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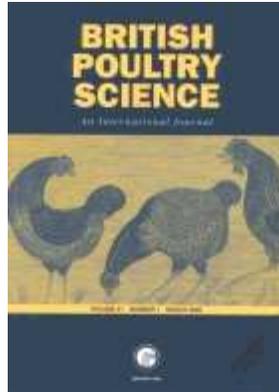
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The effect of previous exposure to dietary microbial phytase on the endogenous excretions of energy, nitrogen and minerals from turkeys

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4 1 **The effect of previous exposure to dietary microbial phytase on the endogenous**
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6 2 **excretions of energy, nitrogen and minerals from turkeys**
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28 11 **Running title: Phytase and endogenous excretions**
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4 22 **Abstract** 1. A precision feeding experiment was conducted with turkeys, which had
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6 23 previously been fed diets with or without phytase, to study the effects on the excretions of
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8 24 endogenous energy (EEL), nitrogen (ENL), amino acids (EAAL) and minerals.
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11 25 2. Female turkeys (BUT 6) which had been fed one of 4 experimental diets (low P maize-soy
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13 26 diets (control, C), C + 250 international units of phytase/kg diet (FTU), C + 500FTU and C +
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15 27 2500FTU) were used in this study. All birds were fasted and then given 50ml of glucose
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17 28 solution at 46 d of age. Birds were allocated to individual metabolism cages in a randomised
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19 29 block design with 8 replicates for each of the 4 previously-fed diets.
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21
22 30 3. The response of EEL and ENL to phytase pre-exposure was a linear function. An increase
23
24 31 of 100 FTU reduced the EEL and ENL by approximately 1.6 kJ and 20 mg respectively. The
25
26 32 results suggest that a minimum activity of phytase of 500 FTU is needed to initiate the
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28 33 reduction of these losses.
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31 34 4. Pre-exposure to phytase reduced the EAAL, which was best described as a linear response
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33 35 with increasing phytase dose in the pre-study period. An increase of 100 FTU reduced the
34
35 36 losses of total endogenous amino acids by approximately 225 mg.
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37 37 5. In contrast to the results for endogenous energy losses, turkeys pre-exposed to phytase
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39 38 linearly increased their excretions of Ca and Mg with increasing phytase activity in the pre-
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41 39 study period.
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43 40 6. The effects of feeding turkeys with supplementary phytase continued for at least 4 d after
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45 41 the diets were withdrawn. This suggests that exposure to phytase alters the functionality and
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47 42 secretions of the gastrointestinal tract, which may influence the nutritive value of
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49 43 subsequently fed diets.
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56 INTRODUCTION

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4 45 About two-thirds of the phosphorus (P) present in cereals and legumes, which are the main
5
6 46 ingredients of poultry diets, is present as phytate P (NRC, 1994). Phytate chelates minerals
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9 47 and react with protein and starch, reducing their bioavailability (Selle and Ravindran, 2007).
10
11 48 Poultry do not produce sufficient quantities of endogenous phytase, so dietary
12
13 49 supplementation with commercially produced microbial phytase is often used to improve
14
15 50 dietary P availability. The beneficial effect of dietary phytases has been perceived to be due to
16
17 51 the direct hydrolytic effects on dietary phytate and the subsequent improvement in the
18
19 52 availability of minerals, amino acids, and energy (Cowieson *et al.*, 2006; Selle and Ravindran,
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21 53 2007; Pirgozliev *et al.*, 2008). Recent research (Cowieson *et al.*, 2004; Pirgozliev *et al.*, 2007;
22
23 54 Cowieson and Ravindran, 2007), however, suggests that the improvement in apparent nutrient
24
25 55 digestibility might also be mediated in part *via* a reduction in endogenous losses from the
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27 56 gastrointestinal tract (GIT). Using a precision feeding technique, Pirgozliev *et al.* (2009)
28
29 57 found that broiler chickens which had been previously fed on diets containing phytase and
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31 58 then given only glucose had lower excretions of endogenous energy and sodium than those
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33 59 given a low-P diet without phytase supplementation. This suggests that the effects of phytase
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35 60 on endogenous losses may continue for at least 4 d after the diets are withdrawn.
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43 61 However, there is a lack of information on how previous exposure to dietary phytases
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45 62 influences endogenous excretions from the GIT of turkeys. Therefore, the objective of this
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47 63 experiment was to investigate the effects of previous dietary exposure to phytase on the
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49 64 endogenous losses of energy (EEL), nitrogen (ENL), amino acids (EAAL) and minerals in
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51 65 turkeys.
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54 66 MATERIALS AND METHODS

