Phytase in poultry diets
The latest on phytase enzyme technology and use?

Jan Dirk van der Klis
Phytase

the feed additive studied the most, what’s there still to be known?
Contents

- Use of phytase in animal diets
- Requirements for effective commercial use
- Phytase efficacy in the GI tract, potential and limitations
  - Phytate solubility, feedstuff choice
  - Feed(stuff) particle size
  - Dietary calcium source and level
  - Digestibilities of amino acids and other nutrients
- Potential of using high phytase dose levels
- Phytase ranking on efficacy
Phytase use

“Phytase is used in 60% of swine and 80% of poultry diets”
“Global feed enzyme market values 725 million US$“
“Growth trend of roughly 5-7% year-on-year”
Phytase

Hydrolyses phytate-P to
- increase availability of vegetable P
- degrade anti-nutritional effects of phytate

Main classification of commercial phytases
- 3- or 6-phytase
- Fungal or bacterial phytase

Location of phytin:
In cereals: in aleurone layer
In oil seeds: associated with storage proteins throughout the seed
Enzymatic Phytate Degradation

A. niger

D-Ins(1,2,4,5,6)P$_5$

Ins(1,2,3,4,5,6)P$_5$

lily

Ins(1,2,3,4,6)P$_5$

E. coli

D-Ins(2,3,4,5,6)P$_5$

D-Ins(1,2,3,5,6)P$_5$

rye

Ins(1,2,3,4,5,6)P$_6$

D-Ins(1,2,3,4,5)P$_5$
Anti-nutritional effects of phytate

Due to binding of Ca, AA … to phytate

Linear effect sign.; quadratic effect NS

FWG= 916-46.2*X (r^2=0.90)

FCR= 1.429+ 0.0186*X (r^2=0.87)

Schothorst experiment, report 633 (2005)
Commercial type diets
Phytase effects in cereal-based diets

Set up:
- Phytate hydrolyses measured at ileal and faecal level in 3-wk old broilers
- Diets with 57% test cereal with intrinsic phytase
- 0.30% NPP, Ca 0.72-0.87% in diets
- 3-Phytase added
- Diets fed as mash

Result:
- Ileal P contains 70-88% and excreta P contains 26-76% phytate P
- Phytate degradation not completed

Leytem et al. (2008)
Dealing with phytate ...

Phytate utilization by 3-phytase \textit{in vivo} is on average max. 50\% (Selle \textit{et al.} 2006)

- **Phytase source**
- **Phytase dose**

- **Feedstuffs**
  - Physical factors
    - Phytate source
    - Phytate solubility
    - Feed particle size
    - ...

- **Animal**
  - Physiological factors
    - pH
    - Retention time
    - Ca/P requirement
    - ....
New phytases are developed:

- More effective release of phytate P in the GI-tract (irrespective of diet composition)
- More effective elimination of phytate as anti-nutritional factor?
- Improved processing stability (conditioning and pelleting)
- Improved storage stability
- Reduced phytase production costs
More effective release of phytate P

**Phytase product:**
- pH optimum and pH profile
- Substrate affinity and rate of degradation under acidic conditions in proximal GI tract
- Resistance against endogenous proteases

**Other measures:**
- Increased phytase dose level – “super dosing”?
- Lowering dietary Ca contents
- Increasing phytate solubility/accessibility
- Application in combination with other additives
### Characteristics of Commercial Phytases

**pH optimum, pH profile, kinetic constants**

*in vitro determinations*

<table>
<thead>
<tr>
<th>characteristics</th>
<th><em>P. lycii</em></th>
<th><em>A. niger</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH optimum</td>
<td>4.0 - 5.0</td>
<td>2.2 + 5.5</td>
<td>3.5 - 4.5</td>
</tr>
<tr>
<td>pH profile - activity at pH 3.0 (activity at pH 5.5 = 100%)</td>
<td>18 - 25%</td>
<td>38 - 50%</td>
<td>86 - 149%</td>
</tr>
<tr>
<td><strong>kinetic constants at pH 3.0:</strong></td>
<td></td>
<td></td>
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<tr>
<td>$K_M$ [μM]</td>
<td>73 - 81</td>
<td>113 - 135</td>
<td>174 - 386</td>
</tr>
<tr>
<td>$k_{cat}$ [s$^{-1}$]</td>
<td>1216 - 1325</td>
<td>166 - 198</td>
<td>823 - 1076</td>
</tr>
<tr>
<td>$k_{cat}/K_M$ [10$^6$ s$^{-1}$ M$^{-1}$]</td>
<td>15 - 18</td>
<td>1.3 - 1.7</td>
<td>3.5 - 6.2</td>
</tr>
<tr>
<td>pH stability</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>pH 2.0, 37°C, 1 hr</td>
<td>42 - 49%</td>
<td>69 - 83%</td>
<td>88 - 93%</td>
</tr>
<tr>
<td><strong>protease resistance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 2.0, 37°C, 1 hr, 2000 U pepsin</td>
<td>14 - 25%</td>
<td>40 - 50%</td>
<td>92 - 98%</td>
</tr>
<tr>
<td>37°C, 1 hr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ stomach digesta</td>
<td>53 - 60%</td>
<td>60 - 70%</td>
<td>83 - 93%</td>
</tr>
<tr>
<td>+ crop digesta</td>
<td>92 - 97%</td>
<td>93 - 98%</td>
<td>93 - 96%</td>
</tr>
</tbody>
</table>

