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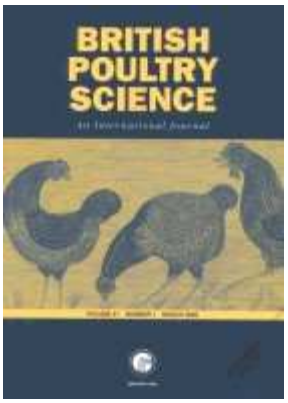

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Effects of dietary phytase on performance and nutrient metabolism in chickens

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Abstract. 1. A broiler growth study was conducted to compare the effect of different concentrations of an *Escherichi coli*-derived phytase on performance, apparent metabolisable energy (AME), nitrogen (N), amino acid and mineral metabolisability, sialic acid excretion and villus morphology when fed to broiler chickens.

2. Female Ross 308 broilers (480) were reared in floor pens from 0 to 28d of age. All birds were fed on nutritionally complete starter (0 to 21d age), and grower (21 to 28d age) with the exception that they were low in P (28 g/kg and 23 g/kg available P, respectively). These maize-soy diets were supplemented with 0, 250, 500 or 2500 phytase units (FTU)/kg feed.

3. Between 21 and 28d of age, two birds from each floor pen were selected, and each pair placed in one of 32 metabolism cages (2 birds per cage). Feed intake was recorded and excreta collected for the last 2 d of the feeding period, and AME, N, amino acid and mineral metabolisability coefficients and endogenous losses were determined following a total collection procedure.

4. Feed intake and weight gain increased in a linear manner in response to phytase dose, with an average increase of approximately 11.7% and 13.5% respectively compared with chickens fed on the low-P diet. Birds given diets with 2500 FTU diet weighed 6.6% more and had a 2.4% higher FCE than those fed on diets containing 500 FTU.

5. Enzyme supplementation increased the intake of AME and metabolisable N by 10.3% and 3.9%, respectively, principally through increases in feed intake. Birds given enzyme-supplemented diets also improved their intake of metabolisable amino acids and P by approximately 14% and 12.4% respectively, compared with birds fed on the control diet. Enzyme supplementation did not affect ileal villus morphometry of the birds.

INTRODUCTION

Approximately 600-700 g/kg of the plant-P is present as phytate. Phytic acid (*myo-inositol hexakis-dihydrogenphosphate, IP6)* is a polyanionic molecule with 6 phosphate groups and is

capable of forming insoluble complexes with divalent cations, starch and protein, reducing their availability for poultry (Johnson and Tate, 1969). Poultry can produce some endogenous phytase (Maenz and Classen, 1998; Applegate *et al*., 2003) but this is insufficient for the effective hydrolysis of dietary phytates. The detrimental effects of phytates in the diets of poultry can be ameliorated by the addition of microbial phytases. Phytases (myo-inositol hexaphosphate phosphohydrolases) are enzymes that can hydrolyse the ester bonds between the phosphate groups and the inositol ring in phytates, increasing the dietary available P (Nelson *et al.*, 1971; Irving and Cosgrove, 1974; Cowieson *et al.*, 2004a; Bryden *et al.,* 2006). However, recent studies demonstrated that the benefits of using dietary phytases are not restricted to the improvement of mineral retention but may improve performance and energy and amino acid availability (Ravindran *et al.*, 1999a, 2001; Selle *et al*., 2000; Rutherfurd *et al*., 2002; Newkirk and Classen, 2001; Cowieson *et al.*, 2006a, b; Pirgozliev *et al*., 2005).

Further, it has recently been demonstrated (Cowieson *et al.*, 2004b; Pirgozliev *et al*., 2005) that supplementation of diets with phytase significantly reduced the endogenous secretions, measured as sialic acid (SA), from the gastrointestinal tract (GIT) of precision fed birds. Sialic acid refers to a family of acidic monosaccharides found at the terminal ends of sugar chains attached to cell surfaces and to soluble glycoproteins (Angata and Varki, 2002). Increased concentrations of sialic acid are often associated with health problems such as cellular senescence, bacterial infections (*e.g.* campylobacter), certain pathological conditions and osmotic fragility. The most widely distributed sialic acid is N-acetylneuraminic acid, which is believed to be a biosynthetic precursor of all other sialic acid molecules. Sialic acid is associated with the gastrointestinal mucin (Montagne *et a*l., 2000) so SA excretion can be used as an indicator of mucin losses from the gastrointestinal tract of experimental animals (Larsen *et al.*, 1993). It has been hypothesised that dietary phytase results in improved intestinal health with concomitant reductions in secretions from the GIT (Cowieson *et al.*,

2004b; Pirgozliev *et al.*, 2005). However, this is based on studies with precision fed birds and it is not clear whether *ad libitum* fed birds will follow the same pattern of response.

