How Ethanol Production Technology Impacts DDGS Value in Animal Nutrition

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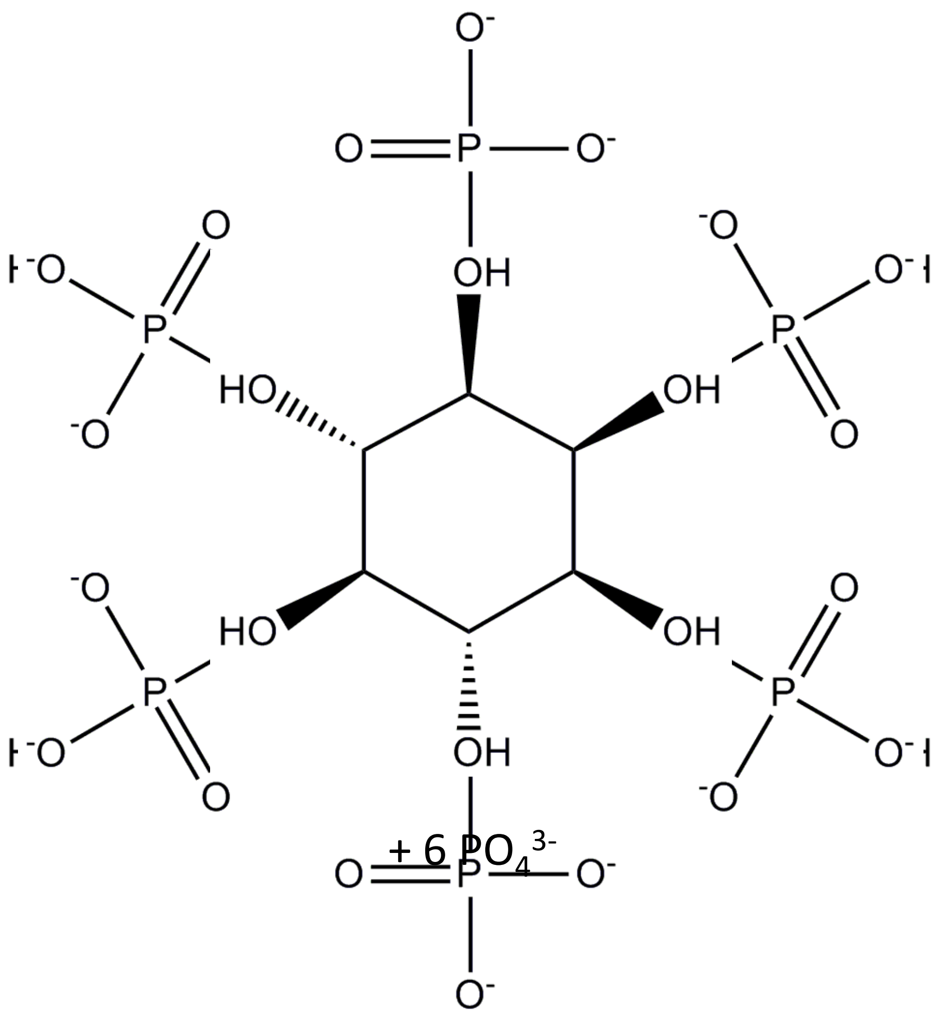
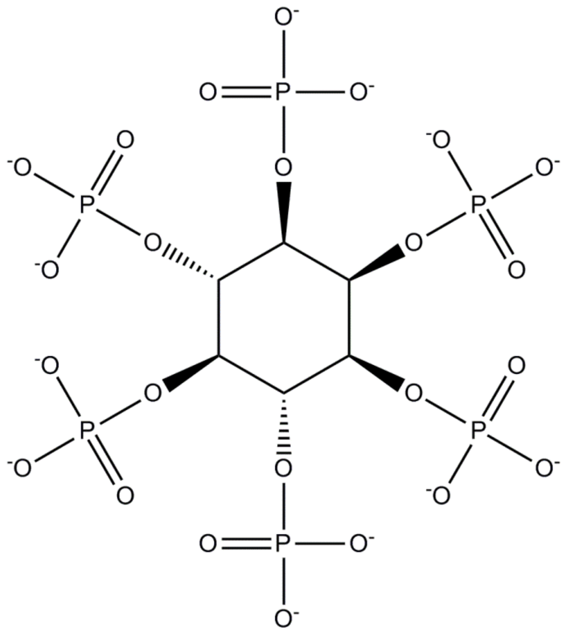
Phytic Acid Molecular Structures of Interest

phytic acid



$+ 6\ PO_4^{3-}$

Some Molecular Facts about Phytic Acid



***myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate**

- phytate (ionic form); phytin (mineral complexed); IP6
- reactive acidic compound that chelates cations
  - more strongly associates with divalent over monovalent
  - 12 protonation sites; 6 pKa's more acidic than the other 6 pKa's
- 1 to 3 %w/w in seeds, cereals, and oilseeds used in animal feed
  - different locations in different grains
  - associated with protein in oilseeds and grain legumes
  - various amounts of endogenous phytase (little to none in corn, high variability in wheat)
- phytin accounts for up to 80% of all P in animal feed
- phytin must be degraded for sufficient P availability (and reduced environmental impact)
- availability of energy and amino acids is reduced when IP6 is present in ingredients and animal feeds
- Phytate alters the secretion of endogenous compounds (animals digestive enzymes, HCl, mucins, etc.)
- DDGS with a reduced phytate content may have a superior nutritional value compared with conventional DDGS
- phytin levels in DDGS can vary greatly – phytase use in fuel ethanol process

Phytate Content and Endogenous Phytase Enzyme Activity in Grains

Grain/Cereal	Phytate [1] (% ds)	Endogenous Phytase [2] (units/kg)
Corn	0.8 -1.2	~20
Sorghum	0.8 -1.1	~20
Wheat	0.25 -1.37	1193
Barley	0.38 -1.16	582
Rye	0.54 -1.46	5130
Triticale	0.50 -1.89	n.d.

Reference: 1) Food Phytases,Edited by Reddy,N. R and Sathe,S. K (2002) CRC Press, New York

2) Ravidran V., W.L. Bryden and E. T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Poultry and Avian Biology Reviews. 6 (2): 125-143

