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Research advances to maximize value from new phytases in poultry and pig diets

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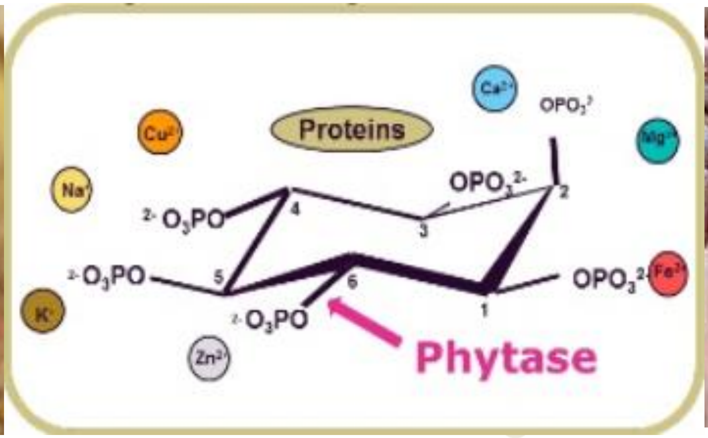
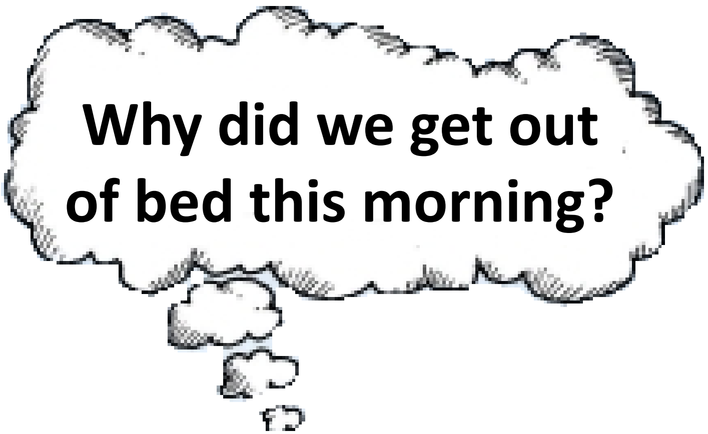
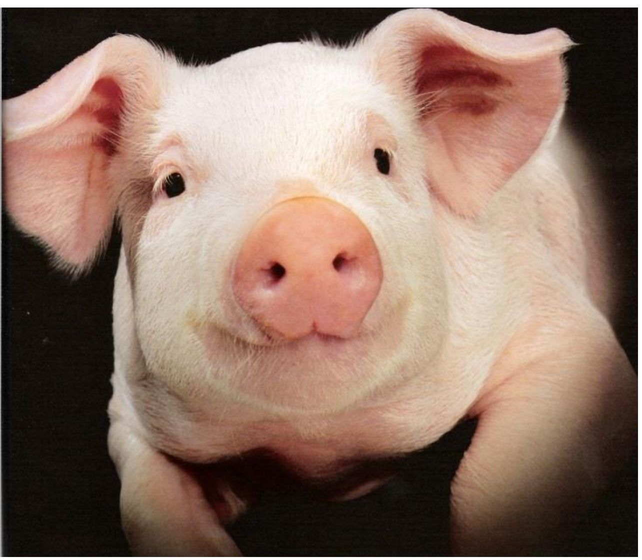


Your Animals, Our Science





DuPont have
$ 8 – 10 Million
Investment in Animal
Nutrition Research
every year to develop
new technologies &
better performance

We are passionate about Using Science to Improve the conversion of Feed to Food, improving efficiency and reduce cost

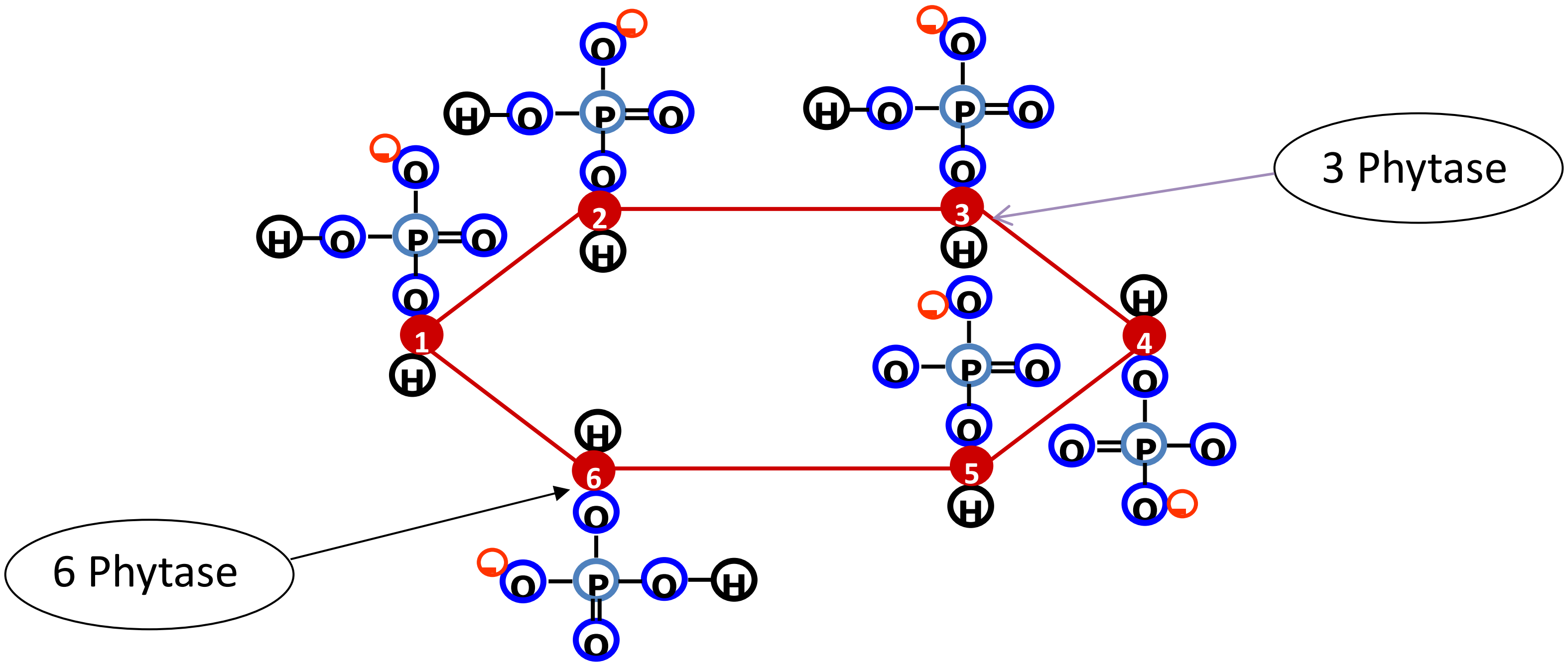
We invest in Research

Understanding how things work physiologically allows Nutritionists to make better decisions on Enzyme source and dose

Our research has produced leading enzymes and probiotics that help our customers achieve their goals

A VERY simplified look at phytate and phytase

- All feed ingredients of plant origin contain phytate phosphorus.
- Average phytate P in wheat/soy diet is about 0.28% (range of 0.22 to 0.35%).
- Phytate phosphorus, is not well digested /available to the animal.



- Adding phytase increases availability of phytate phosphorus
- **Adding phytase** **reduces the amount of inorganic P added to the diet, which saves money.**

The Dilemma of Nutritionists

There are many phytase products out in the market, how does one choose one?

Source organism	Expressed in	Commercial product name
E.coli	*Pichia pastoris*	**Optiphos + Chinese phytase**
Protein Engineered E.coli	*Pichia pastoris*	**Quantum**
E.coli	*Schizosaccharomyces pombe*	**Phyzyme® XP**
Peniophora lycii	*Aspergillus oryzae*	**Ronozyme NP**
Citrobacter braakii	*Aspergillus oryzae*	**Ronozyme HiPhos**
Protein engineered E.coli	*Trichoderma reesei*	**Quantum Blue**
Buttiauxella spp.	*Trichoderma reesei*	**Axtra® PHY**

The Dilemma of Nutritionists

Large differences exist between Phytase suppliers in AvP and digestible P "matrix values"

	E.Coli 1	E.Coli 2	E.Coli 3	Citrobacter	E.Coli 4	Buttiauxella
FTU/kg feed	500 FTU	500 OTU	500 FTU	1000 FYT	500 QU	500 FTU
Digestible P%	0.11	0.11	0.13	0.117	013	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	100	0.80	100	0.92
Calcium %	0.11	0.13	0.14	0.18	0.165	0.134

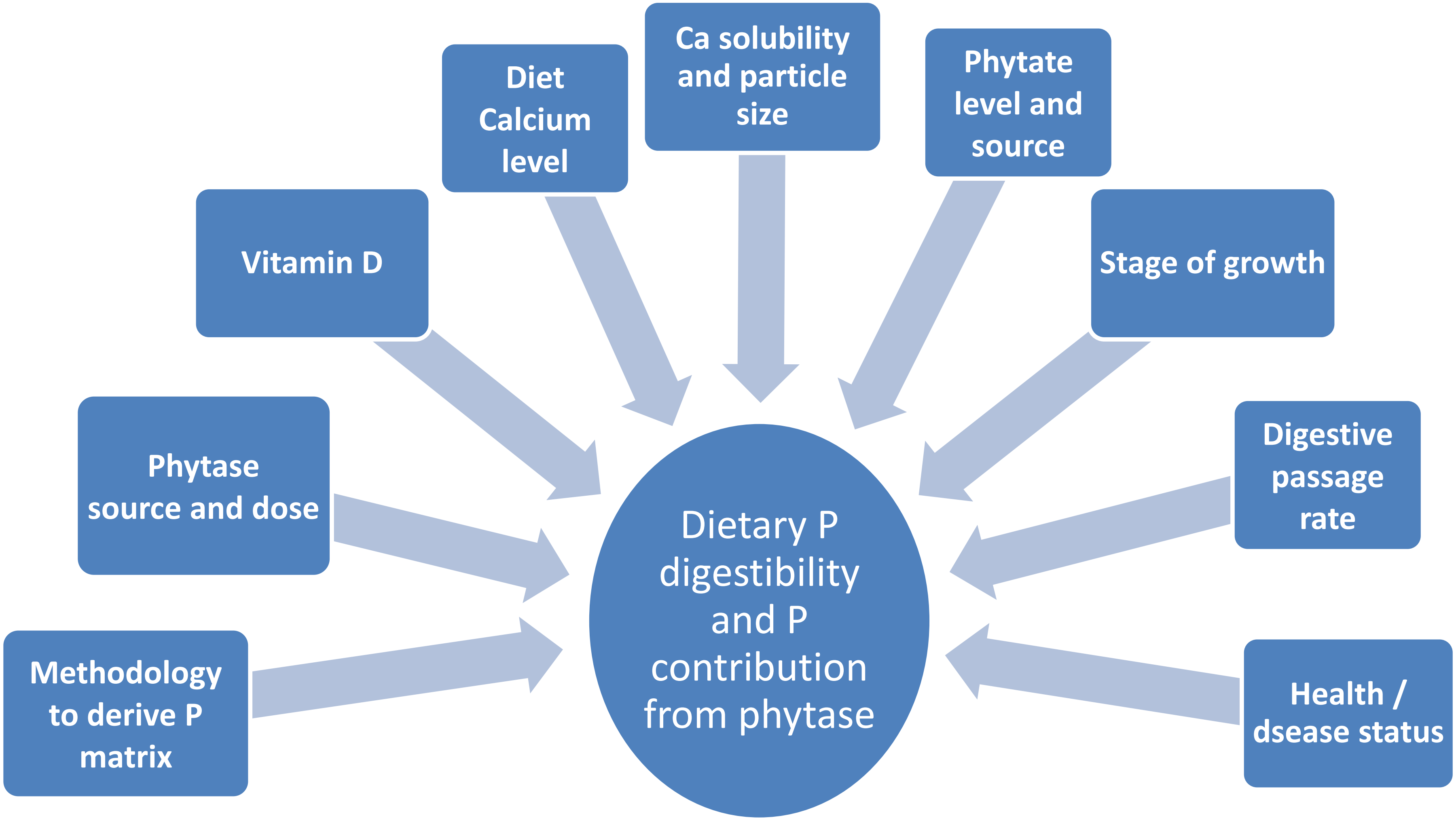
Critical Questions to Ask:

- How were phosphorus (P) matrix values determined?
- How does the P-system used compare to your Ingredient P matrix?
- What about Ca^{2+} matrix values?
- What factors affect the variation in response to phytase?
- How do we predict the response to maximize value?

Understanding phytase matrix values, and what affects variation in Phytase Ca and P contribution from phytase is critical to optimizing the opportunity for feed cost-saving from phytase vs. the risk of incurring a Ca or P deficiency.

Phytase Research & Science behind Matrix values

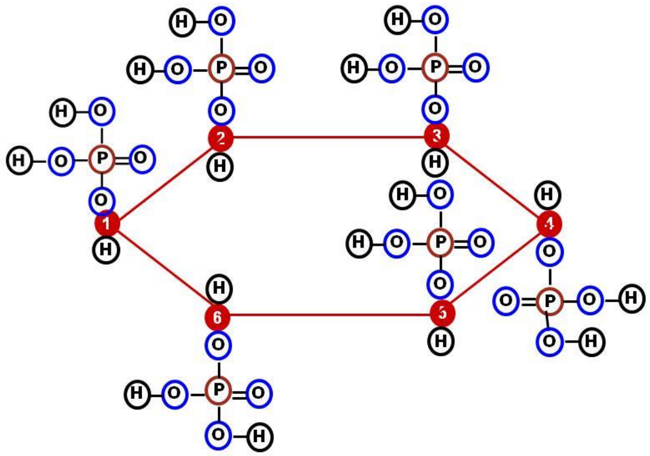
A lot of other factors affect P digestibility and Phytase value. Need to be understood to reduce risk & optimize profit



Thermostability and Recovery in Finished Feed

What Biochemical criteria make a phytase great in terms of in-vivo efficacy?

–For maximum Phytase performance benefits in the animal, IP6 (Phytate) needs to be hydrolysed as **<u>as rapidly as possible</u>, as completely as possible, in the proximal part of the digestive tract** (Acid stomach /Proventriculus/Gizzard) with IP5-IP2 in Duodenum / Early Jejenum.



WHY?

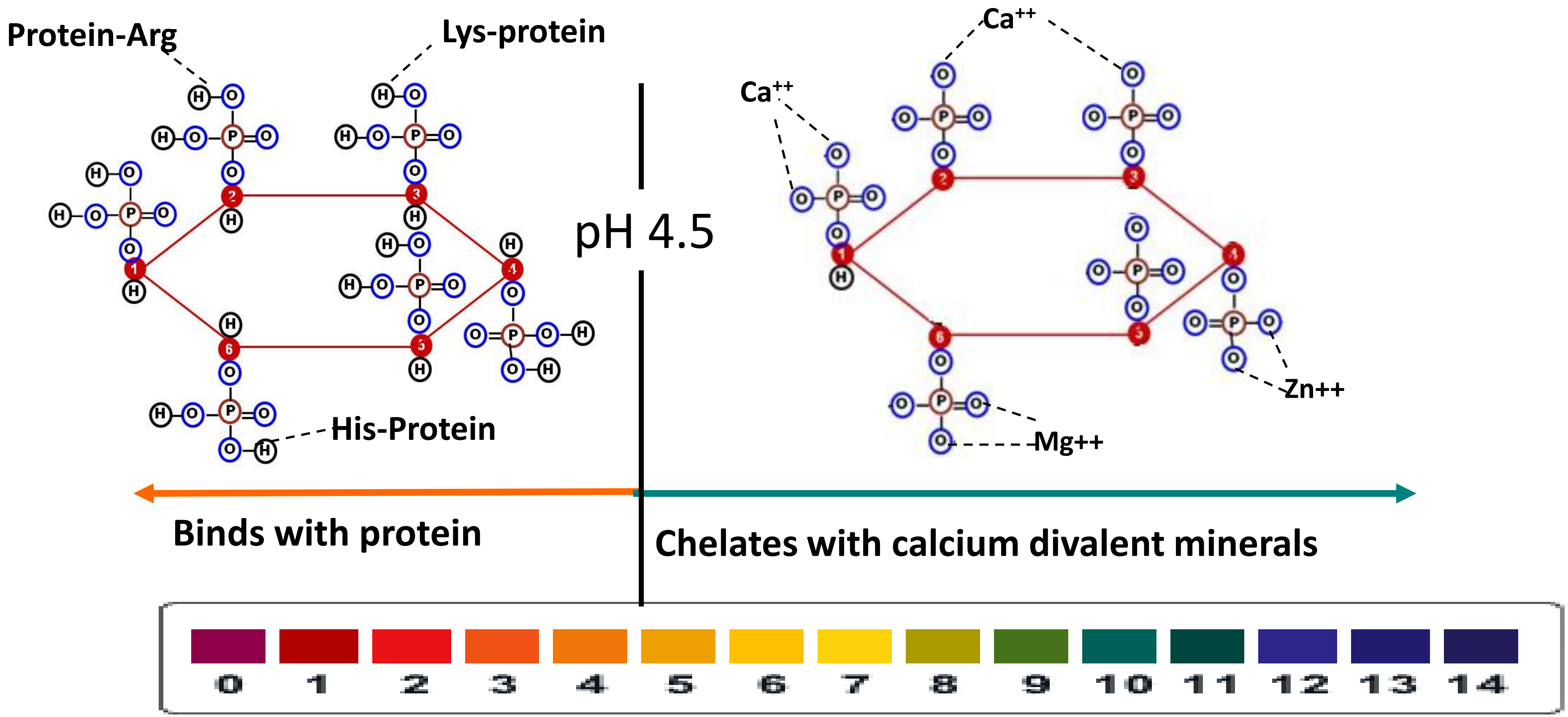
Selection of phytase can be done very effectively in-vivo on this basis:

