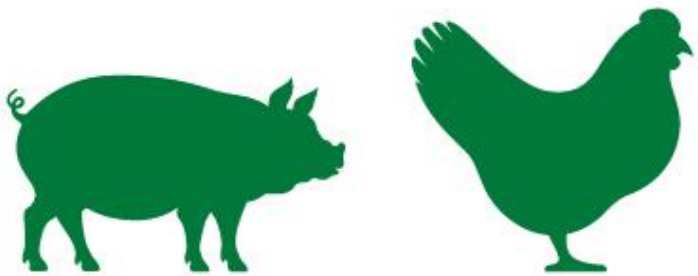
The new phytase

natuphos® E

EFFICIENT BY EXPERIENCE



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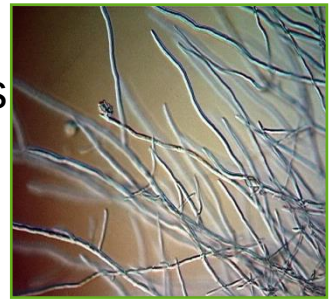
Outline

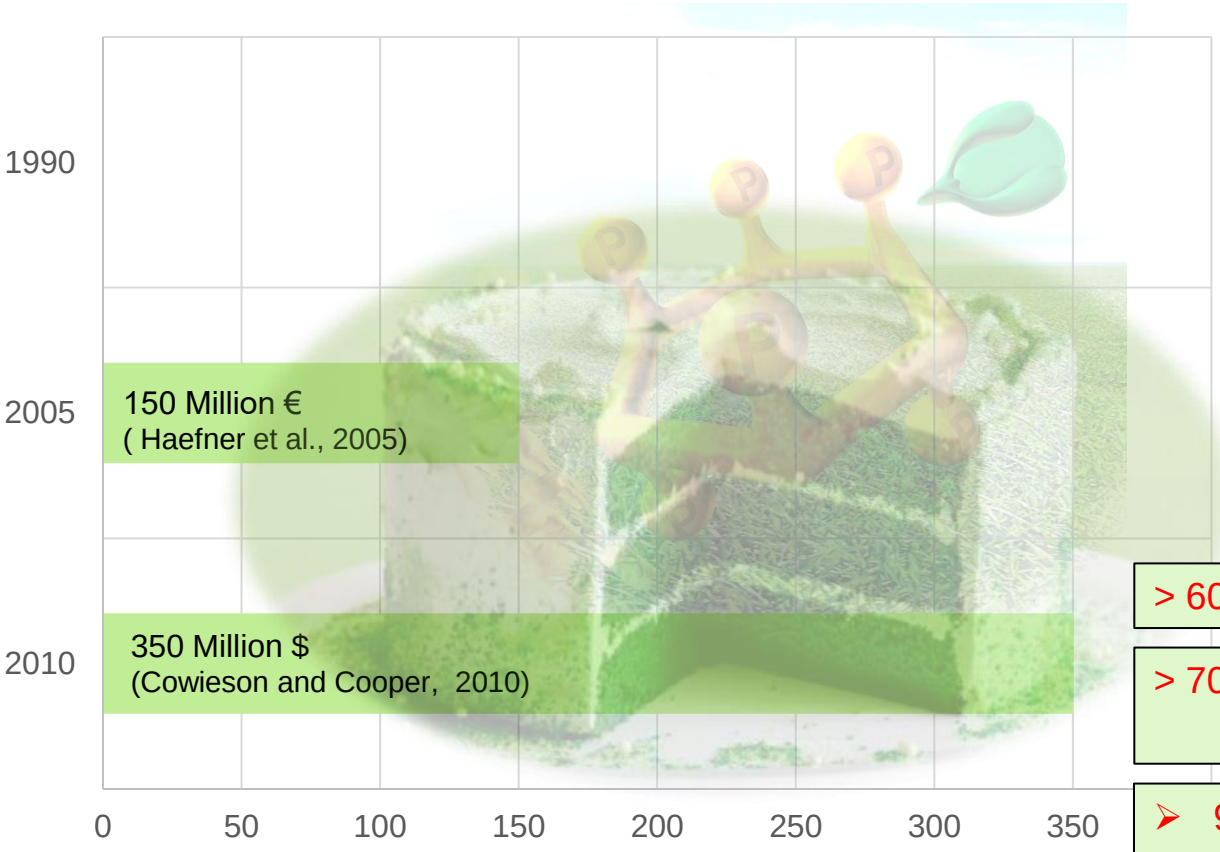
- ✓ Getting started...
- ✓ natuphos® E – BASF ´s new Phytase.
- ✓ Product features
- ✓ Performance in pig and poultry diets.
- ✓ Summary

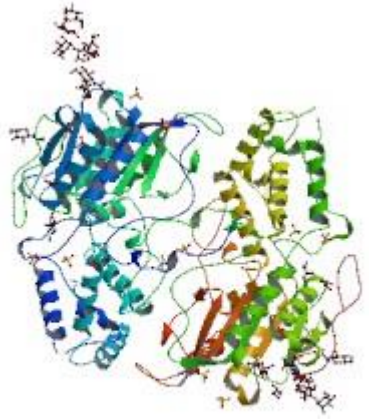
Getting started

- **1903** Phytic acid was discovered by Posternak
- 1907 Natural phytase extracted and described by Suzuki
- 1911 Demonstration of phytase in fungi by Dox & Golden
- 1968 First amounts fermented with non GMO strain by Shieh & Ware
- 1968 First „proof of concept“ feeding trial in broiler by Nelson
- 1985 First few grams of GMO phytase produced
- 1990 Landmark study of Simons et al. published
- 1990/91 First natuphos® sales in the Netherlands by Gist-brocades; BASF took over marketing & sales outside the Netherlands
- 2006 BASF becomes independent enzyme producer and starts own enzyme production



Development of the phytase market





> 60 % of all feed enzymes

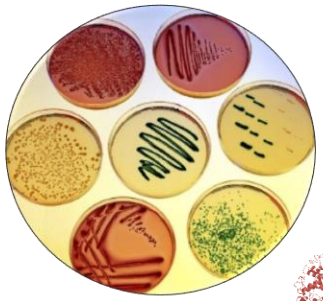
> 70 % in pig and poultry diets worldwide
(Lei et al., 2013)

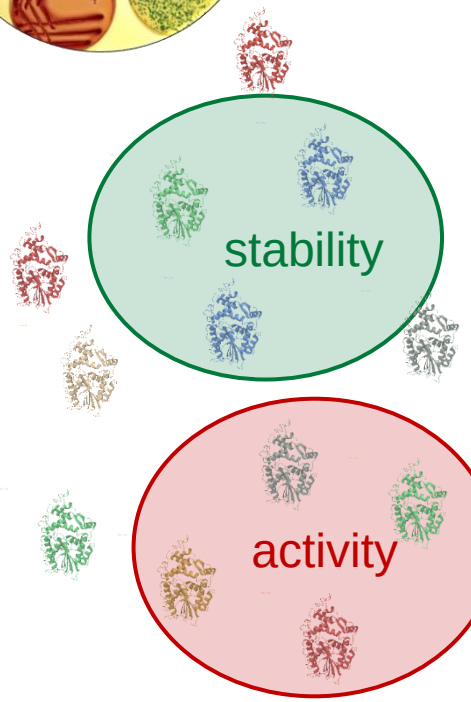
➢ 95 % of the pig feed in D
(Grünewald et al., 2013)

Commercial phytase products

Company	Trade Name	Donor organism	Production organism	Reference
BASF	Natuphos	*Aspergillus niger*	*Aspergillus niger*	(EFSA 2006a)
DSM	Ronozyme P, NP	*Peniophora lycii*	*Aspergillus oryzae*	(EFSA 2010)
DSM	Ronozyme HiPhos	*Citrobacter braakii*	*Aspergillus oryzae*	(Guggenbuhl et al. 2012)
Adisseo	Rovabio PHY	*Penicillium funiculosum*	*Penicillium funiculosum*	(EFSA 2007)
Danisco	Phyzyme XP	*Escherichia coli*	*Schizosaccharo-myces pombe*	(EFSA 2006)
Dupont	Axtra PHY	*Buttiauxella spp.*	Trichoderma reesei	(EFSA 2015)
Huvepharma	OptiPhos	*Escherichia coli*	*Pichia pastoris*	(EFSA 2011)
AB Enzymes	Quantum	*Escherichia coli*	*Pichia pastoris*	(EFSA 2008)
AB Vista	Quantum Blue	*Escherichia coli*	*Trichoderma reesei*	(EFSA 2013)

New Ways Towards a New Phytase





- Screening of nature’s diversity for high efficacy and thermostability
- Several new phytases identified
- No candidate fulfilled **all** requirements

What could make a phytase really different ?

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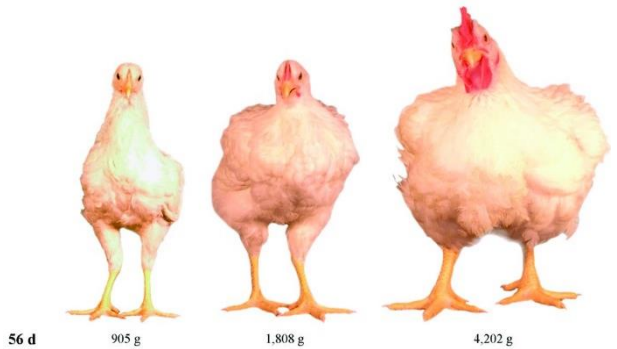
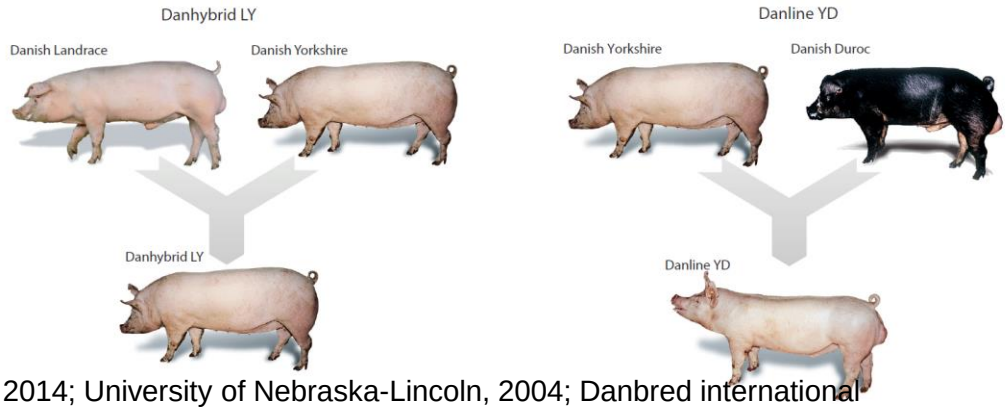
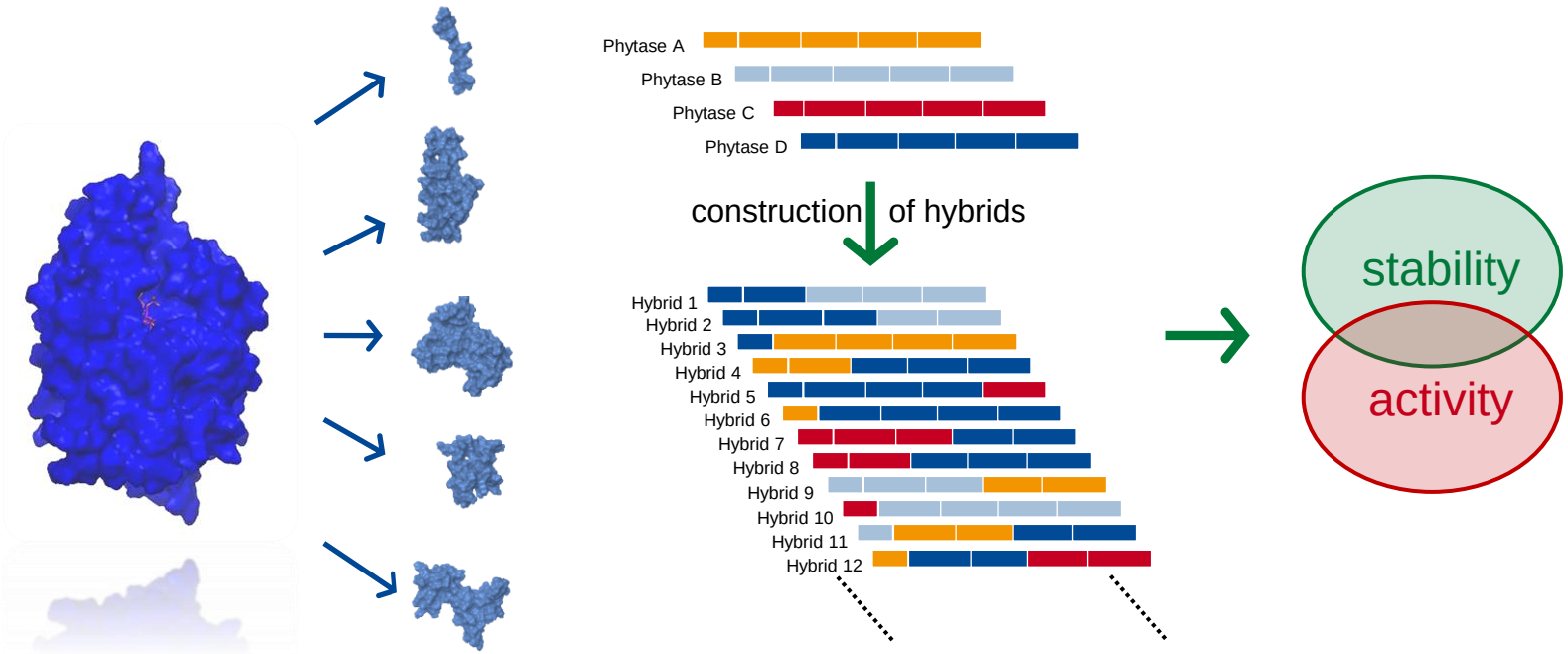


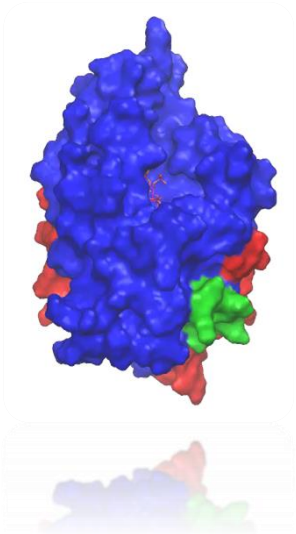


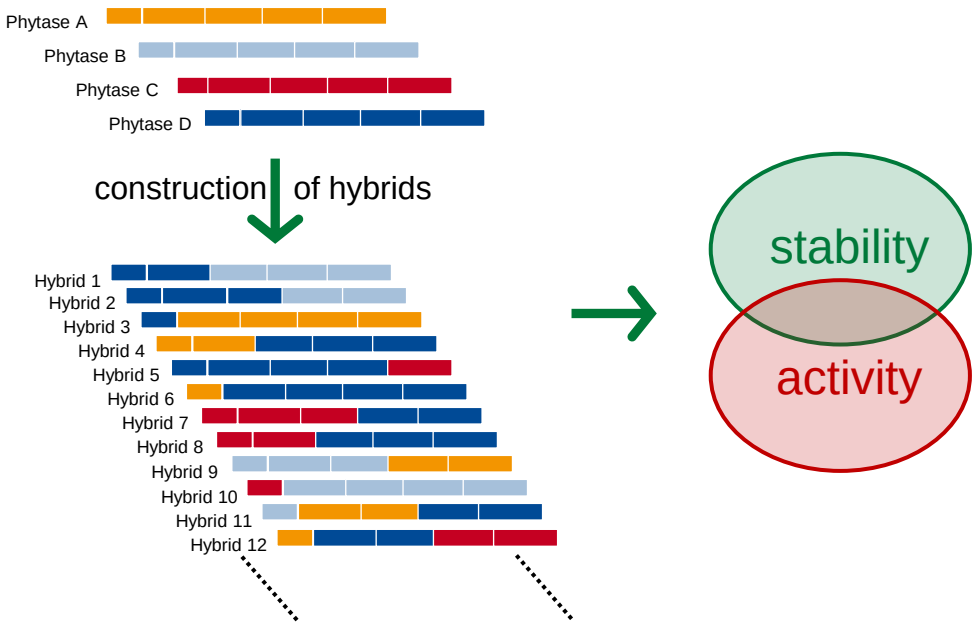
Sources: Zuidhof et al., 2014; University of Nebraska-Lincoln, 2004; Danbred international

Novel Hybrid Phytase Superior to Natural Isolates



Novel Hybrid Phytase Superior to Natural Isolates





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A composite hybrid 6-phytase of bacterial origin created by modern enzyme *E*ngineering

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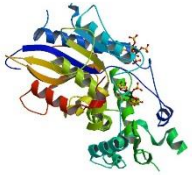
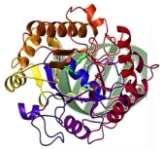
Hafnia sp (Source)



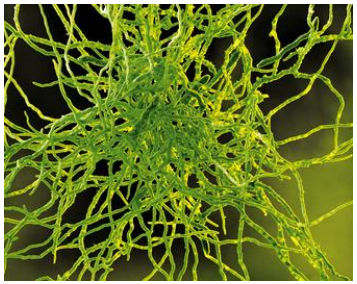
Yersinia sp (Source)

Buttauxiella sp (Source)



Hybrid DNA

Aspergillus niger
(Production strain)



natuphos® E

A more *E*fficient phytase

pH-profile of Natuphos® *E* compared to Natuphos®

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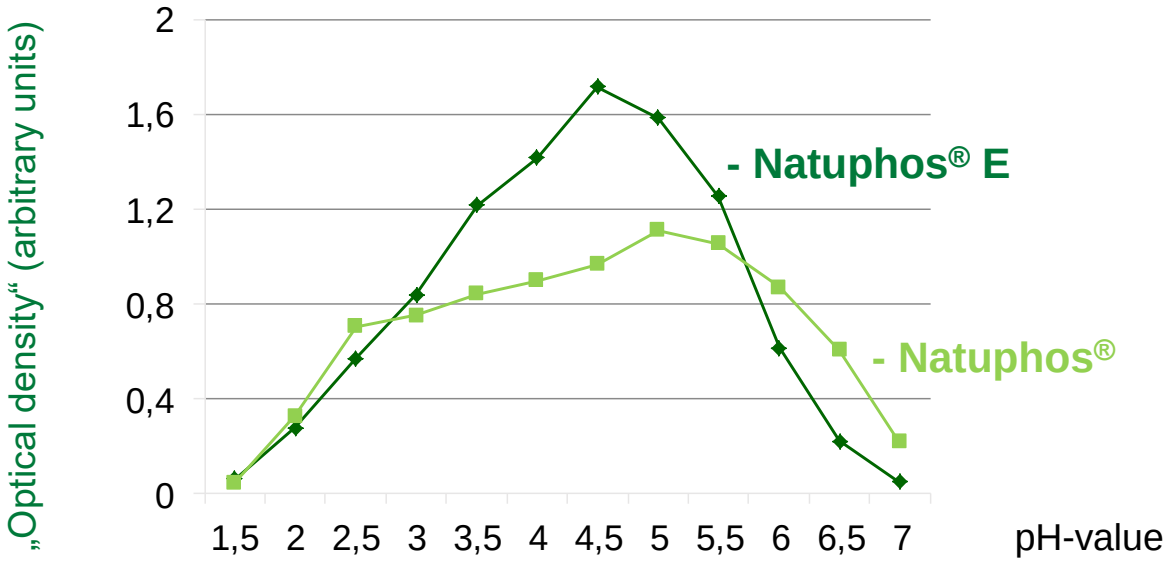
pH profile → larger Area Under the Curve (AUC) indicates higher efficacy within most relevant pH range



Natuphos® E developed for being optimally adapted to GIT conditions.