55 67 **Pre-study diets**

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4 68 Prior to the precision feeding experiment, all turkeys were reared in 32 floor pens on maize-
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6 69 soy based diets (Table 1) from 1-d-old until they reached 46 d of age. Birds were fed in two
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9 70 phases (0-21 d and 21-46 d), where each diet phase was designed to be adequate in all
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11 71 nutrients but to have approximately half the available dietary P recommended by the breeder
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13 72 (Aviagen Turkeys Ltd., UK). The 4 pre-study diets for each phase were prepared by
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15 73 supplementing the basal diet with an evolved *Escherichia coli*-derived phytase (Quantum™
16
17 74 phytase, EC 3.1.3.26; ABVista Feed Ingredients, Wiltshire, UK) at 0, 250, 500 or 2500 units
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19 75 (FTU) of phytase per kg of diet. Each diet was fed to turkeys in 8 replicate floor pens in a
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21 76 randomised block design. These diets had been used for the first 46 d of life, prior to the
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26 77 precision feeding study. Table 1 near here

27 28 78 **Experimental design**

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30 79 A precision feeding technique (McNab and Blair, 1988) was used to determine endogenous
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32 80 losses. A total of 32 female turkeys (BUT 6) were used in the experiment. A bird was selected
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34 81 at random from each of the 32 pens of birds and the blocking structure (randomised block)
35
36 82 maintained as in the pre-study period thereby yielding a total of 8 replicates per treatment.

37
38 83 At 46d, the turkeys were placed on a raised slatted floor pen with no access to feed,
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40 84 litter or excreta. Water was supplied *ad libitum* throughout the study *via* a suspended nipple
41
42 85 drinker line. To alleviate the stress of feed deprivation, after 24 h, the birds were given 50 ml
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44 86 of a glucose solution (600g/l) by gavage. After a further 24 h, the birds were weighed and
45
46 87 given another 50ml of glucose solution and placed in individual metabolism cages (0.5 m x
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48 88 0.8 m floor area) designed for excreta collection. The temperature was maintained at about
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50 89 20°C and birds were provided with 23 h of light per d. Total excreta collection was made over
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54 90 a 48-h period.
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4 91 The study was approved by the Animal Experiments Committee of the Scottish
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6 92 Agricultural College.

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9 93 **Laboratory analysis**

