BENEFITS OF DESTRUCTION OF LOWER PHYTATE ESTERS

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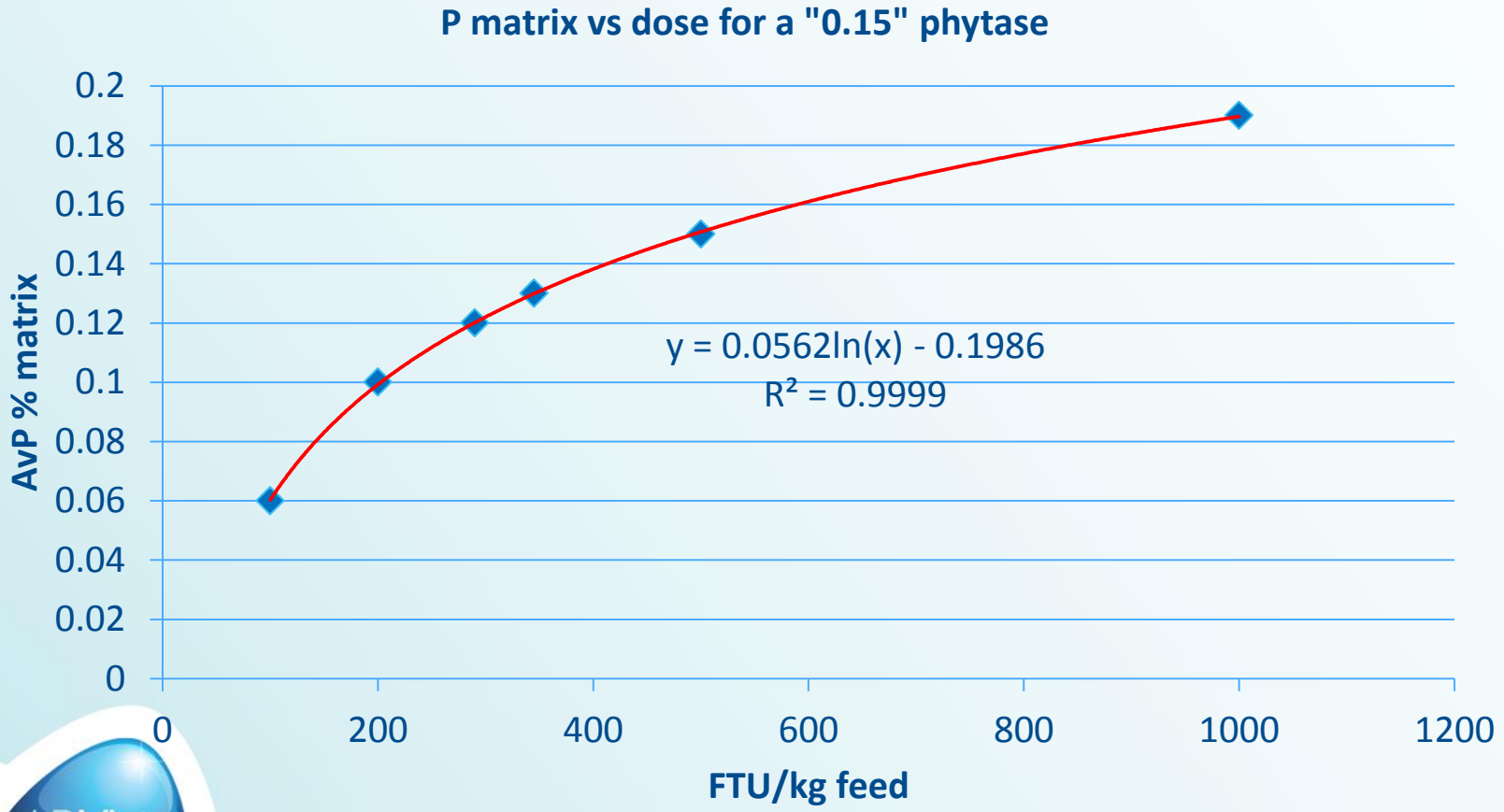
MARLBOROUGH, UK

WPSA Italy, Bologna, 8th June 2017
"Phytate and Phytase:
the value chain of phytate destruction"

TRADITIONAL DOSING OF PHYTASES – MATRIX DRIVEN



IP6 degradation and the role of inositol

Pepsin inhibited at pH 2.5 by very little phytate

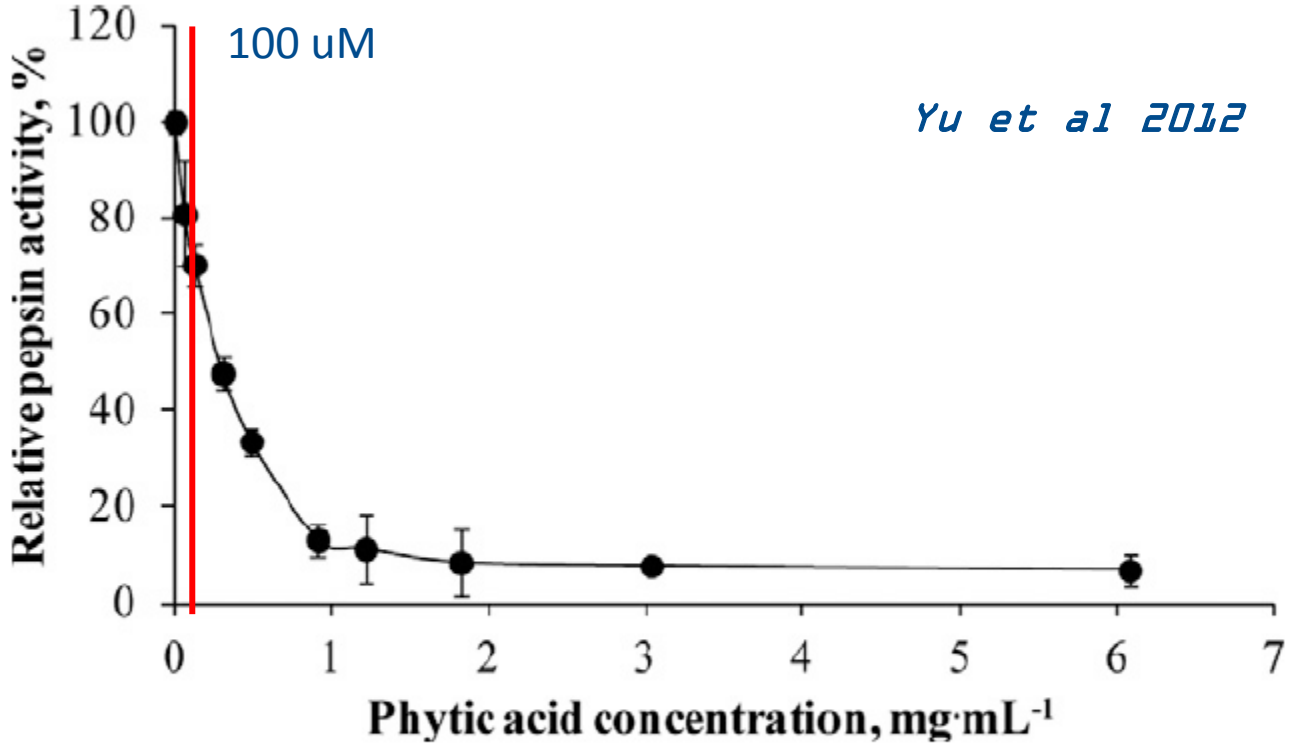
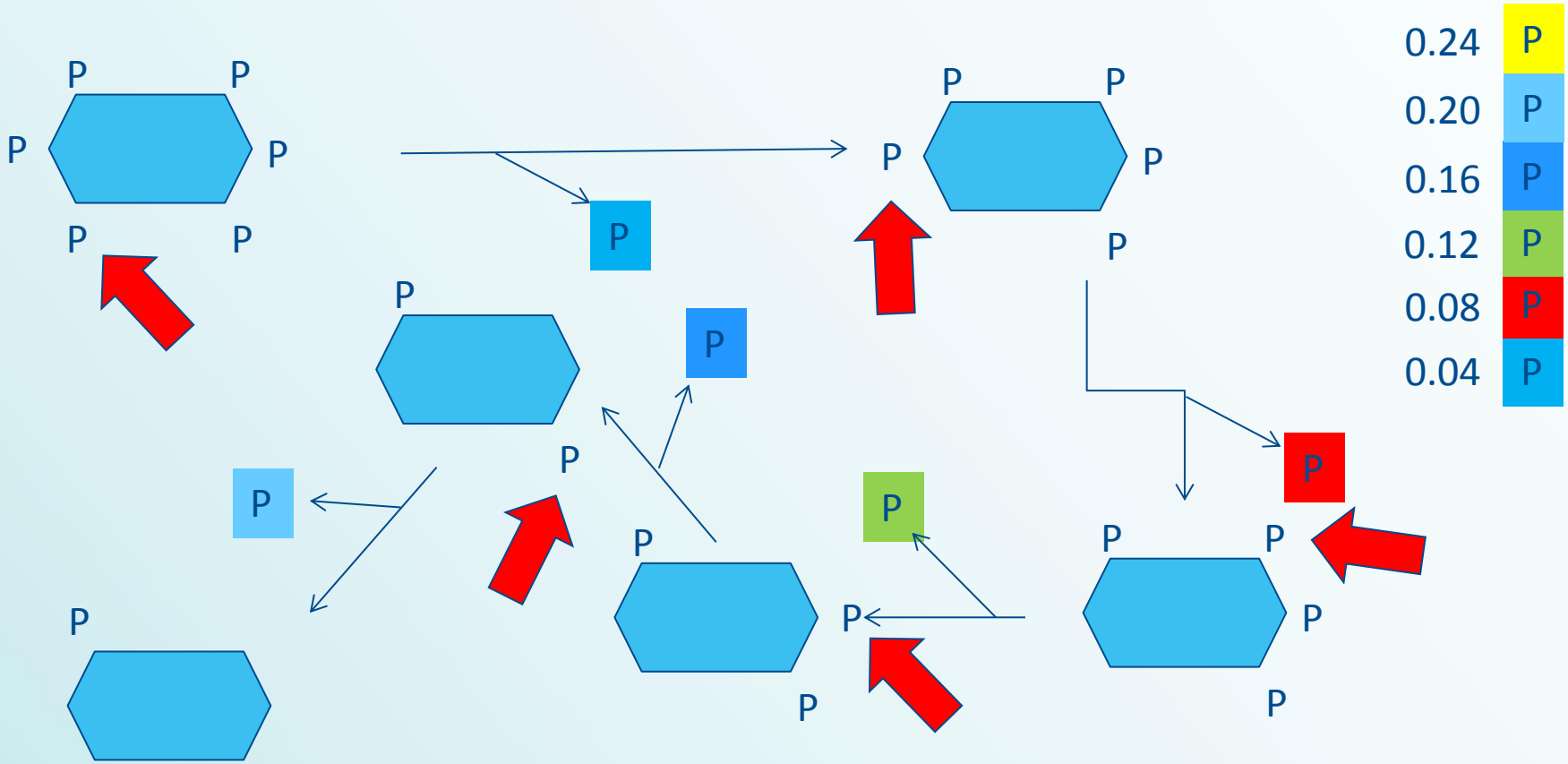


Figure 5. Phytic acid inhibition of porcine pepsin-catalyzed hydrolysis of azurine cross-linked casein. The assay was performed at 40°C. Each data point is the average of duplicate.

Phytase - Traditionally thought of as a means to provide P from IP6



IP6 is not the only problem
Phytase has to get rid of IP5→IP2 as well

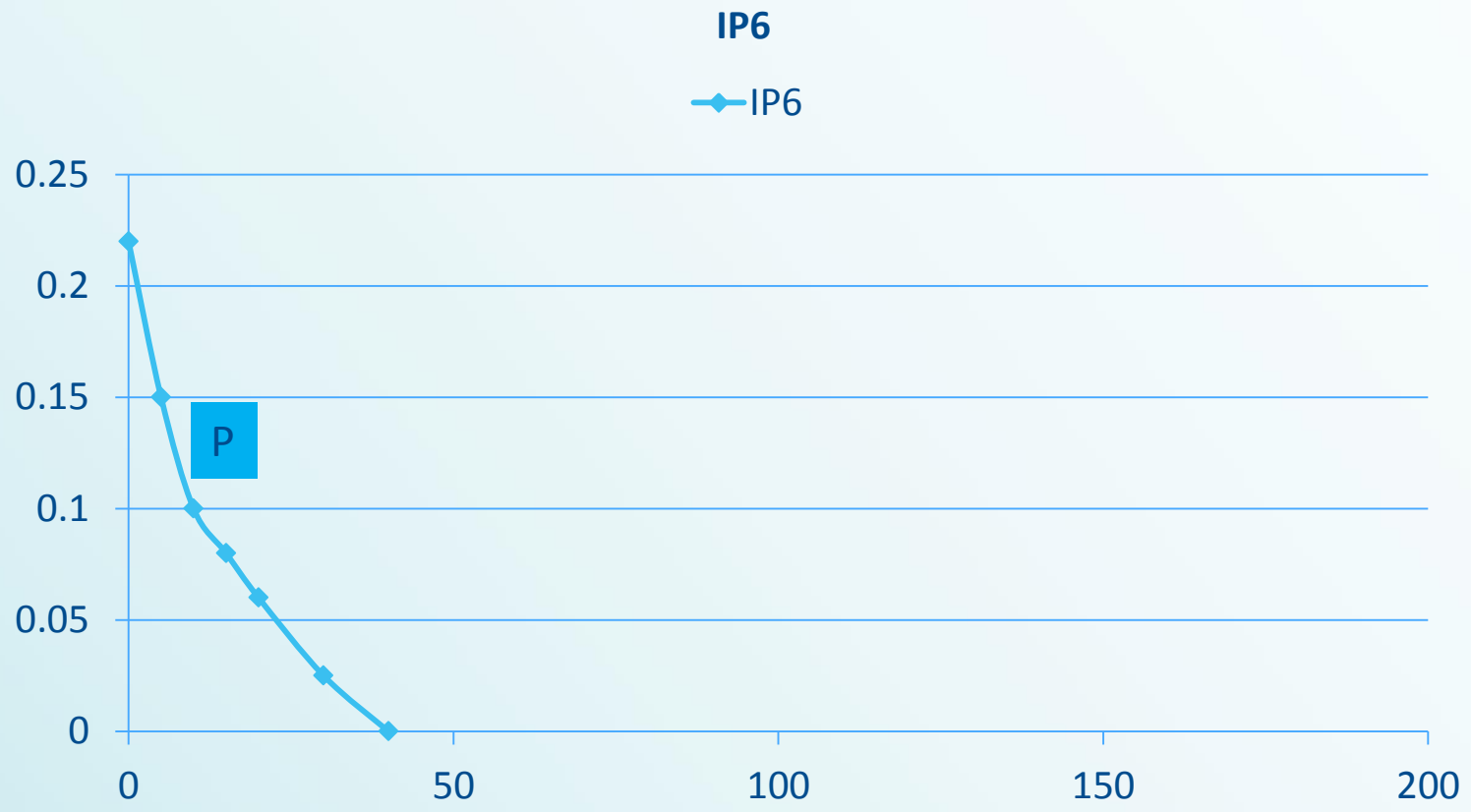


Figure 6. Time course of phytic acid hydrolysis by *E. coli* phytase (Phyzyme XP, Danisco A/S, Brabrand, Denmark; 0.08 phytase unit≅mL^{-1}) and inhibition of porcine pepsin catalyzed azurine cross-linked casein hydrolysis by the hydrolyzates. Phytic acid hydrolysis was performed at 37°C; pepsin activity assay was carried out at 40°C. Each data point is an average of 2 separate experiments.

