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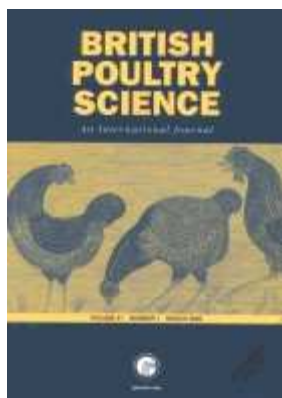
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The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens

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| | |

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3 1 **The effects of supplementary bacterial phytase on dietary energy and total tract amino**
4 2 **acid digestibility when fed to young chickens**
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Running title: Phytase and nutrient digestibility

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2
3 44 **Abstract** 1. Four diets were offered to broiler chickens from 7 to 17 d of age; these included a
4
5 45 phosphorus-adequate positive control (PC) (4.7 g/kg available P), a sub-optimal P negative
6
7 46 control (NC, 2.5 g/kg available P) with (500 and 12500 FTU/kg) and without phytase. Dietary
8
9 47 apparent metabolisable energy (AME), dietary net energy for production (NEp), the
10
11 48 efficiency of AME retention (Kre), heat production and total tract amino acid digestibility
12
13 49 coefficients were determined. The determination of NEp involved a comparative slaughter
14
15 50 technique in which growing chickens were fed the experimental diets *ad libitum*.

16
17
18 51 2. Feed intake, weight gain and feed conversion efficiency increased significantly in a dose
19
20 52 dependent manner in response to dietary phytase activity. Overall, the NEp of the phytase
21
22 53 supplemented diets significantly improved by approximately 15.6% compared with the
23
24 54 negative control, while dietary AME was unaffected. Although phytase did not affect AME,
25
26 55 the large increase in the NEp demonstrated that dietary phytases improves energy utilisation,
27
28 56 *i.e.* diverting more energy, not accounted for in the AME procedure, for production. This is
29
30 57 largely a result of the stimulatory effect that phytase has on feed intake rather than on
31
32 58 digestibility of the diet.

33
34
35 59 3. Overall, the diet supplemented with 12500 FTU had 6.4% significant improvement in total
36
37 60 tract digestibility coefficients of the total amino acids compared with the negative control.
38
39 61 With regard to individual amino acids, the impact of phytase was far more pronounced for
40
41 62 threonine, an important component of the gastrointestinal mucin, than for other amino acids.
42
43 63 4. Dietary NEp was more highly correlated with performance criteria than dietary AME and
44
45 64 seems to be a more sensitive way to evaluate broiler response to phytase supplementation.
46
47

52 65 INTRODUCTION

53
54 66 Over two-thirds of P in cereals and legumes, the main ingredients of poultry diets, are
55
56 67 contained in chemical structures called phytic acid or its salts, collectively known as phytates
57
58 68 (NRC, 1994). Phytate P is not available for poultry unless at least one of the phosphate groups
59
60

1
2
3 69 is removed from the inositol molecule, by intrinsic feed phytase, intestinal phytase or
4
5 70 microbial phytase (Nelson *et al.*, 1971). However, intestinal hydrolysis of dietary phytate in
6
7
8 71 poultry is mainly achieved through use of exogenous microbial phytase, since intrinsic plant
9
10 72 phytases are relatively ineffective and heat labile and intestinal phytase activity is relatively
11
12 73 low (Maenz and Classen, 1998; Applegate *et al.*, 2003). Microbial phytases used in poultry
14
15 74 diets have usually resulted in improvements in growth performance and flock uniformity, and
16
17 75 reduced waste output to the environment (Selle and Ravindran, 2007). While the
18
19 76 improvement in performance is closely associated with destruction of dietary phytate and an
20
21 77 improvement in digestion and absorption of minerals and amino acids, the influence of
22
23 78 phytase on dietary apparent metabolisable energy (AME) has been not consistent (Selle and
24
25 79 Ravindran, 2007; Cowieson *et al.*, 2009). Whereas some authors found an increase in dietary
26
27 80 AME in response to phytase (Ravindran *et al.*, 1999b; Shirley and Edwards, 2003; Cowieson
28
29 81 *et al.*, 2006), others (Tejedor *et al.*, 2001; Pirgozliev *et al.*, 2008) either did not see an
30
31 82 improvement in AME or the response tended to be species dependent (Pirgozliev *et al.*,
32
33 83 2007). Net energy is a more meaningful measure of energy utilisation with regard to
34
35 84 prediction of the nutritive value of poultry diets (De Groote, 1974; Pirgozliev and Rose, 1999;
36
37 85 MacLeod, 2002). Research by Pirgozliev *et al.* (2009) has demonstrated that exposure to
38
39 86 dietary phytase reduces the loss of endogenous energy from bird's GIT suggesting that
40
41 87 phytase supplementation may reduce the energetic costs of growth relative to an
42
43 88 unsupplemented control diet. However, to date, no data has been published on the effect of
44
45 89 supplemental microbial phytase on dietary net energy.

52
53 90 Thus the objective of the present study was to quantify the differences and inter-
54
55 91 relationships in dietary net energy resulting from feeding chickens diets containing various
56
57 92 dosages of phytase. Growth, energy and amino acid metabolism were also determined.

