Microbial Phytase Improves Performance, Apparent Metabolizable Energy, and Ileal Amino Acid Digestibility of Broilers Fed a Lysine-Deficient Diet

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ABSTRACT An experiment was conducted to examine the effects of adding microbial phytase (Natuphos®) on the performance in broilers fed a phosphorus-adequate, lysine-deficient diet. A wheat-soybean meal-sorghum-based diet, containing 1.00% lysine and 0.45% nonphytate phosphorus, was supplemented with L-lysine monochloride to provide 1.06, 1.12, or 1.18% lysine or with 125, 250, 375, 500, 750, or 1,000 phytase units (FTU)/kg diet. Each diet was fed to six pens of 10 chicks each from Day 7 to 28 posthatching. Addition of lysine to the lysine-deficient diet linearly increased (P < 0.001) weight gain and gain per feed of broilers. The response in weight gain to added phytase reached a plateau at 500 FTU/kg diet (quadratic effect, P < 0.001). Phytase had no effect on gain per feed to 250 FTU/kg diet and then increased (quadratic effect, P < 0.05) with further additions. Assuming that the observed responses in weight gain and gain per feed to added phytase were due to the release of lysine alone and by solving linear or nonlinear response equations of lysine and phytase levels, the lysine equivalency value was calculated to be 500 FTU phytase/kg diet = 0.074% lysine. Addition of increasing levels of supplemental phytase to the lysine-deficient diet improved (P < 0.001) the digestibilities of nitrogen and all amino acids. Phytase also increased the AME, and the response reached a plateau at 750 FTU/kg diet (quadratic effect, P < 0.001). These results showed that amino acid and energy responses are responsible for the performance improvements observed when phytase was added to a wheat-soybean meal-sorghum-based diet.

(Key words: phytase, lysine equivalency, amino acid digestibility, apparent metabolizable energy, broilers)

INTRODUCTION

The value of microbial phytase in releasing phytate-bound P and improving P bioavailability of plant ingredients for poultry is well documented (Coelho and Kornegay, 1996). Phytate, in its native state, is also complexed with various cations, protein, lipids (Cosgrove, 1966), and starch (Thompson and Yoon, 1984). The significance of phytate on the utilization by poultry and swine of nutrients other than P, however, has received little attention until recently. By releasing these phytate-bound nutrients and improving their utilization, dietary supplementation with microbial phytase would be expected to have protein/amino acid and energy effects in monogastric animals. This thesis has been confirmed in a number of studies that have demonstrated generally positive effects of supplemental phytase on amino acid digestibility (Yi et al., 1996a; Biehl and Baker, 1997; Sebastian et al., 1997; Ledoux et al., 1999; Ravindran et al., 1999a, 2000) and AME (Farrell et al., 1993; Farrell and Martin, 1998; Ledoux et al., 1999; Namkung and Leeson, 1999; Selle et al., 1999; Ravindran et al., 2000) of poultry diets.

The protein/amino acid effect of microbial phytase is of considerable practical significance and needs to be quantified to enable its inclusion in least-cost diet formulations. In the present study, the effects of adding microbial phytase or lysine on the performance, apparent ileal amino acid digestibility, and AME of broilers fed on a P-adequate, lysine-deficient diet was investigated. Our aim was to generate equations for performance responses obtained with supplemental phytase and lysine and to use them to calculate the equivalency value of phytase for lysine. This approach was similar to that employed by Yi et al. (1996b) to calculate P equivalency values for phytase for broilers fed P-deficient diets.

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Abbreviation Key: AIA = acid-insoluble ash; FTU = phytase unit.
MATERIALS AND METHODS

Enzyme

The microbial phytase (Natuphos® 5000 Granulate) contained 5,000 phytase units (FTU)/g phytase activity. One FTU is the quantity of enzyme that releases 1 µmol of inorganic P/min from 0.00015 mol/L sodium phytate at pH 5.5 at 37 C.

Ingredients

Feed ingredients sufficient for the feeding trial were obtained in bulk and were analyzed for phytate P, total P, Ca, N, and amino acids prior to feed formulation. Monocalcium phosphate and limestone were analyzed for total P and Ca.