DuPont Danisco Animal Nutrition Phytases

granular phytase enzyme for reduced feed costs and a better environment

Phyzyme® XP

Impact of Phytate on Fuel Ethanol Production

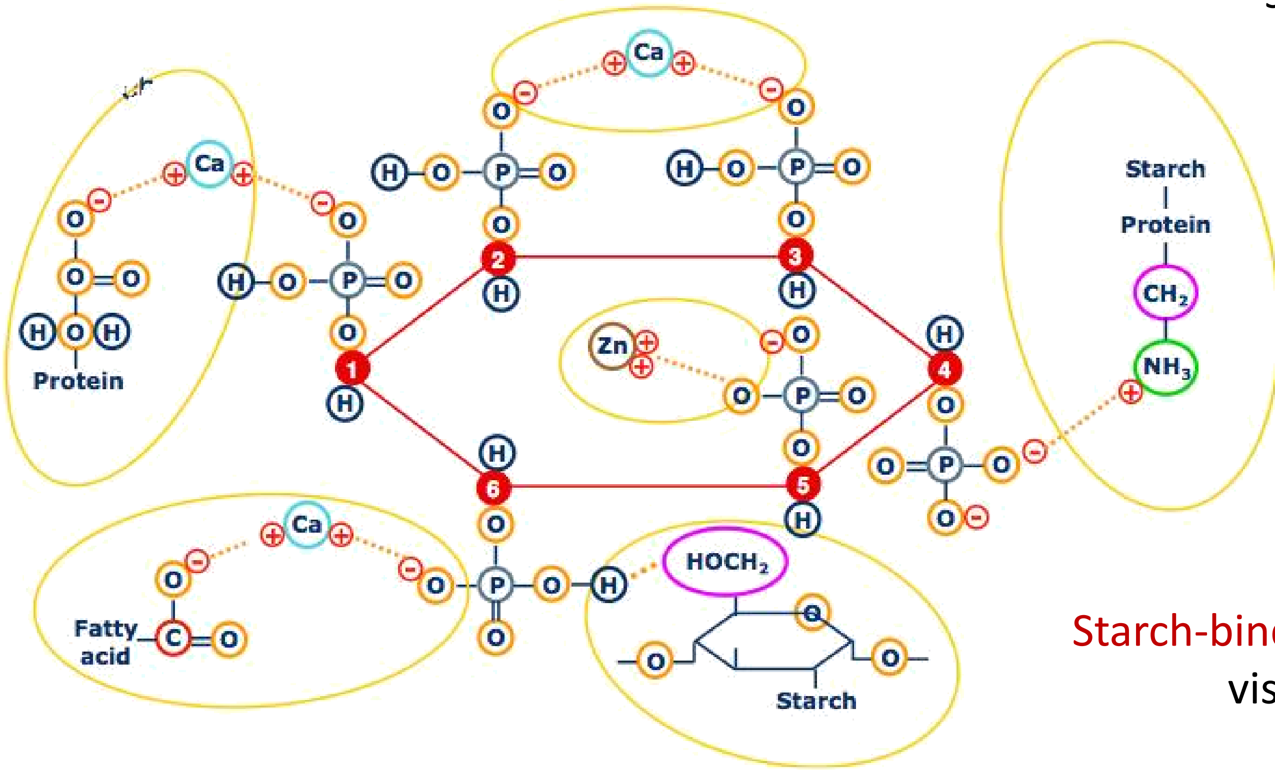
http://en.wikipedia.org/wiki/File:Ethanol_plant.jpg (photo by Steven Vaughn), May 2011

Why Do We Care About Phytate in Fuel Ethanol Production?

calcium is needed for α-amylase stability

direct protein contact can reduce stability of enzymes

starch-protein complexes are less soluble

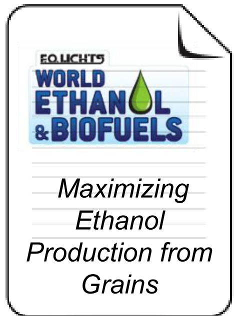


Starch-binding increases viscosity

Zinc is an essential mineral for yeast

The Genencor Timeline for Phytase Application

March: Product Launch GC 491 a phytase product for ethanol production



Paper presented at World Ethanol 2004 & Ethanol Production Workshop by George Virgil

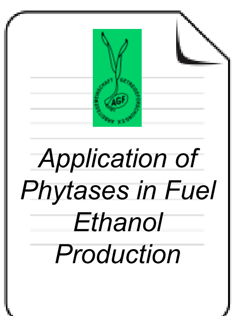
INTERNATIONAL sugar JOURNAL

Article Phytase in fuel ethanol production published in the International Sugar Journal

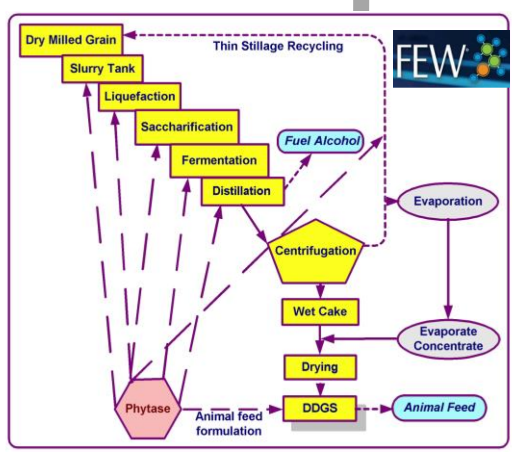
Product Launch MAXALIQ One

Product Launch SPEZYME® RSL

2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011



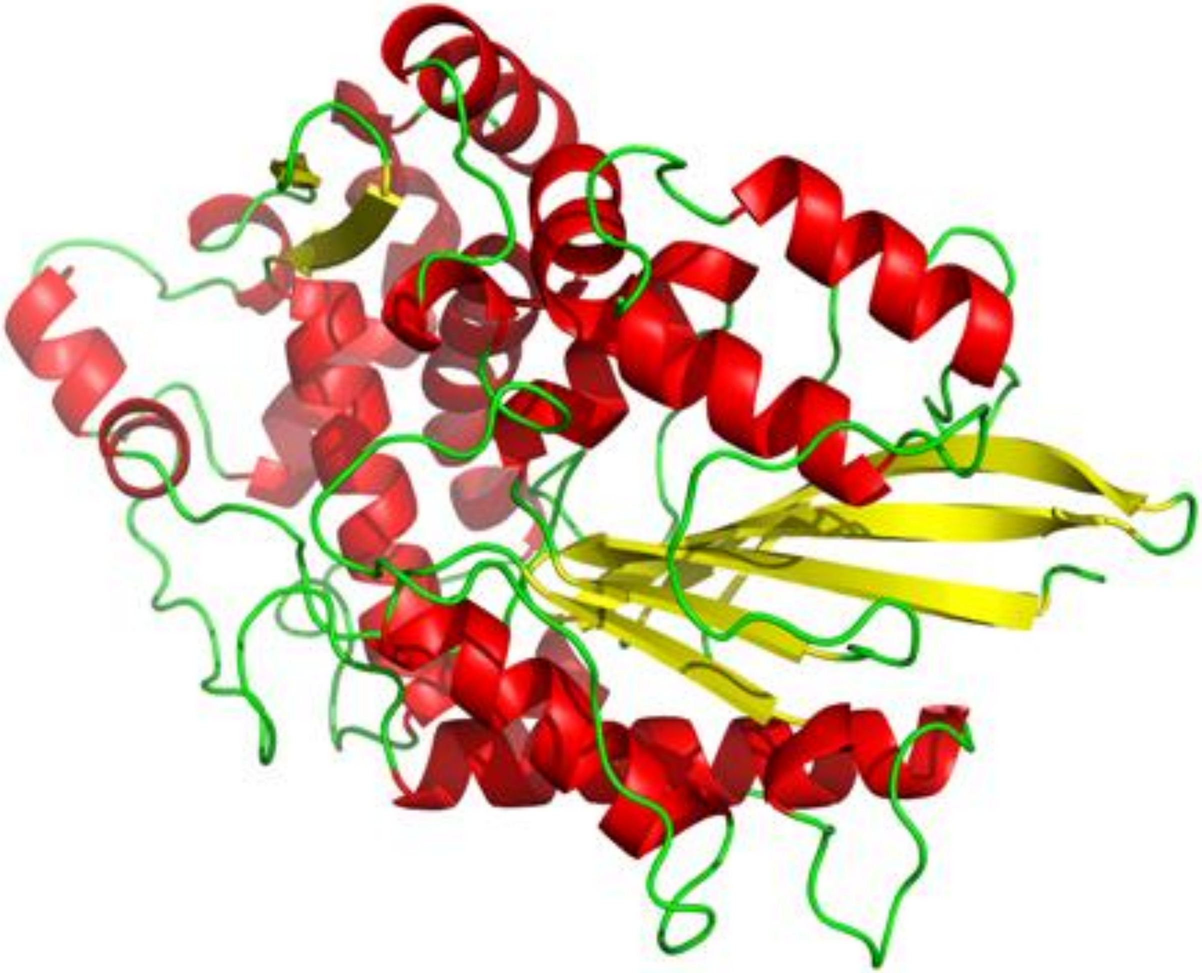
April: Paper presented at the 2001 Detmold Starch Conference by Gerhard Konieczny Janda



