



Original Research Article

Pre-cecal phosphorus digestibility for corn, wheat, soybean meal, and corn gluten meal in growing Japanese quails from 28 to 32 d of age

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ABSTRACT

The optimization of dietary phosphorus (P) depends on precise details of the P availability in feed ingredients to avoid excess or deficient P in a mixed diet. This study was carried out to measure the apparent ileal digestibility of P for corn, wheat, soybean meal, and corn gluten meal in growing Japanese quails from 28 to 32 d posthatch. A total of 400 quail chicks were randomly distributed across 5 treatments with 4 replicates and 20 birds in each floor pen. The P-free diet (PFD) was formulated based on cornstarch to measure the basal endogenous P losses (EPL). Digestibility coefficients were determined by ileal digesta sampling using TiO₂ as an indigestible marker. The EPL was estimated at 384 mg/kg DMI. The apparent ileal P digestibility (AIPD) for corn, soybean meal, wheat, and corn gluten meal were determined to be 0.38, 0.53, 0.38, and 0.78, respectively. The corresponding values for true ileal P digestibility (TIPD) were 0.48, 0.61, 0.50, and 0.83, respectively. The *t*-test analysis showed that the difference of AIPD and TIPD values for corn ($P = 0.031$) and wheat ($P = 0.015$) were statistically significant, however, no significant differences were observed for corn gluten meal ($P = 0.318$) and soybean meal ($P = 0.104$). In conclusion, the correction of AIPD coefficients for EPL in low-P ingredients such as corn and wheat may be much more important than that in high-P feedstuffs such as corn gluten meal and soybean meal in growing quails.

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1. Introduction

Phosphorus (P) is the second most abundant mineral in the body that involves in biochemical reactions in the cell, skeletal development, and metabolism (Corbridge, 2013). Plant originated ingredients such as corn and soybean meal, the most prevalent feed ingredients in poultry diets, contain complex P reserves that are mainly bound with phytic acid (Ballam et al., 1984). Poultry has a limited capacity to hydrolyze the phytate-P (i.e., phytate bond P [PP]) in the gut, but it has been shown that this insoluble portion of

dietary P may be utilized up to 50% in the birds depending on age and microbial diversity of the intestine (Coon et al., 2002). There are different terminologies for P availability such as nonphytate-P, bioavailability of P, and P digestibility. Traditionally, nonphytate-P has been widely used in feed formulation as the available P but the recent studies have shown that partial hydrolysis of PP in the gut may increase the risk of P overfeeding and subsequent environmental issues. Digestibility of P may be a reliable and preferable term of P availability for poultry (Rodehutsord, 2009). In terms of digestibility, many studies showed that apparent ileal amino acid digestibility values (AID), containing both of ingredient origin and endogenous losses of amino acids, may lack of additivity in mixed diets (Stein et al., 2005). Subtracting total endogenous losses (i.e., basal and specific losses) gives true ileal digestibility (TID) values, however, correction AID values for basal endogenous losses (BEL) resulted in standardized ileal digestibility (SID) that should be used in practical feed formulation (Stein et al., 2007). We hypothesized that the same problem may be true for apparent ileal P digestibility (AIPD). The aim of this study was to evaluate the AIPD values for corn, wheat, soybean meal (SBM), and corn gluten meal (CGM) in

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growing Japanese quails from 28 to 32 d of age using a direct method and then estimate the basal endogenous losses of P by a P-free diet (PFD).

2. Materials and methods

2.1. Bird management

The Research Animal Ethic Committee of Ferdowsi University of Mashhad approved this experimental protocol. Day-old Japanese quails (mixed sex) were taken from the meat-type Quail Genetic Stock Centre at the Research Center of the University of Zabol (RCUOZ, Sistan, Iran) and fed a standard diet to meet or exceed the nutritional requirements of quail chicks (NRC, 1994) from hatch to 27 d of age. On d 28, a total of 400 quail chicks with an average initial body weight of 167 ± 8.47 g were randomly assigned to 5 treatments with 4 replicates making 20 floor pens of 20 birds per pen. The temperature and relative humidity of research farm were set at 26 °C and 55%, respectively, during the study. Experimental diets (mash form) and fresh drinking water were offered *ad libitum* from 28 to 32 d of age as the finisher period. The lighting program was 23 h light and 1 h dark (23L:1D).

2.2. Chemical analysis of experimental diets

Experimental diets including PFD and semi-purified diets in which the test ingredients i.e. corn grain, wheat grain, SBM, and CGM were the sole P source, were formulated based on the direct method (Zhang and Adeola, 2017). TiO_2 was added at amount of 5 g/kg in diets as an indigestible marker. The PFD was made based on cornstarch and dextrose. Before the experiment, all feed ingredients were analyzed for dry matter (method 930.15), crude protein (method 968.06), and total P (method 968.08D) according to AOAC (2006) in triplicates. To measure the P and TiO_2 content in feed and digesta samples, each sample was burned at 550 °C and the ash was used to measure P and TiO_2 contents followed by colorimetrically method (UV mini 1240 Shimadzu Co., Japan) at 680 and 410 nm, respectively (Short et al., 1996).