Anti-Nutrient Effects of Phytate are pH Dependent

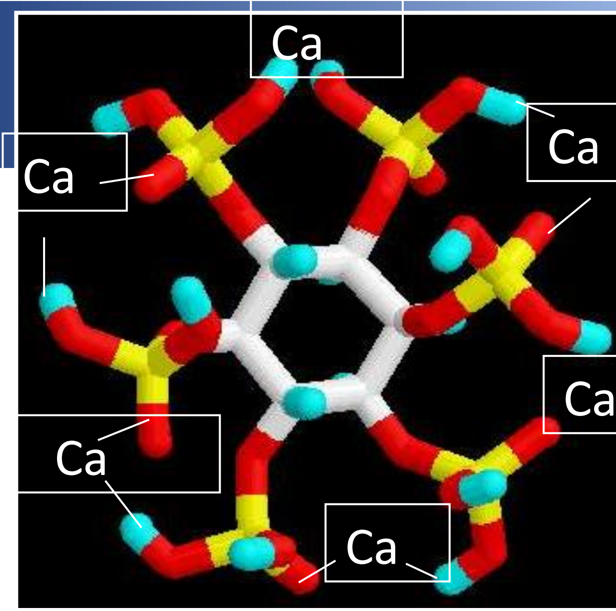
To maximize Phytate Utilisation the phytase must prevent it complexing with both Protein + Cations

Gizzard / Proventriculus

Duodenum / Ileum / Jejenum

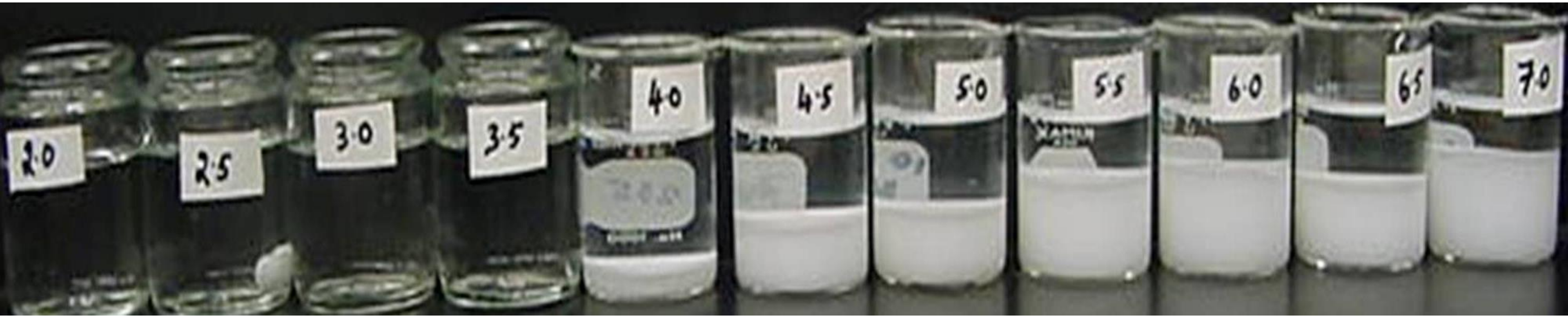


Impact of pH and Calcium on phytate solubility



% soluble Phytate P

100 100 99.3 97.9 84.7 62.1 51.7 26.4 11.1 7.9 1.5

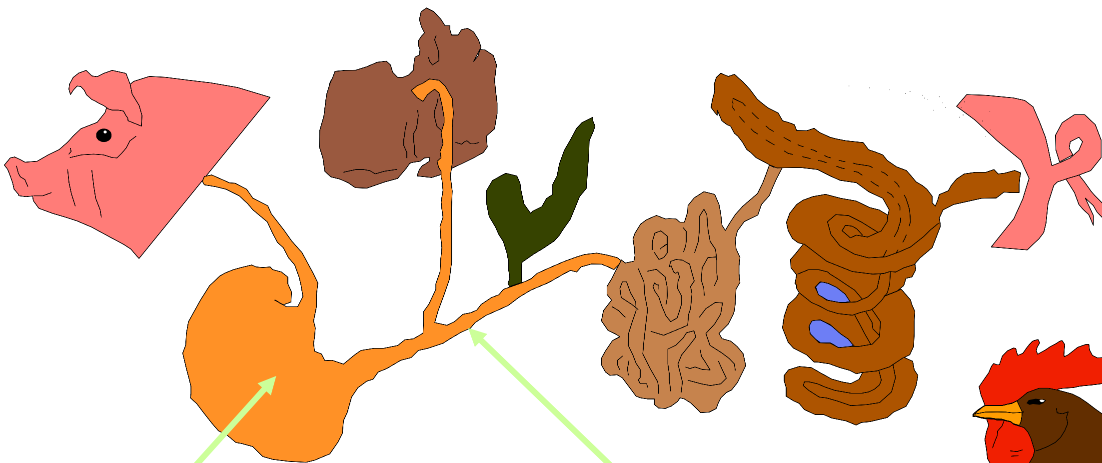


2 mmol Phytic Acid + 30 mmol Calcium
[Corn/SBM dietary phytic acid + 0.9% Calcium (2:1; H_20:feed)]

Used with permission,
Dr. R. Angel et al., 2010

University of Maryland 1856

Putting Phytate Solubility into a practical context



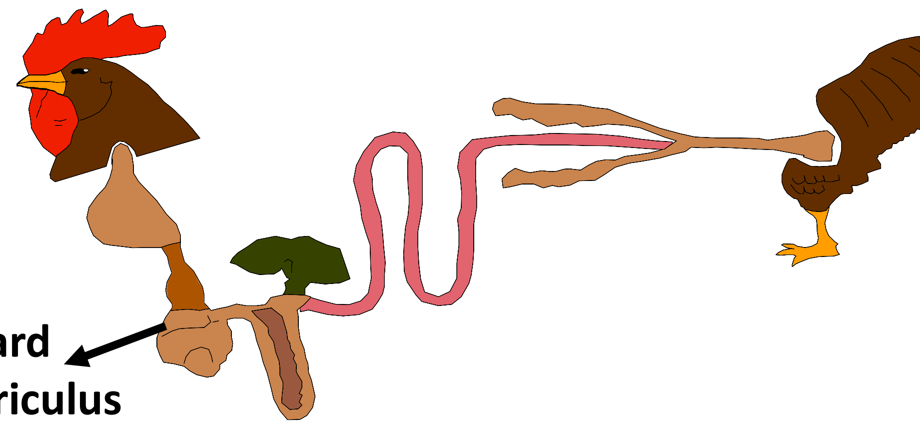
Stomach

pH 2 – 3.5

(3 hrs)

Duodenum

pH 3.5 – 5.5



Gizzard

Proventriculus

pH 2.5 – 3.5

(40-60 min)

% soluble PP

97.9	84.7	62.1	51.7	26.4	11.1	7.9	1.5

In Poultry, Time in Gizzard/ Proventriculus is limiting

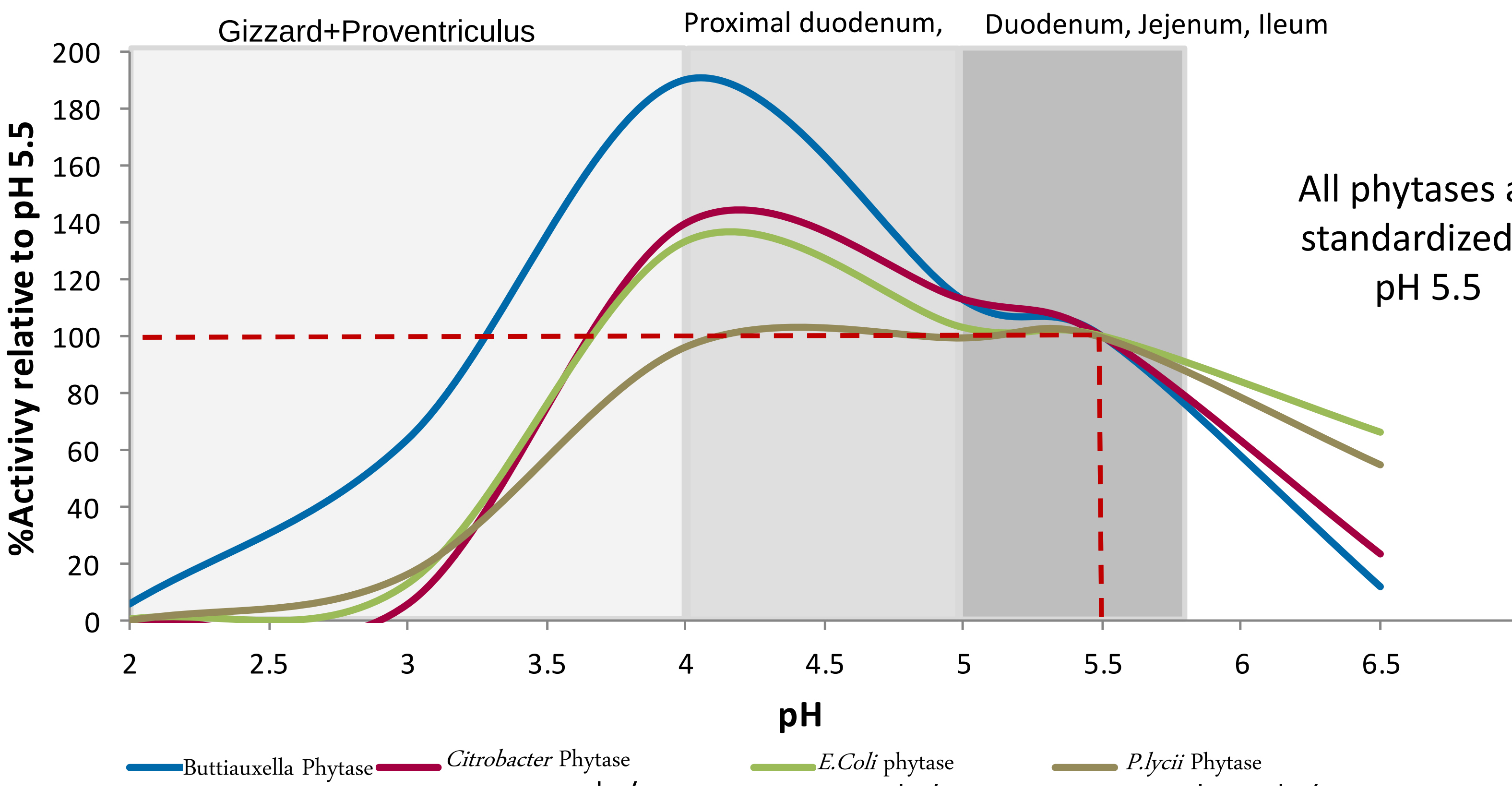
(Slide from R. Angel, 2013)

GIT segment	pH mean (min-max)	Ca-phyate P solubility, %[1]	Mean Retention Time (MRT , min[2]) MRT1	MRT2
Crop	5.7 (3-7)	17.6	41	58
Proventriculus	1.5 (1-3.5)	100	P+G 33	75
Gizzard	2.7 (1.5-4)	100		
Prox. Duod.	4.6 (4.0-4.9)	60.3	2	7
Distal Duod.	6.0 (5.7-6.4)	11.1	3	7
Jejunum	6.3 (5.9-6.8)	8.8		
Prox. Jej.			23	27
Distal Jej.			48	61
Ileum	6.7 (6-7.2)	4.4	90	94

[1]Calculated from regressions done with Ca-phyate P solubilities determined in vitro simulating Corn-SBM starter dt concentrations of PP and Ca (Angel et al; unpub.)

[2]Mean retention time - estimated as MRT1 (steady state) amt Cr in GIT segments as % of daily Cr intake; MRT2 calc. based on exponential curve equation of Cr in different segments between 0 and 4.5 h post marker feed feeding in a system where marked feed is fed for 30 min after a 1 hr withdrawal, followed by feed withdrawal.

Different Phytases have different pH optima and different RELATIVE activity at low pH vs. pH 5.5.

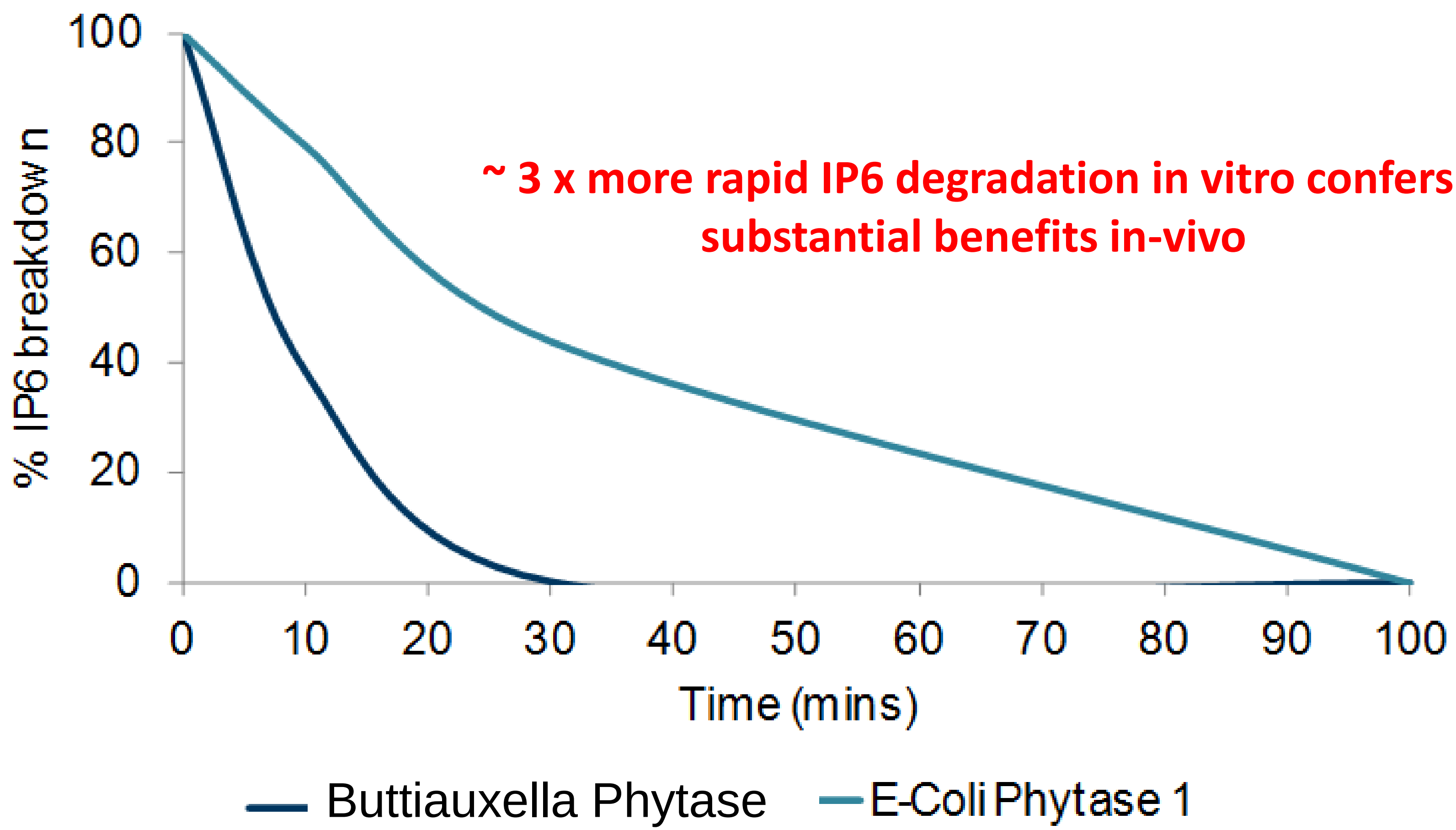


All phytases are standardized at pH 5.5

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

DuPont Laboratory, 2012

Rate of IP6 degradation of Buttiauxella vs. E.coli phytase

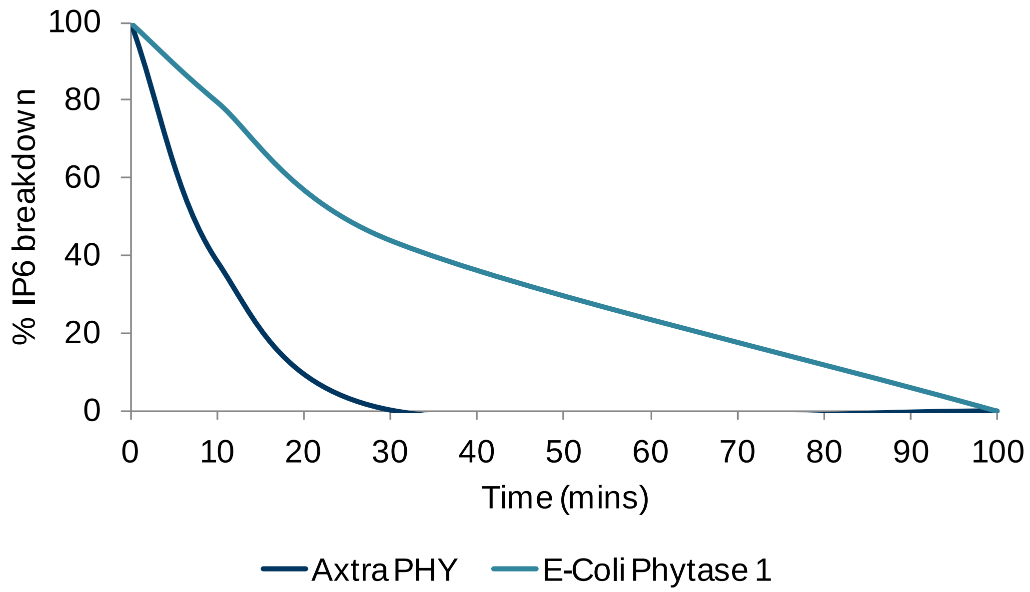


~ 3 x more rapid IP6 degradation in vitro confers substantial benefits in-vivo

*using sodium phytate as a substrate

New Buttiauxella Phytase = Axtra Phy

- Buttiauxella Phytase gene isolated and expressed in *Trichoderma reesei*.
- *In-vitro* comparative efficacy showed Buttiauxella phytase to degrade phytate P 3 x faster from Na-Phytate vs. E-coli Phytase



How does a High Speed of Phytate Hydrolysis affect Efficacy in Broilers?

