‘’Quantifying Nutrient Contributions from Phytase, ‘Fiction vs. Reality?’’

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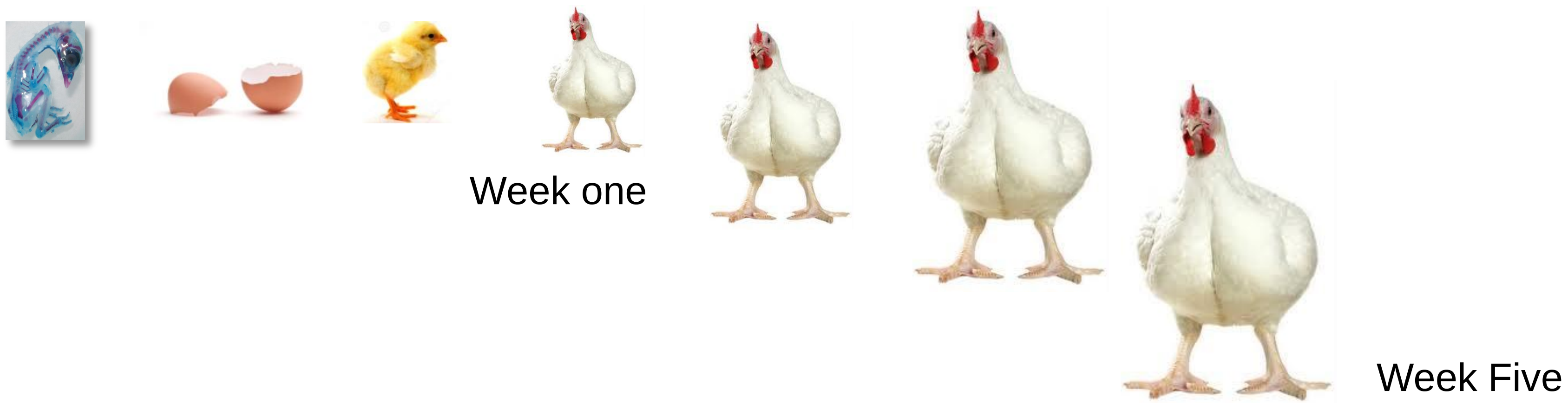
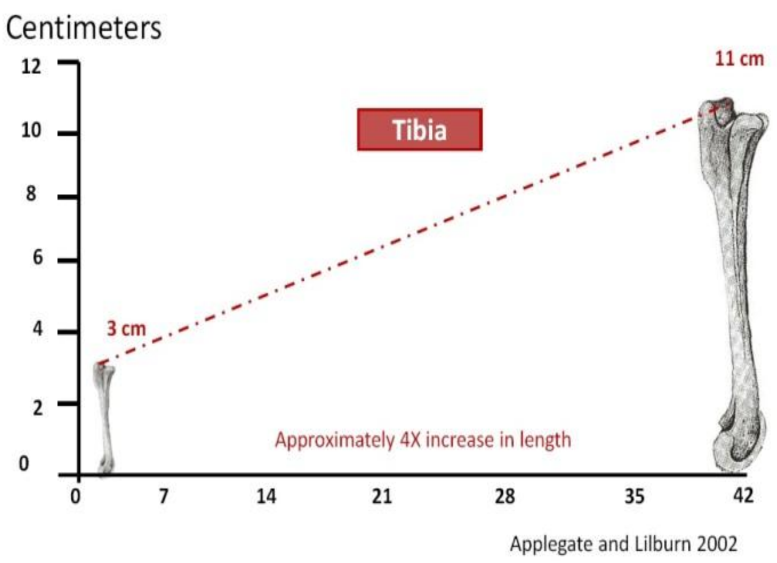
DUPONT SEMINAR, JUNE 2014

Rapid growth means 2- 6% broilers lost due to skeletal issues, with subsequent impact on profitability

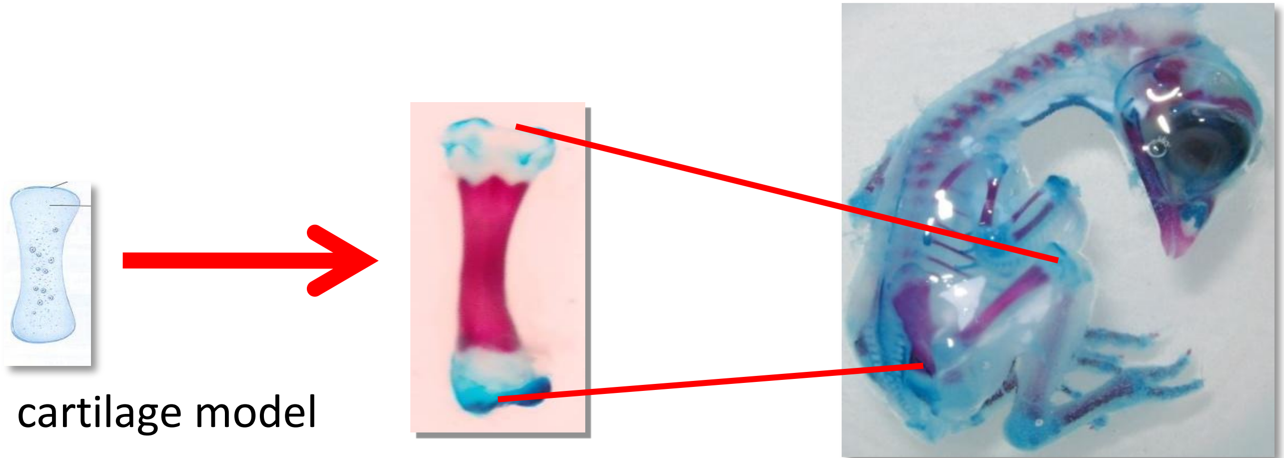




For Optimum Bone Mineralization we Have to Start in the Egg

Bone development begins in the embryo with the formation of a cartilage matrix, which begins to be calcified in the egg.

Corver, 2014



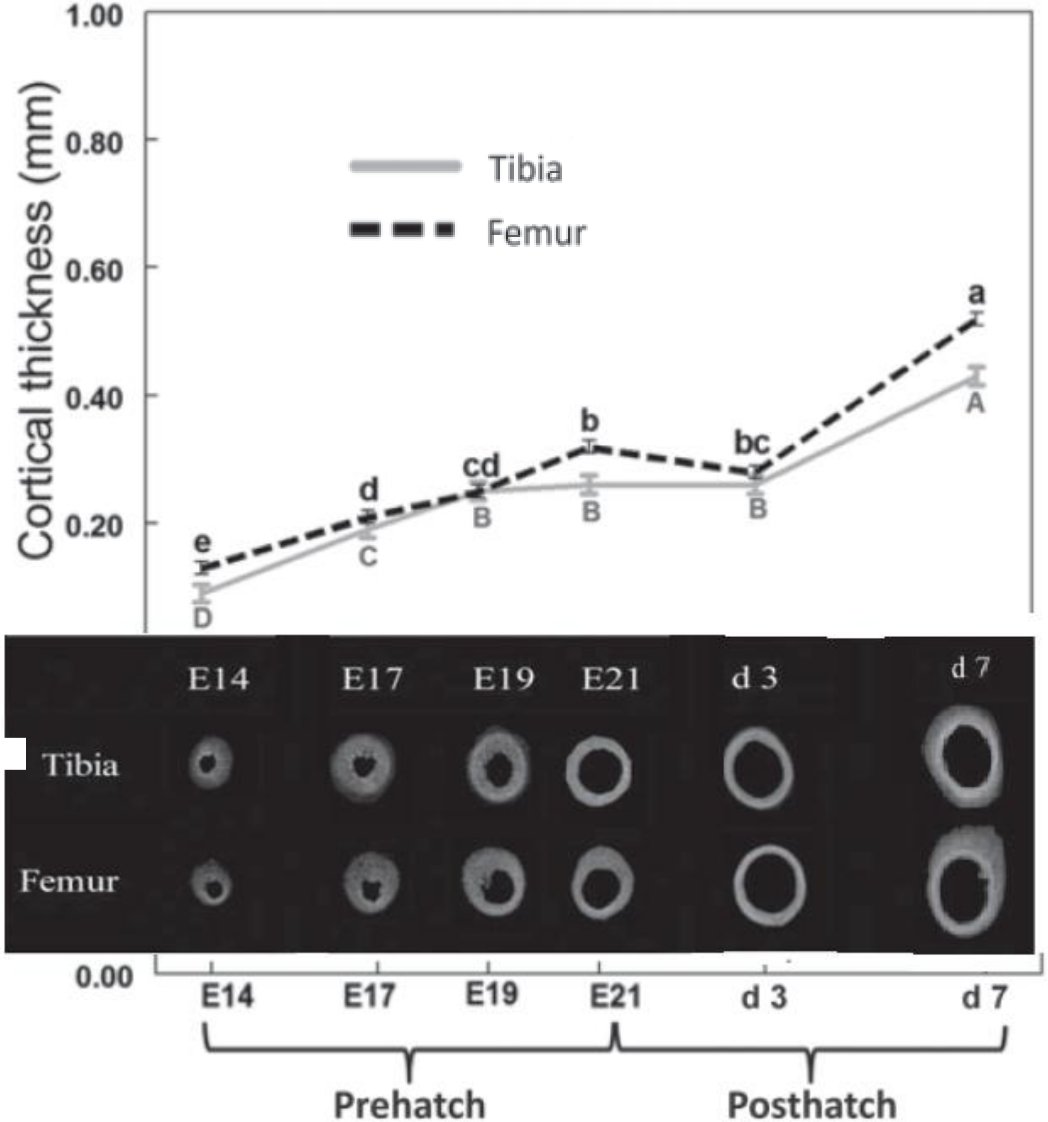
bone cartilage model

Femur– 20 day embryo

20 day embryo

To optimize bone mineralization in Broilers, we need to <u>start</u> with correct Broiler Breeder nutrition (Ca, P, Zn, Mn, Cu, Vit D) as well as focus on correct incubation

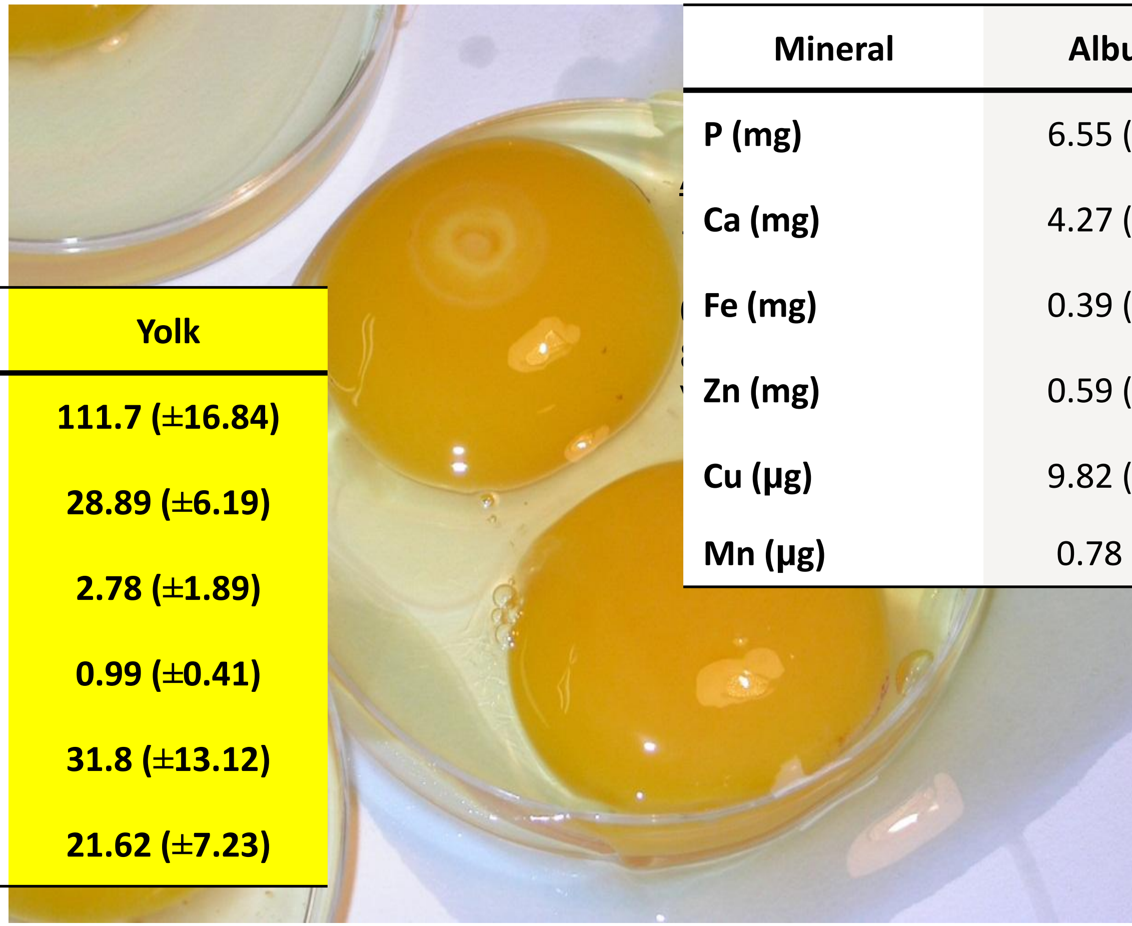
Mineralisation of Tibia and Femur increases rapidly during incubation from E14 to E21



Yair et al., 2012

The yolk is the major mineral reservoir of the egg and developing embryo

Egg

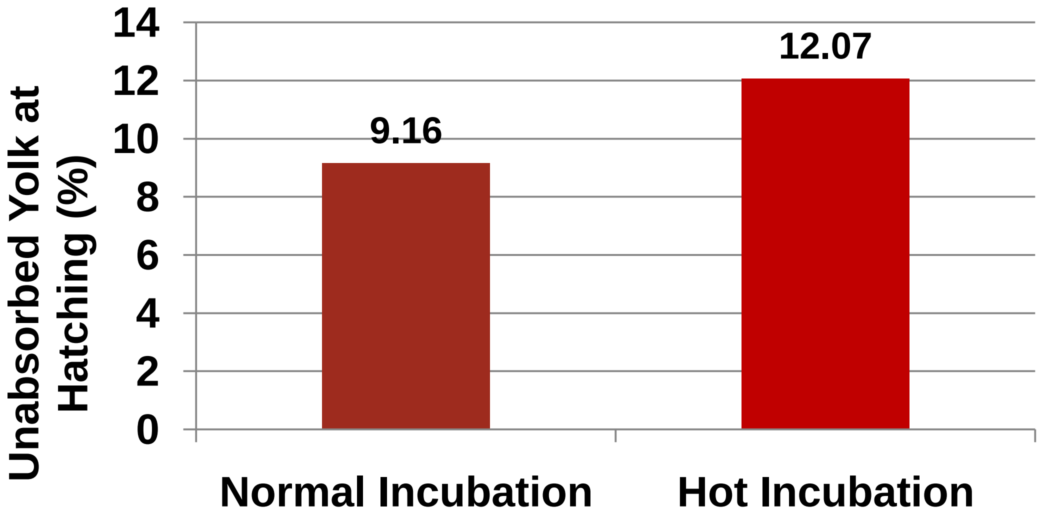


Mineral	Yolk
P (mg)	**111.7 (±16.84)**
Ca (mg)	**28.89 (±6.19)**
Fe (mg)	**2.78 (±1.89)**
Zn (mg)	**0.99 (±0.41)**
Cu (µg)	**31.8 (±13.12)**
Mn (µg)	**21.62 (±7.23)**

