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Johnson Oluwasola Agbede, Holger Kluth, Markus Rodehutschord

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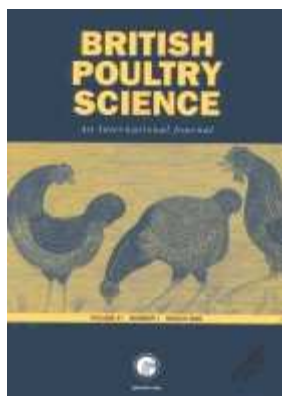
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Studies on the effects of microbial phytase on amino acid digestibility and energy metabolisability in caecectomised laying hens and the interaction with the dietary phosphorus level

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11 **and energy metabolisability in caecectomised laying hens and the**
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13 **interaction with the dietary phosphorus level**
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20 J. O. AGBEDE*, H. KLUTH AND M. RODEHUTSCORD¹
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25
26 *Institut für Agrar- und Ernährungswissenschaften, Martin-Luther-Universität Halle-*
27 *Wittenberg, Halle (Saale), Germany and ¹Institut für Tierernährung, Universität*
28 *Hohenheim, Stuttgart, Germany*
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35 **RUNNING TITLE: PHYTASE IN CAECECTOMISED HENS**
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43 Correspondence to: M. Rodehutsord, Institut für Tierernährung, Universität
44 Hohenheim, 70599 Stuttgart, Germany
45 E-mail: inst450@uni-hohenheim.de
46
47
48
49

50 *Present address: Department of Animal Production and Health, Federal University of
51 Technology, P.M.B.704, Akure, Nigeria
52 E-mail: joagbede@yahoo.com
53
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1 **Abstract** 1. Caecectomised laying hens were used in two assays to study the effects
2 of a microbial 6-phytase on amino acid (AA) digestibility and energy metabolisability
3 using (1) diets with phytate-rich protein sources and (2) with two dietary phosphorus
4 (P) levels. The two assays followed a 2×2-factorial arrangement of treatments using 8
5 hens per treatment. The hens were housed individually in metabolism cages, and
6 excreta were quantitatively collected for a period of 5 d.

7 2. In Assay 1, two dietary P levels (8.0 and 4.0 g/kg dry matter) and two levels of
8 phytase supplementation (0 and 1000 U/kg diet) were used in diets that were mainly
9 based on maize, sunflower meal and wheat gluten. The digestibility of AA was 0.01 to
10 0.02 lower in the diet with the low P than with the high P concentration. The effect of
11 P content was significant for 12 out of the 14 AA studied. The supplementation of
12 phytase significantly improved the degradation of *myo*-
13 inositol(1,2,3,4,5,6)hexakisphosphate (IP6), but affected neither AA digestibility nor
14 energy metabolisability. An interaction between the two factors was not detected.

15 3. In Assay 2, diets contained one main protein source (sunflower meal or rapeseed
16 meal) at a concentration of 250 g/kg. The diets were either supplemented or not with
17 phytase (1500 U/kg diet). On average, AA digestibility was 0.01 lower in the rapeseed
18 meal-containing diet than in the sunflower meal-containing one. The maximum
19 difference was 0.03. The effect of the protein source was significant for 9 of the 14
20 AA studied. The supplementation of phytase had no significant effect on AA
21 digestibility or energy metabolisability.

22 4. Our studies do not substantiate the hypothesis that improved IP6 degradation due to
23 phytase supplementation is associated with improved AA digestibility and energy
24 metabolisability in laying hens. An interaction between phytase efficacy to improve
25 AA digestibility or energy metabolisability and the P level in the diet does not seem to
26 exist.

INTRODUCTION

28 It has been long known that the hydrolysis of phytate in the digestive tract of non-
29 ruminants can be improved by the supplementation of microbial phytase to the diet.
30 The utilisation of phosphorus (P) can be improved and P excretion reduced. The
31 application of phytase as a tool to improve amino acid (AA) digestibility and energy
32 metabolisability remains speculative, especially in laying hens. Various studies using
33 broilers have demonstrated that the application of phytase can improve precaecal AA

