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► To cite this version:

Markus Rodehutschord, Romy Hempel, Peter Wendt. Phytase effects on the efficiency of utilisation and blood concentrations of phosphorus and calcium in Pekin ducks. *British Poultry Science*, 2006, 47 (03), pp.311-321. 10.1080/00071660600732353 . hal-00545297

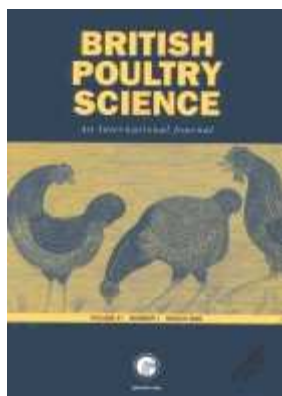
HAL Id: hal-00545297

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Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2005-023.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	28-Jan-2006
Complete List of Authors:	Rodehutsord, Markus; Universitaet Halle-Wittenberg, Institut fuer Ernaehrungswissenschaften Hempel, Romy Wendt, Peter
Keywords:	Ducks, Utilisation, Phytase efficacy, Phosphorus



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**CBPS_2005_023_R1: Duplicate of version in ManuscriptCentral
M MacLeod, Editor (14th March 2006)**

**Phytase effects on the efficiency of utilisation and blood concentrations of
phosphorus and calcium in Pekin ducks**

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Accepted for publication 2nd February 2006

Phytase efficacy in ducks

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2
3 **Abstract** 1. The objective was to study the effects of a supplementation of a 6-phytase
4 derived from the *Peniophora lycii* gene in the White Pekin duck.
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8 2. In two balance studies, low-phosphorus (P) diets consisting mainly of maize, solvent
9 extracted soybean meal and solvent extracted sunflower meal were supplemented with
10 phytase up to concentrations of 1500 U/kg (Study 1) or 2000 U/kg (Study 2). Each diet
11 (phytase level) was fed to 8-10 individually penned ducks. The intake and excretion of each
12 animal was measured for 5 consecutive d when ducks were in their third week of life.
13 Responses were described by nonlinear regression.
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22 3. Although the basal diets from the two studies were similar in ingredient composition,
23 efficiencies of P utilisation ($P \text{ accretion}/P \text{ intake} \times 100$) for the unsupplemented basal diets
24 were 39% in Study 1 and 30% in Study 2. Phytase supplementation significantly improved P
25 utilisation up to levels of about 55% in both studies. A plateau in P utilisation with an increase
26 in phytase supplementation was achieved in Study 2, but not in Study 1. The enzyme was
27 more efficient in Study 2 than in Study 1 at low rates of supplementation. Utilisation of
28 calcium (Ca) was significantly improved by phytase supplementation. Accretions of P and Ca
29 increased at a constant ratio.
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41 4. In a 5-week growth study, diets with an intentionally marginal P level were used. Diets
42 were fed either unsupplemented or supplemented with 1000 or 10000 U/kg of phytase. Eight
43 pens of 10 sex-separated ducks each (4 pens per sex) were allocated to each dietary treatment.
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60 5. Phytase significantly improved the growth of ducks of both sexes between d 1 and 21, but
not between d 22 and 35. Feed conversion rate was not affected by treatment. Blood serum
phosphate concentrations, but not calcium, were significantly increased by phytase
supplementation. Blood concentrations of creatinine, aspartate aminotransferase, and lactate
dehydrogenase remained unaffected while alanine aminotransferase was significantly reduced
by phytase supplementation.

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3 26 6. It was concluded that the efficacy of a microbial phytase varies even under similar
4
5 27 experimental conditions. Differences in intrinsic phytase activity of maize-soybean meal-
6
7 28 based diets may be responsible for this. The 6-phytase used has the potential to improve the
8
9 29 utilisation of plant P in duck feeding. A plateau in response was reached above 1500 U/kg.
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12 INTRODUCTION

13
14
15 31 It is well known that phosphorus (P), present as phytate in plant feedstuffs, is hardly available
16
17 32 to poultry in the absence of the enzyme phytase. Supplementing diets with products
18
19 33 containing microbial phytase is a tool to improving the utilisation of phytate P and, combined
20
21 34 with a reduced need for P supplementation, reducing the amount of P in excreta.
22
23

24 35 Duck meat production is continuously increasing on the global and European markets.
25
26 36 Average annual growth in duck meat production between 1998 and 2003 was 3% in Europe
27
28 37 and 6% worldwide (FAO, 2005). Yet, effective nutrition of ducks must not only meet the
29
30 38 animal's requirements for growth and health; with particular relevance in the case of P,
31
32 39 avoiding excessive intake and minimising excretion is also relevant for economic and
33
34 40 ecological reasons (Rodehutsord, 2005). Consequently, the role of phytase as a feed
35
36 41 supplement in ducks has been of increasing interest, especially the use of a 3-phytase derived
37
38 42 from the *Aspergillus niger* gene (Farrell *et al.*, 1993; Martin *et al.*, 1998; Orban *et al.*, 1999;
39
40 43 Attia, 2003; Wendt *et al.*, 2003). Because 3-phytases and 6-phytases show differences *in vitro*
41
42 44 properties, such as optimum pH, temperature stability, resistance to proteolytic enzymes
43
44 45 (Simon and Igbasan, 2002), and in animal studies in efficacy to improve P utilisation
45
46 46 (Augspurger *et al.*, 2003; Paditz *et al.*, 2004; Payne *et al.*, 2005), each phytase preparation
47
48 47 needs to be studied for its particular efficacy before supplementation levels can be
49
50 48 recommended for diet formulation. Changes in P accretion due to phytase supplementation,
51
52 49 determined at marginal dietary concentrations of P, are commonly regarded as a sensitive
53
54 50 measure of phytase efficacy. Also, growth and blood phosphate concentrations are often taken
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3 51 into consideration. Our objective was to study the efficacy of a 6-phytase derived from the
4
5 52 *Peniophora lycii* gene¹ based on balance, growth, and blood data.
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8 MATERIALS AND METHODS

