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DSM Nutritional Products

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Food and Drug Administration
Division of Animal Feeds (HFV-224)
Office of Surveillance and Compliance
Center for Veterinary Medicine
7519 Standish Place
Rockville, Maryland 20855

July 8, 2013

GRAS Notification of RONOZYME HiPhos® by DSM Nutritional Products

Dear Mr. Wong

In response to the call for voluntary participation in the Notice of Pilot Program published in the Federal Register Vol. 75 31800-31803, DSM Nutritional Products is hereby submitting in triplicate a Notification of the Generally Recognized As Safe use of the 6-phytase, Ronozyme HiPhos®, in swine feed. This enzyme improves the availability of phosphorus found in plant based feeds by cleaving the myo-inositol - phosphate bond.

DSM Nutritional Products gathered the appropriate information on the safety and utility of the notified substance which was provided to an independent panel of experts in the field for their evaluation. The enclosed dossier contains the safety and efficacy study data that was provided to the panel. Information on the identity of the production organism, manufacture of the commercial forms, and the panel's signed conclusion statement were proved to CVM in AGRN#14. Also included are copies of the pertinent literature and the peer reviewed publications addressing the safety of Ronozyme HiPhos® and its performance in a variety of swine feeds indicative of those commercially fed in the United States.

DSM Nutritional Products has concluded that Ronozyme HiPhos® is GRAS through scientific procedures and is therefore exempt from the requirement for premarket approval noted in Section 201 (s) of the Federal Food Drug and Cosmetic Act.

The complete data and information that are the basis of the GRAS Notification are available to the Food and Drug Administration for review and copying upon request during normal business hours.

Sincerely DSM Nutritional Products,

RECEIVED
JUL 11 2013
By

James La Marta, Ph.D.
Senior Manager Regulatory Affairs

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GRAS Notification for
A 6-phytase preparation produced by an *Aspergillus oryzae* strain expressing a synthetic gene coding for a 6-phytase from *Citrobacter braakii*
For Use in Swine Nutrition

James La Marta
Parsippany, NJ

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Kaiseraugst, Switzerland

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1 Introduction

Phosphate is a key nutrient for animals and an increase in dietary phosphate has been shown to be beneficial to swine health, Ref. 1. Phytic acid, present in plant based feed ingredients such as soy beans, is a known anti-nutrient that chelates divalent cations and is a cause of eutrification of waterways by acting as a phosphate source for algae, Ref. 2. In October of 2012 DSM Nutritional Products submitted a GRAS notification to the Center for Veterinary Medicine for a 6-phytase enzyme, RONOZYME® HiPhos, in poultry feeds for the purpose of improving the digestibility of phytic acid present in plant based feed ingredients was GRAS, AGRN#14. DSM has also performed multiple studies to assess the safety and efficacy of the addition of RONOZYME® HiPhos to the feeds of swine for the same purpose. The panel of experts determined that this use is GRAS as noted in their report included in AGRN #14. The results of the target animal studies have been in the public domain for over six months.

1.1 Name and Address of Notifier

DSM Nutritional Products
45 Waterview Blvd.
Parsippany, New Jersey, 07054, USA
Tel: 973-257-8500

Person responsible for the dossier:

Alberto Davidovich, DVM, Ph.D.
45 Waterview Boulevard
Parsippany, New Jersey 07054
Tel: 973-257-8325

1.2 Name and Address of Manufacturer

Novozymes A/S
(b) (4)

Novozymes A/S
(b) (4)

Novozymes A/S
(b) (4)

Novozymes North America Inc.
(b) (4)

1.3 Name and Address of the Exclusive Distributor

DSM Nutritional Products
45 Waterview Blvd.
Parsippany, New Jersey, 07054, USA
Tel: 973-257-8294

1.4 Common or Usual Name of the Substance

DSM's phytase enzyme preparation is obtained from a Genetically Engineered strain of *Aspergillus oryzae* produced by (b) (4) fermentation. The common or usual name of the substance is "phytase". It is produced and sold in three forms; a liquid, a micro-granulate and a thermo-tolerant granulate. The trade name of the enzyme is RONOZYME® HiPhos.

1.5 Applicable Condition of Use

RONOZYME® HiPhos will be included in swine feed for the nutritional purpose of increasing the digestibility of phytate. The recommended use level of RONOZYME® HiPhos is 500 FYT to 4000 FYT/Kg of swine feed; where one FYT is the amount of enzyme that releases 1 µmol of inorganic phosphorous from phytate per minute at 37°C and pH 6.5.

1.6 AAFCO Definition O.P. 2011

Phytase derived from *Aspergillus niger* variants and *Aspergillus oryzae* variants are permissible as feed ingredients in swine and poultry diets. See reference 3.

Table 30.1 Enzymes/Source Organisms Acceptable for Use in Animal Feeds

Phytase	*Aspergillus niger*, var. *Aspergillus oryzae*, var.	Corn, soybean meal, sunflower meal, hominy, tapioca, plant by-products	Hydrolyzes phytate	Increases the digestibility of phytin-bound phosphorus in swine and poultry diets

1.7 Description of ingredient

Three product forms of RONOZYME® HiPhos will be available, two dry forms and a liquid form. RONOZYME® HiPhos (GT) is a granulated thermo-tolerant form with a minimum enzyme activity of 10,000 FYT/gram. RONOZYME® HiPhos (M) is a micro granulated form with a minimum enzyme activity of 50,000 FYT/gram. RONOZYME® HiPhos (L) is an aqueous liquid with a minimum enzyme activity of 20,000 FYT/g. Additional forms may be manufactured with ingredients suitable for feed use if there are additional market needs.

2 Production Organism, Enzyme Manufacturing Process

Detailed information regarding the production organism, the enzyme and the manufacturing methods, raw materials and toxicology studies was provided in the GRAS Notification for the use of RONOZYME® HiPhos, a 6-phytase preparation produced by an *Aspergillus oryzae* strain expressing a synthetic gene coding for a 6-phytase from *Citrobacter braakii* for use in poultry nutrition, AGRN #14.

3 Safety Studies

The summary of the swine safety and efficacy studies presented below was placed in the public domain on 25 May 2012 in the Journal of Animal Science Advances, Ref. 4. The diets conformed to the recommendations of the National Research Council (NRC 1988).

Diet Formulations

Diets were formulated to meet the NRC guidelines for nutrients per class of swine with the exception of phosphorus as noted in the study reports.

3.1 Tolerance study with IPA Mash Phytase [RONOZYME® HiPhos (M)] in weaned piglets (Czech Republic 2008) Report 00000962

The purpose of the trial conducted by [redacted] (b) (4) was to study the tolerance of piglets towards RONOZYME® HiPhos during 6 weeks. The study was conducted in compliance with the requirements of current, international Good Laboratory Practice. See Annex 1.

Experimental conditions

The study was performed with a total 48 early weaned piglets, crossbreeds Large White x Landrace of both sexes. The animals were housed in pairs in litterless boxes at [redacted] (b) (4) facilities that conformed to welfare regulations. Shortly after delivery the weight of piglets was determined and animals were randomly allocated to three groups A, B, C (16 piglets each, 8 castrated males and 8 females).

Pre-starter and starter diets based on wheat, barley and soybean meal as the main feed ingredients were formulated to meet NRC nutrient recommendations except for total and non-phytate P. Diet was provided to animals in mash form. Pre-starter diet was fed from day 0 until day 13 and starter diet from day 14 until day 42 of the Study. Hematological and biochemical examinations were performed in all animals. See also the complete report in the Annexes.

The treatment groups were the following:

Group A: Control non-treated group (basal diet, no enzyme added)

Group B.: basal diet + phytase at 4000 FYT/kg diet

Group C: basal diet + phytase at 40 000 FYT/kg diet

Composition and nutrient content of the diet

Main ingredients (%)	Pre-starter diet	Starter diet
Wheat	31.1	35.0
Barley	25.0	24.0
Soybean meal (48% CP)	14.0	13.0
Nutrients (% – calculated)		
Crude protein	20.08	16.83
Metabolizable energy (MJ/kg)	14.00	-
Digestible energy (MJ/kg)	-	14.35
Lysine	1.40	0.98

Main ingredients (%)	Pre-starter diet	Starter diet
Calcium	0.65	0.70
Total Phosphorus	0.54	0.54
Digestible Phosphorus	0.38	0.22

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Target	Pre-Starter 0	Pre-Starter 4000	Pre-Starter 40,000	Starter 0	Starter 4000	Starter 40,000
Analyzed	477*	5090	43,513	383*	4491	38,201

*: means no contamination but native activity in feed

Results

Performance parameters

Treatments	Initial body weight (kg)	Final body weight (kg) Day 42	Daily weight gain (g) Day 0-42	FCR Day 0 - 42
A (control)	10.57	27.61a	406	2.75a
B (4000 FYT/kg)	10.66	31.09b	486	2.18b
C (40,000 FYT/kg)	10.35	30.93b	490	2.20b

a,b - mean values with a different superscript differ significantly at $P<0.05$

Biochemical and haematological parameters

Treatments	Alkaline phosphatase (µkat/l)	Hematocrit (%)	Monocytes (%)	Lymphocytes (%)
A (control)	3.779d	33.38d	4.063	51.13
B (4000 FYT/kg)	4.546a	36.25a	3.438	54.63
C (40,000 FYT/kg)	4.545a	36.00a	3.563	53.75

a,d: means significant difference versus control ($P<0.05$)

RBC: red blood cell, HCT: hematocrit, MCV: mean corpuscular volume, PLT: blood platelet, WBC: white blood cell, ALB: albumin, GLU: glucose, P: phosphorus, ALT: alanine transaminase, CPK: creatine phosphokinase, HGB: haemoglobin, EO: eosinophil, BA: basophil, LY: lymphocytes, MO: monocytes

Selected haematological and biochemical blood parameters

Treatments	RBC ($x10^6/\mu L$)	HGB (g/100mL)	MCV (fl)	PLT ($x10^3/\mu L$)	WBC ($x10^3/\mu L$)
A (control)	7.03	9.66	47.69	235.8	15.97
B (4000 FYT/kg)	7.18	10.23	50.38	216.4	13.57
C (40,000 FYT/kg)	7.00	10.17	51.56	218.9	16.14
Phys. range	5-7	9-13	52-62	200-500	11-22

Selected haematological and biochemical blood parameters

Treatments	ALB (g/L)	Glu (mmol/L)	P (mmol/L)	ALT (µkat/L)*	CPK (µkat/L)
A (control)	25.8	6.20	3.66	3.52	32.5
B (4000 FYT/kg)	26.5	6.15	3.35	3.53	28.3
C (40,000 FYT/kg)	26.6	5.80	3.35	3.72	38.8
Phys. range	19-42	4-8.1	2.25-3.44	0.55-1.31	1-20.85

*µkat/L – microkatal per liter, one µkat = 59 enzyme units

Selected haematological and biochemical blood parameters

	EO (%)	BA (%)
B (4000 FYT/kg)	2.0	0.38
C (40,000 FYT/kg)	2.8	0.63
A (control)	2.3	0.63
Phys. range	0-11	0-2

Discussion

Dietary administration of RONOZYME® HiPhos resulted in beneficial effects on performance of the piglets. The final body weight of the piglets receiving 4000 and 40,000 FYT/kg diet was significantly increased by more than 12% when compared to the negative control. The feed conversion ratio was also significantly improved from 2.75 (control) to 2.18 and 2.20, respectively. No mortality occurred during the study. Furthermore, no pathological changes were observed in piglets during the post-mortem necropsy. No unfavourable effects due to dietary administration of RONOZYME® HiPhos were observed.

The blood biochemistry examination revealed that the values of ALT were increased in all examined animals of all groups; values of CPK were increased in 15 piglets of Group A, in 9 piglets of Group B and 11 piglets of Group C. The values for ALT and CPK did not differentiate phytase treated pigs from the controls. Although the MCV was below normal for all groups the treatment groups were higher than the control and closer to the normal range.

The amount of serum phosphorus was increased above physiological range in 12 animals of control Group A, in 4 piglets of Group B and 5 piglets of Group C. The other followed biochemical parameters were within physiological ranges or deviations were diagnosed sporadically.

The hematological examination revealed that the amount of RBC in animals of all groups was higher (Group A 10, Group B 12 and Group C 8 piglets), value of HCT was increased in some animals in Groups B and Group C, in animals of Group A corresponded to the physiological range. The amount of WBC in 1 piglet of Group A and one piglet of Group C was above physiological range. The other followed hematological parameters were either in or bellow physiological ranges. These changes were within the normal range.

Conclusions
In spite of significant differences among treatment groups for some biochemical and haematological parameters, no remarkable or significant differences between the maximum recommended dose and the overdose of RONOZYME® HiPhos phytase were noted for all relevant parameters.

Based on the above results it is possible to state that RONOZYME® HiPhos Phytase is well tolerated by weaned piglets when used at the maximum recommended and ten times higher doses.

3.2 Tolerance study with IPA Mash Phytase in gestating and lactating sows (Germany 2009) Report 00003288

The purpose of the trial conducted by [redacted] (b) (4) (b) (4) was to study the tolerance of gestating and lactating sows towards RONOZYME® HiPhos during one reproduction cycle (day 1 of pregnancy to successful service after weaning of the litter). See Annex 2.

Experimental conditions
A total of 36 multiparous sows (EUROC line) in the body weight range 160 to 210 kg and with comparable litter numbers were allocated to the experimental groups. After being assigned to the treatment groups the sows were artificially inseminated with sperm from Pietrain boar. The piglets of the litter were therefore crossbreeds (EUROC x Pietrain). After successful artificial insemination the sows were kept until day 108 of pregnancy at individual sow feeding pens with straw bedding. From 109 days of pregnancy onwards sows were transferred to an environmentally controlled farrowing stable with straw bedding and three compartments (A, B, C) with 12 pens each.

The dose levels of RONOZYME® HiPhos phytase in complete diets for gestating and lactating sows were 0 FYT/kg feed (control), 4,000 FYT/kg of feed (maximum recommended level) and 40,000 FYT/kg of feed (10 times the maximum recommended level).

Composition and nutrient content of the diet

Main ingredients (g/kg)	Gestation diet	Lactation diet
Barley	450.0	645.4
Soybean meal (48% CP)	60.0	168.0
Nutrients (%)		
Crude protein	13.91	17.07
Crude fibre	6.87	5.42
Crude fat	3.88	3.73
Ca	0.75	0.98
P	0.53	0.70

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	A 0 FYT/kg	B 4000 FYT/kg	C 40,000 FYT/kg
Gestation diet	325*	4911	45,895
Lactation diet	409*	4313	40,435

*: does not mean contamination but native activity in feed

Results

Performance of sows during the gestation period and lactation period, performance of piglets during the 28-day suckling period, haematological results in sows at 24 days after farrowing

Treatments	A 0 FYT/kg	B 4000 FYT/kg	C 40,000 FYT/kg	Oneway Anova
Gestation (1^{st} to 114^{th} day)				
Body weight 1^{st} day (kg)	185.3	185.4	182.3	0.705
Body weight 112^{th} day (kg)	235.8	236.8	240.1	0.618
Number of piglets overall	12.7	13.2	13.5	0.669
Body weight (28^{th} day lactation) (kg)	202.9a	211.3ab	214.2b	0.026
Body weight loss (kg)	-32.9a	-25.6b	-25.9b	0.001
Feed intake (1^{st} to 28^{th} d) (total kg)	129.3	127.2	129.1	0.867
Litter size (corrected)	10.5	10.3	10.5	0.640
Rearing losses (%)	3.52	3.80	4.50	0.910
Body weight gain of piglets (1^{st} to 28^{th} day of age) (kg)	4.84a	4.77a	6.02b	<0.001
Feed intake overall (kg)	0.89a	0.87a	1.05b	0.004
Erythrocytes (terra/l)	5.72ab	5.91a	4.91b	0.049
Leukocytes (giga/l)	17.19	16.08	14.87	0.171
Haemoglobin (mmol/l)	7.38	7.31	6.86	0.129
Hematocrit (%)	39.1	38.8	37.0	0.112
Mean corpuscular volume (fl)	68.0	65.7	69.3	0.048

Treatments	A 0 FYT/kg	B 4000 FYT/kg	C 40,000 FYT/kg	Oneway Anova
Alkaline phosphatase (µkat/l)	0.79	0.64	0.79	0.421
Gamma-glutamyl transferase (µkat/l)	1.22	0.91	0.95	0.220
Alanine-amino transferase (µkat/l)	1.03	1.15	1.24	0.121
Urea in plasma (µmol/l)	4.79a	4.11a	7.49b	<0.001
Glucose (mmol/l)	2.64ab	2.45a	2.91b	0.015
Albumin (g/l)	40.8	39.5	69.8	0.693
Inorganic phosphate (mmol/l)	1.93	1.72	2.20	0.006
Weaning to service interval (days)	6.4	6.8	6.5	0.708

a,b mean values with a different superscript differ significantly at $P<0.05$

Discussion

<u>Gestating period</u>:
The sows weighed 184 kg on average at the start of the trial. By day 112 of gestation an average body weight of 237.6 kg was recorded which corresponded to a body weight gain of 53.6 kg per sow. Sows fed with RONOZYME® HiPhos Phytase tended dose dependently to slightly higher body weight gains. However, these changes were not significant. The sows consumed on average 3.13 kg feed per head and day during the 114-day pregnancy period. Consequently, detrimental effects of the overdose on feed intake could be excluded.

<u>Lactation period</u>:
Neither the number of piglets born alive nor the number of stillborn piglets showed any significant treatment-related differences. The body weights at the end of the lactation period were in the range of 209.5 kg. Sows fed with RONOZYME® HiPhos Phytase tended to higher body weights when compared to sows fed without RONOZYME® HiPhos Phytase. Body weight gain of piglets from sows fed without or with RONOZYME® HiPhos Phytase at the level of 4,000 FYT/kg amounted to 4.80 kg. Piglets weight from sows fed with the ten-fold overdose (40,000 FYT/kg) was significantly improved by 25.4% when compared to piglets of sows fed without or with 4,000 FYT/kg RONOZYME® HiPhos Phytase.

<u>Hematological parameters</u>:
To further confirm the safety of IPA Mash Phytase blood examinations were conducted at the 24th day after farrowing which corresponded to a 140-day supplementation period. It was observed that all mean values were within the physiological range. With feeding RONOZYME® HiPhos Phytase at the tenfold level, the mean for erythrocytes was significantly lower than those of sows fed with the maximum recommended dose level. The significantly higher means of inorganic phosphate, total cholesterol, urea and glucose were mainly reflecting the higher performance status when compared to sows fed without or with RONOZYME® HiPhos Phytase at the maximum recommended level (4,000 FYT/kg). However, all differences between the treatments were still within normal limits. Therefore, RONOZYME® HiPhos is characterized by a high level of safety.

Fertility parameters:
In addition to rearing performance, the weaning to service interval, i.e. the number of days from weaning to successful service was measured. The differences did not attain statistical significance. None of the inseminated sows showed signs of return rate up to the 35th day of pregnancy. From this result it can be concluded that with adding RONOZYME® HiPhos Phytase at levels of 4,000 or 40,000 FYT/kg no negative effects on fertility of sows occurred.

Conclusions
It can be concluded that the long term supplementation of RONOZYME® HiPhos at 10X level (40,000 FYT/kg) in sow diets during an overall reproductive cycle, including the successful service after weaning, induced lower estimated body weight losses during the 28-day lactation period and significantly improved body weight gains of piglets compared to the control diet without RONOZYME® HiPhos. Additionally blood examinations and the weaning to service interval of sows fed with the tenfold overdose level of RONOZYME® HiPhos showed no negative health or fertility relevant effects.

3.3 Target animal safety factors calculations

The product RONOZYME® HiPhos is intended for use in swine feeds. The standard recommended dose range of the product is 4,000 FYT/kg feed.

Based on the NOAEL of 860 mg TOS/kg bw-day (590985 FYT/Kg/day) derived from the 13 weeks study in rats and typical feed intake values as derived from NRC[1] feeding tables, the following safety margins can be calculated for the different categories of animals:

Table 1 Consumption estimation and safety factors in target species

Target species	Body weight kg	Typical feed intake kg feed/ day[1]	RONOZYME® HiPhos highest use recommendation		Highest expected enzyme intake		Safety margin
			FYT/ kg feed	mg TOS/ kg feed	FYT/ day	mg TOS/ kg-bw day	(NOAEL / highest intake)
Piglets, 6-7 weeks old	15.0	0.950	4,000	6.6	3,800	0.418	2057
Growing pigs, 13-14 Weeks old	50.0	3.110	4,000	6.6	12,440	0.411	2092
Pregnant sows	200	1.9 -2.5	4,000	6.6	10,000	0.083	10360
Lactating sows	200	5.3 - 7.0	4,000	6.6	28,000	0.231	3723

[1] National Research Council, Nutrient Requirements of Swine. Ninth Revised Edition, National Academy Press, Washington, D.C., 1988. Ref. 5

Discussion:

The safety factors as derived from the NOAEL in rats are comfortably large, in excess of three to four orders of magnitude.

The safety in the target species was confirmed by tolerance studies in piglets for fattening, and gestating/lactating sows using up to 40,000 FYT/kg feed, 10 times the highest recommended dose in FYT. The excessive dose did not produce any adverse effect on body weight gain, reproductive parameters (litter weight), blood cell counts, blood chemistry and gross pathology.

Because of the large safety margins, no regulatory maximum dose for RONOZYME® HiPhos in feed is necessary. However with cost-benefit and marketing considerations and in order to allow flexibility in feed formulation, the following upper dose is recommended: 4000 FYT/kg feed.

4 Efficacy Studies

In addition to the studies presented below another study series was performed by (b) (4) [redacted] (b) (4) using diets indicative of some current US practices; corn /soy with distiller grains products, Ref. 6.

4.1 Diet formulation

A total of nine efficacy studies were performed using a variety of diets in an effort to capture the diversity of possible ingredients used in swine feed production in Europe and North America. All the diets met the NRC recommendations as presented in the tables below.

Piglets:

	Piglet Study 1		Piglet Study 2		Piglet Study 3		NRC
Ingredient	**RPT 2500761**		**RPT 00001788**		**RPT 00003284**		**Piglet**
	w/o P	w/ di CaP	w/o P	w/ di CaP	w/o P	w/ di CaP	Diet
Corn	68.52	68.125	40	40	60.6	60.6	
Corn Starch			0.19		1.65		
Barley			24.68	24.68			
Sweet Milk whey			13.72	13.72			
Soybean Meal 48% CP	15.1	15.1	9.84	9.84	32	32	
Potato protein concentrate			7.23	7.23			
Rapeseed Meal	12.5	12.5					
Salt	0.55	0.55	0.14	0.14	0.4	0.4	
Lard			1.49	1.49			
Soy Oil	1	1			3	3	
Calcium carbonate	1.56	0.355	1.21	0.83	0.9	0.9	
DiCalcium phosphate	0	1.6	0.42	1		1.65	
Micronutrient supplement	0.77	0.77	1.08	1.08	1.45	1.45	
Crude Protein - N x 6.25	15.5	15.5	18.56	18.56	17.96	18.33	18
Lysine %	0.96	0.96	0.91	0.91	1.21	1.18	0.95
Methionine + cysteine %	0.54	0.54	0.84	0.84	0.66	0.6	0.48
Calcium - % Dry Matter	0.82	1.24	0.75	0.75	0.48	0.86	0.69
Phosphorus - % Dry Matter	0.45	0.78	0.42	0.52	0.36	0.66	0.6
Theoretical Available P - %	0.12	0.35					0.32
Observed available P - %	0.11	0.32					
Phytic P - calculated %	0.28	0.54					
Estimated digestible energy - MJ/Kg	13.31	13.31	13.85	13.82	13.81	13.81	
Estimated digestible energy - Kcal/Kg	3180	3180	3300	3300	3300	3300	3220

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Growers:

Ingredient	Grower Study 1 RPT 2500672		Grower Study 2 RPT 00001789		Grower Study 3 RPT 00003283		NRC Grower
	w/o P	w/ di CaP	w/o P	w/ di CaP	w/o P	w/ di CaP	Diet
Corn	53	53	35	35	65.8	65.8	
Corn Starch			0.18		1.05		
Barley	13.9	13.9	41.35	41.35			
Oat Meal	6	6					
Wheat Bran	5.4	5.4					
Soybean Meal 48% CP	18	18	18.95	18.95	29.5	29.5	
Potato protein concentrate			0	0			
Salt			0.35	0.35	0.4	0.4	
Lard			2.3	2.3			
Soy Oil	1	1			2	2	
Calcium carbonate			1.3	0.92	0.95	0.95	
DiCalcium phosphate	0	1.2		0.55		1.05	
Micronutrient supplement	2.7	2.7	0.57	0.57	0.3	0.3	
Crude Protein - N x 6.25	15.5	15.5	16.11	18.56	17.96	18.33	15
Lysine %	0.96	0.96	0.1	0.91	1.06	1.08	0.75
Methionine + cysteine %	0.54	0.54	0.65	0.84	0.59	0.59	0.41
Calcium - % Dry Matter	0.7	0.8	0.6	0.75	0.58	0.79	0.6
Phosphorus - % Dry Matter	0.42	0.62	0.35	0.45	0.33	0.56	0.5
Theoretical Available P - %	0.12	1.86					0.23
Phytic P - calculated %	0.28	0.28					
Estimated digestible energy - MJ/Kg	13.31	13.31	13.4	13.4	13.81	13.81	13.61
Estimated digestible energy - Kcal/Kg	3180	3180	3200	3200	3300	3300	3250

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Sows:

	Sow Study 1	Sow Study 2	Sow Study 3	NRC
Ingredient	**Rpt 00003286**	**RPT 00003285**	**RPT 00003282**	**Sows**
	w/o P	w/o P	w/o P	**Diet**
Maize	47	34.04	30	
Optigrain		23		
Triticale		10		
Barley	20		10	
Oats			4	
Wheat	1	12	8	
Wheat Bran			8.1	
Soybean Meal 48% CP	11.8	12.5	23	
Rapeseed Meal	8			
Sunfower meal	6.4			
Peas			9	
Salt	0.17	0.1	0.51	
Cellulose	1	4.5		
Sunflower Oil	1.5			
Soy Oil		0.5	3.3	
Calcium carbonate		1.66	1.6	
MonoCalcium Phosphate			1.2	
DiCalcium phosphate				
Micronutrient supplement	2.9*	1.2	0.3	
Crude Protein - N x 6.25	17.17	14.44	17.96	13
Lysine %	0.96	0.64	1.06	0.6
Methionine + cysteine %	0.63	0.42	0.59	0.36
Calcium - % Dry Matter	0.8	0.7	0.58	0.75
Phosphorus - % Dry Matter	0.6	0.34	0.33	0.6
Theoretical Available P - %	0.24			0.35
Phytic P - calculated %				
Estimated digestible energy - MJ/Kg	12.7	12.86	13.81	13.43
Estimated digestible energy - Kcal/Kg	3035	3070	3300	3210

*contain Calcium and Phosphorus

4.2 Ingredient Selection

The global economic situation of the past ten years as well as the evolving bio-fuels policy, have permanently altered the once standard corn/soybean meal diet. The drivers of ingredient selection are now formulation economics, availability, geography, milling and processing characteristics, and nutrient quality. All these factors are data inputs for computerized formulation software packages that generate a least cost ration based upon the data, which can vary on a daily basis. First and foremost, the ingredients utilized in the feed formulation software evaluations must meet and/or exceed the minimum NRC requirements for the age and production status of the animal, but economics and ingredient availability quickly take precedence in the assessment of whether or not to feed a certain feedstuff and in what amounts, depending upon the ability to transport, store, and utilize the given ingredient. For example, DDGS did not factor into the formulation equation in North Carolina until the economics were favorable, the mill bin space was available, and the mill had a viable and timely method of unloading the unwieldy ingredient from the railcars. A strong crop of canola along with favorable rail rates can make canola meal available deep into the southeastern parts of the Unted States when it was normally only found in the Dakotas. Nursery pig diets have become so complex that a combination of numerous ingredients (potato proteins, whey and other lactose sources, oat fiber sources, etc.) are regularly utilized to supply the necessary nutrients and gastrointestinal benefits while minimizing the effects of various anti-nutritional factors. Smaller toll mills in the Midwest will typically be able to source grains such as wheat, barley, triticale, milo, and others to meet the needs of the swine customer base that they serve. Pennsylvania feed millers have access to various bakery-type ingredients emanating from the production of potato chips, cookies, candy, bread, and chocolate products. Depending upon the success of the winter wheat crop in the Carolinas, producers in the region will at times feed all wheat as the sole grain source as long as the local crop volume holds out. The variety of feedstuffs across the U.S. continues to increase as pork producers seek out alternatives to expensive corn, soybean meal, and fat. Given the current U.S. climate in terms of available feedstuffs, the goals of a research trial diet formulation are to 1) provide the animal with a diet that nutritionally meets and/or exceeds the minimum requirements for growth and performance and 2) provides ample amounts of the dietary substrate being studied in the specific trial. Educational guides from universities inform farmers and feed mills about which ingredients are suitable and how to utilize them in feed formulation. Wheat, barley, millet and various distiller's grains products are now commonly added to swine feed. See Ref. 7 & 8.

In the case of phytase evaluations, the goal is to provide enough of the phytate-P substrate to confirm the effects of the enzyme being studied.

4.3 OVERVIEW OF EFFICACY STUDIES IN WEANED PIGLETS

Report	Animal Numbers / Species	Trial Duration (wks)	Dosage FYT per kg feed		Negative Control→ RONOZYME® Treatments Effect on Phosphorus Utilization Measured by			
			as targeted	As analyzed				
2500761 Evaluation of the effects of graduated amounts of RONOZYME® HiPhos in the weaned piglet (France 2009) Corn and soybean meal and rapeseed meal diet	120 piglets	5			Daily weight gain (g)	Apparent fecal P digestibility (%)	Fecal P excretion (mg/g)	Bone strength (N)
	n=12		0 (NC)	108*	220	24.1	3.43	272.8
	n=12		250	374	254	26.9	3.80	334.6
	n=12		500	601	249	40.0++	2.73++	476.1++
	n=12		1000	1097	249	42.7++	2.58++	384.1
	n=12		1500	1611	271	50.7++	2.24++	500.3++
	n=12		2000	2225	296	56.0++	2.03++	523.5++
	n=12		3000	3098	300	55.1++	2.02++	476.5++
	n=12		4000	4030	274	61.8++	1.76++	542.2++
	n=12		8000	8238	268	60.1++	1.80++	604.1++
	n=12		0 (PC)	108*	257	40.8++	4.62++	615.5++
00001788 Efficacy of IPA phytase (RONOZYME® HiPhos) in piglets (Spain 2009) Corn, barley and soybean meal	144 piglets	6			FCR Day 0 -42	Apparent fecal P digestibility (%)	Apparent fecal Ca digestibility (%)	P in feces (g/kg DM)
	n=24		0(NC)	<LOQ	1.68bc	37.3e	58.7d	18.7a
	n=24		500	669	1.64bc	60.5c	70.8bc	10.7b
	n=24		1000	1082	1.55ab	68.2b	73.3bc	10.0bc
	n=24		2000	2128	1.54a	71.0b	75.0ab	8.9c
	n=24		4000	4301	1.61ab	79.3a	81.7a	6.6d
	n=24		0 (PC)	137*	1.76c	47.9d	66.5cd	17.2a
00003284 Effects of a novel phytase in corn-soybean meal diets fed to weanling pigs (USA 2009) Corn-soybean meal diet	48 piglets	10 d			Apparent Total tract digestibility (%)	P in feces (%)	P output (g/day)	P absorption (g/day)
			0 (NC)	80*	40.46++	2.30++	1.35++	0.93++
			500	440	61.56++	1.51++	0.87++	1.39++
			1000	958	65.07++	1.46++	0.81++	1.51++
			2000	1743	68.74++	1.22++	0.71++	1.54++
			4000	3974	68.04++	1.10++	0.68++	1.46++
			0 (PC)	91*	60.48++	2.53++	1.68++	2.58++

abcd: means within one column not sharing a common letter index differ with statistical significance
* means no contamination but some native activity in feed
++: means statistically different from non ++ values
NC = negative control   PC =positive control

DSM BRIGHT SCIENCE. BRIGHTER LIVING.

4.4 OVERVIEW OF EFFICACY STUDIES IN GROWING – FINISHING PIGS

Report	Animal Numbers / Species	Trial Duration (wks)	Dosage FYT per kg feed		Negative Control→ RONOZYME® Treatments Effect on Phosphorus Utilization Measured by			
			as targeted	As analyzed				
2500672 Effects of graduated amounts of a microbial phytase (RONOZYME® HiPhos) on the fecal digestibility and excretion of phosphorus, calcium and zinc in growing pigs (France 2009) Corn, soybean and barleyl diet	36 pigs	5			Apparent fecal P digestibility (%)	Fecal Zn digestibility (%)	Fecal P Excretion (mg/g DM)	Apparent fecal Ca digestibility (%)
	n=4		0 NC	225*	29.3	11.4	2.99	60.2
	n=4		500	678	50.4+	25.5+	2.11+	68.8+
	n=4		1000	1179	57.8+	21.4+	1.79+	73.0+
	n=4		1500	1723	59.8+	17.3++	1.71+	72.8+
	n=4		1750	1985	61.3+	25.0+	1.62+	75.7+
	n=4		2000	2232	61.5+	21.6+	1.63+	75.3+
	n=4		2500	2798	66.6+	17.5++	1.40+	86.7+
	n=4		3000	3329	68.0+	18.1++	1.34+	81.8+
	n=4		PC	219*	47.2+	16.7	3.29+	61.1
00003283 Effects of a novel phytase (=RONOZYME® HiPhos) in corn-soybean meal diets fed to growing pigs (USA 2009) Corn, soybean diet	24 pigs	10d			Apparent Total tract p digestibility (%)	P in feces (%)	Apparent Total tract Ca digestibility (%)	Ca in feces (%)
	n=4		0	41*	39.83	2.44	67.30	2.33
	n=4		500	373	59.36**	1.82**	81.44**	2.45**
	n=4		1000	984	65.43**	1.52**	82.62**	1.40**
	n=4		2000	1773	69.09**	1.31**	82.36**	1.29**
	n=4		4000	3681	72.76**	1.09**	85.58**	1.22**
	n=4		PC	39*	59.36**	2.59**	72.90**	0.91**
00001789 Efficacy of IPA phytase (=RONOZYME® HiPhos) in growing pigs (Spain 2009) corn-barley diet	48 pigs	3			Apparent fecal Ca digestibility (%)	P in feces (g/kg DM)	Apparent fecal P digestibility (%)	P in blood (mg/100ml)
	n=8		0	150*	55.3c	13.78c	39.6c	6.69a
	n=8		500	671	62.0bc	10.98b	35.6bc	7.12ab
	n=8		1000	1529	70.6ab	10.49b	42.5b	7.63bc
	n=8		2000	2659	75.9a	8.11a	56.1a	8.04c
	n=8		4000	4448	61.3bc	7.69a	62.4a	7.75c
	n=8		PC	114*	58.0c	14.82c	37.5bc	7.66c

abcd: means within one column not sharing a common letter index differ with statistical significance
* means no contamination but some native activity in feed, + significant differences versus negative control at level P<0.001, ** ++ significant differences versus negative control at level P<0.05

4.5 OVERVIEW OF EFFICACY STUDIES IN SOWS

Report	Animal Numbers / Species	Trial Duration (wks)	Dosage FYT per kg feed		Negative Control→ RONOZYME® Treatments Effect on Phosphorus Utilization Measured by			
			as targeted	As analyzed				
00003282	28 sows	2			P digestibility (%)			Ca digestibility (%)
Dose response study with a new phytase (IPA Mash Phytase) in lactating sows (Germany 2009)	n=7		0	<50	20.5a			33.2
Corn and soybean meal diet	n=7		500	589	23.3ab			37.5
	n=7		1000	1027	32.5bc			37.6
	n=7		2000	2125	34.1c			33.6
00003285	24 sows	2			Fecal conc. Ca (g/kg DM)	Fecal conc. P (g/kg DM)	Ca apparent Digestibility (%)	P apparent Digestibility (%)
Efficacy study with IPA Mash Phytase in gestating sows (Germany 2009)								
Corn, soybean meal, wheat and triticale diet	n=6		0	211*	37.1a	18.6a	30.57a	26.51a
	n=6		500	786	34.3ab	17.8a	35.93b	33.52ab
	n=6		1000	1262	32.4ab	15.4ab	39.08bc	38.59b
	n=6		2000	2440	28.8b	13.9b	41.14c	39.87b
00003286	24 sows	2			DM Digestibility (%)	Fecal conc. P (%)	Ca Digestibility (%)	P Digestibility (%)
Evaluation of the effect of IPA Mash phytase on the nutrient digestibility in gestating sows (Slovak republic 2009)	n=6		0	124*	83.1a	27.90a	35.5a	26.7a
	n=6		500	531	83.7ab	26.1b	41.6b	33.6b
	n=6		1000	898	84.7c	26.1b	47.8c	39.0c
Corn, soybean meal, barley, rapeseed meal, sunflower meal diet	n=6		2000	1890	84.2bc	25.7b	44.1b	37.2bc
00015939	45 sows	3			DM Digestibility (%)	P Digestibility (%)		
Assessment of the effects of phytase (Ronozyme® HiPhos) to improve nutrient digestibility in lactating sows (Canada 2012)	n=15		0 NC	127*	80.5b	33.9b		
	n=15		500	764	81.8a	46.0a		
Wheat, soybean meal, barley, Canola meal	n=15		0 PC	<LOQ	78.8c	29.7b		

^{a,b,c} Values without a common superscript are significantly different according to test (P ≤ 0.05)

* means no contamination but some native activity in feed

4.6 Evaluation of the effects of graduated amounts of RONOZYME® HiPhos in the weaned piglet (France 2009) Report 2500761

The purpose of the trial conducted at Village-Neuf (France) was to determine the effects of graduated amounts of RONOZYME® HiPhos on the performance, blood mineral concentrations, digestibility of phosphorus (P), calcium (Ca), bone mineralization and strength in the weaned piglet. The basal diet, without addition of mineral P, was based on corn, soybean meal and rapeseed meal. See Annex 3.

Experimental conditions
One hundred and twenty Large White x Landrace weaner piglets having an initial body weight of 8.03 ± 1.09 kg were used. The animals were allocated to 10 equal groups of 12 animals each and housed in floor-pen cages.

The piglets were fed, throughout a 32 days observation period, a basal diet in mash form without addition of mineral P (group A) or the diet A supplemented with 16 g/kg of DiCalcium Phosphate (DCP) (group B) or with RONOZYME® HiPhos (L) phytase at the levels of 250 FYT/kg (group C), 500 FYT/kg (group D), 1000 FYT/kg (group E), 1500 FYT/kg (group F), 2000 FYT/kg (group G), 3000 FYT/kg (group H), 4000 FYT/kg (group I) and 8000 FYT/kg (group J).

The basal diet A was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the available P supply, the animals' requirements according to Henry et *al.* (1989) and NRC (1998).

Performance was evaluated for the 32 days of the trial. Blood was collected by jugular puncture from all the animals at the 31st day of the experiment for the determination of the P, Ca, alkaline phosphatase and zinc (Zn) concentrations.

Composition and nutrient content of the diets

Main ingredients (%)	Basal diet (group A)	Basal diet with DCP (group B)
Corn	68.52	68.125
Soybean meal	15.1	15.1
Rapeseed meal	12.5	12.5
DCP	-	1.6
Nutrients		
Crude protein (%)	15.5	15.5
Ca- analysed (% in DM)	0.82	1.24
Lysine (%)	0.96	0.96
P- analysed (% in DM)	0.45	0.78
Theoretical available P (%)	0.12	0.35
Observed available P (%)	0.11	0.32
Phytic P – calculated (%)	0.28	0.54
Estimated digestible energy (MJ/kg)	13.31	13.31

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Target	Basal diet Group A	Basal diet + DCP Group B	C	D	E	F	G	H	I	J
Target (nominal)	0 FYT/kg feed	0 FYT/kg feed	250	500	1000	1500	2000	3000	4000	8000
Analyzed	108*	108*	374	601	1097	1611	2225	3098	4030	8238
Actually added	-	-	266	493	989	1503	2117	2990	3922	8130
Average % of target	-	-	106	99	99	100	106	100	98	102

*: does not mean contamination but native phytase activity in feed

Results

Daily weight gain, total weight gain, feed conversion ratio, fecal P concentration and excretion, apparent fecal digestibility of P, apparent fecal digestibility of Ca and fecal excretion, bone ash and resistance

Target	Basal diet Group A	Basal diet + DCP Group B	C	D	E	F	G	H	I	J
Treatments	0 FYT/kg	0 FYT/kg	250	500	1000	1500	2000	3000	4000	8000
Daily weight gain (g)	220	257	254	249	249	271	296	300	274	268
Total weight gain (kg)	7.04	8.22	8.12	7.96	7.98	8.66	9.46	9.61	8.77	8.56
FCR	2.448	1.914	1.981	1.985	1.931	1.835	1.819	1.793	1.834	1.865
fecal P concentration (mg/g DM)	22.4	24.6 P<0.001	20.8 P<0.05	16.3 P<0.001	16.4 P<0.001	14.1 P<0.001	12.8 P<0.001	12.5 P<0.001	11.5 P<0.001	11.2 P<0.001
fecal P apparent digestibility (%)	24.1	40.8 P<0.001	26.9 NS	40.0 P<0.001	42.7 P<0.001	50.7 P<0.001	56.0 P<0.001	55.1 P<0.001	61.8 P<0.001	60.1 P<0.001
fecal P excretion (mg/g)	3.43	4.62 P<0.001	3.30 NS	2.73 P<0.001	2.58 P<0.001	2.24 P<0.001	2.03 P<0.001	2.02 P<0.001	1.76 P<0.001	1.80 P<0.001
Ca apparent digestibility (%)	58.7	49.2 P<0.001	53.5 P<0.05	62.9 NS	61.8 NS	68.1 P<0.001	73.6 P<0.001	65.7 P<0.05	73.4 P<0.001	68.4 P<0.001
fecal Ca excretion (mg/g)	3.38	6.31 P<0.001	3.81 P<0.05	3.12 NS	3.14 NS	2.63 P<0.001	2.13 P<0.001	2.71 P<0.001	2.04 P<0.001	2.50 P<0.001
Bone strength (N)	272.8	615.5 P<0.001	334.6 NS	476.1 P<0.001	384.1 NS	500.3 P<0.001	523.5 P<0.001	476.5 P<0.001	542.2 P<0.001	604.1 P<0.001
Bone ash (%)	62.17	63.70 P<0.05	62.38 NS	65.19 P<0.001	65.67 P<0.001	65.80 P<0.001	64.85 P<0.001	65.70 P<0.001	66.36 P<0.001	65.24 P<0.001

DCP = Dicalcium phosphate P values are determined in comparison to the negative control A

Discussion

- **Effects on performance**

All the groups receiving phytase supplements and the group supplemented with 16 g/kg of DCP (dicalcium phosphate) had higher daily weight gain (DWG) and lower feed conversion ratio (FCR) than those observed for the control group. The highest DWG and the best FCR were observed for the group with 3000 FYT/kg. The performance of the group supplemented with DCP was equivalent to those of the group receiving 1000 FYT/kg of phytase.

Supplementation with graduated amounts of RONOZYME® HiPhos phytase in piglets induced an increased performance in a dose dependent manner. Inclusion levels over 1000 FYT/kg were more efficient than the DCP supplementation.

- **Effects on Phosphorus digestion**

The mean P fecal concentration of the enzyme supplemented animals was very significantly lower than that measured in the animals receiving the control diet. There was a decrease of the P faecal concentration with the increasing consumption of RONOZYME® HiPhos phytase. The lowest fecal P concentration was observed in the animals receiving phytase at 8000 FYT/kg and represented half of that of the control group.

The P digestibility was dose dependent and highly significantly improved with the exception of the lowest RONOZYME® HiPhos inclusion level. The increases represented in comparison to the control group 12, 66, 77, 110, 132, 129, 156 and 149 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 FYT/kg RONOZYME® HiPhos phytase supplemented groups respectively. The digestibility of P in the DCP supplemented diet was also significantly higher than that of the control by 69 % and very similar to the enzyme supplementation at 500 FYT/kg.

The fecal excretion of P was significantly reduced by 4, 20, 25, 34, 41, 41, 49 and 48 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 FYT/kg phytase supplemented groups respectively. It was increased by 35 % with the DCP supplemented group.

The apparent absorbed P was 1.22, 1.82, 1.93, 2.31, 2.59, 2.48, 2.84 and 2.71 g/kg feed in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 FYT/kg RONOZYME® HiPhos supplemented groups, respectively and 3.18 g/kg feed in the DCP supplemented group. It was significantly increased in all the supplemented groups with the exception of the lowest inclusion level in comparison to the control diet (1.09 g/kg).

- **Effects on Calcium digestion**

The Ca digestibility was improved in the supplemented groups with the exception of the DCP group and the 250 FYT/kg phytase group. The variations were -9, 7, 5, 16, 26, 12, 25 and 17 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 FYT/kg phytase supplemented groups respectively and significant for the five highest concentrations. The Ca digestibility of the DCP supplemented diet was decreased by 16 % comparatively to the control group.

The fecal excretion of Ca was reduced by 8, 7, 22, 37, 20, 40, and 26 % with RONOZYME® HiPhos phytase in the 500, 1000, 1500, 2000, 3000, 4000 and 8000 FYT/kg supplemented groups respectively and significantly with the five highest concentrations. It was significantly increased by 13 % and 87 % with the 250 FYT/kg phytase and DCP groups respectively.

- **Effects on bone resistance and ash**

The phytase supplements strongly influenced the bone strength. For the RONOZYME® HiPhos inclusion level of 8000 FYT/kg the increase of the femur resistance was similar to that of DCP. It represented 121 % and 126 % respectively of that observed for the animals receiving the basal diet. The increases were significant in all supplemented groups except for the 250 FYT/kg and 1000 FYT/kg phytase inclusion levels. The low bone resistance values for the 250 and 1000 FYT inclusion levels do not follow the general trend. The variation in breaking force values within the treatments show the same general coefficient of variability as do the other treatments however the standard deviation for the 250 FYT treatment group is the lowest of all the treatments. Inspection for outliers and reanalysis of the data did not change the outcome. No other measured variables, such as body weight or bone ash reveal a causative relationship. The sensitivity of the force measurements to analytic technique and sample handling are also confounding factors. Consequently it is not possible to assign an exact reason for the recorded differences.

The ash content of the femur was increased in a significant way by the phytase except for the lowest dosage and by the DCP. Nevertheless, the addition of graduated amounts of RONOZYME® HiPhos phytase resulted in a non-linear increase of the ash content of the femur. The addition of 500, 1000, 1500, 3000, 4000 and 8000 FYT/Kg of feed were significantly different, $P<0.05$.

Conclusion
It can be concluded that the RONOZYME® HiPhos phytase improved the digestibility and the apparent absorption of P and Ca, reduced the fecal P excretion, restored phosphataemia, calcaemia and phosphatasaemia to physiological values, increased bone mineralization and resistance and improved the zootechnical performance in the weaned piglet fed on a diet containing P exclusively from vegetable origin. There was a dose dependent effect of RONOZYME® HiPhos phytase on the availability of the dietary P and was significantly different for all doses, $P<0.001$

4.7 Efficacy of IPA phytase (RONOZYME® HiPhos) in piglets (Spain 2009) Report 00001788

The purpose of the trial conducted at [redacted] (b) (4) was to determine the effects of graduated amounts of RONOZYME® HiPhos on the performance, mineral blood concentrations, and digestibility of phosphorus (P), calcium (Ca). The basal diet, without addition of mineral P, was based on corn, soybean meal. See Annex 4.

Experimental conditions
144 (Landrace X Pietrain) piglets having an initial body weight of 7.10 Kg were used. and remained on the experimental treatments for 6 weeks. The animals were divided into eight blocks of 6 pens.
The experimental treatments consisted of a basal, low-P, control diet which was supplemented with RONOZYME® HiPhos (M) at 500, 1000, 2000, or 4000 FYT/kg, and a positive control diet supplemented with 1 g of inorganic P/kg as dicalcium phosphate. Each dietary treatment was assigned to 8 replicate groups.

Body weight gain, feed intake and feed conversion ratio were measured for each pen at 14, 28 and 42 days of trial. At day 14, fresh faeces were sampled from each pen and the apparent digestibility of dry matter, ash, organic matter, Ca and P was measured using titanium dioxide as indicator. At the end of trial, a blood sample was also obtained from each piglet and analysed for alkaline phosphatase activity and inorganic P and Ca concentrations.

Composition and nutrient content of the diets

Main ingredients (%)	Basal diet	Basal diet with DCP
Corn	40.00	40.00
Soybean meal	9.84	9.84
Barley	24.68	24.68
Calculated nutrients		
Crude protein (%)	18.56	18.56
Ca (%)	0.75	0.75
Lysine (%)	1.40	1.40
Total P (%)	0.42	0.52
Non-phytic P (g/kg)	0.26	0.36
Energy (MJ ME /kg)	13.85	13.82

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	Low-P diet T1	T2	T3	T4	T5	Basal diet + DCP T6
Target	0	500	1000	2000	4000	0
Analyzed	<LOQ	669	1082	2128	4301	137*

*: native activity in feed

Results

Feed conversion ratio between 0 and 42 days, apparent fecal digestibility of P and Ca, P in feces

Treatment	FCR 0-42 days	Apparent fecal digestibility P %	Apparent fecal digestibility Ca %	P in feces (g/kg DM)
NC	1.68bc	37.3e	58.7d	18.7a
500 FYT/kg	1.64bc	60.5c	70.8bc	10.7b
1000 FYT/kg	1.55ab	68.2b	73.3bc	10.0bc
2000FYT/kg	1.54a	71.0b	75.0ab	8.9c
4000 FYT/kg	1.61ab	79.3a	81.7a	6.6d
PC	1.76c	47.9d	66.5cd	17.2a

NC: negative control, PC: positive control

a,b,c, d,e: Mean values without a common letter indicate significant differences ($p < 0.05$)

Discussion
Over the whole experimental period (0-42 days), the addition of RONOZYME® HiPhos at 2000 FYT/kg improved feed to gain ratio over the negative and positive control diets. The supplementation with RONOZYME® HiPhos (at all doses) significantly improved the apparent fecal digestibility for ash, P and Ca in a dose response manner, relative to the negative and positive control diets. RONOZYME® HiPhos also reduced the P concentration in feces (statistically significant at all levels of supplementation) in a dose response manner.

Conclusion
It can be concluded that the RONOZYME® HiPhos phytase improved the digestibility of P and Ca, and reduced the fecal P excretion in a dose response manner.

4.8 Effects of a novel phytase (RONOZYME® HiPhos) in corn-soybean meal diets fed to weanling pigs (USA 2009) Report 00003284

The purpose of the trial conducted at the Department of Animal Sciences of University of Illinois was to determine the effects of graduated amounts of RONOZYME® HiPhos on the apparent total tract digestibility (ATTD) of phosphorus in corn-soybean meal diets fed to weaned piglets. See Annex 5.

Experimental conditions
A total of 48 weaned piglets (initial BW: 13.5 ± 2.45 kg) were used in a randomized complete block design. Piglets were crossbreeds from Landrace X Large White x Duroc. Piglets were randomly allotted to the 6 dietary treatments in 8 blocks and placed in metabolism cages equipped with a feeder and a nipple drinker that allowed for total collection of feces.

The positive control diet was a corn-soybean meal diet that contained quantities of Ca and P sufficient to meet the requirement of Ca and P for piglets. This diet contained 0.66% P and 0.86% Ca. The negative control diet that was similar to the positive control diet with the exception that corn starch replaced dicalcium phosphate. This diet contained 0.36% P and 0.48% Ca.

The experimental treatments consisted of a basal, low-P, control diet which was supplemented with RONOZYME® HiPhos at 500, 1000, 2000, or 4000 FYT/kg, and a positive control diet. Feces were collected over a 5-day period after 5 days of adaptation to the diets.

Composition and nutrient content of the diets

Main ingredients (%)	Basal diet	Basal diet with DCP
Corn	60.60	60.60
Soybean meal (48% CP)	32.00	32.00
DCP	-	1.65
Nutrients (analysed)		
Crude protein (%)	17.96	18.33
Ca (%)	0.48	0.86
Lysine (%)	1.21	1.18
Total P (%)	0.36	0.66

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	Low-P diet	Basal diet + DCP	Low-P + 500	Low-P + 1000	Low-P + 2000	Low-P + 4000
Target	0 FYT/kg feed	0 FYT/kg feed	500	1000	2000	4000
Analyzed	80*	91*	440	958	1743	3974

*: native activity in feed

Results

Effects of phytase on apparent total tract digestibility of P

Treatment	P intake (g/day)	P in feces (%)	P output (g/day)	ATTD of P (%)	P absorption (g/day)
Negative control	2.28	2.30	1.35	40.46	0.93
500 FYT/kg	2.26	1.51	0.87	61.56	1.39
1000 FYT/kg	2.33	1.46	0.81	65.07	1.51
2000FYT/kg	2.25	1.22	0.71	68.74	1.54
4000 FYT/kg	2.14	1.10	0.68	68.04	1.46
Positive control	4.26	2.53	1.68	60.48	2.58
P-value	0.494	<0.01	<0.01	<0.01	<0.01

Discussion

The concentration of P excreted in the faeces was lower (P < 0.05) for pigs fed the negative control diet than for pigs fed the positive control diet. Likewise, pigs that were fed phytase containing diets had lower concentration of P in faeces than pigs fed the negative control diet. The daily P output was also lower (P < 0.01) for pigs fed the negative control diet than for pigs fed the positive control diet, and the inclusion of increasing levels of phytase to the negative control diet caused linear and quadratic reductions (P < 0.01) in P output. The ATTD of P was greater (P < 0.01) for pigs fed the positive control diet than for pigs fed the negative control diet, but the ATTD of P increased as RONOZYME® HiPhos phytase was added to the negative control diet (61.56, 65.07, 68.74, and 68.04% for pigs fed diets containing 500, 1,000, 2,000, or 4,000 FYT/kg, respectively). Phosphorus absorption was greater (P < 0.01) for pigs fed the positive control diet than for pigs fed the negative control diet but the addition of RONOZYME® HiPhos phytase to the negative control diet increased P absorption to 1.39, 1.51, 1.54, and 1.46 g/d.

Conclusion

Results from the present experiment show that RONOZYME® HiPhos phytase may be used in corn-soybean meal diets to improve the ATTD of P in weaned piglets. In addition, RONOZYME® HiPhos phytase supplementation may also result in a reduction of P excretion in the faeces of weaned piglets.

4.9 Effects of graduated amounts of RONOZYME® HiPhos on the fecal digestibility and excretion of phosphorus, calcium and zinc in growing pigs (France 2009) Report 2500672

The purpose of the trial conducted at Village-Neuf (France) was to determine the effects of graduated amounts of RONOZYME® HiPhos on phosphorus (P), calcium (Ca) and zinc (Zn) in the growing pig. The basal diet, without addition of mineral P, was based on soybean meal, Corn and barley. See Annex 6.

Experimental conditions

Thirty six Large White × Landrace pigs were used. The animals were housed in floor-pen cages in 9 groups of 4 animals each. The pigs were fed a basal diet without addition of mineral P (diet A) during an adaptive period of 16 days. After that period they were allocated into 9 equal groups and fed for 12 days the basal diet in mash form (group A) or the diet A supplemented with 12 g/kg of dicalcium phosphate (group B), with RONOZYME® HiPhos phytase at the levels of 500 FYT/kg feed (group C), 1000 FYT/kg feed (group D), 1500 FYT/kg feed (group E), 1750 FYT/kg feed (group F), 2000 FYT/kg feed (group G), 2500 FYT/kg feed (group H) and 3000 FYT/kg feed (group I).

The basal diet A was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the available P supply, the animals requirements according to Henry et al. (1989) and NRC (1998).

Composition and nutrient content of the diets

Main ingredients (%)	Basal diet (group A)	Basal diet with DCP** (group B)
Corn	53.0	53.0
Soybean meal	18.0	18.0
Barley	13.9	13.0
Nutrients		
Crude protein (%)	15.5	15.5
Lysine (%)	0.96	0.96
Ca- calculated (% in DM*)	0.66	0.86
Ca- analysed (% in DM)	0.70	0.80
P- calculated (% in DM)	0.41	0.65
P- analysed (% in DM)	0.42	0.62
Theoretical P (%)	0.12	1.86
Phytic P – calculated (%)	0.28	0.28
Estimated digestible energy (MJ/kg)	13.31	13.31

*DM: dry matter

**DCP: DiCalcium Phosphate

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Target	Basal diet Group A	Basal diet + DCP Group B	C	D	E	F	G	H	I
Target (nominal)	0 FYT/kg feed	0 FYT/kg feed	500	1000	1500	1750	2000	2500	3000
Analyzed	225*	219*	678	1179	1723	1985	2232	2798	3329
Actually added	-	-	453	954	1498	1760	2007	2573	3104
Average % of target	-	-	91	94	100	101	100	103	103

*: does not mean contamination but native activity in feed

Results

P, Ca and Zn digestibility and excretion

Treatments	Basal diet Group A	Basal diet + DCP Group B	C	D	E	F	G	H	I
Phytase dose (FYT/kg feed)	0	0	500	1000	1500	1750	2000	2500	3000
Fecal P concentration (% of DM)	1.59	1.67	1.19*	1.08*	0.97*	0.99*	0.94*	0.86*	0.83*
Apparent fecal digestibility of P (%)	29.3	47.2*	50.4*	57.8*	59.8*	61.3*	61.5*	66.6*	68.0*
Fecal P excretion (mg/g DM)	2.99	3.29*	2.11*	1.79*	1.71*	1.62*	1.63*	1.40*	1.34*
Apparent fecal Ca digestibility (%)	60.2	61.1	68.8*	73.0*	72.8*	75.7*	75.3*	86.7*	81.8*
Fecal Zn digestibility (%)	11.4	16.7	25.5*	21.4*	17.3**	25.0*	21.6*	17.5**	18.1**

* significant differences versus negative control at level P<0.001

** significant differences versus negative control at level P<0.05

Results

P, Ca and Zn digestibility and excretion

Treatments	Basal diet Group A	Basal diet + DCP Group B	C	D	E	F	G	H	I
Phytase dose (FYT/kg feed)	0	0	500	1000	1500	1750	2000	2500	3000
Fecal P concentration (% of DM)	1.59	1.67	1.19*	1.08*	0.97*	0.99*	0.94*	0.86*	0.83*
Apparent fecal digestibility of P (%)	29.3	47.2*	50.4*	57.8*	59.8*	61.3*	61.5*	66.6*	68.0*
Fecal P excretion (mg/g DM)	2.99	3.29*	2.11*	1.79*	1.71*	1.62*	1.63*	1.40*	1.34*
Apparent fecal Ca digestibility (%)	60.2	61.1	68.8*	73.0*	72.8*	75.7*	75.3*	86.7*	81.8*
Fecal Zn digestibility (%)	11.4	16.7	25.5*	21.4*	17.3**	25.0*	21.6*	17.5**	18.1**

* significant differences versus negative control at level $P<0.001$
** significant differences versus negative control at level $P<0.05$

Discussion

The animals grew normally during the observation period to reach a final mean body weight of 44.84 ± 3.37 kg. Their daily weight gain was 679 ± 5 g. No mortality was observed during the experiment. Two animals from group H, receiving the diet supplemented with RONOZYME® HiPhos phytase at 2500 FTY/kg feed presented diarrhea during the sampling period, so that no feces could be collected from them. No statistical analysis was performed for this group as the total amount of feces samples was only half (n = 6) of the other groups (n = 12).

- **Effects on Phosphorus digestion** (not all results are displayed in the table above)

The mean fecal P concentration of the enzyme supplemented animals was significantly lower (P<0.001) than that measured in the animals receiving the control diet. There was a decrease of the fecal P concentration with the increasing amount of RONOZYME® HiPhos phytase. The lowest fecal P concentration was observed in the animals receiving phytase at 3000 FYT/kg feed.

The P digestibility was dose dependent and significantly improved by 21.1, 28.5, 30.5, 32.0, 32.2, 37.3 and 38.7 percentage units in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 FYT/kg phytase supplemented groups, respectively (P<0.001). The digestibility of P in the DCP supplemented diet was also significantly higher than that of the control by 17.9 percentage units and very similar to the enzyme supplementation at 500 FYT/kg feed (P<0.001).

The fecal excretion of P was significantly reduced by 29.3, 40.1, 42.8, 45.8, 45.6, 53.0, and 55.2 % with phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 FYT/kg supplemented group, respectively (P<0.001). It was increased by 10.1 % with the DCP supplemented group (P<0.001).

The apparent absorbed P was 2.15, 2.45, 2.54, 2.56, 2.60, 2.80 and 2.84 g/kg feed with phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 FYT/kg feed supplemented groups respectively and 2.93 g/kg feed in the DCP supplemented group. It was significantly increased (P<0.001). in all the supplemented groups in comparison to the control diet (1.24 g/kg). With the exception of the phytase 500 FYT/kg inclusion level, all other supplemented groups were over the recommended requirements of 2.25 g of digestible P per kg feed.

- **Effects on Calcium digestion**

The fecal Ca concentration of the animals receiving the basal diet supplemented or not with DCP was systematically higher than that of the animals receiving the diets supplemented with the phytase. The observed differences were statistically significant for all the enzyme supplemented groups.

