The science and practice of making feed enzyme decisions - the case for and against

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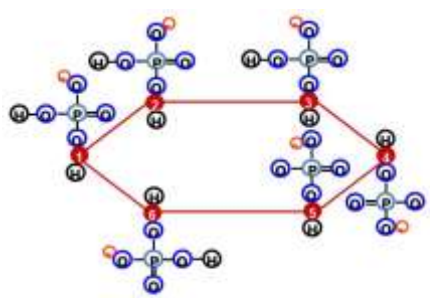
Enzyme Application to Feed

One of the most researched fields in poultry science

More than 2500 independent tests of feed enzymes in broilers
(Rosen, 2010)

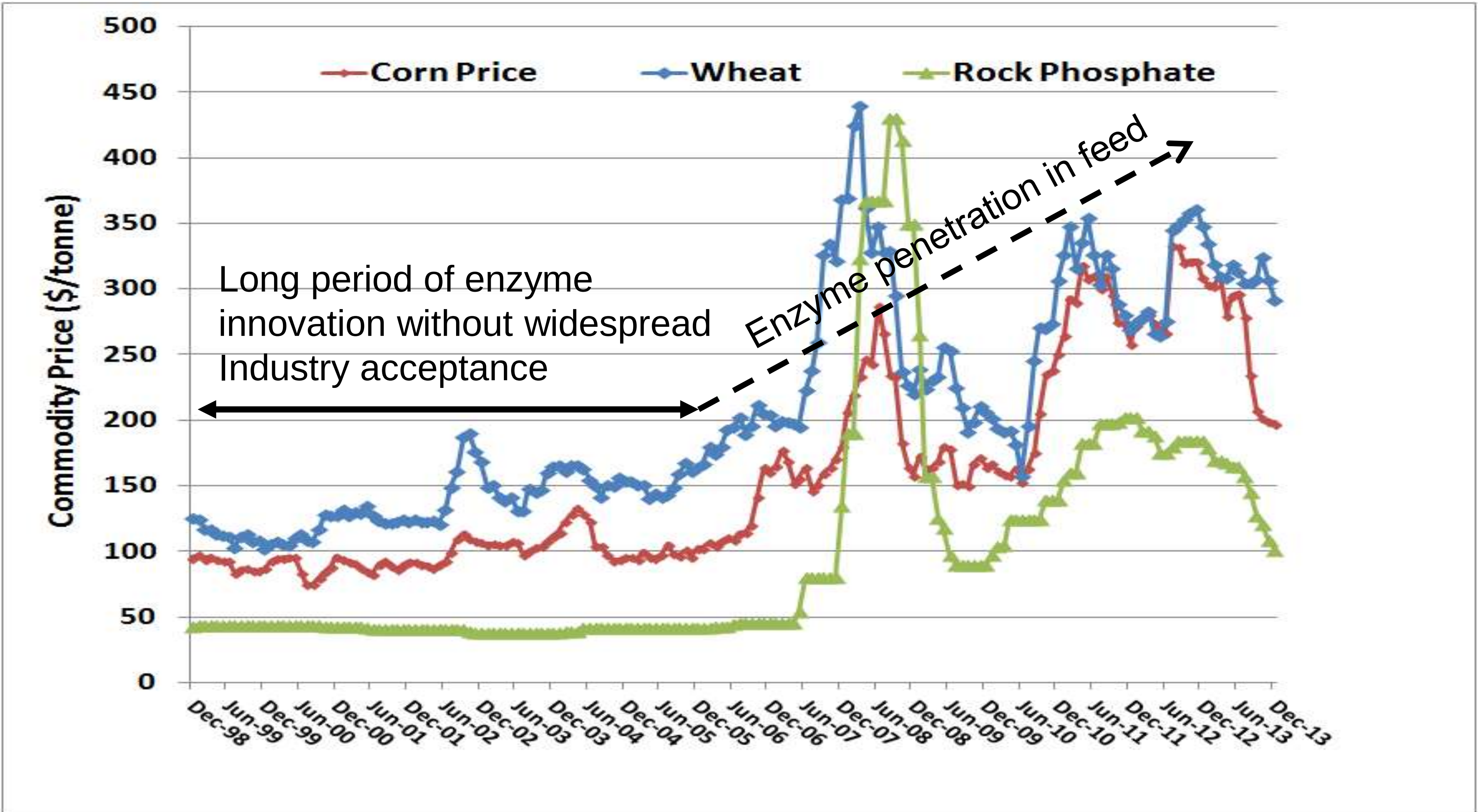
Grown to be a >$550 million Industry that saves the global feed market ~ $3 to 5 billion per year (Adeola & Cowieson, 2011).

Phytase	NSPase	Protease	Other
A.niger *C.braachi* *E.coli* *Citrobacter spp* *Buttiauxella spp*	**Xylanase** **ß-Glucanase**	*Bacillus subtilis* *Bacillus* licheniformis	*Amylase* *Mannanase* *Galactosidase* *Glucoamylase* *Lipase*



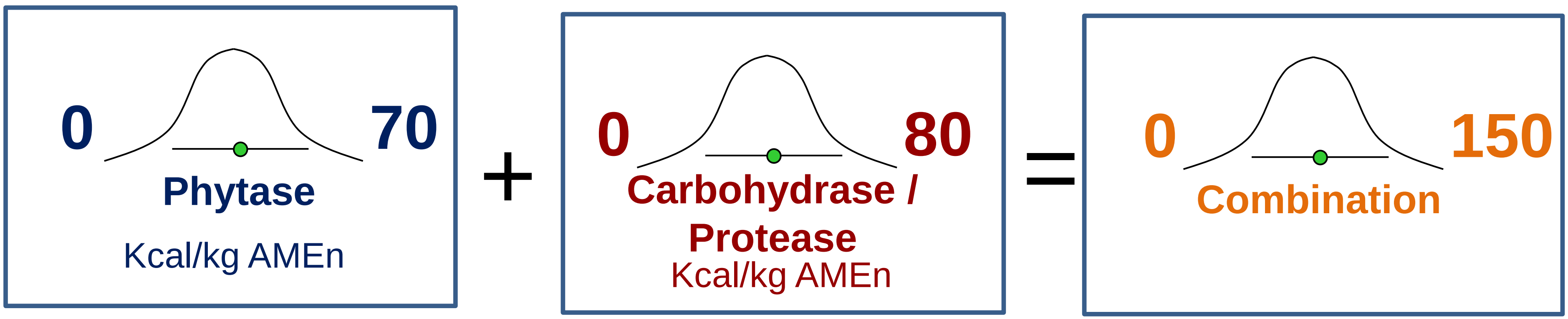
What drove the high penetration of feed enzymes we have today?

"Necessity is the mother of invention" William Horma, 1519



However, in spite of:

- Over >2500 research studies in enzymes by 2010
- Phytase application into >94% of broiler feed
- NSP/Carbohydrase /Protease in majority of broiler feed
- Every enzyme company having “ scientific” matrix values for every conceivable enzyme



Not a lot of clarity on which enzymes are appropriate, factors causing variation in enzyme response, or additivity of enzyme matrix values in energy, let alone amino acid effects

Processes to select enzymes & combinations

1

Knowledge of substrates in feed ingredients

2

Candidate Enzyme selection and dose optimization

Phytase

Xyl

Lip

GA

Amy

Prot

BGI

Man

Gal

Match Enzyme Biochemistry to Substrates and Digestive Physiology *in-vitro& in-vivo*

3

In-Vivo Response

Performance (BW/FCR)

Ileal Digestibility

AMEn

Gut health / Livability

Increased Profitably

Key Decisions: Phytase

Which Phytase? What dose?

How much AvP / Ca^{2+} contribution?

Energy and 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	*A. niger*[*]	*A. niger*	2; 5–5.5	65	Natuphos®
3	*A. niger*[*]	*A. niger*, non-recombinant	6.0	–	Allzyme® SSF
3	*A. niger*[*]	*Trichoderma reesei*	2.5	–	Finase® P/L
6	*Escherichia coli*[*]	*Schizosaccharomyces pombe* (ATCC 5233)	4.5	55	Phyzyme® XP
6	*Escherichia coli*[*]	*Pichia pastoris*	4.5	–	Quantum®
6	*Escherichia coli*	*Trichoderma reesei*	–	–	Quantum Blue®
6	*Escherichia coli*[*]	*Pichia pastoris*	3.4, 5.0	58	OptiPhos®
6	*Peniophora lycii*[*]	*Aspergillus oryzae*	4–4.5	50–55	Ronozyme®
6	*Citrobacter braakii*	*Aspergillus oryzae*	–	–	Ronozyme Hiphos®
6	*Buttiauxella* spp.	*Trichoderma reesei*	3.5–4.5[#]	60[#]	Axtra® PHY

[*] Adapted from Lei *et al.*[1] with modifications;
[†] 3- or 6-phytase; —, no information available;
[#] personal communication (C Evans).

Dersjant-Li et al, 2015

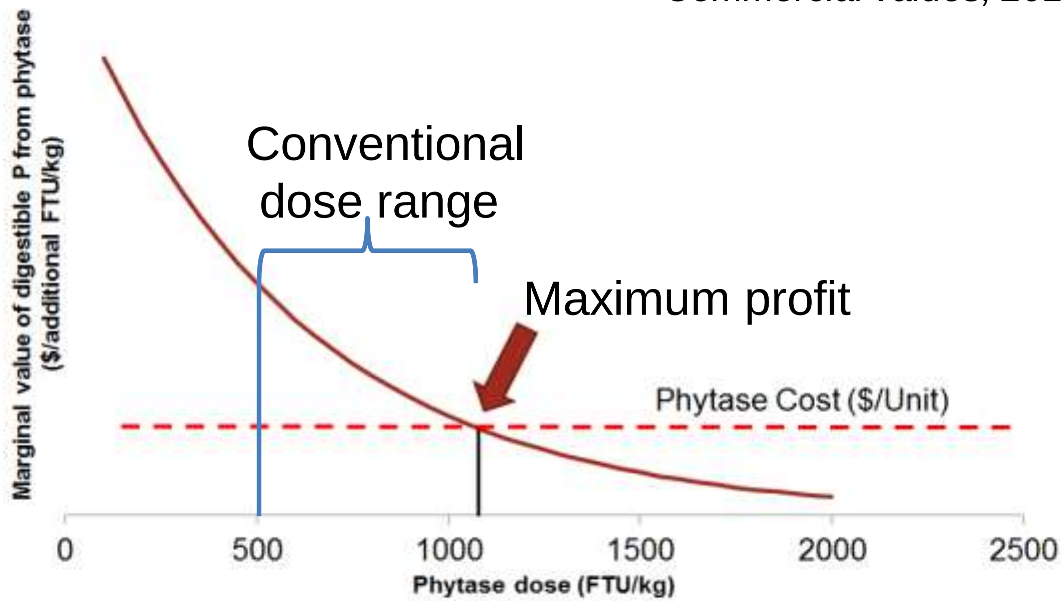
Supplier Recommended Nutrient Contributions from Standard Dose of Phytase

	E.Coli 1	E.Coli 2	E.Coli 3	Citrobacter	E.Coli 4	Buttiauxella
Units/kg feed	500 FTU	500 OTU	500 FTU	1000 FYT	500 FTUQ	500 FTU
Digestible P%	0.11	0.11	-	0.117	-	0.134
"Available" P %	0.12	0.13	0.13	0.146	0.15	0.146
Calcium %	0.11	0.13	0.14	0.18	0.165	0.134
Phytase cost ($/Feed Ton)	0.5	0.5	0.5	0.5	0.5	0.5
Phytase Cost / 0.12% AvP	0.50	0.46	0.46	0.41	0.40	0.41

Commercial values, 2014

In practice, decisions of phytase source and dose are frequently determined on phytase cost /0.10% or 0.12% AvP release

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



Barnard et al., 2014

Methodology of how nutrient contributions from phytase were determined differs between commercially available phytases sources & affects decisions

Ileal vs. Tibia ash method; Adaptation time to test diets; Age broiler; Ca level & source

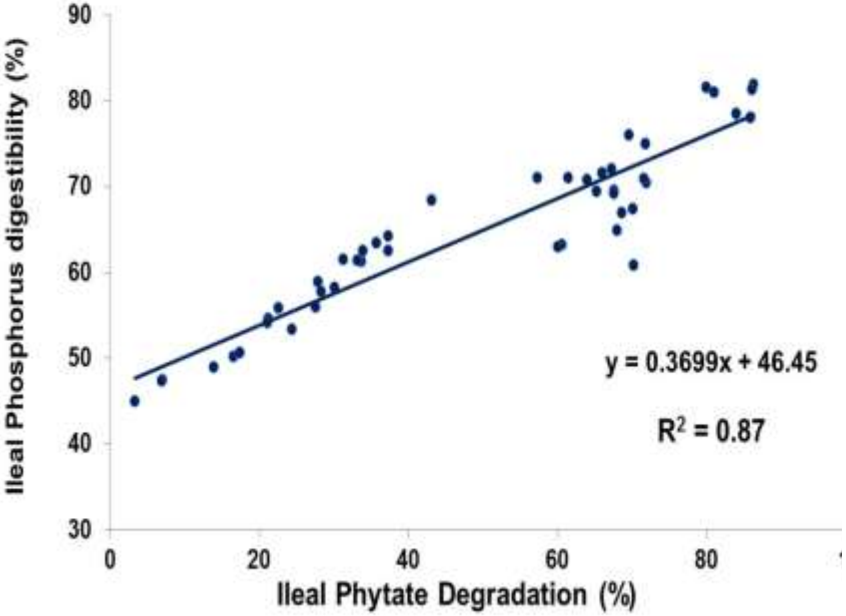
Li et al., 2014

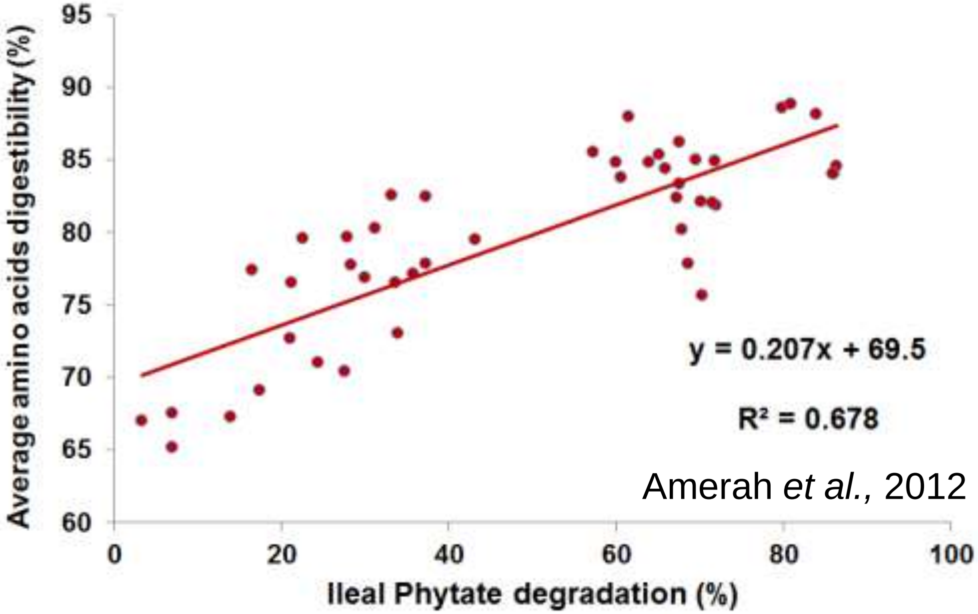
	E.Coli 1	E.Coli 2	E.Coli 3	Citrobacter	E.Coli 4	Buttiauxella
FTU/kg feed	500 FTU	500 OTU	500 FTU	1000 FYT	500 FTU_Q	500 FTU
Digestible P %	0.11	0.11	-	0.117	-	0.134
Av.P %	0.12	0.13	0.13	0.146	0.15	0.146
Dig. P:AvP	0.92	0.85	-	0.80	-	0.92
Calcium %	0.11	0.13	0.14	0.18	0.165	0.134
Ratio Ca:AvP	0.92	1.00	1.08	1.23	1.10	0.92

Critical questions to ask to ensure you are comparing  &  in matrix

- What Research / Methodology was used to derive phosphorus (P) contribution?
- How does the P-system used compare to your Ingredient P matrix?
- What about Ca^{2+} matrix values? How were they determined?
- Is Phosphorus release in the matrix correlated with amino acid release / extra phosphoric effects of amino acids and energy?

