

# Research Note: Phosphorus digestibility in conventional canola meal determined using different balance assays

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**ABSTRACT** Three experiments were conducted to determine ileal P digestibility and excreta P retention values for canola meal (CM) using 3 different types of balance assays. The first experiment was an ad libitum-fed chick experiment which evaluated the effect of phytase on ileal P digestibility and excreta P retention values. Chicks were fed a P-deficient cornstarch-dextrose-45% CM basal diet (0.13% nonphytate P) as diet 1 or that diet plus 125 or 250 FTU/kg of phytase, respectively, from 8 to 21 D of age. The digestibility/retention of P was 38% and phytase linearly increased both ileal digestibility and excreta retention of P ( $P < 0.05$ ). The second experiment was a precision-fed chick assay conducted to determine ileal digestibility of P in CM at 21 D. Mean ileal P digestibility was determined to be 47.5% in chicks fed 6 g and 40.0% in chicks fed 9 g of CM and the values were not

significantly different. Experiment 3 was an ad libitum-fed chick assay to determine ileal P digestibility and excreta P retention for CM with and without increasing levels of dietary supplemental Ca. The chicks were fed P-deficient - dextrose - CM diets containing increasing levels of 13.5, 27, 40.5, or 54% CM, respectively, with Ca:nonphytate P ratio maintained at 2:1 in diets 1–4 and 6:1 in diets 5–8. Based on regression analysis of ileal digesta or excreta P output on dietary P concentration, digestibility/retention of P in CM was 30%. Ileal P digestibility (and to a lesser extent excreta P retention) at 21 D was reduced by increased Ca:P ratio. The results of this study indicated that the 3 balance assays yielded reasonably consistent values of 30–40% for P digestibility/retention and ileal P digestibility was greatly affected by Ca:P ratio.

**Key words:** canola meal, phosphorus, chick, rooster, digestibility, retention

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## INTRODUCTION

There has been little research conducted concerning P digestibility in canola meal (CM) for poultry. This is likely because CM inclusion in poultry diets has traditionally been limited owing to its high fiber and glucosinolate content. However, with new CM varieties that possess higher protein and lower fiber concentrations (Chen et al., 2015), it may be possible, in the future, to feed CM to poultry at greater amounts than has been done in the past. The small amount of research that has been conducted on the digestibility or bioavailability of P in CM has produced varying results. The NRC (1994) lists the total P content in CM at 1.17% and the nonphytate P (NPP) content at 0.30%. By dividing the NPP by the total P content, it can be estimated that

approximately 25% of the total P in CM is digestible or bioavailable. In a chick bioassay conducted by Mutucumarana et al. (2014), ileal digestibility of P in CM was 47% and excreta P retention was 49%, almost double the value calculated from the NRC (1994) total P and NPP values. Other studies on P digestibility and bioavailability for CM have reported highly variable values with Hanna et al. (2018) reporting a P bioavailability value based on chick bone ash of only 15% compared with Combemorel et al. (2015) who reported a true ileal P digestibility value of 43%.

Kim et al. (2011) developed a precision-fed ileal chick assay for determining amino acid digestibility that may also be useful for determining ileal digestibility of P in feed ingredients for poultry. Three-week-old broiler chickens were used in the latter study. The broiler chickens were fasted for at least 8 h before tube feeding and then approximately 10 g of feed ingredient was tube-fed and ileal digesta were collected at 4 h after feeding. This assay was successful in determining digestibility values for amino acids; however, no research has been conducted using this procedure to determine digestibility of P.

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**Table 1.** Ingredient composition of diets in experiment 3 (%) (as-fed basis).

Ingredient	Dietary treatments							
	1	2	3	4	5	6	7	8
Dextrose	81.25	67.75	54.25	40.75	80.85	66.95	53.05	39.15
Canola meal	13.50	27.00	40.50	54.00	13.50	27.00	40.50	54.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	-	-	-	-	0.40	0.80	1.20	1.60
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix <sup>1</sup>	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix <sup>2</sup>	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
NaHCO <sub>3</sub>	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
TiO <sub>2</sub>	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Analysis <sup>3</sup>								
Calcium, calculated	0.08	0.16	0.24	0.32	0.24	0.48	0.72	0.96
Nonphytate P, calculated	0.04	0.08	0.12	0.16	0.04	0.08	0.12	0.16
Total P, analyzed	0.14	0.27	0.42	0.54	0.14	0.28	0.42	0.57

<sup>1</sup>Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- $\alpha$ -tocopheryl acetate, 11 IU; vitamin B<sub>12</sub>, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

<sup>2</sup>Provided as milligrams per kilogram of diet: manganese, 75 from MnSO<sub>4</sub>·H<sub>2</sub>O; iron, 75 from FeSO<sub>4</sub>·H<sub>2</sub>O; zinc, 75 from ZnO; copper, 5 from CuO<sub>4</sub>·5H<sub>2</sub>O; iodine, 75 from ethylenediamine dihydroiodide; selenium, 0.1 from Na<sub>2</sub>SeO<sub>3</sub>.

