Schothorst Feed Research

# Phytase in poultry diets

## The latest on phytase enzyme technology and use?

Jan Dirk van der Klis

4th global feed and food congress South Africa 2013

# 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”

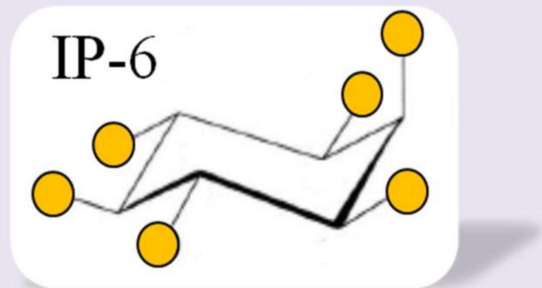
FEEDINFO NEWS SERVICE

Richard Cooper, 29/01/2013

# Phytase

Hydrolyses phytate-P to

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



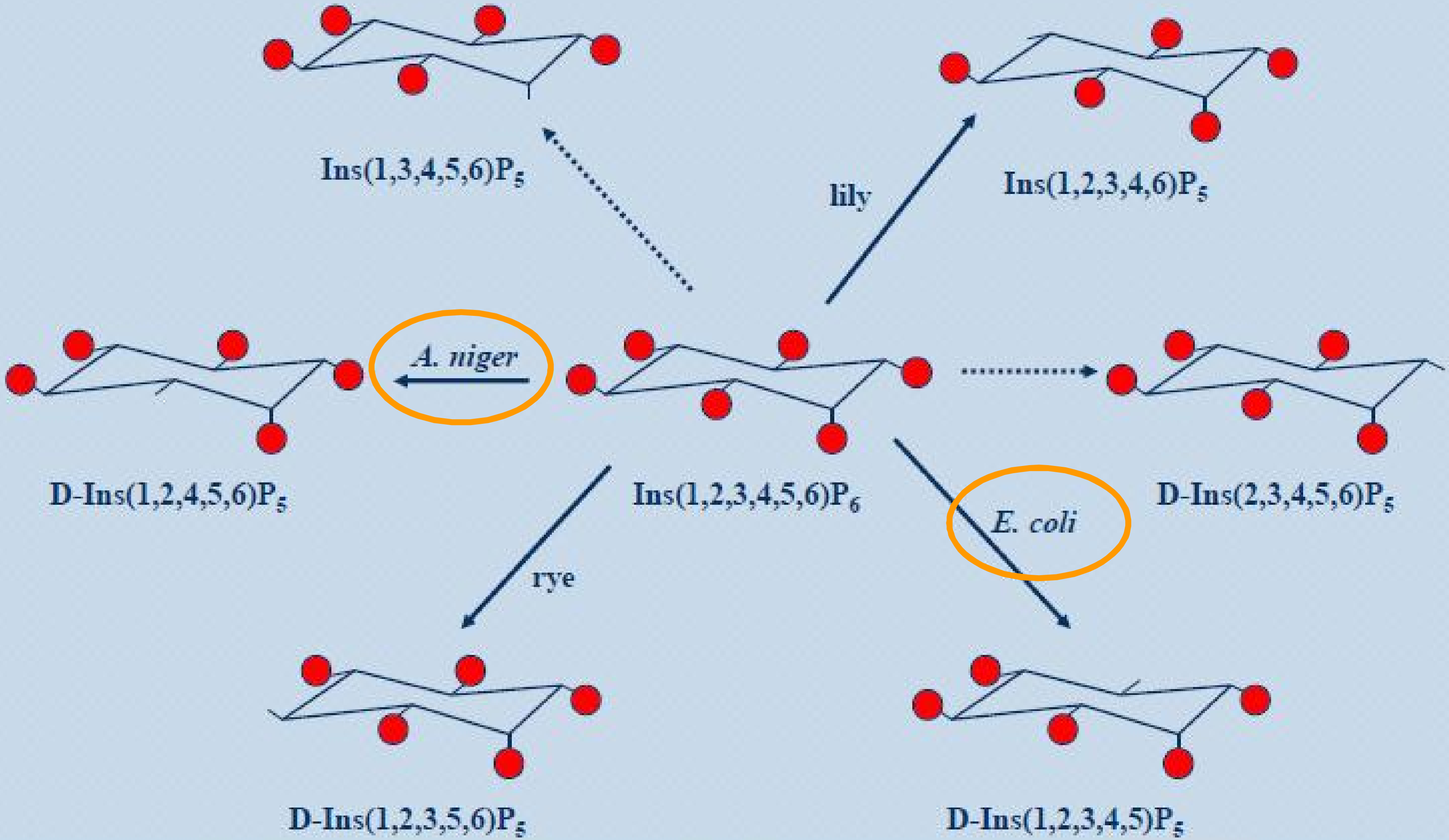

Main classification of commercial phytases

- 3- or 6-phytase
- Fungal or bacterial phytase

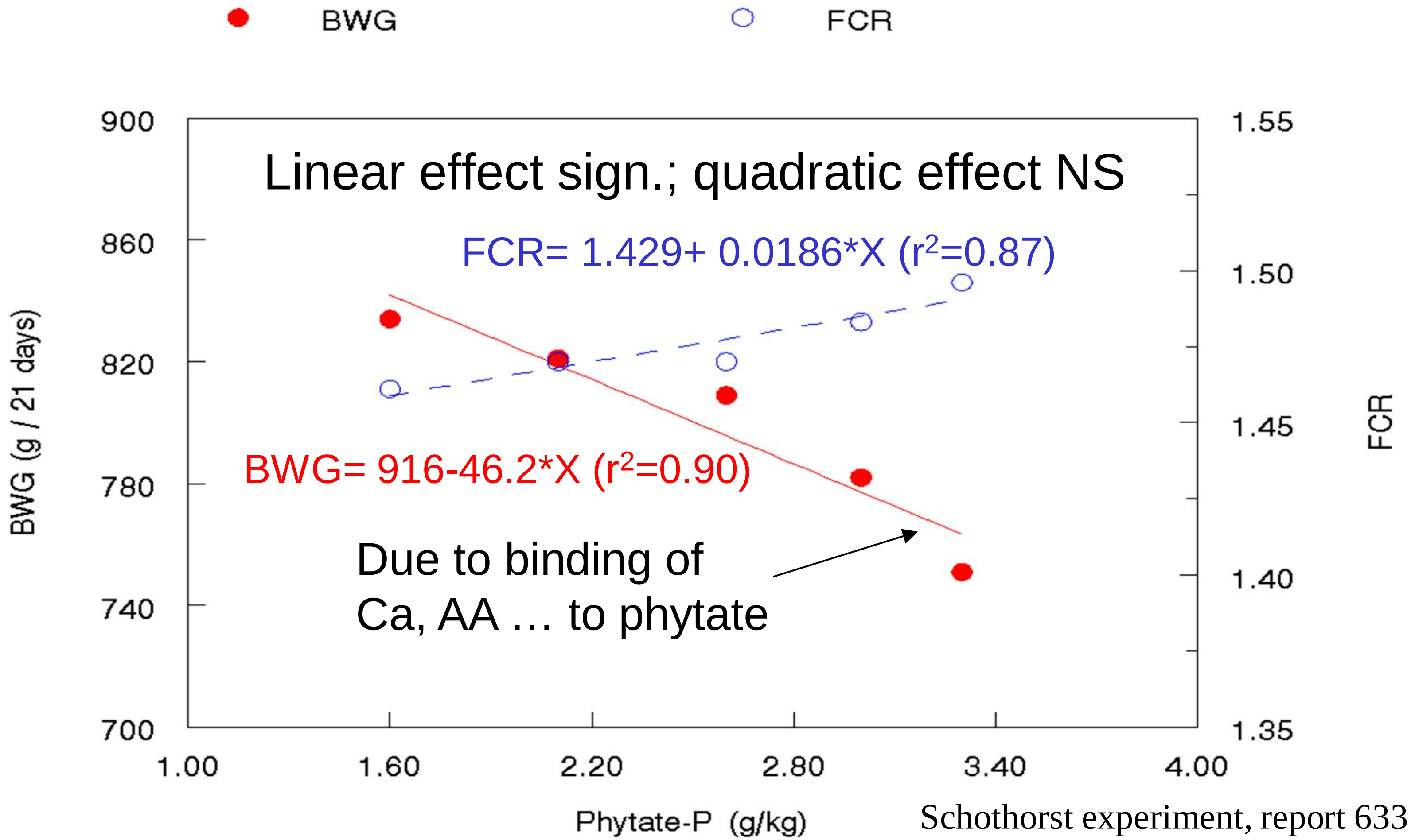
Location of phytin:
*In cereals:* in aleurone layer
*In oil seeds:* associated with storage proteins throughout the seed