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3 34 digestibility in individual feed ingredients (Ravindran *et al.*, 1999; Peter and Baker,
4 35 2001) and in compound diets (Yi *et al.*, 1996; Sebastian *et al.*, 1997; Ravindran *et al.*,
5 36 2000; Rutherford *et al.*, 2002). We are aware of only three studies on the effect of
6 37 phytase on AA digestibility in laying hens. Jalal *et al.* (1999) reported that Met, Cys,
7 38 Ala, and Glu digestibility were improved when layers were fed on maize-soybean
8 39 meal-based diets with supplemental phytase. Recently, Liu *et al.* (2007) studied the
9 40 efficacy of phytases in laying hens fed on 5 diets consisting of a control diet with
10 41 adequate calcium (Ca, 33.0 g/kg), total P (5.0 g/kg), and nonphytate P (NPP, 2.8 g/kg)
11 42 and a diet with Ca, total P and NPP reduced by 12, 14 and 13%, respectively. They
12 43 found that the reduction of Ca and P significantly depressed the digestibility of N and
13 44 AA and that a supplementation of phytase to the diet with reduced Ca and P improved
14 45 the digestibility of AA by 2 to 8%. However, the digestibility of most AA was not
15 46 restored to the levels of the positive control diet by the application of phytase. On the
16 47 contrary, Snow *et al.* (2003) reported that phytase addition to diets based on maize
17 48 and soybean meal, maize, soybean meal and meat and bone meal, or maize, soybean
18 49 meal and wheat middlings fed to moulted laying hens did not have a significant effect
19 50 on AA digestibility. An interaction between feed type and phytase found in the latter
20 51 study indicated that the efficacy of supplemented phytase may depend on the source
21 52 of protein used.

22 53 Previous reports also suggest that phytase effects on energy utilisation are
23 54 variable. For instance, Rojas and Scott (1969) found that AME in chicks fed on
24 55 cottonseed meal and soybean meal were significantly improved following treatment
25 56 with a crude phytase preparation. Ravindran *et al.* (2000) reported that supplemental
26 57 phytase increased AME values from 13.4 to 13.5 MJ/kg dry matter in low NPP diets
27 58 and from 12.7 to 13.4 MJ/kg dry matter in adequate NPP diets. However, Snow *et al.*
28 59 (2003) found that in hens the precaecal digestibility of energy was affected by the diet
29 60 but not by phytase supplementation.

30 61 If the aim is to study the potential of phytase for improving the utilisation of
31 62 P, then diets with a P concentration below the requirement need to be used. Studies on
32 63 phytase effects on AA digestibility or energy metabolisability, however, would use an
33 64 adequate level of P in the diet. The experimental effort can be reduced if an
34 65 interaction between phytase effects on AA and energy on the one hand and the P level
35 66 of the diet on the other hand does not exist and, hence, the utilisation of P, AA
36 67 digestibility and energy metabolisability can be studied in the same assay.

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4 68 The objective of this study, therefore, was to use laying hens for examining
5 69 1) the interaction between the P level in the diet and a microbial 6-phytase with regard
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7 70 to AA digestibility and energy metabolisability, and 2) the phytase effects on AA
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9 71 digestibility of two protein sources that have a high phytate concentration.
10
11 72 Caectomised hens were used in order to reduce the impact of post-ileal microbial
12
13 73 activity on AA excretion. By using caectomised hens for AA digestibility studies,
14
15 74 we also imply that the excretion of AA with urine is negligibly low (Parsons, 1986;
16
17 75 Whittow, 2000).

18 19 20 76 MATERIALS AND METHODS

21 22 77 **General procedures**

23
24 78 Two assays were conducted. All procedures were approved by the animal welfare
25
26 79 authorities in accordance with the German Animal Welfare Regulations. Pullets
27
28 80 (Lohmann Brown) were obtained when they were 16 weeks old from
29
30 81 Geflügelzuchtbetriebe Gudendorf (Ankum, Germany) and housed individually in
31
32 82 metabolic cages in a temperature- and illumination-controlled room. The room
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34 83 temperature was kept at 20-22°C, and 14 h of lighting were provided. The hens were
35
36 84 caectomised when they were between 18 and 22 weeks old. The surgery followed
37
38 85 the procedures of Angkanaporn *et al.* (1997) and Green *et al.* (1987) and was
39
40 86 described in detail by Rezvani *et al.* (2007).

41
42 87 Both assays followed a 2×2-factorial arrangement of treatments and used 16
43
44 88 hens. The hens were weighed and randomly allocated to one of the 4 treatments at the
45
46 89 beginning of each assay. Each assay was carried out in two periods. After the first
47
48 90 period the hens were reallocated to the treatments. Thus, the number of replicates per
49
50 91 treatment was 8.

51
52 92 The daily amount of feed was 120 g and offered in two equal meals at about
53
54 93 0800 and 1500 h. The feed was offered for 5 d of adjustment and for the subsequent 5
55
56 94 d, during which excreta were quantitatively collected from each individual hen. To
57
58 95 minimise volatilisation of excreta components, excreta were removed from the trays
59
60 96 three times per day at about 0700, 1400 and 2000 h. Excreta were collected into
97
98 97 separate and weighed buckets, and immediately frozen at -20°C. Excreta of each hen
99
99 98 were pooled for the 5 d of the collection period. Feathers and spilled feed were
99
99 99 removed from excreta before each collection. Thawed excreta were weighed and

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2
3 100 homogenised. About 200 g of excreta per hen were freeze-dried. Freeze-dried excreta,
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5 101 feed and feed residues were ground through a 0.5-mm screen and stored at room
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7 102 temperature until being analysed.

