



## Effects of Dietary Lysine and Microbial Phytase on Growth Performance and Nutrient Utilisation of Broiler Chickens

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**ABSTRACT :** The effects of offering broilers phosphorus-adequate diets containing 10.0 and 11.8 g/kg lysine, without and with 500 FTU/kg exogenous phytase, on growth performance and nutrient utilisation were determined. Each of the four experimental diets was offered to 6 replicates of 10 birds from 7 to 28 days of age. Effects of treatment on performance, apparent metabolisable energy, apparent ileal digestibility of amino acids and bone mineralisation were examined. Both additional lysine and phytase supplementation improved ( $p < 0.05$ ) weight gain and feed efficiency, with interactions ( $p < 0.05$ ), as phytase responses were more pronounced in lysine-deficient diets. Phytase improved ( $p < 0.05$ ) apparent metabolisable energy, which was independent of the dietary lysine status. Bone mineralisation, as determined by percentage toe ash, was not affected by treatment, which confirms the phosphorus-adequate status of the diets. Phytase increased ( $p < 0.05$ ) the apparent ileal digestibility of the sixteen amino acids assessed. Unexpectedly, however, the dietary addition of 1.8 g/kg lysine, as lysine monohydrochloride, increased ( $p < 0.05$ ) the ileal digestibility of lysine *per se* and also that of isoleucine, methionine, phenylalanine, valine, aspartic acid, glutamic acid and tyrosine. In addition, there were significant interactions ( $p < 0.05$ ) between additional lysine and phytase supplementation for arginine, lysine, phenylalanine, aspartic acid, glutamic acid, glycine and serine digestibilities, with the effects of phytase being more pronounced in lysine-deficient diets. The possible mechanisms underlying the increases in amino acid digestibility in response to additional lysine and the interactions between lysine and microbial phytase in this regard are discussed. Also, consideration is given to the way in which phytate and phytase may influence ileal digestibility of amino acids. (**Key Words :** Amino Acid Digestibility, Broiler Chickens, Lysine, Phytase)

### INTRODUCTION

Phytate (*myo*-inositol hexaphosphate), the mixed salt of phytic acid derived from plant-sourced feed ingredients, is a ubiquitous component of practical poultry diets. Consequently, these diets are frequently supplemented with phytase feed enzymes, which have the capacity to increase availability of phytate-bound phosphorus (P) and reduce P excretion (Simons et al., 1990). More recently, Paik (2003) demonstrated the capacity of phytase to reduce P excretion in broilers and layers in association with reduced dietary P levels and 50% reductions in P excretion in broilers have been recorded (Um et al., 2000). Environmental P pollution from poultry production is a major concern and that phytase feed enzymes can alleviate this problem has contributed to

their growing acceptance. However, the impact of phytase is not limited to reductions in P excreted by broilers as phytase may also enhance growth performance and nutrient retention, as reported by Singh et al. (2003). In addition, microbial phytase enhances energy utilisation in broilers as demonstrated by increases in apparent metabolisable energy, as reviewed by Selle and Ravindran (2007). Exogenous phytase has also been shown to increase ileal amino acid digestibility in broilers offered either complete diets or a range of individual feed ingredients (Selle et al., 2006). The implication is that dietary phytate is an anti-nutritive factor in respect of energy utilisation and protein digestibility, which has obvious economic ramifications for chicken-meat production if these effects are counteracted by exogenous phytase, as demonstrated by Selle et al. (2003). However, the magnitude of the positive influence of phytase on amino acid digestibility reported in poultry is variable and the factors causing this variability are yet to be identified completely (Selle et al., 2006). Amino acid concentrations in the basal diet may be a factor contributing to the observed variability. In the study reported herein, the effects

