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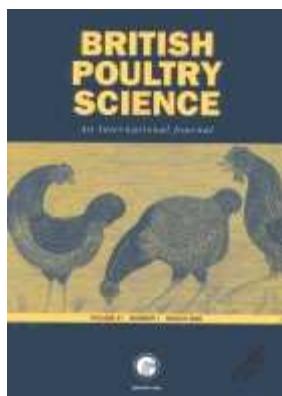
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Effects of dietary phytase on performance and nutrient metabolism in chickens

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15 6 **Effects of dietary phytase on performance and nutrient metabolism in chickens**
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22 9
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RUNNING TITLE: DIETARY PHYTASE

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2
3 29 **Abstract.** 1. A broiler growth study was conducted to compare the effect of different
4
5 30 concentrations of an *Escherichi coli*-derived phytase on performance, apparent metabolisable
6
7 31 energy (AME), nitrogen (N), amino acid and mineral metabolisability, sialic acid excretion
8
9 32 and villus morphology when fed to broiler chickens.
10
11
12 33 2. Female Ross 308 broilers (480) were reared in floor pens from 0 to 28d of age. All birds
13
14 34 were fed on nutritionally complete starter (0 to 21d age), and grower (21 to 28d age) with the
15
16 35 exception that they were low in P (28 g/kg and 23 g/kg available P, respectively). These
17
18 36 maize-soy diets were supplemented with 0, 250, 500 or 2500 phytase units (FTU)/kg feed.
19
20 37 3. Between 21 and 28d of age, two birds from each floor pen were selected, and each pair
21
22 38 placed in one of 32 metabolism cages (2 birds per cage). Feed intake was recorded and
23
24 39 excreta collected for the last 2 d of the feeding period, and AME, N, amino acid and mineral
25
26 40 metabolisability coefficients and endogenous losses were determined following a total
27
28 41 collection procedure.
29
30 42 4. Feed intake and weight gain increased in a linear manner in response to phytase dose, with
31
32 43 an average increase of approximately 11.7% and 13.5% respectively compared with chickens
33
34 44 fed on the low-P diet. Birds given diets with 2500 FTU diet weighed 6.6% more and had a
35
36 45 2.4% higher FCE than those fed on diets containing 500 FTU.
37
38 46 5. Enzyme supplementation increased the intake of AME and metabolisable N by 10.3% and
39
40 47 3.9%, respectively, principally through increases in feed intake. Birds given enzyme-
41
42 48 supplemented diets also improved their intake of metabolisable amino acids and P by
43
44 49 approximately 14% and 12.4% respectively, compared with birds fed on the control diet.
45
46 50 Enzyme supplementation did not affect ileal villus morphometry of the birds.
47
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51 INTRODUCTION

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

1
2
3 54 capable of forming insoluble complexes with divalent cations, starch and protein, reducing
4
5 55 their availability for poultry (Johnson and Tate, 1969). Poultry can produce some endogenous
6
7
8 56 phytase (Maenz and Classen, 1998; Applegate *et al.*, 2003) but this is insufficient for the
9
10 57 effective hydrolysis of dietary phytates. The detrimental effects of phytates in the diets of
11
12 58 poultry can be ameliorated by the addition of microbial phytases. Phytases (myo-inositol
13
14 59 hexaphosphate phosphohydrolases) are enzymes that can hydrolyse the ester bonds between
15
16 60 the phosphate groups and the inositol ring in phytates, increasing the dietary available P
17
18 61 (Nelson *et al.*, 1971; Irving and Cosgrove, 1974; Cowieson *et al.*, 2004a; Bryden *et al.*, 2006).
19
20 62 However, recent studies demonstrated that the benefits of using dietary phytases are not
21
22 63 restricted to the improvement of mineral retention but may improve performance and energy
23
24 64 and amino acid availability (Ravindran *et al.*, 1999a, 2001; Selle *et al.*, 2000; Rutherford *et*
25
26 65 *al.*, 2002; Newkirk and Classen, 2001; Cowieson *et al.*, 2006a, b; Pirgozliev *et al.*, 2005).