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10 54 Two balance studies and one growth study were conducted. They were approved by the
11
12 55 animal welfare authorities in accordance with the German Animal Welfare Act.
13
14

15 **Balance study 1**

16
17 57 A basal diet that was adequate in ME and all nutrients with the exception of P and calcium
18
19 58 (Ca) was formulated. This was based on NRC (1994) recommendations and results of recent
20
21 59 amino acid requirement studies with Pekin ducks (Timmler and Jeroch, 1999; Bons *et al.*,
22
23 60 2002; Timmler and Rodehutschord, 2003). Ingredients were selected to achieve a combination
24
25 61 of low total P concentration with a high proportion of phytate P and low intrinsic phytase
26
27 62 activity (Table 1). Limestone was added to give a Ca concentration of about 6.5 g/kg of diet.
28
29 63 Analysed concentrations of P and Ca were 4.4 and 6.6 g/kg of diet. Ingredients were mixed in
30
31 64 one batch to ensure consistency. This mix was divided into 6 portions. Phytase was then
32
33 65 added to achieve the following activities (U/kg diet): 0, 250, 500, 750, 1000, and 1500. Diets
34
35 66 were mixed again and pelleted through a 3 mm die without using steam. Intended phytase
36
37 67 activities were confirmed by analysis with the exception of the highest supplementary level
38
39 68 (Table 2).
40
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Tables 1 and 2 near here

45
46 69 One-d-old male White Pekin ducklings (Duck-Tec Brüterei GmbH, Belzig,
47
48 70 Germany) were raised in floor pens on straw bedding with a commercial duck starter feed
49
50 71 containing adequate P. After 10 d, 60 of the 130 animals were selected for the experiment
51
52 72 based on appearance and body weight (BW). Each bird was individually penned in a balance
53
54 73 crate that was (in mm) 370 high, 360 wide, and 550 deep. Trays and crates were specially
55
56 74 constructed and closed at the bottom to avoid any loss of excreta. Crates were part of a system
57
58 75 in an animal house at the institute. Ten birds were allocated to each treatment diet (phytase
59
60

¹ The product used was Ronozyme P 5000 (CT), DSM Nutritional Products, Basel.

1
2
3 76 concentration) by BW. Diets were offered for 10 consecutive days with a quantitative
4
5 77 collection of excreta during the last 5 d. Excreta were collected from the trays before the
6
7 78 morning feeding time and bulk-stored at -18°C for each individual bird. During collection
8
9
10 79 and the 3e d prior to collection, feed allowance was slightly restricted in order to avoid feed
11
12 80 refusals. The amount (130 g/d) was chosen on the basis of the previous *ad libitum* intake. The
13
14
15 81 feed was offered in two meals per d (at around 09:00 h and 15:00 h). De-mineralised water
16
17 82 was always available from nipple drinkers with attached cups. The diet was, therefore, the
18
19 83 only source of P and Ca intake. Body weights were determined at the beginning and end of
20
21 84 the excreta collection period prior to the morning feeding time. Data from three ducks (one
22
23 85 from the treatment with 250 U/kg and two from the treatment with 500 U/kg) were not
24
25 86 considered because of incomplete feed intake ($<90\%$ of feed allowance).
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28

29 87 Thawed excreta samples were homogenised. All chemical analyses were run in
30
31 88 duplicate. Diets and excreta samples were analysed for dry matter (105°C) and ash content
32
33 89 (incineration at 550°C). Diets were also analysed for crude protein content (macro Kjeldahl-
34
35 90 N) according to official standards (Naumann and Bassler, 1976). Concentrations of P and Ca
36
37 91 were determined from filtered ash solutions after acid hydrolysis with an Inductively Coupled
38
39 92 Plasma Spectrometer (ICP-OES, JY 24, Jobin Yvon GmbH, Grassbrunn, Germany) as
40
41 93 described by Rodehutschord and Dieckmann (2005). Phytase activity was measured in
42
43 94 accordance with the method of Engelen *et al.* (1994), in which one phytase unit (U) is defined
44
45 95 as the amount of enzyme which, at 37°C and pH 5.5, liberates one μmol of inorganic
46
47 96 phosphate per minute from 0.0051 mol/l sodium phytate.
48
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50
51

52 97 Accretions of P and Ca (mg/d) were calculated as the differences between intake
53
54 98 (mg/d) and the amount recovered in the excreta (mg/d). Efficiency of utilisation is accretion
55
56 99 as a percentage of intake. The content of utilised P (P_u) in the diet is analysed P content (g/kg)
57
58 100 multiplied by the efficiency of P utilisation (%) divided by 100. Unexcreted organic matter
59
60 101 (UOM) was expressed as a percentage of OM intake.

1
2
3 102 Results were subjected to routine ANOVA procedures using the software package
4
5 103 SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA). Responses to phytase
6
7 104 supplementation were described by the following exponential equation as given by Robbins *et*
8
9
10 105 *al.* (1979):

$$11 106 \quad y = a + b (1 - e^{-k x}) \quad \text{[Eq. 1]}$$

12
13
14
15
16 107 where a is estimated response (y -value) at zero phytase supplementation

17
18 108 b is maximum response to supplemented phytase ($a + b =$ upper asymptote)

19
20 109 k is parameter describing the steepness of the curve

21
22 110 y is response criterion (efficiency of P utilisation or P_u concentration in the diet)

23
24
25 111 x is supplemented phytase (U/kg).

26
27
28
29 112 In order to describe the marginal efficacy of phytase, the first derivative of the above equation
30
31 113 was used when applied to the P_u concentration in the diet:

$$32
33
34
35 114 \quad y' = b k e^{(-k x)} \quad \text{[Eq. 2]}$$

36
37 115 This derivative describes the additional amount of P that is released by each incremental unit
38
39 116 of phytase ($\Delta P_u/\Delta U$) (Paditz *et al.*, 2004).

40
41
42 117 A linear regression was calculated to describe the relation between Ca accretion (y ,
43
44 118 mg/d) and P accretion (x , mg/d) using all phytase levels:

$$45
46
47
48 119 \quad y = a + m x \quad \text{[Eq. 3]}$$

49
50
51 120 where a is a constant, and m is the slope of the regression line.

