UNIVERSITÄT HOHENHEIM

Aggiornamento sui pattern di degradazione dei fitati nel tratto gastrointestinale dei polli da carne e sull'interazione tra i minerali e la degradazione dei fitati nel pollame: un problema per i test di digeribilità del fosforo

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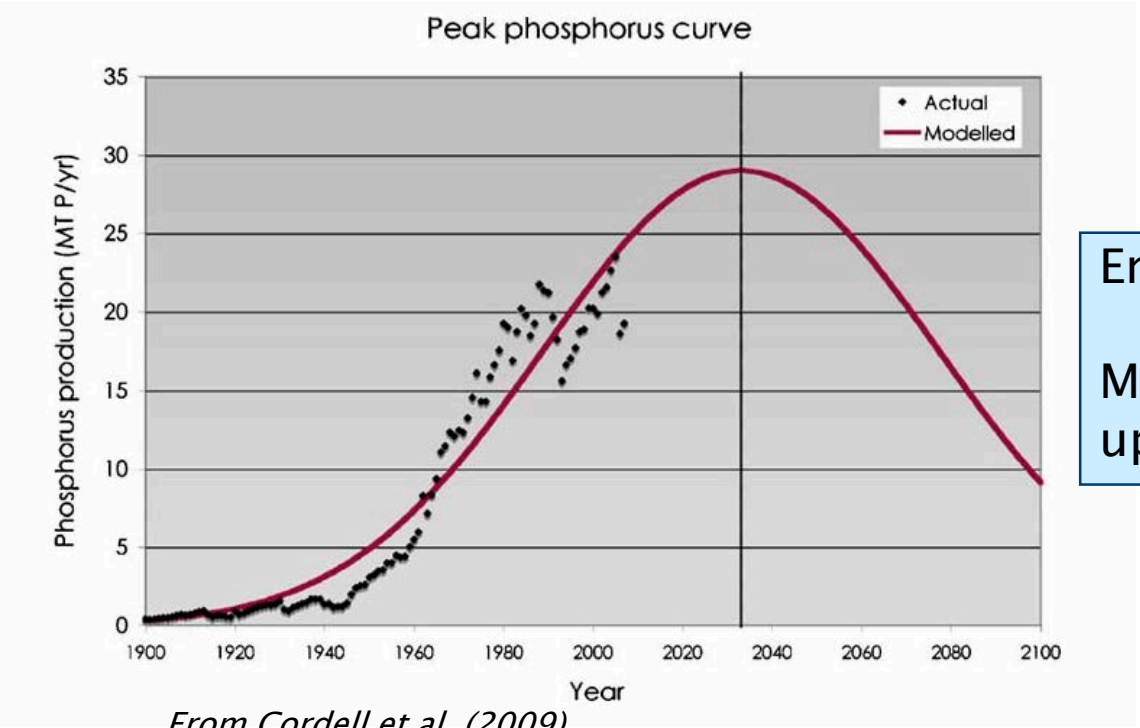
Content

- Why phosphorus again?
- Phytate degradation and origin of enzymes involved
- Amino acids and *myo*-inositol
- Phytate degradation *vs.* P release
- P digestibility of feed raw materials
- The international P digestibility ring test

Why phosphorus again?



From Cordell et al. (2009)

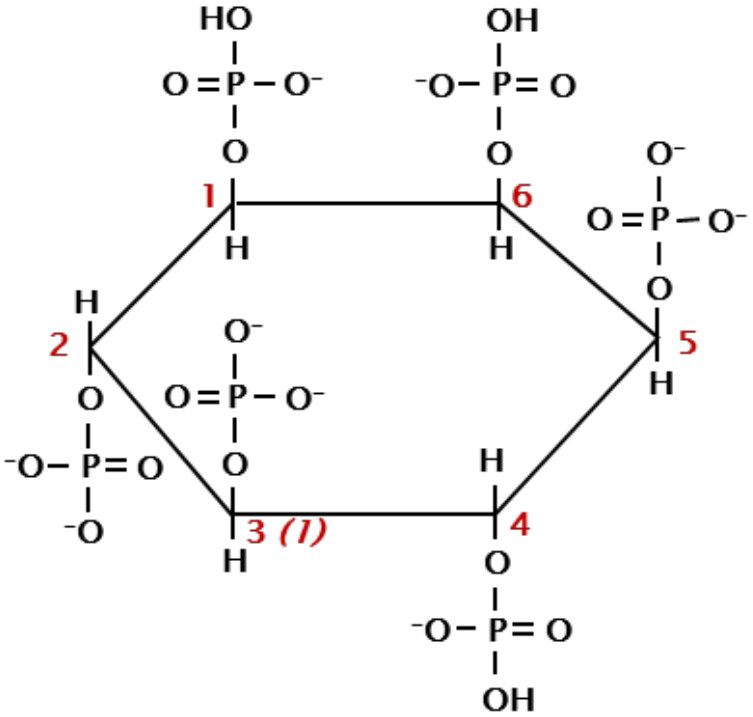
Environmental legislation:

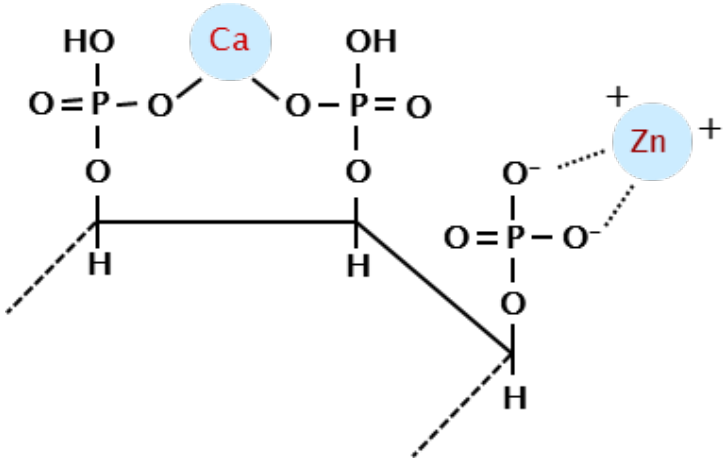
Manure fertilisation –
upper N and P levels reduced

In principle, the approaches are simple:

- Reduce safety margins for P in feed compounding
- Make more out of what is contained in plant feed raw materials

Phytic acid – Phytate





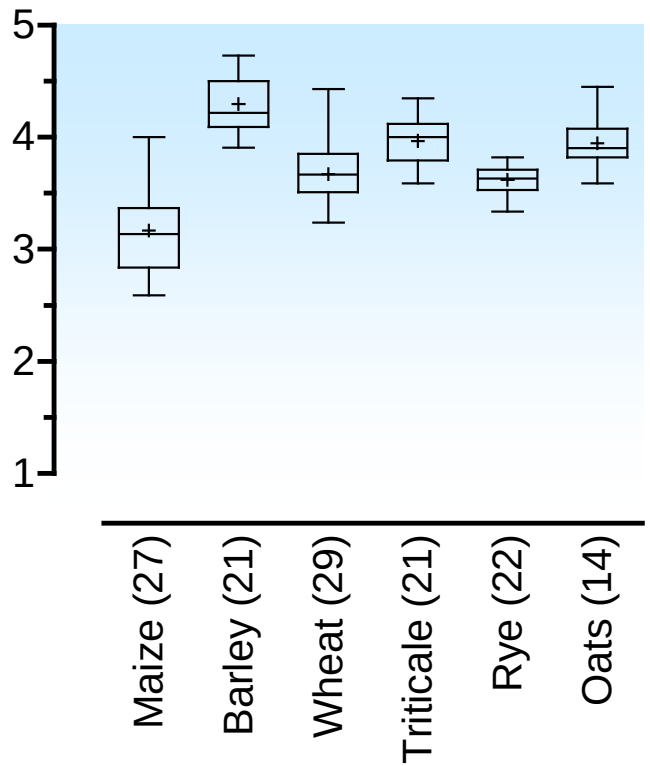
Main storage form of P in
plant seeds and by-products
from food and energy industries

$InsP_6$ *Myo*-Inositol-1,2,3,4,5,6-
Hexakis-Dihydrogenphosphat

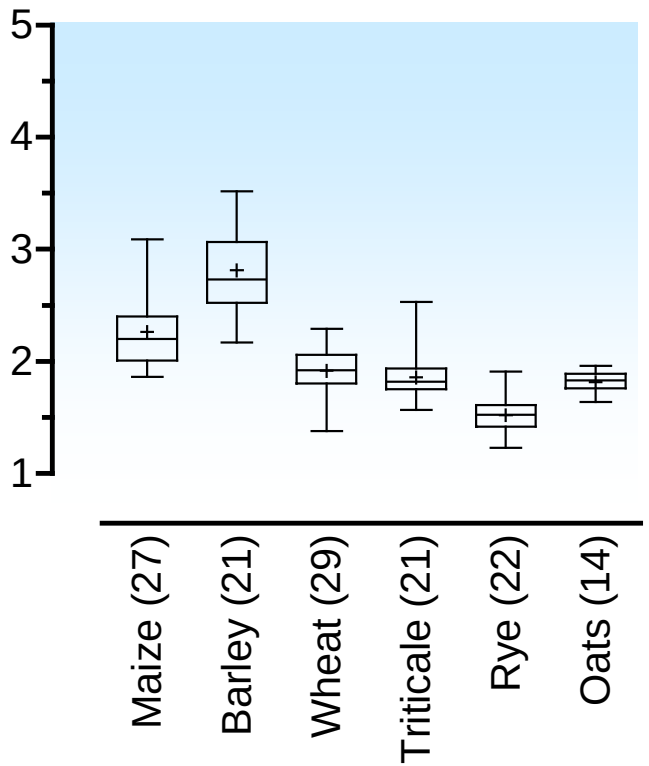
Erdman et al. 1979, Scheuermann et al. 1988

Cereal grains

Total P, g/kg dry matter

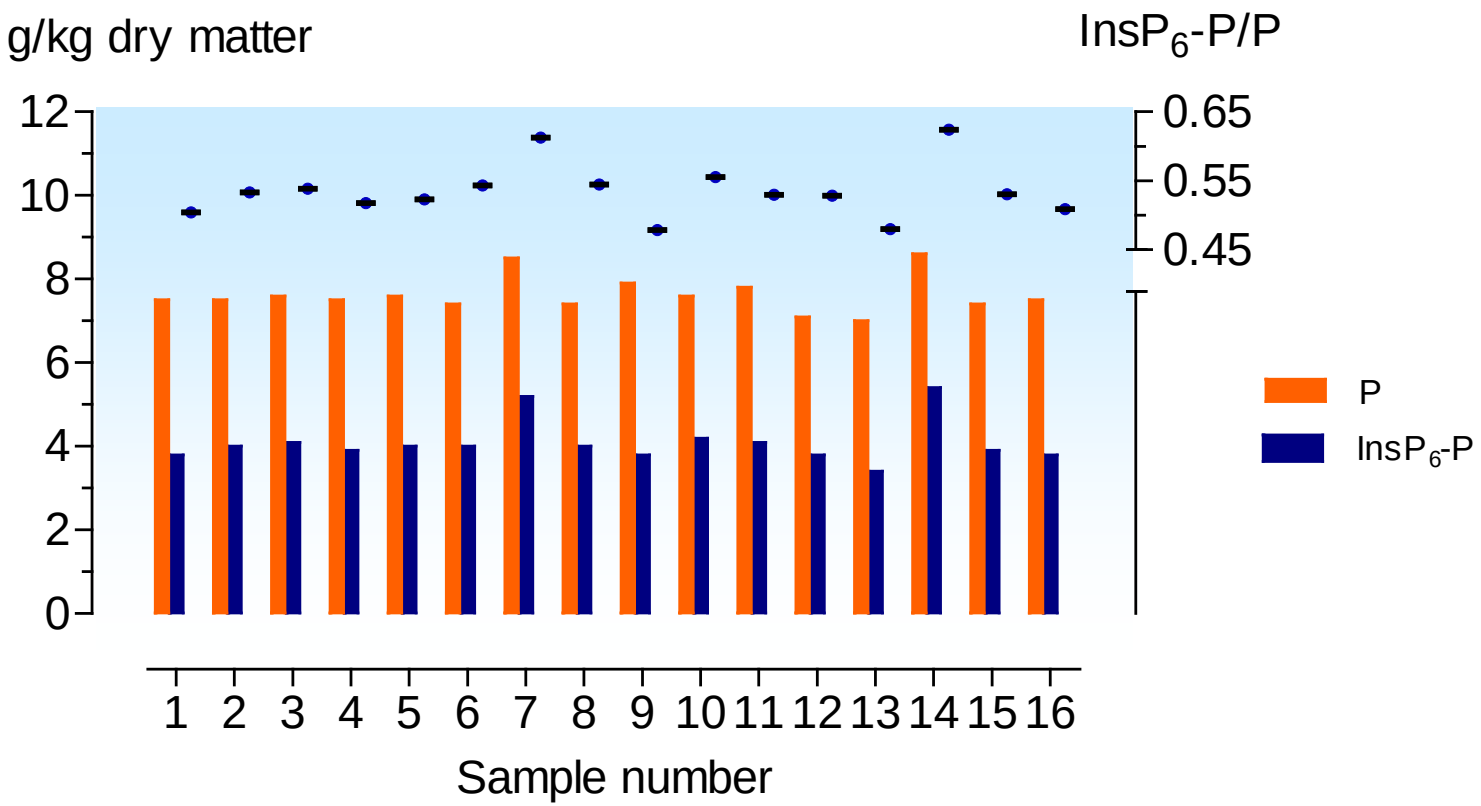


$InsP_6$-P, g/kg dry matter

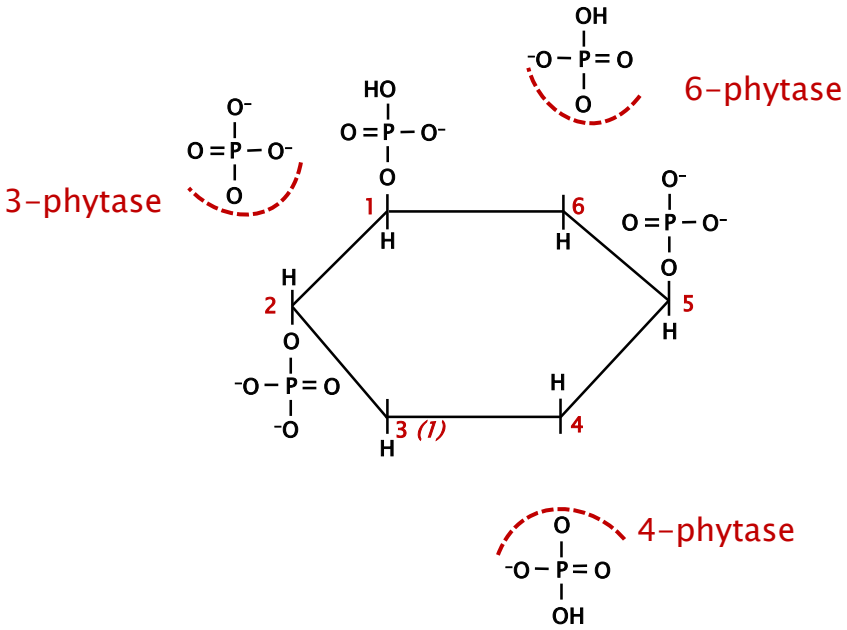


Rodehutscord et al. (2016)

Soybean meal (commercial qualities, 2016)



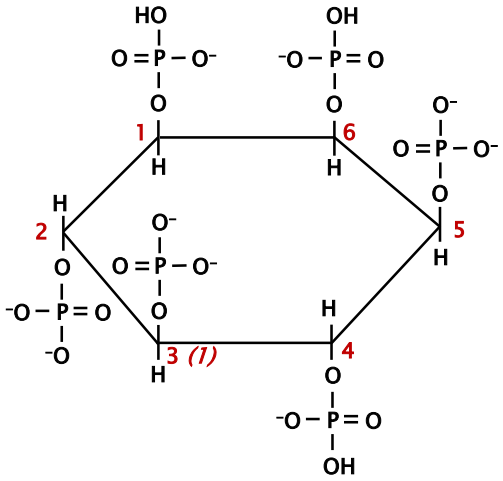
Phytic acid – Dephosphorylation



Nomenclature based on D-numbering

Major starting position of phytases different

Phytic acid – Dephosphorylation



Nomenclature based on D-numbering

Major starting position of phytases different

Dephosphorylation is not complete

Better understand the bottlenecks in the degradation pathways!