Yu et al 2012

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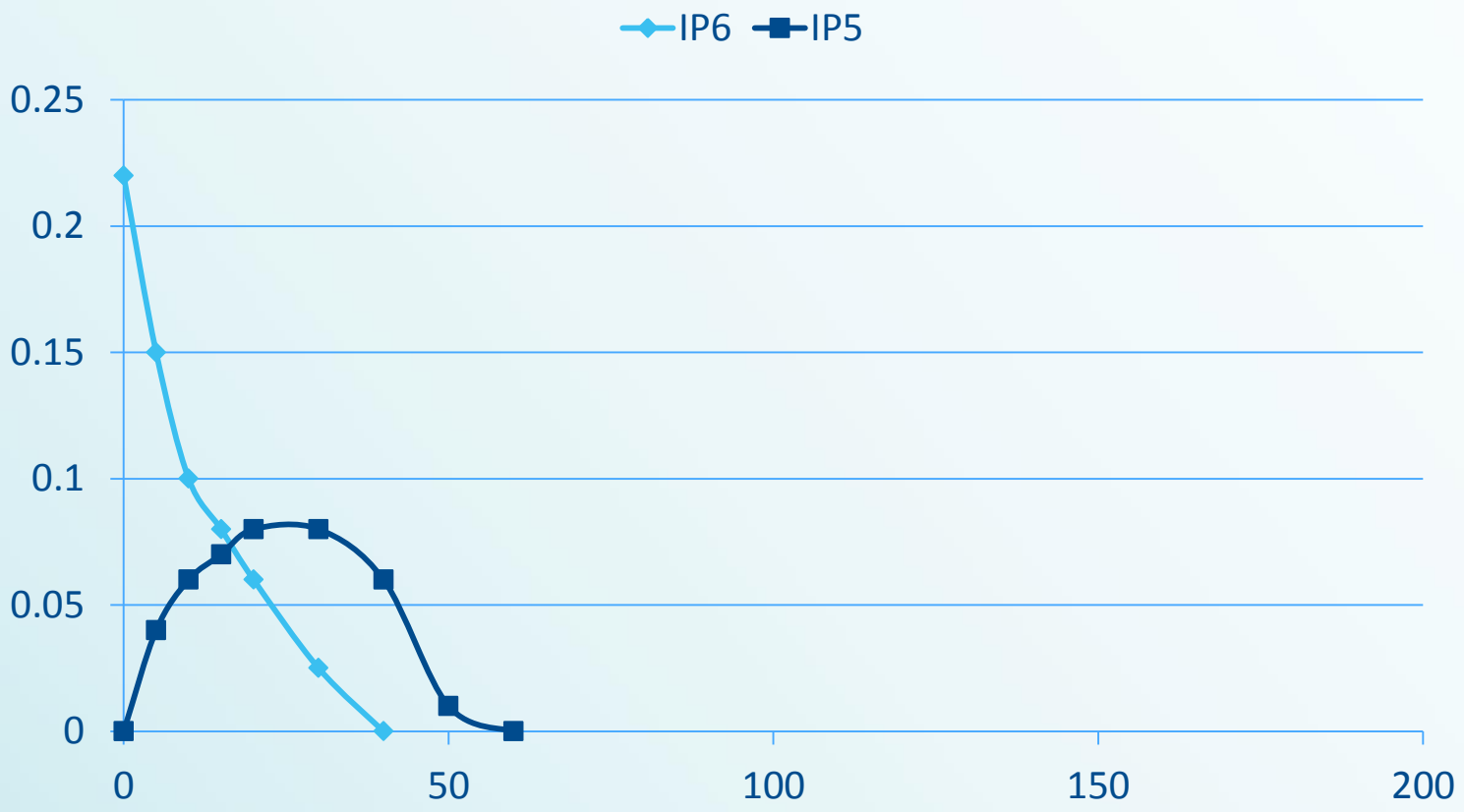

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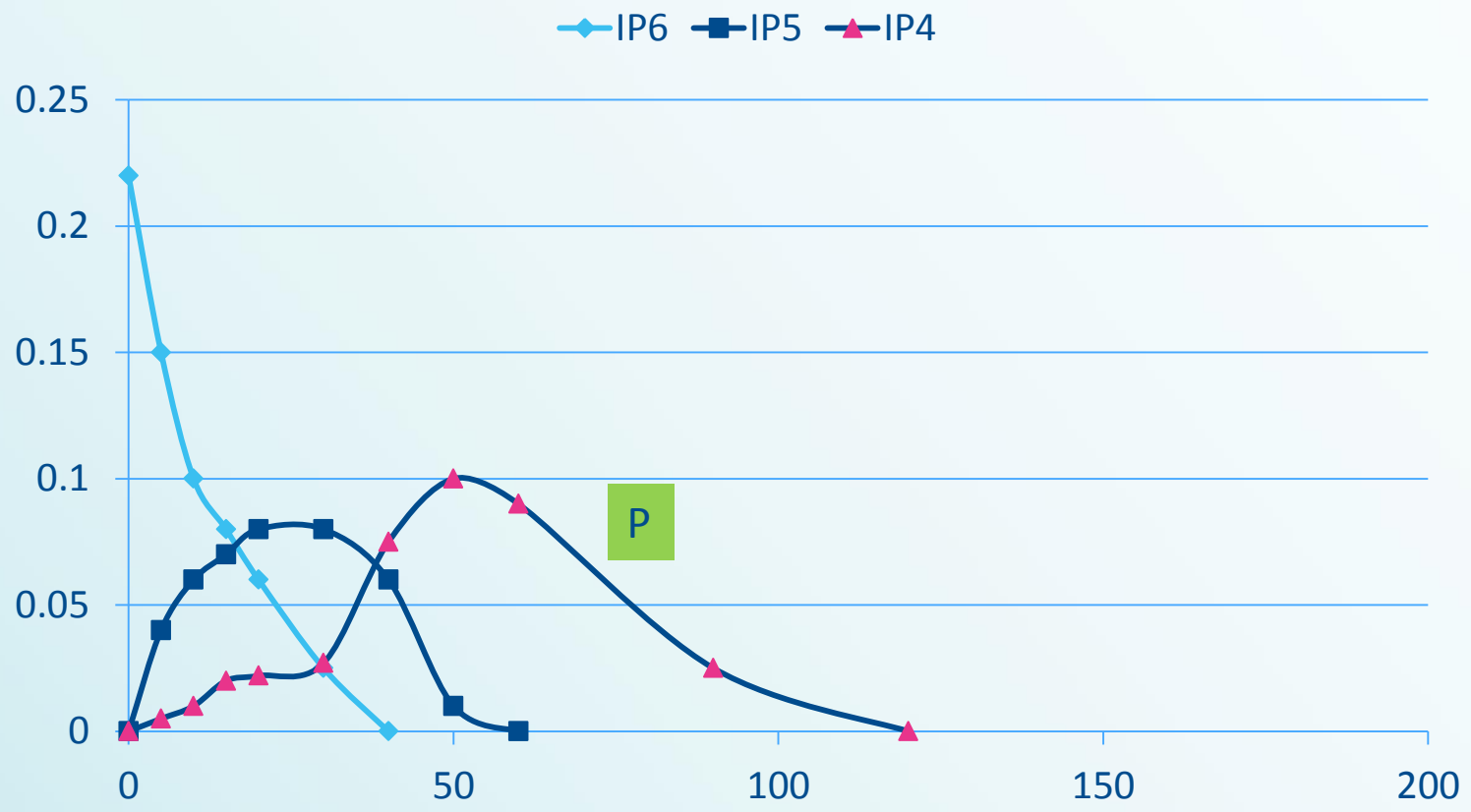

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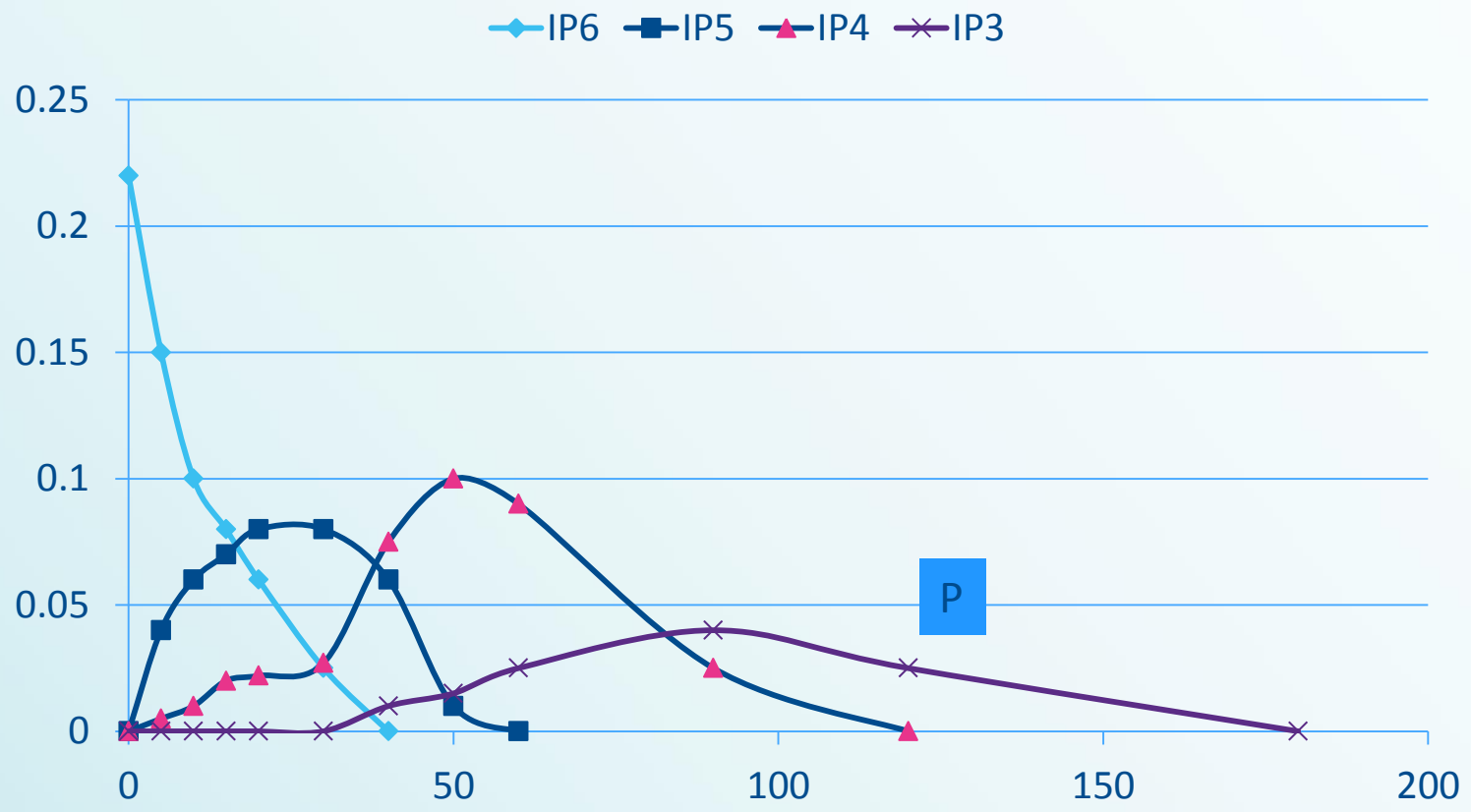

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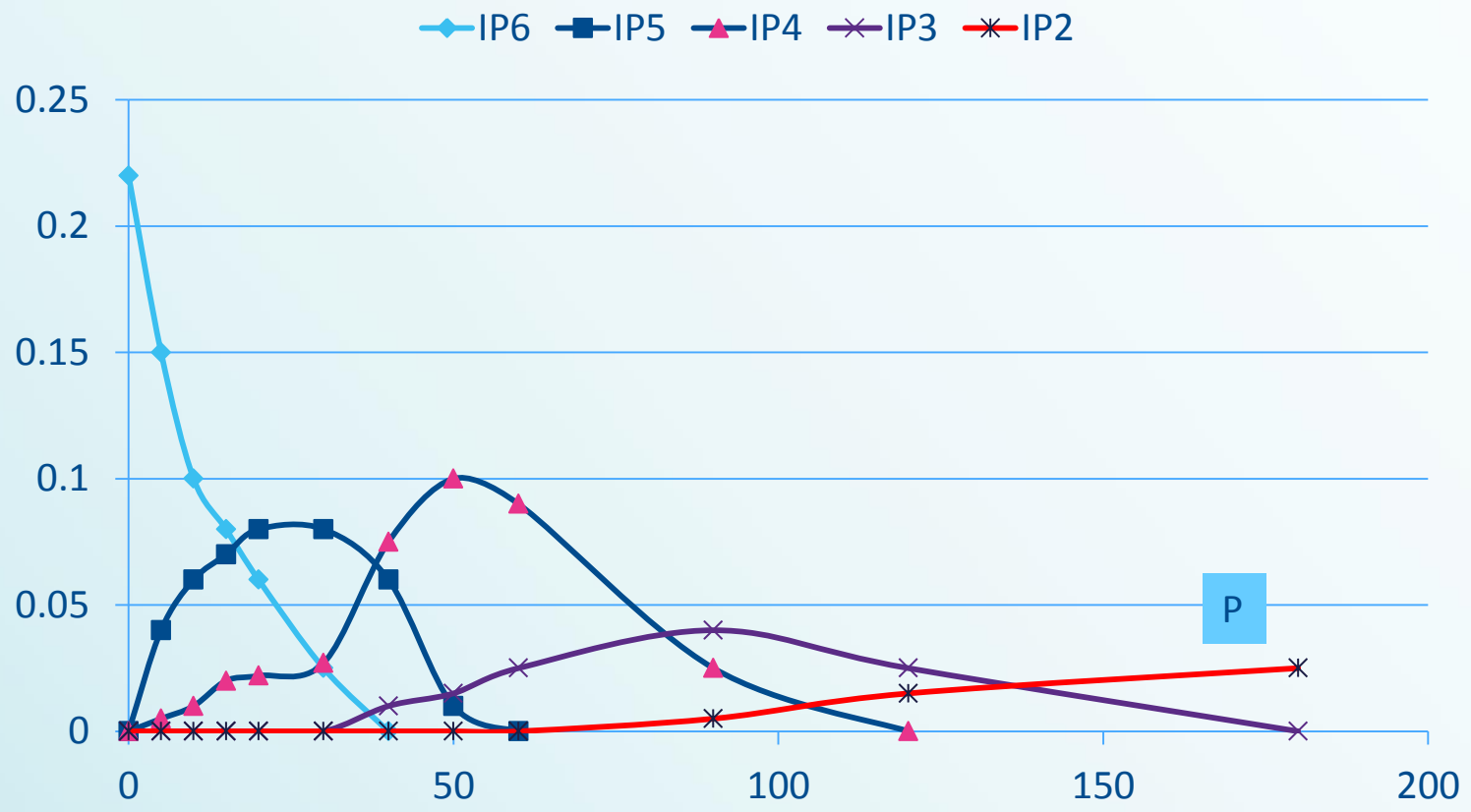

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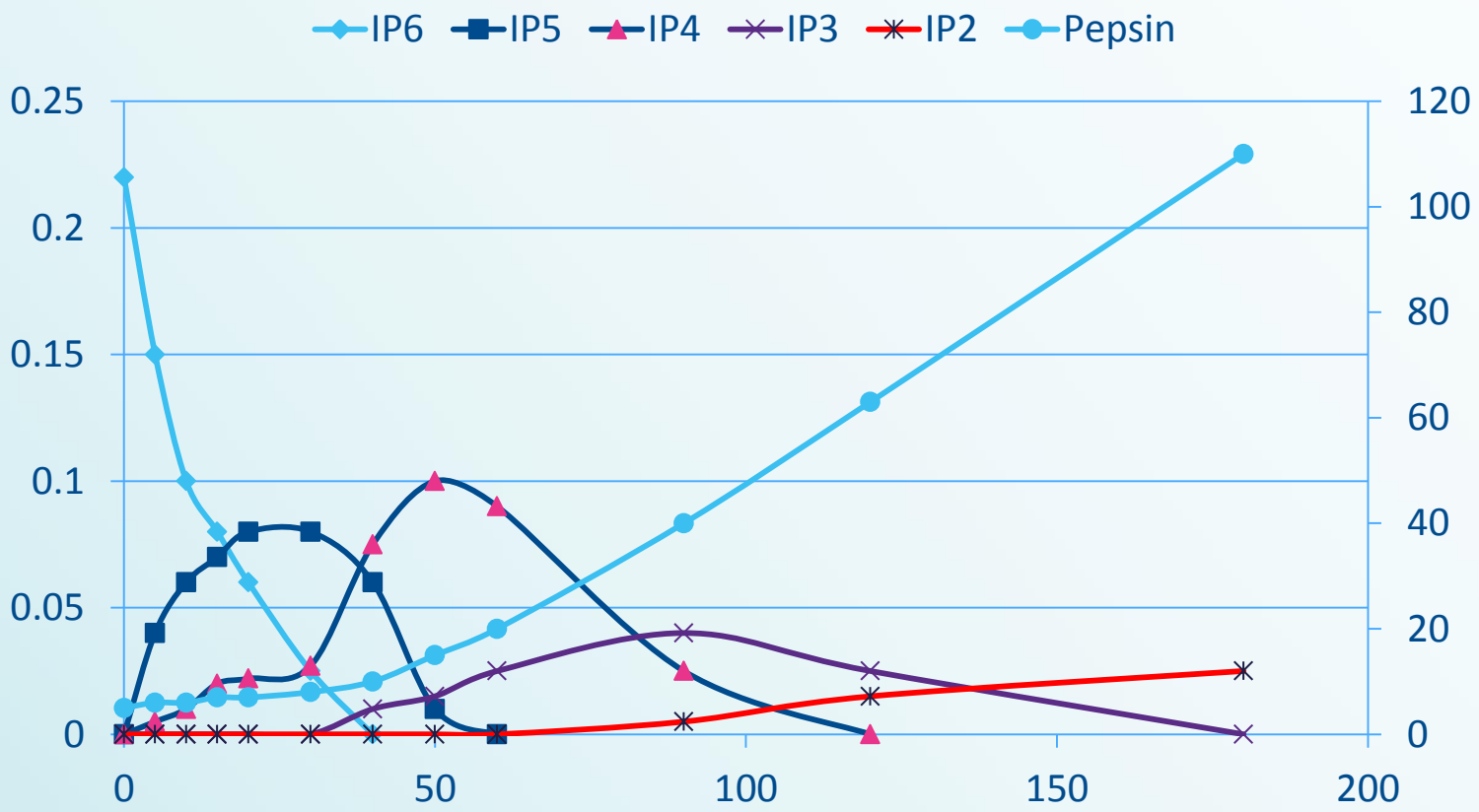

