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Ileal Digestibility of Phosphorus in Plant Origin Feedstuffs Fed for Broiler Chickens: The Effect of Microbial Phytase

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Abstract

The study was conducted to determine the apparent and standardized ileal digestibility (AID and SID) of phosphorus (P) in plant origin feedstuffs of corn distiller's dried grains with solubles (corn DDGS), wheat bran (WB), wheat grain (WG), wheat middling (WM),soybean meal (SBM), canola meal (CNM), cottonseed meal (CSM), and peanut meal (PM), fed to total 612 male arbor acres broilers with or without microbial phytase. A 23 day-old birds with initial body weight (BW) of 939±8.89g were randomly allocated to a factorial 2 × 8 arrangement with a randomized complete block design of sixteen dietary treatments and one P-free diet, with six replicates (cage) and six birds/cage. A purified phosphorus-free (P-free) diet was also prepared to determined endogenous P loss (EPL). The ileal digesta were collected from euthanized birds and analyzed. The study results indicated that the SID of P in WB (38.46%), PM (37.98%), WG (36.90%), and WM (35.69%) was significantly lower *($P < 0.001$)* than SBM (52.01%), corn DDGS (49.41%), CNM (47.02%), and CSM (46.09%) based diets. The Addition of microbial phytase to SBM and WM improved the SID of P by more than 40%, while the others improved by about 34.80%, which is from 31.55% in CNM to 37.77% in CSM. The least improvement was recorded in corn DDGS (5.12%). The feedstuffs ileal digestible P equivalent value of 1000 FTU/kg phytase was ranged from 0.2g to 1.6g. The ileal EPL of the current study was determined to be 46.28 mg/kg DMI. In conclusion, the application of microbial phytase and formulation of plant origin broiler diets based on SID of P can reasonably help to utilize phosphorus resources for reducing feed costs and minimize environmental pollution.

Introduction

Most of the phosphorus (P) in plant origin feedstuffs is represented in the form of phytate P (Ravindran, *et al*., 1994). Depending on the plant endogenous phytase activity and the feed process, the availability of plant P is highly variable (Tran and Sauvant, 2004). The P digestibility in poultry diets can be affected by dietary factors such as type of grain and the amount of calcium and P in the diet which also influences the phytate P excreted and contribute to environmental pollution (Leytem *et al*., 2008). Besides the lower bioavailability of P of the feedstuffs due to phytate (NRC, 1994), the poultry also luck to produce endogenous phytase to efficiently utilize the organic P found in the feedstuffs (Dayyani*et al*. , 2013). Moreover, the presence of endogenous P in the gastrointestinal tract (Fan *et al*., 2001), influenced the apparent value of P digestibility in commonly available feedstuffs fed to pigs (Almeida and Stein, 2010; Rojas and Stein, 2012) and broilers (Dilger and Adeola, 2006a; Liu *et al*., 2012).

To alleviate this problem the apparent digestibility of P should be corrected by endogenous P loss value to get standardized digestibility of P, which is additive in mixed diets of broiler chickens. Nowadays in addition to the feces collection method, the approach of ileal digestibility of P is getting great attention due to no influence of post-ileal microbial activity (Shastak and Rodehutscord, 2013), less urine P contamination (Mutucumarana, 2014), and due to its advantage of being less sensitive to the P level in the diet (Rodehutscord *et al*., 2012). Rutherfurd *et al*. (2004)

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reported that the digestive utilization of P in plant feed ingredients improve when microbial phytase included in the diet of the chickens and phytase activity in feed can be determined with colorimetric assay (Englen *et al*., 2001). To our knowledge, little information is available concerning the effect of microbial phytase supplementation to broiler diets on the ileal digestibility of P. Therefore, the experiment was conducted to assess the ileal digestibility of P in some poultry feedstuffs with the influence of microbial phytase to enrich the feed database of P digestibility.

Materials and Methods

The experiment was conducted at the poultry and feed nutrition laboratory of Feed Research Institute (FRI), the Chinese Academy of Agricultural Sciences (CAAS) following the procedure developed, reviewed, and the approved protocol of the institutional animal care and use committee for the birds handling and data collection.