In Vivo Comparison trials: *Buttiauxella vs. E.coli* Phytase

- Three consecutive 21-d performance + digestibility trials conducted at Schothorst Feed Research, NL.
- Each trial used a similar experimental design consisting of:
- Male Ross 308 broiler chicks fed a P adequate diet from 0-5 d of age.
- Treatment diets fed from 6-21 days of age to 6 replicate cages of four broilers.
- All diets cold-pelleted, and phytase activity confirmed by analysis (LUFA, Oldenburg, Germany)
- Feces collected from 17-21 days for determination of P digestibility (retention).
- Ileal digesta collected at 21d for determination of P digestibility.
- Phosphorus digestibility calculated relative to TiO_2 as an inert marker.
- Left tibia from 4 birds/cage collected at 21 d of age and fat free ash% determined.

Dietary Treatments in 3 broiler trials at Schothorst Feed Research:

1. **Negative Control (NC)**
2. **NC+250 FTU - Axtra Phy**
3. **NC+ 500 FTU - Axtra Phy**
4. **NC+ 750 FTU - Axtra Phy**
5. **NC + 1000 FTU - Axtra Phy**
6. **NC+250 FTU - E.coli Phytase**
7. **NC+ 500 FTU - E.coli Phytase**
8. **NC+ 750 FTU - E.coli Phytase**
9. **NC + 1000 FTU - E.coli Phytase**
10. **Positive Control - +0.18% AvP from Monocalcium Phosphate**

The commercial E.coli Phytase source was varied in each trial:

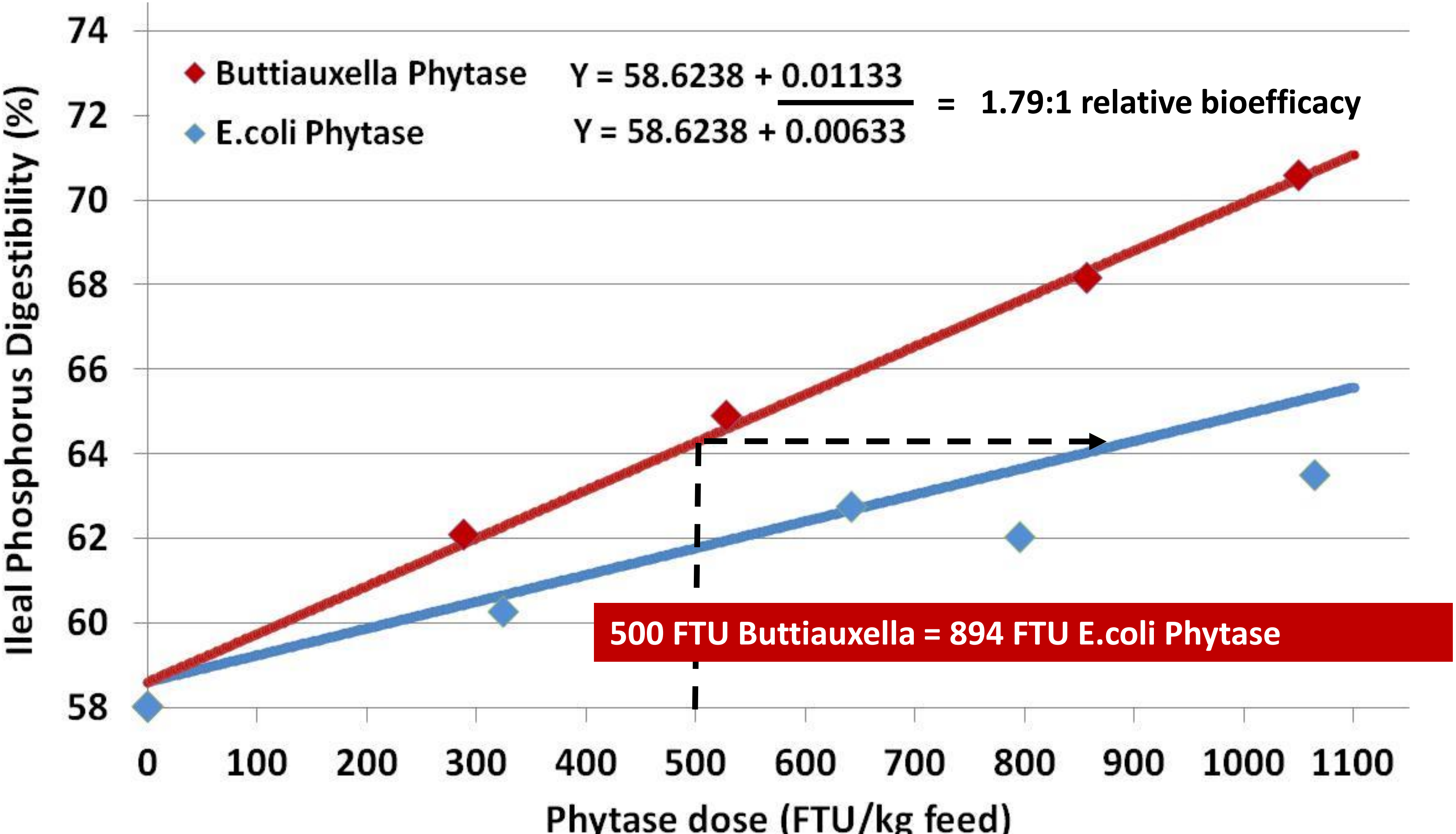
Trial 1: *E.coli* phytase expressed in *P. pastoris_1*

Trial 2: *E.coli* phytase expressed in *P. pastoris*_2

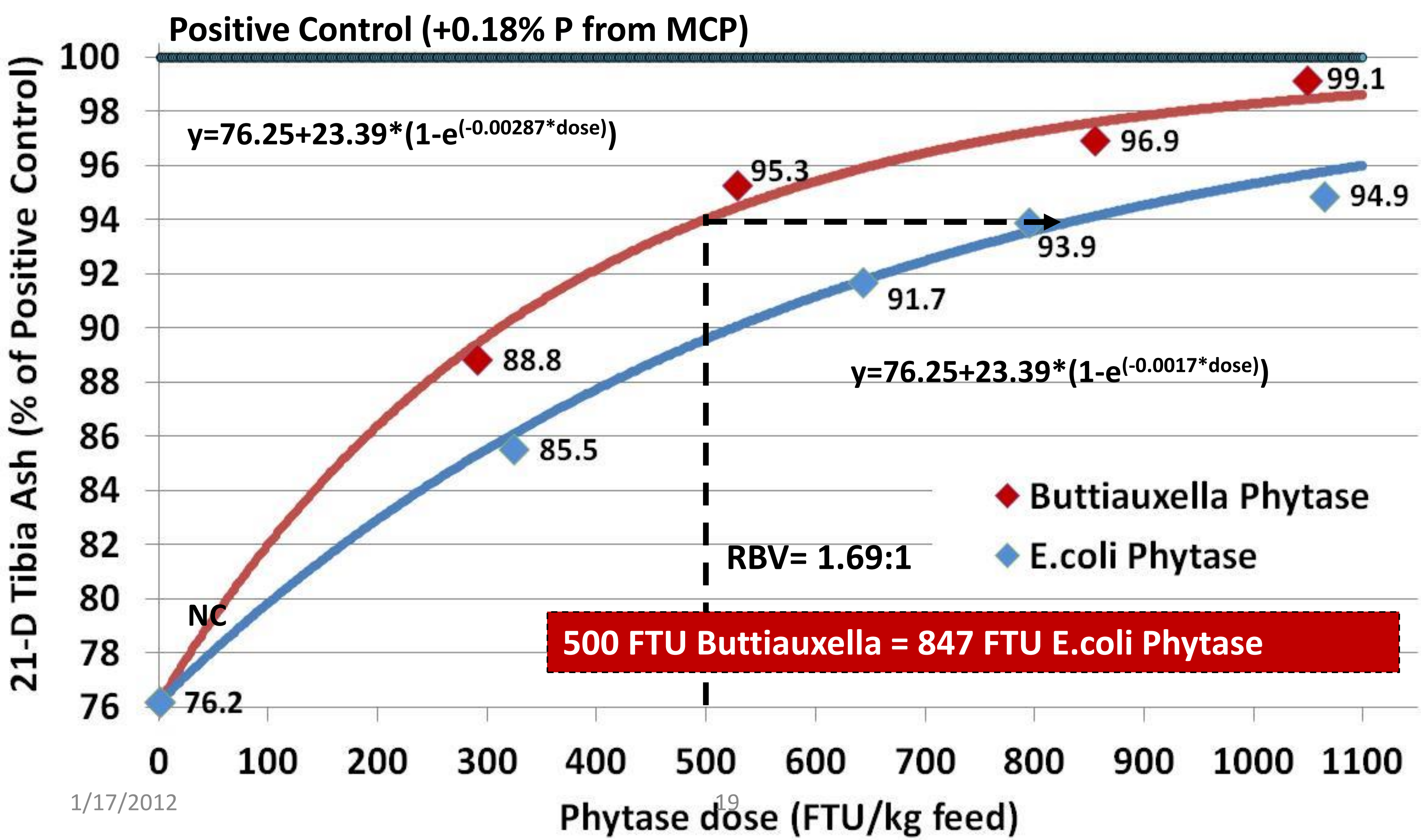
Trial 3: *E.coli* phytase expressed in *S. pombe*

All phytase products were analysed for phytase activity in an independent laboratory (LUFA, Oldenburg, Germany) and dosed based on analyzed FTU/g product.

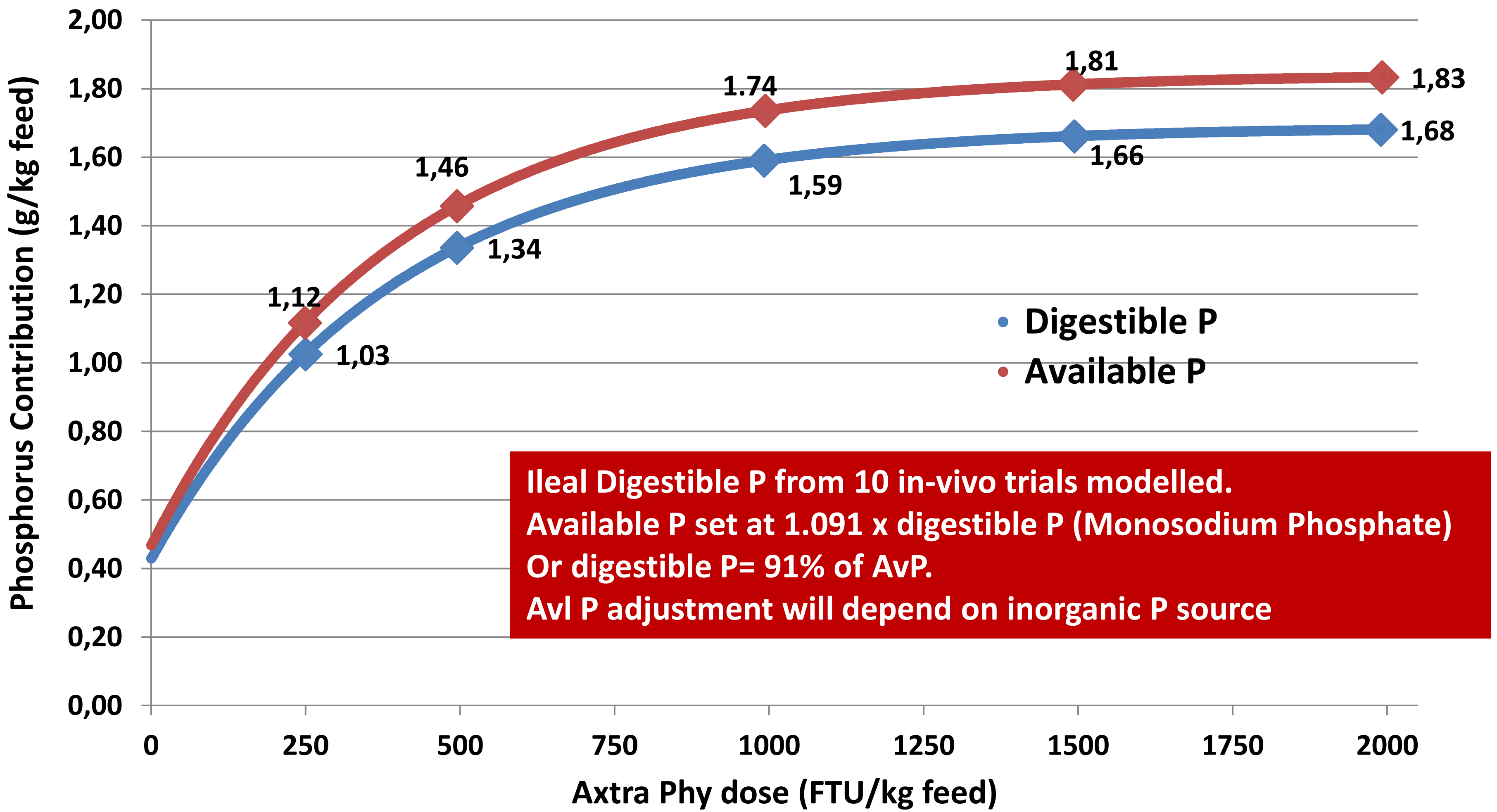
Results: relative bioefficacy of *E.coli* vs. *Butiauxella* phytase in increasing Ileal phosphorus digestibility



Results: relative bioefficacy of *E.coli* vs. *Buttiauxella* *p*hytase using broiler Tibia Ash as the response



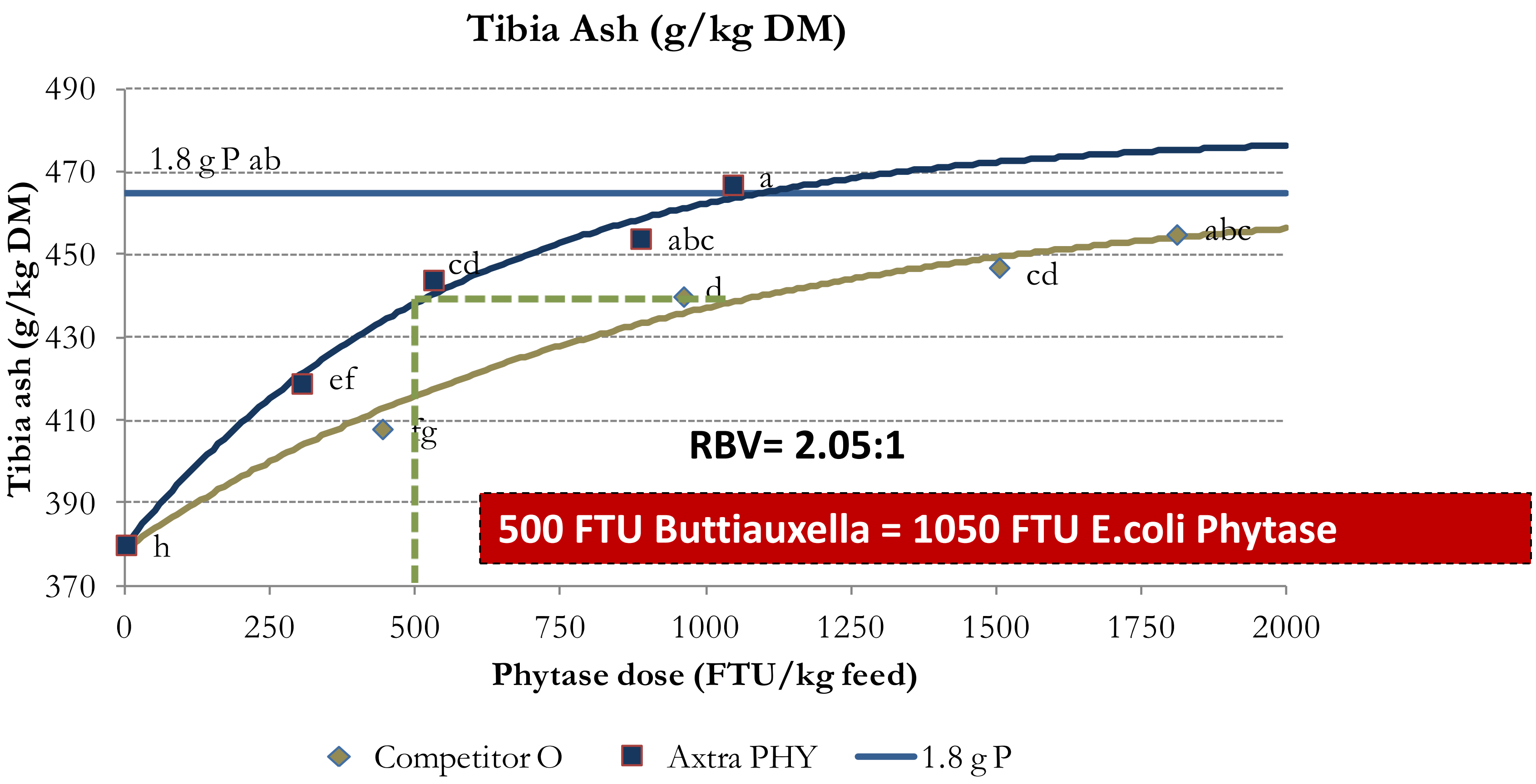
Broiler Matrix values for Digestible P derived from 10 digestibility trials



Model based on 296 data points from 10 broiler ileal digestibility studies.