The Ca digestibility was significantly improved (P<0.001), by the phytase and by all the inclusion levels of phytase. The improvements were 8.6, 12.8, 12.6, 15.5, 15.1, 26.5 and 21.6 percentage units in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 FYT/kg phytase supplemented groups, respectively.

The Ca digestibility of the phytase supplemented diets was more or less dose dependent. The fecal excretion of Ca was significantly reduced (P<0.001) by 23.9, 34.6, 36.8, 42.3, 41.7, 67.9, and 57.0 % with RONOZYME® HiPhos phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 FYT/kg supplemented groups, respectively. It was increased by 12.1 % with the DCP supplemented group.

Conclusion

From the results of the present study, it can be concluded that the RONOZYME® HiPhos phytase improved the digestibility and the apparent absorption of P, Ca and Zn, and reduced the fecal P excretion in the pig fed on a diet containing P exclusively from vegetable origin. There was a dose dependent effect of RONOZYME® HiPhos phytase on the availability of the dietary Phosphorus.

4.10 Effects of a novel phytase (RONOZYME® HiPhos) in corn-soybean meal diets fed to growing pigs (USA 2009) Report 00003283

The purpose of the trial conducted at [redacted] (b) (4) was to determine the effects of graduated amounts of RONOZYME® HiPhos on the digestibility of P in corn soybean meal diets fed to growing pigs. See Annex 7.

Experimental conditions

24 growing barrows were used in a 2 period crossover design. In period 1, barrows had an initial body weight of 36.2 ± 4.0 kg, while in period 2, they had an initial body weight of 47.3 ± 5.3 kg. The animals were placed in metabolism cages and randomly allotted to the 6 dietary treatments. The positive control diet was a corn-soybean meal diet that contained quantities of Ca and P sufficient to meet the requirement of Ca and P for growing pigs (NRC, 1998) with a total concentration of P at 0.56%. The negative control diet was similar to the positive control but contained only 0.33% P.

Composition and nutrient content of the diets

Main ingredients (%)	Basal diet	Basal diet with DCP
Corn	65.80	65.80
Soybean meal (48%)	29.50	29.50
Corn starch	1.05	-
DCP	-	1.05
Nutrients (analysed)		
Crude protein (%)	17.96	18.33
Lysine (%)	1.06	1.08
Ca (%)	0.58	0.79
P (%)	0.33	0.56

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	Basal diet N.C.	Basal diet + DCP P.C.	Basal diet + 500	Basal diet + 1000	Basal diet + 2000	Basal diet + 4000
Target (nominal)	0 FYT/kg feed	0 FYT/kg feed	500	1000	2000	4000
Analyzed	41*	39*	373	984	1773	3681

*: does not mean contamination but native activity in feed

Results

Apparent total tract digestibility (ATTD) of P and Ca

Treatments	NC	PC	500	1000	2000	4000	NC versus HiPhos	*P-value* L	*P-value* Q
P in feces %	2.44	2.59	1.82	1.52	1.31	1.09	<0.01	<0.01	<0.01
P output g/day	2.87	3.41	2.12	1.76	1.54	1.36	<0.01	<0.01	<0.01
P absorption g/day	1.94	5.10	3.00	3.33	3.47	3.66	<0.01	<0.01	<0.01
ATTD of P %	39.83	59.36	58.10	65.43	69.09	72.76	<0.01	<0.01	<0.01
Ca in feces %	2.33	2.45	1.40	1.29	1.22	0.91	<0.01	<0.01	<0.01
Ca output g/day	2.74	3.20	1.62	1.50	1.46	1.13	<0.01	<0.01	<0.01
Ca absorption g/d	5.72	8.82	7.26	7.03	6.80	6.84	<0.01	0.376	0.122
ATTD of Ca %	67.30	72.90	81.44	82.62	82.36	85.84	<0.01	<0.01	<0.01

L: linear contrast, Q: quadratic contrast

Discussion

Throughout the experiment, pigs remained healthy and readily consumed their diets. No differences in feed intake were observed among treatments. Phosphorus intake was lower for pigs fed the negative control than for pigs fed the positive control diet.

The P concentration in feces was lower for pigs fed RONOZYME® HiPhos phytase containing diets than for pigs fed the negative control diet, and there was a linear and quadratic reduction ($P < 0.01$) in fecal P concentration as RONOZYME® HiPhos phytase was included in the diets.

The ATTD of P was lower ($P < 0.01$) for pigs fed the negative control diet than for pigs fed the positive control diet (39.83 vs. 59.36%). The addition of increasing levels of RONOZYME® HiPhos phytase to the negative control diet increased the ATTD of P (linearly and quadratically, $P < 0.01$). Phosphorus absorption was greater for pigs fed the positive control diet than for pigs fed the negative control diet (5.10 vs. 1.94 g/d), but absorption of P increased (linearly and quadratically, $P < 0.01$) as phytase was added to the negative control diet.
Calcium intake was greater for pigs fed the positive control diet than for pigs fed the negative control diet (12.02 vs. 8.47 g/d). Calcium in the feces and total Ca output were lower (linear

and quadratic $P < 0.01$) for pigs fed phytase containing diets than for pigs fed the negative control diet. Addition of phytase to the negative control diet increased (linearly and quadratically, $P < 0.01$) the ATTD of Ca.

Conclusions

Results from the present experiment show that RONOZYME® HiPhos phytase is an effective phytase that may be used in Corn-soybean meal diets to improve the ATTD of P and Ca. The RONOZYME® HiPhos phytase will also result in a reduction in P excretion in the manure from pigs fed diets containing this enzyme.

4.11 Efficacy of IPA phytase (RONOZYME® HiPhos) in growing pigs (Spain 2009) Report 00001789

The purpose of the trial conducted at (b) (4) was to access the effect of RONOZYME® HiPhos on digestibility parameters in growing pigs. See Annex 8.

Experimental conditions

A total of 48 animals (Landrace x Pietrain) were involved. The pigs started on the trial at 51.6 kg body weight and remained on the experimental treatments for 3 weeks. They were divided into eight blocks of 6 animals, taking into account sex and initial body weight. The experimental treatments consisted of a basal, low-P, control diet which was supplemented with RONOZYME® HiPhos at 500, 1000, 2000, or 4000 FYT/kg, respectively, and a positive control diet supplemented with 1 g of inorganic P/kg as dicalcium phosphate. Each dietary treatment was assigned to 8 animals. At the end of the study, fresh feces were sampled for each pig and the apparent digestibility of dry matter, ash, organic matter, Ca and P was measured. A blood sample was also obtained from each pig and analyzed for alkaline phosphatase activity and inorganic P and Ca concentrations.

Composition and nutrient of the diets

Main ingredients (%)	Low P Basal diet	PC diet
Corn	35.00	35.00
Soybean meal (48% CP)	18.95	18.95
Barley	41.35	41.35
DCP	0	0.55
Nutrients (calculated)		
Crude protein (%)	16.11	16.11
Lysine (%)	1.00	1.00
Ca (%)	0.60	0.60
Total P (%)	0.349	0.446
Non phytic P (%)	0.133	0.230

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	Basal diet T1	Basal diet + DCP T6	T2	T3	T4	T5
Target (nominal)	0 FYT/kg	0 FYT/kg feed	500	1000	2000	4000
Analyzed	150*	114*	671	1529	2659	4448

* does not mean contamination but native activity in feed

Results

P in blood, P in feces, apparent fecal digestibility of P and Ca

Treatment	P in blood (mg/dl)	P in feces (g/kg Dry matter)	Apparent P digestibility (%)	Apparent Ca digestibility (%)
Negative Control	6.69a	13.78c	29.6c	55.3c
500 FYT/kg	7.12ab	10.98b	35.6bc	62.0bc
1000 FYT/kg	7.63bc	10.49b	42.5b	70.6ab
2000 FYT/kg	8.04c	8.11a	56.1a	75.9a
4000 FYT/kg	7.75c	7.69a	62.4a	61.3bc
Positive control	7.66c	14.82c	37.5bc	58.0c

a,b,c Mean values without a common letter indicate significant differences ($p < 0.05$)

Discussion

The supplementation of the basal diets with RONOZYME® HiPhos phytase significantly increased P concentration in blood at 1000, 2000 and 4000 FYT/kg diet, respectively. RONOZYME® HiPhos phytase at 500, 1000, 2000 and 4000 FYT/kg diet significantly improved the apparent fecal digestibility of P from 29.6% (negative control) to 35.6, 42.5 ($P<0.05$), 56.1 ($P<0.05$) and 62.4% ($P<0.05$), respectively. The apparent digestibility of Ca was improved as well and the effects were statistically significant for phytase supplementation at 1000 and 2000 FYT/kg diet, respectively. At all inclusion levels RONOZYME® HiPhos phytase reduced P concentration in feces in a statistically significant manner.

Conclusions

Results from the present experiment show that RONOZYME® HiPhos phytase is an effective phytase that may be used in corn meal diets to improve the digestibility of P and Ca and reduce the load of P in feces.

4.12 Dose response study with a new phytase (IPA Mash Phytase, RONOZYME® HiPhos) in lactating sows (Germany 2009) Report 00003282

The objective of the experiment carried out at the [redacted] (b) (4) of the [redacted] (b) (4) (b) (4) was to study the effects of RONOZYME® HiPhos phytase on the digestibility of P in lactating sows. See Annex 9.

Experimental conditions
A total of 28 sows (German Landrace) were used in the experiment. One week before partum the animals were moved into individual cages and were randomly assigned to one of the four treatments. The experimental diets were based on corn (660 g/kg) and soybean meal (270 g/kg) without a mineral P supplementation in order to achieve a sufficiently low basal P level.

In the pre-treatment period (7 days before and 2 to 11 d after parturition) all sows were fed an in-house lactation diet. The sows were then adjusted to the experimental diet by increasing the level of corn and soybean meal in the diet over a period of 3 to 6 days. A 5-day period of faeces collection followed a 7-day period of prefeeding the experimental diets.

Nutrient composition and RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Diet	Phytase activity intended (FYT/kg)	Analysed (FYT/kg)	Ash (g/kg DM)	Crude protein (g/kg DM)	Crude fat (g/kg DM)	P (g/kg DM)	Ca (g/kg DM)
A	0	<50	65	201	85	4.3	11.3
B	500	589	61	206	83	4.2	9.7
C	1000	1027	61	200	87	4.2	10.6
D	2000	2125	61	196	85	4.1	9.4

Results

Digestibility of dry matter, P and Ca

Treatment	Dry Matter digestibility (%)	P digestibility (%)	Ca digestibility (%)
Negative Control	86.9	20.5a	33.2
500 FYT/kg	86.9	23.3ab	37.5
1000 FYT/kg	87.4	32.5bc*	37.6
2000 FYT/kg	86.8	34.1c*	33.6
P	0.38	0.02	0.23

* Means are significantly different from the unsupplemented treatment A according to Dunnett test.

a,b,c Values without a common superscript are significantly different according to t-test ($P \leq 0.05$).

Discussion
Digestibility of P was significantly improved from 21 % to 34 % with increasing RONOZYME® HiPhos supplementation ($P \leq 0.05$). The mean digestibility of Ca was similar in the diets, at an average of 35.5%.

Conclusions
In conclusion, RONOZYME® HiPhos has beneficial effects on phosphorus digestibility in lactating sows.

4.13 Efficacy study with IPA Mash Phytase (RONOZYME® HiPhos) in gestating sows (Germany 2009) Report 00003285

The purpose of the trial run at the [redacted] (b) (4) (Germany) was to assess the efficacy of graduated levels of RONOZYME® HiPhos on apparent digestibility of crude ash, calcium and phosphorus in gestating multiparous sows. See Annex 10.

Experimental conditions
24 multiparous sows (EUROC line) in the body weight range of 190 to 210 kg and the same reproduction stage (29th to 33rd day of pregnancy) were used in the experiment.

Four treatments were imposed to the gestating sows from the 45th to 58th day of pregnancy after a 12-day pre-treatment period (31st to 44th day of pregnancy). Six sows per treatment were used. The first treatment (A) was the negative control diet, a low-phosphorus based diet without RONOZYME® HiPhos phytase. Treatments B, C and D were identical to the negative control but supplemented with RONOZYME® HiPhos Phytase at dose levels of 500, 1000 and 2000 FYT/kg of diet, respectively.

Composition and nutrient content of the diets

Ingredients (%)	Treatment period
Corn	34.04
Optigrain	23.00
Soybean meal	12.50
Wheat	12.00
Triticale	11.00

Nutrients (calculated) (g/kg)	
Metabolizable energy (MJ/kg)	12.86
Crude protein	144.42
Lysine	6.40
Methionine	2.00
Cystine	2.20
Ca	7.00
P	3.40

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	Basal diet A	B	C	D
Target (nominal)	0 FYT/kg feed	500	1000	2000
Analyzed	211*	786	1262	2440

* does not mean contamination but native activity in feed

Results

Fecal concentrations of calcium and phosphorus, apparent digestibility of crude ash, calcium and phosphorus from days 54 to 58 of gestation

Treatment	Fecal concentration of Ca (g/kg DM)	Fecal concentration of P (g/kg DM)	Crude ash apparent digestibility (%)	Ca apparent digestibility (%)	P apparent digestibility (%)
Negative Control	37.1a	18.6a	29.44a	30.57a	26.51a
500 FYT/kg	34.3ab	17.8a	33.76b	35.93b	33.52ab
1000 FYT/kg	32.4ab	15.4ab	35.77bc	39.08bc	38.59b
2000 FYT/kg	28.8b	13.9b	39.29c	41.14c	39.87b
Anova	0.016	0.007	<0.001	<0.001	<0.001

ab Means with different superscripts within the same line differed significantly

Discussion
The apparent crude ash digestibility of sows fed without RONOZYME® HiPhos Phytase supplementation was 29.4%. With supplementation of RONOZYME® HiPhos at the dose levels of 500, 1000 and 2000 FYT/kg of feed the apparent digestibility increased significantly. The highest response was recorded for the addition of 2000 FYT/kg of feed.

The apparent calcium digestibility of sows fed with RONOZYME® HiPhos Phytase was significantly improved with increasing dose levels when compared to sows fed without RONOZYME® HiPhos by 17.5 (500 FYT/kg), 27.8 (1000 FYT/kg) and 34.6% (2000 FYT/kg) when compared to sows fed without RONOZYME® HiPhos. The highest response was shown for sows fed with 2000 FYT/kg feed.

The apparent digestibility of phosphorus in sows fed without RONOZYME® HiPhos amounted to 26.5%. For sows fed with RONOZYME® HiPhos Phytase the respective means were significantly improved ($P<0.001$) with inclusion of 1000 and 2000 FYT/kg of feed by 45.6 and 50.4%, respectively. The addition of RONOZYME® HiPhos Phytase at 500 FYT/kg feed was less effective and due to the high standard deviations the differences compared to sows fed without RONOZYME® HiPhos Phytase were not significant at this concentration.

Conclusions
It can be concluded that RONOZYME® HiPhos phytase is effective in improving the apparent digestibility for crude ash, calcium and phosphorus, when using diets deficient in phosphorus supply.

4.14 Evaluation of the effect of IPA Mash phytase (RONOZYME® HiPhos) on the nutrient digestibility in gestating sows (Slovak Republic 2009) Report 00003286

The purpose of the study run at the [redacted] (b) (4) was to evaluate the effects of RONOZYME® HiPhos on total tract digestibility of organic matter (OM), nitrogen (N), phosphorus (P) and calcium (Ca). See Annex 11.

Experimental conditions
24 gestating sows (Large White x Landrace) with initial mean body weight 239.8 kg at the start of the experiment, in the range from 3rd to 5th parity and in the last third of pregnancy among 98th and 113th day of pregnancy were used for the experiment.

There were four dietary experimental treatments to which the sows were allocated according to body weight and parity. Six sows per treatment were used. The first treatment (F0) was the negative control diet, low-phosphorus basal diet without RONOZYME® HiPhos Phytase. Treatments F1, F2, F3 were identical to the negative control but supplemented with RONOZYME® HiPhos Phytase at dose levels 500, 1000 and 2000 FYT/kg in the diets, respectively.

On day 95 of pregnancy the sows were housed in 24 individual pens. From 95 to 98 day of pregnancy there was an adaptation period in which the sows were fed with a commercial diet for pregnant sows. After the10 day preliminary period (from 98 to 108 day of pregnancy) in which the animals were fed with the experimental diets, followed the 5 days collection period (from 108 to 113 days of pregnancy) during which the feces were collected. The experiment was finished after 15 days.

Composition and nutrient content of the diets

Ingredients (%)	Basal diet
Corn	47.05
Barley	20.00
Soybean meal (46% CP)	11.80
Rapeseed meal (33% CP)	8.00
Calculated nutrients (g/kg)	
Metabolizable energy (MJ/kg)	12.7
Crude protein	171.7
Lysine	9.6
Methionine + Cystine	6.3
P total	6.0
Ca	8.0
P digestible	2.4

RONOZYME® HiPhos assay in FYT/kg feed, after mixing

Treatment	Basal diet F0	F1	F2	F3
Target (nominal)	0 FYT/kg feed	500	1000	2000
Analyzed	124*	531	898	1890

* does not mean contamination but native activity in feed

Results

Digestibility of calcium and phosphorus

Treatment	DM digestibility (%)	P in feces (g/kg DM)	Ca digestibility (%)	P digestibility (%)
F0 - Negative Control	83.1a	27.9a	35.5a	26.7a
F1 - 500 FYT/kg	83.7ab	26.1b	41.6b	33.6b
F2 - 1000 FYT/kg	84.7c	26.1b	47.8c	39.0c
F3 - 2000 FYT/kg	84.2bc	25.7b	44.1b	37.2bc

abc Means within a column followed by the different superscript are significantly different ($P<0.05$)

Discussion

The digestibility of both P and Ca significantly increased as a result of RONOZYME® HiPhos supplementation. The highest effect was observed in diet F2. In comparison to the control group, the total tract digestibility of P increased by 25.81, 46.27 and 39.27 % in diets F1, F2 and F3, respectively. The digestibility of Ca was significantly different from the control diet without added phytase ($P<0.05$), increasing in diets supplemented with 500, 1000 and 2000 FYT/kg by 17.06, 34.52 and 24.23 %, respectively.

As a result of improved nutrient digestibility due to the RONOZYME® HiPhos supplementation, concentration of nutrients in feces was reduced. In comparison to the control diet F0, the reduction of P and Ca concentration in the feces ranged from 6.51% (F1), 6.55% (F2) and 7.9% (F3) in P and 8.3% (F1), 8.6% (F2) and 7.0% (F3) in Ca.

Conclusions

It can be concluded that RONOZYME® HiPhos is efficient in increasing plant phosphorus and calcium digestibility and in reducing the concentration of P in feces.

4.15 Efficacy Study with IPA Mash Phytase (RONOZYME® HiPhos) in gestating sows, Report 00015939

The purpose of the study run at the [redacted] (b) (4) was to evaluate the effects of RONOZYME® HiPhos on nutrient digestibility and performance at 500 FYT/kg feed.

Experimental conditions

45 gestating sows (Large White x Landrace) were individually kept but within five rooms, each having nine sows at the same stage of farrowing and lactation. The sows were allocated to one of the three experimental diets (15 sows per diet). During the experiment the sows were offered lactation obtained from a commercial source) in crumbles form, which were identical except for the amount of total phosphorus.

The three experimental groups were as follows:

PC: Positive control (regular lactation diet containing 0.77% total P)

NC: Negative control diet (containing 0.45 % total P)

NC+P: Negative control diet plus 500 FYT/kg diet (Ronozyme® HiPhos)

Diets were fortified to meet vitamin and mineral requirements.

Composition and nutrient content of the diets

Ingredients (%)	Lactation diets
Wheat	51.7 - 53.5
Soybean meal	13.9 – 13.3
Canola meal	6.5
Analysed nutrients	
Gross energy (Kcal/kg)	4038 - 4051
Crude protein (%)	23.2 – 23.3
Phosphorus (%)	0.590 – 0.860 (PC)
Calculated nutrients (%)	
Lysine	1.070
Methionine	0.360
P total	0.450 – 0.770 (PC)
P digestible	0.202 – 0.517 (PC)

RONOZYME® HiPhos recoveries in FYT/kg feed

Treatment	NC	NC + HiPhos	PC
Target (nominal)	0	500	0
Analyzed	127*	764	<LOQ

* does not mean contamination but native activity in feed

LOQ: limit of quantification

Results

Digestibility of phosphorus and dry matter,

Treatment	DM digestibility d15 (%)	Feed consumed (total) (kg)	P digestibility d15 (%)
Negative Control	80.5b	114.5	33.9b
500 FYT/kg	81.8a	102.4	46.0a
Positive control	78.8c	109.4	29.7b

abc Means within a column followed by the different superscript are significantly different ($P<0.05$)
d15: 15 days post farrowing

Comments

The supplementation of RONOZYME® HiPhos to low P diet increased the Apparent Total Tract Digestibility (ATTD) of phosphorus on day 15 post farrowing. Specifically, supplementation of RONOZYME® HiPhos to the Negative Control diet increased the ATTD of phosphorus by 17% on day 8 and by 35% on day 15 post farrowing as compared with the NC diet.
Feeding the three test diets did not affect ($P>0.05$) total feed consumption, milk production and BW changes of sows during the lactation and liter weight gain of piglets. There was a trend ($P = 0.067$) of 10% reduced daily feed consumption in sows fed with RONOZYME® HiPhos supplemented diets, while keeping all other parameters similar.

Conclusions

It can be concluded that RONOZYME® HiPhos is efficient as to the increase of plant phosphorus digestibility at 500 FYT/kg feed. See Annex 12

4.16 Phytase Usage and its impact on bone properties

Phytases increase the availability of phosphorous in the diet and since phosphorus is a major component of bone, a change in available phosphorous is expected to have an impact on bone composition. Common measurements utilized to define bone quality are bone ash and bone strength. Bone strength is expressed as the force necessary to break a specific bone of the animal; the tibia in poultry and the femur or metatarsal of swine. (Crenshaw et al 1981) Ref. 9 Bone strength values are variable, dependent upon species, age of the animal, diet and analytic technique. (Aerssens et al 1998) Ref..10. Bone ash is determined by ashing the bone sample in a muffle oven after preparing and drying the sample for > 24 hours at 100° C.

RONOZYME® HiPhos is classified as a histidine acetate phytase. A recent article presenting a new phytase notes that the commercial phytases, RONOZYME® P. RONOZYME® NP, Phyzyme and Optiphos also fall within this enzyme classification. (Ariza et al 2013) Ref..11.

Brana et al (2006) Ref. 12 compared the bone ash and mineral digestibility in piglets and growing swine when the feed was fortified with the phytase enzymes Phyzyme, a 6-phytase (as is HiPhos) or Naturphos, a 3-Phytase. Both enzymes were effective in maintaining fibular ash levels similar to the positive control with the effect being more pronounced in the growers. Phosphorous digestibility values for the Phyzyme treatment group were comparable to or

better than the optimum mineral ratio control. The Phyzyme treatment groups experienced a positive effect in both apparent fecal digestibility of phosphorous and bone ash.
Jones et al (2010) Ref. 13 evaluated the bone ash of piglets fed diets containing different commercial phytases; RONOZYME® P, Phyzyme XP and OptiPhos 2000. For each enzyme, in a phosphorous deficient diet, increasing enzyme concentration was correlated to increasing percent bone ash in a statistically significant manner. The enzyme supplemented phosphorus deficient diets all achieved percent bone ash values equivalent to the 0.14% phosphorous control.

A DSM internal comparison study of bone properties revealed that RONOZYME® HiPhos supplemented diets were similar to diets supplemented with other commercial histidine acetate phytases with respect to bone breaking force and percent bone ash. See Annex 13.

Bone strength and mineralization in the growing pigs fed a diet without or with DCP (dicalcium phosphate) or different phytases.
(means per group ± standard deviation, ***% of variation from group Control (-)*****)**

Treatment groups (n = 8 animals)	Control (-)	DCP 12 g/kg	RONOZYME® HiPhos (M)		Phyzyme®XP 4000 TPT		OptiPhos® 2000 PF	
	A	B	C	D	E(1)	F	G	H
Planned phytase addition (U/kg)	0	0	1000	1500	500	750	500	750
Breaking force for the external metacarpal bone (N)	83[a] ± 31	246[d] ± 44 *+197*	148[bc] ± 23 *+79*	182[c] ± 46 *+119*	149[bc] ± 32 *+80*	177[c] ± 24 *+113*	129[b] ± 29 *+55*	138[b] ± 35 *+66*
External metacarpal bone mineralization (% of ash in DM)	57.80[a] ± 2.07	62.53[b] ± 0.85 *+ 8.2*	58.66[a] ± 1.80 *+ 1.5*	61.27[b] ± 0.85 *+ 6.0*	58.68[a] ± 0.55 *+ 1.5*	58.45[a] ± 1.62 *+ 1.1*	57.43[a] ± 3.32 *- 0.6*	59.12[a] ± 1.53 *+ 2.3*
Breaking force for the external metatarsal bone (N)	77[a] ± 32	223[d] ± 53 *+191*	140[bc] ± 38 *+82*	155[c] ± 51 *+102*	122[bc] ± 34 *+59*	129[bc] ± 26 *+68*	96[ab] ± 34 *+25*	125[bc] ± 30 *+62*
External metatarsal bone mineralization (% of ash in DM)	56.96[a] ± 3.19	62.47[d] ± 0.82 *+ 9.7*	57.68[ab] ± 2.38 *+ 1.3*	60.53[cd] ± 1.47 *+ 6.3*	59.26[bc] ± 1.45 *+ 4.0*	59.61[bc] ± 1.56 *+ 4.7*	57.99[ab] ± 2.29 *+ 1.8*	59.70[bc] ± 1.63 *+ 4.8*
Mean breaking force for both bones (N)	80[a] ± 31	235[e] ± 48 *+195*	144[cd] ± 31 *+81*	169[d] ± 49 *+112*	136b[c] ± 35 *+70*	152[cd] ± 35 *+90*	112[b] ± 35 *+40*	132[bc] ± 32 *+66*

(1) n = 7 animals
a, b, c, d, e Means within the same row without a common letter are significantly different (*P*<0.05)

Conclusion

This internal study shows that swine feed low in phosphorous supplemented with RONOZYME® HiPhos achieved bone breaking force and percent bone ash values greater than the negative control and comparable to the phosphate fortified feed and the other commercial phytase supplemented feeds. These results are similar to published results for other histidine acetate phytases, see Ref. 12 & 13.

4.17 Summary

The efficacy studies summarized in the preceding sections support the purpose of RONOZYME® HiPhos, to increase the availability of phytate bound phosphate in swine feed and does so with no adverse effects as noted in the target animal studies.

Published studies of other histidine acetate phytases, including those undertaken by DSM, support the efficacy of phytase supplemented swine diets based upon the more traditional bone property parameters. Since RONOZYME® HiPhos is a histidine acetate phytase it would be anticipated that this enzyme would provide similar results. The recent internal study presented in section 4.16 supports this presumption where swine feed low in phosphorous supplemented with RONOZYME® HiPhos achieved bone breaking force and percent bone ash values greater than the negative control and comparable to the phosphate fortified feed and the other commercial phytase supplemented feeds.

When viewed in total, the safety and efficacy studies show that RONOZYME® HiPhos safely provides the intended effect of increased phosphorus release from phytic acid in swine feed composed of a broad range of plant based ingredients. This broad suitability coupled with demonstrated safety means RONOZYME® HiPhos can be used for swine in all stages of development in all regions of the United States.

5 Human and Environmental Safety

The human and environmental safety of the enzyme was addressed in the GRAS Notification of RONOZYME® HiPhos, a 6-phytase preparation produced by an Aspergillus oryzae strain expressing a synthetic gene coding for a 6-phytase from Citrobacter braakii for use in poultry nutrition, AGRN #14.

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6 Annexes

1. [redacted] et *al.* (2009). Report No. 00000962: Tolerance study with IPA Mash Phytase [RONOZYME® HiPhos (M)] in weaned piglets. 2008

2. [redacted] et *al.* (2009). Report No. 00003288: Tolerance study with IPA Mash Phytase in gestating and lactating sows. 2009

3. Guggenbull et *al.* (2009). Report No. 2500761: Evaluation of the effects of graded amounts of a microbial phytase (RONOZYME® HiPhos) in the weaned piglet. 2009

4. [redacted] and Broz, J. (2009). Report No. 00001788: Efficacy of IPA phytase (RONOZYME® HiPhos) in piglets. 2009

5. [redacted] et *al.* (2009). Report No. 00003284: Effects of a novel phytase (RONOZYME® HiPhos) in corn-soybean meal diets fed to weanling pigs. 2009

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TAB

1

Annex 1

Tolerance study with IPA Mash phytase in weaned piglets

REPORT No. 00000962

REPORT No. 00000962
Regulatory Document

DSM

Document Date: 18 August, 2009

Author(s): [redacted] (b) (4)

[1] [redacted] (b) (4)

[2] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title: **Tolerance study with IPA Mash phytase in weaned piglets**

Project No. 6106

Summary

A 6-week study involving 48 weaned piglets (Large White x Landrace) was conducted in order to evaluate their tolerance to IPA Mash phytase when used at the maximum recommended dose and a 10 times overdose. Prestarter and starter diets based on wheat, barley and soybean meal as the main feed ingredients, formulated to meet NRC nutrient recommendations except for total and digestible P, were used. The experiment involved 3 treatment groups as follows: A – control, non-treated group; B – treated group receiving IPA Mash phytase at 4,000 U/kg diet; C – treated group receiving IPA Mash phytase at 40,000 U/kg diet (overdose group). Each treatment was assigned to 16 piglets (8 times castrated males and 8 times females). Live weight, live weight gain, feed intake, feed conversion ratio (feed/gain) and mortality were monitored as performance parameters during the experimental period. At the termination of study haematological and biochemical examinations were performed using blood samples from all 48 animals. Dietary administration of IPA Mash phytase resulted in beneficial effects of the post-weaning performance. The final body weight of piglets (day 42) receiving the phytase at 4,000 and 40,000 U/kg diet was significantly increased by more than 12% when compared to the negative control. Due to this markedly increased growth rate in both phytase supplemented groups the overall feed conversion ratio was significantly improved from 2.75 (control) to 2.18 and 2.20, respectively. Mild diarrhoea was observed in some animals of all 3 groups during the first weak of trial, but no mortality occurred during this study. Furthermore, no pathological changes were observed in piglets during the post-mortem necropsy. Haematological and biochemical examination did not reveal any obvious changes due to dietary administration of IPA Mash phytase. In conclusion, this particular study confirmed a full tolerance of IPA Mash phytase in weaned piglets when used at the 10 times overdose.

This report consists of Pages I – II and 1 - 46

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Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	19.08.2009

Nomenclature and Structural Formula

IPA Mash phytase (M), enzyme product containing bacterial 6-phytase ([redacted]), produced by a [redacted] fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28656 was used in this study, manufactured by Novozymes A/S, [redacted].

(b) (4) [redacted]

Study Code: 253/2008

Tolerance Study with IPA Mash Phytase in Weaned Piglets

Final Report

Prepared by [redacted] (b) (4)

August 2009

Contents

List of Abbreviations and Terms Used

ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMS	Amylase
AST	Aspartate aminotransferase
BA	Basofiles
bw	Body weight
CPK	Creatine phosphokinase
Cre	Creatinine
CRF	Case Report Form
CV	Coefficient of Variation
D	Day of the Study
EC	European Commission
EFSA	European Food Safety Authority
EMEA	The European Agency for the Evaluation of Medicinal Products
EO	Eosinofiles
g	Gram
GLP	Good Laboratory Practice
GMP	Good Manufacture Practice
HCT	Hematocrit
HGB	Hemoglobin
kg	Kilogram
LY	Lymphocytes
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean cell volume
mL	Millilitre
μg	Microgram
MO	Monocytes
NE	Neutrophils
No.	Number
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development
PLT	Platelets
QA	Quality Assurance
RBC	Red blood cells count
SD	Standard deviation
SG	Segments
SOP	Standard Operating Procedure
T	Temperature
Tbil	Total bilirubin
TI	Test Item
U	Unit
Ur	Urea
VICH	Veterinary International Cooperation on Harmonisation
WBC	White blood cell count

1. Summary

The aim of the Study was to establish that there are no unfavourable effects of the TI to the intended species in maximum recommended and multiplicated (ten times) recommended doses. The TI was admixed to the basal diet and was fed in standard way *ad libitum*. Maximum recommended dose of TI was 4,000 U/ kg of diet, corresponding to 67.2 ppm of the formulated product. Tolerance Study in weaned piglets was performed for 42 consecutive days.

The Study was performed in a total 48 early weaned piglets (crossbreds Large white x Landrace), both sexes, origin [redacted] (b) (4). The animals were housed in pairs in litterless boxes at BIOPHARM facilities conform to welfare regulations. Shortly after delivery the weight of piglets was determined and animals were randomly allocated to three groups A, B, C (16 piglets each, 8 castrated males and 8 females).

Pre-starter and starter diets based on wheat, barley and soybean meal as the main feed ingredients, formulated to meet NRC nutrient recommendations except of total and non-phytate P were used. The basal diet was prepared according to formula approved by the Sponsor prior the start of the Study. Diet was provided to animals in mash form and fed *ad libitum,* namely pre-starter was fed from D 0 till D 13, starter than from D 14 till D 42 of the Study.

Experimental treatments:

A. Control, non-treated group	– basal diet, no enzyme added
B. Treated group, recommended dose of the TI	– basal diet + TI at 4,000 U/kg diet
C. Treated group, ten times multiplicated dose of the TI	– basal diet + TI at 40,000 U/kg diet

Drinking water was available *ad libitum*.

Animals of the Group A served as a negative control and were fed with the basal diet, without enzyme *ad libitum* 24 hour a day for the duration of the Study (42 consecutive days, from D 0 till D 42).

Animals of the Group B were treated at the recommended dosage of 4,000 U of the TI (i.e. 67.2 ppm of IPA Mash Phytase) admixed into 1 kg of feed, administered *ad libitum* 24 hour a day for the duration of the Study (the same way as piglets of group A).

Animals of the Group C were treated at the ten times multiplicated dose of 40,000 U of the TI (i.e. 672 ppm of IPA Mash Phytase) admixed into 1 kg of feed, administered *ad libitum* 24 hour a day for the duration of the Study (the same way as piglets of group A).

The TI dose really obtained was calculated from the total medicated feed consumption during administration period. Consumption of feed was measured daily and calculated in one-week period, i.e. on D 7, 14, 21, 28, 35 and 42. Feed conversion (feed/gain) was calculated for the overall trial period.

Weighing of piglets was performed at the start of administration on D 0, further in 2-week period on D 14, 28 and 42.

The animals were stunned and killed by rapid exsanguination at the BIOPHARM facility. Cadavers were post-mortem examined by a veterinarian and biological samples were collected.

Heamatological and biochemical examinations were performed in all animals, i.e. in 16 piglets per group (total number of examined samples was 48). A standard haematological analysis (amount of red blood cell (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), amount of blood platelets (PLT), amount of white blood cell (WBC)) total and differential and analysis of following serum variables such as chloride, calcium, phosphorus, total protein, albumin, glucose, AMS, urea, creatinine, ALP, ALT, AST, CPK and total bilirubin were performed.

The gross pathological examination was focused mainly on the examination of the gastro-intestinal tract and lymph nodes, liver, kidney, lung, heart and spleen.

Mild diarrhoea was diagnosed in some animals of all groups in time interval from D 4 till D 13, namely in Group A in 11 animals, in Group B in 7 animals and in Group C 7 animals. The symptoms of diarrhoea persisted in piglets for 2 – 8 days. The elevated temperature, changes of behaviour or other disorders were not found out.

Mean body weights of animals of treated groups were higher in comparison with mean body weights of the animals of control group on D 28 and D 42. Dietary administration of IPA Mash phytase at 4,000 and 40,000 U/kg diet increased the mean final weight (D42) of piglets from 27.61 kg (control) to 31.09 and 30.85 kg, respectively.

Mean body weight gains of animals of treated groups were higher in comparison with mean body weight gains of the animals of control group in all followed intervals.

Mean feed consumptions in piglets of treated groups were lower in comparison with mean feed consumptions of the animals of control group in time intervals D 7 – D 14 and D 35 – D 42. As a consequence of markedly increased growth rate and comparable or lower feed intake, the overall feed conversion ratio was improved in both groups receiving IPA Mash phytase. This improvement was significant for treatment group B.

No **pathological changes** were diagnosed in any animals. As macroscopically abnormal organs were not found the histological examination was not performed.

At biochemical examination the values of ALT were increased in all examined animals of all groups, values of CPK were increased in 15 piglets of Group A, in 9 piglets of Group B and 11 piglets of Group C, Tbil values were increased above physiological range in 3 piglets of Group A, in 11 piglets of Groups B and in 11 piglets in Group C, AMS was increased in 4 piglets of Group A, in 8 piglets of Group B and 6 piglets of Group C, TP was decreased in 6 piglets of Group A and in 2 piglets of Groups B. Amount of phosphorus was increased above physiological range in 12 animals of control Group A, in 4 piglets of Group B and 5 piglets of Group C. The values of calcium were lower in 4 animals of Group C, they were in physiological range in Groups A and B. Chlorides showed increase in 10 piglets of Group A, in 9 piglets of Groups B and in 4 piglets in Group C

The others followed biochemical parameters were either in physiological ranges or deviations were diagnosed sporadically.

At haematological examination the amount of RBC in animals of all groups was higher (Group A 10, Group B 12 and Group C 8 piglets), value of HCT was increased in some animals in Groups B and Group C, in animals of Group A corresponded with the physiological range. The amount of WBC in 1 piglet of Group A and one piglet of Group C was above physiological range.

The others followed haematological parameters were either in or bellow physiological ranges.

Based on the abovementioned results it is possible to declare that there were no unfavourable effects of the IPA Mash Phytase (TI) to intended species in maximum recommended and multiplicated (ten times) recommended doses.

2. Identification of the Study, the Test Item and Reference Item

2.1. Descriptive Title

Tolerance Study with IPA Mash Phytase in Weaned Piglets

Study Code: 253/2008

2.2. Statement which Reveals the Nature and Purpose of the Study

The aim of the Study was to establish that there were no unfavourable effects of the IPA Mash Phytase (TI) to intended species in maximum recommended and multiplicated (ten times) recommended doses. The TI was admixed to the basal diet and was fed in standard way *ad libitum* in weaned piglets. Maximum recommended dose of TI was 4,000 U/ kg of diet, corresponding to 67.2 ppm of the formulated product. Tolerance Study in piglets was performed for 42 consecutive days.

2.3. Species

The biological test system weaned piglets was selected because *Sus scrofa domestica* is the likely target species for the use of the Test Item.

2.4. Regulatory Compliance

The Study was conducted by the Study Director and her staff according to the GLP recommendations of the OECD (OECD, 1998), The tolerance of IPA Mash Phytase (M) in the recommended and multiplicated doses in piglets were confirmed to obtain data for brand specific approval according to EC 1831/2003 and Guidelines for the assessment of additives in feedingstuff: Part II. Enzymes and micro-organisms (EFSA, 2006).

This *in-vivo* tolerance study in target animal species (weaned piglets – *Sus scrofa domestica*) aimed to assess the tolerance of the TI in piglets was conducted in accordance with the following guidelines:

Good Clinical Practice (VICH GL9 – GCP) adopted by CVMP in June 2000;
Guidelines for the assessment of additives in feedingstuff: Part II. Enzymes and micro-organisms (EFSA, 2006);

National animal welfare requirements;

Section IV of the Commission Directive 94/40/EC amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition lines out the required studies concerning the safety use of the additive.

2.5. Identification of the Test Item

Name of the TI:	IPA Mash Phytase (M)
Producer:	DSM Nutritional Products Ltd. Wurmisweg 576 CH-4303 Kaiseraugst SWITZERLAND
Batch No.:	PPQ 28656
Date of manufacture:	August 2008
Characterisation:	IPA Mash Phytase (M), enzyme product containing bacterial 6-phytase, expressed in a genetically modified strain of *Aspergillus oryzae*.
Active substance:	Enzyme 6-phytase The used mash form contains 60,700 U/g
Pharmaceutical form:	Dry powder
Administration route:	Oral
Stability:	Minimum 12 months at room temperature
Storage conditions:	Cool storage recommended

Identity of the Test Item was confirmed by analytical laboratory of the Test Facility. Analyses of medicated feed were performed by analytical laboratory of the Sponsor

2.6. Identification of the Reference Item

Not applicable for this kind of Study.

3. Information Concerning the Sponsor and the Test Facility

3.1. Sponsor

DSM Nutritional Products Ltd.
Wurmisweg 576
CH-4303 Kaiseraugst
SWITZERLAND

Phone: +41 61 688 69 09
Fax: +41 61 687 43 99
E-mail: jiri.broz@dsm.com

Responsible Person

Jiri Broz MSc PhD DVM
Phone: +41 61 815 87 35
Fax: +41 61 815 88 70
E-mail: jiri.broz@dsm.com

3.2. Test Facility

[redacted]

[1] Hereinafter referred to as [redacted] only

[redacted]

3.3. Principal Investigators and the Delegated Phases of the Study

[redacted]
Study Director
E-mail: [redacted]

Elaboration of the Study Plan and the Final Report. Responsibility for the overall conduct of the Study. Administration of TI, clinical and physical examinations and clinical observations, blood sampling, tissue sampling, pathological examination, histological examination, data management and evaluation of the results.

[redacted]
Principal Investigator
E-mail: [redacted]cz

Overall conduct of the Study, data management, animals identification, determination of body weights, administration of the TI, clinical observations of animals, blood sampling, tissue sampling, data evaluation, assistance to the Study director.

[redacted]ová
Investigator
E-mail: [redacted]

Biochemical examinations, data evaluation.

[redacted]
Investigator
E-mail: [redacted]

Haematological examinations, data evaluation.

[redacted]
Investigator
E-mail: [redacted]

Statistical evaluation.

All with [redacted]

4. Dates

4.1. Final Report Approval

Name	Date	Signature
Director of the Test Facility [redacted] (b) (4)	----------------	-------------------------------
Study Director [redacted] (b) (4) M	----------------	-------------------------------
Quality Assurance [redacted] (b) (4)	----------------	-------------------------------
Sponsor [redacted] (b) (4)	----------------	-------------------------------

4.2. The Proposed Starting and Completion Dates

Study initiation date:	23-02-2009
Experimental starting date:	04-03-2009
Experimental completion date:	17-04-2009
Study completion date by:	18-08-2009

5. Statements

5.1. Study Director Statement

Study: Tolerance Study with IPA Mash Phytase in Weaned Piglets

Study Code: 253/2008

Test Facility Director: (b) (4) [redacted]

Study Director:

Principal Investigators:

QA Personnel:

The aforementioned Study was done according to the described working procedures and according to the planned schedule set in the Study Plan.

The data stated in protocols are in agreement with reality.

The data in this Final Report correspond with the original protocols.

The study was performed under the OECD/GLP regimen as far as it can be applied to this kind of study.

Date: (b) (4) [redacted]

5.2. Quality Assurance Statement

Study: Tolerance Study with IPA Mash Phytase in Weaned Piglets

Study Code: 253/2008

(b) (4)

Test Facility Director: [redacted]

Study Director:

Principal Investigators:

QA Personnel:

Quality Assurance program for the aforementioned study was designed and performed as follows:

Study Plan inspection
The Study Plan was inspected on 23-02-2009. It was verified that the Study Plan contained all information required for its compliance with the principles of Good Laboratory Practice (OECD/GLP).

Facility and Study-based inspection
Animal handling, housing, labelling, weighing and application of the Test Item were inspected on 06-03-2009. Necropsy procedure and blood sampling were inspected on 15-04-2009. They were found to be in compliance with the principles of OECD/GLP and the Study Plan.

Final Report
The Final Report was inspected on 14-05-2009. It was found that methods and procedures used as well as all observations were completely and accurately described, and the results reflected the raw data obtained during the course of the study. It was stated that the Study was conducted in accordance with the principles of OECD/GLP as far as it can be applied to this kind of study.

The inspection outcomes were reported to the Study Director and the Company Management on the same days.

Date: [redacted] (b) (4)

6. Test Methods, Monitoring, Equipment

The Study was conducted by the Study Director and her staff according to the GLP recommendations *The Guidelines for the Testing of Veterinary Medicinal Products in European Community, The Rules Governing Medicinal Products in the European Community Vol. VI.* Detailed methodical schedule and spectrum of parameters studied were performed according to Sponsor's requests.

6.1. Monitoring

The Study was monitored by the Sponsor on his own decision and charge and by Test Facility Quality Assurance personnel.

6.2. Equipment

Al Cellcounter, model 2000 (Al Systeme, Germany);
Centrifuge Eppendorf 5702R (Eppendorf, Germany);
Disposable Sterican needles Luer Lock (BRAUN, Germany);
Disposable 10-mL syringes (BRAUN, Germany);
Electronic medical thermometer with precision 0.1 °C (Paul Hartmann, Germany);
Finnpipette 2 (200 – 1,000 µL) (Labsystems, Finland);
Liasys – Biochemical analyser (AMS, Assel, Italy);
Laboratory balance Kern (Fisher Scientific, Czech Republic);
Livestock balance, scale up to 1,000 kg with precision 0.1 kg, TRUE-TEST, Czech Republic;
Microscope Opton Standard WL–89 (Opton, Germany);
Plastic tubes 10 mL (Deltalab, Spain);
Plastic tubes 5.0 mL (Eurotubo, Italy);
Suspension digital scale HTR 30 with precision 0.01 kg (Nagata, Taiwan);
Analytic balance, capacity 180 g, precision, 0.1 mg, ER 180 A (A&D, Japan);
Staining machine VARISTAIN (Shandon, UK);
Rotary tissue processor JUNG-HISTOKINETTE 2000 (LEICA, Austria)
Sliding microtome Reichert (Reichert, Austria);
Sliding microtome JUNG SM 2000 (LEICA, Austria);
Microscope Jenamed histology (Carl Zeiss, Germany);
AF Zoom 28-80/3.5-5.6 (Minolta, Japan);
AF Macro Zoom 3x – 1x/1.7 – 2.8 (Minolta, Japan);
Macro Flash 1200 AF set (Minolta, Japan).

7. Issues

7.1. The Justification for Selection of the Test System

The biological test system weaned piglets was selected because *Sus scrofa domestica* is the likely target species for the use of the Test Item.

7.2. Characterisation of the Test System

7.2.1. Experimental Animals

A total of 48 early weaned piglets (crossbreds Large White x Landrace), both sexes [redacted] (b) (4) were included in the Study.

7.2.2. Clinical Signs

Clinical observation of animals was performed daily during the whole Study. Observations and recording of adverse effects after the administration the TI, morbidity and mortality were performed. Moreover, each animal was physically examined at the start of the administration period on D 0, 14, 28 and 42.

7.2.3. Housing

The animals were housed at BIOPHARM facilities conform to welfare regulations of animals (Council Directive 86/609/EEC). Piglets were housed in pairs in litterless boxes throughout the Study. Each box was identified by label containing the Study code, group identification, the animal identification, detailed time schedule of the Study and the name and signature of the Study Director. Maintenance and cleaning of the stable were performed daily. Environmental conditions (temperature and relative moisture) were monitored and recorded daily and these data will be retained in the archives of BIOPHARM.

7.2.4. Inclusion and Exclusion Criteria

Animals must be clinically healthy and none of them should have been treated with any drug before the trial start. In case of illness, the animal was excluded from the Study. Replacement with another healthy animal was consulted with Sponsor. No treatment other than the TI was given during the Study.

7.2.5. Allocation to Groups

Shortly after delivery (D 0) the weight of piglets was determined and animals were randomly allocated to three groups A, B, C (16 piglets each, 8 castrated males and 8 females).

Animals were assigned to groups according to their body weight criteria. At the commencement of the Study the body weight variation did not exceed ± 20 % of the mean body weight of piglets in each group. Each animal was individually identified by an ear tag. Moreover, the animals from each group were marked on their backs by smears of different colours.

Group codes, list of animal numbers and colour were attached to each box. The following identification of animals was used.

Group No.		Animal Identification Number		Colour
		castrated males	females	
A	**Control group (basal diet, no enzyme added)**	1 – 8	9 – 16	Green
B	**Treated group (basal diet + TI at 4,000 U/kg diet)**	17 – 24	25 – 32	Blue
C	**Treated group (basal diet + TI at 40,000 U/kg diet)**	33 – 40	41 – 48	Red

7.2.6. Feeding and Drinking Conditions

Pre-starter and starter diets based on wheat, barley and soybean meal as the main feed ingredients, formulated to meet NRC nutrient recommendations except of total and non-phytate P, were used. The basal diet was prepared according to formula approved by the Sponsor prior the start of the Study. Diet was provided to animals in mash form and fed *ad libitum,* namely pre-starter was fed from D 0 till D 13, starter than from D 14 till D 42 of the Study. Drinking water was available *ad libitum.*

Experimental treatments:

A. Control, non-treated group – basal diet, no enzyme added
B. Treated group, recommended dose of the TI – basal diet + TI at 4,000 U/kg diet
C. Treated group, ten times multiplicated dose of the TI – basal diet + TI at 40,000 U/kg diet

7.2.7. Weighing of Animals

Weighing of piglets was performed on livestock balance (with precision 0.1 kg) at the start of administration on D 0, further in 2-week period on D 14, 28 and 42.

7.2.8. Disposal of Experimental Animals

Slaughtered animals and their products were excluded from consumption and were moved to rendering plant.

7.3. Method of Administration and Reason for Its Choice

The Test Item was administered orally via medicated feed for 42 consecutive days.

7.4. Dose Levels, Frequency and Duration of Administration

7.4.1. Treatment Frequency and Dose Level

Animals of the Group A served as a negative control and were fed with the basal diet, without enzyme *ad libitum* 24 hour a day for the duration of the Study (42 consequent days, from D 0 till D 42).

Animals of the Group B were treated at the recommended dosage of 4,000 U of the TI (i.e. 67.2 ppm of IPA Mash Phytase) admixed into 1 kg of feed, administered *ad libitum* 24 hour a day for the duration of the Study (the same way as piglets of Group A).

Animals of the Group C were treated at the ten times multiplicated dose of 40,000 U of the TI (i.e. 672 ppm of IPA Mash Phytase) admixed into 1 kg of feed, administered *ad libitum* 24 hour a day for the duration of the Study (the same way as piglets of Group A).

The TI dose really obtained was calculated from the total medicated feed consumption during administration period. Consumption of feed was checked daily and calculated in one-week period, i.e. on D 7, 14, 21, 28, 35 and 42. The feed conversion ratio (feed/gain) was calculated for the overall trial period.

7.4.2. Method of Dose Administration

The TI was delivered in its ready-to-use form in original packaging.

Appropriate amount of the TI was admixed into feed and fed up through individual feeders (one feeder per box) during the administration period. Medicated feed was the only source of feed during all trial.

7.4.3. Dose Preparation

Medicated feed was prepared according to recommended dosage by the Manufacturing Unit for Medicated Premixes and Medicated Feedstuffs of BIOPHARM. This unit has GMP Certificate by the State Institute for Control of Drugs of Czech Republic (Detailed formula of diets see Table 11). The control analyses of the added test product in the medicated pre-starter and starter diets were performed by analytical laboratory BIOPRACT GmbH, Berlin, Germany (see Enclosures 13.2.1. and 13.2.2.)

7.5. Concurrent Treatment

No concurrent treatment was allowed during the Study without approval of the Study Director and the Sponsor. Any concurrent medications given were recorded in the raw data, giving identity of the materials, reason for use, route of administration and dosage.

7.6. Sacrifice and Sampling

The animals were stunned and killed by rapid exsanguination at the BIOPHARM facility. Cadavers were post-mortem examined by a veterinarian and biological samples were collected.

7.6.1. Heamatological and Biochemical Examinations

Heamatological and biochemical examinations were performed in all animals, i.e. in 16 piglets per group (total number of examined samples was 48).

Approximately 10-mL blood sample was taken (Groups A, B, C) for haematological and biochemical analyses on D 42.

The blood was collected into one test tube for biochemical examinations and one EDTA test tube for haematological analysis and was transported immediately to haematological laboratories. After centrifugation of biochemical samples their serum was divided in two aliquots. Blood sample processing was performed in accordance with BIOPHARM SOP ODB-001.

One sample of blood serum or EDTA blood was analysed, the other (duplicate) was kept just for case it would be necessary to repeat the analyses. The duplicate samples of blood were stored at +5 °C at BIOPHARM premises for 24 hours. The duplicate serum samples were stored at –20 °C at BIOPHARM premises for 24 hours. Samples were discarded after this period.

Study code, group and animal number were recorded on the test tubes.

A standard haematological analysis (amount of red blood cell (RBC), haemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), amount of blood platelets (PLT), amount of white blood cell (WBC): total and differential) and analysis of following serum variables: Chloride, calcium, phosphorus, total protein, albumin, glucose, AMS, urea, creatinine, ALP, ALT, AST, CPK and total bilirubin was performed.

7.6.2. Pathologicall Examination

The gross pathological examination was focused mainly on the examination of the gastro-intestinal tract and lymph nodes, liver, kidney, lung, heart and spleen.

7.7. Detailed Time Schedule

<u>Administration Period (D 0 till D 42)</u>

D 0	***04–03–2009***
7:00 a.m.	Weighing of individual doses of medicated feed
7:30 a.m.	Clinical observation (general appearance, behaviour and appetite), weighing of animals, allocation to groups, physical examination (state of health, behaviour, body temperature)
till 8:00 a.m.	**Administration of medicated feed** Start of observation of adverse effects
D 1 till D 13	***04–03–2009 till 17–03–2009***
7:00 a.m.	Clinical observation (general appearance, behaviour and appetite, adverse effects)
8:00 a.m.	Measuring of consumption of medicated feed
D 14	***18–03–2009***
7:00 a.m.	Clinical observation (general appearance, behaviour and appetite), weighing of animals, physical examination (state of health, behaviour, body temperature)
8:00 a.m.	Measuring of consumption of medicated feed
D 15 till D 27	***19–03–2009 till 31–03–2009***
7:00 a.m.	Clinical observation (general appearance, behaviour and appetite, adverse effects)
8:00 a.m.	Measuring of consumption of medicated feed
D 28	***01–04–2009***
7:00 a.m.	Clinical observation (general appearance, behaviour and appetite), weighing of animals, physical examination (state of health, behaviour, body temperature)
8:00 a.m.	Measuring of consumption of medicated feed
D 29 till D 41	***02–04–2009 till 14–04–2009***
7:00 a.m.	Clinical observation (general appearance, behaviour and appetite, adverse effects)
8:00 a.m.	Measuring of consumption of medicated feed
D 42	***15–04–2009***
7:00 a.m.	Clinical observation (general appearance, behaviour and appetite), weighing of animals, physical examination (state of health, behaviour, body temperature)
8:00 a.m.	**End of administration of medicated feed** Measuring of consumption of medicated feed Slaughter, gross necropsy, blood and tissue sampling of animals of **Groups A, B, C** Dispatching of blood samples to haematological laboratory

8. Results

8.1. Clinical Examination

Characteristics of the piglets are presented in Table 1.

Mild diarrhoea was diagnosed in some animals of all groups in time interval from D 4 till D 13, namely in Group A in 11 animals (Nos. 1, 2, 4, 5, 6, 7, 8, 9, 12, 15, 16), in Group B in 7 animals (Nos. 20, 21, 22, 24, 30, 31, 32) and in Group C 7 animals (Nos. 33, 34, 36, 39, 40, 43, 46). The symptoms of diarrhoea persisted in piglets for 2 – 8 days. The elevated temperature, changes of behaviour or other disorders were not found out.

8.2. Body Weight

Individual body weights of males and females in Groups A, B and C, mean and SD are given in Tables 2 – 4. Mean values of individual piglet body weights per groups are presented in Table 8. The results of statistical evaluation of initial and final body weight are presented in Table 19. Dietary administration of IPA Mash phytase at 4,000 and 40,000 U/kg diet markedly increased the mean final weight by 12.6% ($P<0.05$) and 12.0%, respectively, when compared to the negative control.

8.3. Body Weight Gains

Individual body weight gains of males and females in Groups A, B and C, mean and SD are given in Tables 2 – 4. Mean values of individual piglet body weight gains per groups are presented in Table 9.

Mean body weight gains of animals of treated groups were higher in comparison with mean body weight gains of the animals of control group in all followed intervals.

8.4. Feed Consumption and Feed Conversion

The feed consumptions (per box – two piglets) during the followed interval are presented in Tables 5 – 7. Mean feed consumptions in control and treated groups (per box) is in Table 10.

Mean feed consumptions in piglets of treated groups were lower in comparison with mean feed consumptions of the animals of control group in time intervals D 7 – D 14 and D 35 – D 42. Feed conversion ratio was calculated for the overall trial period (see Table 19) and it was improved in both groups receiving IPA Mash phytase from 2.75 (control) to 2.18 ($P<0.05$) and 2.20, respectively.

Composition and nutrient content of the used basal diet are given in Table 11 and results of control analysis of selected parameters are presented in Table 12.

8.5. Gross Necropsy

No pathological changes were diagnosed in any animals. As macroscopically abnormal organs were not found the histological examination was not performed.

8.6. Biochemical and Heamatological Examinations

Biochemical and heamatological examinations were performed all 16 piglets per group (total number of examined samples was 48).

The values of biochemical parameters of piglets of Groups A, B and C and their physiological ranges are given in Tables 11 – 13, values of haematological parameters with physiological ranges are presented in Tables 14 – 16.

At biochemical examination the values of ALT were increased in all examined animals of all groups, values of CPK were increased in 15 piglets of Group A, in 9 piglets of Group B and 11 piglets of Group C, Tbil values were increased above physiological range in 3 piglets of Group A, in 11 piglets of Groups B and in 11 piglets in Group C, AMS was increased in 4 piglets of Group A, in 8 piglets of Group B and 6 piglets of Group C, TP was decreased in 6 piglets of Group A and in 2 piglets of Groups B. Amount of serum phosphorus was increased above physiological range in 12 animals of control Group A, in 4 piglets of Group B and 5 piglets of Group C. The values of calcium were lower in 4 animals of Group C, they were in physiological range in Groups A and B. Chlorides showed increase in 10 piglets of Group A, in 9 piglets of Groups B and in 4 piglets in Group C

The others followed biochemical parameters were or in physiological ranges or deviations were diagnosed sporadically.

At haematological examination the amount of RBC in animals of all groups was higher (Group A 10, Group B 12 and Group C 8 piglets), value of HCT was increased in some animals in Groups B and Group C, in animals of Group A corresponded with the physiological range. The amount of WBC in 1 piglet of Group A and one piglet of Group C was above physiological range.

The others followed haematological parameters were either in or bellow physiological ranges.

9. Conclusions

Mild diarrhoea was diagnosed in some animals of all groups in time interval from D 4 till D 13, namely in Group A in 11 animals, in Group B in 7 animals and in Group C 7 animals. The symptoms of diarrhoea persisted in piglets for 2 – 8 days. The elevated temperature, changes of behaviour or other disorders were not found out.

Dietary administration of IPA Mash phytase at 4,000 and 40,000 U/kg diet increased the **mean final weight** (D42) of piglets from 27.61 kg (control) to 31.09 and 30.85 kg, respectively.

Mean body weight gains of animals of treated groups were higher in comparison with mean body weight gains of the animals of control group in all followed intervals.

Mean feed consumptions in piglets of treated groups were lower in comparison with mean feed consumptions of the animals of control group in time intervals D 7 – D 14 and D 35 – D 42. As a consequence of the increased growth rate and comparable or even lower feed intake, the overall **feed conversion ratio** was improved in both groups receiving IPA Mash phytase.

No **pathological changes** were diagnosed in any animals. As macroscopically abnormal organs were not found the histological examination was not performed.

In spite of significant differences among treatment groups for some biochemical and haematological parameters (see Tables 20 and 21) no remarkable and significant differences between the maximum recommended dose (treatment B) and the overdose of IPA Mash phytase (treatment C) were noted for all relevant parameters.

Based on the abovementioned results it is possible to declare that there were no unfavourable effects of the IPA Mash Phytase (TI) in weaned piglets when used in the maximum recommended and multiplicated (ten times) recommended doses.

10. Conduct of the Study

The Study was conducted by the Study Director and her staff according to the GLP recommendations of OECD (OECD, 1998), guidelines for the assessment of additives in feedstuffs – part II. (EC, 2001), guidelines focused to target animal safety for veterinary pharmaceuticals products (VICH, 2006), EFSA´s Proposal on Guidelines/Guidance for the Assessment of Additives for Use in Animals Nutrition (EFSA, 2006) and other regulations, recommendations and guidelines and their principles reported in the Chapter 11 (References) of this Study Plan.

11. Storage of Records and Materials

The Study Plan, raw data, records of all QA inspections and the Final Report of the Study will be stored in the archives of BIOPHARM for a period of 10 years. After this period, the Sponsor will be contacted for instructions of transfer, retention or disposal of all materials. All data will be available for inspections by competent authorities.

The sample of the TI will be stored for 12 months after its expiry period. After this period samples will be discarded. Unused remains of the TI will be disposed immediately after termination of the Study.

12. References

1) EC (1986): Council directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provision of the Member States regarding the protection of animals used for experimental and other scientific purposes. *OJ Eur. Comm., No. L358: 1 – 28.*

2) EC (1994): Commission Directive 94/40/EC of 22 July 1994 amending Council Directive 87/153/EEC fixing guidelines for the assessment of additives in animal nutrition. *OJ Eur. Comm., No. L 208, 15 – 26.*

3) EC (1997A): The rules governing medicinal products in the European Union, Volume 7AE1a: Good clinical practice for the conduct of clinical trials on veterinary medicinal products in the European Union, Brussels, 87 – 100.