59
60 93

94 MATERIAL AND METHODS

95 **Diet formulation**

96 An *Escherichia coli*-derived phytase (Quantum™ EC 3.1.3.26; AB Vista Feed Ingredients,
97 Wiltshire, UK) was used in this experiment. Four experimental diets were prepared, and all
98 were formulated to be adequate in protein and energy, however, the negative control (NC)
99 was lower in non-phytate-P compared to the (PC) positive control (2.5 vs. 4.7 g/kg diet; Table
100 1). The remaining two diets were the negative control supplemented with phytase (500 and
101 12500 FTU respectively; *i.e.* NC+500; NC+12500). The enzyme was added to the diets in
102 powder form and all diets were fed as a mash.

Table 1 near here

103 **Husbandry and apparent metabolisable energy determination**

104 Ross 308 female chickens were obtained from a commercial hatchery (Grampian Ltd,
105 Whitburn, UK) at 1-d-old and were placed in a single floor pen at 32°C. All birds were given
106 a proprietary chicken starter feed during the 6-d pre-study period. On the first day of the
107 experiment (7 d of age), the chicks were individually weighed and the heaviest and lightest
108 birds discarded, leaving 48 birds which were sorted, according to weight, into 6 blocks of 8
109 birds (4 diets and 2 birds per cage), in a randomized block design. Each wire-mesh metabolic
110 cage (0.35 x 0.35 m floor area) was arranged in 4 tiers (tier being the blocking factor) within a
111 controlled environment room. All the cages were equipped with metal feeders and nipple and
112 cup drinkers. Excreta samples were collected in trays under each cage. The room temperature
113 was 28°C at 7d and gradually reduced to 21°C at the end of 10-d feeding period. The light
114 regime was 23 h light and 1 h dark, and the relative humidity was maintained between 50 and
115 70%. Access to feed and water was *ad libitum*. The experiment ended when the birds were 17
116 d of age. The experiment was approved by the Scottish Agricultural College Animal
117 Experimental Committee.

1
2
3 118 All excreta were collected for the last 2 d of the feeding period, stored in a freezer at -
4
5 119 20°C and later freeze-dried and ground. The gross energy of each dried excreta sample and
6
7 120 the experimental diets was determined using an adiabatic bomb calorimeter (Parr Instrument
8
9 121 Company, Moline, IL, 61265, USA). To determine dietary AME of the experimental diets
10
11 122 (MJ/kg DM), a total collection technique was employed for the last 2 d of the study. The
12
13 123 nitrogen in feed and excreta was determined by the method of Sweeney (1989) using a FP-
14
15 124 200 nitrogen analyser (LECO[®], St. Joseph, MI, USA). The coefficients of dry matter
16
17 125 digestibility (DMD) were determined as the difference between intake and excretion of DM,
18
19 126 divided by their respective intake.

127 **Carcase energy retention, heat production and dietary net energy determination**

128 A comparative slaughter technique was applied to determine retention of nutrients. Four birds
129 from the general group (birds from the same batch that were reared in the pre-study period
130 only) were selected at random, killed by cervical dislocation at the beginning of the
131 experiment, and stored in a freezer at -20°C for analysis. At the end of the experimental
132 period, feed was removed for 2 h and the remaining birds weighed and killed by cervical
133 dislocation. The carcasses of the birds, including intestines and feather, from each cage were
134 frozen and minced (Hobart A 200, The Hobart Mfg Co Ltd, London, UK). The minced
135 carcasses of the birds of each pen were pooled, thoroughly mixed and sampled. The carcass
136 samples were freeze-dried, and gross energy (adiabatic bomb calorimeter) and crude protein
137 (N x 6.25) determined. The same procedure was applied to the carcasses of 4 birds taken at the
138 start of the experiment and the data was used to determine the gross energy and protein
139 retention for the experimental period.

140 **Carcass energy retention**

141 The total energy retained in the carcasses (RE_c) was calculated as follows:

$$142 \quad RE_c \text{ (MJ)} = E_{17} - E_7$$

1
2
3 143 where, E_{17} = the total gross energy (MJ) of chicken carcasses at 17 d old, and E_7 = the total
4
5 144 gross energy (MJ) of chicken carcasses at the beginning of the experiment at 7 d.

6
7
8 145 Dietary net energy for production (NE_p MJ/kg DM feed intake) was calculated using the
9
10 146 following equation:

$$NE_p \text{ (MJ kg diet)} = RE_c/FI$$

11
12
13 147
14
15 148 where, FI = dry matter (kg) consumed from 7 to 17 d.

16
17 149 The efficiency of AME used for energy retention (K_{re}) was calculated as the RE_c divided by
18
19 150 the AME intake.

$$K_{re} = RE_c/AME \text{ intake}$$

20
21
22 151
23
24 152 where, AME intake = the feed intake (kg DM) for the experimental period multiplied by
25
26 153 determined metabolisable energy (MJ/kg DM) of the diets.

27
28
29 154 The total carcass protein retention (g/bird) was obtained similarly to RE_c as follows:

$$CPr = (N_{17} - N_7) \times 6.25$$

30
31 155
32
33
34 156 where, N_{17} = the N (g) in chicken carcasses at 17 d, N_7 = the N (g) in chicken carcasses at the
35
36 157 beginning of the experiment at 7 d, and 6.25 is the coefficient used to calculate the protein
37
38 158 retained in the body.

39
40
41 159 The value of the carcass GE retained as protein was obtained:

$$RE_p = CPr \times 23.6$$

42
43 160
44
45
46 161 where, CPr (kg) was multiplied by 23.6 MJ according to Okumura and Mori (1979). It was
47
48 162 assumed that the difference between RE_c and RE_p yields the value for the GE retained as
49
50 163 body fat (RE_f). The amount of retained carcass fat was calculated as the RE_f retained as body
51
52 164 fat divided by the value of 39.12 MJ/kg (Okumura and Mori, 1979).