The objective of this experiment was to compare the effects of different dietary concentrations of an *E. coli* derived phytase on the apparent metabolisable energy (AME), performance, metabolisability of nitrogen (N), amino acids and minerals, endogenous losses, measured as sialic acid and villus morphology, when fed to young broiler chickens.

MATERIALS AND METHODS

Diet formulation

The Animal Experimental Committee of Scottish Agricultural College approved the study. Four maize-soy diets fed in two phases (starter phase:0-21d old; grower phase: 21-28d old), were prepared and were supplemented with an evolved *Escherichia coli*-derived phytase (Quantum: 2500 D, EC 3.1.3.26; Syngenta Biotechnology Inc, Research Triangle Park, NC, USA). Diets were formulated to be nutritionally adequate (NRC, 1994) with the exception of phosphorus (P) (Table 1). The basal diet was supplemented with appropriate quantities of phytase (250, 500 or 2500 phytase units (FTU)/kg diet), respectively. The enzyme was added to the diets in powder form and all diets were fed as mash.

Table 1 near here

Husbandry, dry matter digestibility (DMD), nitrogen metabolisability (NM) coefficients, and apparent metabolisable energy (AME) determination

A total of 480 female broiler (Ross 308) chickens, obtained from a commercial hatchery were used. The birds were allocated to 32 floor pens (193 X 126 cm floor area) from 0 to 28 d of age. Fifteen birds were placed in each pen following recording of their initial weight. The study was designed as a randomised block design, there being 4 blocks of 8 pens, and each treatment was replicated 8 times. Feed and water were available *ad libitum* throughout the experiment. The starter diet was fed from 0 to 21d old and then the grower diet was fed to 28d old. The experimental facility was equipped with a positive pressure ventilation system to

meet commercial recommendations and temperature maintained at 33°C at the beginning of the study and reduced gradually to 20°C at 20 d of age. The light regime was 23 h light:1 h dark.

During the 4th week of the experiment, between 21 and 28 d old, 64 birds were selected, two from each floor pen, and placed in 32 metabolism cages (0.50 x 0.60 m floor area, 2 birds in a cage) in a randomised block design. For the following 5 d birds were fed on the respective treatment diets that they had been fed on previously. Excreta were collected quantitatively for the last 2d of the feeding period, stored in a freezer at -20°C and later freeze-dried. The feed intake for the same period was also measured. The gross energy of each dried excreta sample and the experimental diets was determined using an adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL, USA). The AME of each of the experimental diets (MJ/kg dry matter), was then calculated. The nitrogen in the excreta was determined by method of Sweeney (1989) using a FP-200 nitrogen analyser (LECO®, St. Joseph, MI, USA). Nitrogen metabolisability (NM) and dry matter digestibility (DMD) coefficients of the diets were calculated. The metabolisability coefficients of nutrients are defined as ([nutrient intake –nutrient output]/nutrient intake). The average weight, intake and FCE for birds that remained in each floor pen were also determined at 28d old.

Amino acids in feed and excreta

Amino acids in feed and excreta were determined by high performance liquid chromatography (HPLC) (Jones *et al*., 1981; Alltech, Associates, Carnforth, UK; Cowieson *et al*, 2006a). The HPLC system comprised by a Dionex ASI-100 autosampler fitted with a Dionex P580 pump and a Dionex RF-2000 detector (Sunnyvale, CA). Primary amino acids were derivatised with o-phthaldialdehyde prior to separation by HPLC using an Adsorbosphere OPA-HR column (150 x 4.6 mm; Alltech Associates, Carnforth, Lancs, UK) and fluometric detection. Since the method of hydrolysis destroys methionine, cystine and

tryptophan, data on these amino acids are not reported. Excreta digestibility for glycine is not presented because of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et al*., 1971). The metabolisable amino acid intake (g/bird/d) was obtained as the product of the average total amino acid intake between 21 and 28d of age and their metabolisability coefficient.

Minerals in feed and excreta

Minerals in the samples were determined by inductively coupled plasma emmission spectrometry, ICP (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer, Beaconsfield, UK), (Tanner *et al*., 2002). The metabolisability coefficient of each mineral was calculated. The quantity of metabolisable mineral consumed (mg/bird/d) was obtained as the average mineral intake between 21 and 28d of age was multiplied by their metabolisability coefficient.