4) EC (1997B): The rules governing medicinal products in the Euopean Union, Volume 7AE2a: Evaluation of the safety of veterinary medicinal Products for the Target Animals, Brussels, 103 – 108.

5) EC (2001): Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal product. *OJ Eur. Comm., No. L311: 1 – 27.*

6) EFSA (2006): EFSA's Proposal of Guidelines/Guidance for the Assesment of Additives For Use in Animal Nutrition in accordance with Regulation (EC) No 1831/2003. Consolidated version September 2006.

7) OECD (1998): ENV/MC/CHEM (98)17 OECD Principles of Good Laboratory Practices, In: Series on Principles of Good Laboratory Practice and Compliance Monitoring No. 1. Environment Directorate of the OECD, Paris, 41 pp.

13. Enclosures

13.1. Tables

Table 1 – Characteristics of the Piglets

Group	Box Identification Number	Animal Identification Number		General tolerance	Gross exam
		males	females		
A	1 – 8	1 – 8	9 – 16	Mild diarrhoea from D 4 till D12	No significant lesion
B	9 – 16	17 – 24	25 – 32	Mild diarrhoea from D 5 till D13	No significant lesion
C	17 – 24	33 – 40	41 – 48	Mild diarrhoea from D 5 till D13	No significant lesion

Table 2 – Individual Body Weight and Body Weight Gains of Piglets – Group A

Group	Box No.	Animal No.	Body weight (kg)				Body weight gains (kg)		
			D 0	D 14	D 28	D 42	D 0 till D 14	D 14 till D 28	D 28 till D 42
A	1	1	11.8	13.2	15.5	23.7	1.4	2.3	8.2
		2	10.6	13.5	20.4	31.5	2.9	6.9	11.1
	2	3	11.9	14.8	23.8	35.0	2.9	9.0	11.2
		4	10.2	10.2	15.5	23.5	0.0	5.3	8.0
	3	5	9.1	10.0	15.0	23.7	0.9	5.0	8.7
		6	7.6	11.6	16.2	25.3	4.0	4.6	9.1
	4	7	11.0	12.5	14.8	20.3	1.5	2.3	5.5
		8	11.9	12.6	19.0	26.4	0.7	6.4	7.4
	5	9	11.5	13.0	17.6	27.8	1.5	4.6	10.2
		10	12.1	16.6	22.9	34.5	4.5	6.3	11.6
	6	11	10.9	15.0	23.0	32.7	4.1	8.0	9.7
		12	7.8	9.8	14.0	20.2	2.0	4.2	6.2
	7	13	12.0	17.5	26.2	36.9	5.5	8.7	10.7
		14	11.1	13.7	15.6	25.8	2.6	1.9	10.2
	8	15	9.7	12.3	19.5	29.6	2.6	7.2	10.1
		16	9.9	12.5	17.0	24.8	2.6	4.5	7.8
		Mean	10.6	13.1	18.5	27.6	2.5	5.5	9.1
		SD	1.4	2.2	3.8	5.2	1.5	2.2	1.8

Table 3 – Individual Body Weight and Body Weight Gains of Piglets – Group B

Group	Box No.	Animal No.	Body weight (kg)				Body weight gains (kg)		
			D 0	D 14	D 28	D 42	D 0 till D 14	D 14 till D 28	D 28 till D 42
B	9	17	8.6	11.5	20.1	32.0	2.9	8.6	11.9
		18	8.5	11.2	17.9	28.9	2.7	6.7	11.0
	10	19	9.2	12.5	16.0	24.8	3.3	3.5	8.8
		20	8.9	12.5	19.0	32.1	3.6	6.5	13.1
	11	21	9.8	10.1	16.1	26.8	0.3	6.0	10.7
		22	12.0	13.6	22.7	30.3	1.6	9.1	7.6
	12	23	12.3	17.7	27.9	28.6	5.4	10.2	0.7
		24	12.2	13.2	21.8	31.7	1.0	8.6	9.9
	13	25	7.4	11.6	15.7	22.1	4.2	4.1	6.4
		26	11.8	15.4	23.5	35.2	3.6	8.1	11.7
	14	27	12.0	16.5	25.5	36.1	4.5	9.0	10.6
		28	11.4	15.2	21.4	30.5	3.8	6.2	9.1
	15	29	12.3	17.1	26.2	38.1	4.8	9.1	11.9
		30	10.9	13.7	23.4	32.3	2.8	9.7	8.9
	16	31	11.9	14.5	22.8	36.0	2.6	8.3	13.2
		32	11.3	12.8	21.0	31.9	1.5	8.2	10.9
		Mean	10.7	13.7	21.3	31.1	3.0	7.6	9.8
		SD	1.6	2.2	3.7	4.2	1.4	1.9	3.1

Table 4 – Individual Body Weight and Body Weight Gains of Piglets – Group C

Group	Box No.	Animal No.	Body weight (kg)				Body weight gain (kg)		
			D 0	D 14	D 28	D 42	D 0 till D 14	D 14 till D 28	D 28 till D 42
C	17	33	11.7	12.2	19.6	28.2	0.5	7.4	8.6
		34	10.4	13.1	21.0	31.5	2.7	7.9	10.5
	18	35	10.6	14.5	23.5	33.7	3.9	9.0	10.2
		36	9.2	10.2	12.6	28.1	1.0	2.4	15.5
	19	37	10.1	13.0	20.9	29.3	2.9	7.9	8.4
		38	9.5	11.4	18.3	28.1	1.9	6.9	9.8
	20	39	9.8	11.7	20.8	31.2	1.9	9.1	10.4
		40	11.4	13.3	22.3	34.5	1.9	9.0	12.2
	21	41	8.9	12.5	19.2	28.6	3.6	6.7	9.4
		42	7.1	10.6	19.7	30.8	3.5	9.1	11.1
	22	43	11.8	12.4	20.0	28.1	0.6	7.6	8.1
		44	10.8	13.6	19.5	27.8	2.8	5.9	8.3
	23	45	12.3	18.0	24.9	36.2	5.7	6.9	11.3
		46	10.6	14.7	24.7	34.0	4.1	10.0	9.3
	24	47	11.2	14.2	22.5	33.8	3.0	8.3	11.3
		48	10.2	13.6	21.4	30.9	3.4	7.8	9.5
		Mean	10.4	13.1	20.7	30.9	2.7	7.6	10.2
		SD	1.3	1.9	2.9	2.8	1.4	1.8	1.9

Table 5 – Consumption of Feed per Box – Group A

Group	Box No.	Consumption of feed per box (g)					
		D 0 till D 7	D 7 till D 14	D 14 till D 21	D 21 till D 28	D 28 till D 35	D 35 till D 42
A	1	8,751	14,994	15,978	18,609	20,602	25,623
	2	7,542	12,863	12,264	14,826	20,592	26,679
	3	3,970	9,548	12,678	13,151	16,534	21,480
	4	8,586	12,808	15,303	18,757	20,644	26,911
	5	6,998	10,968	11,872	14,825	18,028	20,835
	6	4,162	7,925	11,822	17,969	21,346	28,987
	7	7,532	10,666	14,047	14,902	20,283	25,261
	8	4,877	9,390	11,902	13,736	17,587	21,387
Mean		6,552.3	11,145.3	13,233.3	15,846.9	19,452.0	24,645.4
SD		1,938.2	2,292.6	1,662.4	2,245.8	1,786.5	3,038.1

Table 6 – Consumption of Feed per Box – Group B

Group	Box No.	Consumption of feed per box (g)					
		D 0 till D 7	D 7 till D 14	D 14 till D 21	D 21 till D 28	D 28 till D 35	D 35 till D 42
B	9	7,992	12,031	13,780	14,214	19,488	22,634
	10	5,017	8,952	10,318	11,945	16,721	20,742
	11	6,582	8,585	11,423	15,174	18,839	22,129
	12	6,829	9,983	14,484	18,511	21,529	25,767
	13	7,904	9,655	12,867	14,974	19,700	21,707
	14	6,342	9,203	14,370	15,789	19,599	22,661
	15	7,272	12,875	15,174	19,139	20,606	25,039
	16	4,639	7,990	14,793	16,960	20,344	23,888
Mean		6,572.1	9,909.3	13,401.1	15,838.3	19,603.3	23,070.9
SD		1,227.9	1,699.6	1,733.7	2,338.6	1,423.1	1,704.6

Table 7 – Consumption of Feed per Box – Group C

Group	Box No.	Consumption of feed per box (g)					
		D 0 till D 7	D 7 till D 14	D 14 till D 21	D 21 till D 28	D 28 till D 35	D 35 till D 42
C	17	7,387	12,002	14,745	17,437	19,868	23,090
	18	6,503	9,333	11,993	15,059	19,939	23,622
	19	6,217	7,804	12,414	13,312	15,826	19,073
	20	7,995	8,832	13,268	18,436	20,629	26,357
	21	5,296	9,464	15,229	18,338	20,592	26,736
	22	6,338	10,997	15,197	18,127	20,768	23,129
	23	8,313	9,768	13,879	16,138	19,720	22,799
	24	4,644	9,254	11,299	15,466	20,075	22,476
Mean		6,586.6	9,681.8	13,503.0	16,539.1	19,677.1	23,410.3
SD		1,267.7	1,294.0	1,507.7	1,854.7	1,604.9	2,391.3

Table 8 – Mean Values of Individual Piglet Body Weights – Summary

Group	D 0		D 14		D 28		D 42	
	MBW	SD	MBW	SD	MBW	SD	MBW	SD
A	10.6	1.4	13.1	2.2	18.5	3.8	27.6	5.2
B	10.7	1.6	13.7	2.2	21.3	3.7	31.1	4.2
C	10.4	1.3	13.1	1.9	20.7	2.9	30.9	2.8

Legend: MBW – Mean of body weights (g)
SD – Standard deviation

Table 9 – Mean Values of Individual Piglet Body Weights Gains – Summary

Group	D 0 till D 14		D 14 till D 28		D 28 till D 42	
	MG	SD	MG	SD	MG	SD
A	2.5	1.5	5.5	2.2	9.1	1.8
B	3.0	1.4	7.6	1.9	9.8	3.1
C	2.7	1.4	7.6	1.8	10.2	1.9

Legend: MG – Mean of body weight gains (g)
SD – Standard deviation

Table 10 – Mean Values of Feed Consumption – Summary

Group	D 0 till D 7		D 7 till D 14		D 14 till D 21		D 21 till D 28	
	MF	SD	MF	SD	MF	SD	MF	SD
A	6,552.3	1,938.2	11,145.3	2,292.6	13,233.3	1,662.4	15,846.9	2,245.8
B	6,572.1	1,227.9	9,909.3	1,699.6	13,401.1	1,733.7	15,838.3	2,338.6
C	6,586.6	1,267.7	9,681.8	1,294.0	13,503.0	1,507.7	16,539.1	1,854.7

Group	D 28 till D 35		D 35 till D 42	
	MF	SD	MF	SD
A	19,452.0	1,786.5	24,645.4	3,038.1
B	19,603.3	1,423.1	23,070.9	1,704.6
C	19,677.1	1,604.9	23,410.3	2,391.3

Legend: MF – Mean of medicated feed consumption per box (g)
SD – Standard deviation

Table 11 – Composition and Nutrient Content of the Used Basal Diets

Feed ingredients (in %)	Pre-starter diet*	Starter diet
Wheat	31.10	35.00
Barley	25.00	24.00
Maize	-	8.00
Soybean meal (48% CP)	14.00	13.00
Wheat flour	8.70	8.89
Dried whey	5.00	-
Potato protein	4.60	-
Soya oil	4.00	4.00
Dried yeast	3.00	4.00
Limestone	0.20	1.50
Salt	0.11	0.43
Monocalcium phosphate	-	0.38
Vitamin & mineral premixes	4.29	0.80
Calculated nutrients		
Crude protein (%)	20.08	16.83
Metabolizable energy (MJ/kg)	14.00	-
Digestible energy (MJ/kg)	-	14.35
Lysine (%)	1.40	0.98
Methionine (%)	0.52	0.29
Calcium (%)	0.65	0.70
Total phosphorus (%)	0.54	0.54
Digestible phosphorus (%)	0.38	0.22

* Seltek COS Medipharm (commercial formula)

Table 12 – Control Analysis of the Used Basal Diet

Analysed items	Pre-starter diet		Starter diet	
	Expected value	Result	Expected value	Result
Lysine (%)	1.40	1.45	0.98	0.97
Ash (%)	4.53	4.23	5.00	4.96
Humidity (%)	12.00	10.40	10.90	9.91
Crude protein (%)	20.08	19.25	16.83	16.79

Table 13 – Biochemical Parameters of Animals – Group A

Animal No.	ALB (g/L)	TP (g/L)	U (mmol/L)	Glu (mmol/L)	ALP (µkat/L)	ALT (µkat/L)	AST (µkat/L)	AMS (µkat/L)	CPK (µkat/L)	Cre (µmol/L)	Ca (mmol/L)	P (mmol/L)	Cl (mmol/L)	TBil (µmol/L)
1	22.4	**49.8**	4.29	5.07	3.35	**3.45**	**1.52**	74.90	**31.42**	114	2.37	**3.49**	**114.0**	0.0
2	27.4	57.1	4.18	6.09	3.74	**3.00**	1.35	**97.30**	**31.19**	84	2.55	**3.52**	**114.9**	**4.9**
3	27.9	55.4	6.28	7.18	3.45	**3.14**	**1.81**	69.92	**30.15**	100	2.53	**4.00**	103.9	0.0
4	23.8	55.0	5.12	6.47	**2.97**	**2.72**	1.28	**97.53**	**32.33**	81	2.33	**3.66**	102.4	0.1
5	24.0	**51.4**	4.94	6.14	3.51	**4.26**	1.15	62.26	**31.50**	**72**	2.60	3.20	**112.4**	0.0
6	25.8	55.6	5.17	5.82	4.73	**4.99**	**2.99**	46.09	**30.39**	78	2.41	3.10	101.0	1.9
7	23.9	53.0	7.88	5.57	4.51	**3.29**	**1.73**	75.13	**31.05**	132	2.75	**4.12**	103.9	0.0
8	25.2	**48.6**	6.22	5.88	3.73	**3.93**	1.15	65.23	**31.67**	103	2.45	**3.53**	**117.1**	**3.5**
9	24.4	**47.2**	4.41	6.62	4.08	**3.67**	1.36	**96.47**	**30.40**	86	2.44	3.43	**115.1**	0.0
10	28.4	57.5	5.17	5.98	3.77	**3.74**	1.26	74.62	**29.86**	109	2.77	**3.90**	**113.1**	0.0
11	30.5	57.5	7.10	6.16	**2.61**	**3.14**	1.09	47.90	**30.65**	99	2.66	**3.64**	101.8	**4.8**
12	27.3	55.8	6.16	5.92	3.58	**3.20**	0.68	52.38	13.40	97	2.64	**4.78**	**112.7**	0.0
13	25.6	**50.5**	6.29	6.44	4.03	**4.13**	**2.23**	62.44	**33.76**	120	2.34	3.26	**114.3**	0.9
14	25.5	56.3	5.01	6.65	4.25	**2.48**	0.99	67.03	**31.34**	**70**	2.80	**3.61**	**115.0**	0.2
15	27.2	56.8	4.53	6.99	4.67	**3.26**	**2.13**	47.31	**30.01**	86	2.52	**3.46**	102.7	0.6
16	22.9	**49.9**	5.75	6.19	3.49	**3.98**	1.15	**101.06**	**71.05**	90	2.35	**3.86**	**113.1**	0.0
Phys. range	**19**	**52**	**2.7**	**4**	**3**	**0.55**	**0.52**	**15.22**	**1**	**77**	**2.16**	**2.25**	**97.1**	**0**
	42	**83**	**9.6**	**8.1**	**13.55**	**1.31**	**1.36**	**77.1**	**20.85**	**165**	**2.92**	**3.44**	**106.4**	**3.4**

Table 14 – Biochemical Parameters of Animals – Group B

Animal No.	ALB (g/L)	TP (g/L)	U (mmol/L)	Glu (mmol/L)	ALP (µkat/L)	ALT (µkat/L)	AST (µkat/L)	AMS (µkat/L)	CPK (µkat/L)	Cre (µmol/L)	Ca (mmol/L)	P (mmol/L)	Cl (mmol/L)	TBil (µmol/L)
17	27.5	63.2	4.88	6.48	4.25	**4.24**	0.69	68.39	**72.47**	90	2.34	3.20	104.6	2.5
18	21.9	**51.5**	**2.65**	6.47	5.32	**4.43**	**1.52**	**97.58**	**27.66**	84	2.38	3.34	**108.0**	**3.7**
19	23.5	**51.3**	5.37	5.64	4.90	**3.29**	0.83	65.33	8.59	100	2.34	3.17	103.3	1.6
20	27.3	64.6	3.57	5.10	3.95	**2.90**	1.11	**93.82**	18.08	89	2.43	3.33	104.2	**5.0**
21	23.8	57.1	5.71	6.18	7.85	**4.21**	**1.54**	**96.84**	**49.32**	100	2.90	**3.67**	**106.6**	**5.0**
22	24.8	58.3	3.53	6.52	3.54	**3.23**	0.85	**90.31**	20.12	90	2.47	**3.60**	**106.5**	**4.2**
23	31.0	63.8	5.51	6.49	4.55	**3.58**	1.10	**94.11**	**33.78**	125	2.27	3.30	104.5	**7.5**
24	26.8	56.3	5.82	6.20	3.59	**3.17**	0.74	36.70	6.39	117	2.45	3.30	**107.8**	**3.6**
25	22.6	58.0	4.45	5.17	4.66	**3.59**	1.19	**95.90**	14.54	104	2.21	3.12	102.6	**4.3**
26	26.0	59.4	3.25	7.11	5.00	**3.00**	1.12	64.73	**36.48**	78	2.30	**3.69**	**108.2**	**4.9**
27	27.8	58.7	4.18	6.52	3.43	**1.92**	0.65	63.78	14.01	104	2.42	3.43	**108.3**	2.5
28	25.7	53.1	3.79	6.14	4.21	**4.73**	1.14	61.82	**23.32**	115	2.43	3.30	**108.6**	**4.0**
29	28.9	61.2	3.69	6.14	4.26	**3.34**	**1.42**	65.94	**27.73**	125	2.38	**3.49**	**109.6**	1.6
30	30.4	65.3	3.60	5.97	4.09	**3.49**	0.85	62.71	7.48	115	2.45	3.16	105.5	**3.8**
31	28.8	60.1	3.77	6.20	5.14	**3.89**	1.02	**87.36**	**31.52**	102	2.39	3.21	**107.3**	**4.9**
32	27.3	62.6	4.23	6.01	4.00	**3.43**	**1.58**	**89.22**	**61.86**	97	2.33	3.33	105.8	2.3
Phys. range	**19**	**52**	**2.7**	**4**	**3**	**0.55**	**0.52**	**15.22**	**1**	**77**	**2.16**	**2.25**	**97.1**	**0**
	42	**83**	**9.6**	**8.1**	**13.55**	**1.31**	**1.36**	**77.1**	**20.85**	**165**	**2.92**	**3.44**	**106.4**	**3.4**

Table 15 – Biochemical Parameters of Animals – Group C

Animal No.	ALB (g/L)	TP (g/L)	U (mmol/L)	Glu (mmol/L)	ALP (µkat/L)	ALT (µkat/L)	AST (µkat/L)	AMS (µkat/L)	CPK (µkat/L)	Cre (µmol/L)	Ca (mmol/L)	P (mmol/L)	Cl (mmol/L)	TBil (µmol/L)
33	27.0	54.3	3.29	5.99	4.19	3.22	0.89	84.34	64.87	98	2.42	3.08	105.6	2.7
34	26.8	58.2	5.60	5.69	6.19	3.68	1.37	79.72	22.31	85	2.44	3.67	102.9	6.4
35	26.4	53.8	4.10	5.89	4.29	3.29	1.74	103.39	106.70	112	2.45	3.91	104.0	4.6
36	22.9	57.4	3.99	5.19	5.16	3.53	0.76	86.84	18.47	66	2.45	3.68	105.2	3.7
37	21.5	57.3	5.41	5.27	4.01	3.75	1.11	66.46	28.63	105	2.04	3.39	107.2	5.1
38	24.0	54.6	3.76	6.17	4.57	5.14	1.11	105.38	23.36	115	2.31	3.56	105.8	4.0
39	26.7	56.9	4.00	6.13	4.06	3.28	0.78	48.80	15.47	95	2.43	3.93	106.7	2.1
40	27.4	56.8	4.84	6.35	5.32	3.78	1.23	49.06	38.21	95	2.41	3.38	105.4	1.2
41	24.8	53.1	5.20	5.18	5.36	4.26	1.20	72.45	23.76	105	2.30	3.24	106.7	2.4
42	27.0	63.9	4.22	5.73	5.25	3.37	0.95	63.12	9.87	99	1.70	3.23	105.6	6.9
43	27.8	56.9	3.40	5.78	4.02	3.96	1.23	39.47	26.28	100	2.52	2.97	104.5	4.6
44	29.2	61.7	2.97	4.43	2.43	3.47	2.02	55.72	126.18	109	2.05	2.86	105.0	8.3
45	26.1	59.4	4.22	6.73	4.07	4.98	1.11	40.27	41.59	116	2.26	3.39	105.3	2.5
46	30.2	64.4	5.04	5.98	4.96	3.58	0.98	69.77	18.49	124	2.25	3.12	104.8	3.7
47	29.2	70.0	3.94	6.78	5.36	2.87	0.74	68.54	37.89	105	2.32	2.97	107.1	3.8
48	27.8	61.3	5.48	5.60	3.48	3.31	0.83	84.09	19.24	94	2.12	3.25	105.8	3.7
Phys. range	19	52	2.7	4	3	0.55	0.52	15.22	1	77	2.16	2.25	97.1	0
	42	83	9.6	8.1	13.55	1.31	1.36	77.1	20.85	165	2.92	3.44	106.4	3.4

Table 16 – Haematological Parameters of Animals – Group A

Animal No.	RBC ($x10^6/ul$)	HCT (%)	MCV (fl)	PLT ($x10^3/ul$)	MCH (pg/l)	MCHC (g/l)	WBC ($x10^3/ul$)	HGB (g/100ml)
1	6.95	32	47	327	12	263	19.9	8.4
2	7.16	34	48	104	13	276	16	9.4
3	7.23	34	47	312	14	291	17.1	9.9
4	7.09	33	47	306	13	288	10.8	9.5
5	6.38	31	49	342	14	290	15	9
6	7.13	34	47	60	14	285	14.3	9.7
7	7.87	32	41	325	12	294	28.4	9.4
8	7.15	33	46	241	13	288	15.5	9.5
9	6.84	34	50	185	14	288	18.6	9.8
10	7.08	35	49	197	15	297	19.8	10.4
11	6.57	33	50	118	15	303	12.9	10
12	6.89	34	50	229	15	294	12	10
13	7.07	33	47	212	14	303	20.9	10
14	6.74	32	47	282	14	294	13.8	9.4
15	7.21	36	50	275	14	283	9.4	10.2
16	7.08	34	48	257	14	294	11.1	10
Phys. range	5	26	52	200	17.0	290	11.0	9.0
	7	35	62	500	24.0	340	22.0	13.0

Animal No.	NE		EO (%)	BA (%)	LY (%)	MO (%)
	SG (%)	T (%)				
1	45	1	2	1	48	3
2	37	0	5	1	50	7
3	47	1	2	0	47	3
4	46	1	1	1	46	5
5	39	0	2	0	56	3
6	45	0	2	0	50	3
7	50	1	0	1	46	2
8	46	0	1	0	49	4
9	44	0	2	1	45	8
10	35	0	2	0	59	4
11	34	0	2	0	61	3
12	45	1	4	1	45	4
13	34	0	3	0	58	5
14	42	0	2	0	52	4
15	39	0	2	0	56	3
16	46	0	0	0	50	4
Phys. range	28	0	0	0	36	2
	47	4	11	2	92	10

Table 17 – Haematological Parameters of Animals – Group B

Animal No.	RBC ($x10^6/ul$)	HCT (%)	MCV (fl)	PLT ($x10^3/ul$)	MCH (pg)	MCHC (g/l)	WBC ($x10^3/ul$)	HGB (g/100ml)
17	7.1	38	54	207	14	253	11.6	9.6
18	7.03	35	50	264	14	283	14.3	9.9
19	6.84	34	49	208	14	285	10.2	9.7
20	7.8	38	48	330	14	284	11.3	10.8
21	7.37	37	50	218	15	289	13.3	10.7
22	7.33	35	48	248	14	291	12.1	10.2
23	7.29	38	52	208	15	279	13.2	10.6
24	7.34	36	49	264	14	286	18.3	10.3
25	6.68	34	51	242	15	285	11	9.7
26	7.4	37	50	235	14	278	14.4	10.3
27	7.31	38	52	211	15	279	14.1	10.6
28	7.85	36	45	233	13	281	16.4	10.1
29	6.79	36	53	110	15	289	14.8	10.4
30	7.06	37	52	183	15	289	18.7	10.7
31	6.46	36	55	148	16	283	10.1	10.2
32	7.15	35	48	154	14	283	13.3	9.9
Phys. range	5	26	52	200	17.0	290	11.0	9.0
	7	35	62	500	24.0	340	22.0	13.0

Animal No.	NE SG (%)	NE T (%)	EO (%)	BA (%)	LY (%)	MO (%)
17	33	0	1	1	62	3
18	33	0	3	0	59	5
19	42	0	6	1	48	3
20	37	0	3	0	56	4
21	35	0	5	0	56	4
22	33	0	1	1	62	3
23	46	0	1	0	46	7
24	53	0	2	0	41	4
25	51	0	2	2	42	3
26	42	0	1	1	52	4
27	36	0	4	0	58	2
28	33	1	6	1	57	2
29	33	0	2	0	62	3
30	50	1	3	1	43	2
31	29	0	4	0	64	3
32	28	0	1	2	66	3
Phys. range	28	0	0	0	36	2
	47	4	11	2	92	10

Table 18 – Haematological Parameters of Animals – Group C

Animal No.	RBC ($x10^6/ul$)	HCT (%)	MCV (fl)	PLT ($x10^3/ul$)	MCH (pg/l)	MCHC (g/l)	WBC ($x10^3/ul$)	HGB (g/100ml)
33	6.8	35	52	163	15	283	16	9.9
34	7.04	37	53	148	15	281	14.5	10.4
35	7.18	38	53	172	15	279	16.1	10.6
36	7.22	36	51	232	14	289	16.8	10.4
37	7.68	38	49	222	14	276	15.6	10.5
38	7.03	36	51	270	14	278	13.5	10
39	7	35	49	204	14	274	18.9	9.6
40	6.79	34	51	176	15	291	14.1	9.9
41	6.36	33	53	179	15	285	18.9	9.4
42	6.69	36	53	262	14	269	17.1	9.7
43	6.85	35	51	317	14	280	14.3	9.8
44	6.51	34	52	272	15	288	22.1	9.8
45	7.38	36	49	190	14	283	16.6	10.2
46	7.04	38	54	177	16	297	15.4	11.3
47	6.82	37	54	189	15	281	11.8	10.4
48	7.62	38	50	329	14	284	16.5	10.8
Phys. range	5	26	52	200	17.0	290	11.0	9.0
	7	35	62	500	24.0	340	22.0	13.0

Animal No.	NE		EO (%)	BA (%)	LY (%)	MO (%)
	SG (%)	T (%)				
33	24	0	2	2	68	4
34	45	0	1	2	49	3
35	22	0	5	1	67	5
36	50	0	3	2	43	2
37	27	0	1	0	68	4
38	43	0	1	0	53	3
39	47	0	2	0	49	2
40	40	0	2	0	54	4
41	50	1	1	1	43	4
42	47	0	2	2	47	2
43	45	0	2	0	48	5
44	56	1	3	0	38	2
45	20	0	5	0	70	5
46	43	0	4	0	49	4
47	40	0	1	0	55	4
48	35	0	2	0	59	4
Phys.	28	0	0	0	36	2
range	47	4	11	2	92	10

Table 19 – Statistical Evaluation of Body Weight and Feed Conversion

	Body Weight		Feed Conversion
	D0	**D42**	**D 0 till D42**
P (ANOVA)	0.831	**0.039**	**0.044**
P (Kruskal-Wallis ANOVA)	0.563	0.068	**0.037**
Group A			
N	16	16	8
Mean	10.57	27.61 [b]	2.75 [b]
Median	10.95	26.10	2.50
SD	1.43	5.21	0.72
Group B			
N	16	16	8
Mean	10.66	31.09 [a]	2.18 [a]
Median	11.35	31.80	2.09
SD	1.65	4.25	0.26
Group C			
N	16	16	8
Mean	10.35	30.93	2.20
Median	10.50	30.85	2.10
SD	1.29	2.78	0.33

P probability with significance level α=0.05 highlighted in bold
[a] significant difference between group and control group A
[b] significant difference between group and group B

Table 20 – Statistical Evaluation of Biochemical Parameters

	ALB	TP	U	Glu	ALP	ALT	AST
P (ANOVA)	0.586	**0.001**	**0.000**	0.100	**0.023**	0.628	**0.018**
P (Kruskal-Wallis ANOVA)	0.546	**0.001**	**0.002**	0.112	**0.012**	0.591	**0.032**
Group A							
N	16	16	16	16	16	16	16
Mean	25.76	53.59 [d]	5.531 [d]	6.198	3.779 [d]	3.524	1.492 [d]
Median	25.55	55.20 [d]	5.170 [d]	6.150	3.735 [c]	3.370	1.315
SD	2.21	3.49	1.055	0.527	0.584	0.635	0.572
Group B							
N	16	16	16	16	16	16	16
Mean	26.51	59.03 [a]	4.250 [a]	6.146	4.546 [a]	3.528	1.084 [a]
Median	27.05	59.05 [a]	3.985 [a]	6.190	4.255	3.460	1.105
SD	2.67	4.43	0.952	0.510	1.050	0.681	0.307
Group C							
N	16	16	16	16	16	16	16
Mean	26.55	58.75 [a]	4.341 [a]	5.806	4.545 [a]	3.717	1.128 [a]
Median	26.90	57.35 [a]	4.160 [a]	5.835	4.430 [a]	3.555	1.110
SD	2.31	4.54	0.824	0.601	0.913	0.617	0.352

	AMS	CPK	Cre	Ca	P	Cl	TBil
P (ANOVA)	0.513	0.438	0.355	**0.001**	**0.012**	**0.004**	**0.000**
P (Kruskal-Wallis ANOVA)	0.665	0.329	0.289	**0.002**	**0.031**	0.207	**0.000**
Group A							
N	16	16	16	16	16	16	16
Mean	71.10	32.51	95.06	2.532 [c]	3.660 [d]	109.8 [d]	1.056 [d]
Median	68.48	31.12	93.50	2.525 [c]	3.570	112.9	0.050 [d]
SD	18.72	11.23	17.53	0.158	0.410	5.9	1.757
Group B							
N	16	16	16	16	16	16	16
Mean	77.16	28.33	102.19	2.406	3.353 [a]	106.3 [a]	3.838 [a]
Median	77.88	25.49	101.00	2.385	3.315	106.6	3.900 [a]
SD	18.07	19.18	14.19	0.150	0.179	2.1	1.523
Group C							
N	16	16	16	16	16	16	16
Mean	69.84	38.83	101.44	2.279 [a]	3.352 [a]	105.5 [a]	4.106 [a]
Median	69.16	25.02	102.50	2.315 [a]	3.315	105.5	3.750 [a]
SD	20.21	33.19	13.65	0.212	0.327	1.1	1.874

P probability with significance level $\alpha=0.05$ highlighted in bold
[a] significant difference between group and control group A
[b] significant difference between group and group B
[c] significant difference between group and group C
[d] significant difference between group and both other groups

Table 21 – Statistical Evaluation of Haematological Parameters

	RBC	HCT	MCV	PLT	MCH	MCHC	WBC
P (ANOVA)	0.334	**0.000**	**0.000**	0.670	**0.010**	**0.034**	0.073
P (Kruskal-Wallis ANOVA)	0.264	**0.000**	**0.000**	0.448	**0.025**	**0.010**	**0.033**
Group A							
N	16	16	16	16	16	16	16
Mean	7.028	33.38 [d]	47.69 [d]	235.8	13.75 [d]	289.4 [d]	15.97
Median	7.080	33.50 [d]	47.50 [d]	249.0	14.00	290.5 [c]	15.25
SD	0.328	1.26	2.24	85.4	0.93	9.9	4.79
Group B							
N	16	16	16	16	16	16	16
Mean	7.175	36.25 [a]	50.38 [a]	216.4	14.44 [a]	282.3 [a]	13.57
Median	7.220	36.00 [a]	50.00 [a]	214.5	14.00	283.5	13.30 [c]
SD	0.372	1.39	2.58	52.3	0.73	8.7	2.60
Group C							
N	16	16	16	16	16	16	16
Mean	7.001	36.00 [a]	51.56 [a]	218.9	14.56 [a]	282.4 [a]	16.14
Median	7.015	36.00 [a]	51.50 [a]	197.0	14.50	282.0 [c]	16.05 [b]
SD	0.362	1.59	1.71	55.8	0.63	6.8	2.44

	HGB	SG	T	EO	BA	LY	MO
P (ANOVA)	**0.002**	0.439	0.306	0.300	0.529	0.451	0.376
P (Kruskal-Wallis ANOVA)	**0.006**	0.348	0.300	0.506	0.666	0.504	0.441
Group A							
N	16	16	16	16	16	16	16
Mean	9.66 [d]	42.13	0.313	2.000	0.375	51.13	4.063
Median	9.75 [d]	44.50	0.000	2.000	0.000	50.00	4.000
SD	0.49	5.08	0.479	1.265	0.500	5.28	1.569
Group B							
N	16	16	16	16	16	16	16
Mean	10.23 [a]	38.38	0.125	2.813	0.625	54.63	3.438
Median	10.25 [a]	35.50	0.000	2.500	0.500	56.50	3.000
SD	0.39	7.97	0.342	1.760	0.719	8.27	1.263
Group C							
N	16	16	16	16	16	16	16
Mean	10.17 [a]	39.63	0.125	2.313	0.625	53.75	3.563
Median	10.10 [a]	43.00	0.000	2.000	0.000	51.00	4.000
SD	0.50	10.94	0.342	1.352	0.885	10.00	1.094

P probability with significance level α=0.05 highlighted in bold
[a] significant difference between group and control group A
[b] significant difference between group and group B
[c] significant difference between group and group C
[d] significant difference between group and both other groups

13.2. Certificates

13.2.1. Control Analysis of Diet – Pre-starter

[redacted] (b) (4)

Report of Analysis

09. Mrz. 09

DSM Nutritional Products Ltd
Dr. J. Broz
P.O. Box 3255, VFA, B. 241/867
CH-4002 **Basel**
Switzerland

Parameter: Phytase
Product: IPA Mash Phytase
Batch used: PPQ28656

Request N°: 05/2009
Theme N°: 6106

Registration date: 04.03.2009 **Customer/Manufacturer:** Biopharm a.s.

Sample Number	Sample Label	Declaration U/kg	Found U/kg	Average	STDEV	CV
01	Group A - M	< 50	544 404	**474**	99	21%
01 rep.	Group A - M	< 50	462 496	**479**	24	5%
02	Group B - M	4000	4602 5579	**5090**	691	14%
03	Group C - M	40000	45495 41531	**43513**	2802	6%

M - mash E - expanded Page 1 of 1
P - pellet F - flour
C - crumb TQ -Tel Quel LOD - Limit of Detection
PM - premix LOQ - Limit of Quantification

Responsible Analyst
J. König

13.2.2. Control Analysis of Diet – Starter

[redacted] (b) (4)

Report of Analysis

27. Mrz. 09

DSM Nutritional Products Ltd
Dr. J. Broz
P.O. Box 3255, VFA, B. 241/867
CH-4002 **Basel**
Switzerland

Parameter: Phytase
Request N°: 08/2009 **Product:** IPA Mash Phytase
Theme N°: 6106 **Batch used:** PPQ28656

Registration date: 25.03.2009 **Customer/Manufacturer:** Biopharm a.s.

Sample Number	Sample Label	Declaration U/kg	Found U/kg	Average	STDEV	CV
01	Group A - M	< 50	397			
			368	**383**	20	5%
02	Group B - M	4000	4008			
			4974	**4491**	680	15%
03	Group C - M	40000	38068			
			38343	**38206**	190	0%

M - mash E - expanded
P - pellet F - flour
C - crumb TQ -Tel Quel
PM - premix

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LOD - Limit of Detection
LOQ - Limit of Quantification

Responsible Analyst
J. König
J. König

efsa

European Food Safety Authority

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA Mash phytase (M)** Batch number: **PPQ 28656**

Trial ID: **253/2008** Location: (b) (4)

Start date and exact duration of the study: **04/03/2009, 6 weeks**

Number of treatment groups (+ control(s)): **3** Replicates per group: **8**

Total number of animals: **48** Animals per replicate: **2**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **<50, 4000, 40 000 U/kg** Analysed: **479/383 (native activity) 5090/4491, 43 513/38 206 U/kg**

†

Substances used for comparative purposes:

Intended dose: Analysed:

Animal species/category: **weaned piglets**

Breed: **Large White x Landrace** Identification procedure: **ear number, box label**

Sex: **both sexes** Age at start: **4 weeks** Body weight at start: **10.6 kg**

Physiological stage: **post-weaning** General health: **good**

Additional information for field trials:

Location and size of herd or flock:

Feeding and rearing conditions:

Method of feeding:

Diets (type(s)): **prestarter and starter diets**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **wheat, barley, soybean meal, wheat flour**

Nutrient content (relevant nutrients and energy content)

Intended values: **prestater: 20.08% CP, 1.40% lysine; starter: 18.83% CP, 0.98% lysine**

Analysed values: **prestater: 19.25% CP, 1.45% lysine; starter: 16.79% CP, 0.97% lysine**

Date and nature of the examinations performed: **blood analyses, post mortem necropsy**

Method(s) of statistical evaluation used: **analysis of variance, LSD procedure**

Therapeutic/preventive treatments (reason, timing, kind, duration): **none**

Timing and prevalence of any undesirable consequences of treatment: **none**

Date **August 2009**	Signature Study Director

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

2

Annex 2

Tolerance study with IPA Mash Phytase in gestating and lactating sows

REPORT No. 00003288

Report

Title:

Tolerance study with IPA Mash Phytase in gestating and lactating sows

Institute number: SL 1/09
Notification number: A 0298/97

Sponsor:
DSM Nutritional Products Ltd
Animal Nutrition and Health R & D
CH-4002 Basel

Investigator:
(b) (4)
[redacted]

Responsibilities

Study director:

(b) (4) [redacted]

Study monitor:

(b) (4) [redacted]

Feed producers:

(b) (4) [redacted]

Trial site and research facility personnel:

(b) (4) [redacted]

Veterinary surgeon:

(b) (4) [redacted]

Documentation and biostatistics:

(b) (4) [redacted]

Analytical Lab:

(b) (4)

[redacted]

Table of contents

Tolerance study with IPA Mash Phytase in gestating and lactating sows

1 Abstract

A tolerance study was conducted for IPA Mash Phytase in diets for sows during one reproduction cycle (day 1 of pregnancy to successful service after weaning of the 3rd lactation period). The enzyme test product was containing a 3-phytase derived from *Citrobacter braakii*, expressed in a genetically modified strain of *Aspergillus oryzae*. For this reason standard diets during pregnancy, lactation and the following service period after weaning were offered during a overall 152-day feeding period unsupplemented or supplemented with the maximum recommended level (4 000 U/kg corresponding to 70.1 ppm) or with a tenfold overdose (40 000 U/kg corresponding to 701 ppm) of IPA Mash Phytase, respectively. The tolerance study was carried out on a commercial farrows-to-weaned piglet farm with a breeding stock of 500 sows. Two days before the expected beginning of return of heat after the 2nd farrowing a total of 36 multiparous sows were randomly assigned to three groups of 12 animals each. After successful artificial insemination the sows were kept until day 108 of pregnancy at individual sow feeding pens with straw bedding. From 109 days of pregnancy onwards sows were transferred to an environmentally controlled farrowing stable with straw bedding and three compartments with 12 pens each. The potential preventing of cross-contamination as far as possible was assured by one free row between each occupied compartment. Housing systems and climate quality were in accordance to standard conditions in commercial pig breeding farms. The feed allowance during pregnancy was geared towards the optimal body conditioning score (BCS: 3.5). During the first 6 days after farrowing the amount of lactation diet was enhanced continuously from 3.1 kg in daily increments of 0.5 kg per sow until maximum feeding capacity was reached. From day 25 of lactation onwards the lactation diet was continuously reduced to 2 kg at the day of weaning. During the following 10-day service period after weaning sows were transferred to the pregnancy house and offered daily 3.1 kg of the lactation diet. The piglets (EUROC x Pietrain) were weaned at 28 days and received a supplement without IPA Mash Phytase from day 11 to day 28 of the suckling period. Tolerance of IPA Mash Phytase was assessed by weight development of the sows (pregnancy: days 1, 112; lactation: days 28), feed intake and, in the piglets, litter weight development (at 1 and 28 days of age) and consumption of prestarter diet. In addition, faecal consistency of sows and piglets was analysed daily and haematological and biochemical blood parameters were analysed for sows at day 24 of lactation. With regard to the overall reproductive cycle the overdose of IPA Mash Phytase (40000 U/kg) led to no detrimental effects on relevant performance and health parameters when compared to sows fed without or with IPA Mash Phytase at the maximum recommended level (4000 U/kg). Body weight losses during the lactation period were significantly 88.6% lower than those of sows fed without IPA Mash Phytase. Body weight gains of suckling piglets from sows fed with the overdose level were significantly improved by 24.4% when compared to sows fed without IPA Mash Phytase. Additionally the tenfold overdose level of IPA Mash Phytase showed no negative effects on the interval from weaning to onset of oestrus as well as on faecal consistency. Haematological and biochemical blood parameters were also within the respective reference range. It summary it can be concluded that IPA Mash Phytase fed at the tenfold overdose level is a safe feed additive with positive effects on sow performance (significantly reduced body weight losses during lactation as well as significantly improved litter weight gains) without any negative health or fertility relevant effect characterized by blood examination, faecal consistency and weaning to service interval.

Tolerance study with IPA Mash Phytase in gestating and lactating sows

2 General information

2. 1 Study	Efficacy and tolerance study with IPA Mash Phytase at the maximum recommended and tenfold overdose level in diets for sows offered during the complete reproductive cycle (day 1 of pregnancy to successful service after weaning of the third litter) involving performance parameters, health status and blood haematology.
2.2 Animals:	• 36 sows (EUROC line) (Hülsenberger Zuchtschweine GmbH, 33803 Steinhagen); • 380 piglets of both sexes (EUROC x Pietrain).
2.3 Feed:	• Complete diet for gestating sows pregnancy: days 1 to 108 • Complete diet for lactating sows pregnancy: days 109 to 114; lactation: days 1 to 28 weaning to successful service: days 1 to 10 • Prestarter diet for suckling piglets 11th to 28th day of age.
2.4 Test product:	IPA Mash Phytase (bacterial 3-phytase derived from *Citrobacter braakii* expressed in *Aspergillus oryzae)* Batch: LOT PPQ 28656 Dose levels in complete diets for gestating and lactating sows: 4 000 U/kg of feed (corresponding to 70.1 ppm) 40 000 U/kg of feed (corresponding to 701 ppm).
2.5 Trial site:	[redacted] (b) (4)
2.6 Study personnel:	[redacted] (b) (4)
2.7 Time schedule:	Start of experiment: 15th July 2009 End of experiment: 30th October 2009.

Tolerance study with IPA Mash Phytase in gestating and lactating sows

3 Introduction

The objective of the present study was to evaluate the tolerance of IPA Mash Phytase in diets for sows during one reproduction cycle (day 1 of pregnancy to successful service after weaning of the 3rd litter). This study was required for an EU registration in accordance with the Commission Regulation (EC) No. 429/2008. The enzyme test product was containing a 3-phytase derived from *Citrobacter braakii*, expressed in a genetically modified strain of *Aspergillus oryzae*. For this reason standard basal diets for sows during pregnancy, lactation and successful service after weaning were offered unsupplemented or supplemented at the maximum recommended level (4 000 U/kg) or at the tenfold overdose level (40 000 U/kg) of IPA Mash Phytase, respectively. Performance data were monitored during the complete reproductive cycle of sows and their piglets. Additionally blood haematology of sows as well as health status of sows and piglets was recorded.

4 Study descriptions

The study was carried out on a commercial farrows-to-weaned piglet farm with a breeding stock of 500 sows. Two days before the expected beginning of return of heat after farrowing a total of 36 multiparous sows with comparably litter numbers were allocated to the experimental groups. Details of the study design are shown in Table 1. Additionally three sows were assigned for each treatment group as resources in the case of possible negative ultrasonic pregnancy test with the experimental sows after 22 days of insemination.

Table 1: Study design

Treatment group	**A**	**B**	**C**
Total number of sows (n)	12	12	12
Litter size (number of piglets)	10 to 12	10 to 12	10 to 12
Overall feeding period (days)	152	152	152
Feeding schedule for sows			
• diet for gestating sows (days of experiment)	1 to 108	1 to 108	1 to 108
• diet for lactating sows (days of experiment)	109 to 152	109 to 152	109 to 152
Feeding schedule for piglets			
• prestarter diet (days of age)	11 to 28	11 to 28	11 to 28
IPA Mash Phytase (U/kg feed)			
• diet for gestating sows	0	4 000	40 000
• diet for lactating sows	0	4 000	40 000
• prestarter diet	0	0	0

5 Animals and methods

5.1 Animals

A total of 36 multiparous sows (EUROC line) in the body weight range 160 to 210 kg were obtained before service after weaning from a pool of fifty sows of the breeder farm after the 2nd to 4th lactation period with regard to body weight as well as litter number and litter size during the foregoing lactation period. After assigning to the treatment groups and determine of heat the sows were artificially inseminated with sperm from Pietrain. The piglets of the following litter number were therefore crossbreds (EUROC x Pietrain).

5.2 Housing

After successful artificial insemination the sows were kept until day 108 of pregnancy at individual sow feeding pens with straw bedding. From 109 days of pregnancy onwards sows were transferred to an environmentally controlled farrowing stable with straw bedding and three compartments (A, B, C) with 12 pens each. The potential preventing of cross-contamination as far as possible was assured by one free row between each occupied compartment. Housing system and climate quality were in accordance to standard conditions in commercial pig breeding farms. The average house temperature at sow level was maintained at the temperature range 20 to 22 °C. The temperature in the piglet lying area was set at about 32° C for the entire 28-day suckling period using infrared heaters. In order to avoid overheating of the sows the heaters were mounted on the side wall in the far third of each pen. The relative humidity was in the range of 55 to 60%. Details of daily measured environmental temperature as well as relative humidity are presented in Figures 1 to 4. The calculated ventilation capacity was in the range of 2.0 changes per hour. Feed was offered in automatic feeders. Fresh drinking water was continuously supplied by drinking bowls for sows and piglets. In order to eliminate potential handling-related contaminations all daily management tasks were performed starting with the unsupplemented control group.

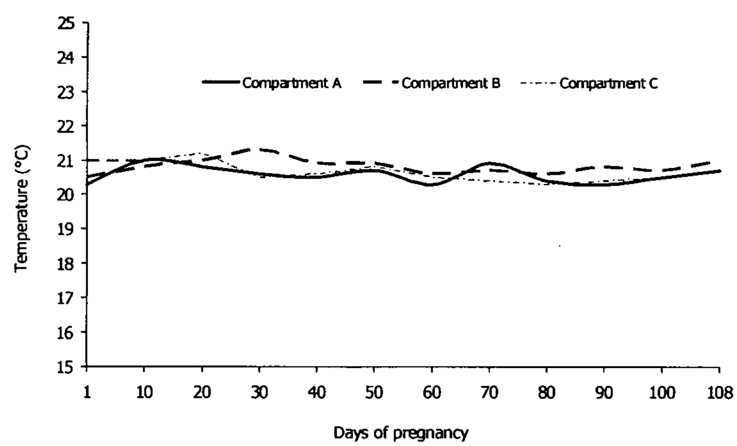

Figure 1. Temperature in the pregnancy stable

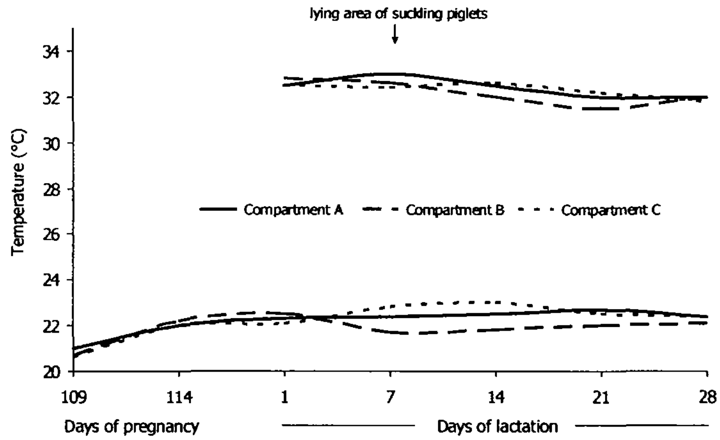

Figure 2. Temperature in the farrowing stable

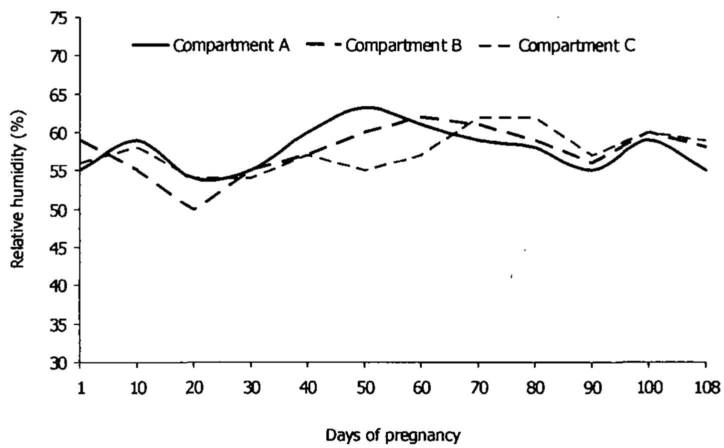

Figure 3. Relative humidity in the pregnancy stable

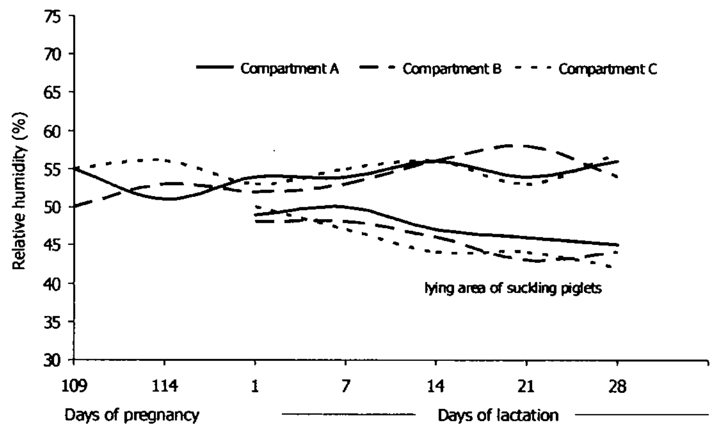

Figure 4. Relative humiditiy in the farrowing stable

5.3 Feeding

During the complete reproductive cycle sows were fed with basal diets for gestating and lactating sows which were following the recommendations of the German Society for Nutrition Physiology for pigs (GfE 2006). All experimental diets for sows were manufactured in a compound feed mill (Gerswalder Mühle GmbH). The composition of the diets for sows is given in Tables 2 and 3, respectively. From day 1 until day 114 of pregnancy 3.1 kg (diet for pregnant sows: days 1 to 108; diet for lactating sows: days 109 to 114) was offered semi-daily per sow. The feed allowance during pregnancy was geared towards the optimal body conditioning score (BCS: 3.5). During the first 6 days after farrowing the amount of lactation diet was enhanced continuously from 3.1 kg in daily increments of 0.5 kg per sow until maximum feeding capacity was reached, which was defined in this trial as the amount resulting in daily leftovers in the trough of about 100 g after the 1:00 pm feeding. Amounts of feed dispensed and any leftovers and losses were recorded daily. From day 25 of lactation onwards the lactation diet was continuously reduced to 2 kg at the day of weaning. During the following 10-day service period after weaning sows were offered daily 3.1 kg of the lactation diet.

The piglet prestarter diet was manufactured in the feed mill owned by the institute (registration number: DE-BE-100001) and offered ad libitum from day 11 of the suckling period via automatic feeders accessible only for the piglets. The composition of the piglet prestarter is shown in Table 4. The diet was manufactured in one batch of 500 kg without phytase enzyme supplementation or other feed additives with exception of trace minerals, amino acids and vitamins.

Basal diets for sows were also prepared without inclusion of feed additives with exception of trace elements, amino acids as well as vitamins and were mixed in three lots of 4500 and 1800 kg each and were subsequently divided into equal parts for IPA Mash Phytase addition either at the maximum recommended dose level of 4 000 U/kg or at the tenfold overdose level (40 000 U/kg), respectively. All diets for sows and piglets were offered in mash form and made at least two weeks before the trial start to allow time for checking the content of IPA Mash

Phytase in the sow diets and were stored in a cool dry place until required. Representative samples (2 x 800 g) from each diet batch manufactured for the trial were collected, labelled and identified. One sample was analysed for nutritional composition. The second sample was analysed for phytase activity.

Table 2. Composition and calculated metabolisable energy (ME) and nutrient concentrations of the basal diet for gestating sows

Treatment group		**Basal diet (A, B, C)**
Composition:		
Barley	g/kg	450.00
Wheat	g/kg	180.00
Wheat bran	g/kg	140.00
Soybean meal (CP: 48%)	g/kg	60.00
Oat bran	g/kg	60.00
Dried sugar beet pulp	g/kg	40.00
Premix*	g/kg	27.00
Plant oil	g/kg	15.00
Calcium carbonate	g/kg	13.00
Beet molasses	g/kg	10.00
Sodium chloride	g/kg	5.00
Calculation:		
ME	MJ/kg	11.57
Crude protein	g/kg	135.11
Crude fiber	g/kg	75.00
Crude fat	g/kg	35.00
Starch	g/kg	347.10
Total sugar	g/kg	35.90
Lysine	g/kg	6.80
Methionine	g/kg	2.20
Methionine/Cystine	g/kg	4.66
Threonine	g/kg	4.60
Tryptophan	g/kg	1.60
Calcium	g/kg	6.80
Phosphorus	g/kg	4:50
Digestible phosphorus	g/kg	1.90
Sodium	g/kg	2.20

** Contents per kg feed: 14.500 IU vit. A; 1600 IU vit.D_3; 80 mg vit. E; 2,7 mg vit. K_3; 2 mg vit. B_1; 6,2 mg vit. B_2; 25,5 mg niacin ; 4,0 mg vit. B_6; 34 µg vit. B_{12}; 567 µg Biotin; 15 mg pantothenic acid ; 1,5 mg folic acid; 200 mg betaine; 1,6 g Mg; 121 mg Zn; 220 mg Fe; 120 mg Mn; 16 mg Cu; 2,7 mg J; 0,8 mg Co; 0,28 mg Se.*

Table 3. Composition and calculated metabolisable energy (ME) and nutrient concentrations of the basal diet for lactating sows

Treatment group		**Basal diet (A, B, C)**
Composition:		
Barley, clean	g/kg	645.40
Soybean meal (CP: 48%)	g/kg	168.00
Wheat bran	g/kg	80.00
Wheat, cleaned	g/kg	50.00
Monocalcium phosphate	g/kg	11.00
Beet molasses	g/kg	10.00
Soya oil	g/kg	10.00
Premix*	g/kg	10.00
Calcium carbonate	g/kg	8.60
Sodium chloride	g/kg	5.20
L-Lysine	g/kg	1.80
Calculation:		
ME	MJ/kg	12.37
Crude protein	g/kg	165.30
Crude fiber	g/kg	55.00
Crude fat	g/kg	35.50
Starch	g/kg	374.20
Total sugar	g/kg	40.10
Lysine	g/kg	8.98
Methionine	g/kg	2.48
Methionine/Cystine	g/kg	5.46
Threonine	g/kg	5.75
Tryptophan	g/kg	2.08
Calcium	g/kg	8.80
Phosphorus	g/kg	6.70
Digestible phosphorus	g/kg	2.90
Sodium	g/kg	2.30

** Contents per kg feed: 17.996 IU vit. A; 2000 IU vit.D_3; 100 mg vit. E; 2,7 mg vit. K_3; 2 mg vit. B_1; 6,2 mg vit. B_2; 25,5 mg niacin ; 4,0 mg vit. B_6; 34 µg vit. B_{12}; 567 µg Biotin; 15 mg pantothenic acid ; 1,5 mg folic acid; 200 mg betaine; 1,6 g Mg ; 121 mg Zn; 220 mg Fe; 120 mg Mn; 20 mg Cu; 2,7 mg J; 0,6 mg Co; 0,38 mg Se.*

Table 4. Composition and calculated metabolisable energy (ME) and nutrient concentrations of the prestarter for suckling piglets (11th to 28th day of age)

Treatment group		**Prestarter diet (A, B, C)**
Composition:		
Wheat	g/kg	440.70
Soybean meal (HP: 48%)	g/kg	274.00
Skimmed-milk powder	g/kg	120.00
Rolled oats	g/kg	100.00
Calcium carbonate	g/kg	12.70
Premix*	g/kg	12.00
Monocalcium phosphate	g/kg	11.30
Soya oil	g/kg	10.00
L-Lysine	g/kg	3.00
DL-Methionine	g/kg	1.60
L-Tryptophan	g/kg	0.70
Sucrose	g/kg	14.00
Calculation:		
ME	MJ/kg	13.97
Crude protein	g/kg	249.30
Crude fibre	g/kg	22.90
Crude fat	g/kg	30.10
Starch	g/kg	334.80
Total sugar	g/kg	111.70
Lysine	g/kg	16.40
Methionine/Cystine	g/kg	9.90
Threonine	g/kg	9.70
Tryptophan	g/kg	3.80
Calcium	g/kg	9.50
Phosphorus	g/kg	7.60
Digestible phosphorus	g/kg	5.80
Sodium	g/kg	1.60

** Contents per kg feed: 20000 IU Vit. A; 3200 IUg/kg Vit. D_3; 91 mg Vit. E; 3 mg Vit. K_3; 3 mg Vit. B_1; 5.5 mg Vit. B_2; 36 mg niacin; 5.2 mg Vit. B_6; 39 µg Vit. B_{12}; 2g/kg60 µg biotin; 23.4 mg pantothenic acid; 2.0 mg folic acid; 40 mg Zn; 210 mg Fe; 150 mg Zn, 110 mg Mn; 24 mg/kgg Cu; 1.3 mg Co; 0.5 mg Se; 2.0 g Mg.*
g/kg

5.4 Measured variables

5.4.1 Body weight

All sows were weighed at the time of insemination (1st day of pregnancy), before farrowing (112th day of pregnancy) and at the end of the lactating period (day 28 after farrowing). All piglets were weighed individually per litter during the 28 day suckling period at 1 and 28 days of age.

5.4.2 Feed intake

During pregnancy, lactation and service after weaning daily feed intakes of sows were calculated with regard to possible leftovers. The averaged prestarter intake of suckling piglet was recorded daily per litter between 11 and 28 days of age.

5.4.3 Health status

All sows and piglets were checked daily throughout the trial for their health status (visual inspection). Any necessary treatments and deviations from normal health status were recorded. Finally, a diarrhoea score on litter basis was calculated after a daily monitoring of each litter.

Faecal consistency scores was used as follows:

1	=	normal	(DM > 25%)
2	=	pasty	(DM 24 to 18%)
3	=	watery	(DM < 18%)
4	=	watery with colour changes	(DM < 18%)

5.4.4 Blood examination

In order to exclude possible health risks of IPA Mash Phytase blood examinations in sows receiving normal standard diets with phytase activities at 4 000 or 40 000 U/kg were compared to those which were fed with the normal standard diet without supplementation of IPA Mash Phytase. Blood was taken by all experimental sows from the vena cranialis at the 24^{th} day after farrowing for measuring cell counts of erythrocytes, leukocytes and thrombocytes as well as means corpuscular volume (MCV), mean corpuscular haemaglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Additionally electrolytes (sodium, potassium, chloride, phosphorus, and calcium), triglycerides, cholesterol, urea, glucose, albumin, total protein, and enzymes (alanine-amino-transferas = ALAT, aspartate-amino-transferase = ASAT, gamma-glutamyl-transferase = GGT, alkaline phosphatase) were analysed in blood or plasma, respectively. The samples were obtained by "Landeslabor Berlin Brandenburg".

5.5 Analyses

Diets were analysed after milling (sieve size: 0.25 mm) by the Weender technique, including starch, total sugars, calcium, phosphorus and sodium determination, in accordance to the official VDLUFA methodology (dry matter: VDLUFA 3.1; crude protein: VDLUFA 4.1.2 modified according to macro-N determination (vario Max CN); crude fibre: VDLUFA 6.1.1; crude ash: VDLUFA 8.1; crude fat: VDLUFA 5.1.1; starch: VDLUFA 7.2.5; total sugars: VDLUFA 7.1.1; calcium: VDLUFA 10.3.1 modified according to DIN EN ISO 11885; phosphorus: VDLUFA 10.6.1 modified according to DIN EN ISO 11885; sodium: VDLUFA modified according to DIN EN ISO 11885) by the institute. Parallel feed samples of diets for pregnant and lactating sows were used for measuring the activity of IPA Mash Phytase by DSM-Biopract GmbH.