53
54
55 165 The efficiency of AME used for protein (K_{rep}) and fat retention (K_{ref}) were calculated as
56
57 166 follow:

$$K_{rep} = RE_p/AME \text{ intake}$$

1
2
3 168 $K_{ref} = RE_f/AME \text{ intake}$
4

5
6 169 **Heat production**
7

8 170 The total heat production of the birds from 7 to 17 d (HPt), which consists of the energy for
9
10 171 tissue retention, maintenance and the heat increment of production, was calculated as
11
12 172 difference between dietary AME intake and REc:
13

14
15 173 $HP_t \text{ (MJ)} = AME \text{ intake} - RE_c$
16

17 174 The heat production (HPf MJ/kg DM feed intake) per kg feed intake was also calculated:
18

19
20 175 $HP_f \text{ (MJ/kg DM feed intake)} = HP_t/FI$
21

22 176 where, HP_t = the total heat production of the birds from 7 to 17 d (MJ) and FI = dry matter
23
24 177 (kg) consumed.
25

26
27 178 The NEp/HPf ratio was used as criteria for the use of heat production for body energy
28
29 179 retention, assuming that higher ratio indicates that most of the released heat was used for
30
31 180 carcass energy retention and maintenance, and not for heat increment.
32

33
34 181 **Amino acids in feed and excreta**
35

36 182 Amino acids in feed and excreta were determined by high performance liquid
37
38 183 chromatography with fluorimetric detection of the orthophthaldehyde derivatives (Jones *et al.*,
39
40 184 1981). Since this method of hydrolysis destroys methionine, cystine and tryptophan, data on
41
42 185 these amino acids are not reported. Excreta digestibility for glycine is not presented because
43
44 186 of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et al.*, 1971).
45
46

47
48 187 The total tract digestibility coefficient of each amino acid was calculated as the
49
50 188 difference between amino acid intake and the amino acid excreted divided by the amino acid
51
52 189 intake. The digestible amino acids intake (g/bird/d) was obtained as the product of amino acid
53
54 190 intake and their total tract digestibility coefficient.
55
56

57
58 191 **Statistical analyses**
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60

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3 192 Statistical analyses were performed with *Genstat 11th Edition* statistical software package
4
5 193 (Lawes Agricultural Trust, 2008). The performance results, dietary energy, amino acids and
6
7 194 other variates were compared statistically by ANOVA. The relationship between results on
8
9
10 195 growth performance, dietary energy and amino acids responses were determined by
11
12 196 correlation. In all instances, differences were reported as significant at $P < 0.05$ and trends
13
14
15 197 were noted at $P > 0.05 < 0.10$.

17 198 RESULTS

19 199 **Performance and energy metabolism**

20 200 All birds remained healthy throughout the study period. Feeding enzyme supplemented diets
21
22 201 significantly increased dry matter intake by approximately 17%, weight gain by 27% and FCE
23
24 202 by approximately 7.6% compared with birds fed the negative control diet (Table 2). The
25
26
27 203 improvement was more noticeable for the birds fed on the diet containing 12500 FTU,
28
29 204 suggesting a dose relationship existed. Birds given 12500 FTU, also tended ($P = 0.066$) to
30
31 205 metabolise energy more effectively (*i.e.* a higher AME:GE ratio) than the birds fed the other
32
33 206 diets (Table 2). This in part was reflected by the higher ($P < 0.05$) dry matter digestibility
34
35 207 coefficient (DMD) for the 12500 FTU/kg diet in comparison with other diets (Table 2).
36
37 208 Metabolisability coefficients for N were not affected ($P > 0.05$) by the diets. Birds fed on PC
38
39 209 and NC+12500 FTU supplemented diets consumed approximately 26.1 and 20.3 %,
40
41 210 respectively, more ($P < 0.05$) AME than those fed the NC, which was more a reflection in
42
43 211 differences in intake than metabolisability, although no differences ($P > 0.05$) existed between
44
45 212 the NC and NC+500 FTU.

Tables 2 & 3 near here

52 213 There was no difference in the GE/kg of the carcass tissue from birds fed different
53
54 214 diets (Table 3) and the GE values were similar to previous data (Pirgozliev *et al.*, 2001). The
55
56 215 difference in total carcass energy retained (RE_c), however, was a result of differences in body
57
58 216 weight between birds fed different diets, with the NC being significantly lower than PC,
59
60

1
2
3 217 NC+500 FTU or NC+12500 FTU. Energy retained as carcass protein or fat followed a similar
4
5 218 path, with NC being lower for protein ($P < 0.001$) and fat ($P < 0.05$) than for the remaining
6
7
8 219 diets.

9
10 220 The values for Kre, Krep and Kref, were significantly lower for the NC diet, relative
11
12 221 to the other diets, although there was no significant difference between the PC and phytase
13
14 222 supplemented diets (Table 3). As expected, the supplementation of phytase to the NC
15
16 223 significantly improved the determined value of NEp. The NEp values were in the expected
17
18 224 range (Pirgozliev *et al.*, 2001; McCracken and Clements, 2001) and the NEp of the NC+500
19
20 225 FTU, PC and NC+12500 FTU were 11.3%, 15.6% and 19.9%, respectively, significantly
21
22 226 higher than the NC.