MRI

# Enzymatic Phytate Degradation



# Anti-nutritional effects of phytate




Schothorst experiment, report 633 (2005)
Commercial type diets

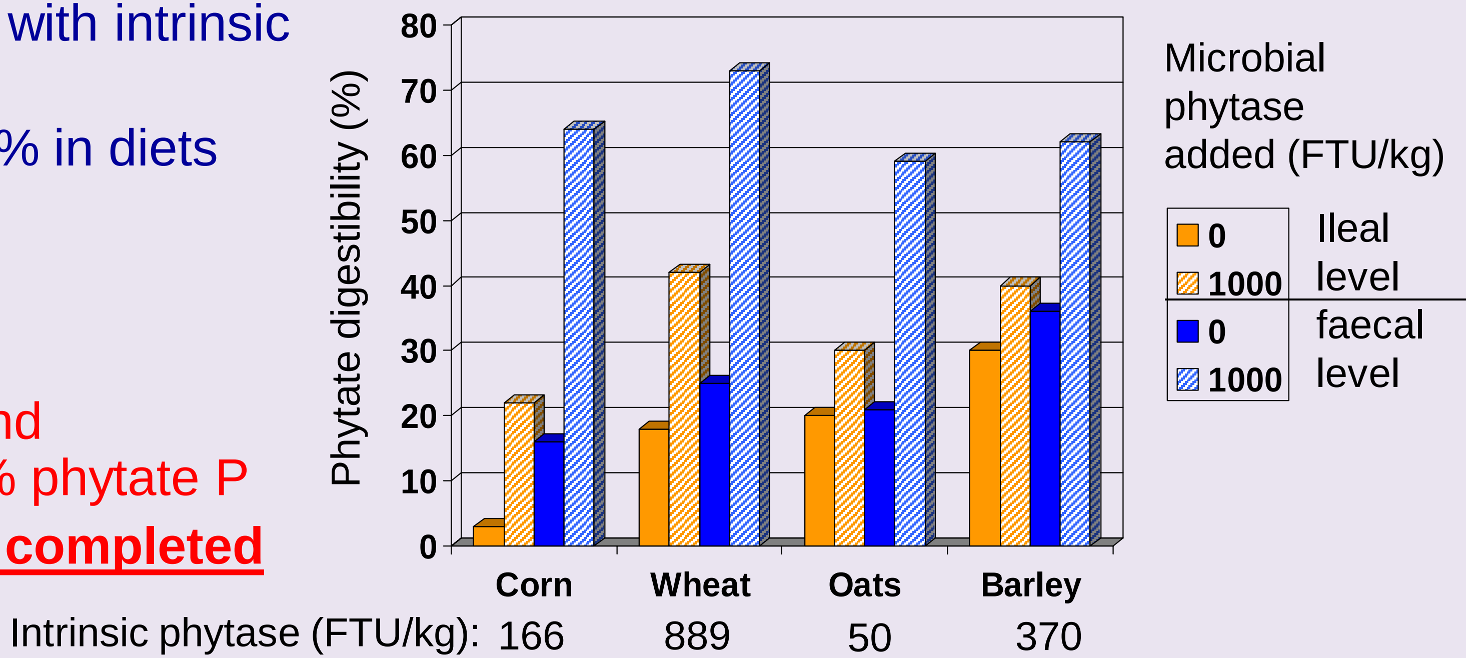
# Phytase effects in cereal-based diets

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
- **<u>Phytate degradation not completed</u>**




Leytem et al. (2008)

# Dealing with phytate …

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

**Feedstuffs**

Physical factors

**Phytase source**

**Phytase dose**

**Animal**

Physiological factors

- Phytate source
- Phytate solubility
- Feed particle size
- …

- 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

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



(adapted from Greiner, IPS-2)