Sialic acid determination

The concentration of excreta sialic acid was determined by the periodate-resorcinol method as described by Jourdian *et al*. (1971). The sialic acid excretion was reported as excreted per kg metabolic body weight ($W^{0.75}$) and is assumed to give a measure of endogenous losses.

Ileal villus morphometry

On d 28, one bird from each of 8 pens of each treatment was killed by cervical dislocation. Approximately 4 cm of the middle part of the ileum were sampled and stored in formal saline histological fixative (Gurr ®, BDH, Poole, UK) until required for analyses. The samples were embedded in paraffin wax, sectioned at approximately 5µm and 4 gut segments were fixed in each slide. Morphometric measurements were determined on 20 villi for each slide (Stereo Binocular Microscope, Olympus, Japan; CCD Camera & Monitor, JVC, Japan; Image Analysis software Bioscan Optimas 3.01 for Windows). The length of the villus was defined

as the distance from the crypt opening to the tip on the right side of the villus, as explained by Sigleo *et al*. (1984). Villus thickness was measured at a point one-third from the villus tip.

Statistical analysis

Statistical analyses were performed using the Genstat VII statistical software package (IACR Rothamstead, Hertfordshire, England). The comparison between treatments for performance and other parameters was examined by analysis of variance (ANOVA). A regression analysis was employed to test the relationship between performance, AME, enzyme activity and excreted sialic acid with enzyme dosage. In all instances, differences were reported as significant at $P \leq 0.05$.

RESULTS

All animals were healthy and survived until the end of the study. It is clear (Table 2) that birds given enzyme supplemented diets grew faster ($P<0.001$) and consumed more ($P<0.05$) than those fed the unsupplemented (NC) diet. Chickens fed on diets containing phytase increased feed intake and weight gain on average by 11.7% and 13.5% respectively compared to chickens fed the unsupplemented diet. The improvements ($P<0.001$) obtained for the 500 FTU compared with the unsupplemented diet for these parameters were 14.5 and 14.1%, respectively and the increases were even more pronounced (22 and 17% respectively; $P<0.001$) for the NC+2500 FTU. In addition, the highest level of enzyme supplementation, (2500 FTU), improved FCE ($P<0.05$) by approximately 3.9%. The linear response to enzyme (feed intake, $r^2=0.29$; $P<0.05$, weight gain $r^2=0.55$; $P<0.001$, and FCE, $r^2=0.12$; $P<0.05$. Table 7) was exemplified by the fact that feeding 2500 FTU significantly improved the weight gain (7.3%, $P<0.05$) and FCE (4.3%, $P<0.05$) compared to the 500 FTU dose.

Table 2 near here

Phytase did not influence ($P>0.05$) dry matter digestibility and nitrogen metabolisability coefficients (Table 2). However phytase increased the retention of N as chickens receiving enzymes retained approximately 16.9% more N than those fed on the

unsupplemented diet. Birds given 500 FTU retained 16% more N than those fed the unsupplemented diet while feeding enzyme supplemented diets tended (P=0.055) to increase dietary AME and gross energy metabolisability coefficient (AME:GE). The intake of AME was improved (P<0.001) by approximately 14% and 18.3% for birds fed on the diets supplemented with 500 and 2500 FTU diets, respectively, compared to birds fed the unsupplemented diet, the majority of this effect being driven by intake rather than differences in metabolisability. No significant changes (P>0.005) in excreta moisture were determined in this study (Table 2). High enzyme supplementation (2500 FTU) decreased (P<0.05) the concentration of excreted sialic acid (SA). Although the effect was not significant (P>0.05), supplementation with 2500 FTU decreased daily excretion of SA by approximately 12% compared to the NC diet. A weak negative linear relationship was detected between AME and sialic acid excretion (r^2=0.17; P<0.05) (Table 7). Birds fed on the NC+500 FTU diet had thicker villi (P<0.05) compared to those fed the unsupplemented diet (Table 2). However, on average enzyme supplementation did not significantly affect ileal villus morphometry.

Enzyme supplementation tended to improve metabolisability coefficients of histidine (P=0.082) and tyrosine (P=0.086), by 5.6 and 3.4% respectively when comparison was made between unsupplemented and 2500 FTU supplemented diet (Tables 3 & 4). The intake of metabolisable amino acids was influenced by both metabolisability coefficients and feed intake of the birds (Table 4) but the majority of this effect was due largely to intake. Birds fed enzyme-supplemented diets on average consumed 17.4 % and 16.8% more indispensable (P<0.05) and dispensable (P<0.05) amino acids respectively than the control diet. Intake of histidine, leucine, valine, serine and tyrosine increased by 19.0 %, 15.1 %, 15.3 %, 15.3 % and 15.9 %, respectively (P<0.05). When comparison was made between the negative control and birds fed 500 and 2500 FTU, the intake of indispensable metabolisable amino acids increased by approximately 18% (P<0.05) and 22.6% (P<0.05), respectively. No differences

were observed between the coefficient of amino acid metabolisability and metabolisable amino acid intake when comparison was made between diets supplemented with 500 and 2500 FTU.

