

« Research Note »

Measurement of Endogenous Phosphorus Losses in Broiler Chickens

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The experiment reported herein was conducted to estimate the ileal and excreta endogenous phosphorus (P) losses in broiler chickens. Three purified diets, namely a P-free diet, a gelatin-based diet containing negligible amounts of P, and a casein-based diet with 100% available P, were formulated. Test diets were offered *ad libitum* from day 25 to 28 post hatch and ileal digesta were collected. Excreta samples were also collected to estimate total tract endogenous P losses. Ileal endogenous P losses in birds fed the casein-based diet were higher ($P < 0.05$) than those in birds fed P-free and gelatin-based diets. The ileal endogenous losses of P in birds fed P-free, gelatin-based, and casein-based diets were 25, 104 and 438 mg/kg dry matter intake, respectively. The endogenous P loss values estimated at the excreta level were 830, 560 and 372 mg/kg dry matter intake, respectively. Ileal and excreta endogenous losses of P in birds fed a casein-based diet were similar ($P > 0.05$), but ileal losses were lower ($P < 0.05$) than the excreta values in birds fed P-free and gelatin-based diets, resulting in a significant ($P < 0.001$) assay diet by site of measurement interaction. The present data demonstrate that values determined for endogenous P losses in broiler chickens vary widely depending on the assay diet used.

Key words: broilers, endogenous losses, excreta, ileal, phosphorus

J. Poult. Sci., 58: 58–63, 2021

Introduction

The main function of the gastrointestinal tract is the digestion and absorption of nutrients in the food, but there are also a significant amount of endogenous nutrients secreted into the gut (Ravindran, 2016). It is recognized that the amount of nutrients leaving the ileum represents the net balance between dietary nutrient intake and nutrient secretion minus the absorption of dietary nutrient and reabsorption of endogenous nutrients. Accurate measurement and correction for these inevitable losses is necessary for the estimation of true ileal digestibility and to predict the net nutrient requirement for maintenance based on dry matter intake (Boisen and Moughan, 1996, Moughan and Fuller, 2003).

Estimates of endogenous P losses have been reported for pigs (Lopes *et al.*, 1999a, 1999b), ruminants (Salviano and

Vitti, 1998) and equines (Furtado *et al.*, 2000). There have been no systematic studies conducted on ileal endogenous phosphorus (P) losses in poultry. The primary sources of endogenous P are bile, enzyme secretions and sloughed epithelial cells (Coleman, 1987; Cross *et al.*, 1987; Fan *et al.*, 2001). Although not strictly endogenous, gut microbes also contribute to endogenous materials. Different approaches have been employed to measure endogenous P losses in animals and include the regression method (Fan *et al.*, 2001; Shen *et al.*, 2002), feeding P-free diets (Petersen and Stein, 2004) or diets with minimal P content (Rutherford *et al.*, 2002, 2004) and the radio-isotope dilution technique (Al-Masri, 1995). The aim of the present study was to determine the endogenous losses of P using three different assay diets. Broiler chickens were fed purified diets containing no P, negligible P (gelatin-based diet) or 100% available P (casein-based diet) and endogenous losses were estimated at the ileal and excreta levels. Gelatin contains only trace amounts of P and casein contains 6.8 g/kg P but its P is 98% digestible (NRC, 2012). These materials, therefore, are theoretically suitable for the measurement of endogenous P losses.

Materials and Methods

The experimental procedures were approved by the Massey

Received: October 7, 2019, Accepted: March 7, 2020

Released Online Advance Publication: June 25, 2020

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University Ethics Committee (approval number: MUAEC 1104) and were in accordance with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Day-old male broilers (Ross 308) were raised in the floor until day 14 and fed a commercial broiler starter diet (12.9 MJ/kg metabolizable energy, 262 g/kg crude protein, 11.0 g/kg Ca, 6.7 g/kg total P). On day 14, birds were transferred to grower cages and were maintained on the same diet until the introduction of test diets on day 25 post hatch. Both the floor facility and cages were in an environmentally controlled broiler house. Each cage (60×60×40 cm; length×width×height) were equipped with a linear feeder in the front and a nipple drinker with a cup in the back. The temperature was maintained at 31°C on day 1 and reduced to 20°C by day 25. Temperature control was achieved through thermostatically controlled fans and wall-mounted electric heaters. Daily maximum and minimum temperatures, at the level of birds, inside the broiler house were recorded throughout the study. A lighting schedule of 20 hours per day was applied by fluorescent tubes (58 Watts). Light

intensity at the height of the birds was about 160 lux. The house was ventilated by four exhaust fans (capacity 0.3 m³/s), mounted in pairs on opposite walls. The location of the fans ensured air movement through the whole house and cooling for the birds. To allow for a sufficient amount of inlet air, two air inlets were also positioned in the roof. Feed was provided *ad libitum* and water was available at all times.