Dietary Treatments

Ten experimental diets, containing varying levels of lysine or microbial phytase, were formulated. A wheat-sorghum-based diet containing 91% of the recommended level of lysine for broiler starters (NRC, 1994) served as the basal diet (Diet 1). The composition of the basal diet is shown in Table 1. This diet met or exceeded the recommended requirements of all amino acids, except lysine, (NRC, 1994) for broiler starters and contained recommended levels of nonphytate P (0.45%). The phytate-P level in the diet was 0.30%. The Ca:nonphytate P ratio was maintained at 2.3:1. Celite, a source of acid-insoluble ash (AIA), was added at 2% as a digesta marker. Wheat was steam-pelleted prior to mixing the diets to lower the inherent phytase activity.

Diets containing 96, 102, and 107% of recommended lysine levels (Diets 2 to 4) were formulated by addition of L-lysine monochloride to Diet 1. Diet 1 was also supplemented with a xylanase (Natugrain® Blend Granulate) at levels recommended by the manufacturer (80 mg/kg). This product contained 55,000 endoxylanase units/g xylanase activity and 1,200 β-glucanase units/g β-glucanase activity. One endoxylanase unit is the enzyme activity required to liberate 1.00 µmol reducing sugars (measured as xylose equivalents) per minute at pH 3.5 and 40 C, from a 1.0% xylan solution. One β-glucanase unit is the enzyme activity required to liberate 0.28 µmol reducing sugars (measured as glucose equivalents) per min at pH 3.5 and 40 C, at a substrate concentration of 0.5% β-glucan from barley. Addition of xylanase preparations in wheat-based diets is a routine practice to ameliorate the adverse effects of wheat nonstarch polysaccharides on broiler performance (Ravindran et al., 1999b). In diets with added phytase, nonphytate P and calcium levels were lowered by adjusting the inclusion rates of monocalcium phosphate and limestone and by taking into account the P equivalency for phytase (500 FTU phytase/kg = 1 g P from monocalcium phosphate) as recommended by the manufacturer. Sorghum levels were increased in the phytase diets in place of monocalcium phosphate and limestone.

Birds and Conduct of the Trial

Experimental procedures were approved by the University of Sydney Animal Care and Ethics Committee and

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
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<tr>
<td>Wheat</td>
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</tr>
<tr>
<td>Sorghum</td>
<td>22.26</td>
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<tr>
<td>Soybean meal</td>
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<tr>
<td>Canola meal</td>
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<tr>
<td>Dextrose</td>
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<tr>
<td>Celite</td>
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<tr>
<td>Monocalcium phosphate</td>
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<tr>
<td>Limestone</td>
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<tr>
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<tr>
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<tr>
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<td>Methionine + cysteine</td>
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<tr>
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<tr>
<td>Crude protein (Nitrogen × 6.25)</td>
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<tr>
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<tr>
<td>Arginine</td>
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</tbody>
</table>

1Diets 2 to 4 were formulated by addition of L-lysine monochloride to provide 1.06, 1.12, or 1.18% lysine, and Diets 5 to 10 were formulated by the supplementation of 125, 250, 375, 500, 750, or 1,000 phytase units of Natuphos® phytase/kg diet. All diets contained xylanase (Natugrain® blend granulate).

2OMYA Southern Ltd., Lindfield, NSW 2070, Australia.

3Kemira Kemi AB, Helsingborg, Sweden.

4OMYA Southern Ltd., Lindfield, NSW 2070, Australia.

5Supplied per kilogram diet: trans-retinol, 3.3 mg; cholecalciferol, 87.5 µg; dl-α-tocopheryl acetate, 20 mg; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 8 mg; calcium pantothenate, 15 mg; niacin, 30 mg; pyridoxine, 5 mg; folic acid, 2 mg; cyanocobalamine, 15 g; biotin, 100 µg; Mn, 75 mg; Zn, 50 mg; Cu, 5 mg; Mo, 1.6 mg; Co, 300 µg; I, 1 mg; Fe, 20 mg; Se, 100 µg; choline chloride, 300 mg; ethoxyquin, 125 mg.

6All amino acids, except lysine, were supplied to meet or exceed the recommended requirements for broiler starters (NRC, 1994).

