

# Proceedings of the Arkansas Nutrition Conference

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Volume 2022

Article 14

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2022

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### Recommended Citation

Hampton, Jay; Li, Wenting; Mussini, Franco; Hilton, Katie; Remus, Janet; and Rochell, Samuel J. (2022) "Recent Findings on Phosphorus Digestibility of Feed Ingredients in Broilers," *Proceedings of the Arkansas Nutrition Conference*: Vol. 2022, Article 14.

Available at: <https://scholarworks.uark.edu/panc/vol2022/iss1/14>

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## Recent Findings on Phosphorus Digestibility of Feed Ingredients in Broilers

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## Recent Findings on Phosphorus Digestibility of Feed Ingredients in Broilers

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### Introduction

Accurately meeting the dietary P needs of broilers is critical to ensure optimal bird performance, health, and welfare without causing undue environmental burdens associated with excess dietary P excretion. Phosphorus is commonly supplied in broiler diets from inorganic phosphates derived from non-renewable sources, but it has been estimated that peak P production will occur between 2030-2040 and that the global supply of P could be depleted within this century (Cordell and Drangert, 2009; Nest and Cordell, 2012). To address these concerns, inorganic P use in agriculture, including use in broiler diet formulation, requires careful stewardship. To this end, the dietary inclusion of animal protein meals can help reduce or eliminate the need for inorganic phosphate use, especially when paired with phytase (van Harn et al., 2017). However, the proportion of P available to the bird within commonly used inorganic phosphate and animal protein sources is often not well-defined.

Historically, P availability for feed ingredients has been expressed as availability relative to a reference source assumed to have high availability, with outcomes based bone measurements or growth differences among birds fed graded levels of different test sources (Nelson et al., 1990). However, relative bioavailability values generated from such assays can be difficult to directly translate into feed ingredient matrix values when quantitative P availability from the reference source is not measured (Leske and Coon, 2002; Shastak and Rodehutsord, 2015). To address this, the WPSA (2013) presented suggested guidelines for conducting P availability experiments as well as definitions to help standardize commonly used nomenclature of total P, digestible P, available P, and prececal digestibility. It was proposed that prececal, or ileal, digestibility should

be the preferred method for determining P availability. The WPSA (2013) method involves a regression-based approach for determining ileal P. Furthermore, the direct method based on feeding the test sources as the only source of P in the diet can also be used (An et al., 2020). As with amino acids, ileal P digestibility values can be determined without accounting for endogenous losses and reported as apparent ileal digestibility (AID) values, or they can be corrected for estimated basal endogenous losses and reported as standardized ileal digestibility values (SID).

With increasing adoption of ileal digestibility of P in feed formulation, additional data are needed to develop robust databases for commonly used and important sources of P. Therefore, the aim of this study was to generate values of AID and SID of P for different inorganic phosphates and meat and bone meal sources using the direct method. In addition, relative P digestibility of these sources was determined using monosodium phosphate as the reference ingredient to provide a basis for comparing quantitative digestibility results with relative values.

## **Approach**

### ***Test Ingredients and Diet Formulation***

The P sources evaluated in this experiment were monocalcium phosphate (MCP), dicalcium phosphate dihydrate (DCP), monocalcium phosphate (MDCP), defluorinated phosphate (DFP), and bovine and porcine meat and bone meals (MBM) (Table 2). Additionally, anhydrous monosodium phosphate (MSP) was used as a reference source for determination of relative digestibility. A semi-purified P and N free diet was formulated to allow for determination of ileal endogenous P flow. All inorganic phosphates were included in this P-free semi-purified basal diet to provide 0.31% P at the expense of Celite. The 2 MBM sources replaced dextrose and corn starch in addition to Celite. To establish a linear regression for comparison of digestibility values relative to MSP, a series of diets was formulated to contain 0.13, 0.23, and 0.33% total P from MSP. Calcium levels were balanced to 0.65% in all diets by varying limestone inclusions. Titanium dioxide was used as a digestibility marker in all diets.

### ***Bird Husbandry***

This experiment was conducted using Cobb 500 breeder male by-product chicks placed in battery cages at 8 chicks per cage. A common diet that met or exceeded primary breeder nutrient recommendations was provided until 20 d post-hatch. At 20 d post-hatch, 6 birds were re-allotted to equalize body weight across treatment groups weight and then provided randomly-

assigned experimental diets. Birds consumed experimental diets ad libitum for 48 hours and were then euthanized and sampled for ileal digesta at 22 d.

### ***Determination of Apparent and Standardized Ileal Digestibility***

Digesta samples were flushed from the lower one-half of the ileum into specimen cups and frozen shortly after. Ileal samples were freeze dried, ground, and analyzed for complete mineral profiles and  $\text{TiO}_2$  concentrations. Experimental diets were analyzed in a similar manner to allow for calculation of AID and SID of P. For statistical analyses, AID of Ca and P and SID of P of treatment groups 2-10 were compared using a one-way ANOVA followed by a Tukey's HSD test. Statistical significance was considered at  $P < 0.05$ .