52
53 121 Nonlinear and linear regression analysis was performed with the program GraphPad
54
55 122 Prism 4.02 (GraphPad Software Inc., San Diego, CA, USA). As parameters for the goodness
56
57 123 of fit, r^2 and $s_{y,x}$ are presented. The $s_{y,x}$ values are the standard deviations of the residuals,
58
59 124 which are the distances between the individual points and the calculated line.

Balance study 2

A basal diet very similar to that of Balance Study 1 was used (Table 1). Phytase was applied at the following concentrations (U/kg): 0, 250, 500, 750, 1000, 1500, and 2000. The highest level was included because no saturation of response was detected in Study 1. Intended differences in the supplementary levels were confirmed by analysis (Table 2). Analysed concentrations of P and Ca in the diets were 4.2 and 6.0 g/kg of diet. Details of diet and pellet preparation were as described for Balance Study 1.

One-d-old male White Pekin ducklings (Seddiner Zucht- und Mastenten GmbH, Westerscheps, Germany) were raised until they were 10 d old as described for Balance Study 1. Birds were then individually penned in the balance crates. With the exception of the unsupplemented basal diet, for which 10 ducks were used, each diet was allocated to 8 ducklings. The daily feed allowance during the collection period and the 3 d preceding was 90 g. All other experimental details, including excreta sampling, sample treatment and chemical and statistical analysis, were as described for Balance Study 1. Two animals were excluded from data evaluation due to incomplete feed intake (<90 % of feed allowance; one from the treatment without phytase supplementation and one belonging to the highest phytase level).

Growth study

The growth study comprised two phases: phase one from hatch to d 21 and phase 2 from d 22 to the end (d 35). Two basal diets were mixed for each phase (Table 1). They were calculated to meet the requirements as specified for the balance studies with the exception of P and Ca. Phosphorus concentrations were calculated to be slightly below the estimated requirement, as suggested by Rodehutschord (2005). The calculated concentrations of ME were 12.2 and 12.5 MJ/kg in phase one and two, respectively. In order to ensure consistency of the mixes, the total amount of feed needed for the respective phase was mixed in one lot and subsequently divided into three portions. Phytase was then supplemented to achieve the following activities

Phytase efficacy in ducks

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3 150 (U/kg diet): 0, 1000 and 10000. Diets were mixed again and pelleted through a 3 mm die
4
5
6 151 without using steam. Intended phytase activities were confirmed by analysis (Table 2).

7
8 152 One-d-old ducklings (120 of each sex) of a White Pekin duck hybrid (Duck-Tec
9
10 153 Brüterei GmbH, Belzig, Germany) were penned in groups of 10, sex-separated, on straw
11
12 154 bedding. Eight pens (4 with male and 4 with female ducklings) were randomly allocated to
13
14
15 155 each dietary treatment. Feed was offered *ad libitum* throughout the study. Troughs were
16
17 156 manually re-filled on demand from pre-weighed buckets. During the first week, pellets were
18
19 157 also offered from a flat plastic tray on the ground. Tap water was available from three
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21
22 158 drinkers per pen. Drinkers had an attached cup. The room temperature was 32°C from d 1 to
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24 159 4, and was then successively reduced to 16°C on d 16, and kept constant from then on.
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26
27 160 Continuous lighting was provided during the first week, and a 20 h light:4 h dark pattern was
28
29 161 applied from d 8 onwards.

30
31 162 Ducks were weighed at the onset of the experiment and at weekly intervals
32
33 163 thereafter. Feed consumption was simultaneously determined on a per-pen basis. Ducks were
34
35 164 continuously monitored for general health status, and no abnormalities were observed during
36
37 165 the trial. On d 35, blood samples were taken from two ducks per pen by wing vein puncture.
38
39 166 Blood samples were centrifuged at 2000 g and 20°C for 10 min. The serum samples were
40
41 167 spectrophotometrically analysed² for inorganic phosphate (P_i) after conversion to ammonium
42
43 168 phosphomolybdate, for Ca after conversion to a Ca-o-cresolphthalein complex, and for
44
45 169 creatinine after a Jaffé reaction with alkaline picrate. The enzymes alanine aminotransferase
46
47 170 (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined
48
49 171 with commercial test kits. Due to some technical problems, which were probably caused by
50
51 172 the high viscosity of the serum samples, measurements could not be made for all birds. The
52
53 173 minimum number of replicates per treatment and sex was, however, less than 6 in only three
54
55 174 cases. Diets were analysed according to the methods described for Balance Study 1.
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57
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² Conducted by Labor für klinische Diagnostik GmbH&Co. KG, Bad Kissingen, Germany

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3 175 Data were subjected to two-way ANOVA procedures using the software package
4
5 176 SPSS for Windows (version 11.0, SPSS Inc., Chicago, IL, USA).

177 RESULTS

178 Balance studies

179 In Balance Study 1, ducks weighed 780 g (SD 30 g) and 1079 g (SD 30 g) at the beginning
180 and end of the collection period, respectively. Ducks in Balance Study 2 had lower BW than
181 those in Balance Study 1 (601 g (SD 30 g) at the beginning and 828 g (SD 31 g) at the end of
182 the collection period). Neither study showed any significant effect of phytase supplementation
183 on growth, which was what we expected considering the short period (5 d) and restricted feed
184 supply.

Tables 3, 4 and 5 near here

185 In Balance Study 1, the efficiency of P utilisation was 39% when no phytase was
186 added (Table 3). Phytase supplementation significantly reduced the excretion of both P and
187 Ca ($P \leq 0.001$). Accordingly, both accretion and efficiency of utilisation of P and Ca were
188 significantly improved by phytase supplementation ($P \leq 0.001$). Results were similar in
189 Balance Study 2 (Table 4). However, efficiency of P and Ca utilisation on the
190 unsupplemented basal diet was lower than in Study 1. While a plateau in the efficiency of P
191 utilisation was not attained in Study 1, it did become obvious with the higher range of phytase
192 supplementation in Study 2 (Figure 1). The greatest P utilisation found in Study 2 was about
193 54% (Figure 1, Table 5). A higher plateau in efficiency of P utilisation was indicated by a
194 regression analysis for Study 1 (63%, sum of parameters a and b), but this estimate is based
195 on an extrapolation of the curve without the necessary data for phytase levels above 1500
196 U/kg. Accordingly, the SE of estimate for parameter b was high in Balance Study 1 (Table 5).