8
9 103 The phytase product used was Ronozyme P 5000 (CT), DSM Nutritional
10 104 Products, Basel. It contained a 6-phytase derived from a *Peniophora lycii* gene at a
11 105 level of 5000 U/g of product. The phytase activity analysed in the complete diets is
12 106 summarised in Table 3.

Tables 1, 2, 3 near here

17 **Assay 1**

18 108 The objective of this assay was to investigate whether an interaction exists between
19 109 the dietary P level (high (HP) and low (LP)) and the phytase supplementation (Phy-
20 110 and Phy+) in regard to AA digestibility. The IP6 degradation, utilisation of P and N as
21 111 well as energy metabolisability were also studied. The hens were 25 weeks old when
22 112 the assay started. Two basal diets mainly based on maize, maize starch, wheat gluten
23 113 and sunflower meal were used with the ingredient and analysed compositions as
24 114 shown in tables 1 and 2. The sunflower meal was chosen because of its high
25 115 concentration of phytate. Diet HP contained 8.0 g total P/kg of DM achieved by the
26 116 inclusion of monobasic calcium phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) and it met or exceeded, in
27 117 all nutrients, the requirements recommended by the Gesellschaft für
28 118 Ernährungsphysiologie (1999) for laying hens. The total P concentration in diet LP
29 119 was reduced to 4.0 g/kg of DM by the non-inclusion of MCP. Each diet was mixed in
30 120 one lot. Thereafter, each batch was divided into two equal parts. One part was
31 121 supplemented with phytase (1000 U/kg diet intended) and mixed again while the other
32 122 remained without a supplement. The 4 complete diets were pelleted without using
33 123 steam through a 3-mm die to prevent selective pecking of the birds.

48 **Assay 2**

49 125 The objective of this assay was to examine the effect of phytase on AA digestibility
50 126 and energy metabolisability using diets containing either sunflower meal (SF) or
51 127 rapeseed meal (RS) as the main protein source at a level of 250 g/kg (Table 1). They
52 128 were chosen because of their high phytate content. The utilisation of P was not studied
53 129 in this assay, because P was not a limiting factor. The two basal diets were formulated
54 130 to meet the recommendation of the Gesellschaft für Ernährungsphysiologie (1999).
55 131 Diet preparation, phytase supplementation (1500 U/kg diet intended) and pelleting

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4 132 were as in Assay 1. The analysed phytase concentrations were lower than intended,
5
6 133 but in each diet the supplementation was confirmed (Table 3).
7

8 134 **Chemical analyses**

9
10 135 The dry matter (105°C), crude nutrients, AA, gross energy, P and Ca were determined
11
12 136 according to the Verband Deutscher Landwirtschaftlicher Untersuchungs- und
13
14 137 Forschungsanstalten (VDLUFA) standard procedures used in Germany (Naumann
15
16 138 and Bassler, 1976). Total N was determined using method 6.1.1 as described by
17
18 139 Bassler (1993) with a Kjeltec Auto 1030 Analyser (Tecator AB, Höganäs, Sweden),
19
20 140 crude ash using method 3.1 (Naumann and Bassler, 1976), crude fat using method
21
22 141 5.1.1 (Bassler, 1988) and crude fibre using method 6.1.1 (Bassler, 1993). The amino
23
24 142 acids were analysed following standard procedures (Naumann and Bassler, 1976) with
25
26 143 laboratory details as described by Rodehutschord *et al.* (2004). In brief: after an
27
28 144 oxidation step, samples were hydrolysed in 6 M hydrochloric acid. Norleucine was
29
30 145 used as the internal standard. Amino acids were separated and detected by an AA
31
32 146 analyser (Biochrom 30, Biochrom Ltd., Cambridge) using various buffer solutions
33
34 147 and ninhydrin. Because tryptophan is destroyed during acid hydrolysis, uric acid from
35
36 148 excreta can be converted into glycine upon hydrolysis, and histidine and tyrosine are
37
38 149 degraded during performic acid oxidation, these AA were not be determined. The
39
40 150 energy contents of the samples were measured by a bomb calorimeter (IKA-
41
42 151 Calorimeter C7000 isoperibolic, Janke & Kunkel Analysetechnik, Staufen,
43
44 152 Germany). Concentrations of P and Ca were determined in accordance with
45
46 153 Rodehutschord and Dieckmann (2005). Samples were incinerated at 550°C, the
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48 154 minerals then solubilised using 6 N HCl, and the solution was taken to dryness.
49
50 155 Samples were subsequently heated after the addition of 10 ml of dilute HNO₃ and
51
52 156 then filtered. Concentrations of P and Ca were determined with an Inductively
53
54 157 Coupled Plasma Spectrometer (ICP-OES, JY 24, Jobin Yvon GmbH, Grassbrunn,
55
56 158 Germany). The concentration of IP₆ and lower inositol phosphates (IP₅-IP₃) were
57
58 159 measured in extracted samples of feed and excreta using HPLC according to the
59
60 160 method of Sandberg and Ahderinne (1986). Phytase activity was measured in
161
162 161 accordance with the method of Engelen *et al.* (1994). One phytase unit (U) is defined
163
164 162 therein as the amount of enzyme which, at 37°C and pH 5.5, liberates one micromole
165
166 163 of inorganic phosphate per minute from 0.0051 mol/l sodium phytate.

