Measurement of Ileal and Excreta Endogenous Losses of Phosphorus in Broiler Chickens

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Abstract

An experiment was conducted to estimate the ileal and excreta endogenous phosphorus (P) losses in broiler chickens. Three purified diets, namely a P-free diet and a gelatine-based diet containing negligible amount of P and a casein-based diet having 100% available P, were developed. They were offered *ad libitum* from day 25 to 28 post hatch and ileal digesta were collected. Excreta samples were collected to estimate total tract endogenous losses. The ileal endogenous flow of P in birds fed P-free, gelatin-based and casein-based diets were 25, 104 and 438, mg/kg dry matter intake (DMI), respectively. Corresponding values estimated at the excreta level were 830, 560 and 372 mg/kg DMI, respectively. Ileal and excreta endogenous flow of P in birds fed casein-based diet were similar (P>0.05), but ileal flows were lower (P<0.05) than excreta values in birds fed P-free and gelatin-based diets. In conclusion, the present data showed that values determined for endogenous P losses in broiler chickens widely varied depending on the methodology employed.

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Introduction

The main function of the gastrointestinal tract is the digestion and absorption of nutrients in the food. However, a considerable amount of endogenous nutrients is secreted into the gut lumen simultaneously. It is recognized that the amount of a particular nutrient leaving the ileum represents the net balance between its dietary intake plus its endogenous secretion minus the absorption of that particular dietary nutrient and re absorption of its endogenous material. Accurate measurement of and correction for these inevitable losses are necessary for the estimation of true ileal digestibility and to predict the net nutrient requirement for maintenance based on dry matter intake (Moughan and Fuller, 2003).

Estimates of endogenous phosphorus (P) losses have been reported for different animal species. But there have been no systematic studies conducted on ileal endogenous P losses in poultry. The primary sources of endogenous P are bile, enzyme secretions and sloughed epithelial cells. Although not strictly endogenous, gut microbes are also normally considered as components of endogenous materials. Different approaches that have been employed to measure endogenous P flow in animals include regression method, feeding P-free diets or diets with minimal P content and radio-isotope dilution technique (Fan *et al.*, 2001; Rutherfurd *et al.*, 2004). The aim of the present study was to determine the illeal and excreta level endogenous losses of P of broiler chickens fed purified diets containing no P, negligible P (gelatin-based diet) or 100% available P (casein-based diet).

Materials and Methods

Dietary treatments

Three purified diets were developed. The first diet was a P-free diet. The second diet was based on gelatin, which is known to contain almost no P (NRC, 1994). The third diet was a diet based on casein, which had negligible Ca and P contents. Phosphorus in casein was assumed to be 100% available (NRC, 1994). All diets contained 3 g/kg titanium dioxide as an indigestible marker.

Birds

Male broilers (Ross 308), 25-day old were individually weighed and, a total of 72 birds (average weight ± SD, 1265 ± 12 g) were assigned to 12 cages of six birds each. After four-hours of feed withdrawal, the test diets were introduced, and offered *ad libitum*. Water was available at all times. A lighting schedule of 20 hours per day was applied.

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 and the contents from the lower ileum were collected and lyophilized. Daily excreta collections were pooled within a cage, mixed well, sub-sampled and lyophilised.

Chemical analysis

Samples of diets, digesta and excreta were ground to pass through 0.5-mm sieve and representative samples were analysed for dry matter (DM), Ca, P and titanium (Ti) according to the standard procedures.

Calculations

The flows of P were calculated, as mg lost/kg ingestion of feed DM, using the formula described below (Rutherfurd *et al.*, 2004).

Endogenous phosphorus flow (mg/kg)

$$= \text{P in digesta or excreta (mgkg)}\ x$$

$$\left(\frac{\text{Titanium in diet (mg/kg)}}{\text{Titanium in digesta or excreta (mg/kg)}}\right)$$

Statistical analysis

Data were subjected to ANOVA procedures using SAS statistical software package. Means of endogenous flow of P in the ileal digesta and excreta between diets were separated using the least significant difference. The differences between ileal and excreta endogenous P flows for each diet were compared by paired t-test at P<0.05 level of significance.