# The gastrointestinal tract

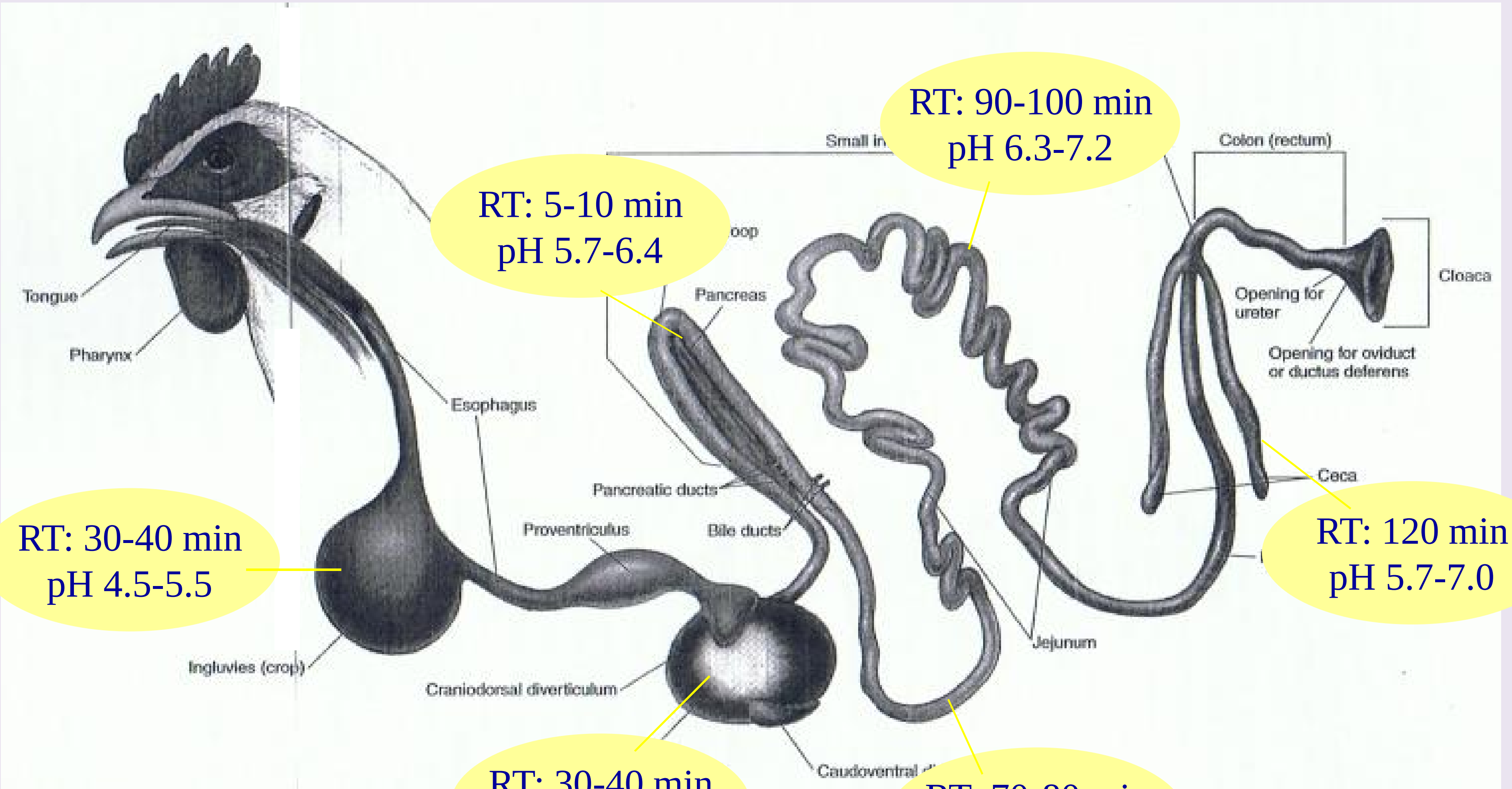


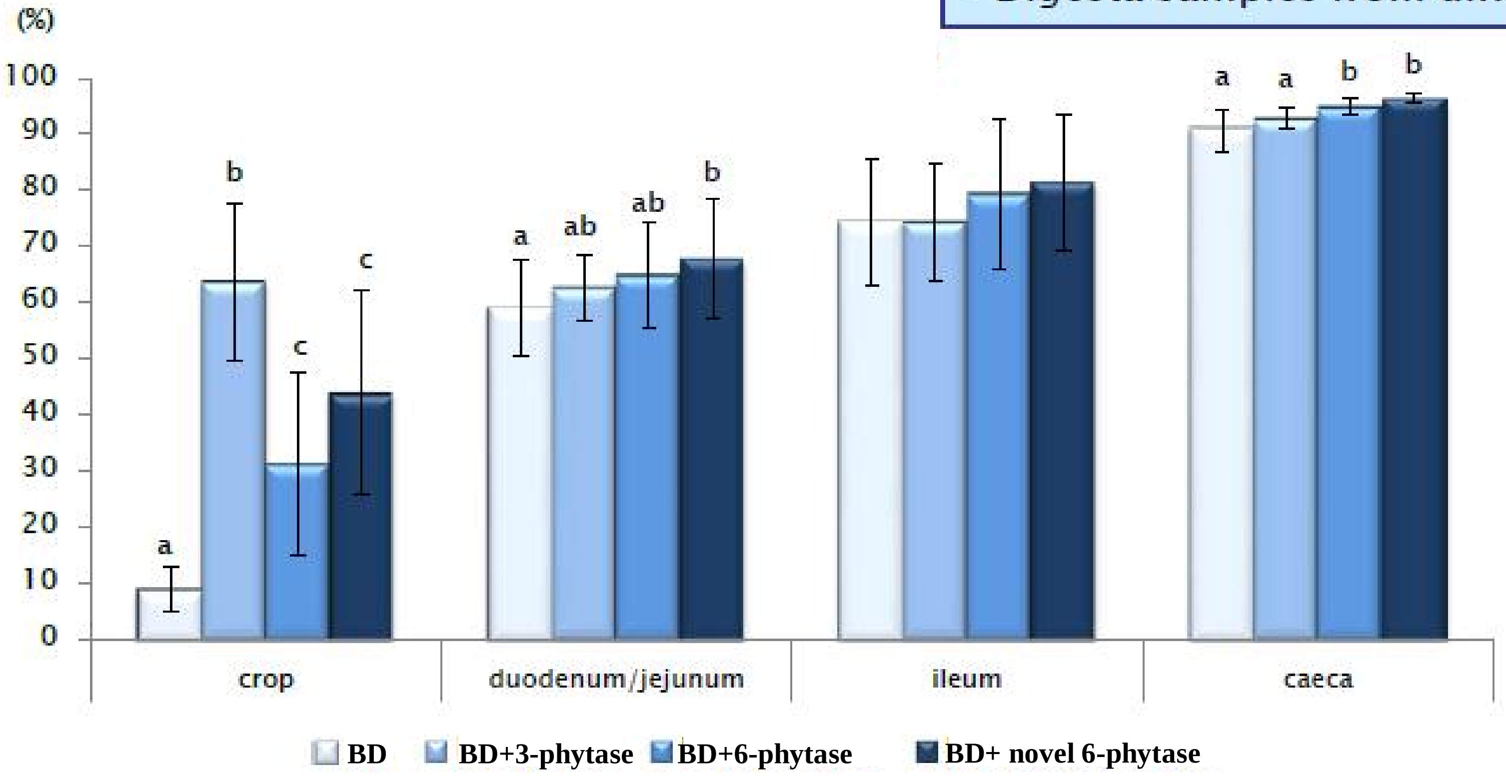

**Figure 30-3.** The gastrointestinal tract of the … sion of Wiley-B… Kainer, R.A., and Spurgeon, T.S. *Spurgeon's Color Atlas of Large Animal Anatomy.* Ba… & Wilkins, 1999.