Mineral	Albumen
P (mg)	6.55 (±5.19)
Ca (mg)	4.27 (±3.78)
Fe (mg)	0.39 (±0.47)
Zn (mg)	0.59 (±0.45)
Cu (µg)	9.82 (±7.03)
Mn (µg)	0.78 (±0.8)

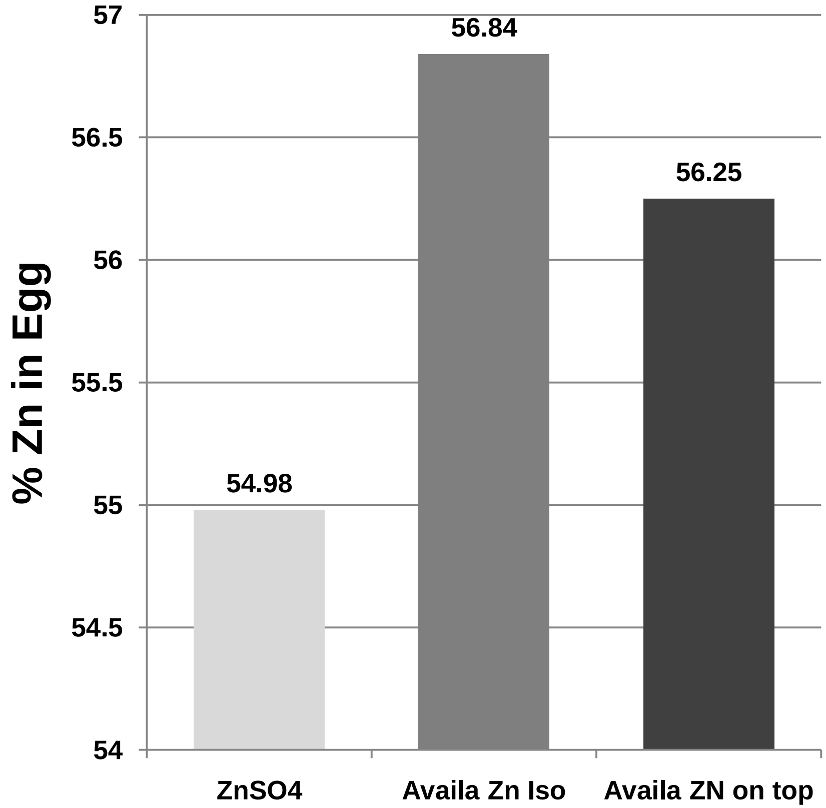
Yair R. and Z. Uni (2011) Poultry Sci.

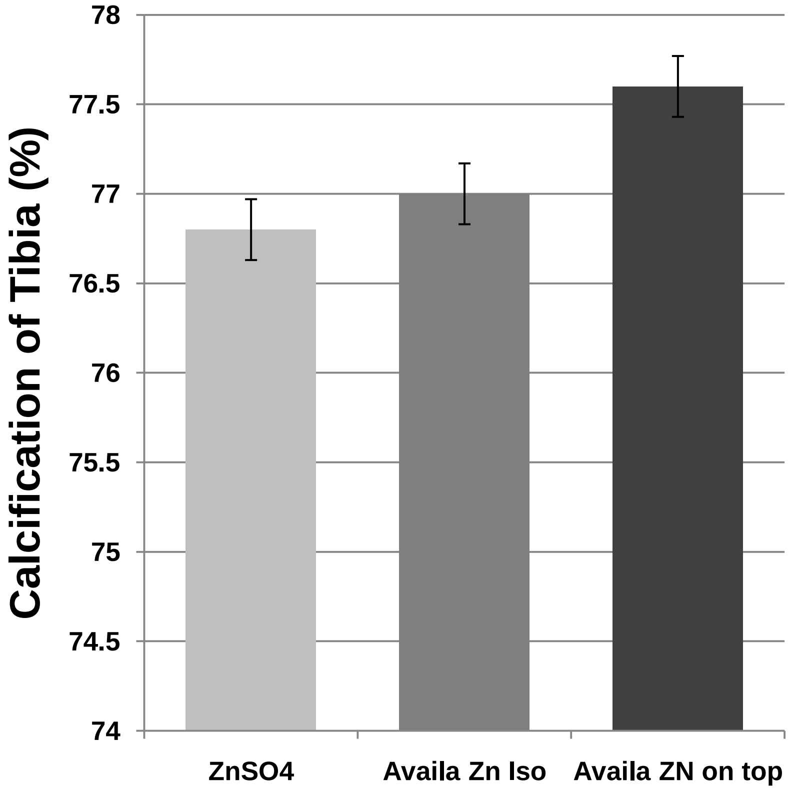
Yolk absorption at point of hatching is critical for bone mineralization and affected by incubation temperature



Leksrisompong et al., 2006

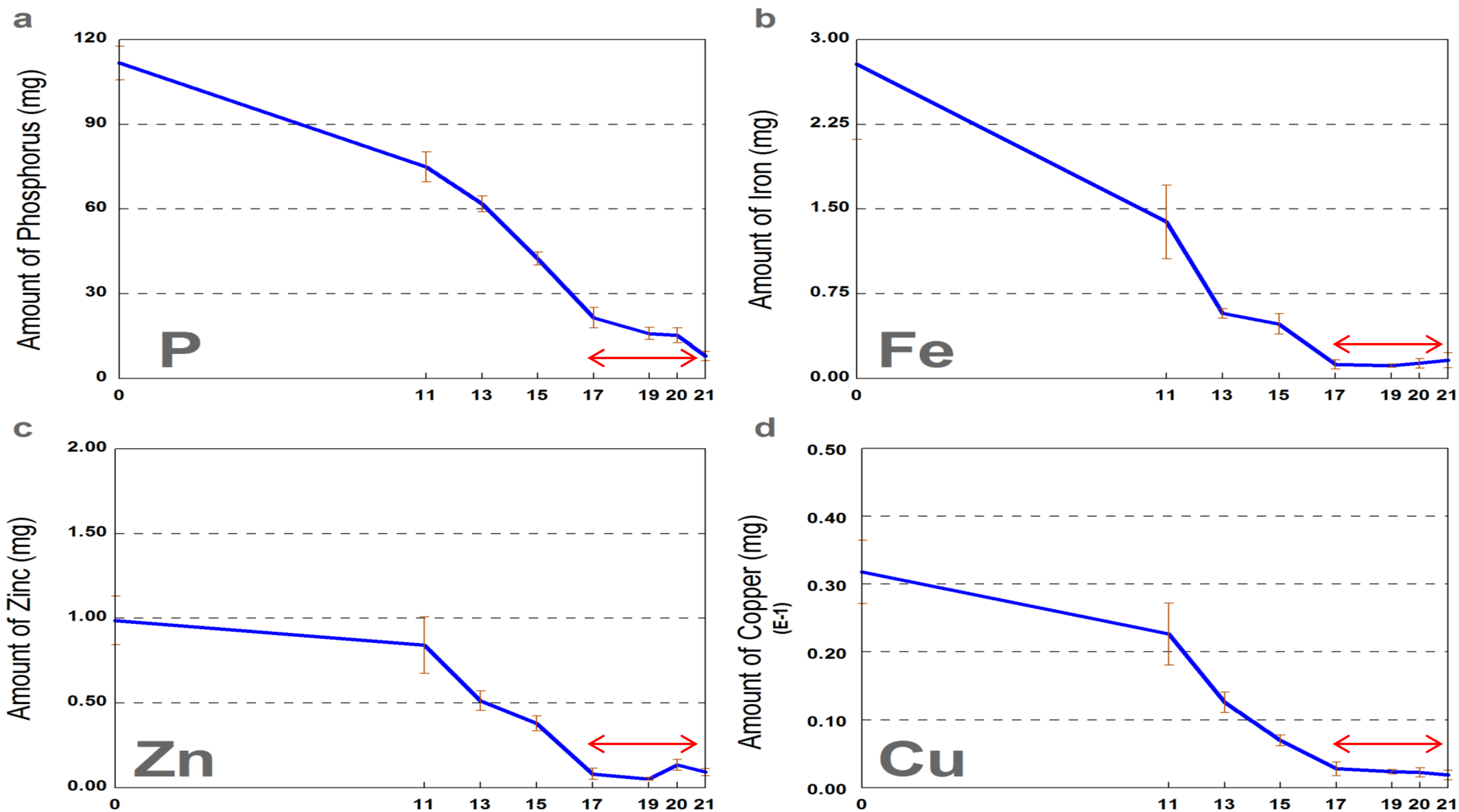
Changing Egg Zn with organic minerals altered % calcification of tibia of chicks at hatch



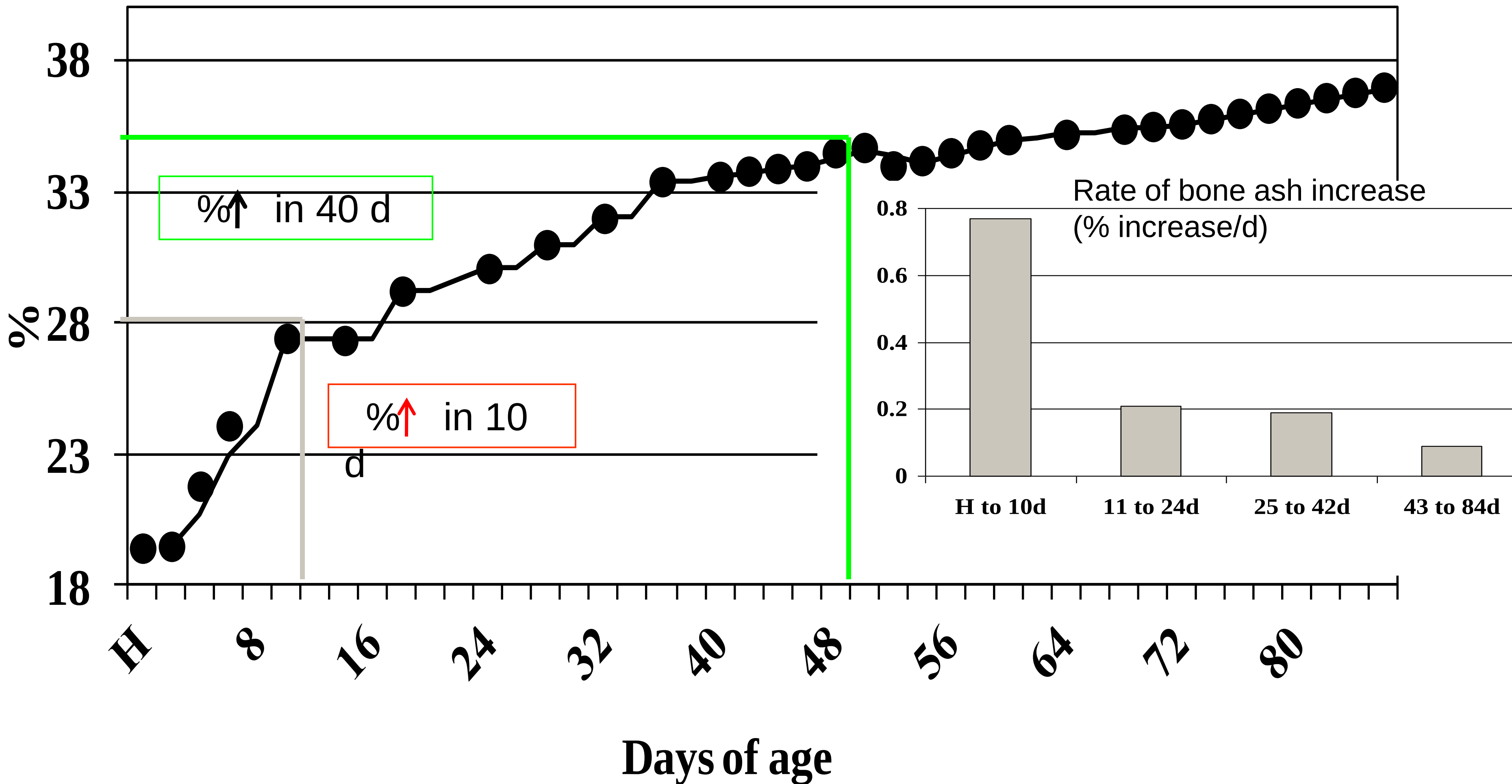


Favero et al., 2013

At the point of Hatching, the Yolk P, Zn, Fe, and Cu reserves are almost depleted…



… further, skeletal mineralization data suggests that H to 10 d is critical



Slide from R Angel, 2013

There is large variation in formulated Ca and NPP levels. What are the Ca and P requirements in pre-starter diets?