164 **Calculations and statistical analysis**

165 The digestibility of AA was calculated, for each hen, according to the following
166 equation:

$$167 \text{Digestibility} = (\text{DI} - \text{DE}) / \text{DI},$$

168 where DI = daily intake (mg) and DE = daily excretion (mg).

169 Utilisation of N, P and Ca, energy metabolisability and IP6 degradation were
170 calculated accordingly. Retention of P and Ca is the difference between quantitative
171 intake and the amount contained in excreta.

172 The data were analysed using the two-way ANOVA procedure of the
173 statistical software package Statistica for Windows (StatSoft, Inc., Tulsa, Oklahoma,
174 US). Differences between the treatment means were tested for significance using the
175 Tukey test with a level of significance of $P < 0.05$.

176 **RESULTS**

177 **Assay 1**

178 The digestibility of AA in the HP diet ranged from 0.81 (threonine) to 0.96 (glutamic
179 acid) (Table 4). The digestibility was 0.01 to 0.02 lower in the LP than in the HP diet
180 for most of the AA studied. This effect of the P level was significant ($P < 0.05$) for all
181 AA with the exception of lysine and serine. The supplementation of phytase did not
182 significantly affect AA digestibility, and a significant interaction between the two
183 factors was not detected. Degradation of IP6 was significantly improved by phytase
184 supplementation ($P < 0.001$, Table 4). In the diets without phytase supplementation,
185 IP6 degradation was lower in the HP diet than in the LP diet, but this difference was
186 not statistically significant. The concentration of lower inositol phosphates (IP5-IP3)
187 in excreta was significantly lower ($P < 0.001$) at the low than at the high P level (Table
188 5).

Tables 4 and 5 near here

189 Dry matter intake averaged 109 g/d, the efficiency of N utilisation was 0.51,
190 and energy metabolisability was on average 0.82 (Table 6). These traits were affected
191 neither by the dietary P level nor by phytase supplementation. The reduction in the P
192 concentration of the diet led to a significant reduction in intake and excretion of P,
193 irrespective of the phytase level ($P < 0.001$, Table 7). The efficiency of P utilisation
194 was significantly higher in the LP diet than in the HP diet ($P = 0.034$). The efficiency
195 of P utilisation was also higher in the Phy+ than in the Phy- diets, but a significant

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3 196 phytase effect was not detected. The efficiency of Ca utilisation was not significantly
4 197 affected by the two factors.

Tables 6, 7, 8 near here

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8 **Assay 2**

9
10 199 The digestibility of AA in the unsupplemented SF diet ranged from 0.70 (cystine) to
11 200 0.90 (arginine, methionine) (Table 8). On average, AA digestibility was 0.01 lower in
12 201 the RS than SF diet, with a maximum difference of 0.03. The effect of the protein
13 202 source was significant ($P < 0.05$) for 10 of the AA studied. The supplementation of
14 203 phytase did not significantly affect AA digestibility. A significant interaction between
15 204 the two factors was not detected, with the exception of a trend in methionine
16 205 digestibility. Feed intake was similar to that in Assay 1, but showed a greater variation
17 206 between treatments (Table 6). The efficiency of N utilisation was significantly lower
18 207 ($P = 0.005$) and energy metabolisability significantly higher ($P < 0.001$) in the RS than
19 208 in the SF diet. A significant negative phytase effect ($P = 0.029$) on the efficiency of N
20 209 utilisation was detected. In contrast to Assay 1, differences in egg production became
21 210 obvious.

30
31 **DISCUSSION**

32
33 212 One objective of this study was to investigate whether phytase has an effect on AA
34 213 digestibility using protein sources high in phytate. For this reason, sunflower meal and
35 214 rapeseed meal were chosen as the main protein sources. In Assay 1 the significant
36 215 effect of phytase on IP6 degradation proved that the phytase was effective in the
37 216 digestive tract. However, in both assays, phytase did not have an effect on AA
38 217 digestibility.