<sup>3</sup>The canola meal was analyzed to contain 0.61% Ca, 1.10% total P, and 0.29% nonphytate P.

Therefore, the purpose of the experiments conducted in this study was to evaluate and compare 2 ad libitum-fed chick assays and a precision-fed chick assay for determining ileal P digestibility values and/or excreta P retention values for CM and also the effect of phytase enzyme on ileal digestibility and excreta retention values.

## MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use committee.

### Chemical Analyses

A sample of solvent-extracted CM was obtained from a commercial company and the same sample was used in all animal assays. The CM sample was the same as the conventional CM used in experiment 2 and 3 of the study by Hanna et al. (2018). A detailed nutrient analysis of the CM is presented in the latter paper. In summary, the CM contained 40.2% CP, 18.9% NDF, 14.3% ADF, 0.62% Ca, 1.20% total P, and 0.29% NPP on an 88.9% DM basis (as-fed). The P analyses of the experimental diets, ileal digesta, and excreta were determined using inductively coupled plasma spectroscopy (Method 985.01 A, B, and C; AOAC International, 2007) after wet ashing (Method 975.03 B[b]; AOAC International, 2007). Titanium content of diets, ileal digesta, and excreta were determined using UV spectroscopy (Myers et al., 2004). All analyses were conducted at the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratory.

### Diets and Experimental Design

In experiment 1, the apparent ileal digestibility and excreta P retention values for CM and the effect of

phytase enzyme on the values were determined in ad libitum-fed chicks. A total of 75 New Hampshire x Columbian male chicks were fed a nutritionally complete corn-soybean meal starter diet for 7 D. On day 7, the chicks were fasted overnight before being placed on the experimental diets. On day 8, the chicks were weighed, wing banded, and allotted to the 3 dietary treatments via a complete randomized design with a similar mean body weight across treatments. There were 5 chicks per pen with 5 replicate pens per treatment. Diet 1 was a P-deficient cornstarch-dextrose-45% CM basal diet calculated to contain 0.13% NPP and CM was the only source of P in the diet. The detailed composition of this diet is shown in the Hanna et al. (2018) study (conventional CM diet in experiment 3, Table 1 of that study). This diet was used because it contains a high level of CM and it was the same diet used earlier by Hanna et al. (2018) to evaluate the effects of phytase enzyme on P bioavailability in CM using a slope-ratio chick bone ash assay. Thus, using this diet and feeding it for the same amount of time (8–21 D of age) allowed for a direct comparison between 2 different experimental methods, namely the chick bone assay used by Hanna et al. (2018) vs. the ileal digestibility/excreta retention balance assay used herein. Diets 2 and 3 were the basal diet plus 125 or 250 FTU/kg of phytase, respectively. The phytase was OptiPhos from Huvepharma, Sofia, Bulgaria, and one unit of phytase (FTU) is equal to the amount of enzyme catalyzing the release of 1.0 µmol/min of P from an excess of sodium phytate substrate at pH 5.5 and 37°C. Titanium dioxide was added to each diet at a level of 0.4% as an indigestible marker. The chicks were housed in an environmentally controlled room and in thermostatically controlled starter batteries with raised wire flooring. From days 8–21 of age, the experimental diets and water were available for ad libitum consumption. On day 22 of age, chicks were euthanized by CO<sub>2</sub> inhalation. Ileal digesta

from Meckel's diverticulum to the ileal-cecal junction were collected. Excreta were also collected once from pans under the cages on the same day. Ileal digesta and excreta were frozen and stored at  $-20^{\circ}\text{C}$ , freeze-dried, ground with mortar and pestle, and analyzed for P and Ti.