Tables 3,4,5 near here

Contrary to expectations, enzyme supplementation decreased the metabolisability coefficients of Ca (P<0.05) and K (P<0.001) compared to the unsupplemented diet (Tables 4 & 5). In general the results were variable and supplementation with 500 FTU did not result in pronounced changes in mineral metabolisability coefficients for P, Na, Mn and Zn, compared to 2500 FTU supplemented diets. The intake of metabolisable P was enhanced (Table 6) in birds fed enzyme supplemented diets (P<0.05). Their intake of metabolisable P increased by approximately 14% compared to control and a similar trend was observed for S (P=0.089). However, the intake of total metabolisable minerals, and thus electrolyte balance, was not affected by dietary phytase.

Tables 6 & 7 near here

DISCUSSION

The benefits in bird performance due to phytase supplementation to the diets is well documented in the literature (Ravindran *et al.*, 1999a; Selle *et al.*, 2000; Rutherfurd *et al.*, 2002; Cowieson *et al.*, 2006a). In this study, the significant improvements on bird performance by phytase supplementation of the diets can be largely explained by the increased intake of energy and digestible nutrients. The improvements seen with phytase supplementation, on the intake of DM, N, AME, metabolisable amino acids, and metabolisable P, are functions of improved digestibility coefficients and greater feed intake, the majority of the effect being due to intake. The improved performance, N and AME intakes seen when birds were fed on diets containing 500 FTU, compared to the birds fed on the unsupplemented diet suggest that supplementation at this level is practically valuable. However, it should also be noted that in this study birds fed on diets with 2500 FTU gained 7% more and converted feed 4.4% more efficiently compared with birds receiving the diet

containing 500 FTU. This suggests that improvements can be obtained at inclusion levels well in excess of current commercial practice (approximately 500 FTU). This observation is similar to data in previous reports with chickens (Cowieson *et al.*, 2006a) and turkeys (Esteve-Garcia *et al*., 2005; Ledoux *et al*., 2005). Ledoux *et al*. (2005) reported that turkeys given diets low in P but supplemented with 10000 FTU outperformed birds fed on the diet adequate in P by 7 and 2.6% for gain and feed efficiency, respectively. Cowieson *et al*. (2006a) found that chickens fed on low P diets supplemented with either 2400 FTU or 24000 FTU had 14% better feed efficiency compared to birds fed a diet with adequate supplies of P. Similarly, a positive linear relationship existed between bird performance and phytase activity in the present study (Table 7).

The lack of improvement in amino acid metabolisability coefficients and reduction in sialic acid excretion when enzyme supplemented diets were fed could be due to the age of the birds as well as masking by the influence of microbial activity in the gut. It is known that intestinal microflora can change mucin production and amino acid metabolism (Sharma and Schumacher, 1995; Ravindran *et al*., 1999b). Whilst this study was designed to investigate the effects of supplemental phytases, overall, on amino acid retention including the effects of the microbiota within the gut it is recognised that hindgut microflora will influence amino acid excretion form the birds. Ravindran *et al*. (1999b) demonstrated that amino acid metabolism by microflora in the lower gut of chickens may be substantial and that amino acid digestibilities measured in the terminal ileum are more accurate measures of amino acid availability than those measured in the excreta. This may account for the lack of differences in amino acid digestibilities found in this study. It is also known (Bedford, 2000; Acamovic, 2001) that the effects of dietary enzymes are more obvious in younger birds and this might also account for the relatively poor effects on metabolisability coefficients. Nevertheless, the benefit of the enzyme on intake was so large that it resulted in increased metabolisable amino

acid intake and this provides an indicative assessment of the effect of treatment on the retention of amino acids including the effects on the microflora.

It should be noted that in the present study the superior effect of 2500 FTU compared to 500 FTU was observed only with respect to weight gain and FCE. The lack of effect of phytase on energy, amino acids or mineral metabolisability coefficients is in accord with other published data (Cowieson *et al.,* 2006a). It was reported that lower enzyme activities, (150-300 FTU/kg), were sufficient to improve AME and amino acid digestibility coefficients, with higher doses having relatively little additional effect (Cowieson *et al*., 2006a). The same authors found that higher dietary phytase supplementation improved P retention, mediated through an improvement in the digestibility of phytate-bound P. In this study, enzyme supplementation increased the intake of metabolisable P compared to the control diet, however no difference existed between diets supplemented with 500 and 2500 FTU. The improved gain seen in birds fed the diet containing 2500 FTU compared with that seen for the birds fed the diets containing 500FTU cannot be explained by any of the digestibility indices measured and suggests that growth was influenced by some other factor associated with phytase treatment. The present results may suggest that the improvements in bird performance with incremental doses of phytase are the result of an improved use of dietary nutrients for production rather than maintenance, which is consistent with the differences in size of the birds on the various treatments.

