ASCERTAINING IN VIVO PHOSPHATE SOLUBILIZATION: MINERAL RECOVERY TECHNIQUES AND PHOSPHORUS SOURCES

RANDALL W. GORDON and DAVID A. ROLAND, SR.
Department of Poultry Science and Alabama Agricultural Experiment Station, Auburn University, AL 36849
Phone: (334) 844-2605
FAX: (334) 844-2641

Primary Audience: Nutritionists, Researchers, Poultry Extension Specialists

SUMMARY

In vivo mineral source solubilization is determined by a decanting procedure in which unsolubilized mineral sources are recovered from the excreta. These studies were conducted to improve the accuracy and broaden the use of this procedure. These studies examined the influence of excreta sample wet weight on percentage mineral source recovered; compared results from wet samples and dried, ground samples of comparable original wet weights; and evaluated the influence of three P sources on percentage in vivo P source solubilization in broilers and layers.

Reducing sample weights significantly (P < .001) improved percentage mineral source recovered. No differences resulted from using wet vs. dried, ground samples. Suggested wet and dry sample weights are 5 and 1 g respectively. Phosphorus source significantly (P < .001) influenced phosphorus source solubilization, with approximately 1.75, 5.5, and 10.25% of Biofos, Dynafos, and Multifos, respectively, passing through the bird without being solubilized.

Key words: Calcium, limestone, phosphate, phosphorus, solubilization

1999 J. Appl. Poultry Res. 8:48-53

DESCRIPTION OF PROBLEM

Because the available phosphorus content of feedstuffs commonly used in poultry rations is not sufficient to meet the phosphorus requirement of the bird, inorganic phosphorus supplements are typically added to the diet. Although studies have clearly demonstrated that the phosphorus bioavailability of monocalcium-, dicalcium-, and defluorinated phosphates differs [1, 2], many nutrition programs still assume 100% phosphorus availability regardless of phosphorus source.

Attempts have been made to correlate the bioavailability of phosphates with their solubilization in various solutions. Day et al. [3], using broiler chicks, reported very little agreement between phosphate solubilization in 0.4% HCl, 2.0% citric acid, or neutral ammonium citrate and phosphate availability.

1 Alabama Agricultural Experiment Station Journal Series No. 12-985964.
2 To whom correspondence should be addressed
However, Sullivan et al. [2] reported that the 2% citric acid and neutral ammonium citrate tests provided reliable estimates of the biological availability of dicalcium and defluorinated phosphates in turkey poult. These tests were not reliable in predicting the biological availability of monocalcium phosphates. Because of the risks involved, Sullivan et al. [2] appropriately caution against totally removing the bird from the evaluation process. Furthermore, because in vitro procedures are incapable of determining the influence of environmental factors or nutritional interactions on phosphate availability, these methods are limited in their research capabilities.

Since inorganic phosphates must first be solubilized within the digestive system before the phosphorus they contain can be absorbed by the bird [4, 5], it has been hypothesized that differences in phosphorus availability among phosphate sources may be attributed to differences in their solubilization within the digestive tract. Rao et al. [6], using a decanting procedure to separate unsolubilized phosphate from laying hen excreta, determined the in vivo solubilization of various phosphate sources. Percentage in vivo P source solubilization (PS) of Biofos (2/3 monocalcium phosphate and 1/3 dicalcium phosphate), Dynafos (1/3 monocalcium phosphate and 2/3 dicalcium phosphate), and Multifos (defluorinated tricalcium phosphate) was 96, 94, and 92, respectively. Solubility values of these phosphates in broilers were 99.8, 99.3, and 97.3%, respectively [7].

The higher solubilization values in broilers were unexpected but may be related to the greater weight of decanted excreta samples used. Rao and Roland [6] reported using a sample wet weight of 20 to 40 g while Gordon et al. [7] based phosphate solubilization values on an 80-g wet weight sample. Increasing the concentration of excreta in the decanting medium may limit the amount of unsolubilized mineral material that can be recovered because of physical interference from organic material as denser materials settle. As a result, phosphate solubility could be overestimated if inorganic phosphates are inadvertently removed with organic material during decanting. Determination of optimal sample weight may also be useful in addressing the discrepancy between the actual P content of waste lagoons analyzed by Roland et al. [8] and the lower P content calculated from reported solubilization values [9]. The greater recovery of phosphate from waste lagoon sediment may have been related to recrystallization of P in a basic, high Ca environment or to an actual increased excretion of unsolubilized P due to lower egg production than was reported by Rao and Roland [7]. However, confirmation of the decanting procedure seemed appropriate before pursuing other possible causes.

When birds are housed under high environmental temperatures (whether young chicks or heat-stressed older birds), the excreta becomes harder and more resistant to mixing, and is more difficult to decant and accurately analyze. It may be possible, under these conditions, to dry and grind the excreta before washing in order to liberate the unsolubilized mineral sources that would otherwise remain in the aggregates.