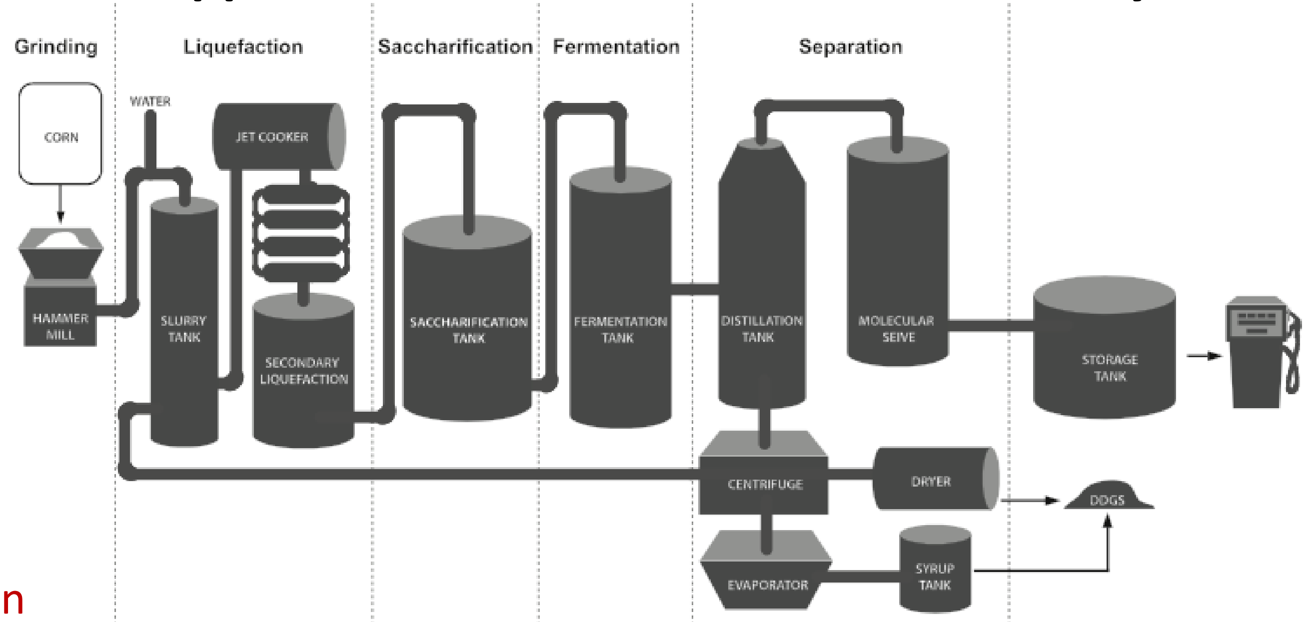
Fuel Ethanol Workshop presentation given by Dr. Jay Shetty

Product Launch DISTILLASE SSF+ a blend for SSF which contains phytase

Engineered Phytases *with Enhanced Thermostability*



Where Phytase is Applied in Fuel Ethanol Production Today



In Liquefaction

- To enhance the pH and temperature stability of alpha amylases
- Liquefaction at pH 5.0 to 5.3 reduces the amount of H_2SO_4 and anhydrous ammonia needed
  - Lower total cost of liquefaction
  - Decreasing the sulfur present in wet distillers grains and DDGS

In Fermentation

- To generate inositol*, an important yeast nutrient
- To maximize the level of free phosphate in residuals (DDGS)

Reference:

* Determining the effects of inositol supplementation and the *opi1* mutation on ethanol tolerance of *Saccharomyces cerevisiae Erin L. Krause, Manuel J. Villa-García, Susan A. Henry, Larry P. Walker. Industrial Biotechnology. October 2007, 3(3): 260-268. doi:10.1089/ind.2007.3.260.*

Dry Grind Liquefaction Has Some Issues

- Liquefaction has typically required two pH adjustments
  - From 5.1 to 5.8
  - Back to the chosen fermentation pH (5.8 to 4.5)
- It requires tight pH and temperature control
  - A pH variance of 0.2 can hurt performance
  - A temperature variance of +2° can hurt performance
- Adds significant sulfur to the system and eventually to DDGS (0.08 to 0.12% dmb)
- At many plants, two enzyme doses are necessary – adding complexity
- Poor liquefaction reduces ethanol yields and plant performance

Plant	Backset	Backset, Water and Corn
A	4.3	5.4
B	4.2	5.7
C	4.3	5.1
D	3.4	5.6
E	3.7	5.2
F	4.2	4.8
G	4.3	5.1
H	3.7	4.7
I	4.3	5.1
J	4.5	5.0
K	3.6	4.9
L	3.4	5.1
M	3.8	4.7
Average	4.0 ± 0.4	5.1 ± 0.3

Recommended Conditions for Liquefaction

Conventional α-amylase use:

Solids	30 to 36% wt/wt
pH Range	5.6 to 6.0
Temperature Range	170 to 190° F
Liquefaction Time	90 to 140 minutes

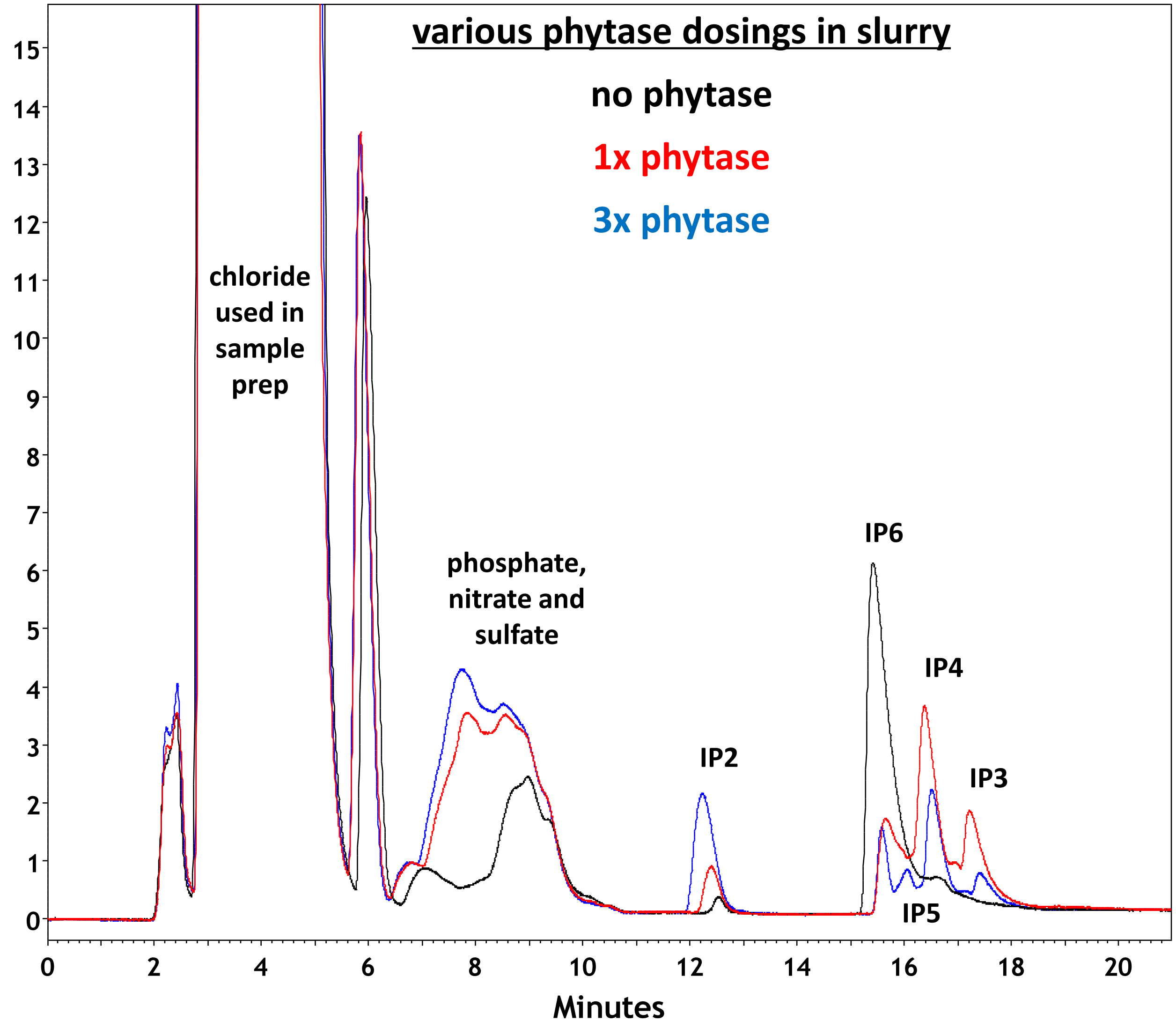
Recommended Dose: 0.020 to 0.024 %wt/wt as-is grain