5.6 Statistical evaluation

Results are presented as means ± standard deviation. Data analysis was based on "Oneway ANOVA". Statistical analyses was performed with the software package SPSS (SPSS, Inc. Chicago, IL). After checking the homogeneity of the variances means were compared by the usual test procedures (Sheffe test, Tukey test). The significance level was set at $p < 0.05$.

6 Results

The trial proceeded without incidents on the whole. The results of the feed analyses are presented in Table 5. They confirm the values assumed for ration formulation purposes. Table 6 shows the averaged enzyme activity for IPA Mash Phytase in both sow diets. The enzyme equivalents were only slightly higher (gestation) or lower than intended (lactation), but especially with regard to the native phytase activity of 325 (gestation) and 409 U/kg (lactation) still within the intended range.

Table 5. Analysed nutrient concentrations in the sow diets and prestarter diet (means referred to original matter)

Diets (treatment A, B, C)		Gestation	Lactation	Prestarter
Dry matter	%	89.10	90.17	90.79
Crude protein	%	13.91	17.07	24.97
Crude fiber	%	6.87	5.42	2.20
Crude ash	%	6.15	5.16	6.12
Crude fat	%	3.88	3.73	3.10
Starch	%	36.01	37.62	33.43
Total sugar	%	4.05	4.21	10.12
Calcium	%	0.75	0.98	0.97
Phosphorus	%	0.53	0.70	0.79
Sodium	%	0.19	0.21	0.25

Table 6. Analytical results of the sow diets

Treatment		A	B	C
ISP Mash Phytase	U/kg			
Gestation				
• intended		0	4 000	40 000
• analysed		325 (native)	4 911	45 895
Lactation				
• intended		0	4 000	40 000
• analysed		409 (native)	4 313	40 435

6.1 Performance data during pregnancy

The performance data generated during the overall pregnancy period are presented in Table 7. The sows weighed 184 kg on average at the start of the trial. By day 112 of pregnancy an average body weight of 237.6 kg was recorded which corresponded to a body weight gain of 53.6 kg per sow. Sows fed with ISP Mash Phytase tended dose dependently to slightly higher body weight gains. However, significant treatment effects could not be monitored.

The sows consumed on average 3.13 kg feed per head and day during the 114-day pregnancy period. Consequently, detrimental effects of the overdose on feed intake could be excluded.

The scores for daily faecal consistency were within a range that did not indicate any adverse health effects at any time. The overall average was slightly above 1 thus reflecting only few changes in the physiological faecal consistency (dry matter > 25%). Diarrhoea with liquid faeces was not seen in either feeding group.

The incidence of sickness was mainly characterized by claw injuries and absesses. Respective treatments were in accordance to the veterinarian practice. Treatment-related effects could be excluded.

Table 7. Performance and health status of sows during the pregnancy period

Treatment		**A**	**B**	**C**	**Oneway Anova**
ISP Mash Phytase	U/kg	0	4 000	40 000	
Sows	n	12	12	12	**P**
Replicates	n	12	12	12	
• **Pregnancy**					
1st to 114th day of pregnancy					
→ Body weight	kg				
- 1st day		185.3 ± 12.2	185.4 ± 9.9	182.3 ± 9.2	0.705
- 112th day		235.8 ± 9.9	236.8 ± 11.8	240.1 ± 11.3	0.618
→ Feed intake (1st to 114th day)	kg				
- overall		357 ± 0	357 ± 0	357 ± 0	1.000
- daily		3.13 ± 0	3.13 ± 0	3.13 ± 0	1.000
→ Faecal score* (1st to 114th day)	n	1.12 ± 0.06	1.04 ± 0.08	1.07 ± 0.08	0.064
→ Incidences of sickness					
- Claw injuries	n	2	1	2	
- Abscesses	n	1	1	2	

*Scores: 1 = normal (DM > 25%); 2 = pasty (DM 24 -18%),3 = liquid (DM < 18%); 4 = liquid (DM < 18%) + colour changes

6.2 Performance data during lactation period

6.2.1 Sows

The performance data recorded during the 28-day lactation period have been summarised in Table 8. Neither the number of piglets born alive nor the number of stillborn piglets showed any significant treatment-related differences. In order to minimize litter size effects during the suckling period litter sizes were slightly equalized. The body weights at the end of the lactation period were in the range of 209.5 kg. Sows fed with ISP Mash Phytase tended to higher body weights when compared to sows fed without ISP Mash Phytase. Taking into account, that the conceptus losses are normally in the range of 25 kg (GfE 2006) the overall body weight losses during the 28-day lactation period amounted to 7.9 kg in sows fed without and 0.75 kg in sows fed with ISP Mash Phytase at both dose levels. However, the differences were not significant. The reduced body weight losses of sows fed with ISP Mash Phytase were not characterized by different amounts of feed intake, as presented in Figure 5. The overall fed intake was 128.5 kg or 4.59 kg per sow and day, respectively. Sows fed with IPA Mash Phytase at the level of 4 000 U/kg tended to slightly lower feed intake by 1.6% when compared to sows fed without or with IPA Mash Phytase at the level of 40 000 U/kg.

Rectal body temperatures measured on the first three days after farrowing averaged 39.23 °C and were therefore 0.6 °C higher when compared to the respective rectal body temperatures at the end of pregnancy. Differences between sows fed without or with IPA Mash Phytase could not be found.

The scores for daily faecal consistency were within a range that did not indicate any adverse health effects at any time. The overall average was slightly above 1 thus reflecting only few changes in the physiological faecal consistency (dry matter > 25%). Diarrhoea with liquid faeces was not seen in either feeding group.

The incidence of sickness was mainly characterized by MMA and claw injuries. Respective treatments were in accordance to the veterinarian practise. Sows fed with ISP Mash Phytase tended to a lower MMA incidence. However, the overall number of MMA was too small for characterizing a positive health potential of ISP Mash Phytase at both dose levels. Therefore treatment-related effects could be excluded.

Table 8. Performance and health status of sows during the lactation period

Treatment		**A**	**B**	**C**	**Oneway Anova**
ISP Mash Phytase	U/kg	0	4 000	40 000	
Sows	n	12	12	12	**P**
Litter number	n	3.2 ± 0.6	3.3 ± 0.6	3.2 ± 0.7	
Replicates	n	12	12	12	
• Number of piglets					
Overall	n	12.7 ± 2.0	13.2 ± 2.2	13.5 ± 2.6	0.669
- - stillborn		2.0 ± 1.8	2.6 ± 20.4	2.3 ± 2.1	0.796
- born alive	n	10.7 ± 0.9	10.6 ± 1.3	11.2 ± 0.8	0.339
- corrected		10.8 ± 0.8	10.7 ±.0.5	11.0 ± 0.7	0.517
Weaned		10.5 ± 0.5	10.3 ± 0.6	10.5 ± 0.8	0.640
• Body weight	kg				
- 112th day of pregnancy		235.8 ± 9.9	236.8 ± 11.8	240.1 ± 11.3	0.618
- 28th day of lactation		202.9 ± 9.8[a]	211.3 ± 7.6[ab]	214.2 ± 12.1[b]	0.026
• Body weight loss	kg	- 32.9 ± 4.7[a]	- 25.6 ± 6.0[b]	- 25.9 ± 3.6[b]	0.001
• Feed intake (1st to 28th d)	kg				
- total		129.3 ± 12.6	127.2 ± 5.5	129.1 ± 11.9	0.867
- per sow and day		4.6 ± 0.5	4.5 ± 0.2	4.6 ± 0.4	0.867
• Body temperature (rectal)	°C				
- 114th day of pregnancy		38.9 ± 0.3	38.6 ± 0.3	38.6 ± 0.4	0.426
- 1st day of lactation		38.5 ± 0.3	39.2 ± 0.4	39.2 ± 0.5	0.999
- 2nd day of lactation		39.2 ± 0.4	39.3 ± 0.4	39.3 ± 0.6	0.724
- 3rd day of lactation		39.2 ± 0.4	39.1 ± 0.4	09.2 ± 0.3	0.781
• Faecal score*	n				
- 1st week of lactation		1.08 ± 0.08[a]	1.13 ± 0.06[ab]	1.16 ± 0.06[b]	0.029
- 2nd week of lactation		1.10 ± 0.10	1.13 ± 0.10	1.12 ± 0.07	0.772
- 3rd week of lactation		1.01 ± 0.03[a]	1.07 ± 0.08[ab]	1.09 ± 0.07[b]	0.010
- 4th week of lactation		1.10 ± 0.08	1.08 ± 0.06	1.08 ± 0.08	0.623
• Incidence of sickness					
- Mastitis, Metritis, Agalacty	n	2	1	1	
- Claw injuries	n	2	1	2	
- Abscess	n	1	1	2	

* Scores: 1 = normal (DM > 25%); 2 = pasty (DM 24 -18%),3 = liquid (DM < 18%); 4 = liquid (DM < 18%) + colour changes

[ab] Means with different superscripts within a row differ significantly (P<0.05)

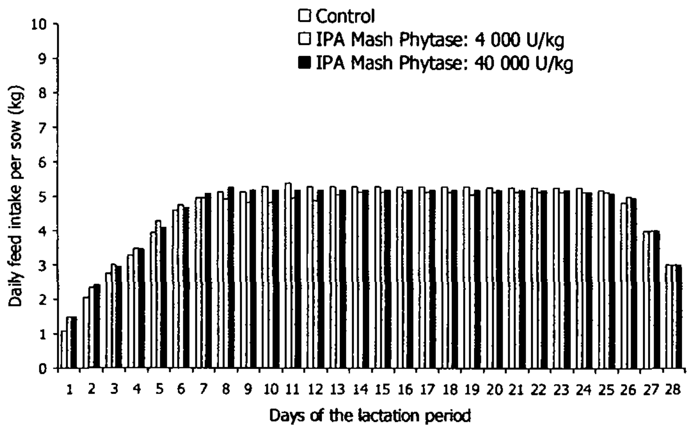

Figure 5. Daily feed intake of sows during the 28-day lactation period

6.2.2 Piglets

The performance of piglets from sows fed without or with supplementation of IPA Mash Phytase at levels of 4 000 and 40 000 U/kg feed is shown in Table 9.

Table 9. Performance of piglets during the 28-day suckling period

Treatment		A	B	C	Oneway Anova
Sows: ISP Mash Phytase	U/kg	0	4 000	40 000	
Litters	n	12	12	12	P
Overall piglets (corrected)	n	130	128	132	
• Litter size (corrected)	n				
- 1st suckling day		10.8 ± 0.9	10.7 ± 0.5	11.0 ± 0.7	0.517
- 28th suckling day		10.5 ± 0.5	10.3 ± 0.6	10.5 ± 0.8	0.640
• Rearing losses	%	3.52 ± 0.22	3.80 ± 0.24	4.50 ± 0.2	0.910
• Body weight	kg				
- 1st day of age		1.48 ± 0,22[a]	1.46 ± 0.29[a]	1.78 ± 0.13[b]	< 0.001
- 28th day of age		6.32 ± 0.56[a]	6.23 ± 0.78[a]	7.80 ± 0.26[b]	< 0.001
• Body weight gain	kg				
- 1st to 28th day of age		4.84 ± 0.54[a]	4.77 ± 0.78[a]	6.02 ± 0.24[b]	< 0.001
• Feed intake					
- overall	kg	0.89 ± 0.12[a]	0.87 ± 0.16[a]	1.05 ± 0.10[b]	0.004
- daily	g	49.4 ± 6.8[a]	48.2 ± 9.1[a]	58.1 ± 5.3[b]	0.004
• Faecal score*					
- 1st week of age		1.41 ± 0.10	1.56 ± 0.22	1.45 ± 0.11	0.057
- 2nd week of age		1.39 ± 0.12	1.38 ± 0.20	1.46 ± 0.09	0.350
- 3rd week of age		1.46 ± 0.10	1.48 ± 0.16	1.43 ± 0.08	0.545
- 4th week of age		1.54 ± 0.24	1.43 ± 0.12	1.45 ± 0.11	0.216
• Incidence of sickness					
- Funiculitis	n	3	4	3	
- Polyarthritis	n	2	1	1	

*Scores: 1 = normal (DM > 25%); 2 = pasty (DM 24 -18%),3 = liquid (DM < 18%); 4 = liquid (DM < 18%) + colour changes

[ab] Means with different superscripts within a line differ significantly (P<0.05)

In order to minimize litter size effects during the suckling period litter sizes were slightly equalized (s. Table 8). The rearing losses of only 3.94% were reflecting the optimal management and health conditions at the farm. Body weight gain of piglets from sows fed without or with IPA Mash Phytase at the level of 4 000 U/kg amounted to 4.80 kg. Piglets from sows fed with the tenfold overdose (40 000 U/kg) were significantly improved by 25.4% when compared to piglets of sows fed without or with 4 000 U/kg IPA Mash Phytase. As shown in Table 9 and Figure 6 the daily intake of the prestarter diet by piglets from sows fed with the tenfold overdose (40 000 U/kg) was on average 19.3% higher than in piglets from sows without or with supplementation of IPA Mash Phytase at the maximum recommended level (4 000 U/kg). When calculated with reference to the content of metabolisable energy in the supplement (13.97 MJ ME/kg), this means that each piglet consumed 2.37 MJ ME more energy. As the estimated energy requirement per kg of weight gain is 21.74 MJ ME (GfE 2006), the feed induced higher body weight gain of piglets from sows fed with IPA Mash Phytase at the overdose level (40 000 U/kg) amounted about to 110 g. Therefore the significantly improved body weight gain was only by about 9% attributable to the higher consumption of the prestarter diet.

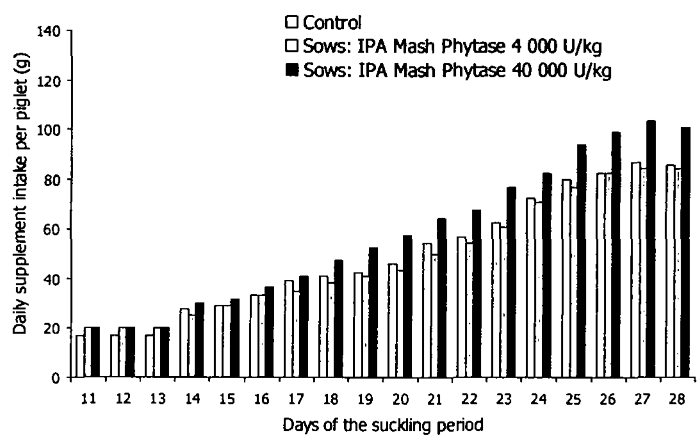

Figure 6. Daily feed intake of the prestarter diet averaged per piglet and litter

The faecal consistency score showed no health-relevant differences. Consequently differences attained no statistical significance. Some piglets showed signs of funiculitis and polyarthritis. Treatment effects could be excluded.

6.3 Blood constituents

For further identifying safety of IPA Mash Phytase blood examinations were used at the 26th day after farrowing which was corresponding to a 140-day supplementation period The results are given in Table 10. For better appraisal the respective reference values given by the literature are additionally shown in Table 11. It was observed that all means were within the physiological range. With feeding IPA Mash Phytase at the tenfold overdose level means for erythrocytes were significantly lower than those of sows fed with the maximum recommended dose level. The significantly higher means of inorganic phosphate, total cholesterol, urea and glucose were mainly reflecting the higher performance status when compared to sows fed without or with IPA Mash Phytase at the maximum recommended level (4 000 U/kg). However, all differences between the treatments were still within the reference range. Therefore, IPA Mash Phytase is characterized by a high safety potential.

Table 10. Haematological results in sows fed control or IPA Mash Phytase supplements at 24 days after farrowing

Treatment		A	B	C	Oneway Anova
Sows	n	12	12	12	
Replicates	n				P
ISP Mash Phytase	U/kg	0	4000	40000	
• Blood cell numbers					
- Erythrocytes	terra/l	5,72 ± 0.84[ab]	5.91 ± 0.43[a]	4.91 ± 1.41[b]	0.049
- Leukocytes	giga/l	17.19 ± 3.84	16.08 ± 1.76	14.87 ± 2.60	0.171
- Thrombocytes	giga/l	241.3 ± 162.8	234.0 ± 84.7	161.8 ± 66.3	0.261
• Blood					
- Haemoglobin	mmol/l	7.38 ± 0.49	7.31 ± 0.49	6.86 ± 0.22	0.129
- haematocrit	%	39.1 ± 4.2	38.8 ± 2.5	37.0 ± 9.9	0.112
- MCV [1)]	fl	68.0 ± 4.4[ab]	65.7 ± 2.8[a]	69.3 ± 2.6[b]	0.048
- MCH [2)]	fmol	1.30 ± 0.08[ab]	1.24 ± 0.07[a]	1.30 ± 0.05[b]	0.046
- MCHC [3)]	Mmol/l	19.1 ± 0.5[ab]	18.8 ± 0.4[a]	18.7 ± 0.3[b]	0.046
• Electrolytes (plasma)					
- Sodium	mmol/l	138.3 ± 2.7	137.5 ± 2.8	136.5 ± 2.5	0.329
- Potassium	mmol/l	7.83 ± 2.89	9.60 ± 3.35	8.25 ± 3.81	0.414
- Chloride	mmol/l	100.7 ± 2.3	99.8 ± 2.2	99.3 ± 2.2	0.326
- Calcium	mmol/l	2.31 ± 0.32	1.83 ± 0,86	2,24 ± 0.21	0.131
- Inorganic phosphate	mmol/l	1.93 ± 0.35[a]	1.72 ± 0.40[a]	2.20 ± 0.21[b]	0.006
• Enzymes (plasma)					
- ALAT*	µkat/l	1.03 ± 0.24	1.15 ± 0.27	1.24 ± 0.19	0.121
- ASAT**	µkat/l	0.76 ± 0.30	0.61 ± 0.17	0.53 ± 0.16	0.054
- GGT***	µkat/l	1.22 ± 0.46	0.91 ± 0.13	0.95 ± 0.65	0.220
- Alkaline Phosphatase	µkat/l	0.79 ± 0.34	0.64 ± 0.23	0.79 ± 0.39	0.421
• Metabolites (plasma)					
- Total cholesterol	mmol/l	1.40 ± 0.16[a]	1.59 ± 0.31[a]	1.84 ± 0.22[b]	0.001
- Triglycerides	mmol/l	0.87 ± 0.46	0.56 ± 0.25	0.66 ± 0.33	0.110
- Creatinine	mmol/l	176.8 ± 28.2	157.9 ± 19.8	152.9 ± 26.1	0.065
- Urea	µmol/l	4.79 ± 1.17[a]	4.11 ± 0.72[a]	6.93 ± 2.15[b]	< 0.001
- Bilirubin	µmol/l	1.75 ± 0.68	1.85 ± 0.87	1.57 ± 0.52	0.644
- Glucose	mmol/l	2.64 ± 0.23[ab]	2.45 ± 0.40[a]	2.91 ± 0.40[b]	0.015
- Albumin	g/l	40.8 ± 3.7	39.5 ± 5.6	36.8 ± 10.0	0.693
- Total protein	g/l	81.1 ± 8.3	86.0 ± 8.8	83.1 ± 4.9	0.295

* Alanine-Amino-Transferase ** Aspartate-Amino-Transferase *** Gamma-Glutamyl-Transferase

[1)] Mean cellular volume; [2)] mean cellular haemoglobin; [3)] mean haemoglobin concentration

[ab] Means with different superscripts within the same line differed significantly

Table 11. Reference values for selected blood parameters in sows

Treatment		Reference range	Literature
• Blood cell numbers			
- Erythrocytes	terra/l	5.5 - 8.1	Kraft and Dürr (2005)
- Leukocytes	giga/l	10 - 25	Kraft and Dürr (2005)
- Thrombocytes	giga/l	100 - 320	Kraft and Dürr (2005)
• Blood			
- Haemoglobin	mmol/l	6.7 - 9.2	Kraft and Dürr (2005)
- Haematocrit	%	33 45	Kraft and Dürr (2005)
- MCV [1)]	fl	50 - 65	Lahrmann (2009)
- MCH [2)]	fmol	1.0 - 1.3	Lahrmann (2009)
- MCHC [3)]	mmol/l	19 - 22	Lahrmann (2009)
• Electrolytes (plasma)			
- Sodium	mmol/l	140 - 160	Kraft and Dürr (2005)
- Potassium	mmol/l	4.0 - 5.0	Kraft and Dürr (2005)
- Chloride	mmol/l	102 - 106	Kraft and Dürr (2005)
- Calcium	mmol/l	2.4 - 3.0	Kraft and Dürr (2005)
- Inorganic phosphate	mmol/l	2.1 - 3.3	Kraft and Dürr (2005)
• Enzymes (plasma)			
- ALAT*	µkat/l	< 1.1	Kraft and Dürr (2005)
- ASAT**	µkat/l	< 0.6	Kraft and Dürr (2005)
- GGT***	µkat/l	< 4.3	Kraft and Dürr (2005)
- Alkaline Phosphatase	µkat/l	< 2.9	Kraft and Dürr (2005)
• Metabolites (plasma)			
- Total cholesterol	mmol/l	1.5 - 3.3	Plonait und Bickhardt (1988)
- Triglycerides	mmol/l	0.2 - 2.0	Kraft and Dürr (2005)
- Creatinine	mmol/l	100 - 2000	Kraft and Dürr (2005)
- Urea	mmol/l	3.3 - 8.3	Kraft and Dürr (2005)
- Bilirubin	mmol/l	< 4.3	Kraft and Dürr (2005)
- Glucose	mmol/l	2.2 - 6.4	Kraft and Dürr (2005)
- Albumin	g/l	18 - 45	Plonait und Bickhardt (1988)
- Total protein	g/l	< 86	Kraft and Dürr (2005)

* Alanine-Amino-Transferase; ** Aspartate-Amino-Transferase; *** Gamma-Glutamyl-Transferase
[1)] Mean cellular volume; [2)] mean cellular haemoglobin; [3)] mean haemoglobin concentration

6.4 Fertility parameters of sows after weaning

In addition to rearing performance the weaning to service interval, i.e. the number of days from weaning to successful service was measured. The results are presented in Table 12. The differences did not attain statistical significance. None of the inseminated sows showed signs of return rate up to the 35th day of pregnancy. From the results it could be concluded, that with adding IPA Mash Phytase at levels of 4 000 or 40 000 U/kg no negative effects on fertility of sows occurred.

Table 12. Effect of ISP Mash Phytase on fertility parameters of sows

Group		**A**	**B**	**C**	**Oneway Anova**
Sows	n	12	12	12	
Replicates	n	12	12	12	P
ISP Mash Phytase	U/kg	0	4 000	40 000	
Weaning to service interval	days	6.4 ± 1.1	6.8 ± 1.0	6.5 ± 1.0	0.708

7 Discussion

The objective of this long-term study, which extended over the third complete reproductive cycle up to the successful service after weaning, was to investigate whether the continuous overdosed supplementation of IPA Mash Phytase at the level of 40 000 U/kg in sow diets (gestation, lactation) is without any negative performance-, reproductive- or health-related effect when compared to sows fed without supplementation of IPA Mash Phytase or with IPA Mash Phytase at the maximum recommended level of 4 000 U/kg, respectively. Performance data of sows were demonstrating that adding overdosed IPA Mash Phytase in diets for sows showed with regard to the tested health- and performance-relevant parameters not any negative effect. The body weight losses during the lactation period were significantly by 88.6% lower than those estimated for sows fed without IPA Mash Phytase. Additionally the body weight gain of piglets from sows fed with the tenfold overdose level were significantly improved when compared to sows fed without or with IPA Mash Phytase at the maximum recommended level of 4 000 U/kg. As the estimated energy requirement per kg of weight gain is 21.74 MJ ME (GfE 2006), the feed induced higher body weight gain of piglets from sows fed with IPA Mash Phytase at the overdose level (40 000 U/kg) amounted only to 110 g body weight gain during the suckling period. Therefore the significantly improved body weight gain was only by about 9% attributable to the higher consumption of the prestarter diet. Reasons for the significantly improved body weight gain of piglets remain speculative, because the trial was only designed for excluding possible negative effects at the overdose level. By the fact that the feed intake of sows was nearly identical between the different treatment groups, and the body weight losses of sows fed with IPA Mash Phytase were lower when compared to sows without feeding IPA Mash Phytase, the results were obviously reflecting additionally positive enzyme effects in sows.

The potential for efficacy of IPA Mash Phytase at the maximum recommended level (4 000 U/kg was only focussed on lower body weight losses of sows during the 28-day lactation period. In contrast to the tenfold overdose level no further positive response on body weight gain of suckling piglets was monitored. The positive effects on reduced body weight losses of sows during the 28-day lactation period were obviously reflecting the enzyme induced improvements. However a definitive elucidation of the mode of action must be part of further experiments, because the tested enzyme induced improvements, as found in former trials, on the availability of calcium, phosphorus, zinc and iron can only partly be responsible for the reduced body weight losses during lactation. Probably effects of improvements in apparent ileal digestibility of amino acids have to be taken into account.

The results of the blood examination, faecal score and the weaning to service interval showed that IPA Mash Phytase had no negative health or fertility relevant effects at the tenfold overdose level. Differences in blood parameters (partly significant) found between IPA Mash Phytase treated sows and sows fed without IPA Mash Phytase were still within the reference range given by the literature.

8 Conclusions

It summary it can be concluded that the long term supplementation of IPA Mash Phytase at the overdose level (40 000 U/kg) in sow diets during a overall reproductive cycle including the successful service after weaning induced lower estimated body weight losses during the 28-day lactation period and significantly improved body weight gains of piglets which were fed a prestarter diet without IPA Mash Phytase. Additionally blood examinations, faecal consistency and the weaning to service interval of sows fed with the tenfold overdose level of IPA Mash Phytase showed no negative health or fertility relevant effects. To that extend a sufficient safety-factor for possible metering mistakes by adding IPA Mash Phytase in diets for sows can be guaranteed.

[redacted] (b) (4) ovember 2006

[redacted] (b) (4)

TAB

3

Annex 3

Evaluation of the effects of graded amounts of a microbial phytase in the weaner piglet

REPORT No. 2500761

REPORT No. 2500761
Regulatory Document

DSM

Document Date:	11-Jun-2009
Author(s):	P. Guggenbuhl, C. Simões Nunes, [redacted] (b) (4)
	DSM Nutritional Products France, BP 170, 68305 Saint Louis, France
Title:	Evaluation of the effects of graded amounts of a microbial phytase in the weaner piglet.
Project No.	6106
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Summary

The aim of the present study (S12-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the zootechnical performance, mineral blood concentrations, digestibility of phosphorus (P) and calcium (Ca) and bone mineralisation and resistance in the weaned piglet. The basal diet, without addition of mineral P, was based on maize, soybean meal and rapeseed meal. IPA phytase was included in the diet at the levels of 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg. A dietary treatment was based in the very slightly modified control diet containing the recommended available P by addition of dicalcium phosphate (diCaP).Supplementation with graded amounts of IPA phytase in piglets induced an increased performance in a dose dependant manner. Inclusion levels over 1000 U/kg were more efficient than the diCaP supplementation. IPA phytase restored dose dependently phosphataemia, calcaemia and phosphatasaemia to physiological levels comparatively to the controls. The mean P faecal concentration of the enzyme supplemented animals was significantly lower than that observed for the animals ingesting the control diet. All the phytase inclusion levels increased the bioavailability of P and accordingly reduced the piglet quantitative faecal excretion of P comparatively to the basal diet. The P digestibility was dose dependant and highly significantly improved with the exception of the lowest phytase inclusion level. The increases represented in comparison to the control group 12, 66, 77, 110, 132, 129, 156 and 149 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively. The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control, of 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg of the phytase were 0.13, 0.73, 0.84, 1.22, 1.50, 1.39, 1.75 and 1.62 g of full available P/kg feed respectively. Ca digestibility was improved by all the inclusion levels of the phytase. IPA phytase supplements improved bone mineralisation and bone resistance comparatively to the non-supplemented animals. In conclusion the IPA phytase improved the digestibility and the apparent absorption of P and Ca, reduced the P faecal excretion, restored phosphataemia, calcaemia and phosphatasaemia to physiologic values, increased bone mineralisation and resistance and improved the zootechnical performance in the weaned piglet fed on a diet containing P exclusively from vegetable origin.

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Nomenclature and Structural Formula (if available)

Liquid form IPA phytase expressed in *Aspergillus oryzae,* batch PPQ28432, activity at pH 5.5 of 26500 U/g.

1. INTRODUCTION

The aim of the present study (S12-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the zootechnical performance, mineral blood concentrations, digestibility of phosphorus (P) and calcium (Ca) and bone mineralisation and resistance in the weaned piglet.
The experiment was performed in July-August 2008 in the facilities of the Centre de Recherche en Nutrition Animale (CRNA), DSM Nutritional Products France, BP 170, 68305 Saint-Louis cedex, France. It has been performed according to the French legal regulations on experiments with live animals.

2. MATERIAL AND METHODS

2.1. Test compounds

The used IPA phytase was expressed in *Aspergillus oryzae*, batch PPQ28432, had an activity at pH 5.5 of 26500 U/g and was in a liquid form.

NRD/CM measured the phytase activity in the enzyme preparation and in the feed. One unit of phytase is defined as the quantity of enzyme which sets free 1 µmole of inorganic phosphate per minute from 0.005 moles per litre sodium phytate at pH 5.5 and at 37°C.

Di-calcium phosphate (diCaP), batch S1784 6212, was supplied by TIMAC Industries and had a P concentration of 18.2 % and a Ca concentration of 24 %.

2.2. Animal trial

One hundred and twenty Large White × Landrace weaner piglets having an initial body weight of 8.03 ± 1.09 kg were used. The animals were allocated to 10 equal groups of 12 animals each and housed in floor-pen cages in two sub-groups (1 of 7 animals and 1 of 5 animals) in an environmentally controlled room. Each pen had a plastic-coated welded wire floor and was equipped with four water nipples and four stainless-steel individualised feeders. Room temperature was initially 27°C and was lowered weekly by about 2°C until 21-22° C and humidity percentage was 50 %.

The piglets were fed, throughout a 32 days observation period, a basal diet without addition of mineral P (group A) or the diet A supplemented with 16 g/kg of diCaP (group B) or with IPA phytase at the levels of 250 U/kg (group C), 500 U/kg (group D), 1000 U/kg (group E), 1500 U/kg (group F), 2000 U/kg (group G), 3000 U/kg (group H), 4000 U/kg (group I) and 8000 U/kg (group J).

The basal diet A was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the available P supply, the animals' requirements according to Henry *et al.* (1989) and NRC (1998). The basal diet A (table 1) had a theoretical P content of 0.41 % and an analysed content of 0.45 %. The theoretical available P in the diet was 1.20 g/kg and the observed availability of 1.09 g/kg.

An indigestible tracer (chromium oxide) was added at a concentration of 0.4 % to all the diets allowing calculation of the digestibility of P and Ca. The feed was distributed *ad libitum* in mash form, under pen feed consumption control, and the animals had free access to drinking water.

The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water in the region is 120 mg/L.

Performance was evaluated for the 32 days of the trial duration. Blood was collected by jugular puncture from all the animals at the 31st day of the experiment for the determination of the P, Ca, alkaline phosphatase and zinc (Zn) concentrations.

Faecal P, Ca and Cr concentrations were measured at the 32nd day of the second period. Faeces were sampled per pen, in approximately the same amount at the same time of the day, during the last 3 days preceding that date. Thus, for each dietary treatment and for each criterion a total of 6 individual determinations have been performed. All minerals were determined according to standard Association of Official Analytical Chemists (1990) methods using a Vista-MPX ICP-OES spectrometer (Varian Australia Pty Ltd, Mulgrave Victoria, 3170 Australia). The apparent digestibility (% of the intake) of the minerals was calculated for the mentioned 3 day period.

At the end of the evaluation all animals were slaughtered after tranquilization and stunning for the right femur collection. Samples of the collected bones were prepared immediately after slaughter. After careful dissection and removal of the soft tissue, a diaphysis section was obtained by sawing each bone. The obtained sections of about 3.5-cm long were immediately subjected to compression in order to determine the force in Newton necessary to break them (maximal-breaking force at the fracture point). The measurements were performed with a LR10K compression machine, using a XLC/10K/A1 force captor and a compression device TH23-196/AL (Lloyd Instruments, Fareham, UK). The broken bones were then used for the determination of the ash content, which was measured after 72-h incineration at 550°C.

2.3. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu + Ai + Bij + Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Snedecor and Cochran, 1989). These calculations were performed using StatGraphics Plus 5.1 (Manugistics, Rockville, U.S.A. 2001).

3. RESULTS AND DISCUSION

3.1. Phytase and animals

The observed phytase activity in the supplemented feed used was in general excellent agreement with the programmed inclusion levels (table 2). The basal diet without addition of mineral P (group A) and with diCaP (group B) had an endogenous phytase activity of 108 ± 34 U/kg.

The animals grew normally during the observation period to reach a final mean body weight of 16.45 ± 2.85 kg. Three animals, one in the control group, one in the 2000 U/kg and one in the 3000 U/kg phytase supplemented groups had to be euthanized during the early stage of the trial after leg injuries. No mortality was observed during the rest of the experiment.
All the groups ingesting phytase supplements and the group supplemented with 16 g/kg of diCaP had higher daily weight gain (DWG) and lower feed conversion ratio (FCR) than those observed for the control group (table 3). The highest DWG and the best FCR were observed for the group ingesting 3000 U/kg. The performances of the group supplemented with diCaP were equivalent to those of the group receiving 1000 U/kg of phytase.

Supplementation with graded amounts of IPA phytase in piglets induced an increased performance in a dose dependant manner. Inclusion levels over 1000 U/kg were more efficient than the diCaP supplementation.

3.2. Effects on plasma mineral and alkaline phosphatase concentrations

Phosphataemia was increased dose dependently in the phytase supplemented groups in comparison to the control group (table 4). The increases were highly significant with the exception of the lowest inclusion level. The group supplemented with diCaP presented also a high significant increase of the phosphataemia but at a lower level than the 4000 and 8000 U/kg phytase supplemented groups. The consumption of phytate rich diets like the control one induced hypophosphataemia. IPA phytase restored the physiological P blood level confirming the sensitiveness of phosphataemia to the dietary available P.

Comparatively to the control group, calcaemia was decreased in all the phytase supplemented animals (table 4). The effects of the phytase were dose dependant although the curve levelled off from the 2000 U/kg inclusion and highly significant with the exception of the lowest inclusion level. As observed in the control group, hypophosphataemia is generally associated with hypercalcaemia in swine. In the present study, calcaemia in the animals ingesting the basal diet supplemented with diCaP or with phytase was within the normal piglet values.

Zincaemia was not significantly influenced by the supplementation of phytase or diCaP, although these treatment groups presented higher mean concentrations than the control group (table 4). Zn is well known to bind to phytate and generally its digestibility is improved by phytases in growing pigs. Nevertheless, it seems that in piglet the blood Zn concentration is not altered by the dietary treatments used in the present experiment.

Compared to the control group, phosphatasaemia was decreased dose dependently in the phytase supplemented groups (table 5). The decreases were only significant at the 500 and 8000 U/kg inclusion levels. The group supplemented with diCaP presented also a decrease of the phosphatasaemia at a level similar to that observed with 1500 U/kg phytase supplemented group. Alkaline phosphatase plays an important role in bone metabolism. As observed in the control non-supplemented group, hypophosphataemia induces osteoblasts heperphosphatasaemia in response to an increased activity of osteoclasts in bone. In the present study, phosphatasaemia of the phytase supplemented animals was systematically lower than that of the control clearly indicating restored normal bone function.

3.3. Effects on phosphorus digestion

The mean P faecal concentration of the enzyme supplemented animals was very significantly lower than that measured in the animals ingesting the control diet (table 6). There was a decrease of the P faecal concentration with the increasing allowance of IPA phytase. The lowest P faecal concentration was observed in the animals ingesting phytase at 8000 U/kg and represented the half of that of the control group.

The P digestibility was dose dependant and highly significantly improved with the exception of the lowest phytase inclusion level. The increases represented in comparison to the control group 12, 66, 77, 110, 132, 129, 156 and 149 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively (table 7, figure 1). The digestibility of P in the diCaP supplemented diet was also significantly higher than that of the control by 69 % and very similar to the enzyme supplementation at 500 U/kg.

The faecal excretion of P was significantly reduced by 4, 20, 25, 34, 41, 41, 49 and 48 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively. It was increased by 35 % with the diCa-P supplemented group (table 8, figure 2).

The apparent absorbed P was 1.22, 1.82, 1.93, 2.31, 2.59, 2.48, 2.84 and 2.71 g/kg feed in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively and 3.18 g/kg feed in the diCaP supplemented group (table 9). It was significantly increased in all the supplemented groups with the exception of the lowest phytase inclusion level in comparison to the control diet (1.09 g/kg). The highest inclusion levels of IPA phytase were in accordance with the recommended requirements of 2.80 g of digestible P per kg feed for piglets.

The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control, of 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase were 0.13, 0.73, 0.84, 1.22, 1.50, 1.39, 1.75 and 1.62 g of full available P/kg feed respectively (table 10, figure 3). In comparison the P equivalency of diCaP supplemented diet was 2.10 g of full available P/kg feed.
In the present study, using the equation of the tendency curve the calculated inclusion level to reach 1.5 g of full available P/kg feed was 3109 U/kg feed of IPA phytase ($y = 167.21e^{1.9466x}$, $R^2 = 0.8897$) but was reached experimentally with the 2000 U/kg inclusion level.

In general on all the P parameters, IPA phytase showed high dose dependant potency.

3.3. Effects on calcium digestion

The Ca faecal concentration of the animals ingesting the non-supplemented diet was higher than that of the animals ingesting the phytase, with the exception of the lowest inclusion level (table 11). The observed differences were statistically significant for the enzyme supplemented groups excepted for the 250 and 1000 U/kg inclusion levels. The highest Ca faecal concentration was observed in the diCaP supplemented group.

The Ca digestibility was improved in the supplemented groups with the exception of the diCaP group and the 250 U/kg phytase group (table 12). The variations were -9, 7, 5, 16, 26, 12, 25 and 17 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively and significant for the five highest concentrations. The Ca digestibility of the diCaP supplemented diet was decreased by 16 % comparatively to the control group.

The faecal excretion of Ca was reduced by 8, 7, 22, 37, 20, 40, and 26 % with the IPA phytase in the 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg supplemented groups respectively and significantly with the five highest concentrations. It was significantly increased by 13 % and 87 % with the 250 U/kg phytase and diCaP groups respectively (table 13).

The apparent absorbed Ca was 4.38, 5.28, 5.08, 5.61, 5.94, 5.20, 5.63 and 5.42 g/kg feed with the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively and 6.10 g/kg feed in the diCa-P group (table 14). It was significantly increased in all the 1500, 2000, 4000 and 8000 U/kg phytase and diCaP supplemented groups and significantly decreased in the 250 U/kg phytase inclusion level in comparison to the Ca apparent absorption in the control diet (4.79 g/kg).

3.4. Bone resistance and bone ash

The phytase supplements strongly influenced the bone strength (table 15). For the IPA phytase inclusion level of 8000 U/kg the increase of the femur resistance was similar to that of diCaP. It represented 121 % and 126 % respectively of that observed for the animals ingesting the basal diet. The increases were significant in all supplemented groups excepted for the 250 U/kg and 1000 U/kg phytase inclusion levels.
The ash content of the femur was increased in a significant way by the phytase excepted for the lowest dosage and by the diCaP (table 15). Nevertheless, the addition of graded amounts of IPA phytase resulted in a non-linear increase of the ash content of the femur.

In the present study IPA phytase supplements in young pigs confirmed the positive effects of phytases on the improvement of bone resistance and the positive but moderate effect on bone mineralisation of animals fed diets containing P exclusively from vegetable origin.
The bone mineralisation data were in agreement with the improvements in P digestibility and with P and Ca blood concentrations.

4. CONCLUSION

It can be concluded that the IPA phytase improved the digestibility and the apparent absorption of P and Ca, reduced the P faecal excretion, restored phosphataemia, calcaemia and phosphatasaemia to physiologic values, increased bone mineralisation and resistance and improved the zootechnical performance in the weaned piglet fed on a diet containing P exclusively from vegetable origin. There was a dose dependant effect of the IPA phytase on the availability of the dietary P.

Table 1 - Composition (%) of the basal diet (A) and of that supplemented with diCa-P (B)

INGREDIENTS	Basal diet without P (%)	Basal diet with diCA-P (%)
Maize	68.52	68.125
Soybean meal	15.1	15.1
Rapeseed meal	12.5	12.5
Salt	0.55	0.55
Soya oil	1.0	1.0
Calcium carbonate	1.56	0.355
Di-calcium phosphorus	-	1.6
Minerals[1] , vitamins and synthetic aa	0.77	0.77
Crude Protein – N x 6.25	15.5	15.5
Lysine - %	0.96	0.96
Methionine + cystine - %	0.54	0.54
Ca – analyzed - % in DM	0.82	1.24
P – analyzed - % in DM	0.45	0.78
Theoritically available P - %	0.12 [2]	0.35 [3]
Observed available P - %	0.11	0.32
Phytic P – calculated - %	0.28	0.54
Estimated digestible energy – MJ/kg	*13.31*	*13.31*
Phytase activity – U [4]/kg	*108 ± 34*	*108 ± 34*

[1] Mixture without mineral P;
[2] Estimated from the mean P digestibility of the previous realized trials
[3] Sum of the theoretically available P and 80 % of added mineral P as generally accepted
[4] Quantity of enzyme that sets free 1 μmole of inorganic phosphate per minute from 0.005 mole per litre sodium phytate at pH 5.5 and at 37°C.

Table 2 - Phytase activity (U[(a)]/kg) and % of the target in the different diets.

Treatment groups	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A	B	C	D	E	F	G	H	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Measured phytase addition (U/kg) [(1)]	108 ± 34	108 ± 34	374 ± 111	601 ± 25	1097 ± 21	1611 ± 41	2225 ± 45	3098 ± 104	4030 ± 208	8238 ± 283
Actually added phytase (U/kg)	-	-	266	493	989	1503	2117	2990	3922	8130
% of target	-	-	106	99	99	100	106	100	98	102

[(a)] Quantity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 3.2 and at 37°C.
[(1)] Mean ± standard deviation of 4 determinations.

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Table 3 – **Effects on the zootechnical performances in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Initial Body Weight (kg)	8.03 ± 1.13	8.02 ± 1.13	8.02 ± 0.84	8.03 ± 1.27	8.03 ± 1.52	8.03 ± 1.08	8.03 ± 0.86	8.02 ± 1.04	8.03 ± 1.19	8.02 ± 1.16
Final Body Weight (kg)	15.20 [(1)] ± 2.10 *(100)*	16.24 ± 3.11 *(107)*	16.14 ± 1.99 *(106)*	15.98 ± 4.13 *(105)*	16.01 ± 3.58 *(105)*	16.69 ± 3.14 *(110)*	17.44 [(1)] ± 2.86 *(115)*	17.48 [(1)] ± 2.29 *(115)*	16.79 ± 2.51 *(110)*	16.58 ± 2.38 *(109)*
Total Weight Gain (kg)	7.04 [(1)] ± 2.32 *(100)*	8.22 ± 2.25 *(117)*	8.12 ± 1.64 *(115)*	7.96 ± 3.09 *(113)*	7.98 ± 2.71 *(113)*	8.66 ± 2.30 *(123)*	9.46 [(1)] ± 2.24 *(134)*	9.61 [(1)] ± 1.86 *(137)*	8.77 ± 1.94 *(125)*	8.56 ± 2.03 *(122)*
Daily Weight Gain (g)	220 [(1)] ± 73 *(100)*	257 ± 70 *(117)*	254 ± 51 *(115)*	249 ± 97 *(113)*	249 ± 85 *(113)*	271 ± 72 *(123)*	296 [(1)] ± 70 *(134)*	300 [(1)] ± 58 *(137)*	274 ± 61 *(125)*	268 ± 63 *(122)*
Feed intake (g/day) [(2)]	468 [(1)] ± 16 *(100)*	484 ± 55 *(103)*	499 ± 40 *(107)*	475 ± 136 *(101)*	478 ± 34 *(102)*	489 ± 99 *(104)*	510 [(1)] ± 42 *(109)*	523 [(1)] ± 57 *(112)*	497 ± 36 *(106)*	491 ± 55 *(105)*
Feed Conversion Ratio (kg/kg) [(2)]	2.448 [(1)] ± 0.220 *(100)*	1.914 ± 0.014 *(78)*	1.981 ± 0.032 *(81)*	1.985 ± 0.048 *(81)*	1.931 ± 0.002 *(79)*	1.835 ± 0.068 *(75)*	1.819 [(1)] ± 0.064 *(74)*	1.793 [(1)] ± 0.013 *(73)*	1.834 ± 0.021 *(75)*	1.865 ± 0.023 *(76)*
Mortality	1	0	0	0	0	0	1	1	0	0

[(1)] n = 11 animals ; [(2)] n = 2 pens

Table 4 – Effects on plasma mineral concentrations in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P plasma levels (mg/dl)	5.11 ± 1.15 *(100)*	7.69 ± 1.03 *(151)*	5.53 ± 0.86 *(108)*	6.23 ± 0.82 *(122)*	6.59 ± 1.00 *(129)*	7.07 ± 1.03 *(138)*	7.23 ± 1.32 *(141)*	7.33 ± 0.86 *(144)*	8.10 ± 0.91 *(159)*	7.97 ± 0.90 *(156)*
Statistical analysis										
	A -	P<0.001	NS	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	NS	NS	NS	NS	NS	NS
			C -	NS	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	NS	NS	P<0.001	P<0.001
					E -	NS	NS	NS	P<0.001	P<0.001
						F -	NS	NS	P<0.05	P<0.05
							G -	NS	P<0.05	P<0.05
								H -	NS	NS
									I -	NS
Ca plasma levels (mg/dl)	13.34 ± 0.69 *(100)*	11.06 ± 0.21 *(83)*	13.17 ± 0.87 *(99)*	12.18 ± 0.60 *(91)*	12.05 ± 0.85 *(90)*	11.67 ± 0.48 *(88)*	11.76 ± 0.47 *(88)*	11.61 ± 0.55 *(87)*	11.62 ± 0.86 *(87)*	11.56 ± 0.41 *(87)*
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	NS	NS	NS	NS	NS
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS
Zn plasma levels (µg/dl)	65.99 ± 12.65 *(100)*	70.93 ± 14.72 *(108)*	78.28 ± 7.03 *(119)*	71.99 ± 14.94 *(109)*	70.50 ± 12.65 *(107)*	75.27 ± 9.52 *(114)*	69.82 ± 11.27 *(106)*	70.50 ± 10.62 *(107)*	68.20 ± 11.62 *(103)*	71.01 ± 10.78 *(108)*
	No statistical differences									

(1) n = 11 animals NS : non significant

Table 5 – Effects on plasma alkaline phosphatase concentrations in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[1]	B	C	D	E	F	G[1]	H[1]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
ALP plasma levels (U/L)	325.1 ± 96.1	239.4 ± 50.4	285.9 ± 46.7	223.4 ± 48.0	280.3 ± 78.4	237.4 ± 90.7	255.5 ± 104.8	255.7 ± 85.8	251.5 ± 57.5	202.3 ± 47.5
Variation from A (%)	100.0	73.7	87.9	68.7	86.2	73.0	78.6	78.6	77.4	62.2
Statistical analysis										
	A -	NS	NS	P<0.05	NS	NS	NS	NS	NS	P<0.001
		B -	NS	NS	NS	NS	NS	NS	NS	NS
			C -	NS	NS	NS	NS	NS	NS	NS
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

[1] n = 11 animals NS : non significant

<u>Table 6</u> - **Effects on the faecal concentration of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal concentration (mg/g DM)	22.4 ± 0.6	24.6 ± 2.6	20.8 ± 1.3	16.3 ± 1.1	16.4 ± 0.6	14.1 ± 1.0	12.8 ± 0.6	12.5 ± 1.1	11.5 ± 1.2	11.2 ± 0.7
Variation from A (%)	100.0	110.0	93.2	73.1	73.5	63.0	57.1	55.8	51.6	49.9
Statistical analysis										
	A -	P<0.001	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.001
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

[(1)] n = 11 animals NS : non significant

Table 7 - Effects on the total apparent digestibility of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[1]	B	C	D	E	F	G[1]	H[1]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal apparent digestibility (%)	24.1 ± 1.5	40.8 ± 5.1	26.9 ± 3.5	40.0 ± 3.6	42.7 ± 2.4	50.7 ± 5.4	56.0 ± 1.9	55.1 ± 3.6	61.8 ± 3.5	60.1 ± 4.8
Variation from A (%)	100	169	112	166	177	210	232	229	256	249
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	NS	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.001
							G -	NS	P<0.05	NS
								H -	P<0.05	NS
									I -	NS

[1] n = 11 animals NS : non significant

Table 8 - Effects on the faecal excretion of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal excretion (mg/g)	3.43 ± 0.07	4.62 ± 0.40	3.30 ± 0.16	2.73 ± 0.17	2.58 ± 0.11	2.24 ± 0.25	2.03 ± 0.09	2.02 ± 0.16	1.76 ± 0.16	1.80 ± 0.22
Variation from A (%)	100	135	96	80	75	66	59	59	51	52
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.001
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

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Table 9 - Effects on the faecal apparent absorption of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal apparent absorption (mg/g)	1.09 ± 0.07	3.18 ± 0.40	1.22 ± 0.16	1.82 ± 0.17	1.93 ± 0.11	2.31 ± 0.25	2.59 ± 0.09	2.48 ± 0.16	2.84 ± 0.16	2.71 ± 0.22
Variation from A (%)	100	292	112	168	177	212	238	228	261	249
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.05
							G -	NS	NS	NS
								H -	P<0.05	NS
									I -	NS

(1) n = 11 animals NS : non significant

Figure 1

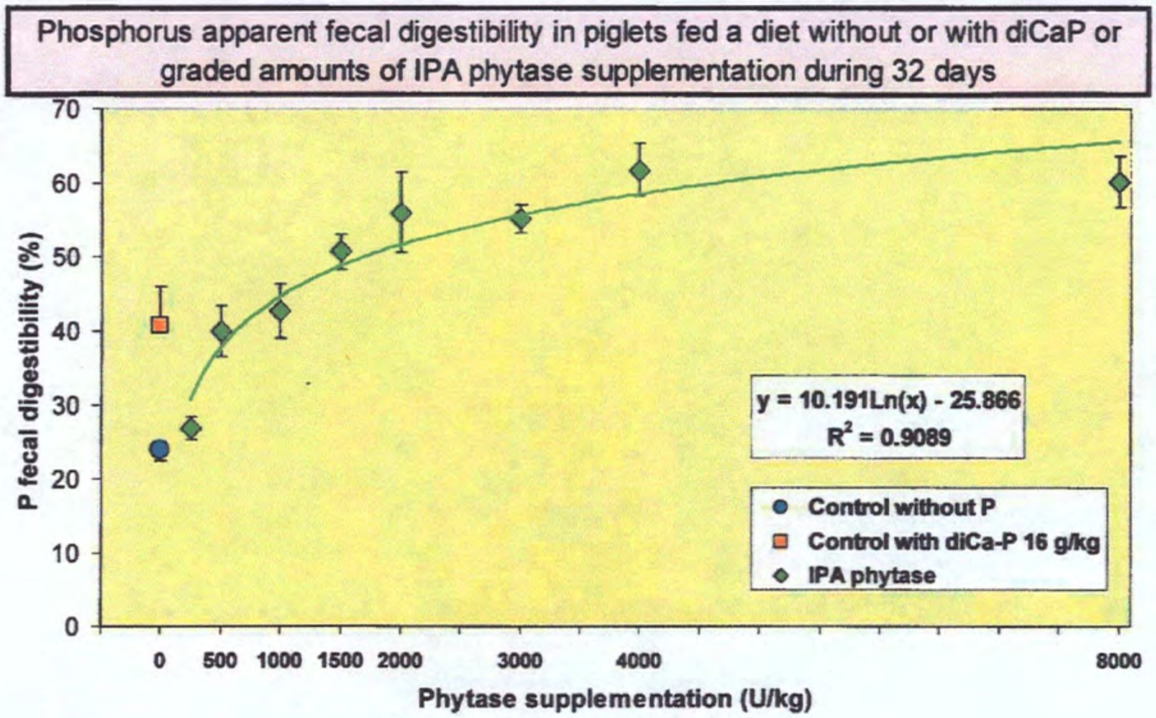

Figure 2

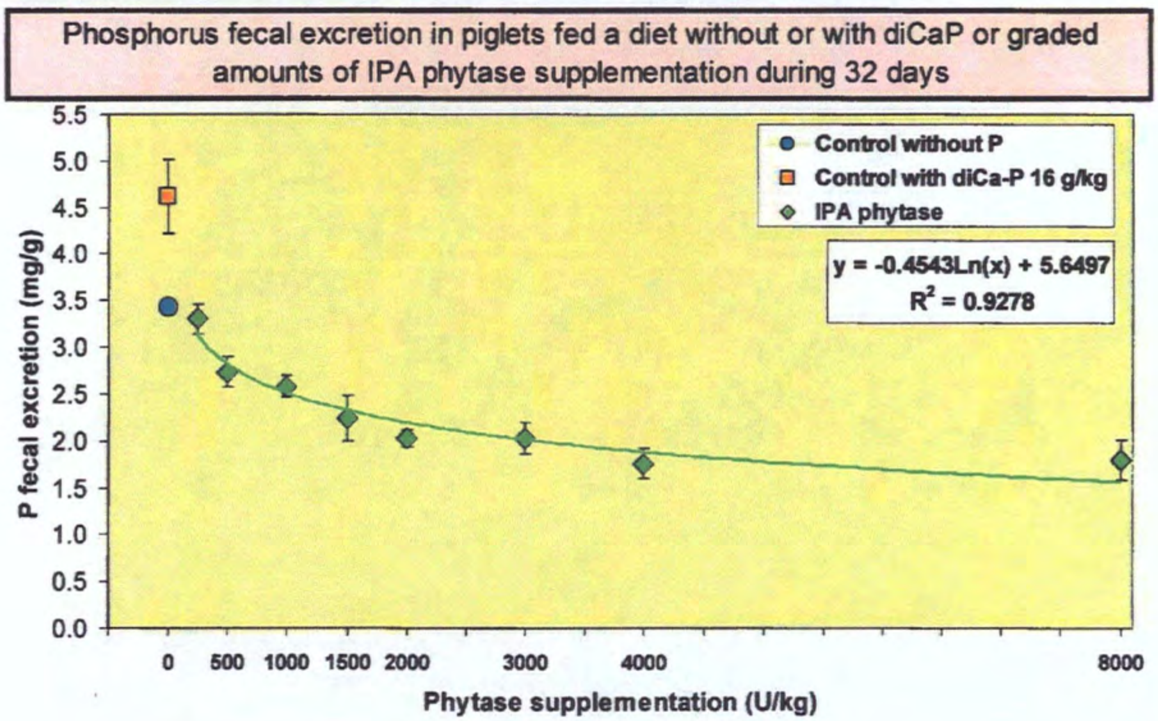

Table 10 - **Phosphorus equivalencies (g of full available supplemental P per kg of feed comparatively to the non-supplemented control) in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P equivalence (mg/g)	0.00 ± 0.07	2.10 ± 0.40	0.13 ± 0.16	0.73 ± 0.17	0.84 ± 0.11	1.22 ± 0.25	1.50 ± 0.09	1.39 ± 0.16	1.75 ± 0.16	1.62 ± 0.22
Variation from C (%)	-	-	100	577	659	957	1177	1096	1378	1272
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.05
							G -	NS	NS	NS
								H -	P<0.05	NS
									I -	NS

(1) n = 11 animals NS : non significant

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Figure 3

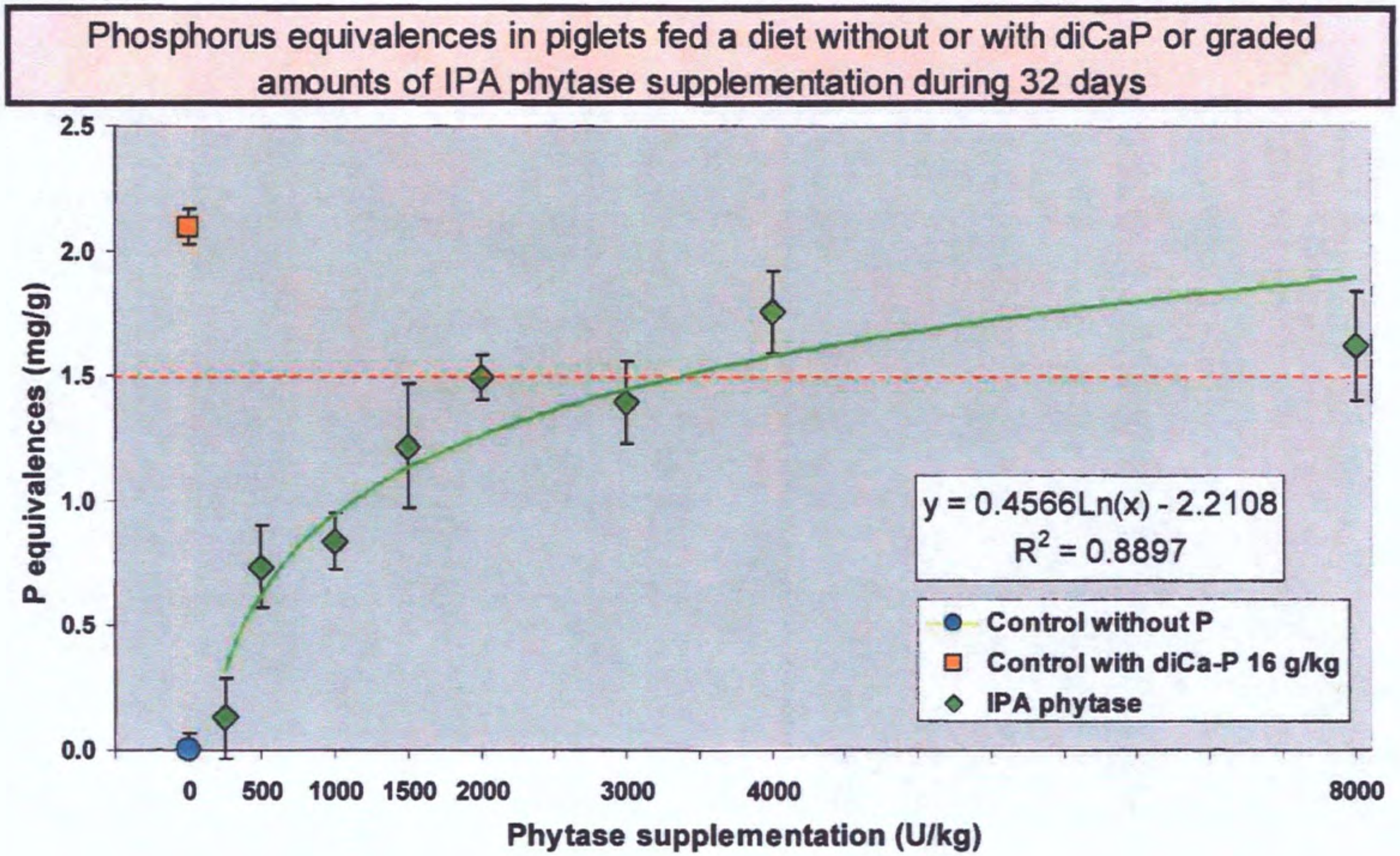
Phosphorus equivalences in piglets fed a diet without or with diCaP or graded amounts of IPA phytase supplementation during 32 days
P equivalences (mg/g)
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
3000
4000
8000
Phytase supplementation (U/kg)
y = 0.4566Ln(x) - 2.2108
R² = 0.8897
Control without P
Control with diCa-P 16 g/kg
IPA phytase

Table 11 - Effects on the faecal concentration of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[1]	B	C	D	E	F	G[1]	H[1]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal concentration (mg/g DM)	22.1 ± 1.3	33.6 ± 3.3	24.0 ± 1.6	18.6 ± 2.1	20.0 ± 0.8	16.5 ± 1.4	13.4 ± 0.7	16.6 ± 1.4	13.3 ± 1.2	15.6 ± 2.2
Variation from A (%)	100.0	152.4	108.7	84.4	90.5	74.8	60.5	75.3	60.4	70.5
Statistical analysis										
	A -	P<0.001	NS	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	P<0.001	P<0.05
					E -	P<0.05	P<0.001	P<0.05	P<0.001	P<0.001
						F -	P<0.05	NS	P<0.05	NS
							G -	P<0.05	NS	NS
								H -	P<0.05	NS
									I -	NS

[1] n = 11 animals NS : non significant

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Table 12 - Effects on the total apparent digestibility of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[1]	B	C	D	E	F	G[1]	H[1]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal apparent digestibility (%)	58.7 ± 1.2	49.2 ± 3.7	53.5 ± 3.5	62.9 ± 4.3	61.8 ± 3.6	68.1 ± 3.6	73.6 ± 2.6	65.7 ± 4.5	73.4 ± 3.2	68.4 ± 5.1
Variation from A (%)	100	84	91	107	105	116	126	112	125	117
Statistical analysis										
	A -	P<0.001	P<0.05	NS	NS	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001
		B -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	P<0.001	NS
					E -	P<0.05	P<0.001	NS	P<0.001	P<0.05
						F -	NS	NS	NS	NS
							G -	P<0.001	NS	NS
								H -	P<0.001	NS
									I -	NS