P digestibility: Pigs *versus* broilers

Maize-based diets, no added P, no added phytase

Pig BW (kg)	P dig. (%)	Reference
6–8	25–28	Lei et al. (1993 a,b)
11	36	Eeckhout und De Paepe (1992)
9–25	29	Pallauf et al. (1992)
13–35	13	Cromwell et al. (1993)
35–62	31	Tossenberger und Kakuk (1992)
37–70	13–28	Jongbloed et al. (1992)
46–66	20–34	Simons et al. (1990)
98	16	Nasi (1990)

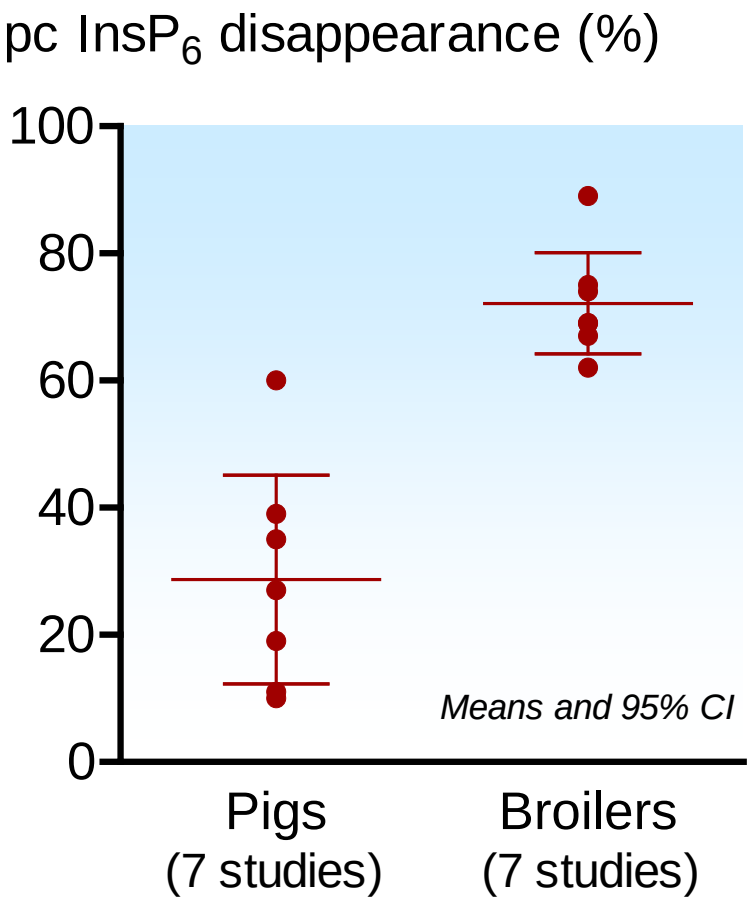
from Düngelhoef u. Rodehutscord (1995)

Literature data on P availability in broilers (%)

	Mean	Min.–Max.
Maize *(n=7)*	42	27 – 73
Soybean meal *(n=20)*	56	27 – 71

n = number of studies *WPSA Working Group (unpublished)*

Precaecal $InsP_6$ disappearance: Literature values



Origin of the enzymes involved?

Measurements at the end/in the lower ileum

Maize-soybean meal-based diets

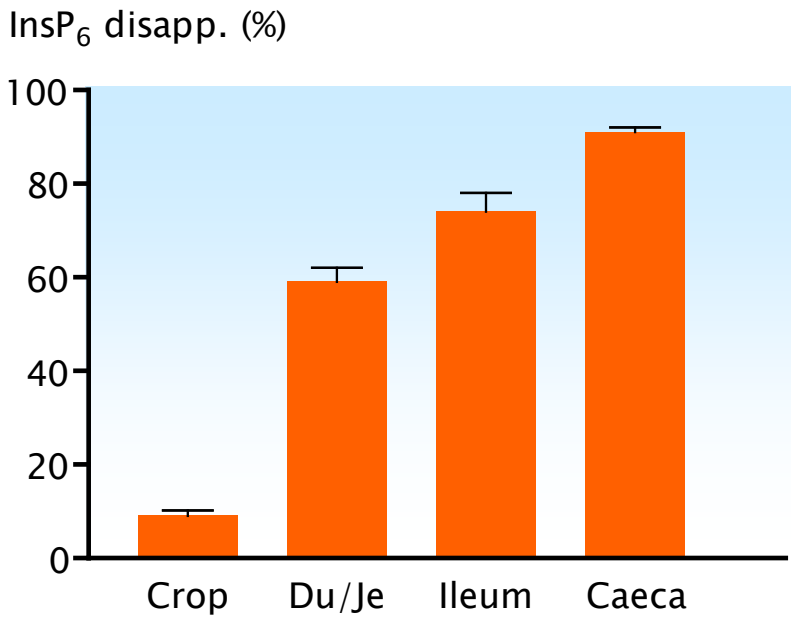
Low-P, low-Ca diets

No phytase supplements

Reviewed by Rodehutscord & Rosenfelder (2016)

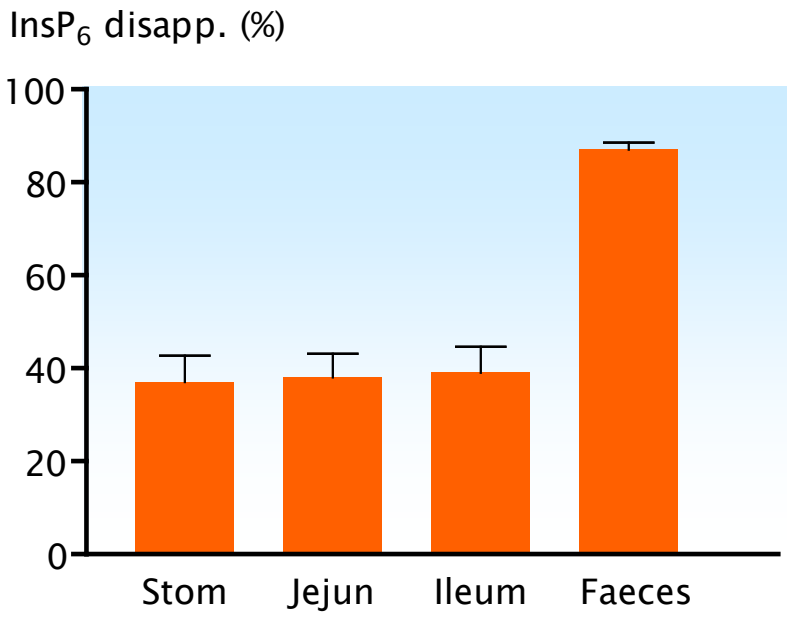
$InsP_6$ disappearance in different GIT sections

Broilers



Zeller et al. (2015b)

Pigs

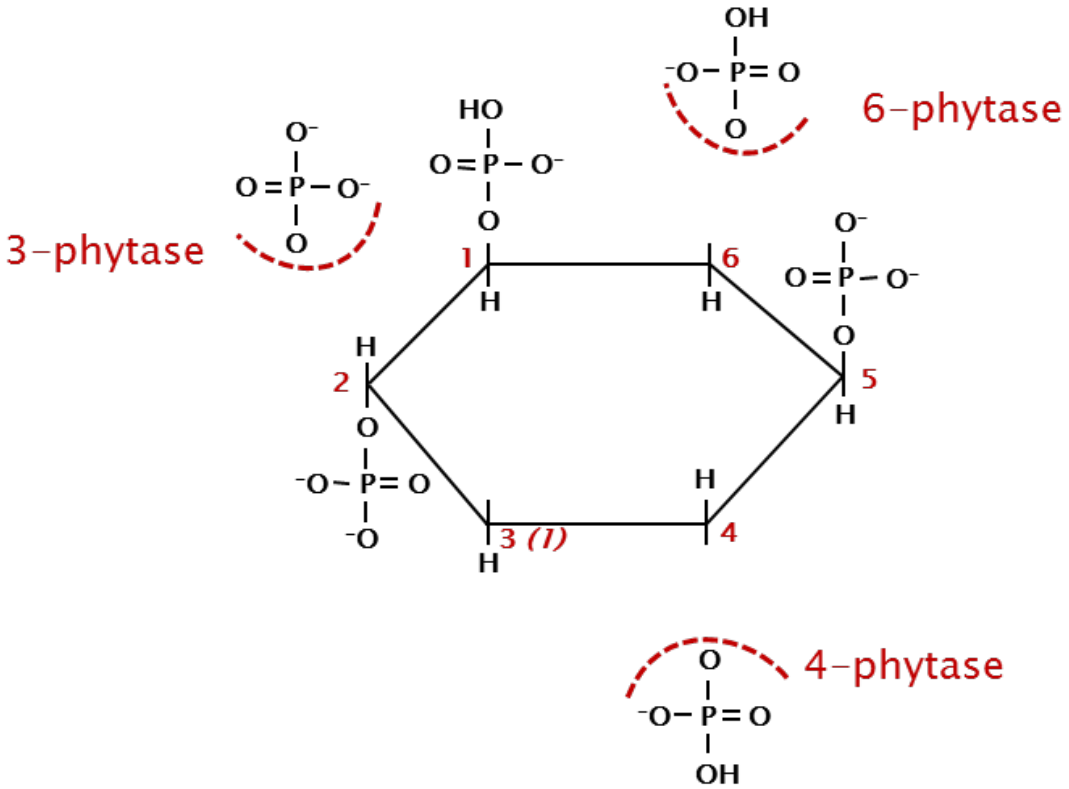


Rutherfurd et al. (2014)

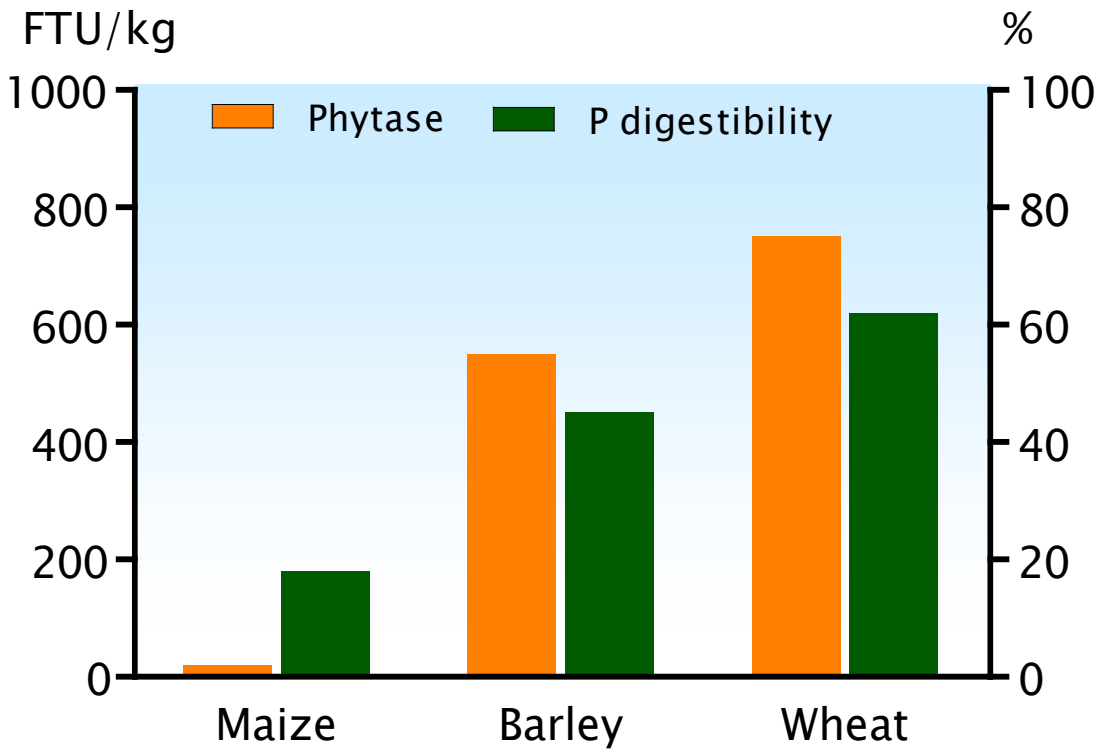
Maize-soybean meal-based diets, no mineral P, no added phytase

