# Running Title: DIGESTIBILITY OF PHOSPHORUS IN BROILERS

# Evaluating Availability of Phosphorus in Feedstuffs of Plant Origin Fed for Broiler Chickens with the Effect of Microbial Phytase

Tesfay Hagos Haile1, 2, She Yue1, Cai Huiyi1 and Liu Guohua1,\*

1Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences,12 Zhongguancun South Street, Haidian District, Beijing, China, [liuguohua@caas.cn](mailto:liuguohua@caas.cn)

2Non-Ruminant Research Directorate, Tigray Agricultural Research Institute, Mekelle, Ethiopia, [eytesfay@yahoo.com](mailto:eytesfay@yahoo.com)

**\*** For correspondence: [liuguohua@caas.cn](mailto:liuguohua@caas.cn); [eytesfay@yahoo.com](mailto:eytesfay@yahoo.com)

# Abstract

*The experimental study was conducted to determine the apparent availability (AA) and true availability (TA) of phosphorus (P) in feed ingredients of soybean meal (SBM), canola meal (CNM), peanut meal (PM), corn distiller’s dried grains with solubles (corn DDGS), wheat bran (WB), wheat grain (WG), and wheat middling (WM) with the effect of phytase. In the experiment, 504 male Arbor Acres broiler chickens aged 23 days with similar body weights were assigned randomly into 14 treatments with six replicate (cage) and six birds per cage. Fourteen diets were designed by combining two experimental factors that include seven ingredients and phytase (addition or not). Dietary P was only provided by the tested feed ingredient. A purified phosphorus-free (P-free) diet was prepared to determine endogenous P loss (EPL) using six birds. The availability of P was evaluated by collect the feces’ trial. Results showed that the EPL value was 97.45 mg/DMI. The TA of P in SBM, CNM, and corn DDGS was 53.90%, 53.22%, and 49.45%, respectively, which was much higher than PM (42.76%), WB (38.26%), WG (36.77%), and WM (36.95%). The addition of phytase increased the AA or TA of P significantly (P<0.001). Phytase had greatest improvement for wheat ingredients (WM 41.35%; WG 41.34%, and WB 35.94%) and SBM (31.52%), followed by CNM (23.07%), PM (11.51%), and corn DDGS (10.23%). Application of phytase and formulation of broiler diets based on the true P availability can reasonably help to utilize phosphorus resources for reducing feed costs and minimize environmental pollution.*

***Keywords:*** *feedstuff; availability; phosphorus; microbial phytase; broiler chickens*

# Introduction

Phosphorus (P) is a necessary nutrient for animals. Most of the body’s P is stored in bones in the form of phosphate. A small part of P is distributed in cells and body fluids which plays crucial roles in cellular metabolism, the integrity of cellular membranes, and electrolyte balance (Veum 2010). Lack of P could retard the development of bone, induce joint deformity, reduce appetite, and restrain the growth of growing poultry (NRC 1994). In general, feed is the sole source of P for animal and the commercial feed could provide adequate or even excessive amounts of P to meet the animal’s requirements. However, in recent decades, the public pays more attention to the remarkable negative impact of excessive dietary P on the environment and non-renewal of inorganic P resources. As a result, concerned about the efficient and precise utilization of P from the plant ingredients has been increasing. Therefore, assessment of utilization efficiency of P in feedstuffs has become the basis of the whole strategy for optimizing dietary P nutrition of animals.

Most of the phosphorus in plant feedstuffs exists in the form of phytate P (Ravindran et al., 1994), and non-ruminant animals lack enough endogenous phytase activity that leads to poor utilization of organic P in the feedstuffs (Dayyani et al. 2013). Besides, depending on the plant endogenous phytase activity and the feed technological process the bioavailability of P in plant ingredients is highly variable (Tran and Sauvant 2004). Furthermore, P availability can also be affected by dietary factors (Leytem et al., 2008). Therefore, it is necessary to assess the bioavailability of P in feed ingredients of plant origin at regular bases.

Nowadays, evaluate the bio-availability of plant feed ingredients with classical methods in vivo include the indirect method which determinates relative bioactivity of ingredients to the standard inorganic P source by growth response of P deposition in bone. The direct method assesses the availability (metabolizability) of P in feed ingredients by the digestion experiments. The former will obtain the relative bio-availability based on the growth rate, biochemical makers and mineralization of bone of target animals, which takes a longer time and more labour to feed animal and become difficult to evaluate multiple samples at a single trial. The latter can get the availability of P in feed ingredients based on collecting feces trial, which takes less time and is helpful to assess multiple samples once, and is thought as a classical and the most frequently used method. Using the classical digestion experiment, we can examine the apparent digestibility for swine or the availability for poultry. Considering the existence of endogenous P loss (Fan et al., 2001) has an inevitable influence on apparent digestibility (Almeida and Stein 2010; Fang et al., 2007; Rojas and Stein 2012). The value of apparent availability (Dilger and Adeola, 2006b; Liu et al., 2012b), the data of apparent digestibility could be corrected into true digestibility and true availability by determining and deducting the endogenous P loss value. The true digestibility or availability is more reliable and additive because of the influence from dietary factors and interference from endogenous loss of P has been eliminated. However, evaluation of the true availability of P, especially for broilers chicken is lacking.