SPEZYME® RSL (α-amylase + phytase) use:

Solids	30 to 36% wt/wt
pH Range	5.0 to 6.0
Temperature Range	170 to 195° F
Liquefaction Time	90 to 140 minutes

Recommended Dose: 0.020 to 0.024 %wt/wt as-is grain

Ion Chromatography for Inositol Phosphates



Dionex OmniPac PAX-100 column

0.20M NaOH gradient (2.5% IPA isocratic)

- 5%-25% in 8 min
- 25%-70% in 7 min
- regen and equil steps

suppressed conductivity detection

supernatant dilution to 0.5M HCl

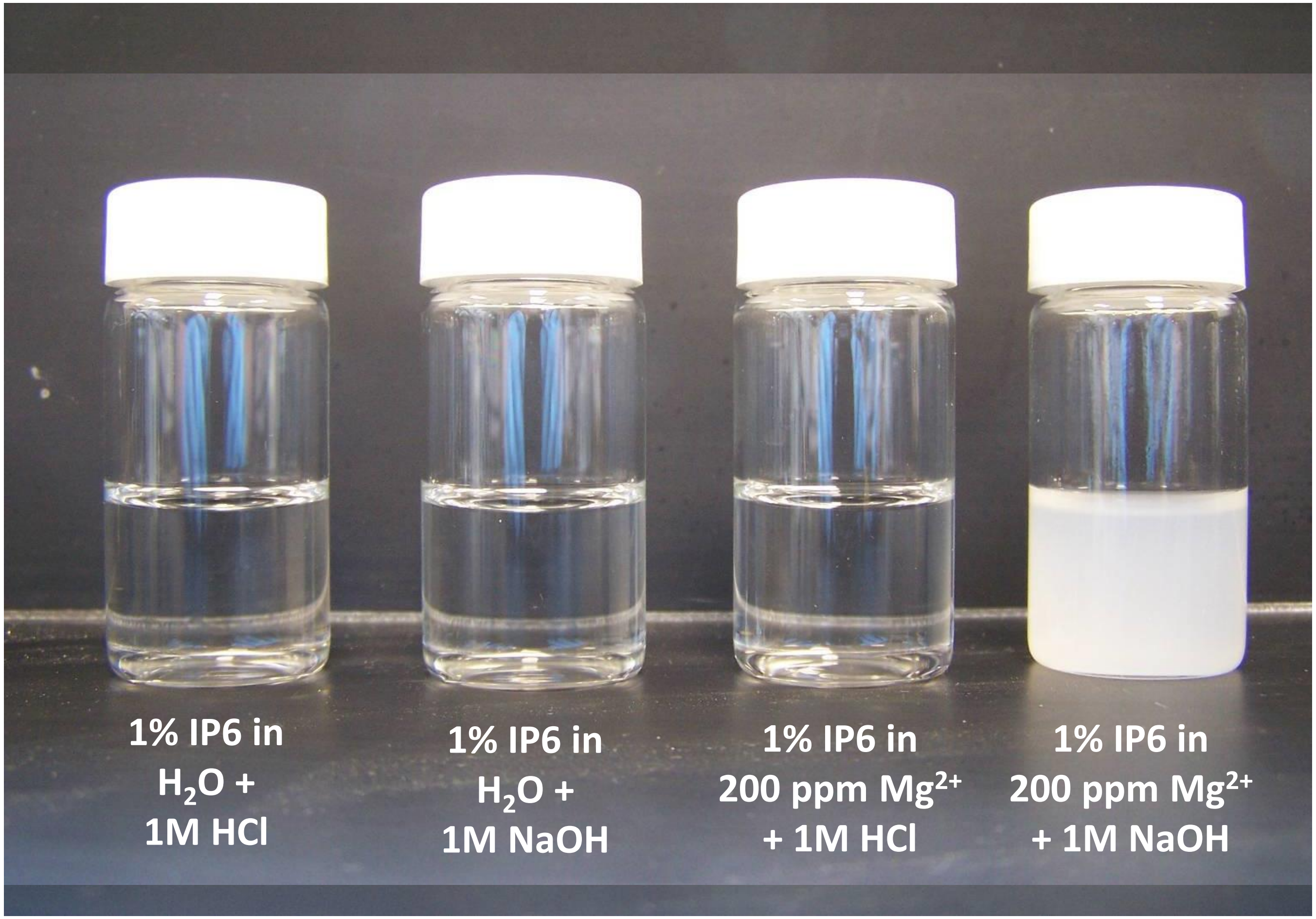
liquefaction supernatant: 0.25 %w/v supernatant, (31.6 DS% equates to 0.8 %w/w corn as is basis)

The Analytical Method: sample preparation

multiple chemical equilibriums to consider:

K_a's…K_{chel}'s…K_{sp}'s

$IP_6H_{12} \leftrightarrow IP_6^{12-}$; $IP_6^{12-} + M^{2+} \leftrightarrow IP_6M_6$; $\underline{IP_6}_{\ s} \leftrightarrow IP_{6\ aq}$



Phytate is Soluble in Acidic Environments

(i.e. phytate is NOT soluble in many conditions)