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





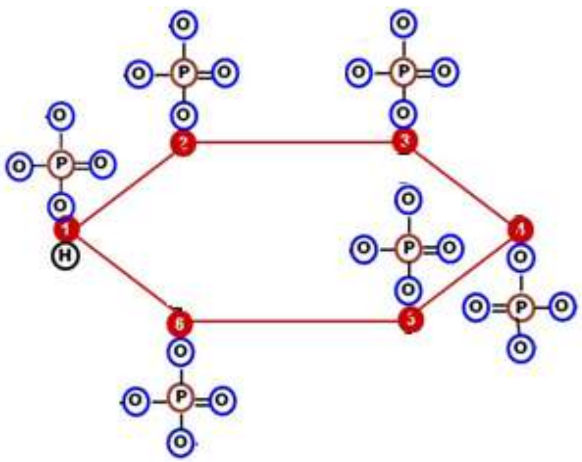
Amerah *et al.*, 2012

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)

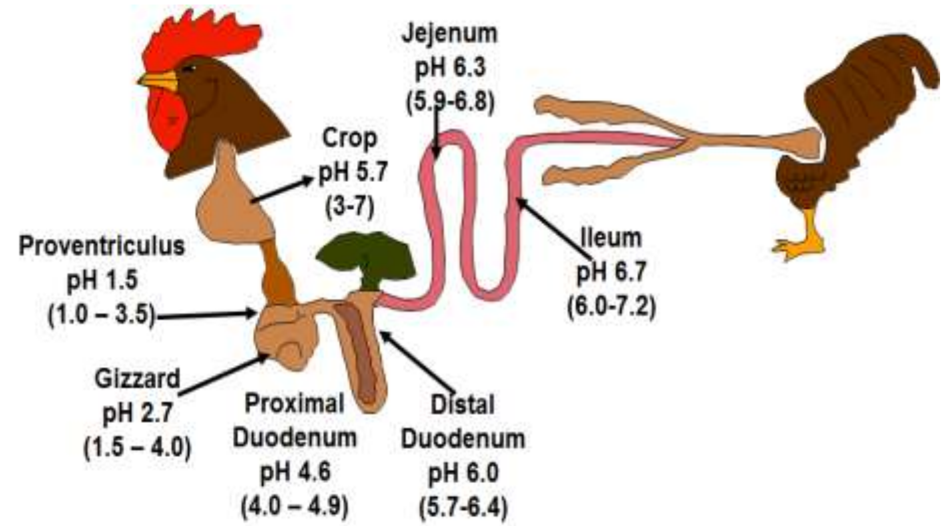
Phytase decisions on **Source** and **Dose** also need be based on phytate interactions with nutrients and understanding differences in biochemistry between phytase enzymes in the context of digestive physiology



Enzyme pH, kinetics

Interactions

Ca^{2+} Zn^{2+}
Protein, AA
Sodium



1. **Interactions of Phytate, Calcium, and Phytase Enzymes – affects P contribution**

2. **Interactions of Phytate with Protein, Starch, and Na – Anti-nutrient effects on live performance & drives ME& AA digestibility improvement from phytase**

3. **Differences in phytase enzyme pH optima and kinetics - affect in-vivo results**

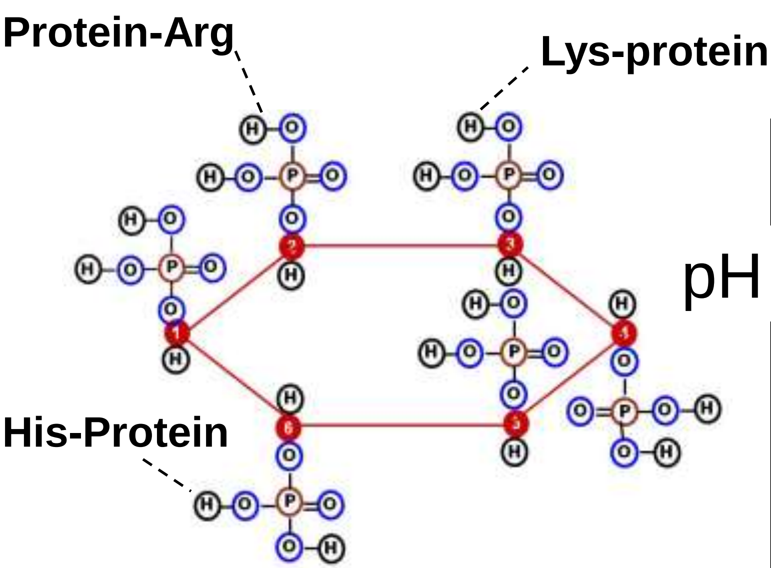
Interactions of phytic acid with dietary nutrients are pH dependent

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

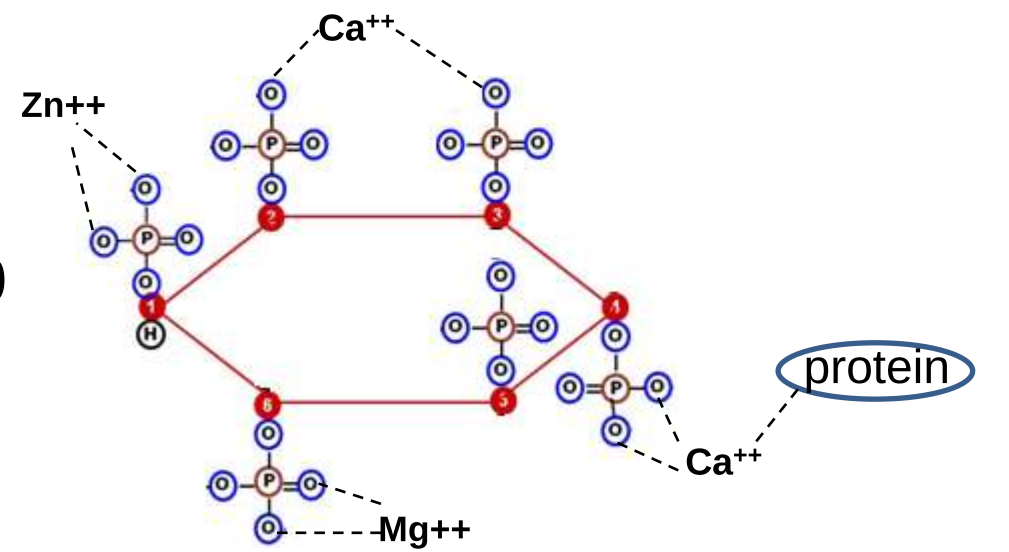
Proteins and phytate acid also interact at higher pHs >6 in presence of Ca^{2+} Briggs (1959, **Saio** et al. (1967,1968)

Gizzard / Proventriculus

Duodenum / Ileum / Jejenum



pH 4.0



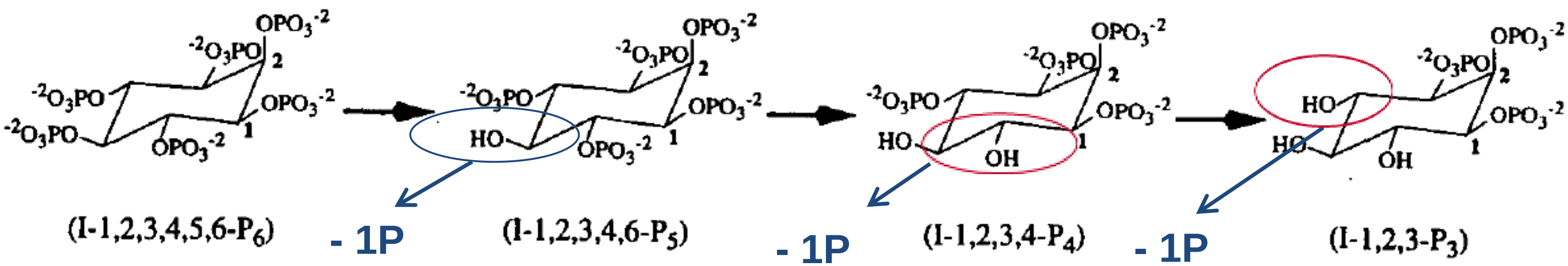
Binds with basic AA of protein

Binds w/ divalent mineral cations

Nelson et al., 1968; Maga, 1982; Angel et al., 2002, Selle et al.,2009,2012; Walk et al.,2012

Interactions of Phytate and Ca^{2+}

- Potential for phytate binding Ca^{2+} and other minerals increases with pH and dependent on Calcium source solubility Angel et al., 2002, Li et al., 2014



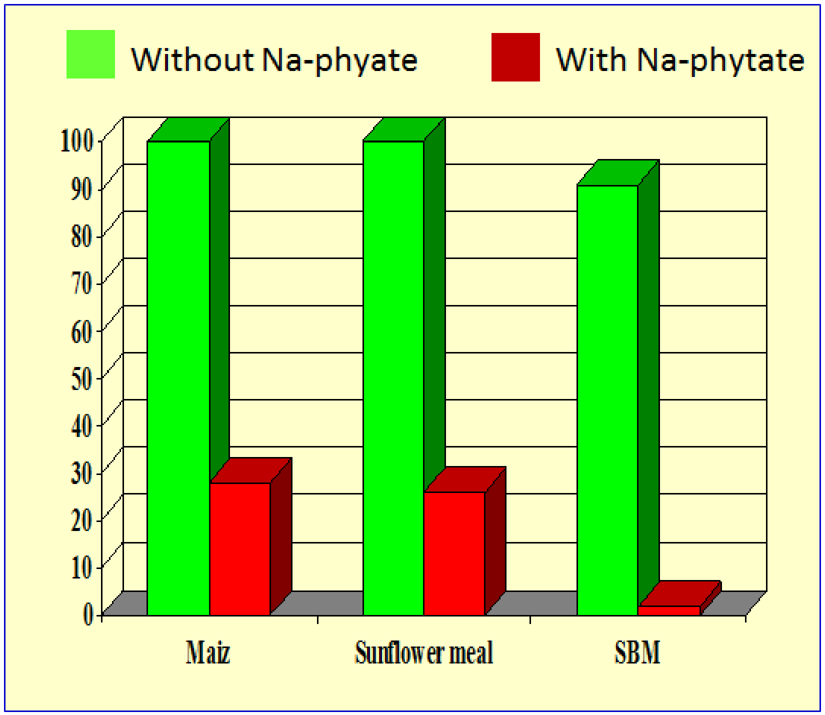
IP6 >>> IP5 >> IP4 > IP3

- Affinity and binding strength of phytate esters with Ca^{2+} decreases IP6-IP3 Luttrell (1993)

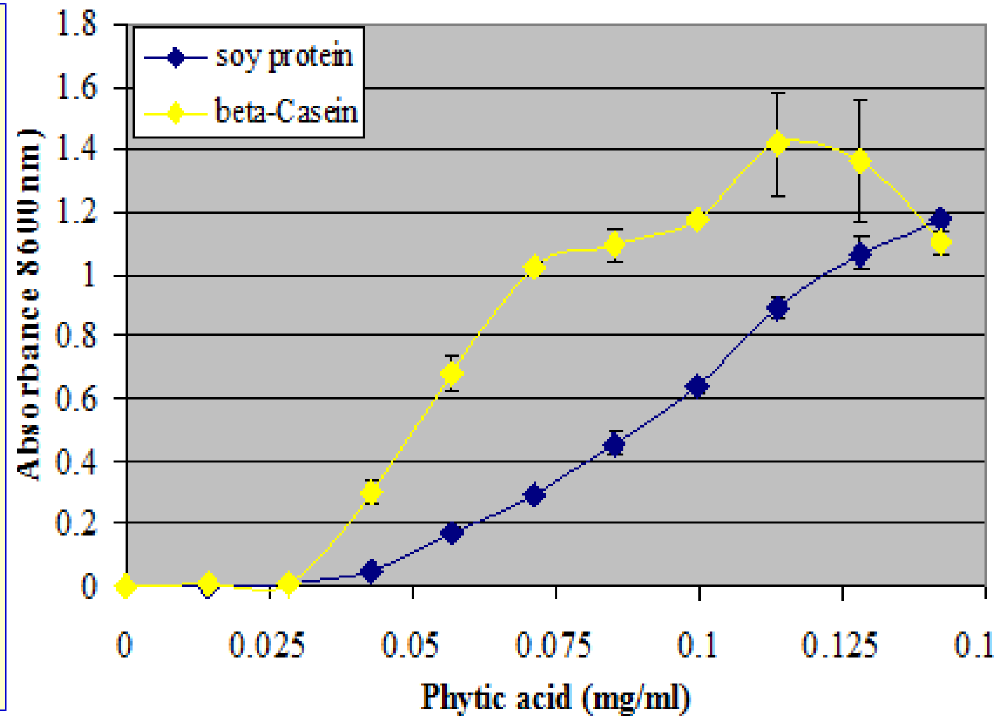
Phytase enzymes that can rapidly hydrolyze IP6-IP3 in Gizzard/ proventriculus will be less inhibited by Ca-phytate interactions

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.