As a new route to improve the bio-availability of phosphorus in the animal feed, phytase has widely used as a feed additive to increase the P digestibility of feeds in monogastric animals (Englen et al., 2001). To understand the exact effect of phytase, different research works were tried building the equivalent relationship between phytase and inorganic P (Adedokun et al., 2004; Selle & Ravindran, 2007). Nevertheless, these results were obtained from experiments conducted using complete diets, which lacked variation among different plant ingredients. The above literature data shows there is a big variation between the responses of various feed ingredients to phytase addition. Unfortunately, there are scarce reports which compare the effect of phytase on digestion of P in different single feed ingredients. Therefore, this study aims at evaluating the availability of P in several feed ingredients for broiler chickens and investigates the effect of microbial phytase addition on digestion of P in these single ingredients. These studies significantly contributed for enriching the feed database of availability of P for broiler chickens and understand the nutrient value of microbial phytase profoundly.

# Materials and Methods

The experiments were conducted at the poultry nutrition laboratory of Feed Research Institute (FRI) of Chinese Academy of agricultural sciences (CAAS) following the procedure developed, reviewed and approved by the protocol of the Animal Care and Use Committee of FRI, CAAS for the birds handling and data collection.

## Test feedstuffs and experimental diets

The test feedstuffs of SBM, CNM, PM, Corn DDGS, WB, WG, and WM were purchased from a feedstuff company in China. The chemical composition of these feedstuffs was analyzed in the feed research institute laboratory as shown in Table 1. Fourteen semi-purified experimental diets were formulated by seven test ingredients with and without microbial phytase. For each experimental diet, the test ingredient was the sole source of P, and CaCO3 was used to adjusting the ratio of Ca to total P (1.3:1). The composition of experimental diets was summarized in Table 2. The 1000FTU/kg microbial phytase was added to each of the eight experimental diets. The microbial phytase was produced by *E. coli* production system in Challenge Bio-Tec Company, Beijing, China. Each unit of phytase is deﬁned as the quantity of enzyme required to release 1 µmol of inorganic phosphorus/min from 0.00015 mol/L sodium phytate at pH 5.5 and 37°C (Rutherfurd et al., 2004). Before the feeding trials, key nutrients level and phytase activity of all experimental diets were detected as shown in Table 3.

To determinate endogenous loss of P, a purified P-free diet was prepared by 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 is the same as the experimental diets. Silicon dioxide (SiO2) was added as an indicator (acid-insoluble ash) at 1.07% of all diets. All diets were pelleted to size of 2mm x 3mm before feeding.

## Animals, housing management, and experimental design

One thousand male Arbor Acres broiler chickens aged 1 day were obtained from Beijing Arbor Acres Poultry Breeding CO., LTD and raised in heated, thermostatically controlled, stainless cage coated with plastic (100 x 50 x 45 cm) and equipped with feeder and waterer. During the preparation period (1-23 day-old), birds were fed 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 23:1h light: darkness scheduled. Birds accessed randomly tap water from a low-pressure drinking nipple.

A total of 504 male chicken aged 23 days with similar body weight (930-960 g) were selected and allotted to fourteen dietary treatments by a randomized block design with two factors (7 ingredients & phytase addition level: 0 or 1000 FTU/kg). Each treatment has six replicates (cages) with 6 chickens per cage. After 4 days of adaptation period (24-27 day-old) to corresponding semi-purified diet, the feces were collected in 4 days (28-31 day-old) from the tray underneath the cage.

At the same time, six other male chickens were selected to fix a plastic cups around their anus to determine the endogenous P loss (EPL). After an adaptation period of 4 days, the chicken fasted for 12h to evacuate the digestive tract content following the procedure reported by Liu et al. (2012a) with a slight modification of fasting hours. Then each chicken was forced-fed 30g of purified P-free diet and their feces were collected for 18 h. The collected feces samples from a cage were pooled and put in a zip-lock plastic bag and stored immediately in -20 oC for analysis.

## Chemical analysis

For the chemical analysis, after air-dried of air oven 65oC, samples of ingredient, diet, and feces were grounded through a 0.45-mm sieve using a grinding mill. Samples of feed (triplicate), and feces (duplicate) were analyzed for dry matter (DM, AOAC 930.15) (AOAC, 2007), crude protein (CP, Dumatherm, Gerhardt company, Germany). Following the ashing process at 550°C for 6 hours in a muffle furnace, samples of diets, feedstuffs and feces were extracted with 4N HCl solution and the P concentration was determined by spectrometry (Model UV-1780, Shimadzu, Japan). The content of Ca in the samples was determined by flame spectrometry (Model novAA® 400P, Analytikjena, Germany). The content of phytate-P was analyzed 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 phosphorus concentration was calculated by the difference between the total phosphorus and phytate-P. Acid-insoluble ash (AIA ) of samples of the diets and feces were analyzed following the procedure of AOAC Method Ba 5b-68 (Keulen and Young 1977).