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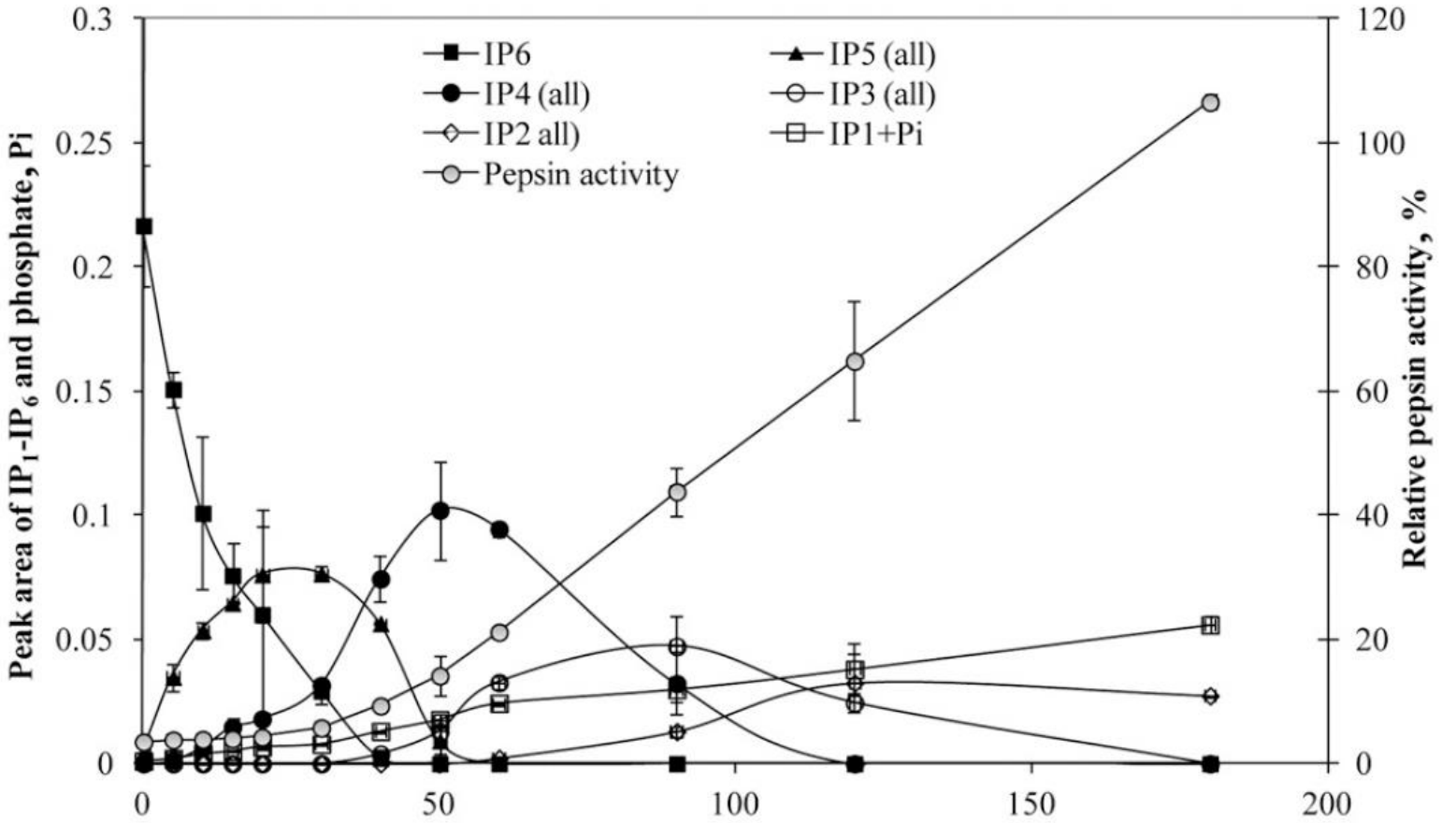

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Yu et al 2012

Knuckles 1989

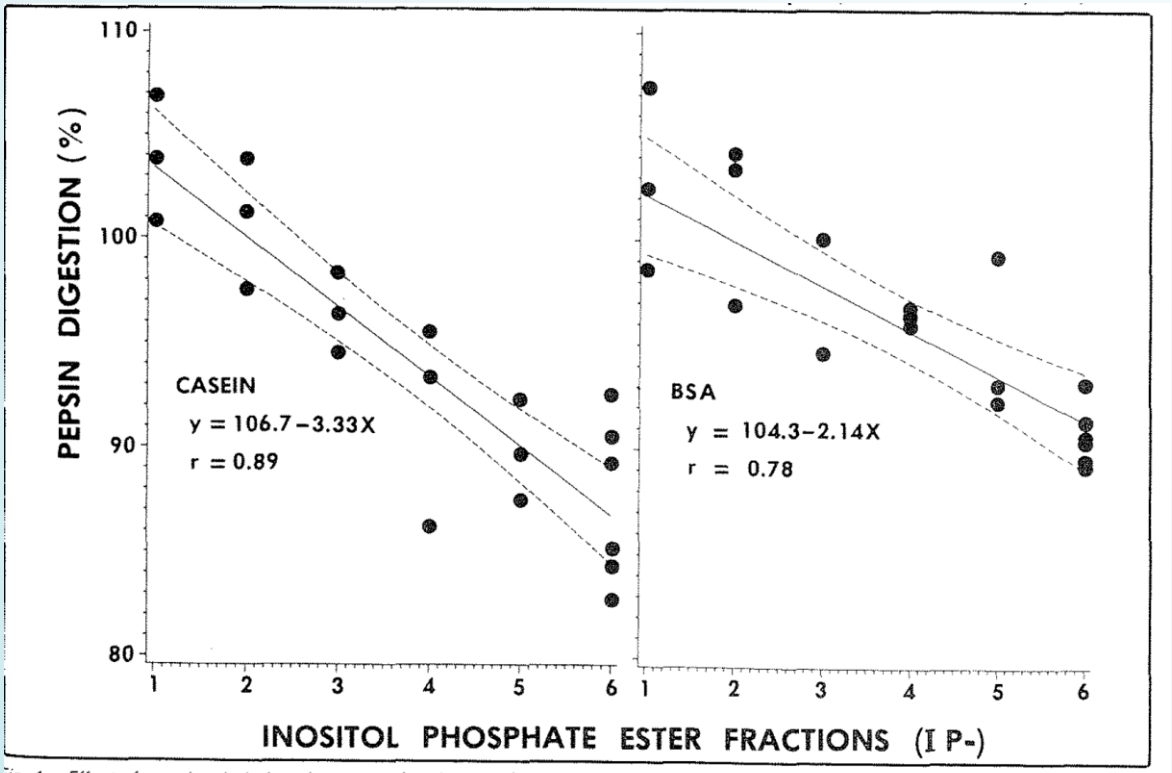

Fig. 1. – Effect of myo-inositol phosphate ester fractions on pepsin digestion of casein and BSA. Dashed lines indicate 95% confidence intervals.

Lower IP esters are not innocuous

pH 1.2!!

PERSSON ET AL 1998

Lower IP esters are not innocuous

Table 1. Number of Metal Ions Bound per Inositol Molecule at pH ≈5−6

IP*x*	$n_{Cu^{2+}}$	$n_{Zn^{2+}}$	$n_{Cd^{2+}}$
IP6	5.8	4.9	5.3
IP5	5.7	4.8	5.1
IP4	3.3	3.0	3.3
IP3	3.1	3.0	2.4

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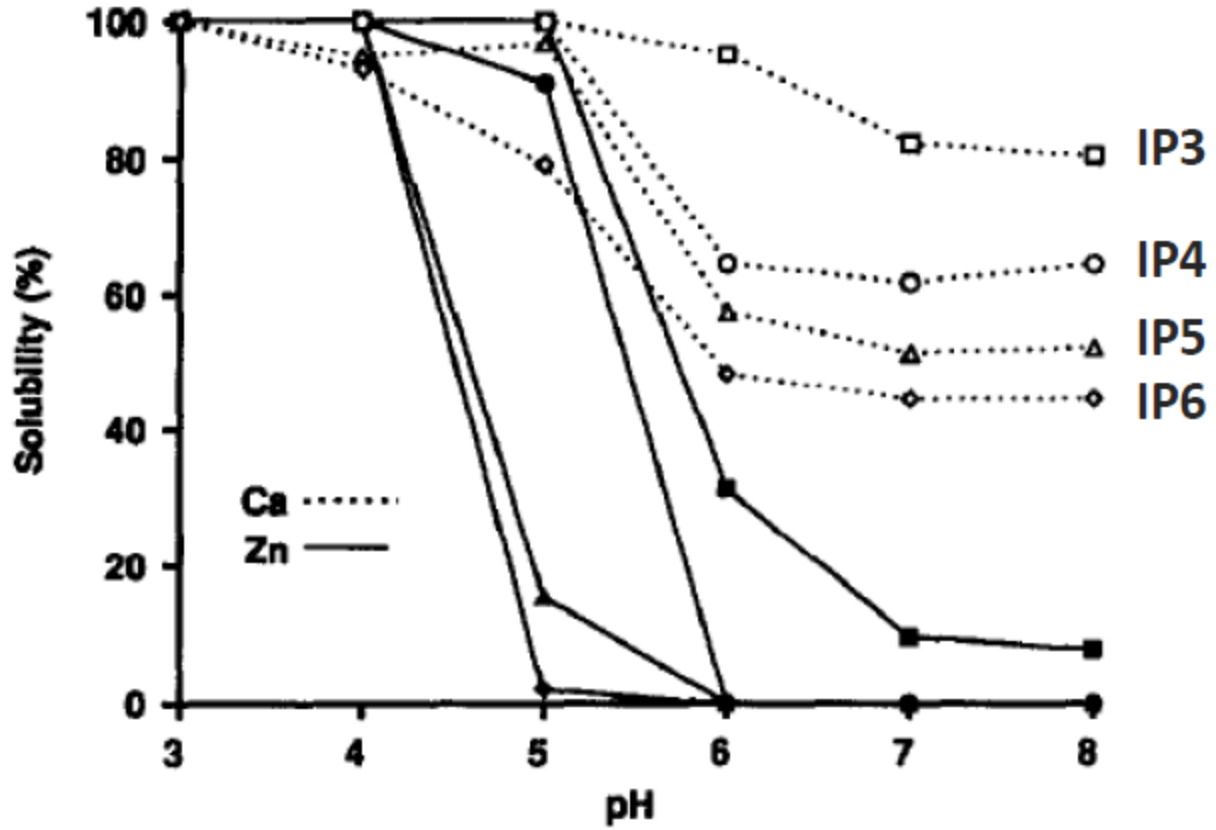
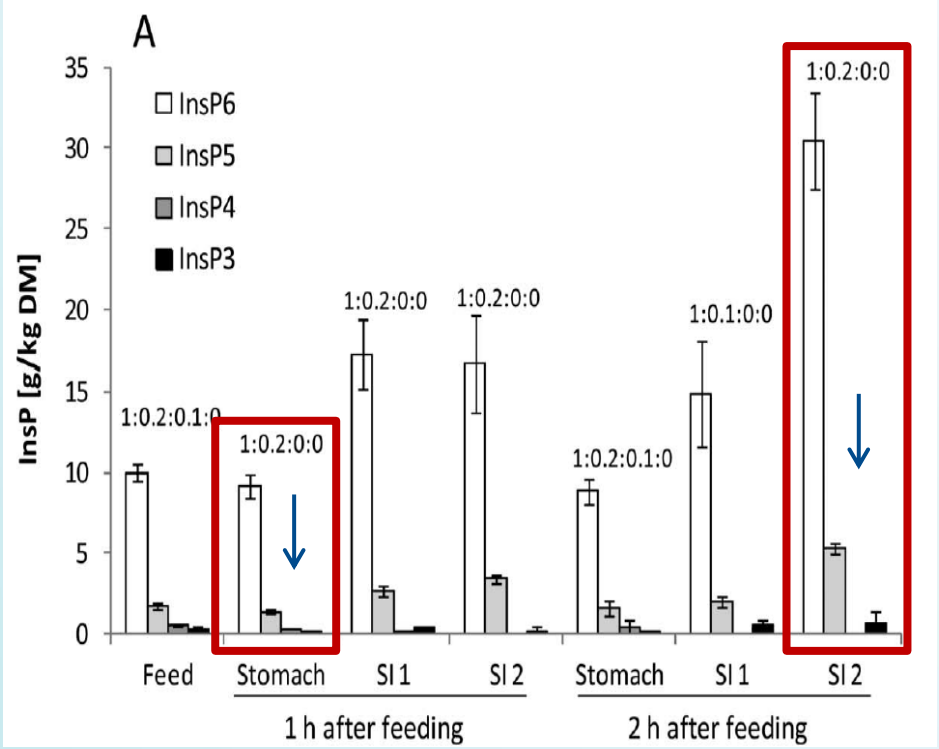


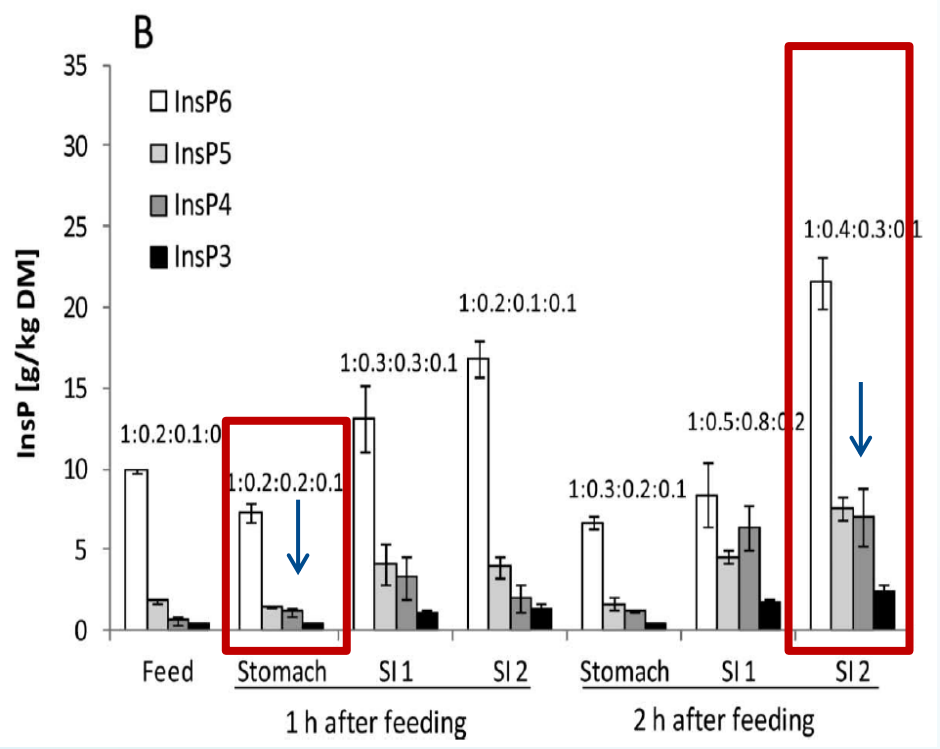
Xu, J. Price, A. Wise and P.J. Aggett, "Interaction of Inositol Phosphates with Calcium, Zinc, and Histidine," J. Inorgonk Biochemirt, 1992, 41,119- 130

Supplementation with a novel *C. braakii* phytase: Results in a 'pool' of IP4

No phytase



Phytase (500 FTU)



Pontopiddan et al 2012. 25kg pigs fed 0 or 500 FTU HiPhos and after starving for 24hrs then fed treats for 30 mins, slaughtered 1 and 2 hours post feed. A=control, B =500FTU/kg

Pontoppidan et al., 2012

If it is present and soluble in the stomach, it will ppt with something in the SI

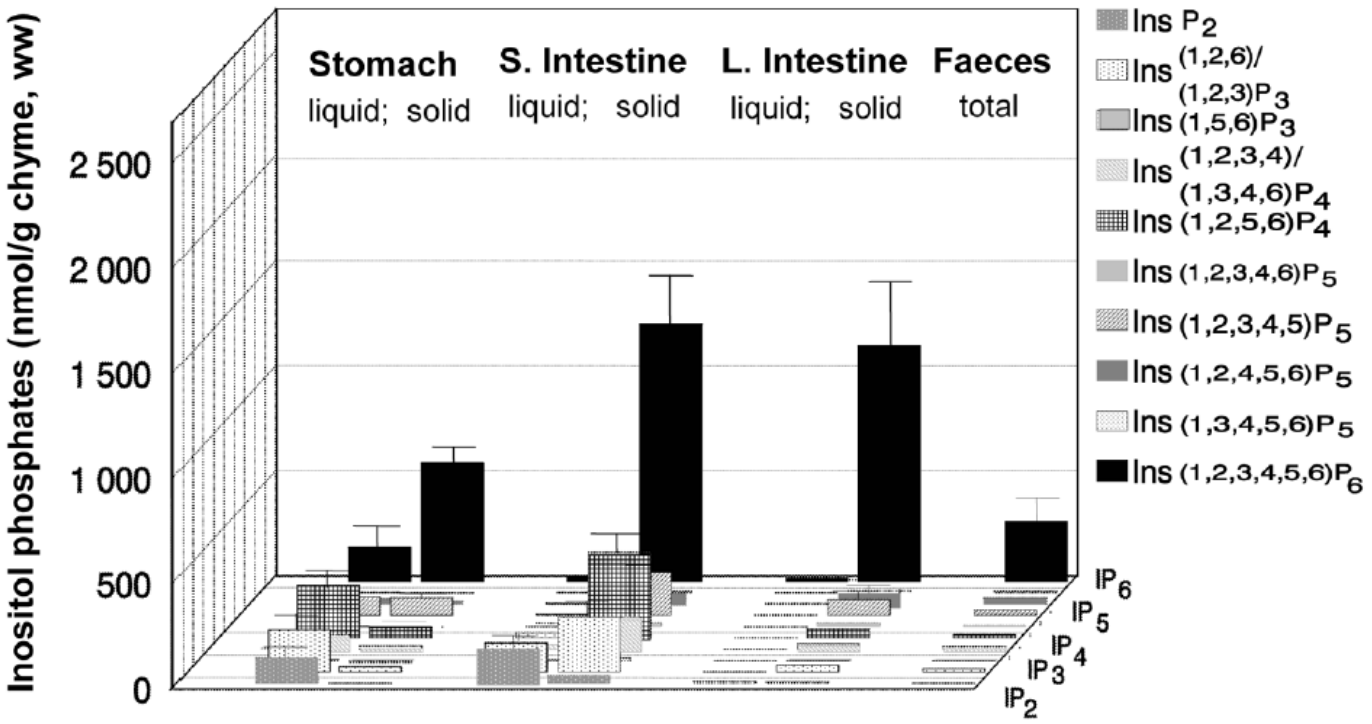


Figure 2. Hydrolysis of phytate from a diet rich in intrinsic feed phytases during the passage throughout the stomach, small intestine and large intestine as well as in the faeces of pigs 5 h after feeding [159]. Inositol phosphates are listed from the front to the back in the following row:
InsP_2, Ins(1,2,3)P_3/Ins(1,2,6)P_3, Ins(1,5,6)P_3, Ins(1,2,3,4)P_4/Ins(1,3,4,6)P_4, Ins(1,2,5,6)P_4, Ins(1,2,3,4,6)P_5, Ins(1,2,3,4,5)P_5, Ins(1,2,4,5,6)P_5, Ins(1,3,4,5,6)P_5 and InsP_6.