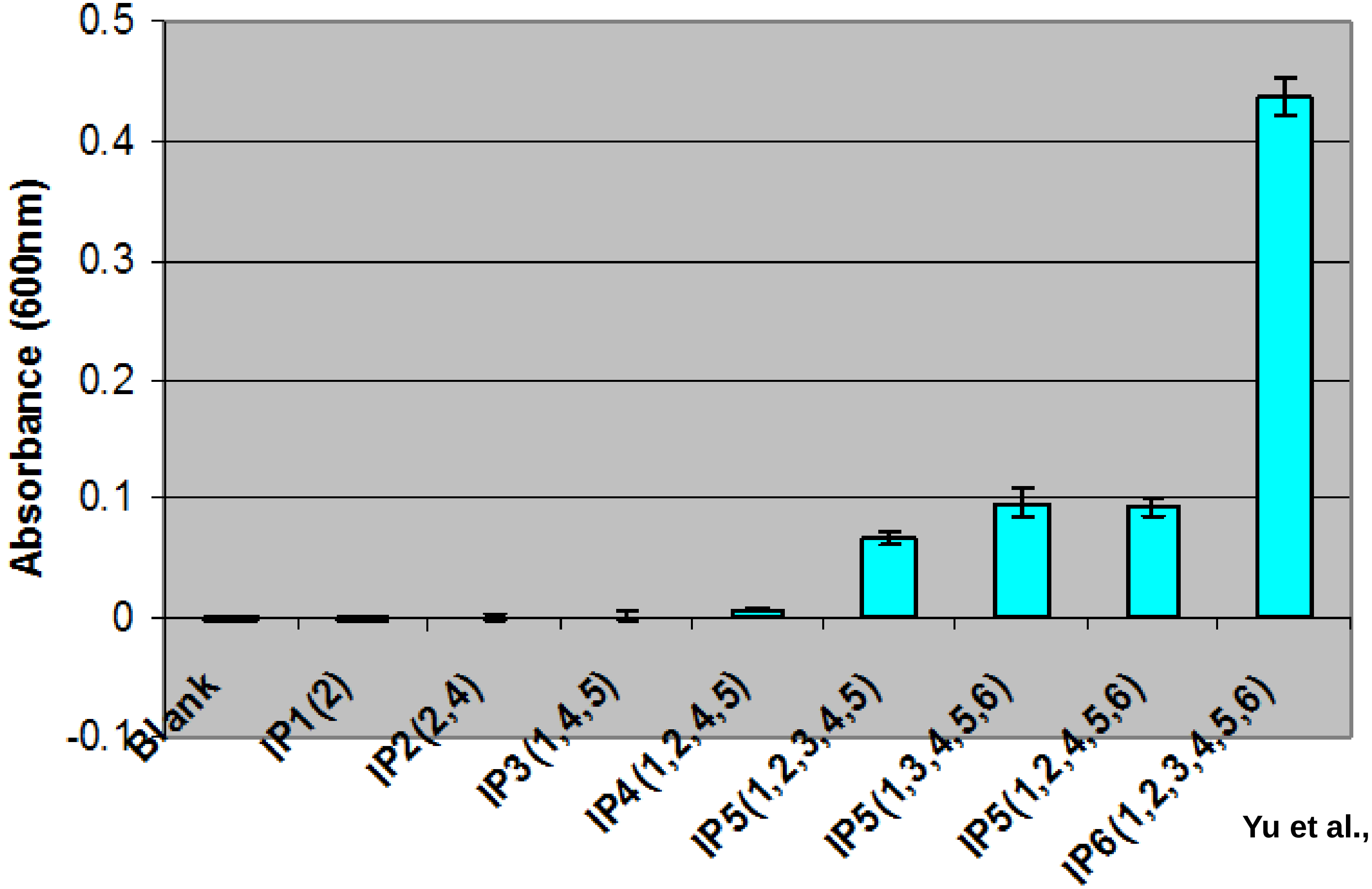


Yu et al.,2012 J. *Anim Sci.* 90:1824-32.

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

Selle et al., 2012, Adeola&Cowieson, 2014

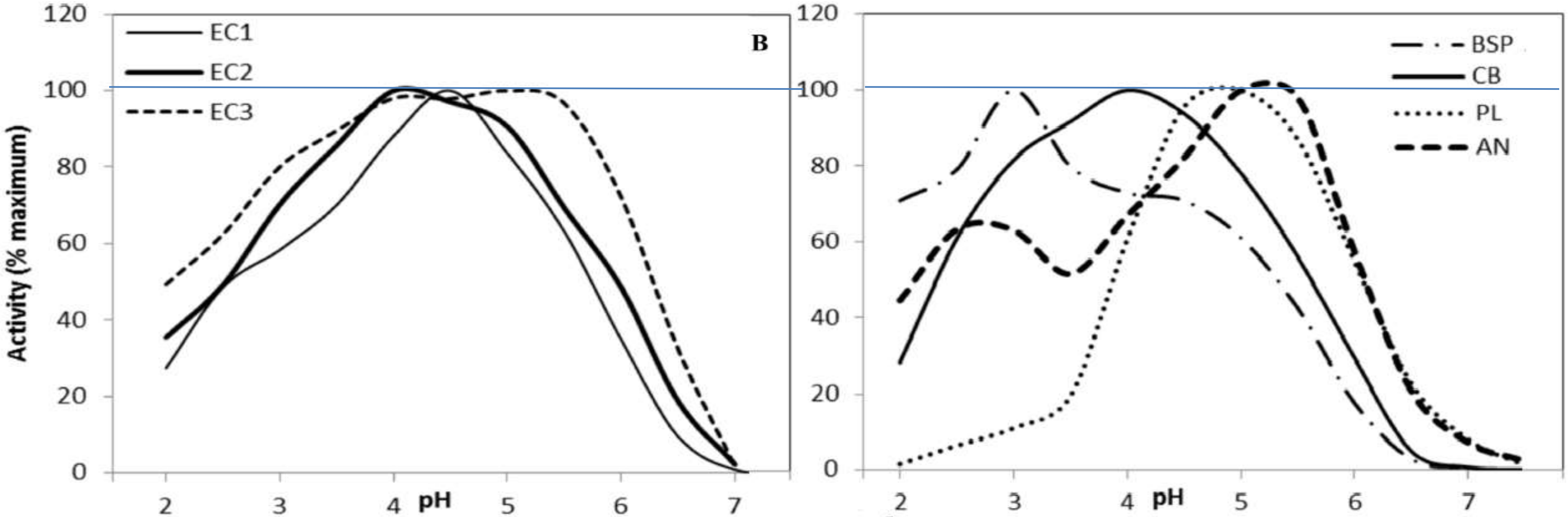
Only IP6 and to a lesser extent IP5 has the ability to aggregate with soluble proteins at a pH of 2.5



Yu et al., 2012

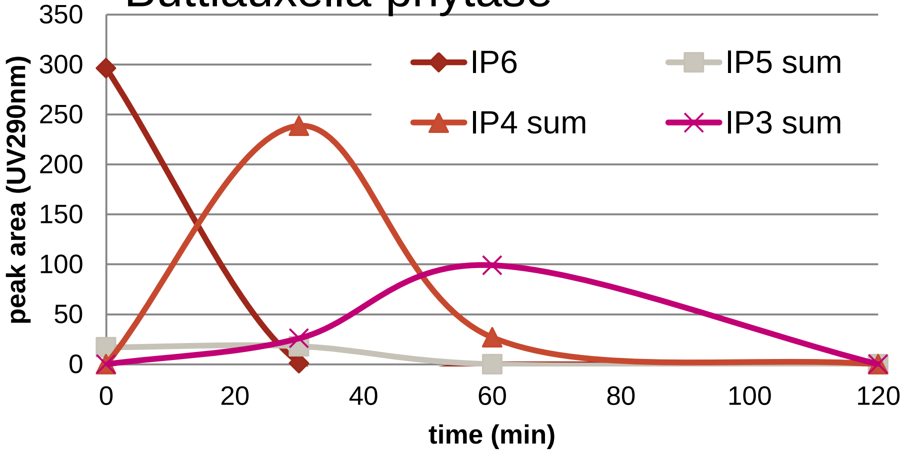
Phytase that can effectively degrade IP6-protein complexes rapidly at low pH will be more effective at

Large differences exist between phytase enzymes in optimum pH and enzyme kinetic properties

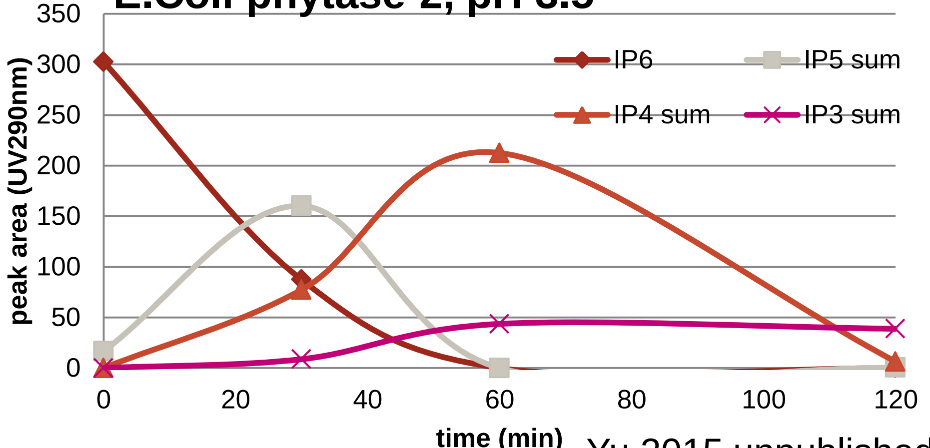


Mendez et al., 2015, J.Agric.Chem

Buttiauxella phytase

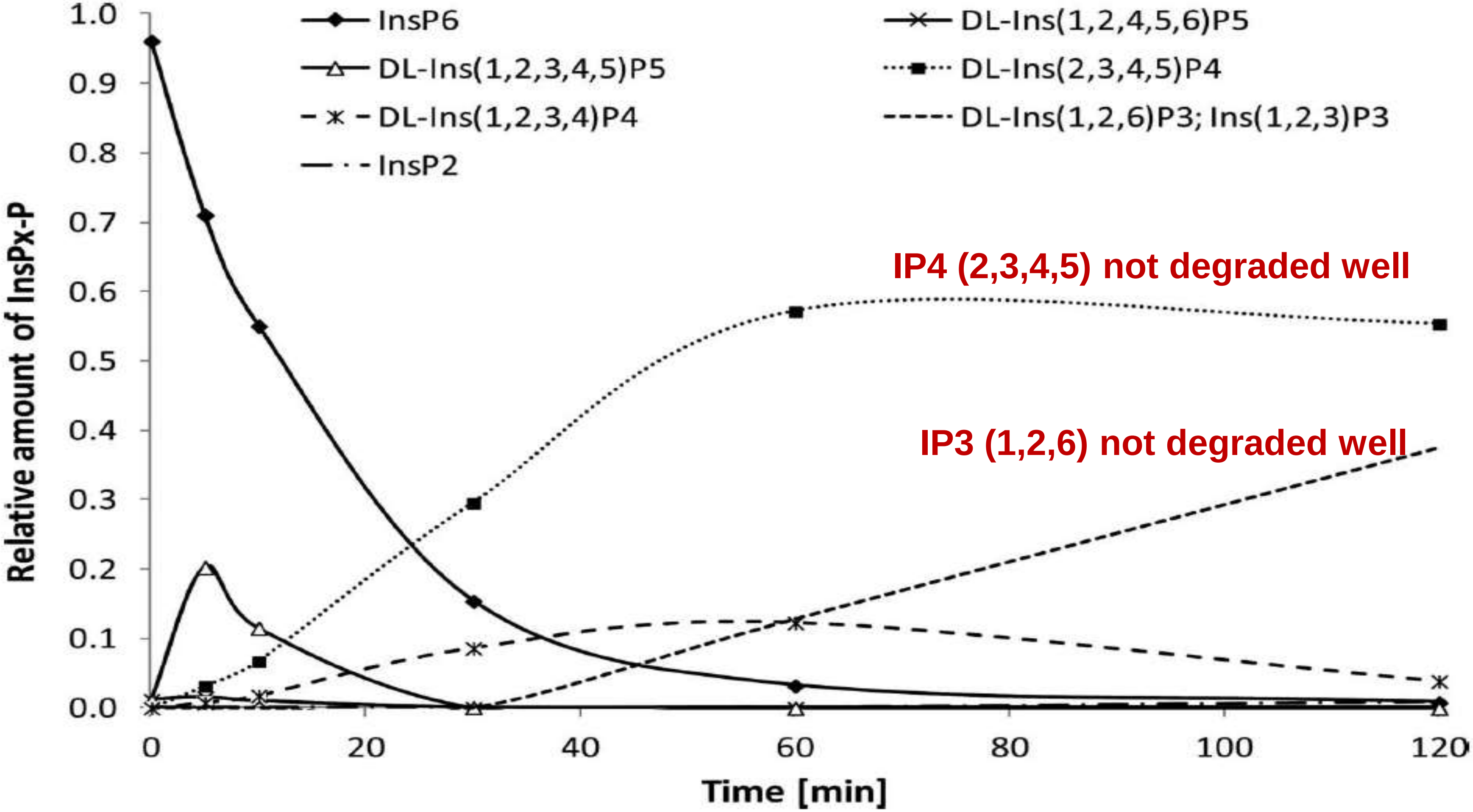


E.Coli phytase 2, pH 3.5



Yu 2015 unpublished

Published work on Citrobacter phytase shows this phytase seems to struggle degrading IP4 (DL-Ins(2,3,4,5) and IP3 esters.

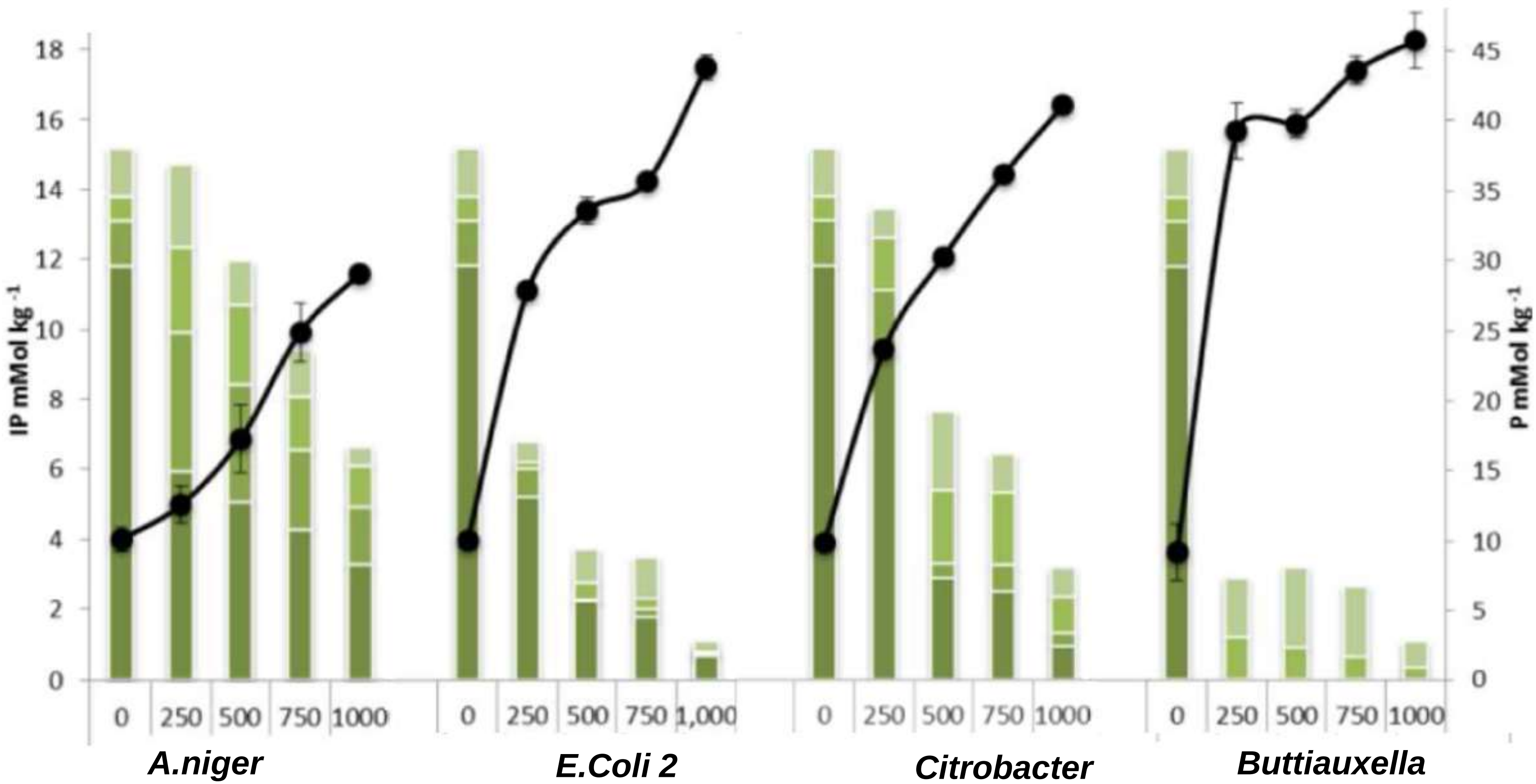


Pontoppidan et al., 2012 in Arch.An.Nutrition, 66:6, 431–444

Differences in enzyme kinetics and pH optima of phytases result in very different phytate dephosphorylation patterns and phopsphate release during in-vitro simulation of digestion