27
28 66 Further, it has recently been demonstrated (Cowieson *et al.*, 2004b; Pirgozliev *et al.*,
29
30 67 2005) that supplementation of diets with phytase significantly reduced the endogenous
31
32 68 secretions, measured as sialic acid (SA), from the gastrointestinal tract (GIT) of precision fed
33
34 69 birds. Sialic acid refers to a family of acidic monosaccharides found at the terminal ends of
35
36 70 sugar chains attached to cell surfaces and to soluble glycoproteins (Angata and Varki, 2002).
37
38 71 Increased concentrations of sialic acid are often associated with health problems such as
39
40 72 cellular senescence, bacterial infections (*e.g.* campylobacter), certain pathological conditions
41
42 73 and osmotic fragility. The most widely distributed sialic acid is N-acetylneuraminic acid,
43
44 74 which is believed to be a biosynthetic precursor of all other sialic acid molecules. Sialic acid
45
46 75 is associated with the gastrointestinal mucin (Montagne *et al.*, 2000) so SA excretion can be
47
48 76 used as an indicator of mucin losses from the gastrointestinal tract of experimental animals
49
50 77 (Larsen *et al.*, 1993). It has been hypothesised that dietary phytase results in improved
51
52 78 intestinal health with concomitant reductions in secretions from the GIT (Cowieson *et al.*,

79 2004b; Pirgozliev *et al.*, 2005). However, this is based on studies with precision fed birds and
80 it is not clear whether *ad libitum* fed birds will follow the same pattern of response.

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

85 MATERIALS AND METHODS

86 Diet formulation

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

Table 1 near here

95 Husbandry, dry matter digestibility (DMD), nitrogen metabolisability (NM) coefficients, 96 and apparent metabolisable energy (AME) determination

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

1
2
3 104 meet commercial recommendations and temperature maintained at 33°C at the beginning of
4
5
6 105 the study and reduced gradually to 20°C at 20 d of age. The light regime was 23 h light:1 h
7
8 106 dark.

9
10 107 During the 4th week of the experiment, between 21 and 28 d old, 64 birds were
11
12 108 selected, two from each floor pen, and placed in 32 metabolism cages (0.50 x 0.60 m floor
13
14 109 area, 2 birds in a cage) in a randomised block design. For the following 5 d birds were fed on
15
16 110 the respective treatment diets that they had been fed on previously. Excreta were collected
17
18 111 quantitatively for the last 2d of the feeding period, stored in a freezer at -20°C and later
19
20 112 freeze-dried. The feed intake for the same period was also measured. The gross energy of
21
22 113 each dried excreta sample and the experimental diets was determined using an adiabatic bomb
23
24 114 calorimeter (Parr Instrument Company, Moline, IL, USA). The AME of each of the
25
26 115 experimental diets (MJ/kg dry matter), was then calculated. The nitrogen in the excreta was
27
28 116 determined by method of Sweeney (1989) using a FP-200 nitrogen analyser (LECO®, St.
29
30 117 Joseph, MI, USA). Nitrogen metabolisability (NM) and dry matter digestibility (DMD)
31
32 118 coefficients of the diets were calculated. The metabolisability coefficients of nutrients are
33
34 119 defined as $([\text{nutrient intake} - \text{nutrient output}] / \text{nutrient intake})$. The average weight, intake and
35
36 120 FCE for birds that remained in each floor pen were also determined at 28d old.

