Research Note: Phosphorus digestibility of corn, wheat, soybean meal, and corn gluten meal in quail chicks

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ABSTRACT A series of experiments was designed and conducted to determine the apparent and standardized ileal phosphorus digestibility (i.e., AIDP and SIDP, respectively) of some grains and protein meals in Japanese quail at different age classes during the growing period from the hatch to 35 d of age. Experimental diets included a PFD, to measure basal endogenous P losses (**EPL**), corn, wheat, soybean meal (**SBM**), and corn gluten meal (**CGM**), so as to be each ingredient the sole source of P, were developed and fed to five experimental groups with 5 replicates of 30 chicks each. Titanium dioxide as an indigestible marker was added to the diets at the rate of 5 g/kg of diet. The EPL in birds fed on PFD was estimated at 201 mg/kg dry matter intake (**DMI**) and quail chicks fed on PFD exhibited the lowest performance compared to chicks received dietary P regardless of P sources ($P = 0.001$). The estimated coefficients of AIPD ($P = 0.001$) | SIDP ($P = 0.004$) for CGM, corn, SBM, and wheat were 49.2 | 51.9%, 38.8 | 44.9%, 41.4 | 45.9%, and 33.2 | 40.1%, respectively. The mean differences between AIPD and SIPD coefficients with each ingredients including CGM ($P = 0.245$), corn ($P = 0.169$), and SBM ($P = 0.169$) were not statistically significant, while the comparison of those estimations for wheat ($P = 0.022$) showed significant differences. The present work showed that the use of direct method could successfully estimate the coefficients of P digestibility in growing quail for both the cereals and protein meals that were studied. The high relative contribution of endogenous P in young quail fed on wheat during the first 2 wk posthatch makes it inevitable to correct AIDP for EPL and evoke the implementation of SIDP coefficients in the feed formulation matrix.

Key words: Basal endogenous losses, direct method, phosphorus, quail chick

INTRODUCTION

Cereal grains and protein-meals are the main sources of metabolizable energy, protein, and phosphorus (P) for poultry species that may provide more than 60% of P requirement of the birds. Use of costly nonrenewable inorganic phosphate supplements is not only associated with the risk of depletion in P reserves around the world (Li et al., 2016), but also the increasing environmental issues caused by P accumulation in soil and groundwaters (Pavlidis and Tsihrintzis, 2018; Whalen and Chang, 2001). In this case, poultry nutritionists were recently encouraged to increase their knowledge about the organic P reserves in the feed ingredients and assessment the bioavailability of the P resources for domestic poultry. Provided precise information on the P features in the cereal grains and protein meals as the main backbone of poultry diets, the possibility of either underfeeding or overfeeding of P could be minimized in the least cost or maximized profit feed formulation.

Because of low digestible P content of the most feed ingredients such as cereal grains, the researchers have usually implemented regression method for determination of P digestibility in poultry and swine (Fan et al., 2001; Chen et al., 2011; Mutucumarana et al., 2015), which is based on increasing levels of P in a series of experimental diets and endogenous phosphorus losses (**EPL**) were estimated by the intercept of the relationship between excreted and ingested P (Fan et al., 2001). The regression method estimates total EPL, which is a theoretically estimated value and correcting apparent ileal phosphorus digestibility (**AIPD**) for total EPL gives true ileal P digestibility (**TIPD**). The worst character of this methodology would be high variability in the estimated EPL across individual animals (She et al., 2017). In addition, because of subtracting total EPL (i.e., basal + specific endogenous P losses) by extrapolating regression line to zero P intake in regression method, TIPD values failed to estimate the specific endogenous losses and could not

evaluate the feeding value of the feed ingredients based on their specific characteristics (Stein et al., 2007).

In the present study, we aimed to estimate AIPD and SIPD coefficients for corn, wheat, soybean meal (**SBM**), and corn gluten meal (**CGM**) in quail chicks during the first 2 wk of age.

MATERIALS AND METHODS

Bird Management

The Research Animal Ethics Committee of the University of Zabol, Iran, approved this experimental protocol. Day-old straight-run Japanese quail were taken from the meat-type Quail Genetic Stock Centre at the Research Center of the Research Institute of Zabol (RCRIZ, Sistan, Iran) and fed standard diet to meet or exceed the nutritional requirements (e.g., ME: 11.7 MJ/kg and CP: 240 g/kg) recommended by NRC (1994) from hatch to 9 d of age. On d 10, a total of 750, 10-d old quail chicks were randomly allocated to 25 floor pens including 5 treatments with 5 replicates of 30 birds per pen. The temperature of the experimental house was set at 35 and 29°C for the first and second wk of age, respectively, with a relative humidity of 55% to 60%. The birds had free access to the water and experimental diets (mash form) and lighting program was 23L:1D during the study.

