

## Measurement of total tract retention of phosphorus in several feedstuffs of plant origin for broiler chickens: effect of microbial phytase

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### ABSTRACT

An experiment was conducted to compare the true total tract retention of phosphorus (P) in soybean meal, canola meal, cottonseeds meal, peanut meal, corn distiller's dried grains with soluble (DDGS), wheat bran, wheat grain, and wheat shorts with or without phytase addition. A total of 576 male Arbor Acres broilers aged 23 days of similar live weight were randomly assigned into 16 treatments with 6 replicates each and 6 birds in each replicate. Sixteen diets were designed by combining 2 factors that include 8 ingredients and phytase level (0 or 1000 FTU/kg). Dietary P was only provided by the ingredient to be tested. A purified P-free diet was prepared to determine endogenous P loss (EPL). The retainability of P was evaluated by collecting an excreta trial. The results showed that basal EPL was 97.45 mg/DMI. The true retainability of P in soybean meal, canola meal, cottonseed meal, and DDGS was 53.90%, 53.22%, 54.73%, and 49.45%, respectively, much higher than peanut meal (42.76%), wheat bran (38.26%), wheat grain (36.77%), and wheat shorts (36.95%). Phytase significantly increased apparent or true retainability of P ( $p < .05$ ). Phytase showed the greatest improvement of retainability of P for wheat ingredients (wheat shorts 41.35%; wheat grain 41.34%; wheat bran 35.94%) and soybean meal (31.52%), followed by canola meal (23.07%), cottonseed meal (14.43%), peanut meal (11.51%), and DDGS (10.23%). The retainable P equivalent of 1000 FTUs of microbial phytase corresponding to ingredients was between 0.4 g (DDGS and peanut meal) and 1.3 g (wheat bran).

### HIGHLIGHTS

- Phytase significantly increased apparent or true retainability of P in feed ingredients of plant origin.
- Phytase showed the greatest improvement of retainability of P for wheat ingredients, more than 30%.
- The retainable P equivalent of 1000 FTUs of microbial phytase corresponding to different ingredients was between 0.4 and 1.3 g.

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## Introduction

Phosphorus (P) is an essential nutrient for animals. Most of the P in an animal's body is stored in bones in the form of phosphate. A small part of P is distributed in cells and body fluids and plays crucial roles in cellular metabolism, the integrity of cellular membranes, and electrolyte balance (Robert et al. 2018). Lack of P could retard the development of bone, induce joint deformity, reduce appetite, and restrain the growth of growing animals. In general, feed is the sole source of P for animals, and commercial feed

could provide adequate or even excessive amounts of P to meet animals' requirements. However, in recent decades, the public pays more attention to the remarkable negative impact of excessive dietary P on the environment, and the cost of inorganic P resources increased continuously for its non-renewal. Thus, in recent years there is a growing concern on efficient and precise utilisation of P from plant ingredients. Here, assessment of utilisation efficiency of P in feedstuffs is the basis of the whole strategy for optimising dietary P nutrition of animals.

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Most of P in plant feedstuffs exist in the form of phytate P (Cheng 2001), and non-ruminant animals lack enough endogenous phytase activity so that organic P in the feedstuffs was poorly utilised (Dayyani et al. 2011). Besides, depending on the plant's endogenous phytase activity and the feed technological process, the bioavailability of P in plant ingredients is highly variable (Tran and Sauvant 2004). In addition, P bioavailability can also be affected by dietary factors (Leytem et al. 2008; Li et al. 2021). Therefore, it is necessary to regularly assess the bioavailability of P in feed ingredients of plant origin.

Current methods to evaluate the bioavailability of plant feed ingredients *in vivo* include the indirect method, which determinates relative bioactivity of ingredients to the standard inorganic P source by growth response or P deposition in bone, and the direct method, which assesses the bioavailability (metabolizability) of P in feed ingredients by digestion experiments. The former will obtain the relative bioavailability based on the growth rate, biochemical markers, and mineralisation of bone in target animals; however, feeding animals is relatively time- and labor-consuming, and it is generally difficult to evaluate multiple samples in a single trial. The latter can provide information on P retainability in feed ingredients based on collecting excreta samples, which is simple and can be performed to assess multiple samples at one time, and thus has been the most frequently used method. By a classical digestion experiment, we can assay the apparent digestibility for swine or the retainability for poultry. Considering that endogenous P loss (Fan et al. 2001) has an inevitable influence on apparent digestibility (Almeida and Stein 2010; Rojas and Stein 2012; Babatunde and Adeola 2021) and apparent retainability (Dilger and Adeola 2006a; Liu et al. 2012), the data on apparent digestibility and retainability could be corrected into true digestibility and true retainability by determining and deducting endogenous loss of P. Several reports have shown that true digestibility or retainability is more reliable and

additive because the influence of dietary factors is eliminated by removing the interference from endogenous loss of P (Babatunde and Adeola 2021).