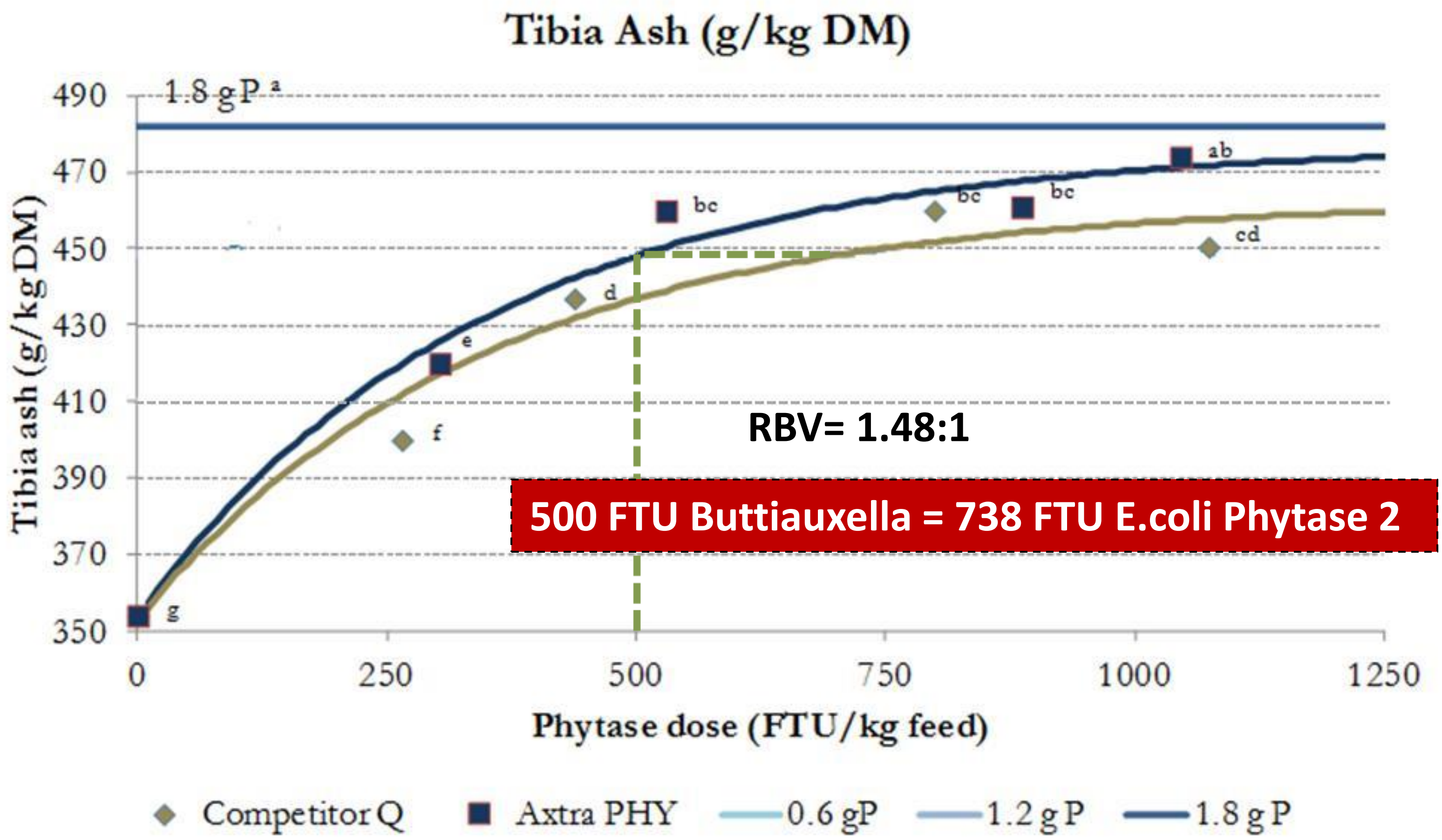
Validation vs. Positive Control diets using Tibia ash% :

Schothorst Trial 1: Axtra PHY versus E.coli Phytase 1



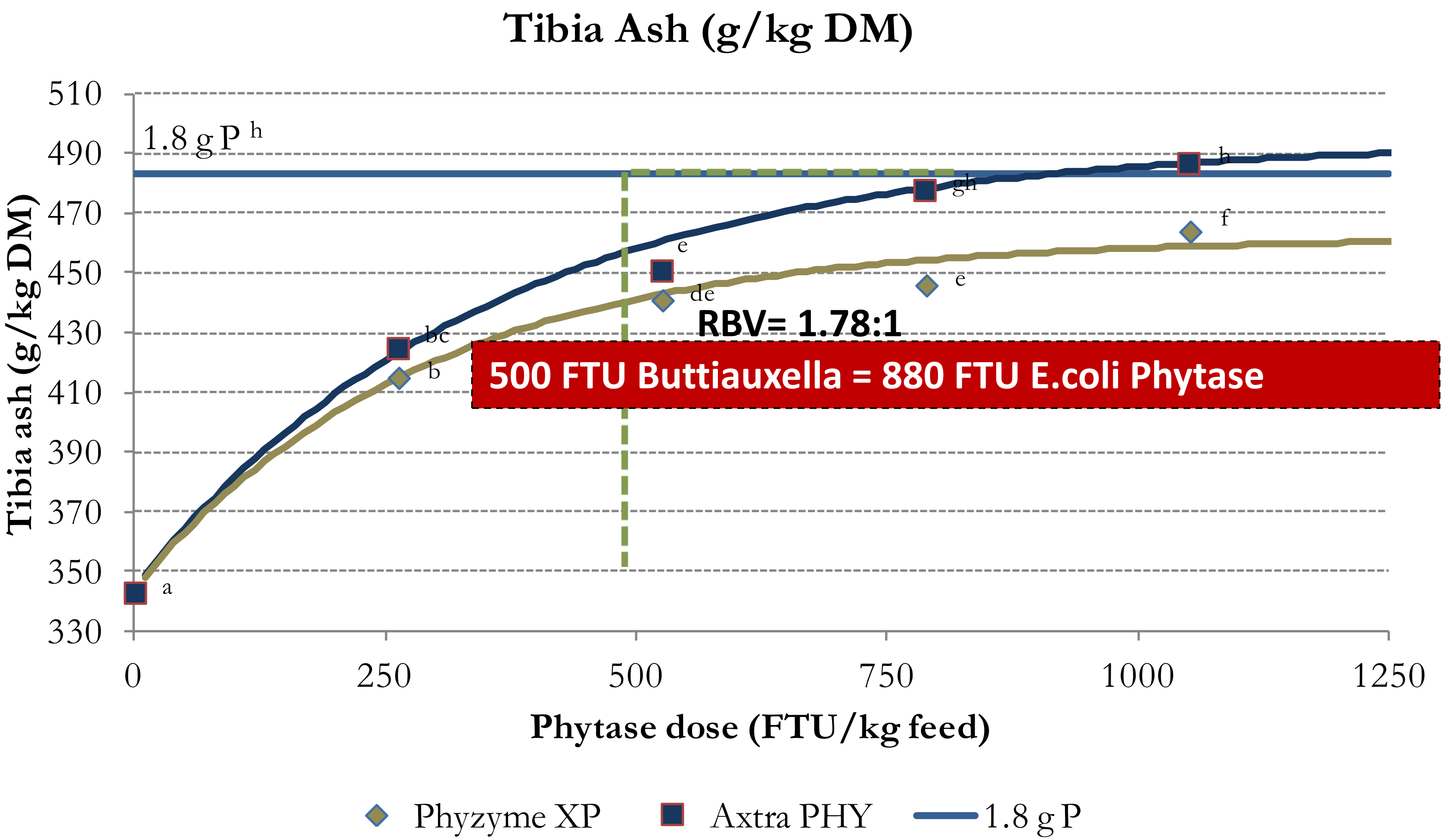
Schothorst Trial 2: Axtra PHY versus E.coli phytase 2

Schothorst Trial 1: Axtra PHY versus E.coli Phytase 2

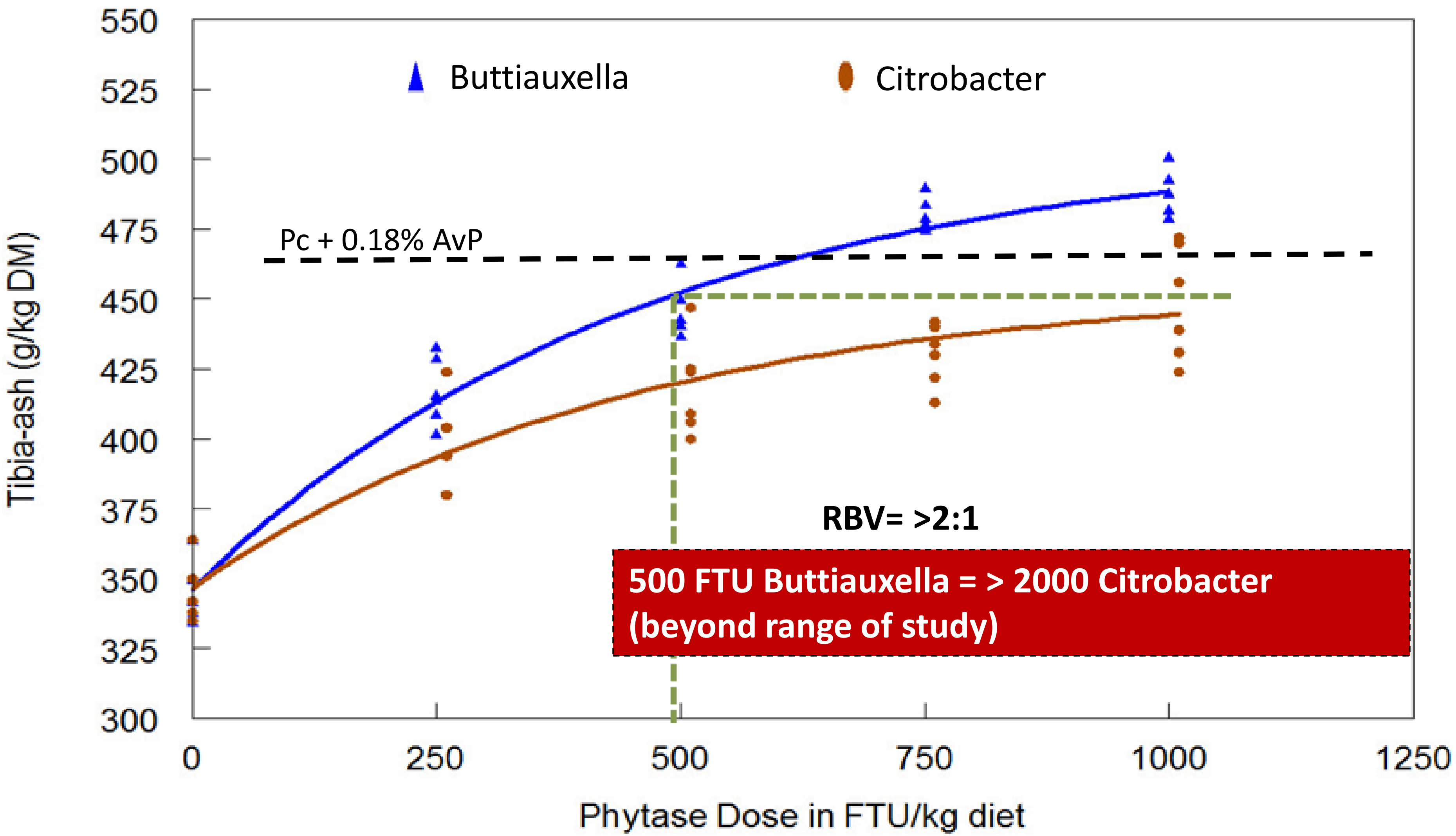


Schothorst trial 3: Axtra PHY versus Phyzyme XP

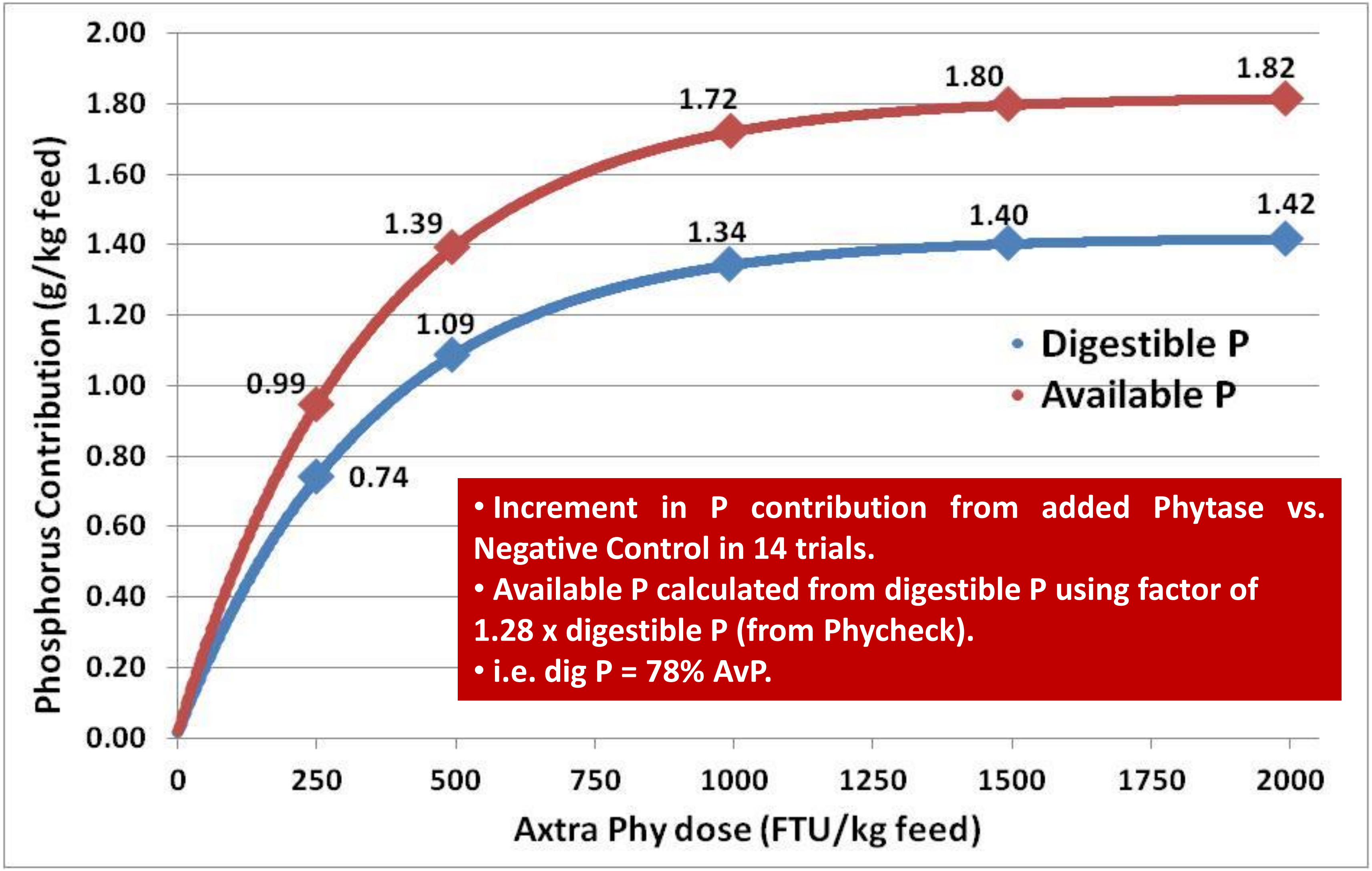
Schothorst Trial 3: Axtra PHY versus E.coli Phytase 3



Schothorst trial 4: Axtra PHY versus Citrobacter



Swine digestible P Matrix values derived from 14 studies



- Increment in P contribution from added Phytase vs. Negative Control in 14 trials.
- Available P calculated from digestible P using factor of 1.28 x digestible P (from Phycheck).
- i.e. dig P = 78% AvP.

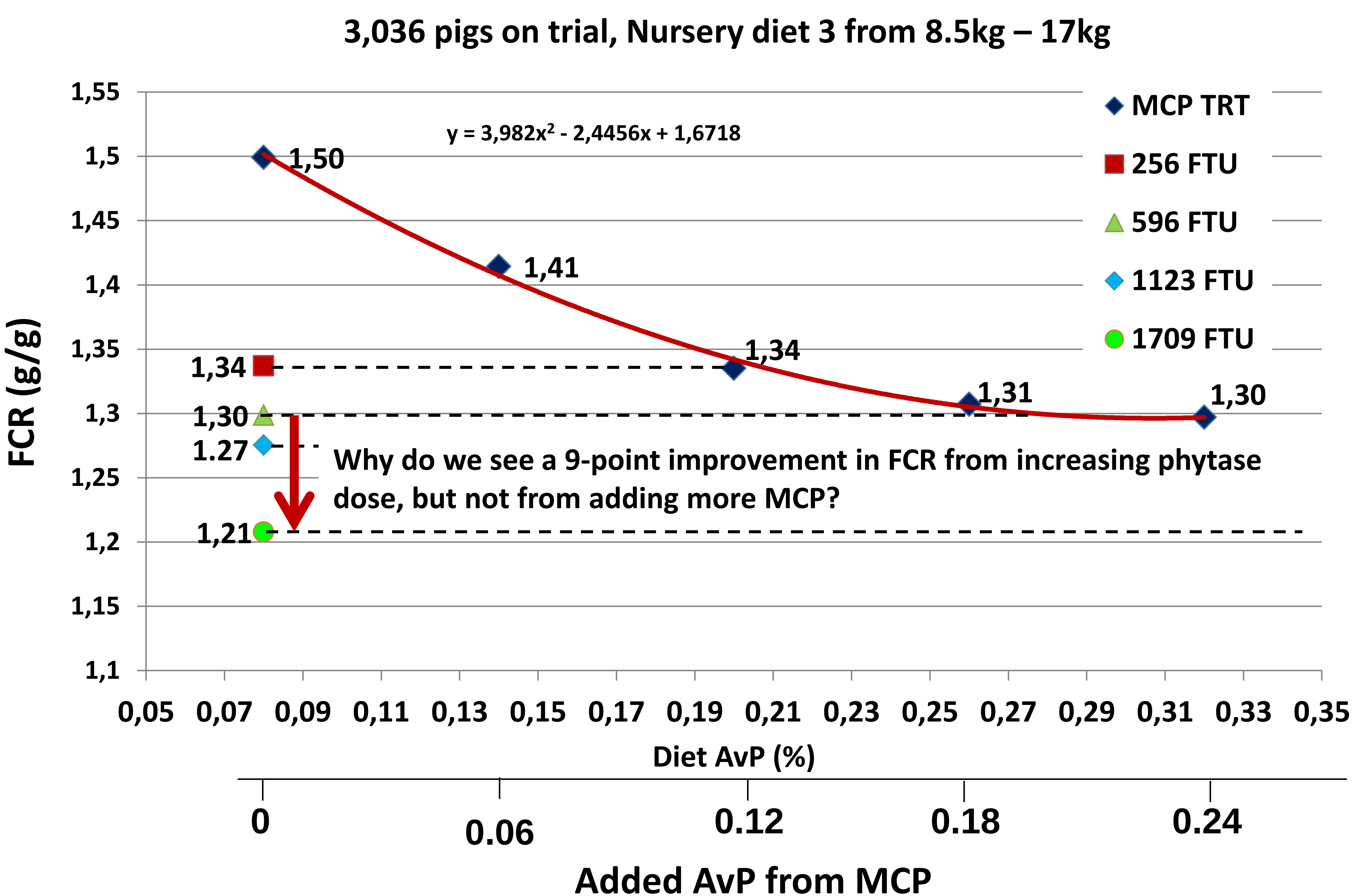
Based on 14 swine digestibility studies, 563 datapoints

Our research to understand Energy and Amino Acids effects from Phytase?