Test feedstuffs and experimental diets

Test feedstuffs, which include corn distiller's dried grains with solubles (corn DDGS), wheat bran (WB), wheat grain (WG), wheat middling (WM),soybean meal (SBM), canola meal (CNM), cottonseed meal (CSM), and peanut meal (PM) were purchased from the Longwei Feed Company of China. Two *semi*-purified experimental diets for each test feedstuff were formulated with or without 1000FTU/kg microbial phytase. Dietary P was only provided by the test feedstuff.

A total of sixteen diets were formulated (Table 1).Vitamins and mineral premix other than P was also included in all diets based on the recommendation of NRC (1994). The *semi*-purified experimental diets were formulated to have the same ratio of calcium to total P of (1.3:1) adjusted using $CaCO_3$. The external indigestible marker of silicon dioxide (SiO_2) was adding at 1.07% to diets. The chemical composition of *semi*-purified diets is presented in Table 2. The microbial phytase was produced by an E. coli production system challenge biotech company, Beijing, China. The phytase activity is defined by phytase units (FTUs) and one FTU is the phytase activity that is required to release 1 μmol of inorganic phosphorus per minute from an excess of 15 M sodium phytate at pH 5.5 and 37°C (Rutherfurd *et al*., 2004). The determined test diet phytase activity of CSM, WB, WG, and WM diet was 1604, 1445, 505, and 1570 FTU/kg, while SBM, CNM, PM, and C-DDGS test diets had no detected phytase activity (Table 2).

The ileal endogenous P loss was determined using a purified phosphorus-free diet which was prepared from corn starch 68.53%, glucose 9.64%, soybean oil 4.00%, table salt 0.30%, limestone 0.98%, cellulose 2.88%, L-lysine hydrochloride 0.96%, DL-methionine 0.53%, L-threonine 0.32%, L-tryptophan 0.17%, L-arginine 0.58%, L-glutamine 2.70%, L-histidine 0.16%, L-leucine 0.87%, L-isoleucine L-0.44%, L-phenylalanine 0.34%, L-tyrosine 0.30%, L-valine 0.42%, L-glycine 0.52%, L-serine 0.52%, L-asparagine 0.96%, L-proline 1.91%, feed binder 0.40% and premix 0.50%. The premix composition was the same as the experimental diets. All diets were pelleted as size as 2mm × 3mm before feeding. The birds could access freely to tap water all times from a low-pressure drinking nipple.

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

Ingredient (%)	Corn DDGS	WB	WG	WM	SBM	CNM	CSM	PM
Test Ingredients	56.00	65.00	85.00	85.00	40.00	42.99	40.00	35.00
Corn Starch	30.20	16.04	3.12	2.56	22.00	12.10	19.80	30.00
Sucrose	5.00	5.00	-	-	29.00	33.00	27.11	25.00
Soybean oil	3.00	3.00	2.00	3.00	3.00	5.00	5.00	1.50
Table salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	2.25	2.37	2.16	2.38	2.05	1.51	2.3	2.37
L-lysine	0.91	0.85	0.97	0.75	-	0.63	0.56	0.71
DL-Methioine	0.12	0.37	0.27	0.27	0.20	0.19	0.28	0.31
L-Cystine	0.13	0.18	0.12	0.10	0.09	-	0.04	0.22
L-Theronine	0.17	0.41	0.41	0.25	-	0.04	0.18	0.28
L-Tryptophan	0.1	0.07	0.07	0.03	-	0.02	0.03	0.04
L-Arginine	0.35	0.28	0.41	0.18	-	0.14	-	-
L-Glutamine	0.07	4.73	3.77	3.78	0.08	1.35	1.00	0.04
Choline chloride	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix[2]	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_2	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

[1]Corn DDGS: corn distiller's dried grains with solubles; WB: wheat bran; WG: wheat grain; WM: wheat middling; SBM: soybean meal; CNM: canola meal; CSM: cottonseed meal; PM: peanut meal

[2]Provided the following nutrients (per kg of diet): vitamin A, 10000IU; vitamin D_3, 2000IU; vitamin E, 10IU; vitamin K_3, 2.5 mg; vitamin B_1, 1.8 mg; vitamin B_2, 40 mg; vitamin B_6, 5.0 mg; vitamin B_{12}, 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 2. Analyzed composition of the *semi*-purified experimental diets (as an air-dried basis)