## Calculations

The apparent availability (AA%) of P was estimated based on the indicator marker method equation of Dilger and Adeola (2006b):

Where: Pfeces is the content of P in feces; the Pfeed is the content of phosphorus in the diet; Markerfeed is the content of acid insoluble ash (AIA) in diet and Markerfeces is the content of AIA in feces.

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

**Where: Fintake is the average daily dry matter intake of P-free diet (mg of DMI) during the feces collection period. Pfeces is the content of phosphorus in the feces originated from the birds during the feces collection period based on dry matter.

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



Where: EPL is endogenous P loss of P, and Pfeed is the content of phosphorus in the diet.

## Statistical analysis

The normality of the data was analyzed and confirmed using the general linear model (GLM) procedure (SAS Inst. Inc., Cary, NC). Outliers were tested using the UNIVARIATE procedure in SAS, but no outlier was observed. All data were analyzed by a two-way analysis of variance (ANOVA) procedure of SAS statistical software. Multiple comparisons among means were carried out by Tukey’s test. The α-level of 0.05 was used to determine significance among means.

# Results

## Chemical components of ingredients

The ingredients composition of test diets from this experiment is presented in Table 1. Corn DDGS have the highest percentage of non-phytate P (NPP) in a total P (93.33%), and peanut meal has the lowest percentage (18.42%). The remaining ingredients have about 30.01% NPP of total P, which is from 20.90% of wheat bran to 37.29% of soybean meal. The phytase activity of test diets was determined (Table 3) and calculated as 1445 for WB, 505 for WG, and 1570 for WM. No phytase activity was detected for SBM, CNM, PM, and Corn DDGS.

## Endogenous loss of phosphorus

The determined average endogenous phosphorus loss (EPL) was 97.45 mg/kg DMI. The intake of P from a P-free diet was extremely lower levels which were (only 1.5 mg), while the excrete P content was 0.087g.

## Availability of phosphorus in feedstuffs for broilers chicken

All birds remained healthy during the whole experimental period. The apparent and true availability of phosphorus varies significantly depending on the type of ingredient (P<0.001). Canola meal, soybean meal, and corn DDGS had higher availability of P which range from 46.91% to 50.95% for apparent availability and 49.45% to 53.22% for true availability. The other four ingredients had lower values which is lower than 40% for apparent availability and 36.77% to 42.76% for true availability (Table 4).

The addition of 1000 FTU/kg microbial phytase to diets indicated a significant improvement of P availability for all feed ingredients (P<0.01). Phytase showed the most noticeable relative improvement of apparent availability in wheat grain, wheat middling, wheat bran and soybean meal, with an increase of 45.08%, 45.00%, 38.08% and 34.41% respectively. Canola meal and peanut meal was followed by 24.30% and 12.19%, respectively. The least improved corn DDGS was recorded only at 10.51% (Table 4). The same is true for true P availability. The absolute improvement of apparent P availability expressed by percentage points from high to low value was soybean meal (17.15), wheat middling (15.42), wheat grain (15.17), wheat bran (13.80), canola meal (12.38), corn DDGS (4.93) and peanut meal (4.79) (Table 4). The equivalent relationship between phytase and available P is described in Table 5. It shows the equivalent value of 1000 FTU/kg phytase varied with different ingredients from 0.13% in wheat bran to 0.04% in corn DDGS and peanut meal.

# Discussion

## Composition of ingredients

The content of calcium, phosphorus and relative chemical components of most ingredients measured in this study were mostly consistent with or close to those reported in the literature (Eeckhout and De Paepe 1994; NRC, 1994; She et al., 2015). The non-phytate P content in SBM, CNM, PM, WG, and WB was comparable to the reported value of NRC (1994), while, WM and corn DDGS had a higher value. There was no detected phytase activity in soybean meal, canola meal, peanut meal and corn DDGS. This result is in agreement with the previous studies (Eeckhout and De Paepe 1994) which reported a ranged value of phytase activity from 0 to 120 (SBM), 0 to 36 (CNM), and 0 to 8 FTU/kg (PM) whilst, there are no reports about DDGS. In general, the absence of phytase activity in these feed ingredients could be related to the inactivating effect of overheating and some chemical reagents during their process flow.

The three kinds of wheat-based ingredients showed high phytase activity. The phytase activity of wheat origin ingredients is in agreement with previous studies (Selle et al., 2003) and wheat bran (Eeckhout and De Paepe 1994). In contrast, some studies (Eeckhout and De Paepe, 1994; Viveros et al., 2000) showed a higher value of wheat grain and wheat middling. The reason for the inconsistency may be related to the different processing temperatures of different feedstuffs. Some feed ingredients were detoxified at lower temperature (about 80℃), while the detoxification temperature of other feed ingredients was up to 130℃. It was also reported that heating to 70-80 ℃ and the pH value < 2.5 causes partial or total inactivation of endogenous plant phytase (Nernberg, 1998).