37 121 **Amino acids in feed and excreta**

38
39 122 Amino acids in feed and excreta were determined by high performance liquid
40
41 123 chromatography (HPLC) (Jones *et al.*, 1981; Alltech, Associates, Carnforth, UK; Cowieson *et*
42
43 124 *al.*, 2006a). The HPLC system comprised by a Dionex ASI-100 autosampler fitted with a
44
45 125 Dionex P580 pump and a Dionex RF-2000 detector (Sunnyvale, CA). Primary amino acids
46
47 126 were derivatised with o-phthaldialdehyde prior to separation by HPLC using an
48
49 127 Adsorbosphere OPA-HR column (150 x 4.6 mm; Alltech Associates, Carnforth, Lancs, UK)
50
51 128 and fluometric detection. Since the method of hydrolysis destroys methionine, cystine and
52
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2
3 129 tryptophan, data on these amino acids are not reported. Excreta digestibility for glycine is not
4
5 130 presented because of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et*
6
7 131 *al.*, 1971). The metabolisable amino acid intake (g/bird/d) was obtained as the product of the
8
9 132 average total amino acid intake between 21 and 28d of age and their metabolisability
10
11 133 coefficient.

15 134 **Minerals in feed and excreta**

16
17 135 Minerals in the samples were determined by inductively coupled plasma emission
18
19 136 spectrometry, ICP (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer,
20
21 137 Beaconsfield, UK), (Tanner *et al.*, 2002). The metabolisability coefficient of each mineral was
22
23 138 calculated. The quantity of metabolisable mineral consumed (mg/bird/d) was obtained as the
24
25 139 average mineral intake between 21 and 28d of age was multiplied by their metabolisability
26
27 140 coefficient.

31 141 **Sialic acid determination**

32
33 142 The concentration of excreta sialic acid was determined by the periodate-resorcinol method as
34
35 143 described by Jourdian *et al.* (1971). The sialic acid excretion was reported as excreted per kg
36
37 144 metabolic body weight ($W^{0.75}$) and is assumed to give a measure of endogenous losses.

41 145 **Ileal villus morphometry**

42
43 146 On d 28, one bird from each of 8 pens of each treatment was killed by cervical dislocation.
44
45 147 Approximately 4 cm of the middle part of the ileum were sampled and stored in formal saline
46
47 148 histological fixative (Gurr ®, BDH, Poole, UK) until required for analyses. The samples were
48
49 149 embedded in paraffin wax, sectioned at approximately 5µm and 4 gut segments were fixed in
50
51 150 each slide. Morphometric measurements were determined on 20 villi for each slide (Stereo
52
53 151 Binocular Microscope, Olympus, Japan; CCD Camera & Monitor, JVC, Japan; Image
54
55 152 Analysis software Bioscan Optimas 3.01 for Windows). The length of the villus was defined
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3 153 as the distance from the crypt opening to the tip on the right side of the villus, as explained by
4
5 154 Sigleo *et al.* (1984). Villus thickness was measured at a point one-third from the villus tip.

8 155 **Statistical analysis**

9
10 156 Statistical analyses were performed using the Genstat VII statistical software package (IACR
11
12 157 Rothamstead, Hertfordshire, England). The comparison between treatments for performance
13
14 158 and other parameters was examined by analysis of variance (ANOVA). A regression analysis
15
16 159 was employed to test the relationship between performance, AME, enzyme activity and
17
18 160 excreted sialic acid with enzyme dosage. In all instances, differences were reported as
19
20 161 significant at $P \leq 0.05$.

24 162 **RESULTS**

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26
27 163 All animals were healthy and survived until the end of the study. It is clear (Table 2) that
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29 164 birds given enzyme supplemented diets grew faster ($P < 0.001$) and consumed more ($P < 0.05$)
30
31 165 than those fed the unsupplemented (NC) diet. Chickens fed on diets containing phytase
32
33 166 increased feed intake and weight gain on average by 11.7% and 13.5% respectively compared
34
35 167 to chickens fed the unsupplemented diet. The improvements ($P < 0.001$) obtained for the 500
36
37 168 FTU compared with the unsupplemented diet for these parameters were 14.5 and 14.1%,
38
39 169 respectively and the increases were even more pronounced (22 and 17% respectively;
40
41 170 $P < 0.001$) for the NC+2500 FTU. In addition, the highest level of enzyme supplementation,
42
43 171 (2500 FTU), improved FCE ($P < 0.05$) by approximately 3.9%. The linear response to enzyme
44
45 172 (feed intake, $r^2 = 0.29$; $P < 0.05$, weight gain $r^2 = 0.55$; $P < 0.001$, and FCE, $r^2 = 0.12$; $P < 0.05$.
46
47 173 Table 7) was exemplified by the fact that feeding 2500 FTU significantly improved the
48
49 174 weight gain (7.3%, $P < 0.05$) and FCE (4.3%, $P < 0.05$) compared to the 500 FTU dose. Table 2 near here