Item	Corn DDGS		WB		WG		WM		SBM		CNM		CSM		PM	
Phytase addition[1]	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
AME[2] (Kcal/kg)	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046	3046
Crude protein[2] (%)	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	18.00	18.00
Dry matter[3] (%)	92.51	92.18	92.43	92.54	90.82	91.07	90.22	90.82	93.97	93.84	94.04	94.17	94.60	94.75	93.34	93.58
Calcium[3] (%)	0.50	0.49	0.63	0.62	0.41	0.40	0.51	0.53	0.31	0.32	0.56	0.58	0.51	0.53	0.36	0.35
Total P[3] (%)	0.38	0.36	0.48	0.49	0.31	0.31	0.36	0.38	0.24	0.25	0.43	0.45	0.40	0.41	0.28	0.27
Calcium: P Ratio	1.32	1.36	1.31	1.27	1.32	1.29	1.42	1.39	1.29	1.28	1.30	1.29	1.28	1.29	1.29	1.30
Phytase activity[3] (FTU/kg)	0	935	1445	2515	505	1480	1570	2625	0	1426	0	1041	1604	2507	0	1335

Corn DDGS: corn distiller's dried grains with solubles; WB: wheat bran; WG: wheat grain; WM: wheat middling; SBM: soybean meal; CNM: canola meal; CSM: cottonseed meal; PM: peanut meal

[1]Microbial phytase addition (-: no addition; +: added 1000 FTU/kg diet)

[2]Calculated value

[3]Analyzed value

Animals, housing management, and experimental design

A day-old male arbor acres (AA) breed broiler chickens were obtained from Beijing arbor acres poultry breeding CO., LTD, and raised in a thermostatically controlled room of wire cages (100 × 50 × 45 cm) equipped with a feeder and nipple waterer. During the preparation period of 1 to 23 days old, 10 chicks per cage was placed and fed a commercial broiler starter diet containing 2981kcal/kg of ME, 21.45% of crude protein, 1.0% of calcium, 0.45% of non-phytate P, vitamins and minerals. The room temperature was maintained on a 23:1h light: darkness scheduled. A temperature of 32°C was maintained in the first week and gradually reduced to a constant temperature of 26°C.

A total of 612 male chickens aged 23days with relatively similar initial body weight (BW) of 939±8.89g were selected and allotted to a factorial 2 × 8 arrangement of randomized complete block design with sixteen dietary treatments and one P-free diet with six replicate cages per treatment and six chicks/cage. After 5-d of adaptation period for their respective *semi*-purified and purified P-free diet, birds were fasting for 6h to maximize feed intake and again feeding for 3h according to the procedure of Rutherfurd *et al*. (2004) with a slight modification of the fasting period. In the end, all chickens were euthanized asphyxiated using carbon dioxide exposure based on the procedure of Rodehutscord *et al*. (2012) and dissected for ileal digesta collection from distal two-thirds of the ileum (from Meckel's diverticulum to 2-cm anterior to the ileo-ceca-colonic junction) and carefully emptied the separated gut segment by flushing with distilled water to avoid the dissected portion. The collected ileal digesta samples were pooled per each cage and put in a clean sealed plastic bag and stored immediately at -20 °C for the next analysis.

Chemical analysis

For the chemical analysis of the feedstuff, diets, and ileal digesta were grounded through a 0.45-mm sieve using a grinding mill to facilitate analysis after samples were dried on forced air oven at 65°C. Sample*s* of feed (triplicate) and ileal digesta (duplicate) were analyzed for dry matter (DM*)* based on the procedure of 930.15 in AOAC (AOAC International, 2007). Crude protein, CP% (6.25 × %N) was determined using (Dumatherm, Gerhardt, and Germany). Following the ignition process at 550°C in a muffle furnace of diet and digesta, the leftover ash was extracted with 4N-HCl and the P concentration was determined using a UV-visible spectrophotometer (Model UV-1780, Shimadzu, Japan). The atomic absorption spectrometer (Model novAA® 400P, Analytikjena, Germany) was also implemented to determine the calcium concentration of the feedstuffs and diets. Phytate P was analyzed based on the procedure of Akinmusire and Adeola (2009). The concentration of phytate in the test materials was calculated as 28.2% of the phytic acid concentration (Tran and Sauvant, 2004). The non-phytic phosphorus concentration is the difference between total phosphorus and phytate. Diet and ileal digesta samples were analyzed for acid-insoluble ash (AIA) following the procedure of Keulen and Young (1977).

