

Winning with phytase

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Winning Strategy Requires Key Decisions To Be Made

**Which Phytase?
What dose?**

**How much AvP / Ca²⁺
contribution?**


**Energy & Amino
Acids from Phytase?**

Table 1. Some examples of currently commercially available 3- and 6-phytases and their characteristics						
Type [†]	Protein origin		Expression	pH optima	Temperature optima (°C)	Trade name
3	<i>A. niger</i> *	<i>A. niger</i>		2; 5–5.5	65	Natuphos [®]
3	<i>A. niger</i> *	<i>A. niger</i> , non-recombinant		6.0	–	Allzyme [®] SSF
3	<i>A. niger</i> *	<i>Trichoderma reesei</i>		2.5	–	Finase [®] P/L
6	<i>Escherichia coli</i> *	<i>Schizosaccharomyces pombe</i> (ATCC 5233)		4.5	55	Phyzyme [®] XP
6	<i>Escherichia coli</i> *	<i>Pichia pastoris</i>		4.5	–	Quantum [®]
6	<i>Escherichia coli</i>	<i>Trichoderma reesei</i>		–	–	Quantum Blue [®]
6	<i>Escherichia coli</i> *	<i>Pichia pastoris</i>		3.4, 5.0	58	OptiPhos [®]
6	<i>Peniophora lycii</i> *	<i>Aspergillus oryzae</i>		4–4.5	50–55	Ronozyme [®]
6	<i>Citrobacter braakii</i>	<i>Aspergillus oryzae</i>		–	–	Ronozyme Hiphos [®]
6	<i>Buttiauxella</i> spp.	<i>Trichoderma reesei</i>		3.5–4.5 [#]	60 [#]	Axtra [®] PHY
* Adapted from Lei <i>et al.</i> ¹ with modifications;						
[†] 3- or 6-phytase; —, no information available;						
[#] personal communication (C Evans).						
Dersjant-Li <i>et al</i> , 2015						

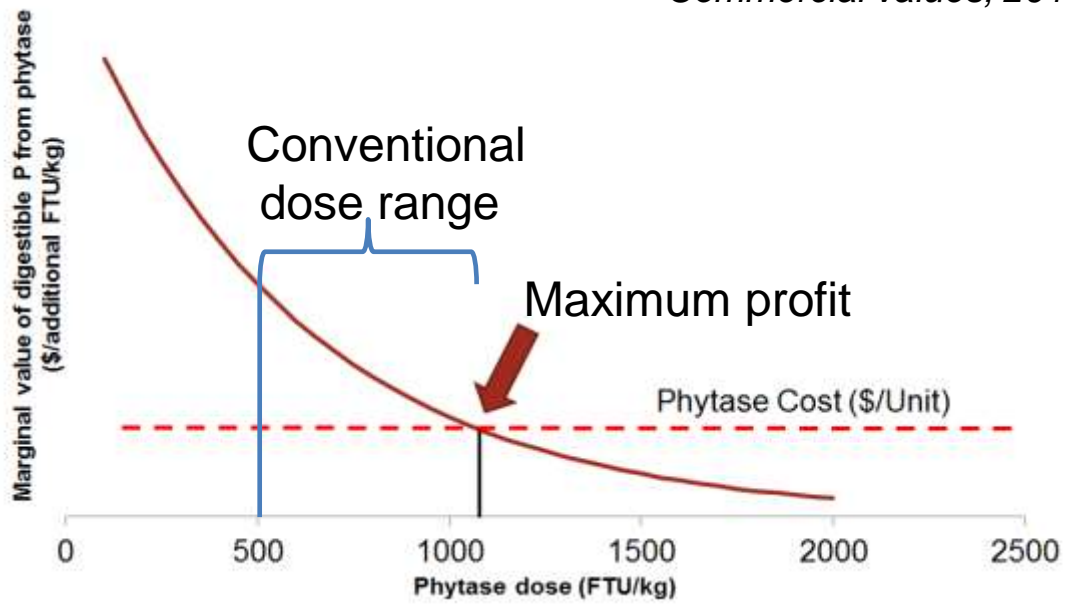
Supplier Recommended Nutrient Contributions From Standard Dose Of Phytase

	E.Coli 1	E.Coli 2	E.Coli 3	Citrobacter	E.Coli 4	Buttiauxella
FTU/kg feed	500 FTU	250 OTU	500 FTU	1000 FYT	500 QU	500 FTU
Digestible P%	0.11	0.11	0.13	0.117	0.13	0.134
Av.P %	0.12	0.13	0.13	0.146	0.15	0.15
Ratio of Dig. P:AvP	0.92	0.85	1.00	0.80	1.00	0.92
Calcium %	0.11	0.13	0.14	0.18	0.165	0.134

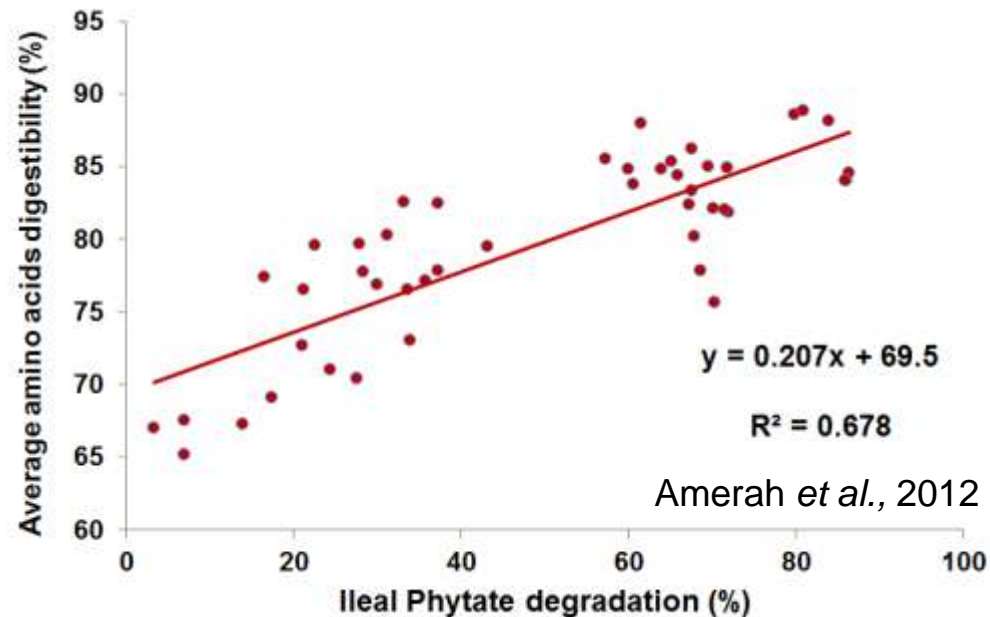
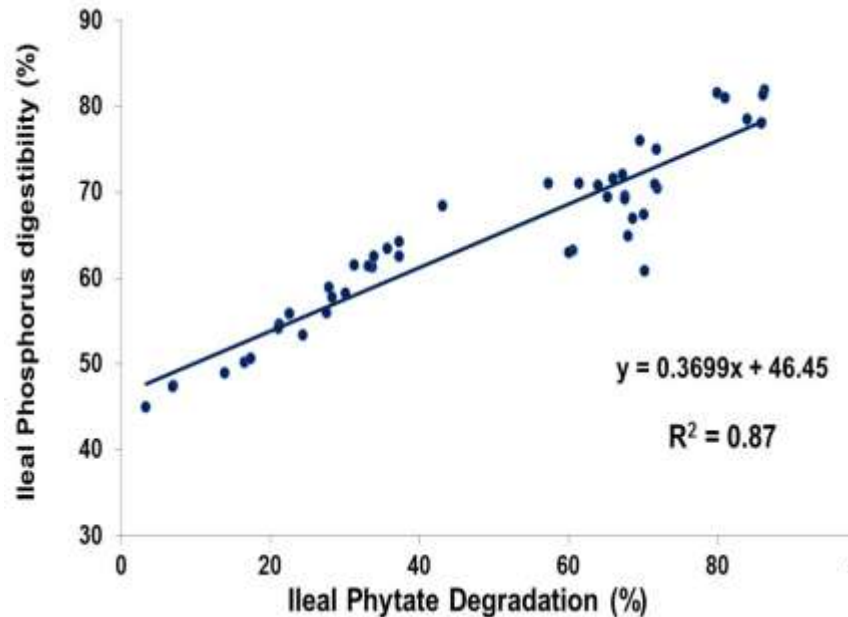
In practice, decisions of phytase **source and dose** are frequently determined on phytase cost /0.10% AvP release

Dose is usually < Max. profit from P replacement to  risk

Commercial values, 2014



Phytate not only affect phosphorus digestibility, but also amino acid digestibility, starch digestibility, endogenous losses, and live-performance