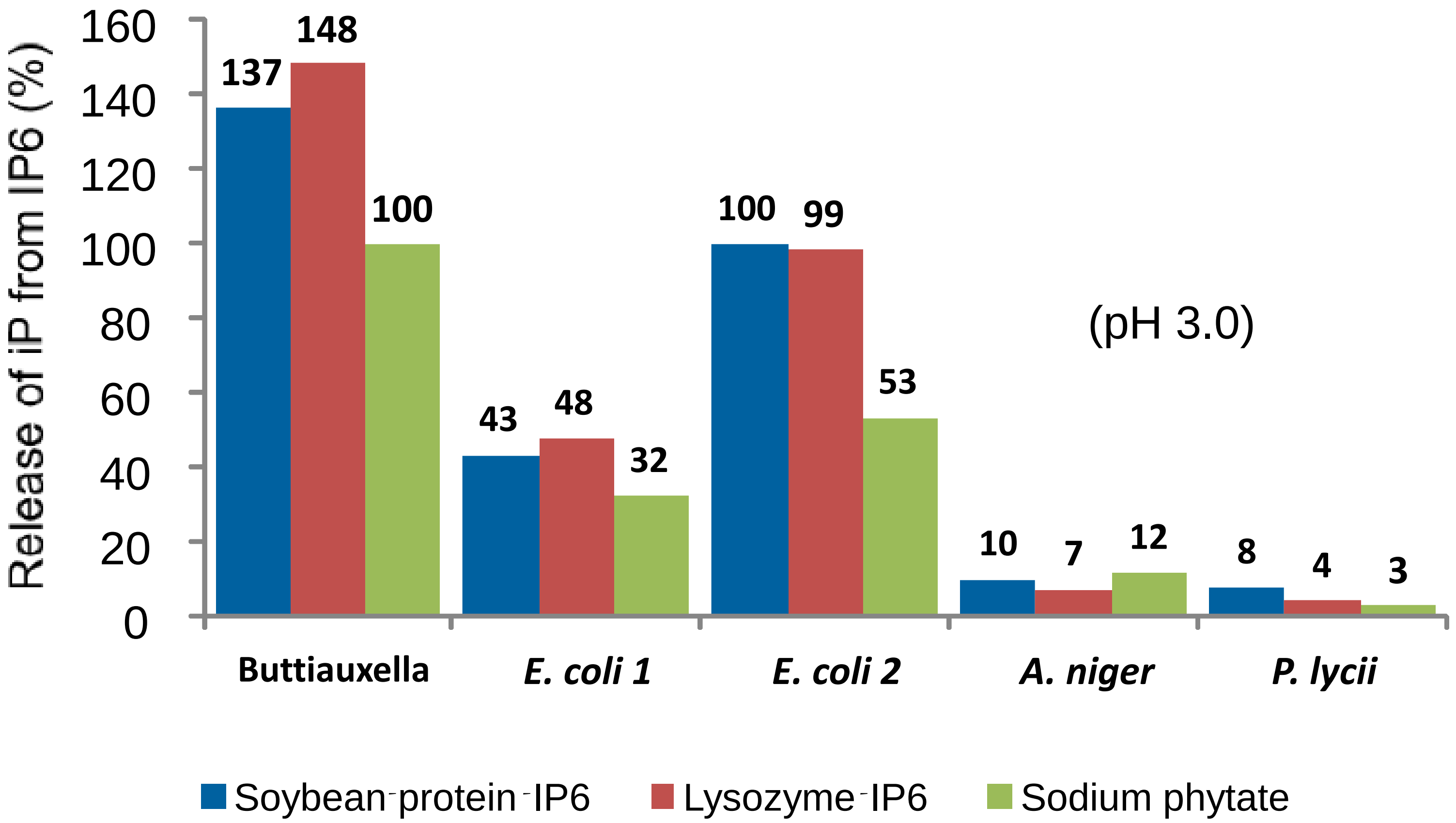
Mendez et al., **2015**, J.Agric Chem.

Intestine



Enzymatic phytate dephosphorylation of wheat during in vitro simulation of poultry digestive tract in a high buffer system

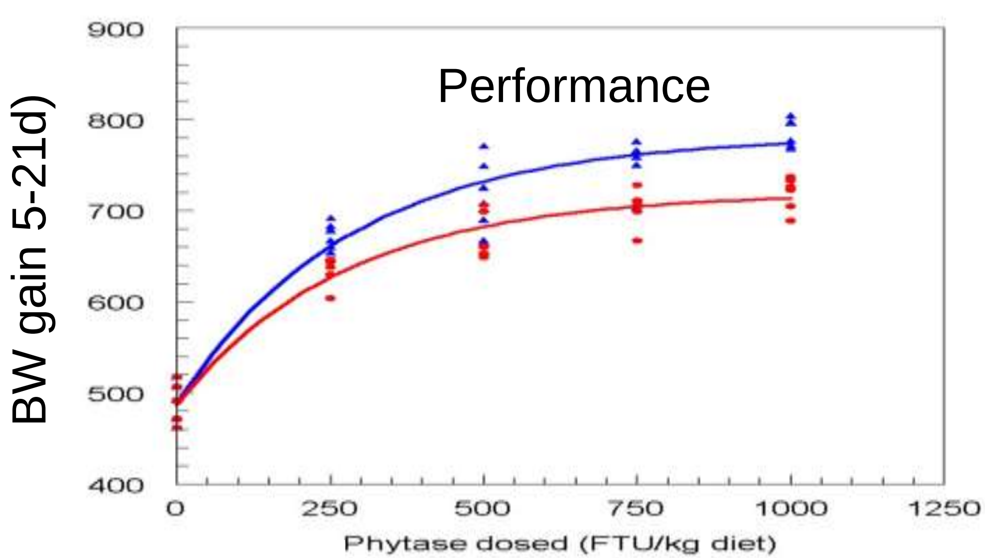
Degradation of protein-phytate complexes or Na-phytate by phytase



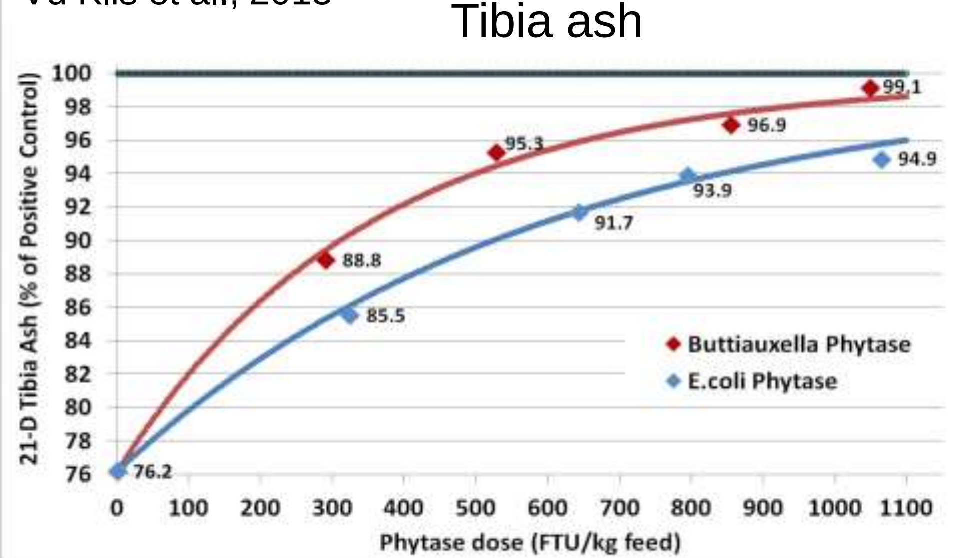
All values expressed relative to release of iP by Buttiauxella phytase on sodium phytate substrate as 100%

DuPont Laboratory, 2012

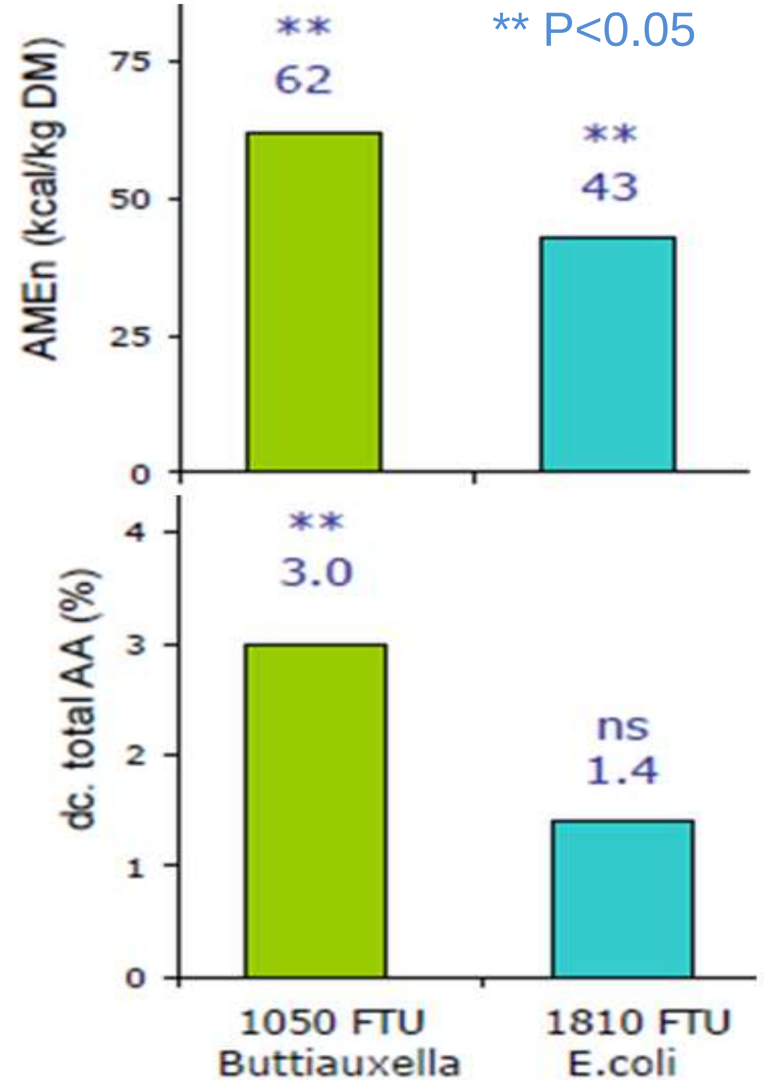
Differences in In-vitro phytase chemistry , IP6 hydrolysis rate & protein-phytate degradation need to be supported by repeatable in-vivo responses



Vd Klis et al., 2013



Plumstead et al., 2012



Kwakernak et al., 2013 ESPN

What about other Enzymes other than Phytase?

1

Knowledge of substrates in feed ingredients

2

Candidate Enzyme selection and dose optimization

Phytase

Xyl

Lip

GA

Amy

Prot

BGl

Man

Gal

Match Enzyme Biochemistry to Substrates and Digestive Physiology *in-vitro& in-vivo*

3

In-Vivo Response

Performance (BW/FCR)

Ileal Digestibility

AMEn

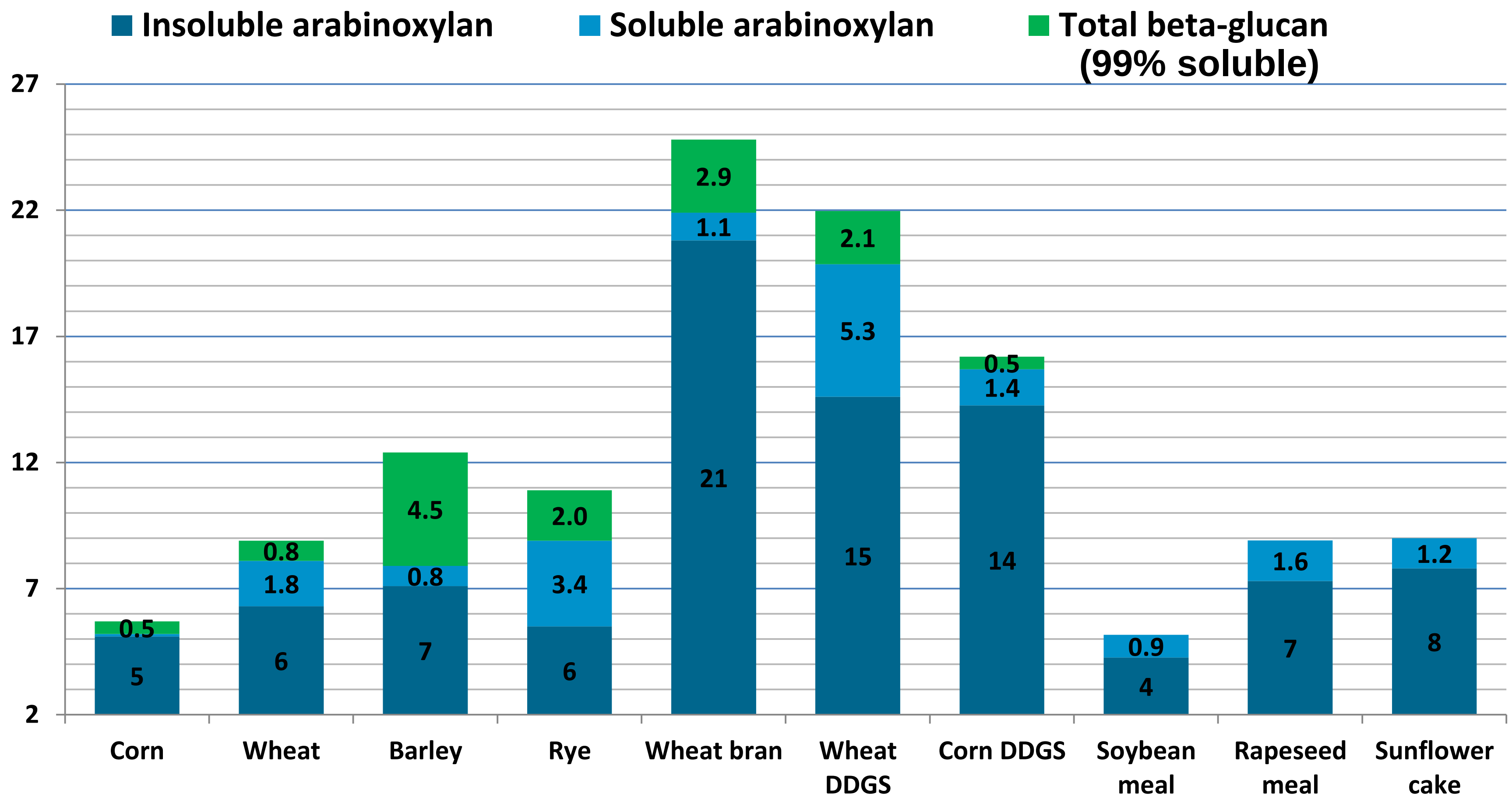
Gut health / Livability

Increased Profitably

Xylanase / B-glucanase is normally the first enzyme considered for wheat/barley based diets… and corn