Calculations

The apparent ileal digestibility (AID) of P was estimated based on the external indigestible marker method equation of Dilger and Adeola (2006b) representing for each testing diet with or without phytase supplementation

$$AID,\% = 100 - ((\frac{P_{ilealdigesta}}{P_{feed}}) X (\frac{Marker_{feed}}{Marker_{ilealdigesta}}) X 100) \quad \text{.....(1)}$$

Where: $P_{ilealdigesta}$ is the value of ileal digesta phosphorus, the P_{feed} is the value of the feed phosphorus, $Marker_{feed}$ is the external indigestible marker of the feed (g) and the $Marker_{ilealdigesta}$ is the external indigestible marker of the ileal digetsa (g).

The ileal endogenous phosphorus loss (EPL) was determined from the phosphorus content of ileal digesta collected from broilers fed a phosphorus-free diet based on the formula used by Rutherfurd *et al*. (2002)

$$EPL\ (mg/kg\ DMI) = (\frac{Total\ P_{digesta}(mg/kg)\ XMarker_{digesta}(mg/kg)}{Marker_{P-free\ diet}(mg/kg)} \quad \text{.....(2)}$$

Where total $P_{digesta}$ is the total phosphorus of digesta (mg/kg), $marker_{digesta}$ is the digesta of external indigestible marker (mg/kg); marker P-free diet is an external indigestible marker of the phosphorus-free diet (mg/kg).

The AID of P was corrected using the ileal EPL value and the standardized ileal digestibility (SID) of P calculation was obtained based on the formula used by Fan *et al*. (2001)

$$SID\% = AID\% + (\frac{Basal\ ileal\ EPL}{P_{feed}}) \quad \text{.....(3)}$$

Where the ileal EPL determined from ileal digesta is measured by (mg/kg DMI) and P_{feed} is also calculated as (mg/kg DMI) and both changed to percentage and added to the AID% value.

Statistical analysis

The normality of data was analyzed using the GLM procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Experimental cages were served as an analysis unit for all statistical analyses and alpha levels of 0.05 were used to determine significance among means. Two way ANOVA procedures of SAS were used to analyze all data and outliers were tested using the UNIVARIATE procedure in SAS, but no outlier was observed. Tukey's adjustment was used to separate multiple comparisons among means if differences were observed.

Results

Composition of ingredients

The chemical composition of experimental feed ingredients is presented in Table 3. The phytate P% in total P content of the feed ingredients was ranged from the least content of 6.67% in corn DDGS to the highest value of 81.58% in PM followed by 79.12% in WB. The others had about 67.45% of phytate P, which has from 62.80% in SBM to 73.00% in CNM.

Table 3. The chemical composition of experimental feed ingredients fed to broilers (air-dried basis)

Item, %	Corn DDGS	Wheat bran	Wheat grain	Wheat middling	Soybean meal	Canola meal	Cottonseed meal	Peanut meal
Dry matter	90.74	91.56	89.69	89.57	91.42	89.68	92.58	92.01
Crude Protein	17.06	16.60	15.60	17.16	44.31	35.71	39.64	47.43
Calcium	0.19	0.16	0.09	0.13	0.33	0.65	0.28	0.27
Total P	0.75	0.91	0.42	0.56	0.59	1.00	0.98	0.76
Phytate	0.18	2.55	1.03	1.31	1.30	2.60	2.30	2.20
Phytate P[1]	0.05	0.72	0.29	0.37	0.37	0.73	0.65	0.62
Phytate P of total P	6.67	79.12	69.05	66.07	62.80	73.00	66.33	81.58
Non-phytate P[2]	0.70	0.19	0.13	0.19	0.22	0.27	0.33	0.14
Non-phytate P of total P	93.33	20.90	30.95	33.93	37.29	27.00	33.67	18.42

[1]Phytate P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).
[2]Non-phytate P was calculated as the difference between total P and phytate P.
P: phosphorus; Corn DDGS: corn distiller's dried grains with solubles.

Ileal endogenous loss of phosphorus

The ileal endogenous phosphorus loss (EPL) was 46.28mg/DMI (Table 4). The intake of P from a P-free diet was ignored because of the extremely low levels (only 1.5 mg).

