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Abstract

This study aimed to measure the endogenous losses, the apparent (AID), and the standard ileal digestibility (SID) of phosphorus (P) using a diet containing corn, soybean meal, and wheat bran in 45-week-old Hy-line Brown laying hens. Hens were fed experimental diets containing inorganic phosphate supplements and plant-based phosphorus sources. Experimental diets included: (i) inorganic phosphate supplements (monocalcium phosphate [MCP], dicalcium phosphate [DCP], monosodium phosphate [MSP]); (ii) plant-based phosphorus sources (corn [CRN], soybean meal [SBM], and wheat bran [WB]); and (iii) a phosphorus-free diet [P-free]. Measurements were collected from the ileum and excreta over three days with six replicates per dietary treatment and four hens per cage, under *ad libitum* feeding conditions. The study demonstrated interactions ($p < 0.05$) between diet type and sampling locations on endogenous phosphorus losses. Higher endogenous phosphorus losses ($p < 0.05$) were observed in the excreta of hens fed P-free, MCP, DCP, MSP, and WB diets. Increased endogenous P losses in the ileum ($p < 0.05$) were noted in hens-fed CRN, SBM, and WB diets compared to inorganic phosphate supplement diets. The AID and SID of P were significantly lower ($p < 0.05$) in the CRN diet compared to SBM, WB, MCP, DCP, and MSP diets. Among inorganic phosphate supplements, MCP exhibited the highest SID of P, while for plant-based sources, SBM and WB demonstrated higher SID values than CRN. The AID of P was higher ($p < 0.05$) in hens fed the MCP diet compared to SBM and WB diets, but diet type did not affect the AID of dry matter ($p > 0.05$). In conclusion, diet type and sampling location influence endogenous P losses in laying hens, with differences between ileal and excreta measurements. The results highlight the variability in P bioavailability among plant-based and inorganic phosphorus sources, emphasizing the need to account for ingredient-specific P digestibility when formulating diets for optimal P utilization.

Keywords: Digestibility, endogenous losses, excreta, laying hens, phosphorus

INTRODUCTION

Phosphorus (P) is noteworthy in poultry nutrition, playing key roles in bone formation, energy metabolism, cellular structure, and egg production [1,2]. However, not all phosphorus is utilized by the gastrointestinal tract of poultry; a certain portion is lost endogenously. Thus, a comprehensive understanding of the mechanisms behind phosphorus absorption and utilization is essential for optimizing the chickens' performance.

Recent advances in animal nutrition have focused on optimizing nutrient utilization and lowering nutrient loss in the excreta due to its potential environmental effects. In this regard, many studies were conducted to estimate the amount of endogenous loss of essential nutrients such as amino acids and minerals to facilitate the precise formulation of diets that meet the animal's requirements, thereby reducing waste and environmental leakage of hazardous micro and macro components originating from animals [3-5]. The nutrients existing in the ileum reflect actual results of dietary nutrient intake, endogenous nutrient secretion, and the extent of nutrient absorption and reabsorption. To accurately assess true ileal digestibility and determine the net nutrient requirements for maintenance, it is essential to account for dry matter intake. This approach allows for more precise measurement and correction of inevitable nutrient losses [6].

It was reported that endogenous losses are attributed to saliva, digestive enzymes, bile, sloughed epithelial cells, and mucins and can be further increased by antinutritional factors in feeds, such as lectins, trypsin inhibitors, tannins, and fiber [7]. Though several approaches have been employed in previous trials, such as using diets free from phosphorus, regression methods, or diets with negligible amounts of phosphorus [6,8], determining the accurate measure of endogenous P losses in animals is still inconclusive. Therefore, the current study aimed to measure the endogenous losses, apparent, and standardized digestibility estimates of phosphorus of 45-week-old Hy-line Brown laying hens using seven experimental diets, specifically phosphorus-free (P-free), monocalcium phosphate (MCP), dicalcium phosphate (DCP), monosodium phosphate (MSP), corn-based (CRN), soybean meal-based (SBM), and wheat bran-based (WB) diets, and the effects of these different diets on the endogenous P losses were measured at the ileal and excreta stages. The tested diets were hypothesized to be suitable for measuring the endogenous losses and ileal digestibility of P in laying hens.

MATERIALS AND METHODS

The experiment procedures and protocol were reviewed and approved by the Animal Ethics Committee of Chungnam National University (Protocol number: 202401A-CNU-003).