Schlemmer 2001

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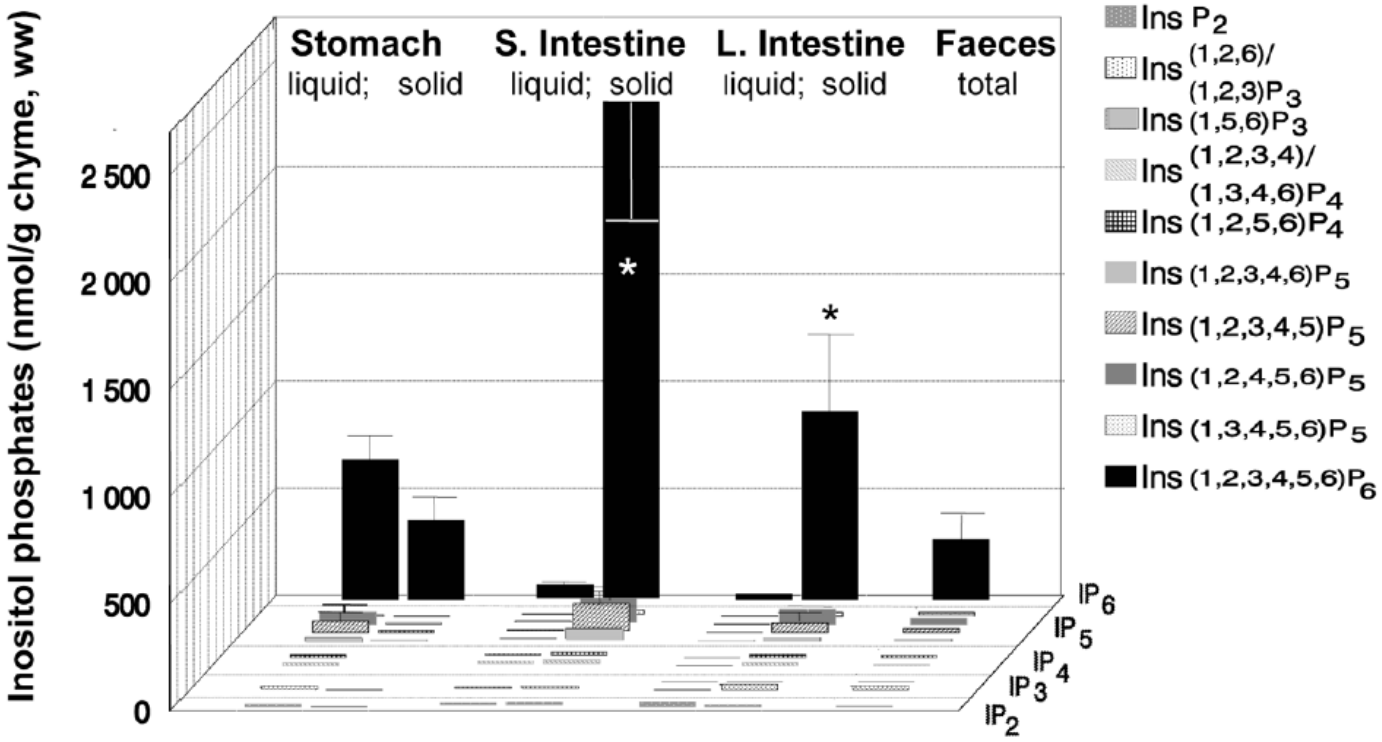


Figure 3. Hydrolysis of phytate from an extruded diet with inactivated phytases, during the gastro-intestinal passage throughout the stomach, small intestine and large intestine as well as in the faeces of pigs 5 h after feeding [159]. *InsP_6 concentrations were different ($p < 0.05$). Inositol phosphates are listed from the front to the back in the following row: InsP_2, Ins(1,2,3)P_3/Ins(1,2,6)P_3, Ins(1,5,6)P_3, Ins(1,2,3,4)P_4/Ins(1,3,4,6)P_4, Ins(1,2,5,6)P_4, Ins(1,2,3,4,6)P_5, Ins(1,2,3,4,5)P_5, Ins(1,2,4,5,6)P_5, Ins(1,3,4,5,6)P_5 and InsP_6.

Schlemmer 2001

IP4 AND IP3 ARE NOT INNOCUOUS

Ester	Nutrient	R	P value	n
Ileal iP3	Ileal AME, kcal	-0.849	<0.0001	40
	Ileal DM digestibility	-0.848	<0.0001	40
	Ileal N digestibility	-0.693	<0.0001	40
	Ileal Na digestibility	-0.675	<0.0001	40
Ileal iP4	Ileal Mg digestibility	-0.688	<0.0001	40
	Ileal Fe digestibility	-0.606	<0.0001	40

Beeson, 2016

IP3 and IP4 hydrolysis may have been overlooked

- What about provision of inositol??

Inositol Provision
Zyla et al 2004

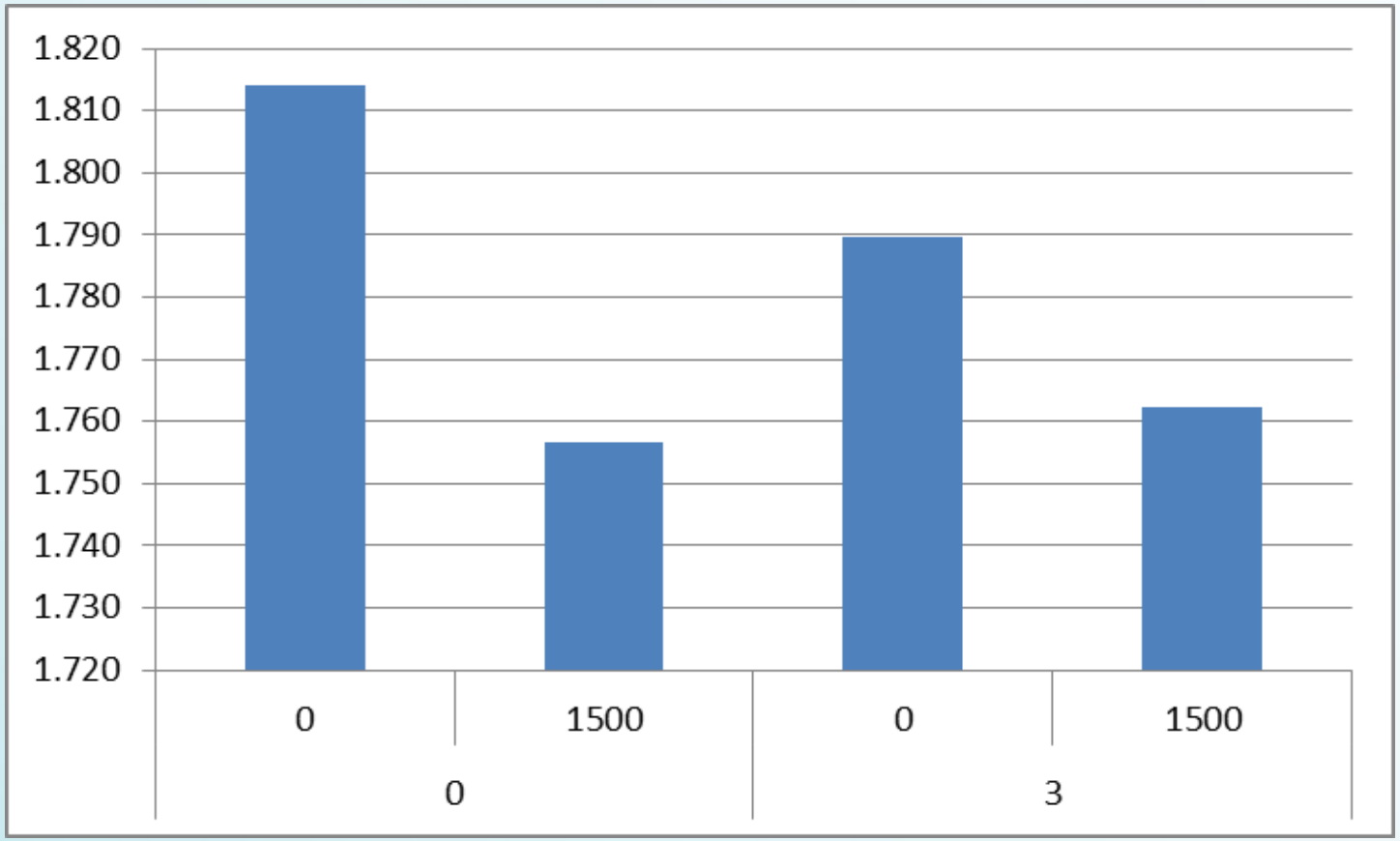
Myo-inositol	None	0.10%	p value
Intake	838	861	NS
Gain	537	579	**0.0199**
FCR	1.57	1.47	**0.0573**
Toe ash %	11.9	12.2	NS
P retention	56	50	**0.0015**
Ca retention	61	59	NS

Broilers, 1-21d post hatch, main effects on 0.65/0.27 and 0.8/0.47 Ca AvP diets

Inositol interacts with phytase

It is likely part of the superdosing effect

- FCR
  - Inositol* Phytase interaction p<0.0143
  - LSD = 0.017



Grower Pigs - SD reduces IP4 and 3 and increases inositol in 37kg pigs

Table 1 Effect of dietary phytase level on ileal $InsP_{2-6}$ and MYO concentration (nmol/g DM).

Diet	MYO	$InsP_2$	$InsP_3$	$InsP_4$	$InsP_5$	$InsP_6$
PC	4,422	5,330	917	1,711	3,052	21,616
NC	4,309	5,142	657	1,204	2,950	25,189
500	7,729	5,634	1,236	2,658	2,075	15,217
1000	9,964	4,663	1,195	1,972	1,271	12,505
2000	12,812	5,841	1,172	1,771	987	11,038
8000	14,086	5,149	413	304	574	7,868
SEM	1,744	821	177	400	311	2,817
P value						
Linear	0.001	0.907	0.002	<0.001	<0.001	0.001
Quadratic	0.008	0.449	0.012	0.162	<0.001	0.007

Laird et al 2016

Grower Pigs - Increased ileal inositol correlates with increased plasma inositol - especially portal

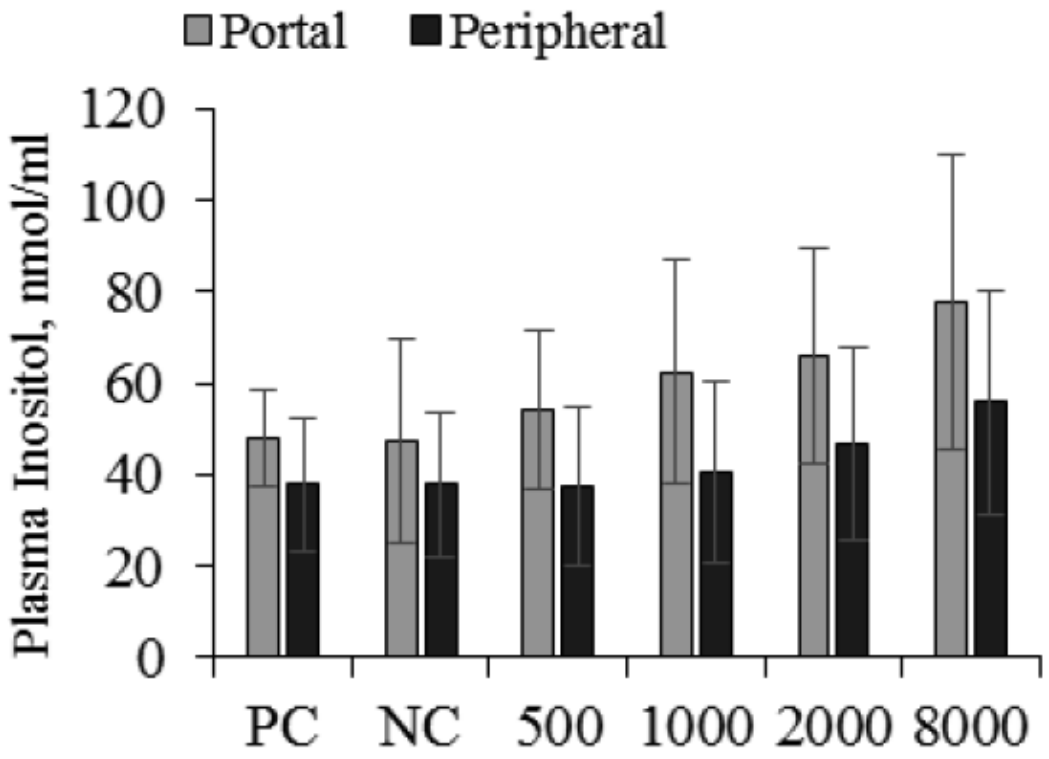


Figure 1 Effect of dietary phytase level on portal and peripheral plasma MYO concentration.

Laird et al 2016

Piglet IP6 in ileum

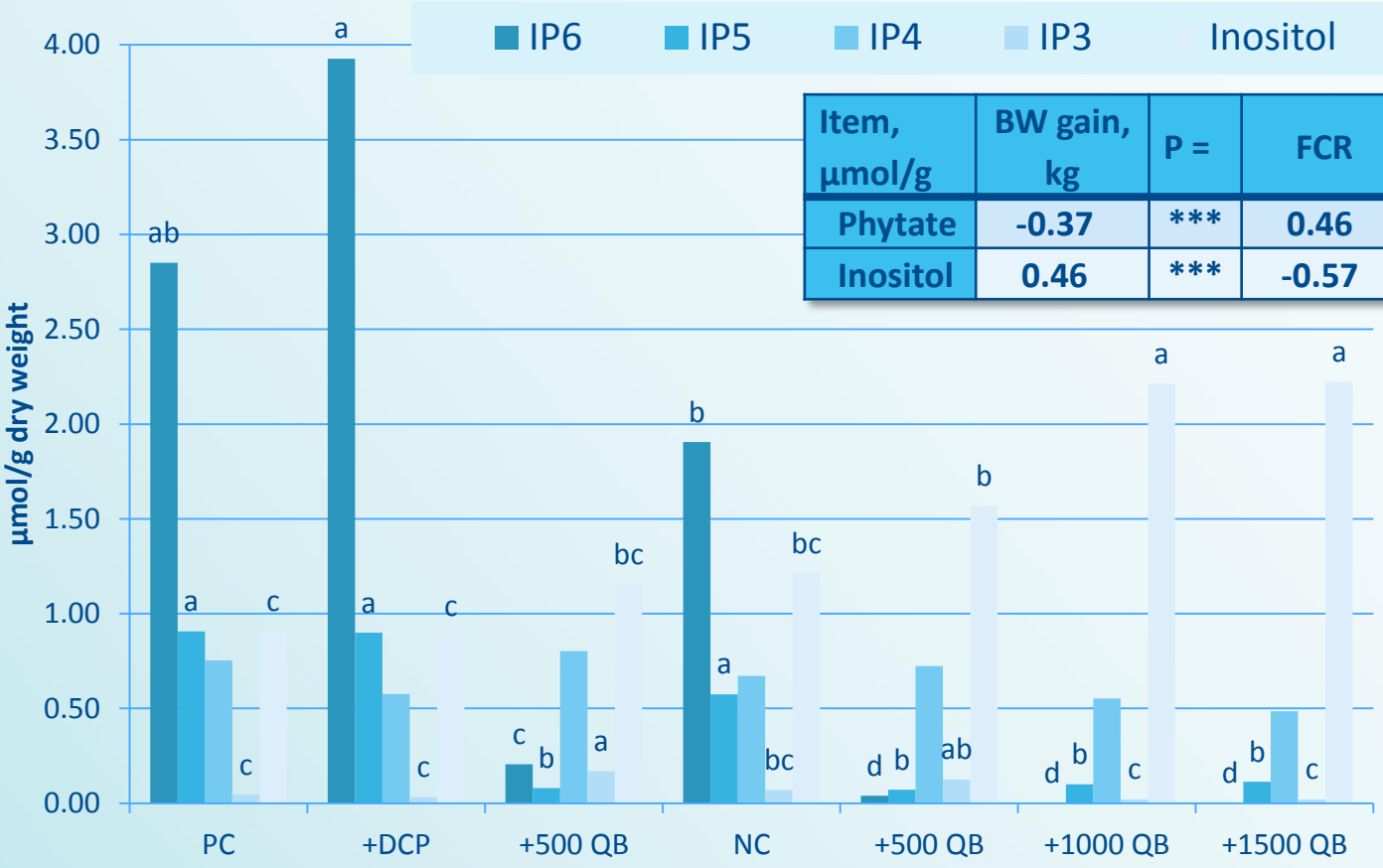
Treatment	Fe, mg/kg	PHY, FTU/kg	∑IP5 + IP6	IP6	IP5	IP4	IP3	IP2	Inositol
LO-0	110	0	28435	23985	4450	3620	1949	3272	3184
LO-500	110	500	14476	12307	2169	5200	3617	5293	6780
LO-2500	110	2500	6953	6267	797	1613	1428	4670	10297
HI-0	360	0	21033	17518	3515	5011	2668	3812	4305
HI-500	360	500	14849	12509	2341	4917	2837	3505	4314
HI-2500	360	2500	9865	8739	1125	2850	1767	4418	8795
SEM			2516	1896	329	769	454	568	1096
Main effects	Fe								
		110	16658	14186	2472	3478	2332	4412	6753
		360	15249	12922	2327	4259	2424	3912	5805
	SEM		*1494*	*1334*	*195*	*457*	*269*	*337*	*651*
	PHY	0	24734	20751^{a}	3983^{a}	4316^{a}	2308^{ab}	3542	3744^{a}
		500	14662	12408^{b}	2255^{b}	5059^{a}	3227^{a}	4399	5547^{a}
		2500	8464	7654^{b}	961^{c}	2231^{b}	1598^{b}	4544	9546^{b}
	SEM		*1779*	*1636*	*233*	*544*	*322*	*402*	*775*
P-value									
	Fe		0.483	0.480	0.578	0.220	0.808	0.263	0.295
	PHY		<0.001	<0.001	<0.001	0.002	0.004	0.174	<0.001
	Fe x PHY		0.128	0.140	0.132	0.496	0.245	0.130	0.253