50
51 175 Phytase did not influence ($P > 0.05$) dry matter digestibility and nitrogen
52
53 176 metabolisability coefficients (Table 2). However phytase increased the retention of N as
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55 177 chickens receiving enzymes retained approximately 16.9% more N than those fed on the

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3 178 unsupplemented diet. Birds given 500 FTU retained 16% more N than those fed the
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5 179 unsupplemented diet while feeding enzyme supplemented diets tended ($P=0.055$) to increase
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7
8 180 dietary AME and gross energy metabolisability coefficient (AME:GE). The intake of AME
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10 181 was improved ($P<0.001$) by approximately 14% and 18.3% for birds fed on the diets
11
12 182 supplemented with 500 and 2500 FTU diets, respectively, compared to birds fed the
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14
15 183 unsupplemented diet, the majority of this effect being driven by intake rather than differences
16
17 184 in metabolisability. No significant changes ($P>0.005$) in excreta moisture were determined in
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19
20 185 this study (Table 2). High enzyme supplementation (2500 FTU) decreased ($P<0.05$) the
21
22 186 concentration of excreted sialic acid (SA). Although the effect was not significant ($P>0.05$),
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24 187 supplementation with 2500 FTU decreased daily excretion of SA by approximately 12%
25
26
27 188 compared to the NC diet. A weak negative linear relationship was detected between AME and
28
29 189 sialic acid excretion ($r^2=0.17$; $P<0.05$) (Table 7). Birds fed on the NC+500 FTU diet had
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31 190 thicker villi ($P<0.05$) compared to those fed the unsupplemented diet (Table 2). However, on
32
33
34 191 average enzyme supplementation did not significantly affect ileal villus morphometry.

35
36 192 Enzyme supplementation tended to improve metabolisability coefficients of histidine
37
38 193 ($P=0.082$) and tyrosine ($P=0.086$), by 5.6 and 3.4% respectively when comparison was made
39
40
41 194 between unsupplemented and 2500 FTU supplemented diet (Tables 3 & 4). The intake of
42
43 195 metabolisable amino acids was influenced by both metabolisability coefficients and feed
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45
46 196 intake of the birds (Table 4) but the majority of this effect was due largely to intake. Birds fed
47
48 197 enzyme-supplemented diets on average consumed 17.4 % and 16.8% more indispensable
49
50 198 ($P<0.05$) and dispensable ($P<0.05$) amino acids respectively than the control diet. Intake of
51
52
53 199 histidine, leucine, valine, serine and tyrosine increased by 19.0 %, 15.1 %, 15.3 %, 15.3 %
54
55 200 and 15.9 %, respectively ($P<0.05$). When comparison was made between the negative control
56
57
58 201 and birds fed 500 and 2500 FTU, the intake of indispensable metabolisable amino acids
59
60 202 increased by approximately 18% ($P<0.05$) and 22.6% ($P<0.05$), respectively. No differences

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3 203 were observed between the coefficient of amino acid metabolisability and metabolisable
4
5 204 amino acid intake when comparison was made between diets supplemented with 500 and
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7
8 205 2500 FTU. Tables 3,4,5 near here