Layer chickens, housing, and management

A total of 168, 45-week-old Hy-line Brown® laying hens were used in this study. All hens were fed on a standard layer diet until the beginning of the study. Experimental hens were randomly assigned to experimental diets in a completely randomized design with four hens per cage and six replicates per dietary treatment for three days. All hens had access to feed and water on an *ad libitum* basis. Each cage ($60 \times 25 \times 45$ cm^3) had two nipple drinkers and a metal feeder. The lighting program employed 16 hours of continuous light and 8 hours of darkness. In addition, hens were reared in a windowless and temperature-controlled (around 20°C to 22°C) environment.

Diets

Table 1 shows the ingredients and chemical compositions of the diets fed for three days. The test ingredients included MCP (22.8% P, 17.0 % Ca), DCP (21.0% P, 28.0 % Ca), MSP (25.5 % P, 0 % Ca), corn, soybean meal, and wheat bran as the sole source of phosphorus. A phosphorus-free diet was subsequently formulated to estimate the basal endogenous phosphorus losses. All seven experimental diets contained 0.3% chromium oxide as an indigestible marker. Table 1 illustrates the formulation of the seven experimental purified diets used in the current study with the analyzed Ca and P compositions.

Postmortem procedures, sample collection, and analysis

On day 3, collection trays were introduced, and samples of fresh excreta were collected from each cage for two days. Thereafter, 24 hens per group (four hens per replicate) were randomly selected and euthanized by carbon dioxide asphyxiation for the digesta sample collection. The ileal digesta was gently finger-stripped into labeled plastic containers and stored at –80°C until further nutrient digestibility analysis.

Samples of diets, digesta, and excreta were analyzed for dry matter (DM), calcium (Ca), P, and chromium oxide composition. DM content was determined following method 930.15 of AOAC [9]. The concentration of chromium oxide was determined following the procedure of Fenton and Fenton [10], Ca (method 927.02) and P (method 965.17) composition in the feed, ileal digesta, and excreta were analyzed according to the procedures of the AOAC [9].

Calculations

The apparent ileal digestibility (AID, %), endogenous P losses (mg/kg DM intake), and standard ileal digestibility (SID, %) were calculated as milligrams lost per kilogram (mg/kg) of feed DM ingestion, using the following formula stipulated by Oketch et al. [5].

$$\text{AID (\%)} = 100 - \left[100 \times \frac{Chromium\ oxide\ (Diet)(mg/kg) \times phosphorus\ (digesta)\ (mg/kg)}{Chromium\ oxide\ (Digesta)\ (mg/kg) \times phosphorus\ (diet)\ (mg/kg)}\right]$$

Endogenous phosphorus losses (mg/kg DM intake)

$$= \text{phosphorus (Digesta or excreta) (mg/kg)} \times \frac{\text{Chromium oxide (Diet) (mg/kg)}}{\text{Chromium oxide (Digesta or excreta) (mg/kg)}}$$

$$\text{SID (\%)} = [\text{AID} + (100 \times \text{basal endogenous losses /phosphorus (digesta)}]$$

Statistical analyses

The statistical analyses of the data were performed using SPSS (version 26; IBM SPSS 2019). The data obtained from the experiment, except for the AID and SID of phosphorus, were analyzed using the general linear model (GLM) procedure for two-way ANOVA to evaluate the main effects (type of diet and site of measurement). The data of AID and SID values of phosphorus were analyzed using the GLM procedure for the one-way ANOVA technique. The experimental unit for this trial was defined as the individual birds. Statistical significances were determined at a significance level of $p < 0.05$. When treatment effects were significant ($p < 0.05$), the means were further analyzed and compared using Tukey's multiple-range test procedures implemented in SPSS software.