Retention time (RT) can be increased by antiperistalsis!

…nimals, 2009

# $InsP_6$ 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




[a-c] 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)*

**(Adapted from Rodehutscord)**

# 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

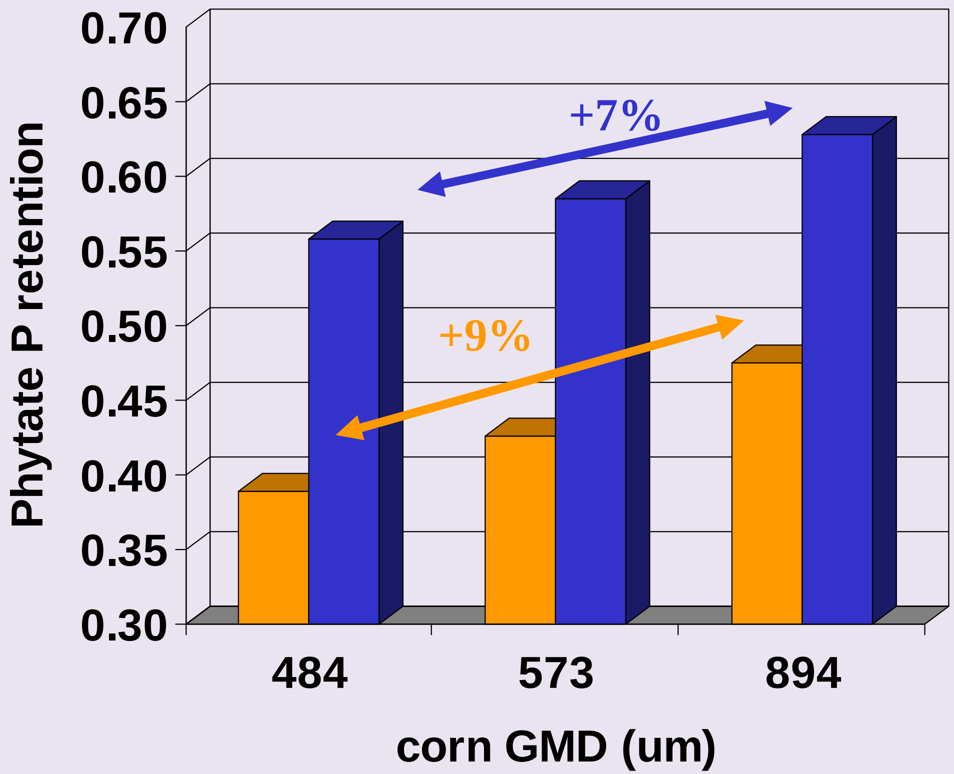
    ⬇

    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:**

- 0 FTU
- 600 FTU

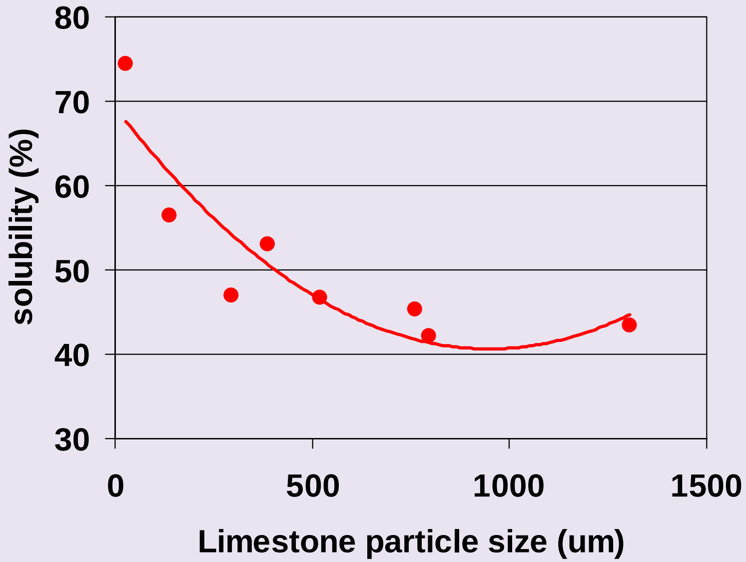
Caused by:

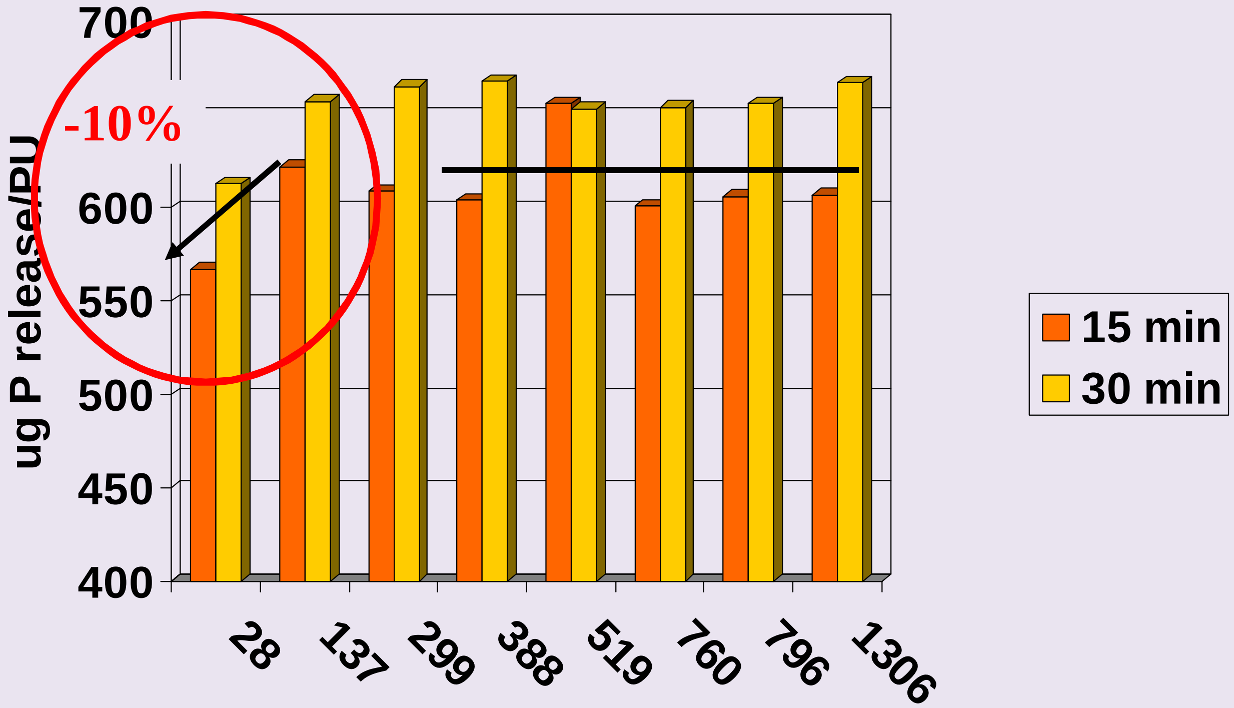
- Longer retention time in the crop and gizzard?
- Larger gizzard with lowered pH?