9
10 206 Contrary to expectations, enzyme supplementation decreased the metabolisability
11
12 207 coefficients of Ca ($P<0.05$) and K ($P<0.001$) compared to the unsupplemented diet (Tables 4
13
14
15 208 & 5). In general the results were variable and supplementation with 500 FTU did not result in
16
17 209 pronounced changes in mineral metabolisability coefficients for P, Na, Mn and Zn, compared
18
19
20 210 to 2500 FTU supplemented diets. The intake of metabolisable P was enhanced (Table 6) in
21
22 211 birds fed enzyme supplemented diets ($P<0.05$). Their intake of metabolisable P increased by
23
24 212 approximately 14% compared to control and a similar trend was observed for S ($P=0.089$).
25
26
27 213 However, the intake of total metabolisable minerals, and thus electrolyte balance, was not
28
29 214 affected by dietary phytase. Tables 6 & 7 near here

215 DISCUSSION

216 The benefits in bird performance due to phytase supplementation to the diets is well
217 documented in the literature (Ravindran *et al.*, 1999a; Selle *et al.*, 2000; Rutherford *et al.*,
218 2002; Cowieson *et al.*, 2006a). In this study, the significant improvements on bird
219 performance by phytase supplementation of the diets can be largely explained by the
220 increased intake of energy and digestible nutrients. The improvements seen with phytase
221 supplementation, on the intake of DM, N, AME, metabolisable amino acids, and
222 metabolisable P, are functions of improved digestibility coefficients and greater feed intake,
223 the majority of the effect being due to intake. The improved performance, N and AME intakes
224 seen when birds were fed on diets containing 500 FTU, compared to the birds fed on the
225 unsupplemented diet suggest that supplementation at this level is practically valuable.
226 However, it should also be noted that in this study birds fed on diets with 2500 FTU gained
227 7% more and converted feed 4.4% more efficiently compared with birds receiving the diet

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2
3 228 containing 500 FTU. This suggests that improvements can be obtained at inclusion levels well
4
5 229 in excess of current commercial practice (approximately 500 FTU). This observation is
6
7
8 230 similar to data in previous reports with chickens (Cowieson *et al.*, 2006a) and turkeys
9
10 231 (Esteve-Garcia *et al.*, 2005; Ledoux *et al.*, 2005). Ledoux *et al.* (2005) reported that turkeys
11
12 232 given diets low in P but supplemented with 10000 FTU outperformed birds fed on the diet
13
14
15 233 adequate in P by 7 and 2.6% for gain and feed efficiency, respectively. Cowieson *et al.*
16
17 234 (2006a) found that chickens fed on low P diets supplemented with either 2400 FTU or 24000
18
19 235 FTU had 14% better feed efficiency compared to birds fed a diet with adequate supplies of P.
20
21
22 236 Similarly, a positive linear relationship existed between bird performance and phytase activity
23
24
25 237 in the present study (Table 7).

26
27 238 The lack of improvement in amino acid metabolisability coefficients and reduction in
28
29 239 sialic acid excretion when enzyme supplemented diets were fed could be due to the age of the
30
31 240 birds as well as masking by the influence of microbial activity in the gut. It is known that
32
33
34 241 intestinal microflora can change mucin production and amino acid metabolism (Sharma and
35
36 242 Schumacher, 1995; Ravindran *et al.*, 1999b). Whilst this study was designed to investigate the
37
38 243 effects of supplemental phytases, overall, on amino acid retention including the effects of the
39
40
41 244 microbiota within the gut it is recognised that hindgut microflora will influence amino acid
42
43 245 excretion from the birds. Ravindran *et al.* (1999b) demonstrated that amino acid metabolism
44
45
46 246 by microflora in the lower gut of chickens may be substantial and that amino acid
47
48 247 digestibilities measured in the terminal ileum are more accurate measures of amino acid
49
50
51 248 availability than those measured in the excreta. This may account for the lack of differences in
52
53 249 amino acid digestibilities found in this study. It is also known (Bedford, 2000; Acamovic,
54
55 250 2001) that the effects of dietary enzymes are more obvious in younger birds and this might
56
57
58 251 also account for the relatively poor effects on metabolisability coefficients. Nevertheless, the
59
60 252 benefit of the enzyme on intake was so large that it resulted in increased metabolisable amino