[1] n = 11 animals NS : non significant

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Table 13 - Effects on the faecal excretion of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal excretion (mg/g)	3.38 ± 0.10	6.31 ± 0.46	3.81 ± 0.29	3.12 ± 0.36	3.14 ± 0.13	2.63 ± 0.29	2.13 ± 0.21	2.71 ± 0.36	2.04 ± 0.25	2.50 ± 0.41
Variation from A (%)	100	187	113	92	93	78	63	80	60	74
Statistical analysis										
	A -	P<0.001	P<0.05	NS	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	P<0.001	P<0.05
					E -	NS	P<0.001	NS	P<0.001	P<0.05
						F -	P<0.05	NS	P<0.05	NS
							G -	P<0.05	NS	NS
								H -	P<0.001	NS
									I -	NS

(1) n = 11 animals NS : non significant

<u>Table 14</u> - **Effects on the faecal apparent absorption of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A(1)	B	C	D	E	F	G(1)	H(1)	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal apparent absorption (mg/g)	4.79 ± 0.10	6.10 ± 0.46	4.38 ± 0.29	5.28 ± 0.36	5.08 ± 0.13	5.61 ± 0.29	5.94 ± 0.21	5.20 ± 0.36	5.63 ± 0.25	5.42 ± 0.41
Variation from A (%)	100	127	92	110	106	117	124	109	109	113
					Statistical analysis					
	A -	P<0.001	P<0.05	NS	NS	P<0.001	P<0.001	NS	P<0.001	P<0.05
		B -	P<0.001	P<0.001	P<0.001	NS	NS	P<0.001	NS	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	NS	NS
					E -	NS	P<0.001	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	P<0.001	NS	P<0.05
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 15 – **Effects on bone ash and bone resistance in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Bone resistance maximal strength (N)	272.8 ± 88.7	615.5 ± 179.1	334.6 ± 76.0	476.1 ± 124.4	384.1 ± 77.2	500.3 ± 118.7	523.5 ± 157.7	476.5 ± 97.1	542.2 ± 108.7	604.1 ± 119.2
Variation from A (%)	100	226	123	175	141	183	192	175	199	221
Statistical analysis										
	A -	P<0.001	NS	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	NS	P<0.001	NS	NS	NS	NS	NS
			C -	NS	NS	NS	P<0.05	NS	P<0.05	P<0.001
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	P<0.001
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS
Bone ash (%)	62.17 ± 1.91	63.70 ± 1.58	62.38 ± 1.88	65.19 ± 0.94	65.67 ± 0.95	65.80 ± 1.70	64.85 ± 1.55	65.70 ± 1.06	66.36 ± 1.21	65.24 ± 1.78
Variation from A (%)	100	103	100	105	106	106	104	106	107	105
Statistical analysis										
	A -	P<0.05	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.05	NS	P<0.05	P<0.05	NS	P<0.05	P<0.001	NS
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

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23.02.2010

Groupe A	Porcelet	Poids	GP	GMQ	Cons. Moy. Ind./Jour	IC/ Lot
A 1	551	17.057	7.778	0.243		
	529	13.074	3.877	0.121		
	524	17.914	8.995	0.281		
	525	11.010	2.293	0.072		
	534	18.103	9.446	0.295		
	526	14.292	5.668	0.177		
	535	15.153	6.567	0.205	0.457	2.292
A 11	527	15.007	7.400	0.231		
	531	14.508	7.063	0.221		
	569	16.285	9.348	0.292		
	528					
	530	14.818	8.971	0.280	0.479	2.604
B 2	586	20.933	10.750	0.336		
	543	21.077	11.513	0.360		
	545	18.604	10.052	0.314		
	538	16.933	8.534	0.267		
	595	17.028	8.670	0.271		
	547	13.936	5.800	0.181		
	542	14.257	6.237	0.195	0.523	1.904
B 12	537	17.251	9.350	0.292		
	539	17.085	9.924	0.310		
	566	12.808	5.748	0.180		
	541	11.147	4.466	0.140		
	540	13.816	7.536	0.236	0.445	1.923
C 3	557	15.389	6.179	0.193		
	556	17.656	8.503	0.266		
	550	19.748	10.630	0.332		
	555	15.734	7.238	0.226		
	532	17.917	9.705	0.303		
	549	15.760	7.682	0.240		
	553	16.841	9.044	0.283	0.528	2.004
C 13	552	17.498	9.807	0.306		
	559	14.828	7.321	0.229		
	554	12.005	4.825	0.151		
	558	15.858	8.807	0.275		
	548	14.474	7.691	0.240	0.471	1.958
D 4	561	23.364	13.012	0.407		
	567	17.659	8.465	0.265		
	642	19.957	10.926	0.341		
	570	17.668	8.869	0.277		
	571	18.034	9.422	0.294		
	565	15.279	6.842	0.214		
	568	16.134	7.951	0.248	0.571	1.952
D 14	563	13.474	6.224	0.195		
	564	14.196	7.297	0.228		
	560	17.515	10.737	0.336		
	533	10.798	4.180	0.131		
	544	7.723	1.573	0.049	0.379	2.019
E 5	588	20.147	9.765	0.305		
	621	20.443	10.803	0.338		
	577	18.385	9.193	0.287		
	579	11.647	2.466	0.077		
	581	21.223	12.341	0.386		
	576	15.762	7.260	0.227		
	573	14.136	6.349	0.198	0.502	1.932
E 15	572	16.857	9.367	0.293		
	578	17.255	9.821	0.307		
	575	12.013	5.850	0.183		
	580	12.414	6.560	0.205		
	583	11.843	6.028	0.188	0.454	1.930
F 6	590	22.327	12.850	0.402		
	593	19.510	10.119	0.316		
	594	19.230	10.379	0.324		
	536	16.214	7.503	0.234		
	584	15.576	6.934	0.217		
	591	17.981	9.691	0.303		
	631	16.986	8.969	0.280	0.558	1.883
F 16	574	15.707	7.937	0.248		
	589	18.000	10.428	0.326		
	585	15.848	9.018	0.282		
	592	11.884	5.356	0.167		
	587	10.991	4.760	0.149	0.419	1.787
G 7	604	20.936	11.509	0.360		
	600	21.090	11.869	0.371		
	605	22.392	13.664	0.427		
	601	17.931	9.229	0.288		
	607					
	598	14.229	6.410	0.200		
	599	15.830	8.080	0.253	0.540	1.865
G 17	606	15.489	7.951	0.248		
	596	16.169	8.679	0.271		
	597	14.179	6.960	0.218		
	639	17.811	10.817	0.338		
	603	15.816	8.889	0.278	0.480	1.774
H 8	612					
	611	21.615	12.470	0.390		
	609	19.252	10.612	0.332		
	619	18.103	9.706	0.303		
	618	20.422	12.061	0.377		

	617	17.545	9.242	0.289		
	608	16.738	8.763	0.274	0.563	1.783
H 18	**614**	15.200	7.250	0.227		
	582	14.012	6.099	0.191		
	615	17.438	10.320	0.323		
	610	16.611	9.599	0.300		
	613	15.302	9.567	0.299	0.483	1.802
I 9	**630**	20.113	10.500	0.328		
	625	18.614	9.404	0.294		
	562	16.777	7.691	0.240		
	626	17.032	7.950	0.248		
	616	17.977	9.300	0.291		
	546	19.335	11.183	0.349		
	629	16.133	8.332	0.260	0.523	1.819
I 19	**627**	18.284	10.562	0.330		
	628	16.478	8.822	0.276		
	620	11.662	4.370	0.137		
	623	16.508	10.411	0.325		
	637	12.589	6.662	0.208	0.472	2.868
J 10	**622**	19.107	9.177	0.287		
	640	16.330	6.986	0.218		
	624	14.946	5.777	0.181		
	602	18.971	10.115	0.316		
	643	19.117	10.756	0.336		
	634	17.568	9.535	0.298		
	638	19.740	11.901	0.372	0.530	1.849
J 20	**635**	13.779	6.172	0.193		
	633	17.025	9.816	0.307		
	632	13.374	6.170	0.193		
	641	15.744	9.169	0.287		
	636	13.290	7.156	0.224	0.453	1.881

Traitement	ALP (U/L)	PHOSPHORE mg/dl	CALCIUM (mg/dl)	Zinc (mg/dL)
A	197.10	6.05	12.88	0.06403
A	406.58	6.55	12.43	0.06767
A	232.39	6.65	13.32	0.08153
A	328.08	5.03	13.68	0.06679
A	287.52	5.87	13.88	0.09008
A	301.20	4.90	13.61	0.06588
A	202.01	5.77	12.33	0.07292
A	349.51	4.52	13.36	0.06328
A	472.13	3.70	13.13	0.04488
A	328.41	3.60	13.36	0.05312
A				
A	471.18	3.59	14.80	0.05572
B	201.91	8.12	11.24	0.07588
B	174.50	9.54	10.78	0.07316
B	203.48	7.86	10.89	0.10568
B	234.97	7.93	11.02	0.07648
B	209.87	8.55	10.75	0.07624
B	338.96	7.20	10.93	0.06288
B	311.92	8.13	11.00	0.06564
B	192.74	8.09	11.32	0.05928
B	237.69	7.90	11.34	0.07764
B	289.05	6.26	11.36	0.06452
B	252.83	5.61	11.00	0.04312
B	225.36	7.12	11.11	0.07068
C	247.85	5.40	13.75	0.07152
C	246.38	6.54	11.49	0.08032
C	317.77	6.17	12.55	0.08116
C	315.11	6.42	12.65	0.08532
C	300.62	5.97	13.84	0.09372
C	217.59	6.03	12.08	0.07468
C	330.44	6.06	13.28	0.08404
C	307.47	4.75	14.03	0.07684
C	315.89	5.65	14.01	0.07584
C	194.15	5.33	12.58	0.06784
C	331.96	4.18	13.97	0.07412
C	305.57	3.91	13.78	0.07416
D	186.44	7.40	12.26	0.08232
D	281.86	6.15	11.80	0.06784
D	209.97	7.42	12.10	0.09392
D	236.47	5.98	12.94	0.08532
D	181.51	6.79	11.87	0.08368
D	144.16	6.02	11.77	0.08028
D	223.03	7.14	12.51	0.07832
D	254.58	5.51	12.78	0.07644
D	267.09	6.16	12.35	0.07208
D	268.59	5.40	12.69	0.06440
D	273.13	5.96	12.46	0.06144
D	154.01	4.81	10.71	0.03780
E	205.74	7.89	11.59	0.07992
E	339.14	7.87	11.02	0.08692
E	174.16	5.91	12.27	0.06376
E	246.97	5.46	10.90	0.03904

E	316.12	8.01	11.64	0.08448
E	336.17	6.09	12.92	0.07248
E	232.89	4.92	12.24	0.06892
E	317.19	6.81	12.18	0.06908
E	455.35	6.45	14.00	0.07404
E	280.24	6.84	12.15	0.07176
E	194.77	7.03	11.42	0.07600
E	264.86	5.78	12.34	0.05964
F	255.08	6.64	11.94	0.07844
F	194.47	7.72	11.50	0.07516
F	180.88	8.18	11.63	0.08888
F	219.67	5.54	11.48	0.07336
F	260.24	5.91	11.02	0.06496
F	512.17	7.62	11.59	0.07828
F	205.23	6.56	12.66	0.08768
F	225.21	7.77	12.14	0.07984
F	213.06	8.09	10.91	0.07464
F	190.60	8.55	11.43	0.08096
F	169.27	6.12	11.89	0.06500
F	222.57	6.14	11.92	0.05604
G	497.55	6.35	11.33	0.08268
G	185.40	7.83	12.57	0.07824
G	233.11	6.87	12.26	0.08420
G	412.84	6.09	11.27	0.06508
G				
G	277.09	5.17	12.44	0.07100
G	176.53	8.83	11.81	0.06024
G	198.81	9.52	11.75	0.07620
G	189.82	7.65	11.60	0.06284
G	221.58	5.76	11.67	0.04900
G	233.53	7.66	11.18	0.05956
G	184.06	7.78	11.51	0.07896
H				
H	444.61	8.08	11.70	0.05916
H	171.77	8.53	11.25	0.05456
H	346.47	7.06	11.59	0.06900
H	204.17	7.47	12.25	0.07204
H	273.47	8.01	10.68	0.08304
H	257.50	5.83	11.36	0.06040
H	168.62	8.05	11.28	0.08764
H	255.67	7.33	11.61	0.07050
H	267.03	7.01	12.60	0.07548
H	192.62	6.27	11.48	0.06700
H	230.48	7.05	11.93	0.07664
I	281.58	8.59	11.44	0.08556
I	231.98	8.45	11.23	0.08108
I	209.13	8.22	11.05	0.06108
I	312.47	8.16	10.62	0.06856
I	252.58	8.52	11.91	0.07836
I	289.83	7.44	11.15	0.06980
I	356.04	7.84	10.55	0.06700
I	256.24	8.26	12.38	0.07604
I	196.20	7.72	12.38	0.06276
I	139.10	6.86	11.02	0.04148
I	219.94	10.31	13.33	0.06532

I	273.48	6.88	12.46	0.06136
J	166.06	7.63	11.31	0.07812
J	210.52	7.03	11.66	0.07236
J	202.04	6.09	12.01	0.06408
J	215.30	8.42	11.42	0.07412
J	199.44	7.55	11.59	0.07628
J	198.55	9.20	11.94	0.07548
J	197.00	8.28	11.10	0.08844
J	325.61	8.15	11.48	0.07616
J	147.96	9.13	10.79	0.05056
J	220.65	8.87	11.29	0.07712
J	134.12	7.67	12.21	0.05348
J	210.67	7.60	11.92	0.06588

Diet	Treatment	FECES (mg/g DM)	DIGESTIBILITY (%)	Absorbed (mg/g)	Excreted (mg/g)
A1	Control without P	23.522	57.5	4.700	3.472
A1		20.524	59.8	4.889	3.283
A1		22.861	58.5	4.778	3.394
A11		23.152	56.8	4.641	3.532
A11		20.083	59.9	4.894	3.279
A11		22.335	59.4	4.857	3.315
B12	Control with diCa-P 16 g/kg	38.592	44.7	5.552	6.864
B12		35.725	46.4	5.758	6.659
B12		31.685	50.2	6.237	6.179
B2					
B2		29.055	55.4	6.881	5.535
B2		33.152	49.1	6.091	6.325
C13	IPA Phytase 250 U/kg	25.321	49.1	4.021	4.171
C13		24.771	51.9	4.248	3.944
C13		24.661	49.8	4.076	4.116
C3		24.971	55.8	4.568	3.624
C3		23.502	55.8	4.572	3.620
C3		20.718	58.9	4.823	3.369
D14	IPA Phytase 500 U/kg	18.704	63.3	5.313	3.080
D14		19.429	59.2	4.970	3.424
D14		17.398	64.2	5.387	3.007
D4		22.444	55.7	4.673	3.721
D4		15.429	67.8	5.691	2.703
D4		18.351	67.1	5.629	2.765
E15	IPA Phytase 1000 U/kg	19.319	61.3	5.043	3.177
E15		19.615	63.2	5.193	3.028
E15		20.150	60.8	5.000	3.221
E5		21.621	59.2	4.864	3.356
E5		19.730	63.3	5.207	3.014
E5		19.426	63.1	5.191	3.029
F16	IPA Phytase 1500 U/kg	18.203	65.7	5.411	2.825
F16		14.611	74.2	6.110	2.126
F16		17.973	67.5	5.556	2.680
F6		15.786	69.3	5.706	2.530
F6		17.277	62.5	5.151	3.085
F6		15.209	69.3	5.704	2.531
G17	IPA Phytase 2000 U/kg	13.105	75.5	6.097	1.976
G17		12.035	77.7	6.274	1.799
G17		13.778	74.7	6.033	2.040
G7		14.162	70.2	5.665	2.407
G7		13.369	72.7	5.866	2.206
G7		13.671	71.0	5.731	2.341
H18	IPA Phytase 3000 U/kg	17.467	64.2	5.083	2.835
H18		13.509	74.5	5.901	2.017
H18		17.903	66.9	5.299	2.619
H8		16.764	60.5	4.794	3.124
H8		17.082	61.8	4.896	3.021
H8		17.090	66.3	5.246	2.672
I19	IPA Phytase 4000 U/kg	14.068	74.7	5.721	1.941
I19		10.763	78.6	6.023	1.639
I19		14.697	70.8	5.425	2.237
I9		13.691	70.0	5.364	2.298
I9		13.555	70.5	5.403	2.259
I9		13.277	75.9	5.818	1.844
J10	IPA Phytase 8000 U/kg	18.050	59.8	4.734	3.181
J10		14.826	67.6	5.349	2.566
J10		15.392	71.2	5.635	2.281
J20		11.471	77.0	6.093	1.822
J20		15.504	67.7	5.361	2.554

J20		18.140	67.3	5.327	2.589

Diet	Treatment	P in FEES (mg/g DM)	DIGESTIBILITY (%)	Absorbed (mg/g)	Excreted (mg/g)	equivalence (mg/g)
A1	Control without P	23.143	24.3	1.099	3.416	0.010
A1		22.165	21.5	0.969	3.546	-0.119
A1		22.730	25.3	1.140	3.375	0.052
A11		22.637	23.5	1.062	3.453	-0.027
A11		21.114	23.7	1.068	3.447	-0.021
A11		22.370	26.5	1.194	3.321	0.106
B12	Control with diCa-P 1g/kg	28.366	35.3	2.756	5.045	1.667
B12		26.519	36.6	2.859	4.943	1.770
B12		24.149	39.6	3.092	4.709	2.003
B2						
B2		20.690	49.5	3.860	3.942	2.771
B2		23.304	43.0	3.355	4.446	2.267
C13	IPA Phytase 250 U/kg	20.864	24.0	1.083	3.437	-0.006
C13		21.555	24.1	1.088	3.432	-0.001
C13		20.470	24.4	1.103	3.416	0.014
C3		22.466	27.9	1.259	3.260	0.170
C3		21.356	27.2	1.230	3.289	0.141
C3		18.360	33.9	1.534	2.986	0.445
D14	IPA Phytase 500 U/kg	15.963	42.3	1.929	2.629	0.840
D14		16.233	37.2	1.697	2.861	0.608
D14		14.475	45.1	2.056	2.502	0.967
D4		17.895	34.9	1.591	2.967	0.502
D4		16.252	37.5	1.710	2.847	0.621
D4		17.258	42.9	1.957	2.600	0.869
E15	IPA Phytase 1000 U/kg	16.450	40.0	1.803	2.705	0.714
E15		16.356	44.0	1.983	2.525	0.894
E15		15.592	44.7	2.016	2.492	0.927
E5		17.542	39.6	1.785	2.723	0.696
E5		15.861	46.3	2.086	2.423	0.997
E5		16.787	41.9	1.891	2.618	0.802
F16	IPA Phytase 1500 U/kg	15.252	48.0	2.184	2.367	1.095
F16		12.924	58.7	2.671	1.880	1.582
F16		12.861	57.9	2.633	1.918	1.544
F6		14.904	47.5	2.162	2.388	1.073
F6		13.580	46.7	2.126	2.425	1.037
F6		14.933	45.4	2.065	2.485	0.976
G17	IPA Phytase 2000 U/kg	12.771	58.3	2.690	1.925	1.601
G17		12.705	58.9	2.716	1.899	1.628
G17		13.975	55.2	2.546	2.069	1.458
G7		12.138	55.3	2.552	2.063	1.463
G7		12.501	55.3	2.552	2.063	1.463
G7		12.573	53.3	2.462	2.153	1.373
H18	IPA Phytase 3000 U/kg	14.327	48.4	2.181	2.325	1.092
H18		12.242	59.4	2.679	1.828	1.590
H18		13.358	56.6	2.552	1.954	1.463
H8		11.460	52.6	2.371	2.136	1.282
H8		11.210	56.0	2.524	1.983	1.435
H8		12.234	57.6	2.593	1.913	1.505
I19	IPA Phytase 4000 U/kg	13.426	59.7	2.745	1.853	1.656
I19		9.403	68.9	3.166	1.432	2.077
I19		12.051	60.1	2.763	1.834	1.674
I9		11.329	58.6	2.696	1.902	1.607
I9		11.020	60.1	2.761	1.837	1.672
I9		12.058	63.6	2.922	1.675	1.834
J10	IPA hytase 00 U/kg	12.463	51.2	2.306	2.196	1.217
J10		10.780	58.6	2.636	1.866	1.547
J10		10.812	64.4	2.900	1.602	1.811
J20		10.231	63.9	2.877	1.625	1.788

J20	P 80	11.476	58.0	2.612	1.891	1.523
J20		11.146	64.7	2.912	1.590	1.823

Matières sèches
Cendres

Box Traitement	N° Porc	N° Creuset	% Cendres 100% MS
A	530	1	60.85
	527	2	61.14
	531	3	62.39
	569	4	58.03
	524	5	62.41
	534	6	63.20
	551	8	65.18
	525	11	64.52
	526	12	62.62
	529	13	61.33
	535	14	62.18
B	539	15	63.33
	541	16	66.00
	540	17	63.14
	566	18	60.89
	537	19	63.01
	586	20	62.92
	595	21	63.14
	543	22	63.82
	542	23	66.26
	547	25	62.02
	545	26	65.06
	538	27	64.82
C	552	28	64.15
	548	29	64.05
	559	30	60.78
	558	31	61.32
	554	32	65.66
	550	33	64.14
	549	35	59.85
	556	36	60.93
	532	38	62.49
	555	39	60.27
	553	42	61.36
	557	44	63.60
D	564	45	65.63
	570	47	65.87
	563	48	63.56
	560	49	63.99
	533	50	65.08
	567	51	65.48
	561	52	66.53
	565	53	65.64
	544	54	65.35
	568	46	63.83
	642	55	65.08
	571	56	66.19
E	578	57	65.33
	583	58	66.36
	575	59	65.92
	580	60	64.07

Traitement	Force maximale (N)	
535 Tr:A	256.7426249	
529 Tr:A	340.1596004	
526 Tr:A	258.093239	
525 Tr:A	186.7291519	
551 Tr:A	530.3761638	
534 Tr:A	384.5513903	
524 Tr:A	323.8680103	
569 Tr:A	115.666211	
531 Tr:A	266.7461561	
527 Tr:A	164.2895492	
530 Tr:A	173.7323481	
		272.81404
538 Tr:B	540.0311813	
545 Tr:B	834.7405529	
547 Tr:B	427.9822428	
542 Tr:B	591.1987166	
543 Tr:B	921.8980087	
595 Tr:B	778.6862945	
586 Tr:B	539.8671732	
537 Tr:B	439.0113013	
566 Tr:B	334.4434488	
540 Tr:B	845.074253	
541 Tr:B	361.5372753	
539 Tr:B	771.7388637	615.517443
557 Tr:C	402.801209	
553 Tr:C	344.8880304	
555 Tr:C	470.3058998	
532 Tr:C	312.6604302	
556 Tr:C	297.0565641	
549 Tr:C	263.1132889	
550 Tr:C	528.383459	
554 Tr:C	235.6361231	
558 Tr:C	354.6941817	
559 Tr:C	362.5725794	
548 Tr:C	185.7726641	
552 Tr:C	257.5002348	334.615389
565 Tr:D	363.321649	
561 Tr:D	688.0720657	
567 Tr:D	532.6939528	
571 Tr:D	648.9839943	
642 Tr:D	341.1408643	
568 Tr:D	487.1100546	
570 Tr:D	663.1119311	
564 Tr:D	537.1049144	
563 Tr:D	257.0583716	
560 Tr:D	475.6459217	
533 Tr:D	521.6086111	
544 Tr:D	197.1909669	
621 Tr:E	481.3788921	
581 Tr:E	456.2503067	
577 Tr:E	400.4909892	
579 Tr:E	497.5923086	

	572	61	64.41	588 Tr:E	491.1624503
	576	62	65.03	573 Tr:E	279.9124038
	573	63	66.28	576 Tr:E	332.075501
	588	65	65.89	572 Tr:E	339.4116251
	579	66	67.40	580 Tr:E	225.6324171
	577	67	65.86	575 Tr:E	323.5987187
	581	68	66.56	583 Tr:E	340.8468524
	621	69	64.89	578 Tr:E	441.0039327
F	594	70	66.23	590 Tr:F	733.3963922
	593	74	67.11	631 Tr:F	732.8905364
	536	73	65.67	584 Tr:F	381.1834395
	584	76	66.58	536 Tr:F	441.4039541
	631	78	67.75	593 Tr:F	575.2005417
	590	79	66.44	594 Tr:F	480.4039022
	589	80	66.22	591 Tr:F	366.3464737
	574	81	65.38	587 Tr:F	355.7700955
	585	82	63.74	592 Tr:F	442.8848729
	592	96	66.22	585 Tr:F	471.6250791
	587	5	66.85	574 Tr:F	350.7080104
	591	20	61.42	589 Tr:F	672.300791
G	639	25	66.03	605 Tr:G	677.2170074
	603	29	64.90	600 Tr:G	674.3742296
	596	38	65.40	601 Tr:G	346.47432
	606	75	63.87	599 Tr:G	579.8156437
	604	79	65.48	598 Tr:G	771.2172716
	598	80	66.78	604 Tr:G	419.0117661
	597	82	65.19	606 Tr:G	340.948089
	599	86	60.91	596 Tr:G	437.8630664
	601	88	64.20	597 Tr:G	366.4934948
	600	94	65.93	603 Tr:G	363.1637289
	605	103	64.68	639 Tr:G	782.2427814
H	613	107	64.57	618 Tr:H	651.9685548
	610	109	65.77	611 Tr:H	474.3866234
	615	110	66.69	609 Tr:H	456.6356886
	582	111	63.84	619 Tr:H	278.1951272
	614	113	65.98	608 Tr:H	382.0124437
	617	117	66.06	617 Tr:H	478.0334909
	618	119	65.10	614 Tr:H	832.5413146
	611	148	66.14	582 Tr:H	413.9346984
	608	155	65.77	615 Tr:H	346.0081756
	619	175	65.00	610 Tr:H	450.7266936
	609	197	67.77	613 Tr:H	477.3702603
I	629	199	65.49	629 Tr:I	386.0671629
	562	221	67.45	562 Tr:I	737.3985065
	546	280	66.35	546 Tr:I	713.7054483
	628	304	67.12	630 Tr:I	544.540216
	627	308	66.84	618 Tr:I	649.0371071
	630	331	69.05	625 Tr:I	525.0902763
	616	336	66.68	626 Tr:I	588.7252952
	625	338	65.67	637 Tr:I	448.852972
	637	339	65.56	620 Tr:I	317.7936126
	626	342	66.47	623 Tr:I	454.9275191

	620	343	64.71	627 Tr:I	468.0993745
	623	352	64.90	628 Tr:I	672.3203289
J	638	355	65.55	643 Tr:J	811.7108032
	624	357	63.06	640 Tr:J	623.5242283
	602	362	62.64	622 Tr:J	630.5928393
	633	365	67.79	634 Tr:J	649.8534942
	640	390	67.05	624 Tr:J	481.5921059
	643	25	67.37	638 Tr:J	670.7008961
	622	52	64.99	602 Tr:J	410.3670993
	634	55	65.44	633 Tr:J	927.3685516
	632	68	63.47	636 Tr:J	488.385717
	641	70	63.68	635 Tr:J	506.7045849
	636	80	64.88	632 Tr:J	418.3187809
	635	58	66.97	641 Tr:J	629.8908135

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA phytase**	Batch number: **PPQ28432**
Trial ID: **S 12-08 VN**	Location: **DSM Nutritional Products France** **Centre de Recherche en Nutrition Animale** **BP 170** **68305 Saint-Louis cedex, France**
Start date and exact duration of the study: **July 3rd 2008 - 32 days**	
Number of treatment groups (+ control(s)): **8 + (2)**	Replicates per group: **2**
Total number of animals: **120**	Animals per replicate: **7 + 5 = 12**
Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)	
Intended: **0 / 250 / 500 / 1000 / 1500 / 2000 / 3000 / 4000 / 8000 U/kg**	Analysed: **108 (endogenous activity) / 374 / 601 / 1097 / 1611 / 2225 / 3098 / 4030 / 8238 U/kg**
Substances used for comparative purposes: **Dicalcium phosphate**	
Intended dose: **16 g per kg of feed. Equivalent to 3.2 g of additional P per kg of feed in a dry matter basis**	Analysed: **3.3 g of additional P per kg of feed in a dry matter basis**
Animal species/category: **Swine / weaners**	
Breed: **Large White x Landrace**	Identification procedure: **Pen and individual earring**
Sex: **Males** Age at start: **28 days**	Body weight at start: **8.03 ± 1.09 kg**
Physiological stage: **Weaned piglets**	General health: **Three animals presented leg injuries and were euthanized. No clinical sings were observed in the rest of the animals**
Additional information for field trials:	
Location and size of herd or flock:	
Feeding and rearing conditions:	
Method of feeding:	
Diets (type(s)): **Basal diet formulated to provide P exclusively from vegetable origin and according to the NRC**	
Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other	
Composition (main feedingstuffs): **Maize - 68.52%, soybean meal - 15.1% and rapeseed meal - 12.5%**	
Nutrient content (relevant nutrients and energy content)	
Intended values: **Crude protein - 15.5%, lysine - 0.96%, methionine + cystine - 0.54%, P - 0.41% in D.M. and digestible energy - 13.31 MJ/kg**	
Analysed values: **Ca - 0.82% in D.M. and P - 0.45% in D.M.**	

[1] Please submit this form using a common word processing format (e.g. MS Word).

Date and nature of the examinations performed: **July 3rd and August 4th - weight measurement** **July 29th, 30th and 31st - faecal sampling per pen** **July 31st - individual blood sampling**
Method(s) of statistical evaluation used: **Two-factor analysis of variance (diet and diet + animal or pen) followed by a Duncan multiple range test**
Therapeutic/preventive treatments (reason, timing, kind, duration): **No therapeutic / preventive treatments were used**
Timing and prevalence of any undesirable consequences of treatment: **Nothing to report**

Date **22.02.2010**	Signature Study Director Dr P. GUGGENBUHL

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

TAB

4

Annex 4

Efficacy of IPA phytase in piglets

REPORT No. 00001788

REPORT No. 00001788
Regulatory Document

DSM

Document Date: 18 September, 2009

Author(s): [redacted] (b) (4) and J. Broz[2]

[1] [redacted] (b) (4)

[2] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title: **Efficacy of IPA phytase in piglets**

Project No. 6106

Summary

A trial was conducted to study the efficacy of IPA phytase at different doses in weaned piglets. A total of 144 animals (*Landrace x Pietrain*) were involved. The piglets started on the trial at 7.1 kg live weight and remained on the experimental treatments for 6 weeks. The animals were divided into eight blocks of 6 pens (3 animals per pen), as similar as possible, taking into account initial body weight and pen location. The experimental treatments consisted of a basal, low-P, control diet (T-1), which was supplemented with IPA phytase (M) at 500, 1000, 2000, or 4000 U/kg (T-2, T-3, T-4 and T-5, respectively), and a positive control diet supplemented with 1 g of inorganic P/kg as dicalcium phosphate (T-6). Each dietary treatment was assigned to 8 replicate groups. Body weight gain, feed intake and feed conversion ratio were measured for each pen at 14, 28 and 42 days of trial. At day 14, fresh faeces were sampled from each pen and the apparent digestibility of dry matter, ash, organic matter, Ca and P was measured using titanium dioxide as indicator. At the end of trial, a blood sample was also obtained from each piglet and analysed for alkaline phosphatase activity and inorganic P and Ca concentrations. Over the whole trial, the addition of IPA phytase at 2000 and 4000 U/kg significantly improved average daily weight gain when compared to both controls. IPA phytase significantly improved the apparent faecal digestibility of ash, P and Ca in a dose response manner. The supplementation of the basal diet with different doses of IPA phytase increased P blood concentration and reduced the activity of alkaline phosphatase to the values comparable with the positive control. Finally, IPA phytase also significantly reduced the P concentration in faeces at all inclusion levels.

This report consists of Pages I – II and 1 - 28

Distribution

Dr. M. Eggersdorfer, NRD
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Approved

Name	Signature	Date
Main Author Dr. J. Broz, NRD/CA	signed by J. Broz	18.09.2009
Principal Scientist / Competence Mgr Dr. J. Broz, NRD/CA	signed by J. Broz	18.09.2009
Research Center Head Dr. A.-M. Klünter, NRD/CA	signed by A.-M. Klünter	21.09.2009
Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	23.09.2009

Nomenclature and Structural Formula

IPA phytase (M), enzyme product containing bacterial 6-phytase ([redacted] (b) (4)), produced by [redacted] (b) (4) fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28656 was used in this study, manufactured by Novozymes A/S, [redacted] (b) (4).

[redacted]

FINAL REPORT OF THE CONTRACT SIGNED WITH:

Company: DSM Nutritional Products

Title: EFFICACY OF IPA PHYTASE IN PIGLETS

Experiment number: P-393

Contract Code: 22539

Organic Code: 0602

Author: [redacted]

Center: [redacted]

Number of pages: 28

Date: 14/09/2009

TABLE OF CONTENTS

(b) (4) [redacted]

Contract code: *2 2 5 3 9*

SUMMARY

A trial was conducted to study the efficacy of IPA phytase at different doses in weaned piglets. A total of 144 animals (*Landrace x Pietrain*) were involved. The piglets started on the trial at 7.1 kg BW (4 weeks of age) and remained on the experimental treatments for 6 weeks. Pigs were divided into eight groups (blocks) of 6 pens (3 animals per pen), as similar as possible, taking into account initial body weight and pen location, to which six experimental treatments were assigned. The experimental treatments consisted of a basal, low-P, control diet (T-1), which was supplemented with 500, 1,000, 2,000, or 4,000 U/kg of IPA phytase (M) (T-2, T-3, T-4 and T-5, respectively), and a positive control diet supplemented with 1 g of inorganic P/kg as dicalcium phosphate (T-6). Body weight gain, feed intake and feed conversion rate were measured for each pen at 14, 28 and 42 days of trial. At day 14, fresh faeces were sampled from each pen and the apparent faecal digestibility of dry matter, ash, organic matter, Ca, and P was measured using titanium oxide as indigestible marker. At the end of the trial (day 42), a blood sample was also obtained from each pig and analysed for alkaline phosphatase activity and inorganic phosphorous and calcium concentrations. Over the whole trial, the addition of 2,000 and 4,000 U/kg IPA phytase improved average daily weight gain over the negative control diet. The addition of 2,000 U/kg IPA phytase also improved feed to gain ratio over the negative and positive control diets and that of 4,000 U/kg IPA phytase improved feed to gain ratio over the positive control diet. IPA phytase significantly improved the apparent faecal digestibility for ash, P and Ca in a dose response manner, relative to the negative and positive control diets. The supplementation of the negative control diet with different doses of IPA phytase reduced Ca blood concentration (at all levels of supplementation), and it increased P blood concentration in a dose response manner. The increased alkaline phosphatase activity in the negative control treatment was significantly reduced to the positive control values at all levels of IPA phytase supplementation. Finally, IPA phytase reduced the P concentration in faeces (at all levels of supplementation) in a dose response manner. It is concluded that supplementing a low-P diet with IPA phytase improves weight gain and feed to gain ratio in weaned piglets, it improves the apparent faecal digestibility for ash, P and Ca, it increases the P concentration in blood and it reduces the P concentration in faeces.

[redacted] (b) (4)

Contract code: 2 2 5 3 9

RESPONSIBILITIES

Study researcher

[redacted] (b) (4)

Study monitor

Dr. Jiri Broz
DSM Nutritional Products
Animal Nutrition and Health R&D, CH-4002 Basel, Switzerland

Daily monitors

[redacted] (b) (4)

Stockworkers

[redacted] (b) (4)

Feed preparation

[redacted] (b) (4)

Laboratory analysis

[redacted] (b) (4)

In feed enzyme analysis

[redacted] (b) (4)

OBJECTIVES

The objective of this experiment was to evaluate the efficacy of **IPA phytase** in the feeding of weaned piglets at the different dosages when compared to negative and positive controls. A low phosphorous diet was used as basal diet.

METHODOLOGY

Site of the experiment

The trial was conducted in the post-weaning unit of the [redacted] (b) (4).

Location and housing

The trial was conducted using piglets from [redacted] (b) (4)s experimental farm at [redacted] (b) (4) site. The piglets were housed in two weaning rooms with 24 pens each. The rooms are provided with automatic heating, forced ventilation and completely slatted floors. Feed was distributed *ad libitum.*

Animals

A total of one hundred and forty four piglets (*Landrace x Pietrain*) of four weeks of age were used. Their average initial body weight was 7.1 (SD 1.49) kg. Piglets were randomly distributed by initial body weight into eight blocks, and each block consisted of six pens with three piglets each.

Feeding program

There was a unique dietary composition (13.8 MJ ME; 1.4% Lysine) for the whole experiment. Feed was presented in mash form and offered *ad libitum*. The composition of the diets is presented in Tables 1 and 2. During the first two weeks of trial, feed included 0.5% of titanium oxide as indigestible marker.

Tested product

Name:	IPA phytase (M)
Description:	bacterial 6-phytase expressed in *Aspergillus oryzae*
Produced by:	Novozymes A/S, [redacted] (b) (4)
Provided by:	DSM Nutritional Products Ltd, Basel, Switzerland
Lot No:	PPQ 28656
Activity:	57 085 U/g product
Dosages:	500, 1000, 2000 and 4000 U/kg diet, corresponding to 8.8, 17.6, 35.2, and 70.4 ppm, respectively.

Treatments and experimental design

There were six experimental treatments:

T-1: Negative control (NC; a low-P basal diet)
T-2: NC + **IPA phytase** at 8.8 mg/kg, corresponding to 500 U/kg diet
T-3: NC + **IPA phytase** at 17.6 mg/kg, corresponding to 1000 U/kg diet
T-4: NC + **IPA phytase** at 35.2 mg/kg, corresponding to 2000 U/kg diet
T-5: NC + **IPA phytase** at 70.4 mg/kg, corresponding to 4000 U/kg diet
T-6: Positive control (PC, diet with an additional 1 g of inorganic P/kg from DCP)

The negative control diet was low in available phosphorous, and different doses of phytase were added via a premix using maize starch as the carrier to create the different experimental treatments.

For the first two weeks of trial, 0.5% titanium oxide was added to the diet as indigestible marker in order to perform Ca and P faecal digestibility measurements. In-feed analytical determination of the added phytase was conducted by Biopract GmbH, Berlin (Germany), on behalf of DSM Nutritional Products.

The piglets were housed in 48 pens of 3 piglets each. The animals were randomly distributed by initial weight into 8 blocks. Each block therefore consisted of 6 pens (3 pigs per pen). Within each block, one of the six treatments was randomly adjudicated to each pen.

Controls

Feed and piglets were weighed at the start, at 14 days, 28 days and at the end of the experiment (42 days). Initial and final body weight, daily weight gain, feed intake and feed conversion rate were calculated.
At the end of the second week of trial, fresh faeces were sampled from each pen. Diet and faeces were analysed for TiO_2, ash, Ca and P, and the apparent faecal digestibility was calculated.

After the conclusion of the trial, on day 42, a blood sample was obtained from one piglet from each pen and was analysed for alkaline phosphatase activity and inorganic phosphorous and calcium concentrations.

Dates

The trial started on September 18^{th} 2008 and was completed on October 30^{th}, 2008, lasting a total of 42 days.

STATISTICAL ANALYSIS

The parameters measured were compared among treatments using the GLM procedure of the statistical package SAS. Average pen values were used as the experimental unit. For statistical analysis, a randomized block design was used. The mean values for each treatment were calculated and they were compared taking into account the block effect (initial weight and pen location).

INCIDENCES

Three piglets died due to pneumonia during the second and third weeks of trial. They belonged to treatments T-4, T-6 and T-2. The data of these animals was not used for the calculations. Their feed intake was estimated from the feed intake of the pen until their withdrawal, their weight gain and the weight gain of their pen mates.

RESULTS AND DISCUSSION

The analysed composition of the experimental diets is shown in Table 3. The results of phytase analytics confirmed the proper addition of test product. The negative and positive basal diets fed to control pigs contained either non-detectable (T-1) or a low level of phytase (137 U/kg) which represents native phytase activity present in the used feed ingredients. Phytase activity in the supplemented diets were 669 (treatment T-2), 1082 (treatment T-3), 2128 (treatment T-4), and 4301 (treatment T-5) U/kg.

The performance results are shown in Tables 4-12.

Between 0-14 days, no significant differences in performance were observed among treatments. Feed to gain ratio, however, was numerically improved with the addition of 2,000 U/kg IPA phytase (T-4) relative to the positive control diet (T-6).

Between 14-28 days, the addition of 2,000 and 4,000 U/kg IPA phytase (T-4) improved average daily weight gain over the negative control diet (T-1) and the diet supplemented with 500 U/kg IPA phytase. Similarly the addition of 2,000 U/kg IPA phytase (T-4) resulted in better weight gain than the addition of 1,000 U/kg IPA phytase. No differences were observed between the low-P diets supplemented with either 2,000 or 4,000 U/kg IPA phytase and the positive control diet.

Between 28-42 days, the addition of 2,000 or 4,000 U/kg IPA phytase (T-4 and T-5) improved average daily weight gain over the negative control diet (T-1) and the diet supplemented with 1,000 U/kg IPA phytase.

Between 14-42 days, the addition of 2,000 or 4,000 U/kg IPA phytase (T-4 and T-5) improved average daily weight gain over the negative control diet (T-1) and the diets supplemented with 500 or 1,000 U/kg IPA phytase. Furthermore, the addition of 1,000 or 2,000 U/kg IPA phytase (T-3 and T-4) improved feed to gain ratio over the negative and positive control diets (T-1 and T-6).

Over the whole experimental period (0-42 days), the addition of 2,000 U/kg IPA phytase (T-4) improved average daily weight gain over the negative control diet (T-1) and the diets supplemented with 500 or 1,000 U/kg IPA phytase, and it improved feed to gain ratio over the negative and positive control diets (T-1 and T-6) and the diet supplemented with 500 U/kg IPA phytase (T-2).

The effect of the different doses of IPA phytase on the apparent faecal digestibility of dry matter, ash, organic matter, phosphorous and calcium is shown in Table 13. The apparent faecal digestibility of the negative control diet (T-1) was significantly lower than that of the positive control diet (T-1) for P (P<0.05) and numerically lower for ash and Ca. This was probably due to the removal of dicalcium phosphate from the positive control diet. The supplementation with IPA phytase (at all doses) significantly improved the apparent faecal digestibility for ash, P and Ca in a dose response manner, relative to the negative and positive control diets.

The effect of the different doses of IPA phytase on alkaline phosphatase activity, Ca and P concentration in blood and P concentration in faeces is shown in Table 14. The negative control diet increased Ca and reduced P concentration, and increased alkaline phosphatase activity in blood (P<0.05), relative to the positive control diet (T-6). No difference was observed between these two diets in the P concentration in faeces. The supplementation of the negative control diet with different doses of IPA phytase resulted in a reduced Ca concentration in blood (statistically significant at all levels of supplementation), that was not significantly different from that in the positive control diet at inclusion doses of 1,000, 2,000 and 4,000 U/kg. The supplementation of the negative control diet with IPA phytase also increased P concentration in blood (statistically significant at all levels of supplementation) in a dose response manner. The concentrations observed were equivalent to or higher than those found for the positive control diet. The increased alkaline phosphatase activity in the negative control diet was significantly reduced to the positive control diet values with all levels of IPA phytase supplementation. Finally, IPA phytase also reduced the P concentration in faeces (statistically significant at all levels of supplementation) in a dose response manner.

It is concluded that the supplementation of a low-P diet with IPA phytase improves weight gain and feed to gain ratio in weaned piglets. Under the current conditions the best performances were obtained with the addition of 2,000 U/kg of IPA phytase. IPA phytase improved the apparent faecal digestibility for ash, P and Ca, increased P concentration in blood and reduced the P concentration in faeces, in a dose response manner, relative to both control diets.

[redacted] (b) (4)

Contract code: 2 2 5 3 9

Signatures:

[redacted] (b) (4)

Date: 5-8-2009 Date: 11/8/2009 Date: 7.08.2009

Best Copy Available

TABLES AND FIGURES

Table 1 Composition of the basal experimental diets (%)

Ingredients	Low P Basal diet	STD P diet
Maize	40.00	40.00
Barley	24.68	24.68
Sweet milk whey	13.72	13.72
Soybean meal, 48% CP	9.84	9.84
Potato protein concentrate	7.23	7.23
Lard	1.49	1.49
Dicalcium phosphate	**0.42**	**1.00**
Calcium carbonate	**1.21**	**0.83**
Salt	0.14	0.14
L-Lysine-HCl	0.40	0.40
DL-Methionine	0.18	0.18
L-Threonine	0.08	0.08
L-Tryptophan	0.02	0.02
Vit-Min complex*	0.40	0.40
Maize starch	**0.19**	-

* Providing per kg of diet: vitamin A: 10000 IU; vitamin D_3: 2000 IU; vitamin E: 15 mg; thiamin: 1,3 mg; riboflavin: 3,5 mg; vitamin B_{12}: 0.025 mg; vitamin B_6: 1,5 mg; calcium pantothenate: 10 mg; nicotinic acid: 15 mg; biotin: 0.1 mg; folic acid: 0.6 mg; vitamin K_3: 2 mg; Fe: 80 mg as iron sulfate; Cu: 140 mg as copper sulfate; Co: 0.75 mg as cobalt sulfate; Zn: 185 mg as zinc oxide; Mn: 60 mg as manganese sulfate; I: 0.75 mg as potassium iodate; Se: 0.10 mg as sodium selenite; ethoxiquin: 0.15 g.

Table 2 Estimated nutritive composition of the experimental diets

Nutrients	Low P Basal diet	STD P diet
Moisture (%)	12.23	12.77
Crude Protein (%)	18.56	18.56
Crude Fibre (%)	2.45	2.45
Fat (%)	3.78	3.78
Ash (%)	4.63	4.81
Energy (MJ ME/kg)	13.85	13.82
Calcium (g/kg)	7.50	7.50
Total phosphorous (g/kg)	4.21	5.21
Non-phytic P (g/kg)	2.60	3.60
Lysine (g/kg)	14.00	14.00
Threonine (g/kg)	9.10	9.10
Methionine (g/kg)	5.13	5.13
Methionine+Cystine (g/kg)	8.40	8.40
Tryptophan (g/kg)	2.52	2.52

Table 3 Analyses of the experimental diets

Nutrients	T-1	T-2	T-3	T-4	T-5	T-6
Dry matter (%)	89.05	89.02	89.06	89.07	89.02	89.10
Crude protein (%)	19.02	18.91	18.72	18.83	18.53	18.75
Crude fibre (%)	2.14	2.19	2.17	2.12	2.18	2.06
Fat (%)	3.72	3.59	3.64	3.49	3.63	3.56
Ash (%)	4.52	4.66	4.52	4.58	4.53	4.65
Phosphorous (g/kg)	4.08	3.94	3.97	4.05	4.02	5.03
Calcium (g/kg)	6.85	6.90	6.85	7.00	7.05	6.90
Phytase activity (U/kg)	BDL*	669	1082	2128	4301	137

* BDL = Below detection limit

Table 4 Productive parameters of animals between 0-14 days of experiment

	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed intake (g/d)	Feed to gain ratio
T-1 Negative control (low P)	7.70	9.66	140	250	1.84
T-2 IPA phytase (500 U/kg)	7.76	10.05	164	266	1.76
T-3 IPA phytase (1,000 U/kg)	7.75	9.97	158	264	1.79
T-4 IPA phytase (2,000 U/kg)	7.70	10.27	184	296	1.68
T-5 IPA phytase (4,000 U/kg)	7.66	9.83	155	255	1.84
T-6 Positive control (DCP)	7.74	9.95	158	310	2.12
Root MSE	*0.086*	*0.827*	*57.9*	*70.6*	*0.428*
Block Effect (Pr>F)	***	***	**	*	**
Treat. Effect (Pr>F)	NS	NS	NS	NS	NS

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

[redacted] (b) (4)

Table 5 Productive parameters of animals between 14-28 days of experiment

	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed intake (g/d)	Feed to gain ratio
T-1 Negative control (low P)	9.66	16.04	456c	694	1.53
T-2 IPA phytase (500 U/kg)	10.05	16.23	441c	662	1.50
T-3 IPA phytase (1,000 U/kg)	9.97	16.50	467bc	654	1.41
T-4 IPA phytase (2,000 U/kg)	10.27	17.74	533a	735	1.38
T-5 IPA phytase (4,000 U/kg)	9.83	17.03	515ab	740	1.45
T-6 Positive control (DCP)	9.95	16.78	488abc	754	1.55
Root MSE	*0.827*	*1.469*	*57.0*	*95.4*	*0.145*
Block Effect (Pr>F)	***	***	***	*	NS
Treat. Effect (Pr>F)	NS	NS	*	NS	NS

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Table 6 Productive parameters of animals between 28-42 days of experiment

	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed intake (g/d)	Feed to gain ratio
T-1 Negative control (low P)	16.04	24.08c	574b	1011	1.76
T-2 IPA phytase (500 U/kg)	16.23	24.90bc	619ab	1076	1.73
T-3 IPA phytase (1,000 U/kg)	16.50	24.95bc	603b	980	1.62
T-4 IPA phytase (2,000 U/kg)	17.74	27.29a	682a	1119	1.64
T-5 IPA phytase (4,000 U/kg)	17.03	26.54ab	679a	1158	1.71
T-6 Positive control (DCP)	16.78	25.36abc	613ab	1125	1.86
Root MSE	*1.469*	*2.299*	*71.5*	*157.0*	*0.173*
Block Effect (Pr>F)	***	***	***	†	NS
Treat. Effect (Pr>F)	NS	†	*	NS	NS

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Table 7 Productive parameters of animals between 14-42 days of experiment

	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed intake (g/d)	Feed to gain ratio
T-1 Negative control (low P)	9.66	24.08c	515b	852	1.66bc
T-2 IPA phytase (500 U/kg)	10.05	24.90bc	530b	869	1.64abc
T-3 IPA phytase (1,000 U/kg)	9.97	24.95bc	535b	817	1.53a
T-4 IPA phytase (2,000 U/kg)	10.27	27.29a	608a	927	1.53a
T-5 IPA phytase (4,000 U/kg)	9.83	26.54ab	597a	949	1.60ab
T-6 Positive control (DCP)	9.95	25.36abc	550ab	940	1.72c
Root MSE	*0.827*	*2.299*	*58.9*	*114.9*	*0.122*
Block Effect (Pr>F)	***	***	***	*	NS
Treat. Effect (Pr>F)	NS	†	*	NS	*

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Table 8 Productive parameters of animals between 0-28 days of experiment

	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed intake (g/d)	Feed to gain ratio
T-1 Negative control (low P)	7.70	16.04	298	472	1.60
T-2 IPA phytase (500 U/kg)	7.76	16.23	303	464	1.55
T-3 IPA phytase (1,000 U/kg)	7.75	16.50	313	459	1.49
T-4 IPA phytase (2,000 U/kg)	7.70	17.74	359	516	1.44
T-5 IPA phytase (4,000 U/kg)	7.66	17.03	335	497	1.51
T-6 Positive control (DCP)	7.74	16.78	323	532	1.67
Root MSE	*0.086*	*1.469*	*51.8*	*79.5*	*0.165*
Block Effect (Pr>F)	***	***	***	*	†
Treat. Effect (Pr>F)	NS	NS	NS	NS	NS

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Table 9 Productive parameters of animals between 0-42 days of experiment

	Initial weight (kg)	Final weight (kg)	Weight gain (g/d)	Feed intake (g/d)	Feed to gain ratio
T-1 Negative control (low P)	7.70	24.08c	390c	652	1.68bc
T-2 IPA phytase (500 U/kg)	7.76	24.90bc	408bc	668	1.64bc
T-3 IPA phytase (1,000 U/kg)	7.75	24.95bc	410bc	633	1.55ab
T-4 IPA phytase (2,000 U/kg)	7.70	27.29a	466a	717	1.54a
T-5 IPA phytase (4,000 U/kg)	7.66	26.54ab	450ab	717	1.61ab
T-6 Positive control (DCP)	7.74	25.36abc	420abc	730	1.76c
Root MSE	*0.086*	*2.299*	*54.2*	*93.9*	*0.128*
Block Effect (Pr>F)	***	***	***	*	†
Treat. Effect (Pr>F)	NS	†	†	NS	*

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Table 10 Body weight of animals at different days of experiment calculated using individual values

	BW day 14 (kg)	BW day 28 (kg)	BW day 42 (kg)
T-1 Negative control (low P)	9.73	16.13b	24.20c
T-2 IPA phytase (500 U/kg)	10.08	16.38b	25.15bc
T-3 IPA phytase (1,000 U/kg)	9.96	16.53b	24.96bc
T-4 IPA phytase (2,000 U/kg)	10.36	17.84a	27.42a
T-5 IPA phytase (4,000 U/kg)	9.96	17.19ab	26.74ab
T-6 Positive control (DCP)	9.99	16.76ab	25.36bc
Root MSE	*1.082*	*2.177*	*3.328*
BW day 0 Effect (Pr>F)	***	***	***
Sex Effect (Pr>F)	NS	NS	NS
Treat. Effect (Pr>F)	NS	†	*

abc Values in the same column with different letters are significantly different (P<0.05).