natuphos® E

A more *E*fficient phytase

Resistance of Natuphos® *E* and Natuphos® in the GIT against inactivation

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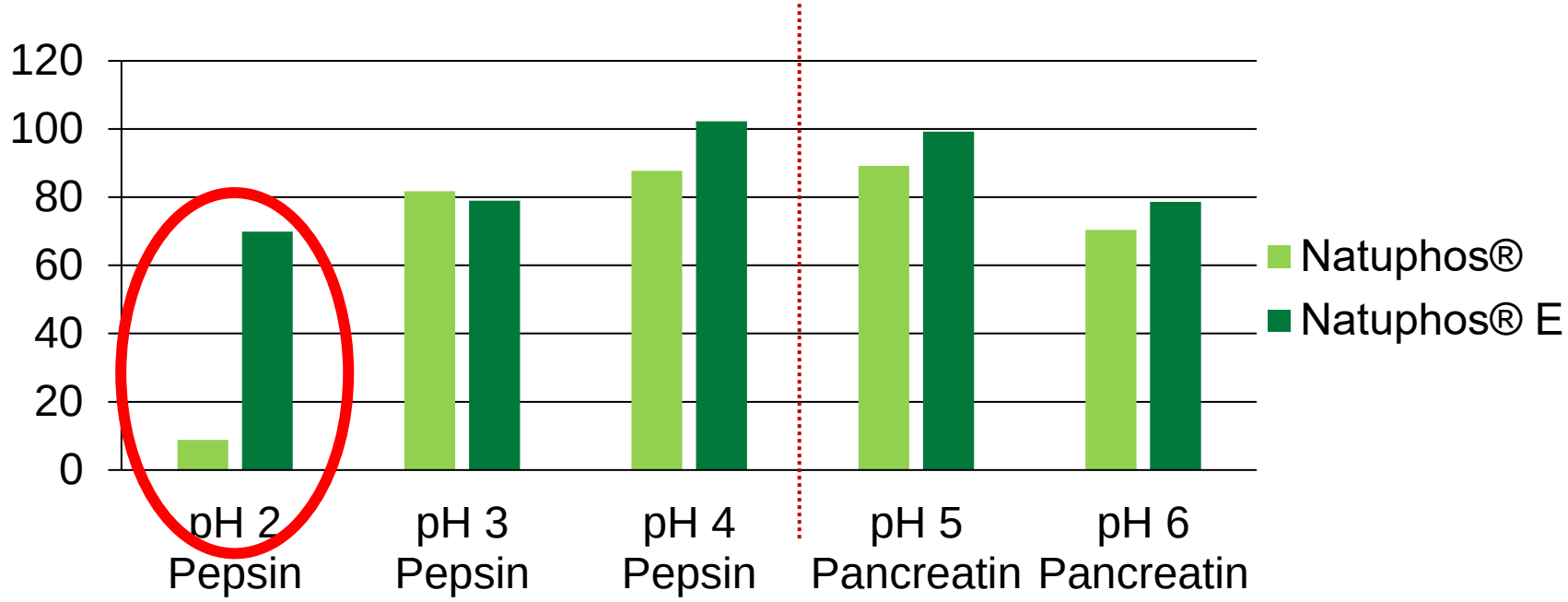
Residual phytase activity [%] was measured after *in vitro* treatment for 45 min at various conditions*



Natuphos® E more stable and longer active under gastric conditions.

*Results were expressed against the *phytase* analysed after pre-incubation at pH 6 without any digestive enzyme supplementation.

The new phytase molecule

Characterization and properties (IVb)

Relative activity of *phytases* (% of reference) after incubation with pepsin at pH 2, 3, and 4 and pancreatine at pH 5 and 6 (n=2). The activity of each phytase after incubation at pH 6 without enzyme addition was used as reference.

Enzyme	Pepsin				Pancreatin	
pH	2	3	4		5	6
Phytase						
RNP	4.9	86.7	98.7		99.4	96.7
QB	48.4	46.1	33.8		108.7	98.2
OP	17.7	46.0	83.2		90.8	61.7
NP E	69.9	79.0	102.2		99.2	78.6

Natuphos E has high stability & activity at conditions in the upper GIT.

TID-32-12

natuphos® E

Excellent improvements in Stability

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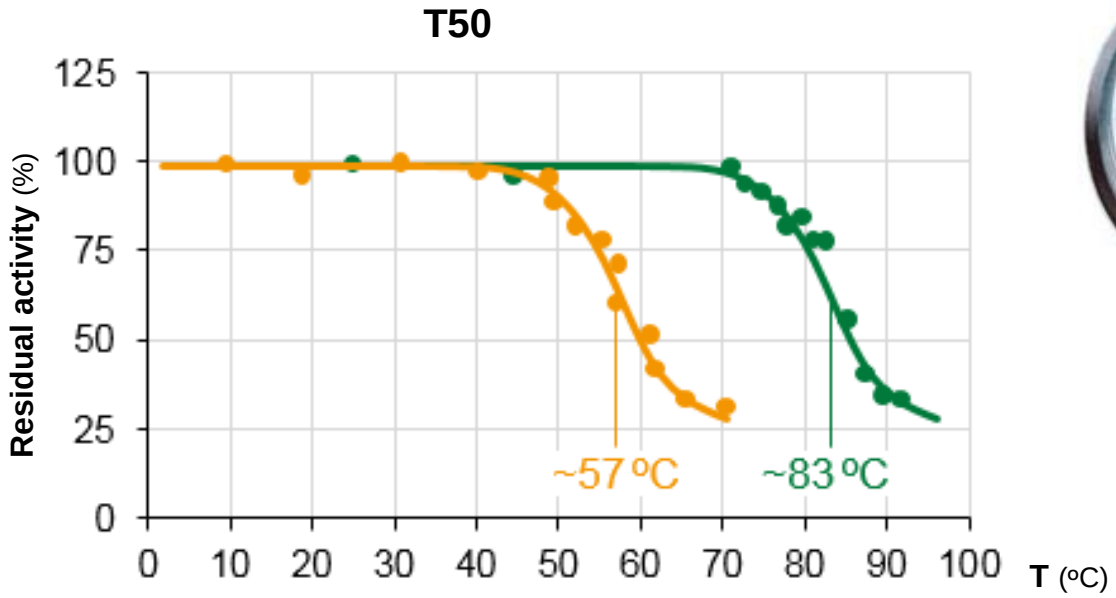
T 50 –Test (*In vitro*):

T50 is the temperature required to reduce the initial enzyme activity by 50% after a fixed incubation period.

Natuphos® E compared to Natuphos®



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Pelleting stability of unprotected “classic”formulation

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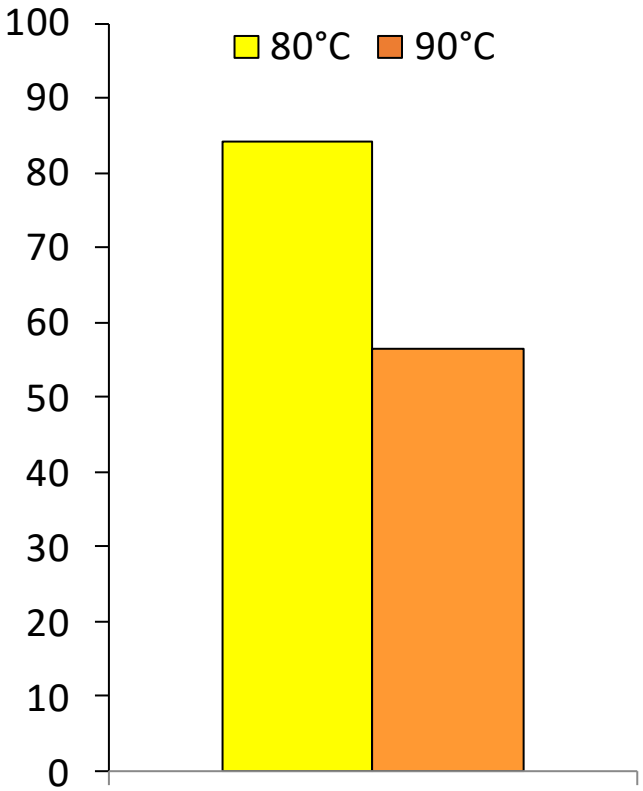
TID-7A-12



- pelleting test in regular feed mill with broiler feed (n = 3) (50% corn, 27% SBM, 10% soybean, 5% peas, 3.5% soy oil)
- conditioning time ~20s
  die: diameter of holes: 4mm, length: 45 mm
  capacity: 630 kg/h
  dry steam: 140°C at 2.8 to 3.0 bar
  pelleting temperature: measured in pellet after die
- dosed activity: 1000 FTU/kg

Natuphos E powder (“classic”) formulations recommended for pelleting temperatures up to the range of 80°C.

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Our Experience in Formulation Technology

IP protected granulation process

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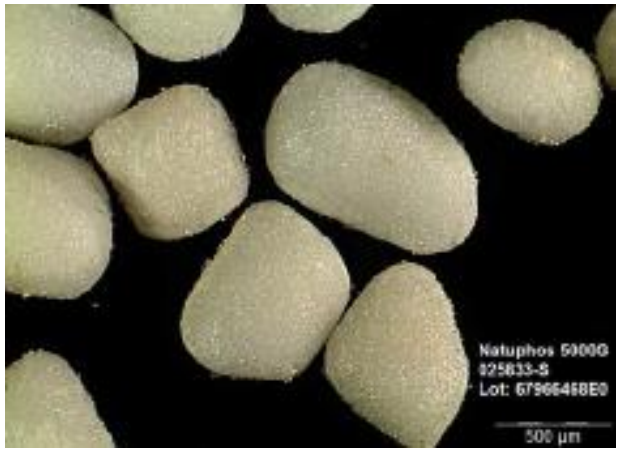
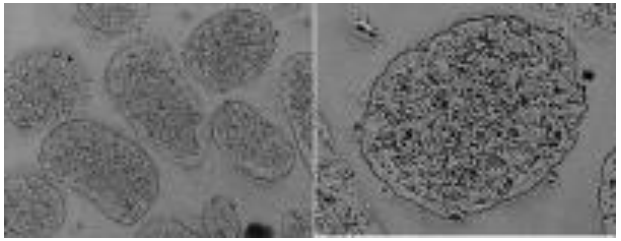
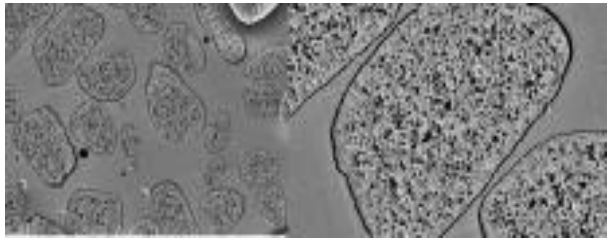


Natuphos 5000 / 10000 G

Natuphos E 5000 / 10000 G





Natuphos E G appears visually and structurally the same as Natuphos G.

Proven technology for superior stability in premix and mineral feed under stress conditions and compound feed up to 95°C pelleting temperature

natuphos® E

Excellent improvements in Stability

Pelleting stability of Natuphos® *E* 5000 G & 10000 G

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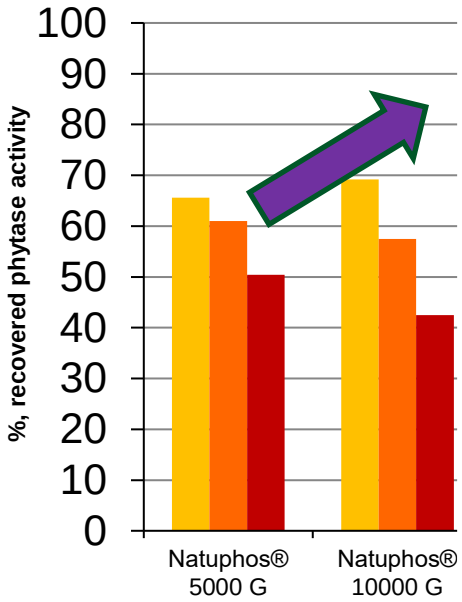

Pelleting temperature at: 85°C 90°C 95°C

Conditions: corn/SBM-based broiler diet, 20s conditioning time, die holes: 40 mm x 3 mm, Capacity: 600-650kg/h, Gap width: 1 mm

Superior pelleting stability of Natuphos® E 5000 G & 10000 G at pelleting temperatures up to 95°C.

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Excellent improvements in Stability

Premix stability of Natuphos® *E* G

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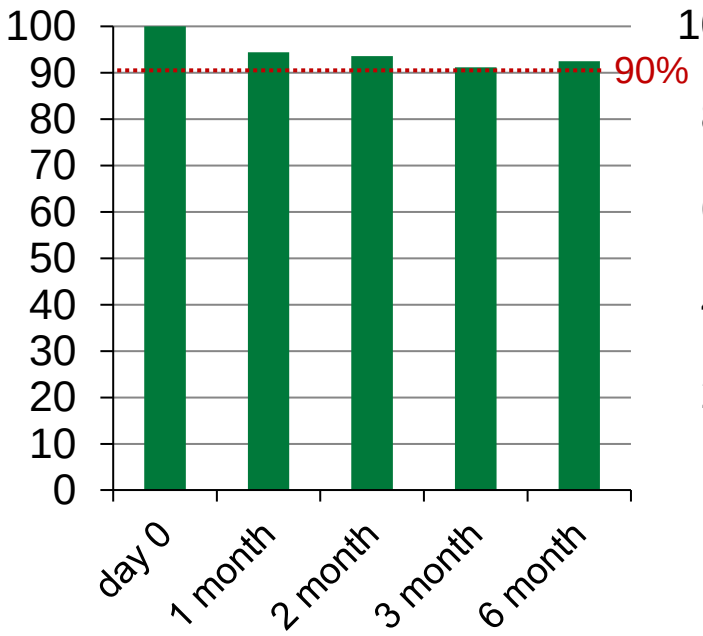
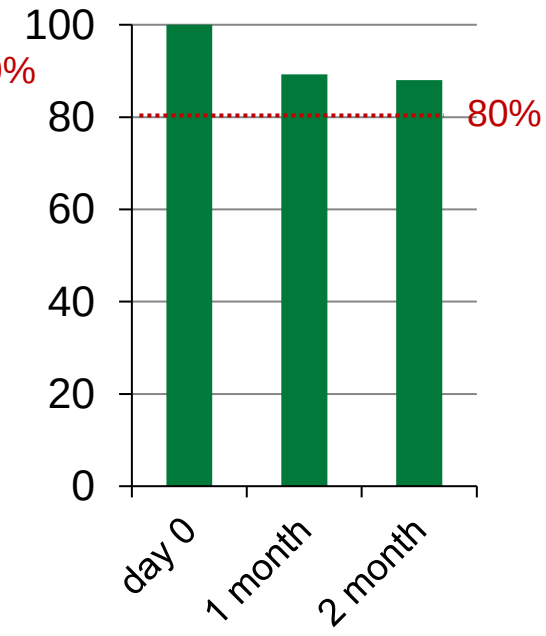
Storage at 20°C



30°C / 75% humidity



Composition of broiler premix:

Ingredients	Contend in %
Vitamin A 100	0,1350
Vitamin D3 500	0,0800
Vitamin E 50	1,0000
Biotin 2%	0,1250
Iron sulphate 32%	1,5625
Copper sulphate 25 %	0,6000
Manganese-(II)-Oxide 62%	1,6129
Zinc oxide 75%	0,9333
Limestone	91,30127
Vit.B-conc. poultry	2,5000
Mineral traces	0,1500

Natuphos® E Granule shows unique stability in premix: “Industry Leading Premix Stability”.

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Our Experience in Formulation Technology

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Natuphos® E 2500 L

Natuphos® E 5000 L

Natuphos® E 10000 L

Natuphos® E 5000

All formulations with a guaranteed shelf life of 18 months* from production.

Natuphos® E 10000

Natuphos® E 5000 G

Natuphos® E 10000 G

*up to use storage recommended at max. 20° C in originally sealed package

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Formulations

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- ✓ Natuphos E 5000
- ✓ Natuphos E 10000

For use in premixes and mineral feed, compound feed up to 80 °C pelleting temperature.

- ✓ Natuphos E 5000 G
- ✓ Natuphos E 10000 G

For use in concentrated premixes and mineral feed under stress conditions, compound feed up to 95 °C pelleting temperature

- ✓Natuphos E 2500 L
- ✓Natuphos E 5000 L
- ✓Natuphose E 10000L

For use via post pelleting application or in mash feed

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A more *E*fficient phytase

Natuphos® *E* in Pigs

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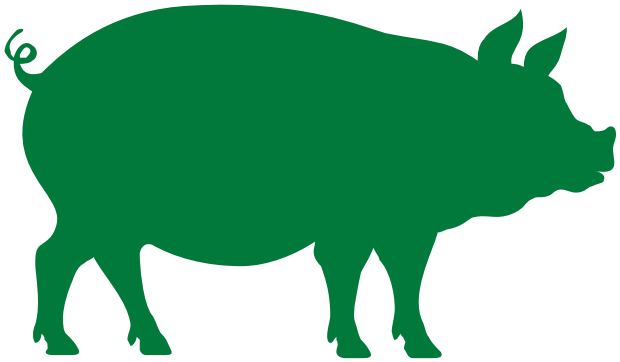

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A more Efficient phytase

Natuphos® *E* in Piglets

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Objective of the study:
To document the efficacy of Natuphos *E* 5000 L on performance and P digestibility in weaned piglets (26 d of age)

Set up

			Prestarter 1-21 d			Starter 22-42 d		
	Treatment	Added phytase (FTU/kg)	tP	Dig P	Ca	tP	Dig P	Ca
T 1	Positive Control	-	6.1	3.2	8.0	5.7	2.8	7.4
T 2	Negative control (NC)	-	4.2	1.8	5.5	4.0	1.4	5.2
T 3	NC + Natuphos ***E***	125						
T 4	NC + Natuphos ***E***	250						
T 5	NC + Natuphos ***E***	500						
T 6	NC + Natuphos ***E***	1000						

- Diet: corn, soybean based, DCP ,
- DCP in negative control was reduced by 10.5 kg, Ca:tP 1.3
- 8 replicates (3 piglets) per treatment

Torrallardona and Ader, 2015

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A more Efficient phytase

Natuphos® *E* in Piglets

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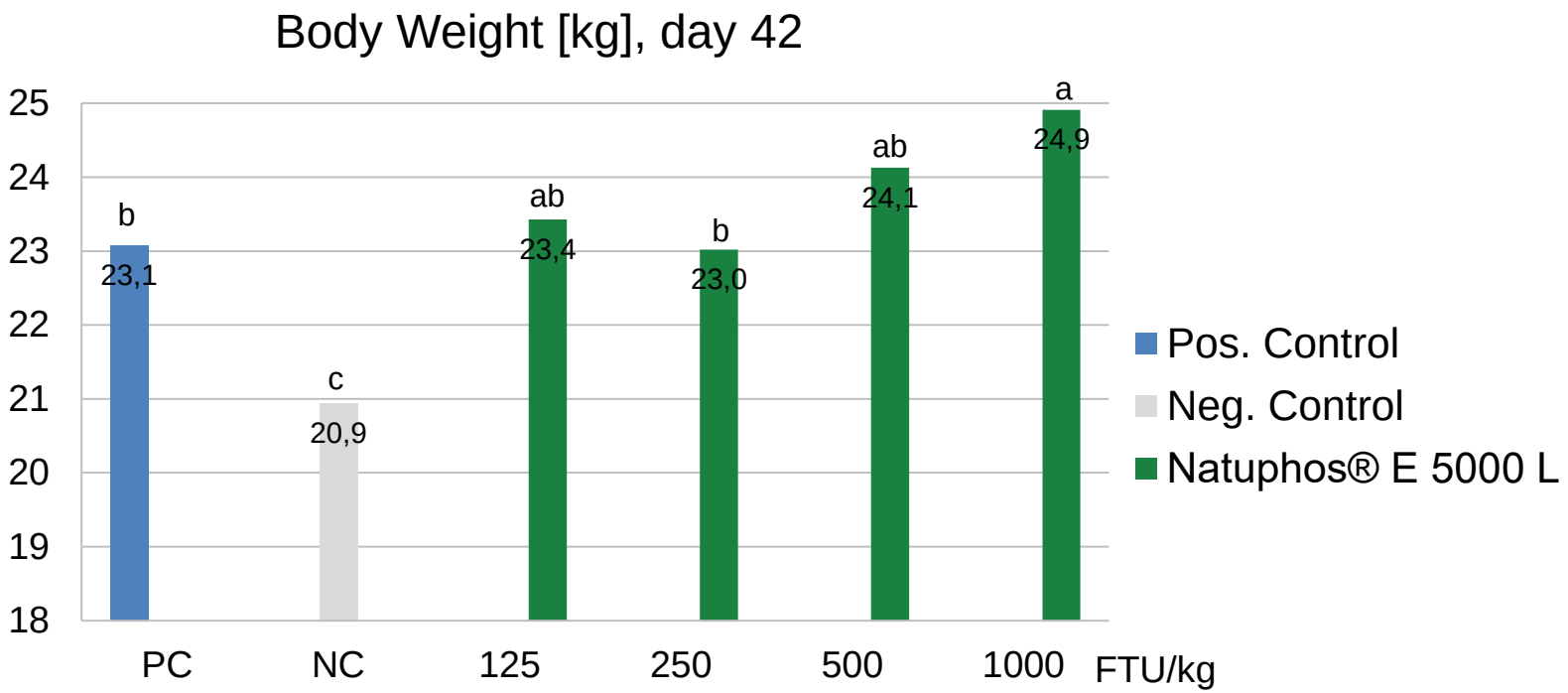

Reduction of DCP even more than balanced out by Natuphos® E at high dose levels.

abc Mean values without a common superscript letter within a column are significantly different (P≤0.05).