23
24
25
26
27 227 No significant difference existed in terms of the HPt values. However, when presented
28
29 228 on a per kg of DM intake basis, *i.e.* HPf of birds fed, the NC was 0.7 MJ significantly greater
30
31 229 than birds fed the other three diets (Table 3). The HPf values from the birds fed the PC and
32
33 230 phytase supplemented diets were almost identical. Chicks fed the NC lost 26.4%, 22.2% and
34
35 231 31.8% significantly more energy per kg feed intake to the surroundings than birds fed the PC,
36
37 232 NC+500 FTU or NC+12500 FTU respectively

Tables 4 & 5 near here

233 **Total tract amino acid digestibility**

234 The effects of dietary treatments on total tract amino acid digestibility coefficients and
235 digestible amino acid intake are presented in Tables 4 and 5. The total tract digestibility
236 coefficients of most amino acids were significantly reduced by feeding the NC, and only
237 when the highest dosage of phytase was offered were the coefficients similar to the PC.
238 Digestible amino acid intake tended to follow the same pattern, although due to the beneficial
239 effect of the lower dose of phytase on intake, equilibration with the PC was achieved at 500
240 rather than 12500 FTU, as observed for the digestibility coefficients alone. Overall, the diet
241 supplemented with 12500 FTU improved the digestibility coefficient of the indispensable and

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2
3 242 total amino compared to the negative control and even the 500 FTU supplemented diets. A
4
5 243 similar trend ($P = 0.091$) was observed for the dispensable amino acid digestibility
6
7 244 coefficient.

Table 6 near here

9
10 245 Table 6 shows the correlation coefficients among the various experimental results.
11
12 246 Weight gain was more highly correlated with NEp ($r = 0.854$; $P < 0.001$) than with AME ($r =$
13
14 247 0.577 ; $P < 0.05$). FCE was correlated with the NEp ($r = 0.546$; $P < 0.05$) although there was no
15
16 248 significant correlation with the dietary AME. Weight gain was also more highly correlated
17
18 249 with the digestibility of dietary indispensable amino acids ($r = 0.626$; $P < 0.001$) compared
19
20 250 with the digestibility of dispensable ($r = 0.590$; $P < 0.05$). In addition, the correlation
21
22 251 coefficient between weight gain and the digestibility of dietary threonine was relatively higher
23
24 252 ($r = 0.615$; $P < 0.001$) than the digestibility coefficient of dietary lysine ($r = 0.550$; $P < 0.05$).

253 DISCUSSION

254 The beneficial effect of phytase supplementation of low P diets is well documented (Selle and
255
256 Ravindran, 2007; Cowieson *et al.*, 2009). In this study, birds given 12500 FTU were 23%
257
258 heavier and converted feed 37% more efficiently than birds fed the NC. Similar to previous
259
260 research with the same phytase (Cowieson *et al.*, 2006; Pirgozliev *et al.*, 2007, 2008), birds
261
262 fed on diets with high phytase activity, *i.e.* over 2000 FTU, tended to outperform those fed the
263
264 PC, provided the P reduction employed in the NC was not overly ambitious.

265 The improvement in growth performance with phytase supplementation is mirrored
266
267 somewhat by the increase in dietary energy and amino acid availability and intake. Phytase-
268
269 driven improvements in carcass protein and fat deposition is in agreement with Boekholt *et*
270
271 *al.* (1994) who found that, when protein is not limiting in the diets of broilers, extra energy
272
273 available in the diet is used for both fat and protein retention. However, the effect of treatment
274
275 on AME and total tract digestibility amino acids was relatively minor compared with the

1
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3 266 responses achieved in gain and FCR, suggesting that phytase addition influences more than
4
5 267 nutrient digestibility.
6
7

8 268 The efficiency of dietary AME energy use for tissue energy retention was slightly
9
10 269 higher compared with the previous findings of Olukosi *et al.* (2008), who observed the Kre
11
12 270 values of 0.377 to 0.387 with growing broiler chickens fed phytase or a cocktail of xylanase,
13
14 271 amylase and protease from 0 to 21 d age. The same authors also reported that the Krep was
15
16 272 higher than for carcass fat deposition, whereas the reverse was seen in this study. However,
17
18 273 the use of different diet formulations, exogenous enzyme supplementation, different strains
19
20 274 and flocks of birds, and feeding regimes may be responsible for these differences. The large
21
22 275 improvement in Kre due to phytase supplementation is in accord with the higher NEp values,
23
24 276 which is not particularly well correlated with effects on dietary AME. The literature suggests
25
26 277 that although phytase supplementation does not always improve dietary metabolisable energy,
27
28 278 it usually improves intake and gain (Ravindran *et al.*, 1999a; Pirgozliev *et al.*, 2007, 2008) the
29
30 279 former being an implicit part of AME intake, which was well correlated with NEp. With
31
32 280 increased AME intake, birds will have consumed more energy per d per unit of body weight,
33
34 281 resulting in proportionately more of total intake being available for production than for
35
36 282 maintenance. The close relationship between AME intake and Kre suggests that this
37
38 283 hypothesis may have validity. Thus, feeding phytase may improve energy retention as much,
39
40 284 if not more, through increasing total energy intake, thereby increasing the supply of energy in
41
42 285 excess of maintenance, as through increasing the efficiency of energy metabolisability. As a
43
44 286 consequence, there was a better relationship between dietary NEp rather than AME with bird
45
46 287 weight gain and FCR, indicating that dietary NEp is a more predictive measure of animal
47
48 288 performance.
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53 289 Although birds fed the NC retained less total carcass energy, they released more heat
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55 290 per kg feed intake compared with all other diets. It is known that animals use energy to
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58
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2
3 291 maintain not only muscle, fat and bone tissues, but also respiratory tissues, liver and the
4
5 292 gastro-intestinal tract, all of which can be energetically very demanding (Spratt *et al.*, 1990).
6
7
8 293 It has been demonstrated by Spratt *et al.* (1990) that, despite the fact that the liver and the gut
9
10 294 account for approximately only 3% of the body weight of a hen, they may contribute up to
11
12 295 26% of the total heat production, suggesting that a relatively small reduction in the GIT size
13
14
15 296 could account for a significant saving in maintenance energy. Wu *et al.* (2004) reported a
16
17 297 reduction of the weight and the relative proportion of the gastrointestinal tract and other
18
19 298 related organs when phytase was fed to broilers. This suggests that birds fed phytase may
20
21 299 have a lower heat increment, thereby allowing them to divert relatively more energy towards
22
23
24 300 growth rather than maintenance.