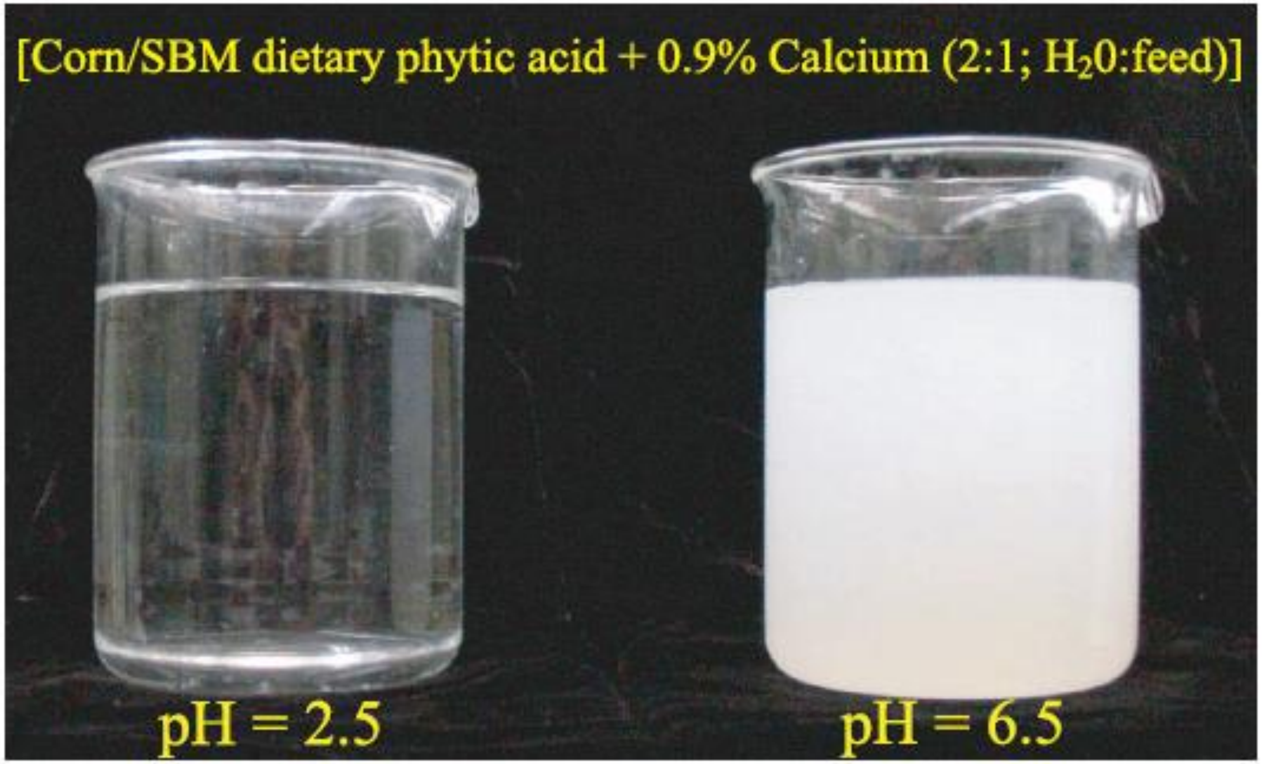
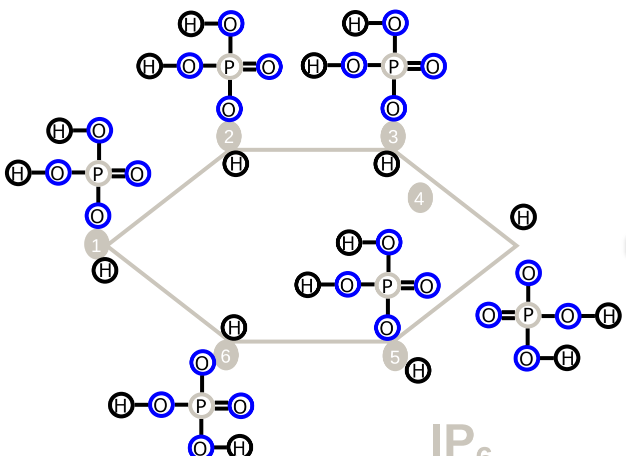


Figure 2: Demonstration of what occurs to phytin-Ca complex in the stomach (pH 2.5) and small intestine (pH 6.5). At the higher pH, phytase cannot work as easily on the substrate phytin because the subtstrate is precipitated.

Applegate, T.J. and Angel, R.; "Phytase: Basics of Enzyme Function," Purdue Univ. Extension, *Farm Animal Management @ Purdue*, **2004**, *Doc. AS-560-W*.

Phytase Use at Low pH Reduces the Antinutrient Effects of Phytate

Phytate is more soluble at low pH and more susceptible to phytase attack



IP_6

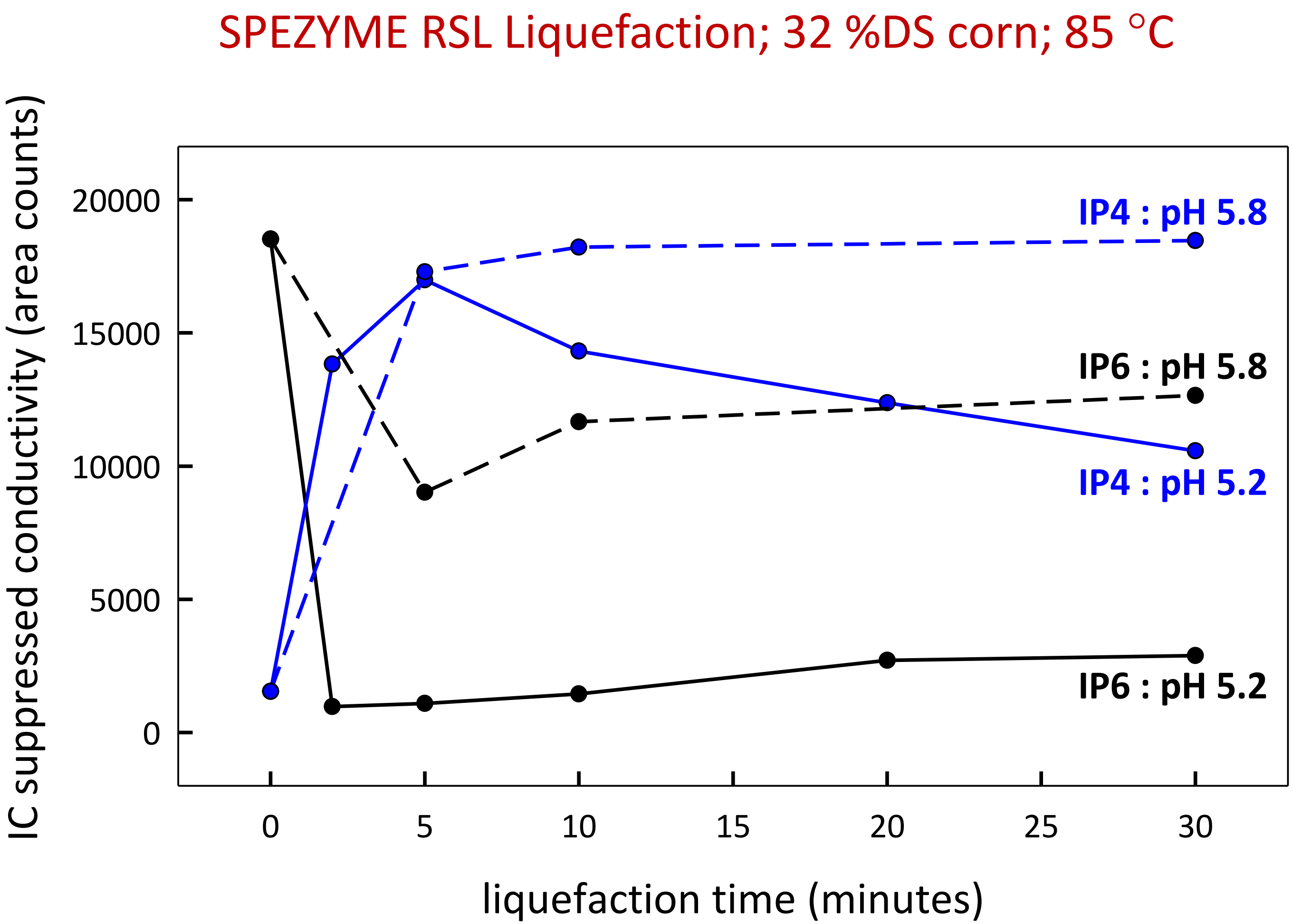
Phytase →

Results in Lower order esters (IP5, IP4 etc.)