The second experiment was a precision-fed chick assay (Kim et al., 2011) which was conducted to determine the apparent ileal digestibility of P in CM. Ross 308 broiler chicks were fed a nutritionally complete corn-soybean meal starter diet from days 1 to 20 of age and housing was the same as for experiment 1. After feed withdrawal for 10 h on day 21, the chicks were tube-fed 3, 6, or 9 g of CM with 4 pens of 4 chicks assigned to each CM level. Different levels of CM were tube-fed in an attempt to evaluate the effect of P intake level on P digestibility and to use regression analysis to estimate P digestibility. Titanium dioxide was added to the CM at a level of 0.4% as an indigestible marker. Chicks were euthanized by  $\text{CO}_2$  inhalation at 4 h after feeding and ileal digesta from Meckel's diverticulum to the ileal-cecal junction were collected. Ileal digesta were freeze-dried, ground, and analyzed for P and Ti.

Experiment 3 was an ad libitum-fed chick assay conducted to determine ileal P digestibility and excreta P retention for CM and to evaluate the effect of the Ca:NPP ratio on P digestibility and retention. Two hundred Ross 308 broiler chicks were fed a nutritionally complete corn-soybean meal starter diet from days 1 to 14. On day 15, the chicks were weighed, wing banded, and allotted to the 8 dietary treatments via a complete randomized design with a similar mean body weight across treatments. There were 5 chicks per pen with 5 replicate pens per treatment. Diet 1 was a P-deficient dextrose-CM basal diet calculated to contain 0.04% NPP (Table 1). Diets 1–4 contained graded concentrations of CM which were 13.5, 27, 40.50, and 54% and the Ca:NPP ratio was 2:1. Diets 5–8 were the same as diets 1–4 but had additional Ca from 0.4, 0.8, 1.2, or 1.6% added limestone, respectively, to maintain a 6:1 ratio of Ca:NPP. Diets 1–4 were formulated to be the same as those used by Mutucumarana et al. (2014) and the 6:1 Ca:NPP ratio in diets 5–8 is similar to the Ca:NPP ratio in the negative control diet used in the slope-ratio bone ash assay in the Hanna et al. (2018) study. Thus, the diets in the present study were designed so that the digestibility results obtained herein could be directly compared to the CM digestibility results of Mutucumarana et al. (2014), to evaluate the effects of increasing dietary Ca on P digestibility values for CM, and to compare the digestibility results herein to the chick bone-ash assay results of Hanna et al. (2018). On day 22 of age, chicks were euthanized by  $\text{CO}_2$  inhalation. Ileal digesta from Meckel's diverticulum to the ileal-cecal junction were collected. Excreta were also collected on days 21 and 22. Ileal digesta and excreta were frozen and stored at  $-20^{\circ}\text{C}$ , freeze-dried, ground with mortar and pestle, and analyzed for P and Ti.

**Table 2.** Apparent ileal digestibility and excreta retention values for P in canola meal in experiment 1.<sup>1</sup>

Dietary treatment	Ileal digestibility (%) <sup>4</sup>	Excreta retention (%) <sup>4</sup>
1. Basal diet (B) <sup>2</sup>	38.0 <sup>a</sup>	38.7 <sup>b</sup>
2. B + 125 FTU/kg <sup>3</sup>	44.8 <sup>a</sup>	47.3 <sup>a</sup>
3. B + 250 FTU/kg <sup>3</sup>	47.6 <sup>a</sup>	51.0 <sup>a</sup>
Pooled SEM	3.33	1.82

<sup>a-b</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 5 pens per dietary treatment with 5 chicks per pen at 21 D of age.

<sup>2</sup>45% conventional canola meal diet in Table 1, experiment 3, of Hanna et al. (2018).

<sup>3</sup>Level of phytase provided from Optiphos, Huvepharma, Sofia, Bulgaria.

<sup>4</sup>Significant linear effect of phytase ( $P < 0.05$ ).

## Statistical Calculations and Analysis

Apparent ileal P digestibility and apparent excreta P retention values for individual diets in experiments 1–3 were calculated using the indicator ratio equation of Mutucumarana et al. (2014). Data from all 3 experiments for individual dietary treatments were analyzed using the ANOVA procedure of SAS (SAS Institute, Inc., 2011) with pen of chicks as the experimental unit. Differences among treatment means were assessed using the least significant difference test. Significance was assessed at  $P < 0.05$ . In addition, linear regression analysis was used in experiment 1 to determine if there was a significant linear effect of phytase level on ileal P digestibility and excreta P retention. Linear regression analysis was also used for experiment 3 where regression of ileal or excreta P excretion on total P intake was computed. The regression coefficient (P indigestibility) was then subtracted from one to calculate ileal P digestibility and excreta P retention values. This was the same procedure that was used by Mutucumarana et al. (2014) for estimating ileal digestibility and excreta retention values of P in CM.

