Superdosing of phytases

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Traditional dosing of phytases – matrix driven

**P matrix vs dose for a "0.15" phytase**

\[
y = 0.0562\ln(x) - 0.1986
\]

\[R^2 = 0.9999\]
Quantum® Blue ‘super-dosing’ – 4 Point Improvement in FCR

Figure 3. Composite analysis of 6 trials: Body weight corrected FCR of broilers from d 0 to 35/42 (n = 35)

$y = -0.0255x + 1.638$
$R^2 = 0.9538$

4 points in FCR currently worth $7 per tonne of feed
QUANTUM SUPERDOSE - TRIAL SUMMARY – ADG

(FROM WEANING THROUGH TO 21/35 DAYS POST-WEANING)

+6.26% ADG IMPROVEMENT
80% POSITIVE I.E. > 100% OF CONTROL
QUANTUM SUPERDOSE - TRIAL SUMMARY – FCR

(FROM WEANING THROUGH TO 21/35 DAYS POST-WEANING)

+3.80% FCR IMPROVEMENT
88% POSITIVE I.E. < 100% OF CONTROL
Why

1. Does superdosing work?
   1. IP6 destruction
   2. IP4 and 3 destruction
   3. Inositol provision

2. Do we have to have high P
   1. Superdosing makes animals grow faster – higher P RQ?
   2. Rephosphorylation of inositol
Activity at low substrate: In the gut, not all phytate is soluble and susceptible to the enzyme

Leslie, 2006
Pepsin inhibited at pH 2.5 by very little phytate

Figure 5. Phytic acid inhibition of porcine pepsin-catalyzed hydrolysis of azurine cross-linked casein. The assay was performed at 40°C. Each data point is the average of duplicate.

Yu et al 2012
Protein – phytate precipitation depends upon pH and protein

Kies et al 2006
Protein – phytate precipitation depends upon pH and protein

- pH 2 Protein
- pH 2 Pr+ Phyt
- pH 3 Protein
- pH 3 Pr+ Phyt

Percent soluble protein varies across different pH levels and protein sources, such as casein, corn, canola meal, rice pollards, SFM, and SBM.
Protein – phytate precipitation depends upon pH and protein

- pH 2 Protein
- pH 2 Pr+ Phyt
- pH 2 P+P+P
- pH 3 Protein
- pH 3 Pr+ Phyt
- pH 3 P+P+P

Percent soluble protein

Casein  Corn  Canola meal  Rice pollards  SFM  SBM

June 11, 2008
IP6 is not the only problem
Phytase has to get rid of IP5→IP2 as
Yu et al. 2012

Figure 6. Time course of phytic acid hydrolysis by *E. coli* phytase (Phyzyme XP, Danisco A/S, Brabrand, Denmark; 0.08 phytase unit/mL) and inhibition of porcine pepsin catalyzed azurine cross-linked casein hydrolysis by the hydrolyzates. Phytic acid hydrolysis was performed at 37°C; pepsin activity assay was carried out at 40°C. Each data point is an average of 2 separate experiments.
IP6 is bad, but IP4 is not much better

added together in the molar ratio Ca:IP:Zn = 100: 10: 1

Supplementation with a novel *C. braakii* phytase: Results in a ‘pool’ of IP4

Pontoppidan et al., 2012. 25kg pigs fed 0 or 500 FTU HiPhos and after starving for 24hrs then fed treats for 30 mins, slaughtered 1 and 2 hours post feed. A=control, B =500FTU/kg
If it is present and soluble in the stomach, it will ppt with something in the SI

Figure 2. Hydrolysis of phytate from a diet rich in intrinsic feed phytases during the passage throughout the stomach, small intestine and large intestine as well as in the faeces of pigs 5 h after feeding [159]. Inositol phosphates are listed from the front to the back in the following row:

InsP$_3$, Ins(1,2,3)P$_3$/Ins(1,2,6)P$_3$, Ins(1,5,6)P$_3$, Ins(1,2,3,4)P$_2$/Ins(1,3,4,6)P$_4$, Ins(1,2,5,6)P$_4$, Ins(1,2,3,4,6)P$_5$, Ins(1,2,3,4,5)P$_5$, Ins(1,2,4,5,6)P$_5$, Ins(1,3,4,5,6)P$_5$ and InsP$_6$. 
If it is present and soluble in the stomach, it will ppt with something in the SI

![Graph showing hydrolysis of phytate from an extruded diet with inactivated phytases, during the gastro-intestinal passage throughout the stomach, small intestine and large intestine as well as in the faeces of pigs 5 h after feeding [159]. InsP6 concentrations were different (p < 0.05). Inositol phosphates are listed from the front to the back in the following row: InsP₂, Ins(1,2,3)P₃/Ins(1,2,6)P₃, Ins(1,5,6)P₃, Ins(1,2,3,4)P₄/Ins(1,3,4,6)P₄, Ins(1,2,5,6)P₄, Ins(1,2,3,4,6)P₅, Ins(1,2,4,5,6)P₅, Ins(1,3,4,5,6)P₅ and InsP₆.](image)