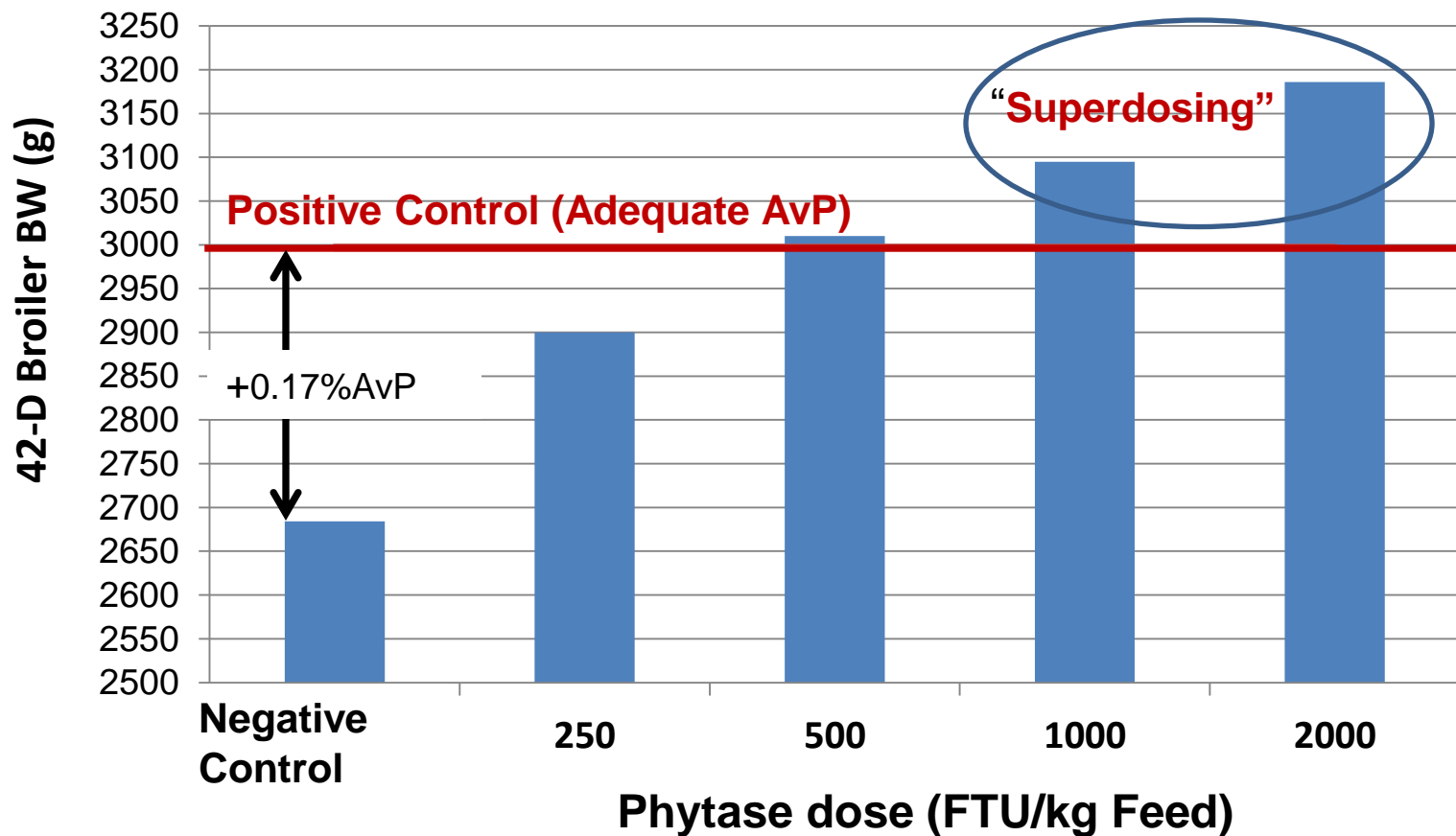


Higher phytate has also been shown to decrease live performance

Woyengo *et al.*, 2014

Animal type	Initial age (d)	PA ^z content in control diet (%)	PA content in PA diet (%)	Response criterion ^y	Decrease in performance due to PA (%)	Reference
Broiler	0	0.78	1.57	BWG	3	Liu et al. (2009)
Broiler	0	0.78	1.57	BWG	3	Liu et al. (2008a)
Broiler	0	0.78	1.57	BWG	7	Liu et al. (2008b)
Broiler	7	1.04	1.57	BWG	7	Cabahug et al. (1999)
Broiler	8	0.00	1.65	BWG	28	Onyango and Adeola (2009)

High Doses Of Phytase Have Also Been Shown To Improve Performance Beyond What Can Be Explained By Phosphorus



Unpublished, 2015

Interactions Of Phytic Acid With Dietary Nutrients

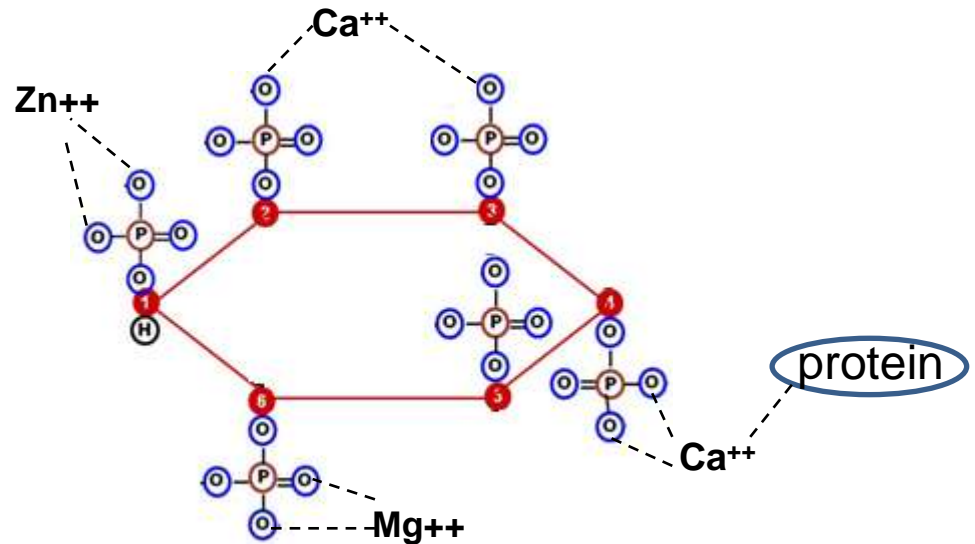
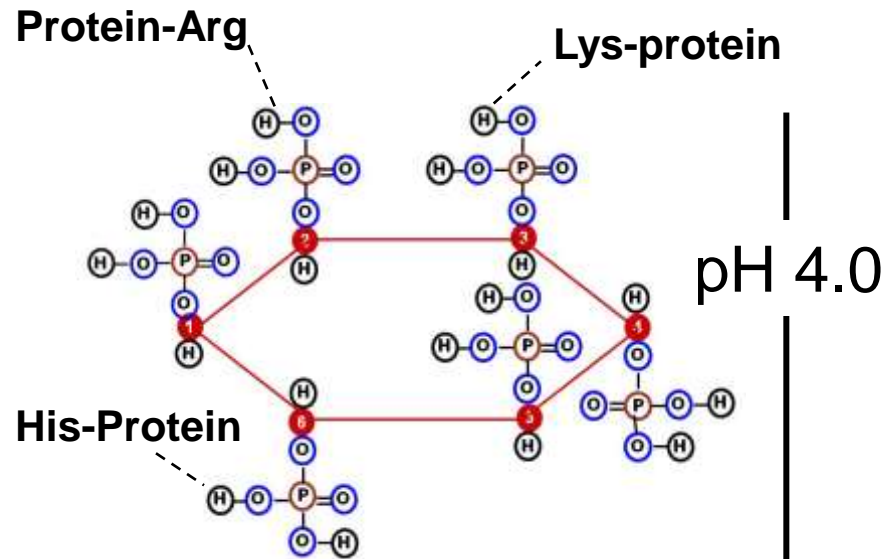
Are pH Dependent

Mineral cations also chelate at low pHs if soluble (Tamin et al., 2003)

Proteins and phytate acid also interact at higher pHs >6 in presence of Ca^{2+} Briggs (1959, Saio et al. (1967,1968)