7Determined values.
complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

One-day-old male broiler chicks (Cobb) were obtained from a commercial hatchery and fed a commercial broiler starter diet (23% crude protein) to Day 7. On Day 7, the birds were individually weighed, and the heaviest and lightest were discarded. Six hundred chicks of uniform weights were randomly allotted to 60 pens (10 chicks/pen) in electrically heated, raised wire-floored starting cages in an environmentally controlled room. Each of the 10 dietary treatments was randomly assigned to six pens of 10 chicks each. The diets, in mash form, were fed from Days 7 to 28. Feed and water were available ad libitum. On Day 18, the birds were transferred to cages with facilities for excreta collection.

### Measurements

Individual body weights and pen feed intake were recorded at weekly intervals. Mortality was recorded daily. During the third week of the trial, feed intake and excreta output were measured quantitatively per pen over four consecutive days (Days 24 to 27) to obtain AME data. The excreta was collected daily at 0900 h, dried for 24 h at 80°C in a forced-air oven, and pooled within a pen for analysis. Care was taken to avoid contamination from feathers, scales, and debris. The dried excreta were allowed to equilibrate to atmospheric conditions before being weighed. Representative samples were taken and ground to pass through a 0.5-mm sieve.

On Day 28, all surviving birds were killed by intracardial injection of sodium pentobarbitone. The small intestine was immediately exposed, and digesta contents were collected from the lower half of ileum, pooled within a pen, and processed as described previously (Ravindran et al., 1999a). Toe samples were obtained by severing the left middle toe through the joint between the second and third tarsal bones from the distal end. The toes of all birds within a pen were pooled, and the composite samples were dried to a constant weight at 100°C and then ashed in a muffle furnace at 600°C for 6 h. Toe ash was expressed as a percentage of dry weight. Toe ash has been shown to be a good indicator of P status and is accurate in determining P availability of diets for poultry (Potter, 1988).

### Chemical Analysis

The gross energy of diet and the excreta samples were determined using an adiabatic bomb calorimeter (Gallenkamp) standardized with benzoic acid. Nitrogen content was determined by the method of Sweeney (1989) using an FP-428 nitrogen determinator.4

Amino acid concentrations in the diet and ileal digesta samples were determined using a Shimadzu amino acid analysis system5 after acid hydrolysis (Ravindran et al., 1999a). Tryptophan contents were determined after alkaline hydrolysis of samples according to the procedures of Ravindran and Bryden (1996). Separate sulfur amino acid analyses were not carried out. The AIA contents of diet and ileal digesta samples were measured after ashing the samples and treating the ash with boiling 4 M hydrochloric acid (Siriwan et al., 1993). All diets were analyzed for phytase activity by using procedures described elsewhere (Selle et al., 1996).

### Calculations

The AME values of the diets were calculated using the following formula. Appropriate corrections were made for differences in moisture content.

\[
AME_{\text{diet}} (\text{kcal/kg}) = \left( \frac{\text{feed intake} \times \text{gross energy}_{\text{diet}} - (\text{excreta output} \times \text{gross energy}_{\text{excreta}})}{\text{feed intake}} \right)
\]

Apparent ileal nitrogen and amino acid digestibilities were calculated, using AIA as the indigestible marker, as shown below.

\[
\text{apparent digestibility} \, (\%) = \frac{(\text{amino acid/AIA})_d - (\text{amino acid/AIA})_i}{(\text{amino acid/AIA})_d} \times 100.
\]

where (amino acid/AIA)_d = ratio of amino acid or nitrogen to acid-insoluble ash in the diet, and (amino acid/AIA)_i = ratio of amino acid or nitrogen to acid-insoluble ash in the ileal digesta.

### Statistical Analysis

The data were analyzed by the general linear models procedure of the SAS® (SAS Institute, 1990) with pen means as the experimental unit. Linear and quadratic effects of lysine (Diets 1 to 4) and supplemental phytase (Diet 1 and Diets 5 to 10) on gain, feed intake, gain per feed, toe ash, AME, and ileal digestibility of nitrogen and amino acids were tested using orthogonal polynomials. Linear and nonlinear response functions of body weight gain, feed intake, and gain per feed that best fit the data were derived for lysine levels (Diets 1 to 4) and for phytase levels (Diet 1 and Diets 5 to 10). The models used were as follows:

- Linear function \( Y = a + bX \)
- Nonlinear function \( Y = a(1 - be^{-kX}) \)

where \( Y \) = the response measurement, and \( X \) = lysine (percentage of diet) or phytase added (units per kilogram of diet). The nonlinear or linear response equations with the higher \( r^2 \) value for added lysine and the equations for added phytase were set to be equal and were solved using the procedures described by Yi et al. (1996b). Linear and nonlinear response functions of AME and ileal digestibility
of nitrogen and amino acids that best fit the data were also derived for phytase levels (Diet 1 and Diets 5 to 10).