DU PONT®

The miracles of science™

Response in FCR to added inorganic P (MCP) or 'Superdoses' of Axtra Phy

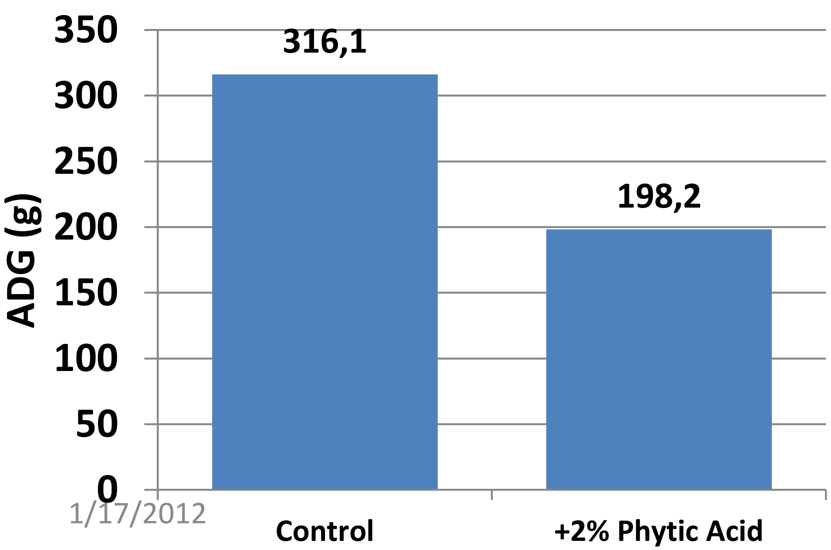


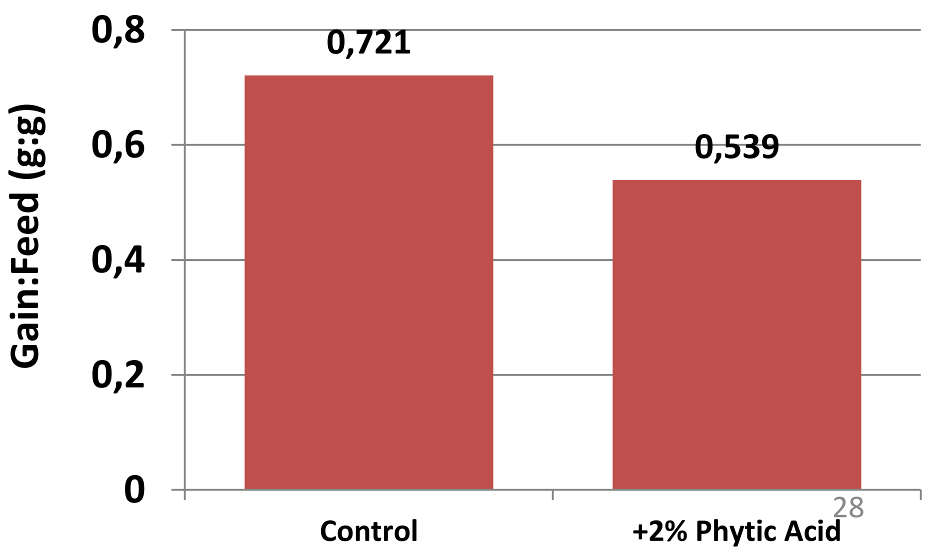
Phytate has been shown to have negative effects on live performance

Table 1. Results from studies on the effect of dietary phytic acid on performance of poultry and pigs

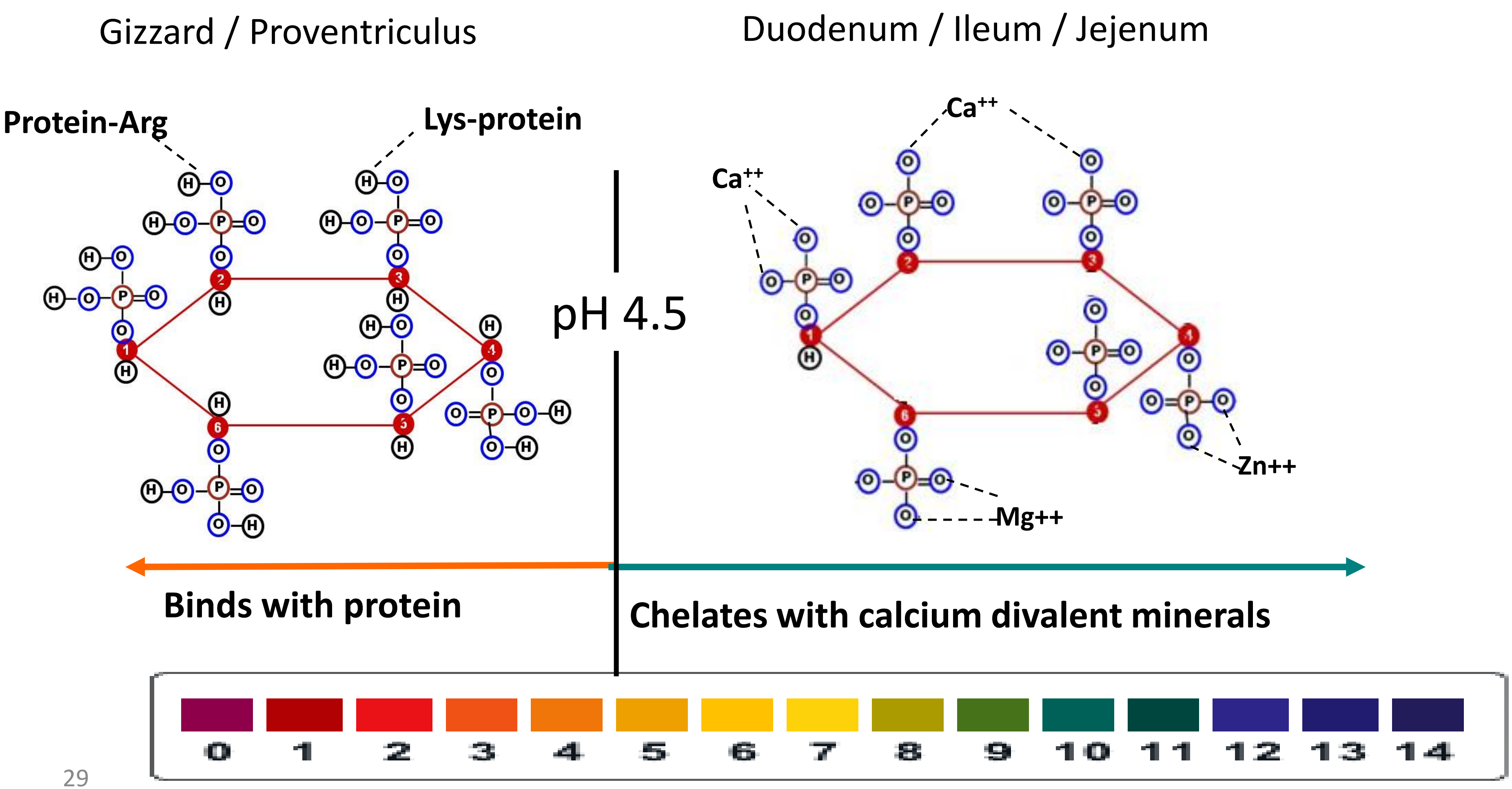
Animal type	Initial age (d)	PA[z] content in control diet (%)	PA content in PA diet (%)	Response criterion[y]	Decrease in performance due to PA (%)	Reference
Broiler	0	0.78	1.57	BWG	3	Liu et al. (2009)
Broiler	0	0.78	1.57	BWG	3	Liu et al. (2008a)
Broiler	0	0.78	1.57	BWG	7	Liu et al. (2008b)
Broiler	7	1.04	1.57	BWG	7	Cabahug et al. (1999)
Broiler	8	0.00	1.65	BWG	28	Onyango and Adeola (2009)
Chicks[w]	28	0.00	1.65	BWG	44	Shan and Davis (1994)
Laying hens	140	0.57	0.71	Egg productio	–	Ceylan et al. (2003)
Piglets	25	0.00	2.00	BWG	37	Woyengo et al. (2012)

Woyengo and Nyachoti, 2013. Can. J. Anim. Sci. (2013) 93: 921





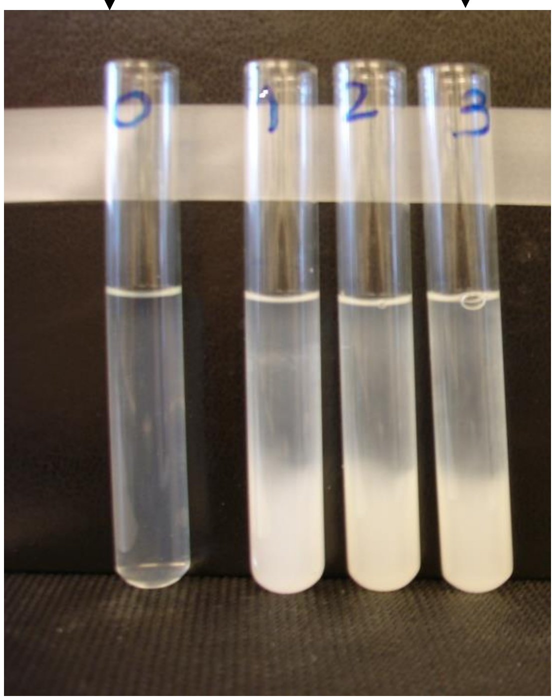
To maximize Phytate Utilisation the phytase must prevent it complexing with both Protein + Cations

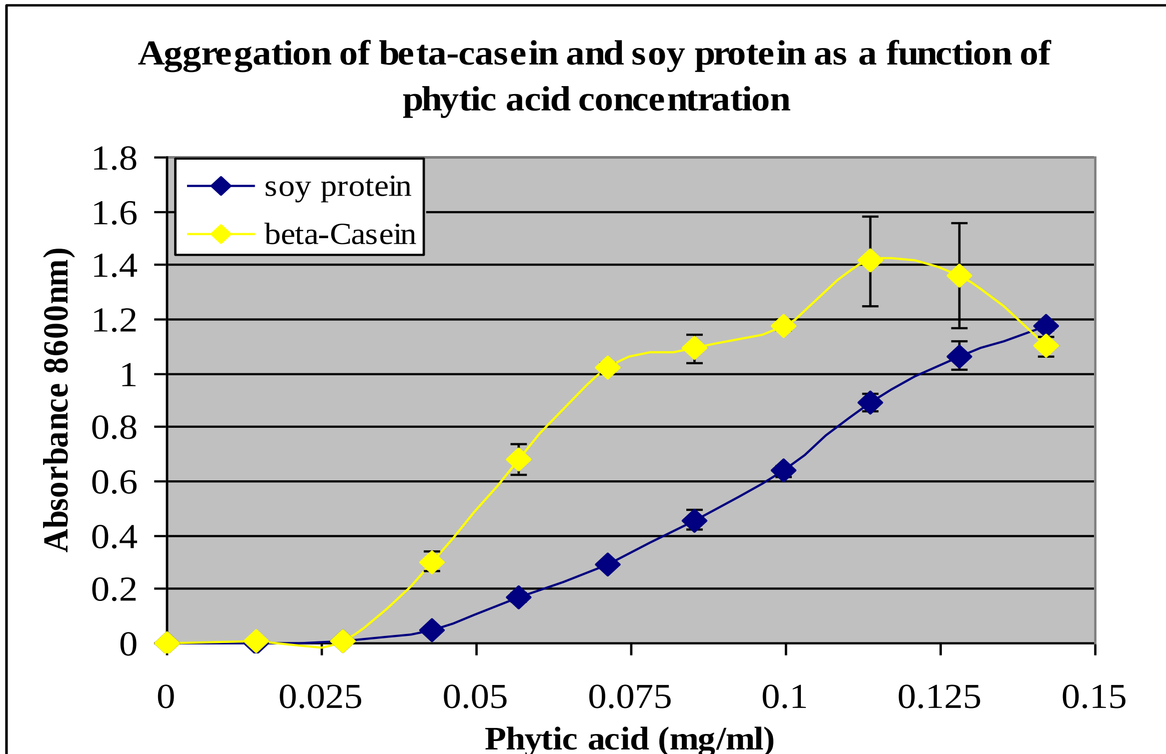


Phytate reduces protein solubility at low pH

Soy Protein Isola
pH 2.5

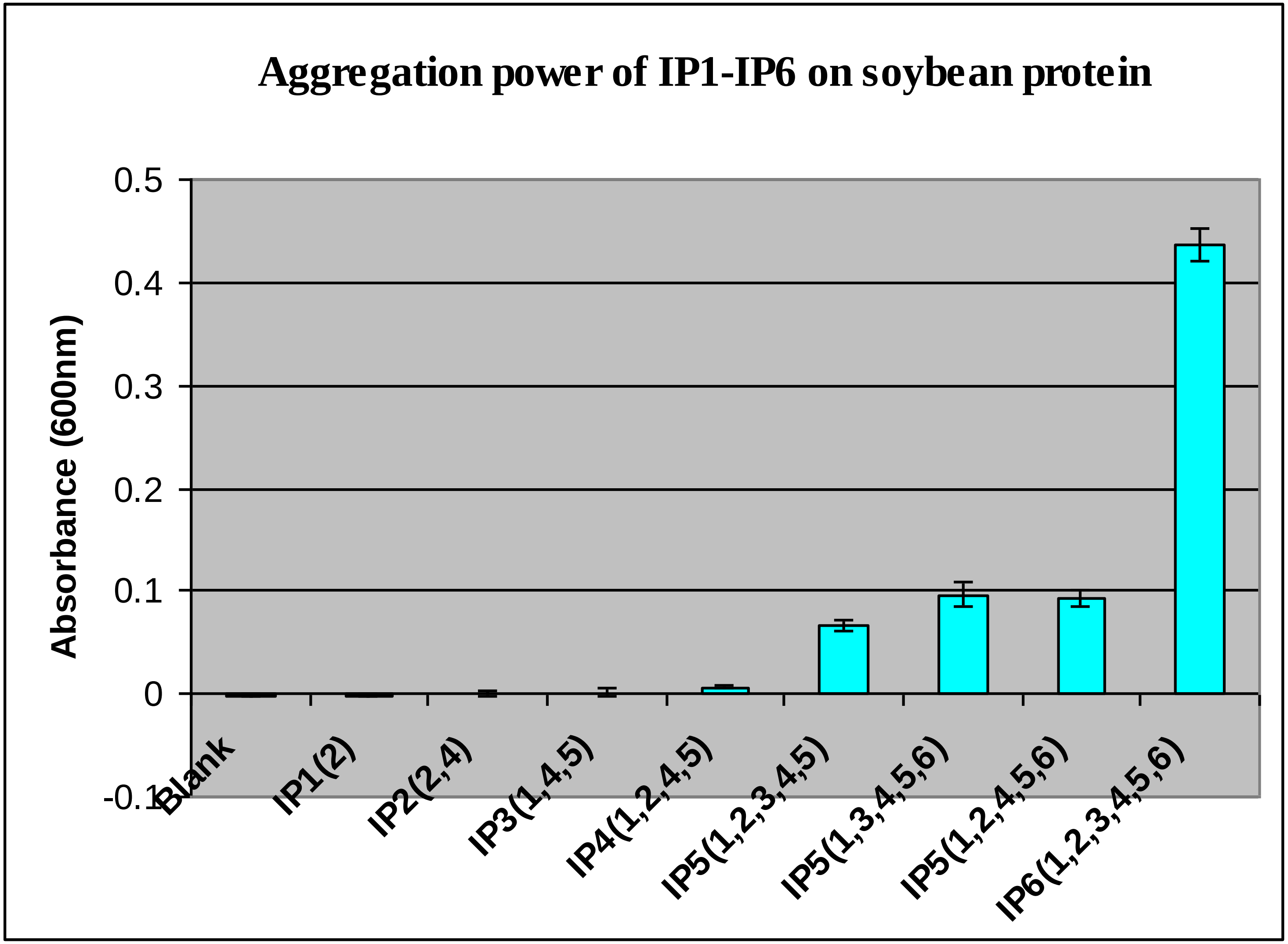
Soy Protein
Isolate
+ Phytic acid
(0.15 mg/ml)