Results and Discussion

Analysed P contents of P-free, gelatin-based and casein-based diets were <0.09, <0.09 and 1.6 g/kg (as fed), respectively. Analysed Ca contents of P-free, gelatin-based and casein-based diets were 0.5, 0.8 and 0.5 g/kg (as fed), respectively. Ileal endogenous flow of P (mg/kg DMI) in birds fed casein-based diet (438) were higher (P<0.05) than those fed P-free (25.1) and gelatin-based (104) diets (Table 1). The present results demonstrate that the ileal endogenous flow of P is diet-dependant. Phosphorus-free diet was devoid of protein and the absence of protein may reduce enzyme secretions which in turn lowers the endogenous P secretion into the gut lumen. In gelatin-based and casein-based diets, the presence of protein (crude protein, 176 and 174 g/kg as fed, respectively)is expected to increase the secretion of proteolytic enzymes and may explain, at least in part, the higher endogenous P flow estimated in birds fed those diets. This calculation was based on the assumption that the casein-P is 100% available (NRC, 1994), but it is possible that casein P may not be 100% digestible and the values generated with casein diet may have been overestimated. But the ileal endogenous P flow determined with the casein- based diet in the present study (438 mg/kg DMI) was in close agreement with the finding (446 mg/kg DMI) of Rutherfurd *et al.* (2004) reported using a minimal P diet. Gastric, biliary and pancreatic secretions together with sloughed enterocytes are the main contributors of endogenous P (Fan *et al.*, 2001). It is known that the secretion of pancreatic proteolytic enzymes is sensitive to the nature of the protein source ingested (Snook and Meyer, 1964). The presence of protein has also been found to increase cell slough-off and mucous secretion (Snook and Meyer, 1964). According to them, dietary proteins with high biological value, such as casein, are potent stimulators of the synthesis and secretion of pancreatic enzymes.

High microbial turnover may also have contributed to the higher ileal endogenous P losses in birds fed casein- and gelatin-based diets. The endogenous flow of P in the excreta of birds fed P-free, gelatin-based and casein- based diets were830, 560 and 372 mg/kg DMI, respectively (Table 1). Excreta endogenous P flows in birds fed casein-based and gelatin-based diets were similar (P>0.05), but it was lower (P<0.05) in birds fed casein-based diet than that of P-free diet. Considerable numerical difference in excreta endogenous P losses between birds fed casein-based and gelatine-based diets was not significant due to high variability between replicates. Ileal and excreta endogenous flows of P in birds fed casein-based diet were similar (P>0.05), but ileal flow was lower (P<0.01) than excreta flows of P in birds fed P-free and gelatin-baseddiets (Table 1).

The comparison of ileal and excreta endogenous flows provides interesting insight into the P homeostasis in poultry. Markedly higher endogenous P in the excreta of birds fed P-free diet suggests an increase P output *via* urine when diets contain little or no Ca. Published data on endogenous losses of P in poultry are not only limited, but also highly variable. These data are difficult to interpret as researchers have rarely employed similar methodology which would allow for direct comparisons of data. The discrepancy among published reports may also be due to confounding factors, including differences in animal and dietary factors. In conclusion, values obtained for endogenous P

Table 1: Comparison of ileal and excreta endogenous P flow (mg/kg dry matter intake) in broiler chickens[1]

Diet	Endogenous P flow (mg/kg DMI)		Significance[2]
	Ileal	Excreta	
Phosphorus-free	$25.1^a \pm 11.1$	$830^b \pm 139.1$	P<0.01
Gelatin-based	$104^a \pm 41.3$	$560^{ab} \pm 49.2$	P<0.01
Casein-based	$438^b \pm 67.1$	$372^a \pm 40.7$	NS[3]
Pooled SEM[4]	45.9	88.4	

[1]Each value represents the mean of four replicates (6 birds per replicate) ± standard error
[2]Endogenous ileal P flow vs. excreta P flow for respective diets
[3]Not significant
[4]Pooled standard error of mean
[a, b]Means in a column not sharing a common superscript are significantly different at P<0.05

losses showed a wide variability depending on the methodology employed.

Endogenous flow 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. Perhaps this can be used in the calculation of true P digestibility of ingredients for poultry. In contrast, the estimates from casein- and gelatin-based diets also include specific losses which are influenced by the presence of protein that stimulates endogenous secretions. To the author's knowledge, the current work is the first study comparing different methodologies to determine endogenous P flow in poultry. Clearly further research is warranted to confirm the present findings.

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