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**Table 1.** Composition and specifications of the basal diet

| Ingredient                   | g/kg  |
|------------------------------|-------|
| Wheat                        | 330.4 |
| Sorghum                      | 222.5 |
| Soyabean meal                | 225.0 |
| Canola meal                  | 50.0  |
| Rice bran                    | 26.0  |
| Corn gluten meal             | 25.0  |
| Vegetable oil                | 47.0  |
| Dextrose                     | 7.5   |
| Celite <sup>1</sup>          | 20.0  |
| Monocalcium phosphate        | 16.4  |
| Limestone                    | 16.6  |
| DL-methionine                | 2.9   |
| L-threonine                  | 0.7   |
| Vitamin-trace mineral premix | 5.0   |
| Salt                         | 3.0   |
| Choline chloride             | 2.0   |
| Calculated analysis          |       |
| Phytate-P                    | 3.0   |
| Total P                      | 7.5   |
| Calcium                      | 10.3  |
| AME (MJ/kg)                  | 13.1  |
| Crude protein                | 197.5 |
| Lysine                       | 10.0  |
| Methionine+cystine           | 9.3   |
| Determined values            |       |
| Arginine                     | 12.6  |
| Histidine                    | 4.9   |
| Isoleucine                   | 11.1  |
| Leucine                      | 18.0  |
| Lysine                       | 9.7   |
| Threonine                    | 9.3   |
| Tryptophan                   | 2.3   |
| Valine                       | 14.6  |
| Protein (N×6.25)             | 196.2 |

<sup>1</sup> Source of acid insoluble ash.

of offering P-adequate diets to broilers containing 10.0 and 11.8 g/kg lysine, without and with 500 FTU/kg exogenous phytase, on apparent ileal digestibility (AID) of protein and amino acids, apparent metabolisable energy (AME), growth performance and bone mineralisation were investigated.

## MATERIALS AND METHODS

The study was conducted in compliance with the guidelines set down by the Animal Ethics Committee of The University of Sydney. The composition and specifications of the basal, lysine-deficient diet are shown in Table 1. The diet was based on a blend of sorghum and pre-pelleted wheat and was formulated to contain adequate P (4.5 g/kg nonphytate P) and, with the exception of lysine, recommended levels of amino acids. Lysine monohydrochloride was added to the basal diet to increase the lysine content from 10.0 to 11.8 g/kg and the basal diet

was supplemented with 500 FTU/kg phytase activity (Natuphos<sup>®</sup> 5000 Granulate; BASF Animal Nutrition) to provide a 2×2 factorial array of dietary treatments. The diets were analysed for phytase activity by the method of Engelen et al. (1994), which confirmed the accurate addition of microbial phytase. In phytase-supplemented diets, monocalcium phosphate and limestone levels were reduced, and replaced with sorghum, to adjust P and calcium (Ca) levels in accordance with recommendations of the manufacturer. Celite, a source of acid insoluble ash, was used as the dietary marker to determine the ileal digestibility of amino acids.

The four dietary treatments were randomly assigned to 24 pens (10 birds per pen) with six replicates per treatment. The diets in mash form were offered to male broiler chicks (Cobb) from 7 to 28 days of age on an *ad libitum* basis under continuous fluorescent lighting in an environmentally controlled facility. During this period, individual body weights and pen feed intakes were recorded at weekly intervals to determine growth performance. From days 24 to 27 total excreta output per pen was collected and feed intakes recorded in order to generate AME data. On day 28 the birds were euthanased by intracardiac injections of sodium pentobarbitone and digesta samples were taken from the distal half of the ileum and pooled within each pen to determine AID of amino acids and protein (nitrogen). Toe samples were taken on a pooled pen basis to determine percentage toe ash (Potter, 1998). The methodology employed for the determinations of gross energy and amino acids and for the calculations of AME and AID of amino acids, have been previously described by Selle et al. (2003a). The data were statistically assessed by analyses of variance using a general linear models procedure (SPSS Inc. Chicago, IL.).

## RESULTS

Additional lysine increased ( $p < 0.001$ ) weight gain by 7.2% and feed efficiency by 7.4% (Table 2). Similarly, supplemental phytase increased ( $p < 0.01$ ) weight gain and feed efficiency by 3.4% and 2.4%, respectively. There were, however, treatment interactions for both weight gain ( $p < 0.02$ ) and feed efficiency ( $p < 0.05$ ) as responses to phytase were more pronounced in lysine-deficient diets. Feed intake was not affected by dietary treatments.

