Action of different phytase types & doses in different segments of the broiler digestive tract

Danisco Animal Nutrition Phytase Workshop,

ESPN, Prague, August 2015

Loek de Lange,
Manager Poultry Cluster
Ingredients of this talk about phytase

- Introduction and a brief history
- Efficacy and economy
- ANF effect of phytate
- Interaction Ca, P and N
- DuPont SFR experiment
- Conclusions
- Take Home Messages
Introduction

Phytase has become an indispensable ingredient in feeds for poultry and pigs:

- To diminish P-excretion and improve P-utilisation
- To improve animal performance
- To make extra money
Brief history phytase development

Started in the eighties in The Netherlands by:
- Gist Brocades
- Dutch Board for Animal Feeds

Because of excessive emission of phosphorous (P) to the environment
- First generation: Aspergillus 3-phytase, Natuphos
- Second generation: E.Coli 6-phytases
- Third generation: Buttiauxella, Axtra® PHY
Improvement in efficacy

1.98 Kg

2.19 Kg

2.77 Kg


Aspergillus-3

Peniophora lycii

Citrobacter braakii

E. Coli-6

E. Coli (Trichoderma reesei)

Buttiauxella spp (Trichoderma reesei)

Based on Rauw 1998 and Ross info
©2004-2015 Schothorst Feed Research. All rights reserved
Economic benefits of phytase above animal performance and environmental benefits

<table>
<thead>
<tr>
<th></th>
<th>1991</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Price of a 5,000 FTU phytase product</td>
<td>~ 25 USD/kg</td>
<td>~ 9 USD/kg</td>
</tr>
<tr>
<td>Cost of using 500 FTU</td>
<td>2.50 USD/ton</td>
<td>0.90 USD/ton</td>
</tr>
<tr>
<td>Price of inorganic phosphorus (as MCP)</td>
<td>~ 200 USD/ton</td>
<td>~ 650 USD/ton</td>
</tr>
<tr>
<td>Typical phytase “P contribution” at 500 FTU dose</td>
<td>0.10% av.P</td>
<td>0.13% av.P</td>
</tr>
<tr>
<td>MCP replacement in the formulation</td>
<td>4.42 kg</td>
<td>5.75 kg</td>
</tr>
<tr>
<td>Savings from MCP replacement in feed</td>
<td>0.88 USD/tonne</td>
<td>3.74 USD/tonne</td>
</tr>
<tr>
<td><strong>Net profit</strong></td>
<td><strong>- 1.62 USD/ton</strong></td>
<td><strong>+ 2.84 USD/ton</strong></td>
</tr>
</tbody>
</table>
Phytate: Anti-Nutritional Factor (ANF)

- Contains P and binds minerals as Ca, Mg, Fe, Cu and Zn
- More endogenous losses of amino acids
- Reduces sodium pump activity (Ha)
- Suggested to form complexes with proteins
- More protein and energy used to compensate for endogenous losses
- ANF-effect mostly related to IP-6 and IP-5, but impact from IP-3 and IP-4 also important
Criteria for a “highly effective” phytase

Need to hydrolyze phytate (IP6) in the stomach and upper part of the small intestine:
- As completely as possible
- As quickly as possible

A good phytase needs to have the following attributes:
- Highly active in low and wide pH range
- Resistance to protease
- High affinity for and fast degradation of IP6
- Heat stable
Differences in enzyme kinetics and pH optima of phytases result in very different phytate dephosphorylation patterns and phosphate release during *in vitro* simulation of digestion.

Menezes-Blackburn *et al.*, 2015
Interaction between Ca, P and N

- Calcium: most abundant mineral in the body; 75 - 95% is found in the skeleton and also plays a major role in many enzyme systems, e.g. control of nerve impulses, muscle contractions.
- Phosphorus: predominantly present in the skeleton (50 - 80%) also occurs in soft tissues: phosphoproteins, nucleic acids, phospholipids in animal cell walls; plays a vital role in energy metabolism
- Ca & P stored in bone (mineral hydroxy-apatite) at constant ratio of 2.1 to 1.
- N is stored mainly in the soft tissues as meat
Interaction of Ca and P with N/protein

- Calcium (Ca) 40.078
- Phosphorus (P) 30.973
- Nitrogen (N) 14.007

Bone +  Meat -

P-requirements are also related to protein-requirements
Interaction of Ca and P with N/protein

- Ca (Calcium, 40.078) - Bone -
- P (Phosphorous, 30.973) -
+ N (Nitrogen, 14.007) + Meat +