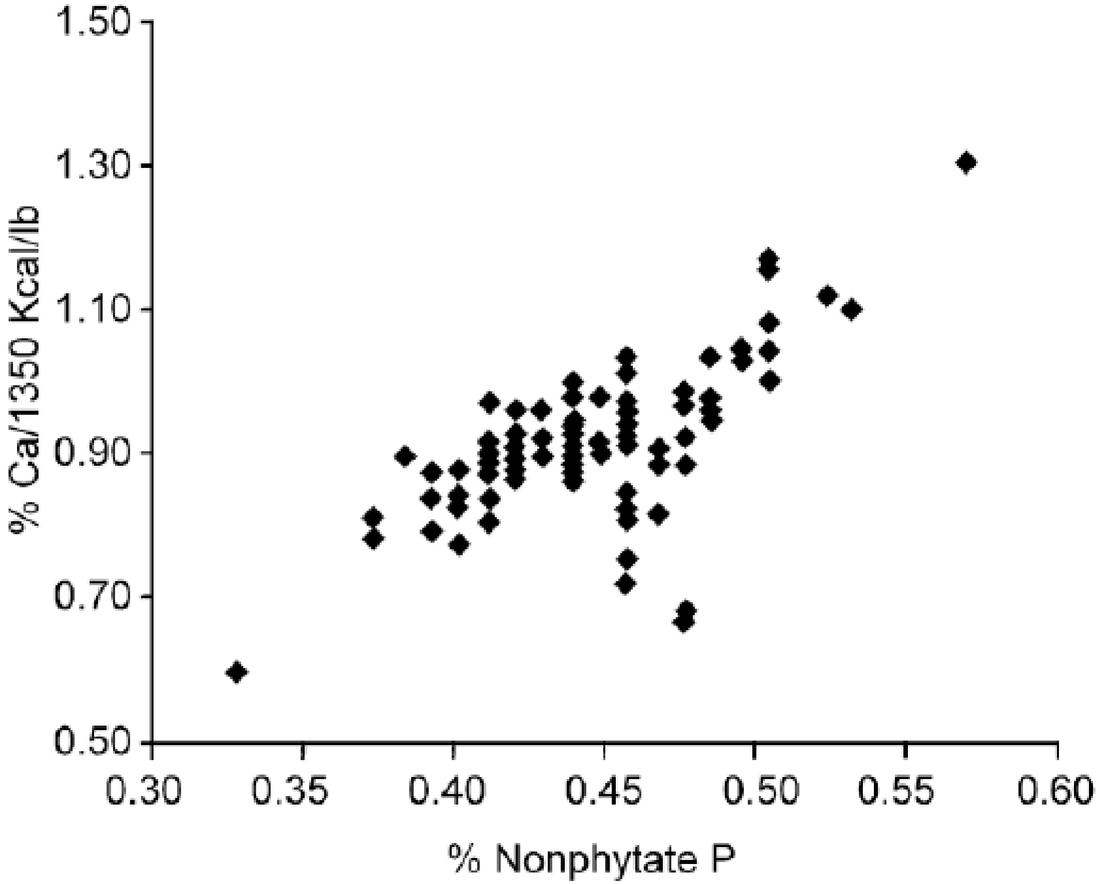
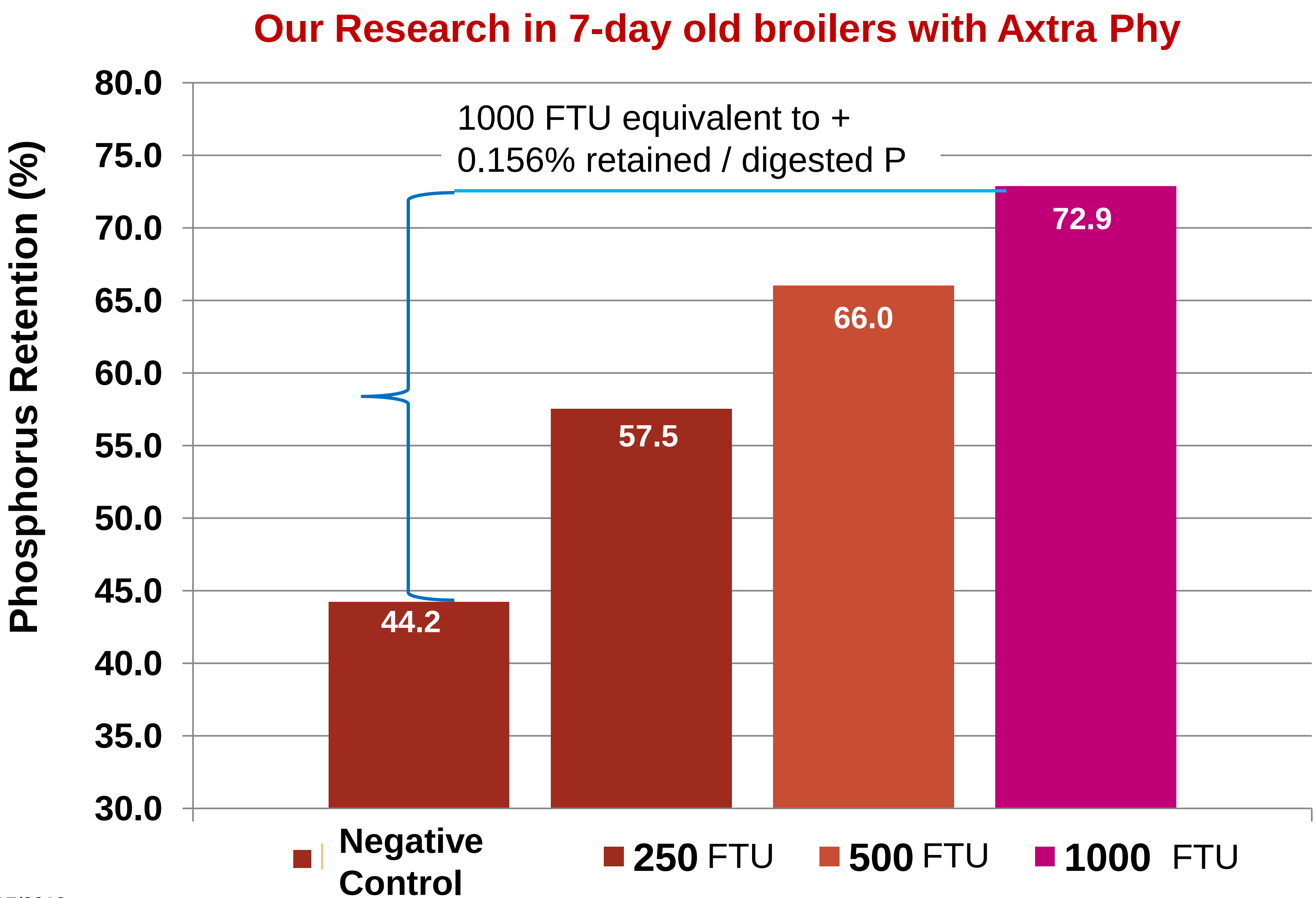


Figure 1.5: Current industry usage levels of calcium and nonphytate phosphorus in broiler starter diets (Survey by Agri Stats, Fort Wayne, Indiana).

Ca + P nutrition, and Recommendations PreStarter diet (Hatch to 10d of age) *R. Angel, 2013*

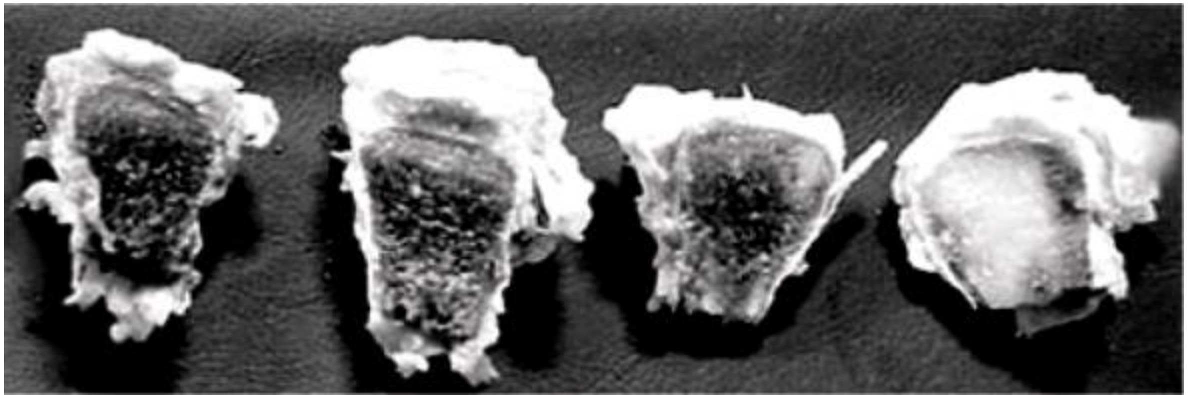
	CVB		Brazilian Tables , 2011 H-8d	Cobb 500, ‘12	Ross, 708, ‘09	Ind US[1]	Lit Avg, 2012[2] H-21d	Angel et al .,		
	‘08 H-7d	’12 H-10d						2007 H-18d Ross	‘13 Ross BWG/Tib ash	‘13 Hubbard Cross BWG/Tib ash
Ca, %	0.9-1.0	0.88-0.92	0.91	0.9	1.05	1.0	(0.91)	(0.92)	**1.05/ 1.10**	**0.95/ 1.05**
nPP, %						0.48	(0.38)	0.42	**0.55/0.60**	**0.56/0.63**
aP, %			0.44	0.45	0.50	0.48	(0.38)	0.42	**0.55/0.60**	**0.56/0.63**
dP, %	0.42-0.44		0.37						**0.46/0.50**	**0.47/0.52**
rP, %		0.40								
dCa, %									**0.58/0.66**	**0.60/0.65**
BW, g	165		218	277	276		(550)	723	**250**	**257**
ME, Kcal/kg	2830	2938	2960	3035	3025	3000-3050		3025	3025	3025

Is it safe to use Phytase in Pre-starter diets?

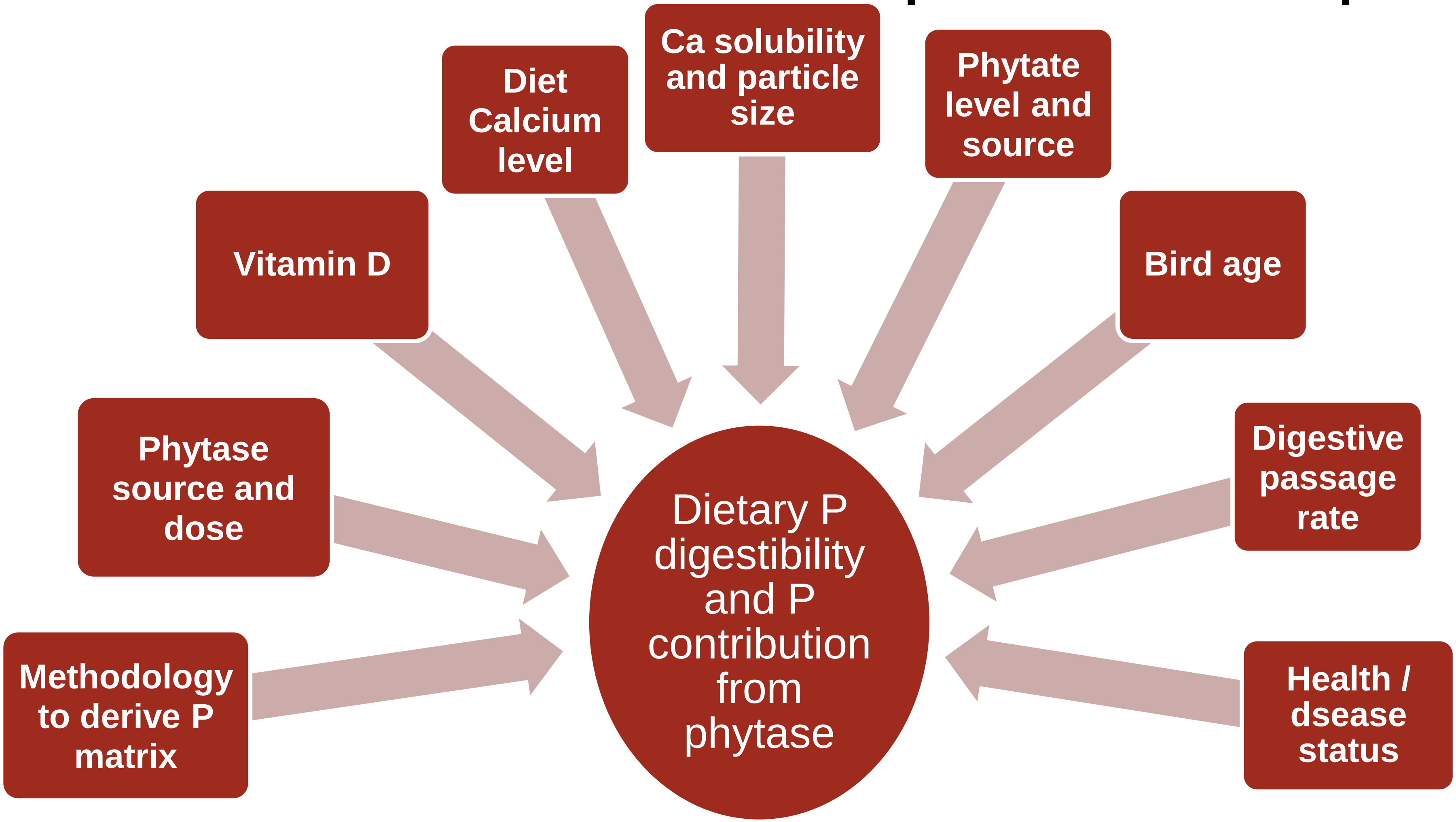


Plumstead et al., Schothorst Feed Research , Buttiauxella Phytase, 2012, unpublished

Understanding how Phytase works and what affects Phytase Ca and P contribution is critical to maximize the opportunity for feed cost-saving from phytase vs. the risk of incurring a Ca or P deficiency!



A lot of other factors affect P digestibility and Phytase value, Need to be understood for maximum performance and profit



Thermostability and Recovery in Finished Feed

Large Differences In AvP and Digestible P ''Matrix Values'' Exist Between Phytase Suppliers…

	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	0.15	0.134
Av.P %	0.12	0.13	0.13	0.146	0.15	0.146
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

Large $$ Incentive for bigger numbers…

- Phytase releases 0.1% AvP **= $3.16** value*.
- Phytase releases 0.12% AvP = **$3.79** value*
- Phytase releases 0.15% AvP = **$4.74** value*
- Phytase releases 0.18% AvP = **$5.69** value*

… But what P value from phytase is realistic?

How are Phosphorus Matrix Values Determined for Phytase?

Option 1: Calculating your phytase matrix based on log linear response.

- *‘’The relationship between phytasedose and the biological response has previously been established as log-linear, i.e. a logarithmic increase in dose is required to maintain a linear increment in response (Rosen, 2001; Kornegay, 2001; Rosen, 2002). This is in line with a similar relationship noted for NSP enzymes (Zhang et al., 1996;; Zhang et al., 2000).”*
- *Given this observation, it is possible to calculate the expected nutrient sparing effect of any dose of phytase from the equations published in the literature*
- *ME and Amino acid values are linked to Phosphorus values*

Bedford & Cowieson, 2009 ESPN

Option 1: Calculating your matrix based on the assumption of a Log linear response

Table 1. Illustration of the log-linear dose response to microbial phytases.

Dose	Relative matrix
500	100%
600	108%
700	115%
750	118%
800	121%
900	126%
1000	130%

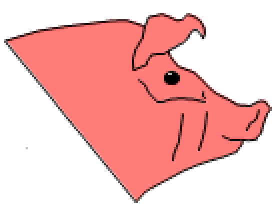
	500 FTU/kg	750 FTU/kg	1000 FTU/kg
Increment (relative to 500 FTU/kg)	100%	118%	130%
Available P (kg/t)	1.50	1.75	1.95
Ca (kg/t)	1.65	1.92	2.15
Sodium (kg/t)	0.35	0.40	0.45
Lysine (g/t)*	170	200	230
Methionine (g/t)*	39	44	50
Cysteine (g/t)*	351	396	450
Methionine + Cysteine (g/t)*	390	440	500
Threonine (g/t)*	330	390	440
Tryptophan (g/t)*	190	225	250
Glycine + Serine (g/t)*	570	665	740
Arginine (g/t)*	130	155	170
Valine (g/t)*	230	265	300
Isoleucine (g/t)*	255	300	335
Crude Protein (g/t)*	4210	4910	5480
ME (MJ/t)	217	255	284
ME (kcal/t)	52000	61000	68000

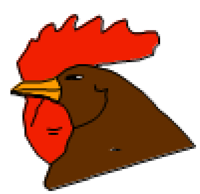
*Apparent ileal digestible values

- **Note that the Rosen models were based only on phosphorus and calcium, and mainly used published 1st generation phytase data (Natuphos)**
- **Highly questionable if the response in AA and ME follows the same response as AvP.**

Interestingly, the same phytase supplier recommends using the same matrix values for swine and chickens (!)