The present study was conducted with the following objectives: 1) to determine whether wet sample weight influences percentage unsolubilized mineral sources recovered (MSR) from the excreta (broiler and layer) and, if so, what is the optimal sample weight; 2) to compare the two methods (using wet vs. dried, ground excreta) in order to determine the legitimacy of drying and grinding excreta when conditions warrant; and 3) to determine the in vivo mineral source solubilization of mono-, di-, and tricalcium phosphates by layers and broilers using the optimal sample weight.

**MATERIALS AND METHODS**

**EXPERIMENTAL PROTOCOL**

Experiment 1 consisted of two trials. In Trial 1, 84 male Peterson × Arbor Acres 5-wk-old broilers were randomly divided into 12 battery cages. The experimental unit was two cages. Birds were fed a commercial corn-soy diet containing a defluorinated tricalcium phosphate (Multifos), which provided 0.3% added phosphorus. After collection, excreta were thoroughly mixed and 11 sample weights per experimental unit (100, 80, 60, 40, 20, 10, 5, 4, 3, 2, and 1 g) were recovered for analysis (six replicates of each weight). A 120-mL solution (50% ethanol and 50% water) was added to each manure sample in a 250-mL beaker with a diameter of 6.5 cm.
Beaker contents were stirred gently until all clumps were broken. After heavier particles settled to the bottom, the supernatant and suspended particulate matter were gradually decanted until only heavier particles remained. It was assumed that all P and Ca in this collected sediment were from phosphates or limestones that had passed through the digestive system without being solubilized. The recovered sediment was dried at 100°C for 2 hr, weighed, and analyzed for P and Ca content using an inductively coupled argon plasma spectrophotometer (ICAP) [10]. Percentage MSR was determined through the following equation:

$$\text{MSR} = \frac{a}{b} \times 100$$

where a is the P or Ca collected from the sediment and b is the dry weight of the sample.

The design of Trial 2 was similar to that of Trial 1 except that the 0.3% added P was provided as a di-monocalcium phosphate blend (Dynafos). In addition, the excreta collected were pooled and six samples of five weights (80, 40, 20, 10, and 5 g) were analyzed for percentage MSR.

In Experiment 2, a commercial corn-soy diet containing 0.6% total P and 4% Ca was fed to 20, 75-wk-old W-36 Hy-Line hens. Phosphorus was added in the form of Dynafos. Excreta collection, treatment, and analysis were identical to Trial 2 of Experiment 1.

In Experiment 3, excreta were collected for 48 hr from six replicates of 5-wk-old broilers fed a diet containing 0.3% added P from Dynafos. After mixing, 5 g (wet weight) samples were collected for analysis and 20 g (wet weight) samples were oven dried. Dried samples were ground and 1 g (dry weight) samples were collected. Washing procedure, treatment, and analysis were the same as in previous experiments.

In Experiment 4, 72, 4-wk-old broilers were randomly assigned to treatment diets containing either Biofos, Dynafos, or Multifos as the P source. Each treatment was replicated six times and the phosphorus source provided 0.3% added P. Phosphoric acid was used as a control since it is already in a solubilized form. Excreta collection took place for 72 hr and feed consumption during this period was determined. After thorough mixing, 5 g (wet weight) samples were washed and analyzed as in previous experiments.

In Experiment 5, feed was removed from 200 individually caged W-36 Hy-Line hens at 16:00 hr. The following morning, birds with ovipositions between 07:30 hr and 09:30 hr were given preweighed treatment feeds at oviposition. Treatment diets consisted of a corn-soy basal diet to which either Biofos, Dynafos, or Multifos and limestone were added to provide 0.6% total P (480 mg) and 3.75% Ca (3 g) in 80 g of feed. Excreta collection began at oviposition and was completed at oviposition the next morning. Only birds with two consecutive ovipositions were used in this study. Each treatment was replicated 12 times. Three birds receiving only the basal diet (no added phosphate) after feed removal were used as controls. In addition, three birds per treatment were killed at seconds oviposition by cervical dislocation and GI tracts collected for recovery of unsolubilized phosphorus sources. Percentage in vivo P source solubilization (PS) was determined through the following equation:

$$\text{PS} = \frac{(a-b)-c}{a} \times 100$$

where a is the amount of phosphorus consumed, b is the amount of unsolubilized phosphorus recovered from the GI tract of treatment hens, and c is the amount of unsolubilized phosphorus recovered from the excreta.

**STATISTICAL ANALYSIS**

All percentage data were arc sin transformed and tested for significance by ANOVA using the GLM procedure of SAS [11]. Significant differences between treatment means were determined by Tukey's studentized range test [12] based on a 5% probability level.