Table 4. Ileal endogenous phosphorus losses in the ileal digesta were calculated by phosphorus-free diet method[1]

Parameter	Intake of P, g	Ileal digesta of P, g	Ileal digesta of P, mg/kg DMI	Ileal endogenous loss of P, mg/kg DMI
P-free diet	0	0.027±0.02	46.28±12.79	46.28±12.79

[1]Means±SD (standard deviation); P: phosphorus.

Phosphorus digestibility of feedstuffs

Birds of all experimental cages remained healthy and readily consumed their diets. The current study finding indicated that there was a significant difference *($P < 0.001$)* of SID of P among the different s*emi*-purified experimental diets with and without microbial phytase. CSM, CNM, corn DDGS, and SBM showed higher P digestibility, which is from 46.09% to 52.01% for SID. However, the other feedstuffs had below 40% of P digestibility, which was from 35.69% to 38.46% of SID (Table 5). The addition of 1000FTU/kg microbial phytase to the *sem*i-purified diets was reported significant improvement *($P < 0.001$)* of P digestibility. The higher relative improvement of SID of P with the application of phytase was recorded in SBM (52.43%), followed by WM (43.67%). The least improvement was observed in corn DDGS meal (5.12%) (Table 5). The trend was similar for the AID of P. In the current study, the ileal digestible P equivalent relationship between digestible P and phytase was established. The equivalent value of ileal digestible P at 1000FTU/kg microbial phytase was shown great variation within the different feedstuffs, which is ranged from 0.02% in corn DDGS to 0.16% cottonseed meal (Table 6).

Table 5. Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of phosphorus for corn DDGS, wheat bran, wheat grain, wheat middling, soybean, canola, cottonseed, and peanut meals with or without phytase fed to 28-29 day old broiler chickens (%)[1]

Ingredient	Phytase[2]	Apparent ileal digestibility of P	Increment[3]	Standardized ileal digestibility of P[4]	Increment
Corn DDGS	-	48.21^{a}	-	49.41^{a}	-
	+	50.66^{c}	5.11	51.93^{c}	5.12
Wheat bran	-	37.49^{bcd}	-	38.46^{bcd}	-
	+	51.68^{c}	38.41	52.61^{c}	37.36
Wheat grain	-	35.42^{cd}	-	36.90^{cd}	-
	+	48.24^{c}	36.59	49.74^{c}	35.15
Wheat middling	-	34.42^{d}	-	35.69^{d}	-
	+	49.92^{c}	45.50	51.13^{c}	43.67
Soybean meal	-	50.08^{a}	-	52.01^{a}	-
	+	76.38^{a}	54.73	78.23^{a}	52.43
Canola meal	-	45.94^{ab}	-	47.02^{ab}	-
	+	60.29^{b}	32.44	61.32^{b}	31.55
Cottonseed meal	-	44.93^{abc}	-	46.09^{abc}	-
	+	61.61^{b}	38.93	62.71^{b}	37.77
Peanut meal	-	36.32^{bcd}	-	37.98^{bcd}	-
	+	46.34^{c}	33.89	48.06^{c}	32.15
P-value	-	***	-	***	-
	+	***	**	***	**
SEM	-	1.12	-	1.12	-
	+	1.49	3.26	1.50	3.09
Probability of ANOVA					
Ingredients		***	-	***	-
Phytase		***	-	***	-
Ingredient*phytase		***	-	***	-

SEM: the standard error of the mean; P: phosphorus; Corn DDGS: corn distiller's dried grains with solubles

$^{**}P < 0.01$; $^{***}P < 0.001$

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

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

[3)] The increment represents the relative magnitude of the improvement by microbial phytase addition.

[4)] Values for SID were calculated by correcting values of AID for average ileal EPL value of 46.28 (mg/kg DMI) determined using a P-free diet (n=36)

Table 6. The equivalency of phytase on standardized ileal digestibility of phosphorus (%) in feedstuffs of plant origin with 1000FTU/kg or without phytase addition for 28-29 day old broiler chickens[1]

Ingredient (%)	Phytase[2]	Standardized ileal digestible P	Equivalency of phytase[3]
Corn DDGS	-	0.37^{bc}	0.02
	+	0.39^{cd}	-
Wheat bran	-	0.35^{cd}	0.13
	+	0.48^{b}	-
Wheat grain	-	0.16^{e}	0.05
	+	0.21^{f}	-
Wheat middling	-	0.20^{e}	0.09
	+	0.29^{e}	-
Soybean meal	-	0.31^{cd}	0.15
	+	0.46^{bc}	-
Canola meal	-	0.47^{a}	0.14
	+	0.62^{a}	-
Cottonseed meal	-	0.45^{ab}	0.16
	+	0.61^{a}	-
Peanut meal	-	0.29^{d}	0.08
	+	0.36^{d}	-