Phytase addition increased the AME by 2.4% (14.56 versus 14.22 MJ/kg DM;  $p < 0.001$ ). Additional lysine did not influence ( $p > 0.05$ ) energy utilisation and the treatment interaction was not significant ( $p > 0.05$ ). Phytase increased the ileal digestibility of crude protein by 2.9% (0.812 versus 0.789;  $p < 0.001$ ), but additional lysine had no effect ( $p > 0.05$ ) on protein digestibility. Bone mineralisation, as

**Table 2.** Effects of lysine addition and phytase supplementation of lysine-deficient broiler diets on growth performance (7-28 days of age), apparent metabolisable energy (AME), apparent ileal digestibility (AID) of protein and percentage toe ash<sup>1</sup>

| Treatment         | Phytase      | Weight gain | Feed intake | Feed per gain | AME        | AID of      | Toe ash |
|-------------------|--------------|-------------|-------------|---------------|------------|-------------|---------|
| Lysine (g/kg)     | (500 FTU/kg) | (g/bird)    | (g/bird)    | (g/g)         | (MJ/kg DM) | protein (N) | (%)     |
| 10.0              | 0            | 823         | 1476        | 1.79          | 14.22      | 0.781       | 12.93   |
| 10.0              | 500          | 868         | 1497        | 1.73          | 14.55      | 0.812       | 13.19   |
| 11.8              | 0            | 899         | 1473        | 1.64          | 14.21      | 0.798       | 13.19   |
| 11.8              | 500          | 913         | 1484        | 1.62          | 14.56      | 0.812       | 12.94   |
| SEM <sup>2</sup>  |              | 5.73        | 12.0        | 0.010         | 0.048      | 0.005       | 0.21    |
| Main effects      |              |             |             |               |            |             |         |
| Lysine            |              |             |             |               |            |             |         |
| 10.0 g/kg         |              | 845         | 1,486       | 1.76          | 14.38      | 0.797       | 13.06   |
| 11.8 g/kg         |              | 906         | 1,478       | 1.63          | 14.39      | 0.805       | 13.06   |
| Phytase           |              |             |             |               |            |             |         |
| 0 FTU/kg          |              | 861         | 1,474       | 1.72          | 14.22      | 0.789       | 12.93   |
| 500 FTU/kg        |              | 890         | 1,490       | 1.68          | 14.56      | 0.812       | 13.19   |
| Significance (p<) |              |             |             |               |            |             |         |
| Lysine (L)        |              | 0.000       | 0.52        | 0.000         | 0.93       | 0.12        | 0.99    |
| Phytase (P)       |              | 0.000       | 0.20        | 0.001         | 0.000      | 0.000       | 0.97    |
| L×P interaction   |              | 0.02        | 0.68        | 0.04          | 0.80       | 0.11        | 0.24    |

<sup>1</sup> Mean of six replicates. <sup>2</sup> Pooled standard error of means.

**Table 3.** Effects of lysine addition and phytase supplementation of lysine-deficient broiler diets on apparent ileal digestibility (AID) of essential amino acids<sup>1</sup>