## Endogenous loss of phosphorus

The endogenous loss of phosphorus (EPL) (97.45mg/kgDMI) of the this study is lower than 235 mg/kg DMI compare to the finding of Dilger and Adeola (2006a) who applied the regression method to determine the P loss. Similarly, the obtained P loss is lower than the value (830mg/kg DMI) compare to the report of Mutucumaranata (2014) on broilers fed P-free diet and higher than the value ranged between -107 and 63 mg/kg DMI reported by Liu et al. (2014). The factors such as dietary, animal, and research protocol approaches may contribute to the diverse value of the EPL (Dilger and Adeola 2006a; Rutherfurd et al., 2004).

## Phosphorus availability of feedstuffs

Since one-third of phosphorus in the plant feedstuffs are in the form of non-phytate phosphorus, and the remaining 2/3 is in the form of phytate phosphorus, it is generally believed that the availability of phosphorus in plant feedstuffs is about 1/3(NRC 1994). However, most of the tested ingredients showed higher availability of P which ranged from 33.65% for wheat grain to 50.95% for canola meal. According to Nernberg (1998), the variation in P availability between the different ingredients may be related to the concentration phytate P and non-phytate P of the feedstuffs, and there was a strong negative relationship between the concentration of phytate P and P digestibility in pigs (Almaguer et al., 2014). However, the findings of this study do not support the above views. It seems that the existing form, physical properties, chemical structure and endogenous phytase activity of phosphorus phytate affect the utilization rate of phosphorus. The availability of phosphorus could be related to the bond form of phytate, endogenous phytase activity, physical properties, the chemical structure of ingredients, and other unknown factors.

The apparent availability of P in SBM (49.84%) is also in agreement with the existing literature who reported a value ranged from 14 to 62.7% (Mutucumaranata et al., 2014) at ileal level, but lower compared to the report by Dilger and Adeola (2006a) who showed a range value from 71.2 to 88.8% and from 64 to 90% (Liu et al., 2013). Similarly, the true availability of P in SBM (53.90%) was slightly lower than the values observed in the existing literature who reported a data of 59 % (Rutherfurd et al., 2002), 59.8% (Dilger and Adeola 2006a). The determined apparent availability of P in canola meal (50.95%) was in close agreement with the ranged value of 51.2-67.9% (Mutucumarana et al., 2014). The true availability of P in wheat grain (36.77%) fed to broilers is lower compare to Rutherfurd et al. (2002) and Leske and Coon (1999) for wheat middling (36.95%). A higher value of true availability of P (74%) in peanut flour was reported by Iyayi and Adeola (2013) compared to this study (42.76%). As to the knowledge of the authors, there is no any previous study about availability of P in corn DDGS except true ileal P digestibility of “72.7%” reported in broilers by Mutucumaranata (2014), which is higher than our result of true P availability (54.51%). The availability of plant P is variable in different studies for the fact that the variation of endogenous plant phytase activity, phytate P, and the undergone technological process of the diets (Sauvant et al., 2004).

## Effect and phosphorus equivalency of phytase

In order to facilitate the application of phytase and compare the efficiency of different phytase products, researchers have been trying to establish the equivalent relationship between phytase and inorganic phosphorus (available phosphorus) in spite of the possible differential effect of phytase on different ingredients. The finding of this study confirmed that the efficiency of phytase for different feed materials was very different. Phytase increased the availability of phosphorus in wheat and its by-products and soybean meal by more than 30% and had a significant effect on canola meal. But, the phytase effect in corn DDGS and peanut meal was very limited. The result of peanut meal disagrees with the general knowledge that responses to phytase could be more pronounced with increasing dietary phytate levels (Selle et al., 2003). According to report by Selle and Ravindran (2007), the microbial phytase phosphorus equivalency in poultry diets was found that the phosphorus equivalency of 1,000 FTU of different phytase was between 1.03 and 2.5g for complete diets, which was estimated based on broilers' growth, ash of toe or bone, and phosphorus retention. Noticeably, the difference in dietary composition is an important reason for the large variation of phytase phosphorus equivalency. In current finding, the phosphorus equivalency of 1000 FTU phytase in different feed ingredients was measured as 0.4 - 1.3g, of which the phosphorus equivalent in soybean meal was 1.0g, higher than the 0.87g measured by Yi et al. (1996) based on a semi-pure diet. The phosphorus 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 addition in different feed ingredients for the precise and accurate application of phytase.