P: phosphorus; Corn DDGS: corn distiller's dried grains with solubles.
1) The equivalency of phytase on ileal digestible phosphorus is calculated by the ileal digestibility of phosphorus and total phosphorus in ingredients.
2) Microbial phytase was added in the diets containing phytase at 1000FTU/kg complete feed (-, no phytase; +, with 1000FTU/kg microbial phytase).
3) Equivalency of phytase shows an increase of ileal digestible phosphorus on account of 1000 FTU/kg phytase addition.

Discussion

Composition of ingredients

The analyzed value of calcium, P, and phytate P in most experimental feed ingredients was within the range reported in the previous literature (Eeckhout and De Paepe 1994; NRC, 1994; She *et al*., 2015). Consistent value of non-phytate P was reported for WM and corn DDGS (Eeckhout and De Paepe, 1994) and SBM, CNM, CSM, PM, WG, and WB (NRC, 1994). In the current study, the phytase activity was not detected in SBM, CNM, PM, and corn DDGS based diets. This value was in line with reported data from 0 to120 in SBM, from 0-36 in CNM, and 0-8 FTU/kg in PM (Eeckhout and De Paepe, 1994). For the corn DDGS meal from 260-850 FTU/kg phytase activity was reported in previous research studies (Eeckhout and De Paepe, 1994; Mutucumarana*et al*., 2014; She *et al*., 2015). Plant phytase activity is not effective as exogenous phytase for the reason of in vivo narrow pH spectrum and inactive at lower pH (Phillippy, 1999) and destruction of phytase in feed ingredients at high pelleting temperatures (Eeckhout and De Paepe, 1994). It was reported that the phytase activity of the wheat origin ingredients concurred with the published data for WG (Selle *et al*., 2003),WB, and WM (Eeckhout and De Paepe, 1994).

However, there are previous research studies that reported a higher value of phytase activity for WG, WB, and WM (Eeckhout and De Paepe, 1994; Viveros *et al*., 2000). The current study was determined higher phytase activity in the CSM (1604 FTU/kg) compared to the report of 5-80 FTU/kg (Selle *et al*., 2003; Selle and Ravindran, 2007). This variation maybe created due to the difference in the processing method undertaken for CSM products. The CSM used in the current study was detoxified below 80□, while the detoxification temperature of traditional CSM was up to 130□. The partial or total inactivation of plant endogenous phytase activity was reported through the heating process of 70-80℃ and the pH value < 2.5 (Nernberg, 1998).

Ileal endogenous loss of phosphorus

The determined ileal endogenous phosphorus loss (EPL) of the current study was higher than the reported literature of (25.1mg/kg DMI) (Mutucumarana, 2014), that determined using P-free dextrose based diet fed for male broiler chickens of Ross 308 and (-290 mg/kg DMI) (Iyayi and Adeola, 2013), which was detected usin gregression of peanut flour fed for broiler chickens of Ross 708. On the other hands, higher ileal endogenous phosphorus loss of 104 mg/kg DMI (Mutucumarana, 2014) and 209 mg/kg DMI (Dilger and Adeola, 2006a) have been reported on broiler chickens of Ross 308, which was detected using minimal P gelatin-based and regression of conventional soybean meal-based diets, respectively. As indicated above the main factors that created discrepancy among the different published experimental reports related to ileal EPL value maybe including the variation of dietary, animal, and research protocol approaches (Rutherfurd *et al*., 2004; Dilger and Adeola, 2006a; Mutucumarana, 2014;).

Phosphorus digestibility of feedstuffs

The determining apparent and standardized ileal P digestibility value at different feed ingredients of the current study was showed wide variation. Commonly the two-third of P in plant origin feed ingredients is in the form of phytate P and only one third was considered as available phosphorus value. However, most of the feedstuffs used in the present studies have higher AID of P ranged from 34.42% in WM to 50.08% in SBM. According to the previously reported literature data, the phytate P concentration and P digestibility had a strong negative relationship (Almaguer *et al*., 2014) and the variation of P digestibility among the different feed ingredients can be associated with phytate P and non-phytate P concentration of feedstuffs (Nernberg, 1998). However, the current study results had no maintained the above concept. Our observation explains that the digestibility of P maybe related to the chemical structure of ingredients, physical properties, endogenous phytase activity, the bond form of phytate, quality of raw materials, type of technological processing, and other unidentified factors. The observed difference in P digestibility between wheat-based diets and oilseed based diets except for peanut meal of the current study was supported by the previous research study of Selle and Ravindran (2007), which had reported as cereals had low P digestibility for the reason of their phytate P form and location (aleurone or fiber) compared to the oilseed diets.