1
2
3 253 acid intake and this provides an indicative assessment of the effect of treatment on the
4
5 254 retention of amino acids including the effects on the microflora.
6
7

8 255 It should be noted that in the present study the superior effect of 2500 FTU compared
9
10 256 to 500 FTU was observed only with respect to weight gain and FCE. The lack of effect of
11
12 257 phytase on energy, amino acids or mineral metabolisability coefficients is in accord with other
13
14 258 published data (Cowieson *et al.*, 2006a). It was reported that lower enzyme activities, (150-
15
16 259 300 FTU/kg), were sufficient to improve AME and amino acid digestibility coefficients, with
17
18 260 higher doses having relatively little additional effect (Cowieson *et al.*, 2006a). The same
19
20 261 authors found that higher dietary phytase supplementation improved P retention, mediated
21
22 262 through an improvement in the digestibility of phytate-bound P. In this study, enzyme
23
24 263 supplementation increased the intake of metabolisable P compared to the control diet,
25
26 264 however no difference existed between diets supplemented with 500 and 2500 FTU. The
27
28 265 improved gain seen in birds fed the diet containing 2500 FTU compared with that seen for the
29
30 266 birds fed the diets containing 500FTU cannot be explained by any of the digestibility indices
31
32 267 measured and suggests that growth was influenced by some other factor associated with
33
34 268 phytase treatment. The present results may suggest that the improvements in bird
35
36 269 performance with incremental doses of phytase are the result of an improved use of dietary
37
38 270 nutrients for production rather than maintenance, which is consistent with the differences in
39
40 271 size of the birds on the various treatments.
41
42
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48 272 The theory of enzymatic breakdown of phytate compounds distinguishes between
49
50 273 liberation of phytate molecules from complexes with other tissue components and enzymatic
51
52 274 cleavage of phosphate residues on the *myo*-inositol ring (Zyla *et al.*, 2004). The stepwise
53
54 275 manner of dephosphorylation of IP6 (Venekamp *et al.*, 1995; Greiner *et al.*, 2000) will lead to
55
56 276 a release of different *myo*-inositol isomers and phosphates. The conditions and the limited
57
58 277 time spent in the GIT of poultry are not ideal for phytase to function effectively, so it is
59
60

1
2
3 278 unlikely that exogenous phytase will be able to completely dephosphorylate dietary phytates,
4
5
6 279 particularly when used at low activities. However, with increased phytase use there are likely
7
8 280 to be increased amounts of the *myo*-inositol isomers of lower molecular masses which may be
9
10 281 considered as potential growth promoters. Waagbo *et al.* (1998) reported that feeding fish
11
12 282 with inositol-supplemented diets improved their performance while Zyla *et al.* (2004) also
13
14
15 283 found that addition of *myo*-inositol to P deficient diets improved the weight gain of broiler
16
17 284 chickens. These results suggest that *myo*-inositol by itself acts as a growth promoter. The
18
19 285 biological importance of the inositol phosphates is well documented (Irvine and Schell, 2001;
20
21 286 Beemster *et al.*, 2002; Fisher *et al.*, 2002), however there is a lack of information about the
22
23 287 effect of inositol on nutrient availability and performance when fed to poultry. The
24
25 288 performance results from this study suggest that increased phytase activity increased the
26
27 289 dephosphorylation of IP6, increasing the concentration of *myo*-inositol isomers of lower
28
29 290 relative molecular mass (RMM). The linear relationship between dietary enzyme activity and
30
31 291 weight gain, as birds fed 2500 FTU increased their weight gain by 22%, 11% and 7% relative
32
33 292 to control, NC+250 and NC+500FTU, respectively, may be due to the reduced adverse effects
34
35 293 of phytate but also to beneficial effects if inositol esters of lower RMM.