Phytase is a new route to improve the bio-availability of P in animal feed (Engelen et al. 2001; Butani and Parnerkar 2015). To exactly understand the nutritional value of phytase, researchers tried investigating the equivalent relationship between phytase and inorganic P. Nevertheless, most of these results were from experiments based on complete diets, which omitted the difference among various plant ingredients. In fact, there are distinct variations in the responses of various feed ingredients to phytase addition. Unfortunately, studies comparing the effect of phytase on utilisation of P in different single feed ingredients are limited. This study aimed to evaluate the retainability of P in various feed ingredients for broiler chickens and investigate the effect of microbial phytase addition on utilisation of P in these single ingredients and enrich the feed database of retainability of P for broiler chickens and precisely understand the nutritional value of microbial phytase.

## Materials and methods

### Test feedstuffs and experimental diets

The test feedstuffs of soybean meal (SBM), canola meal (CNM), cottonseed meal (CSM), peanut meal (PM), distillers dried grains with solubles from corn (corn DDGS), wheat bran (WB), wheat grain (WG), and wheat shorts (WS) were purchased from a feedstuff company in China. The chemical components of these feedstuffs were analysed and shown in Table 1. Eight isocaloric and isonitrogenous semi-purified diets were formulated using the eight test ingredients, corn starch, sucrose, soybean oil, and crystal amino acids, premix, and binder, which contain 12.75 MJ/kg metabolisable energy, 18% crude protein, 1.05% lysine, 0.45% methionine, 0.72% threonine, and 0.20 tryptophan.

**Table 1.** Chemical component of feed ingredients under test (as air-dried basis).

|                                | Soybean meal | Canola meal | Cottonseed meal | Peanut meal | Corn DDGS | Wheat bran | Wheat grain | Wheat-shorts |
|--------------------------------|--------------|-------------|-----------------|-------------|-----------|------------|-------------|--------------|
| DM, %                          | 91.42        | 89.68       | 92.58           | 92.01       | 90.74     | 91.56      | 89.69       | 89.57        |
| CP, %                          | 44.31        | 35.71       | 39.64           | 47.43       | 17.06     | 16.60      | 18.60       | 17.16        |
| Ca, %                          | 0.33         | 0.65        | 0.28            | 0.27        | 0.19      | 0.16       | 0.09        | 0.13         |
| Total P, %                     | 0.59         | 1.00        | 0.98            | 0.76        | 0.75      | 0.91       | 0.42        | 0.56         |
| Phytate, %                     | 1.30         | 2.60        | 2.30            | 2.20        | 0.18      | 2.55       | 1.03        | 1.31         |
| Phytate P <sup>a</sup> , %     | 0.37         | 0.73        | 0.65            | 0.62        | 0.05      | 0.72       | 0.29        | 0.37         |
| Phytate P of total P, %        | 62.80        | 73.00       | 66.33           | 81.58       | 6.67      | 79.12      | 69.05       | 66.07        |
| Non-phytate P <sup>b</sup> , % | 0.22         | 0.27        | 0.33            | 0.14        | 0.70      | 0.19       | 0.13        | 0.19         |
| Non-phytate P of total P, %    | 37.29        | 27.00       | 33.67           | 18.42       | 93.33     | 20.90      | 30.95       | 33.93        |

<sup>a</sup>Phytate P was calculated as 28.2% of phytate (Tran and Sauvant 2004); <sup>b</sup>Non-phytate P was calculated as the difference between total P and phytate. Abbreviations. DM, dry matter; DDGS, distiller's dried grains with soluble; CP, crude protein.