| Treatment         | Phytase      | Arginine | Histidine | Iso-leucine | Leucine | Lysine | Methionine | Phenylalanine | Threonine | Tryptophan | Valine |
|-------------------|--------------|----------|-----------|-------------|---------|--------|------------|---------------|-----------|------------|--------|
| Lysine (g/kg)     | (500 FTU/kg) |          |           |             |         |        |            |               |           |            |        |
| 10.0              | 0            | 0.822    | 0.797     | 0.762       | 0.764   | 0.794  | 0.910      | 0.771         | 0.749     | 0.762      | 0.769  |
| 10.0              | 500          | 0.856    | 0.825     | 0.795       | 0.791   | 0.830  | 0.917      | 0.808         | 0.785     | 0.795      | 0.808  |
| 11.8              | 0            | 0.831    | 0.805     | 0.793       | 0.776   | 0.837  | 0.920      | 0.813         | 0.760     | 0.779      | 0.802  |
| 11.8              | 500          | 0.838    | 0.817     | 0.825       | 0.783   | 0.846  | 0.924      | 0.827         | 0.794     | 0.789      | 0.839  |
| SEM <sup>2</sup>  |              | 0.006    | 0.007     | 0.005       | 0.005   | 0.005  | 0.002      | 0.005         | 0.005     | 0.007      | 0.005  |
| Main effects      |              |          |           |             |         |        |            |               |           |            |        |
| Lysine            |              |          |           |             |         |        |            |               |           |            |        |
| 10.0 g/kg         |              | 0.839    | 0.811     | 0.778       | 0.777   | 0.812  | 0.914      | 0.790         | 0.767     | 0.778      | 0.789  |
| 11.8 g/kg         |              | 0.835    | 0.811     | 0.809       | 0.779   | 0.842  | 0.922      | 0.820         | 0.777     | 0.784      | 0.820  |
| Phytase           |              |          |           |             |         |        |            |               |           |            |        |
| 0 FTU/kg          |              | 0.826    | 0.801     | 0.777       | 0.770   | 0.816  | 0.915      | 0.792         | 0.754     | 0.770      | 0.785  |
| 500 FTU/kg        |              | 0.847    | 0.821     | 0.810       | 0.787   | 0.838  | 0.920      | 0.818         | 0.789     | 0.792      | 0.824  |
| Significance (p<) |              |          |           |             |         |        |            |               |           |            |        |
| Lysine (L)        |              | 0.47     | 0.91      | 0.000       | 0.72    | 0.000  | 0.01       | 0.000         | 0.08      | 0.40       | 0.000  |
| Phytase (P)       |              | 0.001    | 0.01      | 0.000       | 0.01    | 0.000  | 0.04       | 0.000         | 0.000     | 0.01       | 0.000  |
| L×P interaction   |              | 0.03     | 0.24      | 0.88        | 0.09    | 0.02   | 0.52       | 0.03          | 0.84      | 0.09       | 0.89   |

<sup>1</sup> Mean of six replicates. <sup>2</sup> Pooled standard error of means.

assessed by percentage toe ash, was not influenced ( $p>0.05$ ) by either lysine or phytase supplementation.

Supplemental phytase significantly increased the ileal digestibility of all sixteen amino acids assessed (Table 3 and 4). Phytase increased the average AID coefficients by 2.9% (0.817 vs. 0.794), with responses ranging from a 0.6% increase in methionine (0.920 versus 0.915;  $p<0.05$ ) to a 5.0% increase in valine (0.824 versus 0.785;  $p<0.001$ ) digestibility.

The main effect of additional lysine on average amino acid AID coefficients was negligible. However, lysine

significantly increased AID of eight amino acids (values shown in parentheses), which included isoleucine (4.0%;  $p<0.001$ ), lysine (3.7%;  $p<0.001$ ), methionine (0.9%;  $p<0.01$ ), phenylalanine (3.8%;  $p<0.001$ ), valine (3.9%;  $p<0.001$ ), aspartic acid (2.5%;  $p<0.001$ ), glutamic acid (1.4%;  $p<0.04$ ), tyrosine (2.6%;  $p<0.01$ ). Additional lysine, however, decreased ( $p<0.05$ ) the AID of glycine by 1.7%. Moreover, significant ( $p<0.05$ ) treatment interactions were observed for the digestibilities of arginine, lysine, phenylalanine, aspartic acid, glutamic acid, glycine and serine, because responses to phytase were more pronounced in lysine-deficient diets. For these seven amino acids,