Torrallardona and Ader, 2015

FEU - SA 01032016/23

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A more Efficient phytase

Natuphos® *E* in Piglets

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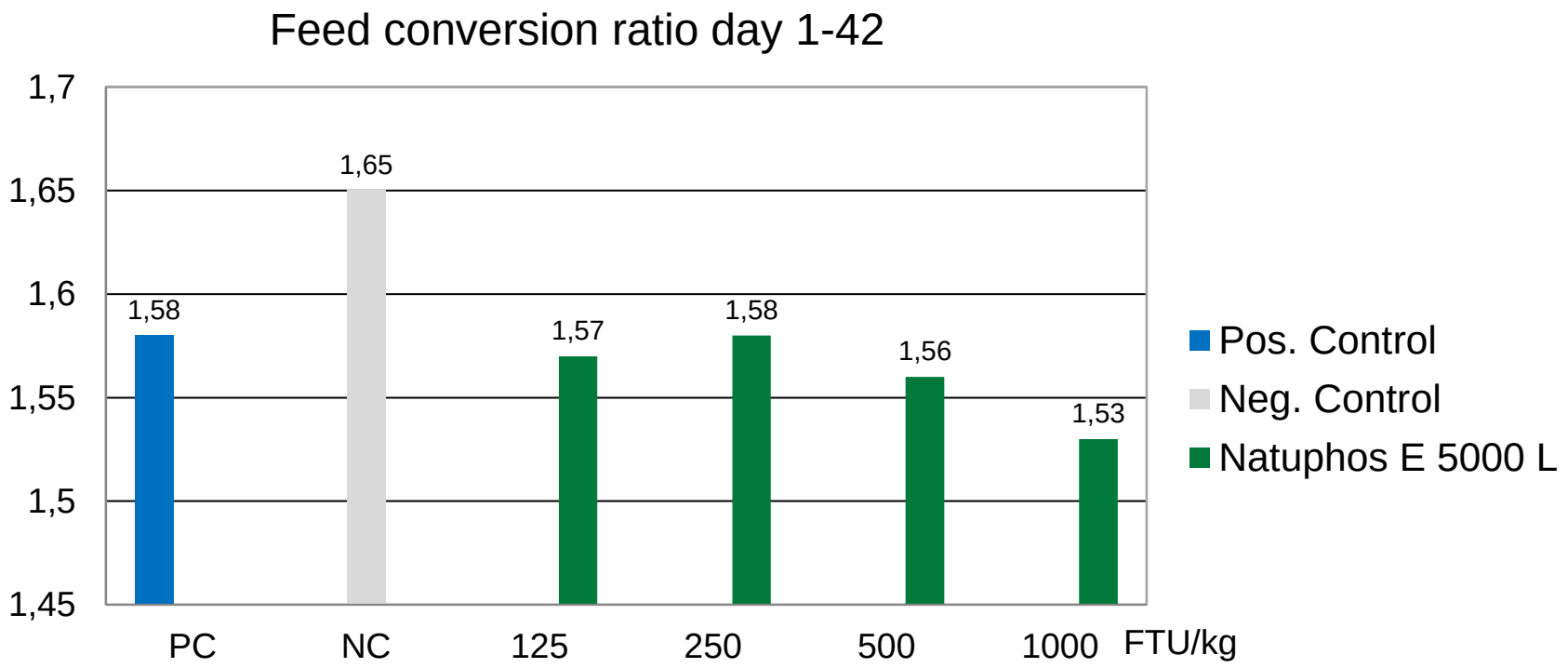

Torrallardona and Ader, 2015

FEU - SA 01032016/24

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A more *E*fficient phytase

dP and dP improvement by Natuphos® *E* at day 21 (g/kg)

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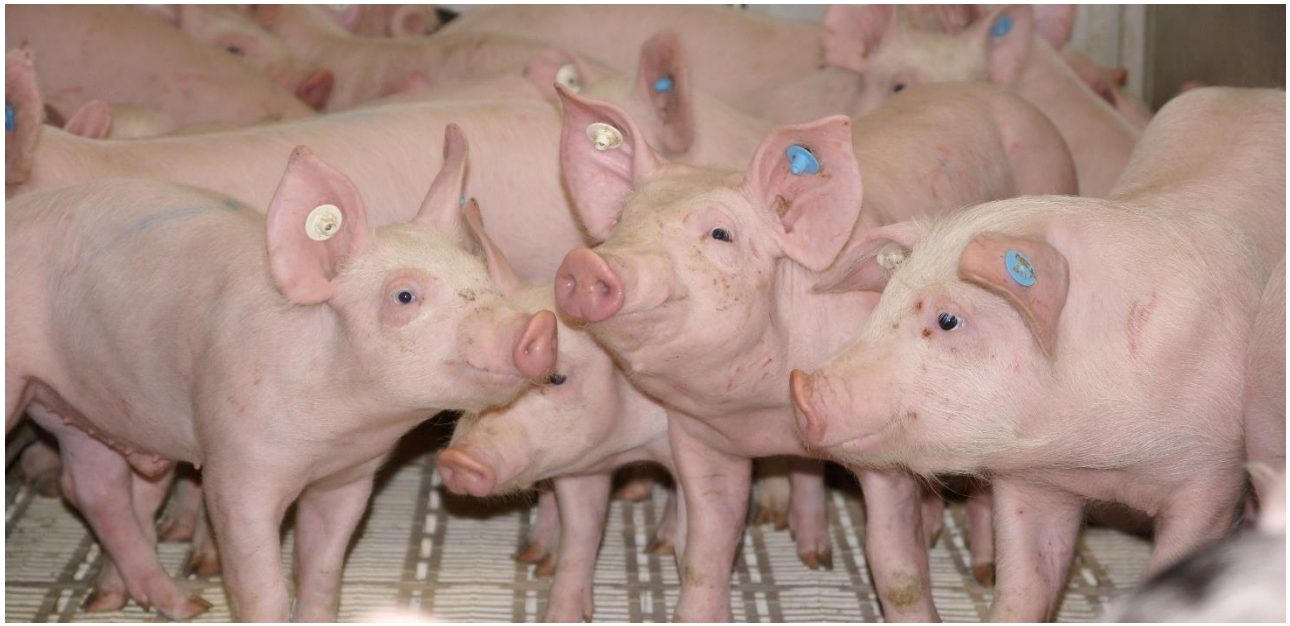

Torrallardona and Ader, 2015

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A more Efficient phytase

Natuphos® *E* in Piglets

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Objective of the study:
To compare the efficacy of Natuphos 5000 G and Natuphos *E* 5000 G on P and Ca digestibility in weaned piglets (23.5 kg start weight)

Set up

	Treatment	Exogenous phytase (FTU/kg	Total P (g/kg)	Dig P (g/kg)	IP-P (g/kg)	Total Ca (g/kg)	Ca/P
T 1	NC without mineral P	-	4,42	1,25	2,8	6,19	1,42
T 2	NC + 250 FTU/kg Natuphos ***E***	250					
T 3	NC + 500 FTU/kg Natuphos ***E***	500					
T 4	NC + 1000 FTU/kg Natuphos ***E***	1000					
T 5	NC + 500 FTU/kg Natuphos	500					
T 6	NC + 1000 FTU/kg Natuphos	1000					

- Diet: corn, sunflower meal, corn gluten meal, soy based
- 6 replicates per treatment
- d 26-28 faecal collection

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A more Efficient phytase

Effect on apparent faecal P & Ca digestibility in piglets

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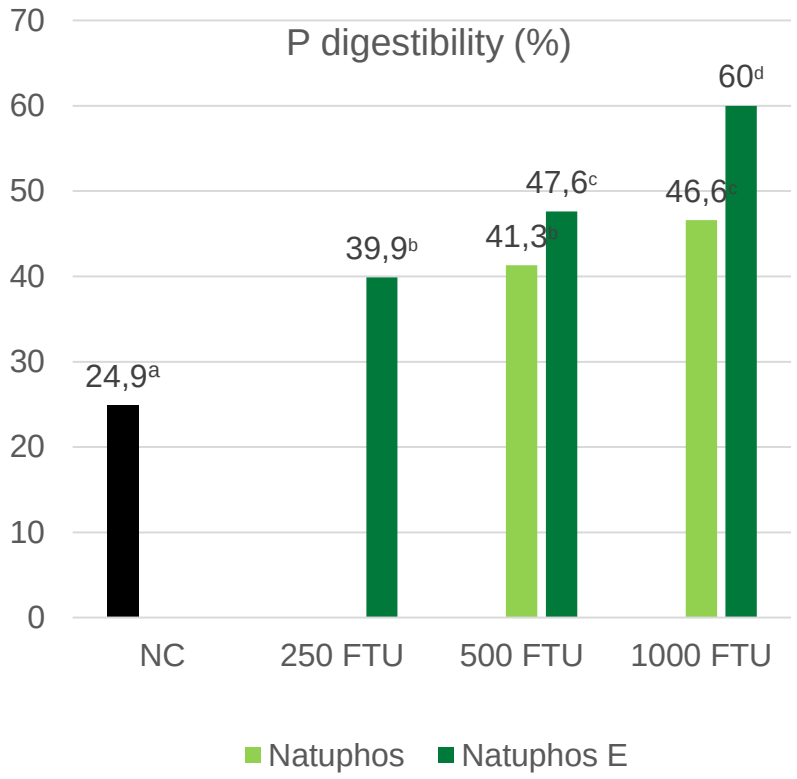

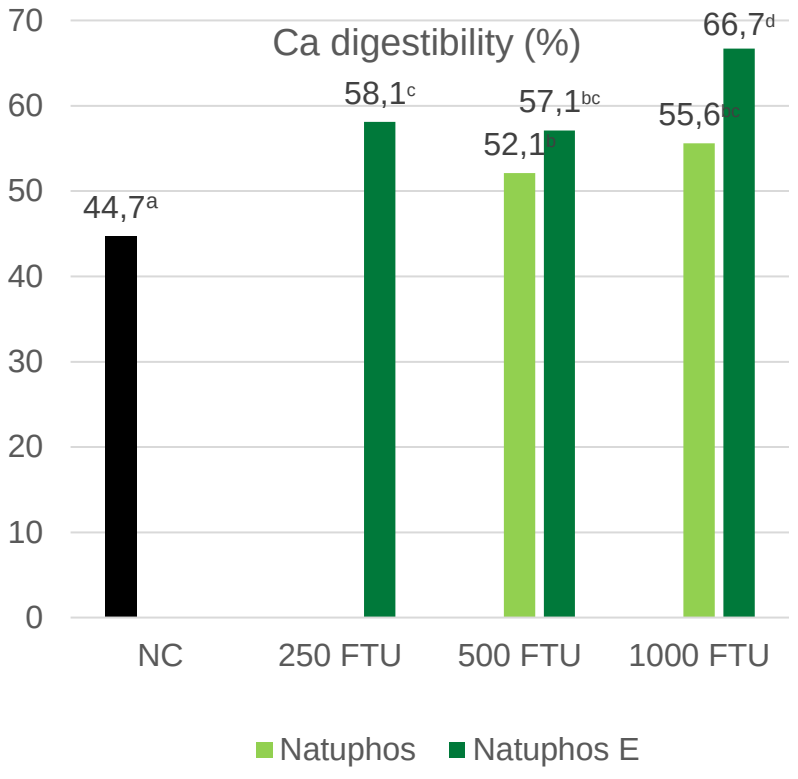

abc Mean values without a common superscript letter within a column are significantly different (P≤0.05).

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A more Efficient phytase

Dose-response-relationship on apparent fecal digested P (dP g/kg; 90 % DM)

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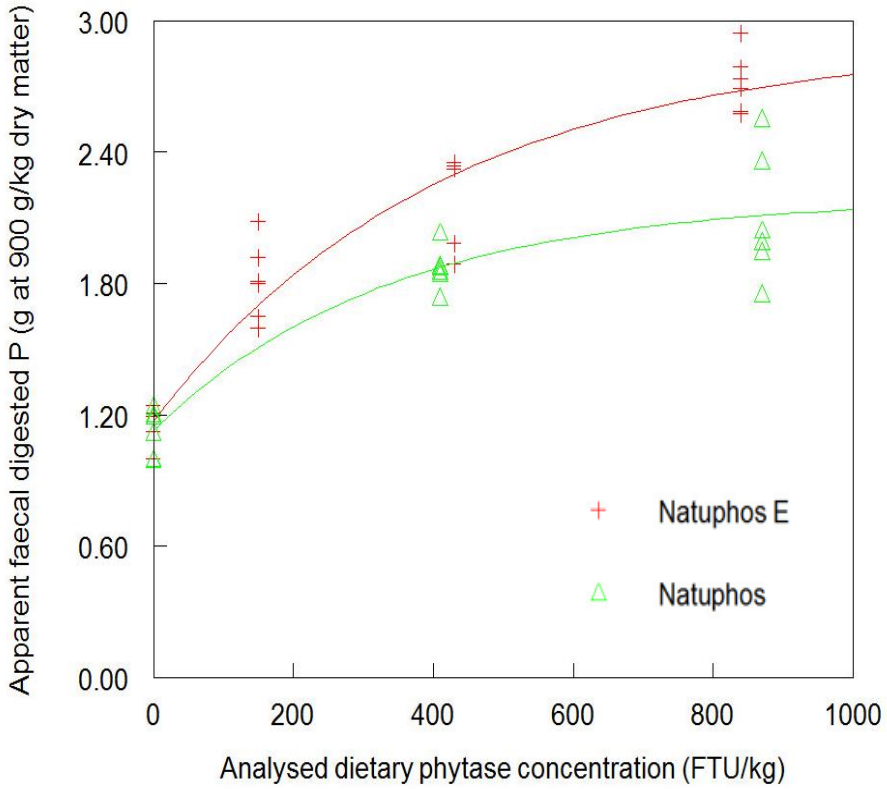

Amount of digestible P released by Natuphos E	
Natuphos® E (FTU/kg)	dP release (g/kg)
250	0,82
500	1,25
1000	1,61

Calculated dose of Natuphos® E needed to generate equal amounts of dP at … FTU/kg dietary Natuphos® supplementation based on the fitted response curves on analysed dietary phytase concentration.

Natuphos®	Natuphos® E	Natuphos® E in % Natuphos®
250	**158**	**- 37 %**
500	**265**	**- 47 %**
1000	**361**	**- 64 %**

natuphos® E

The *Ecological* Phytase

Reduction of P excretion by using Natuphos® *E* in piglets and fattening pigs

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Trial design	
Treatment	**Enzyme dose**
Piglet (12-25 kg LW)	
A	NC without phytase, DCP, MCP
B	NC + 250 FTU Natuphos® E/kg
C	NC + 500 FTU Natuphos® E/kg
D	NC + 750 FTU Natuphos® E/kg
E	NC + 1000 FTU Natuphos® E/kg
Fattening pigs (40-80 kg LW)	
A	NC without phytase, DCP, MCP
B	NC + 250 FTU Natuphos® E/kg
C	NC + 500 FTU Natuphos® E/kg
D	NC + 750 FTU Natuphos® E/kg

- **Digestibility study piglets:** 20 castrates(Topigs x Pietrain) in 2 sets.
- Live weight during the trial between 12 and 25 kg, average starting weight 16 kg.

- **Digestibility study fattening pig** as latin squaret, 8 castrates (Topigs x Pietrain), 4 sets.
- Live weight at start 46 kg and between 40 and 80 kg over the entire trial.

- Feed based on maize, soybean meal, rapeseed meal, isonitrogenic, isoenergetic

Winkler et al. 2017

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The *Ecological* Phytase

Using Natuphos® *E* in piglets and fattening pigs

Phosphorus digestibility (%)

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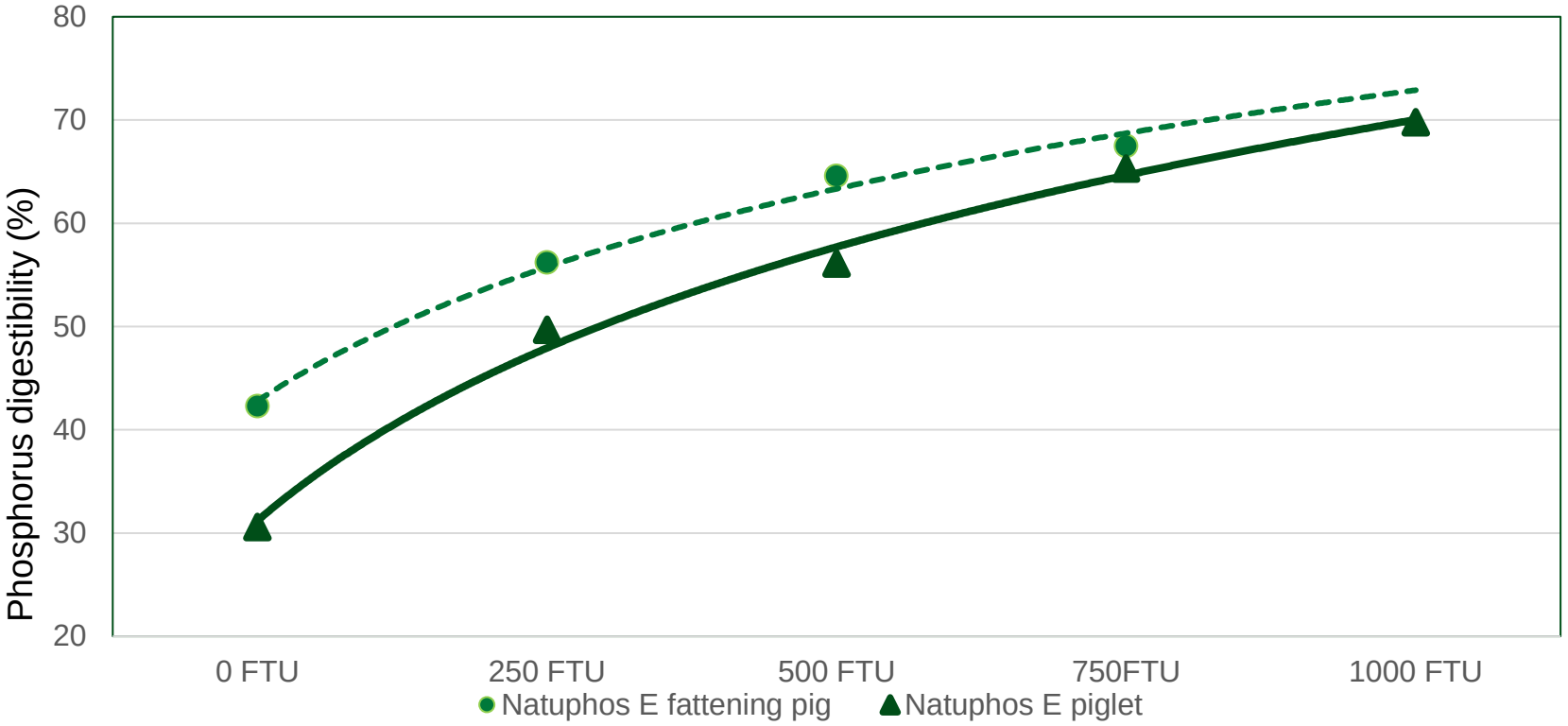

P digestibility in piglets was increased by Natuphos E addition of 1000 FTU/kg up to 70 % and in fattening pigs by the addition of 750 FTU/kg up to 68 %.