**Table 2.** Composition of the semi-purified experimental diets (as air-dried basis).

| Ingredient, %       | SBM   | CNM   | CSM   | PM    | Corn DDGS | WB    | WG    | WS    |
|---------------------|-------|-------|-------|-------|-----------|-------|-------|-------|
| Test ingredients    | 40.00 | 42.99 | 40.00 | 35.00 | 56.00     | 65.00 | 85.00 | 85.00 |
| Corn starch         | 22.00 | 12.10 | 19.80 | 30.00 | 30.20     | 16.04 | 3.12  | 2.56  |
| Sucrose             | 29.00 | 33.00 | 27.11 | 25.00 | 5.00      | 5.00  |       |       |
| Soybean oil         | 3.00  | 5.00  | 5.00  | 1.50  | 3.00      | 3.00  | 2.00  | 3.00  |
| Table salt          | 0.30  | 0.30  | 0.30  | 0.30  | 0.30      | 0.30  | 0.30  | 0.30  |
| Limestone           | 2.05  | 1.51  | 2.3   | 2.37  | 2.25      | 2.37  | 2.16  | 2.38  |
| L-lysine            |       | 0.63  | 0.56  | 0.71  | 0.91      | 0.85  | 0.97  | 0.75  |
| DL-methionine       | 0.20  | 0.19  | 0.28  | 0.31  | 0.12      | 0.37  | 0.27  | 0.27  |
| L-cysteine          | 0.09  |       | 0.04  | 0.22  | 0.13      | 0.18  | 0.12  | 0.10  |
| L-threonine         |       | 0.04  | 0.18  | 0.28  | 0.17      | 0.41  | 0.41  | 0.25  |
| L-tryptophan        |       | 0.02  | 0.03  | 0.04  | 0.1       | 0.07  | 0.07  | 0.03  |
| L-arginine          |       | 0.14  |       |       | 0.35      | 0.28  | 0.41  | 0.18  |
| L-glutamine         | 0.08  | 1.35  | 1.00  | 0.04  | 0.07      | 4.73  | 3.77  | 3.78  |
| Choline chloride    | 0.20  | 0.20  | 0.20  | 0.20  | 0.20      | 0.20  | 0.20  | 0.20  |
| Premix <sup>a</sup> | 0.13  | 0.13  | 0.13  | 0.13  | 0.13      | 0.13  | 0.13  | 0.13  |
| Feed binder         | 1.88  | 1.33  | 2.00  | 2.83  |           |       |       |       |
| SiO <sub>2</sub>    | 1.07  | 1.07  | 1.07  | 1.07  | 1.07      | 1.07  | 1.07  | 1.07  |
| Total               | 100   | 100   | 100   | 100   | 100       | 100   | 100   | 100   |

Abbreviation. SBM, soybean meal; CNM, canola meal; CSM, cottonseed meal; PM, Peanut meal; Corn DDGS, corn distiller's dried grains with solubles; WB, wheat bran; WG, wheat grain; WS, wheat shorts

<sup>a</sup>Provided the following nutrients (per kg of diet): vitamin A, 10,000 U; vitamin D<sub>3</sub>, 2000 U; vitamin E, 10 U; vitamin K<sub>3</sub>, 2.5 mg; vitamin B<sub>1</sub>, 1.8 mg; vitamin B<sub>2</sub>, 40 mg; vitamin B<sub>6</sub>, 5.0 mg; vitamin B<sub>12</sub>, 0.71 mg; biotin, 0.12 mg; folic acid, 0.5 mg; nicotinic acid, 50 mg, D-pantothenic acid, 11 mg; Cu (copper sulphate), 8 mg; Fe (ferrous sulphate), 80 mg; manganese sulphate, 60 mg; Zn (zinc sulphate), 40 mg; I (potassium iodide), 0.35 mg; Se (sodium selenite), 0.15 mg

Sixteen experimental diets were prepared by eight semi-purified diets with or without microbial phytase. For each experimental diet, the test ingredient was the sole source of P, and CaCO<sub>3</sub> was used to adjusting the ratio of Ca/P (1.3:1) same.

The composition of experimental diets is presented in Table 2. Approximately 1000 FTU/kg microbial phytase were added to the eight experimental diets. Microbial phytase was produced by an *Escherichia coli* production system (Challenge Bio-Tec Company, Beijing, China). Each unit of phytase is defined as the quantity of enzyme required to release 1 µmol of inorganic P/min from 0.00015 mol/L sodium phytate at pH 5.5 and 37 °C (Rutherford et al. 2004). Before the feeding trial, key nutrient levels and phytase activity of all experimental diets were determined (Table 3). To determinate endogenous loss of P, a purified P-free diet was prepared using corn starch (69.60%), glucose (9.64%), soybean oil (4.00%), table salt (0.30%), limestone (0.98%), cellulose (2.88%), lysine hydrochloride (0.96%), DL-methionine (0.53%), threonine (0.32%), tryptophan (0.17%), arginine (0.58%), glutamine (2.70%), histidine (0.16%), leucine (0.87%), isoleucine (0.44%), phenylalanine (0.34%), tyrosine (0.30%), valine (0.42%), glycine (0.52%), serine (0.52%), asparagine (0.96%), proline (1.91%), feed binder (0.40%), and premix (0.50%). The composition of premix was the same as the experimental diets. Silicon dioxide (SiO<sub>2</sub>) was used as an indicator (acid-insoluble ash) at 1.07% of

all diets. All diets were pelleted (size: 2 mm × 3 mm) before feeding.