Origin of phytases acting in the digestive tract

- Plant intrinsic
- Endogenous
  - mucosal
  - microbial
- Added exogenous



Plant intrinsic phytase is effective in pigs



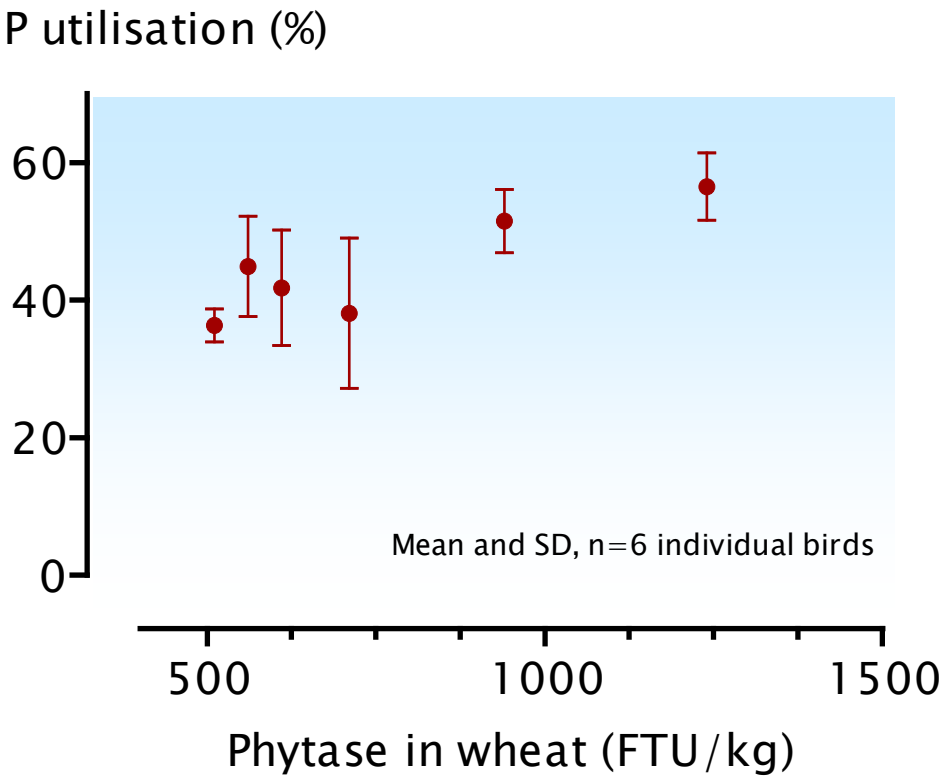
Phytase inactivation through extrusion resulted in strong reduction of lower InsPs in the stomach of pigs (Schlemmer et al. 2001)

Pig studies, faecal collection

According to standard protocol of GfE (1994)

Düngelhoef et al. 1994
Rodehutscord et al. 1996

Plant intrinsic phytase effective in broilers?

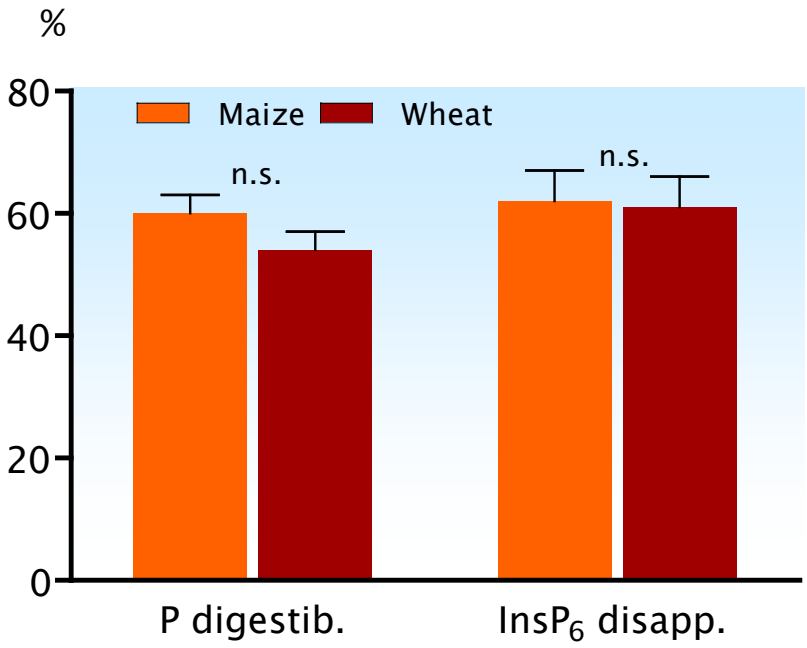


Here: Wheat

- Broiler balance study
  excreta collection d 16-20 of age
- Wheat (almost) only-diet
- Wheat: 3 varieties, 2 locations

Oloffs et al. (2000)

Plant intrinsic phytase effective in broilers?



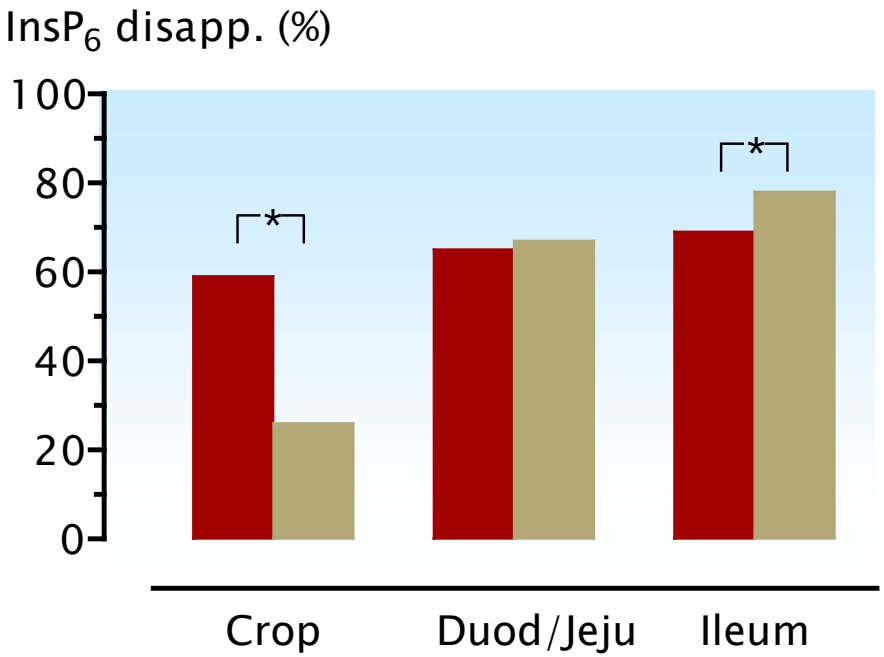
Broiler study
precaecal measurements

2 diets based on:
Maize: <100 FTU/kg
Wheat: 702 FTU/kg

No mineral P supplementation

Shastak et al. (2014)

Plant intrinsic phytase effective in broilers?



Broiler study

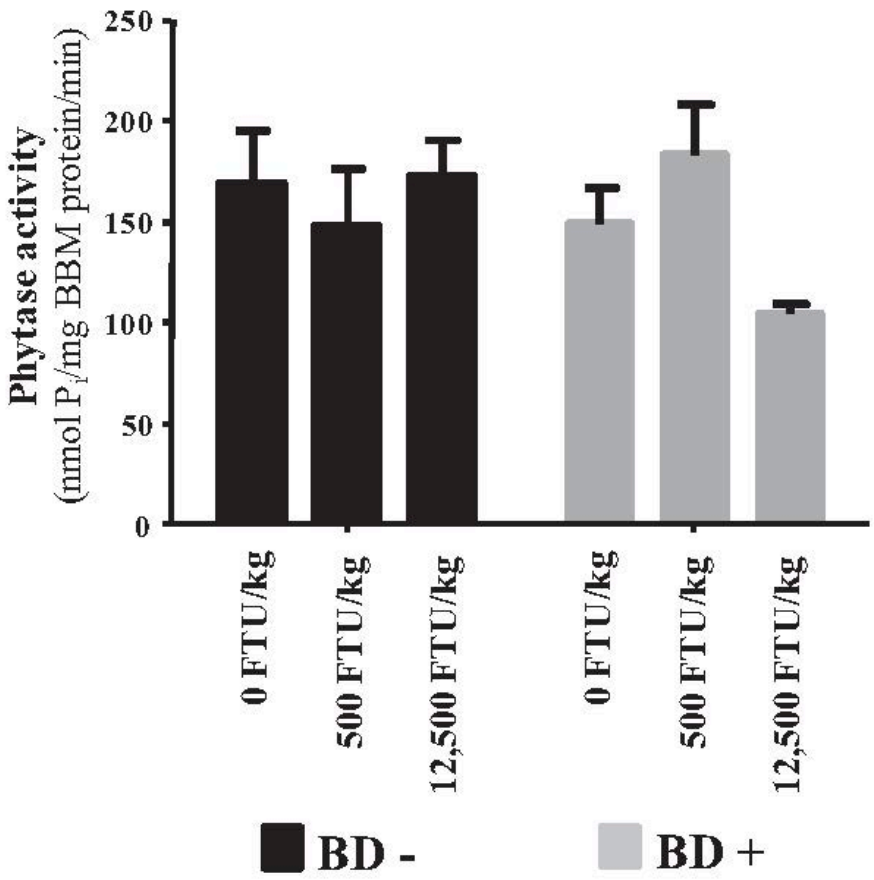
2 diets based on wheat:
Wheat untreated: 632 U/kg
Wheat microwave-treated: 121 U/kg

No mineral P supplementation

Zeller et al. (2015b, 2016)

- Intrinsic plant phytase acts primarily in the crop
- If not present, enzymes of other origin are able to compensate

Phytase activity in jejunal mucosa of broilers



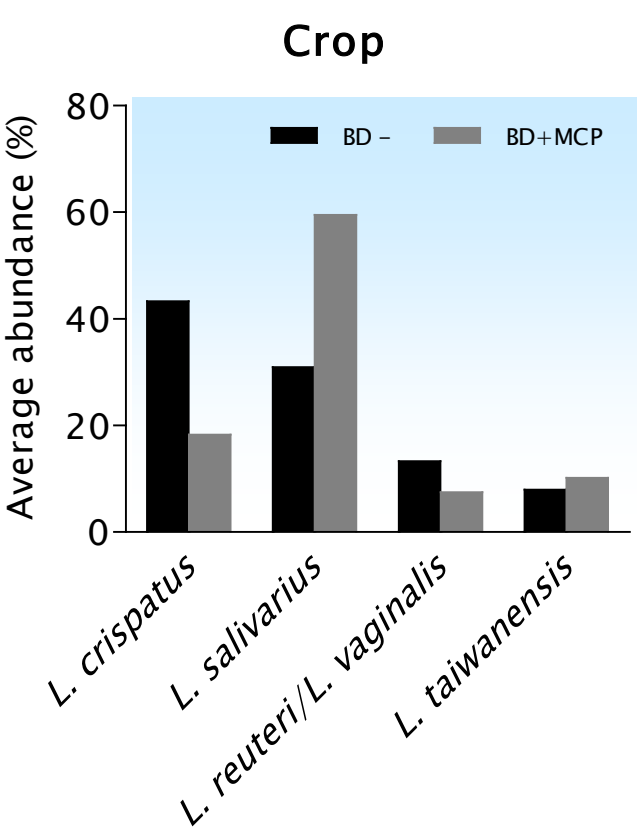
3 week-old broilers
Purified brush border membrane vesicles from the jejunum

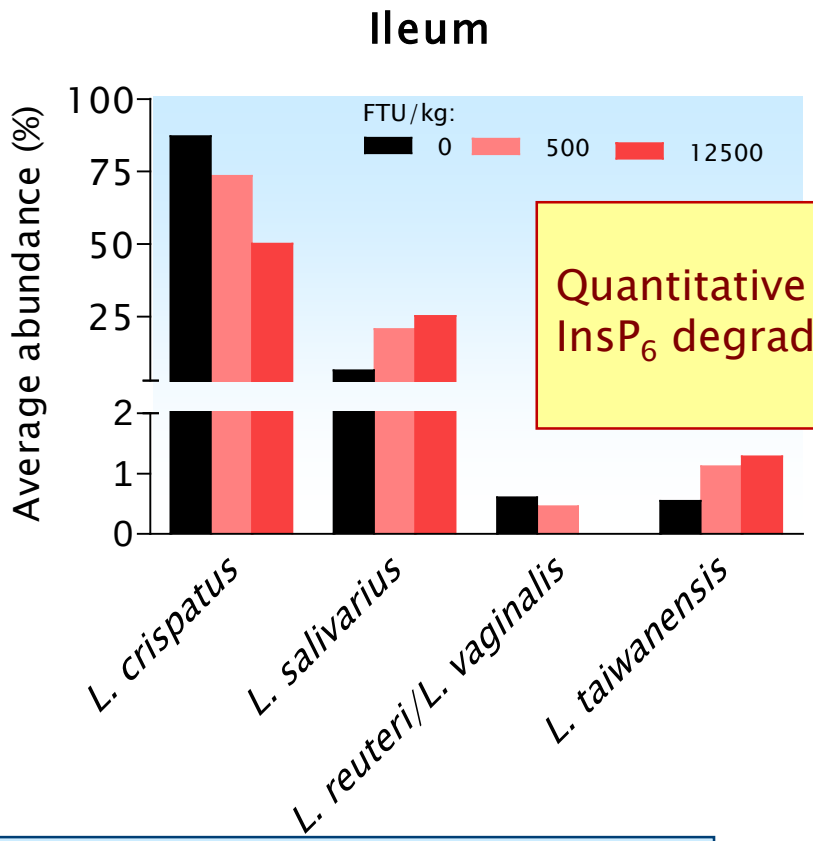
Maize-soybean meal-based basal diet without (BD-) and with MCP (BD+)

Quantitative relevance for $InsP_6$ degradation?

Huber et al. (2015)

Contribution of microbiota?