**RESULTS AND DISCUSSION**

In Trial 1 of Experiment 1, decreasing sample weight resulted in a highly significant (P < .001) improvement to percentage MSR (Table 1). Reducing the sample weight below 10 g did not result in any additional recovery of Ca. In Trial 2, sample weight also significantly influenced (P < .001) the percentage unsolubilized limestone and Dynafos collected from broiler excreta (Table 1). In this trial, reducing sample weight below 20 g yielded no additional response in percentage Ca collected. In Experiment 2, sample weight
significantly influenced the percentage unsolubilized limestone and Dynafos recovered in laying hen excreta (Table 1). Percentage Ca recovered was greatest at 20 g with no additional response below this point.

While there were no additional benefits to reducing sample weight below 10–20 g in Experiments 1 and 2, there were no adverse effects to making such reductions. Furthermore, optimal P source recovery took place below 5–10 g wet sample weight (Table 1). It is therefore recommended that 5 g (wet weight) samples be used when analyzing for either percentage Ca or P source solubilization.

In Experiment 3, there were no differences between 5-g wet weight and 1-g dry weight samples, either in percentage Ca (0.36±0.031) or P (0.07±0.009) collected. Therefore, when the condition of the excreta is not conducive to breaking up aggregates during the washing procedure, samples can, instead, be dried and mechanically ground before washing without compromising mineral source recovery. When this is done, it is recommended that 1-g dry samples be used since this is approximately the amount of dry matter in a 5-g wet sample of excreta. The similar Ca and P values from the 1-g dry weight and 5-g wet weight samples of Experiment 3 indicate that the 1-g dry weight yield results as accurate as the 5-g wet sample weight.

Since beaker diameter and sample weight are both related to the optimal recovery of unsolubilized material, any reduction in diameter should be compensated for by a corresponding reduction in sample weight. While not tested, increasing beaker diameter is not expected to influence recovery at the suggested weights.

In Experiment 4, percentage PS in broilers was significantly influenced (P < .001) by P source, with the order of solubilization being Biofos > Dynafos > Multifos (Table 2). Likewise, in Experiment 5, PS in laying hens was significantly influenced (P < .001) by P source with the same order of solubilization. These findings confirm the earlier reports of Rao et al. [6] for laying hens and Gordon et al. [7] for broilers.
More importantly, however, the increased recovery of unsolubilized mineral sources due to this procedural improvement resulted in lower PS values than previously reported, particularly in broilers [7]. These corrections indicate that approximately 10.25, 5.5, and 1.75% of Multifos, Dynafos, and Biofos, respectively, pass through the digestive tract of the bird without being solubilized and are, therefore, not available for utilization.

Furthermore, because of the improved efficiency of this procedure, the solubilization values of the present study more closely correspond to the P availability data presented by Sullivan et al. [2]. This appears to indicate the potential for using this procedure as an alternative to slower and more costly P availability assays, while still maintaining the research capabilities of an in vivo procedure.

### TABLE 2. Influence of P source on percentage in vivo P source solubilization in broilers and layers (Experiments 4 and 5)

<table>
<thead>
<tr>
<th>P SOURCE</th>
<th>BROILERS (EXPERIMENT 4)</th>
<th>LAYERS (EXPERIMENT 5)</th>
<th>( \bar{x} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>***</td>
<td>99.34 ( ^a )</td>
<td>97.14 ( ^a )</td>
<td>98.24</td>
</tr>
<tr>
<td>Biofos</td>
<td>94.94 ( ^b )</td>
<td>94.05 ( ^b )</td>
<td>94.49</td>
</tr>
<tr>
<td>Dynafos</td>
<td>90.91 ( ^c )</td>
<td>88.54 ( ^c )</td>
<td>89.73</td>
</tr>
<tr>
<td>SEM</td>
<td>0.66</td>
<td>1.23</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) Biofos is approximately \( \frac{1}{3} \) monocalcium phosphate and \( \frac{1}{3} \) dicalcium phosphate. Dynafos is approximately \( \frac{1}{2} \) dicalcium phosphate and \( \frac{1}{2} \) monocalcium phosphate. Multifos is a defluorinated tricalcium phosphate.

\( ^b \) Values within a column that do not share a common superscript are significantly different (P < .05). Broiler means represent six replicates and layer means represent 12 replicates.

\( *** \) P < .001.

### CONCLUSIONS AND APPLICATIONS

1. The in vivo solubilization of supplemental feed phosphates was influenced by the form of the phosphate. Biofos was more solubilized than Dynafos, while Multifos was the least solubilized of the three sources tested.
2. The solubilization values of the phosphorus sources tested were similar to their reported biological availability values, indicating the potential for using this procedure as an assay for determining the phosphorus availability of feed phosphates.
3. Weights of excreta samples decanted had a significant effect on the percentage of mineral source recovered. Wet and dry sample weights of 5 and 1 g, respectively, are recommended for optimal recovery of unsolubilized mineral sources.

### REFERENCES AND NOTES


10. ICAP-9000 Thermo Jarrell Ash Corp., Franklin, MA 02038.


ACKNOWLEDGEMENT
This research was partially supported by Mallinckrodt Feed Ingredients, Inc., Mundelein, IL 60060.