The AID of P determined for SBM (50.08 %) concurred to the reported data range of 14-67% (Mutucumarana *et al*., 2014) and 49% (Rutherfurd *et al*., 2002). However, it is lower than the range value of 71.2 to 88.8% (Dilger and Adeola 2006a) and reported data from 64 to 90% (Liu *et al*., 2013). Similarly, the SBM SID of P agreed with the report of Rutherfurd *et al*. (2002). The standardized ileal digestibility of P in CNM was lower than the report of Mutucumarana *et al*. (2014) but higher than the reported data of Rutherfurd *et al*. (2002). A higher value of true digestibility of P with and without phytase in peanut flour (Iyayi and Adeola, 2013) and in wheat grain (Rutherfurd *et al*., 2002; Wu *et al*., 2004) had been reported. Compared to the current study, it has been reported that higher true P digestibility in corn DDGS (Mutucumarana *et al*., 2014). This maybe related to the physical, chemical, and nutritional variability of DDGS supply from different sources (Cromwell *et al*., 1993) for the reason of the implemented ethanol fermentation method and drying process temperature difference among industries (Salim *et al*., 2010). To our knowledge, there is no published literature data related to the ileal digestibility of P in CSM, WB, and WM fed to broiler chickens by now.

The superior ileal P digestibility value observed in SBM and corn DDGS meals can be explained to the readily available of phytate P in more soluble form and evenly distributed in SBM (Selle and Ravindran, 2007) and the partial hydrolysis of phytate due to the fermentation process undergo for corn DDGS (Eeckhout and De Paepe, 1994; Salim *et al*., 2010). Moreover, the variation of feedstuffs P digestibility observed in different research studies maybe because of variability in phytate P content, the composition, physical and chemical properties of diets (Mutucumarana *et al*., 2014), feed quality (Selle and Ravindran, 2007), technological processing, and plant phytase activity (Eeckhout and De Paepe, 1994), the type and age of animals (NRC, 1994), and other unknown factors.

To improve the digestibility of P of different feedstuffs, phytase supplementation is effective in the diet of poultry. Expressing of P equivalency of phytase as the total non-phytase P from an inorganic source of phosphate was commonly practiced to estimate the potential amount of P made available through the application of phytase to a diet. In the current study, the effect of phytase on different plant origin ingredients was addressed and a great variation of phytase efficiency among the feedstuffs was observed. Similar to our study findings, digestible P equivalence of 1.48g (Adeola and Walk, 2013) at the ileal level and ranged value from 0.87g to 2.5g (Adedokun *et al*., 2004; Selle and Ravindran, 2007) with response criteria of weight gain, toe ash has been reported at 1000 U/kg phytase addition. The variation of phytase phosphorus equivalence between feedstuffs within the current study and other published data can be explained by the phytate bond phosphorus concentration and dietary composition, respectively. The application of microbial phytase to different plant origin feedstuffs fed to broiler chickens greatly

influenced the digestibility of P, which is economically and environmentally feasible in the poultry sector.

Conclusion

The findings of the current study indicated that the SID value of P for SBM (52.01%), corn DDGS (49.41%), CNM (47.02%), and CSM (46.09%) was higher than values for WB (38.46%), PM (37.98%), WG (36.90%), and WM (35.69%). Application of microbial phytase to the *semi*i-purified diets has significantly improved the SID of P in the feedstuffs, even though the susceptibility of the feedstuffs to phytase was greatly varied and more than 40% improvement was observed in SBM and WM followed by CNM, PM, WG, WB, and CSM based diets with improvement range from 31.55% to 37.77%. The least improvement was shown in corn DDGS (5.12%). The ileal digestible P equivalence of 1000FTU/kg related to the different feedstuffs was from 0.2 g in corn DDGS to 1.6g in CSM.

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