Substrates	Examples	Main anti-nutritive effects
Soluble, non-viscous, α-galactosides	Stachyose Raffinose	Increased activity of intestinal flora Increased osmolarity and reduced DM of digesta
Soluble, viscous, NSPs	Arabinoxylans and β-glucans (low molecular weight)	Increased digesta viscosity Increased mean retention time of digesta Reduced absorption rate of nutrients Increased activity of intestinal flora
Insoluble, non-viscous, NSPs	Arabinoxylans and β-glucans (high molecular weight) Cellulose	Reduced accessibility of nutrients (e.g. physical entrapment of starch granules)
Starch	Starch, Resistant Starch Varyable Amylose:Amylopectin, Starch-protein complexes	Reduced ME value of ingredients Increased substrate for gut microbiota
Protein	Variable digestibility of protein / AA, especially in poorer quality ingredients	Reduced ME + AA value of ingredients Increased substrate for gut microbiota
Phytate	Variable amounts in feed, antinutritive effects other than Phosphorus	Reduced Ca, P, ME, AA digestibility Interactions with gut microbiota

Arabinoxylan and beta-glucan in some feed ingredients (% dry matter)

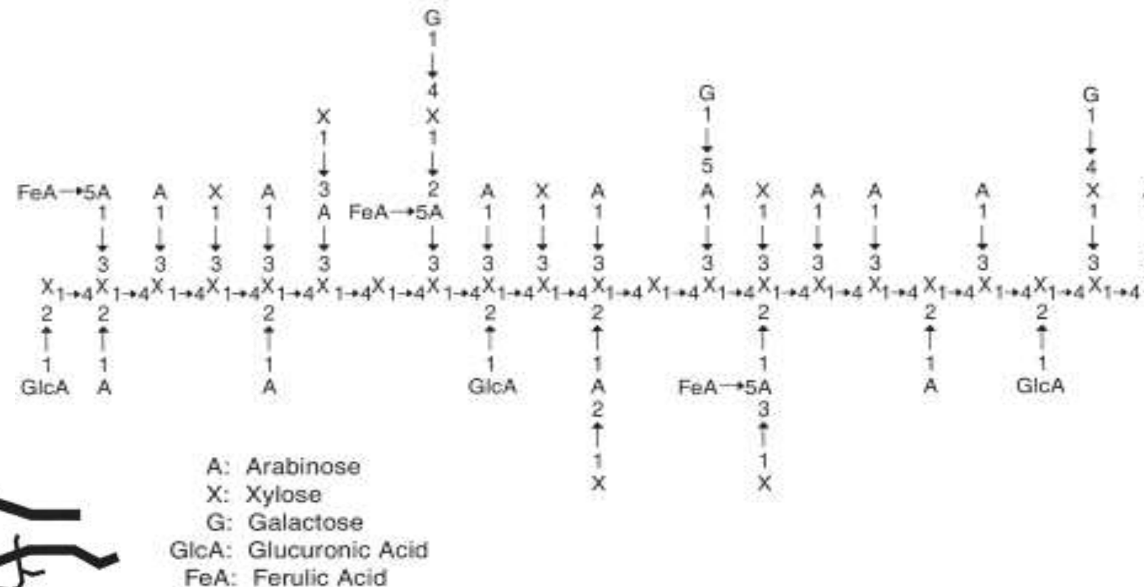


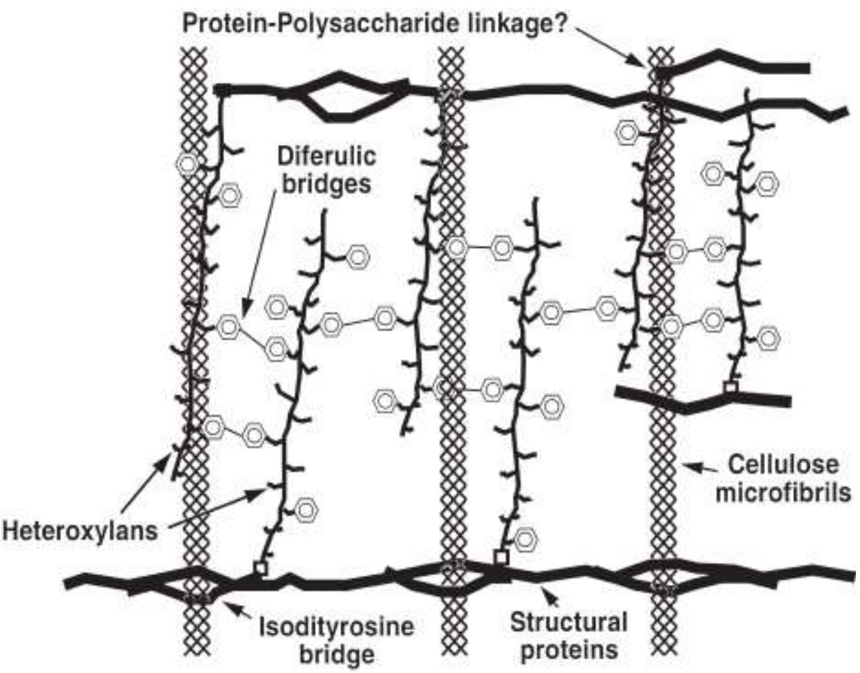
To be effective in reducing Viscocity of soluble NSPs, a Xylanase needs to be able to hydrolyze both Insoluble and soluble arabino-xylan fractions

Choct et al., 2004; Adeola and Cowieson, 2014)

NSP database Source: Choct (2006); Danisco Non Starch Polysaccharide (NSP) database (2012)

Arabinoxylans from cereals are structurally complex and differ between feed ingredients in structure of Arabinose side chains and Diferulic bridges

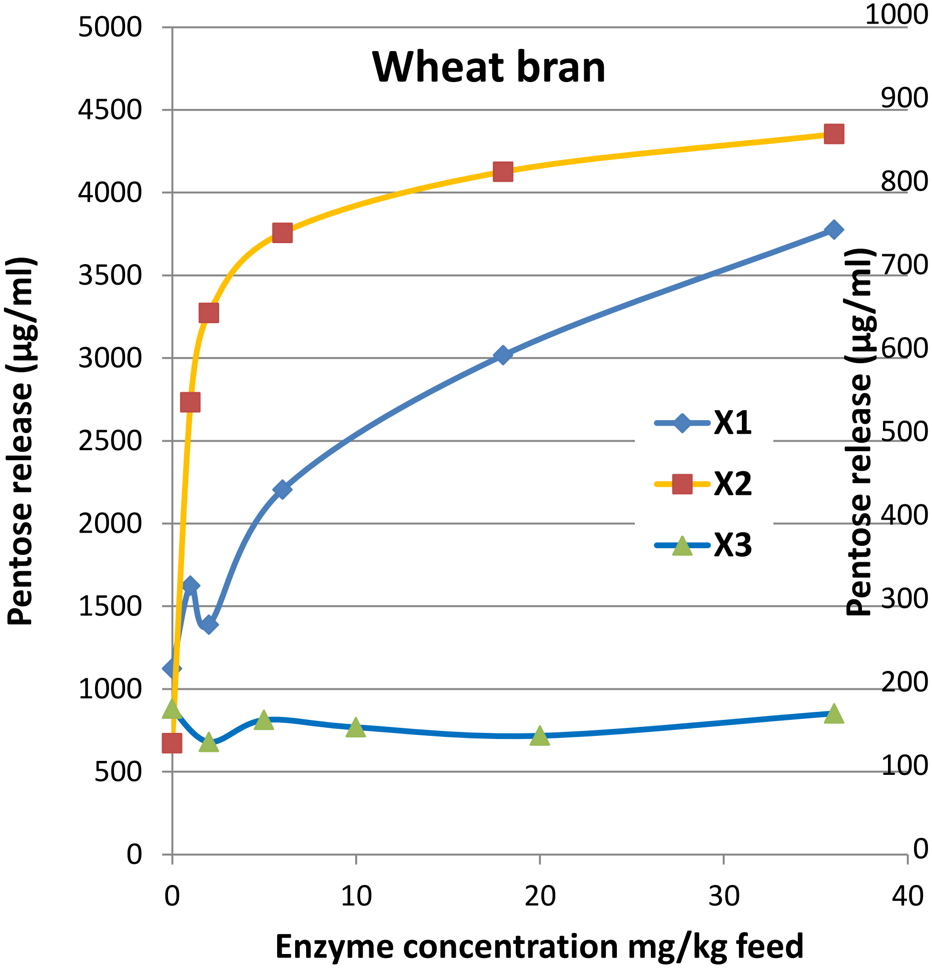


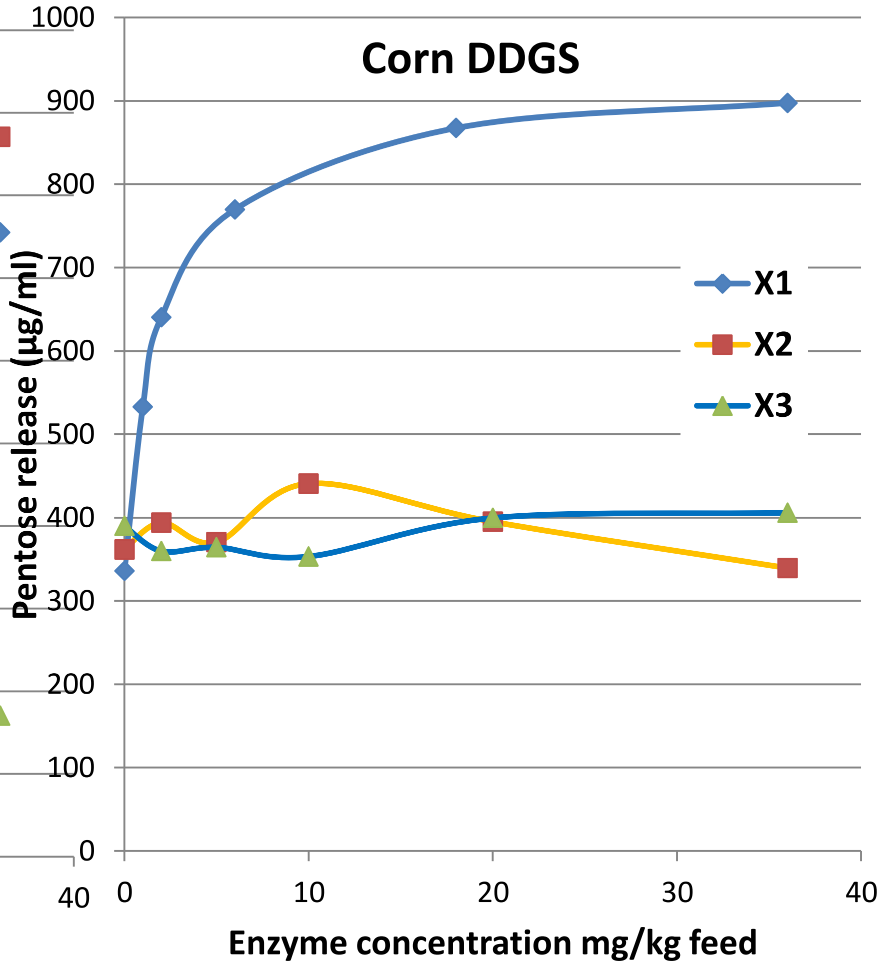


	Diferulic acids/ xylose units	Arabinose/ xylose
Wheat	1/217	0.58
Corn	1/41	0.72

(Bunzel et al., 2001)

Although Xylanase targets ArabinoXylan substrate… there seem to be source-dependent differences in response





DuPont Laboratory, 2010 unpublished

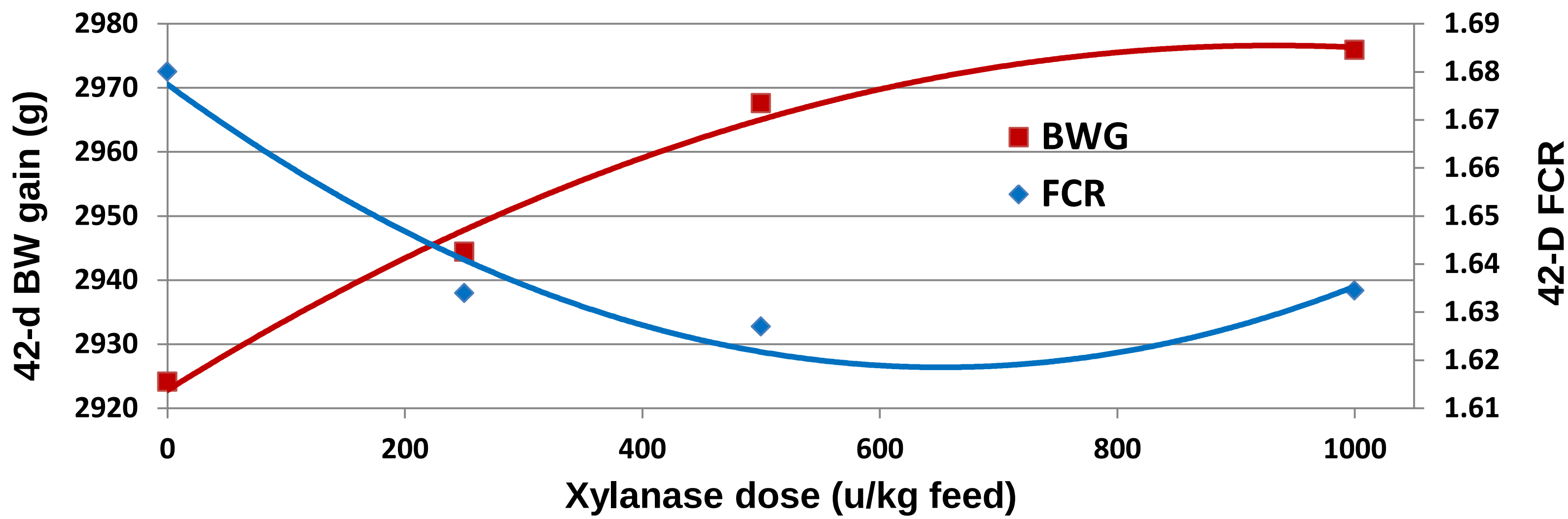
In-Vivo support of xylanase being effective in both corn and wheat-based diets is required