10 94 Excreta were freeze-dried, weighed and milled to pass through a 0.75 mm mesh. The gross
11
12 95 energy (GE) of excreta was determined using an adiabatic bomb calorimeter (Parr Instrument
13
14 96 Company, Moline, IL, USA) and the EEL calculated. The nitrogen in the excreta was
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16 97 determined by the method of Sweeney (1989) using an FP-200 nitrogen analyser (LECO[®], St.
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18 98 Joseph, MI, USA). Amino acid content in feed and excreta was determined by high
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20 99 performance liquid chromatography following oxygen-free hydrochloric acid digestion (Jones
21
22 100 *et al.*, 1981). The system comprised a Dionex ASI-100 autosampler fitted with a Dionex P580
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24 101 pump and a Dionex RF-2000 detector (Sunnyvale, California, USA). The flow rate used was 1
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26 102 ml min⁻¹ and the column used was a Spherisorb ODS2 (150 x 4.6mm fitted with a Waters
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28 103 guard cartridge). Since this method of hydrolysis destroys methionine, cystine and tryptophan,
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30 104 data on these amino acids are not reported. Metabolisability coefficient for glycine is not
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32 105 presented because of the glycine yield from acid hydrolysis of uric acid in excreta (Soares *et*
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34 106 *al.*, 1971). Minerals, including S, were determined by inductively coupled plasma emission
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36 107 spectrometry (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer,
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38 108 Beaconsfield, UK), (Tanner *et al.*, 2002). The endogenous nitrogen, amino acid and mineral
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40 109 losses (ENL, EAAL, EML respectively) were thus calculated. The loss of cations from
41
42 110 endogenous sources is expressed as milli-equivalents (mEq) of cations as measured in the
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44 111 excreta (Pirgozliev *et al.*, 2009).

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49 113 The cation concentration measured as milli-equivalent/kg (mEq) of excreta dry matter;

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$$\text{Total mEq} = \text{mEq} (\text{Ca}^{2+} + \text{Mg}^{2+} + \text{Mn}^{2+} + \text{K}^{+} + \text{Na}^{+} + \text{Zn}^{2+})$$

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$$\text{For each ion } mEq = \frac{(mg \times valence)}{\text{atomic mass}}$$

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120 Statistical analysis

121 Statistical analyses were performed using the Genstat 11 statistical software package (IACR

122 Rothamstead, Hertfordshire, UK). The comparison between the experimental results was

123 performed by ANOVA, testing orthogonal polynomials for linear, quadratic and cubic

124 responses to previous phytase exposure with the body weight at the beginning of the study

125 included as a covariate. In all instances, differences were reported as significant at $P < 0.05$

126 and trends were noted when P was < 0.10 .

127 RESULTS

128 All birds remained healthy throughout the study period. There were no significant quadratic

129 or cubic deviations and no quadratic or cubic responses to pre-exposure to phytase, so these

130 have been omitted from the results tables. The effect of treatment on EEL, GE and N

131 excretion from turkeys was best described as a linear relationship between increasing dose

132 and reduced losses (Table 2). An increase of 100 FTU reduced the EEL, GE and ENL by

133 approximately 1.6 kJ, 30 kJ and 20 mg respectively. The response of DM excreted to phytase

134 pre-exposure approached significance ($P = 0.074$).

Tables 2 & 3 near here

135 Overall, excretion of amino acids, which was reduced significantly in a linear manner

136 (Table 3) with increasing phytase dose in the pre-feeding period, was in agreement with the

137 ENL data. An increase of 100 FTU reduced the losses of endogenous indispensable,

138 dispensable and total amino acids by approximately 122, 101 and 225 mg respectively. An

139 activity of at least 500 FTU was needed to initiate the reduction of the losses in most of the

140 data.

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4 141 Mineral analyses determined that dietary Ca levels were slightly lower in the starter
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6 142 and slightly higher in the grower diets, compared with the expected values. However, the
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9 143 content of dietary P was in the predicted range. In contrast to most of the results, excretions of
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11 144 Ca and Mg increased linearly with increasing phytase activity in the pre-study period (Table
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14 145 4). The data suggest that an increase of 100 FTU increases the losses of endogenous Ca and
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16 146 Mg by 1.1 and 0.7 mg respectively. Interestingly, excretion of S tended to decrease linearly (P
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18 147 = 0.056) with increasing phytase activity in the pre-study diets. However, this may relate to
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21 148 endogenous excretions of sulphur-containing amino acids, which put the response in
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23 149 agreement with the most of the other amino acids. No responses were detected in the
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26 150 excretion of the other elements and cations.