Dietary Treatments

Three purified diets were formulated (Table 1). The first diet was a P-free diet. The second diet was gelatin-based, which is known to contain almost no P (NRC, 1994; Dänner *et al.*, 2006). The third diet was a diet based on casein. Casein contains very low concentrations of Ca and P, which are 100% available (NRC, 1994). All three diets contained 3 g/kg titanium dioxide as an indigestible marker.

Birds

On day 25, birds were individually weighed and 72 birds (average weight±SD, 1265±12 g) were assigned to 12 cages of 6 birds each. After 4 hours of feed withdrawal, the assay diets were introduced, and offered *ad libitum*. Water was available at all times.

Table 1. **Ingredient composition and analysis (g/kg as fed) of the assay diets**

	Phosphorus-free Diet	Gelatin-based diet	Casein-based diet
Dextrose	856.0	655.8	658.0
Casein	—	—	200.0
Gelatin ¹	—	200.0	—
Soybean oil	50.0	50.0	50.0
Cellulose ²	50.0	50.0	50.0
L-tryptophan	—	0.2	—
Sodium bicarbonate	30.0	30.0	30.0
Magnesium oxide	2.0	2.0	2.0
Titanium dioxide	3.0	3.0	3.0
Sodium chloride	4.0	4.0	2.0
Trace mineral-premix ³	2.5	2.5	2.5
Vitamin premix ⁴	2.5	2.5	2.5
Calculated composition			
Metabolizable energy, MJ/kg	15.3	14.1	15.6
Crude protein	—	176	174
Lysine	—	7.4	15.9
Methionine	—	1.36	5.3
Methionine + Cysteine	—	1.54	6.0
Threonine	—	2.6	8.6
Calcium	—	1.0	1.2
Total phosphorus	—	—	1.6
Available phosphorus	—	—	1.6
Analyzed values			
Ca, g/kg as fed	0.5	0.8	0.5
Total P, g/kg as fed	<0.09	<0.09	1.6

¹ Davis Food Ingredients, Petone, New Zealand.

² Asahi Kasei Chemicals Corporation, Chiyoda-Ku, Tokyo, Japan.

³ Supplied per kg diet: Co, 0.3 mg, I, 1.5 mg, Mo, 0.3 mg, Se, 0.3 mg, Mn, 100 mg, Cu, 10 mg, Zn, 80 mg, Fe, 60 mg, anti-oxidant, 100 mg.

⁴ Supplied per kg diet: vitamin A, 37,500 IU, vitamin D₃, 12,500 IU, thiamine, 9 mg, riboflavin, 28 mg, pyridoxine, 31 mg, folic acid, 9 mg, biotin, 0.78 mg, vitamin B₁₂, 0.06 mg, vitamin E, 250 mg, choline chloride, 1.88 g, nicotinic acid, 187.5 mg, Ca pantothenate, 47 mg, menadione, 12.5 mg.

Digesta and Excreta Collection

On day 26, collection trays were introduced, and grab samples of fresh excreta were collected for two days and pooled within a cage. On day 28, birds were euthanized by intravenous injection of sodium pentobarbitone and the contents of the lower ileum were collected. The ileum was divided into two halves and the contents were collected from the lower half, towards the ileo-cecal junction (Ravindran *et al.*, 1999). Digesta were flushed out with reverse-osmosis water, pooled within a cage and lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Daily excreta collections were pooled within a cage, mixed well, subsampled and lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand). Samples of diets, digesta and excreta were ground to pass through 0.5-mm sieve and stored in air-tight plastic containers till analysis for dry matter (DM), Ca, P and titanium (Ti).

Chemical Analysis

Representative samples of diets, digesta and excreta were analyzed for DM, Ca, P and Ti. Dry matter was determined by drying samples at 105°C for 16 hours in a pre-weighed dried crucible in a convection oven (AOAC International, 2005; method no: 930.15). Samples were ashed and P was

determined colorimetrically (UV mini 1240 Shimadzu Corp., Kyoto, Japan) at 680 nm (AOAC International, 2005; method no: 968.08D). Titanium dioxide was determined by the colorimetric method (UV mini 1240 Shimadzu Corp., Kyoto, Japan) at 410 nm as described by Short *et al.* (1996).