197 The content of P_u in the diet increased by up to 1 g/kg at a high level of phytase
198 supplementation (Figure 2). Marginal efficacy was greater by a factor of 3 in Study 2 than it
199 was in Study 1 at a low level of supplementation (Figure 3). Differences in marginal
200 efficiency between studies decreased with increasing supplementation.

Figs 1, 2, 3, 4 near here

Phytase efficacy in ducks

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3 201 There was a linear relationship between accretions of P and Ca, calculated at all
4
5 202 phytase levels (Figure 4). The Ca:P ratio in accretion caused by phytase supplementation was
6
7
8 203 1.63:1 in Study 1 and 1.90:1 in Study 2, as indicated by the slopes of the regression lines.
9

10 204 In relation to organic matter intake, about 73% (Study 1) and 70% (Study 2) of
11
12 205 organic matter was not recovered in excreta. This proportion was not significantly affected by
13
14
15 206 phytase supplementation (Tables 3 and 4).

Tables 5, 6 and 7 near here

207 **Growth study**

208 A total of 5 ducks had to be culled in the course of the experiment because of mobility
21
22 209 restrictions and standing ability (one male in the treatment without phytase, one male and one
23
24
25 210 female each in the treatments with 1000 and 10000 U/kg).

26
27
28 211 Initial BW was 58.9 g (SE 1.4 g) and 58.1 g (SE 1.1 g) for male and female
29
30 212 ducklings, respectively. Growth rate was high, overall. Males grew significantly more rapidly
31
32 213 than females, and final BWs of about 2.6 kg (males) and 2.4 kg (females) were achieved at d
33
34
35 214 35 (Table 6). Phytase supplementation significantly increased growth rate in phase one but
36
37 215 not in phase two. The effect on growth for the total experimental period was significant. In
38
39 216 both sexes, the BW at any given time was always greatest in birds that were given the diet
40
41
42 217 with 10000 U of phytase per kg. The feed/BW gain ratio was 1.52 in phase one and 2.27 in
43
44 218 phase two, without a significant effect of sex and phytase supplementation.

45
46 219 Phosphate concentration in blood serum, but not Ca concentration, was significantly
47
48 220 increased by phytase supplementation (Table 7). The concentration of ALT was significantly
49
50
51 221 reduced by phytase supplementation. Concentrations of creatinine, AST and LDH were not
52
53 222 significantly affected by phytase supplementation, nor were they significantly different
54
55
56 223 between males and females.

224 **DISCUSSION**

225 The results of Balance Studies 1 and 2 indicated that the efficiency of P utilisation in the basal
226
226 diet is a critical factor in phytase efficacy studies. In Balance Study 1, the P utilisation values

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3 227 found for all phytase supplemented diets were similar to those measured in Study 2 for the
4
5 228 corresponding treatments but the basal level was much higher (39 vs. 30%). Rodehutschord
6
7 229 and Dieckmann (2005) described a range in P utilisation for plant-based diets of from 28 to
8
9 230 49% in ducks, 40 to 73% in broilers, and 46 to 63% in turkeys. This was based on a review of
10
11 231 studies using 3 to 5-week-old birds and diets with low P content, as well as low or
12
13 232 undetectable intrinsic phytase activity. The wide ranges in P utilisation are, therefore, difficult
14
15 233 to explain. The differences found between the two basal diets in the present studies were
16
17 234 remarkable because of the very similar experimental conditions and diets. Dietary ingredients
18
19 235 were, however, from different batches. Concentrations of phytate P, which were not analysed,
20
21 236 may have been different. A difference existed in intrinsic phytase activity, which was higher
22
23 237 in Study 1 than in Study 2, yet close to the detection limit. This difference corresponds to the
24
25 238 ranking found in the efficiency of P utilisation for the two basal diets. Phytase was most
26
27 239 effective at a low level of supplementation. Therefore, small differences in the intrinsic
28
29 240 phytase activity of the ingredients may become relevant for P utilisation. Furthermore,
30
31 241 intrinsic phytase activity may be underestimated by the method of Engelen *et al.* (1994),
32
33 242 which was applied in the present study and was developed for supplemental microbial
34
35 243 phytase. Zimmermann *et al.* (2002) determined intrinsic phytase activity either in direct
36
37 244 incubation or after extraction in a buffer with pH 5.5. Extraction caused phytase activities that
38
39 245 were only about 0.3 (wheat) and 0.5 (barley, rye) of those measured in direct incubation. We
40
41 246 hypothesise that variation in intrinsic phytase activity of maize, although at a low level,
42
43 247 contributes to the variation found in P utilisation of maize/soybean meal-based diets. As the
44
45 248 present studies indicate a great relevance for efficacy studies with microbial phytases – not
46
47 249 only in ducks – this hypothesis should be investigated in future studies.

57 250 Intestinal phytase activity was detected in brush border vesicles obtained from
58
59 251 duodenal and jejunal mucosa cells of 18-d-old Pekin ducks (Rush *et al.*, 2005). Alkaline
60
252 phosphatase, which is involved in phytate hydrolysis, was also found in the small intestine

Phytase efficacy in ducks

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3 253 mucosa of Pekin ducks (King *et al.*, 2000). Whether these intestinal enzyme activities are
4
5 254 high enough to be relevant for intestinal phytate hydrolysis is unclear. Along this line, it
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7
8 255 remains uncertain whether the differences in P utilisation found between Studies 1 and 2 can
9
10 256 be explained by differences in intestinal enzyme activities.