The theory of enzymatic breakdown of phytate compounds distinguishes between liberation of phytate molecules from complexes with other tissue components and enzymatic cleavage of phosphate residues on the *myo*-inositol ring (Zyla *et al*., 2004). The stepwise manner of dephosphorylation of IP6 (Venekamp *et al.*, 1995; Greiner *et al.*, 2000) will lead to a release of different *myo*-inositol isomers and phosphates. The conditions and the limited time spent in the GIT of poultry are not ideal for phytase to function effectively, so it is

unlikely that exogenous phytase will be able to completely dephosphorylate dietary phytates, particularly when used at low activities. However, with increased phytase use there are likely to be increased amounts of the *myo*-inositol isomers of lower molecular masses which may be considered as potential growth promoters. Waagbo *et al.* (1998) reported that feeding fish with inositol-supplemented diets improved their performance while Zyla *et al*. (2004) also found that addition of *myo*-inositol to P deficient diets improved the weight gain of broiler chickens. These results suggest that *myo*-inositol by itself acts as a growth promoter. The biological importance of the inositol phosphates is well documented (Irvine and Schell, 2001; Beemster *et al.*, 2002; Fisher *et al*., 2002), however there is a lack of information about the effect of inositol on nutrient availability and performance when fed to poultry. The performance results from this study suggest that increased phytase activity increased the dephosphorylation of IP6, increasing the concentration of *myo*-inositol isomers of lower relative molecular mass (RMM). The linear relationship between dietary enzyme activity and weight gain, as birds fed 2500 FTU increased their weight gain by 22%, 11% and 7% relative to control, NC+250 and NC+500FTU, respectively, may be due to the reduced adverse effects of phytate but also to beneficial effects if inositol esters of lower RMM.

No relationship between dietary AME and performance was found in the present experiment. It is not unusual that AME values do not predict adequately the nutritive quality of poultry feeds. Rose and Bedford (1995) and Ravindran *et al.* (1999a) did not observe a correlation between dietary AME and performance when broilers were fed untreated or enzyme supplemented wheat-based diets. The poor relationship between bird performance and dietary AME in this study supports previous finding that, although convenient, the measurement of metabolisable energy is not sufficiently sensitive to evaluate the nutritive value of poultry feed (Emmans, 1994; Pirgozliev and Rose, 1999). This is likely to be because AME does not account for the partitioning of energy between maintenance and growth (De

Groote, 1974; Hoffmann and Shiemann, 1980). Thus the improved performance of the birds fed phytase suggest that these birds can utilise more energy and protein from the feed, however this cannot be detected by dietary determining AME and suggests that intermediary metabolism is influenced by phytases thereby increasing dietary available (net) energy.

The results for ileal villus morphometry were in the expected range and similar to those measured in other growing broiler experiments (Langhout *et al.*, 1999; Pirgozliev *et al.*, 2001). However Koutsos *et al.* (2005) reported that dietary phytase increased the duodenal villus width when fed to laying hens. Perhaps the phytase influences the type of microflora within the GIT (Steer and Gibson, 2002) and thus influences concomitant adherence and any damage to the GIT and intestinal villi.

The negative relationship between dietary AME and excreted sialic acid per kg metabolic body weight was in accord with previous results suggesting that the health of the gastrointestuinal tract may be enhanced by the presence of phytase (Cowieson *et al.*, 2004b; Pirgozliev *et al.*, 2005). Sialic acid is involved in many physiological functions (Kongtawelert *et al.*, 2003; Browning *et al.*, 2004; Nayak and Bhaktha, 2005). Sialic acids are also involved in the binding of pathogenic and other microflora to mucin (Ryan *et al.*, 2001; Deplanske *et al.,* 2002; Edelman *et al.*, 2002; Edelman *et al.*, 2003; Kettunen *et al.*, 2003), thus SA excretion may provide valuable information about the health of the gut. The epithelium of the GIT is covered by protective mucus gel composed predominantly of mucin glycoproteins that are synthesised and secreted by goblet cells. This mucus layer acts as a medium for protection, lubrication, and transport between the intestinal content and the epithelial cells. However, the presence of nutrients with low digestibility in the gut might increase the number of bacteria in the lumen. Some of those bacteria may be bind to the mucus layer and later be excreted as a defence reaction of the gut. Thus changes in nutrient supply, *e.g.* phytase supplementation, might change nutrient utilisation and affect mucin dynamics. Smirnov *et al.*

(2004) also found that starvation changed mucin dynamics and decreased thickness of the mucous layer of young chickens, suggesting that this may affect intestinal digestive function and defence.