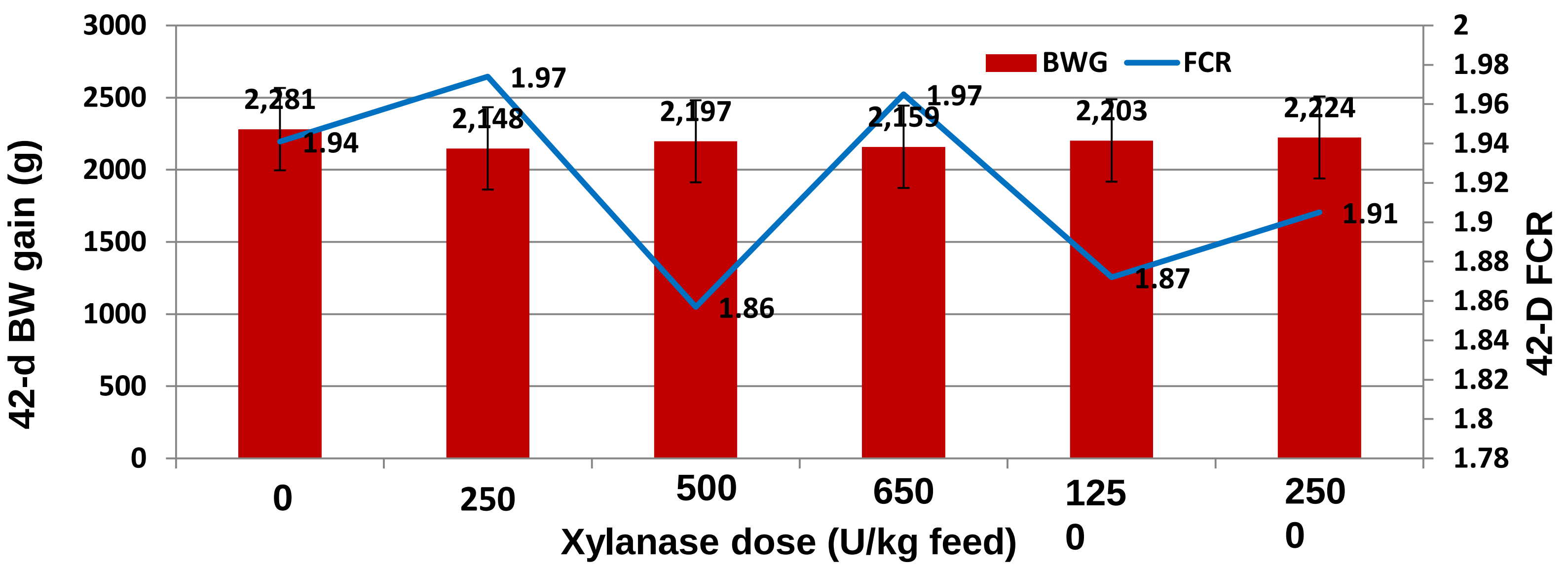
Main effect	NDF retention%	ADF retention%	AMEn Kcal/kg
Control	27.9^{b}	9.57^{b}	2995^{b}
+ Xylanase	32.3^{a}	16.6^{a}	3059^{a}
Probability			
Diet	<0.01	0.04	<0.001
Xylanase	<0.01	0.01	<0.001
Diet x Xylanase	0.95	0.86	0.63

Retention, % of intake		
NDF^{1}	ADF^{1}	AME_{n}, kcal/kg
31.7	11.9	3,005
36.2	19.4	3,061
24.0	7.23	2,985
28.4	13.9	3,057
1.022	2.29	16.18
[illegible]	15.7^{a}	3,033
[illegible]	10.6^{b}	3,026
[illegible]	1.62	11.44
27.9^{b}	9.57^{b}	$2,995^{b}$
32.3^{a}	16.6^{a}	$3,059^{a}$
0.72	1.62	11.44
<0.01	0.04	0.45
<0.01	0.01	<0.01
0.95	0.86	0.63

Kiarie, Romero, and Ravindran, 2014

Dose response trials to Xylanase in 42-d Broilers are sometimes frustrating with variable responses that are hard to predict

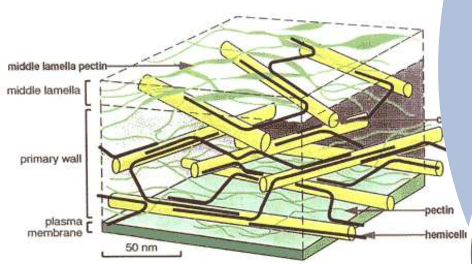


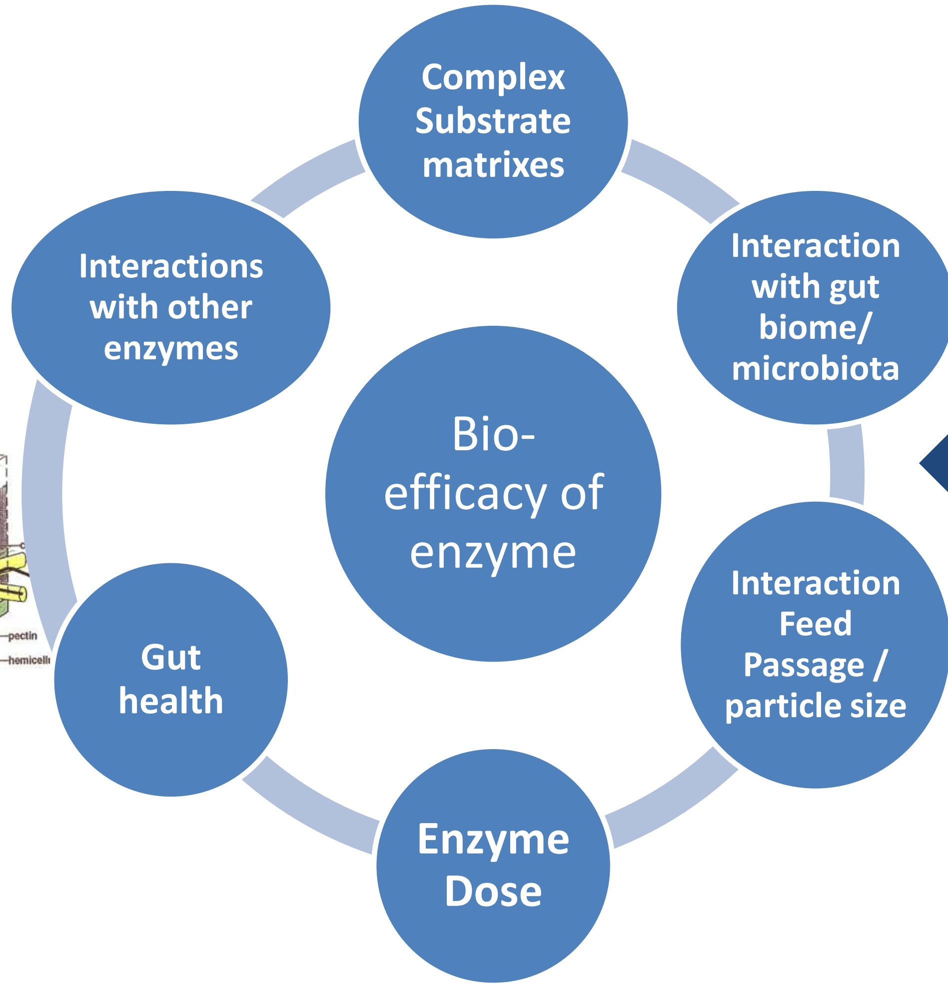


Simple corn-soy diets, single Xylanase dose

Plumstead, 2009, unpublished

Bio-efficacy of exogenous Xylanase and other enzymes may be affected by complex interactions between substrates in the feed ingredient and with the gut biome





Consequently, reported performance responses have been variable

In addition to NSP's do we also need to consider other substrates when selecting enzymes for corn/soy –based diets?

Substrates	Examples	Main anti-nutritive effects
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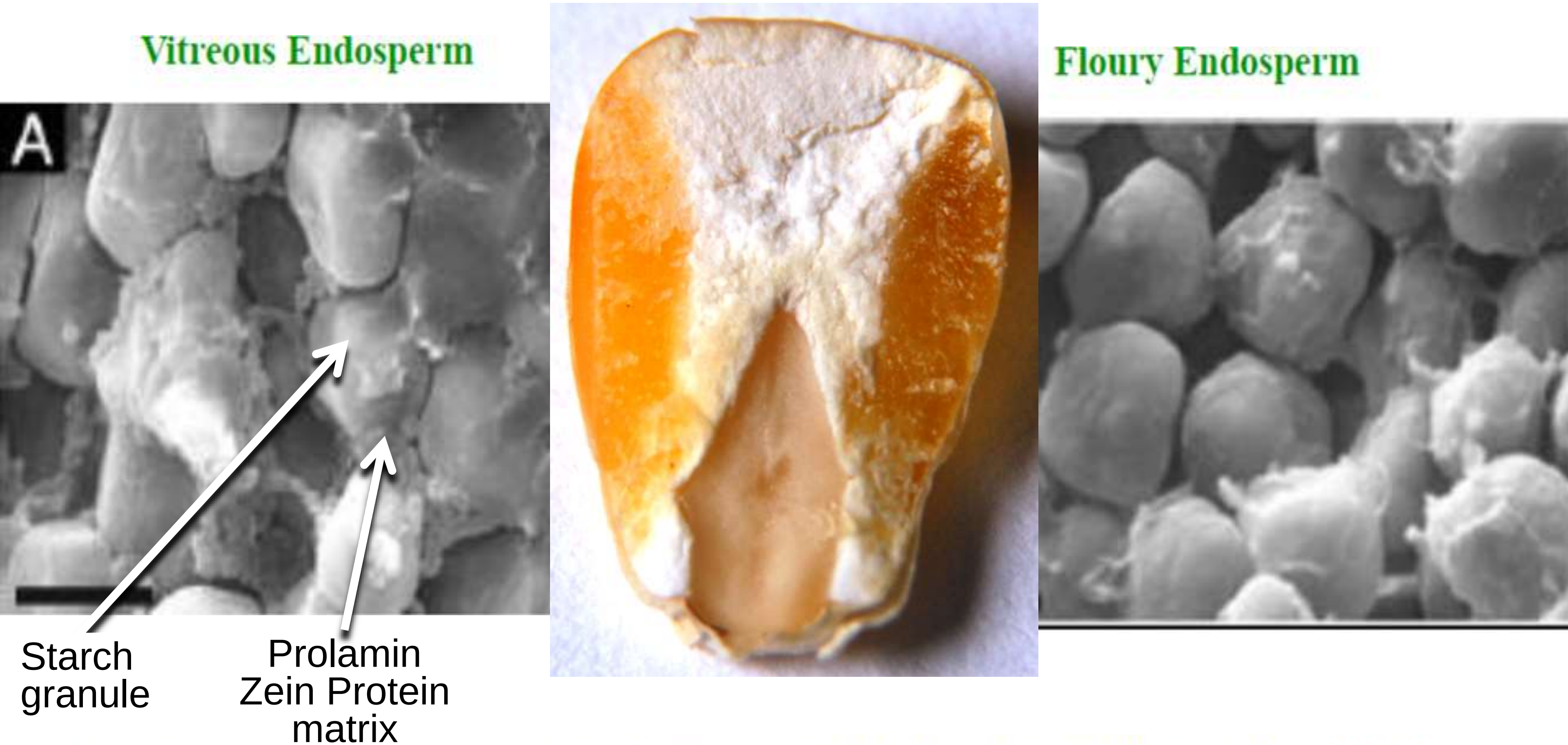
In addition to ''NSP's, Undigested Starch and Protein account for the largest amount of undigested ''Substrate'' available in mixed corn/soy-based diets

Corn morphology is important to degree of starch-protein binding, degree of starch digestion and responsiveness to enzymes



Scanning electron microscopy of starch granules in corn: A) starch granules heavily imbedded in prolamin-protein matrix, B) starch granules in opaque corn endosperm with less extensive encapsulation by prolamin-proteins (Gibbon et. al., 2003).

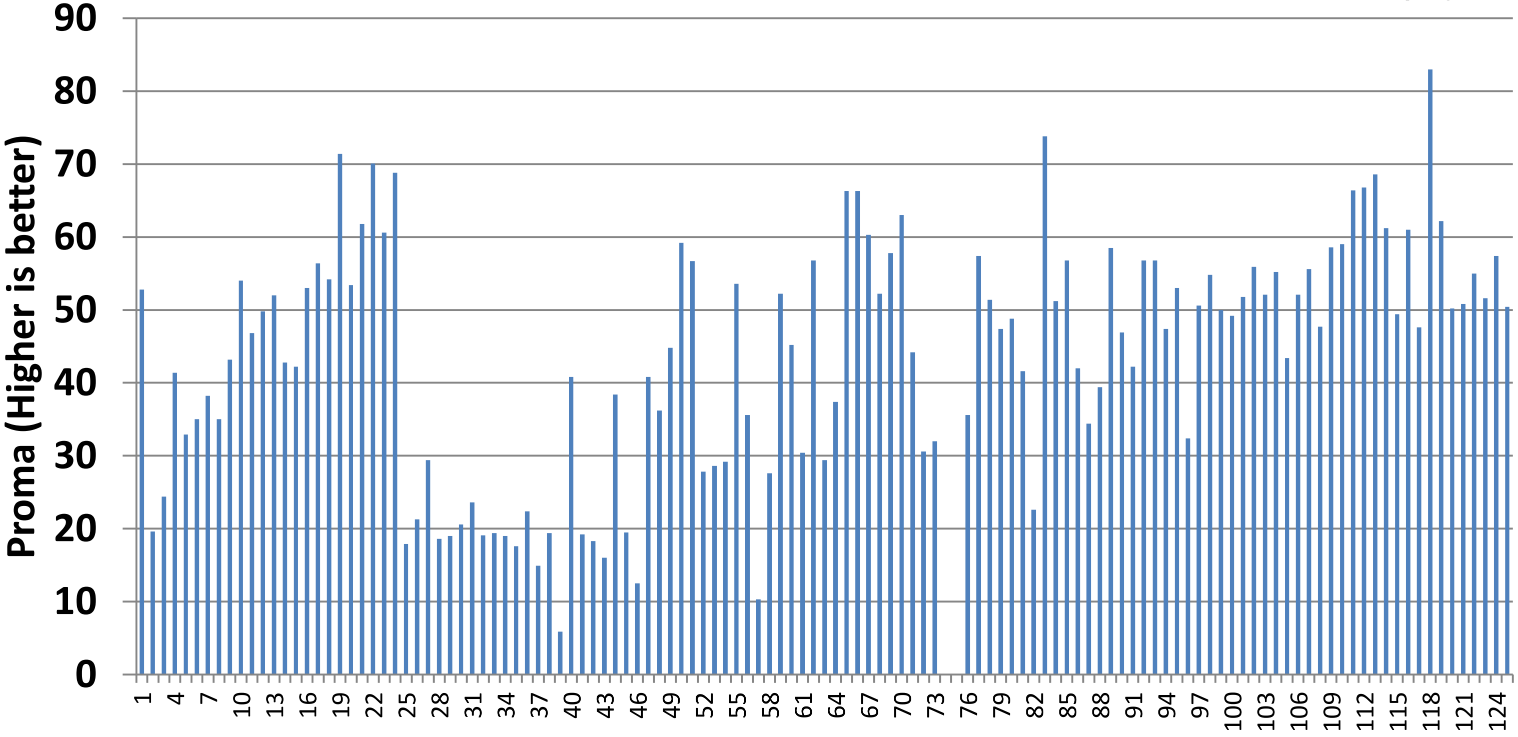
Assessing variation in Corn protein Composition