Gizzard / Proventriculus

Duodenum / Ileum / Jejunum



Binds with basic AA of protein

Binds w/ divalent mineral cations

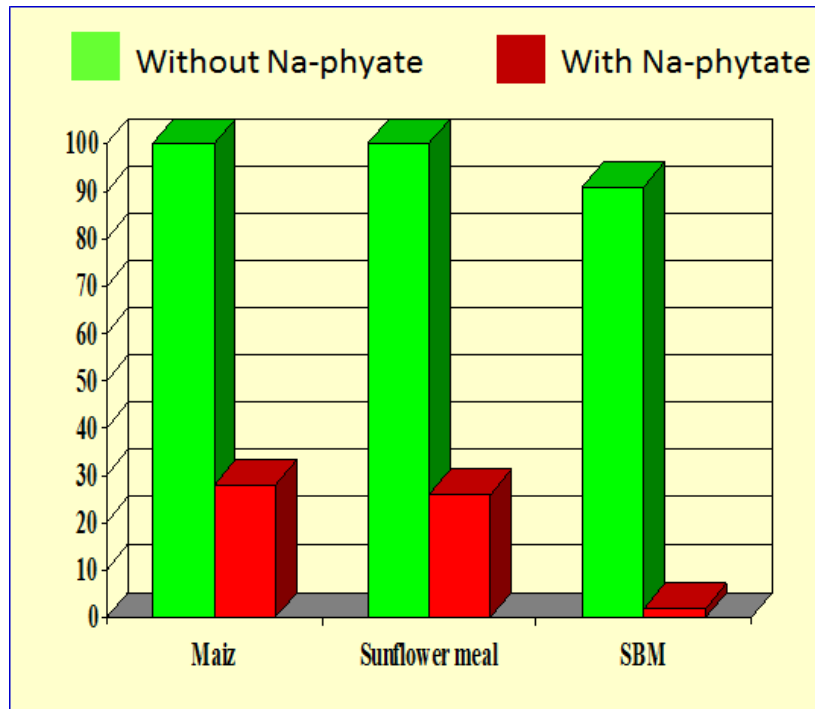
pH

pH

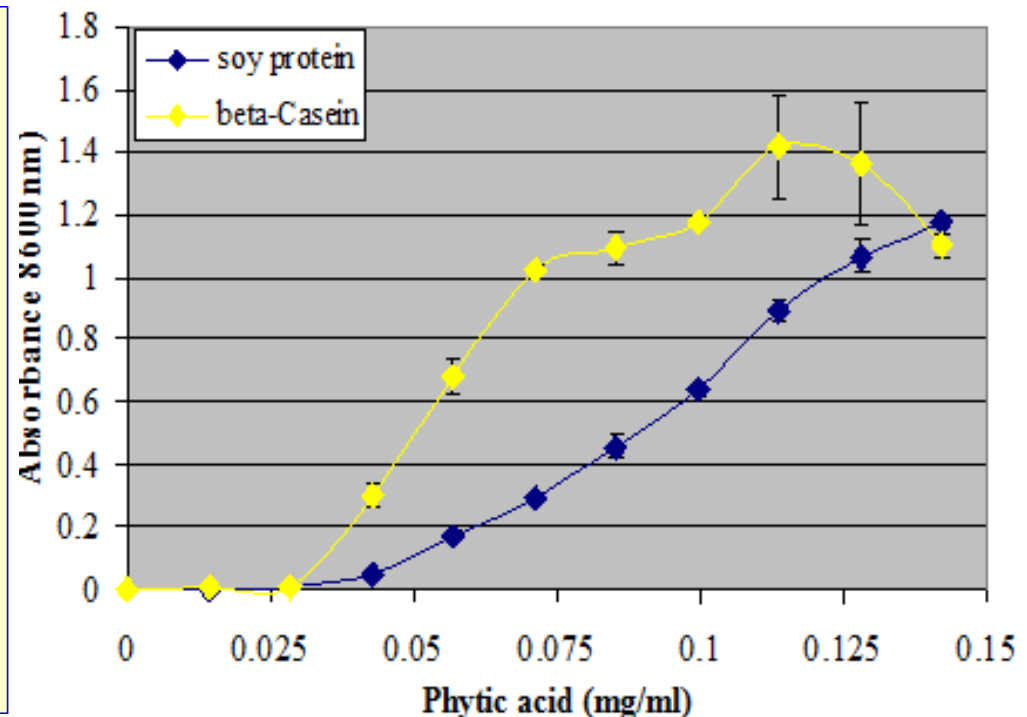


Phytic Acid Interactions with Protein

- Protein-phytate complexes – form directly with phosphate group at low pH
- Tertiary bridges – via Ca and basic residues in the protein, at pHs > 6
- Protein-phytate formation proportional to the ratio of phytate:protein



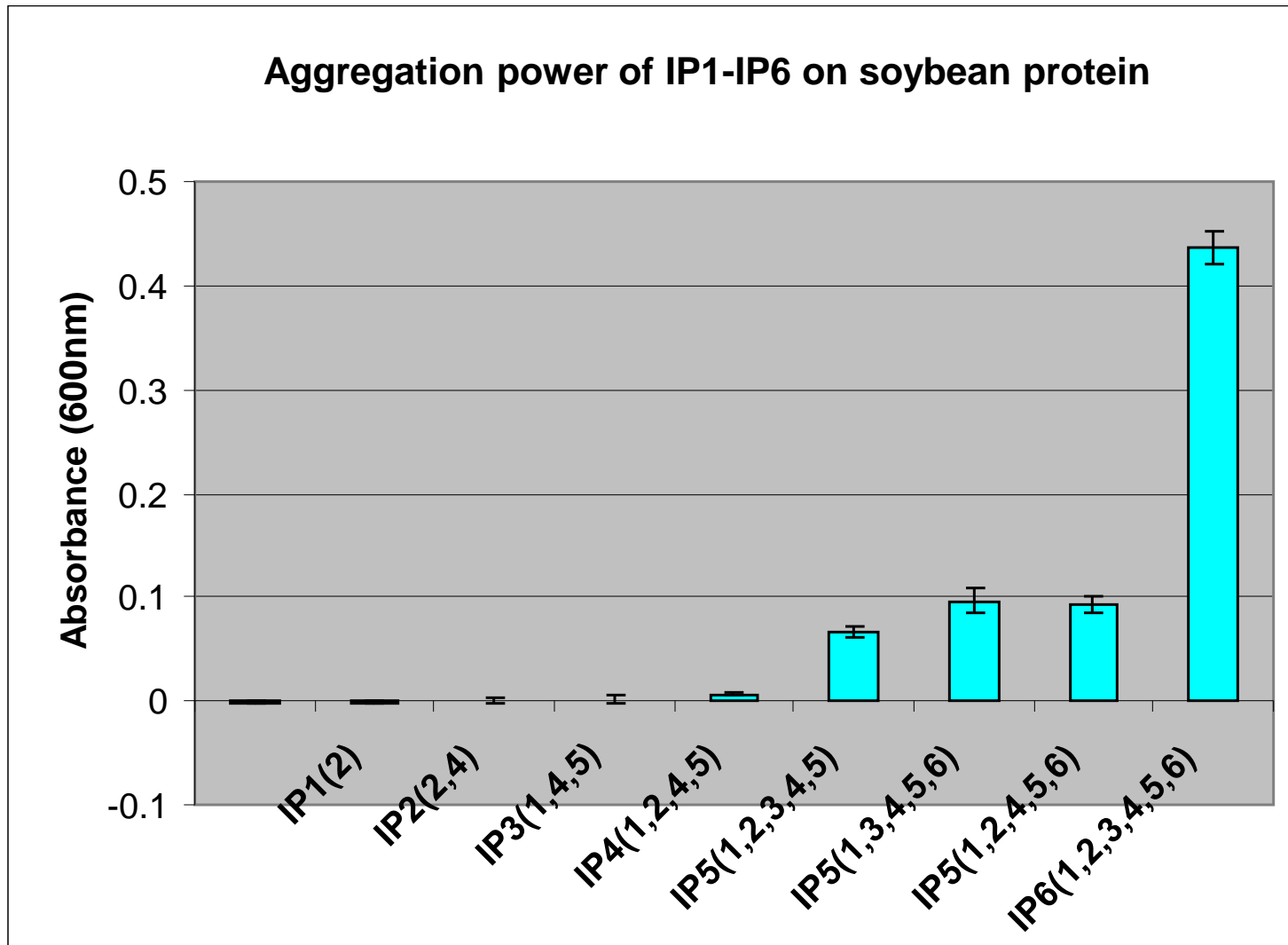
Kies *et al.*, 2006.



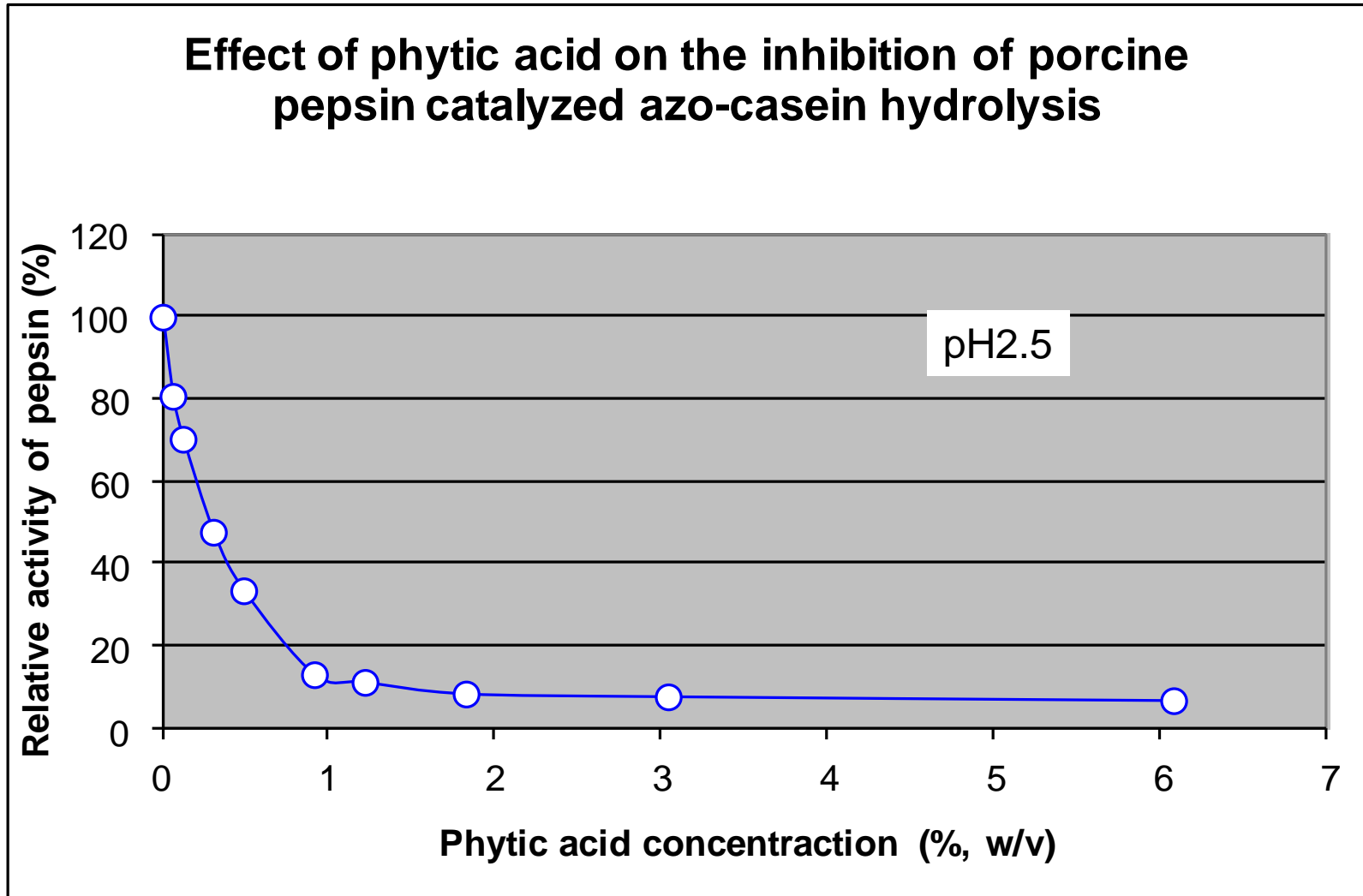
Yu *et al.*, 2012 *J. Anim Sci.* 90:1824-32.