### Animals, housing management and experimental design

A total of 1000 1-day-old male Arbour Acres broiler chickens were obtained from Beijing Arbour Acres Poultry Breeding, Ltd., and raised in heated, thermostatically controlled, stainless cages coated with plastic (100 × 50 × 45 cm) and equipped with a feeder and drinking nipple. During the preparation period (1- to 23-day-old), the birds were fed a commercial broiler starter diet containing 12.48 MJ/kg of ME, 20.45% of CP, 1.0% of Ca, 0.45% of non-phytate P, and abundant vitamins and minerals. The room was maintained on a 23:1 h light:darkness schedule. The birds had access to tap water from a low-pressure drinking nipple *ad libitum*.

A total of 576 male chickens aged 23 days of similar body weight (948 ± 9 g) were selected and allotted to 16 dietary treatments using a factorial experiment design with two factors (8 ingredients & phytase addition level: 0 or 1000 FTU/kg). Each treatment had six replicates (cages) with 6 birds per cage. After 4 days of adaptation period (24- to 27-day-old) to corresponding semi-purified diet, their excreta were daily collected for 4 days (28- to 31-day-old) from the tray underneath the cage.

At the same time, six other male chickens (BW: 942 ± 11 g) were selected, and a plastic cup was fixed around their anus to determine endogenous P loss (EPL). After an adaptation period of 4 days, the chickens were fasted for 12 h to evacuate the digestive tract content following the procedure reported by Liu et al. (2012), with slight modification in fasting hours. Then, each chicken was force-fed 30 g of purified P-free diet, and its excreta were collected for 18 h by the plastic bags attached to cloaca. The excreta in the bags were collected per 3 h. The collected excreta samples from a cage were pooled and placed in a Ziploc plastic bag and stored immediately at -20 °C for analysis.

### Chemical analysis

For chemical analysis, after air-drying in a 65 °C air oven, samples of ingredients, diets, and excreta were ground through a 0.45-mm sieve using a grinding mill. Samples of feed (triplicate) and excreta (duplicate) were analysed for dry matter (DM, AOAC 2007, 930.15), crude protein (CP, Dumatherm, Gerhardt Co,

Germany). Following the ashing process at 550 °C for 6 h in a muffle furnace, samples of diets, feedstuffs, and excreta were extracted with 4 N HCl solution, and P concentrations were determined by spectrometry (AOAC 2007, 965.17). The content of Ca of the samples was determined by flame spectrometry (Model novAA® 400 P, Analytikjena, Germany). The content of phytate-P was analysed based on the procedure of Akinmusire and Adeola (2009). The content of phytate in the test ingredients was calculated as 28.2% of the phytic acid concentration (Tran and Sauvant 2004). The non-phytate P concentration was calculated by the difference between total P and phytate-P. Acid-insoluble ash (AIA) of samples of the diets and excreta was analysed following Keulen and Young (1977). The activity of phytase was determined following Engelen et al. (2001). One phytase unit (FTU) represents the amount of enzyme needed to release 1 µmol of inorganic phosphate/min from 5.0 mmol/L sodium phytate at pH 5.5 and 37 °C.

### Calculations

The apparent retainability of P was estimated based on the indicator marker method equation of Dilger and Adeola (2006a):

$$\begin{aligned} \text{Apparentretainability} &= 100 - (P_{\text{Excreta}}/P_{\text{feed}}) \\ &\times (\text{Marker}_{\text{feed}}/\text{Marker}_{\text{excreta}}) \\ &\times 100, \end{aligned} \quad (1)$$

where  $P_{\text{excreta}}$  is the content of P in excreta;  $P_{\text{feed}}$  is the content of P in the diet;  $\text{Marker}_{\text{feed}}$  is the content of acid-insoluble ash (AIA) in the diet, and  $\text{Marker}_{\text{excreta}}$  is the content of AIA in the excreta.

The endogenous P loss (EPL) was calculated based on the formula of Almeida and Stein (2010):

$$\text{EPL (mg/kgDMI)} = (P_{\text{excreta}}/F_{\text{intake}}) \times 1000 \times 1000, \quad (2)$$

where  $F_{\text{intake}}$  is the average daily dry matter intake of P-free diet (mg of DMI) during the excreta collection

period.  $P_{\text{excreta}}$  is the content of P in the excreta that originated from the birds during the excreta collection period based on dry matter.

True retainability of P was obtained by correcting apparent retainability with EPL value as the formula provided by Fan et al. (2001):

$$\text{Trueretainability} = \text{Apparentretainability} + (\text{EPL}/P_{\text{feed}}), \quad (3)$$

where EPL is endogenous P loss of P, and  $P_{\text{feed}}$  is the content of P in the diet.