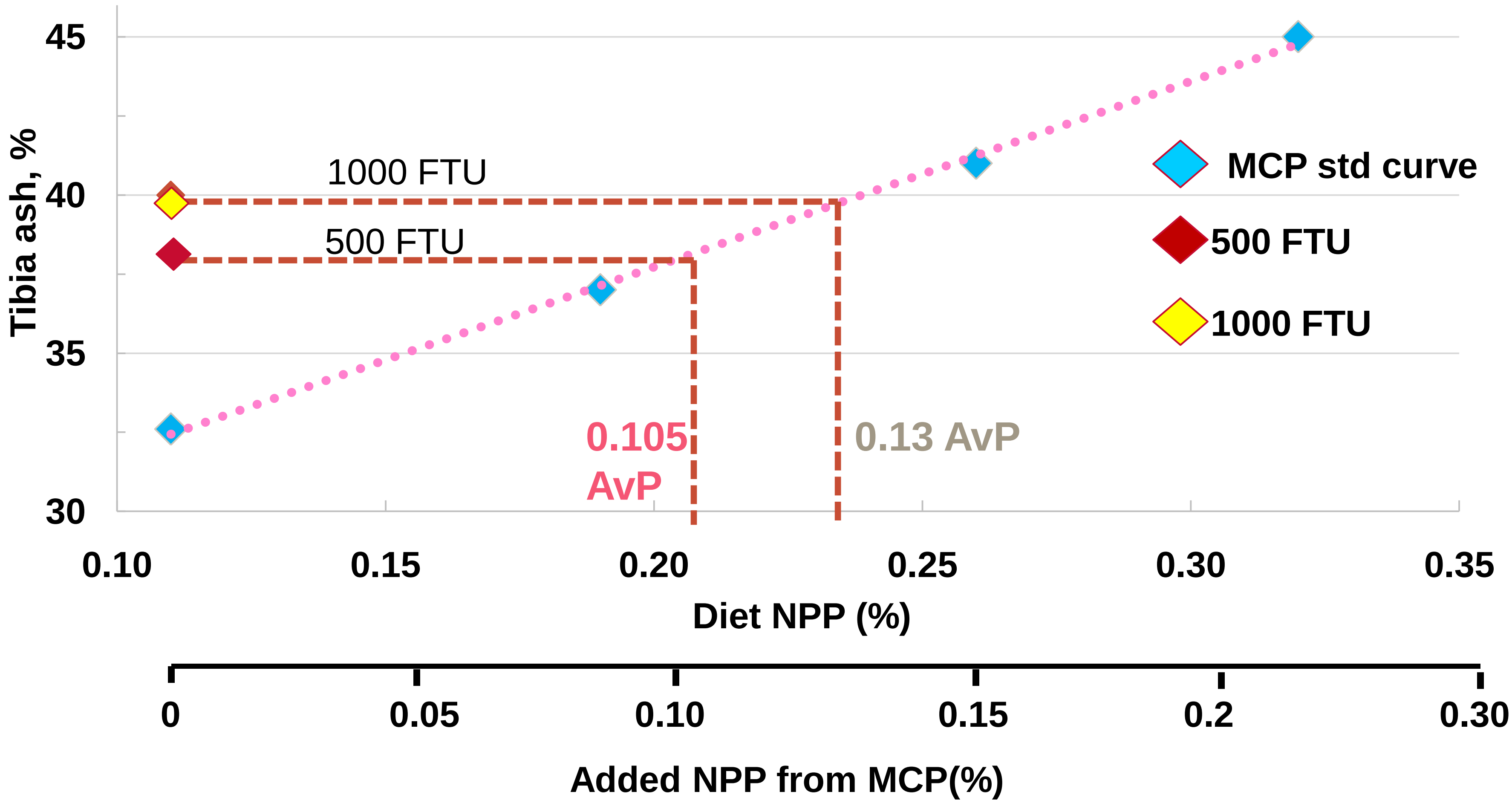
Nutrient	Value % broiler [11]	Value % swine [11]
Available P	0.15	0.15
Calcium	0.165	0.165
Protein	0.421	0.421
Dig. Threonine	0.033	0.033
Dig. Lysine	0.017	0.017
ME kcal/kg	52	52
Sodium	0.035	0.035
Methionine	0.0039	0.0039
Tryptophan	0.019	0.019
Iso-leucine	0.0255	0.0255
Arginine	0.013	0.013
Valine	0.023	0.023





Pigs ≠ Chickens!

Option 2: Most other Phytase suppliers determine "Available P" relative to an inorganic P standard (MCP/DCP)

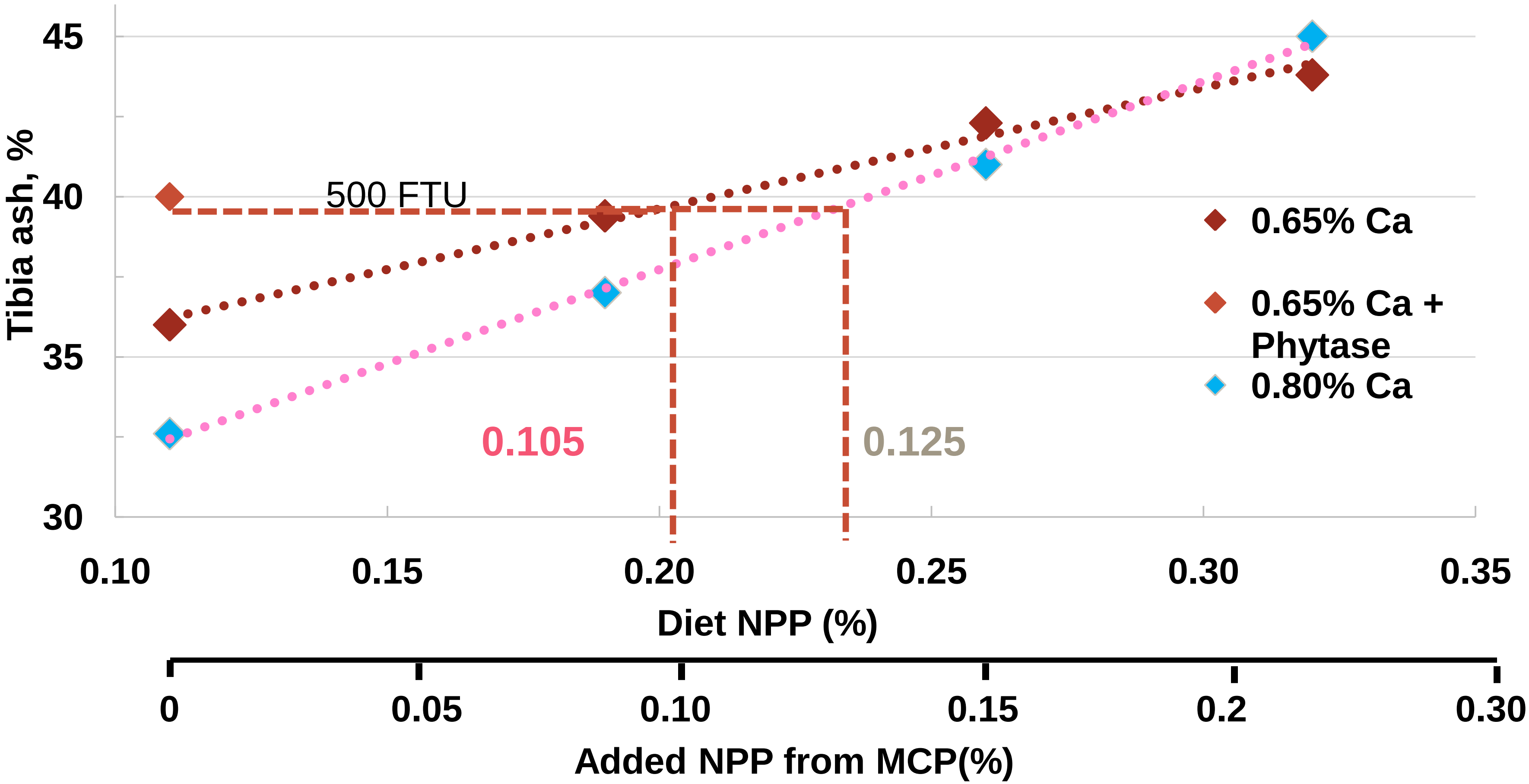


Digestible P ≠ Available P

Phosphorus source	MCP	MDCP	DCP
Total P	**23**	**21**	**18**
Available P (Coefficient)	**100.00%**	**0.98%**	**0.95%**
Digestible P (Coefficient)	**0.81**	**0.79**	**0.78**
Calculated AvP	**23**	**0.2058**	**0.171**
Calculated Digestible P	**18.63**	**16.59**	**14.04**
Ratio Dig P:AvP	**0.81**	**80.61**	**82.11**

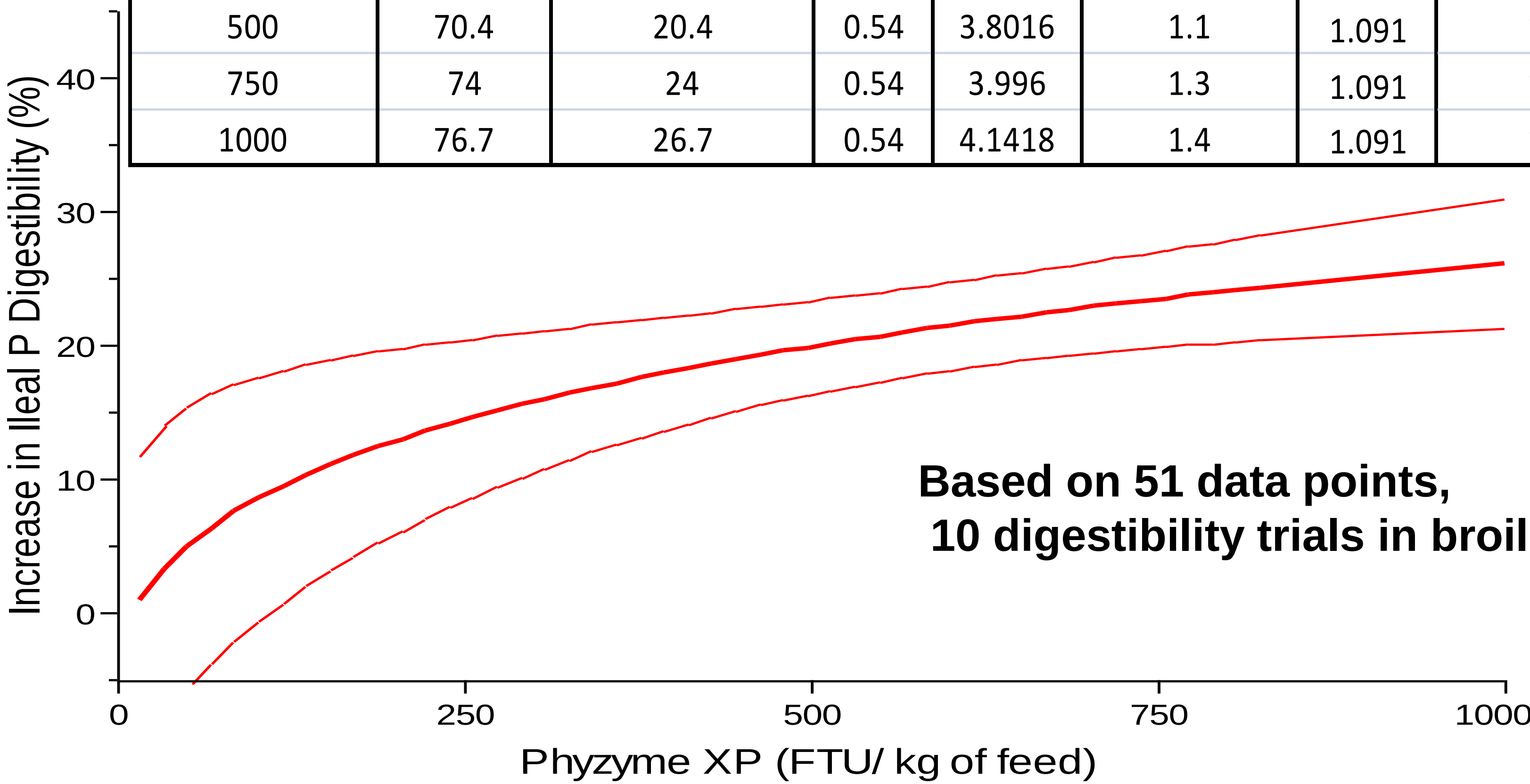
Option 2: Determine ‘’Available P” relative to an inorganic P standard (MCP/DCP)

- *When determining Phytase P release relative to MCP standard, one has to be careful of dietary Ca levels, both in phytase treatments and control diets*



Option 3: Calculating P contribution from Phytase based on absolute increase in ileal P digested from Phytase

Phyzyme XP dose (FTU/kg feed)	Ileal P digestibility %	P digestibility Improvement from Phyzyme XP (%)	Analyzed P in NC (%)	P absorbed (g/kg feed)	Dig. P contribution from Phyzyme XP (g/kg feed)	AvP conversion factor	AvP Contribution from Phyzyme XP (g/kg feed)
0	50	0	0.54	2.7	0.0	1.091	0.0
250	64.9	14.9	0.54	3.5046	0.8	1.091	0.9
500	70.4	20.4	0.54	3.8016	1.1	1.091	1.2
750	74	24	0.54	3.996	1.3	1.091	1.4
1000	76.7	26.7	0.54	4.1418	1.4	1.091	1.6



Top & bottom lines represent 95% confidence intervals of the predicted response

Meta analysis to model Phosphorus Contribution from Buttiauxella Phytase

- 10 Broiler Ileal Digestibility Trials conducted from 2008 to 2011.
- Ross 308 and Cobb 500 broilers
- Range of Phytase dosing from 250 – 2000 FTU/kg feed
- Average Phytate P level = 0.26%
- 296 data points in data set after removing Postive controls and other phytase sources.
- Increments in digestible P vs. Negative control diets calculated
- Available P calculated from digestible P using factor of 0.9167
- Modelling used Non-linear regression; Mitcherlich model.