Winkler et al. 2017

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The *Ecological* Phytase

Effect of Natuphos® *E* on Phosphorus excretion of piglets and fattening pigs (NC = 100)

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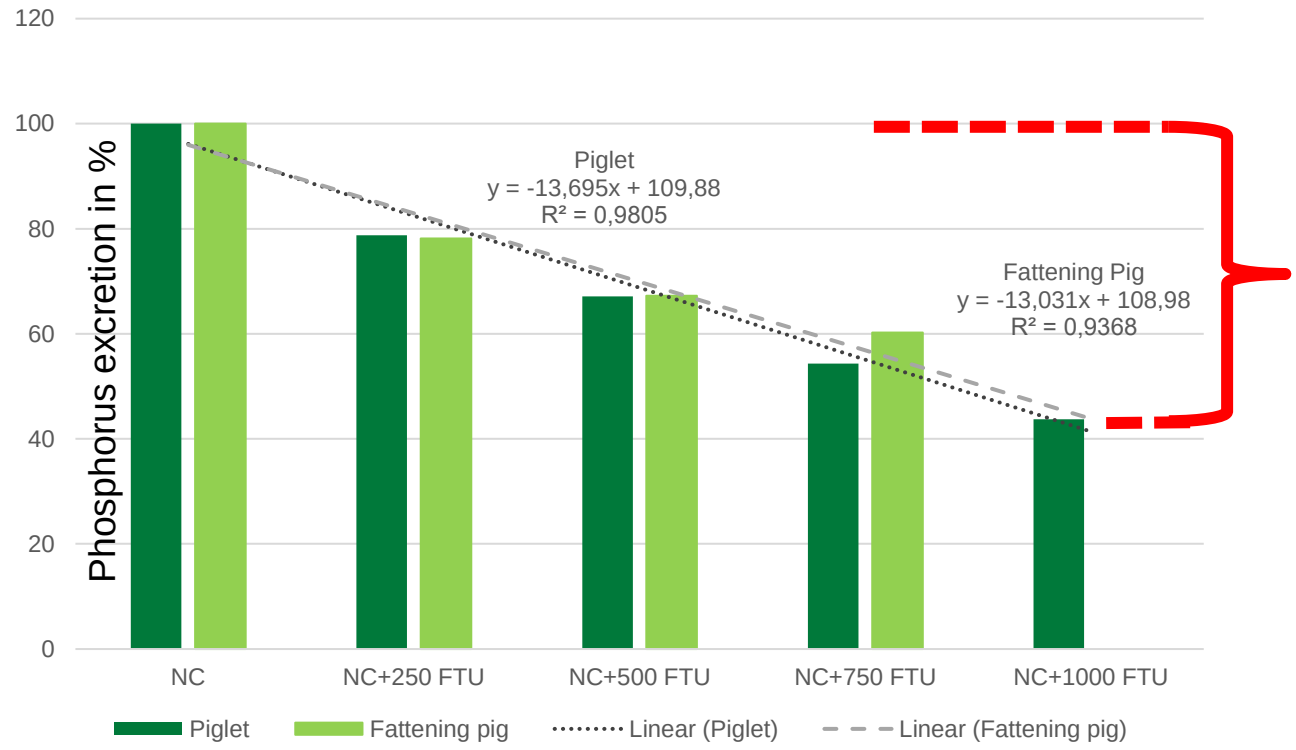

57 % reduced P- Excretion

Compared to a negative control without phytase the Phosphorus excretion could be reduced linearly up to 57 % by a dosage of 1000 FTU/kg Natuphos E

Winkler et al. 2017

natuphos® E

Comparing the efficacy of Natuphos 5000 G and Natuphos E 5000 G on P and Ca digestibility and metabolizable energy in weaned piglets

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Set up

	Treatment	Exogenous phytase (FTU/kg	Total P (g/kg)	Dig P (g/kg)	Total Ca (g/kg)	Ca/P
T 1	Negative control (NC)	-	3,4	0,8	6,4	1,88
T 2	NC + 500 FTU/kg Natuphos E	500				
T 3	NC + 1000 FTU/kg Natuphos E	1000				
T 4	NC + 500 FTU/kg Natuphos	500				
T 5	NC + 1000 FTU/kg Natuphos	1000				

- **Digestibility study piglets:** 20 castrates(Topigs x Pietrain) in 2 sets.
- Live weight during the trial between 12 and 25 kg, average starting weight 16 kg.

- Mash feed based on maize, soybean meal, rapeseed meal, isonitrogenic, isoenergetic

Trautwein et al. 2017

natuphos® E

Comparing the efficacy of Natuphos 5000 G and Natuphos E 5000 G on P and Ca digestibility and metabolizable energy in weaned piglets

Ingredient and calculated nutrient composition of the diet

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Ingredients	%		Nutrients	%
Corn	64.00		ME, (MJ/kg)	13.7
Corn expanded	10.00		NE, (MJ/kg)	10.6
Soybean meal	16.50		Crude protein	16.1
Rapeseed meal	6.00		Crude fiber	3.10
L-Lysine (HCl)	0.455		Lys (dig. Lys)	1.20 (1.07)
DL-Methionine	0.212		M+C (dig. M+C)	0.78 (0.67)
L-Threonine	0.162		Thr (dig. Thr)	0.78 (0.65)
L-Tryptophan	0.10		Trp (dig. Trp)	0.20 (0.17)
Salt (NaCl)	0.30		Calcium	0.64
Limestone ($CaCO_3$)	1.28		Phosphorus	0.34
Oil (rapeseed)	0.50		Phosphorus (dig.)	0.08
Premix (Vit/Min) etc.	0.50			

Trautwein et al. 2017

natuphos® E

Comparing the efficacy of Natuphos 5000 G and Natuphos E 5000 G on P and Ca digestibility and metabolizable energy in weaned piglets

Effect on P and Ca digestibility

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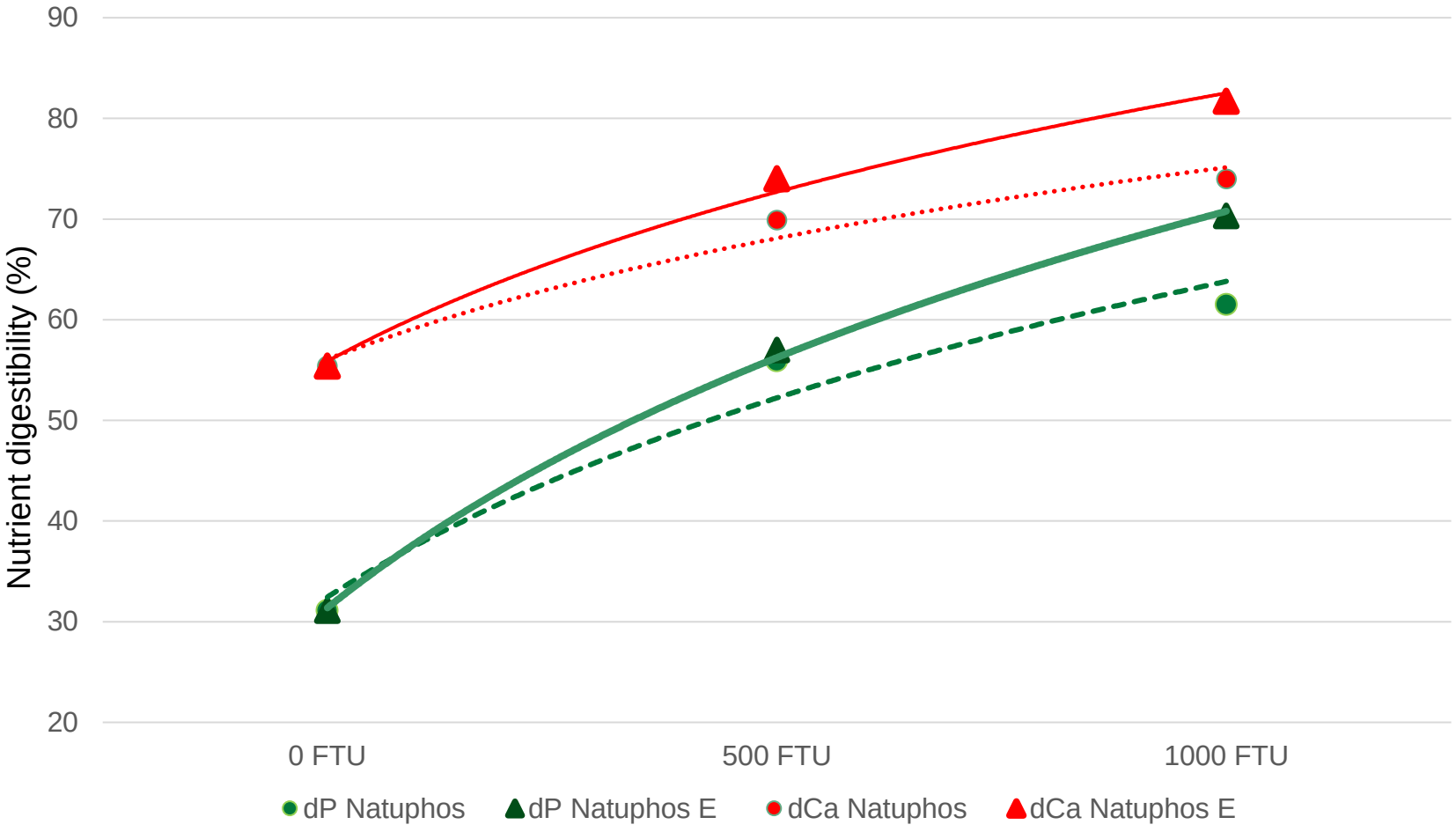

Trautwein et al. 2017

natuphos® E

Comparing the efficacy of Natuphos 5000 G and Natuphos E 5000 G on P and Ca digestibility and metabolizable energy in weaned piglets

Effect on metabolizable energy (ME) content

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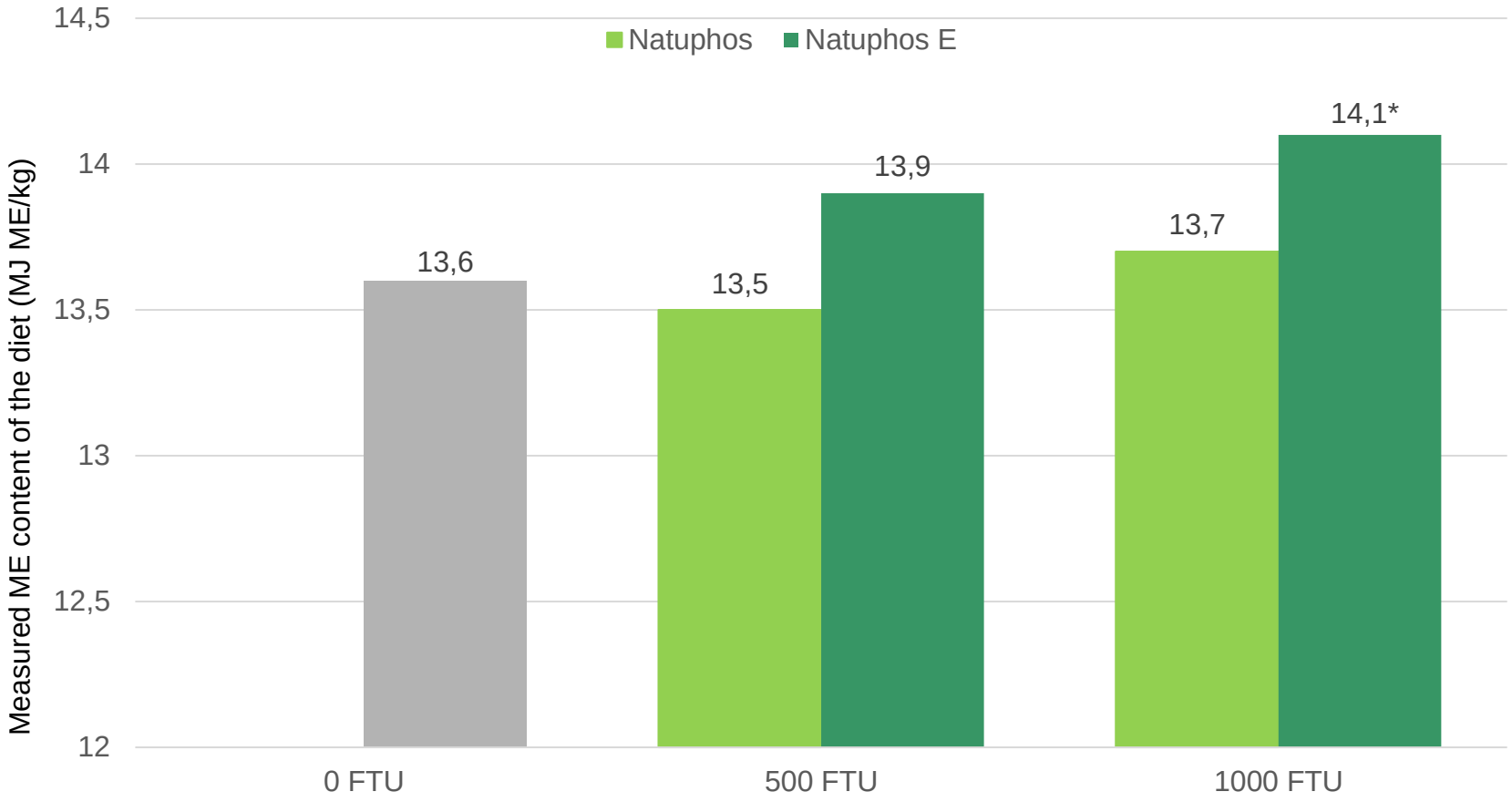

Addition of 1000 FTU/kg Natuphos E increased ME significantly by 0,5 MJ/kg.

Trautwein et al. 2017

natuphos® E

All-Nutrient matrix values (nutrient contribution) for piglets, fattening pigs, sows per kg of feed for different inclusion levels

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Natuphos E nutrient contributions				
	PIGLETS, FATTENING PIGS, SOWS			
Natuphos E incl. rate, FTU/kg feed	300	500	750	1000
Energy *				
ME, kcal/kg	37,1	51,9	63,1	70,5
ME, MJ/kg	0,155	0,217	0,264	0,295
Crude protein, g	2,00	2,80	3,40	3,80
Amino acids **				
Lysine, g	0,080	0,112	0,136	0,152
Methionine, g	0,025	0,035	0,043	0,048
Cysteine, g	0,030	0,042	0,051	0,057
Arginine, g	0,080	0,112	0,136	0,152
Histidine, g	0,020	0,028	0,034	0,038
Isoleucine, g	0,050	0,070	0,085	0,095
Leucine, g	0,120	0,168	0,204	0,228
Phenylalanine, g	0,060	0,084	0,102	0,114
Threonine, g	0,050	0,070	0,085	0,095
Tryptophane,g	0,030	0,042	0,051	0,057
Valine, g	0,040	0,056	0,068	0,076
Phosphorus				
Digestible P, g	0,80	1,12	1,36	1,52
Total P from replacing MCP *** , g	1,00	1,40	1,70	1,90
Total P from replacing DCP *** , g	1,15	1,61	1,96	2,19
Calcium****				
Total Ca when using MCP, g	1,00	1,40	1,70	1,90
Total Ca when using DCP, g	1,15	1,61	1,96	2,19
Other minerals				
Zinc *****, g	0,032	0,045	0,054	0,061
Sodium, g	0,010	0,014	0,017	0,019

* Energy uplifts of phytases and NSP-enzymes are usually not additive and thus need to be respectively corrected when used in combination.

** Apparent ileal digestible

*** Assuming 70% availability of P in DCP and 80% in MCP, according to Dutch CVB 1997.

**** a narrow Ca: tP ratio (e.g. 1,4 - 1,5 to 1) is recommended

***** replacing Zinc sulfate

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Matrix validation

Set up

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The effect of Natuphos E 5000 G was tested in 196 piglets [(Landrace x Duroc) X Pietrain] post weaning, mixed sexes of 26 days of age with 7 replicates per treatment and 4 piglets per pen.
A two phase feeding regime was used (starter I day1-21, starter II day 22-42).
Diets based on corn-soy.

Matrix Validation at 300, 500 and 1000 FTU.

	Treatment	Specification	Dosage (FTU/kg)
1	Pos. Control		0
2	Neg. Control 1	PC minus Matrix values for 300 FTU	0
3	Neg. Control 2	PC minus Matrix values for 500 FTU	0
4	Neg. Control 3	PC minus Matrix values for 1000 FTU	0
5	NC1 + Natuphos E 5000 G	PC minus Matrix values for 300 FTU	300
6	NC2 + Natuphos E 5000 G	PC minus Matrix values for 500 FTU	500
7	NC3 + Natuphos E 5000 G	PC minus Matrix values for 1000 FTU	1000

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Matrix validation

Starter I – calculated nutrient content

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We create chemistry

TID-84-14

	T-1	T-2	T-3	T-4	T-5	T-6	T-7
Nutrients (g)	**PC**	**NC-1 minus „Matrix 300“**	**NC-2 minus „Matrix 500“**	**NC-3 minus „Matrix 1000“**	**NC-1 + 300 FTU**	**NC-2 + 500 FTU**	**NC-3 + 1,000 FTU**
Crude Protein	207.8	- 2.0	-2.8	-3.8	- 2.0	-2.8	-3.8
Energy (MJ ME/kg)	**14.03**	**-0.155**	**-0.217**	**-0.295**	**-0.155**	**-0.217**	**-0.295**
Total calcium	7.50	-6.50	-6.10	-5.60	-6.50	-6.10	-5.60
Total phosphorous	**6.50**	**-1.00**	**-1.40**	**-1.90**	**-1.00**	**-1.40**	**-1.90**
Digestible phosphorus	**3.82**	**-0.8**	**-1.12**	**-1.52**	**-0.8**	**-1.12**	**-1.52**
SID Lysine	12.80	-0.08	-0.112	-0.152	-0.08	-0.112	-0.152
SID Threonine	8.32	-0.05	-0.07	-0.095	-0.05	-0.07	-0.095
SID Methionine	4.53	-0.025	-0.035	-0.048	-0.025	-0.035	-0.048
SID Tryptophan	2.56	-0.03	-0.042	-0.057	-0.03	-0.042	-0.057

natuphos® E

Matrix validation

Starter II – calculated nutrient content

BASF
We create chemistry

TID-84-14

	T-1	T-2	T-3	T-4	T-5	T-6	T-7
Nutrients (g)	**PC**	**NC-1** minus „Matrix 300“	**NC-2** minus „Matrix 500“	**NC-3** minus „Matrix 1000“	**NC-1 + 300 FTU**	**NC-2 + 500 FTU**	**NC-3 + 1,000 FTU**
Crude Protein	195.9	- 2.0	-2.8	-3.8	- 2.0	-2.8	-3.8
Energy (MJ ME/kg)	**13.82**	**-0.155**	**-0.217**	**-0.295**	**-0.155**	**-0.217**	**-0.295**
Total calcium	7.00	-6.50	-6.10	-5.60	-6.50	-6.10	-5.60
Total phosphorous	**5.50**	**-1.00**	**-1.40**	**-1.90**	**-1.00**	**-1.40**	**-1.90**
Digestible phosphorus	**2.88**	**-0.8**	**-1.12**	**-1.52**	**-0.8**	**-1.12**	**-1.52**
SID Lysine	12.00	-0.08	-0.112	-0.152	-0.08	-0.112	-0.152
SID Threonine	7.8	-0.05	-0.07	-0.095	-0.05	-0.07	-0.095
SID Methionine	4.16	-0.025	-0.035	-0.048	-0.025	-0.035	-0.048
SID Tryptophan	2.40	-0.03	-0.042	-0.057	-0.03	-0.042	-0.057