In order to determine relative digestibility of test ingredients using MSP as the reference source, linear regressions were generated by plotting the digestible P (AID P coefficient  $\times$  total P, %) derived from each test against the analyzed total P contributed by the feed ingredient (Rodehutsord et al., 2004). Relative digestibility (%) was calculated by dividing the slopes from the 2 point lines for each test ingredient by the 4 point reference slope established with the MSP diets, and multiplying this value by 100.

### **Findings**

The analyzed total content of diets containing test ingredients ranged from 0.29 to 0.34%, total P and closely matched the target concentration of 0.31%. Similarly, the graded MSP diets contained 0.14, 0.22, and 0.34% analyzed total P which aligned with calculated concentrations. Analyzed Ca concentration was lowest for the porcine MBM diet (0.59%) and highest for the bovine MBM diet (0.79% Ca), whereas all other diets contained analyzed Ca values reasonably close to calculated levels.

The apparent digestibility of P from inorganic P sources resulted in 2 levels of statistical separation, with AID of P from MSP (92.57 to 88.53%) at all dietary concentrations, MCP (89.55%), DCP (90.04%), and the bovine MBM (92.24%) having similar and higher values than DFP (73.59%), MDCP (76.58%) and porcine MBM (77.31%), which were all similar. Recently, An et al. (2020) reported the AID of P to be 86.7% in MCP, 76.2% in DCP, and 86.0% in MDCP in broilers at 19 d of age using the direct method. Cambra-López et al. (2021) also recently reported the ileal P digestibility of MCP to range from 75.2 to 87.4% and that of DCP to range from 80.5 to 86.6% when using regression-based approaches and a basal diet that included corn, potato protein, and soybean meal. On the other hand, Shastak et al. (2012) found much lower

ileal P digestibility values of 30% for DCP and 67% for MSP when estimated based on a regression approach when birds were fed basal diets with based on corn, potato protein, and soybean meal. Thus, it is clear from these studies and others reported in Table 1 that the methods employed as well as the specific source of the test phosphate source will ultimately influence the outcome, making direct comparisons across studies difficult. Nonetheless, though slightly higher, the AID of P values reported herein are in general agreement with many previously reported values.

Despite being traditionally valued as a highly available source of Ca and P, there are currently only a few published studies on the ileal P digestibility of MBM in broilers which report P digestibility values ranging from 23 to 69% (Mutucumarana et al., 2015; Mutucumarana et al., 2016; Munoz et al., 2020; Dilelis et al., 2021). The AID of P in the 2 MBM studies evaluated in the current experiment were found to be 92.24% for the bovine product and 77.31% for porcine product, both of which are higher than previously reported. It is not clear if the wide difference between these 2 values were due to variations in the proportion of soft tissues in the raw material, processing conditions, or a combination of these and other factors. Nonetheless, both sources were found to have P digestibility at least as high as the inorganic sources with the lowest digestibility values (DFP and MDCP), indicating MBM are a good source of digestible P for broilers. However, potential differences in P digestibility among sources should be considered in feed formulation and warrant further investigation.

With the direct method, a P-free basal diet is used to determine ileal endogenous P flow to calculate SID of P. In the current study, ileal endogenous P flow was found to be 75 mg/kg of dry matter intake, which was in general agreement with previous literature for using P and nitrogen-free basal diets basal diet type will certainly influence ileal P flow (Mutucumarana and Ravindran et al., 2021). This relatively low level of endogenous P flow resulted in only minor differences between AID and SID values in the current study.

Relative bioavailability values of P sources are typically based on weight gain or tibia measurements in growing birds fed graded levels of P from the sources of interest and a reference source. Due to the 48 h feeding period of the experimental diets in the current study, there was insufficient time to expect any change in these criteria, so digestibility values relative to a reference P source of MSP were calculated. The linear slopes for MCP, DCP, and the porcine MBM were greater than the slope for the MSP diets, resulting in relative digestibility P

values greater than 100% for these sources. Given that the P within MSP was approximately 90% digestible, expressing the values of all sources relative to MSP increased estimated digestibility values by approximately 10 percentage units compared with the directly determined AID and SID values. This clearly presents the risk and difficulty of trying to use relative data to assign quantitative matrix values of P availability for various sources.

In conclusion, these results add to a growing database of ileal P digestibility values for inorganic phosphates and MBM which are widely used feed ingredient in the commercial poultry industry. Although all sources had generally high digestibility (all >73%), no source was completely available, and differences among sources existed. Furthermore, these data demonstrate the simplicity of interpreting ileal digestibility values compared with relative values, especially when the P in the reference source is not 100% available, which is likely often the case.

