | 50.0 | 50.0 | 50.0 |
| Soybean oil | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Dextrose | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 | 250.0 |
| Salt | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Limestone | 3.9 | 5.3 | 6.6 | 4.1 | 5.5 | 6.9 | 2.3 | 3.7 | 5.1 | 2.5 | 3.9 | 5.3 |
| Chromic dioxide premix[a] | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| Mineral–vitamin premix[b] | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |

[a]Prepared as 1 g chromic dioxide mixed with 4 g of corn starch.
[b]Provided per kilogram diet: retinyl acetate, 688 mg; cholecalciferol, 7.5 μg; DL-α-tocopheryl acetate, 20 mg; menadione, 0.52 mg; thiamine, 4 mg; niacin, 15 mg; riboflavin, 4 mg; pantothenic acid, 12 mg; vitamin $B_{12}$, 15 μg; pyridoxine, 2 mg; D-biotin, 0.1 mg; folic acid, 0.5 mg; choline, 0.6 g; Fe (ferrous sulphate), 90 mg; Mn (manganese oxide), 5 mg; Cu (copper sulphate), 8 mg; I (potassium iodate), 0.20 mg; Se (sodium selenite), 0.21 mg; Zn (zinc sulphate), 90 mg.

**Table 2.** Analysed and calculated nutrient composition of experimental diets (as-fed basis).

| Dietary total P | Moderate-P diets | | | | | | Low-P diets | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Casein | Without casein | | | With casein | | | Without casein | | | With casein | | |
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 | 180 | 320 | 460 | 130 | 270 | 410 |
| Calculated nutrients composition | | | | | | | | | | | | |
| Crude protein, g/kg | 149 | 211 | 272 | 172 | 233 | 295 | 79 | 141 | 202 | 102 | 163 | 225 |
| ME, MJ/kg | 16.2 | 15.7 | 15.2 | 15.6 | 15.1 | 14.6 | 16.7 | 16.3 | 15.8 | 16.1 | 15.7 | 15.2 |
| Ca, g/kg | 2.8 | 3.8 | 4.8 | 2.8 | 3.8 | 4.8 | 1.6 | 2.6 | 3.7 | 1.6 | 2.6 | 3.7 |
| Total P, g/kg | 2.3 | 3.2 | 4.0 | 2.3 | 3.2 | 4.0 | 1.3 | 2.2 | 3.0 | 1.3 | 2.2 | 3.0 |
| Analysed nutrients composition | | | | | | | | | | | | |
| Crude protein, g/kg | 144 | 203 | 270 | 166 | 228 | 297 | 76 | 145 | 198 | 103 | 157 | 221 |
| Ca, g/kg | 2.4 | 4.3 | 4.2 | 3.0 | 3.6 | 5.3 | 1.9 | 2.9 | 3.3 | 1.5 | 2.3 | 3.9 |
| Total P, g/kg | 2.2 | 3.0 | 4.1 | 2.3 | 3.1 | 3.9 | 1.2 | 2.2 | 3.2 | 1.2 | 2.1 | 3.0 |

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:

$$\mathrm{APD},\ \% = 100 - (\mathrm{Cr_I}/\mathrm{Cr_O}) \times (\mathrm{N_O}/\mathrm{N_I}) \times 100$$

where APD is the apparent precaecal digestibility of DM or P; $Cr_I$ is the chromium concentration in diets; $Cr_O$ is the chromium concentration in precaecal digesta; $N_O$ is the DM or P concentration in precaecal digesta; and $N_I$ 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:

$$\mathrm{TTR},\ \% = 100 - (\mathrm{Cr_I}/\mathrm{Cr_O}) \times (\mathrm{P_O}/\mathrm{P_I}) \times 100$$

where TTR is the total tract retention of P; $Cr_I$ is the chromium concentration in diets; $Cr_O$ is the chromium concentration in excreta; $P_O$ is the P concentration in excreta output; and $P_I$ 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:

$$\mathrm{P_{O-DMI}},\ \mathrm{mg/kg} = \mathrm{P_{O-DMO}} \times (\mathrm{Cr_I}/\mathrm{Cr_O})$$

where $P_{O\text{-}DMI}$ and $P_{O\text{-}DMO}$ represent P flow or output concentrations (in precaecal digesta or excreta) on a DMI and DM output (DMO) basis, respectively; $Cr_I$ is the concentration of chromium in diets (mg/kg of DM); and $Cr_O$ 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:

$$P_{D-DMI},\ \mathrm{mg/kg} = P_{I-DM} - P_{O-DMI}$$

where $P_{D\text{-}DMI}$ represents precaecal digested P or total tract retained P on a DMI basis; $P_{I\text{-}DM}$ is the concentration of P in diets on a DM basis; and $P_{O\text{-}DMI}$ 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):

$$P_{D-DMI},\ \mathrm{mg/kg} = (\mathrm{TPD} \times P_{I-DM}) - \mathrm{EPL}$$

where $P_{D\text{-}DMI}$ represents precaecal digested P on a DMI basis; TPD (%) represents true precaecal digestibility of P; $P_{I\text{-}DM}$ 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 × diet type, dietary total P level × SBM level, diet type × SBM level and dietary total P level × diet type × 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 × 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 × SBM levels ($p < .01$). Furthermore, the precaecal digested P and total tract retained P were affected by the interactive effects of dietary total P × casein addition, and SBM level × 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 × SBM level ($p < .01$), and casein addition × 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 × SBM level ($p < .05$). The TTR of P had a linear decrease for

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

| Dietary total P | Moderate-P diets | | | | | | Low-P diets | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Casein | Without casein | | | With casein | | | Without casein | | | With casein | | | Pooled |
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 | 180 | 320 | 460 | 130 | 270 | 410 | SEM |
| BW gain, g/chick | 186 | 284 | 327 | 279 | 353 | 372 | 129 | 180 | 273 | 186 | 270 | 338 | 11.3 |
| Feed intake, g/chick | 455 | 530 | 553 | 502 | 538 | 558 | 388 | 447 | 521 | 438 | 499 | 525 | 15.9 |
| Gain: feed, g/kg | 409 | 535 | 592 | 557 | 655 | 667 | 325 | 401 | 523 | 423 | 539 | 643 | 15.6 |
| Precaecal DM digestibility, % | 80.56 | 76.29 | 72.96 | 81.08 | 77.00 | 73.21 | 80.58 | 79.60 | 77.68 | 82.84 | 81.63 | 79.66 | 1.13 |

| | *p*-Value | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total P | Casein | SBM level | Total P × Casein | Total P × SBM level | Casein × SBM level | Total P × Casein × SBM level |
| BW gain, g/chick | <.001 | <.001 | <.001 | .859 | .007 | .246 | .110 |
| Feed intake, g/chick | <.001 | .002 | <.001 | .377 | .252 | .119 | .508 |
| Gain: feed, g/kg | <.001 | <.001 | <.001 | .796 | <.001 | .291 | .076 |
| Precaecal DM digestibility, % | <.001 | .055 | <.001 | .229 | .016 | .982 | .987 |

| | Moderate-P diets | | | | Low-P diets | | | |
|---|---|---|---|---|---|---|---|---|
| | Without casein | | With casein | | Without casein | | With casein | |
| | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic |
| BW gain, g/chick | <.001 | .049 | <.001 | .047 | <.001 | .129 | <.001 | .572 |
| Feed intake, g/chick | .002 | .175 | .049 | .675 | <.001 | .698 | .004 | .358 |
| Gain: feed, g/kg | <.001 | .070 | .002 | .026 | <.001 | .233 | <.001 | .759 |
| Precaecal DM digestibility, % | .002 | .728 | <.001 | .917 | .084 | .731 | .066 | .786 |

**Table 4.** Dietary P intake, P output and digested P of chicks.

| Dietary total P | Moderate-P diets | | | | | | Low-P diets | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Casein | Without casein | | | With casein | | | Without casein | | | With casein | | | Pooled |
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 | 180 | 320 | 460 | 130 | 270 | 410 | SEM |
| Dietary P intake, mg/kg | 2026 | 2742 | 3756 | 2130 | 2846 | 3600 | 1098 | 2004 | 2941 | 1105 | 1940 | 2751 | – |
| Precaecal P flow, mg/kg | 490 | 680 | 1132 | 425 | 579 | 996 | 313 | 499 | 758 | 206 | 442 | 645 | 33.6 |
| Total tract P output, mg/kg | 667 | 948 | 1493 | 580 | 818 | 1284 | 360 | 675 | 998 | 259 | 599 | 853 | 38.5 |
| Precaecal digested P, mg/kg | 1536 | 2062 | 2624 | 1705 | 2267 | 2603 | 786 | 1505 | 2183 | 899 | 1499 | 2106 | 33.6 |
| Total tract retained P, mg/kg | 1359 | 1794 | 2263 | 1550 | 2028 | 2316 | 738 | 1329 | 1943 | 846 | 1342 | 1898 | 38.5 |