Calculations

The endogenous losses of P were calculated as milligrams lost per ingestion of kilogram of feed DM, by using the following formula (Moughan *et al.*, 1992).

$$\text{Endogenous P losses (mg/kg DM intake)} \\ = \frac{\text{P (Digesta or excreta) (mg/kg)} \times \text{Titanium (Diet) (mg/kg)}}{\text{Titanium (Digesta or excreta) (mg/kg)}}$$

Statistical Analysis

Two-way analysis of variance was conducted to determine the effect of the assay diet and sampling site with their interaction (SAS, 2004). Differences were considered significant at $P < 0.05$ and significant differences between means were separated by the 'pdiff' option.

Table 2. Comparison of ileal and excreta endogenous P losses (mg/kg dry matter intake) in broiler chickens¹, estimated using different assay diets

Assay diet	Site of measurement	Endogenous P loss (mg/kg DMI)
Phosphorus-free	Ileal	25 ^c
	Excreta	830 ^a
Gelatin-based	Ileal	104 ^c
	Excreta	560 ^b
Casein-based	Ileal	438 ^b
	Excreta	372 ^b
SEM ²		70.4
Main Effects		
Assay diet		
Phosphorus-free		428
Gelatin-based		332
Casein-based		405
SEM ²		49.8
Site of measurement		
Ileal		189
Excreta		588
SEM ²		40.7
Probability (P≤)		
Assay diet		NS
Site		***
Diet x Site		***

NS, not significant; *** $P < 0.001$.

Means in a column not sharing a common letter (a, b, c) are significantly different ($P < 0.05$).

¹ Each value represents the mean of four replicates (6 birds per replicate).

² Pooled standard error of mean.

Results

The gelatin-based diet was less readily consumed than the P-free and casein-based diets. Average daily feed intake of birds fed P-free, gelatin-based and casein-based diets was 76.6, 53.7 and 86.7 g/bird, respectively. Analyzed Ca and P contents of three test diets were close to the formulated values (Table 1).

There was a significant ($P < 0.001$) interaction between the assay diet and site of measurement for endogenous P losses (Table 2). Compared to ileal digesta, endogenous P losses were increased in the excreta in birds fed P-free and gelatin-based diets, but no difference was observed between the sites of measurement in those fed the casein-based diet.

Ileal endogenous losses of P in birds fed P-free, gelatin-based and casein-based diets were estimated to be 25.1, 104 and 438 mg/kg DM intake, respectively. Ileal endogenous P losses in birds fed the casein-based diet were higher ($P < 0.05$) than those in birds fed the P-free and gelatin-based diets.

The endogenous losses of P in the excreta of birds fed P-free, gelatin-based and casein-based diets were 830, 560 and 372 mg/kg DM intake, respectively (Table 2). Excreta endogenous P losses in birds fed casein-based and gelatin-based diets were similar ($P > 0.05$) but excreta endogenous P losses in birds fed those two diets were lower ($P < 0.05$) than that in the birds fed the P-free diet. Ileal and excreta endogenous losses of P in birds fed the casein-based diet were similar ($P > 0.05$), but the ileal P losses were lower ($P < 0.01$) than the excreta losses in birds fed the P-free diet (Table 2). The excreta endogenous P losses determined in birds fed the casein-based diet were numerically lower than those in birds fed the gelatin-based diet (372 vs 560 mg/kg DM intake), but this difference was not statistically significant ($P > 0.05$) because of high variability between replicates.

Discussion

Published data on ileal endogenous P losses in poultry are limited. Only two previous studies have reported the ileal endogenous losses of P in poultry, but with variable results. Using a minimal P diet, Rutherford *et al.* (2002, 2004) reported values of 272 and 446 mg endogenous P/kg DMI, respectively.

The present results demonstrate that ileal endogenous losses of P are diet-dependent. Endogenous P losses in birds fed the casein-based diet were found to be higher than in those fed gelatin-based and P-free diets. Phosphorus-free diets are devoid of protein and the absence of protein will reduce enzyme secretions which in turn lowers the endogenous P secretion into the gut lumen. In the casein-based diet, the presence of protein can be expected to increase the secretion of proteolytic enzymes (Ravindran *et al.*, 2009) and may explain, at least in part, the higher endogenous P losses estimated in birds fed this diet. Dietary proteins with high biological values are potent stimulators of the synthesis and secretion of pancreatic enzymes (Snook and Meyer, 1964). Comparatively higher endogenous P losses determined in

birds fed the casein-based diet can therefore be explained partly by the differences in biological values of casein (0.87 to 1.0) and gelatin (0.07) (Asplund, 1987). Endogenous P losses determined in birds fed the casein-based diet yielded the highest estimate and were 4- and 14-folds greater than those estimated for gelatin-based and P-free diets, respectively. This estimation assumed that the casein-P was 100% available (NRC, 1994), but it is possible that the P in casein may not be 100% digestible and the values generated with the casein diet may have been slightly overestimated. The ileal endogenous P losses determined with the casein-based diet in the present study (438 mg/kg DM intake) were in close agreement with the finding (446 mg/kg DM intake) of Rutherford *et al.* (2004), using a minimal P diet supplemented with amino acids.