**RESULTS**

Mortality during the trial was within acceptable levels (less than 2%) and was not related to dietary treatments. The analyzed phytase activities indicated that the determined values agreed well at lower dosages, but there was an overestimation (36 to 42% higher) at dosages of 500 FTU/kg diet and above. The determined microbial phytase activity in Diets 5, 6, 7, 8, 9, and 10 were 123, 253, 378, 688, 1,068, and 1,363 FTU/kg diet, respectively.

The analyzed amino acid contents of Diet 1 (lysine-deficient basal diet) confirmed that the diet met or exceeded the recommended requirements of all essential amino acids, except lysine (Table 1). The determined value for lysine agreed closely with the calculated value.

**Performance Data**

The influence of dietary treatments on broiler performance is summarized in Table 2. Addition of graded levels of lysine, to a wheat-soybean meal-sorghum basal diet containing 1.00% lysine, linearly \((P < 0.001)\) increased weight gain and gain per feed of broilers. Feed intake increased up to 1.12% dietary lysine and then declined (quadratic effect, \(P < 0.05\)) with further addition.

The response in weight gain to the addition of graded levels of phytase reached a plateau at 500 FTU/kg diet (quadratic effect, \(P < 0.001\)). In the case of gain per feed, added phytase had no effect up to 250 FTU/kg diet and then decreased (quadratic effect, \(P < 0.05\)) with further addition of phytase. Feed intake was not influenced (\(P > 0.05\)) by the addition of phytase.

This data set was used to estimate lysine equivalency values for microbial phytase by using the procedure described by Yi et al. (1996b). Linear and nonlinear functions that gave the best fit to the data set (weight gain, feed intake, and gain per feed) were derived for lysine and phytase. The nonlinear or linear response equations with the higher \(r^2\) value for added lysine and the equation of phytase levels were then set to be equal and were solved. Estimates were obtained for weight gain and gain per feed, but equations for feed intake were found to be poor fits and not considered in the calculation of equivalency values.

The following equations were obtained for weight gain (\(Y\)) responses to graded levels of dietary lysine or added phytase. For lysine, \(Y = 404.29 + 420.25 X; r^2 = 0.98\), where \(X = \) dietary lysine level. For phytase, \(Y = 868.2 (1 - 0.0556e^{-0.00359X}); r^2 = 0.92\), where \(X = \) dietary phytase level. By setting these equations for lysine and phytase to be equal and solving them (see Yi et al., 1996b), the following equation was obtained.

\[
\% \text{ lysine} = 1.1039 (1 - 0.1041e^{-0.00059\text{ phytase}})
\]

When resolved, the level of lysine equivalent to 500 FTU phytase/kg diet addition was 1.0848%. Therefore, released lysine = lysine equivalency estimate – lysine in the basal diet (i.e., 1.00%). The gain response from 500 FTU phytase/kg diet is therefore equal to 0.0848% lysine. Because the recommended inclusion rate of Natuphos® phytase in broiler diets is 500 FTU/kg diet, this level was considered in the calculation of lysine equivalency.

Similarly for gain per feed (\(Y_1\)) responses, for lysine, \(Y_1 = 282.2 + 272.8X; r^2 = 0.97\), where \(X = \) dietary lysine level; for phytase, \(Y_1 = 587.9 (1 - 0.0574e^{-0.00157X}); r^2 = 0.79\), where \(X = \) dietary phytase level.
The following equation was derived by setting the above equations for lysine and phytase equal and solving them.

$$\% \text{ lysine} = 1.1206 (1 - 0.1104e^{-0.00157 \text{ phytase}})$$

When resolved, the level of lysine equivalent to 500 FTU phytase/kg diet addition is 1.0641%. The gain per feed response from 500 FTU/kg diet is therefore equal to 0.0641% lysine.