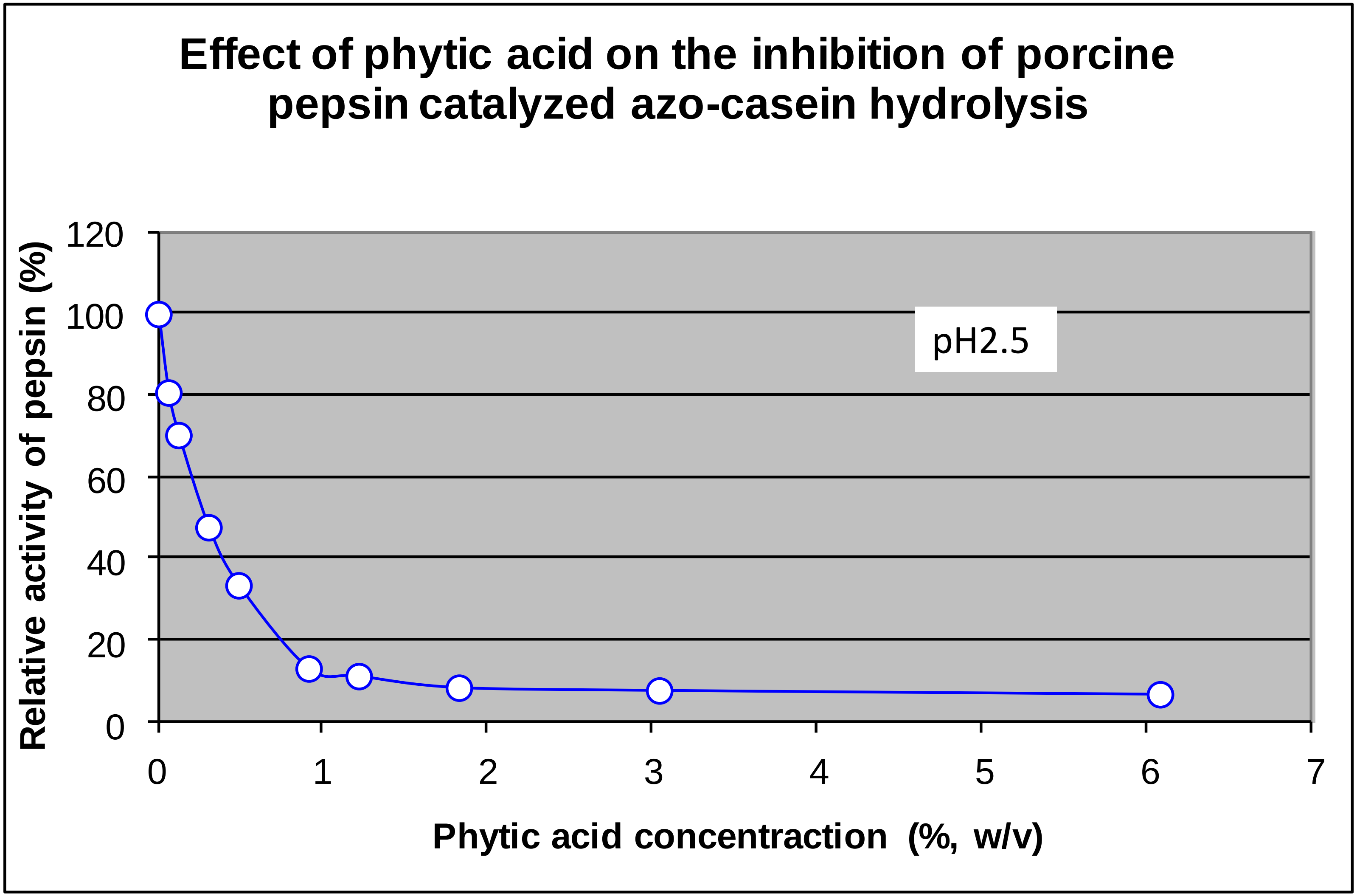


Danisco, 2009

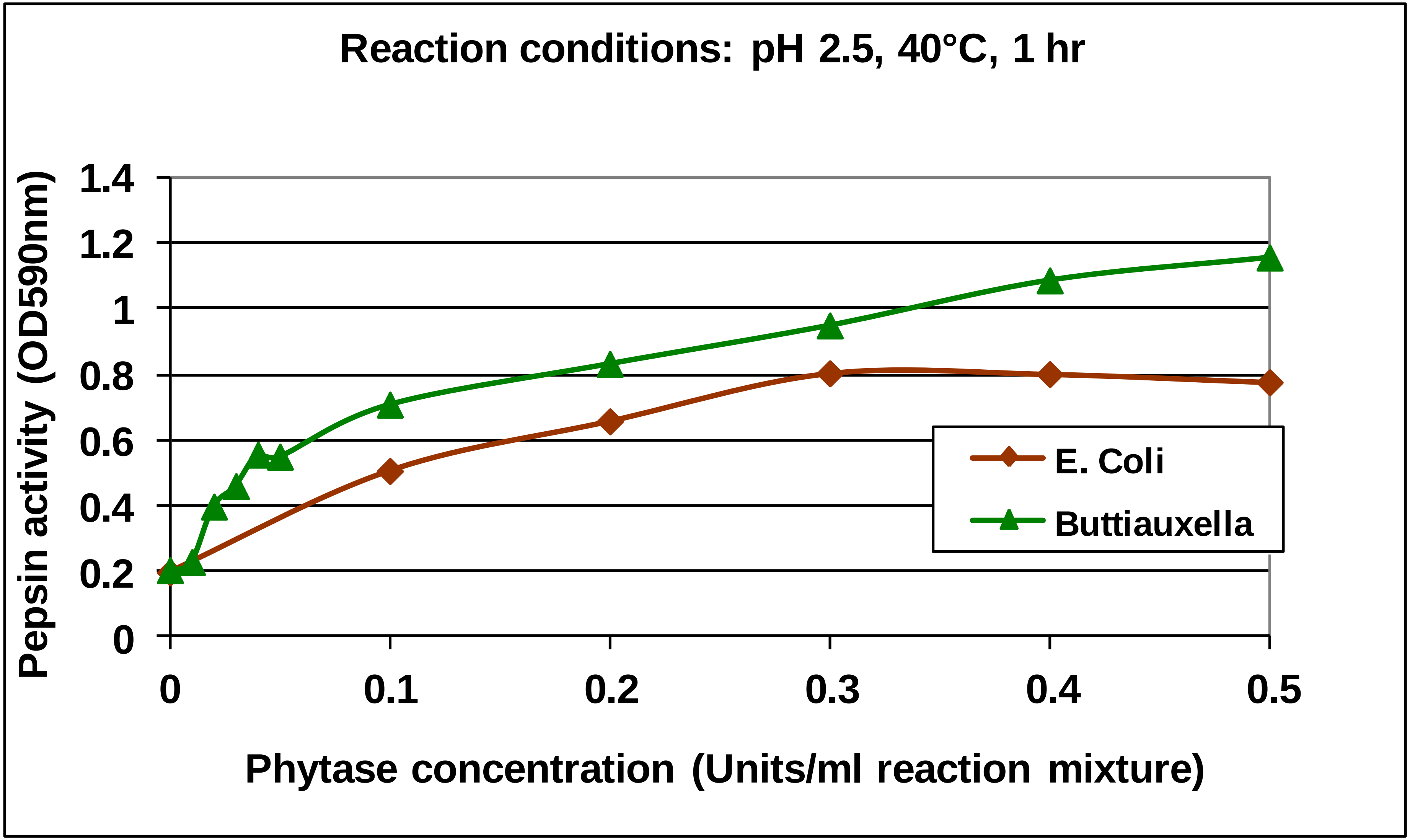
Only IP6 has the ability to bind protein at low pH



Phytate –Protein complexes are not broken down by Pepsin



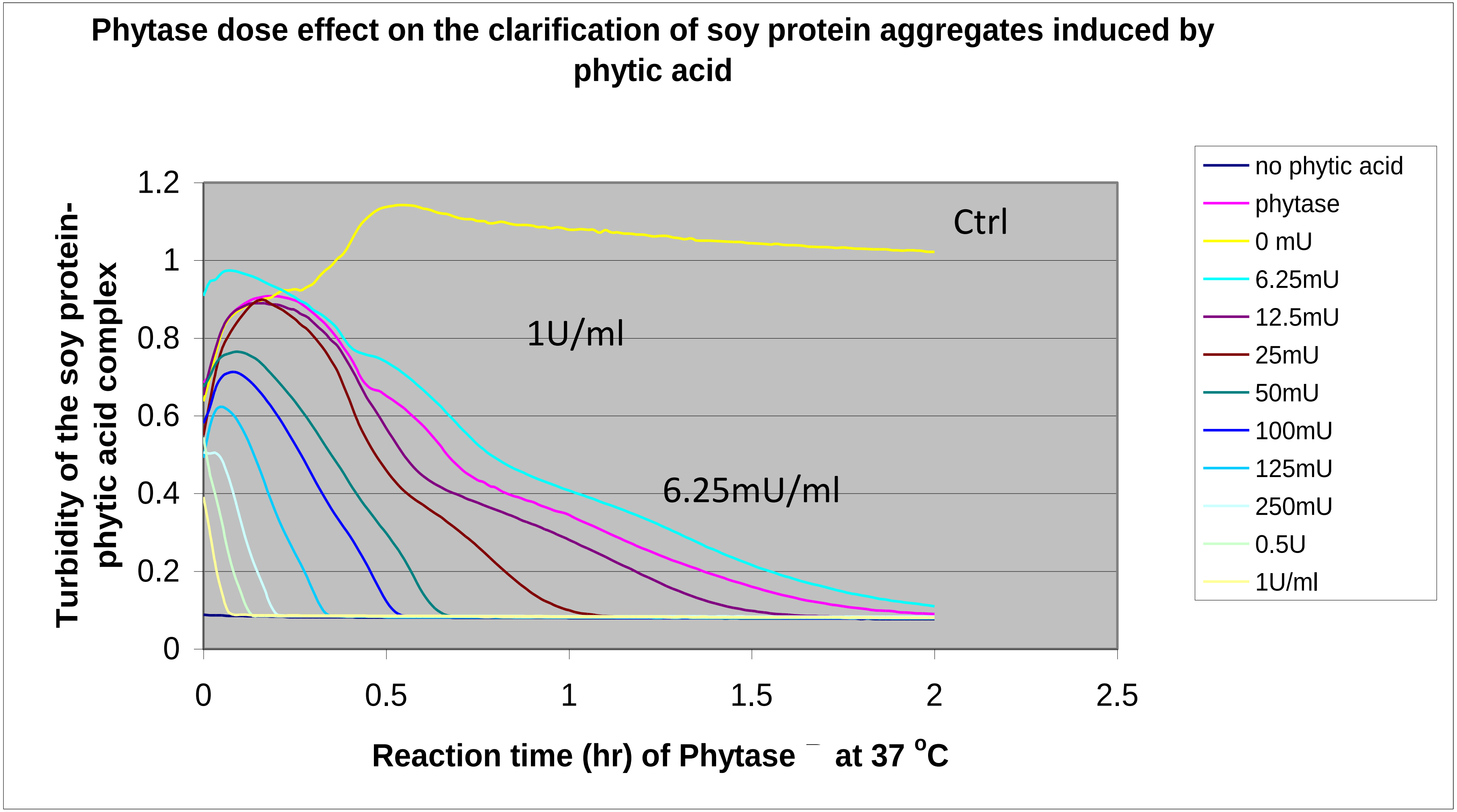
Phytase reverses anti nutritional effects of phytate, allowing Pepsin to degrade protein – dose dependent benefits



Danisco, 2009

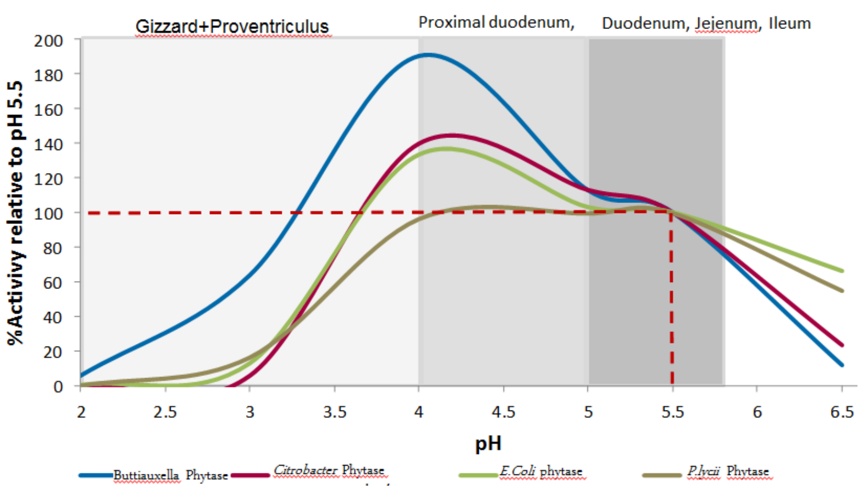
Degradation of protein-phytate aggregates by phytase

Rate of breakdown is dose-dependent

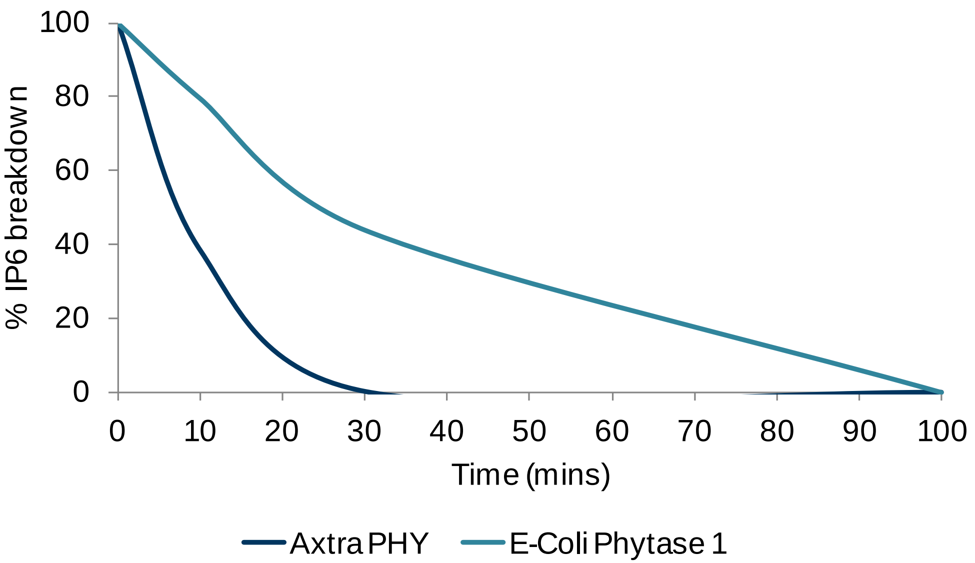


Differences beweeen Phytases in their ability to prevent antinutrient effects of phytate depend on the speed of hydrolysis of IP6 to IP5 of a protein-phytate complex

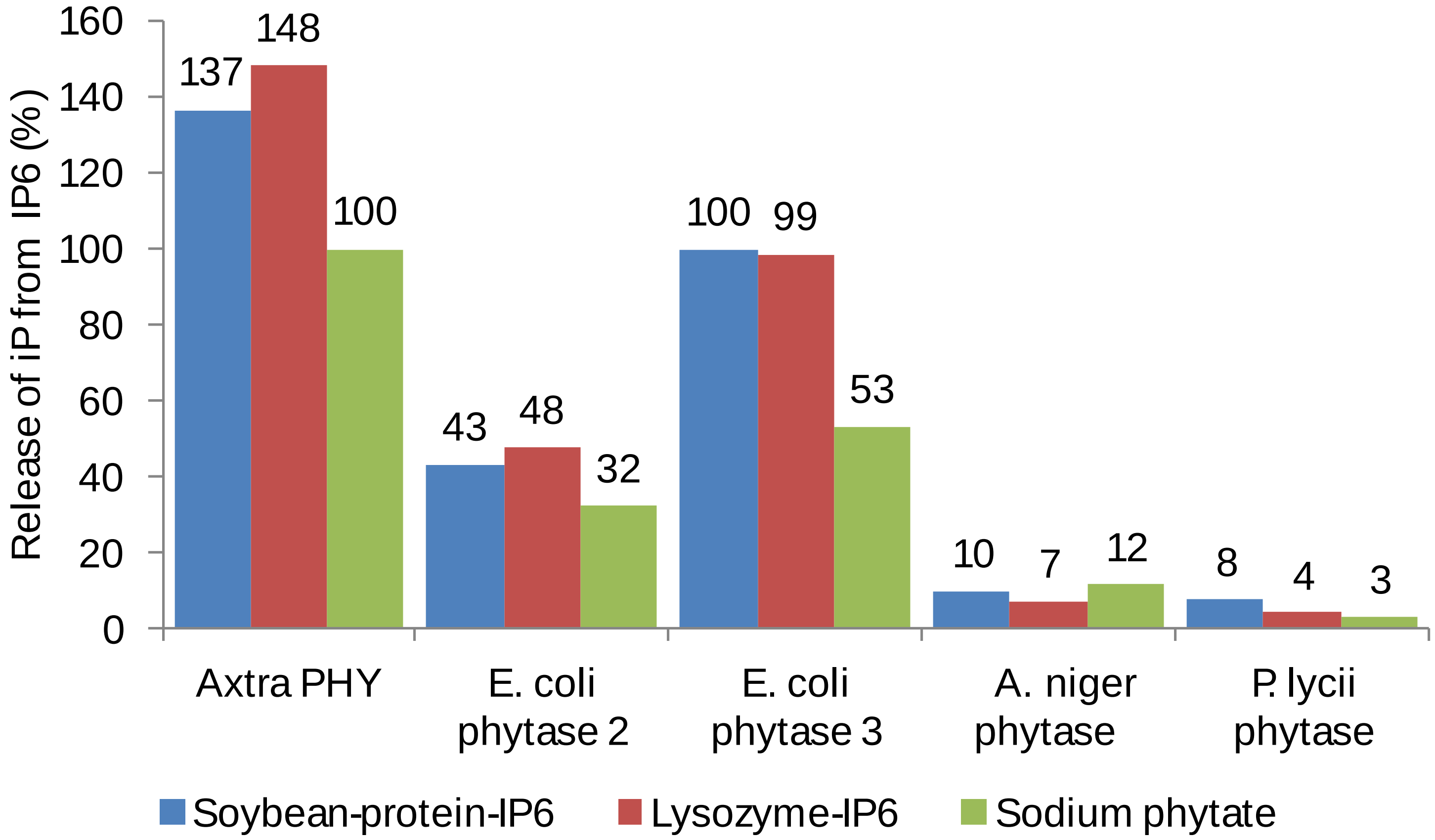
Greater Relative Activity of Buttiauxella Phytase