Gastric, biliary and pancreatic secretions together with sloughed enterocytes are the main contributors of endogenous P (Fan *et al.*, 2001). Bile is the primary source of endogenous P in poultry. In mammals, about 90% of bile lipids are typically composed of phospholipids (Cross *et al.*, 1987). According to Alvaro *et al.* (1986), phosphatidylcholine and phosphatidylethanolamine are the major phospholipids in chicken bile. No published data are available on the mineral content of pancreatic secretions in chickens. Zebrowska *et al.* (1983) reported that about 40% of the endogenous minerals in the duodenal contents of pigs are secreted by the pancreas. However, pancreatic secretions in pigs contain only low concentrations of P and Ca (Partridge *et al.*, 1982; Zebrowska *et al.*, 1983). It is known that the secretion of pancreatic proteolytic enzymes is sensitive to the nature of the protein source ingested (Snook and Meyer, 1964; Valette *et al.*, 1992). The presence of protein has also been found to increase cell slough-off and mucous secretion (Snook and Meyer, 1964). Casein is a phosphoserine rich protein with a higher buffering capacity, and in order to attain optimum pH for enzyme activity, the pancreas secretes more bicarbonate with casein-based diets, leading to increased water secretion and thereby increased volume of pancreatic secretions (Valette *et al.*, 1992). According to Snook and Meyer (1964), dietary proteins with high biological value, such as casein, are potent stimulators of the synthesis and secretion of pancreatic enzymes.

Microbes in the gastrointestinal tract also contribute to endogenous nutrient losses (Cotton, 1972). Diet is a key factor influencing the composition and counts of microflora in the gastrointestinal tract (Barnes, 1972). Microbial cell walls are composed of phospholipids (Cotton, 1972) and high microbial turnover may have contributed to the higher ileal endogenous P losses determined in birds fed casein- and gelatin-based diets.

The comparison of ileal and excreta endogenous losses provides some interesting insight into the P homeostasis in poultry. The higher endogenous P in the excreta of birds fed P-free and gelatin-based diets suggests an increased P output via urine when diets contain little or no Ca. A study by Liu *et al.* (2013) has shown that Ca-deficient diets lead to lower P retention in broilers. As described by Mundy and Guise

(1999), a drop in ionized blood plasma Ca concentration is immediately sensed by the parathyroid gland, which in turn responds with an increase in parathyroid hormone secretion. A rise in parathyroid hormone attempts to normalize serum Ca concentration by (i) increasing bone resorption and releasing Ca and P from bones into the extra cellular fluid, (ii) promoting reabsorption of Ca while inhibiting P reabsorption at renal tubules, and (iii) increasing the absorption of Ca and P via stimulating synthesis of 1,25-dihydroxy-vitamin D₃ (Mundy and Guise, 1999). Since the secretion of parathyroid hormone depends on the serum ionized Ca concentration (Mundy and Guise, 1999), it can be assumed that P excretion in urine will be negatively correlated with dietary Ca concentration. The ileal and excreta endogenous P losses in birds fed casein-based diets were similar. As for Ca, P deprivation in chickens is also known to increase P absorption *via* stimulating production of 1,25-dihydroxy-vitamin D₃ (Baxter and DeLuca 1976). As compared to P-free and gelatin-based diets, improved absorption of highly available P and Ca from casein may perhaps lower the requirement for bone mobilization to compensate for serum P and Ca concentrations in birds.

In conclusion, values obtained for endogenous P losses varied widely depending on the assay diet used. Endogenous losses estimated with the P-free diet may be considered as being representative of “basal” losses, which are related to the dry matter intake and are independent of the raw material or diet composition. This can be used in the calculation of true P digestibility of ingredients. In contrast, the estimates from casein- and gelatin-based diets appear to include specific P losses which are promoted by the presence of protein, which stimulates intestinal endogenous secretions. To the authors’ knowledge, the current work is the first study comparing different assay diets to determine endogenous P losses in poultry.

Conflict of Interest Statement

There is no conflict of interest.

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