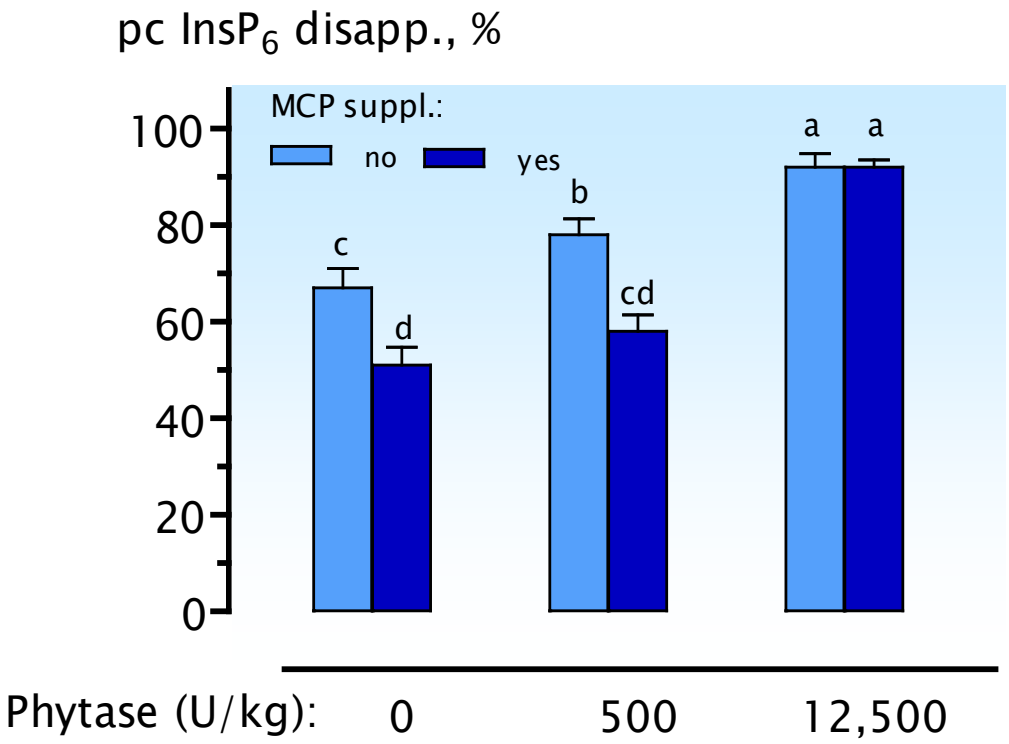


Quantitative relevance for $InsP_6$ degradation?

Maize-soybean meal-based basal diet, without (BD-) und with MCP (BD+)
16S rRNA gen-based T-RFLP fingerprinting
and 454-pyrosequencing of the microbiome

Witzig et al. (2015)

Phytase supplements: Combined effects of Ca/P



Broiler study

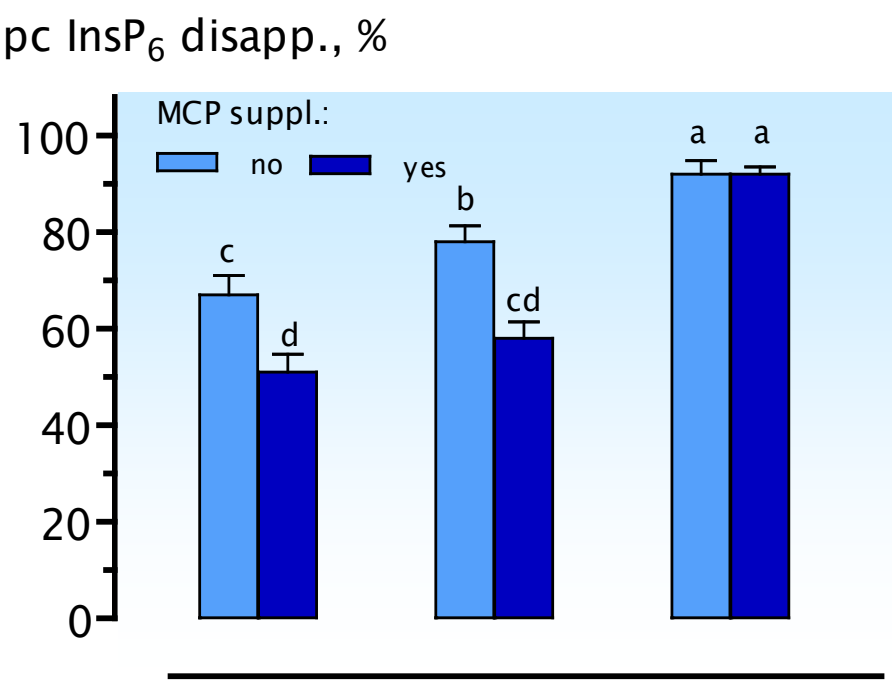
Maize-based diet

MCP and *E. coli* 6-phytase

Measurements in the terminal ileum

Zeller et al. (2015c)

Phytase supplements: Combined effects of Ca/P



Key Challenge:
Find right balance between supplements of Ca, P and phytase

Broiler study

Maize-based diet

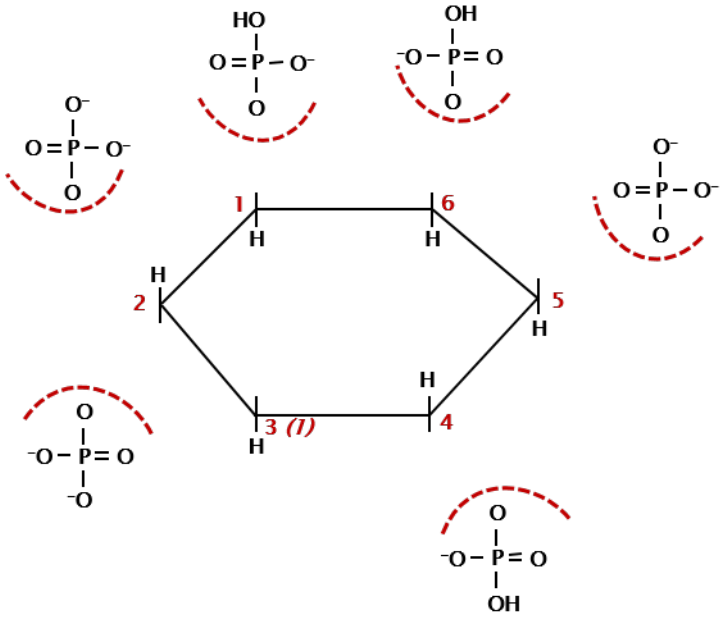
MCP and *E. coli* 6-phytase

Measurements in the terminal ileum

Phytas

- $InsP_6$ breakdown is strongly reduced by Ca/P supplements
  - solubility
  - end product inhibition by phosphate (not myo-inositol)
- The effect can be compensated by added phytase

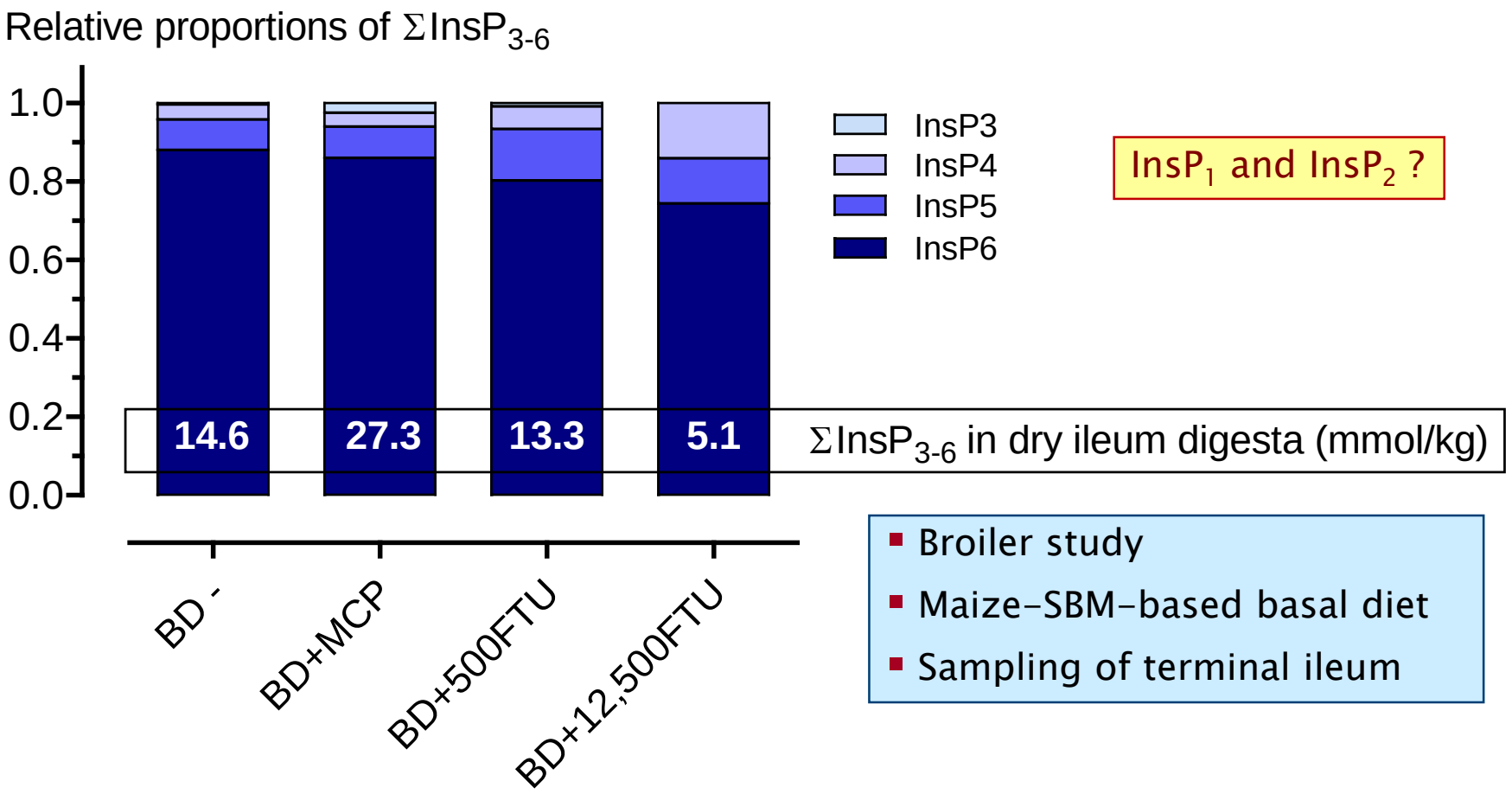
Connect $InsP_6$ degradation and P digestibility



The vision

$InsP_6$ disappearance ≠ Release of six P groups

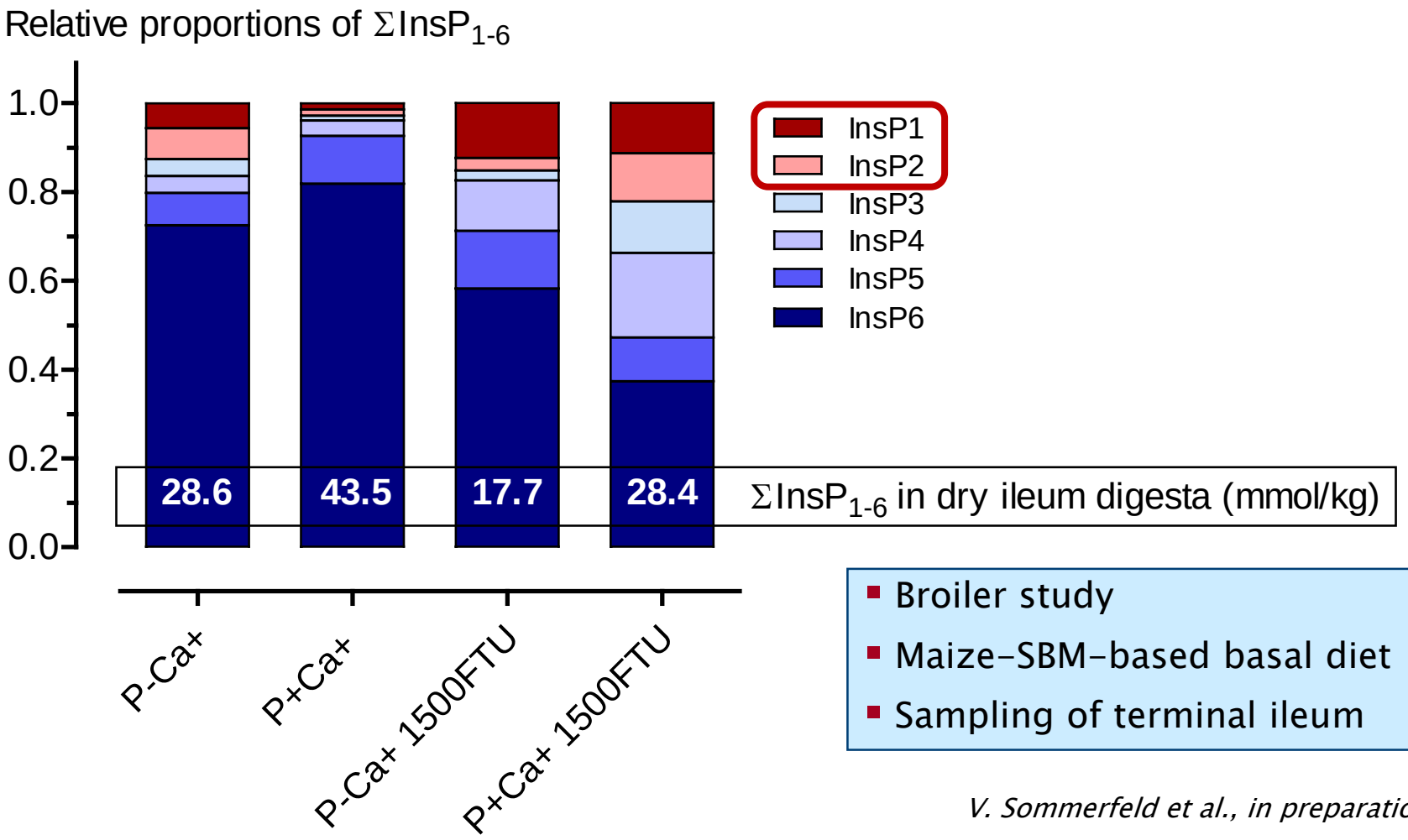
$InsP_6$ disappearance ≠ Release of 6 phosphate groups



$InsP_1$ and $InsP_2$?