25
26
27 301 The fact that the cost of feed accounts for approximately 70% of the cost in poultry
28
29 302 production (Cooke, 1987), and with increasing and volatile feed ingredient prices, the lack of
30
31 303 a relationship between dietary metabolisable energy and growth rate or efficiency emphasises
32
33 304 the need for research to improve the prediction of feeding quality of poultry diets. Whilst the
34
35 305 net energy system is clearly better in this regard, it is subject to relatively high variability and
36
37 306 the values are strongly influenced by diet composition (De Groote, 1974; Pirgozliev and
38
39 307 Rose, 1999; McCracken and Clements, 2001; MacLeod, 2002). Given that feed intake plays
40
41 308 such a large role in describing the energy balance of an animal, as demonstrated in this work,
42
43 309 any assay employing a restricted feeding technique (Priyankarage *et al.*, 2008) to measure net
44
45 310 energy will likely not reflect the full value of a phytase treated feed when utilised
46
47
48 311 commercially.

49
50
51
52 312 The impact of phytase was more pronounced for total tract digestibility of threonine
53
54 313 than all other indispensable amino acids, which is in agreement with previous work by
55
56 314 Ravindran *et al.* (1999a) and Rutherford *et al.* (2002), based on ileal, and Cowieson *et al.*
57
58 315 (2006), based on excreta measurements. Threonine comprises a significant amount of the

1
2
3 316 amino acid composition of mucin, and over half of the absorbed dietary threonine is used by
4
5 317 the enterocytes (Neutra and Forstner, 1987). In clinical situations associated with increased
6
7 318 endogenous excretions from the gastrointestinal tract, *e.g.* relatively high dietary phytate,
8
9 319 threonine availability may limit intestinal mucin synthesis and consequently reduce gut
10
11 320 barrier function. Alternatively, increased mucin secretion will increase threonine losses and
12
13 321 hence reduce its metabolisability. The improvement in threonine metabolisability on feeding
14
15 322 high levels of phytase is probably a result of reduced mucin synthesis and subsequent mucin
16
17 323 loss. However, that lack of effect of exogenous phytase on the total tract digestibility of
18
19 324 dietary serine, another amino acid involved in the mucin composition, may be confounded by
20
21 325 the use of excreta instead of ileal samples for amino acid determination. Ravindran *et al.*
22
23 326 (1999a) demonstrated that amino acid metabolism by microflora in the lower gut of chickens
24
25 327 may be substantial and that amino acid digestibility measured in the terminal ileum would
26
27 328 differ from that measured in the excreta.
28
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34 329 In summary, the present results indicate that dietary phytase improved the nutritional
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36 330 value of maize/soy-based diets that were low in P when fed to young chickens. Whilst the use
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38 331 of phytase, particularly at very high doses, improved utilisation of dietary energy and
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40 332 threonine, its effect on feed intake was proportionately much greater. Much of the growth and
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42 333 efficiency responses noted were therefore more a result of improve digestible nutrient intake
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44 334 rather than nutrient digestibility *per se*. As a result, previous studies which have focussed on
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46 335 the effect of phytase on AME alone may well have under-estimated the full value of a
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Table 1. Diet formulation (g/kg 'as-fed') of the diets

| Feedingstuffs | Negative Control | Positive Control |
|---|------------------|------------------|
| Maize | 699.6 | 689.6 |
| Soybean meal (48) | 192.5 | 192.5 |
| Casein | 70.0 | 70.0 |
| Salt | 4.6 | 4.6 |
| DL Methionine | 3.2 | 3.2 |
| Lysine HCl | 1.9 | 1.9 |
| Limestone | 17.6 | 17.6 |
| Dicalcium Phosphate | 5.6 | 15.6 |
| Vitamin Mineral Premix ¹ | 5.0 | 5.0 |
| <i>Calculated analysis (as fed basis)</i> | | |
| AME (MJ/kg) | 12.93 | 13.05 |
| CP (g/kg) | 214 | 214 |
| Ca (g/kg) | 9.1 | 9.1 |
| Total P (g/kg) | 4.9 | 7.1 |
| Available P (g/kg) | 2.5 | 4.7 |
| Sodium (g/kg) | 1.6 | 1.6 |
| <i>Analyzed values (DM basis)</i> | | |
| GE (MJ/kg) | 17.31 | 17.44 |
| CP (g/kg DM) | 245 | 235 |
| DM (g/kg) | 890 | 892 |
| <i>Amino acid composition (g/kg DM)</i> | | |
| Alanine | 9.29 | 9.00 |
| Arginine | 9.87 | 9.82 |
| Aspartic acid | 18.58 | 17.98 |
| Glutamic acid | 40.78 | 38.64 |
| Histidine | 5.18 | 5.19 |
| Isoleucine | 10.00 | 9.61 |
| Leucine | 19.01 | 18.45 |
| Lysine | 12.65 | 11.70 |
| Phenylalanine | 10.09 | 9.74 |
| Serine | 7.76 | 7.46 |
| Threonine | 7.15 | 6.80 |
| Tyrosine | 5.87 | 5.70 |
| Valine | 11.15 | 10.62 |

¹The 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.