- The amount of Prolamin-Zein protein can be quantified analytically

Hamaker et al., 1995 – Cereal Chemistry

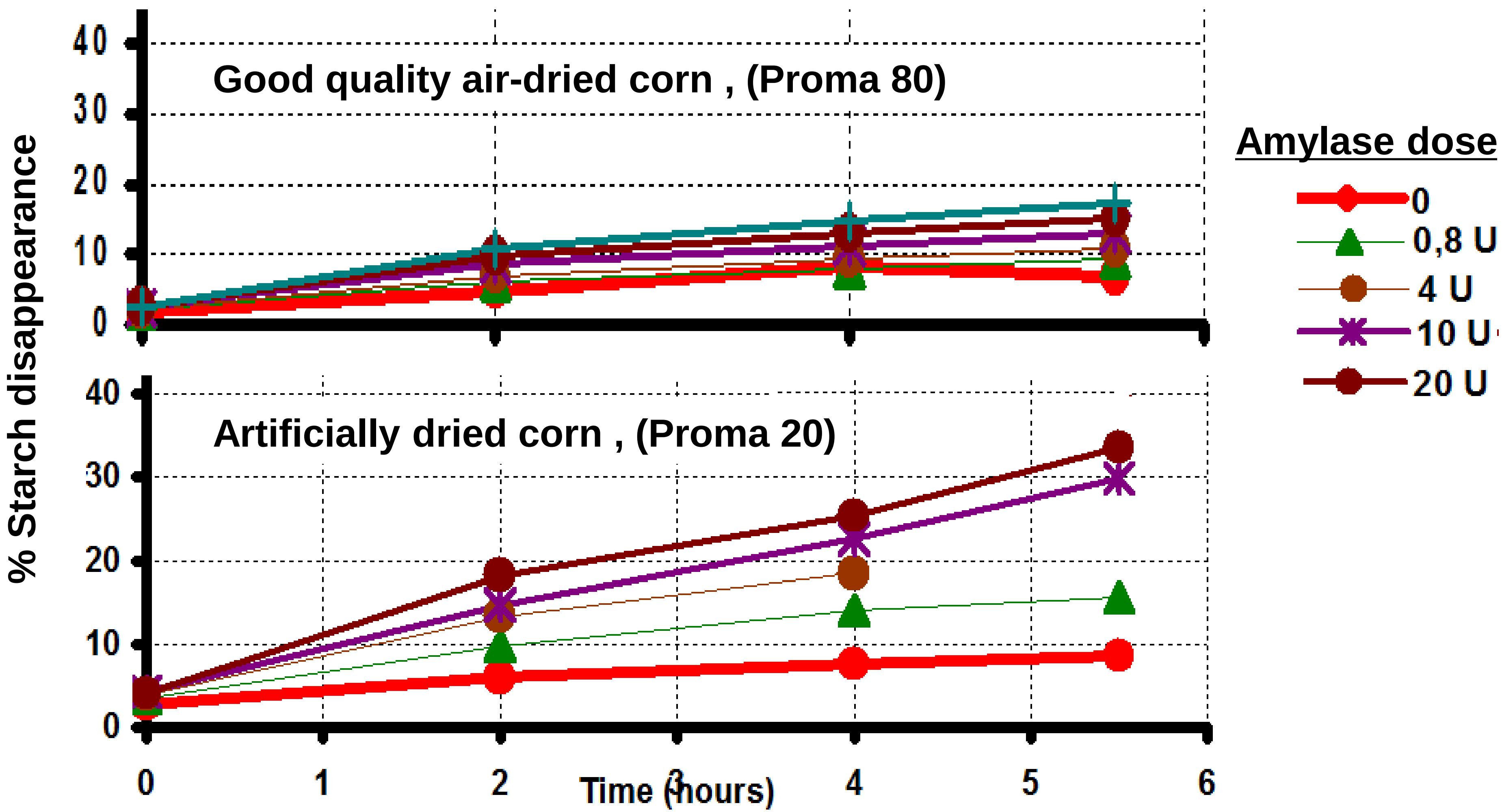
- % prolamin of total protein is affected by growing conditions, maturity, cultivar, and drying conditions of corn

Murphy and Dalby, 1971



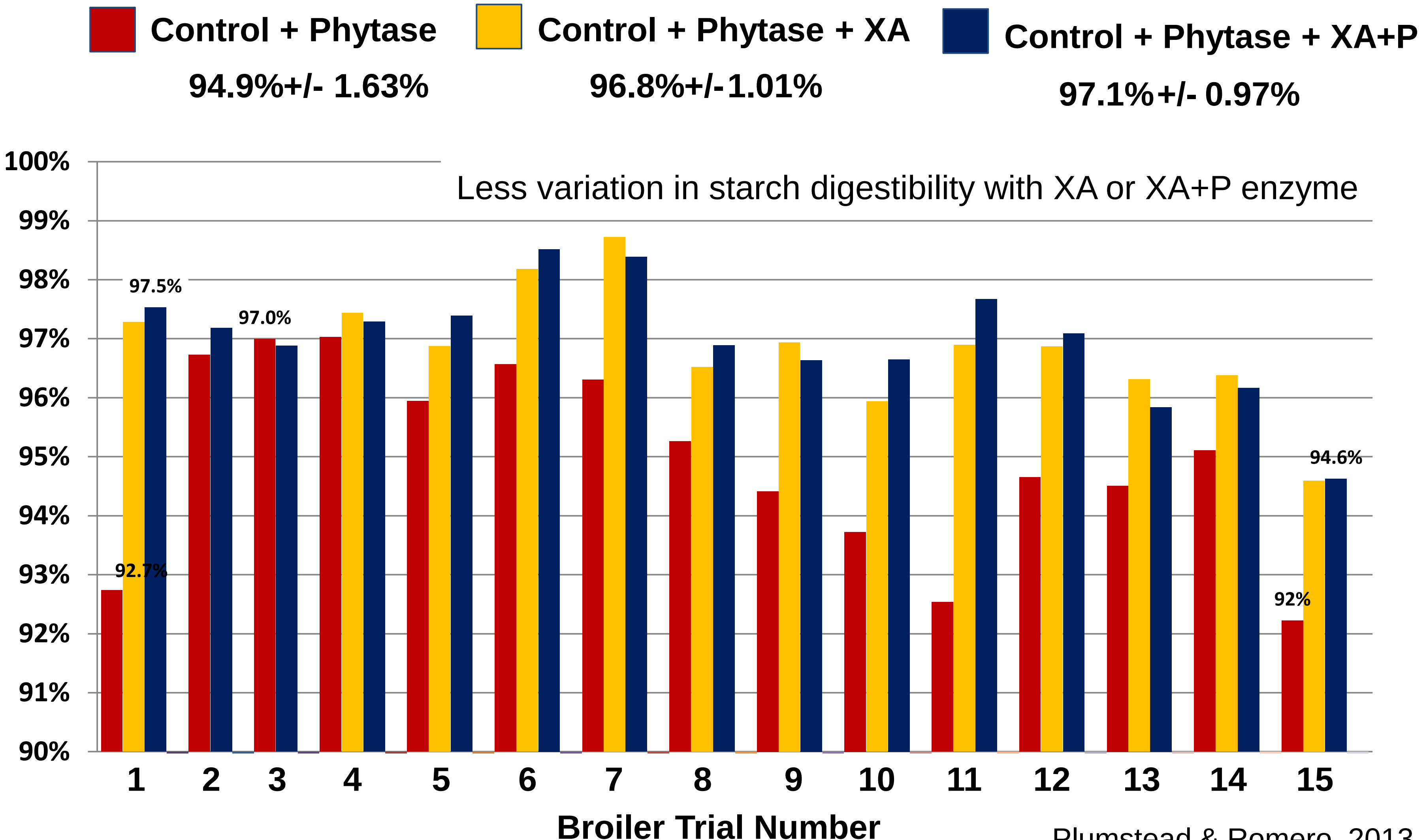
126 corn samples, 8 different countries over 2 years ***DuPont, Internal data***

In vitro effects of graded α-amylase dose on corn with high (80) of low (20) Proma values



DuPont, Internal data

Ileal starch digestibility in broilers: 15 digestibility trials with XA (Xylanase+Amylase) or XA+Protease (XA+P)



Plumstead & Romero, 2013

Decisions on Protease in Broiler diets?

- **Protease effects in feed**

1. Hydrolysis of dietary protein and increased protein solubility

(**Caine et al., 1998)**

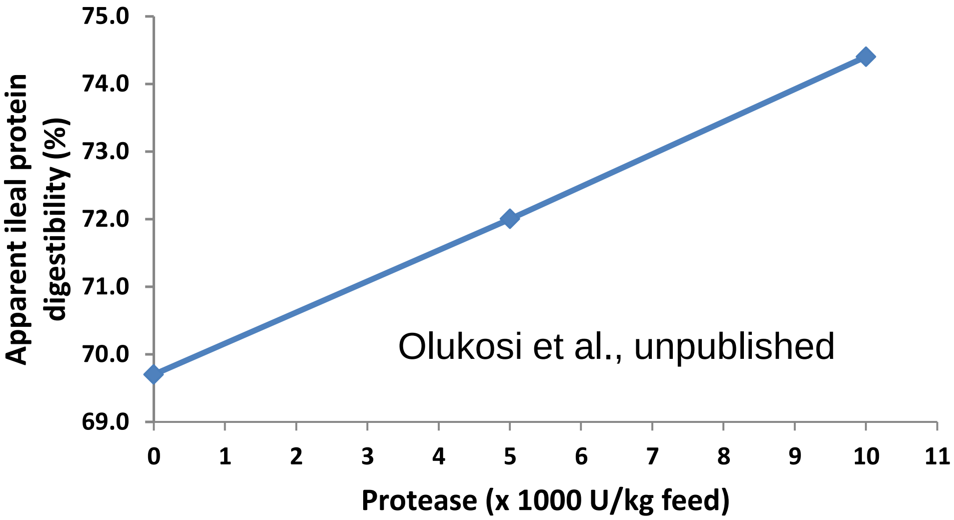
2. Disruption of protein-starch interactions in corn

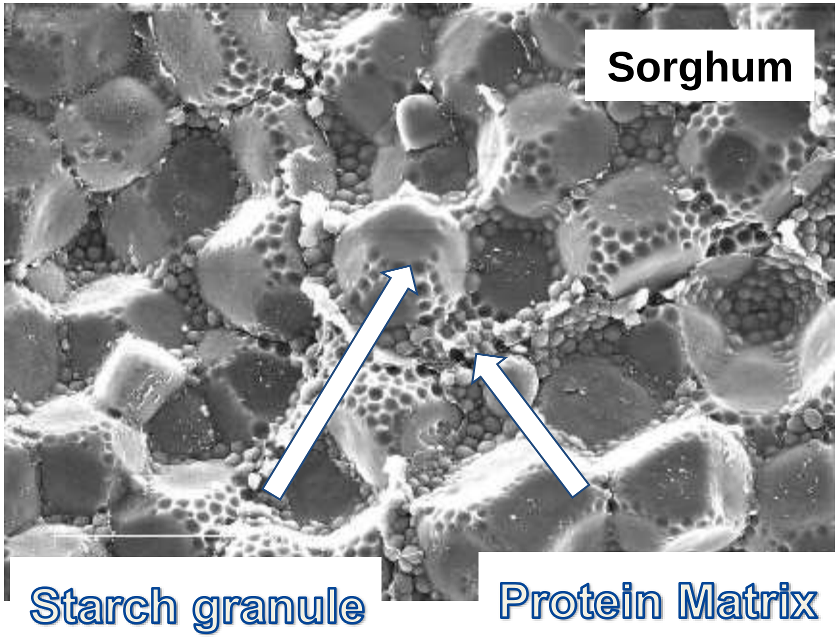
(Mc Allister et al., 1993; Belles et al., 2000)

3. Disrupt Fibre-protein interactions

Colombatto and Beauchemin, 2009

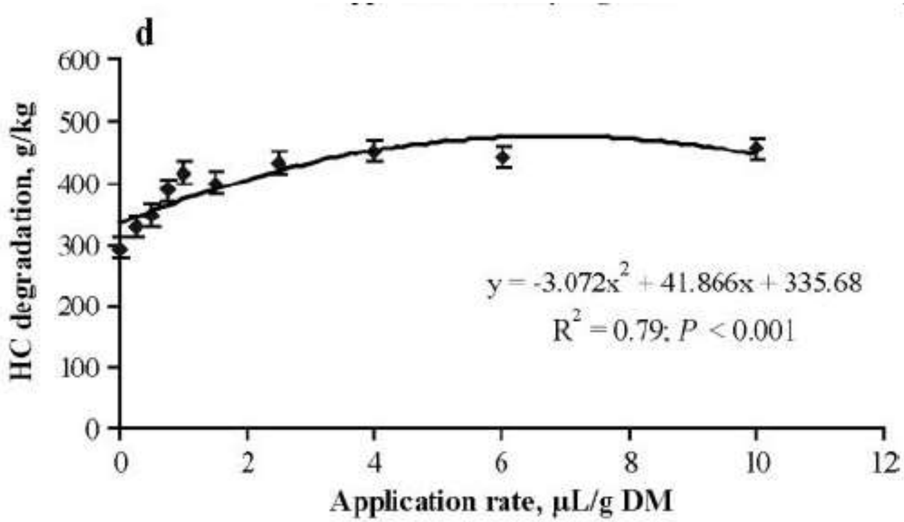
4. Potential gut health benefits of reducing fermentation of undigested protein in ceca/colon

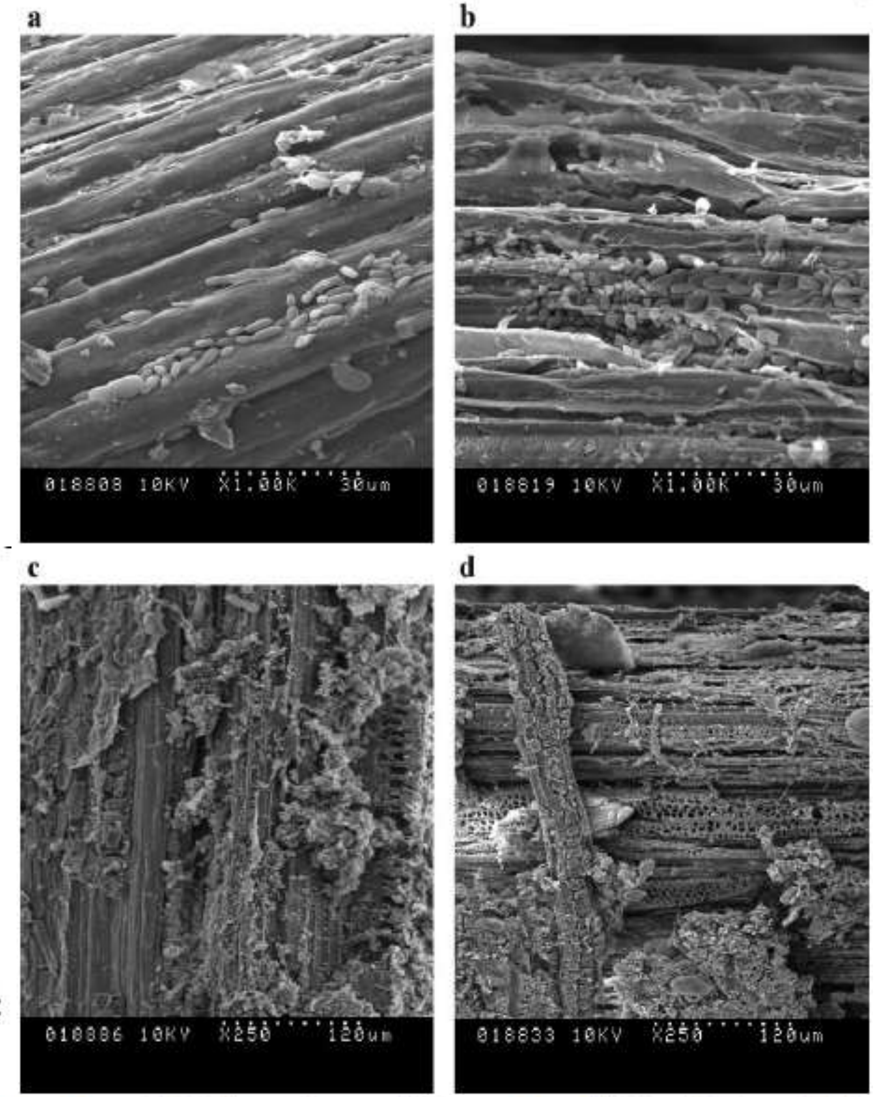




Other benefits of Protease: Fibre Digestion by Xylanase!