- Broiler study
- Maize-SBM-based basal diet
- Sampling of terminal ileum

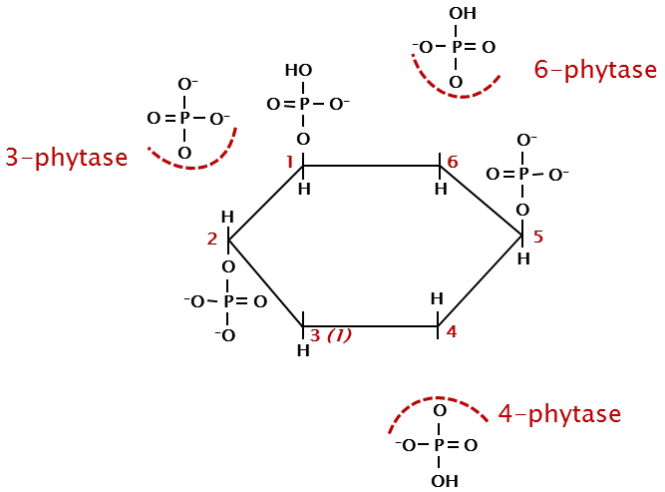
$InsP_6$ disappearance ≠ Release of 6 phosphate groups



- Broiler study
- Maize-SBM-based basal diet
- Sampling of terminal ileum

V. Sommerfeld et al., in preparation

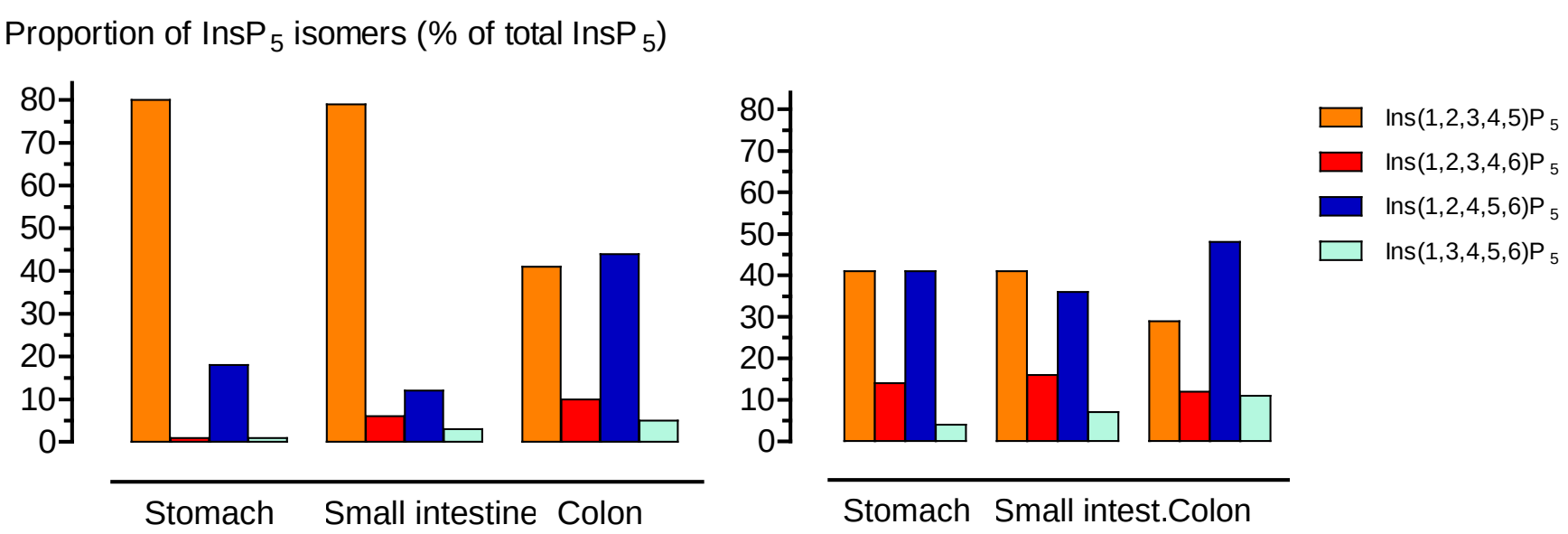
InsP isomer differentiation



$InsP_5$ pattern in the pig GIT change with diet extrusion

Not extruded

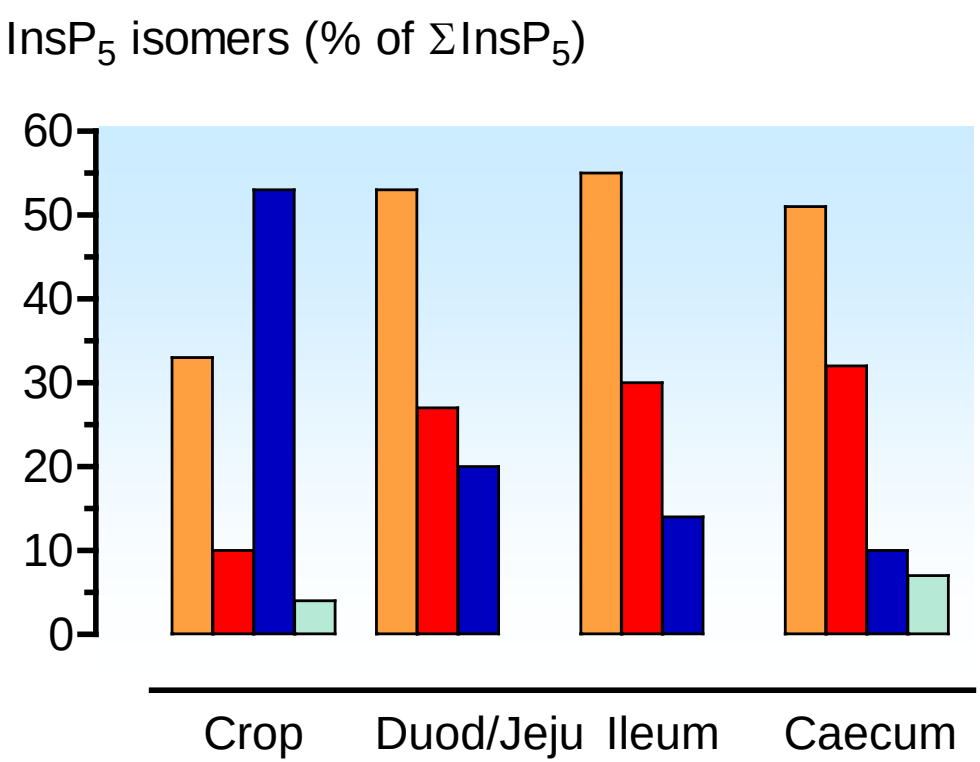
Extruded



Pigs of approx. 100 kg BW at slaughter
Cereal grain/SBM-based diet, cereals either extruded or not

Schlemmer et al. (2001)

$InsP_5$ pattern in broiler GIT change with passage



Broiler chicken

Maize-soybean meal-based diets

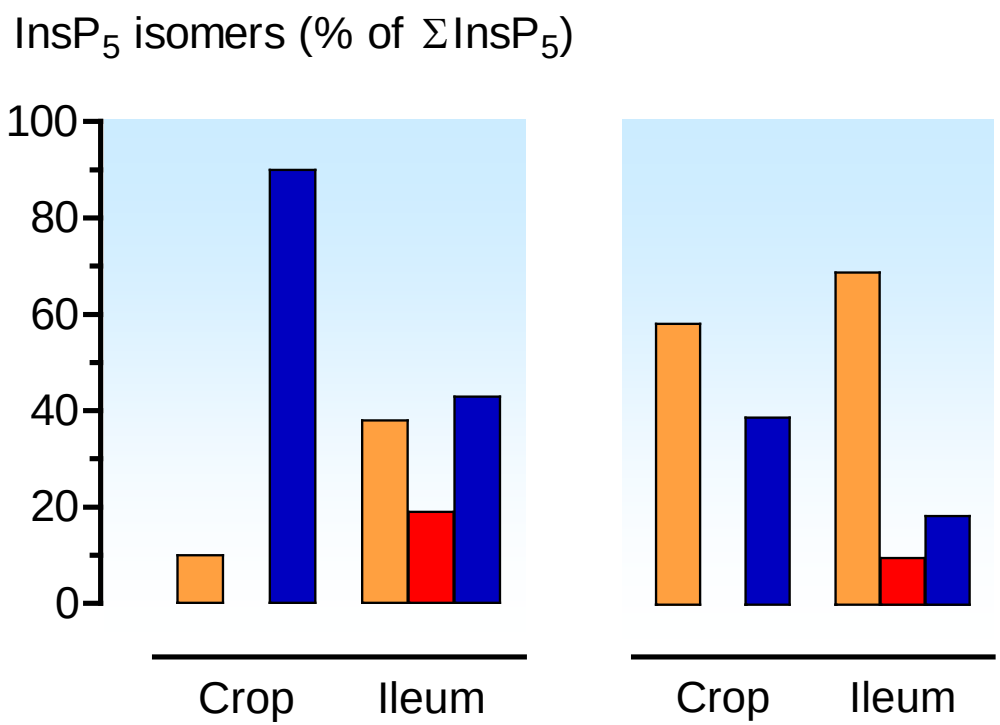
Low-P, low-Ca diets

No phytase supplements

Zeller et al. (2015b)

$InsP_5$ pattern differ with phytase products

A. niger | *E. coli*



Broiler chicken

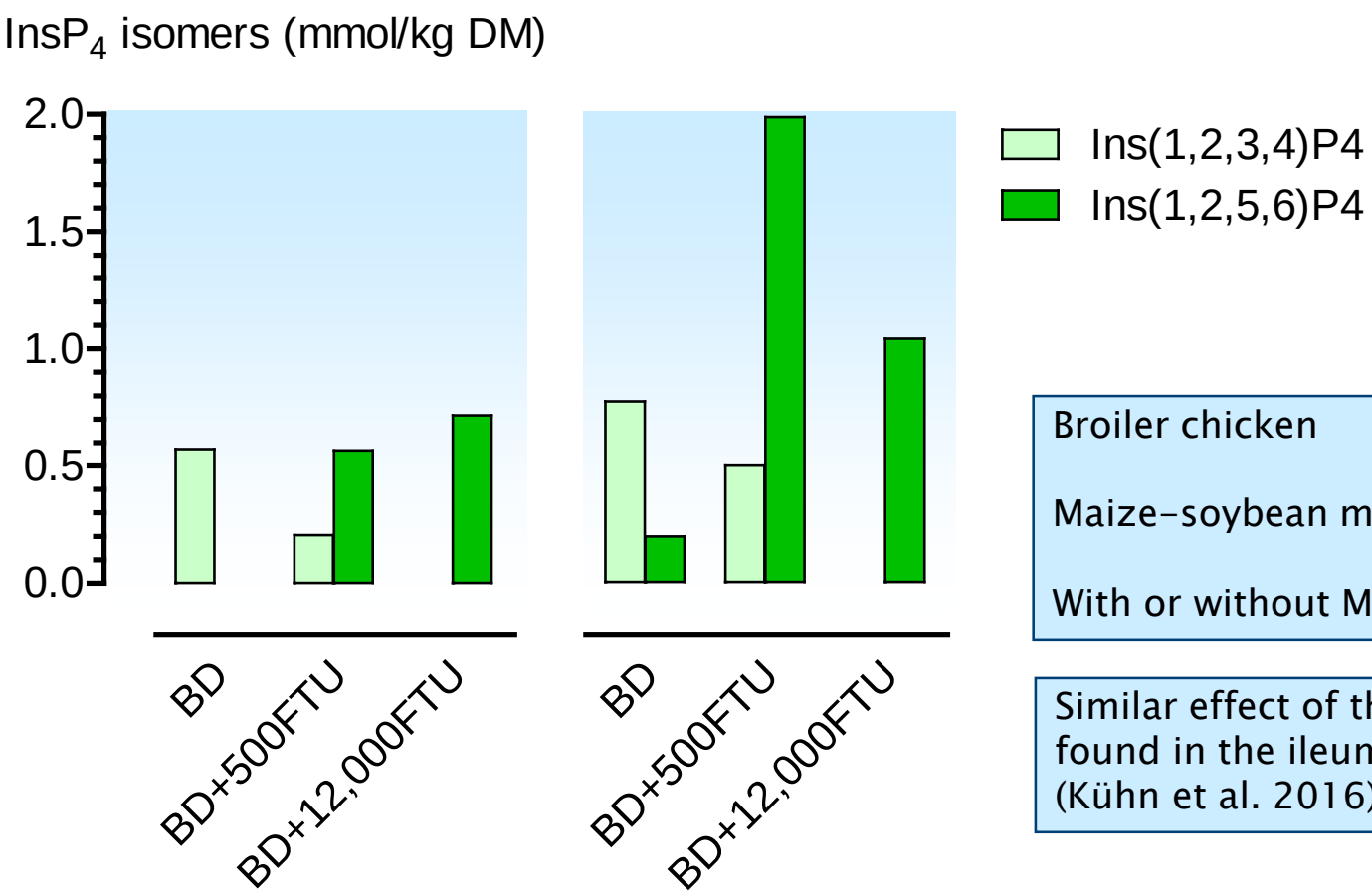
Maize-soybean meal-based diets

With supplemented phytase

Zeller et al. (2015b)

$InsP_4$ pattern in the terminal ileum is affected by MCP

without MCP *with MCP*



Broiler chicken

Maize-soybean meal-based diets

With or without MCP supplement

Similar effect of this phytase also found in the ileum of piglets (Kühn et al. 2016)

Zeller et al. (2015b)

In vitro:

Identification of potential bottlenecks

1 g of feed

↓

step 1 “crop”

+ double-distilled water (and/or enzyme solution)
+ 1.5 M HCl
→pH 5.8

water bath 40°C for 30 min

↓

step 2 “stomachs”

+ 1.5 M HCl
+ 3000 U pepsin
→ pH 2.8

water bath 40°C for 45 min

↓

step 3 “small intestine”

+ 1 M $NaHCO_3$
+ 3.7 mg/ml pancreatin
→ pH 6.1

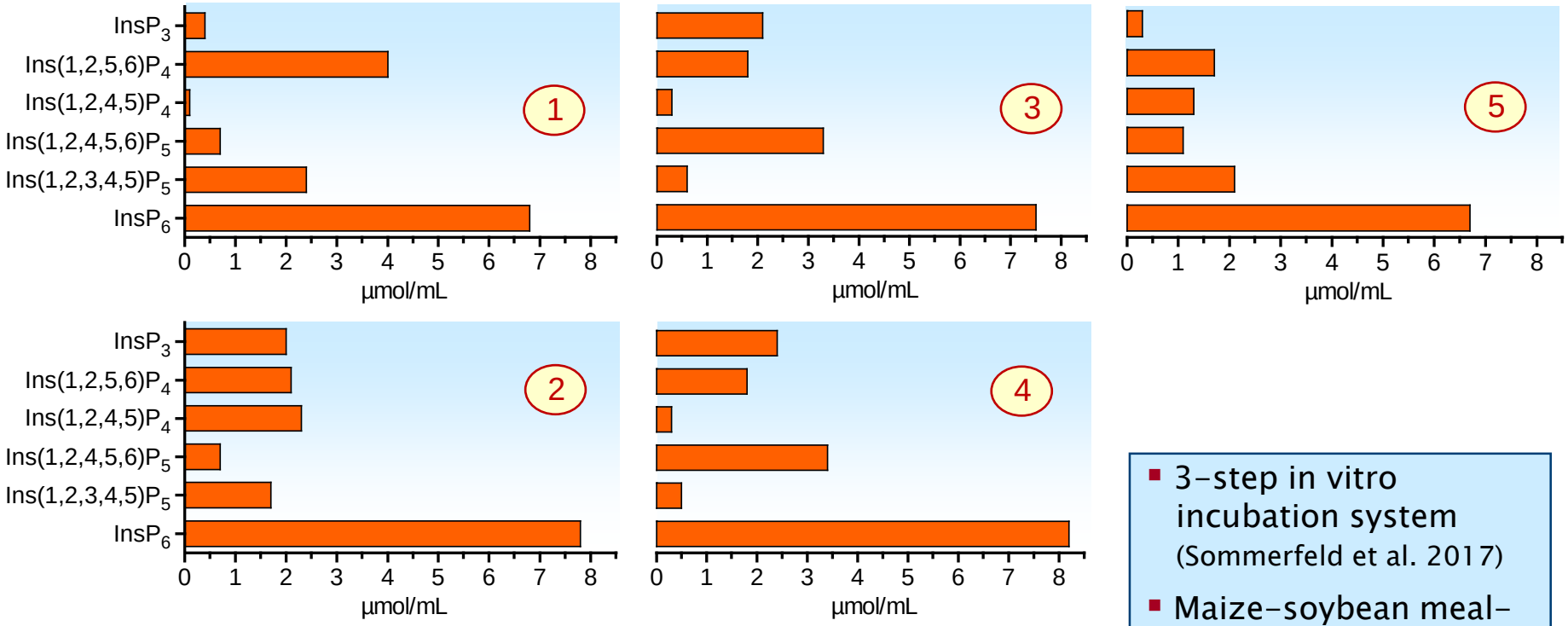
water bath 40°C for 60 min

↓

Extraction and InsP analysis

Sommerfeld et al. (2017)