~ 3 x more rapid IP6 degradation in vitro



Large differences exist between phytases in ability to degrade protein-phytate complexes



All values expressed relative to release of iP by Axtra PHY on sodium phytate substrate

Mechanism of Phytate Anti-nutrient effects

phytate protein

pepsin

Binary protein-phytate complex refractory to pepsin digestion

pepsin + HCl

mucin

+ Na Bicarbonate

Pepsin + Mucin contribute to endogenous amino acid flows

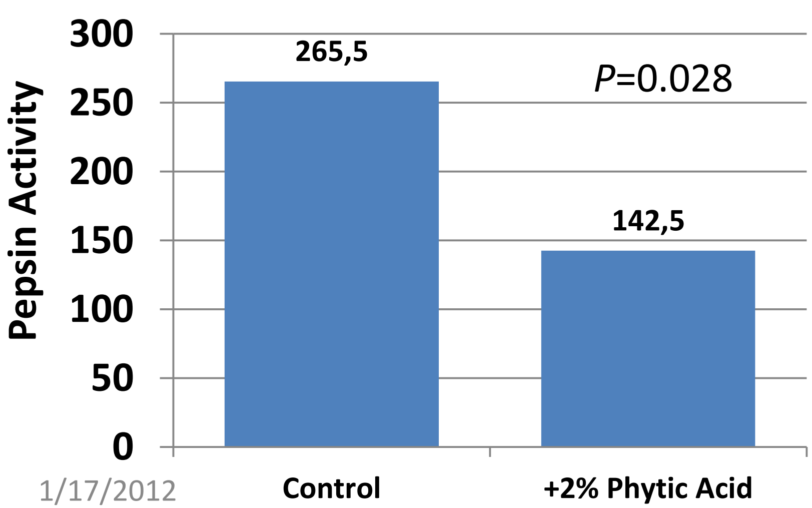
Increased endogenous amino acid flow results in reduced amino acid digestibility and lower efficiency

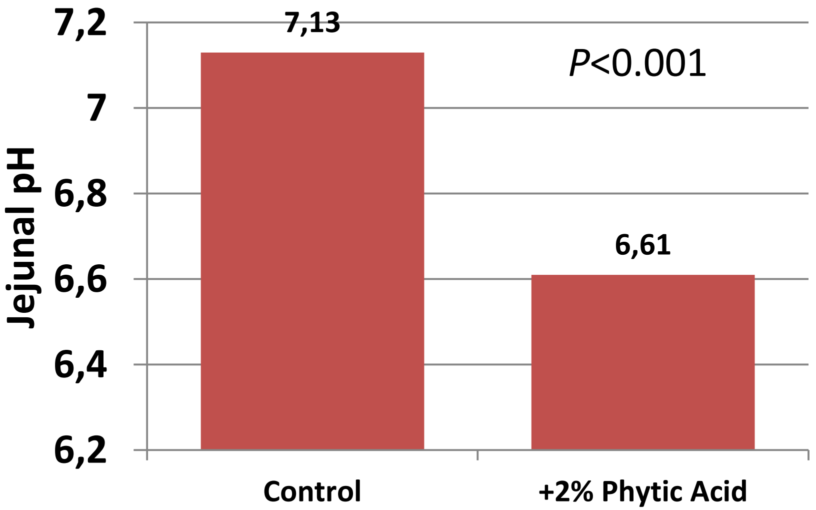
Negative Physiological effects of Phytate have also been shown InVivo

Item	Diet[a]		SEM	Contrasts
	Control	PA		Control vs. 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

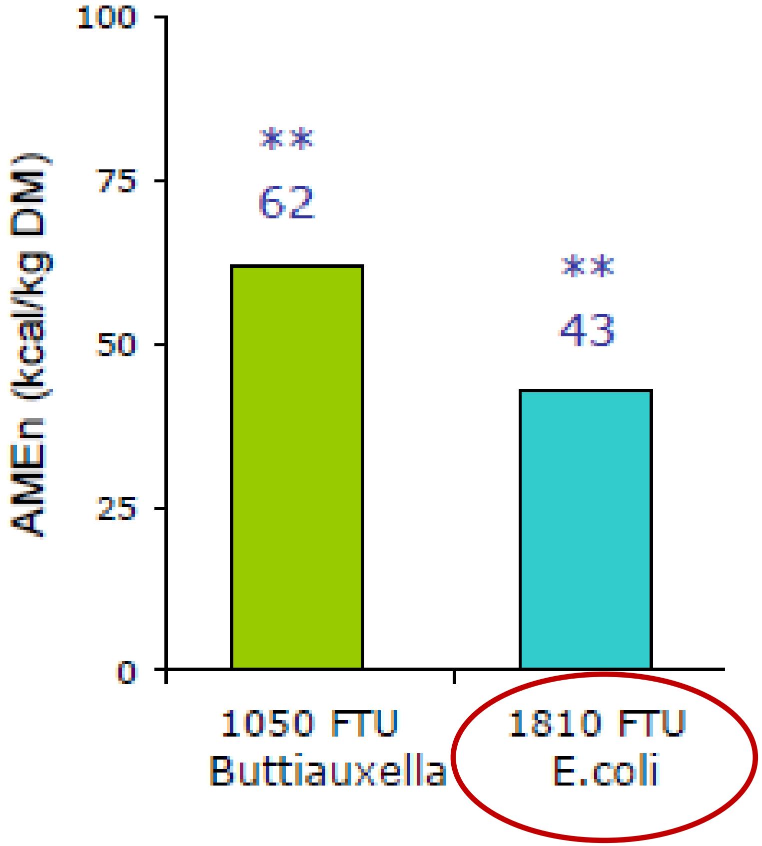
[a] PA = control plus phytic acid.
[b] Determined in stomach digesta.

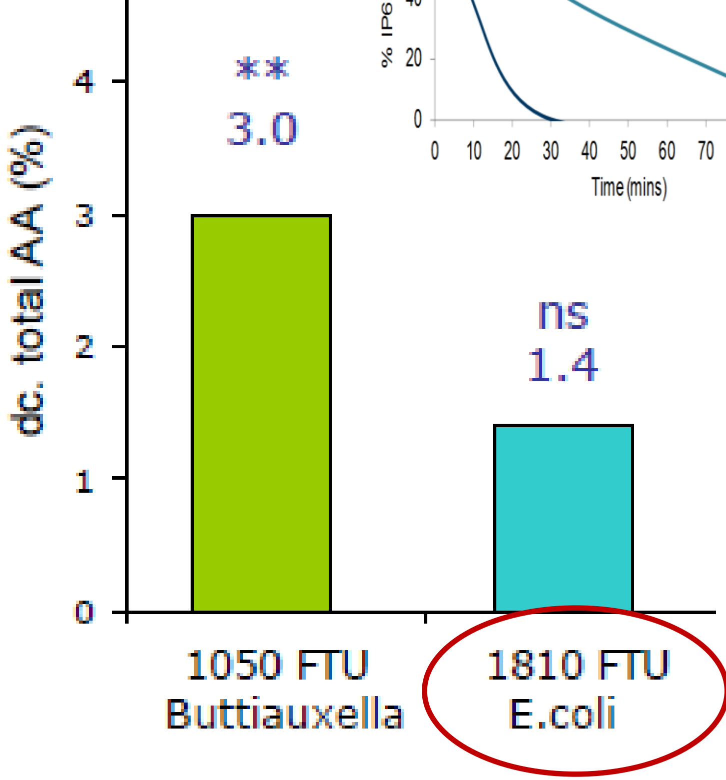
Woyengo et al. / Livestock Science 134 (2010) 91–93

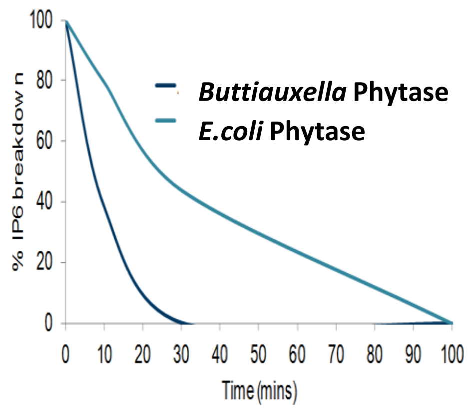




High Phytase dose /speed of IP6 breakdown will improve Energy and amino acid digestibility, independent of Phosphorus release







Kwakernaak et al., 2013 ESPN meeting

Example of a “superdose study in broilers

- Trial run at Queensland University, Au
- 1760 male Ross 308 broiler chicks
- Birds were allocated to 11 treatments (8 replicate pens/treatment, 20 birds/pen)
  - PC1 was based on corn and soybean meal (48% CP)
  - NC1 (reduced by 62 kcal/kg feed and amino acids)
  - NC2 (62 kcal ME and amino acids, 0.9% Calcium, 0.10% total phosphorus and 0.10% available phosphorus)
  - NC3 (62kcal ME and amino acids, 0.13% Calcium, 0.14% total phosphorus and 0.14% available phosphorus)
  - NC4 (0.16% Calcium, 0.18% total phosphorus and 0.17% available phosphorus reduction)
  - NC4 was fed either unsupplemented or supplemented with 250, 500 or 1000 FTU/kg feed of either Axtra Phy or an *E-Coli* phytase
- All diets fed *ad libitum* and contained 25% phytate

Starter Diet Formulations (0-21d)

Ingredients (kg/tonne)	Dietary Treatment				
	PC	NC1[¥]	NC2[*]	NC3[**]	NC4[***]
Corn	595	609	618	622	622
Soybean Meal 48%CP	344	341	339	339	339
Soybean Oil	16.23	5.1	2.34	1	1
L-Lysine HCl	3.44	3.32	3.36	3.37	3.37
DL-methionine	1.73	1.62	1.62	1.62	1.62
L-threonine	0.92	0.71	0.71	0.71	0.71
Salt	3.8	3.8	3.8	3.8	3.8
Limestone	11.62	11.65	12.7	13	13.2
Dicalcium Phosphate	21.4	21.3	15.7	13.4	11.7
Poultry Vits/TE's	2	2	2	2	2
Inert Filler	-	-	-	-	1.26
Buttiauxella or E.Coli Phytase (FTU/kg feed)	-	-	-	-	-/+ 250, 500, 1000

Calculated Analysis	Dietary Treatment				
	PC	NC1[¥]	NC2[*]	NC3[**]	NC4[***]
Crude protein (%)	22.0	22.0	22.0	22.0	22.0
Metabolisable energy (kcal/kg)	2,982	2,920	2,920	2,920	2,920
Lysine (%)	1.44	1.42	1.42	1.42	1.42
Digestible lysine (%)	1.25	1.23	1.23	1.23	1.23
Methionine (%)	0.5	0.49	0.49	0.49	0.49
Digestible methionine (%)	0.46	0.45	0.45	0.45	0.45
Methionine + Cysteine (%)	0.86	0.85	0.85	0.85	0.85
Digestible Met + Cys (%)	0.7	0.7	0.7	0.7	0.7
Total phosphorus (%)	0.78	0.78	0.68	0.63	0.6
Available phosphorus (%)	0.5	0.5	0.4	0.36	0.33
Phytate phosphorus (%)	0.25	0.25	0.25	0.25	0.25
Calcium	1.05	1.05	0.96	0.92	0.89

PC1=Positive Control – Formulated to Ross 308 recommendations for Ca + AvP.

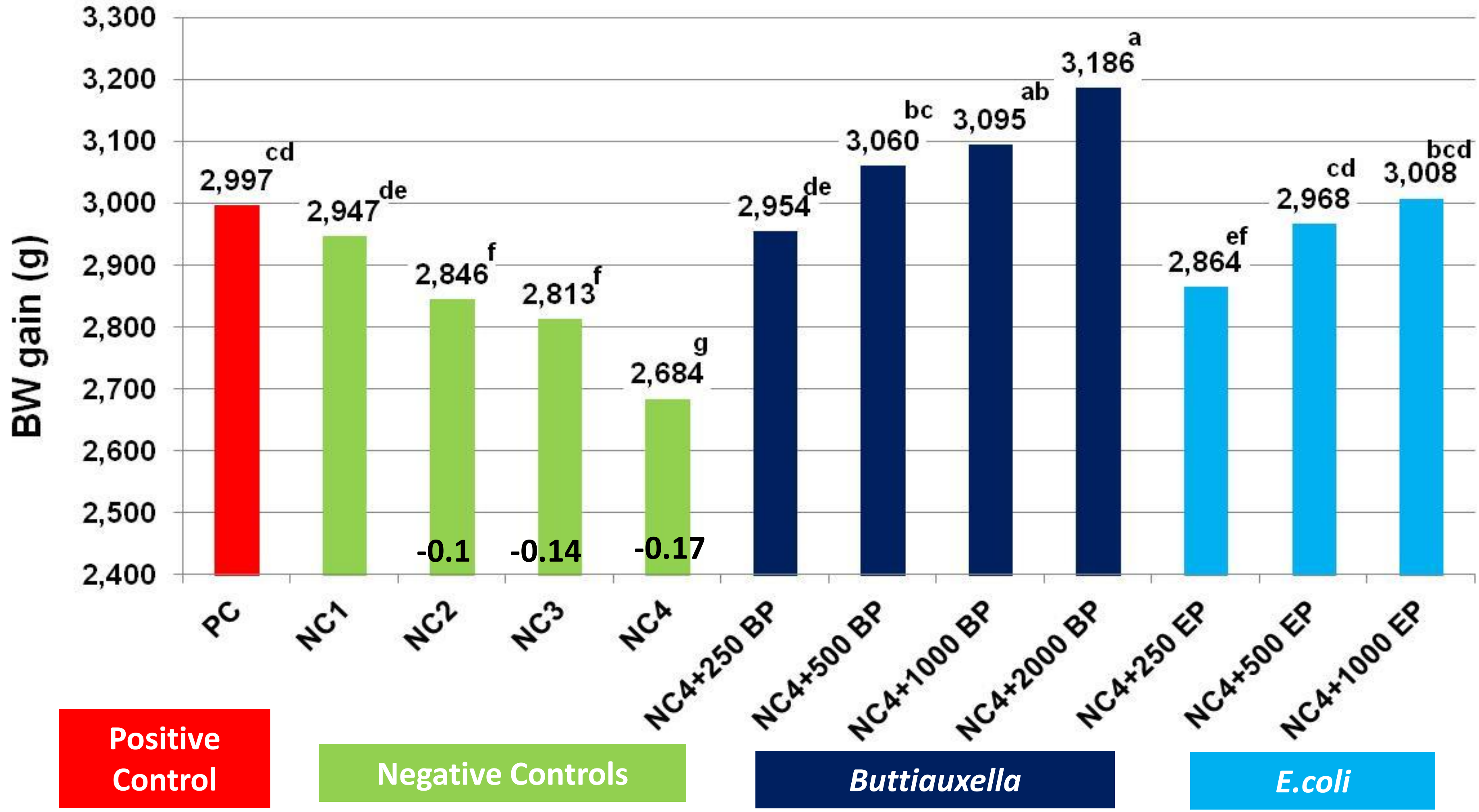
[¥] NC1 Reduced by 62 kcal/kg and 1-2% on digestible amino acid, versus PC

[*] as NC1, plus reductions in calcium (0.09%) and available phosphorus (0.10%)

[**] as NC1, plus reductions in calcium (0.13%) and available phosphorus (0.14%)

[***] as NC1, plus reductions in calcium (0.16%) and available phosphorus (0.17%)

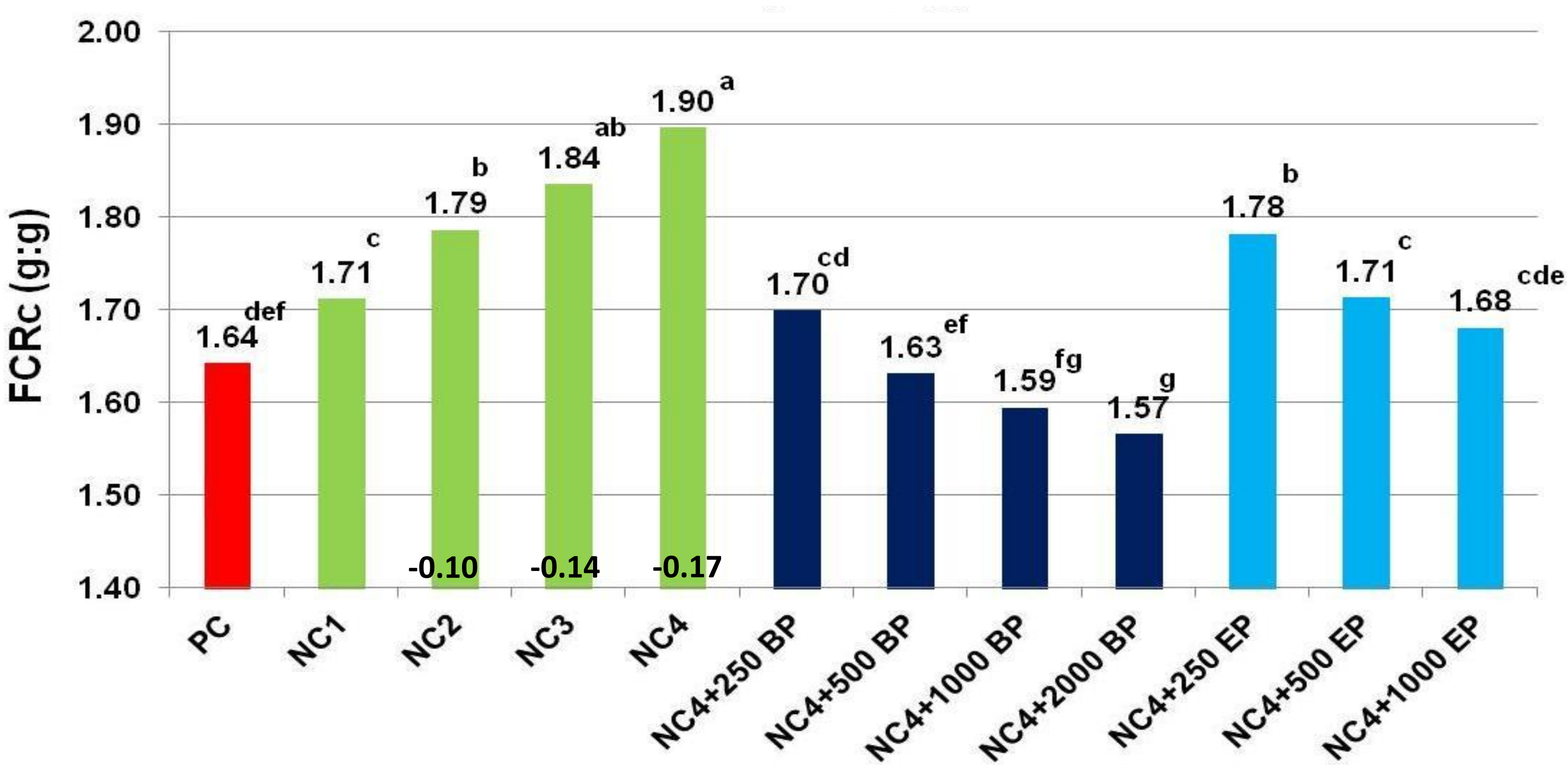
Results: 42-d Broiler Body weight Gain



Phytase source, P<0.05

[a-g] **Values without a common superscript are significantly different (P<0.05),**