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**Table 1.** Summary of experiments reporting ileal digestibility of P in inorganic phosphate sources determined in broilers

Ingredient	Reference	Method / Duration / Bird Age <sup>1</sup>	Dietary Ca, % <sup>2</sup>	Dietary P, % <sup>2</sup>	AID of P, %	SID of P, % <sup>3</sup>
Monocalcium phosphate	An et al., 2020	Direct / 5 d / 19 d	1.05	0.44	86.7	89.8
	Bikker et al., 2016	Direct / 10 d / 28 d	0.44	0.34	78.3	-
	Cambra-Lopez et al., 2021	Regression / 4 d / 25 d	0.40 - 0.47	0.29 - 0.35	-	87.4
	van Harn et al., 2017	Regression / 10 d / 24 d	0.50	0.45	-	88.5
	Trairatapiwan et al., 2018	Regression / 8 d / 29 d	0.39 - 0.48	0.32 - 0.39	-	64.6
	Wang et al., 2022	Substitution / 7 d / 27 d	0.99 - 1.16	0.45-0.61	83.1	-
Dicalcium phosphate	An et al., 2020	Direct / 5 d / 19 d	1.03	0.43	76.2	79.5
	Bikker et al., 2016	Direct / 10 d / 28 d	0.44	0.33	59.0	-
	Cambra-Lopez et al., 2021	Regression / 4 d / 25 d	0.44 - 0.47	0.33 - 0.35	-	86.6
	van Harn et al., 2017	Regression / 10 d / 24 d	0.51	0.45	-	82.4
	Shastak et al., 2012	Regression / 10 d / 21 d	0.82 - 1.16	0.43 - 0.59	-	30.0
	Trairatapiwan et al., 2018	Regression / 8 d / 29 d	0.38 - 0.50	0.31 - 0.40	-	69.3
	Wang et al., 2022	Substitution / 7 d / 27 d	1.07 - 1.13	0.54 - 0.57	75.3	-
Monodicalcium phosphate	An et al., 2020	Direct / 5 d / 19 d	1.08	0.50	86.0	88.7
	Bikker et al., 2016	Direct / 10 d / 28 d	0.44	0.34	70.7	-
	Trairatapiwan et al., 2018	Regression / 8 d / 29 d	0.36 - 0.48	0.30 - 0.39	-	60.2
Tricalcium phosphate	An et al., 2020	Direct / 5 d / 19 d	1.00	0.49	53.8	56.7
Deflourinated phosphate	Bikker et al., 2016	Direct / 10 d / 28 d	0.44	0.33	31.5	-
Monosodium phosphate	Shastak et al., 2012	Regression / 10d / 21 d	0.98-1.28	0.43-0.59	-	67.0

<sup>1</sup>Duration = length of feeding period for experimental diets. Bird age = age of bird at sample collection.

<sup>2</sup>Range is provided when multiple concentrations were used in regression-based assays

<sup>3</sup>Regression-based estimates are included as SID values due to inherent correction for endogenous losses in this approach.

**Table 2.** Test ingredients and experimental treatment groups

Treatment	Description	n	Analyzed value of test ingredient, %	
			Ca	P
1	Nitrogen-phosphorous free	6	-	-
2	0.48% Monosodium phosphate	8		
3	0.85% Monosodium phosphate	8	0.90	27.1
4	1.22% Monosodium phosphate	8		
5	Monocalcium phosphate	7	17.0	20.9
6	Dicalcium phosphate	7	20.7	18.5
7	Defluorinated phosphate	7	33.7	18.5
8	Monodicalcium phosphate	7	16.5	18.3
9	Bovine meat and bone meal	7	10.5	5.0
10	Porcine meat and bone meal	7	9.5	4.6

**Table 3.** Ileal digestibility (%) of P and relative P digestibility in complete diets containing test ingredients as the sole source of P

Diet <sup>2</sup>	AID P <sup>1,2</sup>	SID P <sup>1,2,3</sup>	Linear Regression Equation <sup>4</sup>	Relative P digestibility, % <sup>5</sup>
0.48% MSP	92.57 <sup>a</sup> ± 1.80	92.63 <sup>a</sup> ± 1.80		100.00
0.85% MSP	91.44 <sup>a</sup> ± 1.80	91.48 <sup>a</sup> ± 1.80	$y = 0.9033x - 0.0165$	
1.22% MSP	88.53 <sup>a</sup> ± 1.80	88.55 <sup>a</sup> ± 1.80		
MCP	89.55 <sup>a</sup> ± 1.92	89.58 <sup>a</sup> ± 1.92	$y = 0.9135x - 0.0613$	101.13
DCP	90.04 <sup>a</sup> ± 1.92	90.06 <sup>a</sup> ± 1.92	$y = 0.9198x - 0.062$	101.83
DFP	73.59 <sup>b</sup> ± 1.92	73.61 <sup>b</sup> ± 1.92	$y = 0.7509x - 0.0451$	83.13
MDCP	76.58 <sup>b</sup> ± 2.08	76.60 <sup>b</sup> ± 2.08	$y = 0.7813x - 0.0481$	86.49
Bovine MBM	92.24 <sup>a</sup> ± 1.92	92.26 <sup>a</sup> ± 1.92	$y = 0.9397x - 0.0639$	104.03
Porcine MBM	77.31 <sup>b</sup> ± 2.08	77.34 <sup>b</sup> ± 2.08	$y = 0.79x - 0.049$	87.46
<i>P</i> -value <sup>6</sup>	<0.001	<0.001	-	-