RESULTS

The diet type has not influenced ($p > 0.05$) either ileal or excretory P endogenous loss in layer hens in the current study (Table 2). However, there was a notable interaction effect ($p < 0.05$) between the tested diet and the site of measurement (ileal vs. excreta) for P endogenous losses of laying hens (Table 2). The endogenous P losses were increased ($p < 0.05$) in the excreta in hens fed P-free and MCP diets, while no differences ($p > 0.05$) in endogenous P losses were observed between measurement sites in hens fed the DCP, MSP, CRN, SBM, and WB diet. However, collectively, the measurement site (ileal vs. excreta) has significantly influenced ($p < 0.001$) the endogenous P loss of the experimental layer hens. The endogenous losses of P in the excreta of hens P-free, MCP, DCP, MSP, CRN, SBM, and WB diets were 456.95, 354.52, 267.30, 240.43, 206.00, 225.49, and 366.99 mg/kg DM intake, respectively (Table 2), whereas, ileal endogenous losses of P in hens were estimated to be 65.16, 92.85, 105.56, 125.88, 174.22, 153.18, and 158.51 mg/kg DM intake, respectively.

The numeric values of endogenous P losses in the ileum of hens fed a CRN, SBM, and WB diet were higher ($p > 0.05$) than other diets. The highest numerical increase of endogenous P losses was observed in the excreta of hens fed a P-free diet. The lowest values were observed in the excreta of hens fed CRN and SBM diets. Moreover, for the AID of DM in assay diets, there were no notable differences ($p > 0.05$) (Table 3). The AID and SID of P were significantly lower ($p < 0.05$) in the CRN diet compared to other assay diets. Meanwhile, the MCP, DCP, and MCP diets have shown higher AID and SID of P than the plant-based diets (i.e., CRN, SBM, and WB).

DISCUSSION

Excessive endogenous P loss in laying hens can lead to negative outcomes of health and productivity, leading to issues such as weakened eggshell quality and compromised bone integrity. Understanding these losses is essential for developing a diet that optimizes the nutritional intake of laying hens, improving productivity, and reducing waste and environmental impact. In addition, ileal digestibility is preferred when determining the availability of feed ingredients as it excludes the contribution of urinary P excretion [11]. There are various methods to determine the digestibility and the endogenous P losses in animals, such as the regression method, feeding P-free diets or diets with minimal P content, and the radio-isotope dilution technique [6]. However, previous research on ileal endogenous P losses in poultry, especially in layers, is scarce, with only a couple of studies presenting inconsistent findings. Significant variations were reported in measuring endogenous P losses based on the assay diet employed. The figures derived from the Ca and P-free diet were considered indicative of the fundamental losses, which correlate with the intake of dry matter and were not influenced by the type of raw material or the composition of the diet [6,12].

In our study, the diets' analyzed Ca and P composition closely matched the formulated contents to maintain precise control over dietary variables. The observed significant interaction effects between diet type and measurement site for P endogenous losses suggest that dietary composition influences the gastrointestinal tract section where P losses occur within the hens's body. Notably, increased P losses were observed in the excreta when hens were fed a P-free diet. The high excreta P losses in the P-free diet group highlight the compensatory increase in endogenous P excretion when dietary P is insufficient. This phenomenon has been documented in other studies, where low dietary P levels can lead to increased mobilization of bone P to maintain serum P levels, resulting in higher excretion rates [13,14]. About 80% of the body's P is stored within the bones in the form of hydroxyapatite. This P is liberated from bone during eggshell formation, and the surplus must be eliminated to prevent toxicity in the body [14]. Conversely, the lower ileal endogenous P losses observed in P-free, MCP, DCP, and MSP-based diets can be attributed to the fact that those diets were 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 CRN, SBM, and WB diets, the presence of protein can be expected to increase the secretion of proteolytic enzymes and may be linked to the marginal increase in endogenous P losses estimated in hens fed those diets (Anwar and Ravindran 2020; Mutucumarana and Ravindran 2021).

In addition, mineral P supplements like MCP, DCP, and MSP provide highly available P, reducing the reliance on phytate-bound P and thus leading to lower endogenous P losses in the ileum. This is supported by the work of Rodehutscord et al. [15], who demonstrated that mineral P supplements enhance P digestibility and reduce endogenous P excretion compared to plant-based P sources. The ileal endogenous losses of P varied slightly across the diets, with the CRN, SBM, and WB diets leading to a minimal increase in P losses. This could be attributed to the presence of phytate-bound P in corn, soybean, and wheat bran, which is less available to the hen due to limited phytase activity, thus increasing endogenous losses [16]. However, despite the observed differences in P losses across diets, no significant effects of diet type on endogenous P losses were detected at either measurement site. This could suggest that while specific diets can influence the site-specific excretion of endogenous P, the overall impact on total endogenous P losses may be minimal. The balance between ileal and excreta losses could explain this observation, where a decrease in the other may offset an increase in one site. As was previously reported by Selle et al. [17], the digestive system of poultry can adjust to different dietary compositions by altering the site of P excretion. When a diet causes increased P losses in one part of the digestive tract (i.e., the ileum), there may be a compensatory decrease in another part (i.e., excreta), resulting in an overall stable total P loss.