**Table 4.** Effects of lysine addition and phytase supplementation of lysine-deficient broiler diets on apparent ileal digestibility (AID) of non-essential amino acids<sup>1</sup>

| Treatment         |                      | Alanine | Aspartic acid | Glutamic acid | Glycine | Serine | Tyrosine |
|-------------------|----------------------|---------|---------------|---------------|---------|--------|----------|
| Lysine (g/kg)     | Phytase (500 FTU/kg) |         |               |               |         |        |          |
| 10.0              | 0                    | 0.772   | 0.772         | 0.808         | 0.768   | 0.763  | 0.761    |
| 10.0              | 500                  | 0.801   | 0.807         | 0.856         | 0.801   | 0.795  | 0.787    |
| 11.8              | 0                    | 0.770   | 0.802         | 0.832         | 0.774   | 0.773  | 0.790    |
| 11.8              | 500                  | 0.791   | 0.816         | 0.856         | 0.770   | 0.777  | 0.798    |
| SEM <sup>2</sup>  |                      | 0.007   | 0.004         | 0.006         | 0.005   | 0.005  | 0.006    |
| Main effects      |                      |         |               |               |         |        |          |
| Lysine            |                      |         |               |               |         |        |          |
| 10.0 g/kg         |                      | 0.786   | 0.789         | 0.832         | 0.785   | 0.779  | 0.774    |
| 11.8 g/kg         |                      | 0.780   | 0.809         | 0.844         | 0.772   | 0.775  | 0.794    |
| Phytase           |                      |         |               |               |         |        |          |
| 0 FTU/kg          |                      | 0.771   | 0.787         | 0.820         | 0.771   | 0.768  | 0.776    |
| 500 FTU/kg        |                      | 0.796   | 0.812         | 0.856         | 0.786   | 0.786  | 0.793    |
| Significance (p<) |                      |         |               |               |         |        |          |
| Lysine (L)        |                      | 0.40    | 0.000         | 0.04          | 0.03    | 0.48   | 0.01     |
| Phytase (P)       |                      | 0.001   | 0.000         | 0.000         | 0.02    | 0.01   | 0.01     |
| L×P interaction   |                      | 0.58    | 0.02          | 0.04          | 0.01    | 0.02   | 0.13     |

<sup>1</sup> Mean of six replicates. <sup>2</sup> Pooled standard error of means.

**Table 5.** Effect of lysine addition to non-phytase supplemented broiler diets on apparent ileal digestibility (AID) of amino acids<sup>1</sup>

| Amino acid    | AID coefficients |           | SEM <sup>2</sup> | Significance (p<) |
|---------------|------------------|-----------|------------------|-------------------|
|               | 10.0 g/kg        | 11.8 g/kg |                  |                   |
| Arginine      | 0.822            | 0.831     | 0.007            | 0.36              |
| Histidine     | 0.797            | 0.805     | 0.006            | 0.32              |
| Isoleucine    | 0.762            | 0.793     | 0.004            | 0.000             |
| Leucine       | 0.764            | 0.776     | 0.005            | 0.16              |
| Lysine        | 0.794            | 0.837     | 0.006            | 0.001             |
| Methionine    | 0.910            | 0.920     | 0.002            | 0.02              |
| Phenylalanine | 0.771            | 0.813     | 0.005            | 0.000             |
| Threonine     | 0.749            | 0.760     | 0.006            | 0.19              |
| Tryptophan    | 0.762            | 0.779     | 0.006            | 0.06              |
| Valine        | 0.769            | 0.802     | 0.005            | 0.001             |
| Alanine       | 0.772            | 0.770     | 0.006            | 0.80              |
| Aspartic acid | 0.772            | 0.802     | 0.005            | 0.001             |
| Glutamic acid | 0.808            | 0.832     | 0.007            | 0.02              |
| Glycine       | 0.768            | 0.774     | 0.006            | 0.52              |
| Serine        | 0.763            | 0.773     | 0.006            | 0.24              |
| Tyrosine      | 0.761            | 0.790     | 0.005            | 0.01              |

<sup>1</sup> Mean of six replicates. <sup>2</sup> Pooled standard error of means.

phytase increased average AID coefficients by 4.7% (0.822 vs. 0.785) in the lysine-deficient diets, but by 1.2% (0.819 vs. 0.809) in lysine-adequate diets. In contrast, the effect of phytase on the digestibility of isoleucine, threonine and valine was similar ( $p>0.05$ ), irrespective of the lysine status of the diets.