The impaired performance, N retention and AME intake in birds fed the NC diet compared to those fed phytase-supplemented diets may be due to a reduction in the release of available nutrients which may lead to an unbalanced nutrient supply in the intestinal lumen. It has also been demonstrated that phytic acid, a major component of dietary phytate, irritates the GIT and increases the excretion of endogenous amino acids, minerals and sialic acid (Cowieson *et al.*, 2004b). It can be expected that a combination of irritation and unbalanced supply of nutrients compromised the health of the gut and may explain the negative relationship between the AME and excreted mucin in this study. The thickness of the adherent GIT mucus layer is the result of the balance between the rate of secretion of mucin and its degradation through enzymatic digestion and mechanical shear (Allen, 1989). More mucin excretion will lead to greater mucin synthesis, which is biologically very expensive and demands more energy (Nyachoti *et al*., 1997). However, if there are not enough nutrients to support the demand for increased synthesis, then the thickness of this barrier may decrease and lead to increased exposure of the epithelium to harmful agents in the lumen, e.g. microflora, phytate etc. The exposure of non-protected host intestinal epithelial cells to pathogenic organisms may cause an inflammation, provoking immune response and increased numbers of cytokines (McKay and Baird, 1999). The activation of the immune system of the bird, e.g. increased production of antibodies in response to the invading organism, is an energy demanding process (Klasing and Calvert, 1999; Eraud *et al.*, 2005). It has also been suggested that a strong immune response will increase the risk of tissue damage (Svenson *et al.*, 1998), increased production of free radicals (Finkel and Holbrook, 2000) and further deleterious effects on the organism. Karadas *et al.* (2005, 2006) reported that broilers fed

phytase-supplemented diets had reduced hepatic oxidative stress and increased antioxidant status compared to birds fed non-supplemented, low in P diets. Although, the mechanism is not clear, it suggests that dietary phytases are capable of moderating immune response and free radical production in broiler chickens.

It can be concluded that the exogenous phytase used in this study is effective in improving the nutritional value of maize-soy-based diets for young broiler chickens fed on a diet that is formulated to be sub-optimal in terms of available phosphorus. Although diets supplemented with 500 FTU improved bird performance, it was found that chickens can respond to phytase activity much higher than used in the commercial practice. The negative relationship between dietary AME and sialic acid excretion supports the hypothesis that dietary phytase may influence gut health. However, to understand the mode of action of dietary phytases further research on *myo*-inositol isomers of lower RMM, immune response and antioxidant status needs to be completed.

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625

Table 1. *Ingredient composition (g/kg 'as fed') of the control experimental diets*

Ingredients	Starter diet (0-21d age)	Grower diet (21-28d age)
Maize	600.0	602.7
Soybean meal (48)	300.0	300.0
Maize gluten meal	40.0	25.0
Vegetable oil	20.0	35.0
Limestone	17.2	17.0
Mono dical phosphate	7.0	4.9
Salt	3.6	3.7
Lysine HCl	3.0	3.0
Methionine	4.2	3.7
Vitamin mineral premix[1]	5.0	5.0
Calculated values (as fed)		
ME (MJ/kg)	12.79	13.17
CP (N x 6.25) g/kg	231	221
Lysine g/kg	13.1	13.1
Methionine + cyctine g/kg	9.7	9.3
Ca g/kg	8.6	8.1
Total P g/kg	5.2	4.8
Nonphytate P g/kg	2.8	2.3
Sodium g/kg	1.6	1.6
Determined values (DM)[2]		
DM g/kg	878	901
GE[3] MJ/kg	19.40	19.21
CP (Nx6.25) g/kg	258	244
Total P g/kg	6.2	8.0
Ca g/kg	11.7	19.5

Determined mineral and amino acid composition of grower diet (DM)[2]

Amino acids (g/kg)		Minerals (g/kg)	
Alanine	12.44		
Arginine	12.70	Calcium	14.82
Aspartic acid	26.30	Potassium	12.61
Glutamic acid	51.99	Magnesium	2.17
Histidine	4.67	Manganese	0.135
Isoleucine	12.26	Sodium	2.20
Leucine	23.19	Phosphorus	6.81
Lysine	13.80	Sulphur	4.18
Phenylalanine	12.03	Zinc	0.13
Serine	6.49		
Threonine	7.17		
Tyrosine	5.23		
Valine	12.84		