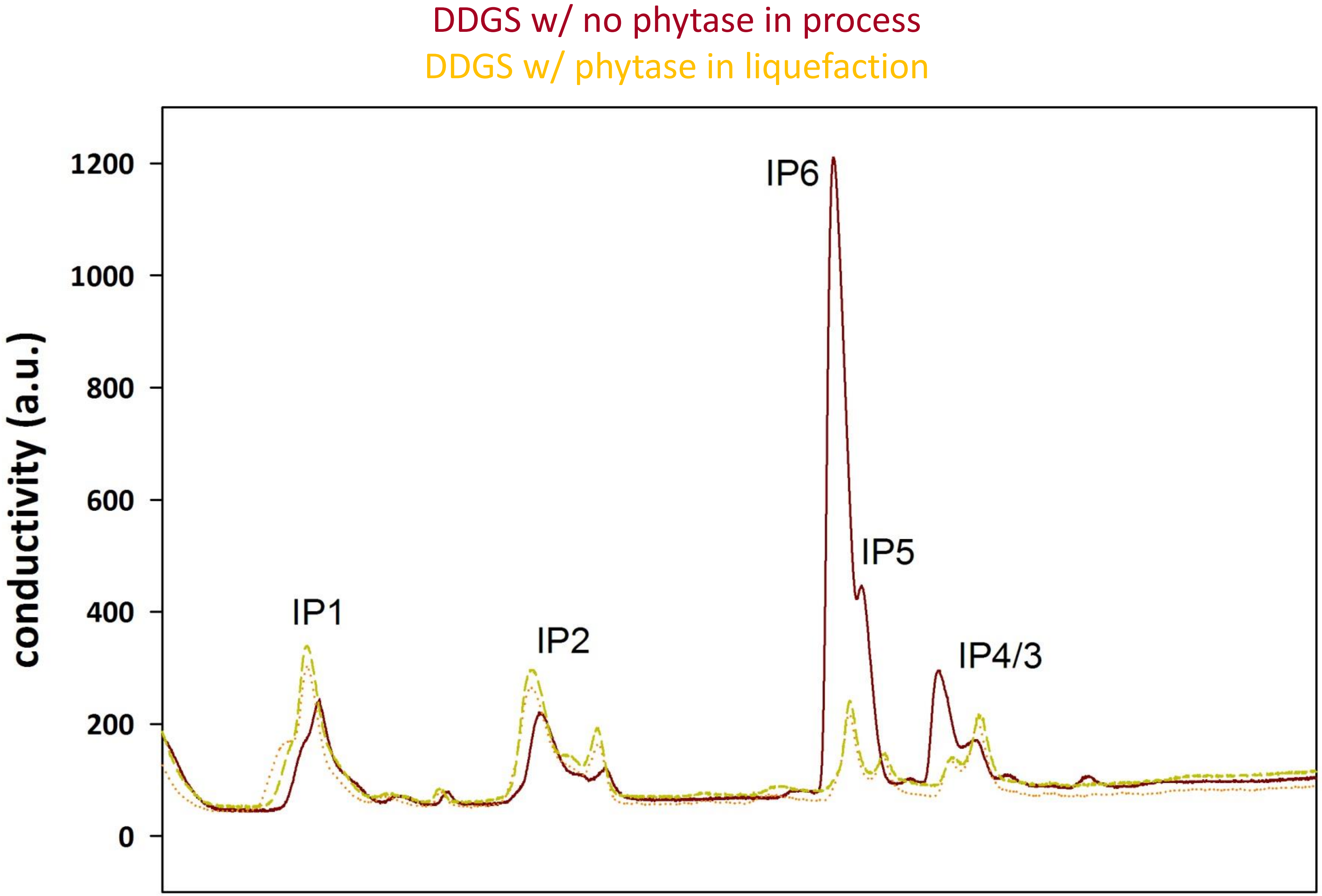
P release, improved utilization of Ca and protein

The most effective phytases will be those that lower **IP_6** concentrations **most rapidly at acidic pH**

Phytic Acid Reduction



Phytic Acid Monitoring in DDGS



Phytate in DDGS using Spezyme® RSL

no phytase in liquefaction

	phytic acid (%w/w)
Plant 1	0.71
	0.71
Plant 1	0.75
	0.68
Plant 1	0.77
	0.80
Plant 2	0.62
	0.63
Plant 2	0.56
	0.60
Plant 2	0.58
	0.56
Plant 3	0.58
	0.62
Plant 3	0.70
	0.68
Plant 3	0.61
	0.62

each DDG was sampled twice,

same plants were sampled at later times

http://www.ethanolproducer.com/articles/8088/
2011-ddgs-exports-to-japan-up-45-likely-to-set-records

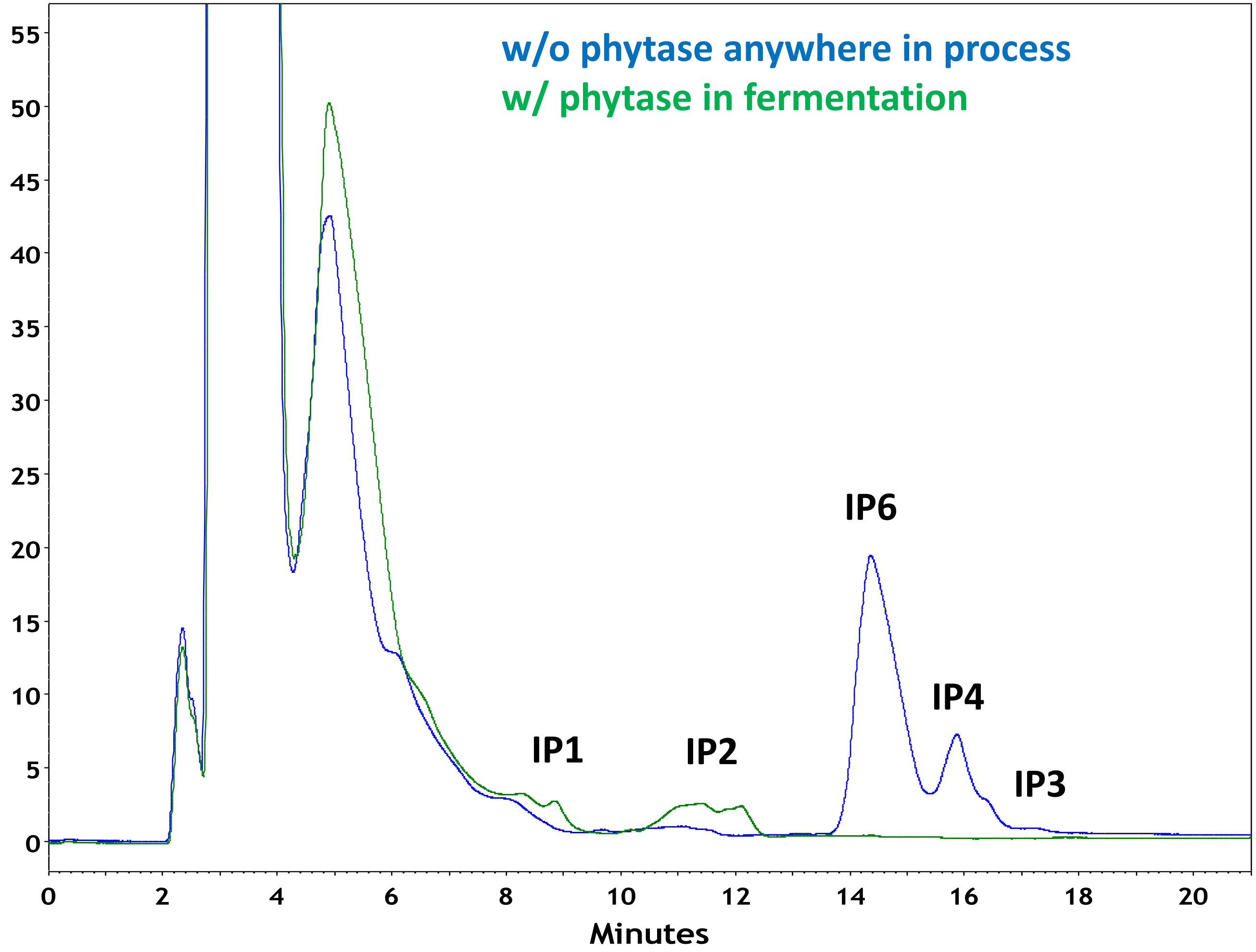
phytase in liquefaction

	phytic acid (%w/w)
Plant A	0.19
	0.21
Plant A	0.23
	0.23
Plant A	0.15
	0.16
Plant B	0.18
	0.17
Plant B	0.13
	0.13
Plant B	0.14
	0.15
Plant C	0.23
	0.24
Plant C	0.26
	0.28
Plant C	0.24
	0.25

DISTILLASE® SSF+ : phytase in fermentation

The DISTILLASE® SSF+ unique composition improves fermentation yield and consistency. It does so by minimizing the fraction of starch that remains unfermented and enhancing yeast nutrition.