**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*



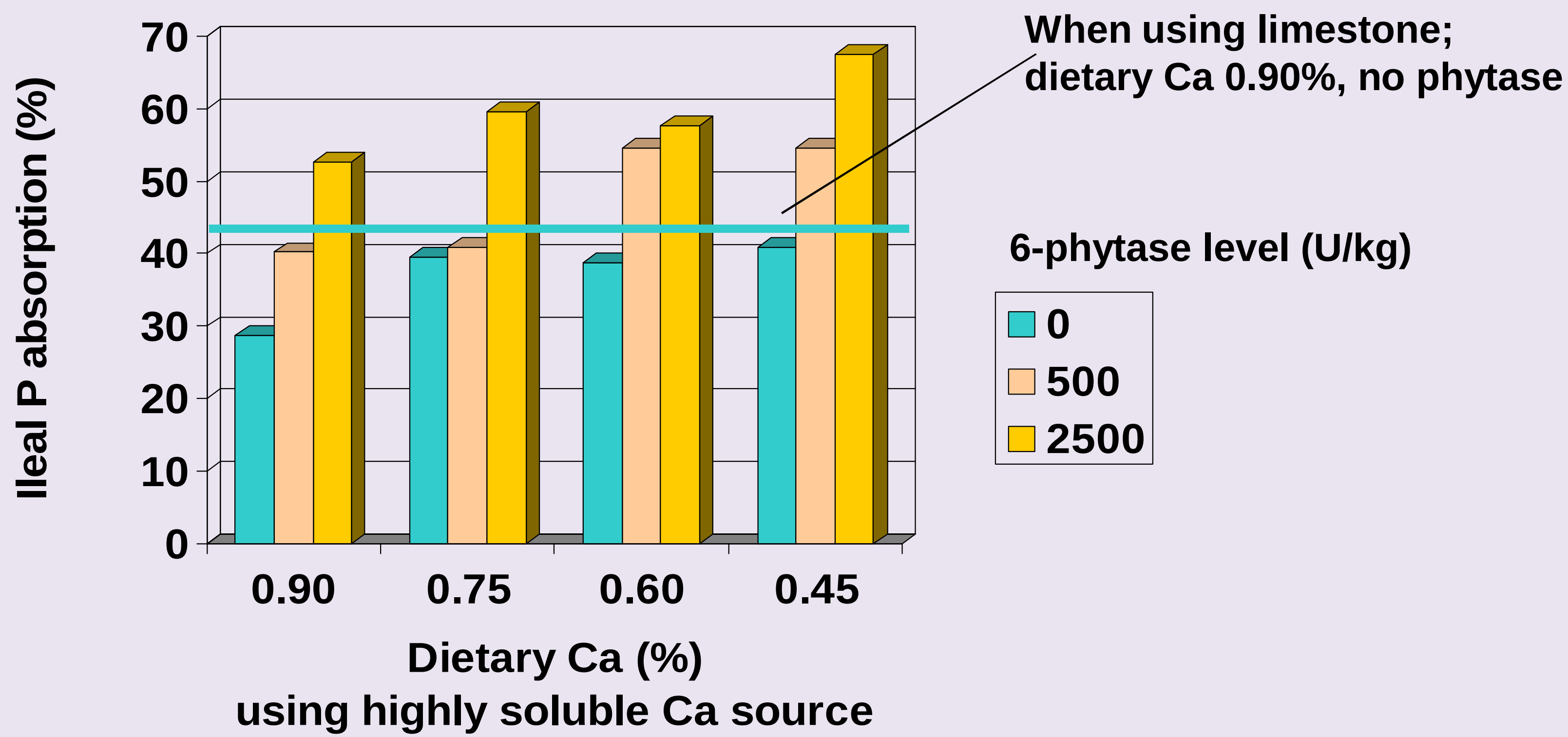

Solubility method not described

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

Very fine limstone reduces phytase efficacy

*Manangi and Coon (2007)*

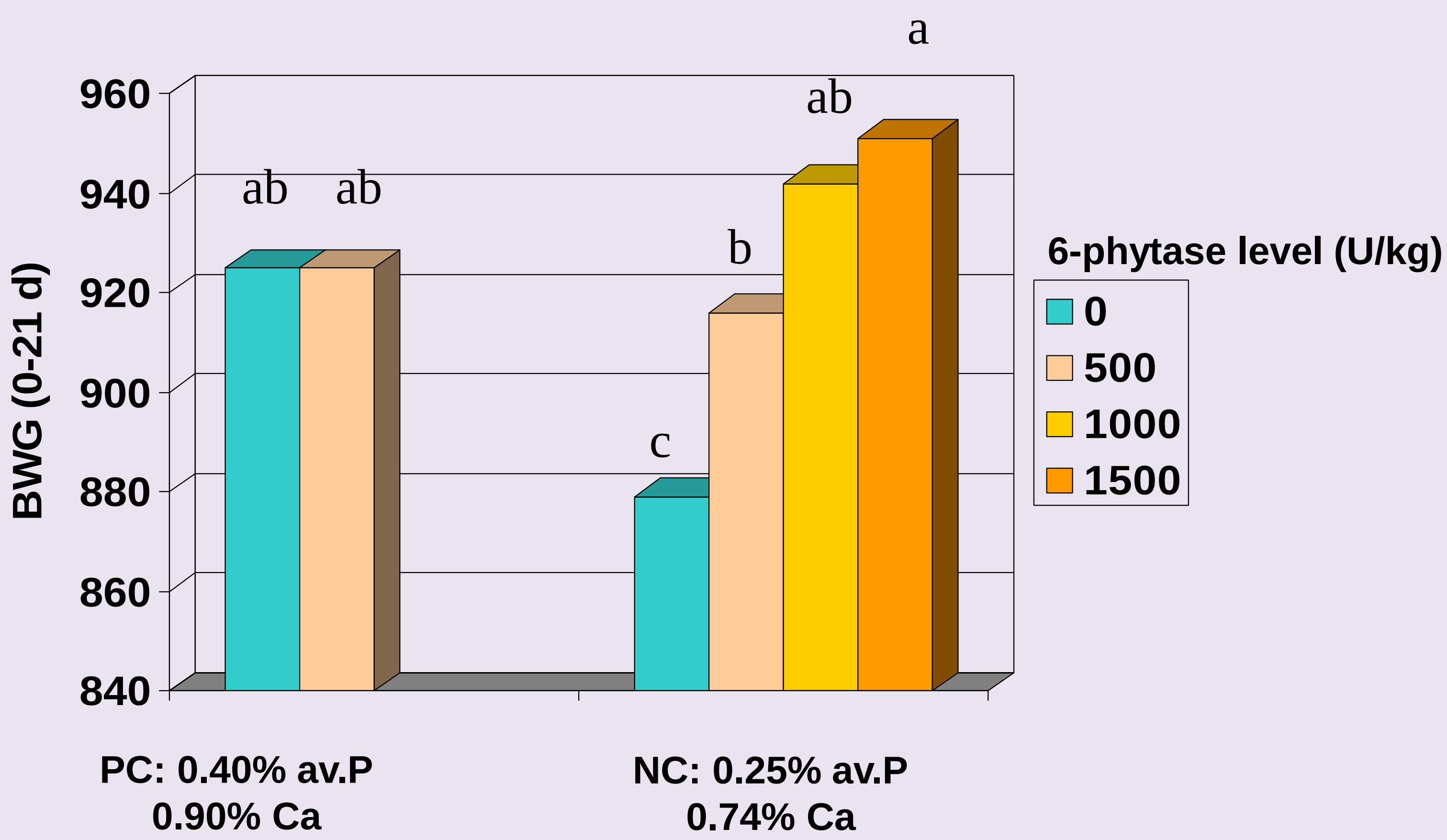
# Formulation on available Ca rather than on total Ca to optimise phytase efficacy?