[1] The vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). All the experimental diets were designed to be low in P. The premix provided (units/kg diet): retinol 3600 µg, cholecalciferol 125 µg, α-tocopherol 34 mg, menadione 3 mg, thiamine 2 mg, riboflavin 7 mg, pyridoxine 5 mg, cobalamin 15 µg, nicotinic acid 50 mg, pantotenic acid 15 mg, folic acid 1 mg, biotin 200 µg, iron 80 mg, copper 10 mg, manganese 100 mg, cobalt 0.5 mg, zinc 80 mg, iodine 1 mg, selenium 0.2 mg and molybdenum 0.5 mg.
[2] Analyses were performed in duplicate.
[3] GE Gross energy.

Table 2. *The effect of bacterial phytase activity on performance (0-28d age), dry matter digestibility (DMD) and nitrogen metabolisability (NM) coefficients, apparent metabolisable energy (AME), metabolisability coefficients of gross energy (AME:GE), excreta moisture, sialic acid excretions and small intestinal villus morphology in chickens*

Variates	Experimental diets				LSD[3]	P
	NC[1]	NC 250 FTU[2]	NC 500 FTU[2]	NC 2500 FTU[2]		
Bird weight (kg/28d age)	1.050	1.148	1.190	1.272	0.0509	<0.001
Feed intake (g DM/bird/d)	47.4	51.2	54.3	55.7	3.53	<0.001
Weight gain (g/bird/d)	36.0	39.6	41.1	44.0	1.81	<0.001
FCE[4] (g/bird/d)	0.761	0.772	0.758	0.791	0.0281	0.093
Feed intake[5] (g DM/bird/d)	86.5	97.5	101.2	104.7	3.47	<0.05
DMD	0.745	0.722	0.744	0.746	0.0211	0.090
NM	0.616	0.565	0.606	0.614	0.0592	NS
N retention (g/bird/d)	0.087	0.098	0.101	0.105	0.0104	<0.05
AME (MJ/kg DM)	14.88	14.59	15.00	15.06	0.357	0.055
AME intake (MJ/bird/d)	0.71	0.75	0.81	0.84	0.059	<0.001
AME:GE	0.774	0.759	0.781	0.784	0.0186	0.055
Moisture in excreta (g/kg)	759	761	772	768	18.6	NS
SA concentration (mg/g/DM)	1.23	1.36	1.15	1.13	0.166	<0.05
SA excreted (mg/bird $W^{0.75}$/d)	18.91	21.07	18.43	16.64	4.598	NS
Villus length (μm)	833	961	695	731	395.9	NS
Villus thickness (μm)	136	169	190	152	54.23	NS

[1]Negative control – diet low in available P.
[2]Phytase activity (units/kg) in diet.
[3]Least significant difference.
[4]Feed conversion efficiency.
[5]Daily feed intake between 21 and 28d of age.

Table 3. *The effect of bacterial phytase activity on the amino acid metabolisability coefficients determined at 28 dof age*

Amino acids	Experimental diets				LSD[3]	P
	NC[1]	NC 250FTU[2]	NC 500 FTU[2]	NC 2500 FTU[2]		
Indispensable						
Arginine	0.898	0.899	0.900	0.910	0.0210	NS
Histidine	0.853	0.877	0.876	0.901	0.0544	NS
Isoleucine	0.865	0.854	0.873	0.873	0.0231	NS
Leucine	0.878	0.872	0.887	0.894	0.0210	NS
Lysine	0.889	0.884	0.901	0.892	0.0258	NS
Phenylalanine	0.850	0.843	0.859	0.862	0.0246	NS
Threonine	0.762	0.735	0.765	0.779	0.0341	0.086
Valine	0.804	0.792	0.812	0.816	0.0368	NS
Dispensable						
Alanine	0.837	0.820	0.844	0.847	0.0333	NS
Aspartate	0.876	0.857	0.882	0.877	0.0222	0.131
Glutamate	0.909	0.898	0.915	0.918	0.0151	0.053
Serine	0.823	0.810	0.833	0.839	0.0308	NS
Tyrosine	0.810	0.818	0.831	0.838	0.0321	NS
Average indispensable	0.850	0.844	0.859	0.866	0.0242	NS
Average dispensable	0.851	0.841	0.861	0.864	0.0246	NS
Average total	0.850	0.843	0.860	0.865	0.0235	NS

[1]Negative control – diet low in available P;
[2]Phytase activity (units/kg) in diet;
[3]Least significant difference.