Protein-phytate complex formation is fundamental to phytate effects on protein/amino acid availability

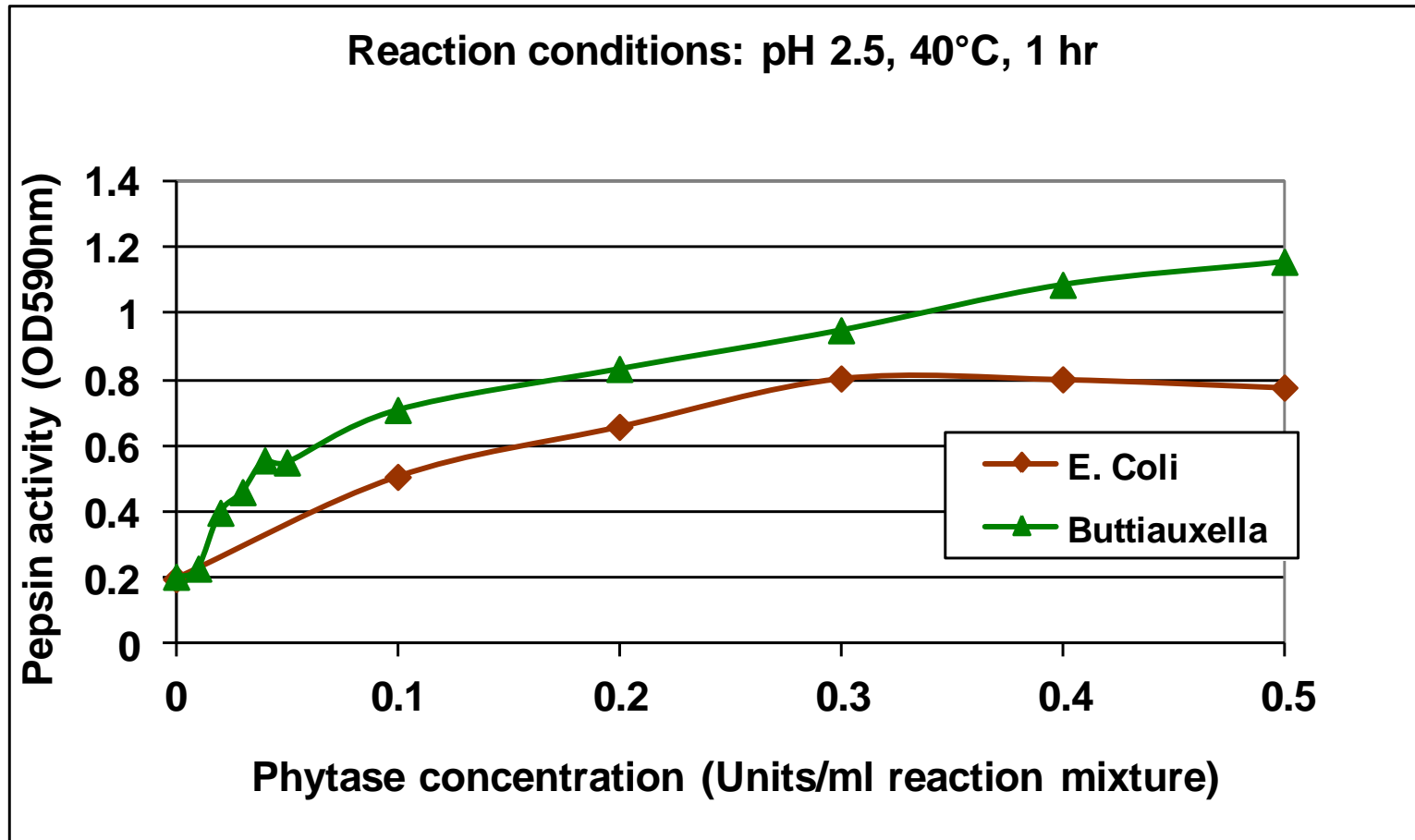
Only IP6 And To A Lesser Extent IP5 Has The Ability To Bind Protein At Low pH



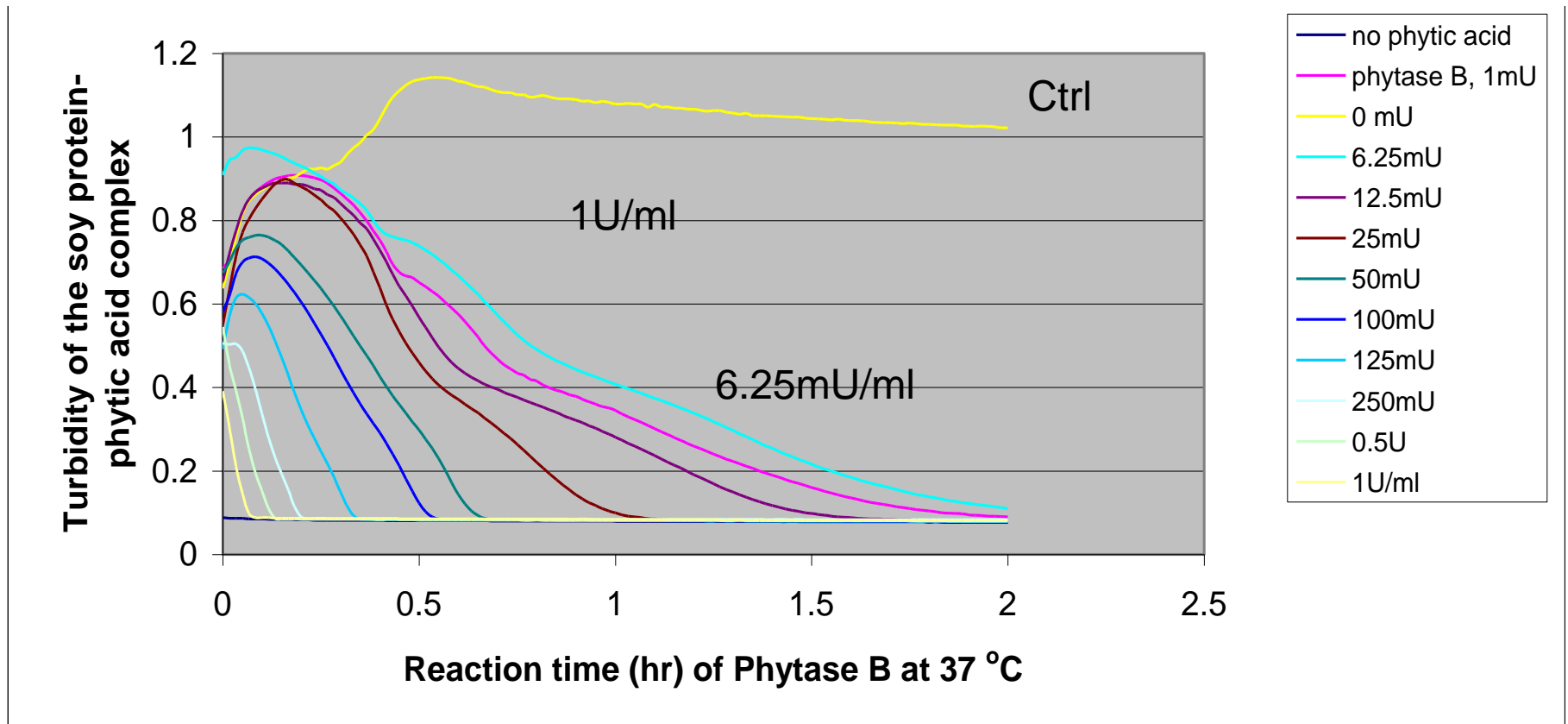
Phytate- Protein Complexes Are Not Broken Down By Pepsin