(adapted from Greiner, IPS-2)
The gastrointestinal tract

Retention time (RT) can be increased by antiperistalsis!
InsP$_6$ hydrolysis in segments of the digestive tract of broilers (% means and SD)

- Maize-soybean meal based diet
- 10 pens per treatment from d16 to d25
- Three different phytase supplements
- Digesta samples from different segments

Values without a common superscript within a segment are significantly different according to t-test ($p \leq 0.05$); BD = basal diet.

Zeller et al. (2012)
More effective elimination of phytate as anti-nutritional factor

**Phytase product:**
- Bacterial 6-phytases preferentially hydrolyse higher molecular weight IPs (see Cowieson *et al.* 2011), i.e. those with highest ANF effects
  - eliminating more IP6 and IP5 per unit of P-release

**Phytase dose:**
- Increased phytase dose level to increase rate and amount of phytate degradation
Grinding: coarse particles increase phytate-P degradation in young broilers

Set-up:
- 1-day-old broiler chicks
- Mixed sex
- Mash diets: 10 g Ca; 5 g tP
- Fecal collection: 14 days

3-Phytase:
- Caused by:
  - Longer retention time in the crop and gizzard?
  - Larger gizzard with lowered pH?

No sign. interaction effect; Similar effects on Ca retention and tibia ash

Kasim & Edwards (2000)
Small limestone particle size limits P release by phytase *in vitro*

In *vitro*, Na phytate, 500 FTU 3-phytase pH 2.5. Data after 60 and 120 min quite variable

Very fine limestone reduces phytase efficacy

Manangi and Coon (2007)
Formulation on available Ca rather than on total Ca to optimise phytase efficacy?

When using limestone; dietary Ca 0.90%, no phytase

6-phytase level (U/kg)

- 0
- 500
- 2500

PC plus 0.10% DCP-P and 0.10% Ca did not improve BWG

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High phytase dose levels improve performance beyond non P-deficient control

PC: 0.40% av.P
0.90% Ca

NC: 0.25% av.P
0.74% Ca

PC plus 0.10% DCP-P and 0.10% Ca no improved BWG

6-phytase level (U/kg)

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Phytase evaluation and ranking (1)

Based on

- *In vivo* efficacy studies:
  - Production performance
  - Tibia ash contents
  - Phytate degradation, P absorption/retention
- *In vitro* efficacy studies
Phytase evaluation (2)

• *In vivo assay for phytase activity* (AOAC)
  
  Definition: One unit phytase (FTU) is the amount of enzyme that hydrolyses 1 µmol P/min from Na-phytate at pH 5.5 at 37°C

• Measurement of *in vivo response per FTU* in target animals fed P-deficient diets
Phytase evaluation (set-up)

- Five commercial phytases (as liquid) tested by SFR: 
  Finase EC, Natuphos, Optiphos, Ronozyme NP, Quantum
- Test diets with 0.65% Ca, 0.26% IP
- Dose levels based on 1.0 g MCP-P equivalence (set as 100%): Used doses 50, 100 and 200%
- 9 replicates (3 exp. x 3 repl.)
- Measurements: 
  BWG 5-21 days, tibia ash 21 days
Efficacy of commercial phytases

- Response based on dietary phytase activity in FTU.
- **ALWAYS** check processing stability of phytase in your feed mill!
- This is more crucial when feeding Ca and P close to requirements.

* Optiphos marketed in OTU using FTU/2
Conclusions/ take home messages

– Conclusions on phytase research are not universal, but may vary from phytase to phytase
– Phytase efficacy varies among feedstuffs due to phytate solubility
– Increased feedstuff particle size can improve phytase efficacy
– Phytate is a potent anti nutrient and phytase value is beyond P release
Conclusions/ take home messages

– Optimum (i.e. lowering) dietary Ca level improves phytase efficacy
– When Ca and dP are close to requirements always verify phytase processing stability in your feedmill
– Recent phytase research will be more focused on its application
Thank you for your attention