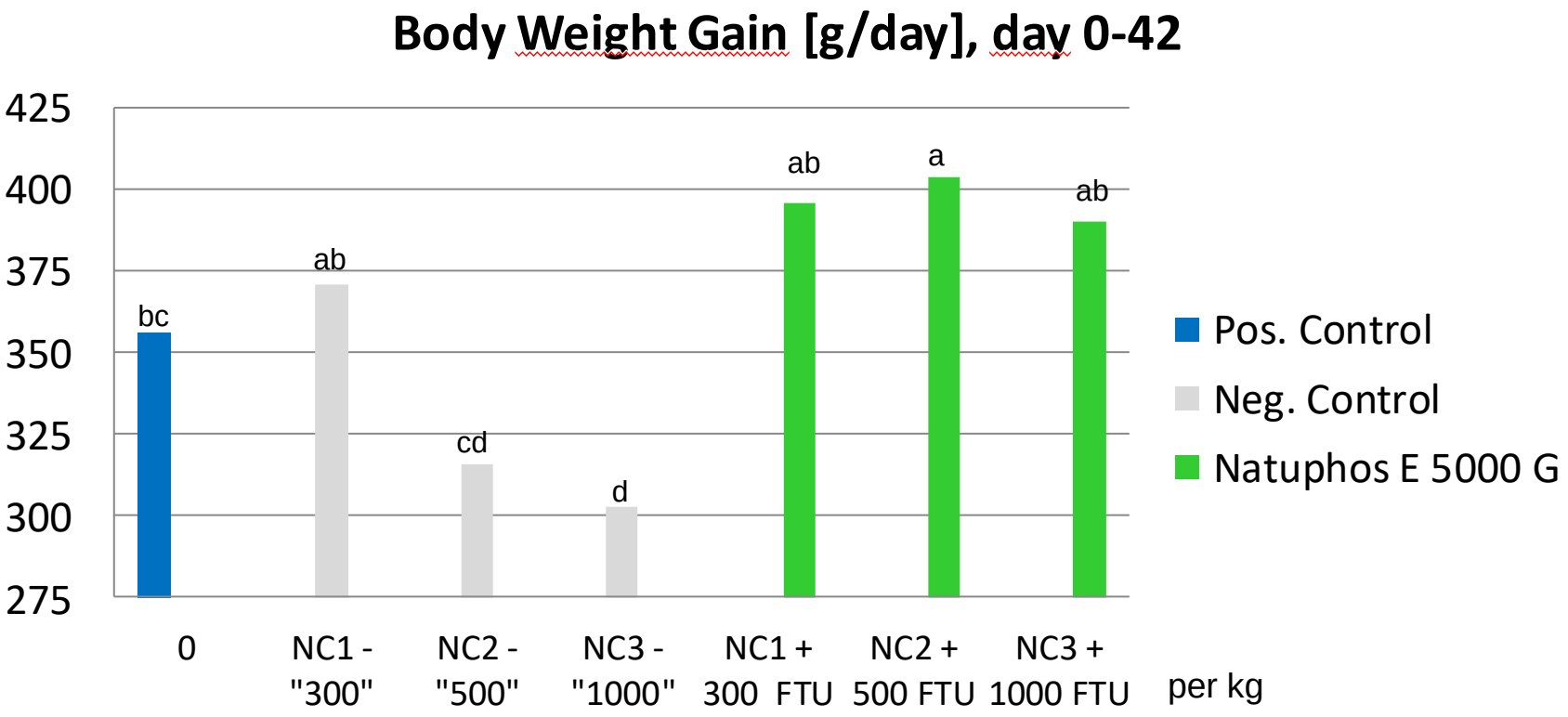
natuphos® E

Matrix validation

Performance data

BASF
We create chemistry

TID-84-14



Nutrient reduction balanced out by Natuphos E supplementation accordingly

a,b,c,d Shows significant difference between the dosages, p<0.05

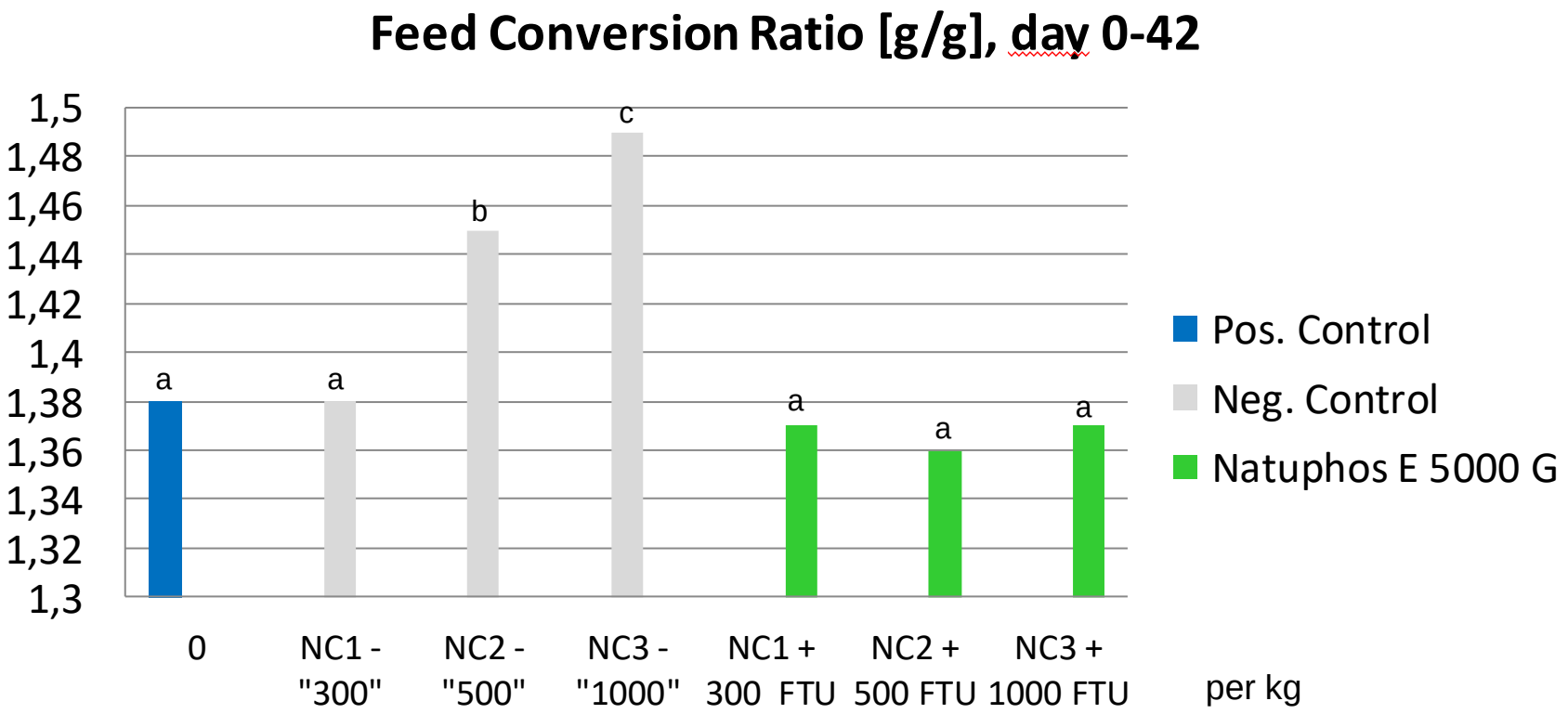
natuphos® E

Matrix validation

Performance data

BASF
We create chemistry

TID-84-14



Nutrient reduction balanced out by Natuphos E supplementation accordingly

a,b,c Shows significant difference between the dosages, p<0.05

natuphos® E

Phosphorus equivalency of Natuphos E in comparison to Natuphos

BASF
We create chemistry

Piglets, sows, fattening pigs	**Natuphos**	**500 FTU/kg**		**1000 FTU/kg**
	P- equivalency	0,8 g dP		1,20 g dP
	Natuphos E	**500 FTU/kg**	**750 FTU/kg**	**1000 FTU/kg**
	P- equivalency	1,12 g dP	1,36 g dP	1,52 g dP

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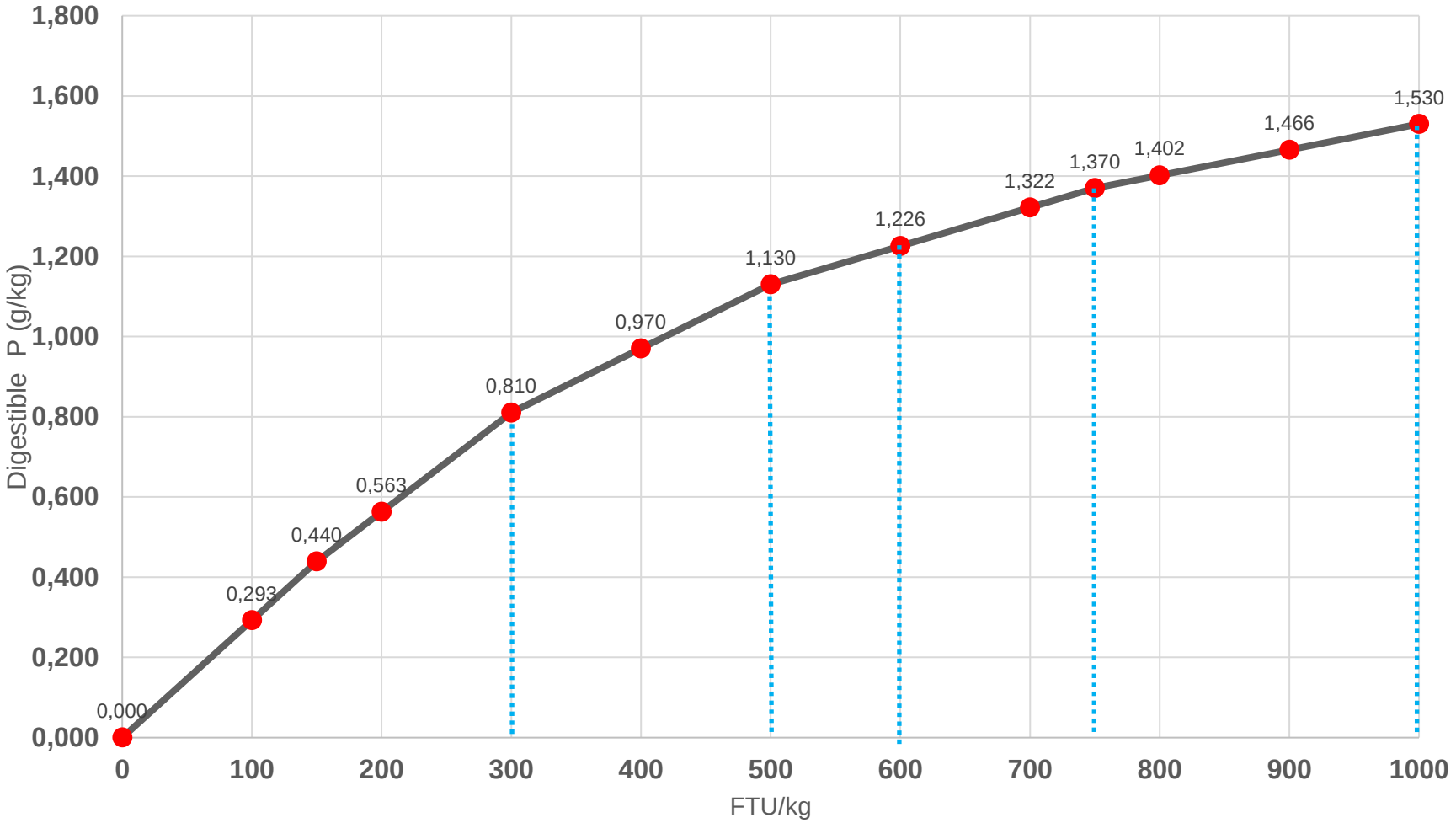
Effect of Natuphos E dose levels on P digestibility in pigs*

BASF
We create chemistry



* incl. piglets, sows

natuphos® E

Matrix values (minerals) for piglets, fattening pigs, sows per kg of feed for different inclusion levels

BASF
We create chemistry

	Natuphos E mineral contributions			
	PIGLETS, FATTENING PIGS, SOWS			
Natuphos E incl. rate, FTU/kg feed	300	500	750	1000
Phosphorus				
Digestible P, g	0,80	1,12	1,36	1,52
Total P from replacing MCP * , g	1,00	1,40	1,70	1,90
Total P from replacing DCP * , g	1,15	1,61	1,96	2,19
Calcium**				
Total Ca when using MCP, g	1,00	1,40	1,70	1,90
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Other minerals				
Zinc ***, g	0,032	0,045	0,054	0,061
Sodium, g	0,010	0,014	0,017	0,019

* Assuming 70% availability of P in DCP and 80% in MCP, according to Dutch CVB 1997.

** a narrow Ca: tP ratio (e.g. 1,4 - 1,5 to 1) is recommended,
that is why increased Ca reduction is recommended with increasing phytase dose level

*** replacing Zinc sulfate

natuphos® E

All-Nutrient matrix values (nutrient contribution) for piglets, fattening pigs, sows per kg of feed for different inclusion levels

BASF
We create chemistry

Natuphos E nutrient contributions				
	PIGLETS, FATTENING PIGS, SOWS			
Natuphos E incl. rate, FTU/kg feed	300	500	750	1000
Energy *				
ME, kcal/kg	37,1	51,9	63,1	70,5
ME, MJ/kg	0,155	0,217	0,264	0,295
Crude protein, g	2,00	2,80	3,40	3,80
Amino acids **				
Lysine, g	0,080	0,112	0,136	0,152
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Arginine, g	0,080	0,112	0,136	0,152
Histidine, g	0,020	0,028	0,034	0,038
Isoleucine, g	0,050	0,070	0,085	0,095
Leucine, g	0,120	0,168	0,204	0,228
Phenylalanine, g	0,060	0,084	0,102	0,114
Threonine, g	0,050	0,070	0,085	0,095
Tryptophane,g	0,030	0,042	0,051	0,057
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Other minerals				
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Sodium, g	0,010	0,014	0,017	0,019

* Energy uplifts of phytases and NSP-enzymes are usually not additive and thus need to be respectively corrected when used in combination.

** Apparent ileal digestible

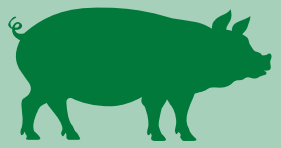
*** Assuming 70% availability of P in DCP and 80% in MCP, according to Dutch CVB 1997.

**** a narrow Ca: tP ratio (e.g. 1,4 - 1,5 to 1) is recommended

***** replacing Zinc sulfate

natuphos® E

EU approval



BASF
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Published 08.03.2018 (Official Journal of the European Union, L65/17-20)
EU - Identification number : **4a 27**
6-phytase EC 3.1.3.26

Species category	Minimum dose rate
• **Pigs for fattening** • **Sows** • **Minor porcine species for growing or reproduction**	100 FTU/kg feed
• **Weaned piglets**	125 FTU/kg feed

natuphos® E

A more *E*fficient phytase

Natuphos® *E* in Poultry

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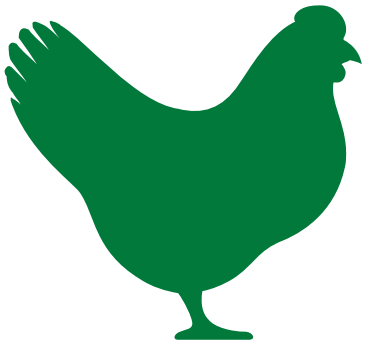


natuphos® E

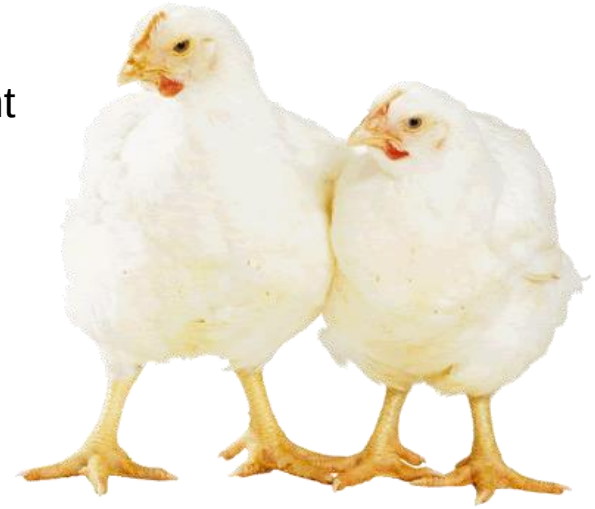
A more *E*fficient phytase

Natuphos® *E* in Poultry

BASF
We create chemistry

Objective of the study: **To compare the efficacy of Natuphos, Natuphos E and MCP on growth and tibia ash content in broiler chicken**

- Ross 308 male broilers were fed the same starter diet (commercial nutrient contents) from 0-8 days of age.
- The experimental diets were fed to the birds from 8-23 days of age.
  Pelleted feed (70-75°C PT) and water was available for ad libitum intake during the entire experiment
- 6 reps a 20 birds/pen per treatment
- All diets ratio Ca/P = 1.5



natuphos® E

A more Efficient phytase

Natuphos® *E* in Poultry (I)

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Set up

	Treatment	Natuphos E (FTU/kg)	Natuphos (FTU/kg)	MCP-P (g/kg)	Total P (g/kg)	Ca (g/kg)
T 1	Negative control (NC) w/o iP	-		-	4,49	6,74
T 2	NC	250		-		
T 3	NC	500		-		
T 4	NC	750		-		
T 5	NC	1000		-		
T 6	NC	1500		-		
T7	NC	-	250	-		
T8	NC	-	500	-		
T9	NC	-	1000	-		
T10	Positive control (PC) 1	-		+ 0,5	4,99	7,48
T11	PC 2	-		+ 0,9	5,39	8,08
T12	PC 3	-		+ 1,3	5,79	8,68
T13	PC 4	-		+ 1,7	6,19	9,28
T14	PC 5	-		+ 2,1	6,59	9,88

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Effect on body weight gain (g, d 7-22)

BASF
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Effect on tibia ash (g/kf FF DM)

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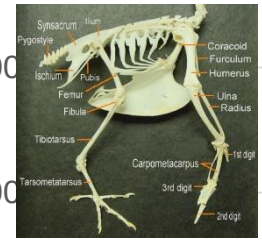

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Equivalence between Natuphos *E* and Natuphos

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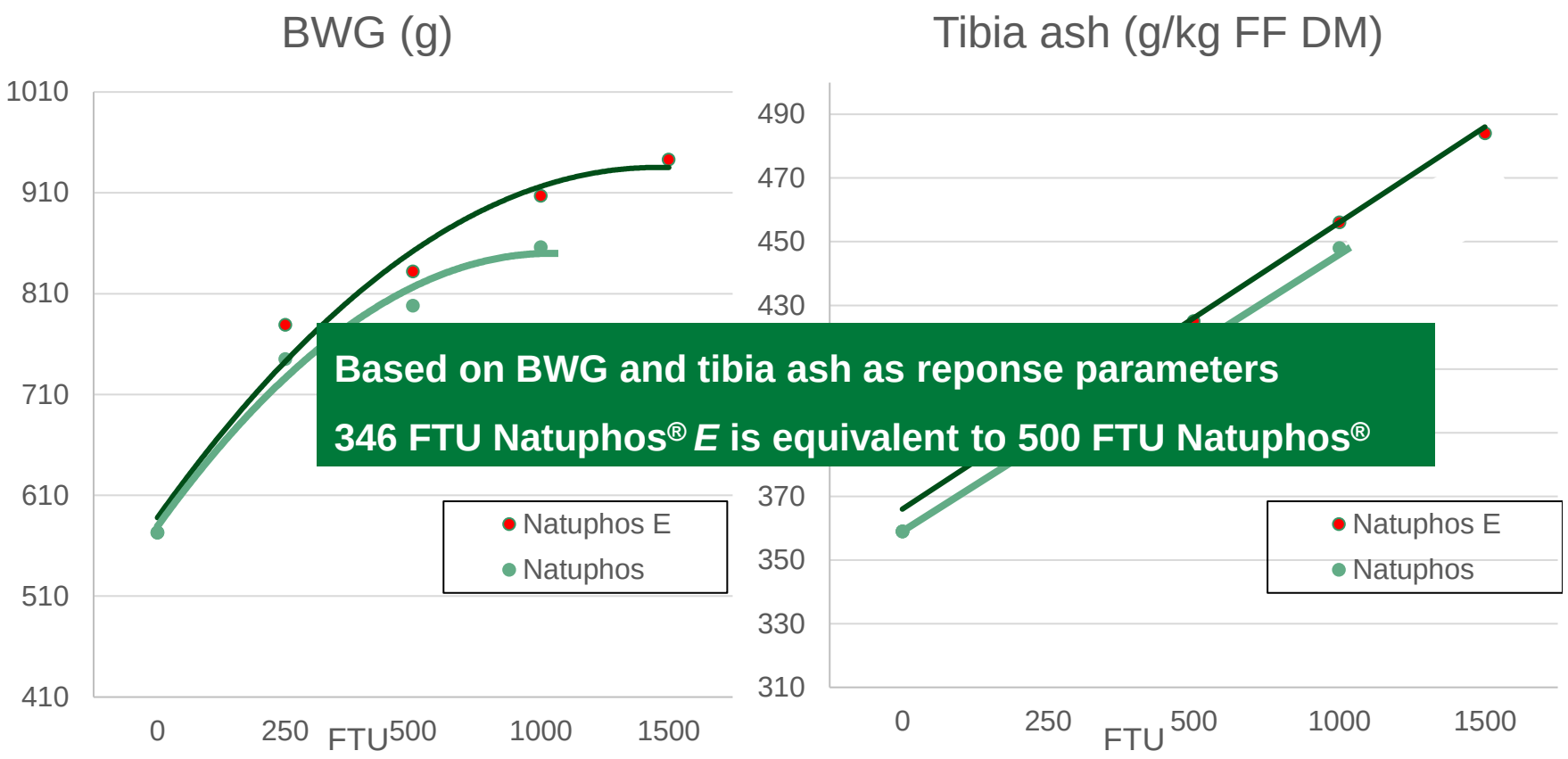

Based on BWG and tibia ash as reponse parameters

346 FTU Natuphos® *E* is equivalent to 500 FTU Natuphos®

natuphos® E

A more Efficient phytase

Natuphos® *E* in poultry (II)

BASF
We create chemistry

Aim of the trial

Comparing Natuphos E and Natuphos in broilers

- Ross 308 male broilers
- Same starter diet (commercial nutrient contents) from 0 - 6. days of age.
- The experimental diets were fed to the birds from 7-22 days of age.