Because of the number of interactions observed, the effects of additional lysine on AID of amino acids in diets not supplemented with phytase are considered (Table 5).

Additional lysine significantly increased (values in parentheses) the digestibilities of lysine (5.4%), phenylalanine (5.4%), valine (4.3%), isoleucine (4.1%),

aspartic acid (3.9%), tyrosine (3.8%), glutamic acid (3.0%) and methionine (1.1%). Also, the increase in tryptophan digestibility (2.2%) approached significance.

## DISCUSSION

Exogenous phytase did not influence percentage toe ash, which confirms the phosphorus-adequate specification (4.5 g/kg nonphytate P) of the experimental diets and indicates that any responses to phytase are not related to the release of phytate-bound P. That both additional lysine and supplemental phytase significantly increased weight gain and feed efficiency, and the effects of phytase were more pronounced in lysine-deficient diets, is noteworthy. The implications are that 10.0 g/kg lysine was limiting performance and that phytase partially addressed this shortfall by enhancing the availability of this critical amino acid.

Exogenous phytase increased AME and the ileal digestibility of amino acids and these findings are consistent with published data (Selle et al., 2006). However, the dietary addition of 1.8 g/kg lysine significantly increased the digestibility of lysine *per se* and isoleucine, methionine, phenylalanine, valine, aspartic acid, glutamic acid and tyrosine. In non-phytase supplemented diets, additional lysine increased the digestibility of certain amino acids with the most pronounced responses being recorded for isoleucine, lysine, phenylalanine, valine, aspartic acid and tyrosine. These findings were unexpected, since it was not anticipated that additional lysine would impact on amino acid digestibility. Moreover, there were significant interactions between additional lysine and phytase

supplementation for seven amino acids.

Croom et al. (1999) advanced the hypothesis that the intestinal capacity to absorb nutrients is limiting on growth performance in broiler chickens. In the discussion that follows, consideration is given to the possibility that both additional lysine and phytase supplementation were impacting on the intestinal uptake of nutrients in the present study.

In the mammalian intestine, the transmembrane sodium (Na) gradient, which is maintained by  $\text{Na}^+, \text{K}^+$ ATPase, or the "sodium pump", drives the active transport of amino acids, sugars and inorganic ions, including phosphate (Ganapathy and Leibach, 1985). Similarly, in broilers the intestinal uptake of glucose and amino acids is largely driven by Na-dependent transport systems and  $\text{Na}^+, \text{K}^+$ ATPase is an indicator of intestinal nutrient uptake. Instructively, the activity of  $\text{Na}^+, \text{K}^+$ ATPase was reduced by 57% in chickens following a reduction in dietary Na levels from 1.4 to 0.5 g/kg (Gal-Garber et al., 2003). In addition, Jaso et al. (1995) reported that low salt diets depressed intestinal glucose transport in chickens. Sklan and Noy (2000) concluded that Na plays a critical role in intestinal uptake of nutrients in young broiler chicks from their study in which low Na diets were associated with strongly depressed  $\text{Na}^+, \text{K}^+$ ATPase activity with minimal intestinal uptakes.

Importantly, therefore, phytate has been shown to increase Na excretion in broilers offered atypical diets (Cowieson et al., 2004) and decrease ileal Na digestibility in conventional, maize-soy diets (Ravindran et al., 2006). The latter workers reported that increasing dietary phytate levels significantly reduced AID coefficients of Na (-0.379 vs. -0.237) and that 1,000 FTU/kg phytase counteracted this loss, as supplementation significantly increased (-0.177 vs. -0.515) Na digestibility at the ileal level. Consequently, it follows that phytate may compromise the efficiency of Na-dependent transport systems and the absorption of amino acids and glucose from the gut by this depletion of Na, which is counteracted by phytase.