Table 2. *The effect of experimental diets on bird performance, dietary apparent metabolisable energy (AME), gross energy (AME:GE) metabolisability coefficient, dietary AME intake, dietary dry matter digestibility (DMD) and nitrogen (NM) metabolisability coefficient*

| Variates | Experimental diets | | | | Significance | |
|--------------------------------|--------------------|-----------------|-------------------------|---------------------------|------------------|----------------------|
| | PC ¹ | NC ² | NC+500 FTU ³ | NC+12500 FTU ³ | LSD ⁴ | P value ⁵ |
| Dry matter intake (g/bird/day) | 38.9 | 32.6 | 36.4 | 40.2 | 4.57 | <0.05 |
| Weight gain (g/bird/day) | 30.8 | 24.1 | 28.1 | 33.1 | 3.99 | <0.05 |
| Feed conversion efficiency | 0.793 | 0.740 | 0.771 | 0.822 | 0.0479 | <0.05 |
| AME (MJ kg DM) | 13.72 | 13.58 | 13.47 | 13.91 | 0.333 | 0.063 |
| AME:GE | 0.787 | 0.786 | 0.778 | 0.804 | 0.0192 | 0.066 |
| AME intake (MJ) | 5.33 | 4.43 | 4.90 | 5.60 | 0.685 | <0.05 |
| DMD | 0.727 | 0.733 | 0.725 | 0.768 | 0.033 | <0.05 |
| NM | 0.542 | 0.564 | 0.532 | 0.624 | 0.0979 | NS |

¹Positive control

²Negative control containing 2.5g/kg available P

³Phytase activity (units/kg) in diet

⁴Least significant differences of means

⁵Results are statistically significant when $P \leq 0.05$ (the P values between 0.05 and 0.1 are described to show a tendency), $P > 0.10$ values = NS

453 **Table 3.** *Energy metabolism of broiler chickens (data based on feeding period from 7 to*
 454 *17d age)*

| Variates ¹ | Experimental diets | | | | Significance | |
|-------------------------------------|--------------------|-----------------|---------------------------|-----------------------------|------------------|-----------------------------|
| | PC ² | NC ³ | NC 500FTU ⁴ | NC 12500FTU ⁴ | LSD ⁵ | <i>P</i> value ⁶ |
| Carcase weight at 7 d (g/DM) | 43.1 | 43.4 | 42.2 | 41.2 | 3.80 | NS |
| Carcase weight at 17 d (g/DM) | 138.4 | 111.4 | 126.3 | 142.9 | 16.40 | <0.05 |
| GE of dry carcass at 7d (MJ/kg) | 24.10 | 24.10 | 24.10 | 24.10 | - | - |
| GE of dry carcass at 17d (MJ/kg) | 24.72 | 24.83 | 25.16 | 24.80 | 0.500 | NS |
| Total carcass GE at 7d (MJ) | 1.04 | 1.03 | 1.02 | 0.99 | 0.092 | NS |
| Total carcass GE at 17d (MJ) | 3.43 | 2.77 | 3.17 | 3.57 | 0.438 | <0.05 |
| Retained carcass protein (g/bird) | 49.3 | 36.8 | 44.0 | 51.9 | 6.42 | <0.001 |
| Retained carcass fat (g/bird) | 31.5 | 22.2 | 28.6 | 34.5 | 5.98 | <0.05 |
| REc (MJ) | 2.40 | 1.74 | 2.16 | 2.57 | 0.368 | <0.05 |
| REp (MJ) | 1.16 | 0.87 | 1.04 | 1.22 | 0.152 | <0.001 |
| REf (MJ) | 1.23 | 0.87 | 1.12 | 1.35 | 0.234 | <0.05 |
| Kre | 0.449 | 0.392 | 0.440 | 0.459 | 0.0185 | <0.001 |
| Krep | 0.218 | 0.196 | 0.212 | 0.219 | 0.0097 | <0.001 |
| Kref | 0.230 | 0.195 | 0.228 | 0.240 | 0.0198 | <0.05 |
| NEp (kg feed DM) | 6.15 | 5.32 | 5.92 | 6.38 | 0.287 | <0.001 |
| HPt (MJ) | 2.94 | 2.70 | 2.74 | 3.02 | 0.338 | NS |
| HPf (kg feed DM) | 7.56 | 8.27 | 7.55 | 7.53 | 0.322 | <0.001 |
| NEp/HPf | 0.814 | 0.644 | 0.787 | 0.849 | 0.0601 | <0.001 |