### Statistical analysis

The normality of the data was analysed and confirmed using the Shapiro-Wilk test in SAS (SAS Institute 2004). Outliers were tested using the UNIVARIATE procedure in SAS, but no outliers were observed. All data were analysed by two-way ANOVA in SAS. Multiple comparisons among means were conducted using Tukey's test. The  $\alpha$ -level of 0.05 was used to determine significance among means.

## Results

### Chemical components of ingredients

The composition of the test diets is presented in Table 1. DDGS showed the highest percentage of non-phytate P (NPP) in total P (93.33%), and PM had the lowest NPP percentage (18.42%). Others excreta had about 30.6% of NPP in total P, which ranged from 20.90% of WB to 37.29% of SBM. We determined the phytase activity of test diets (Table 3) and calculated the phytase activity of the ingredients as follows: 3731 for CSM, 2223 for WB, 594 for WG, and 1847 for WS. No phytase activity was detected for SBM, CNM, PM, and Corn DDGS.

### Retainability of P in feedstuffs of broiler chickens

All birds remained healthy during the entire experimental period. The endogenous phosphorus loss (EPL)

**Table 3.** Analysed composition of the semi-purified experimental diets (as air-dried basis).

| Diet<br>Phytase <sup>a</sup> | SBM   |       | CNM   |       | CSM   |       | PM    |       | DDGS  |       | WB    |       | WG    |       | WS    |       |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                              | –     | +     | –     | +     | –     | +     | –     | +     | –     | +     | –     | +     | –     | +     | –     | +     |
| DM, %                        | 93.97 | 93.84 | 94.04 | 94.17 | 94.60 | 94.75 | 93.34 | 93.58 | 92.51 | 92.18 | 92.43 | 92.54 | 90.82 | 91.07 | 90.22 | 90.82 |
| Ca, %                        | 0.31  | 0.32  | 0.56  | 0.58  | 0.51  | 0.53  | 0.36  | 0.35  | 0.50  | 0.49  | 0.63  | 0.62  | 0.41  | 0.40  | 0.48  | 0.49  |
| P, %                         | 0.24  | 0.25  | 0.43  | 0.45  | 0.40  | 0.41  | 0.28  | 0.27  | 0.38  | 0.36  | 0.48  | 0.49  | 0.31  | 0.31  | 0.36  | 0.38  |
| Ca:P                         | 1.29  | 1.28  | 1.30  | 1.29  | 1.28  | 1.29  | 1.29  | 1.30  | 1.32  | 1.36  | 1.31  | 1.27  | 1.32  | 1.29  | 1.32  | 1.29  |
| Phytase, FTU/kg              | 0     | 1426  | 0     | 1041  | 1604  | 2507  | 0     | 1335  | 0     | 935   | 1445  | 2515  | 505   | 1480  | 1570  | 2625  |

Abbreviations. SBM, soybean meal; CNM, canola meal; CSM, cottonseed meal; PM, Peanut meal; Corn DDGS, corn distiller's dried grains with solubles; WB, wheat bran; WG, wheat grain; WS, wheat shorts; DM, dry matter.

<sup>a</sup>Microbial phytase addition (–: no addition; +: added 1000 FTU/kg diet).

**Table 4.** Endogenous phosphorus losses (EPL) in excreta calculated by P-free diet method.

| Parameter   | Intake of P <sup>a</sup> , g | Excrete of P, g | Excrete of P, mg/kg DMI | EPL, mg/kg DMI |
|-------------|------------------------------|-----------------|-------------------------|----------------|
| P-free diet | 0                            | 0.087 ± 0.02    | 97.45 ± 24.88           | 97.45 ± 24.88  |

Abbreviation. DMI, dry matter intake.

Values are mean ± SD (standard deviation).

<sup>a</sup>Intake of P from P-free diet was ignored because of the extremely low levels (only 1.5 mg).

**Table 5.** Apparent retainability and true retainability of phosphorus in feedstuffs of plant origin with or without phytase addition for 28–31 day old broiler chickens.