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

Table 11 Average daily weight gain of animals at different experimental periods calculated using individual values

	Weight gain 0-14 days (g/d)	Weight gain 14-28 days (g/d)	Weight gain 28-42 days (g/d)
T-1 Negative control (low P)	141	457c	577b
T-2 IPA phytase (500 U/kg)	166	450c	626ab
T-3 IPA phytase (1,000 U/kg)	157	468bc	602b
T-4 IPA phytase (2,000 U/kg)	186	534a	684a
T-5 IPA phytase (4,000 U/kg)	157	517ab	682a
T-6 Positive control (DCP)	159	484abc	614ab
Root MSE	*77.3*	*100.6*	*119.8*
BW day 0 Effect (Pr>F)	***	***	***
Sex Effect (Pr>F)	NS	NS	NS
Treat. Effect (Pr>F)	NS	*	**

Table 12 Average daily weight gain of animals at different experimental periods calculated using individual values

	Weight gain 14-42 days (g/d)	Weight gain 0-28 days (g/d)	Weight gain 0-42 days (g/d)
T-1 Negative control (low P)	517c	299b	391c
T-2 IPA phytase (500 U/kg)	538c	308b	414bc
T-3 IPA phytase (1,000 U/kg)	536c	313b	410bc
T-4 IPA phytase (2,000 U/kg)	609a	360a	468a
T-5 IPA phytase (4,000 U/kg)	600ab	337ab	452ab
T-6 Positive control (DCP)	549bc	322ab	419bc
Root MSE	*96.1*	*77.8*	*79.3*
BW day 0 Effect (Pr>F)	***	***	***
Sex Effect (Pr>F)	NS	NS	NS
Treat. Effect (Pr>F)	**	†	*

Table 13 Effect of different doses of IPA phytase on the apparent faecal digestibility of dry matter, ash, organic matter, phosphorous and calcium in weaned piglets (%)

	Dry matter	Ash	Organic matter	P	Ca
T-1 Negative control (low P)	84.2	51.0c	86.1	37.3e	58.7d
T-2 IPA phytase (500 U/kg)	84.1	60.8b	85.5	60.5c	70.8bc
T-3 IPA phytase (1,000 U/kg)	86.0	61.3b	87.4	68.2b	73.3bc
T-4 IPA phytase (2,000 U/kg)	84.9	63.9ab	86.1	71.0b	75.0ab
T-5 IPA phytase (4,000 U/kg)	85.7	68.6a	86.6	79.3a	81.7a
T-6 Positive control (DCP)	82.8	53.3c	84.5	47.9d	66.5cd
Standard Error	*2.60*	*5.58*	*2.52*	*6.94*	*8.22*
Block Effect (Pr>F)	†	NS	†	NS	NS
Treat. Effect (Pr>F)	NS	***	NS	***	***

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

abc Values in the same column with different letters are significantly different (P<0.05)

Table 14 Alkaline phosphatase (AP) activity, Ca and P concentration in blood and P concentration in faeces of weaned piglets receiving different doses of IPA phytase

	Ca in blood (mg/dL)	P in blood (mg/dL)	AP in blood (U/L)	P in faeces (g/kg DM)
T-1 Negative control (low P)	12.36a	6.72c	900a	18.7a
T-2 IPA phytase (500 U/kg)	11.56b	8.76b	763b	10.7b
T-3 IPA phytase (1,000 U/kg)	11.09bc	8.84b	675b	10.0bc
T-4 IPA phytase (2,000 U/kg)	11.05bc	9.08ab	678b	8.9c
T-5 IPA phytase (4,000 U/kg)	10.30d	9.77a	694b	6.6d
T-6 Positive control (DCP)	10.74cd	8.71b	719b	17.2a
Standard Error	*0.657*	*0.884*	*119.5*	*1.65*
Block Effect (Pr>F)	NS	NS	NS	**
Treat. Effect (Pr>F)	***	***	**	***

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

abc Values in the same column with different letters are significantly different (P<0.05)

ANNEX-I (RAW DATA)

(b) (4)

Contract code: *2 2 5 3 9*

PEN	BLOCK	TREAT	BW 0	BW 14	BW 28	BW 42	ADG 014	ADI 014	FGR 014	ADG 1428
1	1	2	10292	13974	21737	32153	263	388	1.476	554
2	1	6	10171	12910	20534	30247	196	272	1.392	545
3	1	5	9812	12724	20787	31060	208	291	1.397	576
4	1	1	10171	13401	21362	30347	231	359	1.554	569
5	1	4	9887	11790	18989	28300	136	254	1.867	514
6	1	3	10152	14474	22656	33500	309	437	1.415	584
7	2	6	9070	11645	18649	27633	184	320	1.740	500
8	2	4	9059	12328	19599	29753	234	321	1.373	519
9	2	5	9108	12168	20692	31100	219	317	1.451	609
10	2	2	9072	10385	16179	25300	94	183	1.950	414
11	2	3	9132	12808	20782	30680	263	336	1.279	570
12	2	1	9108	10727	17461	26947	116	233	2.018	481
13	3	3	8667	9985	15003	20113	94	217	2.307	358
14	3	4	8670	12522	20627	31160	275	376	1.366	579
15	3	6	8718	11566	18637	28280	203	360	1.768	505
16	3	5	8675	10900	18869	28473	159	315	1.984	569
17	3	1	8676	11432	18363	27000	197	323	1.640	495
18	3	2	8659	13556	21485	32480	350	513	1.466	566
19	4	1	8045	9425	15857	24093	99	171	1.735	459
20	4	3	8037	10027	17000	27173	142	236	1.662	498
21	4	2	7887	9570	16366	24613	120	216	1.797	485
22	4	5	7864	10766	18469	28647	207	300	1.445	550
23	4	4	8016	11001	18928	28640	213	294	1.377	566
24	4	6	7946	10231	17471	27087	163	287	1.756	517
25	5	2	7476	10287	17103	26480	201	282	1.407	487
26	5	3	7482	8875	16267	25533	100	171	1.723	528
27	5	6	7433	9386	16346	24253	140	359	2.570	497
28	5	4	7451	10521	17821	27707	219	376	1.716	521
29	5	5	7431	8670	16587	25980	89	158	1.780	565
30	5	1	7446	8936	14447	22227	106	196	1.839	394
31	6	2	6997	7918	11653	17270	66	137	2.086	267
32	6	1	6859	8183	14151	21420	95	222	2.346	426
33	6	5	6912	8698	14991	23933	128	252	1.974	449
34	6	4	6820	8874	16200	24907	147	241	1.646	523
35	6	6	6908	9408	15339	22513	179	317	1.776	424
36	6	3	6892	8391	13503	20907	107	242	2.264	365
37	7	2	6241	7229	12990	21240	71	156	2.207	411
38	7	1	6086	8011	14589	21893	138	227	1.652	470
39	7	3	6279	8348	15219	22333	148	304	2.059	491
40	7	4	6309	8162	15950	25640	132	251	1.892	556
41	7	5	6164	6914	12121	20173	54	183	3.418	372
42	7	6	6158	7335	13803	21720	84	327	3.894	462
43	8	5	5347	7803	13760	22933	175	220	1.257	426
44	8	2	5460	7486	12343	19667	145	250	1.724	347
45	8	3	5361	6822	11574	19333	104	166	1.593	339
46	8	6	5496	7121	13464	21130	116	239	2.057	453
47	8	4	5354	6966	13813	22190	115	254	2.205	489
48	8	1	5234	7134	12127	18720	136	268	1.975	357

(b) (4)

Contract code: 2 2 5 3 9

PEN	BLOCK	TREAT	ADI 1428	FGR 1428	ADG 2842	ADI 2842	FGR 2842	ADG 028	ADI 028	FGR 028
1	1	2	858	1.548	744	1320	1.773	409	623	1.524
2	1	6	710	1.304	694	1090	1.572	370	491	1.327
3	1	5	754	1.310	734	1253	1.708	392	522	1.333
4	1	1	788	1.387	642	1134	1.768	400	573	1.435
5	1	4	683	1.328	665	1166	1.753	325	468	1.441
6	1	3	817	1.397	775	1327	1.713	447	627	1.403
7	2	6	687	1.373	642	1006	1.568	342	504	1.472
8	2	4	713	1.372	725	1137	1.567	376	517	1.373
9	2	5	839	1.377	743	1143	1.537	414	578	1.397
10	2	2	583	1.409	652	1012	1.553	254	383	1.509
11	2	3	781	1.372	707	1193	1.688	416	559	1.342
12	2	1	716	1.488	678	1243	1.834	298	475	1.591
13	3	3	530	1.478	365	609	1.668	226	373	1.650
14	3	4	772	1.333	752	1139	1.513	427	574	1.344
15	3	6	760	1.505	689	1140	1.656	354	560	1.581
16	3	5	819	1.438	686	1193	1.739	364	567	1.557
17	3	1	750	1.514	617	1107	1.794	346	536	1.550
18	3	2	892	1.576	785	1452	1.849	458	703	1.534
19	4	1	642	1.398	588	940	1.599	279	407	1.458
20	4	3	742	1.490	727	1118	1.539	320	489	1.528
21	4	2	701	1.443	589	1007	1.709	303	458	1.514
22	4	5	792	1.440	727	1231	1.693	379	546	1.441
23	4	4	755	1.333	694	1125	1.622	390	524	1.345
24	4	6	744	1.439	687	1207	1.758	340	515	1.515
25	5	2	712	1.463	670	1167	1.742	344	497	1.447
26	5	3	657	1.245	662	1037	1.567	314	414	1.321
27	5	6	864	1.738	565	1112	1.969	318	611	1.920
28	5	4	901	1.728	706	1252	1.773	370	639	1.725
29	5	5	796	1.407	671	1290	1.923	327	477	1.458
30	5	1	606	1.539	556	906	1.630	250	401	1.603
31	6	2	420	1.575	401	701	1.748	166	279	1.676
32	6	1	637	1.494	519	971	1.871	260	429	1.648
33	6	5	691	1.538	639	1012	1.584	289	472	1.634
34	6	4	679	1.298	622	1038	1.669	335	460	1.374
35	6	6	657	1.551	512	858	1.674	301	487	1.618
36	6	3	568	1.555	529	836	1.580	236	405	1.716
37	7	2	544	1.323	589	1027	1.742	241	350	1.452
38	7	1	745	1.586	522	968	1.855	304	486	1.601
39	7	3	674	1.373	508	849	1.670	319	489	1.532
40	7	4	728	1.308	692	1144	1.653	344	489	1.420
41	7	5	607	1.632	575	1041	1.811	213	395	1.857
42	7	6	935	2.023	566	1127	1.992	273	631	2.311
43	8	5	621	1.459	655	1099	1.677	300	421	1.400
44	8	2	589	1.697	523	920	1.759	246	419	1.705
45	8	3	460	1.355	554	870	1.570	222	313	1.411
46	8	6	675	1.489	548	1462	2.670	285	457	1.605
47	8	4	652	1.333	598	951	1.589	302	453	1.499
48	8	1	668	1.873	471	815	1.731	246	468	1.901

[redacted] (b) (4)

Contract code: 2 2 5 3 9

PEN	BLOCK	TREAT	ADG 042	ADI 042	FGR 042	ADG 1442	ADI 1442	FGR 1442	Blood Ca	Blood P
1	1	2	521	855	1.643	649	1089	1.677	11.2	10.14
2	1	6	478	691	1.446	619	900	1.454	10.7	9.42
3	1	5	506	766	1.514	655	1004	1.533	10	9.04
4	1	1	480	760	1.583	605	961	1.589	12.9	6.36
5	1	4	438	701	1.598	590	924	1.567	10.9	9.29
6	1	3	556	860	1.547	680	1072	1.577	12.5	9.58
7	2	6	442	671	1.518	571	847	1.483	11.1	7.47
8	2	4	493	723	1.468	622	925	1.486	11.2	9.58
9	2	5	524	766	1.463	676	991	1.465	10.9	9.71
10	2	2	386	593	1.534	533	797	1.497	12.4	7.52
11	2	3	513	770	1.501	638	987	1.547	10.7	8.94
12	2	1	425	731	1.720	579	979	1.690	11.5	7.87
13	3	3	273	452	1.658	362	569	1.574	10.9	9.49
14	3	4	535	762	1.423	666	955	1.435	10.8	9.14
15	3	6	466	753	1.618	597	950	1.592	11	8.88
16	3	5	471	776	1.645	628	1006	1.602	10.6	9.25
17	3	1	436	726	1.665	556	928	1.669	12.6	7.14
18	3	2	567	952	1.679	676	1172	1.734	11.1	9.59
19	4	1	382	585	1.530	524	791	1.511	12.7	6.68
20	4	3	456	699	1.534	612	930	1.519	10.8	9.08
21	4	2	398	641	1.610	537	854	1.589	11.9	9.68
22	4	5	495	774	1.564	639	1011	1.584	10.8	9.3
23	4	4	491	725	1.476	630	940	1.492	11.6	8.65
24	4	6	456	746	1.637	602	976	1.621	10.4	9.67
25	5	2	452	720	1.592	578	940	1.625	11	9.27
26	5	3	430	622	1.447	595	847	1.424	11.3	8.06
27	5	6	400	778	1.943	531	988	1.861	10.9	7.42
28	5	4	482	843	1.748	614	1077	1.754	10.4	9.81
29	5	5	442	748	1.694	618	1043	1.687	10.2	9.73
30	5	1	352	569	1.617	475	756	1.592	12.8	6.19
31	6	2	245	420	1.716	334	561	1.679	11.1	7.21
32	6	1	347	610	1.759	473	804	1.701	13	6.41
33	6	5	405	652	1.608	544	851	1.565	10.3	9.44
34	6	4	431	653	1.516	573	859	1.499	11.1	9.09
35	6	6	372	611	1.643	468	757	1.618	9.5	8.59
36	6	3	334	549	1.644	447	702	1.570	9.9	8.95
37	7	2	357	576	1.612	500	785	1.570	12.5	9.54
38	7	1	376	647	1.718	496	856	1.727	12.2	6.58
39	7	3	382	609	1.593	499	761	1.524	10.6	7.99
40	7	4	460	707	1.537	624	936	1.499	10.7	9.12
41	7	5	334	610	1.830	474	824	1.741	9.2	9.72
42	7	6	371	796	2.149	514	1031	2.006	11.5	10.1
43	8	5	419	647	1.544	540	860	1.591	10.4	11.94
44	8	2	338	586	1.733	435	754	1.734	11.3	7.1
45	8	3	333	499	1.499	447	665	1.488	12	8.62
46	8	6	372	792	2.127	500	1068	2.135	10.8	8.14
47	8	4	401	619	1.544	544	801	1.474	11.7	7.98
48	8	1	321	584	1.818	414	742	1.792	11.2	6.53

[redacted] (b) (4)

Contract code: 2 2 5 3 9

PEN	BLOCK	TREAT	Blood AP	Faeces P	Dig DM	Dig Ash	Dig OM	Dig P	Dig Ca
1	1	2	902	10.858	84.90	61.33	86.26	61.86	75.37
2	1	6	873	16.787	87.59	65.89	88.86	62.99	74.66
3	1	5	647	6.109	89.00	75.74	89.76	85.07	84.43
4	1	1	798	18.878	83.84	44.64	86.09	33.65	60.96
5	1	4	565	8.805	87.28	71.16	88.24	75.72	82.73
6	1	3	619	11.645	88.64	60.98	90.20	69.78	69.38
7	2	6	807	16.830	83.22	55.69	84.83	49.82	75.96
8	2	4	857	11.097	85.72	58.90	87.32	65.65	75.29
9	2	5	618	8.707	85.75	62.60	87.07	72.44	75.84
10	2	2	840	9.983	83.67	61.23	84.97	62.09	67.25
11	2	3	536	12.873	89.23	61.82	90.76	68.30	59.04
12	2	1	939	19.058	85.98	53.74	87.83	41.89	59.78
13	3	3	543	10.884	84.09	59.49	85.47	60.43	77.86
14	3	4	611	9.823	87.10	62.59	88.56	72.53	78.44
15	3	6	538	18.865	86.10	55.65	87.88	53.41	59.97
16	3	5	763	6.280	87.55	73.04	88.38	82.63	83.13
17	3	1	852	17.289	81.73	46.58	83.74	31.29	56.74
18	3	2	635	10.513	82.65	53.98	84.30	57.56	61.76
19	4	1	1060	22.159	86.92	56.51	88.66	36.95	75.53
20	4	3	780	9.339	79.01	52.45	80.50	55.20	66.90
21	4	2	551	10.681	85.95	69.73	86.89	65.10	76.77
22	4	5	753	6.201	83.87	66.08	84.88	77.78	80.42
23	4	4	632	5.894	82.29	64.92	83.32	77.37	76.20
24	4	6	843	17.954	81.35	46.46	83.39	40.50	50.16
25	5	2	687	9.382	85.13	63.93	86.35	67.54	72.77
26	5	3	739	9.907	86.32	68.44	87.32	69.02	80.60
27	5	6	579	16.197	81.41	54.09	83.01	46.49	73.34
28	5	4	540	9.272	84.18	59.44	85.65	68.20	68.90
29	5	5	427	6.552	84.13	66.95	85.12	76.91	82.86
30	5	1	950	19.613	86.17	58.07	87.79	41.02	65.40
31	6	2	793	12.903	86.47	63.11	87.82	59.40	75.51
32	6	1	893	20.937	87.07	53.69	88.99	41.14	52.76
33	6	5	907	8.054	86.75	68.96	87.77	76.30	85.28
34	6	4	617	10.524	83.73	63.12	84.96	62.89	64.23
35	6	6	541	18.365	85.40	54.77	87.19	52.36	69.79
36	6	3	664	9.399	85.82	67.52	86.84	69.53	82.05
37	7	2	877	11.331	81.43	53.34	83.05	51.05	62.87
38	7	1	879	19.849	82.57	45.23	84.71	24.75	48.29
39	7	3	737	11.892	87.45	54.95	89.27	65.89	64.68
40	7	4	756	8.005	85.82	67.84	86.89	75.39	86.86
41	7	5	714	6.591	87.80	73.82	88.59	82.13	86.32
42	7	6	666	16.614	75.39	39.43	77.50	27.35	58.79
43	8	5	725	4.348	80.48	61.96	81.53	81.14	75.38
44	8	2	822	10.141	82.88	59.38	84.24	59.62	74.21
45	8	3	783	4.315	87.46	64.66	88.74	87.64	86.26
46	8	6	907	15.756	82.10	54.49	83.71	49.88	69.38
47	8	4	844	8.048	82.91	63.38	84.07	70.18	67.38
48	8	1	832	11.593	79.36	49.44	81.08	47.97	50.29

[redacted] (b) (4)

Contract code: 2 2 5 3 9

PEN	PIG	SEX	BLOCK	TREAT	BW 0	BW 14	BW 28	BW 42	ADG 014	ADG 1428	ADG 2842	ADG 028	ADG 042	ADG 1442
1	3655	2	1	2	10203	14020	21547	27900	273	538	454	405	421	496
1	3664	2	1	2	11060	14375	22275	34540	237	564	876	401	559	720
1	3675	1	1	2	9613	13528	21388	34020	280	561	902	421	581	732
2	3561	2	1	6	9400	12260	21011	31680	204	625	762	415	530	694
2	3652	1	1	6	10184	13171	19495	28460	213	452	640	333	435	546
2	3671	1	1	6	10928	13300	21097	30600	169	557	679	363	468	618
3	3589	2	1	5	10240	11443	17015	28020	86	398	786	242	423	592
3	3639	1	1	5	9450	13480	22560	32100	288	649	681	468	539	665
3	3640	2	1	5	9745	13248	22786	33060	250	681	734	466	555	708
4	3641	2	1	1	9367	12856	20993	29980	249	581	642	415	491	612
4	3647	2	1	1	10096	12867	20204	29520	198	524	665	361	462	595
4	3658	1	1	1	11050	14480	22888	31540	245	601	618	423	488	609
5	3538	2	1	4	9540	11680	19311	28020	153	545	622	349	440	584
5	3543	2	1	4	10260	12514	19023	29400	161	465	741	313	456	603
5	3663	2	1	4	9860	11174	18633	27480	94	533	632	313	420	582
6	3583	2	1	3	9377	17040	26994	38380	547	711	813	629	691	762
6	3654	1	1	3	11269	14730	22060	32400	247	524	739	385	503	631
6	3673	2	1	3	9810	11651	18913	29720	131	519	772	325	474	645
7	3580	1	2	6	9168	12369	20648	29380	229	591	624	410	481	608
7	3585	1	2	6	9047	12465	22600	32120	244	724	680	484	549	702
7	3659	1	2	6	8995	10100	12700	21400	79	186	621	132	295	404
8	3547	1	2	4	9020	11768	17863	29040	196	435	798	316	477	617
8	3595	2	2	4	9240	12200	20120	29460	211	566	667	389	481	616
8	3636	2	2	4	8917	13017	20813	30760	293	557	710	425	520	634
9	3540	1	2	5	9093	11184	20849	32200	149	690	811	420	550	751
9	3553	2	2	5	8964	13388	21724	31020	316	595	664	456	525	630
9	3643	2	2	5	9268	11933	19503	30080	190	541	755	366	496	648
10	3563	1	2	2	9200	10916	18493	27860	123	541	669	332	444	605
10	3565	1	2	2	8984	8738	11400	20880	-18	190	677	86	283	434
10	3649	1	2	2	9032	11500	18643	27160	176	510	608	343	432	559
11	3530	2	2	3	9287	12320	20624	29900	217	593	663	405	491	628
11	3549	2	2	3	9107	13027	20680	32640	280	547	854	413	560	700
11	3633	1	2	3	9003	13077	21043	29500	291	569	604	430	488	587
12	3581	2	2	1	9000	12117	19389	28640	223	519	661	371	468	590
12	3630	2	2	1	9240	10207	18343	28120	69	581	698	325	450	640
12	3661	2	2	1	9084	9856	14652	24080	55	343	673	199	357	508
13	3593	2	3	3	8497	8750	10890	8720	18	153	-155	85	5	-1
13	3642	1	3	3	8745	11152	20178	30560	172	645	742	408	519	693
13	3651	1	3	3	8760	10053	13940	21060	92	278	509	185	293	393
14	3534	1	3	4	8464	11610	18545	28020	225	495	677	360	466	586
14	3552	1	3	4	8685	13655	21917	32380	355	590	747	473	564	669
14	3599	1	3	4	8860	12300	21420	33080	246	651	833	449	577	742
15	3546	2	3	6	8814	12207	20168	31340	242	569	798	405	536	683
15	3564	1	3	6	8650	11220	17591	27160	184	455	683	319	441	569
15	3632	2	3	6	8690	11270	18153	26340	184	492	585	338	420	538
16	3619	1	3	5	8444	10780	18383	27640	167	543	661	355	457	602
16	3662	1	3	5	8856	10543	18497	28140	120	568	689	344	459	628
16	3668	1	3	5	8725	11376	19729	29640	189	597	708	393	498	652

(b) (4)

Contract code: 2 2 5 3 9

PEN	PIG	SEX	BLOCK	TREAT	BW 0	BW 14	BW 28	BW 42	ADG 014	ADG 1428	ADG 2842	ADG 028	ADG 042	ADG 1442
17	3537	2	3	1	8860	11020	18308	27480	154	521	655	337	443	588
17	3590	2	3	1	8457	11026	17555	26140	184	466	613	325	421	540
17	3648	1	3	1	8712	12250	19227	27380	253	498	582	376	444	540
18	3570	1	3	2	8753	13980	21137	31900	373	511	769	442	551	640
18	3656	2	3	2	8760	13233	20736	30880	319	536	725	428	527	630
18	3669	1	3	2	8463	13454	22583	34660	356	652	863	504	624	757
19	3529	2	4	1	7755	9502	16296	24580	125	485	592	305	401	538
19	3558	1	4	1	8420	11106	19329	29020	192	587	692	390	490	640
19	3594	1	4	1	7960	7666	11946	18680	-21	306	481	142	255	393
20	3567	2	4	3	8093	11680	18477	28720	256	486	732	371	491	609
20	3568	1	4	3	7710	9177	17197	27880	105	573	763	339	480	668
20	3615	1	4	3	8307	9223	15327	24920	65	436	685	251	396	561
21	3569	2	4	2	7760	9690	14784	23400	138	364	615	251	372	490
21	3584	1	4	2	8247	9584	16792	24600	96	515	558	305	389	536
21	3629	2	4	2	7655	9437	17523	25840	127	578	594	352	433	586
22	3544	2	4	5	7680	11832	21015	32120	297	656	793	476	582	725
22	3555	1	4	5	8124	10760	19468	28840	188	622	669	405	493	646
22	3607	1	4	5	7788	9706	14923	24980	137	373	718	255	409	546
23	3556	2	4	4	8240	10584	18013	28120	167	531	722	349	473	626
23	3587	1	4	4	8103	12251	20853	30940	296	614	721	455	544	667
23	3609	1	4	4	7703	10168	17918	26860	176	554	639	365	456	596
24	3533	2	4	6	8093	9955	15023	24200	133	362	656	247	383	509
24	3625	2	4	6	7632	10062	17791	26720	174	552	638	363	454	595
24	3660	2	4	6	8113	10676	19600	30340	183	637	767	410	529	702
25	3545	1	5	2	7467	10329	16103	25040	204	412	638	308	418	525
25	3627	1	5	2	7390	10554	17660	27720	226	508	719	367	484	613
25	3628	2	5	2	7572	9977	17548	26680	172	541	652	356	455	597
26	3542	1	5	3	7400	8895	15702	25000	107	486	664	297	419	575
26	3551	1	5	3	7590	9207	16677	26100	116	534	673	325	441	603
26	3605	1	5	3	7456	8523	16423	25500	76	564	648	320	430	606
27	3575	2	5	6	7465	8697	14706	19560	88	429	347	259	288	388
27	3617	2	5	6	7523	10257	18513	27860	195	590	668	393	484	629
27	3644	2	5	6	7310	9205	15819	25340	135	472	680	304	429	576
28	3562	2	5	4	7347	9817	15977	25900	176	440	709	308	442	574
28	3638	1	5	4	7560	10585	18517	27640	216	567	652	391	478	609
28	3667	1	5	4	7447	11160	18969	29580	265	558	758	412	527	658
29	3637	2	5	5	7496	8464	16260	25460	69	557	657	313	428	607
29	3646	2	5	5	7560	9100	16923	26380	110	559	676	334	448	617
29	3672	2	5	5	7236	8447	16577	26100	86	581	680	334	449	630
30	3602	2	5	1	7572	9471	15869	23760	136	457	564	296	385	510
30	3608	1	5	1	7267	8060	13580	21260	57	394	549	225	333	471
30	3650	1	5	1	7500	9277	13892	21660	127	330	555	228	337	442
31	3606	1	6	2	.	.	.	.	.	.	.	.	.	.
31	3645	1	6	2	6833	7890	12656	17980	75	340	380	208	265	360
31	3677	1	6	2	7160	7947	10649	16560	56	193	422	125	224	308
32	3596	2	6	1	7104	8528	14838	22340	102	451	536	276	363	493
32	3635	1	6	1	6620	8757	15733	22520	153	498	485	325	379	492
32	3666	2	6	1	6852	7264	11883	19400	29	330	537	180	299	433

(b) (4)

Contract code: *2 2 5 3 9*

PEN	PIG	SEX	BLOCK	TREAT	BW 0	BW 14	BW 28	BW 42	ADG 014	ADG 1428	ADG 2842	ADG 028	ADG 042	ADG 1442
33	3535	2	6	5	7020	7980	14846	23480	69	490	617	279	392	554
33	3536	2	6	5	7180	8978	15277	23560	128	450	592	289	390	521
33	3621	1	6	5	6536	9137	14850	24760	186	408	708	297	434	558
34	3557	2	6	4	6817	8087	15893	24720	91	558	631	324	426	594
34	3591	1	6	4	6444	8638	15512	24800	157	491	663	324	437	577
34	3598	2	6	4	7200	9897	17195	25200	193	521	572	357	429	547
35	3539	2	6	6	6560	7998	15120	25260	103	509	724	306	445	616
35	3566	1	6	6	7197	9963	16304	26860	198	453	754	325	468	603
35	3600	2	6	6	6967	10263	14593	15420	235	309	59	272	201	184
36	3573	1	6	3	6940	8804	13603	21000	133	343	528	238	335	436
36	3618	1	6	3	7184	8480	11949	17820	93	248	419	170	253	334
36	3626	1	6	3	6552	7889	14957	23900	95	505	639	300	413	572
37	3541	2	7	2	6443	7378	12560	20160	67	370	543	218	327	457
37	3550	2	7	2	6050	6883	13205	22040	59	452	631	256	381	541
37	3670	2	7	2	6230	7427	13205	21520	85	413	594	249	364	503
38	3559	2	7	1	6207	8518	15791	24100	165	520	593	342	426	557
38	3597	1	7	1	6327	8647	15393	23000	166	482	543	324	397	513
38	3612	2	7	1	5725	6869	12583	18580	82	408	428	245	306	418
39	3582	1	7	3	6160	8880	16215	23900	194	524	549	359	422	536
39	3634	1	7	3	6376	7720	13834	20580	96	437	482	266	338	459
39	3653	1	7	3	6300	8443	15607	22520	153	512	494	332	386	503
40	3531	1	7	4	6323	7930	15576	26040	115	546	747	330	469	647
40	3586	2	7	4	6417	8465	15800	25140	146	524	667	335	446	596
40	3610	2	7	4	6187	8091	16475	25740	136	599	662	367	466	630
41	3560	1	7	5	6240	6689	13100	23360	32	458	733	245	408	595
41	3588	1	7	5	6380	7840	12390	18020	104	325	402	215	277	364
41	3604	2	7	5	5873	6212	10872	19140	24	333	591	179	316	462
42	3576	2	7	6	5817	7389	14308	23020	112	494	622	303	410	558
42	3622	2	7	6	6272	7267	13666	21840	71	457	584	264	371	520
42	3676	2	7	6	6387	7349	13435	20300	69	435	490	252	331	463
43	3601	2	8	5	5713	9153	16978	27500	246	559	752	402	519	655
43	3614	2	8	5	5513	8054	14180	22520	181	438	596	310	405	517
43	3674	1	8	5	4813	6203	10122	18780	99	280	618	190	333	449
44	3577	1	8	2	5457	7593	12929	20360	153	381	531	267	355	456
44	3603	1	8	2	5277	7103	11931	19280	130	345	525	238	333	435
44	3623	1	8	2	5647	7763	12170	19360	151	315	514	233	327	414
45	3548	2	8	3	5000	5940	11170	19680	67	374	608	220	350	491
45	3613	1	8	3	5440	7811	12326	19420	169	322	507	246	333	415
45	3616	2	8	3	5643	6713	11227	18900	76	322	548	199	316	435
46	3572	2	8	6	5611	7940	15985	25020	166	575	645	371	462	610
46	3611	2	8	6	5380	6303	10942	17240	66	331	450	199	282	391
46	3631	2	8	6	.	.	.	.	.	.	.	.	.	.
47	3571	1	8	4	5123	6570	12500	20900	103	424	600	263	376	512
47	3592	2	8	4	5584	7363	15126	23480	127	555	597	341	426	576
47	3657	1	8	4	.	.	.	.	.	.	.	.	.	.
48	3532	1	8	1	5580	7330	12635	19900	125	379	519	252	341	449
48	3554	2	8	1	5377	7205	11668	18180	131	319	465	225	305	392
48	3579	1	8	1	4744	6866	12078	18080	152	372	429	262	318	401

ANNEX-II (EFSA)

efsa
European Food Safety Authority

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA phytase (M)** Batch number: **PPQ 28656**

Trial ID: **P-393** Location: [redacted] (b) (4)

Start date and exact duration of the study: **November 18th 2008, 17days**

Number of treatment groups (+ control(s)): **6** Replicates per group: **8**

Total number of animals: **48** Animals per replicate: **3**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **500, 1000, 2000 and 4000 U/kg** Analysed: **669, 1082, 2128 and 4301 U/kg**

†

Substances used for comparative purposes: **Dicalcium phosphate in positive control**

Intended dose: **1 g P/kg** Analysed: **1.02 g P/kg**

Animal species/category: **Weanling pigs**

Breed: **Landrace x Pietrain** Identification procedure: **Ear tags**

Sex: **Males and Females** Age at start: **4 weeks** Body weight at start: **7.1 kg**

Physiological stage: **Weanling** General health: **optimal**

Additional information for field trials:

Location and size of herd or flock:

Feeding and rearing conditions:

Method of feeding:

Diets (type(s)): **Low and adequate P diets for grower pigs**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize, barley, sweet milk whey, soybean meal-48, potato protein concentrate**

Nutrient content (relevant nutrients and energy content)

Intended values: **18.6% CP, 3945 kcal/kg GE, 4.63 & 4.81% ash, 0.42 & 0.52% total P, 0.75% Ca**

Analysed values: **18.8% CP, 3970 kcal/kg GE, 4.56 & 4.65% ash, 0.40 & 0.50% total P, 0.69% Ca**

Date and nature of the examinations performed: **Performance, P and Ca concentration in blood and faeces and apparent faecal digestibility of P and Ca**

Method(s) of statistical evaluation used: **Analysis of variance (GLM procedure)**

Therapeutic/preventive treatments (reason, timing, kind, duration): **not relevant**

Timing and prevalence of any undesirable consequences of treatment: **no adverse effects observed**

Date	Signature Study Director
7-08-2009	[redacted] (b) (4)

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

5

Annex 5

Effects of a novel phytase in corn-soybean meal diets fed to weanling pigs

REPORT No. 00003284

REPORT No. 00003284
Regulatory Document

DSM

Document Date:	9 December, 2009
Author(s):	[redacted] (b) (4), D.-R. Campbell[2] and J. Broz[3]

[1] [redacted] (b) (4)
[2] DSM Nutritional Products, Inc, Parsippany (USA)
[3] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title:	**Effects of a novel phytase in corn-soybean meal diets fed to weanling pigs**
Project No.	6106

Summary

An experiment was conducted in order to evaluate the effects of a novel microbial phytase (IPA Mash phytase) on the apparent total tract digestibility (ATTD) of P in corn-soybean meal diets fed to weanling pigs. Six different diets were formulated for this study. The positive control diet was based on corn and soybean meal and contained dicalcium phosphate to bring the total concentration of P to 0.66%. A negative, low-P control diet without dicalcium phosphate was formulated to contain 0.36% P. Four additional diets were similar to the negative control diet with the exception that IPA Mash phytase was included at 500, 1000, 2000 and 4000 U/kg diet, respectively. A total of 48 weanling pigs (mean body weight of 13.5 kg) were placed in metabolism cages and randomly allotted to the 6 dietary treatments in a randomized complete block design. Faeces were collected over a 5-day period after 5 days of adaptation to the diets. The total P output and P concentration in faeces were reduced (linear and quadratic, $P<0.01$) as phytase was added to the negative control diet. The ATTD of P was greater ($P<0.01$) for the positive control diet (60.5%) than for the negative control diet (40.5%), but increased (linear and quadratic, $P<0.01$) as graded phytase inclusions were added to the negative control diet from 40.5% to 61.6, 65.1, 68.7, and 68.0%, respectively. The breakpoint for ATTD of P (68.4%) was reached at a phytase inclusion level of 1016 U/kg diet. In conclusion, IPA Mash phytase reduced the amount of P excreted in the faeces and increased the ATTD of P in weanling pigs.

This report consists of Pages I – II and 1 – 22, raw data & Annex C

Distribution

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Approved

Name	Signature	Date
Main Author Dr. J. Broz, NRD/CA	signed by J. Broz	09.12.2009
Principal Scientist / Competence Mgr Dr. J. Broz, NRD/CA	signed by J. Broz	09.12.2009
Research Center Head Dr. A.-M. Klünter, NRD/CA	signed by A.-M. Klünter	10.12.2009
Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	11.12.2009

Nomenclature and Structural Formula

IPA phytase (M), enzyme product containing bacterial 6-phytase ([redacted]), produced by [redacted] fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28683 was used in this study, manufactured by Novozymes A/S, [redacted].

Effects of a novel phytase in corn-soybean meal diets fed to weanling pigs[1]

[redacted] (b) (4)

[redacted] (b) (4)

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[1] This research was financially supported by DSM Nutritional Products, Parsippany, NJ.

[2] Corresponding author: [redacted] (b) (4)

ABSTRACT: An experiment was conducted to evaluate the effects of a novel microbial phytase on the apparent total tract digestibility (ATTD) of P in corn-soybean meal diets fed to weanling pigs. Six diets were formulated. The positive control diet was a corn-soybean meal diet that contained dicalcium phosphate to bring the total concentration of P to 0.66%. A negative control diet (0.36% P) without dicalcium phosphate was also formulated. Four additional diets that were similar to the negative control diet were formulated to contain microbial phytase (IPA Mash, DSM Nutritional Products, Parsippany, NJ) at levels of 500, 1,000, 2,000, or 4,000 phytase units (FTU) per kg. Forty eight weanling pigs (initial BW: 13.5 ± 2.45kg) were placed in metabolism cages and randomly allotted to the 6 dietary treatments in a randomized complete block design. Feces were collected over a 5-d period after 5 d of adaptation to the diets. The total P output and P concentration in feces were reduced (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet. The ATTD of P was greater ($P < 0.01$) for the positive control diet (60.48%) than for the negative control diet (40.46%), but increased (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet (40.46 vs. 61.56, 65.07, 68.74, and 68.04%). The breakpoint for ATTD of P (68.39%) was reached at a phytase inclusion level of 1016 FTU/kg. In conclusion, IPA mash phytase reduced the amount of P excreted in the feces and increased the ATTD of P in weanling pigs.

Key words: digestibility, phosphorus, pigs, phytase

INTRODUCTION

Most of the P in cereal grains and oilseeds is in the form of phytate (Erdman, 1979). Because pigs lack endogenous phytases, phytate cannot be digested in the small intestine (Selle and Ravindran, 2008). As a consequence, large amounts of P are excreted in the manure, which may potentially cause environmental pollution. Divalent cations such as Ca likely form insoluble phytate complexes, which may reduce the hydrolysis of phytate. Phytases are enzymes capable of hydrolyzing the phytate molecule, which results in the release of phytate-P as well as Ca, which can then be utilized by pigs (Selle and Ravindran, 2008), and addition of microbial phytase to swine diets improve P utilization by pigs (Akinmusire and Adeola, 2009). Several microbial phytases are commercially available and the inclusion of exogenous phytase to swine diets has become a routine practice, but new and more efficient microbial phytases are constantly being developed. Therefore, an experiment was conducted with the objective of measuring the effect of a novel microbial phytase (IPA Mash, DSM Nutritional Products) on the digestibility of P and Ca in corn-soybean meal diets fed to weanling pigs.

MATERIALS AND METHODS

Diets, Animals, and Experimental Design

Six diets were formulated (Tables 1 and 2). The positive control diet was a corn-soybean meal diet that contained quantities of Ca and P sufficient to meet the requirement of Ca and P for weanling (10-20 kg) pigs (NRC, 1998). Dicalcium phosphate and limestone were used to bring the total concentration of P and Ca in this diet to 0.66 and 0.86%, respectively. A negative control diet that was similar to the positive control diet with the exception that cornstarch replaced dicalcium phosphate was also formulated. This diet contained 0.36% P and 0.48% Ca. Four additional diets that were similar to the negative control diet with the exception that microbial phytase (IPA Mash, DSM Nutritional Products, Parsippany, NJ) was included in the amounts of 500, 1,000, 2,000, or 4,000 phytase units (**FTU**) were also formulated.

A total of 48 weanling pigs (initial BW: 13.5 ± 2.45 kg) were used in a randomized complete block design. Pigs were the offspring of Landrace boars that were mated to Large White x Duroc sows (Pig Improvement Company, Hendersonville, TN). Pigs were blocked by BW and randomly allotted to the 6 dietary treatments in 8 blocks using the Experimental Animal Allotment Program (Kim and Lindemann, 2007). Pigs were placed in metabolism cages equipped with a feeder and a nipple drinker that allowed for total collection of feces.

Feeding and Sample Collection

The amount of feed provided daily was calculated as 3 times the estimated requirement for maintenance energy (i.e., 106 kcal ME per kg $^{0.75}$; NRC, 1998) and divided into 2 equal meals. Water was available at all times. The initial 5 d were considered an adaptation period to the diet. From d 6 to 11, fecal materials were collected according to the marker to marker approach (Adeola, 2001). Chromic oxide and ferric oxide were used to determine the beginning and the conclusion of collections, respectively. Fecal samples were stored at -20°C immediately after collection.

Sample Analysis and Data Processing

At the conclusion of the experiment, fecal samples were dried in a forced air oven and finely ground. Fecal samples and diets were analyzed for Ca and P by inductively coupled plasma (ICP) spectroscopy (method 985.01; AOAC Int., 2007) after wet ash sample preparation (method 975.03; AOAC Int., 2007). Diets were also analyzed for AA (method 982.30 E (a, b, c);

AOAC Int., 2007), ADF (method 973.18; AOAC Int., 2007), NDF (Holst, 1973), DM by oven drying at 135°C for 2 h (method 930.15; AOAC Int., 2007), ash (method 942.05; AOAC Int., 2007), CP (method 990.03; AOAC Int., 2007) using an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ), and for phytase activity (DSM Nutritional Products, Parsippany, NJ).

The apparent total tract digestibility (**ATTD**) of P in each diet was calculated according to the following equation:

$$ATTD\ (\%) = [(Pi - Pf)/Pi] \times 100,$$

where Pi = total P intake (g) from d 6 to 11 and Pf = total fecal P output (g) originating from the feed that was provided from d 6 to 11(Petersen and Stein., 2006).

Data were analyzed as a randomized complete block design using the Proc Mixed Procedure in SAS. The UNIVARIATE procedure was used to verify homogeneity of variances and to identify outliers. The model included diet as the main effect and block as a random effect. The effect of block was not significant and, therefore, removed from the final model. A contrast of the positive control diet vs. the negative control diet was performed to analyze the effects of removing inorganic P from the diets, and orthogonal polynomial contrasts were conducted to test linear and quadratic responses to the inclusion of phytase in the diets. Appropriate coefficients for unequally spaced concentrations of supplemental phytase were obtained using the interactive matrix language procedure (Proc IML) of SAS. Treatments were considered different when $P < 0.05$ and the pig was the experimental unit for all analyses. The minimum level of phytase that was needed to maximize the ATTD of P and Ca were estimated by subjecting the treatment means to a least squares broken-line analysis as described by Robbins et al. (2006). The pig was the experimental unit for all analyses and an alpha level of 0.05 was used to assess significance among means.

RESULTS

There was no difference in feed intake and in fecal output among treatments (Table 3). Phosphorus intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet. The concentration of P excreted in the feces was lower ($P < 0.05$) for pigs fed the negative control diet than for pigs fed the positive control diet. Likewise, pigs that were fed phytase containing diets had lower (linear and quadratic, $P < 0.01$) concentration of P in feces than pigs fed the negative control diet. The daily P output was also lower ($P < 0.01$) for pigs fed the negative control diet than for pigs fed the positive control diet, and the inclusion of increasing levels of phytase to the negative control diet caused linear and quadratic reductions ($P < 0.01$) in P output. The ATTD of P was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (60.48 vs. 40.46%), but the ATTD of P increased (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet (61.56, 65.07, 68.74, and 68.04% for pigs fed diets containing 500, 1,000, 2,000, or 4,000 FTU of phytase, respectively). Phosphorus absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (2.58 vs. 0.93 g/d), but the addition of phytase to the negative control diet increased (linear and quadratic, $P < 0.01$) P absorption to 1.39, 1.51, 1.54, and 1.46 g/d. The breakpoint for phytase concentration resulted in an ATTD of P of 68.39%, which was reached when 1,015.8 FTU/kg of phytase was added to the diet (Figure 1).

Calcium intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (5.55 vs. 3.04 g/d). Pigs that were fed phytase containing diets tended ($P = 0.06$) to have a greater Ca intake than pigs fed the negative control diet.

Concentration of Ca in feces was greater ($P < 0.05$) for pigs fed the positive control diet compared with pigs fed the negative control diet (2.29 vs. 1.86%), but pigs fed phytase containing diets had lower Ca concentration in feces than pigs fed the negative control diet (linear and quadratic, $P < 0.01$). The daily Ca output was also greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (1.52 vs. 1.09 g/d), but the addition of 500, 1,000, 2,000, or 4,000 FTU/kg of phytase to the negative control diet reduced (linear and quadratic, $P < 0.01$) Ca output to 0.80, 0.60, 0.52, and 0.50%, respectively. The ATTD of Ca was greater ($P < 0.05$) for pigs fed the positive control diet than for pigs fed the negative control diet (72.45 vs. 63.90%), but pigs fed diets containing 500, 1,000, 2,000, or 4,000 FTU/kg of phytase had greater (linear and quadratic, $P < 0.01$) ATTD of Ca than pigs fed the negative control diet (73.71, 81.66, 84.81, and 84.63%). The absorption of Ca was reduced ($P < 0.01$) from 4.02 to 1.95 g/d for pigs fed the negative control diet rather than the positive control diet, but Ca absorption was increased (linear and quadratic, $P < 0.01$) for pigs fed phytase containing diets compared with pigs fed the negative control diet (1.95 vs. 2.22, 2.69, 2.97, and 2.74 g/d). The breakpoint for phytase concentration was reached when 1,154.8 FTU/kg of phytase was added to the diet. This inclusion level resulted in an ATTD of Ca of 84.72% (Figure 2).

DISCUSSION

Phytase supplementation increased P digestibility, which was expected because phytase is capable of hydrolyzing the phytate molecule in corn and SBM, and therefore, release some of the P that is bound to the phytate molecule (Cromwell et al., 1993). The beneficial effect of phytase supplementation on the ATTD of P has been demonstrated (Pallauf et al., 1992; Kwon et al., 1995). The values for the ATTD of P measured in the present experiment for weanling pigs are in agreement with those measured by Lei et al. (1993) and Qian et al. (1996). Our research demonstrated that P digestibility reached a plateau at a level of 1,015.8 FTU/kg, which is in agreement with previous work showing maximum responses to phytase at levels around 1,000 FTU/kg in corn-SBM diets (Beers and Jongbloed, 1992; Kornegay and Qian, 1996; Yi et al., 1996). Because P is better utilized by pigs when phytase is supplemented to corn-soybean meal diets it is expected that phytase supplementation also causes a reduction in P excretion. Our results showed that P excretion is reduced if a corn-soybean meal diet containing no inorganic P is supplemented with phytase, which is in agreement with Selle and Ravindran (2008).

The concentration of Ca in the feces was reduced by the addition of phytase to the diets because the ATTD of Ca was increased as phytase was included in the diets. These results are in agreement with results observed by Pallauf et al. (1992), and Lei et al. (1993). The Ca digestibility values that were measured in the present experiments are comparable with those measured by Qian et al. (1996). To our knowledge, no previous studies have shown the effects of graded levels of phytase on Ca digestibility. One possible reason for the increase in Ca digestibility with supplemental phytase is that in the process of phytate hydrolysis, phytate esters are reduced and as a consequence, the ability of phytate to chelate Ca is also reduced. Therefore, Ca digestibility increases when exogenous phytase is supplemented to the diet (Selle et al., 2009). As expected, Ca absorption was increased with the inclusion of exogenous phytase to the diets. Adeola et al. (1995) also reported that Ca absorption is increased when phytase is supplemented to corn-soybean meal diets.

Conclusions

Results from the present experiment show that IPA Mash phytase may be used in corn-soybean meal diets to improve the ATTD of P and Ca in weanling pigs. Apparently, inclusion levels of 1,000 FTU/kg of phytase to diets will result in maximum ATTD of P and no further increases in ATTD of P is achieved by supplementing IPA Mash phytase at levels greater than 1,000 FTU/kg. In addition, IPA Mash phytase supplementation may also result in a reduction of P and Ca excretion in the feces of weanling pigs.

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Table 1. Composition (as-is basis) of experimental diets

Ingredient, %	Diet					
	Positive Control	Negative Control	500 phytase	1000 phytase	2000 phytase	4000 phytase
Ground corn	60.60	60.60	60.60	60.60	60.60	60.60
Soybean meal, 48%	32.00	32.00	32.00	32.00	32.00	32.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Ground limestone	0.90	0.90	0.90	0.90	0.90	0.90
Dicalcium phosphate	1.65	-	-	-	-	-
Cornstarch	-	1.65	1.625	1.60	1.55	1.45
L-lysine HCL	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Phytase premix [1]	-	-	0.025	0.05	0.10	0.20
Vit. mineral premix [2]	0.30	0.30	0.30	0.30	0.30	0.30
Mecadox premix [3]	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]IPA Mash, DSM Nutritional Products, Parsippany, NJ. Produced by mixing 3.4% of concentrated phytase (58,700 units/g) and 96.6% cornstarch.

[2] Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[3] The Mecadox premix (Phibro Animal Health, NJ) provided 55 mg per kg of Carbadox to the complete diet.

Table 2. Analyzed nutrient composition of diets (as-fed basis)

	Diet					
Item	Positive control	Negative control	500 phytase	1,000 phytase	2,000 phytase	4,000 phytase
ADF, %	2.70	2.98	2.79	3.04	2.95	2.72
NDF, %	8.44	9.50	9.84	10.09	8.91	9.55
P, %	0.66	0.36	0.36	0.36	0.36	0.35
Ca, %	0.86	0.48	0.48	0.51	0.56	0.53
CP, %	18.33	17.96	17.24	18.03	19.24	18.27
DM, %	87.42	88.02	87.97	88.08	88.25	88.15
Ash, %	5.74	4.99	4.43	4.27	4.08	4.10
Phytase, FTU/kg[1]	91	80	440	958	1743	3974
Indispensible AA, %						
Arg	1.26	1.30	1.28	1.19	1.22	1.24
His	0.50	0.53	0.52	0.49	0.50	0.51
Ile	0.80	0.84	0.85	0.81	0.81	0.84
Leu	1.60	1.66	1.64	1.57	1.57	1.60

Lys	1.18	1.21	1.20	1.13	1.15	1.20
Met	0.29	0.32	0.31	0.29	0.29	0.30
Phe	0.92	0.95	0.95	0.90	0.90	0.93
Thr	0.71	0.75	0.71	0.67	0.69	0.69
Trp	0.24	0.24	0.24	0.24	0.23	0.24
Val	0.92	0.96	0.97	0.93	0.92	0.96
Dispensible AA, %						
Ala	0.93	0.97	0.94	0.91	0.91	0.93
Asp	1.92	2.03	1.98	1.87	1.91	1.95
Cys	0.31	0.34	0.32	0.29	0.30	0.30
Glu	3.19	3.32	3.26	3.12	3.14	3.20
Gly	0.79	0.83	0.81	0.76	0.77	0.79
Pro	0.94	1.10	1.05	1.02	1.04	1.04
Ser	0.83	0.86	0.79	0.76	0.78	0.76
Tyr	0.63	0.61	0.61	0.56	0.57	0.57

[1]FTU = phytase units.

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Table 3. Effects of phytase on apparent total tract digestibility (ATTD) of P and Ca in weanling pigs[1]

	Diets							*P*-value		*P*-value[2]	
Item	Positive control	Negative control	500 phytase	1,000 phytase	2,000 phytase	4,000 phytase	SEM	Positive vs. Negative	Negative vs. Phytase	L	Q
Feed intake, g/d	645	633	629	646	624	611	18.06	0.646	0.769	0.277	0.685
P intake, g/d	4.26	2.28	2.26	2.33	2.25	2.14	0.08	< 0.01	0.676	0.122	0.494
Fecal output, g/d	66.48	58.79	58.06	56.11	57.15	62.61	3.77	0.157	0.942	0.370	0.361
P in feces, %	2.53	2.30	1.51	1.46	1.22	1.10	0.07	0.023	< 0.01	< 0.01	< 0.01
P output, g/d	1.68	1.35	0.87	0.81	0.71	0.68	0.07	< 0.01	< 0.01	< 0.01	< 0.01
ATTD of P, %	60.48	40.46	61.56	65.07	68.74	68.04	2.34	< 0.01	< 0.01	< 0.01	< 0.01
P absorption, g/d	2.58	0.93	1.39	1.51	1.54	1.46	0.07	< 0.01	< 0.01	< 0.01	< 0.01
Ca intake, g/d	5.55	3.04	3.02	3.30	3.49	3.23	0.10	< 0.01	0.068	0.072	< 0.01

Ca in feces, %	2.29	1.86	1.37	1.11	0.94	0.79	0.13	0.019	< 0.01	< 0.01	< 0.01
Ca output, g/d	1.52	1.09	0.80	0.60	0.52	0.50	0.08	< 0.01	< 0.01	< 0.01	< 0.01
ATTD of Ca, %	72.45	63.90	73.71	81.66	84.81	84.63	2.30	0.012	< 0.01	< 0.01	< 0.01
Ca absorption, g/d	4.02	1.95	2.22	2.69	2.97	2.74	0.12	< 0.01	< 0.01	< 0.01	< 0.01

[1]Data are means of 8 observations per treatment.

[2]L = linear contrast; Q = quadratic contrast.

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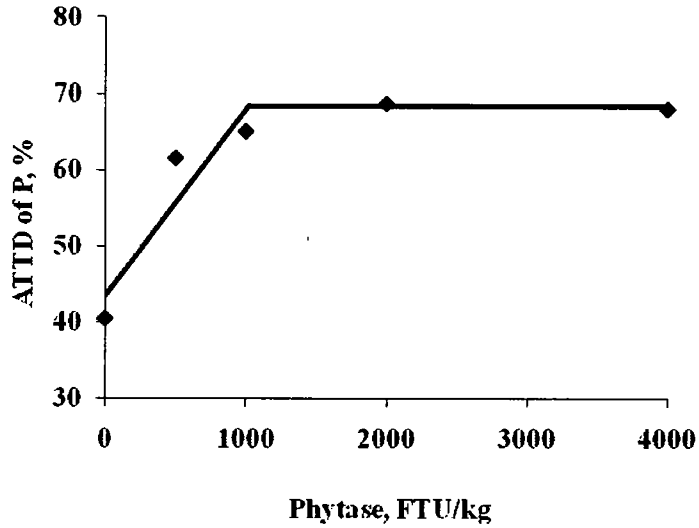

Figure 1. Fitted broken-line plot of ATTD of P as a function of dietary phytase level with observed treatment mean values (n = 8 observations per treatment mean). The minimal dietary phytase level determined by broken-line analysis using least squares methodology was 1015.8 FTU/kg (Y plateau = 68.39; slope below breakpoint = -0.025; Adjusted R^2 = 0.873).

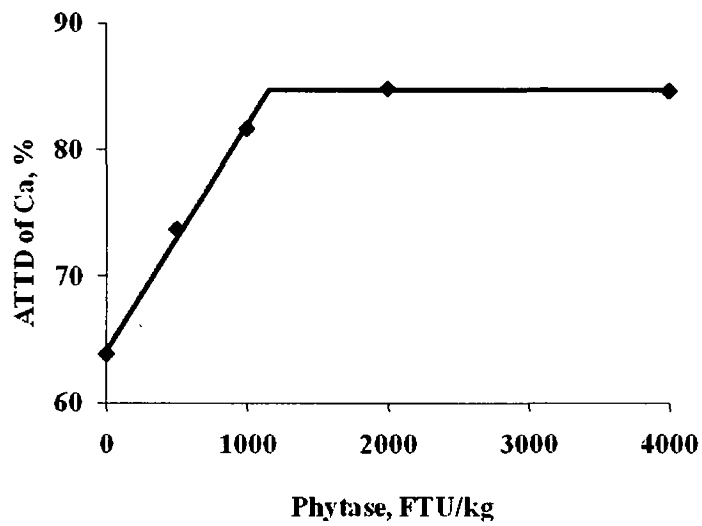

Figure 2. Fitted broken-line plot of ATTD of Ca as a function of dietary phytase level with observed treatment mean values (n = 8 observations per treatment mean). The minimal dietary phytase level determined by broken-line analysis using least squares methodology was 1154.8 FTU/kg (Y plateau = 84.72; slope below breakpoint = -0.0178; Adjusted R^2 = 0.997).

Exp. 186, Data

Trt	Rep	ID	FI_gpd	P in feed	P_intake_gp	Feces_gpd	P in feces	P_out_gpd	ATTD_P	Ca in feed	a_intake_gp	Ca in feces	Ca_out_gpd	ATTD_Ca	P_abs_gpd	Ca_abs_gpd
18601	1	18612	705.64	0.66	4.66	74.74	2.61	1.95	58.11	0.86	6.07	2.30	1.72	71.67	2.71	4.35
18601	2	18616	688.33	0.66	4.54	68.44	2.98	2.04	55.11	0.86	5.92	2.56	1.75	70.40	2.50	4.17
18601	3	18622	664.03	0.66	4.38	59.92	2.08	1.25	71.56	0.86	5.71	1.64	0.98	82.79	3.14	4.73
18601	4	18626	664.76	0.66	4.39	68.72	2.63	1.81	58.81	0.86	5.72	2.52	1.73	69.71	2.58	3.99
18601	5	18634	659.19	0.66	4.35	65.74	2.40	1.58	63.73	0.86	5.67	1.92	1.26	77.73	2.77	4.41
18601	6	18642	607.98	0.66	4.01	75.12	2.29	1.72	57.13	0.86	5.23	2.09	1.57	69.97	2.29	3.66
18601	7	18649	622.02	0.66	4.11	61.46	2.61	1.60	60.93	0.86	5.35	3.14	1.93	63.92	2.50	3.42
18601	8	18651	549.02	0.66	3.62	57.66	2.61	1.50	58.47	0.86	4.72	2.18	1.26	73.38	2.12	3.46
18602	1	18609	713.00	0.36	2.57	57.48	1.74	1.00	61.03	0.48	3.42	1.36	0.78	77.16	1.57	2.64
18602	2	18614	689.49	0.36	2.48	67.48	2.17	1.46	41.01	0.48	3.31	1.91	1.29	61.06	1.02	2.02
18602	3	18620	612.32	0.36	2.20	52.62	2.62	1.38	37.46	0.48	2.94	2.03	1.07	63.66	0.83	1.87
18602	4	18630	637.36	0.36	2.29	62.24	2.32	1.44	37.07	0.48	3.06	1.83	1.14	62.77	0.85	1.92
18602	5	18633	630.41	0.36	2.27	63.44	2.38	1.51	33.47	0.48	3.03	1.88	1.19	60.59	0.76	1.83
18602	6	18640	637.16	0.36	2.29	63.52	2.48	1.58	31.32	0.48	3.06	1.94	1.23	59.71	0.72	1.83
18602	7	18644	572.61	0.36	2.06	51.1	2.35	1.20	41.75	0.48	2.75	1.99	1.02	63.00	0.86	1.73
18602	8	18653	574.03	0.36	2.07	52.48	2.34	1.23	40.57	0.48	2.76	1.93	1.01	63.24	0.84	1.74
18603	1	18613	719.50	0.36	2.59	68.12	1.51	1.03	60.29	0.48	3.45	1.22	0.83	75.94	1.56	2.62
18603	2	18617	650.78	0.36	2.34	75.3	1.44	1.08	53.72	0.48	3.12	1.15	0.87	72.28	1.26	2.26
18603	3	18623	679.89	0.36	2.45	57.38	1.54	0.88	63.90	0.48	3.26	1.17	0.67	79.43	1.56	2.59
18603	4	18631	662.91	0.36	2.39	66.12	1.58	1.04	56.22	0.48	3.18	2.24	1.48	53.45	1.34	1.70
18603	5	18636	584.40	0.36	2.10	49.54	1.63	0.81	61.62	0.48	2.81	2.03	1.01	64.15	1.30	1.80
18603	6	18643	586.63	0.36	2.11	54.62	1.30	0.71	66.38	0.48	2.82	0.97	0.53	81.18	1.40	2.29
18603	7	18645	577.73	0.36	2.08	49.54	1.58	0.78	62.37	0.48	2.77	0.84	0.42	84.99	1.30	2.36
18603	8	18654	566.57	0.36	2.04	43.84	1.49	0.65	67.97	0.48	2.72	1.35	0.59	78.24	1.39	2.13
18604	1	18608	674.43	0.36	2.43	56.84	1.43	0.81	66.52	0.51	3.44	1.45	0.82	76.04	1.62	2.62
18604	2	18618	686.09	0.36	2.47	63.34	1.36	0.86	65.12	0.51	3.50	1.61	1.02	70.86	1.61	2.48
18604	3	18624	707.24	0.36	2.55	71.16	1.48	1.05	58.64	0.51	3.61	0.72	0.51	85.80	1.49	3.09
18604	4	18627	679.16	0.36	2.44	65.66	1.31	0.86	64.82	0.51	3.46	0.59	0.39	88.82	1.58	3.08
18604	5	18635	639.17	0.36	2.30	56.22	1.37	0.77	66.53	0.51	3.26	0.72	0.40	87.58	1.53	2.85
18604	6	18639	636.41	0.36	2.29	53.56	1.55	0.83	63.76	0.51	3.25	1.11	0.59	81.68	1.46	2.65
18604	7	18646	579.58	0.36	2.09	36.02	1.38	0.50	76.18	0.51	2.96	1.37	0.49	83.31	1.59	2.46
18604	8	18655	568.39	0.36	2.05	46.08	1.82	0.84	59.01	0.51	2.90	1.31	0.60	79.18	1.21	2.30
18605	1	18611	618.01	0.36	2.22	77.42	1.33	1.03	53.72	0.56	3.46	0.83	0.64	81.43	1.20	2.82
18605	2	18619	697.22	0.36	2.51	74.02	1.36	1.01	59.89	0.56	3.90	0.79	0.58	85.02	1.50	3.32
18605	3	18621	688.29	0.36	2.48	56.46	1.29	0.73	70.61	0.56	3.85	0.84	0.47	87.70	1.75	3.38
18605	4	18629	620.25	0.36	2.23	48.28	0.93	0.45	79.89	0.56	3.47	1.20	0.58	83.32	1.78	2.89
18605	5	18632	613.02	0.36	2.21	58.42	1.04	0.61	72.47	0.56	3.43	0.74	0.43	87.41	1.60	3.00
18605	6	18641	614.70	0.36	2.21	46.42	1.30	0.60	72.73	0.56	3.44	0.72	0.33	90.29	1.61	3.11
18605	7	18648	598.94	0.36	2.16	50.52	1.38	0.70	67.67	0.56	3.35	0.88	0.44	86.75	1.46	2.91
18605	8	18650	539.04	0.36	1.94	45.7	1.15	0.53	72.92	0.56	3.02	1.55	0.71	76.53	1.42	2.31
18606	1	18610	701.68	0.35	2.46	77.98	1.10	0.86	65.07	0.53	3.72	0.63	0.49	86.79	1.60	3.23
18606	2	18615	572.75	0.35	2.00	57.68	1.15	0.66	66.91	0.53	3.04	1.09	0.63	79.29	1.34	2.41
18606	3	18625	612.65	0.35	2.14	55.72	1.03	0.57	73.23	0.53	3.25	0.64	0.36	89.02	1.57	2.89
18606	4	18628	629.67	0.35	2.20	54.42	0.98	0.53	75.80	0.53	3.34	0.69	0.38	88.75	1.67	2.96
18606	5	18637	640.88	0.35	2.24	70.12	1.00	0.70	68.74	0.53	3.40	1.04	0.73	78.53	1.54	2.67
18606	6	18638	579.88	0.35	2.03	87.64	0.99	0.87	57.25	0.53	3.07	0.80	0.70	77.19	1.16	2.37
18606	7	18647	606.39	0.35	2.12	49.74	1.22	0.61	71.41	0.53	3.21	0.60	0.30	90.71	1.52	2.92
18606	8	18652	542.18	0.35	1.90	47.62	1.36	0.65	65.87	0.53	2.87	0.80	0.38	86.74	1.25	2.49

European Food Safety Authority

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA Mash Phytase** Batch number: **PPQ 28683**

Trial ID: **Experiment 186** Location: [redacted] (b) (4)

Start date and exact duration of the study: **June 16, 2009 for 2 weeks**

Number of treatment groups (+ control(s)): **6** Replicates per group: **8**

Total number of animals: **48** Animals per replicate: **1 per Trt Group**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **500, 1000, 2000, & 4000 FYT/kg Complete Feed** Analysed: **440, 958, 1743, & 3974 FYT/kg**

†

Substances used for comparative purposes:

Intended dose: Analysed:

Animal species/category: **Swine**

Breed: **PIC** Identification procedure: **Ear Notch**

Sex: **Barrows** Age at start: **6-7 Weeks** Body weight at start: **13.5 kg**

Physiological stage: **Weanling Pigs** General health: **Excellent**

Additional information for field trials:

Location and size of herd or flock: **240 Sows Farrow to Finish at th**[redacted] (b) (4)

Feeding and rearing conditions: **Individually and Housed in Metabolism Cages**

Method of feeding: **Limit Feeding**

Diets (type(s)): **Typical Commercial Corn-Soy Diet**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Corn, Soybean meal, & Soy oil**

Nutrient content (relevant nutrients and energy content)

Intended values: **Ca - PC-0.83 & 0.47 % in Others; P-PC-.70 & 0.39 % in Others - ME-3500 Mcal/kg**

Analysed values: **Ca - PC-0.86 NC-0.48 & 0.48, 0.51. 0.56, 0.53 % in Test Diets**
P-PC-0.66 NC-0.36 & 0.36, 0.36, 0.36 & 0.35% in Test Diets

Date and nature of the examinations performed: **None**

Method(s) of statistical evaluation used: **SAS UNIVARIATE & Proc Mixed Procedures**

Therapeutic/preventive treatments (reason, timing, kind, duration): **None**

Timing and prevalence of any undesirable consequences of treatment: **None**

Date **November 23, 2009**	Signature Study Director [redacted] (b) (4)

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

6

Annex 6

Evaluation of graded amounts of a microbial phytase on the faecal digestibility and excretion of phosphorus, calcium and zinc in growing pigs

REPORT No. 2500672

REPORT No. 2500672
Regulatory Document

DSM

Document Date:	10-Jun-2009
Author(s):	Guggenbuhl P, Simões Nunes C, Piñón Quintana A, Portier C, Kurtz N and Lehmann A
Title:	Evaluation of graded amounts of a microbial phytase on the faecal digestibility and excretion of phosphorus, calcium and zinc in growing pigs.
Project No.	6106

Compound No.

Summary

The aim of the present study (S 05-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the digestibility of phosphorus (P), calcium (Ca) and zinc (Zn) in the growing pig.
The basal diet, without addition of mineral P, was based on soybean meal, maize and barley. IPA phytase was included in the diet at the levels of 500 U/kg, 1000 U/kg, 1500 U/kg, 1750 U/kg, 2000 U/kg, 2500 U/kg and 3000 U/kg. A dietary treatment was based in the very slightly modified control diet containing the recommended available P by addition of dicalcium phosphate (diCa-P).
The mean P faecal concentration of the enzyme supplemented animals was significantly lower than that observed for the animals ingesting the control diet.
All the phytase inclusion levels increased the bioavailability of P and accordingly reduced the growing pig quantitative faecal excretion of P comparatively to the basal diet.
The P digestibility was dose dependant and highly significantly improved by 21.1, 28.5, 30.5, 32.0, 32.2, 37.3 and 38.7 % in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. The digestibility of P in the diCa-P supplemented diet was also significantly higher than that of the control.
The faecal excretion of P was significantly reduced by 29.3, 40.1, 42.8, 45.8, 45.6, 53.0, and 55.2 % with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. It was increased by 10.1 % with the diCa-P supplemented group.
The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control of 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg were 0.91, 1.22, 1.30, 1.32, 1.36, 1.56 and 1.60 g of full available P/kg feed respectively. In comparison the P equivalency of the diCa-P supplemented diet was 1.70 g of full available P/kg feed.
Ca and Zn digestibilities were significantly improved by all the inclusion levels of the phytase.
It can be concluded that the IPA phytase improved the digestibility and the apparent absorption of P, Ca and Zn, and reduced the P faecal excretion in the pig fed on a diet containing P exclusively from vegetable origin.
There was a dose dependant increase of the effects of the enzyme on the availability of the dietary P.

This report consists of 22 pages

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Research Center Head Dr. A.-M. Klünter, NRD/CA	[signature]	11.06.09
Project Manager Dr. F.Fru, NRD/PA	[signature]	15.06.09

Nomenclature and Structural Formula (if available)

Liquid form IPA phytase expressed in *Aspergillus oryzae*, batch PPQ27987, activity at pH 5.5 of 24850 U/g.

1. INTRODUCTION

The aim of the present study (S 05-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the digestibility of phosphorus (P), calcium (Ca) and zinc (Zn) in the growing pig. The basal diet, without addition of mineral P, was based on soybean meal, maize and barley. IPA phytase was included in the diet at the levels of 500 U/kg, 1000 U/kg, 1500 U/kg, 1750 U/kg, 2000 U/kg, 2500 U/kg and 3000 U/kg. A dietary treatment was based in the very slightly modified control diet containing the recommended available P by addition of dicalcium phosphate (diCa-P).

The experiment was performed in March-April 2008 in the facilities of the Centre de Recherche en Nutrition Animale (CRNA), DSM Nutritional Products France, BP 170, 68305 Saint-Louis cedex, France. It has been performed according to the French legal regulations on experiments with live animals.

2. MATERIAL AND METHODS

2.1. Test enzymes

The used IPA phytase was expressed in *Aspergillus oryzae*, batch PPQ27987, had an activity at pH 5.5 of 24850 U/g and was in a liquid form.

NRD/CM measured the phytase activity in the enzyme preparation and in the feed. One unit of phytase is defined as the quantity of enzyme which sets free 1 µmole of inorganic phosphate per minute from 0.005 moles per litre sodium phytate at pH 5.5 and at 37°C.