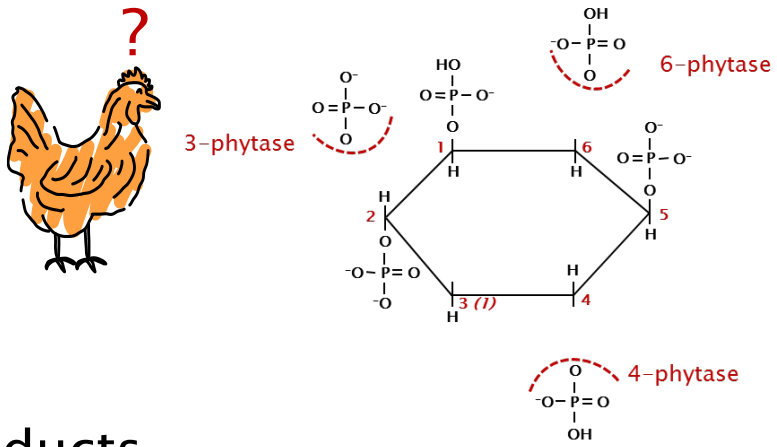
In vitro: Identification of potential bottlenecks



- 3-step in vitro incubation system (Sommerfeld et al. 2017)
- Maize-soybean meal-based diet

- 5 different phytase products
- dosage equivalent to 300 FTU/kg of diet
- FTU defined according to the product-specific pH optimum of $InsP_6$ degradation

$InsP_6$ degradation process is multiple susceptible !



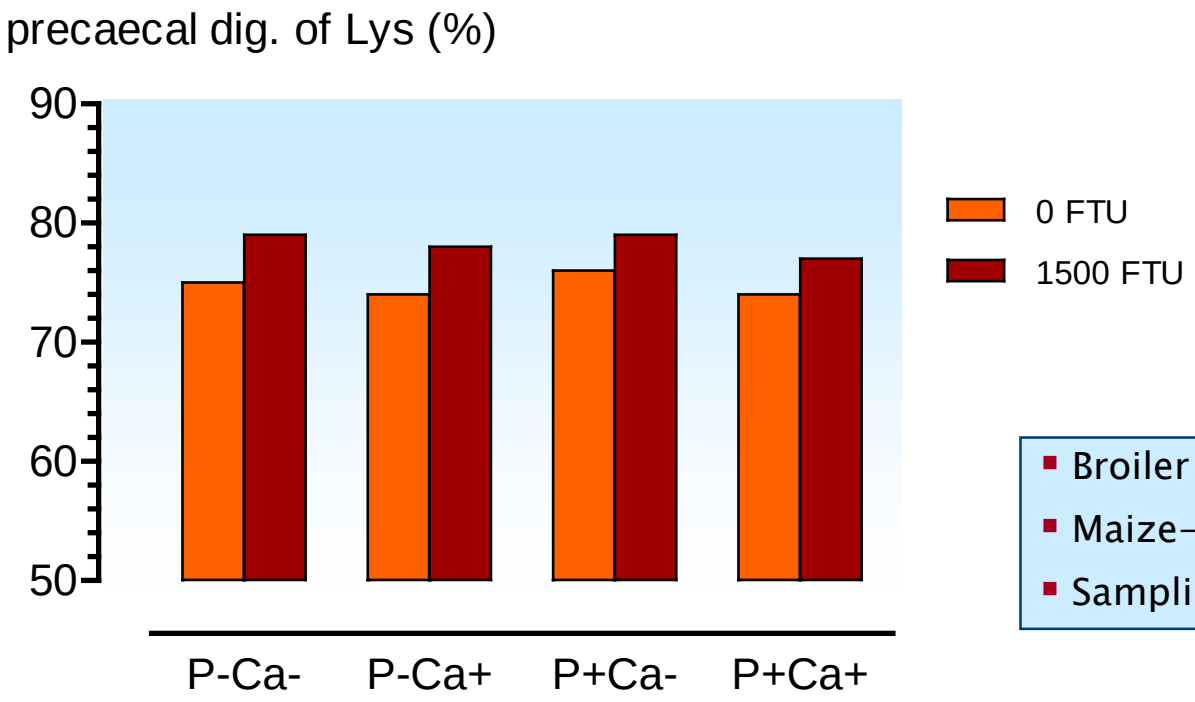
To conclude from this:

(1) Characterise the degradation products

(2) Identify specific bottlenecks of enzymes or enzyme combinations

(3) Improve the degradation process

Phytase and amino acid digestibility

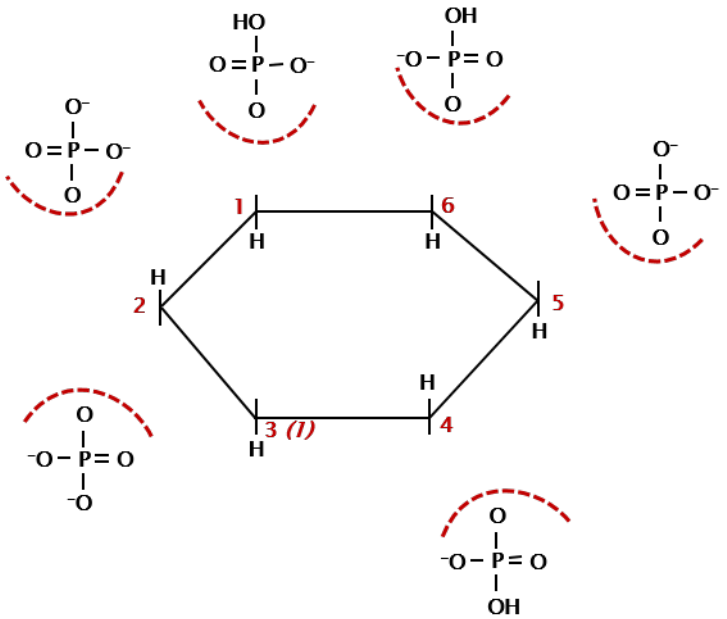


- Broiler chicken
- Maize-soybean meal-based diets
- Sampling of terminal ileum

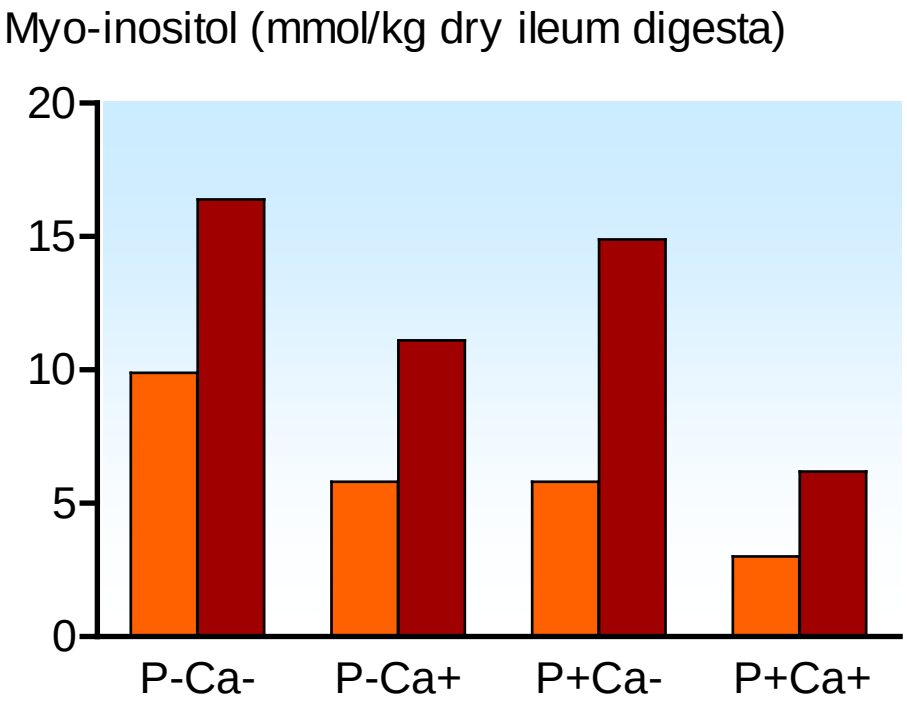
ANOVA *p* values:
P: n.s.; Ca: n.s.; phy: <0.001; No interaction

Sommerfeld et al. 2017

Myo–inositol release



Myo-inositol can be released



- Broiler chicken
- Maize-soybean meal-based diets
- Sampling of terminal ileum
- GC-MS *myo*-inositol analysis

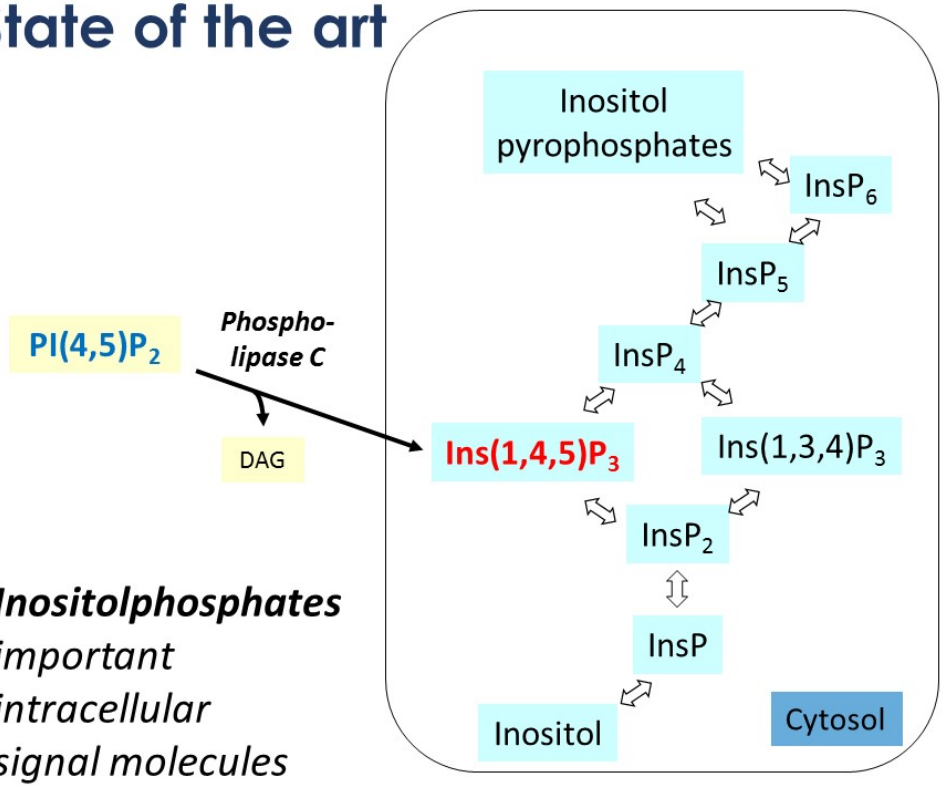
ANOVA *p* values:
P: <0.001; Ca: <0.001; phy: <0.001; Ca×phy: 0.02

Similar treatment effects detected in the gizzard!