<sup>a,b,c</sup> Means with different superscript letters differ ( $P < 0.05$ ) based on Tukey's HSD test.

<sup>1</sup> Values are LSMeans ± SEM of 8 (MSP groups) or 7 (all other sources) replicate pens per treatment.

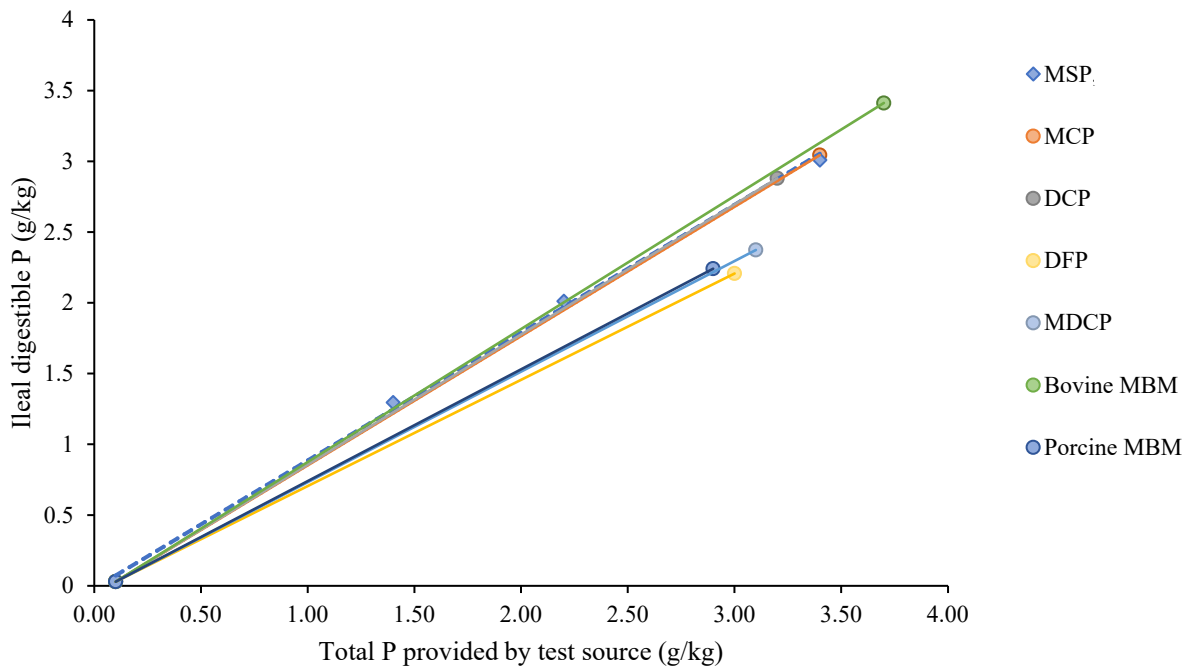
<sup>2</sup> Abbreviations: MSP = monosodium phosphate; MCP = monocalcium phosphate; DCP = dicalcium phosphate; DFP = defluorinated phosphate; MDCP = monodicalcium phosphate; MBM = meat and bone meal; AID = apparent ileal digestibility; SID = standardized ileal digestibility.

<sup>3</sup> For the calculation of SID, values of AID were corrected for the basal endogenous loss of P at 74.6 mg/kg.

<sup>4</sup> Linear regression equation of dietary digestible P (AID of P coefficient × total P, g/kg) as a function of added total P (g/kg) from each P source.

<sup>5</sup> Relative P digestibility based on slope of each test source divided by the slope for MSP and multiplied by 100.

<sup>6</sup> Overall ANOVA *P*-value.



**Figure 1.** Linear regression of dietary digestible P (AID of P coefficient  $\times$  total P, g/kg) as a function of added total P (g/kg) from each P source. Abbreviations: MSP = monosodium phosphate; MCP = monocalcium phosphate; DCP = dicalcium phosphate; DFP = defluorinated phosphate; MDCP = monodicalcium phosphate; MBM = meat and bone meal