## RESULTS AND DISCUSSION

### Experiment 1

Ileal digestibility and excreta retention values for P in CM from the basal diet (no supplemental phytase) were similar (Table 2). There were no significant differences among individual dietary treatments for ileal P digestibility with inclusion of 125 or 250 FTU/kg of phytase. Although there were no significant differences among the individual treatments, there was a linear increase for phytase on ileal P digestibility ( $P < 0.05$ ). The inclusion of phytase had a significant effect on excreta P retention values. The retention value for the basal diet was 38.7% which was significantly lower ( $P < 0.05$ ) than the values obtained from birds fed diets supplemented with phytase. There was also a significant linear effect ( $P < 0.05$ ) of phytase on excreta P retention values. Leske and Coon (1999) and Rutherford et al. (2002) also reported that phytase

**Table 3.** Apparent ileal digestibility values for P determined in chicks tube-fed 6 or 9 g of canola meal in experiment 2.<sup>1</sup>

Canola meal (g)	Digestibility (%)
6	47.5
9	40.0
Pooled SEM	4.25

<sup>1</sup>Values are means of 4 pens of 4 chicks at 21 D of age. There was no significant difference in digestibility values between the 2 canola meal levels.

increased P digestibility or retention in CM and rapeseed meal, respectively. The ileal P digestibility value of 38.0% and excreta P retention value of 38.7% for CM without phytase are somewhat lower than those published by Mutucumarana et al. (2014) who found ileal P digestibility to be 46.9% and excreta P retention to be 48.6% when using regression analysis for birds fed increasing dietary levels of CM.

## Experiment 2

The ileal P digestibility values for chicks tube-fed 6 or 9 g of CM are presented in Table 3. There was not enough ileal digesta present in any of the repetitions of birds fed 3 g of CM to do both P and Ti analysis; thus, this level of feed intake is too low for the tube-feeding ileal digestibility chick assay unless a larger number of birds are used. The ileal P digestibility values for chicks tube-fed 6 or 9 g of CM did not differ significantly from each other. The ileal P digestibility value for chicks fed 6 g of CM was 47.5% and was 40.0% for chicks fed 9 g. These ileal P digestibility values are generally similar to those ranging from 37 to 47% obtained in experiment 1 and are also similar to the ileal P digestibility values reported by Mutucumarana et al. (2014), Rutherford et al., (2002) and Combemorel et al. (2015) for CM fed to broiler chickens.

## Experiment 3

The ileal digestibility and excreta retention values for P in CM are shown in Table 4. The ileal digestibility value for birds fed the 13.5% CM diet was 58.9% and digestibility was significantly decreased ( $P < 0.05$ ) for the 54.0% CM diet. Ileal digestibility of P was decreased ( $P < 0.05$ ) by increasing Ca:NPP ratio at all respective dietary CM levels. A study with the same CM levels as diets 1–4 in the present study was conducted by Mutucumarana et al. (2014) and ileal P digestibility values ranged from 51 to 68% among their 4 diets.

Increasing CM or Ca:NPP ratio did not have as great of an effect on excreta P retention values as for ileal P digestibility values. Thus, there was no consistent effect of CM level on excreta P retention in diets 1–4 which contained the 2:1 Ca:NPP ratio. For diets 5–8 which contained the increased Ca:NPP ratio, excreta P retention was decreased ( $P < 0.05$ ) only at the 40.5 and 54.0% CM levels. Similarly, increased Ca:NPP ratio reduced excreta P retention at the same 40.5 and 54.0% CM