- Serine protease tested in digestion of alfalfa in rumen batch model
- Protease increased in vitro disappearance of DM, NDF, hemicellulose

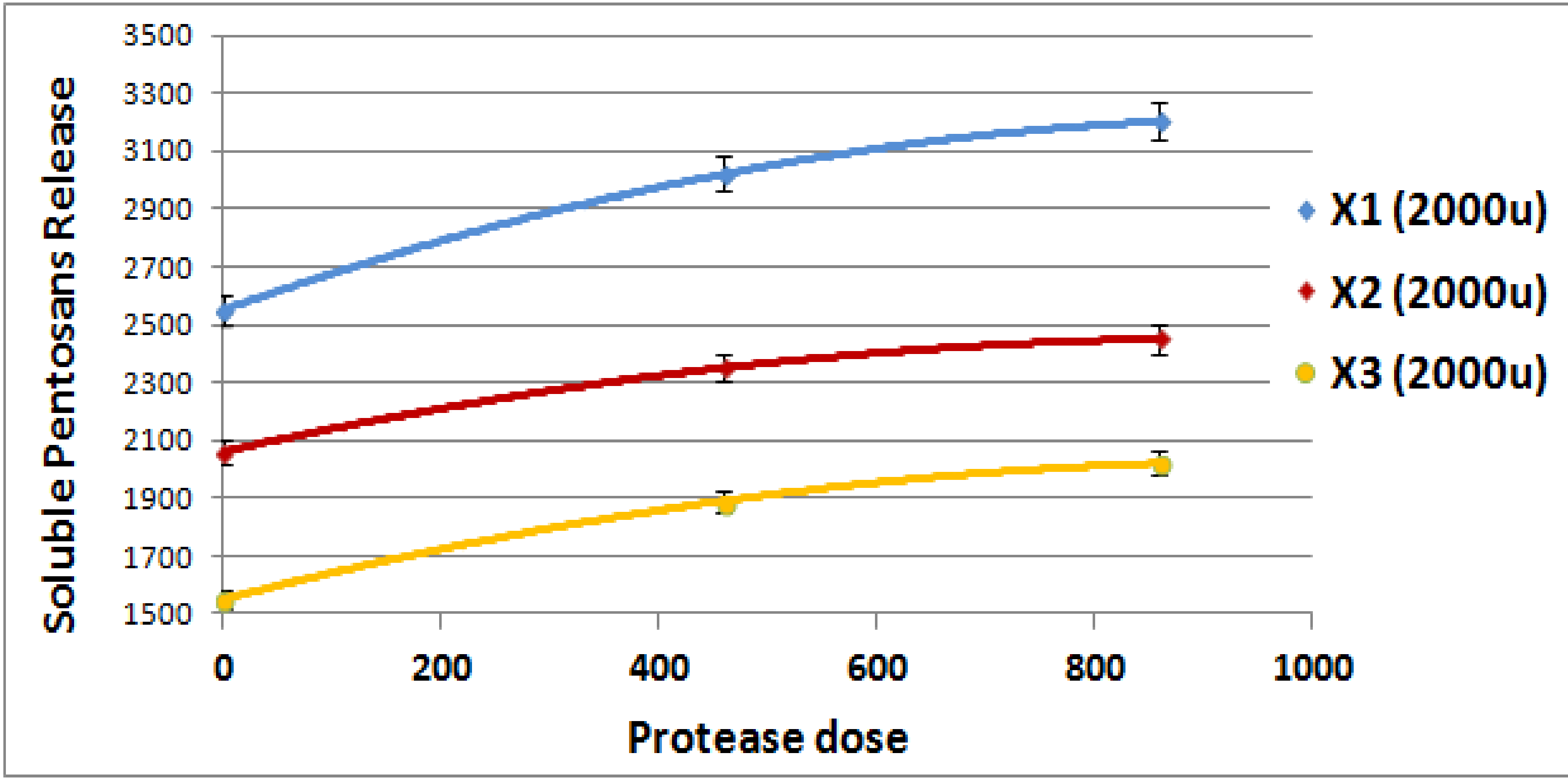




From Colombatto and Beauchemin, 2009

Figure 4. Scanning electron microscopy images of alfalfa hay samples, untreated (a) or enzyme-treated (b; Protex 6L, Genencor Int., Rochester, NY) at 0 h, or untreated (c) or enzyme treated (d) at 18 h postincubation with ruminal fluid in vitro.

Effect of Xylanase Source and Protease dose on Soluble Pensosan release from Corn DDGs



Pedersen et al.Unpublished

Bio-efficacy of exogenous enzymes is not only related to the primary biochemical target of enzymes

Phytase	• P and Ca digestibility • A.A., fat digestibility
Xylanases, B-glucanse	• Fibre disappearance • A.A., fat, starch digestibility
Amylases	• Starch, A.A. digestibility
Proteases	• A.A. / Protein digestibility • Fibre digestibility?
Mannanase	• Galactomannan degradation • Reduction in Innate Immune response

Hsiao et al., 2006; Romero & Plumstead 2014

Decisions on enzyme addition to feed with phytase

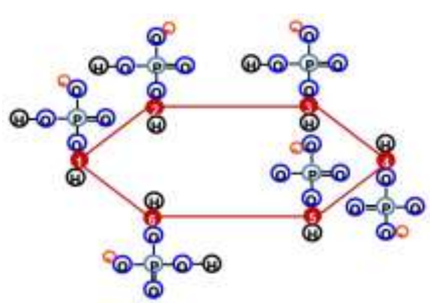
1. General consensus that enzyme effects are **NOT additive with responses ranging from antagonistic to synergystic**
1. Enzyme Response is based on law of diminishing returns.

Cowieson et al., 2012

As phytase is included in >>94% of Broiler feed …

…any other additive need to demonstrate value on TOP of phytase, and each other.

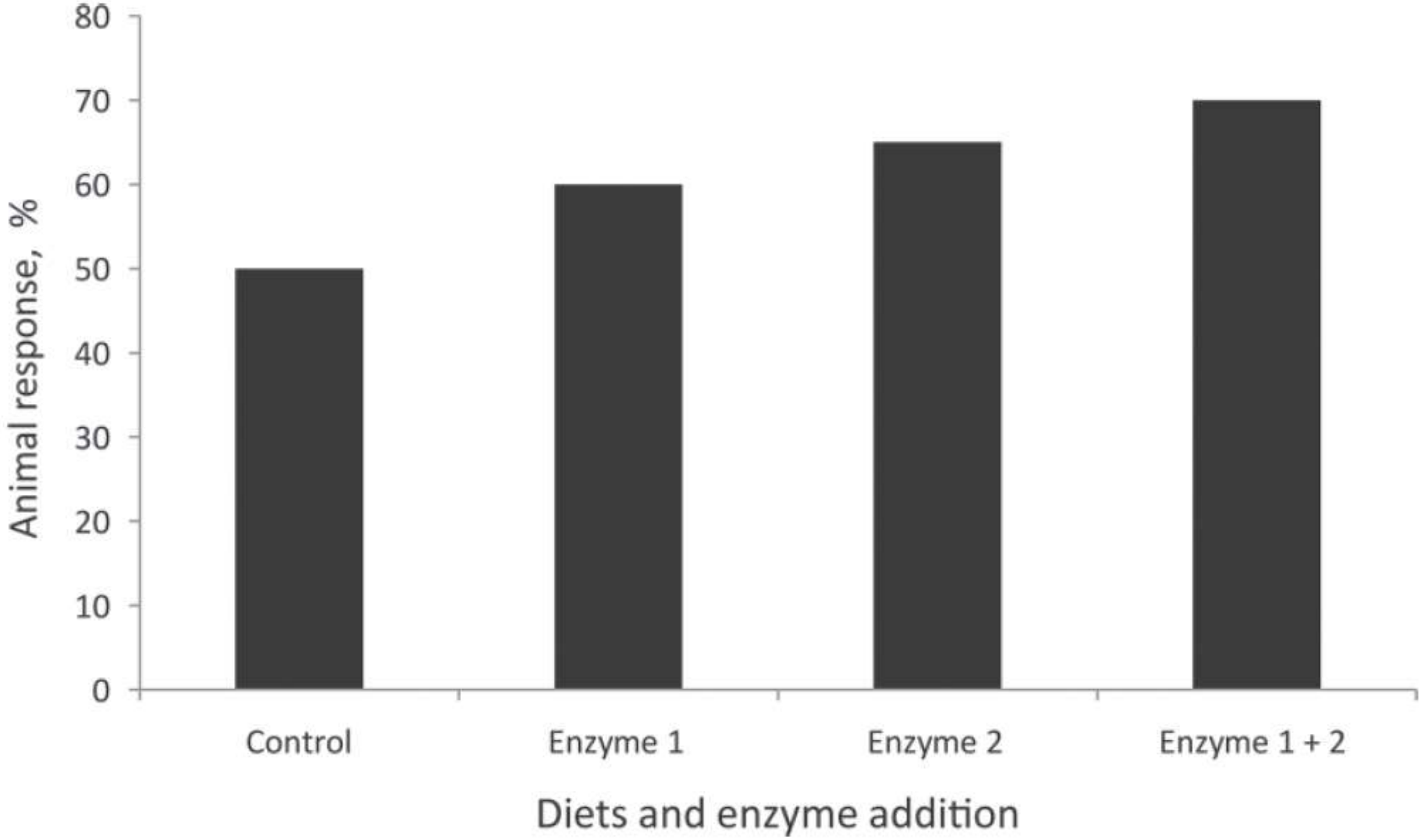
Phytase	NSPase	Protease	Other
A.niger	**Xylanase**	*Bacillus subtilis*	*Amylase*
C.braachi	**ß-Glucanase**	*Bacillus* licheniformis	*Mannanase*
E.coli			*Galactosidase*
Citrobacter spp			*Glucoamylase*
Buttiauxella spp			*Lipase*



Decision Factors

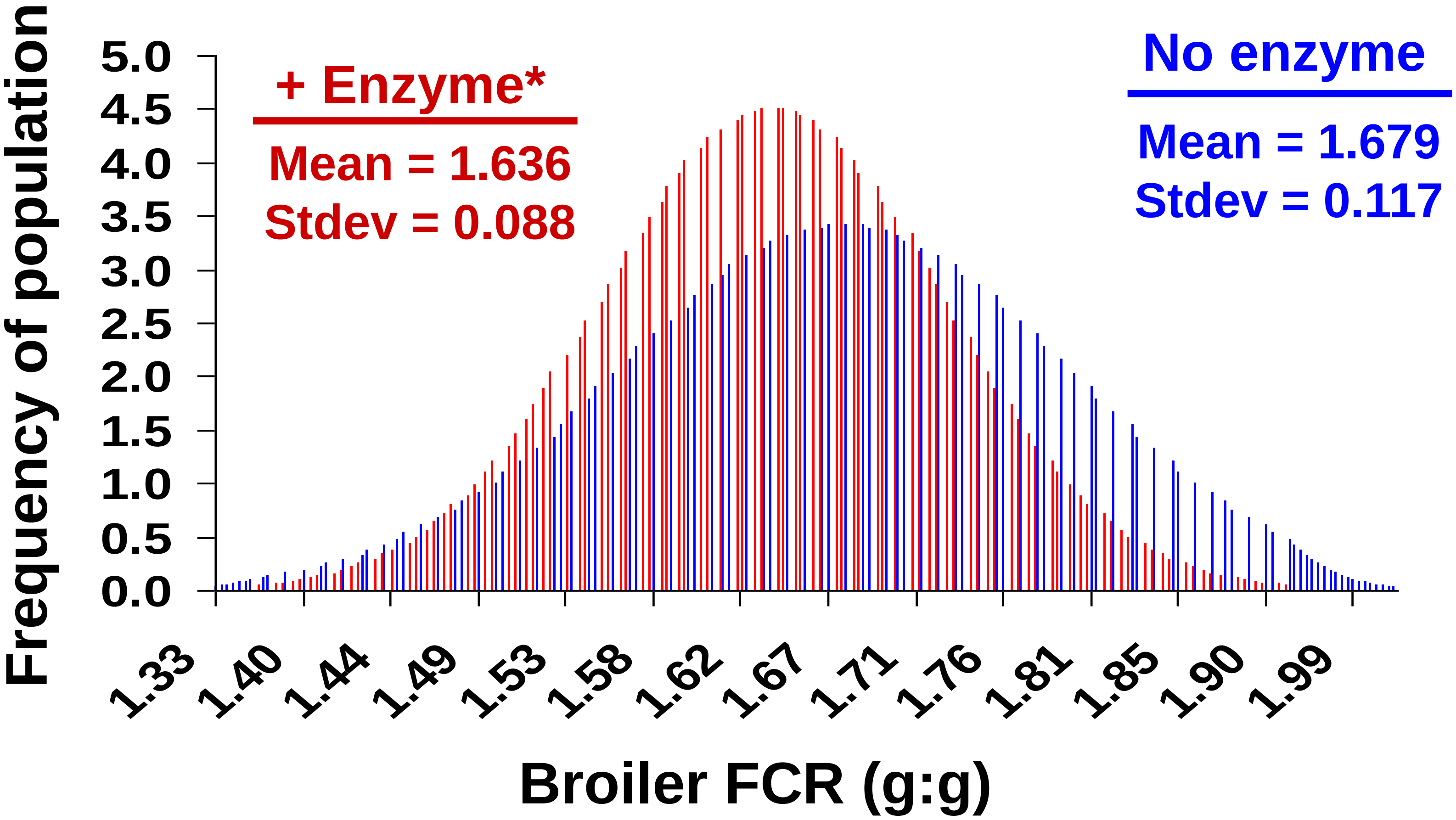
Substrates, Feed Ingredient Quality, Performance

Combining enzyme activities needs to make sense in terms of substrates and be quantifiable in biological trials



Cowieson and Adeola, 2014

Overall objective: address unknown variation by improving mean and consistency of live performance



XA+P enzyme applied to 26 different corn samples fed to broilers

Application of enzymes in poultry diets:

Simplifying complexity

- ***Enzyme responses are dependent on dietary Substrates they target*, which we need to understand better.**
- **Value of Phytase is far greater than improvements in phosphorus availability and negative effects of phytate on nutrient utilization and performance need to be considered in decision making process.**
- Enzyme effects are sub-additive, based on a law of diminishing return
- Value of carbohydrases and other enzymes must be determined on top of phytase
- **Some assessment of feed ingredient quality is required to explain variation in enzyme responses**

QUESTIONS?

University of Pretoria