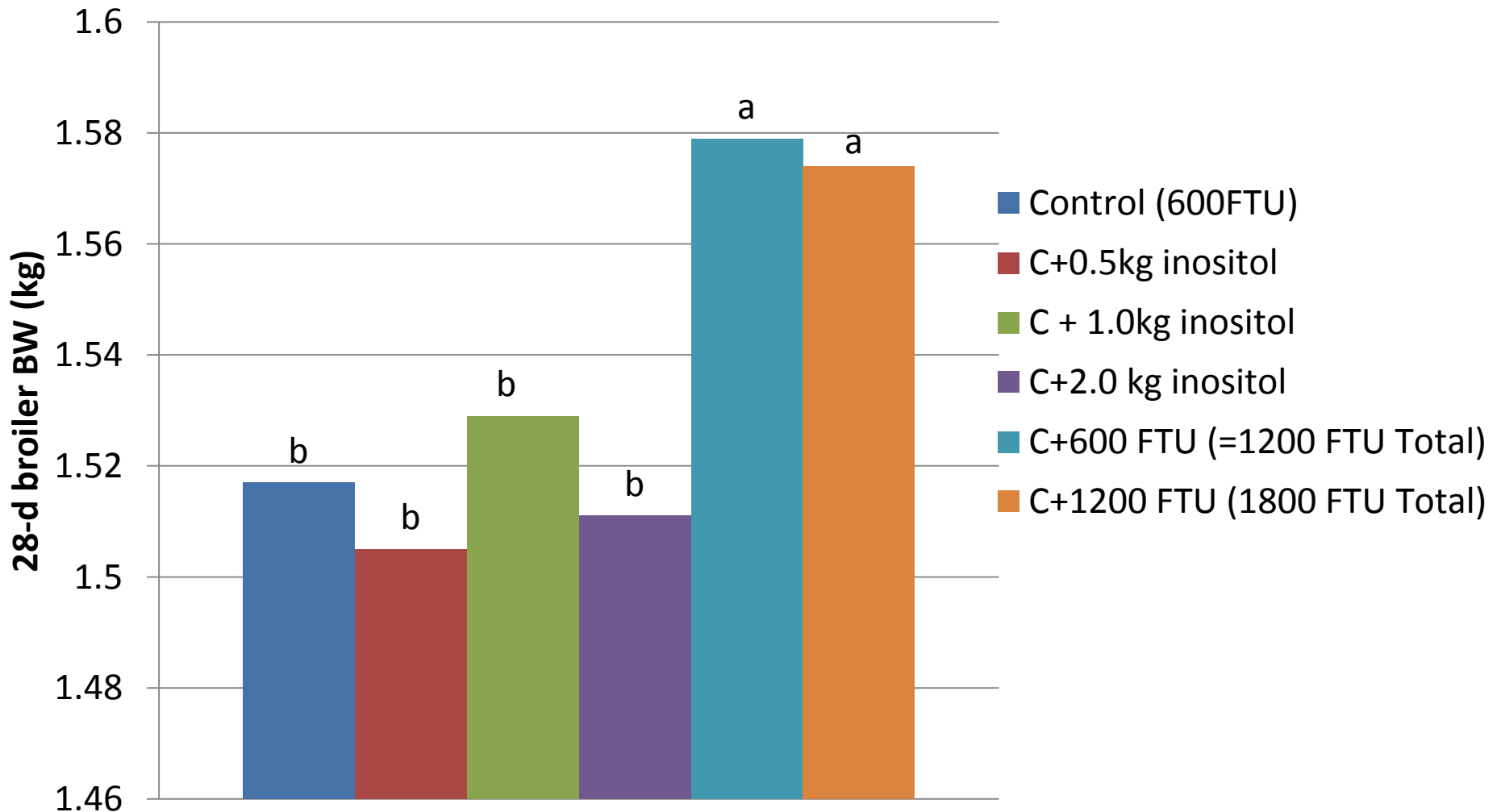
Phytase Reverses Anti Nutritional Effects Of Phytate, Allowing Pepsin To Degrade Protein – Dose Dependent Benefits



Degradation Of Protein-phytate Aggregates By Phytase – Rate Is Dose-dependent



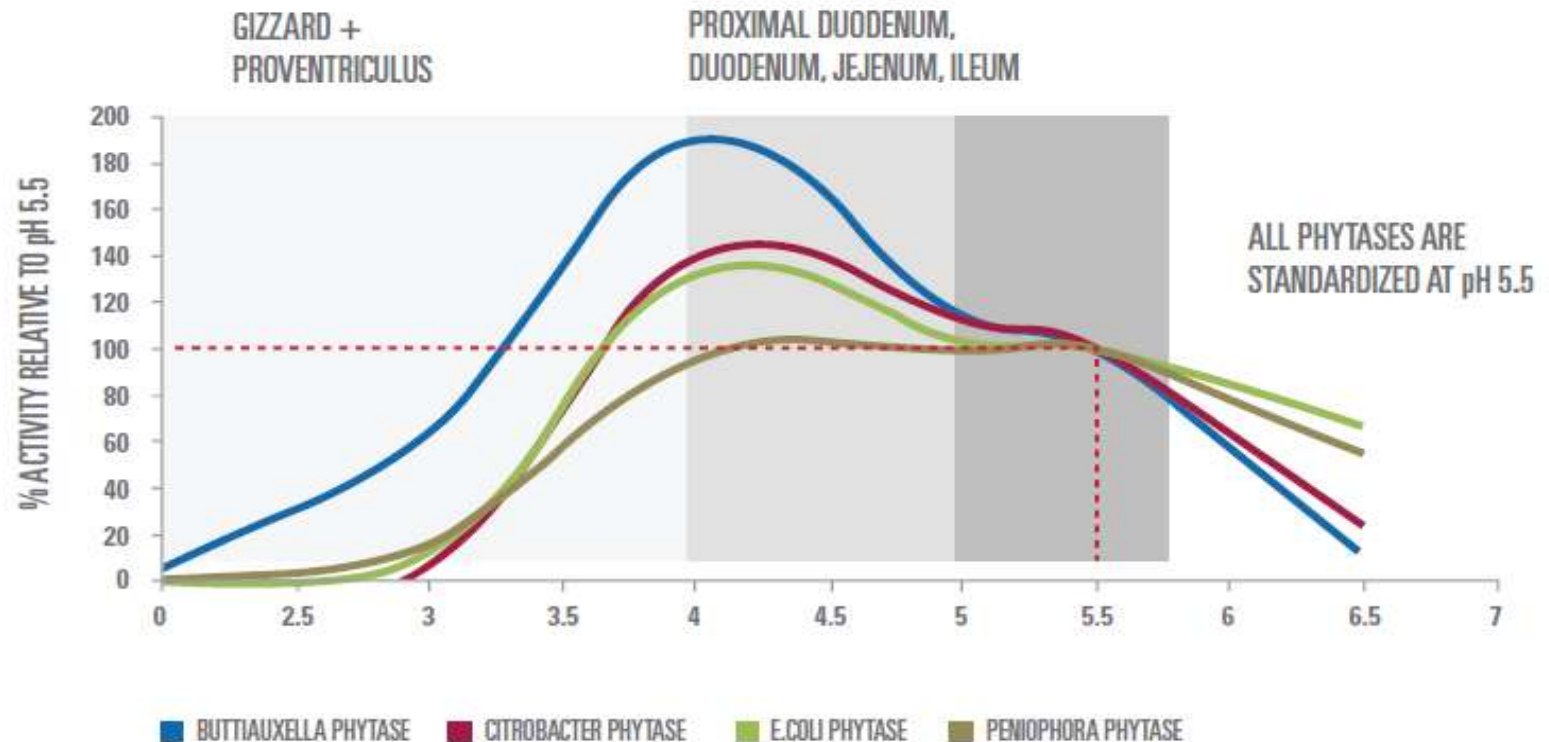
Does Inositol Play A Significant Role In “Superdosing” Effects Of Phytase ?