# Conclusion

In this experiment, the mean value for the EPL of broiler chickens aged 28-31 days found to be 97.45 mg/kg DMI. The true availability of phosphorus in soybean meal, canola meal, corn DDGS was more than 49%, which was significant higher than wheat middling, wheat grain, wheat bran, and peanut meal, that was recorded from 36.77% to 42.76%. Addition of phytase significantly improved the availability of phosphorus in the tested feed ingredients of plant origin. The improvement was varied greatly with the different feed ingredients, among which wheat grain, wheat middling, soybean meal, and wheat bran improved the most, all by nearly or more than 30%, while peanut meal and corn DDGS was improved below 12%. The available phosphorus equivalent of 1000FTU microbial phytase corresponding to different ingredients was between 0.4g (corn DDGS and peanut meal) and 1.3g (wheat bran). In conclusion, formulating diets based on the true availability of P can give a realistic measure of the digestible portion of P in the feedstuffs, which improve the feed base of poultry that provide economically and environmentally benefit. A further research study is a warranty to evaluate the ileal level P digestibility of similar feed ingredients.

**Author Contributions**: Conceptualization and methodology, Tesfay Hagos, She Yue and Liu Guohua; performed the experiment, Tesfay Hagos, data analysis Tesfay Hagos and Liu Guohua, writing—original draft preparation, Tesfay Hagos, supervision and project administration, Liu Guohua and Cai Hyuie. All authors reviewed and approved the final manuscript.

**Funding:** The research was funded by China Agriculture Research System (CARS-41) and China Postdoctoral Science Foundation

**Acknowledgment:** The authors like to forward their acknowledgment to China Agriculture Research System (CARS-41)and China Postdoctoral Science Foundation for the fund support of the project, to the hosting feed research institute (FRI), graduate school of Chinese academy of agricultural sciences (GS-CAAS), Tigray agricultural research institute (TARI) and to all staff of the feed research institute and poultry nutrition laboratory.

**Conflicts of interest:** The authors declare that there is no any conflict of interest.

# References

Adedokun SA, JS Sands, O Adeola (2004). Determining the Equivalent Phosphorus Released by an Escherichia Coli -Derived Phytase in Broiler Chicks. *Journal of Animal Science* 84:437–44.

Akinmusire AS, O Adeola (2009). True Digestibility of Phosphorus in Canola and Soybean Meals for Growing Pigs : Influence of Microbial Phytase. *Journal of Animal Science* 87:977–83.

Almaguer BL, RC Sulabo, Y Liu, HH Stein (2014). Standardized Total Tract Digestibility of Phosphorus in Copra Meal, Palm Kernel Expellers, Palm Kernel Meal, and Soybean Meal Fed to Growing Pigs. *Journal of Animal Science* 92(6):2473–80.

Almeida FN, HH Stein (2010). Performance and Phosphorus Balance of Pigs Fed Diets Formulated on the Basis of Values for Standardized Total Tract Digestibility of Phosphorus. *American Society of Animal Science* 88:2968–77.

AOAC International (2007). *Official Methods of Analysis. 18th Ed. Hortwitz W, Latimer Jr., GW, Editors.* Gaithersburg, MD, USA:AOAC international.

Dayyani Nematollah, Morteza Beyki, Bandar Abadi, Ahmad Amir, Abadi Farhani (2013). Phytate and Phytase in Poultry Nutrition. 1(11):1403–8.

Dilger RN, O Adeola (2006). Estimation of True Phosphorus Digestibility and Endogenous Phosphorus Loss in Growing Chicks Fed Conventional and Low-Phytate Soybean Meals. *Poultry Science* 85:661–68.

Dilger RN, O Adeola (2006). Estimation of True Phosphorus Digestibility and Endogenous Phosphorus Loss in Growing Pigs Fed Conventional and Low-Phytate Soybean Meals. *Journal of Animal Science* 84(3):627–34.

Eeckhout W, MDe Paepe (1994). Total Phosphorus, Phytate-Phosphorus and Phytase Activity in Plant Feedstuffs. *Animal Feed Science and Technology* 47(1–2):19–29.

Englen AJ, FC Heeft van der, PH Randsdrop, WA Somers, Schaefer J, BJ vat van der (2001). Determination of Phytase Activity in Feed by a Colorimetric Enzymatic Method: Collaborative Interlaboratory Study. *Journal of AOAC International* 84(3):629–33.

Fan MZ, T Archbold, WC Sauer, D Lackeyram, T Rideout, Y Gao, CF de Lange, RR Hacker (2001). Novel Methodology Allows Simultaneous Measurement of True Phosphorus Digestibility and the Gastrointestinal Endogenous Phosphorus Outputs in Studies with Pigs. *The Journal of Nutrition* 131(9):2388–96.

Fang RJ, TJ Li, FG Yin, YL Yin, XF Kong, KN Wang, Z Yuan, GY Wu, JH He, ZY Deng, MZ Fan (2007). The Additivity of True or Apparent Phosphorus Digestibility Values in Some Feed Ingredients for Growing Pigs. 20(7):1092–99.