39
40 218 Results from other studies on this subject have not been consistent. In laying
41 219 hens, Jalal *et al.* (1999) reported that only methionine, cystine, alanine, and glutamic
42 220 acid digestibility were improved when phytase was supplemented to a maize-soybean
43 221 meal-based diet, without an effect on crude protein utilisation. Snow *et al.* (2003)
44 222 reported that phytase addition (300 U/kg diet) to diets based on maize and soybean
45 223 meal, maize, soybean meal and meat and bone meal and maize, soybean meal and
46 224 wheat middlings fed to moulted laying hens did not have significant effects on the
47 225 digestibility of any of the AA measured, although an interaction between feed type
48 226 and phytase on the digestibility of four AA was observed. In a third study, phytase
49 227 improved the digestibility of AA by 0.02 to 0.08 when added to a diet with reduced
50 228 contents of P and Ca (Liu *et al.*, 2007). This suggests that improvement in AA

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3 229 digestibility in laying hens could be achieved when the level of Ca and P in the diet is
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5 230 reduced to an amount below the recommended level. While we found indications of a
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7 231 higher phytase effect in low Ca diets (Agbede *et al.*, 2009), data from Assay 1 of the
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9 232 present study clearly showed that phytase did not affect AA digestibility, irrespective
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11 233 of whether the P level was high or reduced. Thus, unlike in broilers, in laying hens a
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13 234 positive effect of phytase supplementation on AA digestibility is hard to expect based
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15 235 on the data published so far. Whether or not the different results between studies are
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17 236 related to different phytase sources or phytase levels remains a subject of speculation.

18 237 The second objective was to study whether an interaction between the P level
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20 238 in the diet and phytase efficacy in regard to AA digestibility and energy
21
22 239 metabolisability exists. The results from Assay 1 suggest that such interaction does
23
24 240 not exist. Phytase did not show any effect on AA digestibility and energy
25
26 241 metabolisability in diets HP and LP. We wanted to answer the question whether in
27
28 242 future studies phytase effects on P utilisation, AA digestibility and energy
29
30 243 metabolisability can be studied with the same diet, *i.e.* a diet that has a low P
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32 244 concentration. The results showed that an effect that is not existent at a high P level
33
34 245 does not exist at low P level. The data do not allow us to judge whether a phytase
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36 246 effect, if existent at a high P level, also exists at a reduced P level.

37 247 Assay 1 showed that the supply of P had a small but significant effect on the
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39 248 digestibility of most of the AA studied. We are not aware of any published data
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41 249 indicating an effect of P deficiency on AA digestibility in poultry that could be
42
43 250 compared with our findings. Digestibility was also reduced in a P reduced diet in the
44
45 251 study of Liu *et al.* (2007), but in their study the reduction of P coincided with a
46
47 252 reduction of Ca. It can be speculated whether P deficiency affects AA absorption. In
48
49 253 rainbow trout, P deficiency caused a reduction in the digestibility of organic fractions
50
51 254 of the diet (Rodehutsord *et al.*, 2000), but the deficiency was more severe than in the
52
53 255 present study.

54 256 In the present study significant differences were detected in cases when the
55
56 257 treatment means for AA digestibility differed by no more than 0.01 or 0.02. This is
57
58 258 unusual for AA digestibility studies, at least when compared with measurements made
59
60 259 at the end of the ileum. Rezvani *et al.* (2008) compared digestibility measurements
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261 based on ileal digesta of hens and on quantitative excreta collection with
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262 caeectomised hens. One result of this comparison was that the variation among the
replicates of one treatment was much smaller when excreta were collected

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3 263 quantitatively, as done in the present study. The authors concluded that this is an
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5 264 advantage when small differences in digestibility between protein sources are to be
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7 265 detected.

8
9 266 The digestibility of AA was slightly lower in the RS than in the SF diet.
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11 267 Digestibilities were, however, relatively high and suggest that both feedstuffs are
12
13 268 suitable protein sources for laying hens. Digestibilities for the RS diet (0.70 to 0.90)
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15 269 were similar to those determined for pure RS with broilers (0.76 to 0.92; Kluth and
16
17 270 Rodehutscord, 2006; and 0.69 to 0.88; Rodehutscord *et al.*, 2004). In the RS diet of
18
19 271 the present study approximately 0.60 of the crude protein originated from the
20
21 272 rapeseed.

22
23 273 In the RS diet, lower values for the efficiency of N utilisation were detected
24
25 274 than in the SF diet. Also, phytase seemed to have a negative effect on N utilisation in
26
27 275 the RS diet although effects of phytase on AA digestibility were not detected. The
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29 276 effects on N utilisation must be seen in a context together with feed intake and egg
30
31 277 performance, because it can be assumed that the AA not needed for egg protein
32
33 278 synthesis are largely recovered in the nitrogenous fraction of the urine. Egg
34
35 279 production was lower in the phytase-supplemented RS diet than in any other treatment
36
37 280 of Assay 2. In view of the short duration of the experiment we consider this a random
38
39 281 rather than a phytase effect. Crude protein intake in relation to egg production was
40
41 282 greater in this treatment, and this led to an increased excretion of N. This suggests that
42
43 283 the efficiency of N utilisation is not a good indicator to study phytase effects on AA.
44
45 284 At least, intake and requirement should be standardised, and the level of supply
46
47 285 preferentially below the requirement. In contrast to the present observation, Panda *et al.*
48
49 286 (2005) and Liu *et al.* (2007) reported an improvement in the utilisation of N with
50
51 287 phytase supplementation in laying hens.