Schlemmer 2001
IP4 and IP3 are not innocuous

<table>
<thead>
<tr>
<th>Ester</th>
<th>Nutrient</th>
<th>R</th>
<th>P value</th>
<th>n</th>
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<tr>
<td>Ileal iP3</td>
<td>Ileal AME, kcal</td>
<td>-0.849</td>
<td>&lt;0.0001</td>
<td>40</td>
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<td></td>
<td>Ileal DM digestibility</td>
<td>-0.848</td>
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<td>Ileal Na digestibility</td>
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<td>&lt;0.0001</td>
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<td>Ileal iP4</td>
<td>Ileal Mg digestibility</td>
<td>-0.688</td>
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<tr>
<td></td>
<td>Ileal Fe digestibility</td>
<td>-0.606</td>
<td>&lt;0.0001</td>
<td>40</td>
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</tbody>
</table>

Beeson et al, 2015 submitted
Supplementation with a novel E. coli phytase:

Superdosing results in near complete destruction of phytate (IP6) and reduced concentrations of IP4 and IP3

Point in time measure of gizzard phytate of 21-day-old broilers

Walk et al., 2013 (in press)
Supplementation with a novel *E. coli* phytase:

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*Point in time measure of gizzard phytate of 21-day-old broilers*
Supplementation with a novel *E. coli* pytase:

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Ileal phytate ester and inositol concentration of piglets 3 weeks post-weaning

Supplementation with a novel *E. coli* pytase: Superdosing results in near complete destruction of phytate (IP6) and reduced concentrations of IP4 and IP3.
Increased phytate hydrolysis may increase inositol production


- Growth responses varying from 18 to 52 g in 4 weeks have been obtained upon the addition of inositol† to several different simplified chick rations (Table I).
Inositol Provision  
Zyla et al 2004

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<th>p value</th>
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<tr>
<td>Intake</td>
<td>838</td>
<td>861</td>
<td>NS</td>
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<tr>
<td>Gain</td>
<td>537</td>
<td>579</td>
<td>0.0199</td>
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<tr>
<td>FCR</td>
<td>1.57</td>
<td>1.47</td>
<td>0.0573</td>
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<tr>
<td>Toe ash %</td>
<td>11.9</td>
<td>12.2</td>
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<tr>
<td>P retention</td>
<td>56</td>
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<tr>
<td>Ca retention</td>
<td>61</td>
<td>59</td>
<td>NS</td>
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</table>

Broilers, 1-21d post hatch, main effects on 0.65/0.27 and 0.8/0.47 Ca AvP diets
Extra-phosphoric effects of phytase

Broiler gizzard phytate, phytate ester and inositol concentration (d21)

Superdosing Quantum Blue decreased phytate and increased inositol concentration

Part of the superdosing response may be associated with inositol provision as well as phytate destruction
Inositol interacts with phytase
It is likely part of the superdosing effect

- FCR
  - Inositol* Phytase interaction $p < 0.0143$
  - LSD = 0.017
Inositol as a source of intracellular phytate (IP6)?

Phytate (IP6) is present in tissues at various concentrations

1. Antioxidant
2. De-phosphorylated for cell signaling, Ca metabolism and inositol production

Rat tissue concentration of IP6, µM

Inositol:

1. Mammalian cell cultures require inositol to grow
2. IP6 can be made in the body from inositol
3. Phytate destruction may increase the requirement for P in the body?

Letcher et al., 2008

www.abvista.com
Red line represents 85% phytate hydrolysis, differences between phytases driven largely by pH profiles and kM
Phytases differ in appetite for lower IP esters
Equilibria to consider

Dietary Phytate $\rightarrow$ Insoluble Phytate $\rightarrow$ Soluble Phytate $\rightarrow$ IP3 and lower

Nuisance factor

Critical phytate concentration

High $k_M$

Low $k_M$
Conclusions

• Superdosing is NOT simply using a higher dose with a matrix .. It is

1) Using a dose \( \geq 0.2\% \) AvP matrix but applying only 0.15\% matrix max

2) Using a phytase which has ability to
   1) Clear all IP6
   2) Clear all IP4
   3) Produce inositol

3) Ranking of phytases in this application is different compared to that of a 0.13 matrix
Beaulac et al 2015 in press

Figure 5.2 The proportional digestibility of essential amino acids (EAA) compared to the digestibility of amino acids prevalent in mucin and biliary secretion (MUC AA) in broilers at 5 (Experiment 1) and 20 (Experiment 2) d of age.

Saskatchewan data with Quantum Blue
% IP6 Hydrolysis vs. FTU/kg Q Blue for 5d and 20d samples.