Extra-phosphoric effects of phytase

Broiler gizzard phytate, phytate ester and inositol concentration (d21)

Superdosing Quantum Blue decreased phytate and increased inositol concentration

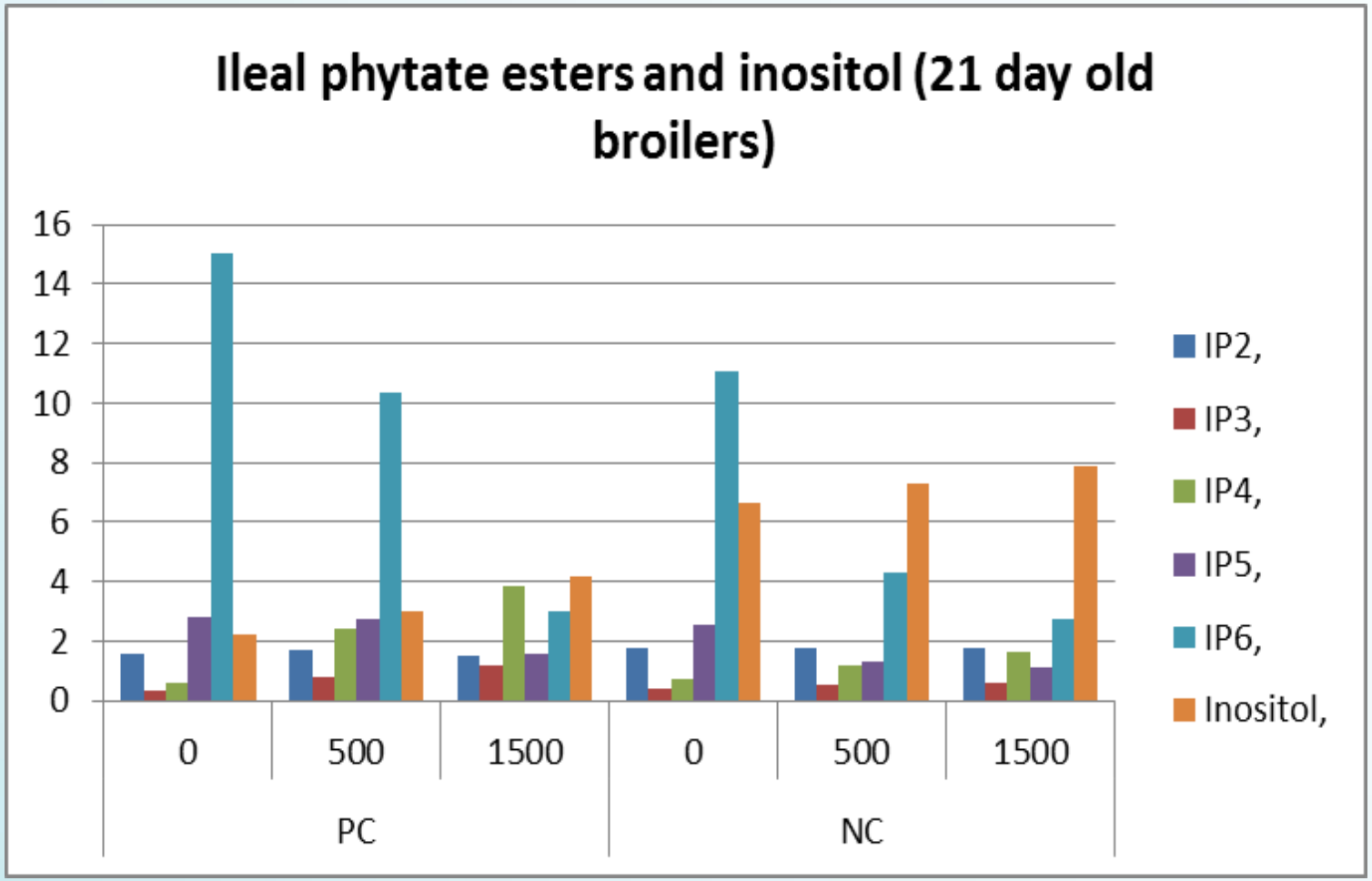
Part of the superdosing response may be associated with inositol provision as well as phytate destruction



Item, µmol/g	BW gain, kg	P =	FCR	P =	Tibia ash, g	P =
Phytate	-0.37	***	0.46	***	-0.21	NS
Inositol	0.46	***	-0.57	***	0.19	NS

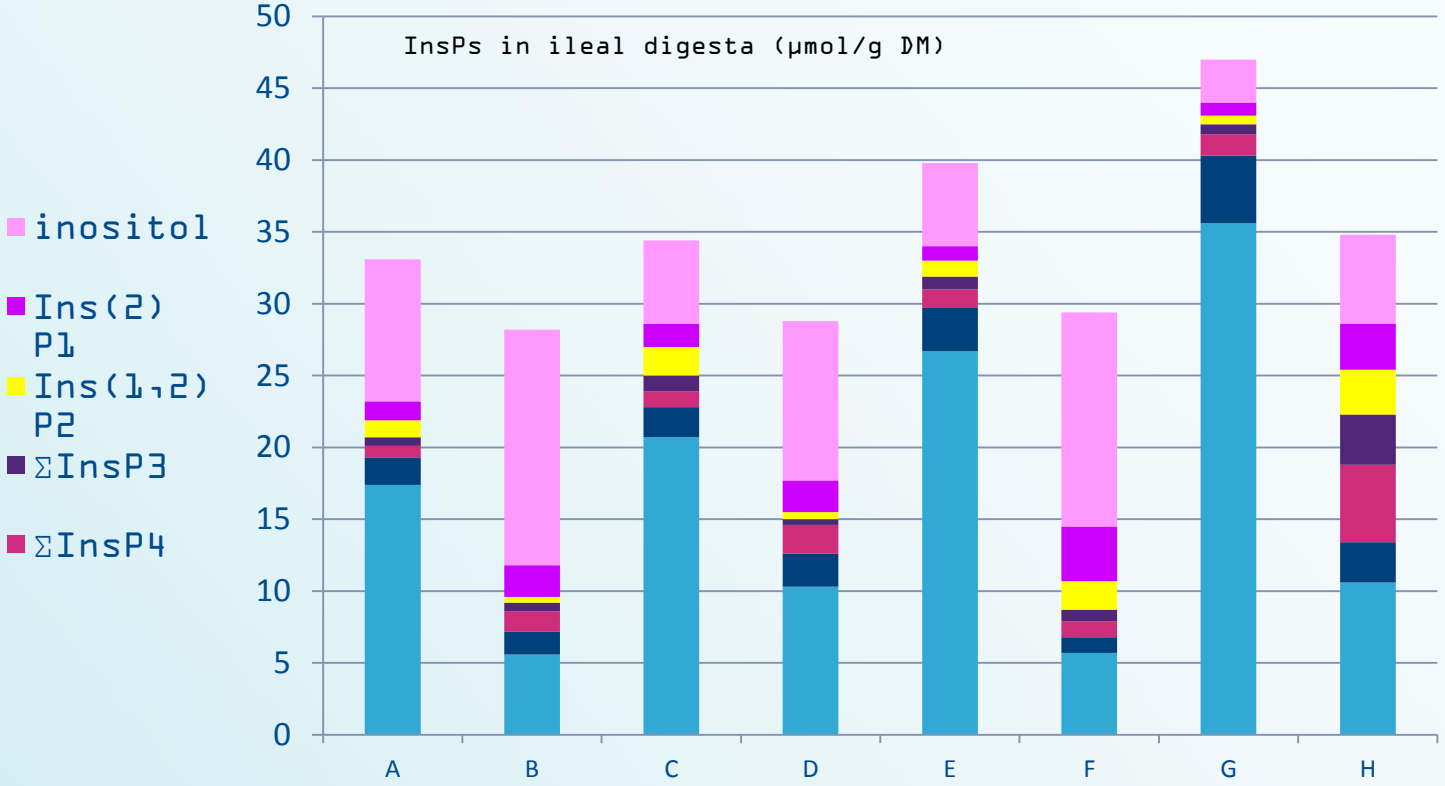
Walk et al., 2014

Phytase more effective in low Ca diets



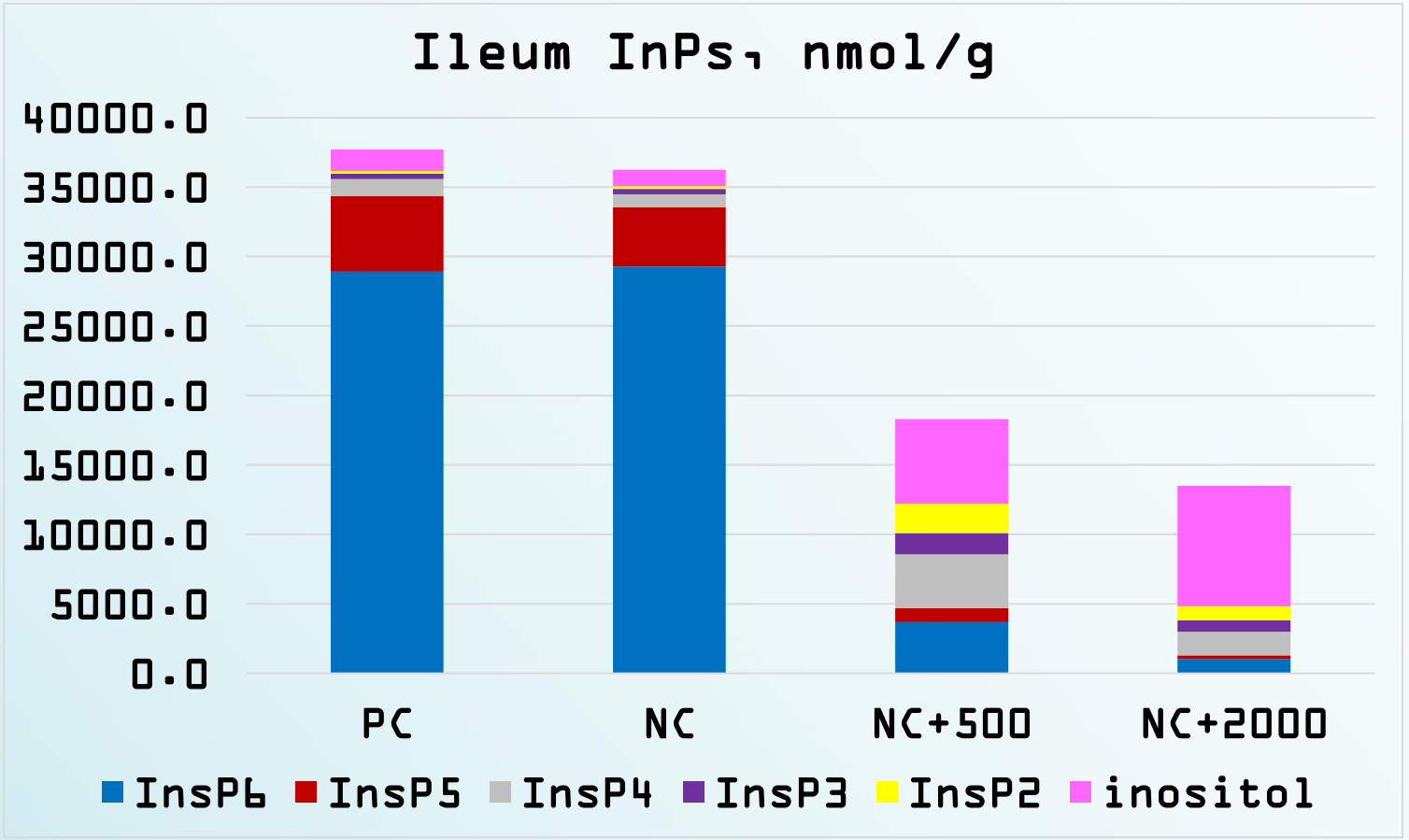
Beeson et al 2016 (QBA 07) Mixed wheat/CS diet. NC 500 matrix on Ca, P and Na

Influence of dietary Ca and P on InsP hydrolysis in broiler ileum



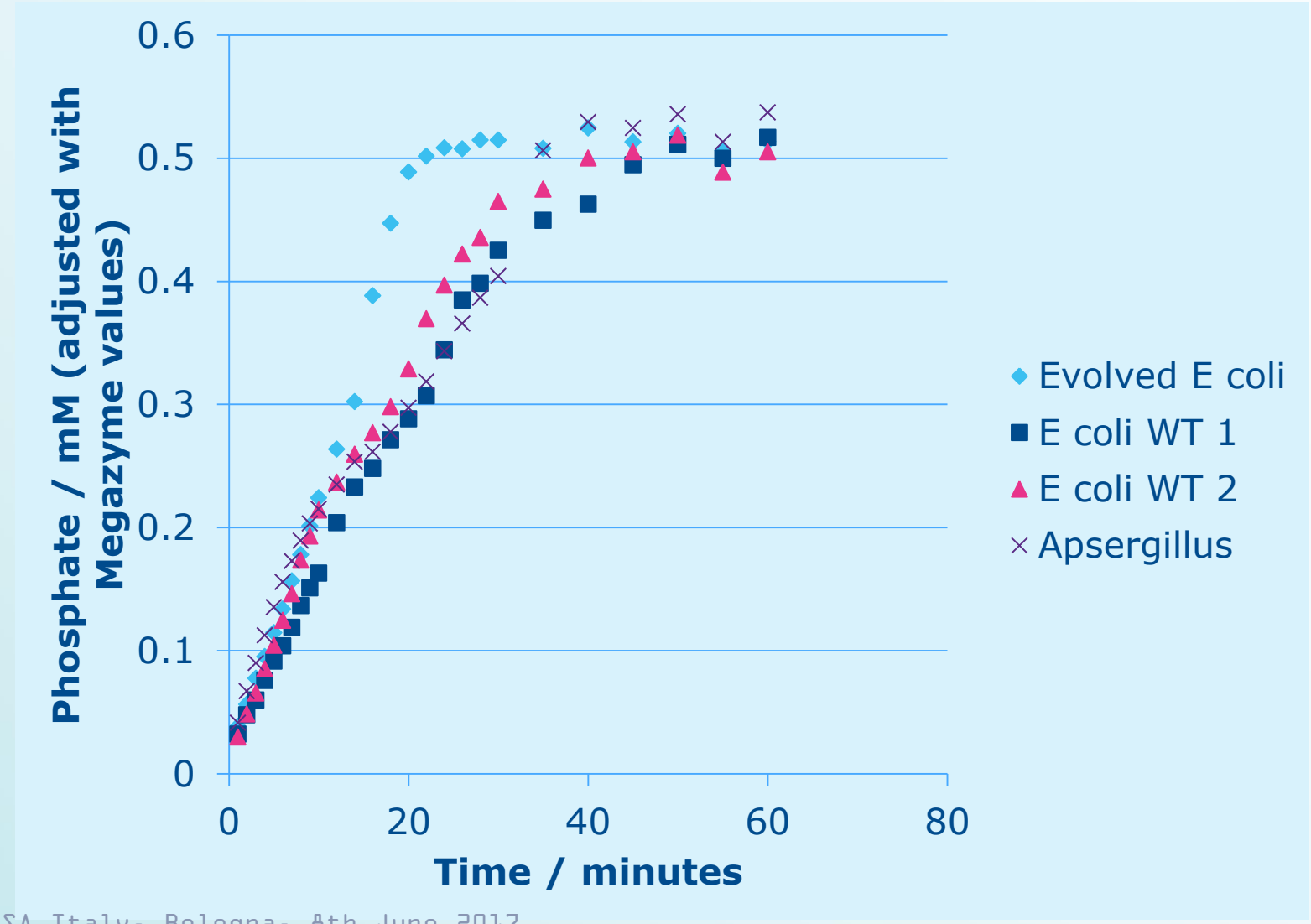
A	B	C	D	E	F	G	H
-	-	-	-	P	P	P	P
-	-	Ca	Ca	-	-	Ca	Ca
-	Phy1500	-	Phy1500	-	Phy1500	-	Phy1500