Table 4. *The effect of bacterial phytase activity on metabolisable amino acid intake (g/bird/d) when fed to broiler chickens from 21 to 28 d of age*

Amino acids	Experimental diets				LSD[3]	P
	NC[1]	NC 250FTU[2]	NC 500 FTU[2]	NC 2500 FTU[2]		
Indispensable						
Arginine	0.99	1.11	1.16	1.21	0.126	<0.05
Histidine	0.34	0.40	0.42	0.44	0.054	<0.05
Isoleucine	0.92	1.02	1.08	1.12	0.109	<0.05
Leucine	1.76	1.97	2.08	2.17	0.216	<0.05
Lysine	1.06	1.19	1.26	1.29	0.123	<0.05
Phenylalanine	0.89	0.99	1.05	1.09	0.111	<0.05
Threonine	0.47	0.51	0.56	0.58	0.060	<0.05
Valine	0.89	0.99	1.06	1.10	0.116	<0.05
Dispensable						
Alanine	0.90	1.00	1.06	1.10	0.107	<0.05
Aspartate	2.00	2.20	2.34	2.41	0.231	<0.05
Glutamate	4.09	4.56	4.81	5.00	0.495	<0.05
Serine	0.46	0.51	0.55	0.57	0.059	<0.05
Tyrosine	0.37	0.42	0.44	0.46	0.048	<0.05
						<0.05
Indispensable	7.33	8.19	8.65	8.99	0.903	<0.05
Dispensable	7.82	8.68	9.20	9.54	0.935	<0.05
Total	15.14	16.86	17.86	18.54	1.836	<0.05

[1]Negative control – diet low in available P.
[2]Phytase activity (units/kg) in diet.
[3]Least significant difference.

Table 5. *The effect of bacterial phytase activity on mineral metabolisability coefficients when fed to 28d old broiler chickens*

Minerals	Experimental diets				LSD[3]	P
	NC[1]	NC 250 FTU[2]	NC 500 FTU[2]	NC 2500 FTU[2]		
Calcium	0.459	0.394	0.361	0.354	0.0958	0.120
Magnesium	0.213	0.131	0.184	0.197	0.0697	0.119
Manganese	0.118	0.121	0.149	0.062	0.0986	NS
Phosphorus	0.471	0.463	0.483	0.440	0.0651	NS
Potassium	0.340	0.199	0.240	0.247	0.0690	<0.05
Sodium	0.399	0.350	0.415	0.325	0.1251	NS
Sulphur	0.623	0.574	0.606	0.561	0.0526	0.082
Zinc	0.162	0.182	0.242	0.151	0.1070	NS

[1]Negative control – diet low in available P.
[2]Phytase activity (units/kg) in diet.
[3]Least significant difference.

Table 6. *The effect of bacterial phytase activity on metabolisable mineral intake (mg/bird/d) when fed to broiler chickens from 21 to 28 d of age*

Minerals	Experimental diets				LSD[3]	P
	NC[1]	NC 250 FTU[2]	NC 500 FTU[2]	NC 2500 FTU[2]		
Calcium	513	492	472	472	115.4	NS
Magnesium	35	24	35	39	11.9	0.087
Manganese	1.2	1.4	1.8	0.7	1.06	NS
Phosphorus	241	267	288	270	36.6	0.094
Potassium	322	210	267	283	72.8	<0.05
Sodium	66	65	79	64	20.0	NS
Sulphur	196	203	222	213	23.3	0.140
Zinc	1.6	2.0	2.8	1.8	1.11	0.150

[1]Negative control – diet low in available P.
[2]Phytase activity (units/kg) in diet.
[3]Least significant difference.

Table 7. *Relationship between bacterial phytase activity and performance, and sialic acid excretion and dietary AME*

Dependant variates	Constant	Explanatory variates	r^2	RSD[1]
		Phytase activity		
Feed intake (g/bird/d)	50.12 (± 0.877)	0.0025 (± 0.00069)	0.29	3.84**
Weight gain (g/bird/d)	38.12 (± 0.517)	0.0025 (± 0.00040)	0.55	2.26 ***
Feed conversion efficiency	0.76 (± 0.064)	0.000012 (± 0.0000050)	0.12	0.0282 **
		Silaic acid excretion		
AME (MJ/kg DM)	15.62 (± 0.284)	- 0.0393 (± 0.01470)	0.17	0.333* *

Statistical significance of regression equation: *** $P<0.001$.
[1] Residual standard deviation.