**Table 4.** Apparent ileal digestibility and excreta retention values for P in canola meal in experiment 3.<sup>1</sup>

Dietary treatment <sup>2</sup>	Ileal digestibility (%)	Excreta retention (%)
1. 13.5% canola meal	58.9 <sup>a</sup>	36.5 <sup>a,b</sup>
2. 27.0% canola meal	54.9 <sup>a</sup>	39.3 <sup>a</sup>
3. 40.5% canola meal	46.5 <sup>a,b</sup>	41.1 <sup>a</sup>
4. 54.0% canola meal	37.8 <sup>b,c</sup>	29.1 <sup>b,c</sup>
5. As 1 + Ca	30.5 <sup>c</sup>	39.1 <sup>a</sup>
6. As 2 + Ca	30.7 <sup>b,c</sup>	42.9 <sup>a</sup>
7. As 3 + Ca	7.8 <sup>d</sup>	26.7 <sup>c,d</sup>
8. As 4 + Ca	11.2 <sup>d</sup>	20.4 <sup>d</sup>
Pooled SEM	5.50	2.98

<sup>a–d</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Values are means of 5 pens per dietary treatment with 5 chicks per pen at 21 D of age.

<sup>2</sup>Diets 1–4 have a Ca:nonphytate P ratio of 2:1 and diets 5–8 have a Ca:nonphytate P ratio of 6:1.

levels but not at the 2 lower CM levels. Interestingly, ileal P digestibility values were numerically higher than excreta P retention values for diets 1–4 (Ca:NPP ratio of 2:1) but the opposite occurred for the high Ca diets 5–8 (Ca:NPP ratio of 6:1). The explanation for these results is unknown. Mutucumarana et al. (2014) reported ileal P digestibility values that were similar to or only slightly lower than excreta P retention values for diets with the same composition as diets 1–4 used in the present study.

Linear regression analysis of P output in ileal digesta or excreta indicated a high linear relationship with  $R^2$  values of 0.91–0.97 (Table 5). The slope values of the regression equation (P indigestibility) yielded digestibility/retention coefficients of 0.29–0.30 for the diets containing a Ca:NPP ratio of 2:1. These results indicated that approximately 30% of the P in the CM was digestible or retainable. By contrast, for diets 5–8 that contained a higher 6:1 Ca:NPP ratio, the regression equations yielded very large slope values, near 1.00, resulting in ileal digestibility or excreta retention values of approximately zero. These results indicated that the high level of Ca in the latter diets greatly reduced the ileal digestibility and excreta retention of the P in CM and that the regression analysis method did not yield reasonable values and may not be appropriate for these types of diets. For the diets containing a 2:1 Ca:NPP ratio, the digestibility/retention values of 29–30% are somewhat lower than the values of 47–49% reported by Mutucumarana et al. (2014) for CM when using the same diet compositions and regression procedure used herein. Part of the reason for this difference between studies could be that different samples of CM were used in the 2 studies.

In summary, when there was no phytase enzyme included in the diets and the dietary Ca:NPP ratio was not artificially high at 6:1, the 3 balance assays evaluated herein generally yielded ileal P digestibility and excreta P retention values of 30–40% for CM (diet 1 in experiment 1, 9 g CM intake in experiment 2, and regression analysis for diets 1–4 in experiment 3). Phytase increased P digestibility/retention values for CM and increasing dietary Ca greatly reduced ileal

**Table 5.** Linear regression of ileal or excreta P output on total dietary P content for canola meal in experiment 3.

Item	Regression equation <sup>1</sup>	SE <sup>2</sup> of the Slope	SE <sup>2</sup> of the Intercept	R <sup>2</sup>	Digestibility/retention Coefficient
Diets 1-4, 2:1 Ca:NPP ratio:					
True ileal P digestibility	Y = 0.70X -0.54	0.05	0.20	0.91	0.30
True excreta P retention	Y = 0.71X -0.23	0.04	0.15	0.95	0.29
Diets 5-8,6:1 Ca:NPP ratio:					
True ileal P digestibility	Y = 1.06X -0.66	0.06	0.23	0.95	-0.06
True excreta P retention	Y = 0.93X -0.66	0.04	0.04	0.97	0.07

<sup>1</sup>Regression of ileal digesta or excreta P output (g/kg dry matter intake) on dietary P content (g/kg) determined by feeding diets containing graded levels of canola meal in diets containing different Ca:nonphytate P (NPP) ratios. See dietary treatments in Table 4. The slope represents true P indigestibility. The ileal digestibility and excreta retention coefficients were calculated by subtracting the slope values from one.

<sup>2</sup>SE = standard error.

P digestibility. The P digestibility and retention values of 30–40% obtained in the balance assays were higher than the relative bioavailability value of 15% obtained in an earlier study for the same CM when using a chick-growth tibia ash assay (Hanna et al., 2018). Thus, P bioavailability values may differ among types of assays and dietary level of Ca.

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

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