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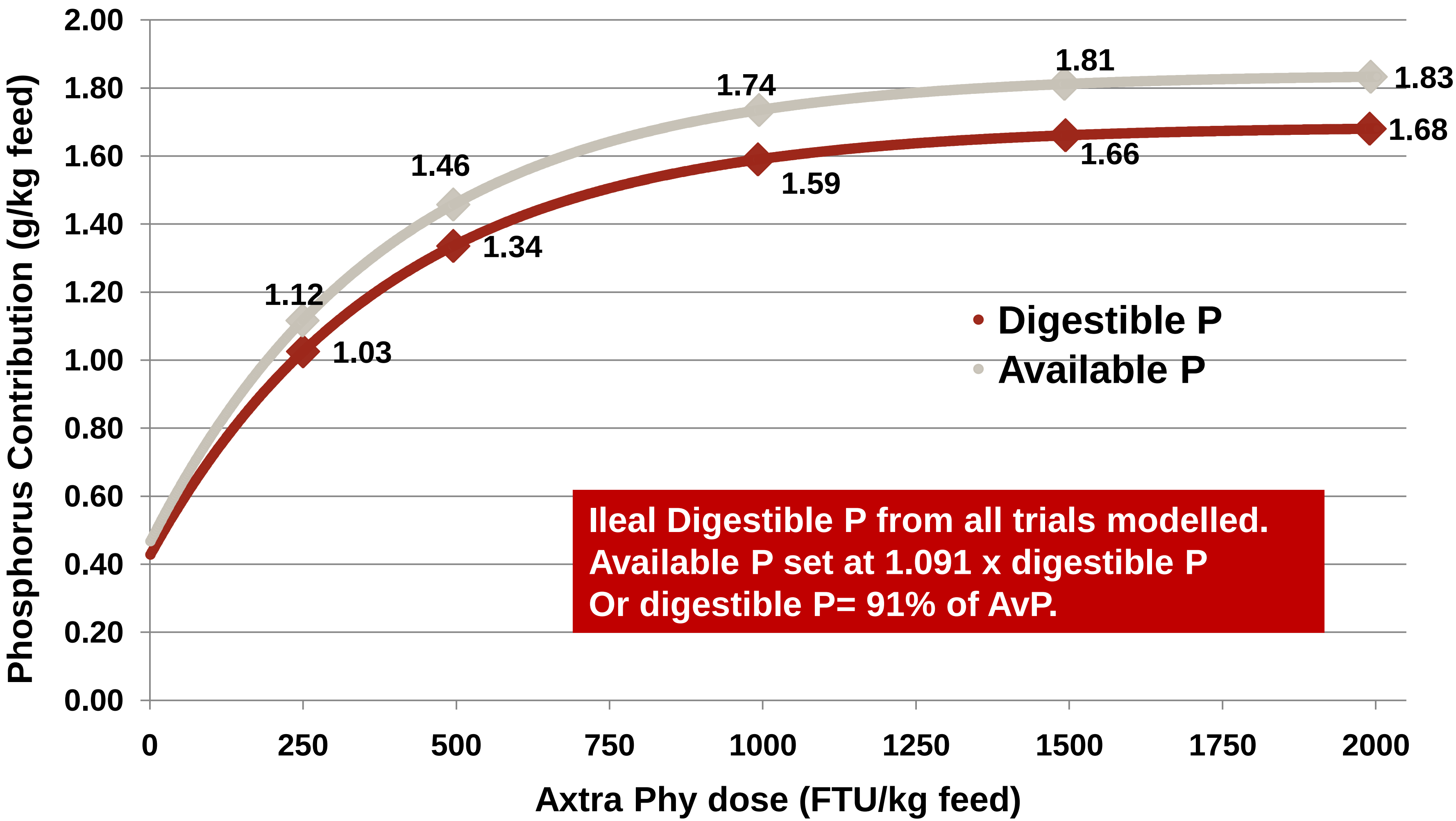
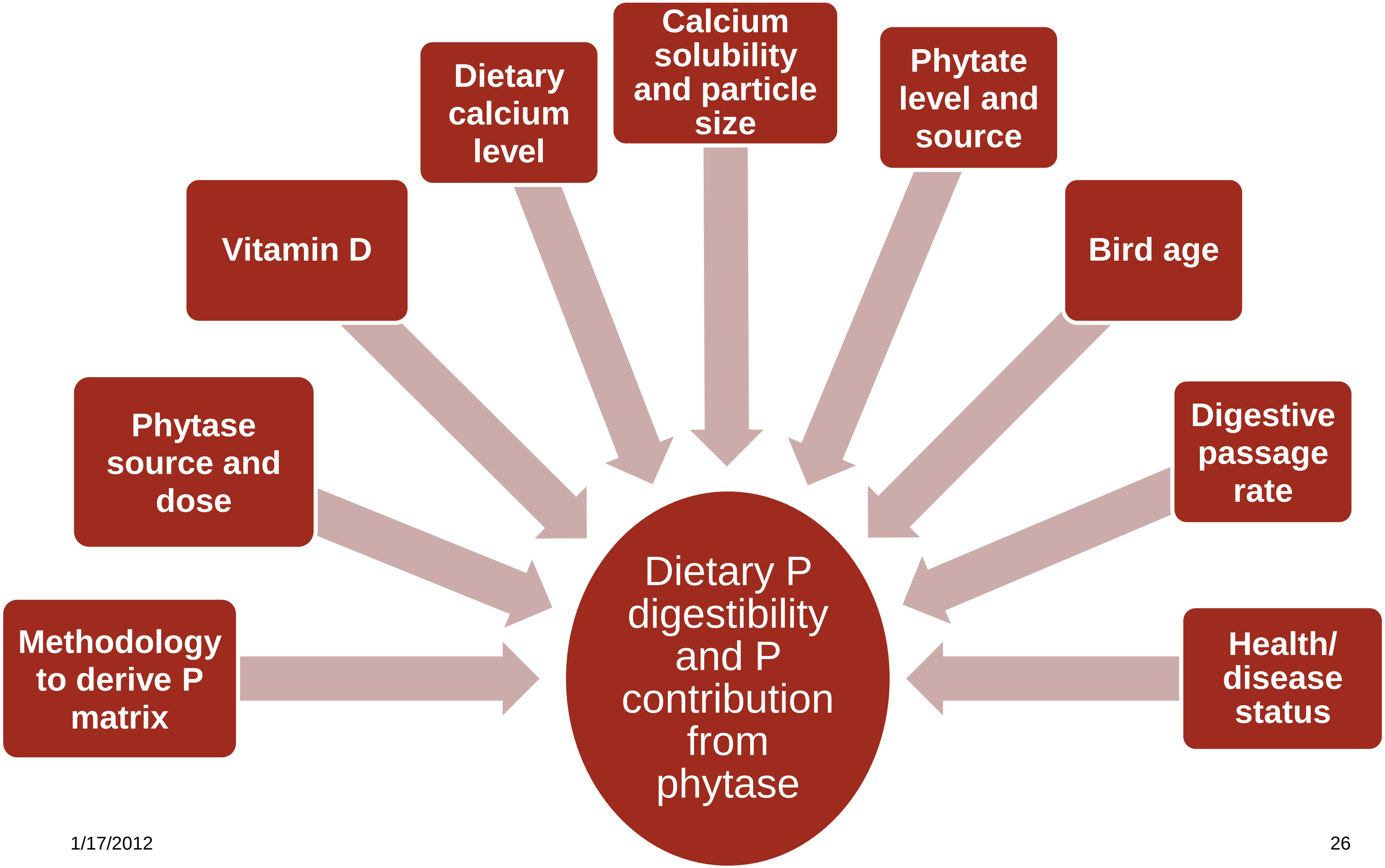
Modeling Digestible and Available P contribution from Axtra Phy



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

A Lot of Factors Impact Phosphorus Digestibility, Phytase Efficacy, and Correct Bone Mineralization



Ca, P, Ca:P Ratio & Skeletal Integrity

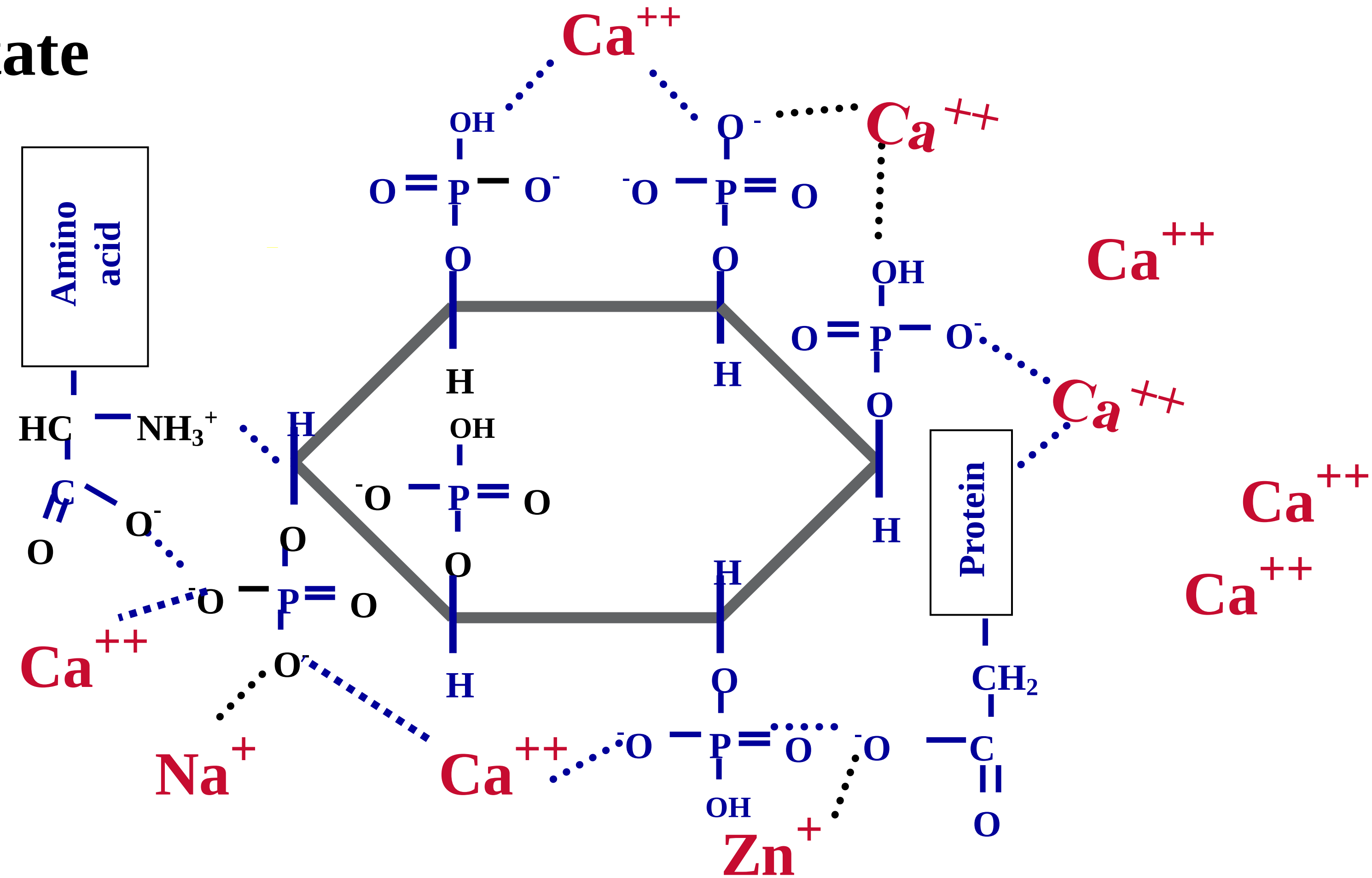
- Calcium and phosphorus make up more than half of a bird's mineral requirement (Cromwell, 1991).
- Mineralization of Hydroxyapatite in Bone requires

  2.14 Ca^{2+} : 1 Available Phosphorus (AvP)
- Concerns with poor mineralization are usually focused on impact of <u>excess calcium on phosphorus metabolism</u>
- <u>Concerns with Phytase are also usually focused on effects of excess Calcium.</u>

Research has shown that 1% phytate (0.28% Phytate P) in a broiler diet could bind 0.36% dietary calcium...

Nelson et al., 1968

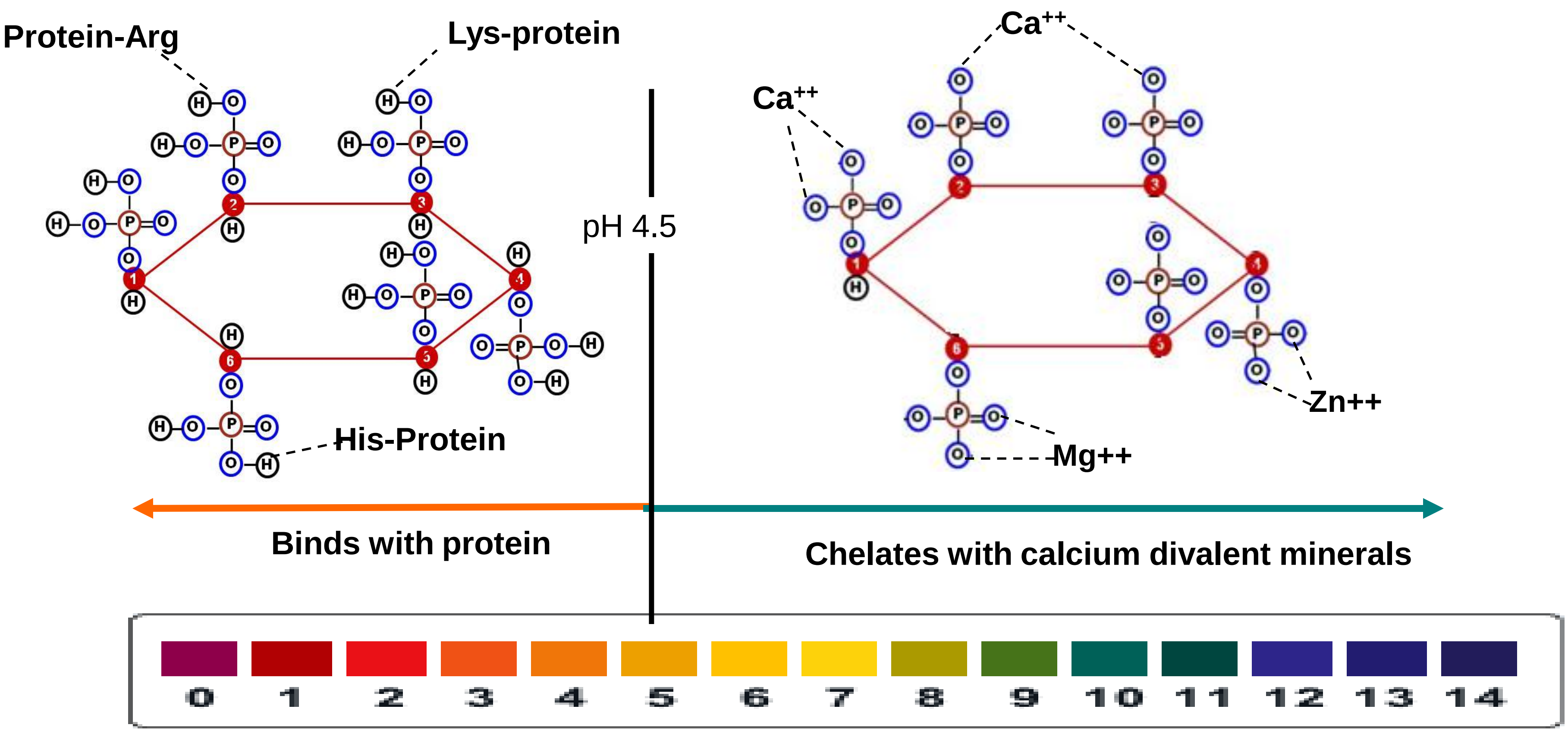
Phytate

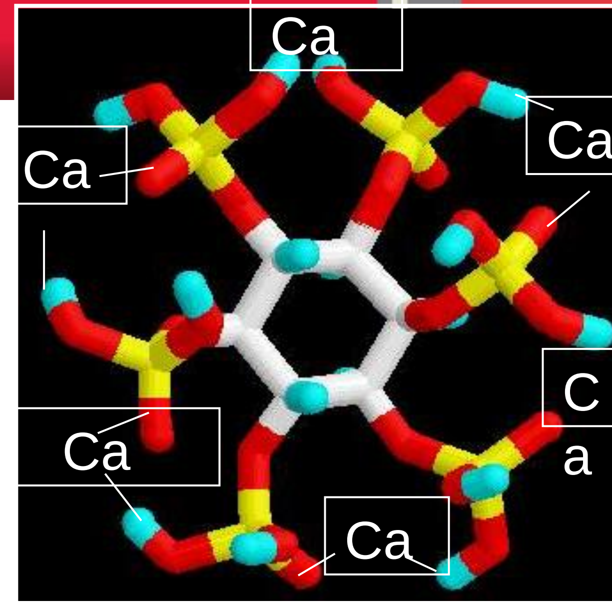


Effect of Ca^{2+} On Phytate P utilization and P digestibility - Ca Binds Phytate at pH > 4.0, reducing P digestibility