a-b indicates a significant difference at $p < 0.05$

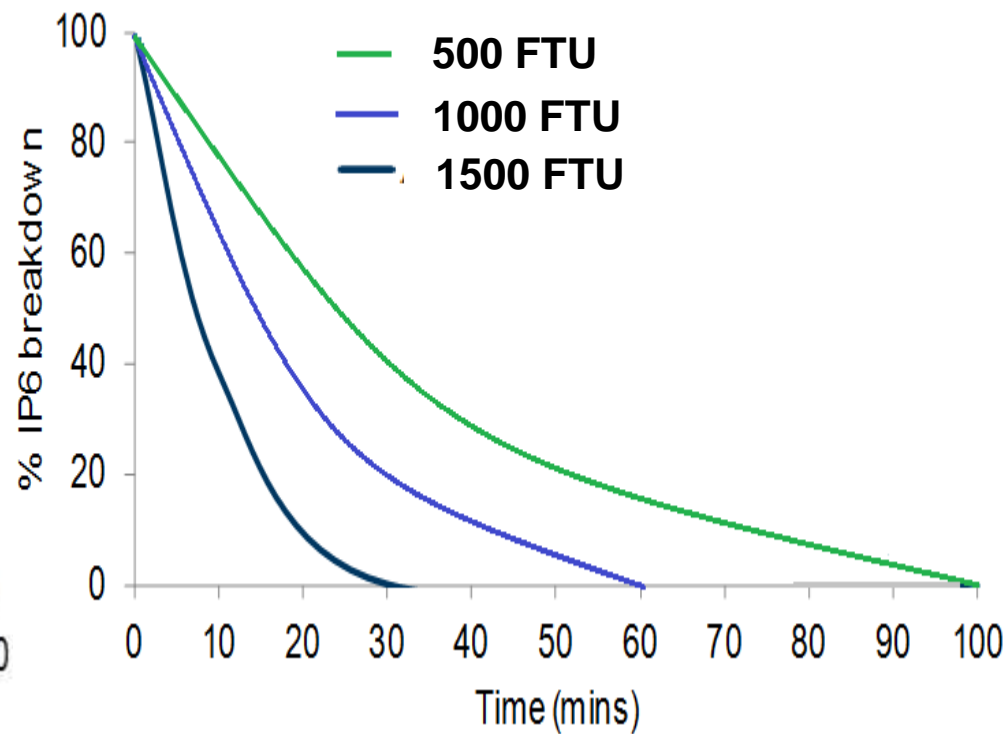
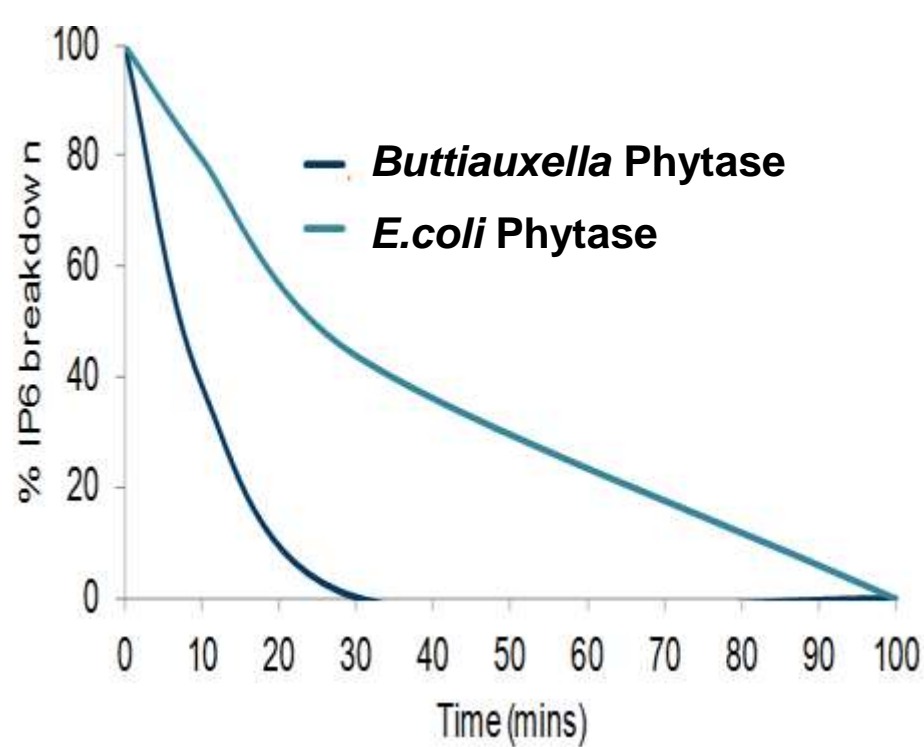
(Walters *et al*, 2015)

Different Phytases Have Different Ph Optima And Different RELATIVE Activity At Low pH Vs. Ph 5.5



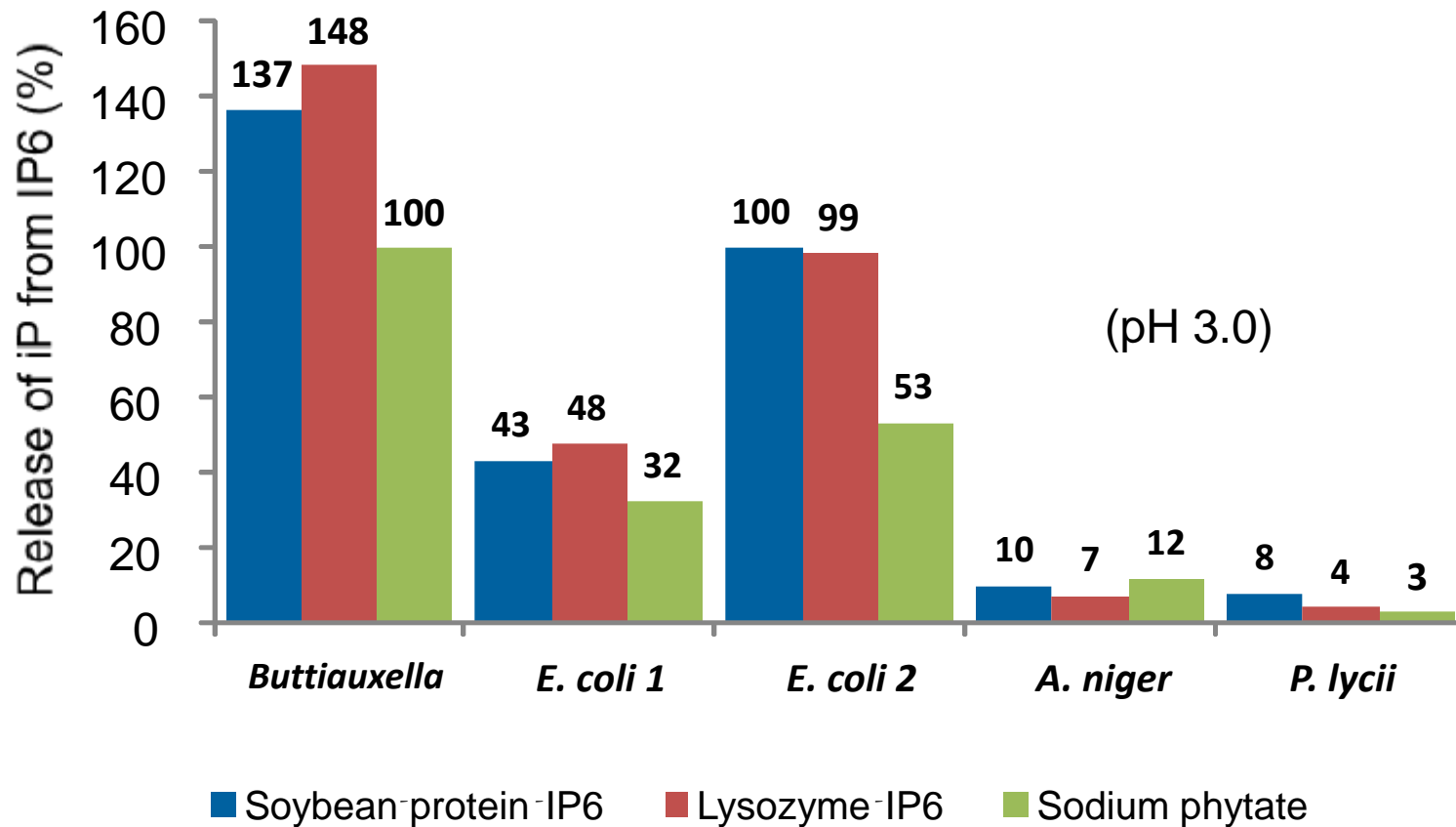
Assay run for 30 minutes at 37 °C, using 5.1 mM Na-phytate as a substrate and 0.02 Ftu/ml