Iyayi EA, O Adeola (2013). True Phosphorus Digestibility of Black-Eyed Pea and Peanut Flour without or with Phytase Supplementation in Broiler Chickens. *Poultry Science* 92(2009):1595–1603.

Keulen J Van, BA Young (1977). Evaluation of Acid-Insoluble Ash as a Natural Marker in Ruminant Digestibility Studies. *Journal of Animal Science* 44(2):282–87.

Leske KL, CN Coon (1999). A Bioassay to Determine the Effect of Phytase on Phytate Phosphorus Hydrolysis and Total Phosphorus Retention of Feed Ingredients as Determined with Broilers and Laying Hens. *Poultry Science* 78(8):1151–57.

Leytem AB, BP Willing, PA Thacker (2008). Phytate Utilization and Phosphorus Excretion by Broiler Chickens Fed Diets Containing Cereal Grains Varying in Phytate and Phytase Content. *Animal Feed Science and Technology* 146(1–2):160–68.

Liu JB, DW Chen, O Adeola (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(4):661–68.

Liu JB, DW Chen, O Adeola (2013). Phosphorus Digestibility Response of Broiler Chickens to Dietary Calcium-to-Phosphorus Ratios. *Poultry Science* 92:1572–78.

Liu SB, SF Li, L Lu, JJ Xie, LY Zhang, XG Luo (2012). Estimation of Standardized Phosphorus Retention for Corn, Soybean Meal, and Corn-Soybean Meal Diet in Broilers. *Poultry Science* 91(8):1879–85.

Liu S, S Li, L Lu, J Xie, L Zhang, Y Jiang, X Luo (2012). Development of a Procedure to Determine Standardized Mineral Availabilities in Soybean Meal for Broiler Chicks. *Biological Trace Element Research* 148(1):32–37.

Mutucumarana RK (2014). Measurment of True Ileal Phosphorus Digestibility in Feed Ingredients for Poultry [Dissertation]. Massey Universtity, Palmerston North, New Zealand.

Mutucumarana RK, V Ravindran, G Ravindran AJ Cowieson (2014). Measurement of True Ileal Digestibility of Phosphorus in Some Feed Ingredients for Broiler Chickens. *American Society of Animal Science* 92:5520–29.

Nernberg LWJ (1998). Improved Phosphorus Availability in Poultry Fed Wheat/Canola Meal-Based Diets Supplemented with Phytase Enzyme [Master’s Thesis]. The University of Manitoba, Canada.

NRC (1994). *Nutrient Requirements of Poultry*. Ninth Revised Edition, National Academy Press, Washington, D.C.USA.

Ravindran V, G Ravindran, S Sivalogan (1994). Total and Phytate Phosphorus Contents of Various Foods and Feedstuffs of Plant Origin. *Food Chemistry* 50:133–36.

Rojas OJ, HH Stein (2012). Digestibility of Phosphorus by Growing Pigs of Fermented and Conventional Soybean Meal without and with Microbial Phytase. *Journal of Animal Science* 90(5):1506–12.

Rutherfurd SM, TK Chung, PCH Morel, PJ Moughan (2004). Effect of Microbial Phytase on Ileal Digestibility of Phytate Phosphorus , Total Phosphorus , and Amino Acids in a Low-Phosphorus Diet for Broilers. *Poultry Science* 83:61–68.

Rutherfurd SM, TK Chung, PJ Moughan (2002). The Effect of Microbial Phytase on Ileal Phosphorus and Amino Acid Digestibility in the Broiler Chicken. *British Poultry Science* 44:598–606.

Sauvant D, J Perez, G Tran (2004). *Tables of Composition and Nutritional Value of Feed Materials*.

Selle PH, V Ravindran (2007). Microbial Phytase in Poultry Nutrition. *Animal Feed Science and Technology* 135(1–2):1–41.

Selle PH, AR Walker, WL Bryden (2003). Total and Phytate-Phosphorus Contents and Phytase Activity of Australian-Sourced Feed Ingredients for Pigs and Poultry. *Australian Journal of Experimental Agriculture* 43(5):475–79.

She Y, Y Su, L Liu, C Huang, J Li, P Li, D Li, X Piao (2015). Effects of Microbial Phytase on Coefficient of Standardized Total Tract Digestibility of Phosphorus in Growing Pigs Fed Corn and Corn Co-Products, Wheat and Wheat Co-Products and Oilseed Meals. *Animal Feed Science and Technology* 208:132–44.

Tran GD, D Sauvant (2004). Chemical Data and Nutritional Value. In: *Tables of composition and nutritional value of feed materials*, edited by D Sauvant, JM Perez, G Tran. Wageningen Academic Publishers, Wageningen, The Netherlands.pp.17-24.