| Ingredient           | Phytase <sup>1</sup> | Apparent retainability of P | Increase rate <sup>2</sup> | True retainability of P <sup>3</sup> | Increase rate <sup>2</sup> |
|----------------------|----------------------|-----------------------------|----------------------------|--------------------------------------|----------------------------|
| Soybean meal         | –                    | 49.84 <sup>bc</sup>         |                            | 53.90 <sup>d</sup>                   |                            |
|                      | +                    | 66.99 <sup>e</sup>          | 34.41                      | 70.89 <sup>f</sup>                   | 31.52                      |
| Canola meal          | –                    | 50.95 <sup>bc</sup>         |                            | 53.22 <sup>d</sup>                   |                            |
|                      | +                    | 63.33 <sup>d</sup>          | 24.30                      | 65.50 <sup>e</sup>                   | 23.07                      |
| Cottonseed meal      | –                    | 52.30 <sup>c</sup>          |                            | 54.73 <sup>d</sup>                   |                            |
|                      | +                    | 60.31 <sup>d</sup>          | 15.32                      | 62.63 <sup>e</sup>                   | 14.43                      |
| Peanut meal          | –                    | 39.28 <sup>ab</sup>         |                            | 42.76 <sup>b</sup>                   |                            |
|                      | +                    | 44.07 <sup>b</sup>          | 12.19                      | 47.68 <sup>c</sup>                   | 11.51                      |
| Corn DDGS            | –                    | 46.91 <sup>b</sup>          |                            | 49.45 <sup>cd</sup>                  |                            |
|                      | +                    | 51.84 <sup>c</sup>          | 10.51                      | 54.51 <sup>d</sup>                   | 10.23                      |
| Wheat bran           | –                    | 36.24 <sup>a</sup>          |                            | 38.26 <sup>ab</sup>                  |                            |
|                      | +                    | 50.04 <sup>bc</sup>         | 38.08                      | 52.01 <sup>d</sup>                   | 35.94                      |
| Wheat grain          | –                    | 33.65 <sup>a</sup>          |                            | 36.77 <sup>a</sup>                   |                            |
|                      | +                    | 48.82 <sup>bc</sup>         | 45.08                      | 51.97 <sup>cd</sup>                  | 41.34                      |
| Wheat shorts         | –                    | 34.27 <sup>a</sup>          |                            | 36.95 <sup>a</sup>                   |                            |
|                      | +                    | 49.69 <sup>bc</sup>         | 45.00                      | 52.23 <sup>d</sup>                   | 41.35                      |
| Pooled SEM           | –                    | 1.17                        |                            | 1.17                                 |                            |
|                      | +                    | 1.16                        |                            | 1.16                                 |                            |
| <i>p</i> -Value      | –                    | ***                         | ***                        | ***                                  | ***                        |
|                      | +                    | ***                         | ***                        | ***                                  | ***                        |
| Probability of ANOVA |                      |                             |                            |                                      |                            |
| Ingredient           |                      | ***                         | ***                        | ***                                  | ***                        |
| Phytase              |                      | ***                         | ns                         | ***                                  | ns                         |
| Ingredient*phytase   |                      | ***                         | *                          | ***                                  | *                          |

Values represent means of 6 replicates with 6 chicks per each cage.

Abbreviations. SEM, standard error of the mean; P, phosphorus; ns, not significant, DDGS, distiller's dried grains with soluble.

\* $p < .05$ ; \*\*\* $p < .001$ .

<sup>1</sup>Microbial phytase was added in the diets containing phytase at 1000 U/kg complete feed (–, no phytase; +, with 1000 U/kg microbial phytase); <sup>2</sup>The increase rate shows the relative magnitude of the improvement; <sup>3</sup>values for true retainability of P were calculated by correcting values of apparent retainability of P with average EPL value of 97.45 (mg/kg DMI), dry matter intake (DMI) determined using P-free diet ( $n = 6$ ).

<sup>a,b,c</sup>Super scripts with different letters in a column showed significant ( $p < .05$ ) difference.

was calculated as 97.45 mg/kg DMI (Table 4). Table 5 shows that the apparent and true retainability of P significantly varied depending on ingredients ( $p < 0.001$ ). CSM, CNM, SBM, and corn DDGS excreta had higher retainability of P, which ranged from 46.91% to 52.30% for apparent retainability and from 49.45% to 54.73% for true retainability. The other four ingredients had excreta lower values, which are lower than 40% for apparent retainability and ranged from 36.77% to 42.76% for true retainability.

The addition of 1000 FTU/kg microbial phytase indicated a significant improvement in P retainability for all feed ingredients ( $p < .01$ ). Phytase showed the most obvious relative improvement of apparent retainability in WG, WS, WB, and SBM, with an increase of 45.08%, 45.00%, 38.08%, and 34.41%, respectively. CNM, cottonseed meal, and peanut meal followed, up to 24.30%, 15.32%, and 12.19%, respectively. The least improvement was recorded as 10.51% in corn DDGS. The same trend was observed for true P retainability.

Absolute improvement in apparent P retainability expressed by percentage points from high to low was as follows: SBM (17.15), WS (15.42), WG (15.17), WB (13.80), CNM (12.38), CSM (8.01), corn DDGS (4.93), and PM (4.79). We also assessed the correlation between phytase and the retainable P (Table 6). The equivalent value of 1000 FTUs/kg phytase varied with an ingredient from 0.13% of wheat bran to 0.04% of corn DDGS and peanut meal.