Effects Of Enzyme Kinetics Or Phytase Dose On Speed Of IP6 Hydrolysis



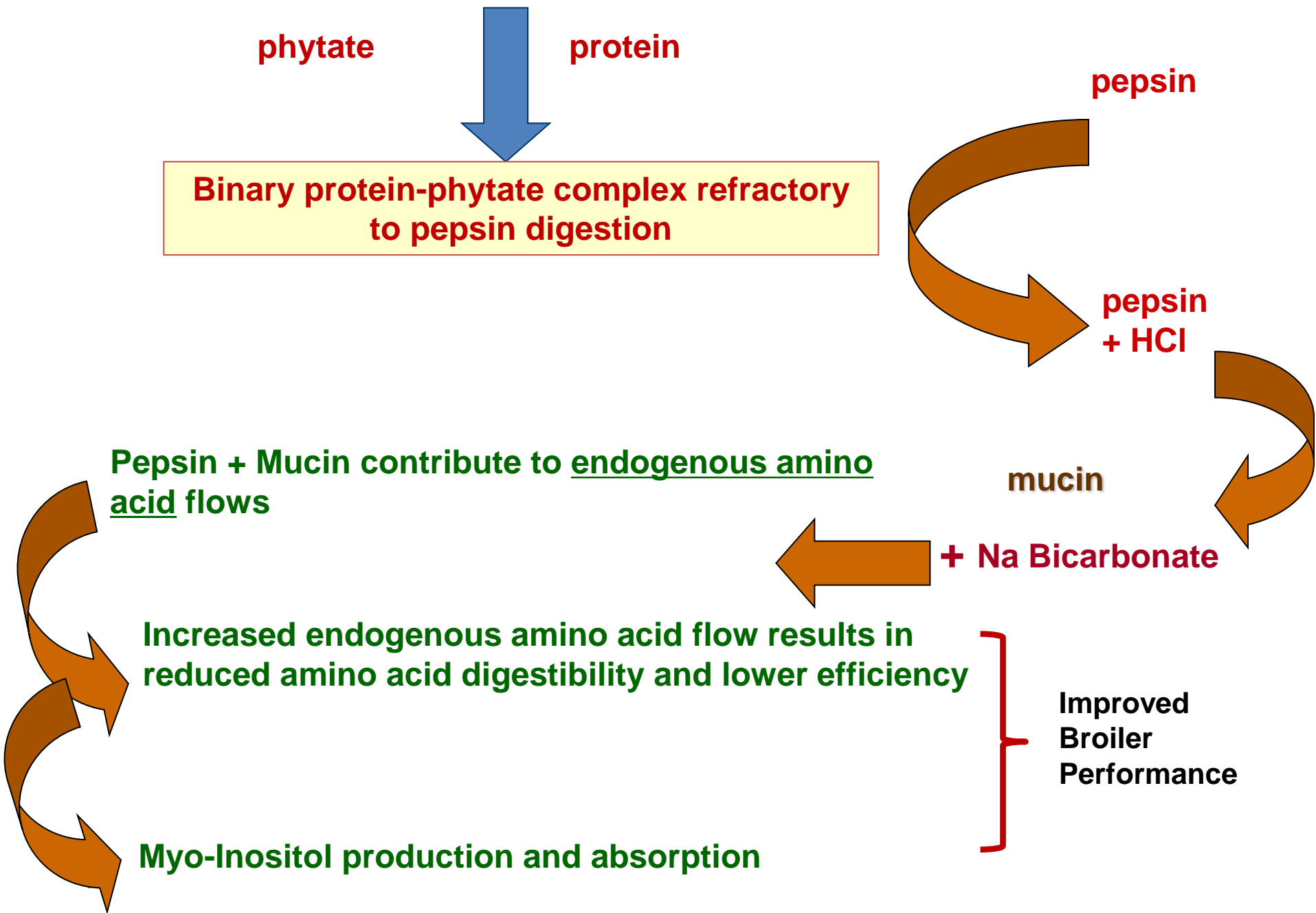
*using sodium phytate as a substrate

Large Differences Exist Between Phytase In Their Ability To Degrade Protein-phytate At Low pH



All values expressed relative to release of IP by *Buttiauxella* phytase on sodium phytate substrate as 100%

Mechanism Of Phytate Anti-nutrient Effects



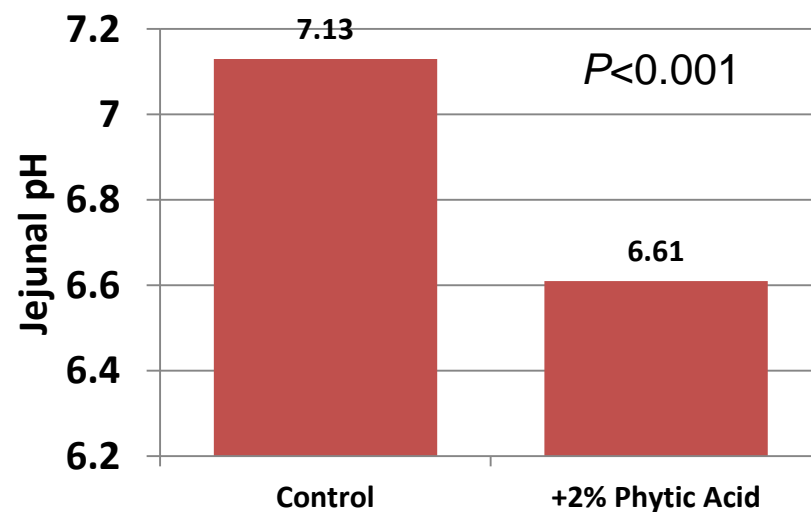
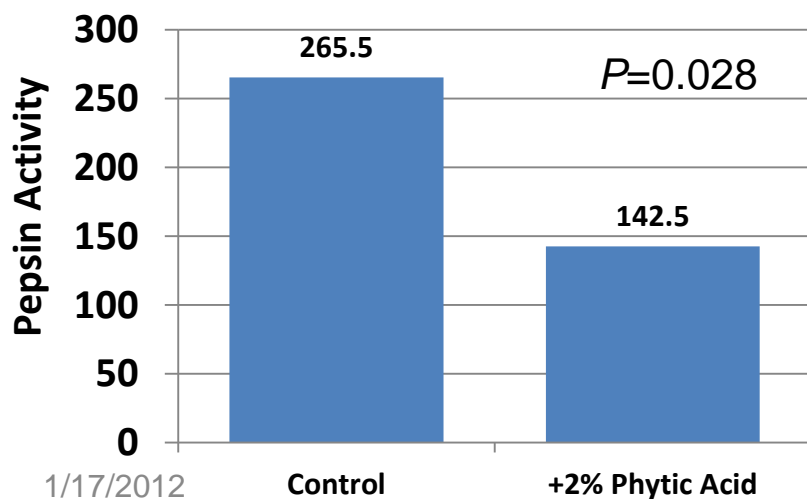
Negative Physiological Effects Of Phytate Have Also Been Shown *in vivo* In Weaner Pigs

Item	Diet ^a		SEM	Contrasts
	Control	PA		
Pepsin activity ^b , PU/ml	265.5	142.5	35.5	0.028
Stomach digesta pH	4.60	4.84	0.277	0.554
Jejunal digesta pH	7.13	6.61	0.122	0.0089
Jejunal mineral content, ppm				
K ⁺	653.4	691.2	75.8	0.737
Mg ²⁺	468.5	69.1	98.7	0.012
Na ⁺	2670.2	4191.9	163.9	<0.0001

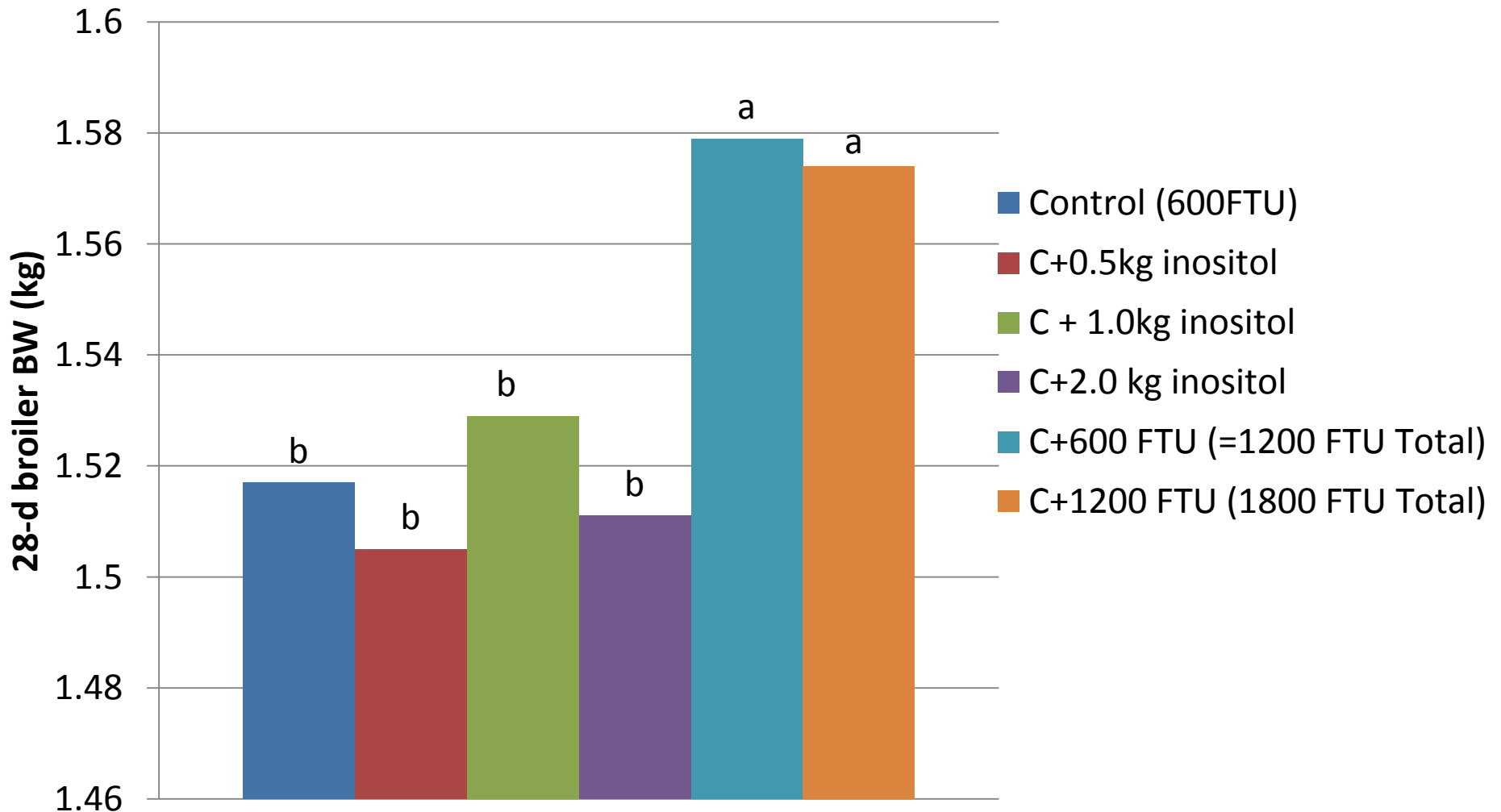
^a PA = control plus phytic acid

^b Determined in stomach digesta.