It contains: glucoamylase, acid stable a-amylase, protease, and phytase



Fermentation is Better Environment for Phytase

Phytic Acid Monitoring in DDGS

DDGS Samples	phytic acid (%w/w)
no phytase added	*0.4 - 1.0*
SpezymeRSL	*0.1 - 0.3*
SSF and prop phytase	0.050 ± 0.006
SSF+	0.005 ± 0.002
SSF++	0.003 ± 0.001
SSF+ and prop phytase	0.001 ± 0.001

Estimating the Value of Improved DDGS

- Substantial Market
  - Swine: 2.8MM tons potential DDGS demand @ 10% inclusion
  - Poultry: 5.6MM tons
- Reduced phosphate supplementation by feed formulator
  - direct savings of added dicalcium phosphate
- Increased digestibility
  - sparing of more costly ingredients such as soybean oil or meal
- Increased DDGS consistency
  - More attractive ingredient, allows higher inclusion rates
- Less phosphorous runoff from feedlots

Estimating the Value of Improved DDGS: *reducing phosphorous waste*

You can't teach pigs to whistle, but you can reduce their impact on the environment

Environmental issues, including waste management, are the number one concern of livestock and poultry producers.

As the size and intensity of swine and poultry production operations have increased, so has the amount of manure produced, often with limited land area available for manure application, says E.T. Kornegay, professor of animal and poultry science. "This can lead to a build-up of nutrients in and on the soil with the potential for runoff into waterways."

Excess environmental phosphorus is suspected as a contributing factor in water-quality degradation and even in the bloom of *Pfiesteria piscicida*, which has caused fish kills in North Carolina and in tributaries to the Chesapeake Bay.

Virginia Tech animal and poultry science researchers, led by Kornegay, have developed feeding regimes for swine and poultry that result in massive reductions of phosphorus and other nutrients in manure. Kornegay's recommendations are being used nationwide, and he lectures about his research worldwide.

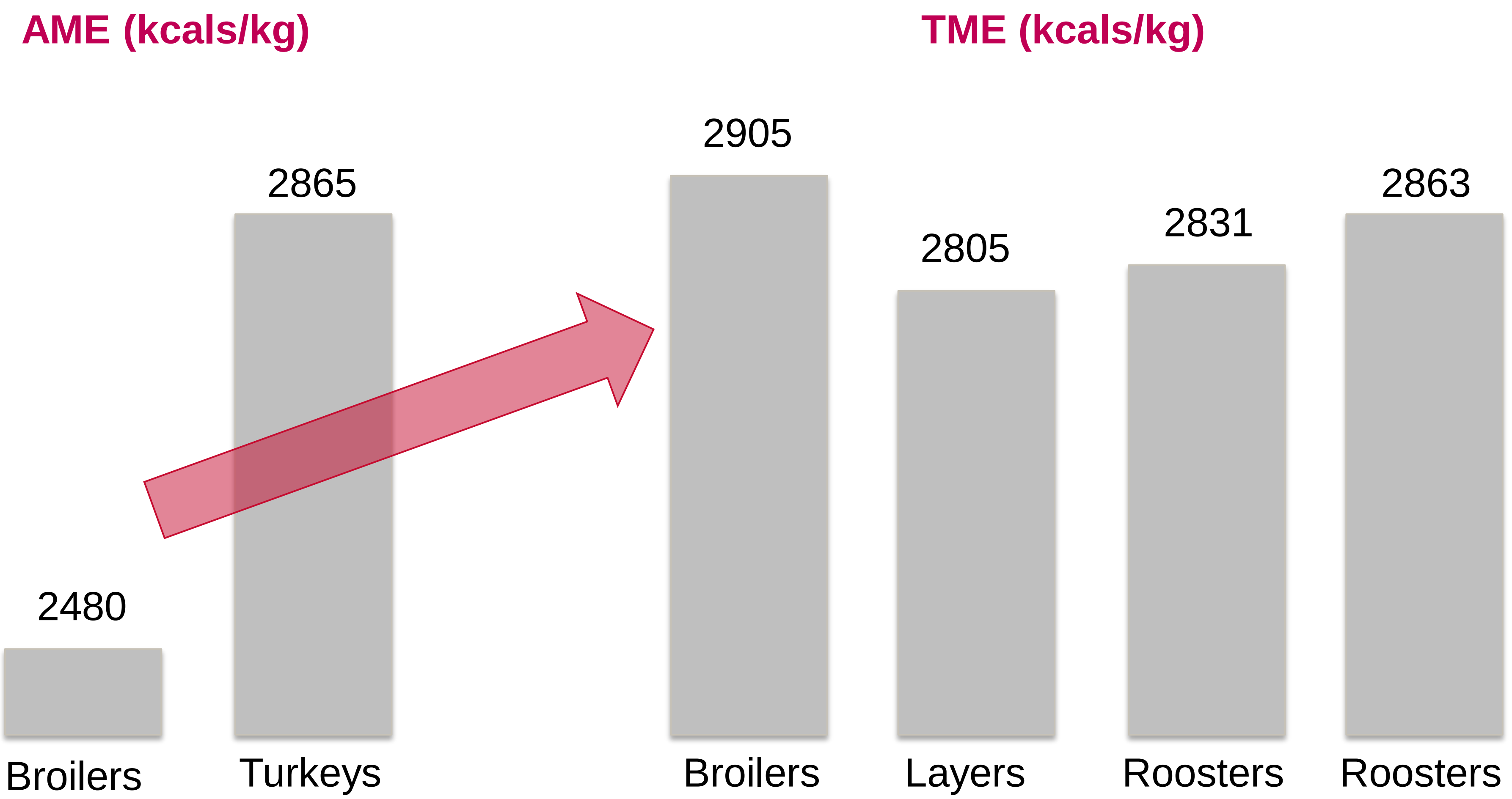
The key is what Kornegay calls "a miracle enzyme," microbial phytase.

Susan Trulove, in Virginia Tech Research magazine

Feed formulation savings example

- Each ton of DDGS saved up to $14
  - Broiler feed formulation
  - Reduced added phosphate, soybean meal and oil
- ~1 kg of Maxaliq® ONE enzyme consumed per ton DDGS
- **Each kg of Maxaliq® ONE added up to $14 / ton in premium DDGS value !!!**
- $1/ton of DDGS premium brings $146K to a 50 MGPY ethanol plant

DDGS can be a highly variable ingredient – difficult for nutritionists to predict its value



References: NRC 1994, Noll *et al* 2004, Lumpkins *et al* 2004, Lumpkins *et al* 2005, Batal & Dale 2004, Parsons *et al* 2006