27 28 151 DISCUSSION

29
30 152 The exact mechanism by which phytase improves dietary feeding quality for poultry is not
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32 153 fully understood. However, recent reports suggest that the improvement in dietary quality
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34 154 after phytase supplementation is achieved by relatively small, and possibly additive,
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36 155 improvements in the apparent digestibility of dietary minerals and amino acids, together with
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38 156 an increase in dietary energy availability as well as a reduction of endogenous losses (Selle
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40 157 and Ravindran, 2007; Cowieson *et al.*, 2009). The results reported here support the hypothesis
41
42 158 that prior exposure of turkeys to phytase reduces EEL, ENL and EAAL. Similar data in terms
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44 159 of EEL were reported in research with chickens (Pirgozliev *et al.*, 2009). However, the results
45
46 160 suggest that a minimum activity of phytase, somewhere between 250 and 500 FTU was
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48 161 needed to initiate a response of these losses. The amount of endogenous losses is influenced
49
50 162 by the chemical composition of the diet. Thus, feeding ingredients containing relatively more
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52 163 phytate, an irritant and poorly digested dietary component, increases losses and, as a result,
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54 164 phytase reduces losses from the GIT of chickens to a significant extent (Cowieson *et al.*,
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4 165 2004; Ravindran *et al.*, 2006). The finding that this effect may continue for at least 4 d after
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6 166 phytase supplemented diets are withdrawn suggests that the impact of dietary phytase on
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8 167 turkey GIT physiology needs further investigation. Previous exposure to phytase may alter the
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10 168 nutritive quality of follow-on diets, indicating a potential commercial impact. Furthermore, if
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12 169 dietary nutrients are made more available upon feeding a phytase (Selle and Ravindran,
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14 170 2007), then the GIT may respond by proportionately decreasing in both size and digestive
15
16 171 enzyme output, effectively reducing endogenous inputs and ultimate losses, as reported by
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18 172 Wu *et al.* (2004). The response of the excretions of endogenous components to previous
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20 173 exposure to phytase suggests that there were changes in terms of GIT functionality and
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22 174 secretions. Intestinal size and GIT enzyme activities were not determined in this study, but
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24 175 such research is warranted.
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30 176 There are several possible mechanisms to explain the reduced endogenous excretions
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32 177 in response to previous exposure to dietary phytase compared to un-supplemented diets. The
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34 178 activity of the enzymes in the intestinal lumen may be reduced due to an interaction of dietary
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36 179 phytate with proteins and minerals (Singh and Krikorian, 1982), and this may induce a
37
38 180 compensatory increment in enzyme secretion, thereby increasing endogenous losses (Green
39
40 181 and Lyman, 1972). This hypothesis agrees with the excretions of endogenous components in
41
42 182 the reported study. Feeding diets with relatively low nutrient availability, *e.g.* presence of
43
44 183 relatively high amounts of phytate without phytase supplementation, by definition means that
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46 184 more undigested material will enter the lower GIT. This can result in an increased enteric
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48 185 microflora population, which may compete with the host for nutrients, secrete toxic
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50 186 compounds, induce an inflammatory response and provoke more rapid turnover of the
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52 187 absorptive epithelial cells in the GIT of the birds (Abrams *et al.*, 1963; Yegani and Korver,
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54 188 2008). The normal life span of the intestinal epithelial cells in chicks is about 5 d, although it
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4 189 can be reduced to approximately 2-3 d in the case of infection or inflammation in the gut
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6 190 mucosa (Fernando and McCraw, 1973). This is in line with the observed changes in the GIT
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9 191 excretions.