455 ¹Carcass weight at 7 d old (g/DM) - the average weight of the dry carcass at the beginning of
 456 the study; Carcass weight at 17 d old (g/DM) - the average weight of the dry carcass at the
 457 end of the study; GE of carcass at 7d old (MJ/kg) – gross energy content in a kilogram of
 458 bird carcass (air dry); GE of carcass 17d old (MJ/kg) – gross energy content in a kilogram of
 459 bird carcass (air dry); Total carcass GE at 7d old (MJ) - gross energy in the whole bird
 460 carcass at 7d of age; Total carcass GE at 17d old (MJ) - gross energy in the whole bird
 461 carcass at 17d of age; REc (MJ) - the total carcass energy retained in a bird from 7 to 17d of
 462 age; Protein retained 7-17d old (g/bird) - the total carcass protein retained from 7 to 17d of
 463 age; REp (MJ) – the carcass gross energy retained as carcass protein; REf (MJ) – the carcass
 464 gross energy retained as carcass fat; Kre - efficiency of dietary apparent metabolisable energy
 465 retention; Krep - efficiency of dietary protein retention; Kref - efficiency of dietary fat
 466 retention; NEc (MJ/kg feed DM) – dietary net energy (carcass energy retained per kilogram
 467 dry matter feed intake); HPt (MJ) – total heat production from 7 to 17d of age; HPf (MJ) –
 468 heat production per a kilogram dry matter feed intake.

469 ²Positive control, ³Negative control containing 2.5g/kg available P.

470 ⁴Phytase activity (units/kg) in diet.

471 ⁵Least significant differences of means.

472 ⁶Results are statistically significant when $P < 0.05$, P values between 0.05 and 0.1 are
 473 described to show a tendency and $P > 0.10$ = not significant (NS).

474
475**Table 4.** *The effect of experimental diets on the total tract amino acid digestibility coefficients of broiler chickens determined between 15 and 17d age*

| Amino acids | Experimental diets | | | | Significance | |
|---------------------------|--------------------|-----------------|-------------------------|---------------------------|------------------|-----------------------------|
| | PC ¹ | NC ² | NC+500 FTU ³ | NC+12500 FTU ³ | LSD ⁴ | <i>P</i> value ⁵ |
| <i>Indispensable</i> | | | | | | |
| Arginine | 0.834 | 0.818 | 0.823 | 0.870 | 0.0304 | <0.05 |
| Histidine | 0.823 | 0.802 | 0.800 | 0.847 | 0.0402 | 0.081 |
| Isoleucine | 0.743 | 0.719 | 0.716 | 0.765 | 0.0385 | 0.054 |
| Leucine | 0.812 | 0.770 | 0.769 | 0.834 | 0.0230 | <0.001 |
| Lysine | 0.829 | 0.772 | 0.775 | 0.832 | 0.0403 | <0.05 |
| Phenylalanine | 0.802 | 0.756 | 0.755 | 0.831 | 0.0293 | <0.001 |
| Threonine | 0.646 | 0.589 | 0.567 | 0.659 | 0.0645 | <0.05 |
| Valine | 0.785 | 0.696 | 0.694 | 0.756 | 0.0721 | <0.05 |
| <i>Dispensable</i> | | | | | | |
| Alanine | 0.742 | 0.665 | 0.653 | 0.745 | 0.0463 | <0.001 |
| Aspartic acid | 0.748 | 0.690 | 0.671 | 0.764 | 0.0588 | <0.05 |
| Glutamic acid | 0.787 | 0.802 | 0.788 | 0.809 | 0.0512 | NS |
| Serine | 0.719 | 0.749 | 0.741 | 0.760 | 0.0456 | NS |
| Tyrosine | 0.809 | 0.747 | 0.756 | 0.829 | 0.0363 | <0.001 |
| Average indispensable | 0.791 | 0.746 | 0.744 | 0.805 | 0.0314 | <0.05 |
| Average dispensable | 0.768 | 0.752 | 0.739 | 0.788 | 0.0395 | 0.090 |
| Average total amino acids | 0.779 | 0.749 | 0.741 | 0.797 | 0.0336 | <0.05 |

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¹Positive control.

477

²Negative control containing 2.5g/kg available P.

478

³Phytase activity (units/kg) in diet.

479

⁴Least significant differences of means.

480

⁵Results are statistically significant when *P* < 0.05, *P* values between 0.05 and 0.1 are described to show a tendency and *P*

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>0.10 = not significant (NS).

Table 5. *The effect of experimental diets on digestible amino acid intake (g/bird/day) when fed to broiler chickens from 7 to 17d age*

| Amino acids | Experimental diets | | | | Significance | |
|---------------------|--------------------|-----------------|-------------------------|--------------|------------------|-----------------------------|
| | PC ¹ | NC ² | NC+500 FTU ³ | NC+12500 FTU | LSD ⁴ | <i>P</i> value ⁵ |
| Indispensable | | | | | | |
| Arginine | 0.317 | 0.267 | 0.293 | 0.343 | 0.0427 | <0.05 |
| Histidine | 0.164 | 0.138 | 0.150 | 0.177 | 0.0229 | <0.05 |
| Isoleucine | 0.287 | 0.230 | 0.250 | 0.295 | 0.0428 | <0.05 |
| Leucine | 0.597 | 0.472 | 0.515 | 0.617 | 0.0807 | <0.05 |
| Lysine | 0.405 | 0.301 | 0.329 | 0.391 | 0.0568 | <0.05 |
| Phenylalanine | 0.312 | 0.245 | 0.267 | 0.325 | 0.0416 | <0.05 |
| Threonine | 0.179 | 0.134 | 0.140 | 0.180 | 0.0333 | <0.05 |
| Valine | 0.338 | 0.258 | 0.281 | 0.339 | 0.0515 | <0.05 |
| Dispensable | | | | | | |
| Alanine | 0.267 | 0.199 | 0.213 | 0.269 | 0.0405 | <0.05 |
| Aspartic acid | 0.540 | 0.411 | 0.437 | 0.552 | 0.0891 | <0.05 |
| Glutamic acid | 1.243 | 1.030 | 1.104 | 1.255 | 0.188 | 0.061 |
| Serine | 0.215 | 0.186 | 0.201 | 0.228 | 0.0320 | 0.065 |
| Tyrosine | 0.183 | 0.141 | 0.157 | 0.190 | 0.0256 | <0.05 |
| ∑ indispensable | 2.60 | 2.04 | 2.23 | 2.67 | 0.360 | <0.05 |
| ∑ dispensable | 2.45 | 1.97 | 2.11 | 2.49 | 0.362 | <0.05 |
| ∑ total amino acids | 5.05 | 4.01 | 4.34 | 5.16 | 0.718 | <0.05 |