  Pelleted feed (70-75°C PT) and water was available for ad libitum intake during the entire experiment
- 6 replicates a 12 birds/pen per treatment
- All diets constant ratio Ca/tP = 1.5

Künzel et al., 2017

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A more *E*fficient phytase

Natuphos® *E* in poultry (II)

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Versuchsaufbau		
T 1	Negative control	Without mineral P and without enzymes
T 2	T 1 + 0,6 g P/kg	from DCP
T 3	T 1 + 1,2 g P/kg	from DCP
T 4	T 1 + 1,8 g P/kg	from DCP
T 5	T 1 + 2,4 g P/kg	from DCP
T 6	**T 1 + 125 FTU/kg**	**Natuphos E 5000 G**
T 7	**T 1 + 250 FTU/kg**	**Natuphos E 5000 G**
T 8	**T 1 + 500 FTU/kg**	**Natuphos E 5000 G**
T 9	**T 1 + 750 FTU/kg**	**Natuphos E 5000 G**
T10	T 1 + 250 FTU/kg	Natuphos 5000 G
T11	T 1 + 500 FTU/kg	Natuphos 5000 G
T12	T 1 + 1000 FTU/kg	Natuphos 5000 G

Künzel et al., 2017

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A more Efficient phytase

Influence on tibia ash content (mg)

BASF
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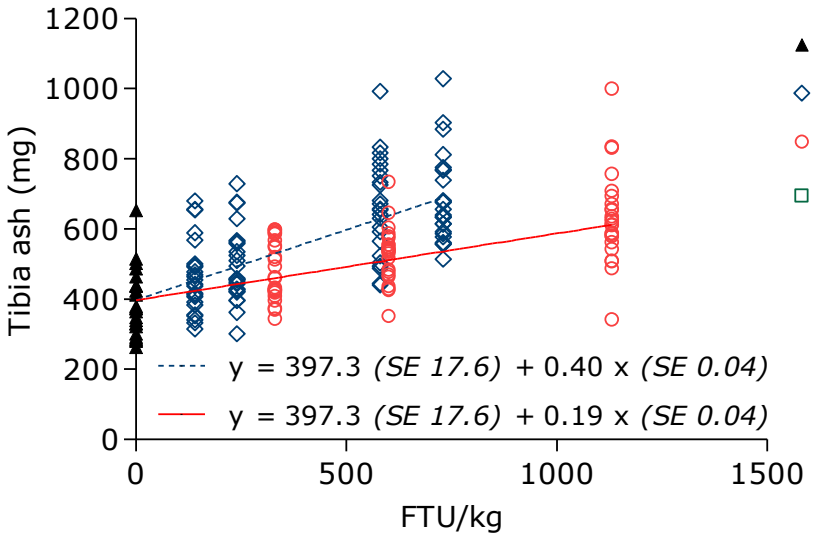

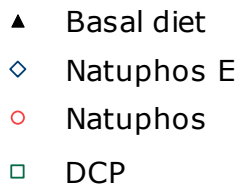

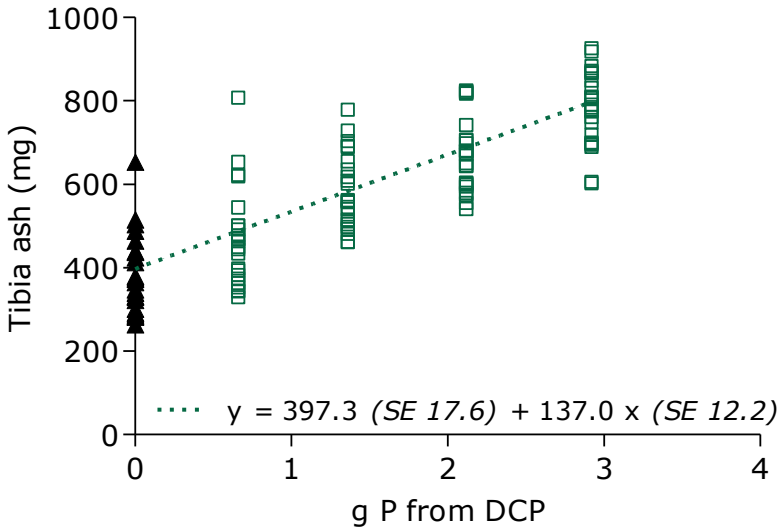

=> Using the dosage of Natuphos E of 750 FTU/kg the same tibia ash content was achieved as using 2,1 gtP aus DCP. (equal to 1.47 g ret P/oP)

Künzel et al., 2017

natuphos® E

A more Efficient phytase

Ratio between increase of DCP and Natuphos E or Natuphos on Tibia ash (g) of different weight classes (FTU/g P from DCP)

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We create chemistry

Weight class	Natuphos E /DCP	Natuphos /DCP
A (light)	349	729
B	403	842
C	310	647
D (heavy)	299	624
All (A+B+C+D)	340	710

=> Efficacy of Natuphos® *E* on tibia ash content was double in comparison to Natuphos® .

Künzel et al., 2017

natuphos® E

A more Efficient phytase

Comparison to proteases

BASF
We create chemistry

Objective of the study:
Evaluation of the effects of two different proteases and Natuphos E on precaecal (pc) amino acid (AA) digestibility in a conventional type broiler diet with adequate P content

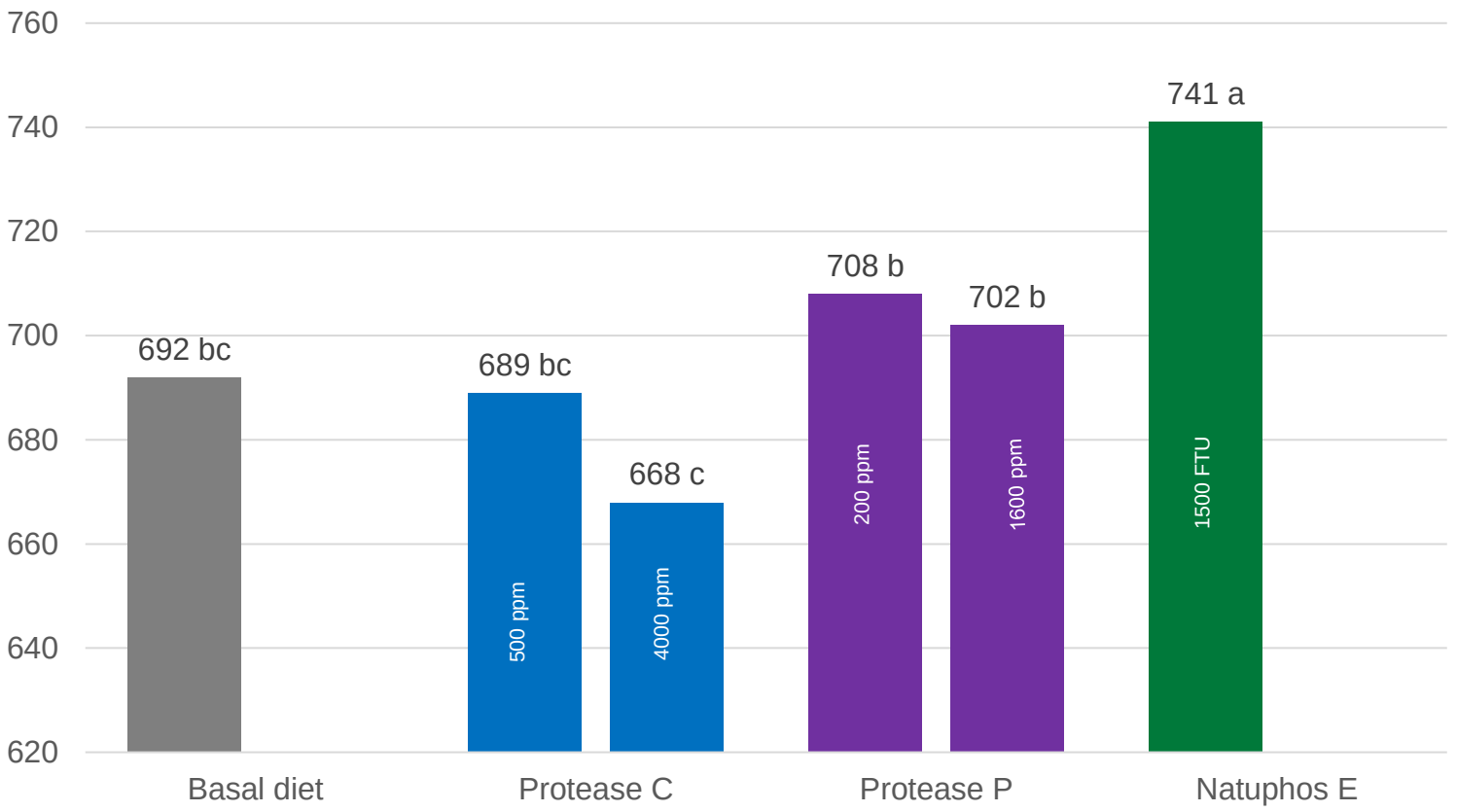
Set up		
Diet 1	Basal diet	without exogenous enzymes
Diet 2	-	+ recommended dose rate of Protease C (500 mg/kg)
Diet 3	-	+ 8 times recommended dose rate of Protease C (4000 mg/kg)
Diet 4	-	+ recommended dose rate of Protease P (200 mg/kg)
Diet 5	-	+ 8 times recommended dose rate of Protease P (1600 mg/kg)
Diet 6	-	+ 1500 FTU/kg Natuphos E

natuphos® E

A more Efficient phytase

Comparison to proteases - body weight (d 21* in g)

BASF
We create chemistry



- Experimental diets were offered from d 14 until slaughter on d 21

[abcde] Values without a common superscript are significantly different according to t-test ($p \leq 0.05$)

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A more Efficient phytase

Comparison to proteases – effect on pc digestibility of methionine (* in %)

BASF
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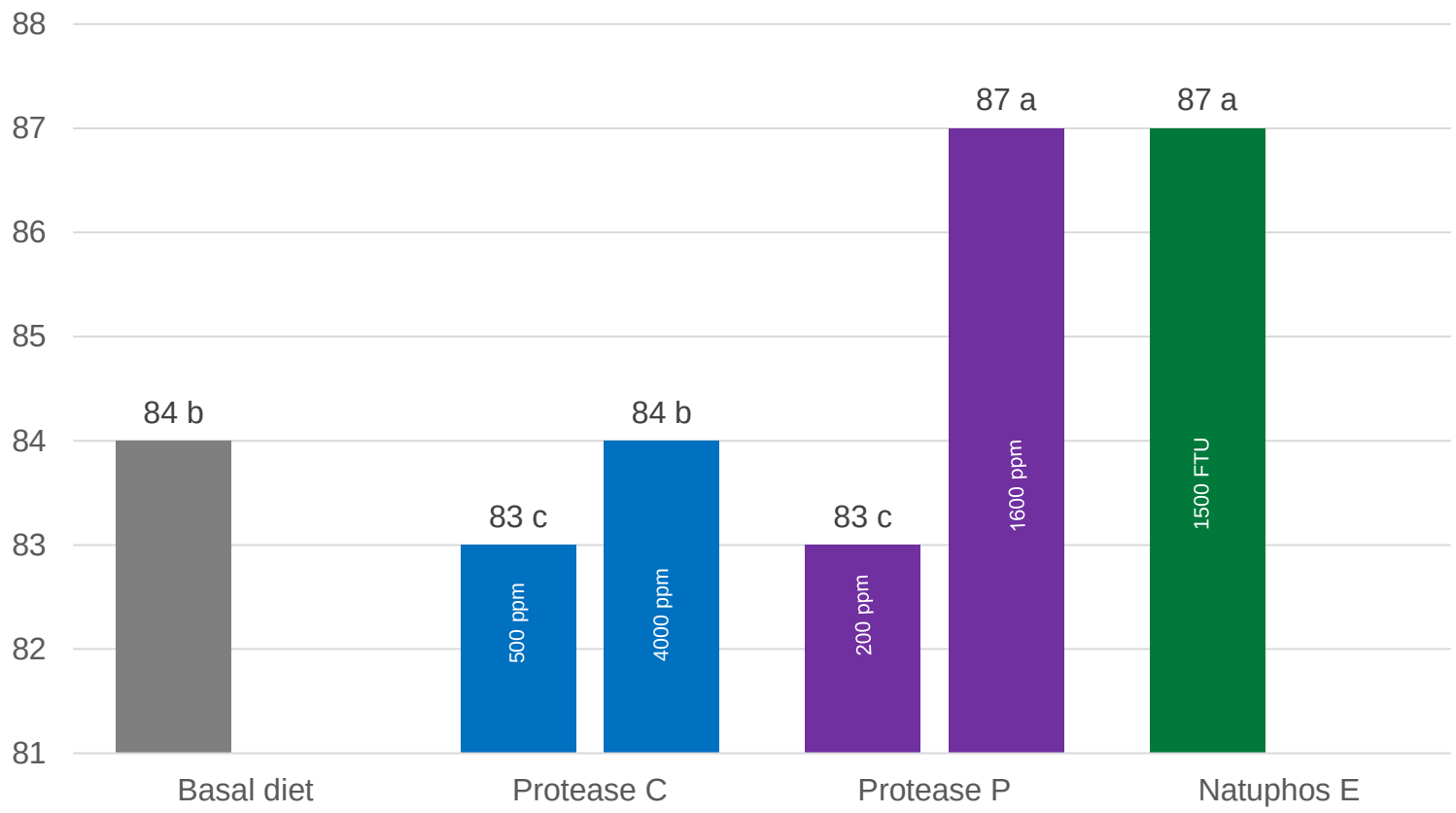

[abcde] Values without a common superscript are significantly different according to t-test ($p \leq 0.05$)

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Comparison to proteases – effect on pc digestibility of lysine (in %)

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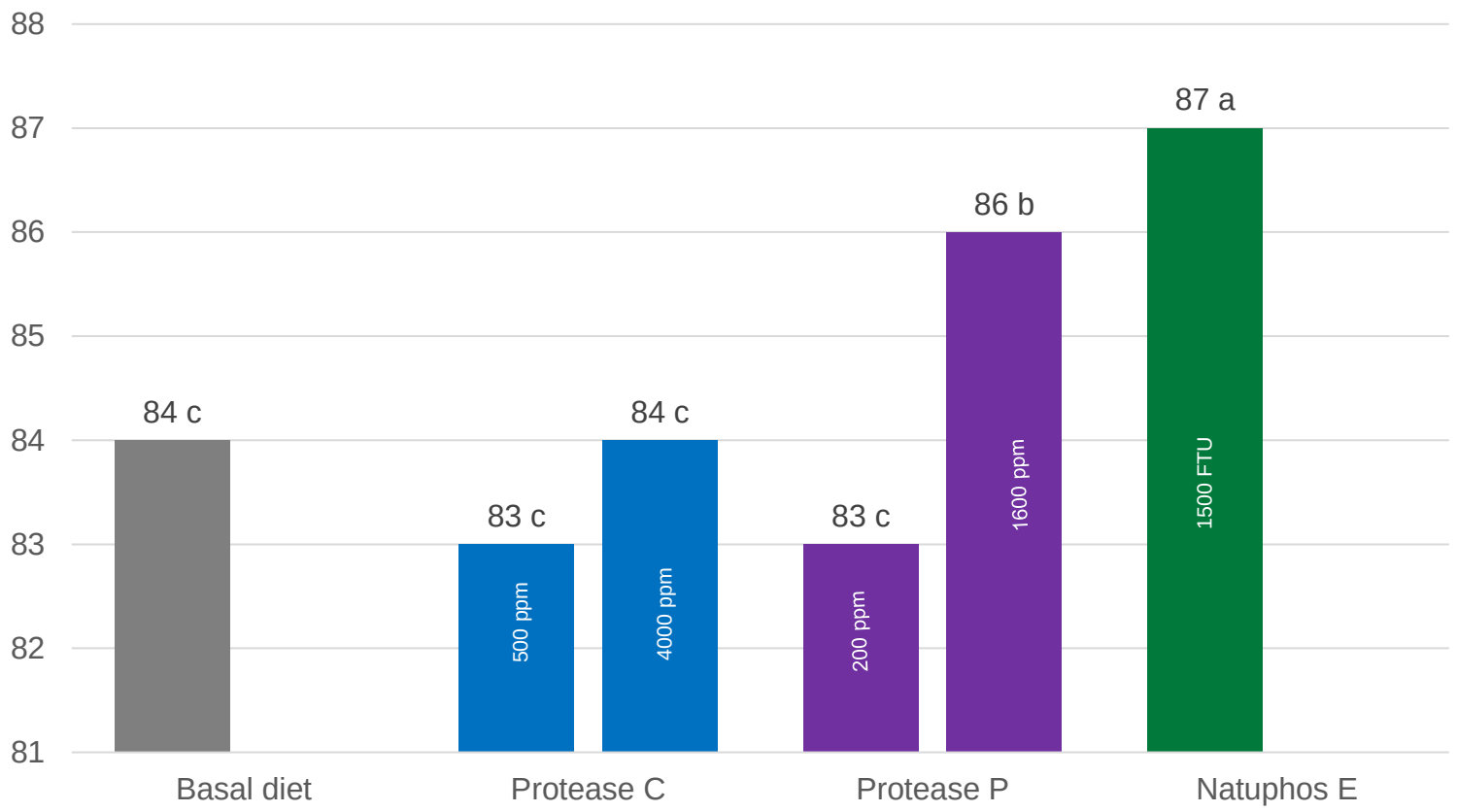