Sommerfeld et al 2016
Ca 5.4 or 9g/1kg
P 3.6 or 6g/kg

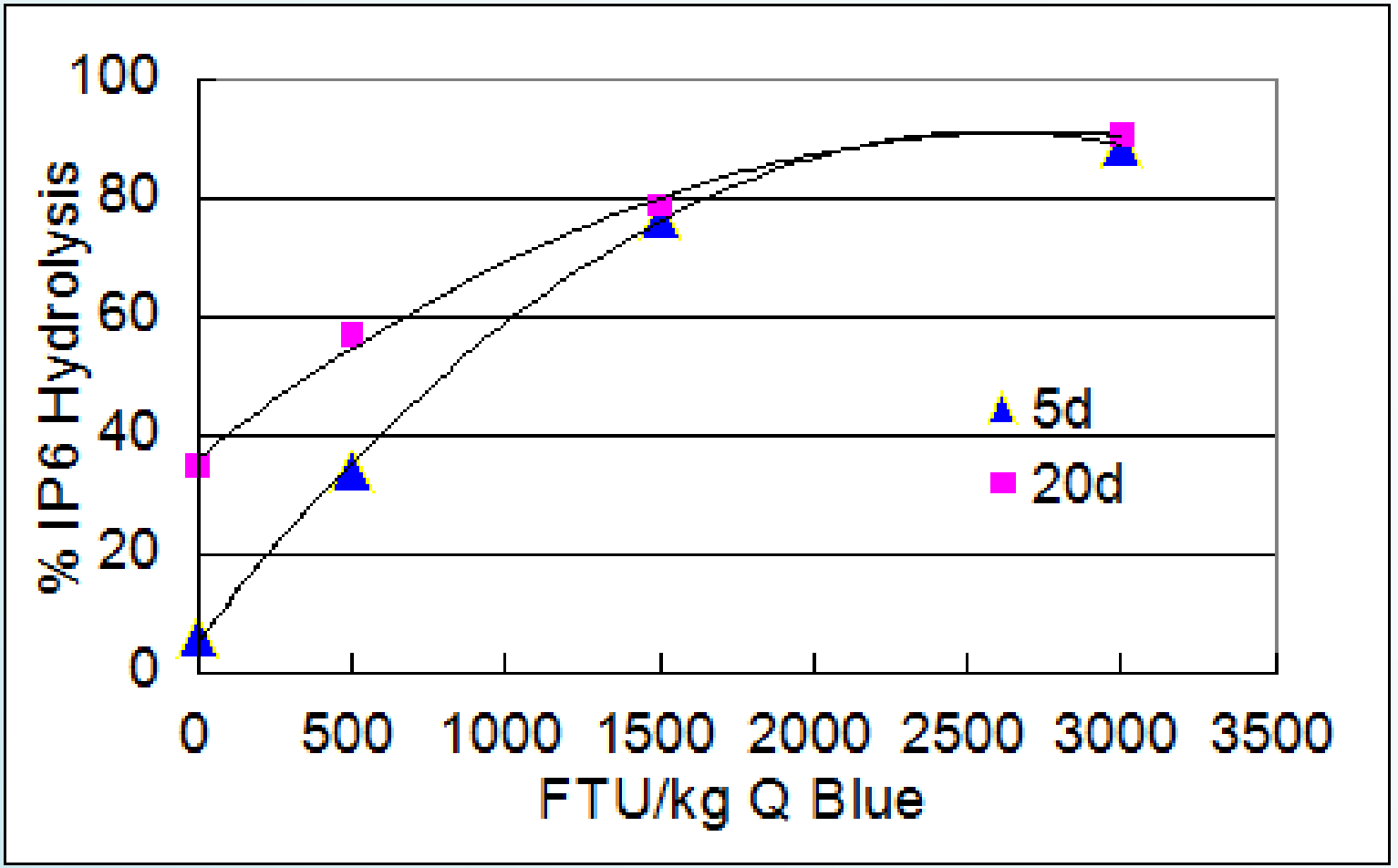

Ileum InPs, nmol/g
40000.0
35000.0
30000.0
25000.0
20000.0
15000.0
10000.0
5000.0
0.0
PC
NC
NC+500
NC+2000
InsP6
InsP5
InsP4
InsP3
InsP2
inositol

SUPERDOSING
OTHER FACTORS TO CONSIDER

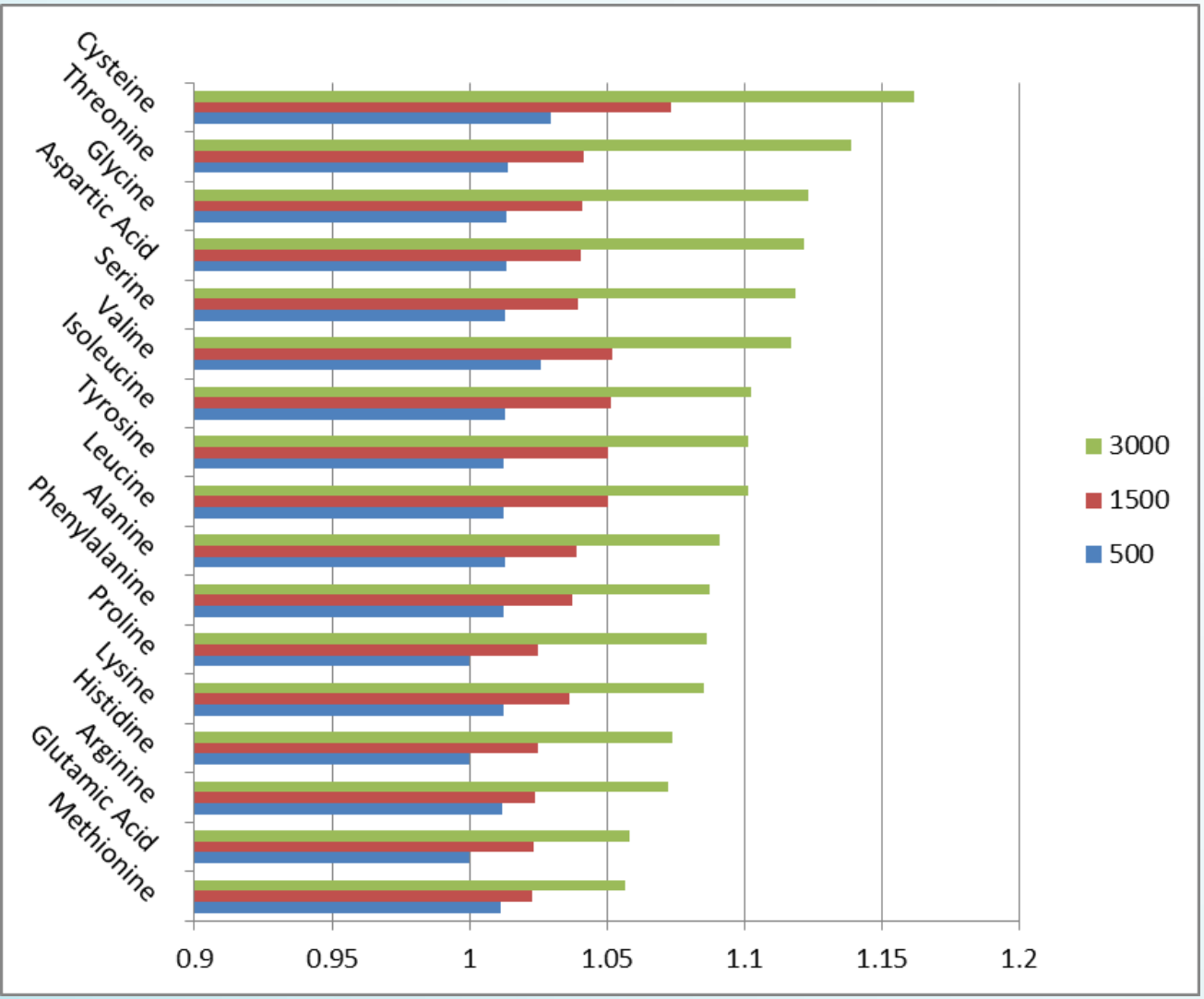
Phytases differ in appetite for lower IP esters



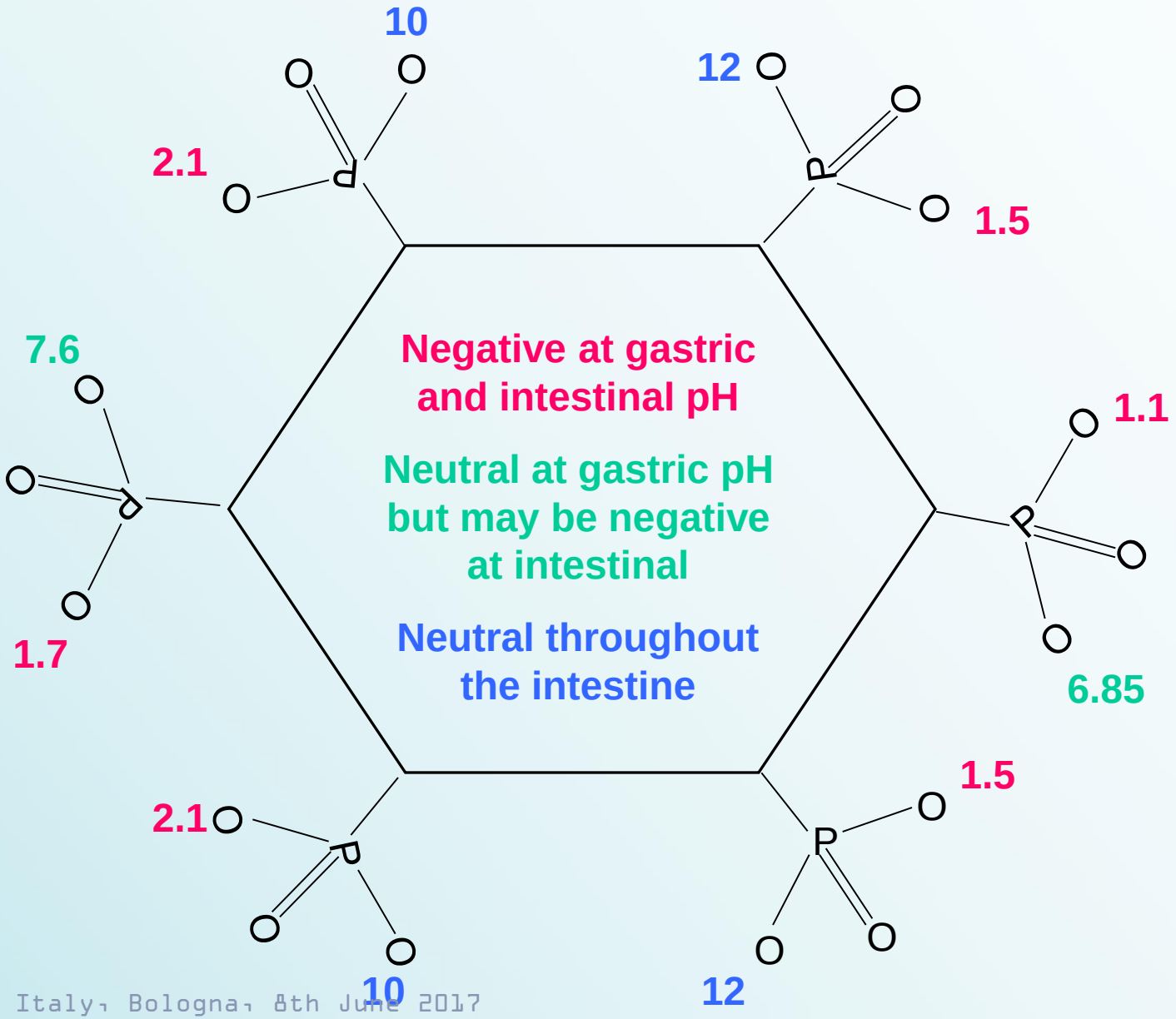
Beaulac et al 2016



Beaulac et al 2015

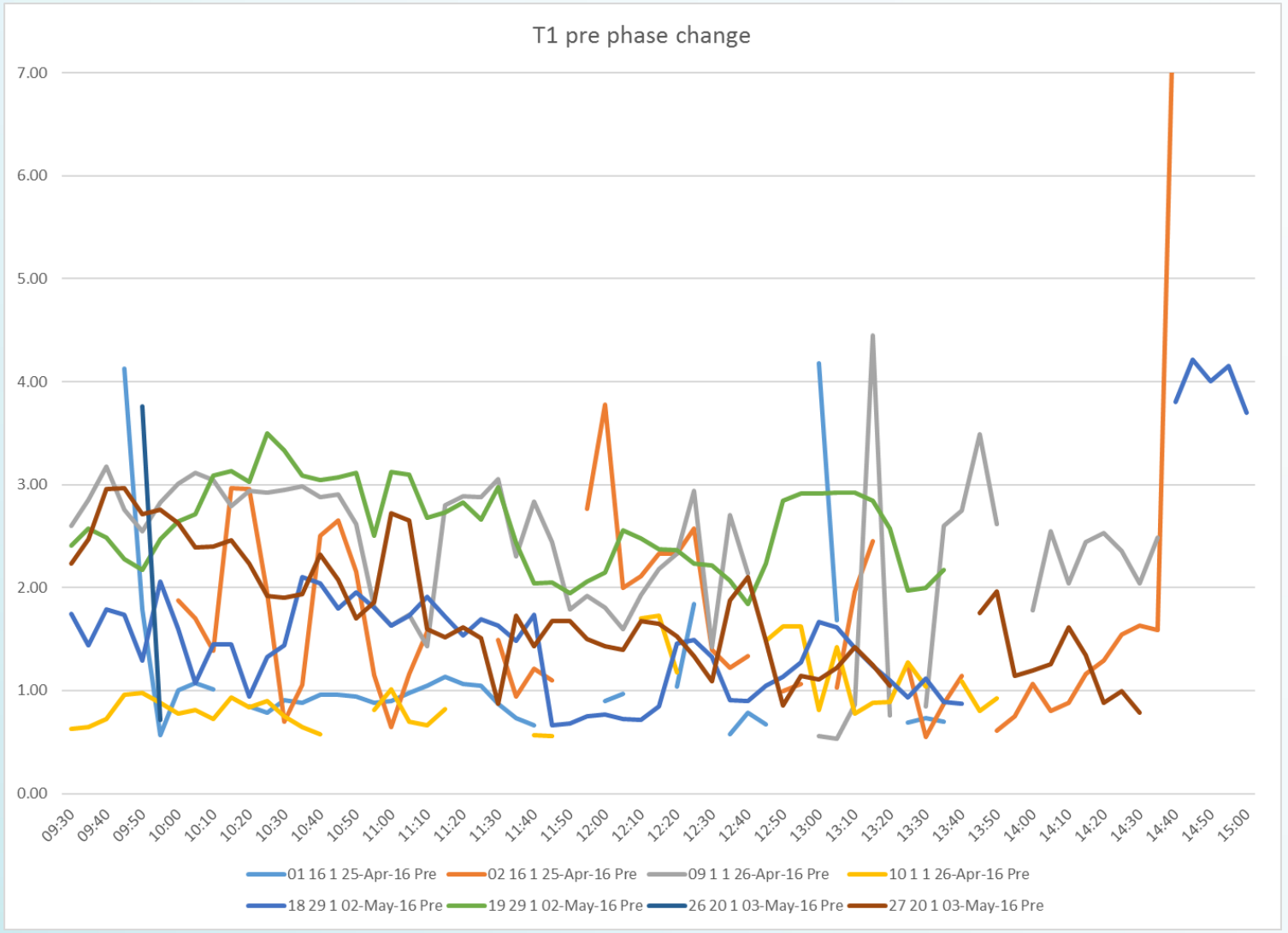


pKa Charges



A. J. R. Costello, T. Glonek, and T. C. Myers (1976). 31P nuclear magnetic resonance-pH titrations of myo-inositol hexaphosphate. Carbohydrate, 46:159-171.

pH of the Gizzard?



Considerations

- A phytate destruction response in performance efficiency is empirically related to IP4 and IP3 reduction.