Less calcium can result in more meat and changed P-deposition
Interaction between Ca and P
Effect of Ca/nPP ratio on BWG day 1-16 in broilers

- A high ratio Ca/nPP has a negative effect on BWG

Driver et al, 2005
Interaction between Ca and P
Effect of Ca on BWG day 1-16 at low and high P-supply

- At a low P-supply, the effect of extra Ca on BWG is negative

Calculations from mathematical broiler model Driver, 2005
Digestion and metabolism of Ca and P in broilers

- Uptake of Ca is regulated at intestinal level depending on supply / requirements

<table>
<thead>
<tr>
<th>Ca in feed</th>
<th>Ileal dig. Ca (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>51.0</td>
</tr>
<tr>
<td>Standard</td>
<td>34.5</td>
</tr>
<tr>
<td>High</td>
<td>31.3</td>
</tr>
</tbody>
</table>

Krimpen et al, 2013

- Excess of P is mainly excreted via urine
Dose and type: set up experiment DuPont at SFR (LPH-51)

<table>
<thead>
<tr>
<th>Product</th>
<th>Phytase FTU/kg</th>
<th>Calc P</th>
<th>Calc Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Buxiauxella</td>
<td>250</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td>E.Coli phytase</td>
<td>250</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td>PC0.6</td>
<td>+0.6 g P</td>
<td>5.4</td>
<td>7.5</td>
</tr>
<tr>
<td>PC1.2</td>
<td>+1.2 g P</td>
<td>6.0</td>
<td>7.5</td>
</tr>
<tr>
<td>PC1.8</td>
<td>+1.8 g P</td>
<td>6.6</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Observations:
- FI, BWG and FCR from day 5 till day 20
- Retention of Ca and P at days 7/8, 13/14 and 18/20
- Ileal absorption of Ca and P at day 21
- Tibia ash at day 14 and day 21
Effect of dose and phytase source on tibia ash at day 21

Phytase from Buttiauxella is more effective on tibia ash than from E. Coli.
Effect of dose and phytase source on BWG day 5 - 20

Phytase from Buttiauxella is more effective on BWG than from E. Coli
Effect of dose and phytases on P-retention

Phytase from Buttiauxella is more effective on P-retention than from E. Coli.
Conclusions on dose and phytase source

- *Butiauxella* phytase is more effective than *E. Coli* phytase regarding bone formation, BWG and P-retention
- Optimal dose: $500 \Rightarrow 1000$ FTU/kg ?!
Effect of phytase on Ca-retention

SFR LPH-51;
All feeds: 7.5 g Ca per kg feed
Retention: day 18-20
Effect of phytase on ileal Ca-absorption and -retention

Phytase has *not* a positive effect on ileal Ca-absorption, but has a positive effect on Ca-retention!

SFR LPH-51;  
All feeds: 7.5 g Ca per kg feed  
Retention: day 18-20  
Absorption: day 21
Effect of mineral P on ileal Ca-absorption and -retention

SFR LPH-51;
All feeds: 7.5 g Ca per kg feed
Retention: day 18-20
Absorption: day 21

Also extra mineral phosphorus from MCP, partly replacing lime stone, has a negative effect on ileal Ca-absorption and a positive effect on Ca-retention!
Why decreases mineral P and phytase ileal Ca-absorption and increases Ca-retention?

- Formation complexes of Ca and phosphates in intestinal tract. Ca-source???
- Increased P-supply below P-requirements increases bone formation and requirements for Ca, and so increase Ca-retention!!!
Conclusions

- The third generation of phytases are more effective per FTU than the older ones, probably related to a high activity at a low and wide pH range.
- To reduce the ANF effect, phytate needs to be degraded rapidly and thoroughly in the upper part of GIT.
- The optimal phytase dose tends to increase depending on source, age, animal performance and economy.
- Requirements for Ca, P and protein are related:
  - Extra Ca at low P-supply seems to reduce P-absorption and Body Weight Gain.
  - Extra mineral P and phytase at a low P-supply do not increase ileal Ca-absorption, but increase retention of Ca.
Take Home Messages

- Use higher phytases doses than in the past depending on economy and age
- Try to develop a new standard phytase measurement \textit{in vitro} at a pH that is better correlated with \textit{in vivo} efficacy than the common standard at pH 5.5, but validation with \textit{in vivo} trials is still necessary
- Try to reduce the Ca-supply and find out the effect of the phytase on Ca-absorption and retention
- Always compare efficacy of phytases in animal trials with graded levels of mineral P from MCP