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11 192 The most abundant excreted endogenous amino acids were glutamic and aspartic
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13 193 acids, which agrees with previous research with chickens (Ravindran and Hendriks, 2004;
14
15 194 Pirgozliev *et al.*, 2009). Whilst the relative proportions of the remaining excreted amino acids
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17 195 follow a similar pattern to that described by Pirgozliev *et al.* (2009), they do differ somewhat
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19 196 from the data of Ravindran and Hendriks (2004). This discrepancy may be attributed to use of
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21 197 animals which differ in their physiological status and also the use of excreta in the present
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23 198 study *vs* ileal digesta in the work by Ravindran and Hendriks (2004). Indeed, it has been
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25 199 demonstrated that amino acid metabolism by microflora in the lower gut of chickens may be
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27 200 substantial and that amino acid digestibility measured in the terminal ileum would differ from
28
29 201 that measured in the excreta (Ravindran *et al.*, 1999). Minner-Williams *et al.* (2009) reported
30
31 202 that the metabolic activity of the intestinal bacteria is linked to the N cycling systems of the
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33 203 gut in monogastric animals and that bacteria can be the source of nearly 45% of the total
34
35 204 digesta N in pigs; presumably this influence is magnified greatly at the faecal level.

36
37 205 In contrast to most of the results, previous exposure to phytase increased the
38
39 206 excretions of endogenous Ca and Mg. Phytate has the potential to form complexes with
40
41 207 divalent cations such as Ca and Mg, and this can negatively affect their retention. Use of a
42
43 208 phytase increases mineral digestibility and feed intake. Thus birds fed phytase would be
44
45 209 expected to retain significantly more minerals than the birds fed the control. It is likely that in
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47 210 the phytase-fed birds, the balance of these mineral ions was considerably in excess when
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49 211 compared with those fed the control. As a result, there was likely to be a higher rate of
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51 212 excretion of these mineral ions in order to maintain a balance with increased absorption. It
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4 213 seems that this excretion process was going on for some time after removing the phytase
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6 214 containing diet and feeding glucose, suggesting that it takes a significant amount of time for
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8 215 the bird to recognise a change in ion status and shut down the excretory pathways
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10 216 accordingly. Further explanation for this observation may rest with the fact that the heavier
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12 217 birds, *i.e.* the phytase-fed turkeys may excrete more minerals than the lighter birds. For
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14 218 example, Dänner *et al.* (2006) found a positive relationship between body weight and
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16 219 excretion of endogenous phosphorus, however, there is a lack of data from similar studies
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18 220 making conclusions particularly difficult to draw.
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23 221 In the current study, precision feeding was used for the determination of endogenous
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25 222 losses from the GIT of turkeys that had previously been exposed to dietary phytase. The
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27 223 results show that some of the effects of feeding phytases may persist for at least 4 d post
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29 224 feeding, thereby influencing secretions from the GIT. This may affect the nutritive value of
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31 225 diets that the birds receive subsequently and may, therefore, have commercial implications.
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Table 1. The composition (g/kg 'as fed') of the pre-study diets fed to the turkeys from 0 to 46 d

	Starter diet (0-21 d)	Grower diet (21-46 d)
<i>Feedingstuffs</i>		
Maize	441.6	475.7
Soybean meal (48)	487.5	444.5
Vegetable oil	20.0	30.0
Limestone	25.0	25.0
Mono-dicalcium phosphate	10.0	10.0
Sodium chloride	3.5	3.5
Lysine HCl	3.0	2.0
Methionine	4.4	4.3
Vitamin mineral premix ¹	5.0	5.0
<i>Calculated values</i>		
ME (MJ/kg)	11.75	12.19
CP (N x 6.25)	281	262
Lysine	18.4	16.2
Methionine + cystine	11.9	14.1
Ca	12.6	12.4
Total P	6.6	6.4
Non-phytate P	3.7	3.6
Phytate P	2.9	2.8
Sodium	1.6	1.6
<i>Determined values</i> ²		
DM g/kg	878	881
Gross energy MJ/kg	17.03	17.10
CP (N x 6.25)	288	258
Total P	6.2	6.3
Ca	11.7	13.3

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.