11
12 257 Supplements of *Aspergillus niger* 3-phytase were found to positively affect P
13
14 258 utilisation in ducks (Farrell *et al.*, 1993; Wendt *et al.*, 2003). The 6-phytase that was used in
15
16 259 the present studies improved the efficiency of P utilisation, although there were some
17
18 260 differences in efficacy between the two studies. The responses to phytase were nonlinear in
19
20 261 the present studies, as they were in the study with 3-phytase by Wendt *et al.* (2003), using a
21
22 262 similar experimental approach. Lowest and highest P utilisation values in their study were 28
23
24 263 and 57%, which basically corresponds with results from Studies 1 and 2 of the present paper.
25
26 264 As outlined above, the efficiency of P utilisation in the basal diet is critical in the
27
28 265 determination of microbial phytase efficacy. Interaction may occur between the efficacy of
29
30 266 supplemented phytase and efficiency of P utilisation in the basal diet. In our opinion, this
31
32 267 weakens the comparison of efficacy values from different studies and makes it almost
33
34 268 impossible to compare phytase efficacy across different papers even when the experimental
35
36 269 approaches are similar.

37
38 270 Supplementing with 1000 U/kg provided an additional 0.5 (Study 1) and 0.9 (Study
39
40 271 2) g P_u per kg of diet. Inorganic P sources were found to differ in P utilisation in ducks
41
42 272 between 77 and 100 % (Wendt and Rodehutschord, 2004). Equivalence values for phytase can
43
44 273 be calculated based on these values and the phytase efficacy determined in the present studies.
45
46 274 Results of the two balance studies and serum P_i concentrations measured in the growth study
47
48 275 indicate that an increase in P utilisation, although small, can still be achieved at inclusions
49
50 276 greater than 1000 U/kg. In studies with turkeys, supplementing the diet with 10000 instead of
51
52 277 1000 U/kg of a so-called consensus 3-phytase still increased the efficiency of P utilisation and

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2
3 278 tibia ash (Esteve-Garcia *et al.*, 2005). Augspurger and Baker (2004) found that chickens still
4
5 279 responded in tibia ash to inclusions 10 to 20 times higher than usual.
6
7

8 280 Phytase supplementation increased the efficiency of Ca utilisation. It is not clear
9
10 281 whether this is a direct phytase effect in the sense of a release of Ca from phytate, especially
11
12 282 because a high proportion of Ca originated from limestone. Accretion of Ca correlated closely
13
14 283 with P accretion (Figure 4). This indicates that Ca excretion was reduced with phytase
15
16 284 supplementation simply because of a higher intermediary demand for Ca as a consequence of
17
18 285 increased P accretion.
19
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22 286 Phytase improved precaecal P digestibility and tibia ash content in some studies, but
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24 287 growth was not always positively effected, depending on the level of P supply and age
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26 288 (Martin *et al.*, 1998; Orban *et al.*, 1999; Attia, 2003). In the present growth study, the P
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28 289 supply in phase one was not high enough to meet the duck's requirement for available P.
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30 290 Consequently phytase supplementation had effects on growth. In phase two, no effect on
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32 291 growth was detected, indicating that P supply was sufficient for growth without phytase.
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34 292 Similarly, Orban *et al.* (1999) found no significant effect on the growth of 3 to 6-week-old
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36 293 ducks when they supplemented a diet containing 4.8 g P/kg with 750 U phytase/kg, but
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38 294 plasma P concentration was almost doubled. While the increase in the serum P_i level indicates
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40 295 that phytate hydrolysis in the present study was further improved when 10000 instead of 1000
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42 296 U/kg were used, no beneficial effects were measured with regard to growth and feed
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44 297 conversion. Lower basal P levels should be applied if growth effects are to be further studied.
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50 298 Data on the activity of liver enzymes or creatinine in the blood of Pekin ducks were
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52 299 not found in the literature, but some data from other ducks were reported. Generally, reported
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54 300 blood concentrations for ducks appear difficult to compare because they show a large
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56 301 variation between and within studies, the reasons for which are only partly known. Mule
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58 302 ducks had significantly increased blood plasma concentrations of LDH and ALT after a 12-d
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60 303 force-feeding period (Tardieu *et al.*, 2004) or after an 11-week application of high

Phytase efficacy in ducks

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3 304 concentrations of fumonisin B₁ (Tran *et al.*, 2005). Laying Tsaiya ducks developed higher
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5 305 blood serum levels of LDH and AST when exposed to dietary supplements of 3-nitro-4-
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8 306 hydroxyphenylarsonic acid (Chen *et al.*, 2000). Olayemi *et al.* (2002) reported that, in the
9
10 307 Nigerian duck, plasma concentrations of ALT and AST were lower in younger ducks than in
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12 308 adults. While overall ALT levels were similar in the Pekin ducks from our study and those
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14 309 from the other papers (Tardieu *et al.*, 2004; Tran *et al.*, 2005), the LDH level was much
15
16 310 lower. In contrast, activities of ALT and AST in our study were lower and activity of LDH
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18 311 was higher level than the values for adult mule ducks given by Samour (2000). We found no
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20 312 explanation as to why phytase supplementation significantly reduced serum ALT
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22 313 concentrations in our study. This observation corresponds, however, with the finding that
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24 314 relative liver weights were reduced due to phytase supplementation (data not shown). Values
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26 315 for creatinine lie within the range reported by Samour (2000) for adult mule ducks.
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316 Conclusions

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33 317 Phytase is a promising tool for improving the utilisation of plant P and reducing P excretion in
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35 318 ducks through a reduced use of inorganic P supplements. The efficacy of microbial phytase
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37 319 decreases with an increasing level of supplementation. A plateau in P utilisation is attainable
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39 320 with this phytase between 1500 and 2000 U/kg. Intrinsic phytase activity in maize/soybean
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41 321 meal-based diets influences the efficacy of supplemental microbial phytase.
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322 ACKNOWLEDGEMENTS

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47 323 The authors gratefully acknowledge the financial support given for this study by DSM
48
49 324 Nutritional Products, Basel. We thank Dr. Woitow for veterinary observation of the birds and
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51 325 taking the blood samples.
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Table 1. *Composition of the basal diets (g/kg unless otherwise stated)*