2.3. Ileal digesta sampling and calculations

At d 32, all birds in each pen were euthanized by intra-cardiac injection of sodium pentobarbitone, and ileal digesta were gently squeezed, pooled within a pen, and lyophilized immediately. The ileum was defined as the section of the small intestine from Meckel's diverticulum to a point approximately 25 mm anterior to the ileocecal junction. The basal EPL, AIPD and TIPD were calculated according to the following equations (Adedokun et al., 2007):

$$\text{EPL (mg/kg DMI)} = P_i \times (T_d/T_i),$$

$$\text{AIPD (g/kg)} = [1 - (T_d/T_i) \times (P_i/P_d)],$$

$$\text{TIPD (g/kg)} = \text{AIPD} + (\text{EPL}/P_d),$$

where EPL was basal endogenous losses of P; AIPD was apparent ileal P digestibility; TIPD was true ileal P digestibility; T_d was titanium dioxide concentration in diet; T_i was titanium dioxide concentration in ileal digesta; P_d was P concentration in diet; and P_i was P output in ileal digesta.

2.4. Statistical analysis

Data were statistically analyzed using one-way ANOVA procedure in Prism Graph Pad 7.0 and Tukey tests were used for mean comparisons. Paired *t*-test was used to compare the AIPD and TIPD values for each ingredient at $P < 0.05$.

3. Results

Chemical analysis of feed ingredients showed that total P of CGM, corn, SBM, and wheat were 7.38, 3.28, 4.46, and 2.89 g/kg DMI, respectively (Table 1). Except for PFD, Ca:P ratio was maintained at 2.28:1 in other experimental diets and each test ingredient was the sole source of P (Table 2). Birds remained healthy during the 5 d of experiment, and no mortality or leg problem were observed. Growth performance of quail chicks during the study are shown in Table 3. The birds fed PFD had the lowest feed intake ($P = 0.001$), gain ($P = 0.037$), and G:F ($P = 0.001$) while the birds received SBM exhibited a better performance compared to the other experimental diets ($P < 0.05$), possibly due to either different crude protein or P contents of each diets. Phosphorous content of the experimental diets including CGM, corn, SBM, and wheat were 3.51, 3.10, 1.78, 2.79 g/kg, respectively. Basal EPL was determined to be 384 mg/kg DMI. As depicted in Table 4, AIPD values in tested ingredients were significantly different ($P < 0.001$), in which the AIPD coefficient for CGM had the highest value, followed by SBM, wheat, and corn grain. True ileal P digestibility values in tested ingredients were statistically different ($P < 0.001$), in which TIPD for CGM was higher than those for SBM, wheat, and corn. Tukey's multiple test (Fig. 1) showed statistical differences between AIPD values for CGM vs. SBM ($P < 0.001$), CGM vs. corn ($P = 0.001$), CGM vs. wheat ($P < 0.001$), SBM vs. corn ($P = 0.025$) and SBM vs. wheat ($P = 0.025$), however, no difference was observed between AIPD values for corn vs. wheat ($P > 0.999$). The differences between TIPD values for SBM vs. wheat ($P = 0.120$) and SBM vs. corn ($P = 0.071$) showed statistical trends (Fig. 1). Although no statistical differences were observed between AIPD and TIPD coefficients in CGM and SBM, differences between those coefficients were significant for corn and wheat (Table 5).

4. Discussion

We have accomplished a series of experiments on P digestibility in quail chicks for age classes including 14 to 18 d and 28 to 32 d of

Table 1
Chemical analysis of feed ingredients.

Ingredient	Dry matter, g/kg	Gross energy, MJ/kg	Crude protein, g/kg	Total P, g/kg	Phytate P, g/kg	NSP ¹ , g/kg
Corn gluten meal	945	20.5	601	7.38	5.31 (0.72) ²	49.0
Corn grain	887	18.5	79.2	3.28	2.35 (0.72)	80.0
Soybean meal	933	21.5	488	4.46	2.61 (0.59)	220
Wheat grain	925	16.9	140	2.89	1.88 (0.66)	110

NSP = non-starch polysaccharides.

¹ Adopted from Sauvant et al. (2002).

² The values in parenthesis represent the relative amount of phytate P to total P.

Table 2
Composition of experimental diets, as-fed basis (g/kg).