Veum TL (2010). Phosphorus and Calcium Nutrition and Metabolism. In: *Phosphorus and calcium utilization and requierments in farm animals*, edited by Vitti DMSS, E Kebreab, CAB International , Oxfordshire, UK. Pp. 94–111

Viveros A, C Centeno, A Brenes, R Canales, A Lozano (2000). Phytase and Acid Phosphatase Activities in Plant Feedstuffs. *Journal of Agricultural and Food Chemistry* 48(9):4009–13.

Yi Z, ET Kornegay, DM Denbow (1996). Improving Phytate Phosphorus Availability in Corn and Soybean Meal for Broilers Using Microbial Phytase and Calculation of Phosphorus Equivalency Values for Phytase. *Poultry Science* 75:240–49.

**Table 1** Chemical component of experimental feed ingredients fed to broilers (air-dried basis)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Item | Soybean meal | Canola meal | Peanut meal | Corn DDGS | Wheat bran | Wheat grain | Wheat middling |
| DM, % | 91.42 | 89.68 | 92.01 | 90.74 | 91.56 | 89.69 | 89.57 |
| CP, % | 44.31 | 35.71 | 47.43 | 17.06 | 16.60 | 18.60 | 17.16 |
| Ca, % | 0.33 | 0.65 | 0.27 | 0.19 | 0.16 | 0.09 | 0.13 |
| Total P, % | 0.59 | 1.00 | 0.76 | 0.75 | 0.91 | 0.42 | 0.56 |
| Phytate ,% | 1.30 | 2.60 | 2.20 | 0.18 | 2.55 | 1.03 | 1.31 |
| Phytate P1, % | 0.37 | 0.73 | 0.62 | 0.05 | 0.72 | 0.29 | 0.37 |
| Phytate P of total P, % | 62.80 | 73.00 | 81.58 | 6.67 | 79.12 | 69.05 | 66.07 |
| Non-phytate P2, % | 0.22 | 0.27 | 0.14 | 0.70 | 0.19 | 0.13 | 0.19 |
| Non-phytate P of total P, % | 37.29 | 27.00 | 18.42 | 93.33 | 20.90 | 30.95 | 33.93 |

1Phytate P was calculated as 28.2% of phytate(Tran and Sauvant 2004)

2Non-phytate P was calculated as the difference between total P and phytate P

Corn DDGS; corn distiller’s dried grains with solubles

**Table 2** Composition of the semi-purified experimental diets (air-dried basis)1

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Ingredient (%) | SBM | CNM | PM | Corn DDGS | WB | WG | WM |
| Test Ingredients | 40.00 | 42.99 | 35.00 | 56.00 | 65.00 | 85.00 | 85.00 |
| Corn Starch | 22.00 | 12.10 | 30.00 | 30.20 | 16.04 | 3.12 | 2.56 |
| Sucrose | 29.00 | 33.00 | 25.00 | 5.00 | 5.00 | - | - |
| Soybean oil | 3.00 | 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 |
| Limestone | 2.05 | 1.51 | 2.37 | 2.25 | 2.37 | 2.16 | 2.38 |
| L-lysine | - | 0.63 | 0.71 | 0.91 | 0.85 | 0.97 | 0.75 |
| DL-Methioine | 0.20 | 0.19 | 0.31 | 0.12 | 0.37 | 0.27 | 0.27 |
| L-Cystine | 0.09 | - | 0.22 | 0.13 | 0.18 | 0.12 | 0.10 |
| L-Theronine | - | 0.04 | 0.28 | 0.17 | 0.41 | 0.41 | 0.25 |
| L-Tryptophan | - | 0.02 | 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 | 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 |
| Premix2 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 |
| Feed binder | 1.88 | 1.33 | 2.83 | - | - | - | - |
| SiO2 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 | 1.07 |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

1SBM: soybean meal; CNM: canola meal; PM: Peanut meal; Corn DDGS: corn distiller’s dried grains with solubles; WB: wheat bran; WG: wheat grain; WM: wheat middling.

2Provided the following nutrients (per kg of diet): vitamin A, 10000IU; vitamin D3, 2000IU; vitamin E, 10IU; vitamin K3, 2.5 mg; vitamin B1, 1.8 mg; vitamin B2, 40 mg; vitamin B6, 5.0 mg; vitamin B12, 0.71 mg; biotin, 0.12 mg; folic acid, 0.5 mg; nicotinic acid, 50 mg, D-pantothenic acid, 11 mg; Cu (copper sulfate), 8 mg; Fe (ferrous sulfate), 80 mg; manganese sulfate, 60 mg; Zn (zinc sulfate), 40 mg; I (potassium iodide), 0.35 mg; Se (sodium selenite), 0.15 mg.