[abcde] Values without a common superscript are significantly different according to t-test ($p \leq 0.05$)

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Comparison to proteases – effect on pc digestibility of threonine (in %)

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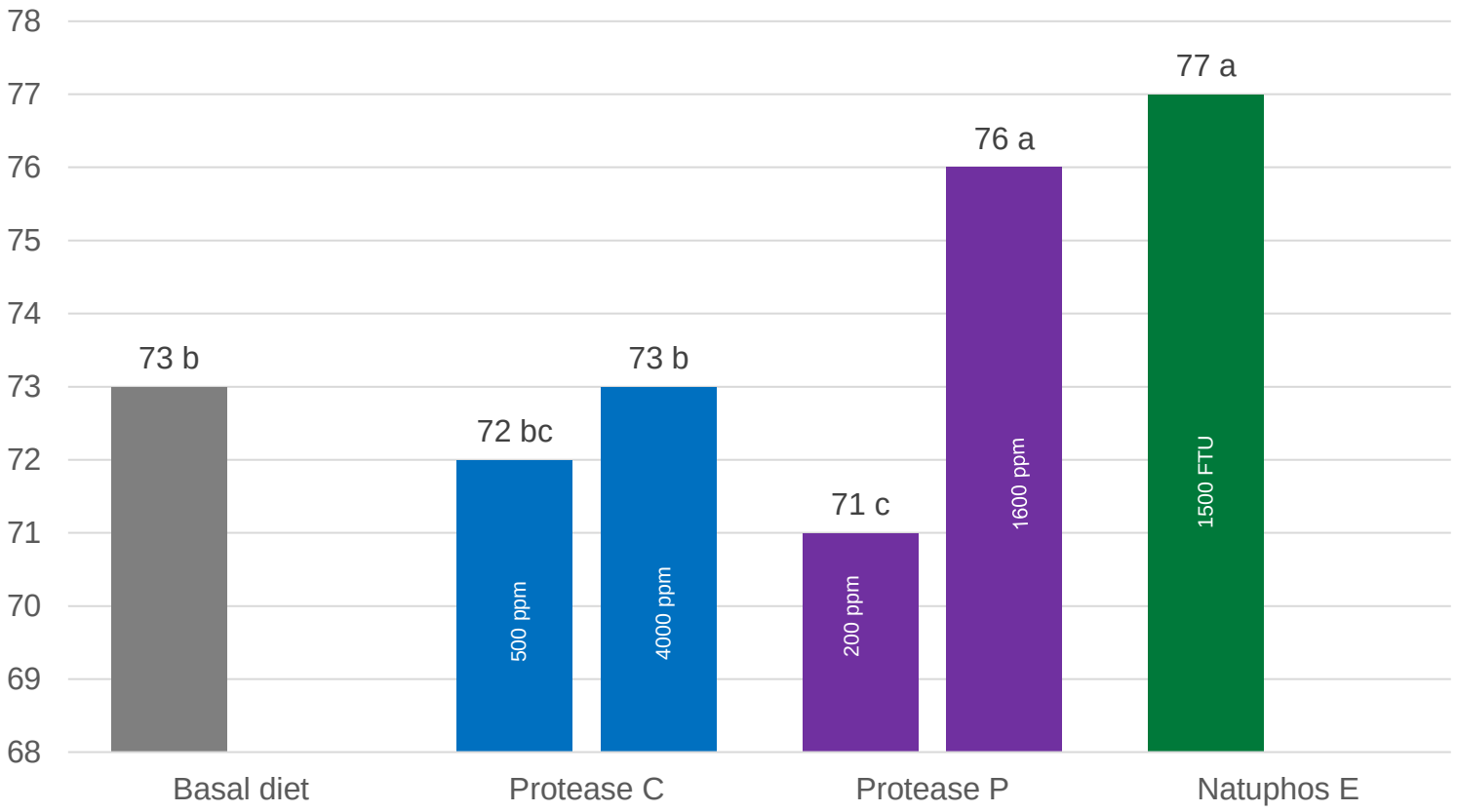

[abcde] Values without a common superscript are significantly different according to t-test ($p \leq 0.05$)

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Comparison to proteases - effect on pc amino acid digestibility (%)

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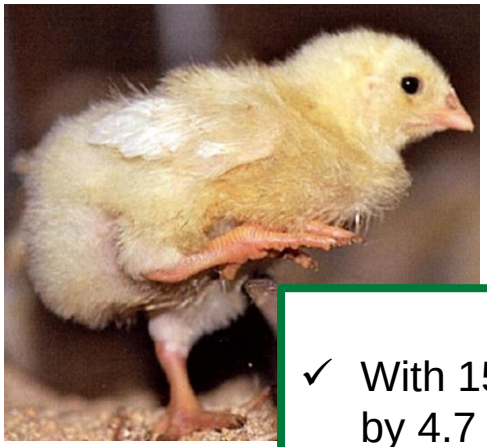

- ✓ With 1500 FTU **Natuphos E** the body weight increased significantly by 4.7 % compared to the highest protease treatment.
- ✓ From the applied proteases only the supplementation of the high dose (8 x recommended) of **Protease P** showed consistent beneficial effects on the pc digestibility of AA.
- ✓ At least the same increasing effect on AA digestibility was achieved when Natuphos E was supplemented at 1500 FTU/kg.

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Effect on broiler performance

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Objective of the study: **To provide efficacy data of Natuphos E 5000 for use in broilers**

Set up		
T 1	Positive control	without exogenous enzymes
T 2	Negative control I	Positive control minus matrix value A
T 3	T2 + 250 FTU/kg	
T 4	T2 + 350 FTU/kg	
T 5	Negative control II	Positive control minus matrix value B
T 6	T5 + 500 FTU/kg	
T 7	T5 + 600 FTU/kg	
T 8	Negative control III	Positive control minus matrix value C
T 9	T8 + 750 FTU/kg	
T10	T8 + 850 FTU/kg	
T11	Negative control IV	Positive control minus matrix value D
T12	T11 + 1000 FTU/kg	

Ross 308, males, day 1 -39, corn-soy diet, 3-phase feeding

Delezie et al., 2016

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Effect on broiler performance

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	Matrix value A	Matrix value B	Matrix value C	Matrix Value D
Treatment	**(T2/T3/T4)**	**(T5/T6/T7)**	**(T8/T9/T10)**	**(T11/T12)**
FTU/kg	**(0/250/350)**	**(0/500/600)**	**(0/750/850)**	**(0/1000)**
Nutrient reduction vs control	g/kg	g/kg	g/kg	g/kg
oP (available P, CVB)	0.8	1.04	1.2	1.36
Total P (DCP)	1.15	1.50	1.725	1.955
Calcium	1.50	1.95	2.25	2.55
Dig Lysine	0.12	0.16	0.18	0.204
Dig Methionine	0.010	0.013	0.015	0.017
Dig Meth +Cyst	0.03	0.04	0.045	0.051
Dig Thr	0.13	0.17	0.195	0.221
Dig Thryp	0.03	0.04	0.045	0.051
Dig Isoleucine	0.12	0.16	0.18	0.204
Crude protein	2.25	2.93	3.375	3.825
ME/Oeslk (MJ/kg)	0.222	0.288	0.332	0.377
ME/Oeslk (kcal/kg)	53	69	79	90

Delezie et al., 2016

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Bone data – tibia ash (%, d26)

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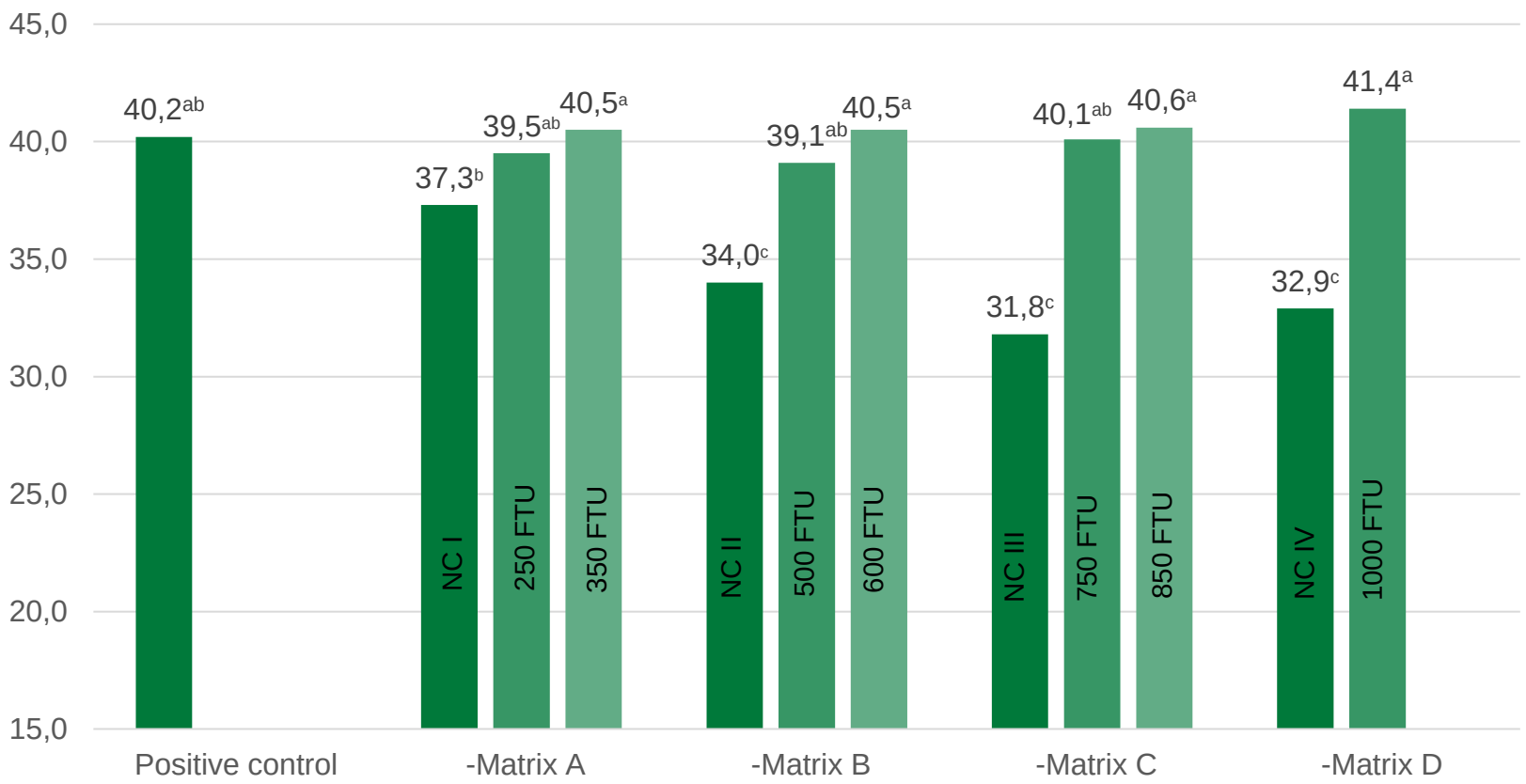

abc Mean values without a common superscript letter within a column are significantly different (P≤0.05).

Delezie et al., 2016

ILVO TID 03-15

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Effect on broiler performance – body weight d 39 (g)

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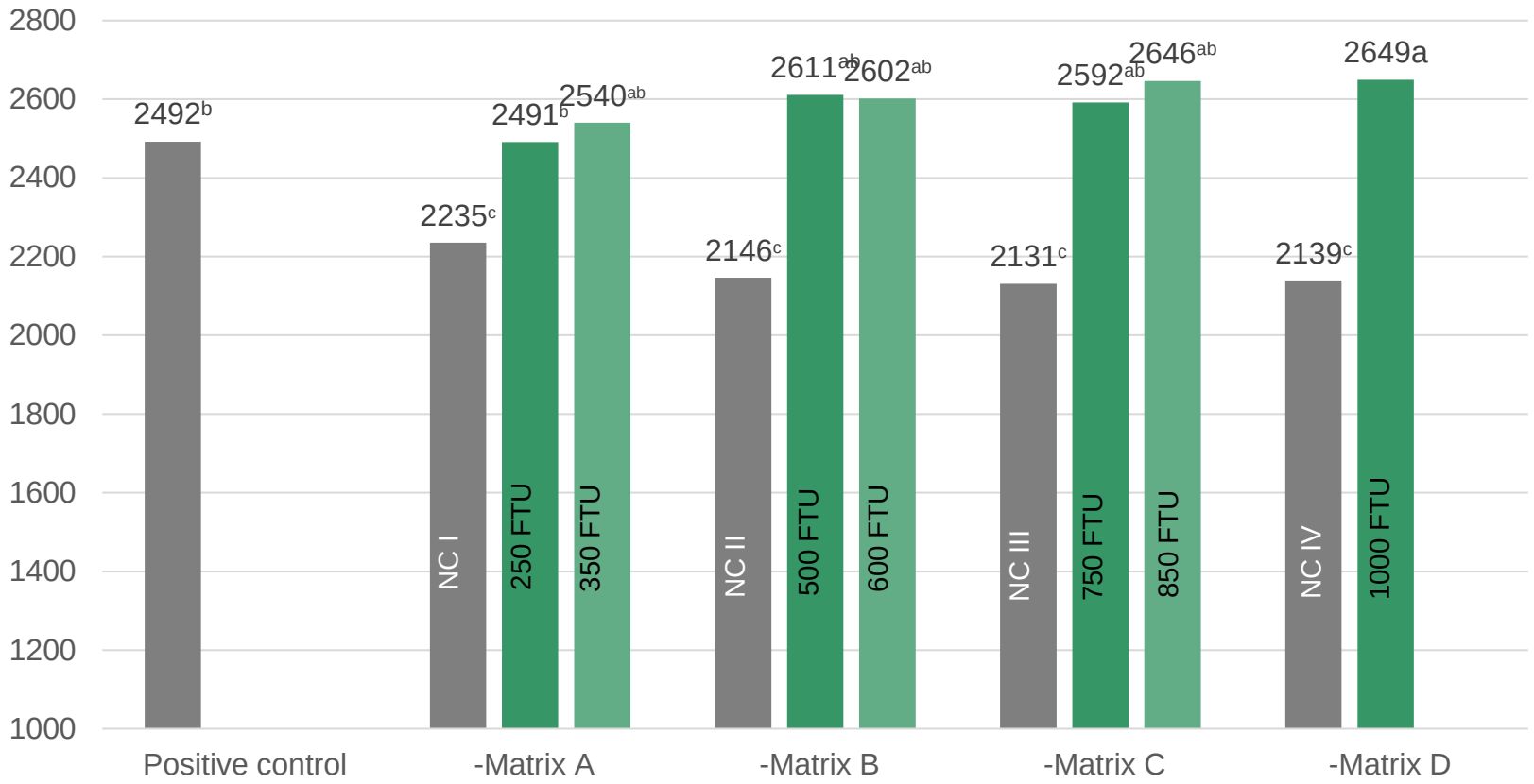

abc Mean values without a common superscript letter within a column are significantly different (P≤0.05).

Delezie et al., 2016

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Effect on broiler performance – feed conversion ratio (d 0-39, g/g)

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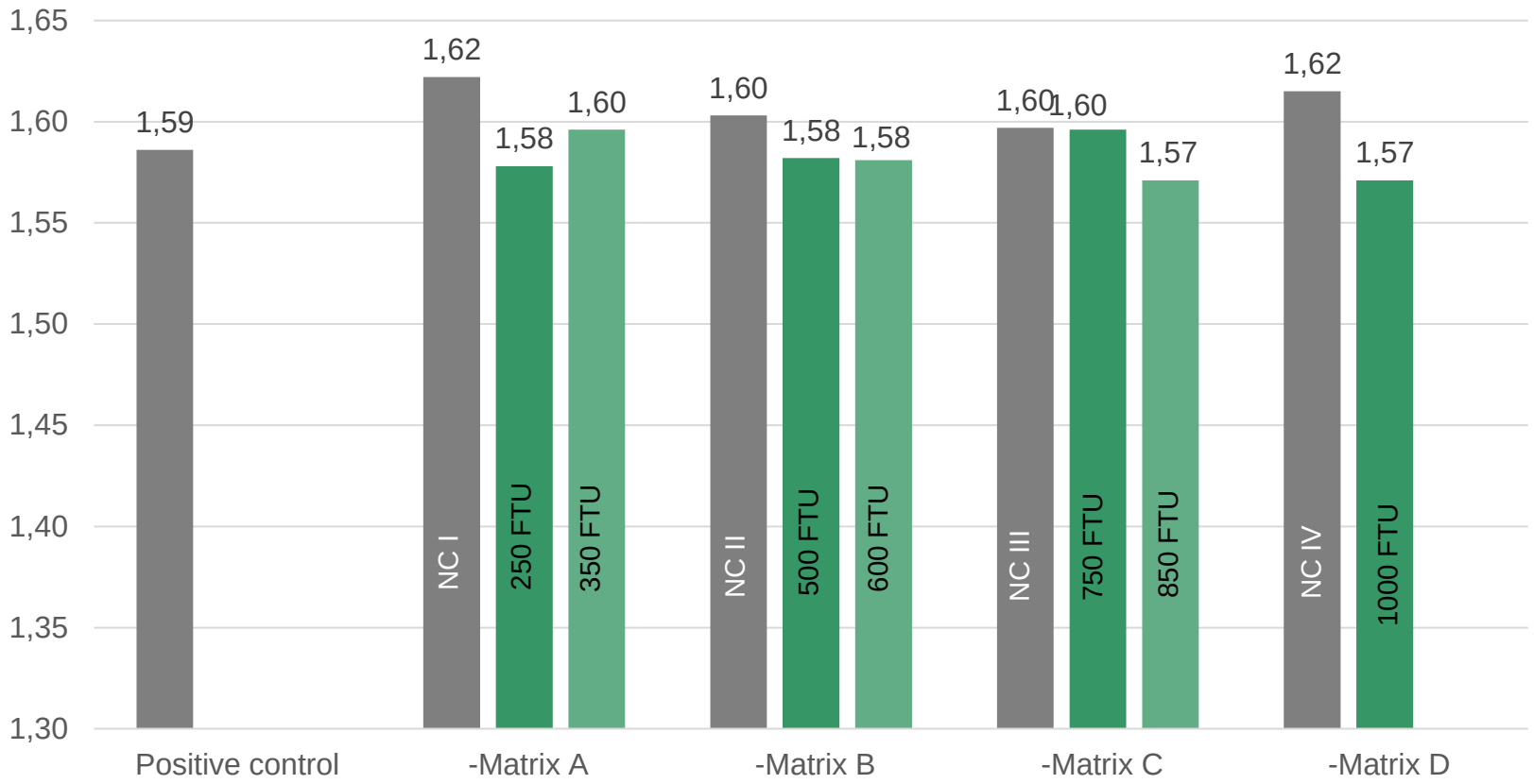

abc Mean values without a common superscript letter within a column are significantly different (P≤0.05).

Delezie et al., 2016

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Phosphorus equivalency of Natuphos E in comparison to Natuphos

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Piglets, sows, fattening pigs	**Natuphos**	**500 FTU/kg**		**1000 FTU/kg**
	P- equivalency	0,8 g dP		1,20 g dP
	Natuphos E	**500 FTU/kg**	**750 FTU/kg**	**1000 FTU/kg**
	P- equivalency	1,12 g dP	1,36 g dP	1,52 g dP

Broiler, ducks, turkeys, pullets	**Natuphos**	**500 FTU/kg**		**1000 FTU/kg**
	P- equivalency	0,8 g op./av.P*		1,04 g op./av. P*
	Natuphos E	**500 FTU/kg**	**750 FTU/kg**	**1000 FTU/kg**
	P- equivalency	1,04 g op./av.P*	1,28 g op./av. .P*	1,36 g op./av. P*

* Opneembar P, according to Dutch CVB 1997, assuming 70% availability of P in DCP and 80% in MCP,.