Item	P-free diet	Corn gluten meal	Corn grain	Soybean meal	Wheat grain
Ingredients					
Test ingredient	–	475.1	944.3	400.0	964.5
Corn starch	650.0	477.5	–	561.2	–
Sucrose	248.5	–	–	–	–
Solka-floc	50.0	–	–	–	–
Limestone	21.05	20.14	20.51	18.0	19.8
K ₂ CO ₃	13.63	8.06	16.24	4.50	–
NaHCO ₃	–	5.11	5.09	2.14	2.05
TiO ₂	5.00	5.00	5.00	5.00	5.00
Vitamin premix ¹	2.50	2.50	2.50	2.50	2.50
Mineral premix ²	2.50	2.50	2.50	2.50	2.50
NaCl	3.82	2.09	1.86	2.16	1.69
MgO	2.00	1.00	1.00	1.00	1.00
Choline chloride	1.00	1.00	1.00	1.00	1.00
Nutrient analysis					
AME, MJ/kg ³	14.5	15.1	14.1	15.3	11.7
Na ³	1.56	2.30	2.30	1.60	1.60
K ³	6.00	4.00	10.0	10.0	4.36
Cl ³	2.30	1.50	1.50	1.50	1.50
NSP ³	–	23.4	75.5	123	106
Crud protein ⁴	–	285	74.8	195	135
Ca ⁴	8.00	8.00	8.00	8.00	8.00
Total P ⁴	–	3.51	3.10	1.78	2.79
DEB, mEq/kg ⁵	210	100	130	232	289
Crude fiber, g/kg ⁶	49.5	38.1	20.8	28.0	28.9

¹ Mineral premix provided per kilogram of diet: Mn (from MnSO₄·H₂O), 65 mg; Zn (from ZnO), 55 mg; Fe (from FeSO₄·7H₂O), 50 mg; Cu (from CuSO₄·5H₂O), 8 mg; I (from Ca (IO₃)₂·H₂O), 1.8 mg; Se, 0.30 mg; Co (from Co₂O₃), 0.20 mg; Mo, 0.16 mg.

² Vitamin premix provided per kilogram of diet: vitamin A (from vitamin A acetate), 11,500 IU; cholecalciferol, 2,100 IU; vitamin E (from dl- α -tocopheryl acetate), 22 IU; vitamin B₁₂, 0.60 mg; riboflavin, 4.4 mg; nicotinamide, 40 mg; calcium pantothenate, 35 mg; menadione (from menadione dimethyl-pyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 10 mg; biotin, 1 mg; choline chloride, 560 mg; ethoxyquin, 125 mg.

³ Calculated values (Sauvant et al., 2002).

⁴ Analyzed values.

⁵ Dietary Electrolyte Balance: represents dietary Na + K – Cl in mEq/kg of diet.

⁶ Calculated value (NRC, 1994).

Table 3
Performance of growing Japanese quails fed on different feed ingredients as the sole sources of P.¹

Ingredients	Feed intake, g		Gain, g		Gain:Feed	
	Means	95% CI	Means	95% CI	Means	95% CI
Corn	70.0 ^c	67.2 to 72.8	–10.3 ^{ab}	–28.9 to 8.39	–0.143 ^a	–0.362 to 0.075
Corn gluten meal	67.4 ^c	64.6 to 70.2	–7.70 ^{ab}	–26.4 to 11.0	–0.115 ^a	–0.333 to 0.103
P-free diet	41.4 ^d	38.6 to 44.0	–32.3 ^b	–50.9 to –13.7	–0.782 ^b	–1.001 to –0.564
Soybean meal	168 ^a	164.8 to 170.4	6.50 ^a	–12.2 to 25.1	0.039 ^a	–0.179 to 0.257
Wheat	89.0 ^b	86.2 to 91.8	6.06 ^a	–12.6 to 24.7	0.066 ^a	–0.152 to 0.284
SEM	1.90		12.6		0.15	
P-value	0.001		0.037		0.001	

CI = confidence interval.

¹Each value is the mean of 80 birds per treatment, 4 replicates and 20 birds each.

^{a-d} Within a column, means that do not share a letter are significantly different ($P < 0.05$).

Table 4
A t-test comparison of apparent ileal P digestibility (AIPD) and true ileal P digestibility (TIPD) coefficients between feed ingredients in growing quail chicks from 28 to 32 d of age.¹

Ingredient	Ileal P digestibility	
	AIPD	TIPD
Corn gluten meal	0.78 ^a	0.83 ^a
Corn grain	0.38 ^c	0.48 ^b
Soybean meal	0.53 ^b	0.61 ^b
Wheat grain	0.38 ^c	0.50 ^b
SEM	0.05	0.05
P-value	0.001	0.001

AIPD = apparent ileal P digestibility; TIPD = true ileal P digestibility.