## Discussion

The content of calcium, P, and relative chemical components of most feed ingredients measured in this study was mostly consistent with or close to that reported in the literature (Eeckhout and De Paepe 1994; NRC 1994; She et al. 2015). The non-phytate P content in SBM, CNM, CSM, PM, WG, and WB was comparable to the reported value of NRC (1994), while WS and corn DDGS had higher values. There was no

**Table 6.** The equivalency of phytase on apparent retainable phosphorus (%) and true retainable phosphorus (%) in feedstuffs of plant origin with or without phytase addition for 28–31 day old broiler chickens<sup>a</sup>.

| Ingredient      | Phytase <sup>b</sup> | Apparent retainable P | Equivalency of phytase <sup>c</sup> | True retainable P | Equivalency of phytase |
|-----------------|----------------------|-----------------------|-------------------------------------|-------------------|------------------------|
| Soybean meal    | –                    | 0.29                  | 0.10                                | 0.32              | 0.10                   |
|                 | +                    | 0.40                  |                                     | 0.42              |                        |
| Canola meal     | –                    | 0.51                  | 0.12                                | 0.53              | 0.12                   |
|                 | +                    | 0.63                  |                                     | 0.66              |                        |
| Cottonseed meal | –                    | 0.51                  | 0.08                                | 0.54              | 0.08                   |
|                 | +                    | 0.59                  |                                     | 0.61              |                        |
| Peanut meal     | –                    | 0.30                  | 0.04                                | 0.33              | 0.04                   |
|                 | +                    | 0.33                  |                                     | 0.36              |                        |
| Corn DDGS       | –                    | 0.35                  | 0.04                                | 0.37              | 0.04                   |
|                 | +                    | 0.39                  |                                     | 0.41              |                        |
| Wheat bran      | –                    | 0.33                  | 0.13                                | 0.35              | 0.13                   |
|                 | +                    | 0.46                  |                                     | 0.47              |                        |
| Wheat grain     | –                    | 0.14                  | 0.06                                | 0.16              | 0.06                   |
|                 | +                    | 0.21                  |                                     | 0.22              |                        |
| Wheat shorts    | –                    | 0.19                  | 0.09                                | 0.21              | 0.09                   |
|                 | +                    | 0.28                  |                                     | 0.29              |                        |

Abbreviation. DDGS, dry distillers grain soluble.

<sup>a</sup>Retainable P is calculated by retainability and total P in ingredients; <sup>b</sup>Microbial phytase was added in the diets containing phytase at 1000 U/kg complete feed (–, no phytase; +, with 1000 U/kg microbial phytase); <sup>c</sup>Equivalency of phytase shows increase of retainable P on account of 1000 FTU/kg phytase addition

detected phytase activity in SBM, CNM, and PM, similar to a previous report (Eeckhout and De Paepe 1994), which ranged the phytase activity from 0 to 120 (SBM), 0 to 36 (CNM), and 0 to 8 FTU/kg (PM). The phytase activity was also not detected in DDGS also, which was inconsistent with the report from Mutucumarana et al. (2014b). They measured the phytase activity in DDGS was 666 FTU/kg. In general, the absence of phytase activity in these feed ingredients is related to the inactivating effect of heating and some chemical reagents during their processing.

The three kinds of wheat-based ingredients and CSM show high phytase activity. Here, the phytase activity of wheat-derived ingredients was in agreement with previously published data (WB, Eeckhout and De Paepe 1994; WG, Selle et al. 2003). However, some reports showed higher values for WG and WB (Viveros et al. 2000). Interestingly, the observed 3731 FTU/kg phytase activity for CSM was markedly higher than other reports (5–80 FTU/kg; Selle et al. 2003; Selle and Ravindran 2007). This discrepancy may be related to the use of different processing temperatures for various cottonseed meal products. The samples used in this experiment were from cottonseed extracted at low temperature (about 40–60 °C), while the expelling temperature of traditional CSM was up to 130 °C.

The EPL value (97.45 mg/kg DMI) of the current study was lower than 235 mg/kg DMI reported by Dilger and Adeola (2006a), which was determined by the regression method. It was also lower than the value (197 mg/kg DMI at day 13 and 159 mg/kg DMI on day 21) reported by Babatunde and Adeola (2021) and the value (830 mg/kg DMI on day 26) reported by

Mutucumarana and Ravindran (2021) on broilers fed on a P-free diet. However, it was higher than the value that ranged from 107 to 63 mg/kg DMI reported by Liu et al. (2014). The factors of dietary, animal, age and research protocol approaches may contribute to these differences in EPL (Rutherford et al. 2004; Dilger and Adeola 2006a; Babatunde and Adeola 2021).