Gizzard / Proventriculus

Duodenum / Ileum / Jejenum





Impact of pH and Calcium on phytate solubility

% soluble Phytate P

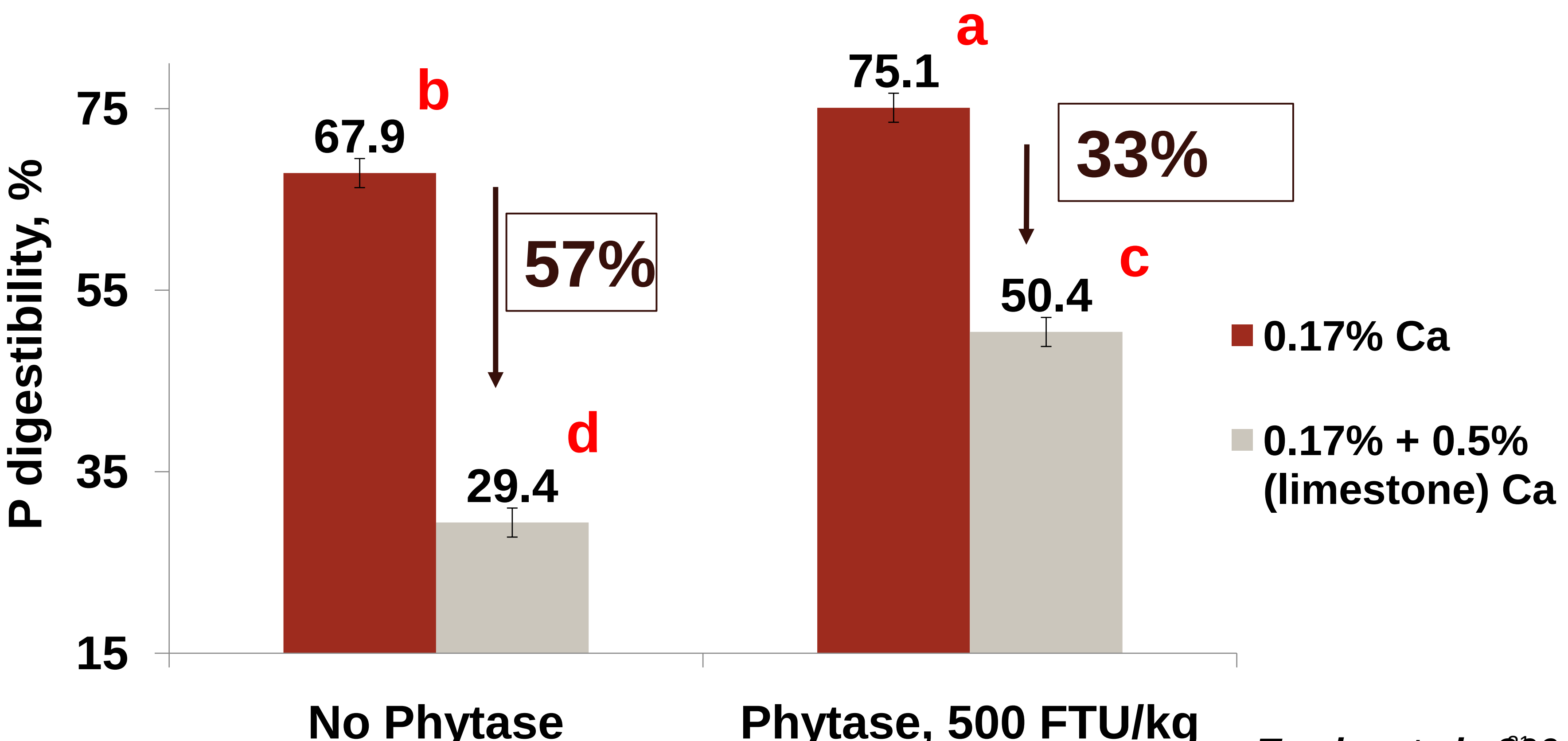
100 100 99.3 97.9 84.7 62.1 51.7 26.4 11.1 7.9 1.5

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

Impact of [Ca] on P digestibility

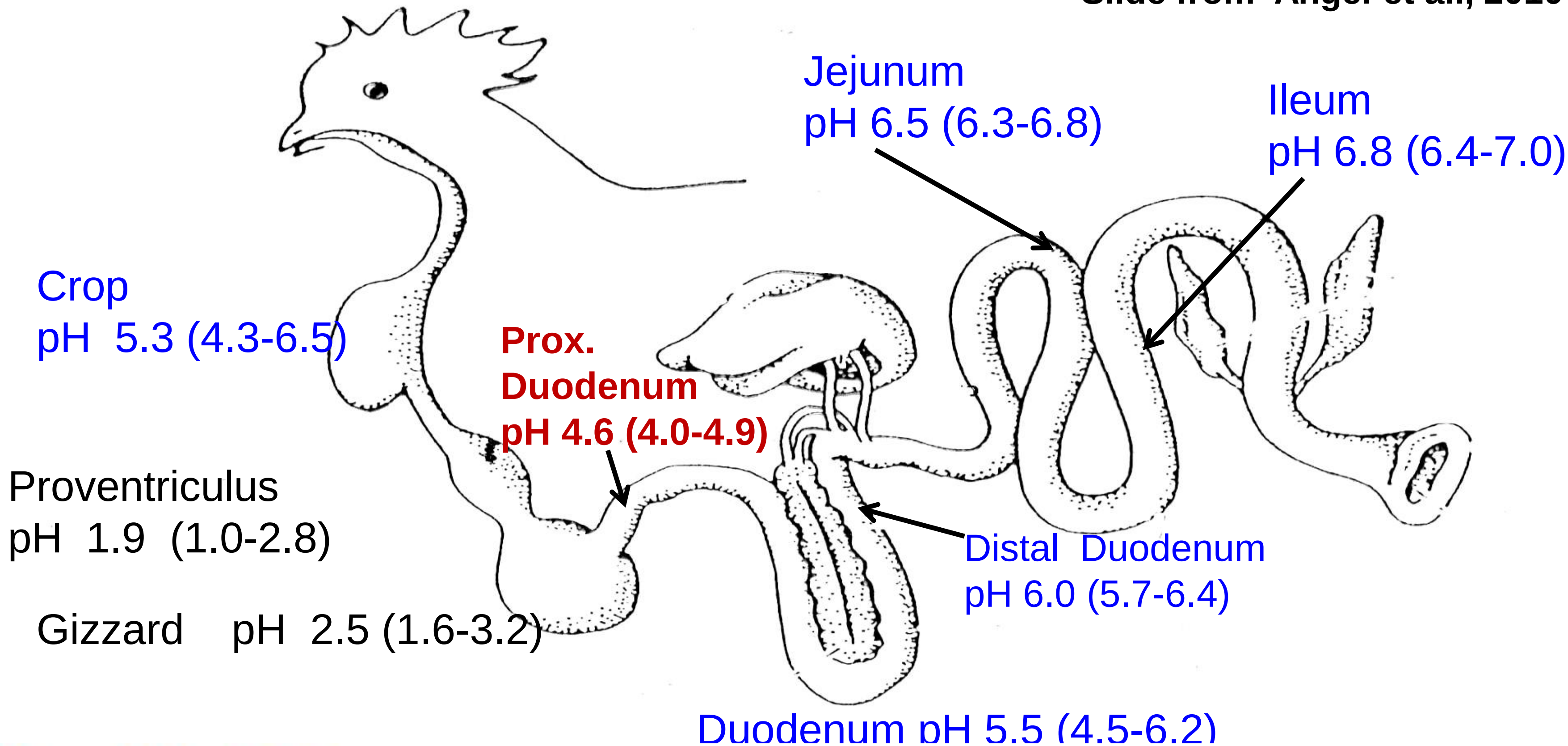
- **Increasing diet [Ca] reduces P digestibility in broilers with/without phytase**
  (Mohammed et al 1991; Tamim et al., 2004; Adeola and Walk, 2013)



Tamim et al., 2004

Phytate will Precipitate with Calcium as soon as digesta enter duodenum

Slide from Angel et al., 2010



% soluble PP

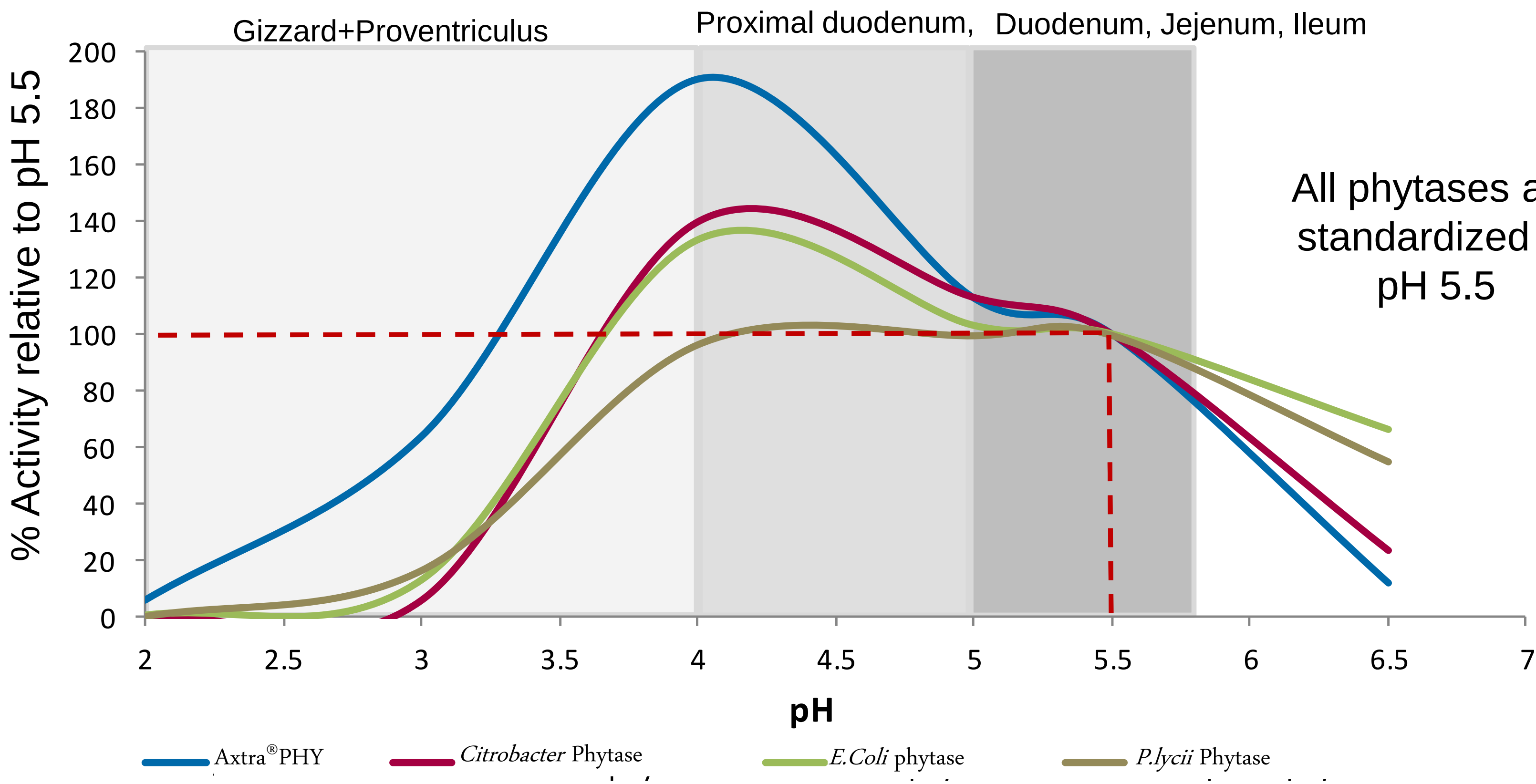
97.9 84.7 62.1 51.7 26.4 11.1 7.9 1.5

Putting information in context (Slide from R. Angel 2013)

GIT segment	pH mean (min-max)	Ca-phyate P solubility, %[1]	Passage rate, min[2]	MRT , min[3]	
				MRT1	MRT2
Crop	5.7 (3-7)	17.6	12	41	58
Proventriculus	1.5 (1-3.5)	100	P+G 37	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	D+J 91	D+J (76	102)
Prox. Jej.				23	27
Distal Jej.				48	61
Ileum	6.7 (6-7.2)	4.4	83	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.)
[1]Determined based % marker in different parts GIT post time of dosing (n=12 per time period)
[3]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 (Van der Klis et al.,)