2.2. Animal trial

Thirty six Large White × Landrace pigs having an initial body weight of 19.06 ± 1.82 kg were used. The animals were housed in floor-pen cages in 9 groups of 4 animals each in an environmentally controlled room. Each pen had a plastic-coated welded wire floor and was equipped with two water nipples and four stainless-steel individualised feeders. Room temperature was 21-22° C and humidity percentage was 50 %.
The pigs were fed a basal diet without addition of mineral P (diet A) during an adaptive period of 16 days. After that period they were allocated into 9 equal groups and fed for 12 days the basal diet (group A) or the diet A supplemented with 12 g/kg of dicalcium phosphate (group B) or with IPA phytase at the levels of 500 U/kg (group C), 1000 U/kg (group D), 1500 U/kg (group E), 1750 U/kg (group F), 2000 U/kg (group G), 2500 U/kg (group H), 3000 U/kg (group I).
The basal diet A was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the available P supply, the animals' requirements according to Henry *et al.* (1989) and NRC (1998). The basal diet A (table 1) had a theoretical P content of 0.41 % and an analysed content of 0.42 %. The theoretical available P in the diet was 1.20 g/kg and the observed availability of 1.24 g/kg.

An indigestible tracer (chromium oxide) was added at a concentration of 0.4 % to all the diets allowing calculation of the digestibility of P, Ca and Zn. The feed was distributed *ad libitum* in mash form, under pen feed consumption control, and the animals had free access to drinking water. The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water in the region is 120 mg/L.

Faecal P, Ca, Zn and Cr concentrations were measured at the 12th day of the second period. Faeces were sampled individually, in approximately the same amount at the same time of the day, during the last 3 days preceding that date. Thus, for each dietary treatment and for each criterion a total of 12 individual determinations have been performed. All minerals were determined according to standard Association of Official Analytical Chemists (1990) methods using a Vista-MPX ICP-OES spectrometer (Varian Australia Pty Ltd, Mulgrave Victoria, 3170 Australia). The apparent digestibility (% of the intake) of the minerals was calculated for the mentioned 3 day period.

2.3. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu + Ai + Bij + Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Snedecor and Cochran, 1989). These calculations were performed using StatGraphics Plus 5.1 (Manugistics, Rockville, U.S.A. 2001).

3. RESULTS

3.1. Phytase and animals

The observed IPA phytase activity in the supplemented feed used was in general in excellent agreement with the programmed inclusion levels (table 2).

The animals grew normally during the observation period to reach a final mean body weight of 44.84 ± 3.37 kg. Their daily weight gain was of 679 ± 5 g. No mortality was observed during the experiment.
Two animals from group H, receiving the diet supplemented with IPA phytase at 2500 U/kg presented diarrhoea during the sampling period, so that no faeces could be collected from them. No statistical analysis was performed for this group as the total amount of faeces samples was only the half (n = 6) of the other groups (n = 12).

3.2. Effects on phosphorus

The mean P faecal concentration of the enzyme supplemented animals was very significantly lower than that measured in the animals ingesting the control diet (table 3). There was a decrease of the P faecal concentration with the increasing allowance of IPA phytase. The lowest P faecal concentration was observed in the animals ingesting IPA phytase at 3000 U/kg feed.

The P digestibility was dose dependant and highly significantly improved by 21.1, 28.5, 30.5, 32.0, 32.2, 37.3 and 38.7 percentage units in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg IPA phytase supplemented groups respectively (table 4, figure 2).
The digestibility of P in the diCa-P supplemented diet was also significantly higher than that of the control by 17.9 percentage units and very similar to the enzyme supplementation at 500 U/kg.

The faecal excretion of P was significantly reduced by 29.3, 40.1, 42.8, 45.8, 45.6, 53.0, and 55.2 % with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. It was increased by 10.1 % with the diCa-P supplemented group (table 5, figure 2).

The apparent absorbed P was 2.15, 2.45, 2.54, 2.56, 2.60, 2.80 and 2.84 g/kg feed with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively and 2.93 g/kg feed in the diCa-P supplemented group. It was significantly increased in all the supplemented groups in comparison to the control diet (1.24 g/kg). With the exception of the IPA phytase 500 U/kg inclusion level, all other supplemented groups were over the recommended requirements of 2.25 g of digestible P per kg feed (Ernandoréna *et al.*, 2008).

The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control, of 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg of IPA phytase were 0.91, 1.22, 1.30, 1.32, 1.36, 1.56 and 1.60 g of full available P/kg feed respectively (table 6, figure 3). In comparison the P equivalency of the diCa-P supplemented diet was 1.70 g of full available P/kg feed.
In the present study, using the equation of the tendency curve the calculated inclusion level to reach 1.5 g of full available P/kg feed was 2412 U/kg feed of IPA phytase ($y = 48.982e^{2.5978x}$, $R^2 = 0.9597$).

3.3. Effects on calcium

The Ca faecal concentration of the animals ingesting the basal diet supplemented or not with diCa-P was systematically higher than that of the animals ingesting the diets supplemented with the phytase (table 7). The observed differences were statistically significant for all the enzyme supplemented groups.

The Ca digestibility was significantly improved by the phytase and by all the inclusion levels of IPA phytase (table 8, figure 4). The improvements were 8.6, 12.8, 12.6, 15.5, 15.1, 26.5 and 21.6 percentage units in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg IPA phytase supplemented groups respectively.
The Ca digestibility of the IPA phytase supplemented diets was more or less dose dependant.

The faecal excretion of Ca was significantly reduced by 23.9, 34.6, 36.8, 42.3, 41.7, 67.9, and 57.0 % with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. It was increased by 12.1 % with the diCa-P supplemented group (table 9, figure 5).

3.3. Effects on zinc

The Zn faecal concentration of the animals ingesting the non-supplemented control diet was systematically higher than that of the animals ingesting the diets supplemented with phytase with the exception of the two highest dosage of IPA phytase (table 10). The observed differences were not statistically significant for all the supplemented groups.

The Zn digestibility was significantly improved by the phytase for all inclusion levels in comparison to the basal diet (table 11). The Zn digestibility of the IPA phytase supplemented diets presented high biological variations from one group to the others giving no regularity in the dose curve.

The faecal excretion of Zn was significantly reduced in the phytase supplemented groups (table 12). IPA phytase presented inconsistency in the faecal Zn excretion reduction in regard to the increasing inclusion levels.

4. CONCLUSION

It can be concluded that the IPA phytase improved the digestibility and the apparent absorption of P, Ca and Zn, and reduced the P faecal excretion in the pig fed on a diet containing P exclusively from vegetable origin. There was a dose dependant effect of the IPA phytase on the availability of the dietary P.

Table 1 - Composition (%) of the basal diet (A) and of that supplemented with diCa-P (B)

INGREDIENTS	Basal diet A without P (%)	Basal diet B with diCa-P (%)
Maize	53	53
Soybean meal	18	18
Barley	13.9	13
Oat meal	6	6
Wheat bran	5.4	5.4
Soya oil	1	1
diCa-P	-	1.2
Minerals [(1)], vitamins and synthetic aa	2.7	2.4
Crude protein - N x 6.25 - %	15.5	15.5
Lysine - %	0.96	0.96
Methionine + cystine - %	0.54	0.54
Ca - calculated - % in DM	0.66	0.86
Ca - analysed in - % in DM	0.70	0.80
P - calculated - % in DM	0.41	0.65
P - analysed - % in DM	0.42	0.62
Theoretically available P - %	0.12 [(2)]	1.86 [(3)]
Phytic-P - calculated - %	0.28	0.28
Estimated digestible energy - MJ / kg	*13.31*	*13.31*
Phytase activity - U[(4)]/ kg	*225 ± 4*	*219 ± 4*

[(1)] Mixture without mineral P;
[(2)] Estimated from the mean P digestibility of the previous realized trials
[(3)] Sum of the theoretically available P and 80 % of added mineral P as generally accepted
[(4)] Quantity of enzyme that sets free 1 μmole of inorganic phosphate per minute from 0.005 mole per litre sodium phytate at pH 5.5 and at 37°C.

Table 2 - Phytase activity (U[a]/kg) and % of the target in the different diets.

Treatment groups	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Measured phytase addition (U/kg) [1]	225 ± 4	219 ± 4	678 ± 6	1179 ± 24	1723 ± 13	1985 ± 8	2232 ± 34	2798 ± 35	3329 ± 54
Actually added phytase (U/kg)	-	-	453	954	1498	1760	2007	2573	3104
% of target	-	-	91	94	100	101	100	103	103

[a] Quantity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 3.2 and at 37°C.
[1] Mean ± standard deviation of 2 determinations.

__Table 3__ - **Effects of the IPA phytase on the faecal concentration of phosphorus in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal P concentration (%of DM) (1)	1.59 ± 0.25	1.67 ± 0.37	1.19 ± 0.13	1.08 ± 0.10	0.97 ± 0.10	0.99 ± 0.17	0.94 ± 0.14	0.86(2) ± 0.18	0.83 ± 0.18
Variation from A (%)	100	105.6	74.8	68.2	61.4	62.2	59.2	54.5	52.6
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	NS	P<0.05		P<0.001
				D -	NS	NS	NS		P<0.05
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 4 - Effects of the IPA phytase on the total apparent digestibility of phosphorus in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal P digestibility (%) (1)	29.3 ± 5.5	47.2 ± 7.9	50.4 ± 5.6	57.8 ± 3.9	59.8 ± 3.5	61.3 ± 3.9	61.5 ± 3.3	66.6(2) ± 4.2	68.0 ± 6.2
Variation from A (%)	-	61.0	72.2	97.5	104.1	109.2	110.0	127.2	132.0
Statistical analysis									
	A -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	NS	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.001
						F -	NS		P<0.05
							G -		P<0.05
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 5 - Effects of the IPA phytase on the faecal excretion of phosphorus in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal P excretion (mg/g DM) [(1)]	2.99 ± 0.23	3.29 ± 0.49	2.11 ± 0.24	1.79 ± 0.16	1.71 ± 0.15	1.62 ± 0.16	1.63 ± 0.14	1.40[(2)] ± 0.18	1.34 ± 0.26
Variation from A (%)	100	110.1	70.7	59.9	57.2	54.2	54.4	47.0	44.8
Statistical analysis									
	A -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.001
						F -	NS		P<0.05
							G -		P<0.05
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
[(1)] Mean ± standard deviation of the mean of 12 determinations. [(2)] Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Figure 1

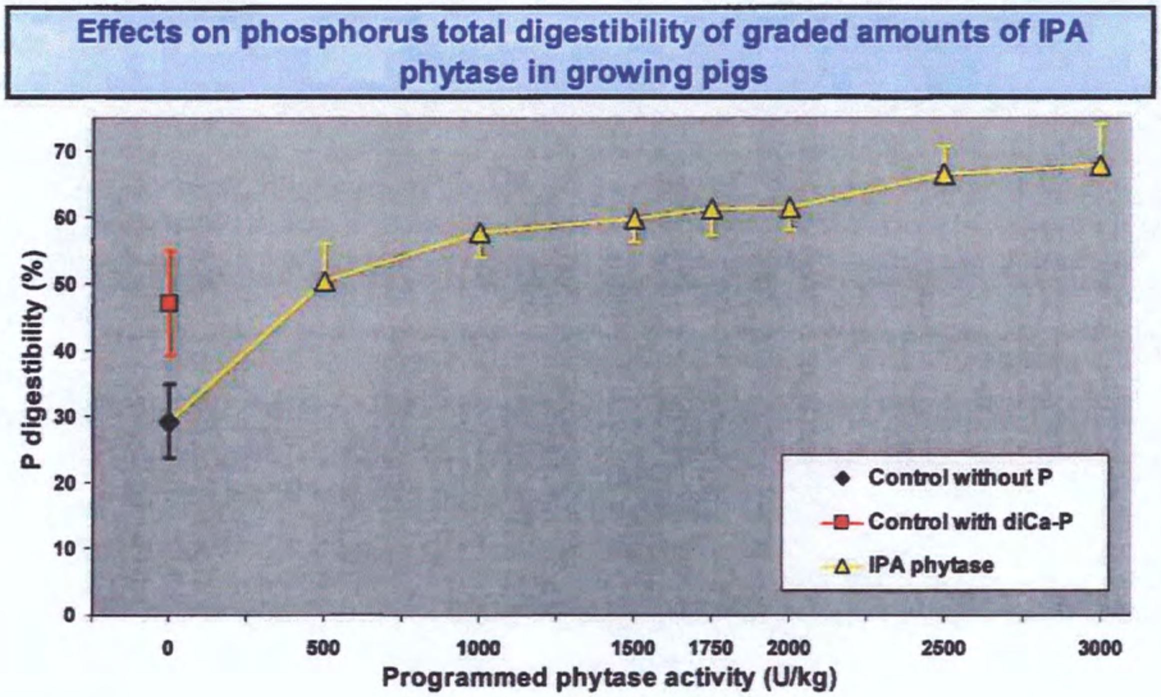

Figure 2

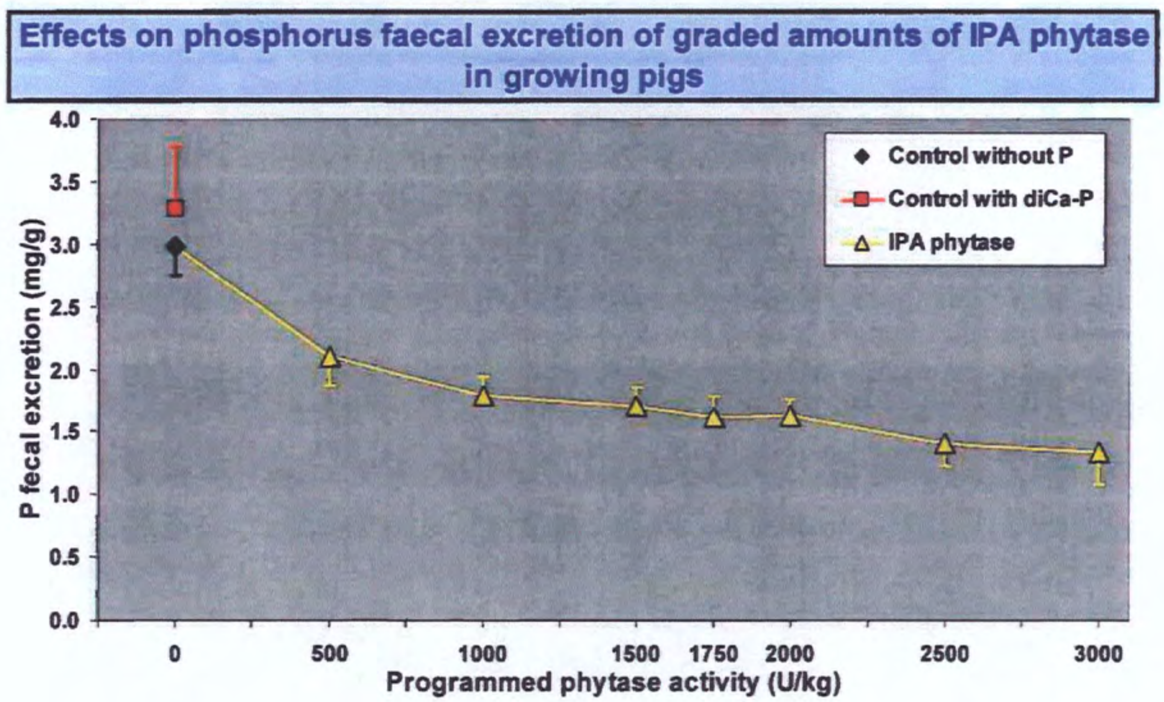

Table 6 - **Phosphorus equivalencies (g of full available supplemental P per kg of feed comparatively to the non-supplemented control) of the IPA phytase in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
P equivalence (g/kg feed)	0.00 ± 0.23	1.70 ± 0.49	0.91 ± 0.24	1.22 ± 0.16	1.30 ± 0.15	1.32 ± 0.16	1.36 ± 0.14	1.56[2] ± 0.18	1.60 ± 0.26
P eq. variation from C (%)	-	-	100	133.5	143.1	145.2	149.3	171.0	175.7
Statistical analysis									
	A -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		NS
			C -	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.05
						F -	NS		P<0.05
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Figure 3

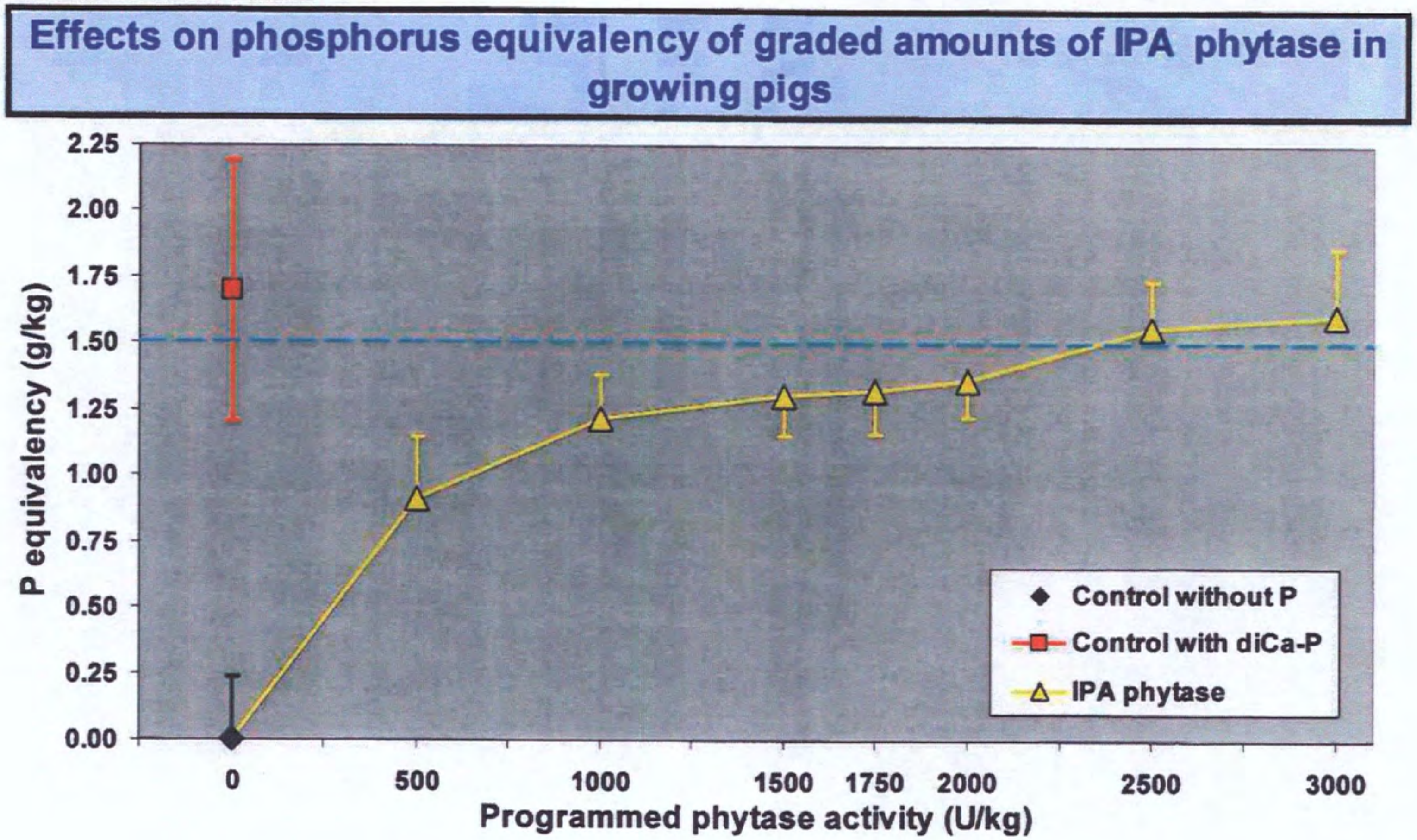
Effects on phosphorus equivalency of graded amounts of IPA phytase in growing pigs
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
P equivalency (g/kg)
0
500
1000
1500
1750
2000
2500
3000
Programmed phytase activity (U/kg)
Control without P
Control with diCa-P
IPA phytase

Table 7 - Effects of the IPA phytase on the faecal concentration of calcium in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Ca concentration (% of DM) (1)	1.48 ± 0.30	1.58 ± 0.38	1.18 ± 0.20	1.09 ± 0.21	1.00 ± 0.22	0.97 ± 0.16	0.93 ± 0.15	0.54(2) ± 0.17	0.74 ± 0.30
Variation from A (%)	100	106.9	79.8	73.7	67.6	65.5	63.0	36.7	49.8
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	NS	NS		P<0.001
				D -	NS	NS	NS		P<0.05
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 8 - Effects of the IPA phytase on the total apparent digestibility of calcium in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Ca digestibility (%) [(1)]	60.2 ± 3.7	61.1 ± 7.1	68.8 ± 5.9	73.0 ± 5.8	72.8 ± 5.5	75.7 ± 3.6	75.3 ± 2.7	86.7[(2)] ± 4.2	81.8 ± 7.9
Variation from A (%)	100	101.5	114.3	121.2	120.9	125.7	125.1	143.9	135.8
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	P<0.05	P<0.05		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.001
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

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Table 9 - **Effects of the IPA phytase on the faecal excretion of calcium in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Programmed phytase addition (U/kg)	**0**	**0**	**500**	**1000**	**1500**	**1750**	**2000**	**2500**	**3000**
Faecal Ca excretion (mg/g DM) (1)	**2.77 ± 0.26**	**3.11 ± 0.57**	**2.11 ± 0.40**	**1.81 ± 0.39**	**1.75 ± 0.35**	**1.60 ± 0.23**	**1.62 ± 0.18**	**0.89**(2) **± 0.28**	**1.19 ± 0.52**
Variation from A (%)	**100**	**112.1**	**76.1**	**65.4**	**63.2**	**57.7**	**58.3**	**32.1**	**43.0**
Statistical analysis									
	A -	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	P<0.05	P<0.05		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.05
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

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Figure 4

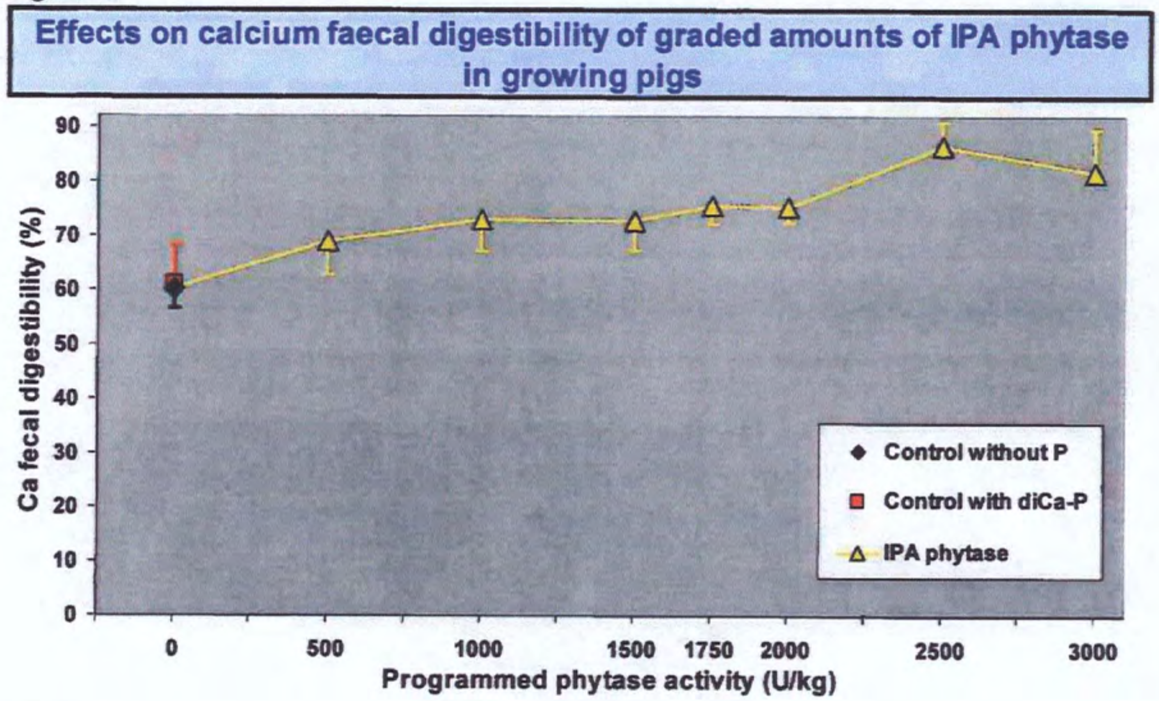

Figure 5

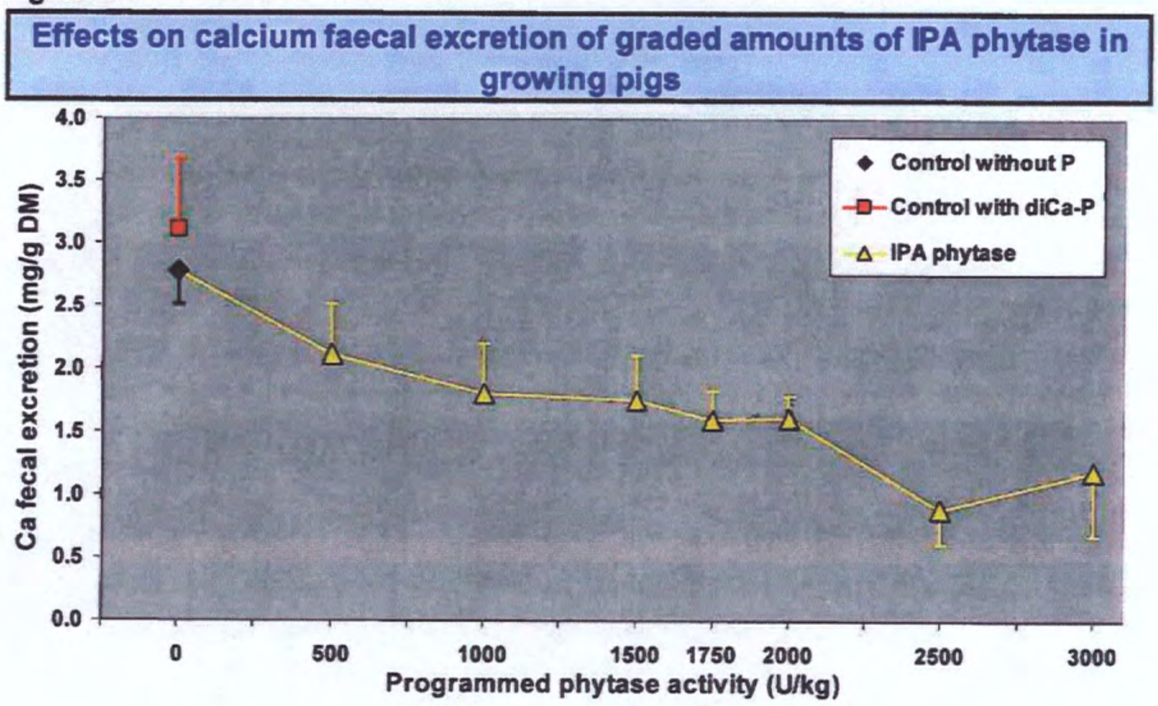

Table 10 - Effects of the IPA phytase on the faecal concentration of zinc in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Zn concentration (% of DM) [(1)]	0.48 ± 0.09	0.46 ± 0.11	0.40 ± 0.06	0.45 ± 0.04	0.42 ± 0.06	0.42 ± 0.06	0.44 ± 0.06	0.49[(2)] ± 0.06	0.49 ± 0.08
Variation from A (%)	100	96.0	83.4	93.5	89.0	89.2	92.1	103.1	102.9
Statistical analysis									
	No significant differences between the groups								

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 11 - Effects of the IPA phytase on the total apparent digestibility of zinc in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Zn digestibility (%) (1)	11.4 ± 5.6	16.7 ± 9.4	25.5 ± 7.0	21.4 ± 6.5	17.3 ± 8.7	25.0 ± 6.2	21.6 ± 4.8	17.5(2) ± 3.7	18.1 ± 9.7
Variation from A (%)	100	146.8	223.6	187.8	151.5	119.1	189.3	153.5	158.4
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001		P<0.05
		B -	P<0.05	NS	NS	P<0.05	NS		NS
			C -	NS	NS	NS	NS		NS
				D -	NS	NS	NS		NS
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

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Table 12 - Effects of the IPA phytase on the faecal excretion of zinc in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	[redacted] (b)(4) phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Zn excretion (mg/g DM) (1)	0.086 ± 0.005	0.083 ± 0.009	0.070 ± 0.007	0.074 ± 0.006	0.074 ± 0.008	0.070 ± 0.006	0.076 ± 0.005	0.080(2) ± 0.004	0.078 ± 0.009
Variation from A (%)	100	97.1	81.6	85.6	86.3	81.1	88.2	93.1	91.2
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.05
			C -	NS	NS	NS	NS		NS
				D -	NS	NS	NS		NS
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.

(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.

NS : non significant

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Diet	Treatment	Pig	P conc feces	DIGESTIBILITY	EXCRETION	E[illegible]Y
A1	Control without P	178	19.187	31.7	2.886	0.102
A1		241	15.024	25.0	3.168	-0.181
A1		236	16.071	31.0	2.916	0.072
A1		223	13.467	27.4	3.066	-0.079
A1		178	16.404	31.1	2.912	0.075
A1		241	15.654	30.0	2.957	0.030
A1		236	17.975	24.2	3.202	-0.214
A1		223	16.308	24.6	3.185	-0.198
A1		178	16.587	34.6	2.761	0.226
A1		241	11.013	39.2	2.570	0.417
A1		236	12.479	34.8	2.756	0.231
A1		223	20.102	17.9	3.470	-0.482
B2	Control with di-CaP	176	13.837	54.5	2.833	2.153
B2		234	18.579	43.4	3.523	1.462
B2		175	13.603	58.0	2.613	2.373
B2		189	15.302	47.8	3.251	1.734
B2		176	18.533	43.6	3.510	1.476
B2		234	17.179	46.5	3.328	1.658
B2		175	15.308	54.3	2.844	2.142
B2		189	13.966	51.2	3.036	1.950
B2		176	29.555			
B2		234	17.068	45.7	3.377	1.609
B2		175	26.962	26.5	4.571	0.415
B2		189	13.884	47.1	3.291	1.695
C3	IPA phytase 500 U/kg	225	9.399	55.9	1.878	1.144
C3		217	12.208	54.1	1.954	1.068
C3		197	11.358	51.5	2.065	0.958
C3		235	13.257	38.0	2.642	0.381
C3		225	12.841	43.7	2.398	0.625
C3		217	11.169	56.5	1.851	1.171
C3		197	10.128	55.0	1.916	1.106
C3		235	11.057	53.4	1.987	1.035
C3		225	14.088	47.8	2.222	0.800
C3		217	12.227	54.5	1.938	1.085
C3		197	12.354	50.4	2.114	0.908
C3		235	12.179	44.4	2.369	0.654
D4	IPA phytase 1000 U/k	212	8.947	66.0	1.442	1.563
D4		232	11.004	56.4	1.849	1.156
D4		237	12.150	56.0	1.866	1.139
D4		208	10.965	54.4	1.936	1.069
D4		212	11.029	54.1	1.947	1.058
D4		232	10.821	63.0	1.568	1.437
D4		237	9.794	59.0	1.738	1.268
D4		208	12.390	54.9	1.914	1.091
D4		212	9.734	59.7	1.711	1.295
D4		232	11.989	51.9	2.041	0.965
D4		237	11.100	58.6	1.758	1.247
D4		208	9.861	60.1	1.691	1.315
E5	IPA phytase 1500 U/k	198	9.496	61.5	1.638	1.375
E5		192	8.921	60.9	1.662	1.351
E5		195	9.277	58.5	1.764	1.249
E5		227	9.381	58.2	1.777	1.236
E5		198	11.231	56.1	1.868	1.145
E5		192	8.647	61.0	1.658	1.355
E5		195	10.438	56.4	1.854	1.159
E5		227	12.158	52.4	2.025	0.988
E5		198	9.383	60.8	1.667	1.346
E5		192	8.864	63.0	1.574	1.439
E5		195	9.039	65.1	1.485	1.528
E5		227	10.026	63.8	1.539	1.474
F6	750 U/k	233	9.341	57.1	1.792	1.149
F6		218	13.480	56.1	1.835	1.107
F6		210	9.751	57.0	1.795	1.146
F6		202	10.848	58.2	1.747	1.194

F6	IPA phytase 1	233	9.422	60.0	1.671	1.270
F6		218	12.510	58.6	1.729	1.212
F6		210	9.288	64.5	1.482	1.459
F6		202	8.302	67.7	1.349	1.593
F6		233	7.052	67.8	1.348	1.594
F6		218	9.947	63.5	1.525	1.417
F6		210	9.289	62.3	1.574	1.367
F6		202	9.204	62.3	1.577	1.365
G7	IPA phytase 2000 U/k	182	10.983	60.7	1.659	1.327
G7		186	7.560	59.7	1.700	1.286
G7		201	8.009	63.4	1.547	1.439
G7		238	10.804	55.5	1.881	1.105
G7		182	11.341	59.1	1.725	1.260
G7		186	9.281	62.3	1.591	1.394
G7		201	9.497	60.0	1.689	1.297
G7		238	9.942	59.4	1.714	1.272
G7		182	10.900	63.2	1.555	1.431
G7		186	8.860	60.2	1.682	1.304
G7		201	7.358	68.4	1.333	1.653
G7		238	8.150	66.2	1.427	1.559
I9	IPA phytase 3000 U/k	203	7.513	68.4	1.319	1.621
I9		191	10.379	58.1	1.752	1.188
I9		216	8.043	69.0	1.297	1.643
I9		228	6.691	75.1	1.040	1.900
I9		203	11.186	63.4	1.530	1.410
I9		191	7.644	73.8	1.096	1.844
I9		216	12.326	56.6	1.813	1.127
I9		228	7.707	72.9	1.132	1.808
I9		203	6.726	70.5	1.231	1.709
I9		191	7.081	66.3	1.407	1.533
I9		216	8.003	64.6	1.478	1.462
I9		228	6.736	76.8	0.970	1.970

Diet	Treatment	Pig	[illegible] conc feces
A1	6ntrol without P	178	0.617
A1		241	0.389
A1		236	0.435
A1		223	0.358
A1		178	0.526
A1		241	0.522
A1		236	0.558
A1		223	0.481
A1		178	0.498
A1		241	0.360
A1		236	0.382
A1		223	0.591
B2	6ntrol with di-6P	176	0.416
B2		234	0.472
B2		175	0.382
B2		189	0.454
B2		176	0.511
B2		234	0.455
B2		175	0.394
B2		189	0.324
B2		176	0.636
B2		234	0.445
B2		175	0.691
B2		189	0.311
C3	IPA phytase 500 U/kg	225	0.297
C3		217	0.511
C3		197	0.432
C3		235	0.322
C3		225	0.396
C3		217	0.433
C3		197	0.350
C3		235	0.361
C3		225	0.496
C3		217	0.429
C3		197	0.411
C3		235	0.332
D4	IPA phytase 1000 U/k	212	0.412
D4		232	0.474
D4		237	0.532
D4		208	0.432
D4		212	0.449
D4		232	0.434
D4		237	0.389
D4		208	0.509
D4		212	0.437
D4		232	0.407
D4		237	0.423
D4		208	0.448
E5	IPA phytase 1500 U/k	198	0.370
E5		192	0.354
E5		195	0.429
E5		227	0.439
E5		198	0.495
E5		192	0.311
E5		195	0.462
E5		227	0.444
E5		198	0.444
E5		192	0.381
E5		195	0.453
E5		227	0.505
F6	750 U/k	233	0.338
F6		218	0.531
F6		210	0.355
F6		202	0.495

F6	IPA phytase 1	233	0.383
F6		218	0.493
F6		210	0.375
F6		202	0.453
F6		233	0.345
F6		218	0.456
F6		210	0.403
F6		202	0.470
G7	IPA phytase 2000 U/k	182	0.520
G7		186	0.356
G7		201	0.356
G7		238	0.476
G7		182	0.504
G7		186	0.440
G7		201	0.367
G7		238	0.449
G7		182	0.553
G7		186	0.406
G7		201	0.407
G7		238	0.434
I9	IPA phytase 3000 U/k	203	0.410
I9		191	0.539
I9		216	0.360
I9		228	0.525
I9		203	0.615
I9		191	0.508
I9		216	0.585
I9		228	0.487
I9		203	0.477
I9		191	0.347
I9		216	0.465
I9		228	0.569

Diet	Treatment	Pig	6 conc feces	DIGSTIBTY	EXBETION
A1	6ntrol without P	178	21.336	54.0	3.209
A1		241	12.427	62.4	2.621
A1		236	17.477	54.5	3.171
A1		223	11.727	61.7	2.670
A1		178	16.467	58.1	2.924
A1		241	13.977	62.1	2.640
A1		236	15.386	60.7	2.740
A1		223	14.497	59.4	2.831
A1		178	17.083	59.2	2.843
A1		241	9.301	68.9	2.170
A1		236	12.362	60.8	2.730
A1		223	15.809	60.9	2.729
B2	6ntrol with di-6P	176	13.976	64.2	2.862
B2		234	18.635	55.8	3.534
B2		175	10.803	74.0	2.075
B2		189	13.075	65.2	2.778
B2		176	19.201	54.5	3.636
B2		234	18.229	55.8	3.531
B2		175	14.121	67.2	2.623
B2		189	11.664	68.3	2.535
B2		176	27.817		
B2		234	18.470	54.3	3.655
B2		175	23.651	49.8	4.010
B2		189	12.441	63.1	2.949
C3	IPA phytase 500 U/kg	225	7.638	77.5	1.526
C3		217	9.837	76.7	1.574
C3		197	14.526	61.0	2.641
C3		235	12.320	63.7	2.455
C3		225	12.918	64.4	2.412
C3		217	10.172	75.1	1.686
C3		197	10.570	70.5	2.000
C3		235	11.404	69.7	2.049
C3		225	13.207	69.2	2.083
C3		217	10.847	74.6	1.719
C3		197	14.809	62.6	2.534
C3		235	13.639	60.8	2.653
D4	IPA phytase 1000 U/k	212	8.469	79.6	1.365
D4		232	7.761	80.6	1.304
D4		237	10.888	75.1	1.672
D4		208	13.246	65.1	2.339
D4		212	13.582	64.2	2.398
D4		232	9.169	80.2	1.329
D4		237	8.329	78.0	1.478
D4		208	14.545	66.5	2.247
D4		212	11.571	69.7	2.034
D4		232	12.311	68.8	2.095
D4		237	9.823	76.8	1.556
D4		208	11.296	71.1	1.937
E5	IPA phytase 1500 U/k	198	12.281	67.1	2.118
E5		192	7.312	78.9	1.362
E5		195	9.466	72.1	1.800
E5		227	9.605	71.8	1.819
E5		198	13.378	65.5	2.225
E5		192	5.460	83.8	1.047
E5		195	12.292	66.1	2.184
E5		227	10.661	72.4	1.776
E5		198	11.869	67.3	2.108
E5		192	8.501	76.6	1.509
E5		195	10.140	74.2	1.666
E5		227	9.186	78.1	1.410
F6	750 U/k	233	9.202	73.2	1.766
F6		218	11.122	77.0	1.514
F6		210	9.710	72.9	1.788
F6		202	12.653	69.1	2.038

F6	IPA phytase 1	233	9.337	74.9	1.656
F6		218	11.409	76.1	1.577
F6		210	7.560	81.7	1.206
F6		202	10.317	74.6	1.676
F6		233	7.299	78.8	1.395
F6		218	8.234	80.9	1.262
F6		210	8.739	77.5	1.481
F6		202	10.832	71.9	1.856
G7	IPA phytase 2000 U/k	182	9.346	78.4	1.411
G7		186	7.755	73.4	1.744
G7		201	7.522	77.8	1.452
G7		238	10.873	71.1	1.893
G7		182	11.464	73.4	1.744
G7		186	9.151	76.0	1.569
G7		201	10.123	72.5	1.800
G7		238	10.358	72.7	1.785
G7		182	11.731	74.4	1.673
G7		186	8.378	75.7	1.591
G7		201	7.316	79.8	1.325
G7		238	8.021	78.5	1.405
I9	IPA phytase 3000 U/k	203	5.349	85.6	0.939
I9		191	15.007	61.3	2.533
I9		216	6.003	85.2	0.968
I9		228	4.263	89.9	0.663
I9		203	9.177	80.8	1.255
I9		191	5.547	87.8	0.795
I9		216	10.626	76.1	1.563
I9		228	5.887	86.8	0.864
I9		203	3.795	89.4	0.695
I9		191	8.966	72.8	1.781
I9		216	6.298	82.2	1.163
I9		228	7.616	83.2	1.097

European Food Safety Authority

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA phytase**	Batch number: **PPQ27987**
Trial ID: **S 05-08 VN**	Location: **DSM Nutritional Products France** **Centre de Recherche en Nutrition Animale** **BP 170** **68305 Saint-Louis cedex, France**
Start date and exact duration of the study: **March 10th 2008 - 38 days**	
Number of treatment groups (+ control(s)): **7 + (2)**	Replicates per group: **1**
Total number of animals: **36**	Animals per replicate: **4**
Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)	
Intended: **0 / 500 / 1000 / 1500 / 1750 / 2000 / 2500 / 3000 U/kg**	Analysed: **225 (endogenous activity) / 678 / 1179 / 1723 / 1985 / 2232 / 2798 / 3329 U/kg**
†	
Substances used for comparative purposes: **Dicalcium phosphate**	
Intended dose: **12 g per kg of feed. Equivalent to 2.4 g of additional P per kg of feed in a dry matter basis**	Analysed: **2.0 g of additional P per kg of feed in a dry matter basis**
Animal species/category: **Swine / growers**	
Breed: **Large White x Landrace**	Identification procedure: **Pen and individual earring**
Sex: **Males** — Age at start: **90 days**	Body weight at start: **19.06 ± 1.82 kg**
Physiological stage: **Growing pigs**	General health: **Normal - no clinical signs were observed**
Additional information for field trials:	
Location and size of herd or flock:	
Feeding and rearing conditions:	
Method of feeding:	
Diets (type(s)): **Basal diet formulated to provide P exclusively from vegetable origin and according to the NRC**	
Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other	
Composition (main feedingstuffs): **Maize - 53%, soybean meal - 18% and barley - 13.9%**	
Nutrient content (relevant nutrients and energy content)	
Intended values: **Crude protein - 15.5%, lysine - 0.96%, methionine + cystine - 0.54%, Ca - 0.66% in D.M., P - 0.41% in D.M. and digestible energy - 13.31 MJ/kg**	
Analysed values: **Ca - 0.70% in D.M. and P - 0.42% in D.M.**	
Date and nature of the examinations performed:	

[1] Please submit this form using a common word processing format (e.g. MS Word).

March 10th and April 17th - weight measurement **March 10th and March 19th - acclimatation period** **March 20th and April 4th - 1st period** **April 5th and April 17th - 2nd period** **April 15th, 16th and 17th - individual faecal sampling**	
Method(s) of statistical evaluation used: **Two-factor analysis of variance (diet and diet + animal or pen) followed by a Duncan multiple range test**	
Therapeutic/preventive treatments (reason, timing, kind, duration): **No therapeutic / preventive treatments were used**	
Timing and prevalence of any undesirable consequences of treatment: **Nothing to report**	
Date **22.02.2010**	Signature Study Director Dr P. GUGGENBUHL

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

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TAB

7

Annex 7

Effects of a novel phytase in corn-soybean meal diets fed to growing pigs

REPORT No. 00003283

REPORT No. 00003283
Regulatory Document

DSM

Document Date: 9 December, 2009

Author(s): [redacted] (b) (4), D.-R. Campbell[2] and J. Broz[3]

[1] [redacted] (b) (4)
[2] DSM Nutritional Products. Inc, Parsippany (USA)
[3] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title: **Effects of a novel phytase in corn-soybean meal diets fed to growing pigs**

Project No. 6106

Summary

An experiment was conducted in order to measure the effect of a novel phytase (IPA Mash phytase) on the digestibility of P in corn-soybean meal diets fed to growing pigs. Six different diets were formulated for this study. The positive control diet was based on corn and soybean meal and contained dicalcium phosphate to bring the total concentration of P to 0.56%. A negative, low-P control diet was formulated by replacing dicalcium phosphate by corn starch and it contained 0.33% P. Four additional diets were similar to the negative control diet with the exception that IPA Mash phytase was included at 500, 1000, 2000 and 4000 U/kg diet, respectively. A total of 24 growing pigs (mean body weight of 36.2 kg) were used in a 2 period crossover design. Pigs were placed in metabolism cages and randomly allotted to the 6 dietary treatments. After a 5-day adaptation period, faeces were collected for 5 days. The P concentration in faeces was lower ($P<0.01$) in pigs fed phytase containing diets than in pigs fed the negative control diet. Pigs fed phytase containing diets also had lower ($P<0.01$) total P output that the control pigs and the inclusion of phytase reduced (linear and quadratic, $P<0.01$) the excretion of P. The addition of graded levels of phytase resulted in a significant increase of the apparent total tract digestibility of P from 39.8 to 72.8% (linear and quadratic, $P<0.01$). Phosphorus absorption was greater ($P<0.01$) in pigs fed the positive control diet than in pigs fed the negative control diets (5.10 vs. 1.94 g/d). Pigs fed phytase containing diets had greater ($P<0.01$) absorption of P than pigs fed the negative control diet, and P absorption increased (linear and quadratic, $P<0.01$) as phytase was added to the control diet from 1.94 to 3.66 g/d. Phytase addition to the negative control diet also increased (linear and quadratic, $P<0.01$) the apparent total tract digestibility of Ca.

This report consists of Pages I – II and 1 – 16, raw data & Annex C

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Approved

Name	**Signature**	**Date**
Main Author Dr. J. Broz, NRD/CA	signed by J. Broz	09.12.2009
Principal Scientist / Competence Mgr Dr. J. Broz, NRD/CA	signed by J. Broz	09.12.2009
Research Center Head Dr. A.-M. Klünter, NRD/CA	signed by A.-M. Klünter	10.12.2009
Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	11.12.2009

Nomenclature and Structural Formula

IPA phytase (M), enzyme product containing bacterial 6-phytase ([redacted] (b) (4)), produced by [redacted] (b) (4) fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28683 was used in this study, manufactured by Novozymes A/S, [redacted] (b) (4).

Effects of a novel phytase in corn-soybean meal diets fed to growing pigs

[redacted]

[1] Corresponding author: [redacted]

ABSTRACT: An experiment was conducted with the objective of measuring the effect of a novel microbial phytase on the digestibility of P in corn soybean meal diets fed to growing pigs. Six diets were formulated. The positive control diet was a corn-soybean meal diet that contained dicalcium phosphate to bring the total concentration of P in this diet to 0.56%. A negative control diet was also formulated. This diet was similar to the positive control diet with the exception that cornstarch replaced dicalcium phosphate and contained 0.33% P. Four additional diets that were similar to the negative control diet with the exception that microbial phytase (IPA Mash, DSM Nutritional Products, Parsippany, NJ) in the amounts of 500, 1,000, 2,000, and 4,000 units per kg was included in the diets were also formulated. A total of 24 growing pigs (initial BW of 36.2 ± 4.0 kg) were used in a 2 period crossover design. Pigs were placed in metabolism cages and randomly allotted to the 6 dietary treatments. Fecal materials were collected for 5 d. The P concentration in feces was lower ($P < 0.01$) for pigs fed phytase containing diets than for pigs fed the negative control diet and there was a linear and quadratic reduction ($P < 0.01$) in fecal P concentration as phytase was included in the diets. Pigs fed phytase containing diets also had lower ($P < 0.01$) total P output than pigs fed the negative control diet, and inclusion of phytase to the negative control diet reduced (linearly and quadratically, $P < 0.01$) the excretion of P. Pigs fed phytase containing diets had greater ($P < 0.01$) apparent total tract digestibility (**ATTD**) of P than pigs fed the negative control diet, and the addition of increasing levels of phytase to the negative control diet increased the ATTD of P from 39.83 to 72.76% (linear and quadratic, $P < 0.01$). Phosphorus absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (5.10 vs. 1.94 g/d). Pigs that were fed phytase containing diets had greater ($P < 0.01$) absorption of P than pigs fed the negative control diet, and P absorption increased (linear and quadratic, $P < 0.01$) as phytase was added to the negative control diet from

1.94 to 3.66 g/d. The addition of phytase to the negative control diet also increased (linear and quadratic, $P < 0.01$) the ATTD of Ca. Pigs fed phytase containing diets had greater ($P < 0.01$) Ca absorption than pigs fed the negative control diet. In conclusion, IPA Mash improved the ATTD of P and Ca and reduced P excretion. At levels of 500 or 1000 units per kg IPA Mash seems to have a greater effect on Ca and P digestibility.

Key words: digestibility, phosphorus, pigs, phytase

INTRODUCTION

Most of the P present in cereal grains and oilseeds is in the form of phytate (Erdman, 1979). Because pigs lack endogenous phytases, phytate cannot be digested (Selle and Ravindran, 2008). As a consequence, large amounts of P are excreted in the manure potentially causing environmental pollution. Phytases are enzymes capable of hydrolyzing the phytate molecule and releasing phytate-P, which can then be utilized by pigs (Selle and Ravindran, 2008), and addition of microbial phytase to swine diets improve P utilization (Akinmusire and Adeola, 2009). Several microbial phytases are available, but new and more efficient phytases are being developed. Therefore, the objective of this experiment was to measure the effect of a novel microbial phytase (IPA Mash, DSM Nutritional Products) on the digestibility of P and Ca in corn-soybean meal diets fed to growing pigs.

MATERIALS AND METHODS

Diets, Animals, and Experimental Design

Six diets were formulated (Tables 1 and 2). The positive control diet was a corn-soybean meal diet that contained quantities of Ca and P sufficient to meet the requirement of Ca and P for growing pigs (NRC, 1998). Dicalcium phosphate was used to bring the total concentration of P in this diet to 0.56%. A negative control diet that was similar to the positive control diet with the exception that cornstarch replaced dicalcium phosphate was also formulated. This diet contained 0.33% P. Four additional diets that were similar to the negative control diet with the exception that microbial phytase (IPA Mash, DSM Nutritional Products, Parsippany, NJ) was included in the amounts of 500, 1,000, 2,000, or 4,000 units per kg were also formulated.

A total of 24 growing barrows were used in a 2 period crossover design. In period 1, barrows had an initial BW of 36.2 ± 4.0 kg, while in period 2, they had an initial BW of 47.3 ± 5.3 kg. Pigs were placed in metabolism cages and randomly allotted to the 6 dietary treatments. The cages were equipped with a feeder and a nipple drinker that allowed for total collection of feces.

Feeding and Sample Collection

The amount of feed provided daily was calculated as 3 times the estimated requirement for maintenance energy (i.e., 106 kcal ME per $kg^{0.75}$; NRC, 1998) and divided into 2 equal meals. Water was available at all times. The initial 5 d were considered an adaptation period to the diet. From d 6 to 11, fecal materials were collected according to the marker to marker approach (Adeola, 2001). Chromic oxide and ferric oxide were used to determine the beginning and the end of collection, respectively. Fecal samples were stored at -20°C immediately after collection.

Sample Analysis and Data Processing

At the conclusion of the experiment, fecal samples were dried in a forced air oven and finely ground prior to analysis. Fecal samples and diets were analyzed for Ca and P by inductively coupled plasma (ICP) spectroscopy method (method 985.01; AOAC, 2005) after wet ash sample preparation (method 975.03; AOAC Int., 2005). Diets were also analyzed for AA (method 982.30 E (a, b, c); AOAC, 2005), ADF (method 973.18; AOAC, 2005), NDF (Holst, 1973), CP by combustion (Elementar, Rapid N cube; method 990.03; AOAC, 2005), and for phytase activity (DSM Nutritional Products, Parsippany, NJ). The ATTD (%) of P in each diet was calculated according to the following equation:

$$ATTD (\%) = [(Pi - Pf)/Pi] \times 100,$$

where Pi = total P intake (g) from d 6 to 11 and Pf = total fecal P output (g) originating from the feed that was provided from d 6 to 11. Data were analyzed as a crossover design using the Proc Mixed Procedure in SAS. The UNIVARIATE procedure was used to verify homogeneity of variances and to identify outliers. One outlier was identified and removed from the data set. The model included diet as the main effect, while block, period, and pig were random effects. Block, period, and pig were, however, not significant and, therefore, sequentially removed from the model. Orthogonal polynomial contrasts were conducted to test linear and quadratic responses to the inclusion of phytase to the negative control diet. Appropriate coefficients for unequally spaced concentrations of supplemental phytase were obtained using the interactive matrix language procedure (Proc IML) of SAS. Treatments were considered different when $P < 0.05$ and the pig was the experimental unit for all analyses.

RESULTS

Throughout the experiment, pigs remained healthy and readily consumed their diets. No differences in feed intake were observed among treatments (Table 3). Phosphorus intake was lower ($P < 0.01$) for pigs fed the negative control than for pigs fed the positive control diet. Fecal output tended ($P = 0.07$) to be greater for pigs fed the positive control diet than for pigs fed the negative control diet. The P concentration in feces was lower ($P < 0.01$) for pigs fed phytase containing diets than for pigs fed the negative control diet, and there was a linear and quadratic reduction ($P < 0.01$) in fecal P concentration as phytase was included in the diets. Phosphorus output was lower ($P < 0.01$) for pigs fed the negative control diet than for pigs fed the positive control diet (2.87 vs. 3.41 g/d). Likewise, pigs fed phytase containing diets had lower ($P < 0.01$) P output than pigs fed the negative control diet, and inclusion of phytase to the negative control diet reduced (linearly and quadratically, $P < 0.01$) the excretion of P. The ATTD of P was lower ($P < 0.01$) for pigs fed the negative control diet than for pigs fed the positive control diet (39.83 vs. 59.36%). The addition of increasing levels of phytase to the negative control diet increased the ATTD of P (linearly and quadratically, $P < 0.01$). Phosphorus absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (5.10 vs. 1.94 g/d), but absorption of P increased (linearly and quadratically, $P < 0.01$) as phytase was added to the negative control diet.

Calcium intake was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet (12.02 vs. 8.47 g/d). Calcium in the feces and total Ca output were lower (linear and quadratic $P < 0.01$) for pigs fed phytase containing diets than for pigs fed the negative control diet. There was also a tendency ($P = 0.07$) for pigs fed the negative control diet to have a lower Ca output than pigs fed the positive control diet. Addition of phytase to the negative control diet increased (linearly and quadratically, $P < 0.01$) the ATTD of Ca. There was also a tendency ($P = 0.07$) for pigs fed the positive control diet to have a greater ATTD of Ca than for pigs fed the negative control diet. Calcium absorption was greater ($P < 0.01$) for pigs fed the positive control diet than for pigs fed the negative control diet. Likewise, pigs fed phytase containing diets had greater ($P < 0.01$) absorption of Ca than pigs fed the negative control diet.

DISCUSSION

The ATTD of P and Ca that were measured in the positive control diet agree with previous values for ATTD of P in corn-soybean meal diets (Johnston et al., 2004; Stein et al., 2008). Phytase supplementation increased P digestibility, which was expected because phytase can hydrolyze the bonds that bind P to the phytate molecule in corn and SBM, and therefore, release some of the P that is bound to the phytate molecule (Cromwell et al., 1993). Our results show that P excretion is reduced if corn-soybean meal diets are supplemented with phytase, which is in agreement with Selle and Ravindran, 2008. However, the linear effect of addition of phytase to the diets indicate that a plateau for P digestibility or P absorption was not reached in the present experiment even though the highest inclusion rate of phytase was 4,000 units per kg. This observation indicates that the present phytase is capable of continuing to hydrolyzing bonds on the phytate molecule and thus continue to release more P as the concentration of phytase is increased. Further experiments are needed to investigate which concentration of phytase is needed to reach a plateau for ATTD and absorption of P.

The ATTD of Ca was increased as phytase was included in the diets. This result is in agreement with results that were observed by Guggenbuhl et al. 2007. Possibly, in the process of phytate hydrolysis, phytate esters are reduced and as a consequence the ability of phytate to chelate Ca is also reduced. Therefore, Ca digestibility increases when exogenous phytase is supplemented to the diet (Selle et al., 2009).

Conclusions

Results from the present experiment show that IPA Mash phytase is an effective phytase that may be used in corn-soybean meal diets to improve the ATTD of P and Ca. The IPA Mash phytase will also result in a reduction in P excretion in the manure from pigs fed diets containing this enzyme.

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Table 1. Composition (as-is basis) of experimental diets [1]

Item	Diet					
	Positive control	Negative control	500 phytase	1,000 phytase	2,000 phytase	4,000 phytase
Ingredient, %						
Ground corn	65.80	65.80	65.80	65.80	65.80	65.80
Soybean meal, 48%	29.50	29.50	29.50	29.50	29.50	29.50
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.95	0.95	0.95	0.95	0.95	0.95
Dicalcium phosphate	1.05	-	-	-	-	-
Cornstarch	-	1.05	1.025	1.00	0.975	0.95
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Phytase premix [1]	-	-	0.025	0.05	0.075	0.10
Vit. mineral premix [2]	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]IPA Mash, DSM Nutritional Products, Parsippany, NJ. Produced by mixing 3.4% of concentrated phytase (58,700 units/g) and 96.6% cornstarch.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A, 11,128 IU; vitamin D_3, 2,204 IU;

vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 2. Analyzed nutrient composition of diets (as-fed basis)

	Diet					
Item	Positive control	Negative control	500 phytase	1,000 phytase	2,000 phytase	4,000 phytase
CP, %	18.33	17.96	17.24	18.03	17.93	18.27
ADF, %	2.64	2.63	2.61	2.64	2.67	2.84
NDF, %	12.11	8.02	8.81	7.71	7.80	8.52
P, %	0.56	0.33	0.34	0.34	0.34	0.34
Ca, %	0.79	0.58	0.59	0.57	0.56	0.54
Phytase, FTU/kg[1]	39	41	373	984	1773	3681
Indispensible AA, %						
Arg	1.23	1.22	1.17	1.23	1.23	1.26
His	0.53	0.50	0.50	0.50	0.50	0.51
Ile	0.84	0.81	0.78	0.81	0.82	0.84
Leu	1.63	1.58	1.54	1.60	1.59	1.62
Lys	1.08	1.06	1.02	1.07	1.07	1.09
Met	0.30	0.29	0.28	0.29	0.29	0.29

Phe	0.92	0.90	0.86	0.90	0.90	0.92
Thr	0.70	0.70	0.65	0.70	0.68	0.69
Trp	0.25	0.24	0.25	0.25	0.24	0.25
Val	0.95	0.91	0.89	0.91	0.94	0.95
Dispensible AA, %						
Ala	0.94	0.92	0.88	0.92	0.91	0.94
Asp	1.91	1.87	1.79	1.88	1.88	1.92
Cys	0.29	0.30	0.28	0.30	0.30	0.29
Glu	3.39	3.31	3.18	3.32	3.32	3.37
Gly	0.79	0.77	0.74	0.77	0.78	0.80
Pro	1.10	1.10	1.01	1.06	1.03	1.07
Ser	0.79	0.80	0.74	0.82	0.76	0.77
Tyr	0.57	0.57	0.57	0.59	0.57	0.59

[1]FTU = phytase units (g/kg).

Table 3. Effects of phytase on apparent total tract digestibility (ATTD) of P and Ca[1]

	Diets							*P*-value		*P*-value[2]	
Item	Positive control	Negative control	500 phytase	1,000 phytase	2,000 phytase	4,000 phytase	SEM	Positive vs. Negative	Negative vs. Phytase	L	Q
Feed intake, g/d	1521	1460	1506	1497	1476	1476	58.08	0.471	0.674	0.930	0.808
P intake, g/d	8.52	4.82	5.12	5.09	5.02	5.02	0.23	< 0.01	0.368	0.825	0.582
Fecal output, g/d	132.86	118.24	116.94	115.95	117.48	123.99	5.48	0.076	0.957	0.356	0.529
P in feces, %	2.59	2.44	1.82	1.52	1.31	1.09	0.07	0.170	< 0.01	< 0.01	< 0.01
P output, g/d	3.41	2.87	2.12	1.76	1.54	1.36	0.10	< 0.01	< 0.01	< 0.01	< 0.01
ATTD of P, %	59.36	39.83	58.10	65.43	69.09	72.76	2.25	< 0.01	< 0.01	< 0.01	< 0.01
P absorption, g/d	5.10	1.94	3.00	3.33	3.47	3.66	0.24	< 0.01	< 0.01	< 0.01	< 0.01
Ca intake, g/d	12.02	8.47	8.89	8.53	8.26	7.97	0.36	< 0.01	0.902	0.128	0.838

Ca in feces, %	2.45	2.33	1.40	1.29	1.22	0.91	0.13	0.539	< 0.01	< 0.01	< 0.01
Ca output, g/d	3.20	2.74	1.62	1.50	1.46	1.13	0.16	0.068	< 0.01	< 0.01	< 0.01
ATTD of Ca, %	72.90	67.30	81.44	82.62	82.36	85.58	2.05	0.069	< 0.01	< 0.01	< 0.01
Ca absorption, g/d	8.82	5.72	7.26	7.03	6.80	6.84	0.39	< 0.01	< 0.01	0.376	0.122

[1]Data are means of 8 observations per treatment.