52
53 288 The supplementation of phytase did not affect energy metabolisability in any
54
55 289 of the 4 diets used in the present study. Similarly, the addition of 0, 600 or 1200 U/kg
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57 290 of phytase to a diet based on maize and soybean meal did not significantly improve
58
59 291 metabolisable energy in caecectomised roosters (Biehl and Baker, 1997). Also, added
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292 phytase (300 U/kg diet) did not significantly influence precaecal digestible energy in
293 moulting laying hens given different types of diet (Snow *et al.*, 2003). Different results
294 have been published for broilers. Rojas and Scott (1969) reported a significant
295 increase in AME for chicks given a phytase preparation from *Aspergillus ficuum*.
296 Ravindran *et al.* (2000, 2001) reported an improvement in AME in broilers fed on a

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2
3 297 diet based on wheat, sorghum and soybean meal due to supplemental phytase. The
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5 298 mechanism of the energy effect is largely unknown, but improved protein digestibility
6
7 299 is thought to be responsible. The data of Ravindran *et al.* (2000) showed that phytase
8
9 300 may improve energy metabolisability, independently of its effect on AA digestibility.
10
11 301 It was proposed that mineral-phytate complexes may contribute to the formation of
12
13 302 insoluble metallic soap in the gut and enhance the utilisation of energy originating
14
15 303 from lipids. Dietary concentrations of Ca and saturated fats would have particular
16
17 304 relevance to this proposed mode of action (Qian *et al.*, 1996). However, in our study
18
19 305 only the feedstuffs used as protein sources in Assay 2 caused differences in energy
20
21 306 metabolisability.

22
23 307 In Assay 1, the efficiency of P utilisation was affected by the P supply as
24
25 308 expected. Diet HP showed lower P utilisation than diet LP although it contained P
26
27 309 from a mineral source. This happened because hens ingested more P than required and
28
29 310 excreted the excessive P intake. In our opinion, the high level of P intake was also the
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31 311 reason that supplemented phytase caused a difference in the P utilisation of diet HP by
32
33 312 only 0.02. P utilisation measured at such high level of P intake is not appropriate to
34
35 313 measure the effects of phytase. Degradation of IP6 in the digestive tract was improved
36
37 314 by 0.35 due to phytase, but hens either did not absorb the released phosphate or
38
39 315 excreted it via urine. The higher concentration of IP5, IP4 and IP3 in excreta of hens
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41 316 fed HP is an indication that the high content of inorganic P in the diet had reduced the
42
43 317 activity of phosphatases in the digestive tract of the hens (Table 5). Inorganic P has
44
45 318 been reported to depress bacterial phytase production as well as to be a competitive
46
47 319 inhibitor of certain bacterial phytases *in vitro* (Konietzny and Greiner, 2002). In diet
48
49 320 LP, P utilisation was 22% higher when phytase was supplemented compared with the
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51 321 unsupplemented diet. Neither was the overall phytase effect significant ($P = 0.14$) nor
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53 322 was an interaction detected, because the variation in P utilisation within one treatment
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55 323 was high. However, together with the significant difference in IP6 degradation, the
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57 324 difference can be regarded as a strong indication that phytase expressed similar effects
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59 325 on the utilisation of P from a phytate-rich diet, as in other studies (van der Klis *et al.*,
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326 1997; Carlos and Edwards, 1998; Boling *et al.*, 2000; Keshavarz, 2003).

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328 In conclusion, our studies do not substantiate the hypothesis that improved
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330 phytate degradation due to phytase supplementation is associated with improved AA
digestibility and energy metabolisability in laying hens. An interaction between
phytase efficacy in improving AA digestibility or energy metabolisability and the P

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3 331 concentration of the diet does not seem to exist in hens. The indication from this study
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5 332 that AA digestibility may be reduced when the P content of the diet is low needs
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7 333 clarification and suggests further research on this subject.
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Table 1. *Ingredient composition of the basal diets (g/kg) (HP =high P, LP = reduced P, SF = sunflower meal, RS = rapeseed meal)*

Diet	Assay 1		Assay 2	
	LP	HP	SF	RS
Maize	390	390	280	280
Maize starch	220	200	170	170
Wheat gluten	90	90	0	0
Sunflower meal	150	150	250	0
Rapeseed meal	0	0	0	250
Soybean meal	0	0	120	120
Soybean oil	36	36	50	50
Monobasic calcium phosphate	-	20	20	20
Sodium chloride	3	3	3	3
Limestone	90	90	90	90
Premix ¹	15	15	15	15
L-lysine·HCl	5	5	1	1
DL-methionine	1	1	1	1

¹ Contained (per kg) 6000 mg Zn; 5000 mg Fe; 7000 mg Mn; 1000 mg Cu; 25 mg Co; 100 mg I; 35 mg Se; 688 mg retinol acetate; 10 mg cholecalciferol; 4000 mg DL- α -tocopherol acetate; 240 mg Vitamin K₃; 100 mg thiamine; 720 mg riboflavin; 300 mg pyridoxine; 2.25 mg cyanocobalamin; 1000 mg Ca-D-pantothenate; 6000 mg nicotinic acid; 150 mg folic acid; 17 mg biotin; 7500 mg butylated hydroxytoluene.