	Balance study		Growth study	
	1	2	Week 1-3	Week 4-5
<i>Ingredients:</i>				
Maize	622.7	656.0	605.5	660.7
Soybean meal, solvent extracted	240.0	200.0	245.0	200.0
Sunflower meal, solvent extracted	100.0	100.0	100.0	100.0
Soybean oil	12.0	25.0	10.0	10.0
Premix ¹ , P-free	10.0	10.0	10.0	10.0
Limestone (CaCO ₃)	8.0	4.0	12.5	11.0
Monocalcium phosphate (Ca(H ₂ PO ₄) ₂)	-	-	10.0	2.0
Salt (NaCl)	3.5	3.5	2.5	3.0
L-lysine·HCl	2.5	-	2.5	1.5
L-threonine	-	-	1.2	-
DL-methionine	1.3	1.5	0.8	1.8
<i>Analysed:</i>				
Total phosphorus	4.4	4.2	7.1	4.9
Phytate P (calculated)	2.8	2.6	2.8	2.4
Calcium	6.6	6.0	10.2	7.8
Crude protein	188	193	192	178
ME (calculated), MJ/kg	12.4	12.5	12.2	12.5

¹Premix contained (per kg): 233 g Ca, 3.6 g Mg, 80 g Na, 2 g Fe, 10 g Zn, 10 g Mn, 3 g Cu, 50 mg Co, 100 mg I, 50 mg Se, 10 g butylated hydroxytoluene (antioxidant), 172 mg retinol acetate, 5 mg cholecalciferol, 3 g DL-alpha tocopheryl acetate, 200 mg menadione sodium bisulphite, 200 mg thiamine mononitrate, 300 mg riboflavin, 200 mg pyridoxine hydrochloride, 1.5 mg cyanocobalamin, 1000 mg Ca-D-pantothenate, 3 g nicotinic acid, 10 mg D-biotin, 100 mg folic acid, 50 g choline chloride.

Table 2. *Intended and analysed phytase activity in the pelleted diets¹ (U/kg)*

Intended	0	250	500	750	1000	1500	2000	10000
Analysed								
Balance study 1	83	131	416	734	822	1126		
Balance study 2	56	191	437	694	862	1520	1759	
Growth study wk 1-3	95				1040			9600
Growth study wk 4-5	50				910			8970

¹ Values given for the supplemented diets are the analysed total activities minus the intrinsic activity determined in the unsupplemented diet.

Phytase efficacy in ducks

Table 3. Intake, excretion and efficiency of utilisation of phosphorus and calcium in three-week-old Pekin ducks given diets with different phytase levels in Balance Study 1

	Phytase supplementation (U/kg)						Pooled SEM	P (ANOVA)
	0	250	500	750	1,000	1,500		
Sample size (n)	10	9	8	10	10	10		
Phosphorus								
Intake (mg/d)	577	577	577	577	577	577	0.0	
Excretion (mg/d)	349	331	312	278	287	257	5.3	≤0.001
Accretion (mg/d)	226	247	264	296	289	320	5.3	≤0.001
Utilisation (%)	39.4	42.8	45.8	51.6	50.2	55.5	0.92	≤0.001
Calcium								
Intake (mg/d)	853	853	853	853	853	853	0.0	
Excretion (mg/d)	512	516	492	424	445	387	9.1	≤0.001
Accretion (mg/d)	340	340	360	426	407	468	9.1	≤0.001
Utilisation (%)	39.9	39.7	42.3	50.1	47.8	54.8	1.07	≤0.001
UOM ¹ (%)	73.1	73.6	73.1	73.6	73.3	73.4	0.13	0.788

¹ UOM = unexcreted organic matter in relation to organic matter intake.

Phytase efficacy in ducks

Table 4. Intake, excretion and efficiency of utilisation of phosphorus and calcium in three-week-old Pekin ducks given diets with different phytase levels in Balance Study 2

	Phytase supplementation (U/kg)							Pooled SEM	P (ANOVA)
	0	250	500	750	1000	1500	2000		
Sample size (n)	9	8	8	8	8	8	7		
Phosphorus									
Intake (mg/d)	374	374	374	374	374	374	374	0.0	
Excretion (mg/d)	263	224	195	190	184	176	171	4.9	≤0.001
Accretion (mg/d)	112	150	179	184	191	199	204	4.9	≤0.001
Utilisation (%)	29.8	40.1	47.9	49.3	50.9	53.1	54.4	1.30	≤0.001
Calcium									
Intake (mg/d)	541	541	541	541	541	541	541	0.0	
Excretion (mg/d)	353	269	208	202	184	169	174	9.5	≤0.001
Accretion (mg/d)	188	272	333	339	357	371	366	9.5	≤0.001
Utilisation (%)	34.7	50.2	61.6	62.6	65.9	68.7	67.8	1.75	≤0.001
UOM ¹ (%)	70.5	69.9	70.4	70.4	69.9	70.5	70.0	0.15	0.826

¹ UOM = unexcreted organic matter in relation to organic matter intake.

Table 5. Results of parameter estimate (\pm SE) when a function of the general type $y = a+b \times (1-e^{-kx})$ was fitted to the response data from Balance Studies 1 and 2

	Estimated parameter			r^2	$s_{y.x}$
	a	b	k		
P utilisation (%) (Figure 1)					
Study 1	39.17 ± 1.29	23.93 ± 9.08	0.000746 ± 0.000487	0.62	4.4
Study 2	29.82 ± 1.67	24.10 ± 2.07	0.002375 ± 0.000513	0.73	5.2
Dietary content of utilised P (g/kg) (Figure 2)					
Study 1	-0.010 ± 0.076	1.320 ± 1.030	0.000534 ± 0.000622	0.94	0.08
Study 2	-0.001 ± 0.035	1.005 ± 0.042	0.002363 ± 0.000243	0.99	0.04

Phytase efficacy in ducks

Table 6. Means (standard deviation in italics) for body weight (BW) and BW gain of ducks given different phytase levels in the growth study (n=4 pens of 10 ducks per treatment)