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

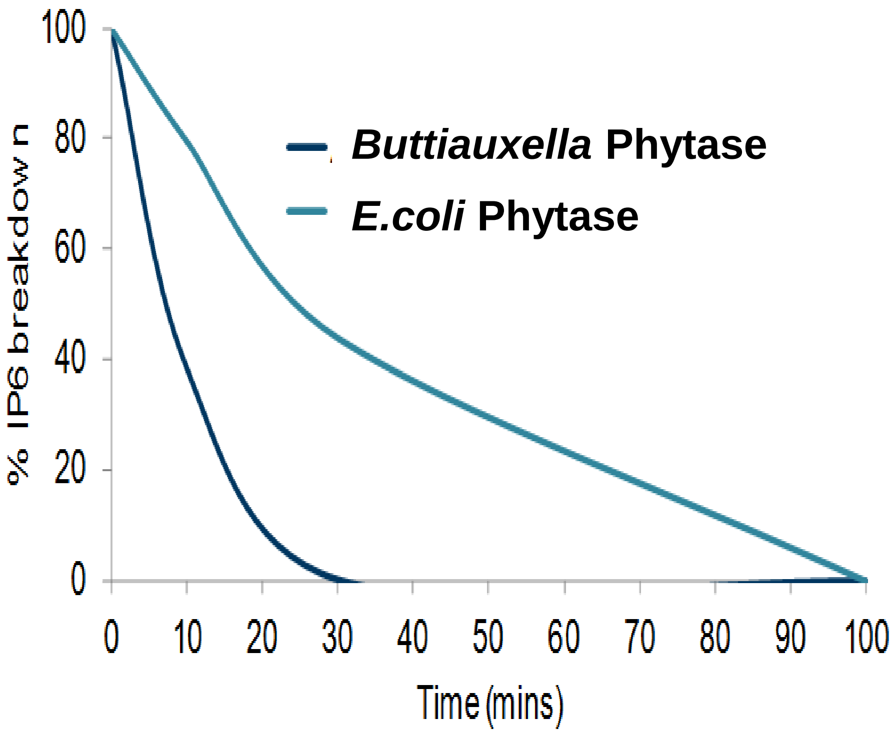


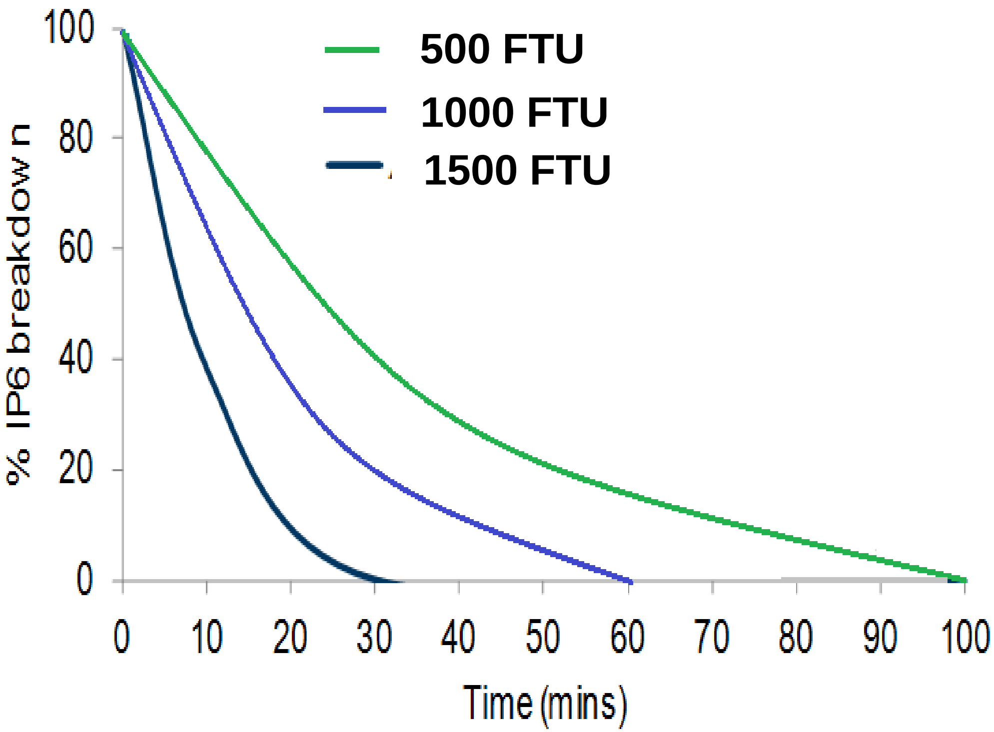
All phytases are standardized at pH 5.5

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

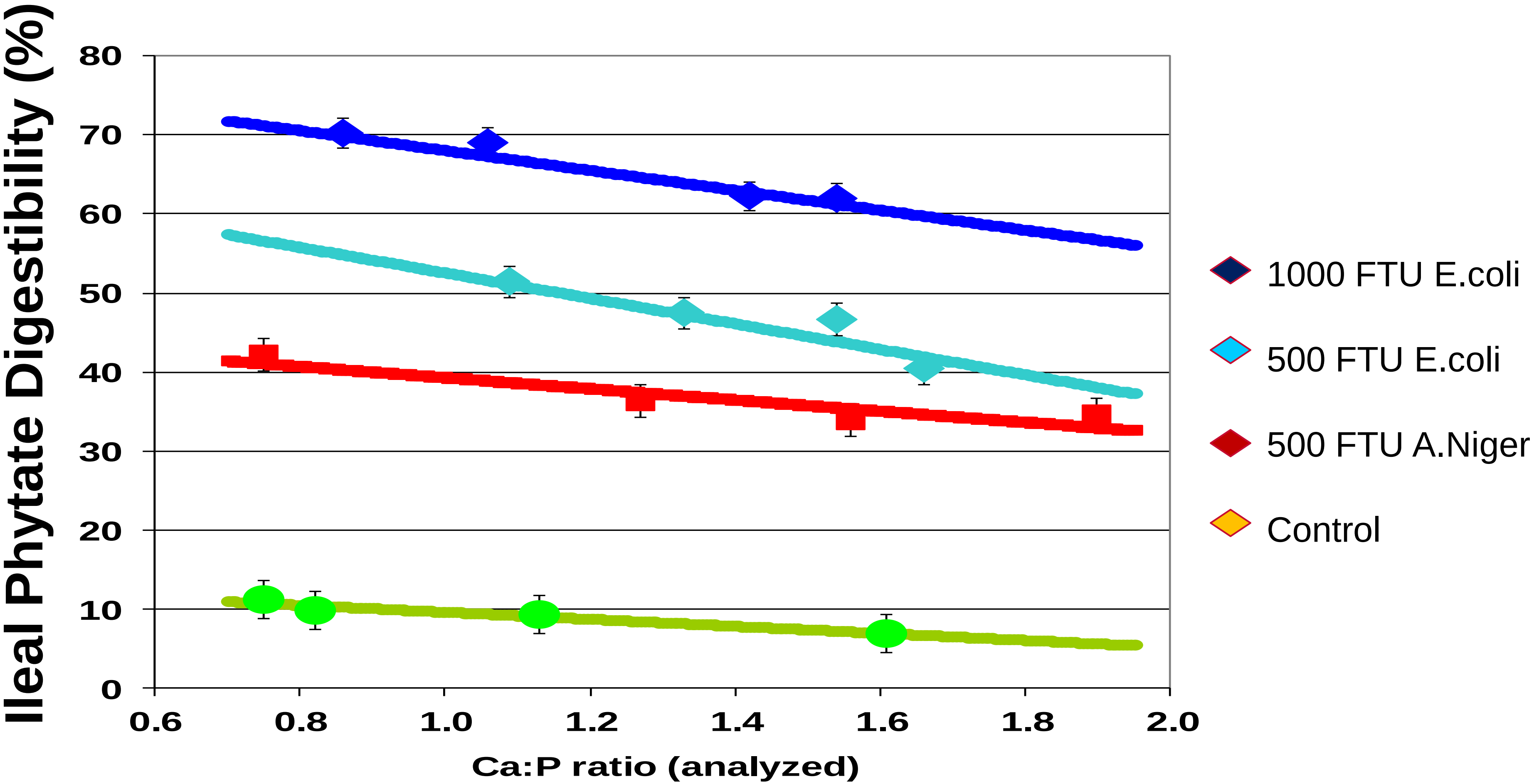
If Time is a limiting factor in the Gizzard / Proventriculus, there will be benefits to either using phytases that work faster, or from increasing the dose of phytase.





*using sodium phytate as a substrate

Ileal Phytate digestibility from E.coli phytase as affected by the Ca:P ratio



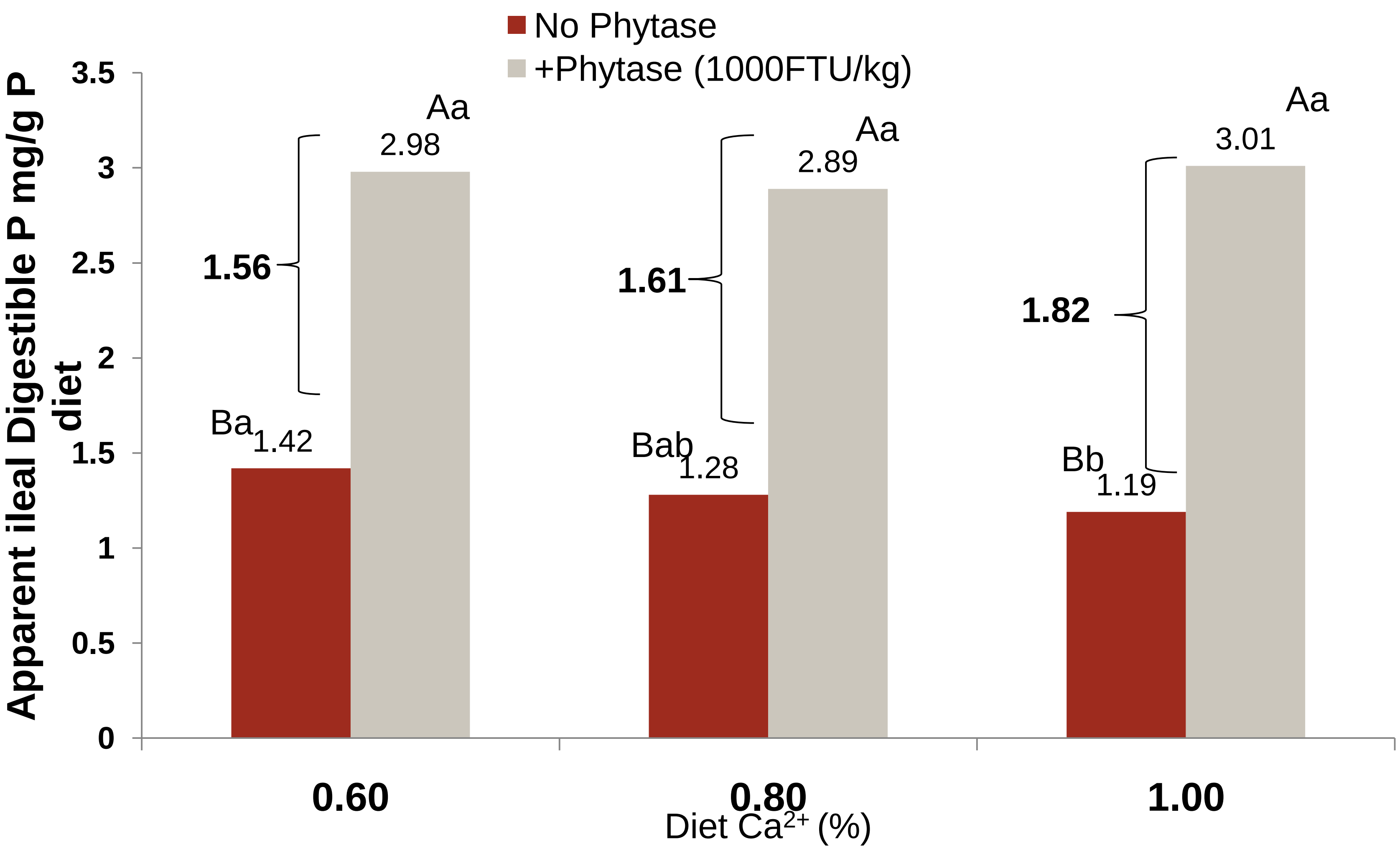
Phytase *P*<0.01

Ca:P = *P<0.01*

Phytase x Ca:P = *NS*

Plumstead et al., 2010

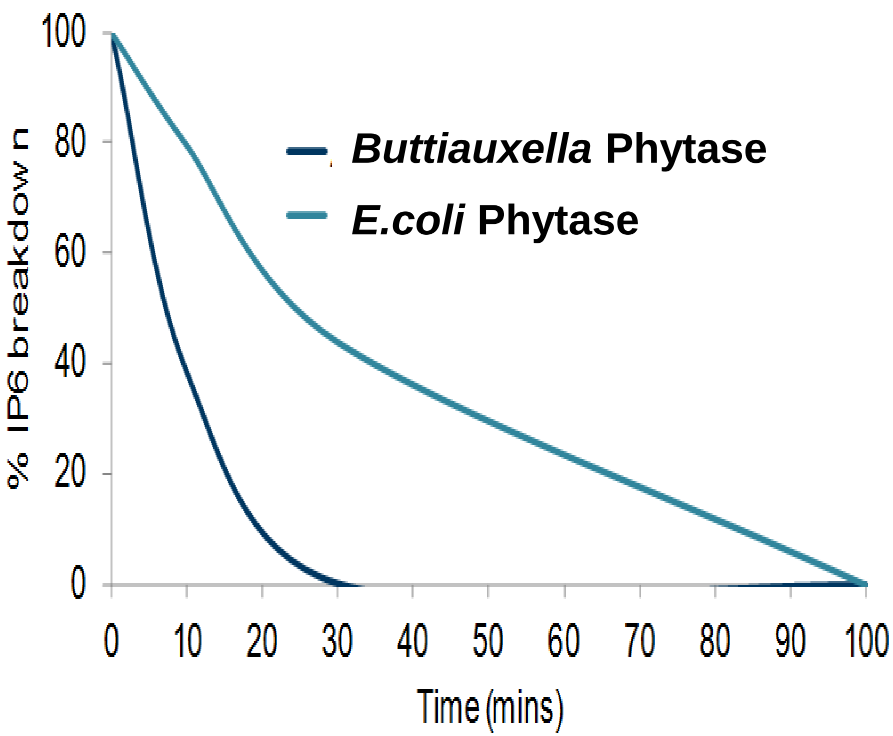
Buttiauxella Phytase Contribution At 1000 FTU/kg Was Not Influenced By Dietary Ca Level (0.42mm Limestone)