Woyengo et al. / Livestock Science 134 (2010) 91–93



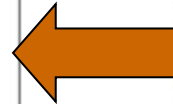
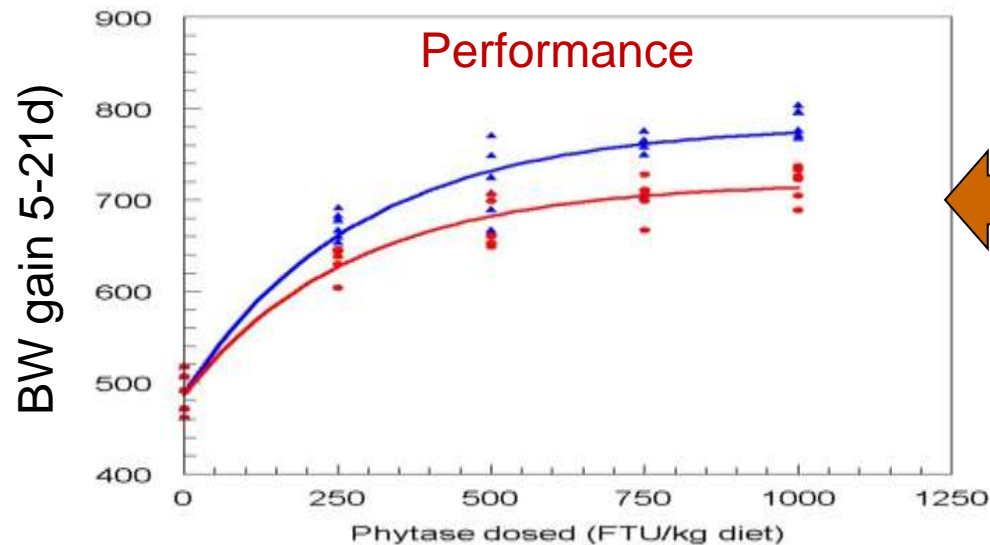
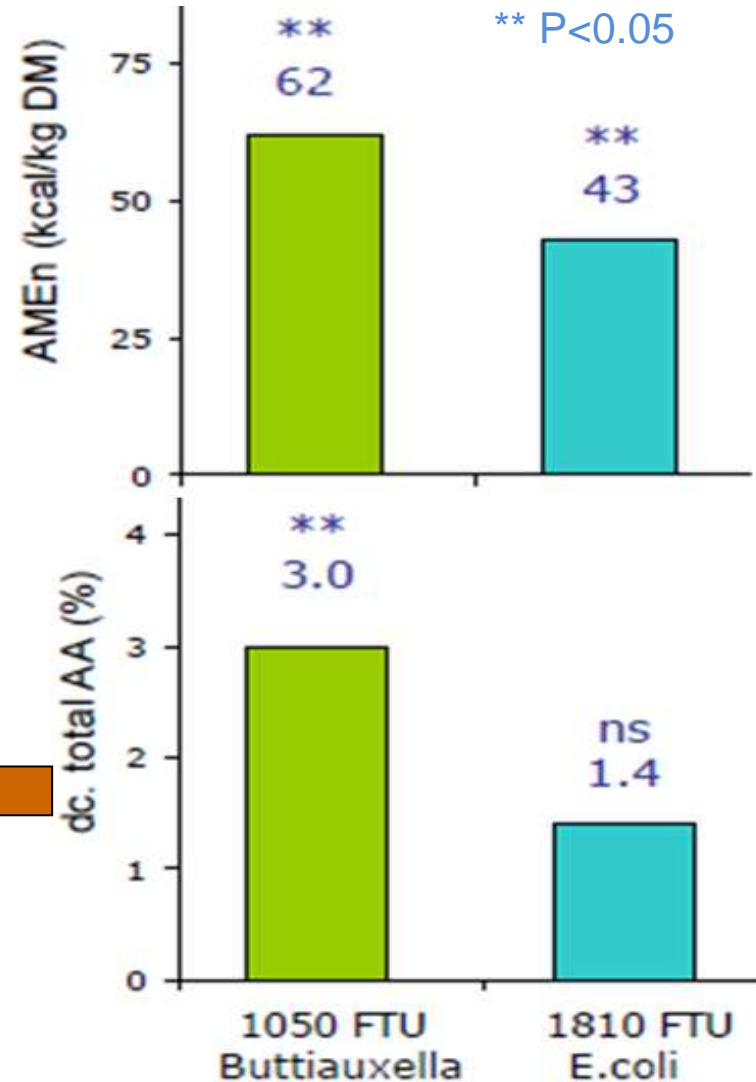
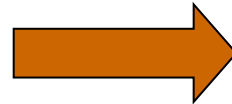
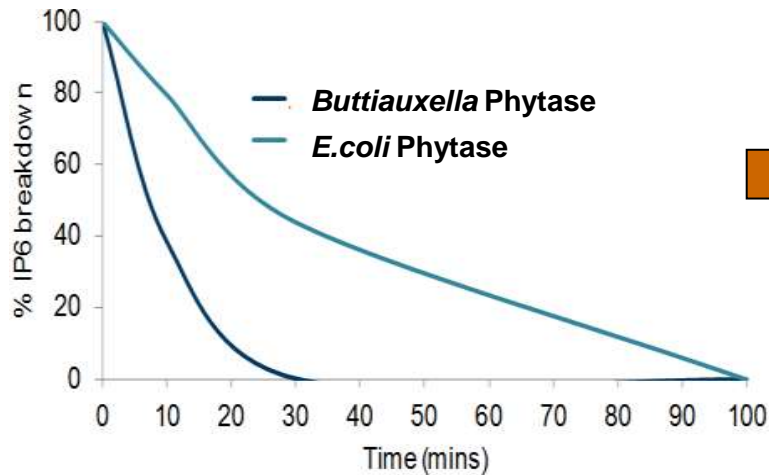
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Walters *et al*, 2015

Differences In In-vitro Phytase Chemistry , IP6 Hydrolysis Rate & Protein-phytate Degradation Support Repeatable In-vivo Responses

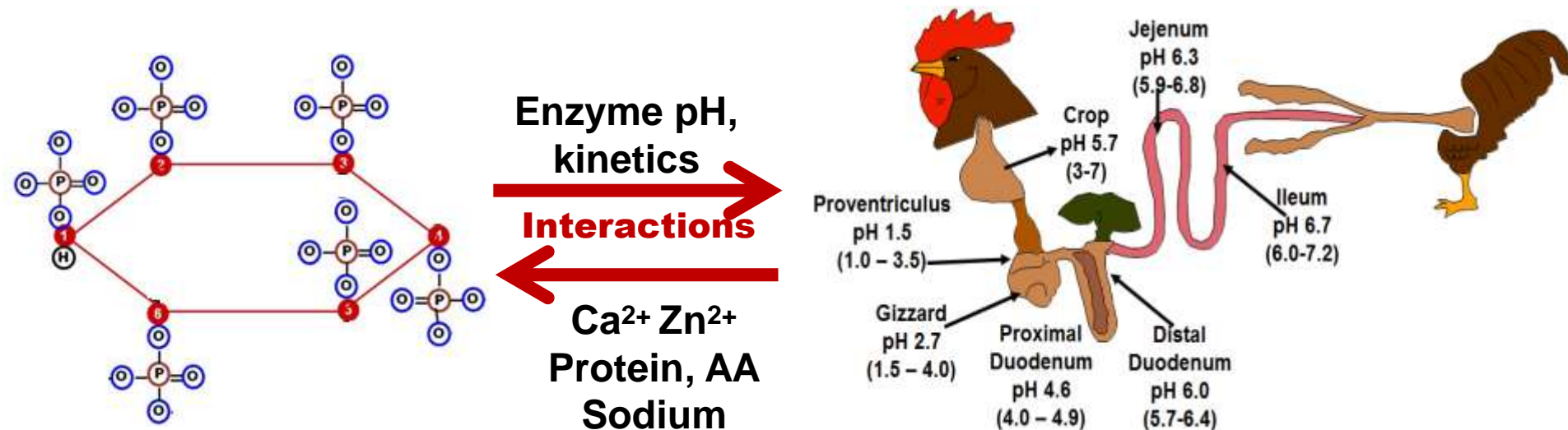


Tibia ash

Kwakernak et al., 2013 ESPN

Vd Klis et al., 2013

Phytase Decisions On *Source* And *Dose* Also Need Be Based On Phytate Interactions With Nutrients And Understanding Differences In Biochemistry Between Phytase Enzymes In The Context Of Digestive Physiology



- Interactions of Phytate, Calcium and Phytase Enzymes – **impacts P contribution**
- Interactions of Phytate with Protein, Starch, and Na – **Anti-nutrient effects on live performance & drives ME & AA digestibility improvement from phytase**
- Differences in phytase enzyme pH optima and kinetics - **impact *in-vivo* results**

Which came first,
the chicken or the egg?



QUESTIONS?