Phytase (U/kg)	0			1000			10000			P (ANOVA)		
	Sex	Male			Female			Phytase	Sex	Phy×S		
Body weight, g/duck												
d 7	226 <i>5.4</i>	229 <i>7.5</i>	236 <i>5.3</i>	215 <i>4.4</i>	216 <i>17.0</i>	223 <i>8.9</i>	0.177	0.004	0.969			
d 14	684 <i>35.7</i>	690 <i>27.0</i>	718 <i>8.3</i>	626 <i>16.7</i>	671 <i>17.5</i>	676 <i>5.6</i>	0.003	<0.001	0.203			
d 21	1334 <i>30.1</i>	1334 <i>19.0</i>	1391 <i>28.2</i>	1215 <i>20.5</i>	1280 <i>13.4</i>	1293 <i>21.0</i>	<0.001	<0.001	0.028			
d 28	1995 <i>52.8</i>	1991 <i>35.3</i>	2099 <i>43.4</i>	1831 <i>20.8</i>	1901 <i>13.0</i>	1956 <i>10.3</i>	<0.001	<0.001	0.102			
d 35	2634 <i>33.0</i>	2605 <i>7.0</i>	2664 <i>64.4</i>	2437 <i>73.5</i>	2469 <i>41.7</i>	2531 <i>27.1</i>	0.024	<0.001	0.325			
BW gain, g/duck												
d 1-21	1,276 <i>30.3</i>	1,274 <i>18.7</i>	1,332 <i>27.0</i>	1,157 <i>21.6</i>	1,222 <i>14.0</i>	1,236 <i>22.0</i>	<0.001	<0.001	0.031			
d 22-35	1,299 <i>62.0</i>	1,271 <i>19.7</i>	1,273 <i>38.4</i>	1,222 <i>61.9</i>	1,189 <i>29.0</i>	1,237 <i>10.6</i>	0.331	0.001	0.490			
d 1-35	2,575 <i>32.9</i>	2,546 <i>6.4</i>	2,605 <i>63.4</i>	2,379 <i>74.0</i>	2,411 <i>42.1</i>	2,473 <i>28.3</i>	0.024	<0.001	0.330			
Feed/BW gain, g/g												
d 1-21	1.52 <i>0.032</i>	1.52 <i>0.008</i>	1.51 <i>0.009</i>	1.53 <i>0.026</i>	1.53 <i>0.024</i>	1.53 <i>0.036</i>	0.818	0.139	0.944			
d 22-35	2.21 <i>0.155</i>	2.25 <i>0.052</i>	2.28 <i>0.048</i>	2.27 <i>0.082</i>	2.37 <i>0.074</i>	2.21 <i>0.202</i>	0.476	0.472	0.289			
d 1-35	1.87 <i>0.080</i>	1.88 <i>0.023</i>	1.89 <i>0.026</i>	1.91 <i>0.048</i>	1.94 <i>0.032</i>	1.87 <i>0.104</i>	0.524	0.247	0.431			

Phytase efficacy in ducks

Table 7. Means (standard deviation in italics) of blood serum concentrations and enzyme activities determined in 35-d-old Pekin ducks given different phytase levels

Phytase (U/kg)	0			1000			10000			P (ANOVA)		
	Sex		Male	Female		Phytase	Sex	Phy×S				
PO ₄ ³⁻ , mmol/l	1.53	1.85	2.49	2.06	1.94	2.63	<0.001	0.017	0.177			
	<i>0.30</i>	<i>0.39</i>	<i>0.21</i>	<i>0.42</i>	<i>0.30</i>	<i>0.31</i>						
Ca, mmol/l	2.93	2.96	3.04	3.07	3.01	3.03	0.559	0.150	0.353			
	<i>0.14</i>	<i>0.15</i>	<i>0.10</i>	<i>0.08</i>	<i>0.13</i>	<i>0.12</i>						
Creatinine, µmol/l	24.0	26.0	23.3	25.0	24.7	24.4	0.193	0.697	0.260			
	<i>2.47</i>	<i>0.99</i>	<i>2.31</i>	<i>1.12</i>	<i>2.39</i>	<i>2.16</i>						
LDH, ¹ U/l	421	404	372	353	363	371	0.883	0.195	0.609			
	<i>96</i>	<i>82</i>	<i>82</i>	<i>55</i>	<i>77</i>	<i>80</i>						
ALT, U/l	19.3	19.3	16.5	23.2	18.0	17.1	0.025	0.399	0.248			
	<i>4.56</i>	<i>4.03</i>	<i>3.25</i>	<i>4.82</i>	<i>2.92</i>	<i>3.83</i>						
AST, U/l	6.6	6.7	6.8	7.4	6.7	6.8	0.948	0.694	0.837			
	<i>1.17</i>	<i>2.99</i>	<i>1.51</i>	<i>0.80</i>	<i>1.76</i>	<i>2.12</i>						

¹ LDH: lactate dehydrogenase, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Figure legends

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- Figure 1. *Effect of phytase supplementation on efficiency of phosphorus utilisation studied in three-week-old Pekin ducks (means and SD). An exponential equation was fitted to the data and the parameter estimates are summarised in Table 5.*
- Figure 2. *Increase in the dietary content of utilised phosphorus (P_u) depending on phytase supplementation. Calculations were made with treatment means based on analysed dietary phosphorus content and measured efficiency of phosphorus utilisation as given in Tables 3 and 4. Estimated parameters of the exponential equations are summarised in Table 5.*
- Figure 3. *Marginal efficacy of supplemented phytase in diets for Pekin ducks. The lines show the first derivatives of the functions from Figure 2 according to Equation 2. Marginal efficacy is the increment in the content of utilised phosphorus (P_u) achieved with each increment in phytase supplementation.*
- Figure 4. *Relationship between phosphorus accretion and calcium accretion in Pekin ducks given diets with variable phytase levels. The equations show the estimated parameters (\pm SE of estimate) of linear regressions fitted to the data.*