Sommerfeld et al. (2016)

Metabolic relevance of *myo*–inositol

State of the art



Abel et al. 2001

Inositolphosphates *important intracellular signal molecules*

via InsP3 receptors

Ca^{2+} release

- Smooth muscle contraction

  Deepak et al. 2005

- Exocytosis of neurotransmitters, hormones and enzymes Williams 2014

Courtesy of Korinna Huber, 2017

To conclude from this

- Biological potential of broilers for $InsP_6$ degradation is high and higher than in pigs: Relevance of the crop?
- P and Ca supplements strongly reduce $InsP_6$ degradation
- Phytase supplements can compensate in a dose-dependent manner
- InsP isomers and *myo*-inositol help understand mode of action of enzymes and (perhaps) further improve enzyme efficiencies
- $InsP_6$ degradation has relevance also for amino acid digestibility and *myo*-inositol supply

Relevance for feed raw material evaluation:

P digestibility

Standard protocol for the determination of available P

- Published

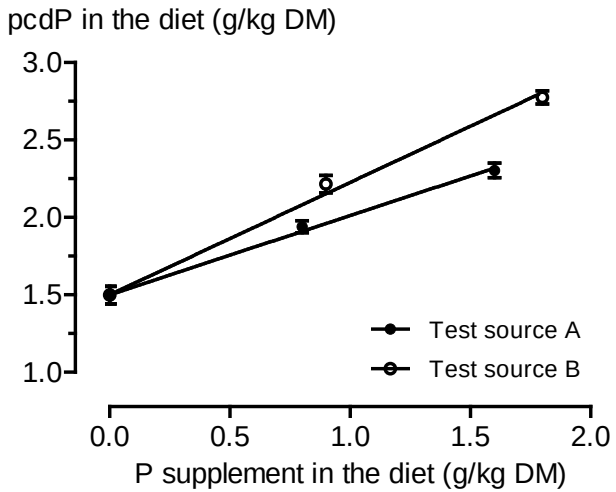
10.1017/S0043933913000688

Working Group Report

Determination of phosphorus availability in poultry

Working Group No 2: Nutrition of the European Federation of Branches of WPSA

- Recommendation: determination of <u>precaecal P digestibility</u> (**pcdP**) as the measure of P availability
- Principle:
  pcdP of a given feedstuff tested by a linear regression approach



- Intended: Differentiated P availability evaluation of
  - Feed raw materials and diets
  - Phytase products
- Approaches:
  - P digestibility until the end of ileum (precaecal digestible P, **pcdP**) (WPSA, 2013)
  - P retention
  - Relative bioavailability (bone criteria)

Review by Shastak and Rodeh. (2013) in WPSJ

- Consensus:
  - Animal studies needed
  - In vitro-techniques not sufficiently accurate (yet)

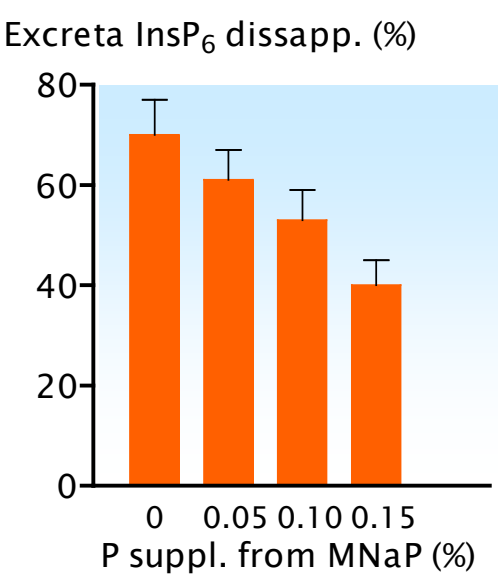
- Relevance of Ca, P, and phytate interactions when evaluating raw materials of
  - mineral or animal origin
  - plant origin
- Insights to gain from the recently completed P digestibility ring test

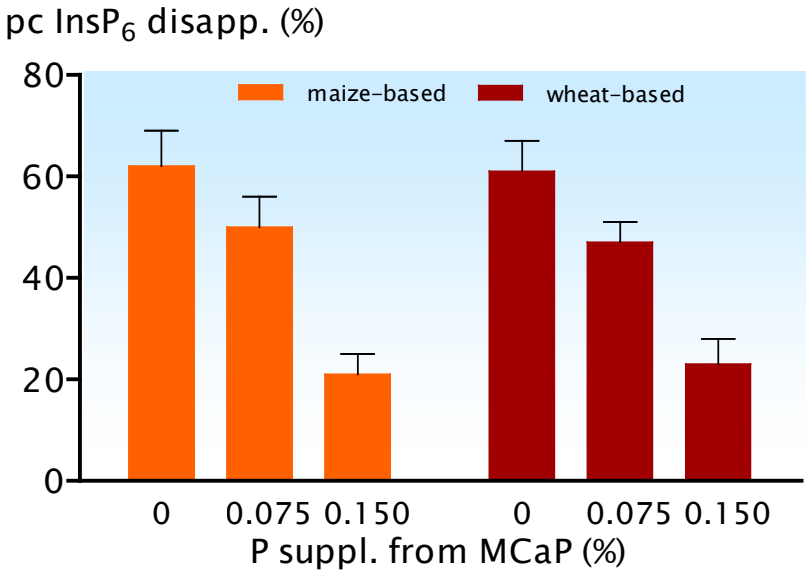
General procedure of animal trials

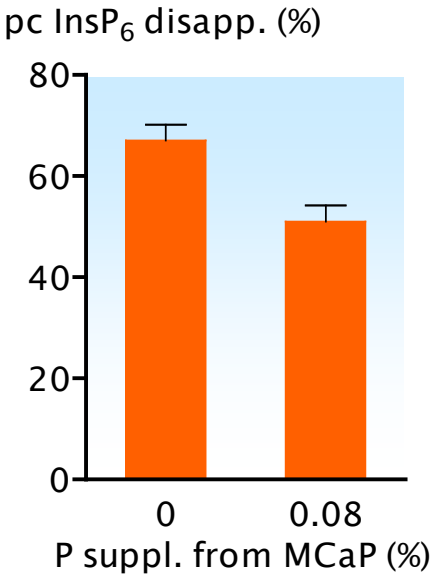
- Use a basal diet with low P and Ca
  - „practical-type“ (contains $InsP_6$)
  - purified or semi-purified (low or no $InsP_6$)
- Use other diets that contain the P source under test at one (or more) levels
- Measure criterion of choice (e.g. P digestibility) of all diets
- Evaluate the P source under test (by regression, difference calculation, etc.)

Assumption implied:
Added P source does not affect digestibility of the basal diet

$InsP_6$ disappearance in broilers: Effects of added Ca/P

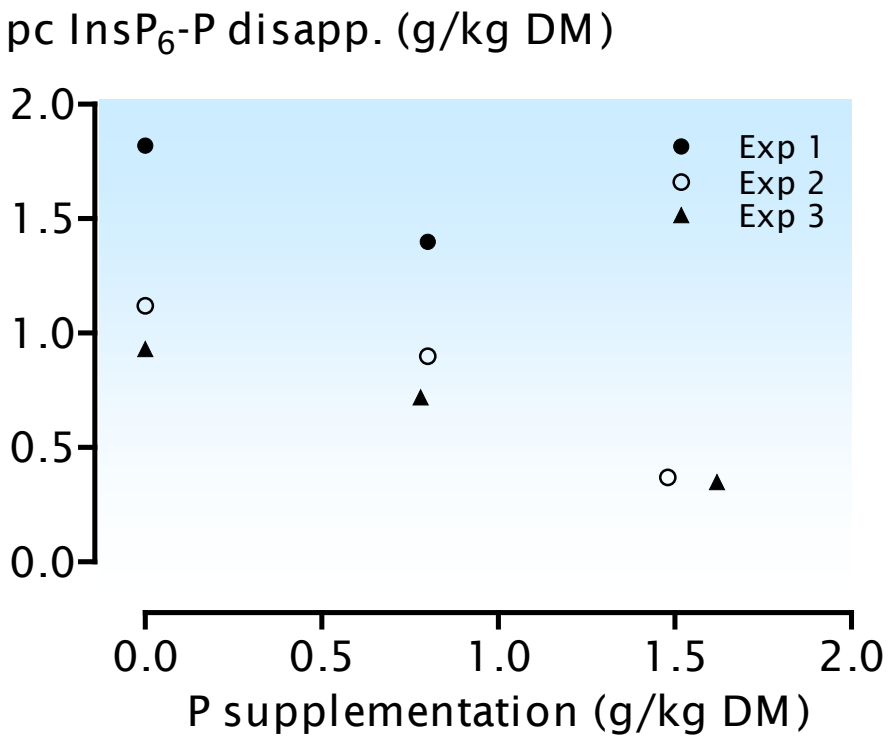






Data from Shastak et al. (2014) and Zeller et al. (2015c)

$InsP_6$ disappearance in broilers: Effects of added Ca/P

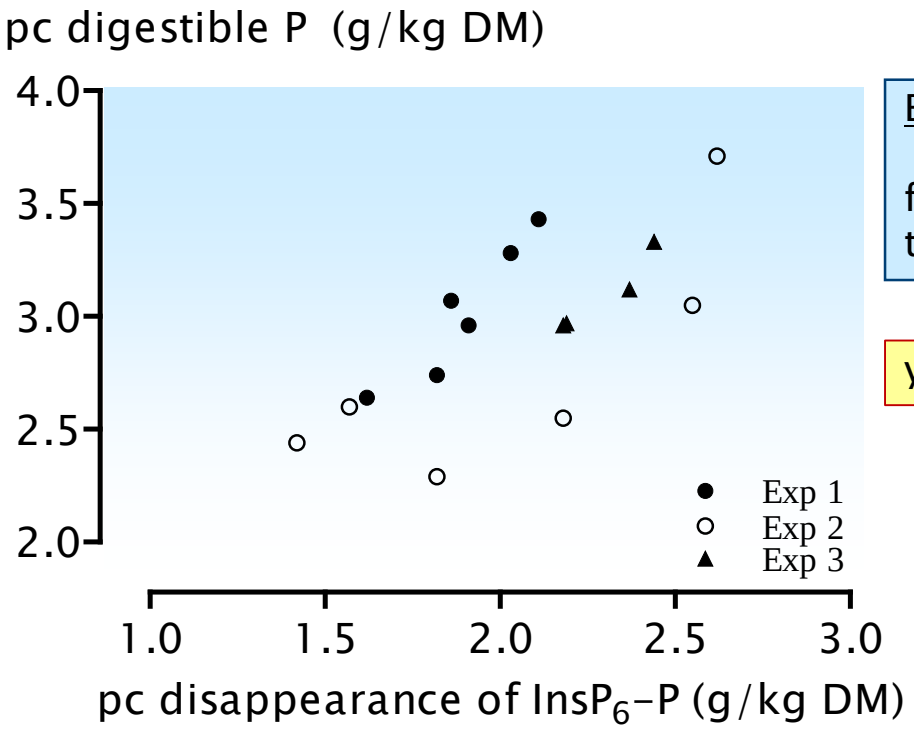


Estimated slopes of linear regressions:

−0.36 to −0.53

Data from Shastak et al. (2014) and Zeller et al. (2015c)

How much of the P released from $InsP_6$ is digested?



Estimate:

from experiments that measured $InsP_6$ and total P at the terminal ileum of broilers

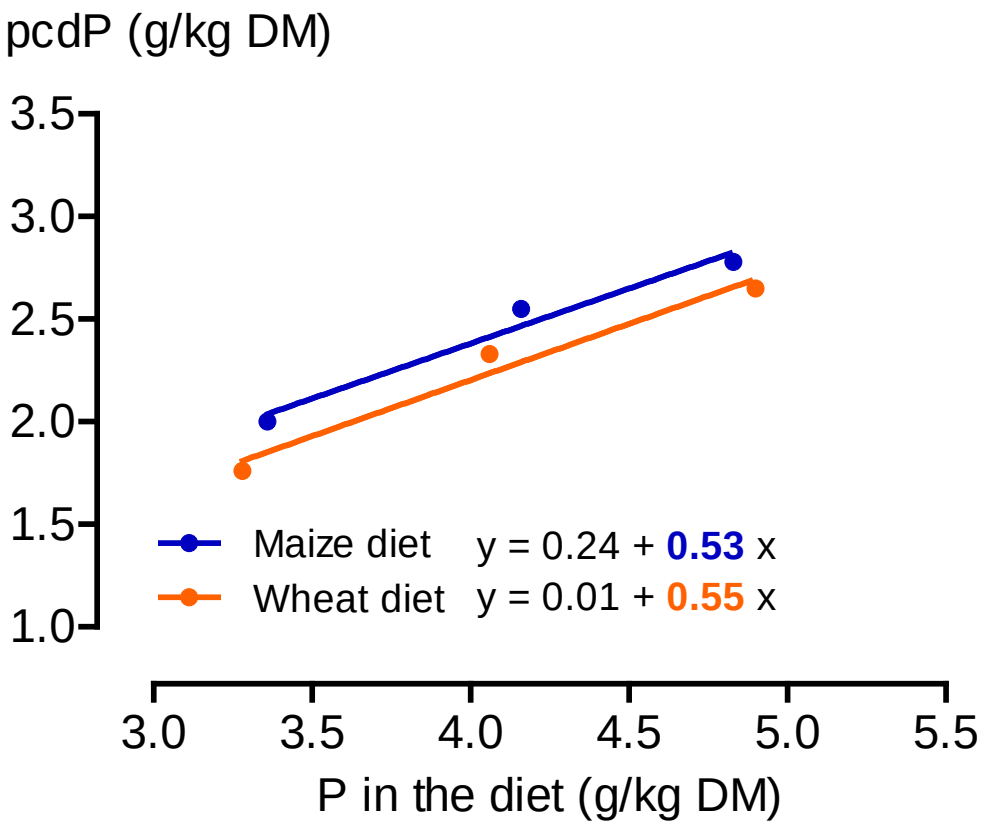
$y = 1.3\ (0.4) + 0.78\ (0.21)\ x$; r^2: 0.52 $s_{y.x}$: 0.27

This calculation does not consider the lower $InsP_x$

Digestibility of P released from $InsP_6$ is very high, probably close to 100%

Data from Zeller et al. (2015a,b,c)

Consequences for P digestibility estimates



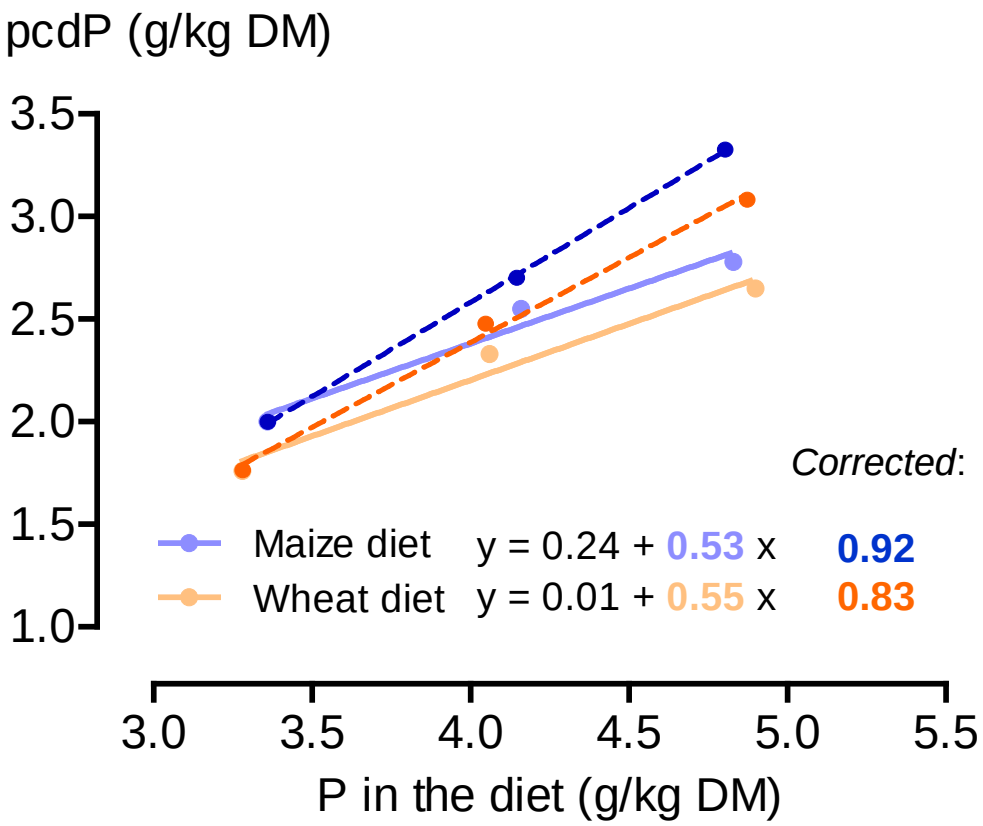
Broiler study

Maize-based or wheat-based basal diets, supplements of monocalcium phosphate (**MCP**)