Since one-third of phosphorus in plant feedstuffs is in the form of non-phytate P, and the remaining 2/3 is in the form of phytate P, it is generally believed that the retainability of P in plant feedstuffs is about 1/3. However, most of the ingredients tested excreta higher retainability of P from 33.65% of WG to 52.30% of CSM. Some reports suggested that variations in P retainability between the different ingredients may be related to the concentration of phytate P and non-phytate P in the feedstuffs (Nernberg 1998), and there was a strong negative correlation between the concentration of phytate P and P digestibility in pigs (Almaguer et al. 2014), but the results of our experiment failed to support the above viewpoint. It seems that the existing form, physical properties, chemical structure, and endogenous phytase activity, and phytate influence the utilisation rate of P.

Compared to the literature, the apparent retainability of P in SBM (49.84%) was in agreement with the report of 51% at the excreta level (Munoz et al. 2020) but was lower than the previous reports that ranged from 71.2% to 88.8% (Dilger and Adeola 2006a) and from 64% to 90% (Liu et al. 2013). Similarly, the true retainability of P in SBM (53.90%) was slightly lower than the reported data of 59% (Rutherford et al. 2002) and 59.8% (Dilger and Adeola 2006a). The determined apparent retainability of P in CNM (50.95%) was in

close agreement with the range of 51.2–67.9% reported by Mutucumarana et al. (2014a) on broilers. In addition, the true retainability of P in WG (36.77%) fed to broilers was lower than the results of Rutherford et al. (2002, 42%) and higher than the report of Leske and Coon (1999, 30.7%). A higher value of true retainability of P (74%) in peanut flour was reported by Iyayi et al. (2013) compared to the current study value (42.76%). There is no report on the retainability of P in corn DDGS, except for the true ileal P digestibility of 72.7% in broilers as reported by Mutucumarana et al. (2014a), which is higher than our results on true retainability (54.51%). No published data excreta been reported for retainability of P in cottonseed meal.

Apparently, the retainability of plant P varies among studies due to differences in endogenous plant phytase activity, phytate P, and the employed technique in processing the ingredients (Sauvant et al. 2004). Thus, it is necessary to assess the retainability of P in different feed ingredient samples to improve the feed database.

Phytase has been globally used in poultry feed. To facilitate the application of phytase and compare the efficiency of different phytase products, people tried to establishing the equivalent relationship between phytase and inorganic P (retainable P) despite possible differences in the effect of phytase on different ingredients (Chen et al. 2019). Our results confirmed that the efficiency of phytase in different feed ingredients is highly variable. Phytase increased the retainability of P in wheat and its by-products and SBM by more than 30% and had a significant effect on CNM. However, the effect on corn DDGS and peanut meal are limited. The effect of phytase in different ingredients should be related to phytic acid content. The significant effect on CNM might depend on the high phytate content. The poor phytase potency in DDGS obviously involved the lack of phytate. However, the results in PM were discordant with the general knowledge that responses to phytase will be more pronounced with increasing dietary phytate levels. The reason is worth further study. According to Selle and Ravindran (2007), the P equivalency of 1000 FTUs of different phytases was between 1.03 g and 2.5 g for poultry complete diets, which was estimated based on broiler growth, ash of toe or bone, and P retention. Obviously, the difference in dietary composition is largely responsible for the observed variations in phytase P equivalency. In our experiment, the P equivalency of 1000 FTU phytase in different feed ingredients was determined to be within the range of 0.4–1.3 g, of which the P equivalent in

soybean meal was 1.0 g, higher than the 0.87 g measured by Yi et al. (1996) based on a semi-pure diet. The P equivalency of phytase in other ingredients has not been reported. The results of this experiment suggest that it is necessary to understand the effects of phytase in different feed ingredients for the precise and accurate application of phytase.

## Conclusions

This study has determined that the EPL of broiler chickens of ages 28–31 days is 97.45 mg/kg DMI. The true retainability of P in SBM, CNM, CSM, PM, corn DDGS, WB, WG, and WS was 53.90%, 53.22%, 54.73%, 42.76%, 49.45%, 38.26%, 36.77%, and 36.95%, respectively. The addition of phytase significantly improved the retainability of P in feed ingredients of plant origin, and the improvement varied greatly with feed ingredient, among which WG, WS, SBM, and WB showed the greatest improvement, all by nearly or more than 30%. The retainable P equivalent of 1000 FTUs of microbial phytase corresponding to different ingredients was between 0.4 g (corn DDGS and PM) and 1.3 g (WB).

## Ethical approval

The experiments were conducted following the procedure developed, reviewed, and approved protocol of the Animal Care and Use Committee of FRI of CAAS for bird handling and data collection.

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## Disclosure statement

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

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