The average of the above two estimates was 0.0744%. Based on the assumption that the observed responses in weight gain and gain per feed to added phytase were due to the release of lysine alone, the lysine equivalency value of the enzyme can be considered as 500 FTU phytase/kg diet = 0.0744% lysine.

**Toe Ash Data**

Although the diets were formulated to contain recommended levels of nonphytate P, to discount any possible P effects, the toe ash contents of the birds were determined. The results summarized in Table 2 show that treatments had no effect on toe ash contents.

**Ileal Digestibility of Nitrogen and Amino Acids**

The influence of microbial phytase on the apparent ileal nitrogen and amino acid digestibilities is presented in Table 3. Because a separate performic acid oxidation was not carried out, values for cystine and methionine are not available.

Addition of increasing levels of supplemental phytase to a lysine-deficient diet caused linear ($P < 0.001$) improvements in the digestibilities of nitrogen and all amino acids. The increases in the digestibility of arginine, lysine, aspartic acid, and glutamic acid also contained quadratic components ($P < 0.05$ to 0.01). The magnitude of response to added phytase varied depending on the level of supplementation and the amino acid considered. The increases in amino acid digestibility were minimal at 250 FTU addition/kg diet. For most amino acids, the highest responses were observed at 1,000 FTU addition/kg.

The percentage improvements in the digestibility of essential amino acids with 500 FTU/kg diet addition were arginine, 4.3; histidine, 3.5; isoleucine, 4.5; leucine, 3.7; lysine, 4.5; phenylalanine, 4.8; threonine, 4.8; tryptophan, 4.2; and valine, 5.1. The corresponding improvements with 1,000 FTU/kg diet addition were arginine, 5.0; histidine, 4.0; isoleucine, 4.6; leucine, 6.4; lysine, 5.9; phenylalanine, 7.0; threonine, 6.3; tryptophan, 4.6; and valine, 6.3.

**AME Data**

The AME content of the diet was not influenced by lysine levels (Table 4) but increased with increasing levels of supplemental phytase to 750 FTU phytase/kg diet and then decreased with further addition (quadratic effect, $P < 0.001$). Addition of 500 FTU/kg improved the AME value by 2.3% (3,399 vs. 3,477 kcal/kg dry matter).

**DISCUSSION**

The relevance of phytate-protein complexes in lowering protein utilization in monogastric animals and the potential of microbial phytase to release the phytate-bound protein have attracted considerable attention in recent years (Kies and Selle, 1998). Although the P equivalency values for microbial phytase is well established (Coelho and Kor-
ensible data show that the addition of phytase to a lysine-deficient diet can significantly improve amino acid digestibility and apparent metabolizable energy (AME) as measured by responses in body weight gain and gain per feed. Note, however, that the diets might not have been deficient in lysine during the last week of the trial because the NRC (1994) recommendation of lysine requirement for 3-to-6-wk-old broilers is 1.00%.

The multifaceted effects of phytase in practical diets are being increasingly appreciated, and it is possible that the observed performance responses may reflect the release of P, available amino acids, and energy by the added phytase. The absence of significant influence of phytase on toe ash content (Table 2) indicates that the diets contained adequate amounts of nonphytate P to support bone mineralization and that the observed performance responses were independent of P effects of the enzyme. The digestibility data show that the addition of phytase to a lysine-deficient diet significantly improved not only the ileal digestibility of lysine but also of other amino acids. The positive influence of microbial phytase on apparent ileal digestibility of amino acid is in agreement with previous reports. In the present study, the addition of 500 FTU phytase/kg diet increased the mean amino acid digestibility by 3.4% units, which is higher than the increments of 1.3 to 2.3% units observed in previous studies (Yi et al., 1996a; Sebastian et al., 1997; Namkung and Leeson, 1999; Ravindran et al., 2000). Possible mechanisms contributing to the observed improvements in amino acid digestibility have been discussed in detail by Ravindran et al. (1999a) and Selle et al. (2000).