MCP-P digestibility calculated by linear regression (WPSA 2013)

Data from Shastak et al. (2014)

Consequences for P digestibility estimates



Broiler study

Maize-based or wheat-based basal diets, supplements of monocalcium phosphate (**MCP**)

MCP-P digestibility calculated by linear regression (WPSA 2013)

Included: correction for changes in $InsP_6$ disappearance

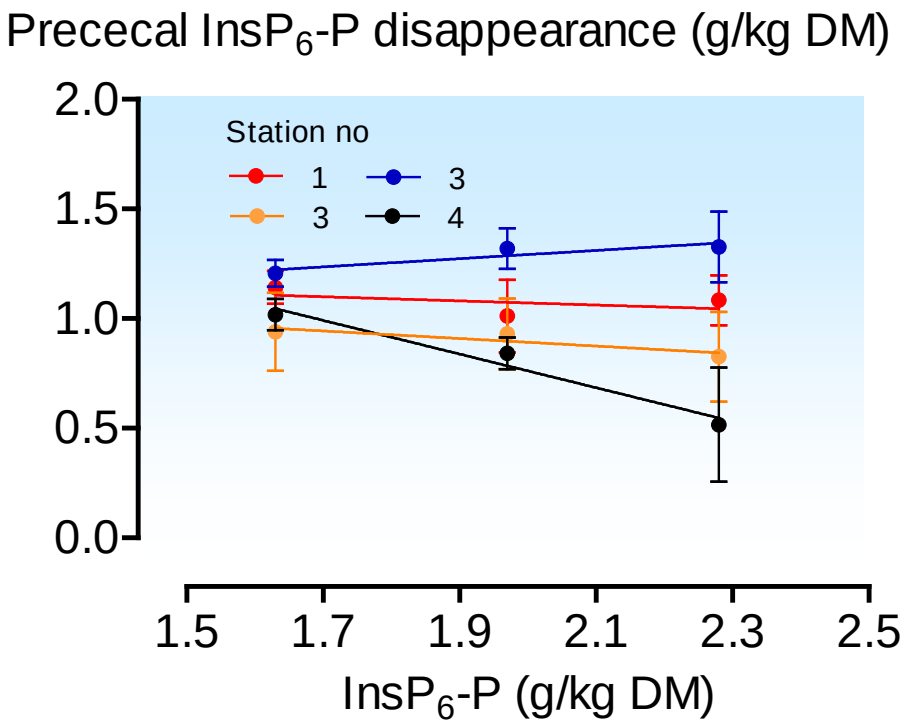
Data from Shastak et al. (2014)

Consequences for P digestibility estimates

- Mineral and animal P sources are underestimated when studied using phytate-containing diets

- Plant P sources are overestimated when using low-P diets

Upper limit of $InsP_6$ hydrolysis?



4 broiler studies in different stations, but using the same diets

low-P basal diet

Increasing $InsP_6$ by soybean meal

Rodehutscord et al. (2017)

How get out of the dilemma?

- Use purified diets?
- Analyse $InsP_6$ along with total P and make corrections?

P digestibility ring test

Participating institutions/persons

Aarhus University	S. Steenfeldt
Adisseo France	A. Preynat
Auburn University	W. A. Dozier
DSM	M. Umar Faruk
Hohenheim University	M. Rodehutscord, M. Witzig
ILVO Belgium	E. Delezie
INRA France	A. Narcy
IRTA Spain	M. Francesch
Manitoba University	C. M. Nyachoti, S. M. Waititu
Maryland University	R. Angel
Purdue University	O. Adeola
SAS Euronutrition	B. Renouf
Schothorst Feed Research	C. Kwakernaak
Scotland's Rural College	O. A. Olukosi
Trouw Nutrition	A. Saiz del Barrio
Vienna University	K. Schedle
Wageningen UR Livestock Research	M. van Krimpen, P. Bikker

Full report published in Poult. Sci. (2017)

P digestibility ring test: Objectives

- To compare the results obtained for P digestibility of soybean meal (SBM) when the WPSA protocol is applied
  - The same diets in all 17 stations
  - All chemical analyses done in only one laboratory
- Hypothesis:
  High reproducibility of determined P digestibility

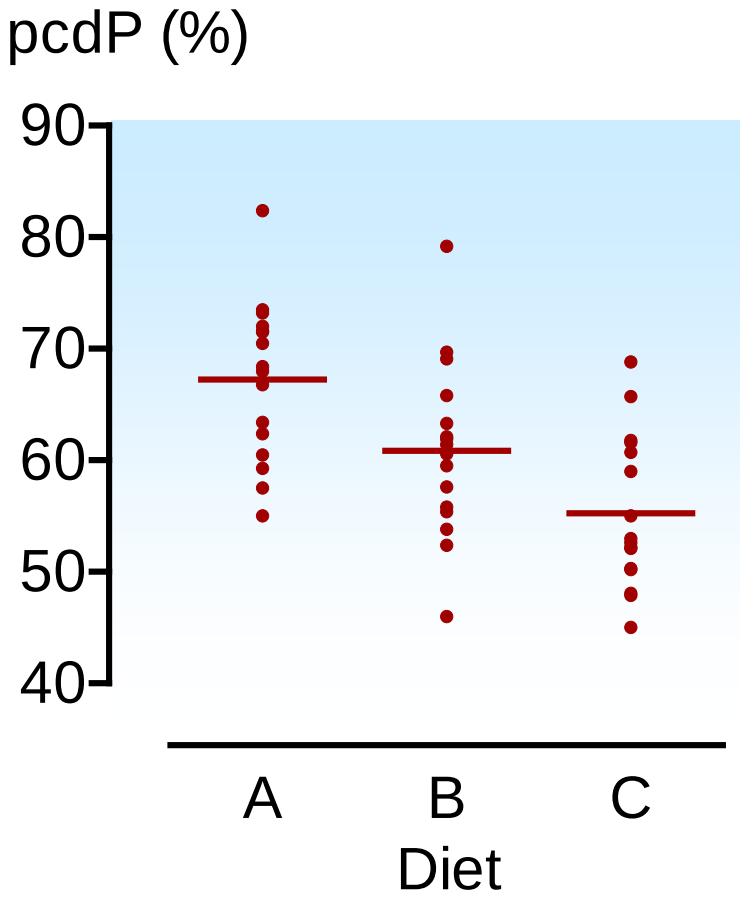
P digestibility ring test: Diets

Diet	A	B	C
Ingredients (g/kg)			
Soybean meal	400	510	620
Corn starch	449	337	225
Limestone (finely ground)	7	9	11
Other	144	144	144
Analysed (g/kg, on DM basis)			
CP	231	288	339
Total P	3.0	3.8	4.6
Ca	4.6	5.7	6.8
$InsP_6$-P	1.6	2.0	2.3
$Ins(1,2,4,5,6)P_5$-P	0.24	0.27	0.32

Procedures used in the collaborating stations

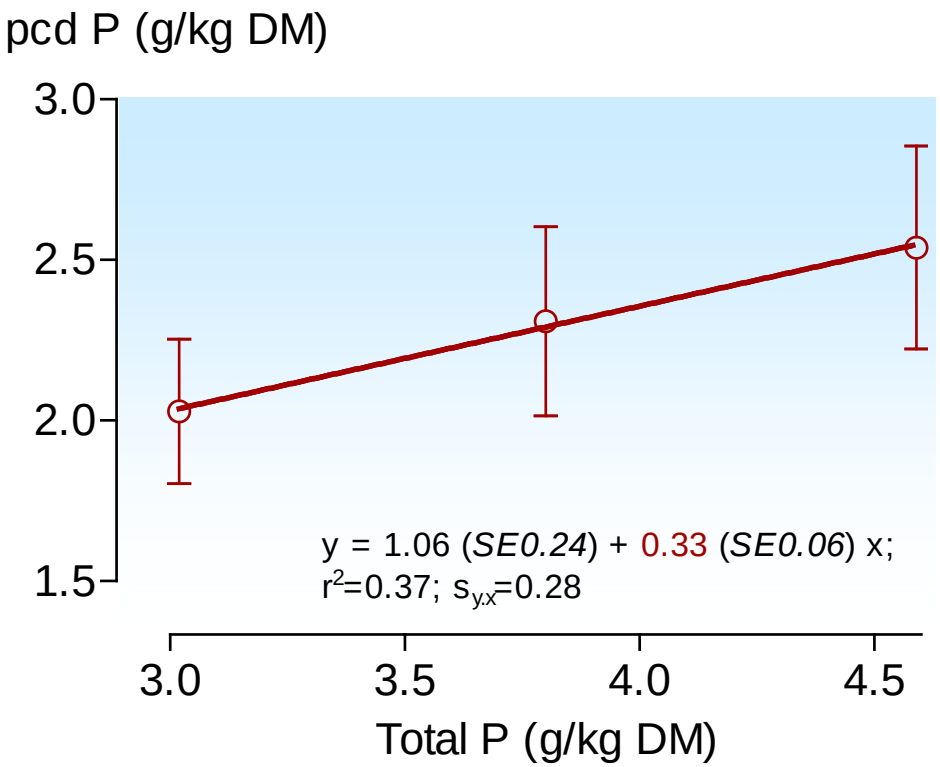
- Broiler chicks (different strains)
  - raised according to station-specific routine
  - pre-experimental starter diet not standardized
- Feeding of the experimental diets (ad libitum) for (a minimum of) 5 days
- Minimum of 6 replicated cages per diet
- Experiment terminated at the age of 21-28 days (CO_2 asphyxiation or cerv. dislocation or pentobarbital inj.)
- Content from the lower half of the ileum

Digestibility of the diets



Each dot is the mean of one station per diet

Digestibility of the soybean meal

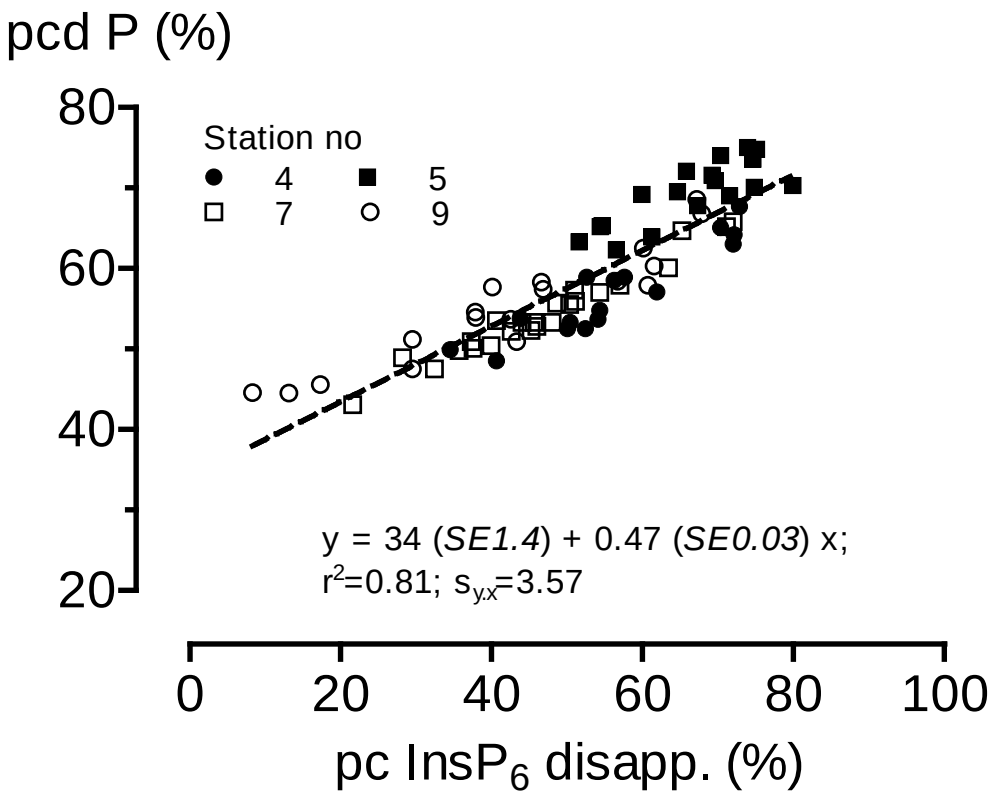


Digestibility of the soybean meal – by station

No of station	Slope		*SE*	R^2	Root MS error
1	0.31	abc	*0.07*	>0.99	0.01
2	0.22	bc	*0.08*	0.97	0.02
3	0.19	c	*0.07*	0.68	0.09
4	0.30	abc	*0.07*	0.98	0.03
5	0.51	a	*0.08*	>0.99	<0.01
6	0.38	abc	*0.08*	0.93	0.07
7	0.33	abc	*0.08*	>0.99	<0.01
8	0.43	ab	*0.07*	0.95	0.06
9	0.19	c	*0.08*	0.91	0.04
10	0.25	bc	*0.07*	0.94	0.04
11	0.27	bc	*0.08*	0.92	0.05
12	0.26	bc	*0.07*	>0.99	0.02
13	0.42	ab	*0.07*	0.95	0.07
15	0.38	abc	*0.07*	0.97	0.04
16	0.34	abc	*0.08*	>0.99	0.02
17	0.41	abc	*0.08*	0.91	0.08

Reject hypothesis!

Relationship: P digestibility and $InsP_6$



Possible explanations for high variation of P digestib.

- High relevance: factors related to $InsP_6$ degradation
  - Endogenous mucosal phytase activity (animal genetic effect)?
  - Endogenous microbial phytase production
    - Housing in the pre-experimental period (contact with litter)?
    - Coccidiostat in the pre-experimental diet?
- P and Ca levels, and phytase in the pre-experimental diet: No clear relationships found
- Age at sampling, growth, feed intake, killing procedure, did not show any clear relationship with P digestibility

Conclusions from the ring test

- Standardization of protocol details must go beyond the experimental period

- Revision of the protocol is needed:
  include pre-experimental starter period

Avoid comparison of data from different studies/labs

Summary and conclusion

The extremely high biological variation in $InsP_6$ degradation –together with the hardly quantified reasons of variation– lets standardised P evaluation remain to be a big scientific and technical challenge.