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Table 2. *The effect of previous exposure to different dietary phytase activities on the excretions of endogenous dry matter, energy (EEL), and nitrogen (N), and excreta energy concentration measured as gross energy (GE) from 50-d old turkeys over a 48-h period*

Endogenous excretions	Previously fed experimental diets				Pooled SEM	L ³ P values
	C ¹	C+250 ²	C+500 ²	C+2500 ²		
Dry matter excreted (g)	14.13	14.14	12.44	11.84	0.973	0.064
EEL (kJ)	217	217	177	173	13.2	<0.05
N excreted (g)	3.08	2.77	2.56	2.35	0.224	<0.05
GE (MJ/kg DM) excreta	15.40	15.32	14.23	14.41	0.337	<0.05

¹ Control maize-based diets is low (2.8-2.9 g/kg) in available phosphorus.

² Phytase activity (units/kg) in diet.

³ Linear effects of previous exposure to dietary phytase activities. There were 8 observations per treatment.

331 **Table 3.** The effect of previous exposure to different dietary phytase activities on endogenous amino
 332 acid excretions (mg) from 50-d old turkeys over 48-h period

Amino acids	Previously fed experimental diets				Pooled SEM	L ³
	C ¹	C+250 ²	C+500 ²	C+2500 ²		
<i>Indispensable</i>						<i>P values</i>
Arginine (mg)	222	215	173	170	15.7	< 0.05
Histidine (mg)	80	76	57	55	6.1	< 0.05
Isoleucine (mg)	228	242	188	196	17.2	0.074
Leucine (mg)	335	353	276	285	24.4	0.056
Lysine (mg)	179	194	152	149	14.9	0.067
Phenylalanine (mg)	223	228	172	175	16.4	< 0.05
Threonine (mg)	244	257	213	195	18.0	< 0.05
Valine (mg)	290	309	242	253	20.8	0.078
<i>Dispensable</i>						
Alanine (mg)	261	274	206	219	18.5	< 0.05
Aspartic acid (mg)	405	436	327	334	29.1	< 0.05
Glutamate (mg)	564	618	472	476	39.2	< 0.05
Serine (mg)	184	187	151	159	13.1	0.082
Tyrosine (mg)	136	147	124	115	10.8	0.097
∑ indispensable (mg)	1799	1873	1472	1479	125.9	< 0.05
∑ dispensable (mg)	1549	1662	1280	1303	107.0	< 0.05
∑ all amino acids (mg)	3350	3540	2750	2780	232.0	< 0.05

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334 ¹ Control maize-based diets is low (2.8-2.9 g/kg) in available phosphorus.

335 ² Phytase activity (units/kg) in diet.

336 ³ Linear effects of previous exposure to dietary phytase activities. There were 8 observations
 337 per treatment.

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345 **Table 4.** *The effect of previous exposure to different dietary phytase activities on endogenous*
 346 *mineral and cation (mEq/bird/48 h) excretion per bird from 50-d-old turkeys over a 48-h*
 347 *period*

<i>Minerals</i>	Previously fed experimental diets				Pooled SEM	L ³ <i>P</i> values
	C ¹	C+250 ²	C+500 ²	C+2500 ²		
Ca (mg)	30	52	39	65	7.4	<0.05
K (mg)	483	540	516	474	47.2	NS
Mg (mg)	29	57	53	59	7.4	<0.05
Mn (mg)	6	9	6	14	3.1	NS
Na (mg)	40	39	38	38	9.1	NS
P (mg)	275	404	355	332	32.4	NS
S (mg)	1214	1210	1048	964	100.0	0.056
Zn (mg)	32	31	19	35	6.3	NS
mEq kg excreta	19	24	22	23	1.8	NS
Total mEq excreted	297	377	308	334	50.6	NS

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349 ¹ Control maize-based diets is low (2.8-2.9 g/kg) in available phosphorus.

350 ² Phytase activity (units/kg) in diet.

351 ³ Linear effects of previous exposure to dietary phytase activities. There were 8 observations
 352 per treatment.

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