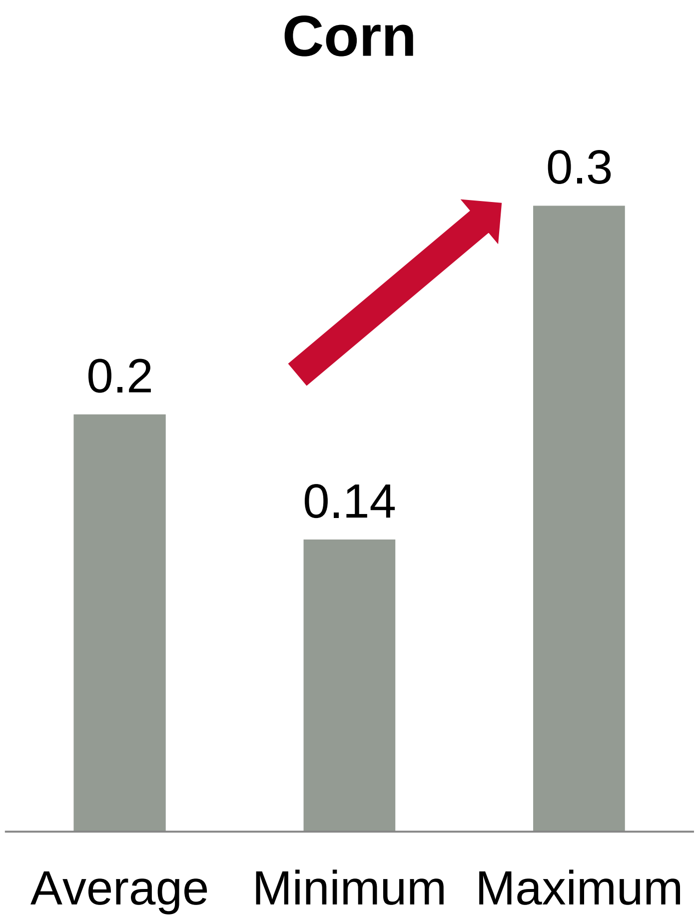
DDGS can be a highly variable ingredient – difficult for nutritionists to predict its value

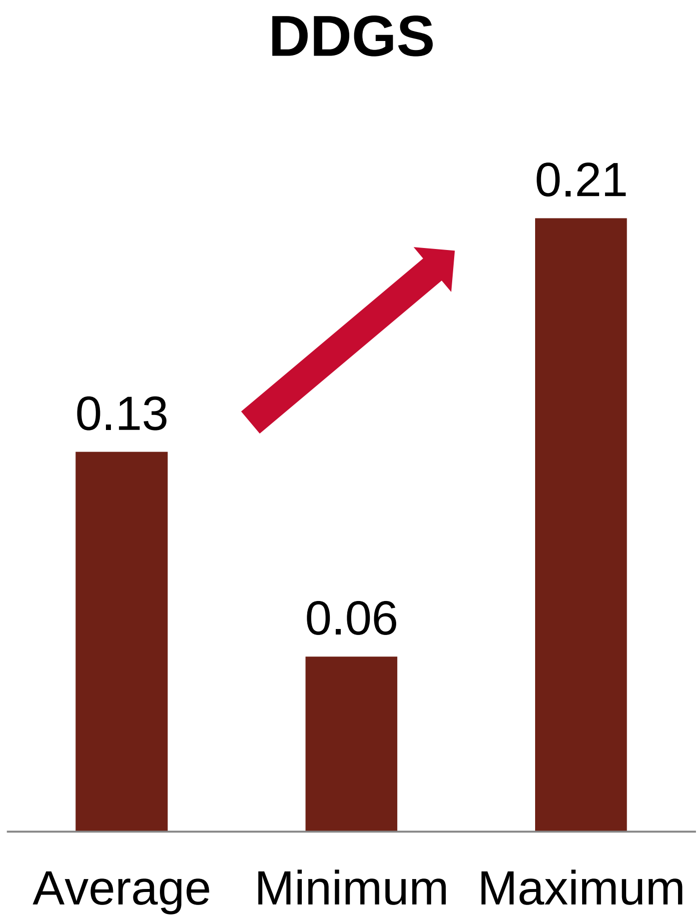
Amino acid	Average	Range	CV (%)
Content (%)			
Lysine	0.73	0.59-0.89	11.6
Methionine	0.49	0.41-0.60	9.7
Threonine	0.98	0.85-1.14	6.0
Cystine	0.52	0.42-0.67	11.3
Digestibility (%)			
Lysine	72	59-84	11.2
Methionine	88	85-92	1.9
Threonine	76	69-83	4.8
Cystine	77	66-87	7.7

20 corn DDGS samples

Reference: Batal & Dale, 2006

Phytate P (%) levels in US corn and DDGS





DuPont database, harvest year 2012, corn (56 samples), DDGS (15 samples)

Nutrient Availability is Highly Variable - Phosphorus

DDGS Sample	Bioavailability (% relative to K_3PO_4)	Total P content (%)	Bioavailable P (%)
1	69	0.72	0.49
2	102	0.74	0.75
3	82	0.72	0.59
4	75	0.73	0.55
5	62	0.67	0.42
6	70	0.76	0.53
7	82	0.72	0.59
8	87	0.77	0.67
9	84	0.74	0.62
Average	79	0.73	0.58
Std deviation	11.8	0.03	0.10
CV%	15.0	3.9	16.8

The values are from a report presented at the 2006 Multistate Nutrition Conference. Ref: Parsons, C.M., C. Martinez, V. Singh, S. Radhakrishman, and S. Noll. 2006. Nutritional value of conventional and modified DDGS for poultry. Multi-State Poultry Nutrition and Feeding Conf., Indianapolis, IN. May 24-25, 2006.

Effect of Phytase in Ethanol Production on Nutritive Value of DDGS

Parameter	DDGS - phytase	DDGS + phytase	% impr.	P value
Dry matter digestibility, %	61.98	66.61	7.5	0.11
TME, kcal/kg DDGS (dmb)				
Total amino acid dig., %				
Essential AA dig., %				
Non-essential AA dig., %				

Essential amino acids: Arg, His, Iso, Leu, Lys, Met, Phe, Thr, Try, Val
Non-essential amino acids: Ala, Asp, Cys, Glu, Ser, Tyr

Same source of corn used to produce both types of DDGS: phytate P levels: 0.05% (phytase used) and 0.34% (no phytase)

Total P digestibility was increased by 27% (28.44 to 36.12%). However, the lower numbers probably reflect the fact that precision feeding method is not the best one to use to evaluate mineral digestibility.

Effect of Phytase in Ethanol Production on Nutritive Value of DDGS

Parameter	DDGS - phytase	DDGS + phytase	% impr.	P value
Lysine dig., %	71.87	75.94	5.7	0.24
Methionine dig., %				
Threonine dig., %				
Isoleucine dig., %				
Leucine dig., %				
Valine dig., %				

Same source of corn used to produce both types of DDGS: phytate P levels: 0.05% (phytase used) and 0.34% (no phytase)

Value of classic and phytase DDGS in a poultry diet

	w/ classic DDGS	w/ phytase DDGS
Corn	52.18	52.46
Classic DDGS	15.00	0.00
Phytase DDGS	**0.00**	**15.00**
SBM 48	24.20	24.16
L-LysHCL	0.21	0.21
DL-Met	0.18	0.18
NaCl	0.30	0.30
Limestone	1.09	1.25
Dical.Phosphate	0.73	0.44
Phytase	0.01	0.01
Vit/Min Premix	0.05	0.05
Fat/oil	6.06	5.96
Cost of diet, $	364.93	363.14

Diet cost difference **$1.8 per ton** of feed (with 15% DDGS) – value of 1 ton of phytase DDGS **~$12** higher than classic DDGS (based on higher P availability only)

Summary

- Phytate is a strong chelator of minerals and interacts with proteins and starch
- Ion chromatography with suppressed conductivity detection is used to resolve inositol phosphates.
- Sample preparation exemplifies the troublesome chemical nature of phytic acid
- Phytase use in dry-grind fuel-ethanol production provides various benefits
- SPEZYME® RSL provides enhanced liquefaction at lower pH’s
- DISTILLASE® SSF+ provides yeast nutrients and is capable of reducing phytate levels in DDGs below detectable limits
- Phytate content in DDGS available to US animal feed industry is variable affecting P availability
- Almost no phytate P detected in DDGS samples from the ethanol processes, which are using phytase in fermentation
- DDGS derived from the phytase treatment can have significantly higher true metabolizable energy (TME) and digestibility of some amino acids, especially those present at high concentrations in endogenous protein
- DDGS with reduced phytate content has a superior nutritional value than classic DDGS

Thank You!