1) Does focus on IP6 and IP5 target the problem incorrectly? IP4, IP3….??
2) More complete IP6→IP1 → Provision of inositol
3) Do pH shifts limit time for

Implications in the field
Trace minerals

Catfish mineral levels

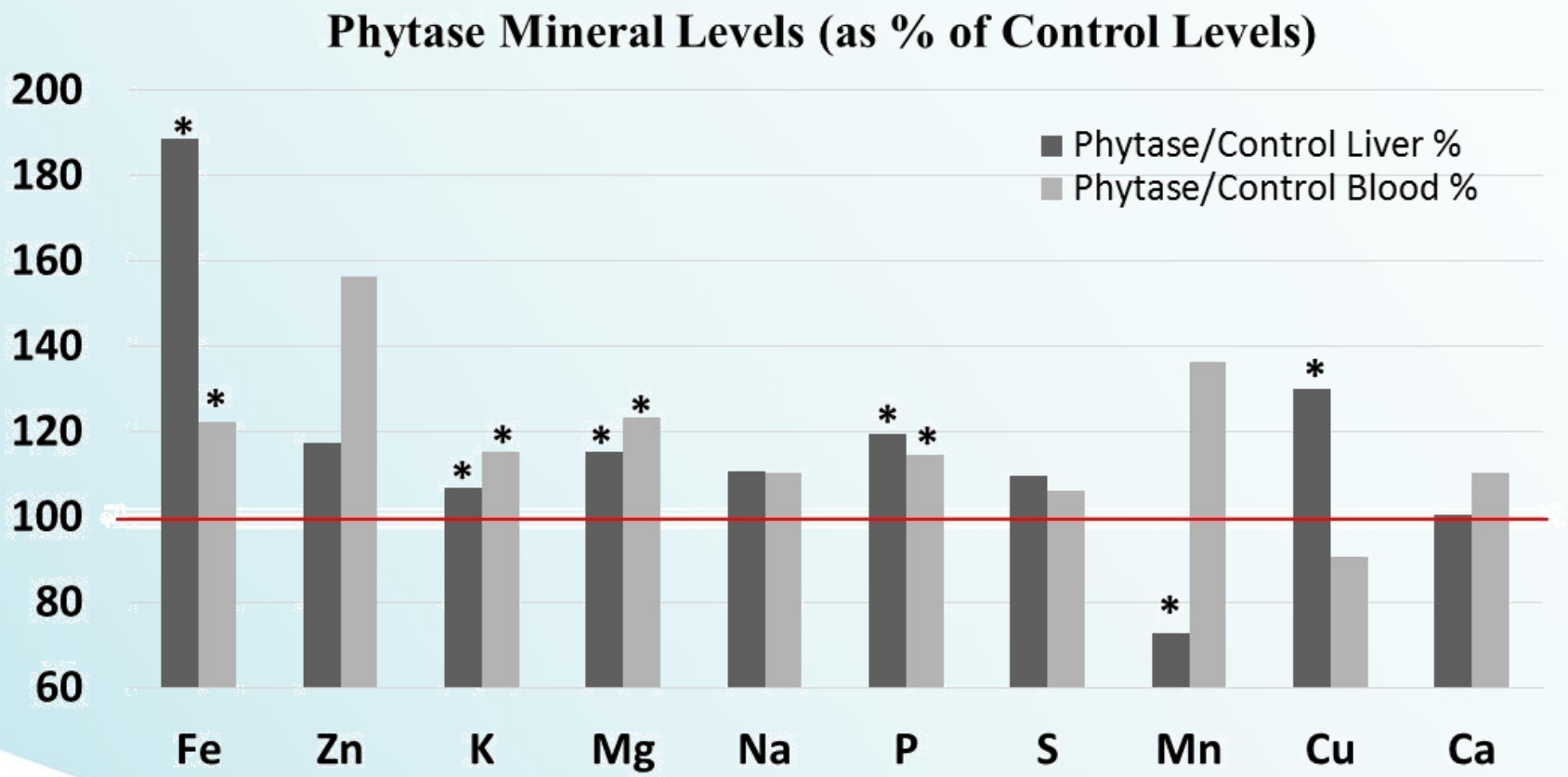
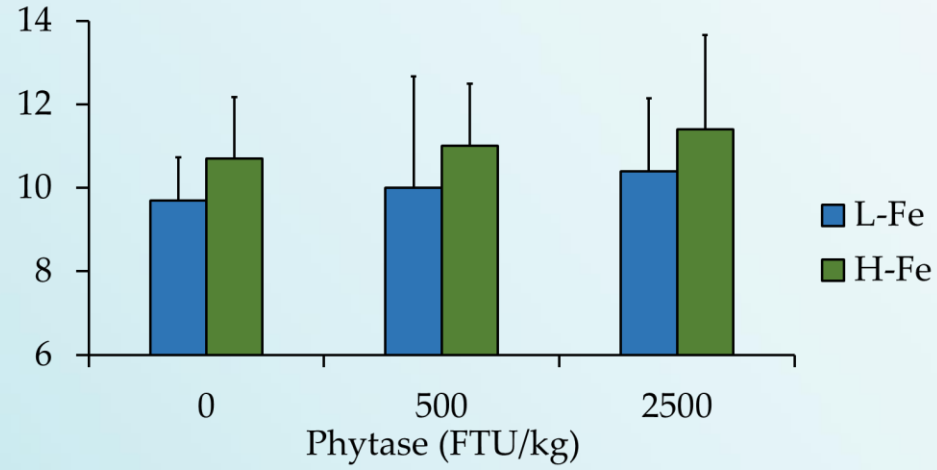
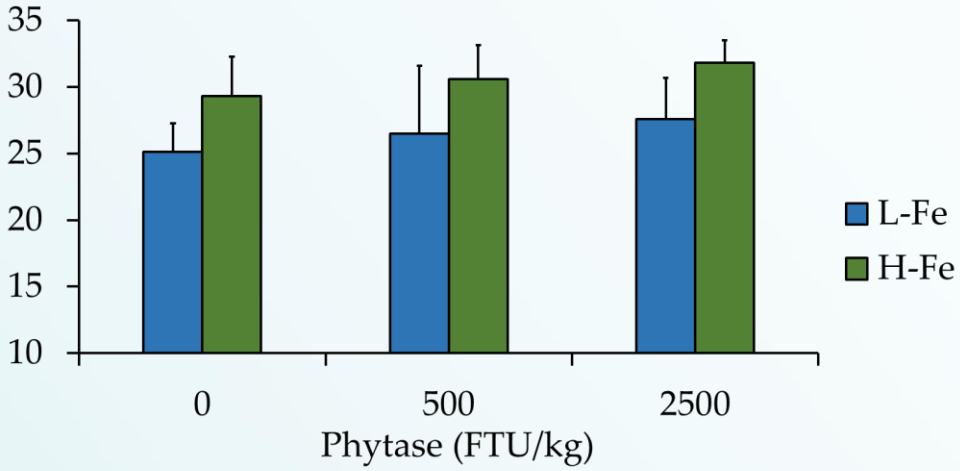


Fig. 2. ICP analysis of liver and blood mineral levels in catfish fingerlings fed a 28% protein control diet or a 28% control diet supplemented with 2500 FTU/kg of phytase for 12 weeks. Data is presented as phytase-fed fish mineral levels as percentage of control diet fish mineral values with the red line noting the 100% level. Asterisks denote statistical significance at the $p<0.05$ level

Effect of Fe and QB on blood parameters in pigs



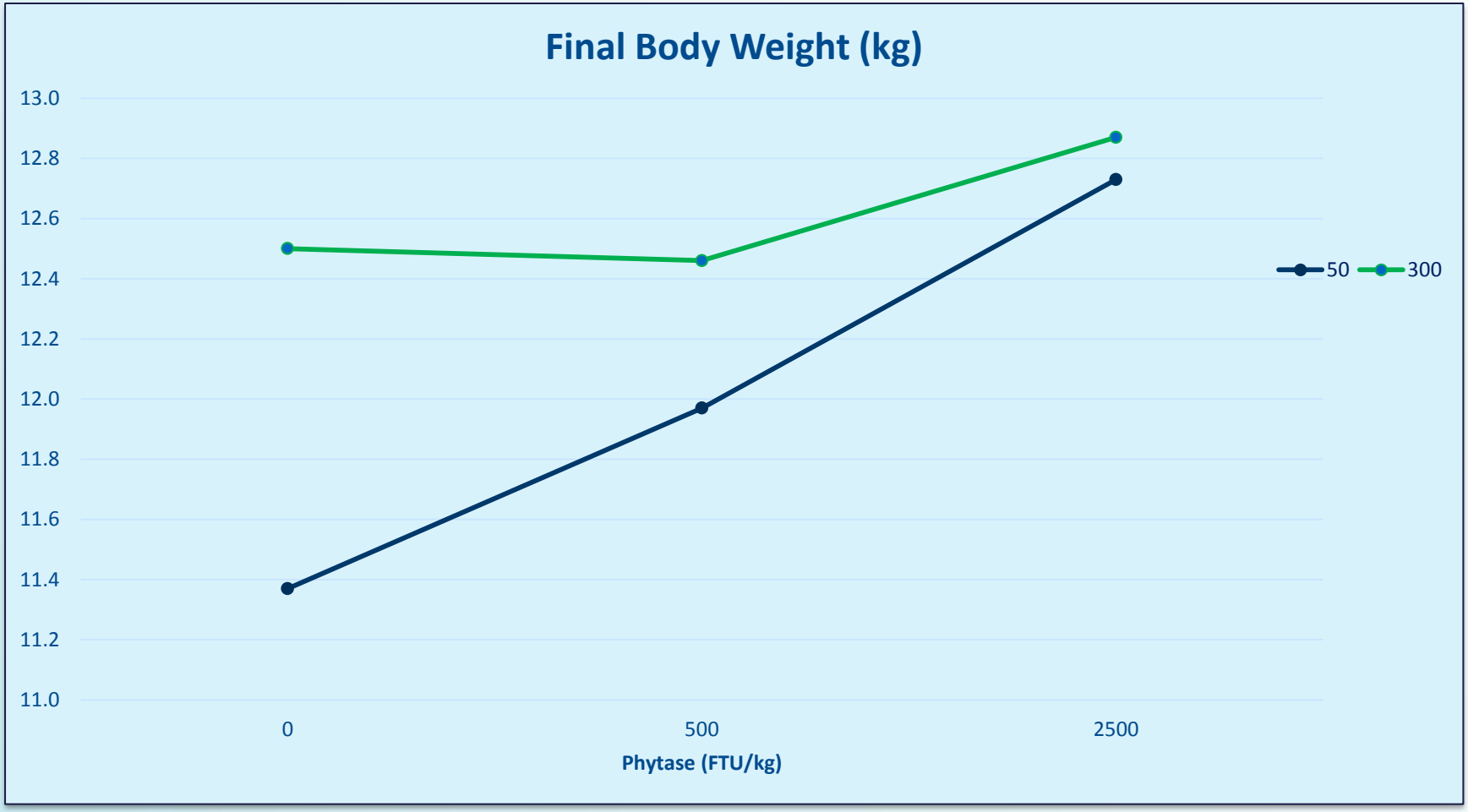
Effect of phytase and Fe supplementation on Hb concentration (g/l)



Effect of phytase and Fe supplementation on haematocrit %

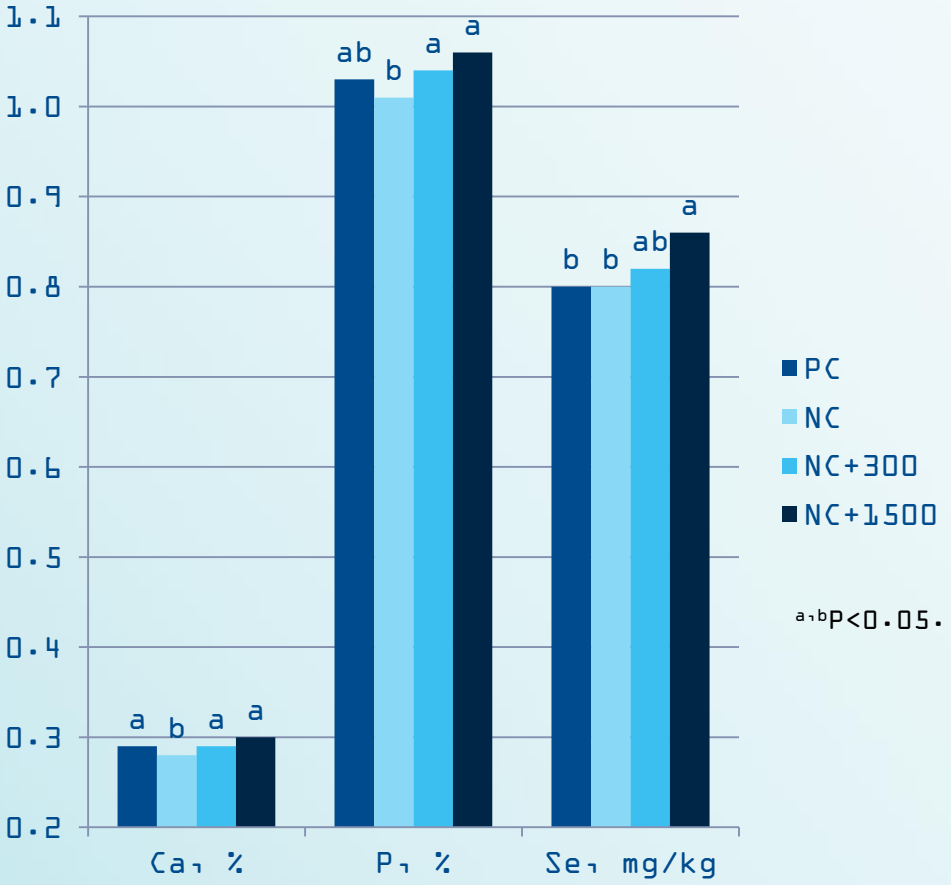
Laird et al 2017 BSAS

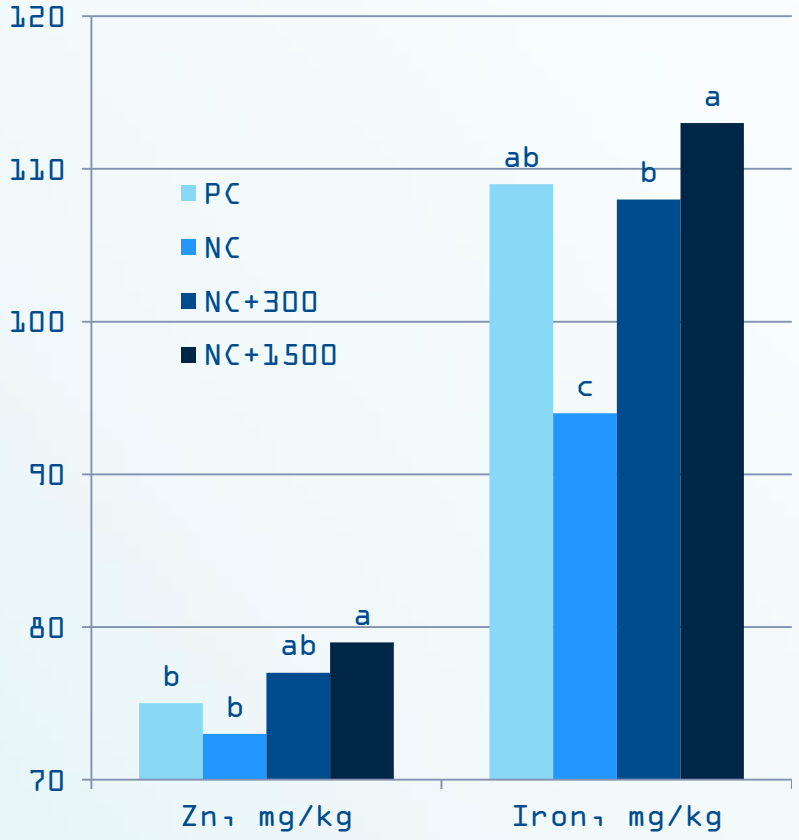
Effect of Fe and Blue on piglet performance



Laird et al , unpublished

Superdosing New Generation phytase:
Mineral deposition in the yolk





- QB superdosing increases mineral deposition in the yolk
- Se and Zn levels of NC+1500FTU/kg were higher than birds fed PC diets.

Increase tissue mineralization

Yolk minerals from 31 to 78 weeks of lay

Treatment	P (%)	Ca (%)	Fe (mg/kg)	Zn (mg/kg)	Se (mg/kg)
PC	1.02^{b}	0.29^{a}	107^{b}	74^{bc}	0.78^{b}
NC	1.01^{b}	0.27^{b}	94^{c}	72^{c}	0.78^{b}
NC+300 FTU QB	1.05^{ab}	0.29^{a}	109^{ab}	78^{ab}	0.79^{ab}
NC+1500 FTU QB	1.07^{a}	0.30^{a}	113^{a}	79^{a}	0.84^{a}

Soto et al., 2013

Implications in the field
Woody breast

Woody Breast: What's causing it?

Relationship between pectoralis major muscle histology and quality traits of chicken meat

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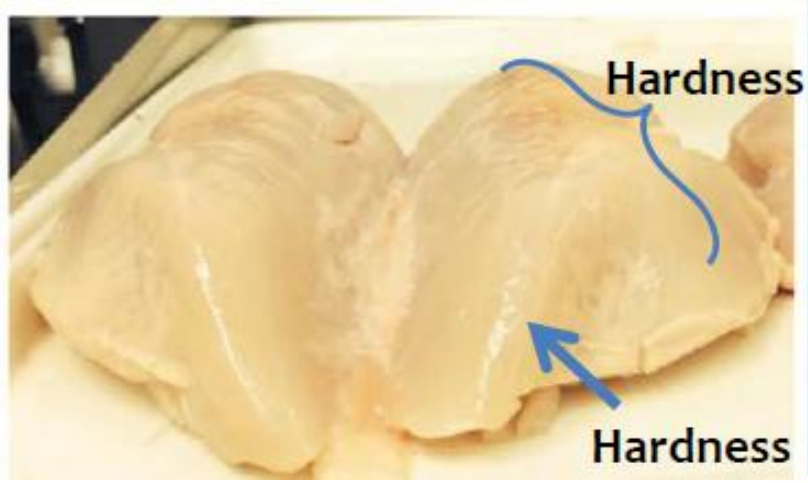
[*]*Department of Agricultural and Food Sciences, Alma Mater Studiorum–University of Bologna, Ozzano dell'Emilia (BO), Italy 40064; and* [†]*Department of Veterinary Medical Sciences, Alma Mater Studiorum–University of Bologna, Ozzano dell'Emilia (BO), Italy 40064*

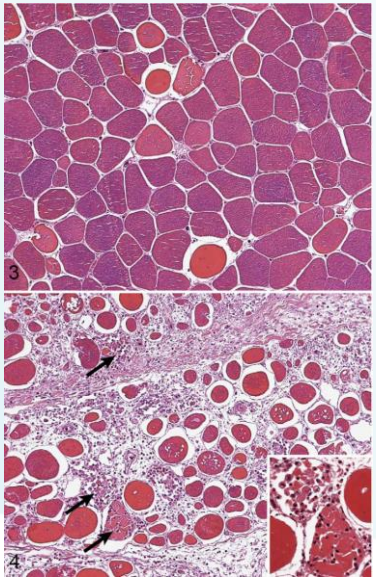
2015 Poultry Science 94:123–130
http://dx.doi.org/10.3382/ps/peu043

malities (Sihvo et al., 2014). The presence of T lymphocytes (confirmed by using of the specific chicken antibody) together with the aforementioned fibrosis and necrosis has confirmed that this is a chronic inflammatory process, which probably represents the undesired result of genetic pressure for increased growth rate of breast muscle.