Experimental Diets

As before stated by Ghazaghi et al. (2019), all feed ingredients were analyzed for gross energy by a bomb calorimeter (Parr 1261 bomb calorimeter, Parr Instruments Co., Moline, IL) using benzoic acid as the standard (AOAC, 2006), dry matter (method 930.15), crude protein (method 968.06), total phosphorus (method 968.08D) according to AOAC (2006) in triplicates. For determination of P and titanium dioxide (TiO_2) in feed and digesta samples, each sample was ashed and then P and TiO_2 (Short et al., 1996) were determined colorimetrically (UV mini 1240 Shimadzu Co., Japan) at 680 and 410 nm, respectively. Calcium was measured by a colorimetric assay following digestion with HCL ($6M$) as described by Pan et al. (2012).

Five dietary treatments including PFD, corn, wheat, SBM, and CGM were developed and TiO_2 was added into the diets as an inert marker (5 g/kg). The PFD was formulated based on cornstarch and dextrose and the ratio of Ca:P ranged from 1.30:1 to 1.42:1 in the experimental diets as recommended by Rodehutscord (2013), except for PFD. In each experimental diets, the test ingredient was substituted for cornstarch to be the sole source of P (Table 1).

Digesta Sampling

At the end of the experiment, all birds in each pen were euthanized by an intra-cardiac injection of sodium pentobarbital, and digesta were gently squeezed out into vials, pooled within a pen, and lyophilized immediately (Anwar et al., 2016). The ileum was defined as the section of the small intestine, which is between Meckel's diverticulum and a point approximately 25 mm anterior to the ileocecal junction.

Calculations

The basal EPL, AIPD and SIPD were calculated according to the following equations (Ghazaghi et al., 2019):

$$EPL_{(mg/kgDMI)} = P'_i(T_d/T_i),$$

$$AIPD_{(g/kg)} = [1 - (T_d/T'_i)(P_i/P_d)]$$

$$SIPD_{(g/kg)} = AIPD + (EPL/P_d),$$

where EPL was basal endogenous losses of P; AIPD and SIPD were the apparent ileal and standardized ileal P digestibility, respectively; T_d and T_i were the concentration of TiO_2 in diets and ileal digesta, respectively; P_d and P_i were the concentration of P in diets and digesta, respectively.

Statistical Analysis

Data were statistically analyzed using one-way ANOVA procedure in Prism GraphPad 7.0 and the Tukey test was used for mean comparisons of AIPD and SIPD values between feed ingredients and differences between AIPD and SIPD values within each feed ingredient were analyzed using t-test at $P < 0.05$.

RESULTS AND DISCUSSION

The experiments concerning nutrient digestibility based on direct method are short-term studies that usually take 5 d, therefore, no mortality and/or leg problem were not observed at the present study. In order to limit the interaction between dietary Ca and P, the ratio of Ca to P maintained in the range of 1.30:1 to 1.42:1 across the experimental diets as suggested by Rodehutscord (2013). Basal EPL was determined to be 201 mg/kg DMI in birds fed on PFD. As shown in Figure 1, the coefficients of AIPD in tested ingredients were significantly different ($P = 0.001$) and the estimated coefficients for CGM, corn, SBM, and wheat were 49.2, 38.8, 41.4, and 33.2%, respectively. Multiple Tukey test showed that the AIPD for CGM was higher than that for corn ($P = 0.006$), SBM ($P = 0.036$), and wheat ($P = 0.001$). The AIPD coefficients for corn and SBM were not statistically different, but higher than that estimated for wheat ($P = 0.001$). The SIPD values between test ingredients were different ($P = 0.004$) and SIPD coefficients for CGM, corn, SBM, and wheat were determined to be 51.9, 44.9, 45.9, and 40.1%, respectively. Figure 2. shows the results of t-test analysis for AIPD and SIPD coefficients in each feed ingredient. Although

Table 1. Composition of experimental diets.