However, the mechanism whereby phytate increases the movement of Na into the gut lumen is not clear. It has been suggested that this movement serves to buffer the polyanionic phytate molecule (Cowieson et al., 2004) but, in conventional diets containing limestone, it is thought that Ca fulfills this role by forming Ca-phytate complexes (Wise, 1983). Thus, there may be other mechanisms underlying the phytate-induced movement of Na into the gut lumen, which should be clarified.

Nevertheless, there are precedents for anti-nutritive factors found in plant-sourced feed ingredients having the capacity to inhibit glucose uptake. For example, Welsch et al. (1989) demonstrated that tannic and chlorogenic acids inhibit Na-dependent glucose uptake in small intestinal

mucosa taken from rats. These workers concluded that these dietary phenolic compounds dissipate the Na electrochemical gradient, which drives active glucose accumulation.

Irrespective of the dietary lysine status, exogenous phytase enhanced energy utilisation in the present study with a significant increase in AME of 0.34 MJ/kg. While this is a quite consistent finding, the underlying mechanisms whereby phytase improves energy utilisation, particularly in relation to starch, still require elucidation (Selle et al., 2000; Selle and Ravindran, 2007). It is relevant that phytate have been shown to reduce blood glucose levels in humans (Thompson et al., 1987) and phytase has been reported to increase blood glucose in pigs (Johnston et al., 2004; Kies et al., 2005). However, addition of phytate to a glucose test meal reduced blood glucose responses in humans (Demjen and Thompson, 1991). As discussed by Rickard and Thompson (1997), this suggests that reductions in glucose absorption generated by phytate may not involve alterations to starch digestion. This raises the possibility that dietary phytate and exogenous phytase may influence the intestinal uptake of glucose *per se*, rather than, or in addition to, impacting on starch digestibility.

As noted earlier, it was not expected that additional lysine would increase the digestibility of amino acids. However, lysine-deficient broiler diets (7.0 vs. 13.0 g/kg lysine) have been shown to reduce the expression of cationic amino acid transporters (CAT1-3) in the liver, pectoralis and bursa of broiler chicks (Humphrey et al., 2006). While this finding is not directly relevant to intestinal absorption of amino acids, it does reflect the importance of dietary lysine concentrations in relation to the transport of lysine. More specifically, Torras-Llort et al. (1998) reported lysine transport across the jejunal brush-border membrane in chickens was increased following dietary lysine enrichment from 9.6 to 13.6 g/kg. Lysine enrichment significantly increased lysine uptake, via two particular,  $\text{y}^+$ - and  $\text{b}^{0,+}$ -like transport systems. It was concluded that lysine was not co-transported with Na but that Na did play an activating role in lysine uptake, which was potentiated in lysine enriched diets. In the present study, therefore, it seems likely that the increase in lysine digestibility, in response to additional dietary lysine, was generated by increased intestinal uptake of lysine, similar to that reported by Torras-Llort et al. (1998).

The above thesis does not explain the impact of additional lysine on AID of amino acids other than lysine observed in the present study, although interactions in the intestinal uptake of cationic and neutral amino acids are recognised (Munck, 1989). As defined by Ganapathy et al. (1994), the two transport systems identified by Torras-Llort et al. (1998) are both Na-independent; the  $\text{y}^+$  system

transports basic amino acids and the  $b^{0+}$  system, in addition to basic amino acids, assimilates dipolar  $\alpha$ -amino acids and cystine. Subsequently, Torras-Llort et al. (2001) described the  $b^{0+}$ -like transport system as having a broad specificity incorporating cationic and neutral amino acids, behaving as an obligatory amino acid exchanger and playing a key role in the intestinal absorption of amino acids. Thus, it follows that when the  $b^{0+}$ -like system, in particular, is up-regulated by dietary lysine enrichment, there is the possibility that absorption of certain additional amino acids will be influenced. For example, lysine has been shown to stimulate tryptophan uptake in jejunal brush-border membrane vesicles of broiler chicks (Iji et al., 2001). In the present experiment, additional lysine tended to increase the ileal digestibility of tryptophan in non-phytase supplemented diets. Curiously, in the present study, additional lysine had no impact on ileal digestibility of arginine and histidine, which, like lysine, are cationic amino acids.