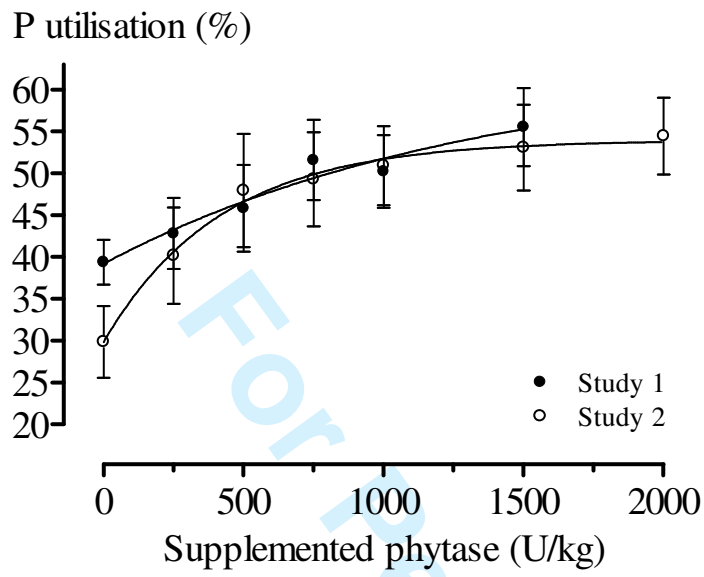


Fig. 1 of Rodehutschord et al., 2005-023

Phytase efficacy in ducks

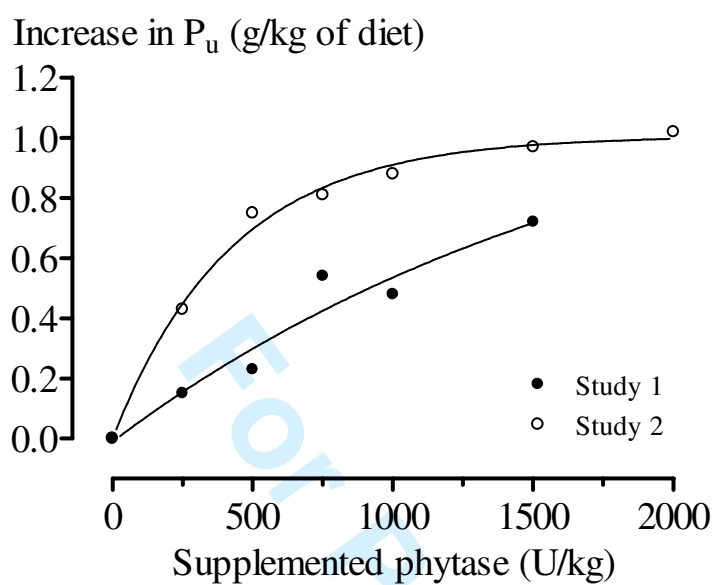


Fig. 2 of Rodehutschord et al., 2005-023

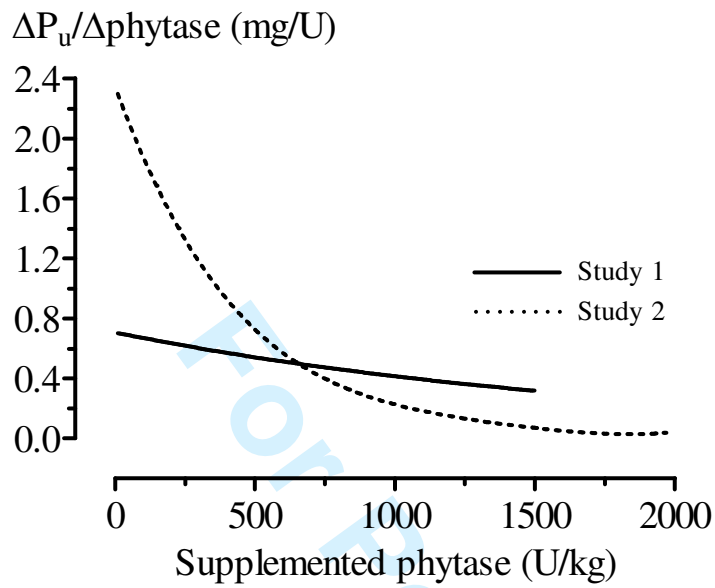
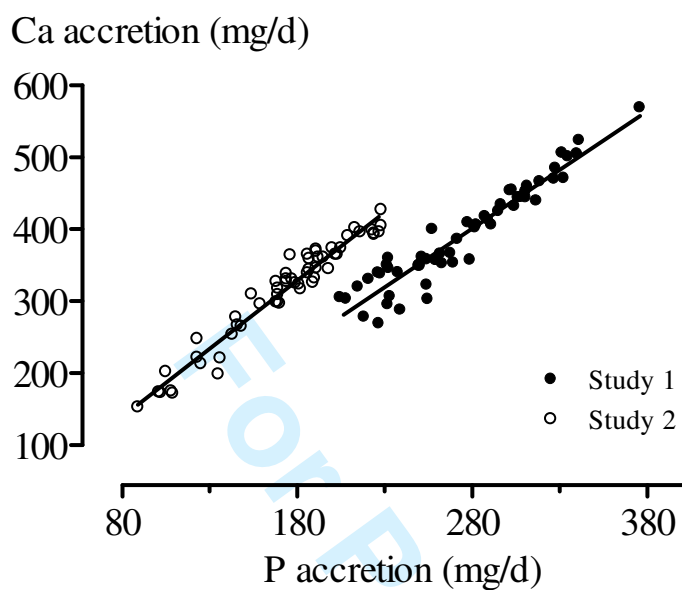


Fig. 3 of Rodehutschord et al., 2005-023

Phytase efficacy in ducks



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$$Y_{\text{Study 1}} = -56(\pm 20) + 1.63(\pm 0.07) x; r^2: 0.91; s_{y,x}: 21$$

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$$Y_{\text{Study 2}} = -13(\pm 11) + 1.90(\pm 0.06) x; r^2: 0.95; s_{y,x}: 16$$

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Fig. 4 of Rodehutschord et al., 2005-023