Table 2. *Analysed chemical composition of the two protein sources and the basal diets (g/kg DM)*
(HP = high P, LP = reduced P, SF = sunflower meal, RS = rapeseed meal)

	Protein sources		Assay 1 diets		Assay 2 diets	
	SF	RS	LP	HP	SF	RS
Crude protein	299	376	160	160	168	187
Crude fibre	289	164	45	44	82	50
Crude lipids	44	43	78	71	68	83
Crude Ash	79	82	134	145	156	162
Calcium	nd	nd	46.7	39.4	45.9	48.0
Phosphorus	nd	nd	4.0	8.0	10.2	10.5
IP6	nd	nd	6.1	6.2	nd	nd
Alanine	13.4	17.4	6.8	6.9	8.4	9.7
Arginine	25.6	23.9	7.7	7.9	11.9	12.3
Aspartic acid	28.2	30.0	9.0	9.2	16.0	17.2
Cystine	5.1	8.6	3.1	3.2	2.8	3.7
Glutamic acid	59.5	66.0	44.6	45.4	32.6	34.6
Glycine	18.4	20.3	6.6	6.7	8.4	9.1
Isoleucine	12.2	14.9	5.7	5.9	7.3	8.1
Leucine	19.4	27.0	12.8	13.1	13.5	15.7
Lysine	11.3	21.4	8.0	8.0	8.8	11.2
Methionine	6.5	7.2	3.7	3.9	3.8	4.1
Phenylalanine	13.6	15.4	7.7	7.9	8.2	9.1
Proline	12.3	22.4	15.3	15.5	8.4	11.5
Serine	13.3	17.3	7.2	7.4	7.8	9.1
Threonine	11.5	17.7	4.9	5.1	6.3	8.0
Valine	13.9	18.1	6.7	6.9	8.1	9.4
Gross Energy (MJ/kg DM)	nd	nd	17.1	16.9	17.4	17.5

nd = not determined.

Table 3. *Analysed phytase activity in the pelleted experimental diets (U/kg diet)*

	Without phytase supplementation	With phytase supplementation
Assay 1		
High P diet	236	640
Reduced P diet	58	590
Assay 2		
Sunflower meal diet	104	1230
Rapeseed meal diet	- ¹	1130

¹ Below the lower limit of detection.

Table 4. *Digestibility of amino acids and IP6 degradation determined with caeectomised hens in Assay 1 using diets different in the level of P and phytase supplementation (n = 8 hens per treatment)*

P level Phytase supplementation	High		Reduced		Pooled SEM	Significance (P value)		
	-	+	-	+		P level	Phytase	P×Phy
Alanine	0.86	0.86	0.84	0.84	0.003	0.002	NS	NS
Aspartic acid	0.83	0.83	0.81	0.82	0.003	0.016	NS	NS
Arginine	0.93	0.93	0.92	0.92	0.002	0.006	NS	NS
Cystine	0.85	0.85	0.84	0.84	0.003	0.049	NS	NS
Glutamic acid	0.96	0.96	0.95	0.95	0.001	< 0.001	NS	NS
Isoleucine	0.91	0.91	0.90	0.90	0.002	< 0.001	NS	NS
Leucine	0.92	0.92	0.91	0.91	0.002	< 0.001	NS	NS
Lysine	0.89	0.89	0.88	0.89	0.002	0.108	NS	NS
Methionine	0.94	0.94	0.93	0.93	0.002	0.002	NS	NS
Phenylalanine	0.93	0.93	0.92	0.92	0.002	0.002	NS	NS
Proline	0.94	0.94	0.93	0.93	0.002	0.008	NS	NS
Serine	0.88	0.88	0.88	0.87	0.004	0.450	NS	NS
Threonine	0.81	0.82	0.79	0.80	0.004	0.009	NS	NS
Valine	0.89	0.89	0.88	0.88	0.002	0.014	NS	NS
IP6 degradation	0.20	0.55	0.34	0.55	0.034	NS	<0.001	NS

Table 5. Concentration of inositol phosphates in the excreta of caeectomised hens in Assay 1 using diets different in the level of P and phytase supplementation (g/kg dry matter; n = 8 hens per treatment)