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

*Walk et al. (2013)*

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




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

*Walk et al. (2013)*

# 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 37ºC

- Measurement of ***in vivo* response per FTU** in target animals fed P-deficient diets

# Phytase evaluation (set-up)

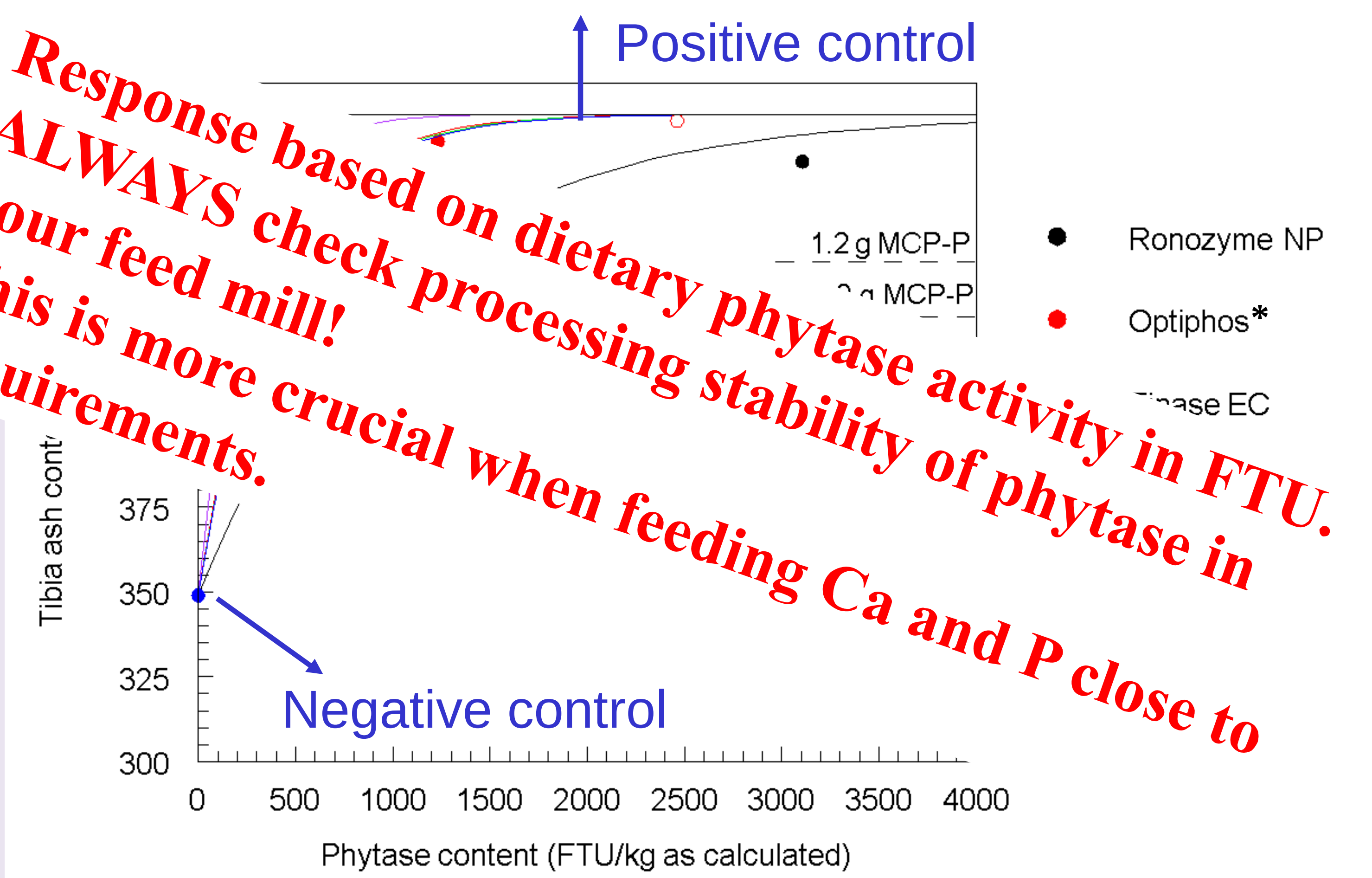
- Five commercial phytases (as liquid) tested by SFR:

  **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 <u>always</u> verify phytase processing stability in your feedmill
- Recent phytase research will be more focused on its application

# Thank you for your attention