WOODEN BREAST: HYPOTHESIS

- Muscle appears to have a reduced capillary blood supply, leading to necrosis of the muscle and macrophage infiltration. In response to necrosis, fibrosis takes place, leading to replacement of muscle specific protein with highly cross-linked collage, giving it the 'wooden appearance". (S. Velleman, 2016)





University of Delaware

"What we found is that there may be **localized hypoxia – a lower oxygen concentration** in the affected tissues. In addition, our findings strongly suggest **presence of oxidative stress** – ***when free radicals build up and there aren't enough antioxidants to detoxify them*** – as well as an increase in calcium in the tissue cells."

"There were lots of similarities in the results of this work and the gene expression work that really confirmed each other," Abasht said. "**The results confirmed that there's oxidative stress in affected muscles.**"

B. Abasht, Dept. of Animal and Food Sciences

– Identified biomarkers for this disorder

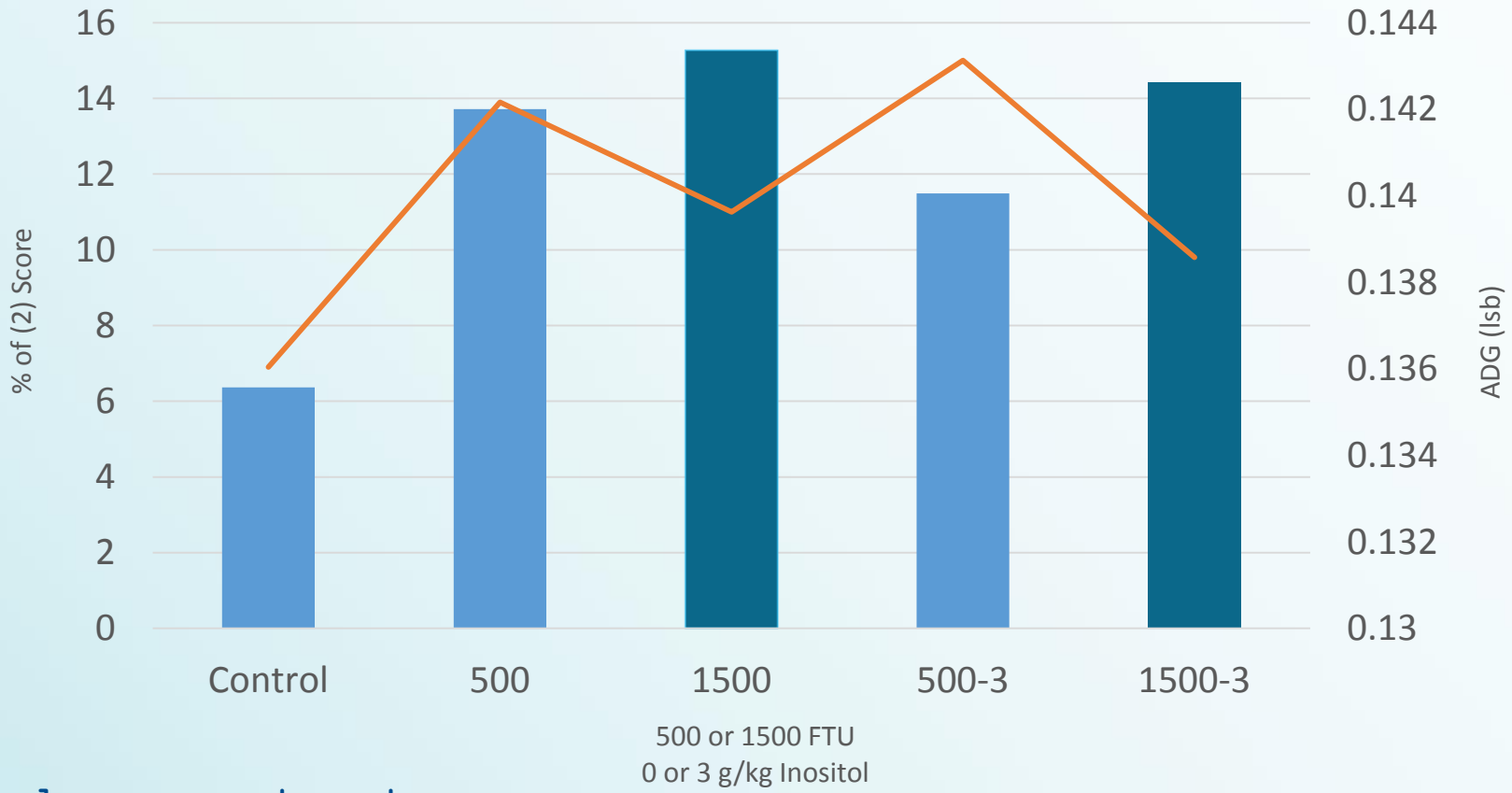
Article, Aug. 15th, 2016 by Adam Thomas CANR News, Research

Initial Research

- Ross 308 Male broilers
- Phytase x Inositol
  - 500, 1500 FTU/kg Phytase
  - 0, 3 g/kg Food Grade Inositol
- 49 days of age

Sirri and Walk, 2014, unpublished

D49 ADG *vs* Severe Form Woody Breast



Control was a phosphorus deficient diet, slower growth expected.

Sirri and Walk, 2014, unpublished

USA trial

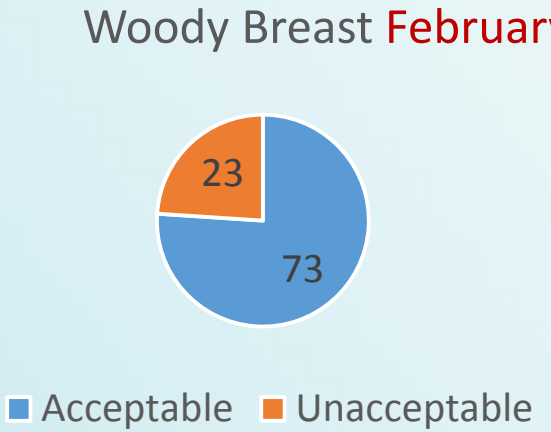
			Day 18				Day 42			
Treatment	Matrix	Phytase (ftu)	Body wt, g	Mort FCR	Liv, %		Body wt, g	Mort FCR	Wt corr FCR	Liv., %
1	Mineral	0	584	1.377	95		3055	1.527	1.533	88.6
2	Mineral	500	597	1.384	94.3		3086	1.492	1.489	87.1
3	Mineral	1500	619	1.328	96.4		3177	1.499	1.467	85
4	Full- AA	0	589	1.375	96.4		3042	1.545	1.555	89.3
5	Full- AA	500	609	1.348	97.1		3171	1.503	1.473	90.7
6	Full- AA	1500	613	1.323	96.4		3162	1.490	1.463	87.1
							32g = 0.01 pts for 3075			

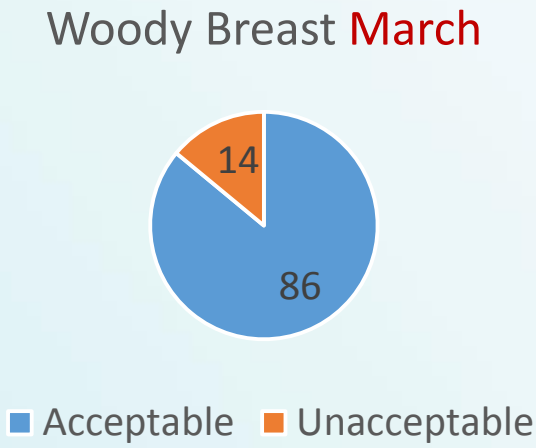
			Day 46								
Treatment	Matrix	Phytase (ftu)	Body wt, g	Mort FCR	Wt corr FCR	Liv., %	Carcass Yield, %	Breast wt, g	Breast yield, %	White meat yield, %	Hemacrit values, %
1	Mineral	0	3466	1.608	1.619	86.4	78.4	886	22.9	27.25	24.99
2	Mineral	500	3475	1.565	1.573	85	78.5	923	23.6	28.1	25.03
3	Mineral	1500	3605	1.566	1.533	83.6	78.5	908	23.2	27.6	26.39
4	Full- AA	0	3486	1.608	1.612	85	78.3	887	22.6	26.9	25.99
5	Full- AA	500	3572	1.57	1.548	88.5	78.4	880	22.5	26.7	28.30
6	Full- AA	1500	3589	1.59	1.562	85	79.3	907	22.7	27.05	26.74
					32g = 0.01 pts for 3500						

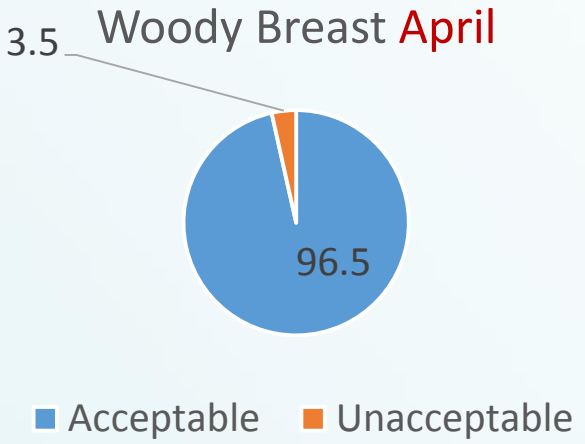
Severity of Woody Breast Reduced

8 wk Field Study

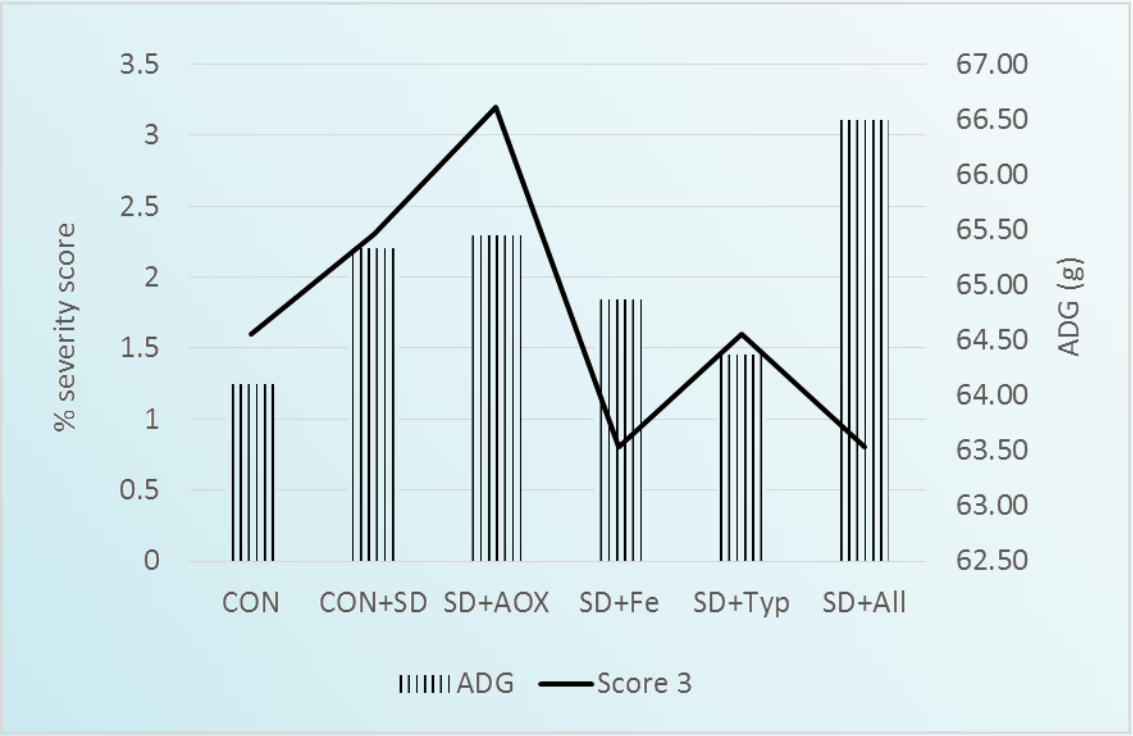
Superdosing Quantum Blue, Organic Zn, Ethoxyquin







CQR Woody Breast Evaluation



TRT	BW	FCR adj	% BMY
CON	8.62	1.624	32.05
CON +SD	8.79	1.600	32.60
SD + AOX	8.80	1.593	32.66
SD + Fe	8.72	1.598	32.81
SD + Tryp	8.66	1.636	32.34
SD + All	8.94	1.564	32.78

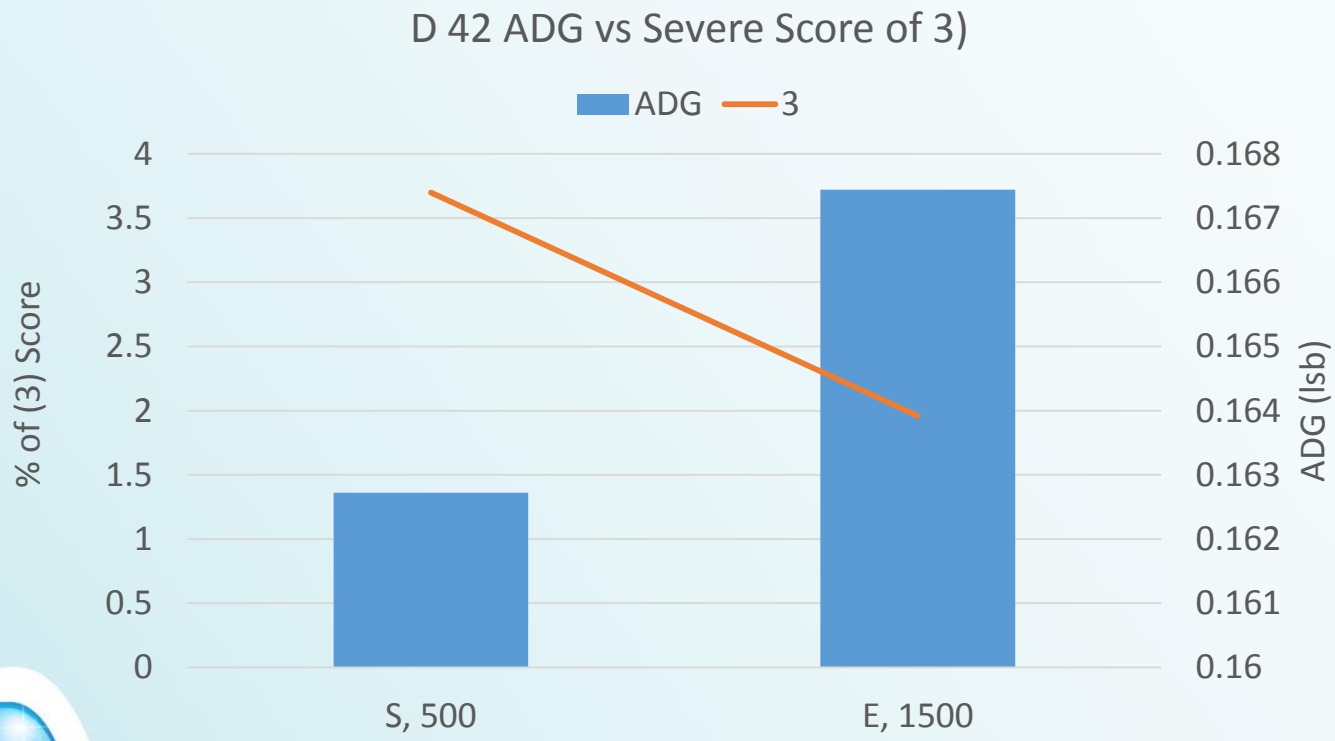
"SD + ALL TRT were the heaviest birds with the lowest FCR and highest ADG.

UARK Woody breast evaluation

- 500 or 1500 FTU Quantum Blue
- 0 or 125 ppm Ethoxyquin
- Industry or Enhance VTM premix
  - Enhanced had higher vitamin levels and more bioavailable forms or Zn, Cu, & Org Se source.

Day 63 Woody Breast Comparison

UARK Severity Score of 3 Cut in Half



Recap

1. Phosphorus
2. Minerals
3. Inositol
4. Ip6→ Ip2/1
5. Microbiome
6. Antioxidant effects

THANK YOU

Mike Bedford
Research Director, AB Vista