¹Each value is the mean of 150 birds per treatment, 5 replicates and 30 birds each.

^{a, b} Within a column, means that do not share a letter are significantly different ($P < 0.001$).

age in our laboratory. Therefore, some comparisons would be presented herein according to our database that the relevant data was submitted to peer-review in a separate report. The basal EPL was determined using PFD in the direct method approach. The basal EPL was 384 mg/kg DMI in the present study, which was significantly higher than that we measured in younger quails from 14 to 18 d of age (219 mg/kg DMI). Generally, body weight of quail chicks from the 2nd to 4th week of age may increase by 8 times and birds with heavier body weight may excrete more endogenous P than smaller birds. The basal EPL is highly correlated to the body weight as reported in previous studies. Bikker et al. (2017) indicated that EPL in pigs substantially increased with increasing body weight. Corn and wheat grains contain lower P content than CGM and SBM and correcting the AIPD values for the basal EPL in the tested cereals, therefore, substantially increased the P digestibility. Low P content of cereals may increase the proportional endogenous

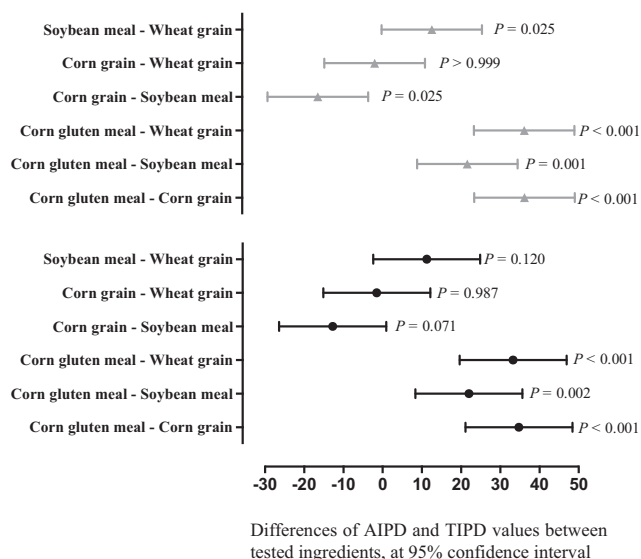


Fig. 1. Tukey's multiple comparisons test of group means of each feed ingredient for apparent (▲) and true (●) ileal digestibility of P in growing quail chicks from 28 to 32 d of age. AIPD = apparent ileal P digestibility; TIPD = true ileal P digestibility.

Table 5

A comparison of apparent ileal P digestibility (AIPD) and true ileal P digestibility (TIPD) coefficients for each feed ingredients in growing quail chicks from 28 to 32 d of age.¹

Ingredient	Ileal P digestibility		P-value
	AIPD	TIPD	
Corn gluten meal	0.78	0.83	0.279
Corn grain	0.38 ^b	0.48 ^a	0.013
Soybean meal	0.53	0.61	0.152
Wheat grain	0.38 ^b	0.50 ^a	0.028

¹Each value is the mean of 150 birds per treatment, 5 replicates and 30 birds each. Pooled SEM = 4.232.

^{a, b} Within a column, means that do not share a letter are significantly different ($P < 0.05$).

losses in digesta resulting in underestimation of AIPD values (Lemme et al., 2004). Although the basal EPL is not affected by feedstuff characteristics while feed intake level may influence the basal endogenous losses (Mutucumarana and Ravindran, 2016). The AIPD coefficients are inconsistent for the same feed ingredient among different studies resulting in high variation in estimated P digestibility (Bohlke et al., 2005). In this situation, correcting AIPD coefficients for basal EPL may help us to formulate dietary P more precisely using TIPD values. In terms of amino acids, it has been documented that apparent digestibility of amino acids are not additive in a mixed diet, especially when low crude protein ingredients constitute a significant portion of dietary protein (Stein et al., 2005). The same concept as amino acids may apply for dietary P as well, and AIPD coefficients and AIPD coefficients in a mixed diet may not be additive, however, this hypothesis should be examined in future studies. Multiple *t*-test analysis revealed more evidence on the importance of correction of AIPD values for basal EPL where the AIPD and TIPD values in high-P ingredients including CGM and SBM were not different but in low-P ingredients including

corn and wheat were substantially different implying the necessity of calculation true P digestibility for quail chicks.

5. Conclusion

The present study clearly showed that AIPD values were underestimated digestible P compared to TIPD values, due to the proportional contribution of the basal EPL. Based on our results, we think that TIPD is a more accurate measurement of digestible P for chicks. This hypothesis should be tested and confirmed in further studies where bird performance will be tested and compared in the diets formulated using either AIPD or TIPD values.

Conflict of interest

The authors do not have any conflict of interest.

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