36
37 294 No relationship between dietary AME and performance was found in the present
38
39 295 experiment. It is not unusual that AME values do not predict adequately the nutritive quality
40
41 296 of poultry feeds. Rose and Bedford (1995) and Ravindran *et al.* (1999a) did not observe a
42
43 297 correlation between dietary AME and performance when broilers were fed untreated or
44
45 298 enzyme supplemented wheat-based diets. The poor relationship between bird performance
46
47 299 and dietary AME in this study supports previous finding that, although convenient, the
48
49 300 measurement of metabolisable energy is not sufficiently sensitive to evaluate the nutritive
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51 301 value of poultry feed (Emmans, 1994; Pirgozliev and Rose, 1999). This is likely to be because
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53 302 AME does not account for the partitioning of energy between maintenance and growth (De
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3 303 Groote, 1974; Hoffmann and Shiemann, 1980). Thus the improved performance of the birds
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5 304 fed phytase suggest that these birds can utilise more energy and protein from the feed,
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8 305 however this cannot be detected by dietary determining AME and suggests that intermediary
9
10 306 metabolism is influenced by phytases thereby increasing dietary available (net) energy.

11
12 307 The results for ileal villus morphometry were in the expected range and similar to
13
14 308 those measured in other growing broiler experiments (Langhout *et al.*, 1999; Pirgozliev *et al.*,
15
16 309 2001). However Koutsos *et al.* (2005) reported that dietary phytase increased the duodenal
17
18 310 villus width when fed to laying hens. Perhaps the phytase influences the type of microflora
19
20 311 within the GIT (Steer and Gibson, 2002) and thus influences concomitant adherence and any
21
22 312 damage to the GIT and intestinal villi.

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26 313 The negative relationship between dietary AME and excreted sialic acid per kg
27
28 314 metabolic body weight was in accord with previous results suggesting that the health of the
29
30 315 gastrointestinal tract may be enhanced by the presence of phytase (Cowieson *et al.*, 2004b;
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32 316 Pirgozliev *et al.*, 2005). Sialic acid is involved in many physiological functions (Kongtawelert
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34 317 *et al.*, 2003; Browning *et al.*, 2004; Nayak and Bhaktha, 2005). Sialic acids are also involved
35
36 318 in the binding of pathogenic and other microflora to mucin (Ryan *et al.*, 2001; Deplanske *et*
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38 319 *al.*, 2002; Edelman *et al.*, 2002; Edelman *et al.*, 2003; Kettunen *et al.*, 2003), thus SA
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40 320 excretion may provide valuable information about the health of the gut. The epithelium of the
41
42 321 GIT is covered by protective mucus gel composed predominantly of mucin glycoproteins that
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44 322 are synthesised and secreted by goblet cells. This mucus layer acts as a medium for
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46 323 protection, lubrication, and transport between the intestinal content and the epithelial cells.
47
48 324 However, the presence of nutrients with low digestibility in the gut might increase the number
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50 325 of bacteria in the lumen. Some of those bacteria may be bind to the mucus layer and later be
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52 326 excreted as a defence reaction of the gut. Thus changes in nutrient supply, *e.g.* phytase
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54 327 supplementation, might change nutrient utilisation and affect mucin dynamics. Smirnov *et al.*

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3 328 (2004) also found that starvation changed mucin dynamics and decreased thickness of the
4
5 329 mucous layer of young chickens, suggesting that this may affect intestinal digestive function
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7
8 330 and defence.