Moreover, the AID and SID values of P were significantly lower in the CRN diet compared to other diets. This could be attributed to the fact that corn may have lower P availability or higher levels of anti-nutritional factors that inhibit P absorption. This aligns with previous studies that suggest that corn has a relatively low P digestibility due to the high phytate content in grains like corn [16]. Phytate-bound P is poorly digested by poultry because chickens lack sufficient phytase activity to break down the phytate molecule effectively. This is also consistent with the findings from Selle et al. [17], who demonstrated that phytate content negatively affects P availability in corn-based diets. Additionally, the lack of significant differences in AID for DM between diets could indicate that the digestibility of other nutrients besides phosphorus is less affected by the diet type, which supports previous research that showed P digestibility is highly diet-dependent, whereas DM digestibility may remain relatively stable across diets [18]. Wheat bran diets, although higher in fiber, are known to also contain moderate levels of phytate, which can bind P and reduce its digestibility. However, the observed higher MCP digestibility over WB, and similarity with other inorganic diets (DCP, MSP) and SBM, could suggest that inorganic P sources provide consistent and higher bioavailability, a pattern observed in several poultry nutrition studies [19]. Our findings show that SBM showed similar P digestibility to MCP, DCP, and MSP diets, which might be explained by the fact that soybean meal, despite being plant-based, contains lower phytate levels than corn or wheat bran. According to studies by Humer et al. [20] and Sommerfeld et al. [21], soybean meal is often considered a moderate source of P, with less bound P compared to grains like corn. The reduced phytate content in SBM improves P digestibility, although it still does not match the highly bioavailable inorganic phosphorus in MCP.

To the best of our knowledge, while most studies have found significant effects of diet type on total endogenous P losses, particularly when extreme differences in dietary P availability are present, our finding that diet type did not significantly affect overall P losses and the AID of DM may contrast with studies that used more varied or extreme diet compositions. This indicates that while the assay diets used in our study were diverse, they might not have sufficient differences to produce significant overall effects or that the adaptive mechanisms in hens were highly effective. Moreover, using inorganic phosphorus sources such as MCP, DCP, and MSP can improve P utilization in laying hens, as were noted with their improved AID and SID of P, which are essential parameters for optimizing feed formulations. In conclusion, our study revealed that dietary variations can influence endogenous P losses within specific segments of the gastrointestinal tract in laying hens. These findings highlight the importance of precise dietary formulations to enhance P utilization efficiency while reducing environmental burdens. Therefore, future research should explore the long-term impacts of such dietary strategies on laying performance and P digestibility, further optimizing poultry nutrition and sustainability.

COMPETING INTERESTS

No potential conflict of interest relevant to this article was reported.

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DATA TABLES

Table 1. Ingredients and chemical compositions of experimental diets (as-fed basis)

Item	Experimental diet[1]						
	P-free	MCP	DCP	MSP	CRN	SBM	WB
Ingredient, %							
Monocalcium phosphate	-	2.37	-	-	-	-	-
Dicalcium phosphate	-	-	2.40	-	-	-	-
Limestone	7.97	6.93	6.24	7.99	8.72	10.05	8.79
Monosodium phosphate	-	-	-	1.95	-	-	-
Corn	-	-	-	-	71.49	-	-
Soybean meal	-	-	-	-	-	45.55	-
Wheat bran	-	-	-	-	-	-	32.55
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Sucrose	28.99	28.32	28.65	28.00	-	-	-
Cornstarch	28.99	28.32	28.65	28.00	-	38.60	38.88
Gelatin	5.00	5.00	5.00	5.00	-	-	-
Cellulose	23.25	23.25	23.25	23.25	13.98	-	13.98
Magnesium oxide	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Sodium bicarbonate	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
L-lysine	1.07	1.07	1.07	1.07	1.07	1.07	1.07
DL-Methionine	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Vit-Min premix[2]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Cr_2O_3	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated composition							
Calcium, %	4.00	4.00	4.00	4.00	4.38	5.03	4.61
Total phosphorus, %	-	0.50	0.50	0.50	0.96	1.01	0.95
Available phosphorus, %	-	0.50	0.50	0.50	0.55	0.63	0.58
Calcium: Available phosphorus	-	8.00:1	8.00:1	8.00:1	7.96:1	7.98:1	7.95:1
Analyzed composition							
Dry matter, %	94.14	93.63	93.47	93.34	87.96	90.47	89.44
Calcium, %	4.03	4.06	4.08	4.02	4.45	5.24	4.93
Total phosphorus, %	0.01	0.51	0.54	0.53	1.03	1.06	1.02