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Item | Diets1 | | | | | | | | | | | | | |
| SBM | | CNM | | PM | | Corn DDGS | | WB | | WG | | WM | |
| Phytase addition2 | - | + | - | + | - | + | - | + | - | + | - | + | - | + |
| AME3 (MJ.kg) | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 | 12.75 |
| Crude protein3 (%) | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 | 18.00 |
| Dry matter4 (%) | 93.97 | 93.84 | 94.04 | 94.17 | 93.34 | 93.58 | 92.51 | 92.18 | 92.43 | 92.54 | 90.82 | 91.07 | 90.22 | 90.82 |
| Ca4 (%) | 0.31 | 0.32 | 0.56 | 0.58 | 0.36 | 0.35 | 0.50 | 0.49 | 0.63 | 0.62 | 0.41 | 0.40 | 0.51 | 0.53 |
| Total P4 (%) | 0.24 | 0.25 | 0.43 | 0.45 | 0.28 | 0.27 | 0.38 | 0.36 | 0.48 | 0.49 | 0.31 | 0.31 | 0.36 | 0.38 |
| Ca: P Ratio | 1.29 | 1.28 | 1.30 | 1.29 | 1.29 | 1.30 | 1.32 | 1.36 | 1.31 | 1.27 | 1.32 | 1.29 | 1.42 | 1.39 |
| Phytase activity4 (FTU/kg) | 0 | 1426 | 0 | 1041 | 0 | 1335 | 0 | 935 | 1445 | 2515 | 505 | 1480 | 1570 | 2625 |

1SBM: soybean meal; CNM: canola meal; PM: Peanut meal; Corn DDGS: corn distiller’s dried grains with solubles; WB: wheat bran; WG: wheat grain; WM: wheat middling

2Microbial phytase addition (-: no addition; +: added 1000 FTU/kg diet), 3calculated value, 4analyzed value.

**Table 4** Apparent availability and true availability of phosphorus in feedstuffs of plant origin with or without phytase addition for 28-31 day old broiler chickens1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ingredient | Phytase2 | Apparent availability of P | Increment3 | True availability of P4 | Increment |
| Soybean meal | - | 49.84a | 17.15a | 53.90a | 16.99a |
| + | 66.99a | (34.41) | 70.89a | (31.52) |
| Canola meal | - | 50.95a | 12.38ab | 53.22a | 12.28ab |
| + | 63.33ab | (24.30) | 65.50b | (23.07) |
| Peanut meal | - | 39.28b | 4.79c | 42.76b | 4.92c |
| + | 44.07d | (12.19) | 47.68d | (11.51) |
| Corn DDGS | - | 46.91a | 4.93c | 49.45a | 5.07c |
| + | 51.84c | (10.51) | 54.51c | (10.23) |
| Wheat bran | - | 36.24b | 13.80a | 38.26b | 13.75a |
| + | 50.04c | (38.08) | 52.01cd | (35.94) |
| Wheat grain | - | 33.65b | 15.17a | 36.77b | 15.20a |
| + | 48.82cd | (45.08) | 51.97cd | (41.34) |
| Wheat middling | - | 34.27b | 15.42a | 36.95b | 15.28a |
| + | 49.69c | (45.00) | 52.23cd | (41.35) |
| SEM | - | 1.17 | 0.77 | 1.17 | 0.76 |
| + | 1.16 | - | 1.16 | - |
| P value | - | **\*\*\*** | **\*\*\*** | **\*\*\*** | **\*\*\*** |
| + | **\*\*\*** | **\*\*\*** | **\*\*\*** | **\*\*\*** |
|  |  | Probability of ANOVA | | | |
| Ingredient |  | **\*\*\*** | **\*\*\*** | **\*\*\*** | **\*\*\*** |
| Phytase |  | **\*\*\*** | **NS** | **\*\*\*** | **NS** |
| Ingredient\*phytase |  | **\*\*\*** | **\*** | **\*\*\*** | **\*** |

SEM, the standard error of the mean; P, phosphorus

NS: not significant; **\*:** p<0.05; **\*\*\***: p< 0.001

1) Values represent means of 6 replicates with 6 chicks per each cage

2) Microbial phytase was added in the diets containing phytase at 1000U/kg complete feed (-, no phytase; +, with 1000U/kg microbial phytase)

3) The increment shows an increase in percentage point by phytase addition. The data [in](javascript:;) [parentheses](javascript:;) represent the relative magnitude of the improvement.

4) Values for the true availability of P were calculated by correcting values of apparent availability of P with an average EPL value of 97.45 (mg/kg DMI), dry matter intake (DMI) determined using P-free diet (n=6)

**Table 5** The equivalency of phytase on apparent available phosphorus (%) and true available phosphorus (%) in feedstuffs of plant origin with or without phytase addition for 28-31 day old broiler chickens1

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ingredient | Phytase2 | Apparent available P | Equivalency of phytase3 | True available 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 |  |
| 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 middling | - | 0.19 | 0.09 | 0.21 | 0.09 |
| + | 0.28 |  | 0.29 |  |

1) Available P is calculated by availability and total P in ingredients.

2) Microbial phytase was added in the diets containing phytase at 1000U/kg complete feed (-, no phytase; +, with 1000U/kg microbial phytase)

3) Equivalency of phytase shows an increase of available P on the account of 1000 FTU/kg phytase addition.