¹Positive control.

²Negative control containing 2.5g/kg available P.

³Phytase activity (units/kg) in diet.

⁴Least significant differences of means.

⁵Results are statistically significant when $P < 0.05$, P values between 0.05 and 0.1 are described to show a tendency and $P > 0.10$ = not significant (NS).

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493**Table 6.** Correlation matrix for the growth performance, dietary energy and amino acids response criteria of broiler chickens to dietary phytase supplementation

| | Intake | Gain | FCE | AME | AME int | HP _f | NE _p | HP _f :NE _p | K _{rep} | K _{ref} | K _{re} | DMD | Thr | Lys | AA dis | AA ind | AA tot |
|----------------------------------|--------|--------|--------|-------|------------|-----------------|-----------------|----------------------------------|------------------|------------------|-----------------|-------|-------|-------|-----------|-----------|-----------|
| Intake | 1.000 | | | | | | | | | | | | | | | | |
| Gain | 0.928 | 1.000 | | | | | | | | | | | | | | | |
| FCE | 0.373 | 0.690 | 1.000 | | | | | | | | | | | | | | |
| AME | 0.490 | 0.577 | 0.480 | 1.000 | | | | | | | | | | | | | |
| AME int | 0.989 | 0.938 | 0.418 | 0.613 | 1.000 | | | | | | | | | | | | |
| HP _f | -0.559 | -0.525 | -0.250 | 0.195 | -0.475 | 1.000 | | | | | | | | | | | |
| NE _p | 0.824 | 0.854 | 0.546 | 0.510 | 0.833 | -0.744 | 1.000 | | | | | | | | | | |
| HP _f :NE _p | 0.768 | 0.769 | 0.446 | 0.238 | 0.737 | -0.905 | 0.956 | 1.000 | | | | | | | | | |
| K _{rep} | 0.452 | 0.665 | 0.792 | 0.214 | 0.445 | -0.605 | 0.676 | 0.682 | 1.000 | | | | | | | | |
| K _{ref} | 0.746 | 0.640 | 0.172 | 0.185 | 0.708 | -0.858 | 0.879 | 0.933 | 0.376 | 1.000 | | | | | | | |
| K _{re} | 0.761 | 0.764 | 0.450 | 0.230 | 0.729 | -0.910 | 0.954 | 0.998 | 0.692 | 0.929 | 1.000 | | | | | | |
| DMD | 0.379 | 0.485 | 0.460 | 0.681 | 0.459 | 0.060 | 0.412 | 0.234 | 0.328 | 0.125 | 0.229 | 1.000 | | | | | |
| Thr | 0.611 | 0.615 | 0.341 | 0.824 | 0.685 | 0.114 | 0.484 | 0.261 | 0.231 | 0.211 | 0.260 | 0.578 | 1.000 | | | | |
| Lys | 0.578 | 0.550 | 0.262 | 0.632 | 0.619 | -0.076 | 0.508 | 0.355 | 0.233 | 0.337 | 0.361 | 0.463 | 0.873 | 1.000 | | | |
| AA dis | 0.522 | 0.590 | 0.440 | 0.790 | 0.603 | 0.119 | 0.457 | 0.251 | 0.296 | 0.154 | 0.241 | 0.602 | 0.790 | 0.628 | 1.000 | | |
| AA ind | 0.582 | 0.626 | 0.428 | 0.781 | 0.651 | -0.059 | 0.598 | 0.409 | 0.333 | 0.348 | 0.410 | 0.582 | 0.907 | 0.905 | 0.798 | 1.000 | |
| AA tot | 0.583 | 0.642 | 0.457 | 0.828 | 0.662 | 0.029 | 0.558 | 0.350 | 0.332 | 0.267 | 0.345 | 0.624 | 0.896 | 0.812 | 0.945 | 0.951 | 1.000 |

494 Key: Intake, Gain and FCE are feed intakes, weight gains and feed conversion efficiencies of birds fed the experimental diets. AME, AME int,
 495 HP_f, NE_p, K_{rep}, K_{ref}, K_{re} and DMD are the determined dietary apparent metabolisable energy, apparent metabolisable energy intake, the heat
 496 production from kilogram dry matter feed intake, dietary net energy, the efficiency of dietary protein, fat and energy retentions, and dry matter
 497 digestibility coefficient. Thr, Lys, AAdis, AAind and AAtot are the determined total tract digestibility coefficients of dietary threonine, lysine,
 498 dispensable, indispensable and total amino acids.

499 Df = 15 Correlation coefficients greater than 0.482 and 0.606 are statistically significant at $P \leq 0.05$ and $P \leq 0.001$ respectively.

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