[1]P-free, phosphorus-free diet; MCP, monocalcium phosphate; DCP, dicalcium phosphate; MSP, monosodium phosphate; CRN, corn; SBM, soybean meal; WB, wheat

bran

[2]Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 3,000 IU; vitamin E, 21 mg; vitamin K_3, 2,400 mg; vitamin B_1, 1,200 mg; vitamin B_2, 4,800 mg; vitamin B_6, 2,400 mg; vitamin B_9, 300 mg; D-pantothenic acid, 10,000 mg; nicotinic acid, 15,000 mg; choline, 20 mg; Fe, 24,000 mg from iron sulfate; Cu, 4.500 mg from copper sulfate; Zn, 60,000 mg from zinc oxide; Mn, 72,000 mg from manganese oxide; I, 1,000 mg from potassium iodide; Se, 200 mg from sodium selenite; Co, 150 mg.

Table 2. Comparison of ileal and excreta basal endogenous phosphorus losses (mg/kg dry matter intake) in laying hens estimated using different assay diets[1]

Type of the diet[2]	Site of measurement	Basal endogenous P loss (mg/kg)
P-free	Ileal	65.16[a]
	Excreta	456.95[c]
MCP	Ileal	92.85[a]
	Excreta	354.52[bc]
DCP	Ileal	105.56[a]
	Excreta	267.30[abc]
MSP	Ileal	125.88[a]
	Excreta	240.43[abc]
CRN	Ileal	174.22[ab]
	Excreta	206.00[ab]
SBM	Ileal	153.18[ab]
	Excreta	225.49[ab]
WB	Ileal	158.51[ab]
	Excreta	366.99[bc]
SEM[3]		19.694
Main effect means		
Type of the diet		
P-free		261.06
MCP		223.68
DCP		186.43
MSP		183.16
CRN		190.11
SBM		189.34
WB		262.75
SEM[3]		30.471
Site of measurement		
Ileal		125.05
Excreta		302.53
SEM[3]		16.288
***p* -values**		
Type of the diet		NS[4]
Site of measurement		<0.001
Diet × Site		0.005

[1]Each value represents the mean of six replicates (4 chickens per replicate).

[2]P-free, phosphorus-free diet; MCP, monocalcium phosphate; DCP, dicalcium phosphate; MSP, monosodium phosphate; CRN, corn; SBM, soybean meal; WB, wheat

bran

[3]Pooled standard error of mean.

[4]Not significant ($P > 0.05$).

[a-c]Values with a different superscript within the column differ significantly ($p < 0.05$).

Table 3. Apparent and standardized ileal digestibility (%) of phosphorus in laying hens estimated using different assay diets[1]

Item	Experimental diets[2]						SEM[3]	*p*-value
	MCP	DCP	MSP	CRN	SBM	WB		
AID DM	72.96	71.81	72.23	73.42	74.81	71.36	0.723	0.937
AID P	98.467^{d}	97.137^{cd}	95.573^{bcd}	73.003^{a}	93.700^{bc}	91.340^{b}	2.110	<0.001
SID P	98.687^{d}	97.357^{cd}	95.790^{bcd}	73.223^{a}	93.917^{bc}	91.557^{b}	2.110	<0.001

AID = apparent ileal digestibility; DM = Dry matter; P = phosphorus; SID = standardized ileal digestibility

[1]Each value represents the mean of six replicates (4 hens per replicate).

[2]P-free, phosphorus-free diet; MCP, monocalcium phosphate; DCP, dicalcium phosphate; MSP, monosodium phosphate; CRN, corn; SBM, soybean meal; WB, wheat bran

[3]Pooled standard error of the mean.

[a-d]Values with a different superscript within the column differ significantly ($p < 0.05$).