Nevertheless, it is relevant, as discussed by Eggum and Jacobsen (1976), that additional lysine has improved the availability of amino acids in rapeseed meal in chickens and similar findings in hens and pigs have been reported. In chickens, an additional 2.5 g/kg lysine significantly increased the total tract digestibility lysine and proline and numerically increased the digestibility of the balance of amino acids (Tao et al., 1971). Thus, these early reports appear to be consistent with our present findings.

There is now convincing evidence that added phytase improves amino acid digestibility in poultry, but the exact mode(s) of action are not understood. The suggested mechanisms include the formation of binary protein-phytate complexes in the gut and that phytate exacerbates endogenous amino acid losses (Selle et al., 2006). Phytate-induced increases in excretion of endogenous amino acids have been demonstrated in broilers (Cowieson et al., 2004), which may reflect increased secretion and/or reduced re-absorption of amino acids (Nyachoti et al., 1997). Interestingly, Gagné et al. (2002) suggested that exogenous phytase might improve the absorption of amino acids. This suggestion was based on their finding that phytase supplementation increased post-prandial plasma concentrations of  $\alpha$ -amino N in growing pigs. Thus there is the possibility that phytate may impede the intestinal uptake of amino acids.

By definition, Na impacts on dietary electrolyte balances (DEB;  $DEB \text{ (meq/kg)} = Na^+ + K^+ - Cl^-$ ) and that phytate induces the transfer of Na into the gut lumen implies that phytate is effectively reducing DEB. Johnson and Karunajeewa (1985) recommended a DEB of from 250 to 300 meq/kg for typical broiler diets. Therefore, it may be relevant that the calculated DEB of the basal diet in the

present study of 155 meq/kg was considerably lower than this recommendation. Instructively, Haydon and West (1990) reported that increasing DEB from -50 to 400 meq/kg (and Na from 1.47 to 6.66 g/kg) in swine diets linearly increased AID of fourteen of sixteen amino acids assessed. Therefore it is tempting to speculate that the enhanced amino acid digestibility with supplemental phytase may have been due, in part, to the positive effect of increasing Na and DEB levels on the intestinal uptake of amino acids via Na-dependent transport systems.

## CONCLUSION

The present data showed that additional lysine increased the ileal digestibility of lysine *per se*, which was probably a consequence of up-regulation of two amino acid transport systems, identified by Torras-Llort et al. (1998). In addition, this up-regulation of lysine absorption may have influenced the uptake of certain other amino acids. To the authors' knowledge, this is the first study reporting improvements in digestibility of amino acids from the dietary addition of an amino acid. Exogenous phytase significantly increased ileal amino acid digestibility and there is the possibility that this is partially due to enhanced amino acid uptake as phytate induces the movement of Na into the small intestine, which might compromise Na-dependent transport systems. Some support for this argument is provided by the significant treatment interactions recorded for seven amino acids, which could imply that additional lysine and supplemental phytase were both impacting on amino acid absorption. Although entirely speculative, the genesis of these interactions may have been that lysine enrichment up-regulated  $Na^+$ -independent transport systems; whereas, phytase supplementation influenced  $Na^+$ -dependent systems. Thus, it appears possible that phytate has a negative impact on the transition of amino acids and glucose across the gut wall, presumably via dissipating Na-electrochemical gradients and reducing Na-dependent transport of these nutrients. If so, phytase supplementation should partially counteract this negative effect on amino acid digestibility and that this may be a previously unrecognised mechanism whereby phytase enhances ileal digestibility of amino acids. In the interests of verification, it may prove instructive to determine the effect of microbial phytase on ileal amino acid digestibility in diets with varying levels of  $Na^+$ ,  $K^+$  and  $Cl^-$  and, therefore, a range of dietary electrolyte balances.

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