9
10 331 The impaired performance, N retention and AME intake in birds fed the NC diet
11
12 332 compared to those fed phytase-supplemented diets may be due to a reduction in the release of
13
14 333 available nutrients which may lead to an unbalanced nutrient supply in the intestinal lumen. It
15
16 334 has also been demonstrated that phytic acid, a major component of dietary phytate, irritates
17
18 335 the GIT and increases the excretion of endogenous amino acids, minerals and sialic acid
19
20 336 (Cowieson *et al.*, 2004b). It can be expected that a combination of irritation and unbalanced
21
22 337 supply of nutrients compromised the health of the gut and may explain the negative
23
24 338 relationship between the AME and excreted mucin in this study. The thickness of the adherent
25
26 339 GIT mucus layer is the result of the balance between the rate of secretion of mucin and its
27
28 340 degradation through enzymatic digestion and mechanical shear (Allen, 1989). More mucin
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30 341 excretion will lead to greater mucin synthesis, which is biologically very expensive and
31
32 342 demands more energy (Nyachoti *et al.*, 1997). However, if there are not enough nutrients to
33
34 343 support the demand for increased synthesis, then the thickness of this barrier may decrease
35
36 344 and lead to increased exposure of the epithelium to harmful agents in the lumen, e.g.
37
38 345 microflora, phytate etc. The exposure of non-protected host intestinal epithelial cells to
39
40 346 pathogenic organisms may cause an inflammation, provoking immune response and increased
41
42 347 numbers of cytokines (McKay and Baird, 1999). The activation of the immune system of the
43
44 348 bird, e.g. increased production of antibodies in response to the invading organism, is an
45
46 349 energy demanding process (Klasing and Calvert, 1999; Eraud *et al.*, 2005). It has also been
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48 350 suggested that a strong immune response will increase the risk of tissue damage (Svenson *et*
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50 351 *al.*, 1998), increased production of free radicals (Finkel and Holbrook, 2000) and further
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52 352 deleterious effects on the organism. Karadas *et al.* (2005, 2006) reported that broilers fed

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3 353 phytase-supplemented diets had reduced hepatic oxidative stress and increased antioxidant
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5 354 status compared to birds fed non-supplemented, low in P diets. Although, the mechanism is
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7
8 355 not clear, it suggests that dietary phytases are capable of moderating immune response and
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10 356 free radical production in broiler chickens.

11
12 357 It can be concluded that the exogenous phytase used in this study is effective in
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14 358 improving the nutritional value of maize-soy-based diets for young broiler chickens fed on a
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16 359 diet that is formulated to be sub-optimal in terms of available phosphorus. Although diets
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18 360 supplemented with 500 FTU improved bird performance, it was found that chickens can
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20 361 respond to phytase activity much higher than used in the commercial practice. The negative
21
22 362 relationship between dietary AME and sialic acid excretion supports the hypothesis that
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24 363 dietary phytase may influence gut health. However, to understand the mode of action of
25
26 364 dietary phytases further research on *myo*-inositol isomers of lower RMM, immune response
27
28 365 and antioxidant status needs to be completed.

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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 + cystine 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	
<u>Determined mineral and amino acid composition of grower diet (DM)²</u>			
<u>Amino acids (g/kg)</u>		<u>Minerals (g/kg)</u>	
Alanine	12.44	Calcium	14.82
Arginine	12.70	Potassium	12.61
Aspartic acid	26.30	Magnesium	2.17
Glutamic acid	51.99	Manganese	0.135
Histidine	4.67	Sodium	2.20
Isoleucine	12.26	Phosphorus	6.81
Leucine	23.19	Sulphur	4.18
Lysine	13.80	Zinc	0.13
Phenylalanine	12.03		
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, pantothenic 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

¹Negative control – diet low in available P.

²Phytase activity (units/kg) in diet.

³Least significant difference.

⁴Feed conversion efficiency.

⁵Daily 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

¹Negative control – diet low in available P;

²Phytase activity (units/kg) in diet;

³Least 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
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

¹Negative control – diet low in available P.

²Phytase activity (units/kg) in diet.

³Least 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

¹Negative control – diet low in available P.

²Phytase activity (units/kg) in diet.

³Least 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

¹Negative control – diet low in available P.

²Phytase activity (units/kg) in diet.

³Least 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.