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Effect of Natuphos E dose levels on P-availability in broilers*

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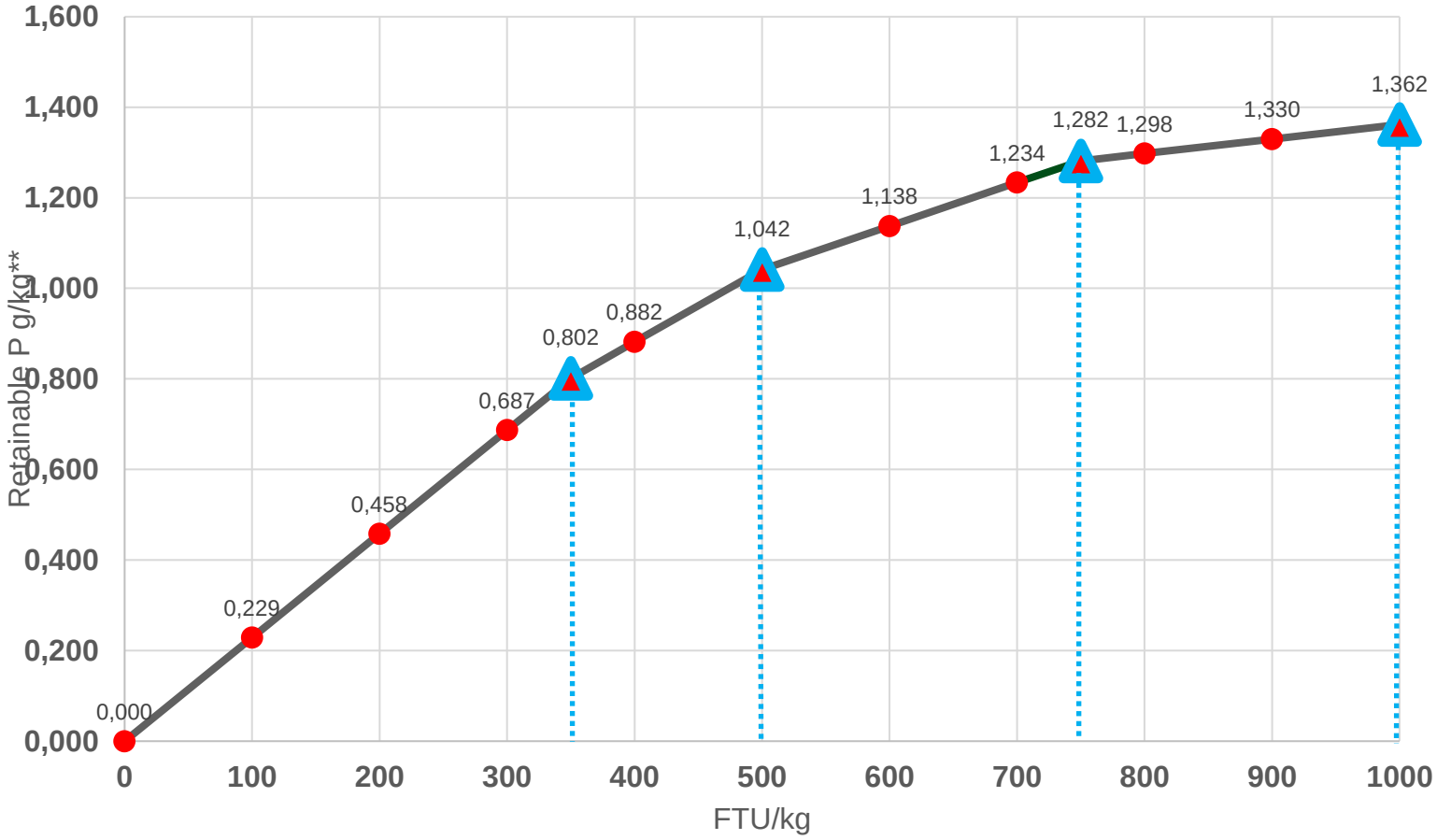

- Fattening poultry
- Retainable P (CVB)

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Matrix values (minerals) for broiler and growing poultry per kg of feed for different inclusion levels

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	Natuphos E mineral contributions			
	BROILER, GROWING PULLET, TURKEY, DUCK , GOOSE			
Natuphos E incl. rate, FTU/kg feed	350	500	750	1000
Phosphorus				
Available/opneembar P*, g	0,800	1,040	1,280	1,360
Total P from replacing MCP * , g	1,000	1,300	1,600	1,700
Total P from replacing DCP * , g	1,150	1,495	1,840	1,955
Calcium**				
Total Ca when using MCP, %	1,100	1,430	1,760	1,870
Total Ca when using DCP, %	1,265	1,645	2,024	2,151
Other minerals				
Sodium, g	0,015	0,020	0,024	0,026

* according to Dutch CVB 1997, assuming 70% availability of P in DCP and 80% in MCP,.

** a narrow Ca: tP ratio (e.g. 1,4 - 1,5 to 1) is recommended
that is why increased Ca reduction is recommended with increasing phytase dose level

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All-Nutrient matrix values (nutrient contribution) for broiler and turkey per kg of feed for different inclusion levels

BASF
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Natuphos E nutrient contributions				
	BROILER, GROWING PULLET, TURKEY, DUCK			
Natuphos E incl. rate, FTU/kg feed	350	500	750	1000
Energy *				
ME, kcal/kg	53	68,9	84,8	90,1
ME, MJ/kg	0,222	0,288	0,355	0,377
Crude protein, g	2,250	2,925	3,600	3,825
Amino acids **				
Lysine, g	0,120	0,156	0,192	0,204
Methionine, g	0,010	0,013	0,016	0,017
Cysteine, g	0,030	0,039	0,048	0,051
Arginine, g	0,130	0,169	0,208	0,221
Glycine,g	0,070	0,091	0,112	0,119
Histidine, g	0,050	0,065	0,080	0,085
Isoleucine, g	0,120	0,156	0,192	0,204
Leucine, g	0,200	0,260	0,320	0,340
Phenylalanine, g	0,130	0,169	0,208	0,221
Serine,g	0,110	0,143	0,176	0,187
Threonine, g	0,130	0,169	0,208	0,221
Tryptophane,g	0,030	0,039	0,048	0,051
Valine, g	0,150	0,195	0,240	0,255
Phosphorus				
Available/opneembar P***, g	0,800	1,040	1,280	1,360
Total P from replacing MCP *** , g	1,000	1,300	1,600	1,700
Total P from replacing DCP *** , g	1,150	1,495	1,840	1,955
Calcium****				
Total Ca when using MCP, g	1,100	1,430	1,760	1,870
Total Ca when using DCP, g	1,265	1,645	2,024	2,151
Other minerals				
Sodium, g	0,015	0,020	0,024	0,026

* Energy uplifts of phytases and NSP-enzymes are usually not additive and thus need to be respectively corrected when used in combination.

** Apparent ileal digestible

*** according to Dutch CVB 1997, assuming 70% availability of P in DCP and 80% in MCP,.

**** a narrow Ca: tP ratio (e.g. 1,4 - 1,5 to 1) is recommended

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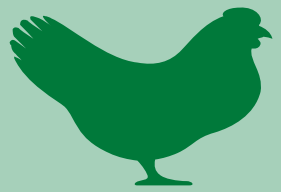
EU approval



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Published 08.03.2018 and 31.07.2019 (Official Journal of the European Union, L65/17-20; EU regulation (EU) 2019/1290)
EU - Identification number : **4a 27**
6-phytase EC 3.1.3.26

Species category	Minimum dose rate
• **Turkeys for fattening** • **Turkeys for breeding** • **All other minor poultry species for fattening or reared for laying/breeding** • **Chickens for fattening** • **Chickens reared for laying/breeding**	125 FTU/kg feed

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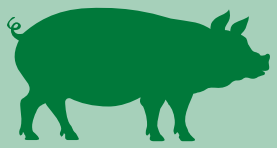
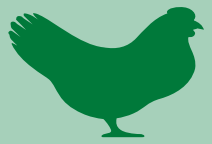
EU approval





BASF
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Published 08.03.2018 and 31.07.2019 (Official Journal of the European Union, L65/17-20; EU regulation (EU) 2019/1290)
EU - Identification number : **4a 27**
6-phytase EC 3.1.3.26

Species category	Minimum dose rate
• **Pigs for fattening** • **Sows** • **Minor porcine species for growing or reproduction**	100 FTU/kg feed
• **Weaned piglets**	125 FTU/kg feed
• **Turkeys for fattening** • **Turkeys for breeding** • **All other minor poultry species for fattening or reared for laying/breeding** • **Chickens for fattening** • **Chickens reared for laying/breeding**	125 FTU/kg feed

natuphos® E

The innovative hybrid 6-phytase

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hybrid 6-phytase

- with improved intrinsic stability and increased efficacy.

- fermented by efficient and proven Aspergillus niger – production technology.

- produced in reliable production facilities by a skilled team.

- formulated with patented, established and reliable formulation technology.

Bilder: BASF

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The innovative hybrid 6-phytase

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- ✓ saves even more phosphorus in feed and further reduces phosphorus excretion into the environment.

- ✓ guarantees safe use in premix and mineralfeed and at high pelleting temperatures in the feed mill.

- ✓ allows additional energy and amino acid savings in feed.

Bilder: BASF; Amandus Kahl

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Importance of quick release of phytase activity and pepsin resistance

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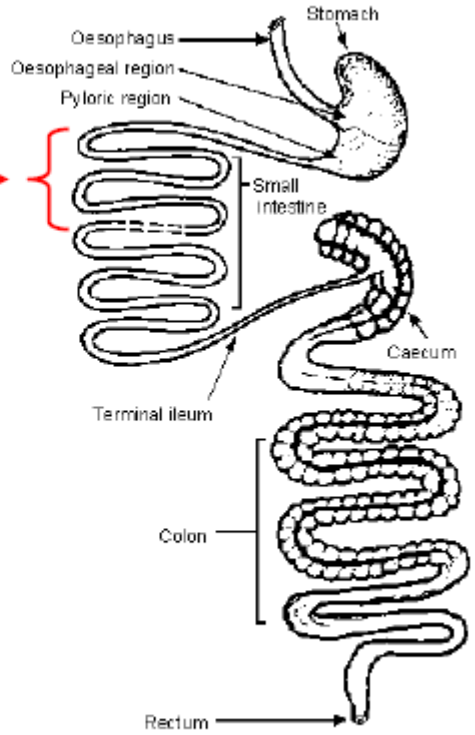
- Proximal small intestine
  - main site for phosphate absorption

- Inositol phosphates
  ($InsP_6 \Rightarrow InsP_5 \Rightarrow InsP_4 \Rightarrow InsP_3 \Rightarrow InsP_2 \Rightarrow InsP_1$)
  must be degraded anterior to the site for absorption of phosphate
  otherwise it's **too** late



Damgaard-Poulsen, 2012

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Efficient Formulation

Activity release from different formulations

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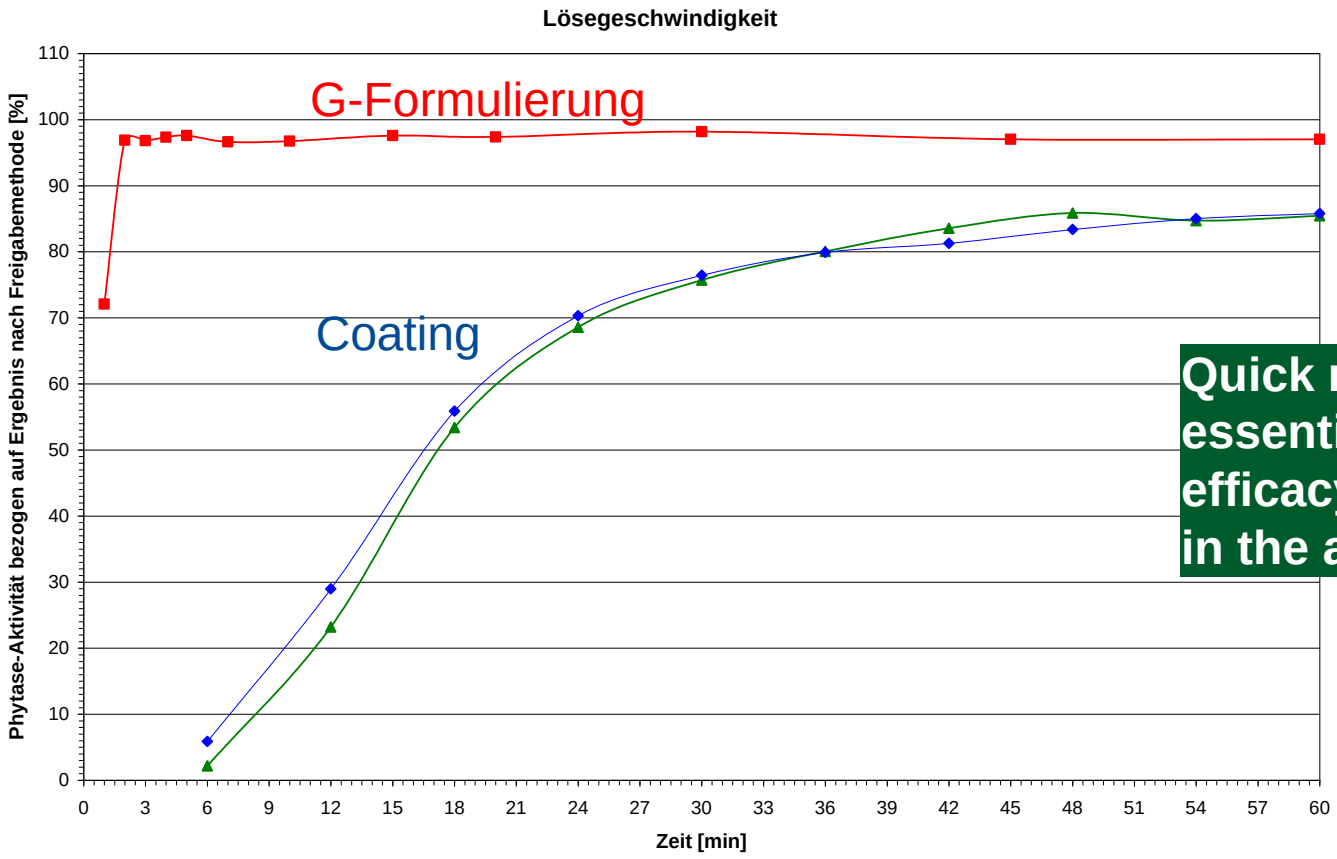

Quick release is essential for high efficacy of the phytase in the animal!

The new phytase

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Use in different P evaluation systems

Dr. Dieter Feuerstein
Senior Technical Manager
BASF SE, Lampertheim

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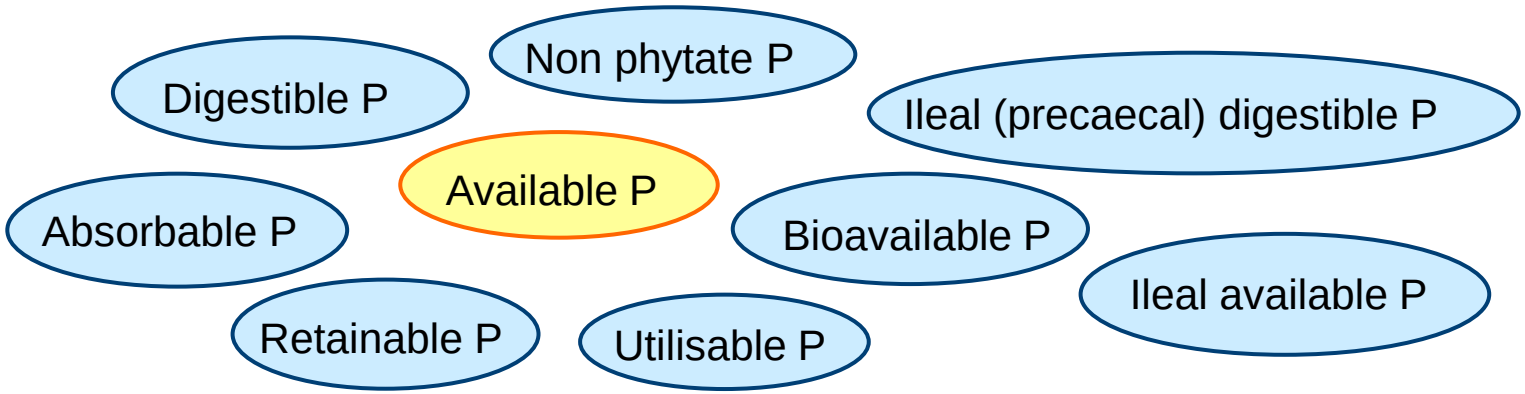
Terminology “available phosphorus



Different definitions of **available P** exist and different approaches are in use to determine P availability. This pluralism in definition not only causes confusion in communication. It also makes it almost impossible to compare data that originate from different laboratories. Compiling comprehensive feeding tables which are needed by the feed and poultry industries is hampered by this lack in harmonisation

Glossary of relevant terms

- The WPSA definition of aP is different from previous interpretations that were linked to the binding form of P or a certain approach of determination.
  - The NRC (1984, 1994) considered only non-phytate P available to poultry, and used the terms available P and non-phytate P interchangeably.
  - The system used in Germany also ignores that phytate P is available to a certain extent and implies that non-phytate P has an availability of 70 %, irrespective of its origin (GfE, 1999).
  - In the French system, the term aP refers to the proportion of P retained in the animal's body compared to a highly available reference source, assumed to have a biological value of 100 (monocalcium phosphate) (Sauvant *et al*., 2004). Bone mineralisation parameters (% tibia or toe ash) were used as response criteria in the French system.
  - The system that is in use in The Netherlands is based on quantitative measurements of retainable P (van der Klis and Blok, 1997).

WPSA Working Group No. 2 (Nutrition), 2013

„Available P“ content in some feedstuffs depending on P evaluation system, g/kg feedstuff

(low , high per feedstuff)

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Feedstuff	Total P, g/kg	CVB[1997]	WPSA[2013]	INRA[2004]	NRC[1994]	GfE[1999]
Corn	2.9	0.8	1.2	0.7	0.8	0.6
Wheat	3.3	1.3	1.8	1.9	1.4	1.0
Rice bran	17.8	2.8	2.5	-	2.6	1.8
Barley	3.7	1.4	1.7	2.2	1.7	1.2
SBM[48% CP]	6.6	2.8	3.7	1.5	2.3	1.8
RSM[38% CP]	11.4	3.8	3.8	2.9	3.0	2.1
SFM[32% CP]	12.1	3.3	4.6	2.1	1.9	1.3
Fish meal[60% CP]	27.0	20.0	20.5	23.0	27.0	18.9
MCP$_h$	226	192	188	194	226	158
DCP$_a$	197	108	101	150	197	138
MDCP	213	168	168	170	213	149

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Using matrix values (phosphorus) for broiler and growing poultry in different international phosphorus evaluation systems for poultry

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P- evaluation system	1 g P from MCP contains	Unit	Rationale
Dutch CVB (1997)	0,85	Opneembar P, g	P from MCP = 85 % available
NRC (1994)	1,0	Available P, g	P from MCP = 100 % available
INRA (2004)	0,91	Available P, g	P from MCP 91% available (reference MSP =100%)
GFE (1999)	0,7	Available P, g	P from MCP 70 % available

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Matrix values (phosphorus) for broiler and growing poultry per kg of feed for different inclusion levels in different phosphorus evaluation systems

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		Natuphos E mineral contributions			
		BROILER, GROWING PULLET, TURKEY, DUCK , GOOSE			
	Natuphos E incl. rate, FTU/kg feed	350	500	750	1000
Replacing total P from MCP * , g		1,000	1,300	1,600	1,700
P- evaluation system	Unit				
Dutch CVB (1997)	Opneembar P, g	0,850	1,105	1,280	1,36
NRC (1994)	Available P , g	1,000	1,300	1,600	1,700
INRA (2004)	Available P, g	0,910	1,183	1,456	1,547
GFE (1999)	Available P , g	0,700	0,910	1,120	1,190

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