P level Phytase supplementation	High		Reduced		Pooled SEM	Significance (<i>P</i> value)		
	-	+	-	+		P level	Phytase	P×Phy
IP6	20.7	11.8	17.0	12.0	0.89	NS	<0.001	NS
IP5	4.8	2.9	2.3	2.5	0.24	<0.001	0.016	0.005
IP4	1.2	1.4	0.5	0.7	0.09	<0.001	NS	NS
IP3	0.6	0.9	0.2	0.4	0.07	<0.001	0.036	NS

Table 6. Feed intake, efficiency of N utilisation and energy metabolisability determined with caecectomised hens using diets different in the level of P in Assay 1 and different in the main protein source in Assay 2 (n = 8 hens per treatment)

Assay 1								
P level	High		Reduced		Pooled	Significance (P value)		
Phytase supplementation	-	+	-	+	SEM	P level	Phytase	P×Phy
Egg Production, %	99	93	96	98	1.2	NS	NS	NS
Dry matter intake, g/d	109	107	107	111	1.5	NS	NS	NS
Nitrogen utilisation	0.51	0.51	0.51	0.51	0.007	NS	NS	NS
Energy metabolisability	0.82	0.82	0.81	0.82	0.003	NS	NS	NS
Assay 2								
Protein source	Sunflower meal		Rapeseed meal		Pooled	Significance (P value)		
Phytase supplementation	-	+	-	+	SEM	Protein	Phytase	Prot×Phy
Egg Production, %	93 ^a	90 ^{ab}	91 ^{ab}	78 ^b	2.1	0.079	0.040	NS
Dry matter intake, g/d	106 ^{ab}	109 ^a	107 ^{ab}	102 ^b	0.8	0.055	NS	0.016
Nitrogen utilisation	0.40 ^a	0.39 ^a	0.38 ^{ab}	0.31 ^b	0.011	0.005	0.029	NS
Energy metabolisability	0.69 ^b	0.69 ^b	0.73 ^a	0.72 ^a	0.004	<0.001	NS	NS

^{a,b} Means in a row not sharing a common superscript are significantly different ($P < 0.05$).

Table 7. Intake, excretion and utilisation of P and Ca determined with caeectomised hens in Assay 1 using diets different in the level of P and phytase supplementation (n = 8 hens per treatment)

P level Phytase supplementation	High		Reduced		Pooled SEM	Significance (P value)		
	-	+	-	+		P level	Phytase	P×Phy
<i>Phosphorus</i>								
Intake, mg/d	867 ^a	851 ^a	382 ^b	394 ^b	43.5	< 0.001	NS	NS
Excretion, mg/d	624 ^a	601 ^a	260 ^b	242 ^b	35.2	< 0.001	NS	NS
Utilisation	0.28	0.30	0.32	0.39	0.015	0.034	NS	NS
<i>Calcium</i>								
Intake, mg/d	4498	4414	4659	4803	68.9	0.049	NS	NS
Excretion, mg/d	1639	1685	1537	1690	100.7	NS	NS	NS
Utilisation	0.64	0.63	0.67	0.65	0.020	NS	NS	NS

^{a,b} Means in a row not sharing a common superscript are significantly different ($P < 0.05$).

Table 8. Digestibility of amino acids determined with caeectomised hens in Assay 2 using diets different in the main protein source and phytase supplementation (n = 8 hens per treatment)

Protein source	Sunflower meal		Rapeseed meal		Pooled SEM	Significance (P value)		
	Phytase supplementation -	+	-	+		Protein	Phytase	Prot×Phy
Alanine	0.80	0.80	0.81	0.80	0.003	NS	NS	NS
Aspartic acid	0.80	0.80	0.79	0.78	0.003	0.016	NS	NS
Arginine	0.90	0.90	0.88	0.88	0.002	< 0.001	NS	NS
Cystine	0.70	0.70	0.70	0.70	0.005	NS	NS	NS
Glutamic acid	0.88	0.88	0.87	0.86	0.002	0.002	NS	NS
Isoleucine	0.86	0.86	0.83	0.83	0.003	< 0.001	NS	NS
Leucine	0.87	0.86	0.86	0.85	0.002	0.064	NS	NS
Lysine	0.82	0.81	0.80	0.79	0.003	< 0.001	NS	NS
Methionine	0.90	0.91	0.90	0.89	0.002	0.025	NS	0.089
Phenylalanine	0.86	0.85	0.84	0.84	0.003	0.010	NS	NS
Proline	0.83	0.84	0.80	0.80	0.004	< 0.001	NS	NS
Serine	0.78	0.77	0.77	0.76	0.004	NS	NS	NS
Threonine	0.74	0.74	0.72	0.71	0.005	0.010	NS	NS
Valine	0.83	0.83	0.80	0.79	0.004	< 0.001	NS	NS