Ingredient (g/kg)	P-free diet	Corn gluten meal	Yellow corn	Soybean meal	Wheat grain
Test ingredient	-	473.0	961.8	400.0	979
Cornstarch	650.0	493.6	-	582.1	-
Sucrose	248.5	-	-	-	-
Solka-Floc	50.00	-	-	-	-
Limestone	21.05	6.10	1.50	18.0	2.70
K_2CO_3	13.63	8.10	16.20	4.50	-
$NaHCO_3$	-	5.10	5.10	2.20	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	2.10	2.20	2.16	1.70
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	14.1	14.3	15.7	11.9
Crude protein (g/kg)[4]	-	284	76.2	195	137
Ca (g/kg)[4]	8.00	2.60	1.30	1.70	1.50
Non-phytate-P (g/kg)[4]	-	1.90	1.00	1.20	1.10
Na (g/kg)[3]	1.56	2.30	2.30	1.60	2.30
K (g/kg)[3]	6.00	4.00	10.0	8.00	8.00
Cl (g/kg)[3]	2.30	1.50	1.50	1.50	1.50
Crude fiber (g/kg)[6]	49.5	37.8	21.2	28.0	28.0
DEB[5] (mEq/kg)	210	100	132	232	232
Ca:nonphytate-P	-	1.37	1.30	1.42	1.36

[1]Mineral premix provided per kilogram of diet: Mn (from $MnSO_4 \cdot H2O$), 65 mg; Zn (from ZnO), 55 mg; Fe (from $FeSO_4 \cdot 7H_2O$), 50 mg; Cu (from $CuSO_4 \cdot 5H_2O$), 8 mg; I [from Ca $(IO_3)2 \cdot H_2O$], 1.8 mg; Se, 0.30 mg; Co (from Co_2O_3), 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 B_{12}, 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 value (Sauvant et al., 2002).

[4]Available phosphorus, analyzed values.

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

[6]Calculated value (NRC, 1994).

no statistical difference was observed between those coefficients in CGM ($P = 0.245$) and corn ($P = 0.169$), and SBM ($P = 0.169$), the SIPD values were higher than AIPD in wheat ($P = 0.022$).

More recently, Ghazaghi et al. (2019) reported the apparent ileal and true ileal P digestibility values for corn, wheat, SBM, and CGM in growing quail from 28 to 32 d of age. Considering non-phytate P content of feed ingredients as the available P may increase the risk of overfeeding of P because the birds can utilize a portion of phytate-bound P in feed ingredients, and subsequently resulting in increasing P excretion (Mutucumarana et al., 2014). The primary concern of this event is the environmental pollution caused by excess excretion of P. Among different methods of the assessment of true P digestibility such as P-free gelatinized diet (Sulabo and Stein, 2013), regression method (Fan et al., 2001) and P-free diet (Ghazaghi et al., 2019), we have used the later method to correct AIPD for basal EPL to determine SIPD. In fact, the former methods may stimulate the endogenous P secretions namely specific endogenous P losses (Mutucumarana, 2014). The SIPD coefficients allow for distinguishing between ingredients in a feed evaluation system (Stein et al., 2007).

As indicated in this experiment, underestimation of AIPD coefficients for tested ingredients could be overcome by correcting for basal EPL that was in agreement with Ghazaghi et al. (2019). In contrast to Ghazaghi et al. (2019), the significant differences among AIPD