Significant improvements in AME were also observed with phytase addition. The energy effects of phytase in wheat and wheat-sorghum based diets have been reported previously (Ravindran et al., 1999b, 2000), and the present results again confirm these effects. Improvements in the AME of TME of poultry diets based on corn (Namkung and Leeson, 1999), sorghum (Farrell et al., 1993; Selle et al., 1999), oats (Farrell and Martin, 1993), and barley (Zhang et al., 1999) have also been reported in the literature. The mechanism of the AME effect is largely unknown, but improved protein digestibility is responsible, at least in part, for these responses. The data of Ravindran et al. (2000) show that phytase may improve energy utilization, independent of its effect on amino acid digestion. It was proposed that mineral-phytate complexes may contribute to the formation of insoluble metallic soaps in the gastrointestinal tract, which is a constraint on lipid utilization. By preventing the formation of mineral-phytate complexes, phytase may reduce the degree of soap formation in the gut and enhance the utilization of energy derived from lipids. Dietary levels of calcium and saturated fats would have particular relevance to this proposed mode of action.

Starch digestibility of poultry diets is not usually considered to be limiting, but another possible facet of the mode of action of phytase is the removal of the adverse effects of phytic acid on starch digestion. It has been demonstrated in humans that manipulation of dietary phytate levels modifies the blood glucose response or glycemic index (Thompson et al., 1987). The glycemic index has been shown to be negatively correlated (r = -0.71; P < 0.01) with phytate concentrations in foods (Yoon et al., 1983), which infers that phytate reduces carbohydrate digestibility. Thompson and Yoon (1984) suggested that phytate may affect starch digestibility via interactions with proteins closely associated with starch or direct binding with starch via phosphate links. It was also suggested that phytate inhibition of amylase may be a factor in the reduced blood glucose responses. Considerable in vitro evidence indicates that phytate is a potent, noncompetitive inhibitor of α-amylase activity, probably as a result of phytate complexing with the enzyme or blocking its active sites (Sharma et al., 1978; Knuckles and Betschart, 1987; Li et al., 1993). Desphande and Cheryan (1984) suggested that the capacity of phytate to inhibit amylase may play a physiological role in relation to starch reserves during seed germination.

In the case of wheat, an additional mode of action has been recently proposed to explain improvements in AME with added phytase (Ravindran et al., 1999b). Based on the observation that phytate is an integral component of the cell wall matrix in wheat (Frolich, 1990), it was postulated that microbial phytase may be acting in a manner similar to that of exogenous xylanases by disrupting cell walls and enhancing contact between digestive enzymes and cell contents.

It is therefore evident that responses in overall amino acid digestibility and AME are responsible for the perfor-

| Table 4. Apparent metabolizable energy (AME) of a lysine-deficient wheat-soybean meal-sorghum diet for broilers as influenced by varying dietary levels of lysine and microbial phytase |
|-----------------|------------------|------------------|
| Diet no. | Lysine (%) | Phytase (FTU/kg diet) | AME (kcal/kg dry matter) |
| 1 | 1.00 | 0 | 3,399 |
| 2 | 1.06 | 0 | 3,406 |
| 3 | 1.12 | 0 | 3,394 |
| 4 | 1.18 | 0 | 3,399 |
| 5 | 1.00 | 125 | 3,403 |
| 6 | 1.00 | 250 | 3,427 |
| 7 | 1.00 | 375 | 3,449 |
| 8 | 1.00 | 500 | 3,477 |
| 9 | 1.00 | 750 | 3,518 |
| 10 | 1.00 | 1,000 | 3,485 |
| SEM | 14.6 |

*Each mean represents six pens of 10 birds each.
One phytase unit (FTU) is equal to the quantity of phytase that releases 1 µmol of inorganic phosphorus per minute from 0.00015 mol/L sodium phytate at pH 5.5 at 37°C.
Represents 91% of the recommended lysine level for broiler starters (NRC, 1994).
Phytase effect (linear, P < 0.001; quadratic, P < 0.001).
mance improvements observed when phytase was added to the lysine-deficient diet. The present results, along with other reports (Loudoux et al., 1999; Namkung and Leeson, 1999; Ravindran et al., 1999b, 2000; Zhang et al., 1999), indicate that the amino acid responses to added phytase are generally associated with energy responses. Because it is not possible to separate the amino acid and energy responses, the difficulty in achieving the original aim of estimating lysine equivalency values for phytase becomes evident. Notwithstanding this difficulty, data are generated on the dose-response to phytase addition of amino acid digestibility and AME values in a wheat-soybean meal-sorghum diet for broiler chickens. The present results also confirm the positive effects of microbial phytase on the digestibility of nutrients other than P.

REFERENCES