| | *p*-Value | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total P | Casein | SBM level | Total P × Casein | Total P × SBM level | Casein × SBM level | Total P × Casein × SBM level |
| Dietary P intake, mg/kg | – | – | – | – | – | – | – |
| Precaecal P flow, mg/kg | <.001 | <.001 | <.001 | .836 | <.001 | .587 | .640 |
| Total tract P output, mg/kg | <.001 | <.001 | <.001 | .432 | <.001 | .242 | .729 |
| Precaecal digested P, mg/kg | <.001 | .002 | <.001 | .007 | <.001 | <.001 | .164 |
| Total tract retained P, mg/kg | <.001 | <.001 | <.001 | .003 | <.001 | .018 | .365 |

| | Moderate-P diets | | | | Low-P diets | | | |
|---|---|---|---|---|---|---|---|---|
| | Without casein | | With casein | | Without casein | | With casein | |
| | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic |
| Dietary P intake, mg/kg | – | – | – | – | – | – | – | – |
| Precaecal P flow, mg/kg | <.001 | .002 | <.001 | .002 | <.001 | .382 | <.001 | .690 |
| Total tract P output, mg/kg | <.001 | .006 | <.001 | .017 | <.001 | .923 | <.001 | .364 |
| Precaecal digested P, mg/kg | <.001 | .662 | <.001 | .007 | <.001 | .612 | <.001 | .920 |
| Total tract retained P, mg/kg | <.001 | .717 | <.001 | .045 | <.001 | .816 | <.001 | .518 |

**Table 5.** Apparent precaecal digestibility and total tract retention of P.

| Dietary total P | Moderate-P diets | | | | | | Low-P diets | | | | | | Pooled |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Casein | Without casein | | | With casein | | | Without casein | | | With casein | | | |
| Soybean meal levels, g/kg | 340 | 480 | 620 | 290 | 430 | 570 | 180 | 320 | 460 | 130 | 270 | 410 | SEM |
| Apparent precaecal digestibility of P, % | 75.83 | 75.21 | 69.87 | 80.05 | 79.67 | 72.31 | 71.54 | 75.09 | 74.21 | 81.36 | 77.22 | 76.56 | 1.38 |
| Total tract retention of P, % | 67.08 | 65.43 | 60.25 | 72.76 | 71.26 | 64.33 | 67.18 | 66.33 | 66.08 | 76.55 | 69.15 | 69.01 | 1.52 |

| | *p*-Value | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total P | Casein | SBM level | Total P × Casein | Total P × SBM level | Casein × SBM level | Total P × Casein × SBM level |
| Apparent precaecal digestibility of P, % | .523 | <.001 | <.001 | .505 | .005 | .046 | .116 |
| Total tract retention of P, % | .015 | <.001 | <.001 | .929 | .029 | .151 | .282 |

| | Moderate-P diets | | | | Low-P diets | | | |
|---|---|---|---|---|---|---|---|---|
| | Without casein | | With casein | | Without casein | | With casein | |
| | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic | Linear | Quadratic |
| Apparent precaecal digestibility of P, % | .001 | .167 | <.001 | .042 | .597 | .194 | .109 | .308 |
| Total tract retention of P, % | .002 | .352 | .001 | .154 | .719 | .877 | .044 | .048 |

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.

**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[c] | SE of the slope[d] | SE of the intercept[d] | $r^d$ | True precaecal digestibility of P, % |
|---|---|---|---|---|---|
| Moderate-P diets | | | | | |
| Diets without casein | Y = 0.6238X + 301.57 | 0.0312 | 92.01 | 0.93 | 62.38[a] |
| Diets with casein | Y = 0.6095X + 449.09 | 0.0422 | 123.21 | 0.90 | 60.95[a] |
| Low-P diets | | | | | |
| Diets without casein | Y = 0.7578X − 35.50 | 0.0305 | 64.10 | 0.91 | 75.78[b] |
| Diets with casein | Y = 0.7332X + 84.79 | 0.0293 | 51.64 | 0.92 | 73.32[b] |

[a–b]Means not sharing a common superscript differed ($p < .05$).
[c]Regression 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.
[d]Standard errors of regression components ($n = 24$ observations).