coefficients in feed ingredients were not disappeared by the correction of AIPD coefficients for EPL. The potential reason for this discrepancy is the lower EPL in the younger birds used in the present study than the older birds used in the study of Ghazaghi et al. (2019). Amount of EPL in the present study was 201 mg/kg DMI that was significantly lower than that reported by Ghazaghi et al. (2019; 384 mg/kg DMI). This big difference between these two values may be related to the body mass. Bikker et al. (2017) reported that the strong correlation exists between body weight and EPL. As Adeola et al. (2016) stated, a possible explanation for the lack of differences between AIPD and SIPD coefficients in CGM and corn are the high level of P in CGM (7.38 g/kg) and low concentration of NSP in CGM (49 g/kg) and corn (80 g/kg) compared to wheat (110 g/kg) as reported by Ghazaghi et al. (2019). The NSP in monogastric nutrition is a well-understood limitation of nutrient digestion (Ai et al., 2007; Fang et al., 2007). Although phytate-P of CGM is relatively high, the high digestibility of P in CGM might be related to the feed processing and increasing nutrient accessibility in the small intestine (Pedersen et al., 2007). In addition, the location and chemical forms of phytate-P may affect the digestibility of P (Selle and Ravindran, 2007). The complex form of phytate in wheat, phytate-protein globoids, makes it resistant to degradation by catabolic enzymes in birds (Bohn et al., 2007), whereas evenly distributed phytate in soybean meals is more soluble than complex form of phytate found in wheat (Selle and Ravindran,
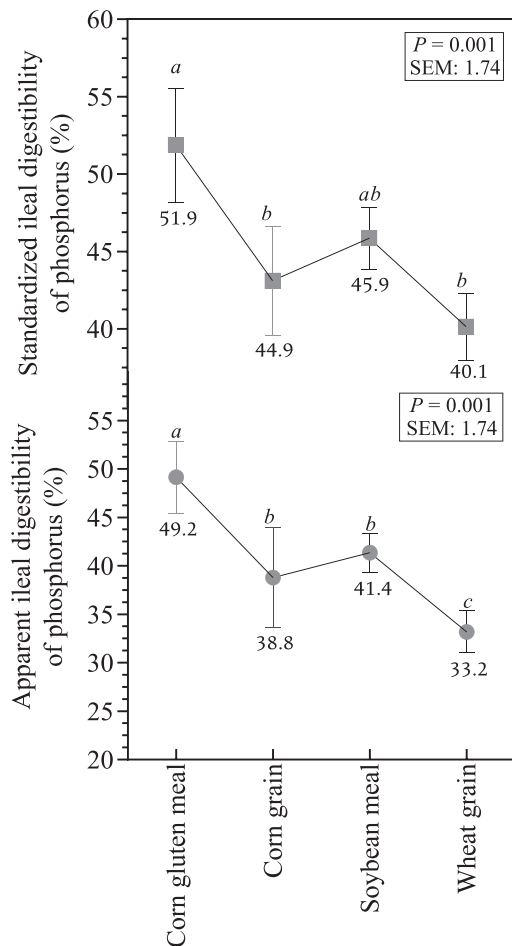
Figure 1. Tukey post hoc test of apparent ileal P digestibility (●) and standardized ileal P digestibility (□) coefficients between feed ingredients in quail chicks from 10 to 14 d of age. Each value is the mean of 150 birds per treatment, 5 replicates and 30 birds each. Means with no common superscripts are significantly different ($P < 0.05$).

Figure 2. A t-test comparison of apparent (AIDP) and standardized ileal digestibility of P (SIDP) coefficients within each feed ingredients in quail chicks from 10 to 14 d of age. Each value is the mean of 150 birds per treatment, 5 replicates and 30 birds each.

In conclusion, the most important achievement of the present study was the estimating of SIPD coefficients to unveil that AIPD values in low-P ingredients such as wheat with relatively high NSP may not be suitable for feed formulation and SIPD values for cereals would be more precise index for P availability than AIPD coefficients.

2007), rendering it to be easily hydrolyzed in gastrointestinal tract.

In terms of amino acid digestibility, basal endogenous secretions are not affected by diet composition but may vary at different levels of DMI (Adeola et al., 2016; Petersen and Stein, 2006). If this concept would be applicable to P digestibility, estimation of SIPD could be effective to precise feed formulation for available P, that could be additive in a mixed diet (Stein et al., 2005). However, this speculation needs to be evaluated in practice for growing quail. The present study clearly showed that low-P ingredients such as wheat had different apparent digestibility coefficients for P and it is of utmost importance to determine standardized P digestibility coefficients to mitigate underestimation of P digestibility and consequently reduce environmental pollution. These differences between AID and SID values in quail chicks may be more critical compared to the adult quail. As indicated by Adedokun et al. (2007), the importance of endogenous losses of amino acids in younger chickens is greater than that in older broiler chicks that could be translated into the higher proportion of endogenous losses in young birds and necessity of correcting for this portion.
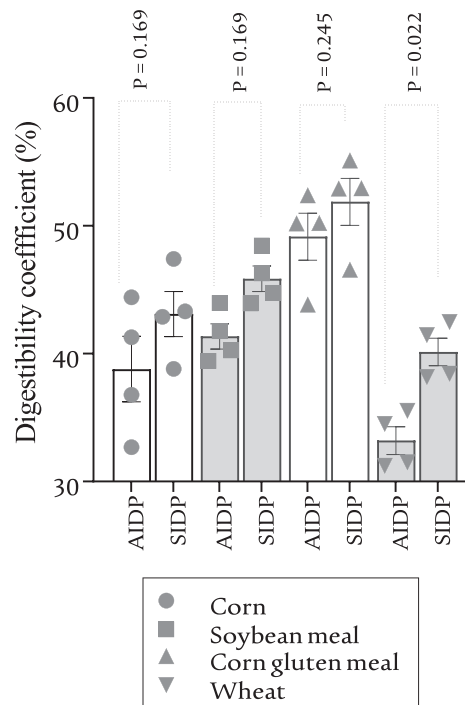
DISCLOSURES

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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