Results: FCR corrected* (0-42 days)



Positive Control

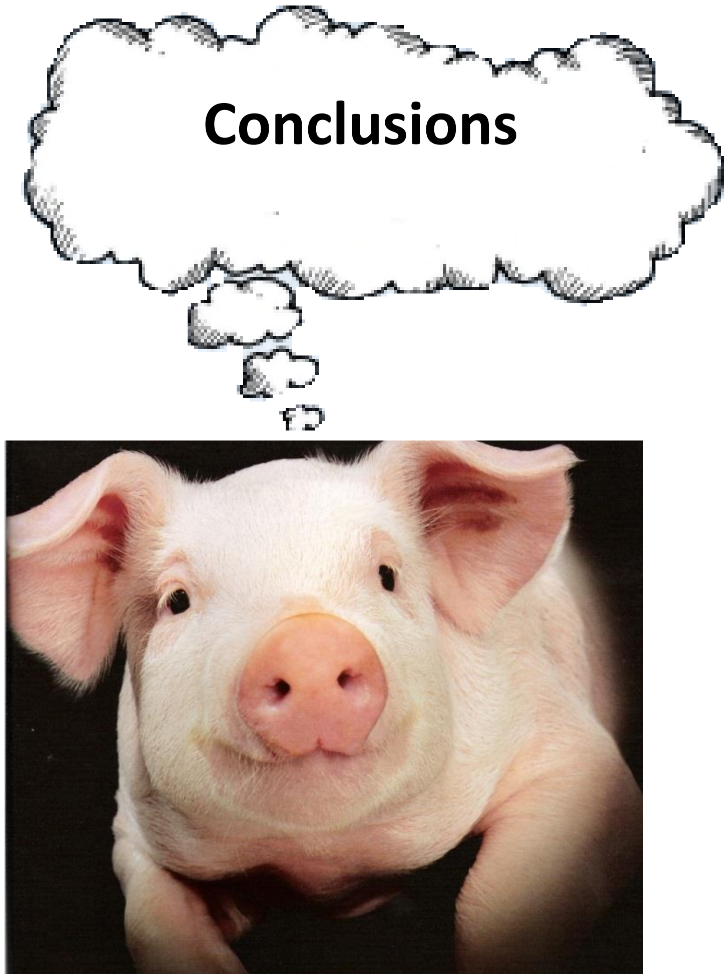
Negative Controls

Buttiauxella

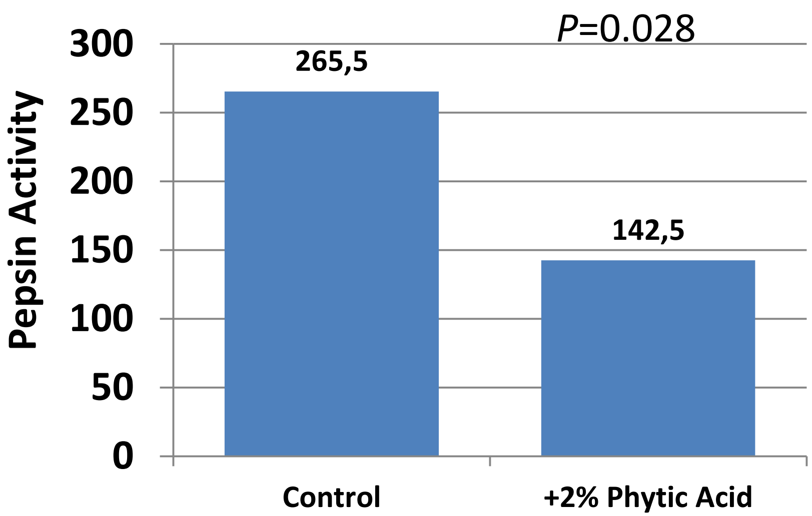
E.coli

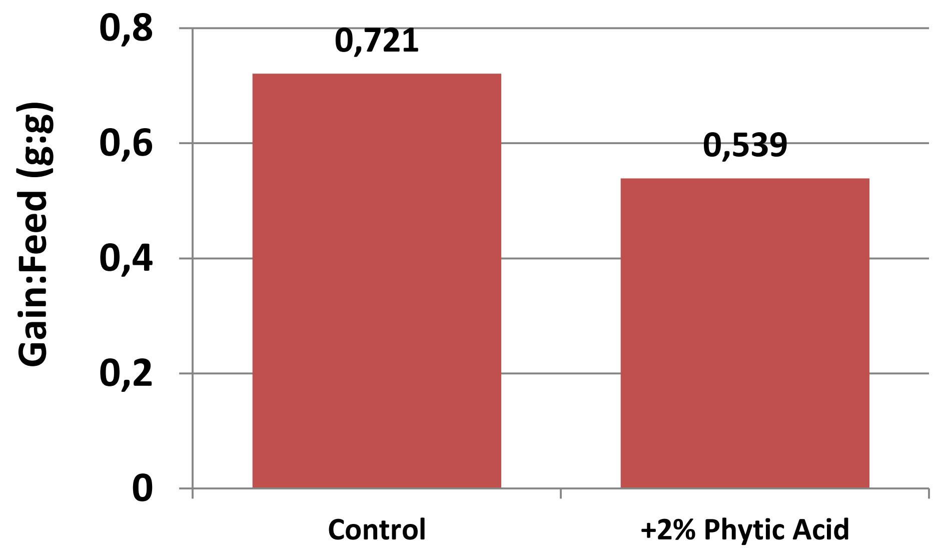
***FCR corrected: 100g BW = 3 pts FCR**

[a-g] **Values without a common superscript are significantly different (P<0.05),**


Conclusions

Dosing Phytase at 500 FTU will not hydrolyze phytate fast enough to overcome phytate antinutrient effects on pepsin activity & performance





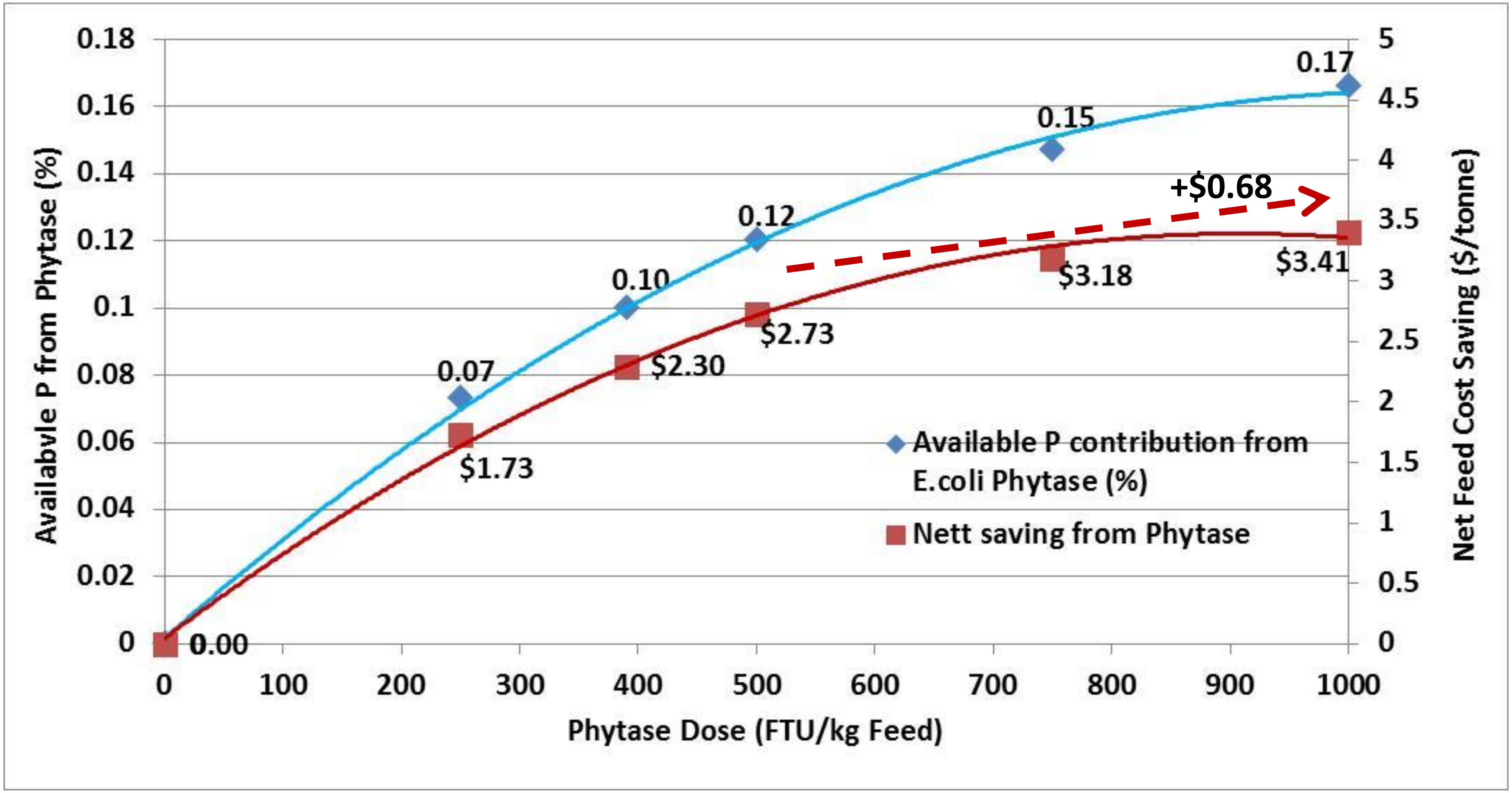
Woyengo et al. 2010

Only Including Phytase at doses of ~0.12 AvP replacement can not fully remove all Phytate

Phytase dig. P replacement	AvP Replacement	% Phytate P made available	% Phytate Remaining*
0.04	0.05	15.4%	84.6%
0.06	0.08	23.1%	76.9%
0.08	**0.10**	**30.8%**	**69.2%**
0.1	**0.13**	**38.5%**	**61.5%**
0.12	**0.15**	**46.2%**	**53.8%**
0.14	0.18	53.8%	46.2%

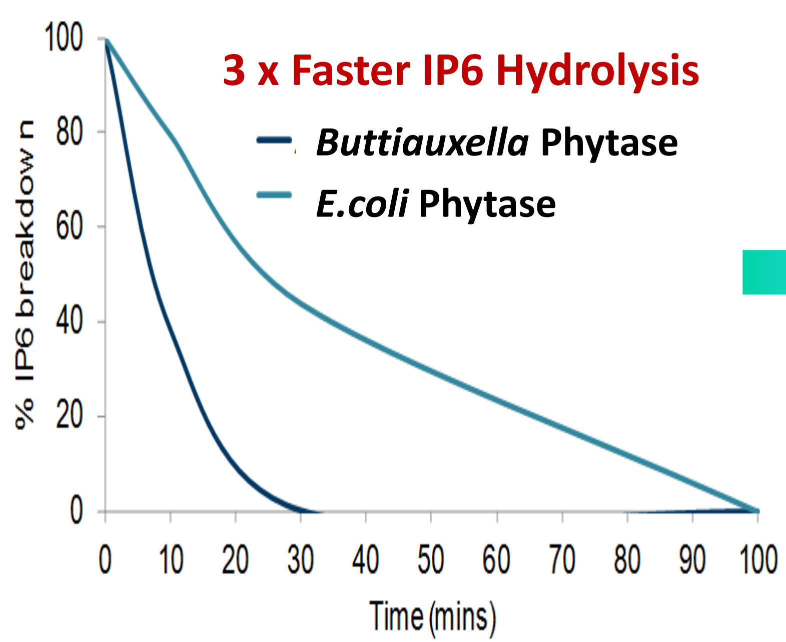
“Dose for Your Profit”

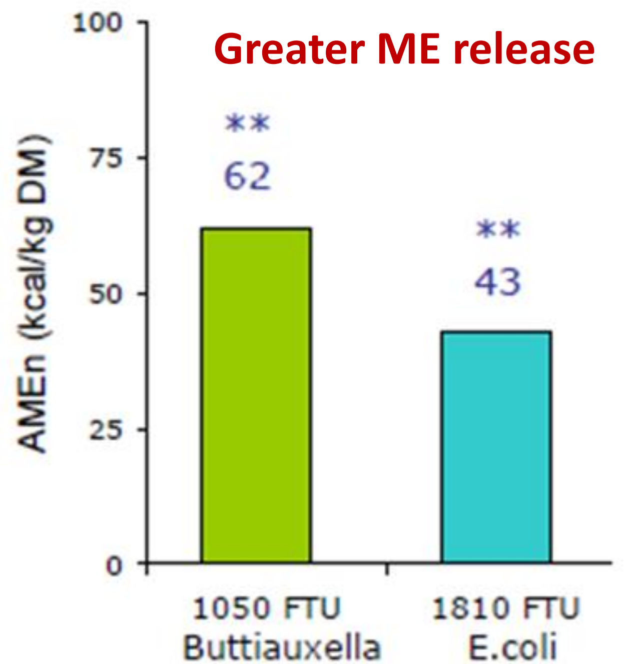
DuPont’s Research Investment gives us confident Matrix values, therefore Increasing Phytase dose also allows greater P replacement and cost-saving

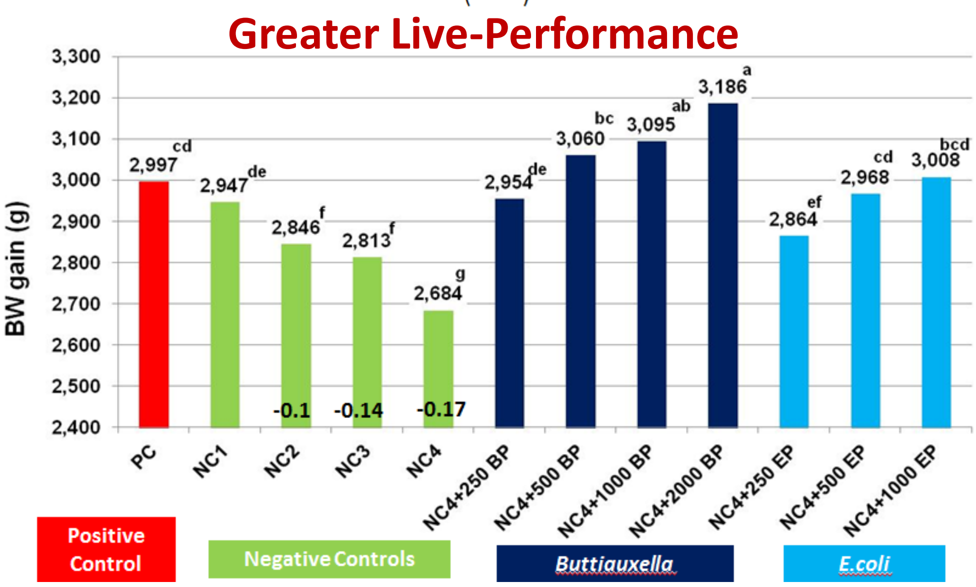


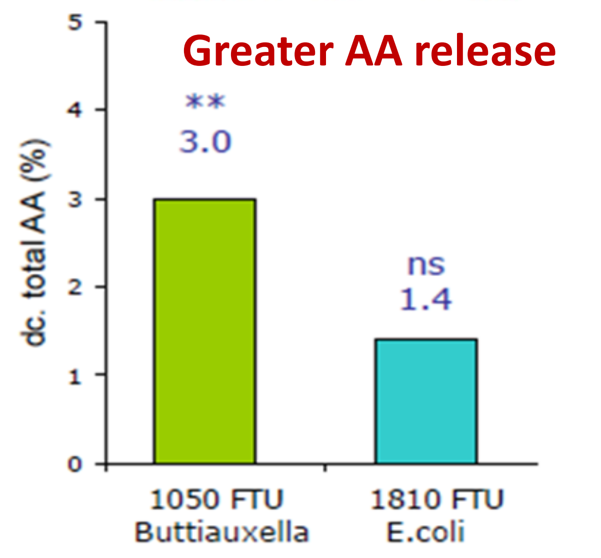
DCP Price = $0.50 / kg; Phytase Price = $0.60/500 FTU; E.Coli Phytase matrix

Speed of IP6 hydrolysis drives ME and Amino acid benefits of phytases









QUESTIONS?

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