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 estimates of APD of P varied from 71.2 to 77.8%. These values were lower than the estimates from 76.1 to 80.7% and 71.2 to 88.8% reported by Mutucumarana et al. (2015) and Dilger and Adeola (2006), respectively. The differences may be due to a constant dietary Ca:P ratio of 1.2 used in the study of Liu et al. (2014) and the present study is greater than the Ca:P ratios of 0.7 and 0.8 used in studies of Mutucumarana et al. (2015) and Dilger and Adeola (2006), respectively. This is because of the P digestibility in SBM decreased with increasing dietary Ca: P ratios (Liu et al. 2013). Furthermore, the estimated values of APD of P in diets for birds fed casein-supplemented diets were consistent with the results of Liu et al. (2013, 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 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.

## Funding

This work was supported by grants from the National Key Research and Development Program of China (2016YFD0500505), the State Key Research and Development Program in 13th Five-Year Plan (2016YFC0502601) and China Agriculture Research System (CARS-40-30).

## References

AOAC. 2006. Official methods of analysis. 18th ed. Gaithersburg (MD): Association of Official Analytical Chemists International.

Dilger RN, Adeola O. 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.

Fan MZ, Archbold T, Sauer WC, Lackeyram D, Rideout T, Gao Y, Delange CF, Hacker RR. 2001. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. J Nutr. 131: 2388–2396.

Iyayi EA, Fru-Nji F, Adeola O. 2013. True phosphorus digestibility of black-eyed pea and peanut flour without or with phytase supplementation in broiler chickens. Poult Sci. 92:1595–1603.

Leytem AB, Willing BP, Thacker PA. 2008. Phytate utilization and phosphorus excretion by broiler chickens fed diets containing cereal grains varying in phytate and phytase content. Anim Feed Sci Tech. 146:160–168.

Liu JB, Chen DW, Adeola O. 2013. Phosphorus digestibility response of broiler chickens to dietary calcium-to-phosphorus ratios. Poult Sci. 92:1572–1578.

Liu JB, Chen DW, Adeola O. 2014. Casein supplementation does not affect true phosphorus digestibility and endogenous phosphorus loss associated with soybean meal for broiler chickens determined by the regression method. Can J Anim Sci. 94:661–668.

Liu JB, Adeola O. 2016. 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. Asian-Australas J Anim Sci. 29:1625–1631.

Mutucumarana R, Ravindran V, Ravindran G, Cowieson A. 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 RK, Ravindran V, Ravindran G, Cowieson AJ. 2015. 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.

National Research Council (NRC). 1994. Nutrient requirements of poultry. 9th rev. ed. Washington (DC): National Academy Press.

Rodehutscord M. 2009. Approaches and challenges for evaluating phosphorus sources for poultry. Paper presented at the Proc. 17th European Symposium on Poultry Nutrition, WPSA UK branch, Edinburgh, Scotland; p. 2–6.

Selle PH, Cowieson AJ, Ravindran V. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest Sci. 124:126–141.

Shen Y, Fan MZ, Ajakaiye A, Archbold T. 2002. Use of the regression analysis technique to determine the true phosphorus digestibility and the endogenous phosphorus output associated with corn in growing pigs. J Nutr. 132: 1199–1206.

Shastak Y, Zeller E, Witzig M, Schollenberger M, Rodehutscord M. 2014. Effects of the composition of the basal diet on the evaluation of mineral phosphorus sources and interactions with phytate hydrolysis in broilers. Poult Sci. 93:2548–2559.

Xue PC, Ajuwon KM, Adeola O. 2016. Phosphorus and nitrogen utilization responses of broiler chickens to dietary crude protein and phosphorus levels. Poult Sci. 95:2615–2623.

Weremko D, Fandrejewski H, Zebrowska T, Han IK, Kim J, Cho W. 1997. Bioavailability of phosphorus in feeds of plant origin for pigs. Asian-Aus J Anim Sci. 10:551–566.

World Poultry Science Association (WPSA). 2013. Determination of phosphorus availability in poultry. Working group No. 2 (Nutrition) of the European Federation of Branches of WPSA. World's Poult Sci J. 69:687–698.