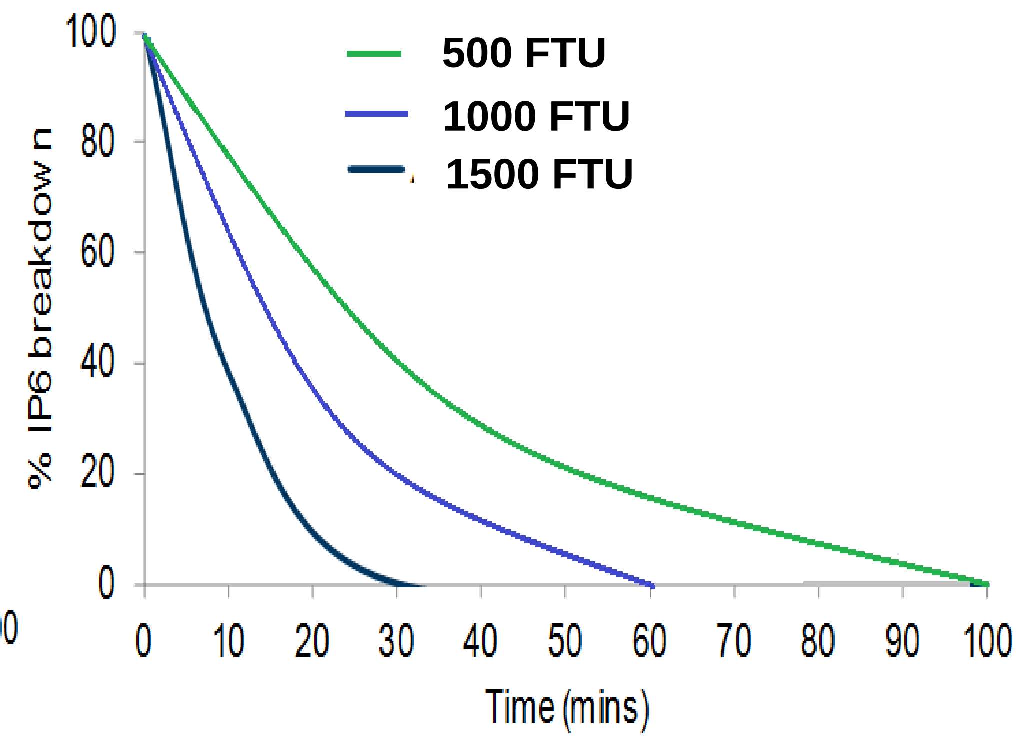


a-c Means within a phytase trt with different superscripts differ ($P < 0.05$). AB Means within the same dietary Ca concentration with different superscripts differ ($P < 0.05$).

Kim *et al,* 2013

One Of The Benefits Of High Phytase Doses, or phytases that are designed to work Rapidly To Degrade IP6 In The Acid Stomach = More Consistent % Phytate Hydrolysis And Less Influence Of Ca^{2+} on phytase efficacy





*using sodium phytate as a substrate

Ca, P, Ca:P Ratio – Lets talk about Calcium

- Calcium and phosphorus make up more than half of a bird's mineral requirement (Cromwell, 1991).

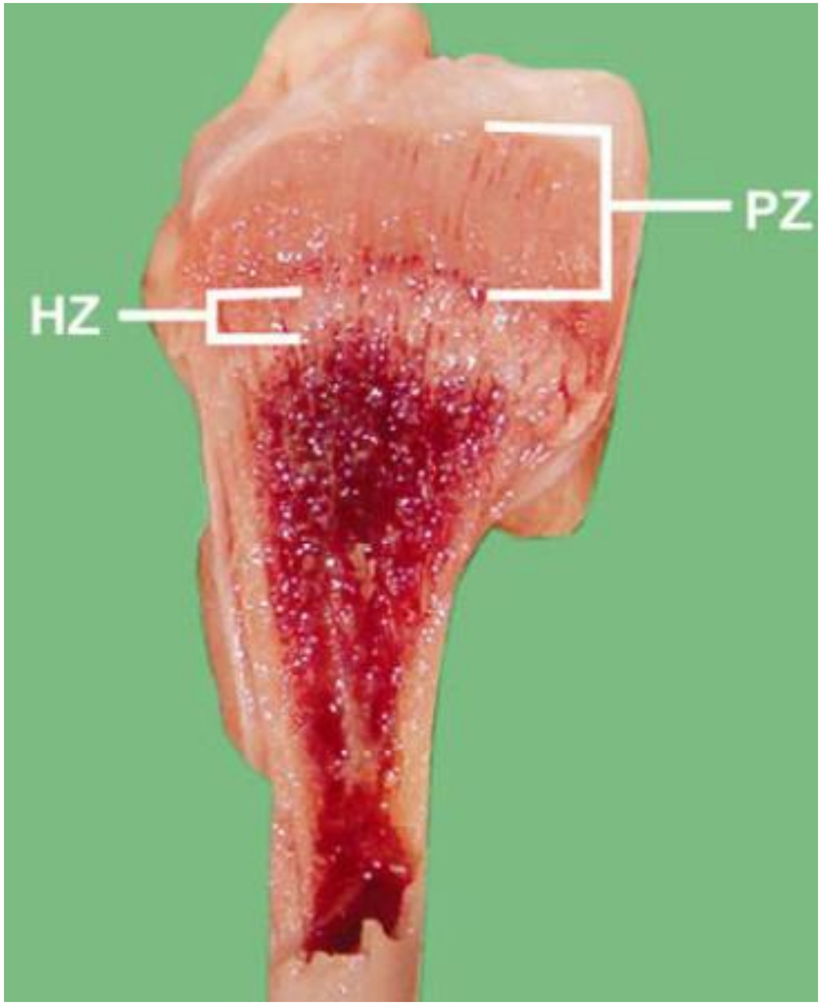
- Concerns about the impact of calcium on phosphorus metabolism
  - Mainly been focused on metabolic imbalances that result from calcium excess.
    - Excretion of both excess Ca and excess P as a Ca-P complex

HOWEVER…..

- Insufficient levels of calcium and inadequate calcium:avaliable phosphorus ratios decrease broiler bone mineralization & performance.
- To mineralize, Hydroxy-Apatite of bone requires 2.14 Ca: 1AvP
- What about Ca^{++} from Phytase?

Too low dietary Ca, or excess P relative to Ca will reduce Ca levels available for mineralization

Hypocalcaemic rickets in a 21-day-old chicken from Farm B. A considerably elongated growth plate proliferative zone (PZ) and widening of the proximal tibiotarsus is seen. The hypertrophic zone (HZ) is not altered.



Large Differences In AvP and Digestible P ''Matrix Values'' Exist Between Phytase Suppliers… but even bigger differences on Ca 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	-	0.117	-	0.134
Av.P %	0.12	0.13	0.13	0.146	0.15	0.146
Ratio of Dig. P:AvP	0.92	0.85	-	0.80	-	0.92
Calcium %	0.11	0.13	0.14	0.18	0.165	0.134

- How were Ca and P matrix values determined?
- How does the P-system used compare to your Ingredient P matrix?
- Why are some Ca^{2+} matrix values so high?
- What factors affect the variation in response to phytase?

Experimental design

- Treatment diets contained 0.25% PP
- 2 Ca concentration 0.65 and 0.80%
- 4 nPP concentrations added from MCP
  0.19, 0.26, 0.33, 0.40%
  - Achieved by adding P from MCP (0, 0.07, 0.14. 0.21%)
- Phytase added (AxtraPhy, 6-phytase) 500 FTU/kg
- 7-21 d, 56 M × Cobb 500 F, straight run broilers, 8 rep, 6 b/rep

Using increment of Ileal digestible Ca or P to determine phytase efficacy of Axtra Phy

9	Digestible Ca, % (Total Ca, 60% dig)	Ratio Ca:dig P from phytase
	500 FTU/kg	
0.65% Ca	0.47 (0.78)	0.79: 1
0.80% Ca	0.44 (0.73)	0.57:1
	Digestible P, %	
	500 FTU/kg	
0.65% Ca	0.99±0.248	
0.80% Ca	1.27±0.248	

Data from Angel et al., 2013

Our data do not support applying greater Ca matrix values vs. dig. P matrix values, if anything, less Ca may be released vs. P.

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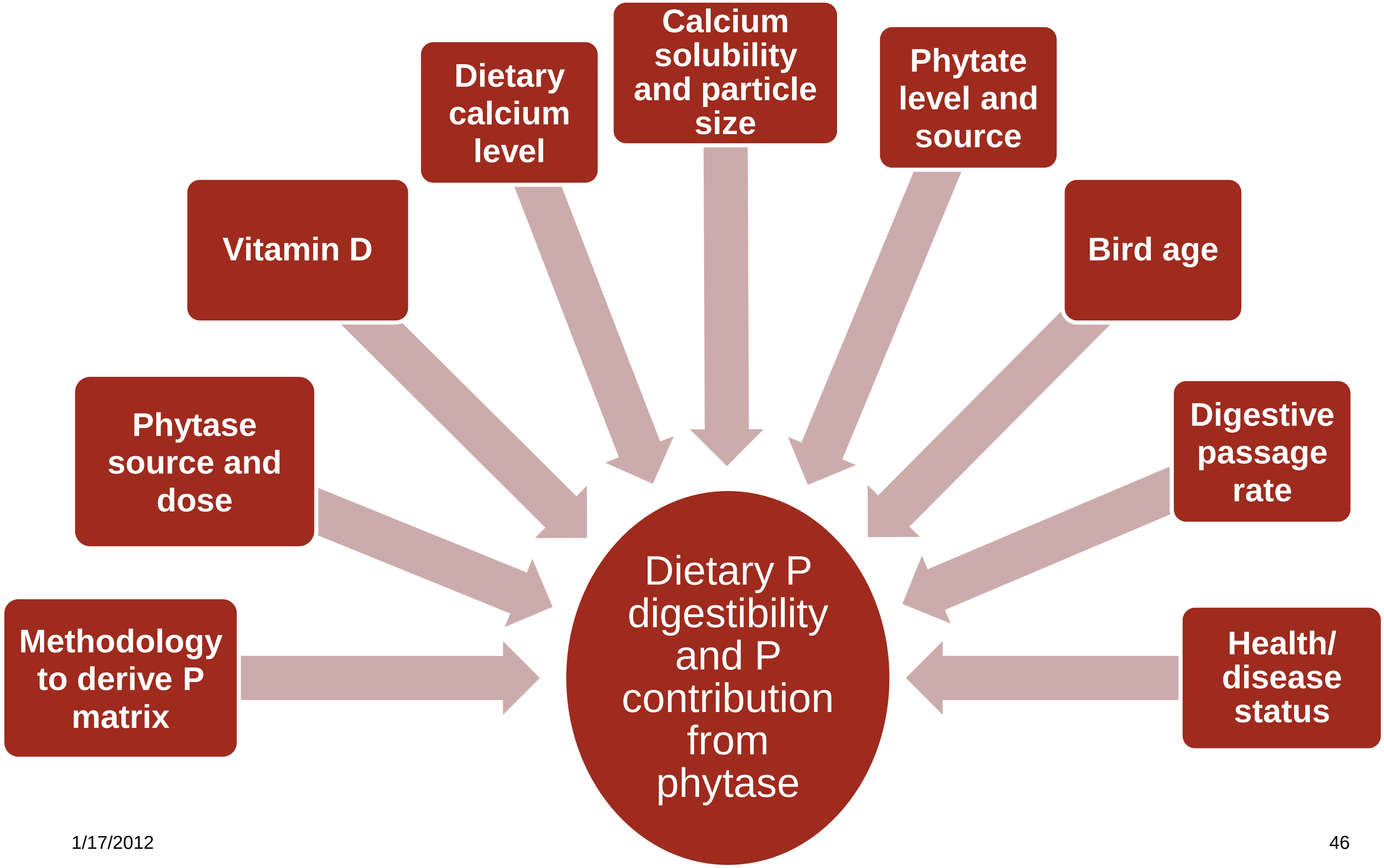
- More needs to be understood how limestone solubility influences the ability of phytase to replace dietary total Ca.

Ca, P, Ca:P Ratio

- Insufficient levels of calcium and inadequate calcium: available phosphorus ratios decrease broiler bone mineralization & performance.
- Our data does not support high Ca contributions from phytase.
- Running trials with too low Ca will impair bone mineralisation and response to phytase
- Too high Ca levels do not seem to have a large impact on efficacy of some phytases provided diets contain >1000 FTU/kg

A Lot of Factors Impact Phosphorus Digestibility, Phytase Efficacy, and Correct Bone Mineralization



Phytate Levels in Feed Need to support the expected P contribution!

- 1 kg AvP from Dicalcium P costs (($550/0.183)/0.95)/ 1000) = $3.16
- Phytase releases 0.1% AvP = 1 kg/tonne feed DCP equivalent **= $3.16** value*.
- Phytase releases 0.12% AvP = 1.2 kg/tonne feed DCP = **$3.79** value* (+ 63 cents)
- Phytase releases 0.15% AvP = 1.5 kg/tonne feed DCP = **$4.74** value* (+ 95 cents)
- Phytase releases 0.18% AvP = 1.5 kg/tonne feed DCP = **$5.69** value* (+ 95 cents)

Corn / Soy diet 0.26% Phytate P

AvP release from Phytase (%)	% of Undigested Phytate P used
0.1	35.2%
0.12	42.3%
0.15	52.8%
0.18	63.4%

Corn/Soy+DDgs+PBY, 0.2% Phytate

AvP release from Phytase (%)	% of Undigested Phytate P used
0.1	45.8%
0.12	55.0%
0.15	68.7%
0.18	82.4%

*Actual savings in feed fomulation are greater due to the ‘’space saving effect of removing dicalcium P

Knowledge of Phytate levels in feed ingredients and diets is critical

Item	N	P%	Phytate P, % of Total P		
		Avg	Avg	SD	Range
Barley	12	0.3	64.4	6.6	52.0-74.4
Corn	56	0.22	88.4	13.5	60.6-95.2
Rye	3	0.25	63.6	5.8	57.0-67.9
Sorghum	29	0.23	83.6	6.2	70.4-93.5
Wheat	27	0.28	77.5	6.5	62.7-90.3
Canola/rapeseed	24	0.95	74.3	11.4	46.3-96.4
Cottonseed meal	6	1.05	69.9	7.8	60.2-78.3
Palm kernel meal	3	0.46	51.8	10	45.1-63.3
Corn gluten meal	1	0.5	86.7	-	-

Item	N	Total P%	Phytate P, % of Total P		
		Avg	Avg	SD	Range
Peas	5	0.39	56.4	2.8	51.6-59.0
Soybean meal	56	0.64	64.3	5.4	46.9-79.1
Sunflower meal	7	1.11	70.5	6.4	63.4-82.6
Corn DDGS	17	0.75	18	5.9	8.3-29.4
Corn germ meal	5	0.61	36.2	2.7	33.5-40.3
Rice bran	14	1.71	93.1	8.8	79.6-98.2
Wheat bran	14	0.94	84.5	20.7	43.8-99.1
Wheat DDGS	2	0.74	5.42	2.7	3.5-7.3
Wheat middlings	8	0.7	86.8	10.2	76.1-98.3

**** Phytate Analyzed by HPLC, DuPont Internal data***

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Questions?

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