[2]L = linear contrast; Q = quadratic contrast.

Exp 195

Exp. 195, Calculations

Diet	Period	Animal	Sample	Sample	FI_5d	CainDiet_pcnt	PinDiet_pcnt	CaIntake_g	Pintake_g	Feces_g	CainFeces_pcnt	PinFeces_pcn	Caout_g	Pout_g	ATTD_Ca	ATTD_P
19501	1	6	19515	19515F	7121.06	0.79	0.56	56.26	39.88	545.40	3.03	2.66	16.51	14.51	70.64	63.61
19501	1	12	19521	19521F	7052.78	0.79	0.56	55.72	39.50	637.60	3.32	2.90	21.18	18.52	61.98	53.12
19501	1	17	19526	19526F	6851.22	0.79	0.56	54.12	38.37	660.80	2.14	2.68	14.13	17.70	73.89	53.88
19501	1	20	19529	19529F	6439.72	0.79	0.56	50.87	36.06	647.90	2.65	2.70	17.14	17.47	66.30	51.56
19501	2	3	19536	19536F	9072.31	0.79	0.56	71.67	50.80	738.60	1.93	1.96	14.28	14.48	80.08	71.49
19501	2	11	19544	19544F	8356.44	0.79	0.56	66.02	46.80	731.30	1.90	2.60	13.89	19.04	78.95	59.32
19501	2	18	19551	19551F	8009.60	0.79	0.56	63.28	44.85	601.50	2.58	2.90	15.51	17.41	75.49	61.18
19501	2	24	19557	19557F	7953.12	0.79	0.56	62.83	44.54	751.40	2.02	2.33	15.17	17.50	75.85	60.71
19502	1	4	19513	19513F	7104.10	0.58	0.33	41.20	23.44	701.20	2.36	2.30	16.53	16.15	59.89	31.12
19502	1	11	19520	19520F	6967.08	0.58	0.33	40.41	22.99	672.50	2.64	2.41	17.75	16.19	56.06	29.57
19502	1	14	19523	19523F	6548.77	0.58	0.33	37.98	21.61	445.70	2.67	2.56	11.91	11.41	68.65	47.20
19502	1	23	19532	19532F	6285.56	0.58	0.33	36.46	20.74	512.10	2.26	2.73	11.55	14.00	68.31	32.53
19502	2	6	19539	19539F	8366.79	0.58	0.33	48.53	27.61	560.80	2.25	2.25	12.62	12.62	74.00	54.28
19502	2	9	19542	19542F	8448.00	0.58	0.33	49.00	27.88	327.90	1.96	2.37	6.43	7.77	86.88	72.11
19502	2	16	19549	19549F	8235.61	0.58	0.33	47.77	27.18	611.50	2.29	2.59	14.01	15.84	70.67	41.72
19502	2	21	19554	19554F	7578.67	0.58	0.33	43.96	25.01	634.70	1.83	2.27	11.62	14.40	73.56	42.42
19503	1	2	19511	19511F	7256.57	0.59	0.34	42.81	24.67	631.40	1.61	1.88	10.14	11.86	76.32	51.94
19503	1	8	19517	19517F	6856.21	0.59	0.34	40.45	23.31	577.30	1.52	1.92	8.80	11.07	78.25	52.50
19503	1	18	19527	19527F	6637.11	0.59	0.34	39.16	22.57	521.60	1.96	2.25	10.22	11.72	73.91	48.06
19503	1	21	19530	19530F	6374.39	0.59	0.34	37.61	21.67	539.10	1.25	1.72	6.73	9.26	82.11	57.27
19503	2	1	19534	19534F	8899.85	0.59	0.34	52.51	30.26	698.50	1.36	1.53	9.49	10.68	81.92	64.71
19503	2	7	19540	19540F	8262.18	0.59	0.34	48.75	28.09	544.80	1.61	1.92	8.78	10.47	81.98	62.73
19503	2	17	19550	19550F	8056.93	0.59	0.34	47.54	27.39	552.70	0.94	1.74	5.20	9.59	89.06	64.97
19503	2	22	19555	19555F	7916.12	0.59	0.34	46.71	26.91	612.20	0.92	1.64	5.61	10.06	87.98	62.63
19504	1	1	19510	19510F	7311.98	0.57	0.34	41.68	24.86	608.40	0.84	1.22	5.11	7.40	87.74	70.24
19504	1	10	19519	19519F	7020.73	0.57	0.34	40.02	23.87	553.30	0.85	1.25	4.70	6.92	88.25	71.00
19504	1	15	19524	19524F	6763.02	0.57	0.34	38.55	22.99	574.70	1.26	1.51	7.22	8.66	81.28	62.34
19504	1	24	19533	19533F	6175.07	0.57	0.34	35.20	21.00	519.00	1.22	1.52	6.31	7.91	82.07	62.33
19504	2	5	19538	19538F	8717.23	0.57	0.34	49.69	29.64	644.40	1.69	1.61	10.92	10.38	78.03	64.97
19504	2	12	19545	19545F	8304.28	0.57	0.34	47.33	28.23	653.10	1.65	1.72	10.76	11.22	77.28	60.26
19504	2	13	19546	19546F	7977.02	0.57	0.34	45.47	27.12	528.30	1.66	1.66	8.75	8.78	80.75	67.65
19504	2	23	19556	19556F	7591.84	0.57	0.34	43.27	25.81	556.90	1.12	1.64	6.23	9.12	85.60	64.68
19505	1	5	19514	19514F	7182.03	0.56	0.34	40.22	24.42	596.20	1.42	1.55	8.45	9.22	78.99	62.23
19505	1	7	19516	19516F	6802.95	0.56	0.34	38.10	23.13	636.10	1.56	1.39	9.92	8.85	73.95	61.72
19505	1	16	19525	19525F	6689.65	0.56	0.34	37.46	22.74	510.40	1.29	1.28	6.57	6.55	82.47	71.19
19505	1	19	19528	19528F	6152.12	0.56	0.34	34.45	20.92	456.10	0.75	1.23	3.44	5.61	90.02	73.16
19505	2	2	19535	19535F	8570.51	0.56	0.34	47.99	29.14	673.30	1.59	1.24	10.71	8.32	77.68	71.46
19505	2	10	19543	19543F	8312.09	0.56	0.34	46.55	28.26	640.20	0.68	1.06	4.38	6.80	90.59	75.94
19505	2	14	19547	19547F	7752.40	0.56	0.34	43.41	26.36	522.80	1.02	1.36	5.35	7.09	87.67	73.08
19505	2	20	19553	19553F	7562.42	0.56	0.34	42.35	25.71	663.90	1.43	1.40	9.51	9.27	77.54	63.93
19506	1	3	19512	19512F	7303.47	0.54	0.34	39.44	24.83	433.40	0.79	0.99	3.42	4.31	91.34	82.65
19506	1	9	19518	19518F	6815.34	0.54	0.34	36.80	23.17	576.50	1.07	1.10	6.17	6.36	83.22	72.56
19506	1	13	19522	19522F	6771.59	0.54	0.34	36.57	23.02	686.00	1.44	1.29	9.90	8.87	72.93	61.47
19506	1	22	19531	19531F	6385.27	0.54	0.34	34.48	21.71	565.10	0.89	1.06	5.01	5.97	85.48	72.49
19506	2	4	19537	19537F	8122.29	0.54	0.34	43.86	27.62	775.90	0.70	0.98	5.41	7.62	87.67	72.41
19506	2	8	19541	19541F	8074.32	0.54	0.34	43.60	27.45	674.80	0.78	1.12	5.26	7.55	87.93	72.49
19506	2	15	19548	19548F	7980.97	0.54	0.34	43.10	27.14	639.30	0.90	1.04	5.74	6.67	86.68	75.40
19506	2	19	19552	19552F	7584.90	0.54	0.34	40.96	25.79	608.60	0.72	1.16	4.36	7.06	89.36	72.62

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FEEDAP UNIT

ANNEX C

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA Mash Phytase** Batch number: **PPQ 28683**

Trial ID: **Experiment 195** Location: [redacted] (b) (4)

Start date and exact duration of the study: **April 2, 2009 for 4 Weeks**

Number of treatment groups (+ control(s)): **6** Replicates per group: **8**

Total number of animals: **24** Animals per replicate: **1 per Trt Group**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg[1] complete feed/L[1] water)

Intended: **500, 1000, 2000 & 4000 FYT/kg in Complete Feed** Analysed: **373, 984, 1773, & 3681 FYT/kg**

+

Substances used for comparative purposes:

Intended dose: Analysed:

Animal species/category: **Swine**

Breed: **PIC** Identification procedure: **Ear Notch**

Sex: **Barrows** Age at start: **12 Weeks** Body weight at start: **36.2 kg**

Physiological stage: **Grower Pigs** General health: **Excellent**

Additional information for field trials:

Location and size of herd or flock: **240 Sow Farrow to Finish at the** [redacted] (b) (4)

Feeding and rearing conditions: **Individually and Housed in Metabolism Cages**

Method of feeding: **Limit Feeding**

Diets (type(s)): **Typical Commercial Corn-Soy Diet**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Corn, Soybean meal, & Soy oil**

Nutrient content (relevant nutrients and energy content)

Intended values: **Ca-PC-0.71 & 0.48 % in Others; P-PC 0.58 & 0.39 % in Others - ME-3450 Mcal/kg**

Analysed values: **Ca-PC-0.79 NC 0.58 & 0.59, 0.57, 0.56, & 0.54 % in Test Diets**
P-PC-0.56 NC 0.33 & 0.34, 0.34, 0.34, & 0.34 % in Test Diets

Date and nature of the examinations performed: **None**

Method(s) of statistical evaluation used: **SAS UNIVARIATE & Proc Mixed Procedures**

Therapeutic/preventive treatments (reason, timing, kind, duration): **None**

Timing and prevalence of any undesirable consequences of treatment: **None**

Date **November 23, 2009**	Signature Study Director [redacted] (b) (4)

[1] Please submit this form using a common word processing format (e.g. MS Word).

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8

Annex 8

Efficacy of IPA phytase in growing pigs

REPORT No. 00001789

DSM

Document Date: 18 September, 2009

Author(s): [redacted] (b) (4)[1] and J. Broz[2]

[1] [redacted] (b) (4)
[2] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title: **Efficacy of IPA phytase in growing pigs**

Project No. 6106

Summary

A trial was conducted to study the efficacy of IPA phytase at different doses in growing pigs. A total of 48 animals (*Landrace x Pietrain*) were involved. The pigs started on the trial at 51.6 kg body weight and remained on the experimental treatments for 3 weeks. They were divided into eight blocks of 6 animals, as similar as possible, taking into account sex and initial body weight. The experimental treatments consisted of a basal, low-P, control diet (T-1), which was supplemented with IPA phytase (M) at 500, 1000, 2000, or 4000 U/kg (T-2, T-3, T-4 and T-5, respectively), and a positive control diet supplemented with 1 g of inorganic P/kg as dicalcium phosphate (T-6). Each dietary treatment was assigned to 8 animals. At the end of trial, fresh faeces were sampled for each pig and the apparent digestibility of dry matter, ash, organic matter, Ca and P was measured using titanium dioxide as indicator. A blood sample was also obtained from each pig and analysed for alkaline phosphatase activity and inorganic P and Ca concentrations. The supplementation of the basal diets with IPA phytase significantly increased P concentration in blood at 1000, 2000 and 4000 U/kg diet, respectively. IPA phytase at 500, 1000, 2000 and 4000 U/kg diet significantly improved the apparent faecal digestibility of P from 29.6% (negative control) to 35.6, 42.5 ($P<0.05$), 56.1 ($P<0.05$) and 62.4% ($P<0.05$), respectively. The apparent digestibility of Ca was improved as well and the effects were significant for phytase supplementation at 1000 and 2000 U/kg diet, respectively. At all inclusion levels IPA phytase also significantly reduced P concentration in faeces.

This report consists of Pages I – II and 1 - 17

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Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	23.09.2009

Nomenclature and Structural Formula

IPA phytase (M), enzyme product containing bacterial 6-phytase [redacted] (b) (4)), produced by [redacted] (b) (4) fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28656 was used in this study, manufactured by Novozymes A/S, [redacted] (b) (4).

[redacted] (b) (4)

FINAL REPORT OF THE CONTRACT SIGNED WITH:

Company: DSM Nutritional Products

Title: EFFICACY OF IPA PHYTASE IN GROWING PIGS

Experiment number: P-396

Contract Code:	*2 2 5 4 7*
Organic Code:	*0 6 0 2*

Author: [redacted] (b) (4)

Center: [redacted] (b) (4)

Number of pages: 17

Date: 14/09/2009

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SUMMARY

A trial was conducted to study the efficacy of IPA phytase at different doses in growing pigs. A total of 48 growing pigs (*Landrace x Pietrain*) were involved. The pigs started on the trial at 51.6 kg BW and remained on the experimental treatments for 3 weeks. Pigs were divided into eight groups (blocks) of 6 animals, as similar as possible, taking into account sex and initial body weight, to which six experimental treatments were assigned. The experimental treatments consisted of a basal, low-P, control diet (T-1), which was supplemented with 500, 1,000, 2,000, or 4,000 U/kg of IPA phytase (M) (T-2, T-3, T-4 and T-5, respectively), and a positive control diet supplemented with 1 g of inorganic P/kg as dicalcium phosphate (T-6). At the end of the trial, fresh faeces were sampled for each animal and the apparent faecal digestibility of dry matter, ash, organic matter, Ca, and P was measured using titanium oxide as indigestible marker. A blood sample was also obtained from each pig and analysed for alkaline phosphatase activity and inorganic phosphorous and calcium concentrations. Increased Ca and reduced P blood concentrations ($P<0.05$) were observed for the negative control diet (T-1), relative to the positive control diet (T-6). The supplementation of the negative control diet with IPA phytase reduced Ca concentration in blood (statistically significant at 4,000 U/kg), increased P concentration in blood (statistically significant at 1,000, 2,000 and 4,000 U/kg), and reduced P concentration in faeces (statistically significant at all levels of supplementation). IPA phytase improved the faecal digestibility of ash (statistically significant at 2,000 U/kg), P (statistically significant at 1,000, 2,000 and 4,000 U/kg), and Ca (statistically significant at 1,000 and 2,000 U/kg). It is concluded that IPA phytase improves the apparent faecal digestibility of phosphorous in growing pigs, resulting in higher P blood concentrations and lower P faecal excretion. It appears that, within the doses tested, the responses to IPA phytase increased with the inclusion level.

(b) (4) [redacted]

Contract code: 2 2 5 4 7

RESPONSIBILITIES

Study researcher

(b) (4) [redacted]

Study monitor

Dr. Jiri Broz
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Daily monitor

(b) (4) [redacted]

Stockworkers

(b) (4) [redacted]

Feed preparation

(b) (4) [redacted]

Laboratory analysis

(b) (4) [redacted]

OBJECTIVE

The objective of this experiment was to evaluate the efficacy of **IPA phytase** in the feeding of grower pigs at the different dosages when compared to negative and positive controls. A low phosphorous diet was used as basal diet.

METHODOLOGY

Site of the experiment

The trial was conducted in the pig experimental farm of the [redacted] (b) (4)

Location and housing

The trial was conducted using grower pigs from [redacted] (b) (4) experimental farm at [redacted] (b) (4) site. The pigs were housed in two rooms with 24 pens each. The rooms are provided with automatic heating, forced ventilation and completely slatted floors. Feed and water were distributed *ad libitum.*

Animals

Forty eight pigs were kept individually in the 48 pens. *Landrace x Pietrain* pigs of 51.6 (SD 5.27) kg body weight were used. Animals were randomly distributed by initial body weight and sex into 8 blocks and each block consisted of six pigs (3 males and 3 females).

Feeding program

There was a unique dietary composition (13.4 MJ ME; 1.0% Lysine) for the whole experiment. Feed was presented in mash form and offered *ad libitum*. The composition of the diets is presented in Tables 1 and 2. Feed included 0.5% of titanium oxide as indigestible marker.

Tested product

Name:	IPA phytase (M)
Description:	bacterial 6-phytase expressed in *Aspergillus oryzae*
Produced by:	Novozymes A/S [redacted] (b) (4)
Provided by:	DSM Nutritional Products Ltd, Basel, Switzerland
Lot No:	PPQ 28656
Activity:	57 085 U/g product
Dosages:	500, 1000, 2000 and 4000 U/kg diet, corresponding to 8.8, 17.6, 35.2, and 70.4 ppm, respectively.

Treatments and experimental design

There were six experimental treatments:

T-1: Negative control (NC; a low-P basal diet)
T-2: NC + **IPA phytase** at 8.8 mg/kg, corresponding to 500 U/kg diet
T-3: NC + **IPA phytase** at 17.6 mg/kg, corresponding to 1000 U/kg diet
T-4: NC + **IPA phytase** at 35.2 mg/kg, corresponding to 2000 U/kg diet
T-5: NC + **IPA phytase** at 70.4 mg/kg, corresponding to 4000 U/kg diet
T-6: Positive control (PC, diet with an additional 1 g of inorganic P/kg from DCP)

The negative control diet was low in available phosphorous, and different doses of phytase were added via a premix using maize starch as the carrier to create the different experimental treatments.

For the whole trial 0.5% titanium oxide was added to the diet as indigestible marker in order to perform Ca and P faecal digestibility measurements. In-feed analytical determination of the added phytase was conducted by Biopract GmbH, Berlin (Germany), on behalf of DSM Nutritional Products.

The pigs were individually housed in 48 pens. The animals were randomly distributed by initial weight and sex into 8 blocks. Each block therefore consisted of 6 pigs (3 males and 3 females). Within each block, one of the six treatments was randomly adjudicated to each pig.

Controls

On the third week of trial, fresh faeces were sampled from the floor of the pens. Diet and faeces were analysed for DM, TiO_2, ash, Ca and P, and the apparent faecal digestibility was calculated. The P concentration per kg DM in faeces was also calculated.

A blood sample was also obtained from each pig on the third week of trial and analysed for alkaline phosphatase activity and inorganic phosphorous and calcium concentrations.

Dates

The animals started the trial on November 18^{th} 2008, and completed it on December 5^{th}, 2008.

STATISTICAL ANALYSIS

The parameters measured were compared among treatments using the GLM procedure of the statistical package SAS. The individual pigs were used as the experimental unit.

For statistical analysis, the GLM procedure of SAS was used, considering the effect of sex and using initial body weight as covariable. The measurements for each pig were used to calculate the mean values for each treatment, and they were compared taking into account the effects of sex and initial body weight. The values presented are least squares means.

Outlier values that were identified with the "Smirnoff-Grubbs's" test[1] were not considered for the analyses.

[1] Grubbs, F.E. (1969) Procedures for Detecting Outlying Observations Samples, Technometrics Vol. 11, No.1, 1-12.

RESULTS AND DISCUSSION

The analysed composition of the experimental diets is shown in Table 3. The results of phytase analytics confirmed the proper addition of test product. The negative and positive basal diets fed to control pigs (treatments T-1 and T-6) contained a low level of phytase (150 and 114 U/kg) which represents native phytase activity present in the used feed ingredients. Phytase activity in the supplemented diets were 671 (treatment T-2), 1529 (treatment T-3), 2659 (treatment T-4), and 4448 (treatment T-5) U/kg.

No statistically significant differences in body weight gain and feed intake were observed for the different doses of IPA phytase (Table 4). Feed to gain ratio was numerically improved in the diets supplemented with IPA phytase relative to the negative control diet.

The effect of the different doses of IPA phytase on alkaline phosphatase activity, Ca and P concentration in blood and P concentration in faeces is shown in Table 5. The negative control diet increased Ca and reduced P concentration in blood ($P<0.05$), relative to the positive control diet (T-6). Also, the alkaline phosphatase activity in blood increased numerically in the negative control diet, and no difference was observed between these two diets in the P concentration in faeces. The supplementation of the negative control diet with different doses of IPA phytase resulted in a reduced Ca concentration in blood (statistically significant at 4,000 U/kg), an increased P concentration in blood (statistically significant at 1,000, 2,000 and 4,000 U/kg), and a reduced P concentration in faeces (statistically significant at all levels of supplementation). A numerical increase in alkaline phosphatase activity was observed at 500 and 1,000 U/kg, whereas this parameter was numerically reduced at 2,000 and 4,000 U/kg of IPA phytase supplementation.

The effect of the different doses of IPA phytase on the apparent faecal digestibility of dry matter, ash, organic matter, phosphorous and calcium is shown in Table 6. No statistically significant differences in digestibility for any of the nutrients studied were observed between the negative (T-1) and the positive (T-6) control diets. There was however, a numerical improvement in ash, Ca and P digestibility for the positive control diet, probably due to the addition of dicalcium phosphate in diet T-6. The supplementation of the negative control diet with different doses of IPA phytase resulted in improved digestibility for ash (statistically significant at 2,000 U/kg), P (statistically significant at 1,000, 2,000 and 4,000 U/kg), and Ca (statistically significant at 1,000 and 2,000 U/kg).

(b) (4) [redacted]

Contract code: 2 2 5 4 7

It is concluded that IPA phytase improves the apparent faecal digestibility of phosphorous in growing pigs, resulting in higher P blood concentrations and lower P faecal excretion. It appears that, within the doses tested, the responses to IPA phytase increased with the inclusion level.

Signatures:

(b) (4) [redacted]

Date: 5 · 8 · 2009 Date: 11/8/2009 Date: 7-08-2009

TABLES AND FIGURES

[redacted] (b) (4)

Contract code: 2 2 5 4 7

Table 1 Composition of the experimental diets (%)

Ingredients	Low P Basal diet	STD P diet
Maize	35.00	35.00
Barley	41.35	41.35
Soyabean meal, 48% CP	18.95	18.95
Lard	2.30	2.30
Dicalcium phosphate	**0**	**0.55**
Calcium carbonate	**1.30**	**0.92**
Salt	0.35	0.35
L-Lysine-HCl	0.16	0.16
DL-Methionine	0.01	0.01
Vit-Min complex*	0.40	0.40
Maize starch	**0.18**	**0**

* Providing per kg of diet: vitamin A: 5000 IU; vitamin D_3: 1000 IU; vitamin E: 15 mg; thiamin: 1,3 mg; riboflavin: 3,5 mg; vitamin B_{12}: 0.025 mg; vitamin B_6: 1,5 mg; calcium pantothenate: 10 mg; nicotinic acid: 15 mg; biotin: 0.1 mg; folic acid: 0.6 mg; vitamin K_3: 2 mg; Fe: 80 mg as iron sulfate; Cu: 140 mg as copper sulfate; Co: 0.75 mg as cobalt sulfate; Zn: 60 mg as zinc oxide; Mn: 30 mg as manganese sulfate; I: 0.75 mg as potassium iodate; Se: 0.10 mg as sodium selenite; ethoxiquin: 0.15 g.

Table 2 Estimated nutritive composition of the experimental diets

Nutrients	Low P Basal diet	STD P diet
Moisture (%)	13.39	13.92
Crude Protein (%)	16.11	16.11
Crude Fibre (%)	3.41	3.41
Fat (%)	4.63	4.63
Ash (%)	4.16	4.34
Energy (MJ ME/kg)	13.4	13.4
Calcium (g/kg)	6.00	6.00
Total phosphorous (g/kg)	3.49	4.46
Non-phytic P (g/kg)	1.33	2.30
Lysine (g/kg)	10.00	10.00
Threonine (g/kg)	6.70	6.70
Methionine (g/kg)	3.00	3.00
Methionine+Cystine (g/kg)	6.47	6.47
Tryptophan (g/kg)	2.14	2.14

Table 3 Analyses of the experimental diets

Nutrients	T-1	T-2	T-3	T-4	T-5	T-6
Dry matter (%)	88.14	88.41	88.40	88.22	88.29	88.40
Crude protein (%)	17.5	18.0	17.9	17.9	18.3	18.5
Crude fibre (%)	3.36	3.32	3.44	3.45	3.28	3.39
Fat (%)	4.12	4.52	4.46	4.04	4.12	4.00
Ash (%)	4.53	4.58	4.56	4.74	4.75	4.91
Phosphorous (g/kg)	3.0	2.9	3.0	2.9	3.3	4.2
Calcium (g/kg)	5.9	6.3	6.4	6.0	6.0	5.9
Phytase activity (U/kg)	150	671	1529	2659	4448	114

Table 4 Performance of the pigs receiving different doses of IPA phytase

	Weight gain (g/day)	Feed intake (g/day)	Feed to gain ratio
T-1 Negative control (low P)	791	2489	3.20
T-2 IPA phytase (500 U/kg)	893	2323	2.63
T-3 IPA phytase (1,000 U/kg)	879	2035	2.31
T-4 IPA phytase (2,000 U/kg)	907	2188	2.43
T-5 IPA phytase (4,000 U/kg)	804	2126	2.67
T-6 Positive control (DCP)	881	2224	2.61
Standard Error	*128.1*	*455.4*	*0.602*
Initial BW Effect (Pr>F)	NS	NS	NS
Sex Effect (Pr>F)	NS	NS	NS
Treat. Effect (Pr>F)	NS	NS	NS

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

abc Values in the same column with different letters are significantly different (P<0.05)

Table 5 Alkaline phosphatase (AP) activity, Ca and P concentration in blood and P concentration in faeces of pigs receiving different doses of IPA phytase

	Ca in blood (mg/dL)	P in blood (mg/dL)	AP in blood (U/L)	P in faeces (g/kg DM)
T-1 Negative control (low P)	10.85 a	6.69a	509	13.78c
T-2 IPA phytase (500 U/kg)	10.67ab	7.12ab	555	10.98b
T-3 IPA phytase (1,000 U/kg)	10.50ab	7.63bc	554	10.49b
T-4 IPA phytase (2,000 U/kg)	10.45ab	8.04c	487	8.11a
T-5 IPA phytase (4,000 U/kg)	9.89c	7.75c	476	7.69a
T-6 Positive control (DCP)	10.21b	7.66c	425	14.82c
Standard Error	*0.454*	*0.502*	*100.9*	*1.685*
Initial BW Effect (Pr>F)	NS	NS	NS	NS
Sex Effect (Pr>F)	NS	NS	NS	NS
Treat. Effect (Pr>F)	**	***	NS	***

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

abc Values in the same column with different letters are significantly different (P<0.05)

Table 6 Effect of different doses of IPA phytase on the apparent faecal digestibility of dry matter, ash, organic matter, phosphorous and calcium in growing pigs (%)

	Dry matter	Ash	Organic matter	P	Ca
T-1 Negative control (low P)	81.6	46.4b	83.5	29.6c	55.3c
T-2 IPA phytase (500 U/kg)	81.1	50.0b	82.8	35.6bc	62.0bc
T-3 IPA phytase (1,000 U/kg)	82.0	52.8ab	83.6	42.5b	70.6ab
T-4 IPA phytase (2,000 U/kg)	81.9	57.7a	83.3	56.1a	75.9a
T-5 IPA phytase (4,000 U/kg)	79.3	51.3ab	80.9	62.4a	61.3bc
T-6 Positive control (DCP)	80.6	48.5b	82.5	37.5bc	58.0c
Standard Error	*2.50*	*6.97*	*2.40*	*8.27*	*9.73*
Initial BW Effect (Pr>F)	NS	NS	NS	NS	*
Sex Effect (Pr>F)	NS	NS	NS	NS	NS
Treat. Effect (Pr>F)	NS	*	NS	***	**

NS P>0.1; † P<0.1; * P<0.05; ** P<0.01; *** P<0.001

abc Values in the same column with different letters are significantly different (P<0.05)

ANNEX-I (RAW DATA)

(b) (4) [redacted]

Contract code: 2 2 5 4 7

PIG	SEX	BL	TR	ADG	ADI	FGR	Blood Ca	Blood P	Blood AP	Faeces P	Dig DM	Dig Ash	Dig OM	Dig P	Dig Ca
1	M	1	3	941	2165	2.300	9.9	8.46	504	10.650	80.99	48.73	82.74	40.33	.
2	M	1	6	951	1779	1.871	10.6	7.72	492	15.647	78.91	39.09	81.25	30.53	62.57
3	M	1	1	973	2376	2.443	11.4	6.88	417	10.913	77.84	49.67	79.37	28.96	57.79
4	M	1	5	896	2591	2.890	10	8.05	457	9.428	82.51	55.20	84.06	55.87	53.83
5	M	1	2	799	2981	3.732	10.4	6.81	628	13.720	82.22	53.38	83.79	25.62	75.25
6	M	1	4	795	1973	2.481	10.9	7.97	439	8.370	85.86	65.67	87.01	63.99	71.44
7	M	2	5	522	1700	3.255	9.2	6.69	372	9.817	75.31	48.91	76.81	.	54.97
8	M	2	2	965	2160	2.239	10.6	7.07	545	11.996	84.79	56.55	86.33	44.37	75.09
9	M	2	3	916	1673	1.825	10.5	7.79	661	10.076	82.85	60.36	84.08	49.09	74.37
10	M	2	6	1186	2107	1.777	9.5	8.09	538	14.167	84.67	62.16	85.99	54.28	75.43
11	M	2	1	746	2056	2.757	11	6.38	630	16.017	83.83	51.35	85.59	23.93	70.07
12	M	2	4	1039	2141	2.061	10.5	8.32	664	7.561	82.85	59.93	84.15	60.55	79.21
13	M	3	1	615	2567	4.172	9.5	7.07	347	13.175	81.45	47.08	83.31	28.18	43.03
14	M	3	3	875	1927	2.202	11	6.83	740	12.063	81.59	46.51	83.50	34.56	57.44
15	M	3	5	896	1860	2.075	10.2	7.38	531	7.399	77.98	42.33	80.01	56.42	71.88
16	M	3	4	1041	2135	2.051	10.7	8.44	533	10.508	84.48	57.18	86.03	50.39	76.62
17	M	3	6	739	2612	3.535	9.9	7.78	389	15.352	78.26	46.12	80.15	29.74	45.78
18	M	3	2	1067	2142	2.008	10.7	6.93	646	10.238	78.20	43.55	80.09	31.96	47.33
19	M	4	2	1016	3219	3.167	10.9	7.38	562	9.650	81.30	55.12	82.73	44.98	75.81
20	M	4	1	782	3067	3.920	10.9	6.48	450	11.784	79.86	46.89	81.65	30.27	53.52
21	M	4	3	892	1992	2.234	10.7	7.32	430	9.115	80.12	58.89	81.27	46.60	81.64
22	M	4	5	.	.	.	9.1	7.09	.	.	76.28	36.40	78.54	.	43.29
23	M	4	4	825	2028	2.459	9.9	8.51	410	6.977	81.78	63.66	82.81	61.33	81.39
24	M	4	6	.	.	.	10.2	6.63	248	.	83.63	49.52	85.63	37.06	73.79
25	F	5	2	.	.	.	11.4	7.52	547	11.749	81.49	48.49	83.30	33.71	72.72
26	F	5	3	829	2145	2.586	10.5	8.38	426	13.455	84.36	51.94	86.13	38.00	59.46
27	F	5	6	869	2629	3.024	9.9	8.32	368	14.435	83.44	55.97	85.05	49.68	62.02
28	F	5	1	964	2080	2.159	11.3	6.59	500	13.884	83.48	49.25	85.34	32.62	59.43
29	F	5	5	827	2820	3.410	10.5	8.71	392	6.564	80.88	57.28	82.22	66.42	58.58
30	F	5	4	821	2412	2.937	10.6	7.56	436	6.585	80.99	60.45	82.16	61.92	79.11
31	F	6	5	972	2426	2.496	9.8	8.01	591	7.391	81.53	55.54	83.01	63.48	62.43
32	F	6	4	894	1912	2.138	10.6	7.41	404	8.480	76.74	44.48	78.58	40.01	.
33	F	6	3	.	.	.	10.2	7.75	584	7.753	83.89	61.94	85.08	.	68.11
34	F	6	1	664	2473	3.727	10.4	7.31	456	11.500	79.27	43.53	81.21	29.97	46.10
35	F	6	2	841	2058	2.446	10.9	7.59	499	8.316	80.42	50.95	82.03	50.35	66.81
36	F	6	6	847	1731	2.043	10.8	7.92	534	15.895	81.35	49.46	83.23	37.61	60.54
37	F	7	5	621	1299	2.091	10	8.27	426	5.559	78.82	56.18	80.11	68.50	79.68
38	F	7	2	748	2146	2.868	10.5	6.96	.	11.024	82.67	54.45	84.21	41.75	61.61
39	F	7	1	719	1772	2.465	10.6	6.51	466	13.911	82.71	44.85	84.76	29.32	53.52
40	F	7	4	848	2240	2.641	9.7	7.89	424	8.512	80.76	55.23	82.21	50.17	68.27
41	F	7	6	674	1809	2.684	10.3	7.33	465	15.077	74.97	34.63	77.35	20.58	35.13
42	F	7	3	836	2022	2.418	10.6	7.45	421	9.835	80.90	47.42	82.73	44.66	75.62
43	F	8	1	869	3342	3.844	11.5	6.31	755	18.041	83.44	37.31	85.94	.	48.35
44	F	8	4	993	2572	2.590	10.6	8.21	558	7.396	81.72	54.63	83.25	58.86	74.14
45	F	8	3	887	2234	2.519	10.4	7.09	603	9.921	80.48	44.87	82.41	42.93	65.37
46	F	8	5	869	2126	2.445	10.2	7.8	537	7.132	80.78	57.84	82.08	63.32	58.91
47	F	8	2	840	1779	2.118	10.4	6.6	599	13.473	80.02	41.01	82.15	17.94	46.12
48	F	8	6	873	3094	3.544	10.6	7.43	397	13.916	80.11	52.41	81.74	41.75	55.08

ANNEX-II (EFSA)

efsa
European Food Safety Authority

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA phytase (M)** Batch number: **PPQ 28656**

Trial ID: **P-396** Location: [redacted] (b) (4)

Start date and exact duration of the study: **November 18th 2008, 17days**

Number of treatment groups (+ control(s)): **6** Replicates per group: **8**

Total number of animals: **48** Animals per replicate: **1**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **500, 1000, 2000 and 4000 U/kg** Analysed: **671, 1529, 2659 and 4448 U/kg**

†

Substances used for comparative purposes: **Dicalcium phosphate in positive control**

Intended dose: **1 g P/kg** Analysed: **1.18 g P/kg**

Animal species/category: **Growing pigs**

Breed: **Landrace x Pietrain** Identification procedure: **Ear tags**

Sex: **Males and Females** Age at start: **15 weeks** Body weight at start: **51.6 kg**

Physiological stage: **Growing** General health: **optimal**

Additional information for field trials:

Location and size of herd or flock:

Feeding and rearing conditions:

Method of feeding:

Diets (type(s)): **Low and adequate P diets for grower pigs**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize, barley, soybean meal-48**

Nutrient content (relevant nutrients and energy content)

Intended values: **16.1% CP, 3932 kcal/kg GE, 4.16 & 4.34% ash, 0.35 & 0.45% total P, 0.60% Ca**

Analysed values: **18.0% CP, 4005 kcal/kg GE, 4.63 & 4.91% ash, 0.30 & 0.42% total P, 0.61% Ca**

Date and nature of the examinations performed: **P and Ca concentration in blood and faeces and apparent faecal digestibility of P and Ca**

Method(s) of statistical evaluation used: **Analysis of variance (GLM procedure)**

Therapeutic/preventive treatments (reason, timing, kind, duration): **not relevant**

Timing and prevalence of any undesirable consequences of treatment: **no adverse effects observed**

Date	Signature Study Director
7-08-2009	[redacted] (b) (4)

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

9

Annex 9

Dose response study with a new phytase (IPA Mash Phytase) in lactating sows

REPORT No. 00003282

REPORT No. 00003282
Regulatory Document

DSM

Document Date: 9 December, 2009

Author(s): [redacted] (b) (4) and J. Broz[2]

[1] [redacted] (b) (4)
[2] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title: **Dose response study with a new phytase (IPA Mash Phytase) in lactating sows**

Project No. 6106

Summary

A digestibility experiment with lactating sows was carried out to study the efficacy of a new bacterial 6-phytase (IPA Mash Phytase). Twenty eight sows (German Landrace) were involved and randomly assigned to one of four dietary treatments. The control treatment was a low-P diet based on maize and soybean meal and was not supplemented with inorganic P source or phytase. This basal diet was formulated to contain 4.2 g total P and 10.2 g Ca per kg. Treatments B, C and D received the same basal diets supplemented with phytase at the inclusion levels of 500, 1000 and 2000 U/kg diet, respectively. The faeces of the sows were collected on 5 consecutive days during the 3rd and 4th week of lactation. The digestibility of P and Ca was calculated using the indigestible marker TiO_2. The apparent faecal digestibility of P in the control diet was on a very low level as expected (20.5%) and it was improved by phytase supplementation in a non-linear manner to a maximum of 34.1% at the highest level of supplementation. The increase in P digestibility was significant for treatments C and D in comparison to treatment A (negative control). The Ca digestibility was similar across all treatments and not significantly affected by phytase supplementation. In conclusion, the new phytase product showed beneficial effects on P digestibility in lactating sows and its dietary supplementation will lead to a reduction of the faecal P excretion.

This report consists of Pages I – II and 1 – 11 & Annex C

Distribution
Dr. M. Eggersdorfer, NRD
Dr. F. Fru, NRD/PA
Mr. J.-F. Hecquet, NBD/RG
Dr. P. Guggenbuhl, NRD/CA
Dr. A.-M. Klünter, NRD/CA
Dr. J. Pheiffer, NRD/CA
Mr. J.-P. Ruckebusch, ANH/GM
Dr. C. Simoes Nunes, NRD/CA

Approved

Name	**Signature**	**Date**
Main Author Dr. J. Broz, NRD/CA	signed by J. Broz	09.12.2009
Principal Scientist / Competence Mgr Dr. J. Broz, NRD/CA	signed by J. Broz	09.12.2009
Research Center Head Dr. A.-M. Klünter, NRD/CA	signed by A.-M. Klünter	10.12.2009
Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	11.12.2009

Nomenclature and Structural Formula

IPA phytase (M), enzyme product containing bacterial 6-phytase [redacted]), produced by [redacted] fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28683 was used in this study, manufactured by Novozymes A/S, [redacted].

(b) (4)

[redacted]

Dose response study with a new phytase (IPA Mash Phytase) in lactating sows

Report to DSM Nutritional Products, Basel

[redacted] (b) (4)

Introduction

The majority of total phosphorus (P) in plant seeds is contained in the form of phytate. Single-stomached animals have only marginal intrinsic phytase activity, which means that such animals are unable to effectively hydrolyse phytate in the digestive tract.

Studies with piglets and growing-finishing pigs showed that microbial phytase added to the feed has a positive effect on P digestibility. Only few reports have been published on the effectiveness of phytases in lactating sows. However, especially during lactation, large quantities of calcium (Ca) and P are required by the animal. Also, the effect of supplemental microbial phytase on P and Ca digestibility may differ in dependence on the source of phytase, the dietary contents of Ca, P and vitamin D_3, and the level of intrinsic phytase activity of the feed ingredients.

It was the objective of the present experiment to study the effects of a new phytase product on the digestibility of P in lactating sows.

Material and methods

Diets

Diet preparation was done in the certified feed mill facilities of [redacted] (b) (4) [redacted]y.

The experimental diets were based on maize (660 g/kg) and solvent-extracted soybean meal from dehulled seed (270 g/kg) without a mineral P supplementation in order to achieve a sufficiently low basal P level in combination with negligibly low intrinsic phytase activity. In addition, soybean oil (36 g/kg), calcium carbonate (4 g/kg), and a P-free vitamin and mineral mix (25 g/kg) were included. The diets contained TiO_2 as indigestible marker (5 g/kg). The diets were calculated to be adequate in ME and all nutrients excluding P according to DLG[1].

The total amount of feed needed for the experiment was mixed in one lot and subsequently divided into 4 equal parts. The basal diet (treatment A) remained without the supplement. Treatments B, C and D comprised supplements of 500, 1000 and 2000 U of phytase per kg of diet, respectively. The product was added in the intended concentrations to the diets and they were mixed again. Diets were not pelleted but fed in mash form.

The test product was a bacterial 6-phytase (IPA Mash Phytase) and was supplied by DSM Nutritional Products, Basel, Switzerland. It was expressed in a genetically modified strain of *Aspergillus oryzae*. The lot number was PPQ 28683 and the product was provided in a powder form containing 58,753 U/g.

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[1] Deutsche Landwirtschafts-Gesellschaft (DLG). 2008. Empfehlungen zur Sauen- und Ferkelfütterung. 1/2008. DLG-Information. DLG-Verlag, Frankfurt/Main.

Intended phytase activities as well as P and Ca concentrations were confirmed by analyses (Table 1).

Table 1: Intended and analysed phytase activity and analysed concentrations of crude nutrients, P and Ca in the experimental diets

Diet	Phytase activity (U/kg)		Ash	Crude protein	Crude fibre	Crude fat	P	Ca
	Intended	Analysed	g/kg dry matter					
A	0	< 50	65	201	26	85	4.3	11.3
B	500	589	61	206	27	83	4.2	9.7
C	1000	1027	61	200	27	87	4.2	10.6
D	2000	2125	62	196	29	85	4.1	9.4

Animals, housing and sampling

The experiment (institutional ID: 2009-10) was conducted in the (b) (4) [redacted] between July 27, 2009 and September 26, 2009. A total of 28 sows (1st to 9th parity), all from the same genotype (German Landrace), were used in the experiment. One week before partum the animals were moved into individual cages with a farrowing rail on a perforated sheet floor and were randomly assigned to one of the four treatments. The animals had free access to water from nipple drinkers. Sows were weighed at the beginning and the end of the faeces collection period.

In the pre-treatment period (7 days before and 2 to 11 d after parturition) all sows were fed an in-house lactation diet (annex table A1). The sows were then adjusted to the experimental diet by increasing the level of maize and soybean meal in the diet over a period of 3 to 6 days.

A 5-day period of faeces collection followed a 7-day period of prefeeding the experimental diets. During these 12 days the experimental diets were offered for *ad libitum* intake twice a day together with some water. The feed consumption was determined during the 5 days of collection. Feed residues were collected twice a day before feeding, pooled for each sow and stored at -20°C. Faeces were sampled immediately after being voided from a rubber inlet on the floor element behind each animal, pooled per sow and stored at -20°C. Because the design of the cages and the floor did not allow for a complete collection of all faeces, the calculation of digestibilities was made applying the indicator technique. Later the samples of feed residues and faeces were thawed, homogenized and dried at 65°C for 48 h prior to analyses.

During the pre-treatment period, a pyretotherapy was done with seven sows (1, 2, 3, and 1 animals in treatment A, B, C, and D, respectively) using antipyretics and antibiotics. No other medical treatment was necessary.

Analyses and data evaluation

Concentrations of dry matter and crude nutrients were determined according to VDLUFA standard methods[2]. Samples of feed and faeces were ground through a sieve with 0.5-mm pore size and treated in the institute's laboratory for analyses of P, Ca, and Ti according to Boguhn et al. (2009)[3].

Measurements of P, Ca, and Ti were made using an inductively coupled plasma spectrometer (ICP-OES). Phytase activity in the feed was determined according to Engelen et al. (1994)[4] by Biopract GmbH, Berlin, Germany.

Digestibility (y) was calculated based on the ratio of P or Ca and TiO_2 in diet and faeces according to the generally accepted equation:

$$y(\%) = 100 - 100 \times \frac{TiO_2 \text{ in the diet (g/kg)}}{TiO_2 \text{ in faeces (g/kg)}} \times \frac{\text{P or Ca in faeces (g/kg)}}{\text{P or Ca in diet (g/kg)}}$$

Data were subjected to *glm* procedure using the software package SAS for Windows 9.2. In case of a significant treatment effect means were compared using t-test. The Dunnett test was used to test the individual differences found between each supplemented diet and the control diet without phytase supplementation.

Non-linear regression analysis was performed with the program GraphPad Prism 5.02. An exponential model of the following type was fitted to the data:

$$y = a \times (1 - e^{(-c \times x)}) + b$$

with a: response (y value) at zero phytase supplementation
b: maximum response to supplemented phytase (a + b = upper asymptote)
c: parameter describing the steepness of the curve
y: concentration of digestible P in the diet (g/kg dry matter)
x: supplemented phytase (U/kg).

Calculations were made with calculated phytase levels. In order to describe the 'marginal' efficacy of the phytase, the first derivative of the equation was used. This derivative describes the amount of digestible P which is additionally released by each incremental unit of phytase[5].

---

[2] Naumann, C. and R. Bassler. 1976. VDLUFA-Methodenbuch, Vol. III. Die chemische Untersuchung von Futtermitteln with supplements 1983, 1988, 1993, 1997, 2004, and 2006. VDLUFA-Verlag, Darmstadt.
[3] Boguhn, J., T. Baumgärtel, A. Dieckmann, and M. Rodehutscord. 2009. Determination of titanium dioxide supplements in different matrices using two methods involving photometer and inductively coupled plasma optical emission spectrometer measurements. Archives of Animal Nutrition 63, 337-342.
[4] Engelen, A. J., F.C. van der Heeft, P.H. Randsdorp, and E.L.C. Smit. 1994. Simple and rapid determination of phytase activity. Journal of AOAC International 77, 760-764.
[5] Paditz, K., H. Kluth, and M. Rodehutscord. 2004. Relationship between graded doses of three microbial phytases and digestible phosphorus in pigs. Animal Science 78, 429-438.

Results

The study could be run and finished without any problems. The temperature in the farrowing units could not be controlled and often followed the high outdoor temperatures during summer. This probably contributed to the relatively low feed intake that was observed.

The sows weighed on average 219 kg (SD = 38.7 kg) during the experimental period. The initial and final body weights as well as the feed consumption were not different between the treatments (Table 2).

Table 2: Number of parity and piglets, body weight (BW) and feed consumption of the sows in the 5-day experimental period (Means and ranges, n = 7 sows per treatment)

Phytase U/kg	Treatment	Number of parity	Number of piglets	Initial BW kg	Final BW kg	Feed consumption kg/day
0	A	4.6	9.7	236	231	7.1
		1 - 8	6 - 12	179 - 284	171 - 287	3.9 - 10.4
500	B	3.6	9.7	215	210	6.4
		2 - 7	7 - 11	155 - 272	149 - 268	3.5 - 9.3
1000	C	3.1	8.9	201	199	5.4
		2 - 5	5 - 11	144 - 243	143 - 244	4.1 - 8.4
2000	D	4.1	10.1	233	231	5.9
		1 - 9	7 - 12	154 - 273	145 - 276	4.9 - 7.6
P				*0.29*	*0.35*	*0.40*

The digestibility of dry matter averaged 87% without any treatment effect (Table 3). However, for one individual sow a dry matter digestibility of only 72% was calculated (see annex table A3) caused by a very low analysed TiO_2 concentration in the faeces. This was identified an outlier and the sow was excluded from the analysis of all digestibility data.

Digestibility of P was significantly improved from 21 % to 34 % with increasing phytase supplementation (Table 3). Similar levels of P digestibility were reported by Jongbloed et al. (2004)[6] using a 6-phytase derived from *Peniophora lycii*. The mean digestibility of Ca was similar across all diets at on average 35.5% (Table 3).

[6] Jongbloed, A.W., J.Th.M. van Diepen, P.A. Kemme, J. Broz. 2004. Efficacy of microbial phytase on mineral digestibility in diets for gestating and lactating sows. Livestock Production Science 91, 143-155.

Table 3: Digestibility of dry matter, P and Ca (Means and SD, n = 7 lactating sows per treatment)

Phytase U/kg	Treatment	Digestibility (%) Dry matter	P	Ca
0	A	86.9	20.5[a]	33.2
		0.87	13.1	3.2
500	B	86.9	23.3[ab]	37.5
		0.46	7.8	4.9
1000	C	87.4	32.5[*bc]	37.6
		0.54	5.4	3.5
2000	D[#]	86.8	34.1[*c]	33.6
		0.62	5.0	7.9
P		0.38	0.02	0.23

[#] n = 6

[*] Means are significantly different from the unsupplemented treatment A according to Dunnett test.

[a,b,c] Values without a common superscript are significantly different according to t-test ($P \leq 0.05$).

The concentration of digestible P in the diet increased from an average of 0.86 g/kg dry matter in the unsupplemented control treatment up to 1.44 g/kg dry matter in the diet containing 2000 U phytase per kg (Figure 1). With each incremental unit of phytase, the increase in digestible P concentration became smaller. The marginal efficacy decreased from about 0.60 mg digestible P per unit of phytase at initial supplementation to about 0.13 mg in the upper range of supplementation.

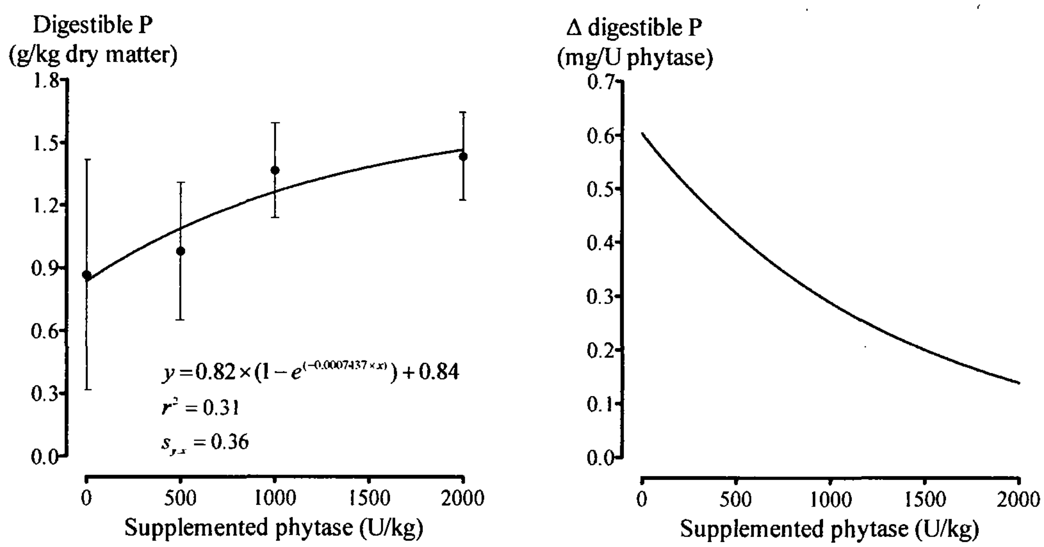

Figure 1: Concentration of digestible P in the diets depending on the level of supplementation of phytase (Means and SD, n = 7 lactating sows per treatment A, B, and C; n = 6 sows per treatment D; left) and marginal efficacy of supplemented phytase (right)

Summary and conclusions

An experiment with lactating sows was carried out to study the efficacy of a new 6-phytase (IPA Mash Phytase) derived from *Aspergillus oryzae*. Twenty eight sows (1st to 9th parity, initial body weight between 144 and 284 kg) were randomly assigned to one of four treatments. The control treatment was a low-P diet based on maize and soybean meal and was not supplemented with inorganic P or phytase. Treatments B, C, and D were the same as treatment A, except that phytase was added at levels of 500, 1000 and 2000 U per kg of diet. The P and Ca concentrations were 4.2 and 10.2 g/kg dry matter, respectively.

The faeces of the sows were collected on 5 consecutive days during the 3rd and 4th week of lactation. The digestibility of P and Ca was calculated based on the indigestible marker TiO_2.

No significant differences were found in the initial and final body weights of the sows as well as the feed consumption and dry matter digestibility. The digestibility of P in the control diet was on a very low level as expected (20.5%). P digestibility was improved by phytase supplementation in a non-linear manner to a maximum of 34.1% at the highest level of supplementation. The increase in P digestibility was significant for the treatments C and D in comparison to treatment A. The Ca digestibility was similar across all treatments at on average 35.5% and not significantly effected by phytase supplementation.

In conclusion, the new phytase product has beneficial effects in the feeding of lactating sows when added to the diet. By supplementing this phytase the faecal excretion of P can be reduced.

[redacted] November 30, 2009

[redacted]

Annex tables 1 to 3 are part of this report.

Annex table 1: Composition and calculated nutrients and energy concentration of the diets before experimental period (g/kg)

Components	Diet 7 d before until at least 2 d postpartum	Diet during adaptation period
Maize	-	300
Soybean meal, solvent extracted	90	230
Soybean oil	10	33
Oats	50	40
Wheat bran	62	80
Peas	100	90
Wheat	123	80
Triticale	200	-
Barley	330	100
Monocalcium phosphate	-	12
Mineral and vitamin mix	35†	35#
Crude protein	156	191
Crude fibre	46	46
P	5.6	6.7
Ca	8.2	10.8
Lysine	9.5	9.8
Methionine and cystine	5.6	5.8
Threonine	6.0	6.9
Tryptophan	1.9	2.3
ME (MJ/kg feed)	12.7	13.4

† per kg: 456 g calcium carbonate; 153 g monocalcium phosphate; 146 g sodium chloride; 30 g magnesium oxide; 32 g wheat bran; 400,000 I.U. vitamin A; 2.5 g vitamin E; 57,000 I.U. vitamin D_3; 0.5 g copper; 17,500 FTU 3-phytase; 14.3 x 10^9 colony-forming unit lactic acid bacteria; with antioxidant food additives

P-free; per kg: 665 g calcium carbonate; 175 g sodium chloride; 40 g magnesium oxide; 5 g rape seed oil; 600,000 I.U. vitamin A; 86,000 I.U. vitamin E; 3 g zinc; 0.33 g copper; 13 mg selenium; with butylated hydroxytoluene as antioxidant food additive

Annex table 2: Number of parity and piglets, body weight (BW), and feed consumption of the sows in the 5-day experimental period (individual data)

Number of sow	Treatment	Number of parity	Number of piglets	Initial BW kg	Final BW kg	Feed consumption kg/d
1	0	6	11	224	222	6.53
2	500	2	11	155	149	3.54
3	1000	2	10	144	143	4.06
4	2000	2	10	206	203	6.37
5	500	2	9	189	189	4.73
6	0	4	11	218	213	6.36
7	2000	2	12	256	256	5.49
8	1000	4	9	194	189	6.42
9	0	5	9	279	269	3.94
10	500	6	11	229	226	5.76
11	1000	5	9	239	232	4.32
12	2000	4	10	232	231	5.17
13	0	2	11	224	216	7.36
14	500	2	10	207	199	4.68
15	1000	2	9	219	218	4.53
16	2000	9	7	242	236	4.89
17	0	8	6	284	287	10.30
18	500	4	9	235	227	7.40
19	1000	3	9	182	179	5.37
20	2000	7	11	272	274	6.61
21	0	6	8	243	239	10.38
22	500	7	7	272	268	9.19
23	1000	2	5	188	192	8.41
24	2000	1	9	154	145	5.24
25	0	1	12	179	171	5.05
26	500	2	11	217	216	9.34
27	1000	4	11	243	244	4.83
28	2000	4	12	273	276	7.56

Annex table 3: Digestibility of dry matter, P and Ca (individual data)

Number of sow	Treatment	Digestibility Dry matter %	P %	Ca %
1	0	87.4	18.9	37.7
2	500	86.3	22.4	46.6
3	1000	87.1	32.6	39.6
4	2000	86.5	38.3	38.3
5	500	86.8	29.5	38.2
6	0	85.9	13.7	32.9
7	2000	86.2	37.9	43.0
8	1000	86.6	35.0	34.9
9	0	88.4	9.2	29.7
10	500	87.0	25.6	32.4
11	1000	87.1	29.9	31.5
12	2000	87.5	33.0	30.3
13	0	87.1	22.7	32.5
14	500	87.8	24.7	39.2
15	1000	88.0	33.0	40.7
16	2000	86.0	27.7	19.9
17	0	86.1	33.4	33.3
18	500	86.7	15.5	34.2
19	1000	87.0	23.9	37.3
20	2000	87.1	28.7	33.8
21	0	87.2	41.3	29.3
22	500	86.7	11.2	33.2
23	1000	87.8	31.1	37.6
24[#]	2000	72.5	-60.8	-25.6
25	0	86.4	4.6	36.8
26	500	87.0	34.0	38.8
27	1000	87.9	41.7	41.5
28	2000	87.3	38.8	36.0

[#] Data were not considered in the statistical analysis.

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ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA Mash Phytase** Batch number: **PPQ 28683**

Trial ID: **2009-10** Location: [redacted] (b) (4)

Start date and exact duration of the study: **2009/07/27 - 2009/09/26**

Number of treatment groups (+ control(s)): **4** Replicates per group: **7**

Total number of animals: **28** Animals per replicate: **1**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **500, 1000, 2000 U/kg feed** Analysed: **589, 1027, 2125 U/kg feed**

Substances used for comparative purposes: **none**

Intended dose: Analysed:

Animal species/category: **Lactating sows**

Breed: **German Landrace** Identification procedure: **ear number**

Sex: **female** Age at start: Body weight at start: **221 kg**

Physiological stage: **normal** General health: **good**

Additional information for field trials:

Location and size of herd or flock:

Feeding and rearing conditions:

Method of feeding:

Diets (type(s)): **Maize/soybean meal-based, low-P**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize (66%), soybean meal (27%), soybean oil (3.6%)**

Nutrient content (relevant nutrients and energy content)

Intended values: **21 % CP in DM, 0.40 % P in DM, 0.92 % Ca in DM**

Analysed values: **20.1 % CP in DM, 0.42 % P in DM, 1.03 % Ca in DM**

Date and nature of the examinations performed: **faeces collection between 3rd and 4th week of lactation**

Method(s) of statistical evaluation used: **Standard analytical methods, ANOVA, Regression analysis**

Therapeutic/preventive treatments (reason, timing, kind, duration): **Seven sows were medicated before the experimental period against fever with Matamizol, Tylosin, Metapyrin, Trimethosel or Pyrogenin (alone or in combination)**

Timing and prevalence of any undesirable consequences of treatment:

Date	Signature Study Director
Nov. 30, 2009	[redacted] (b) (4)

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

10

Annex 10

Efficacy study with IPA Mash Phytase in gestating sows

REPORT No. 00003285

REPORT No. 00003285
Regulatory Document

DSM

Document Date:	15 December, 2009
Author(s):	[redacted] (b) (4)[1] and J. Broz[2]
	[redacted] (b) (4) [2] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel
Title:	**Efficacy study with IPA Mash Phytase in gestating sows**
Project No.	6106

Summary

An experiment involving 24 gestating sows (hybrid line Euroc) in the 3rd parity was conducted in order to evaluate the effects of IPA Mash phytase on the apparent faecal digestibility of crude ash, calcium and phosphorus. The animals were fed a gestation diet based on maize, wheat, triticale and soybean meal as the main ingredients, which provided about 2.2 g of available P per sow and day. This basal diet was supplemented with IPA Mash phytase at the inclusion levels of 0 (negative control), 500, 1000 and 2000 U/kg, respectively. Each dietary treatment was assigned to 6 animals. The basal, P-deficient diet was fed during a 14-day pre-treatment period and subsequently the experimental diets were provided during the following 14-day treatment period. Samples of faeces were collected during the last 5 days, on days 54 to 58 of pregnancy, and the apparent digestibility was measured by an indicator method, using chromium oxide. Dietary supplementation with IPA Mash phytase at 500, 1000 and 2000 U/kg improved the apparent faecal digestibility of P from 26.5% (control) to 33.5, 38.6 ($P<0.05$) and 39.9 % ($P<0.05$), respectively. All 3 phytase inclusion levels also improved significantly the apparent faecal Ca digestibility. The improvement of P digestibility was connected with a dose related reduction of its faecal concentration. At the highest inclusion level of IPA phytase the faecal P concentration was reduced by 25.3% in comparison with the control.

This report consists of Pages I – II and 1 – 20 & Annex C

Distribution

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Approved

Name	Signature	Date
Main Author Dr. J. Broz, NRD/CA	signed by J. Broz	15.12.2009
Principal Scientist / Competence Mgr Dr. J. Broz, NRD/CA	signed by J. Broz	15.12.2009
Research Center Head Dr. A.-M. Klünter, NRD/CA	signed by A.-M. Klünter	17.12.2009
Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	16.12.2009

Nomenclature

IPA phytase (M), enzyme product containing bacterial 6-phytase [redacted] (b) (4)), produced by [redacted] (b) (4) fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28684 was used in this study, manufactured by Novozymes A/S, [redacted] (b) (4).

Report

Title:

Efficacy study with IPA Mash Phytase in gestating sows

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Phytase activity in diets

(b) (4) [redacted]

Efficacy study with IPA Mash Phytase in gestating sows

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1 Abstract

No efficacy studies with the novel IPA Mash Phytase, an enzyme product containing bacterial 3-Phytase, expressed in a genetically modified strain of *Asperigillus oryzae*, regarding the digestibility of crude ash, calcium and phosphorus in breeding sows were reported yet. Therefore, the study was performed with multiparous sows during the 3rd pregnancy period with different levels of IPA Mash Phytase to assess its efficacy and dose response. A total of 24 sows in the body weight range 190 to 210 kg were obtained from a pool of fifty sows of the breeder farm, which were of the same genotype (hybrid line Euroc) as well as in the same parity (3rd parity) and reproduction stage (29th to 33rd day of pregnancy). On day 31 of pregnancy (average value) the sows were allocated in eight blocks of three sows per pen in the gestation stable with individual feeding. Except for calcium and phosphorus the diets were calculated in accordance to the official recommendations for sows. Compared to the official recommendation of 2.4 g available phosphorus per sow and day the supply of sows fed with the phosphorus deficient diet offered in the daily amount of 2.23 kg was with 2.2 g available phosphorus 12.5% lower than the recommended daily supply of available phosphorus. The deficient diet was used for the 14-day depletion period (pre-treatment period) and during the following 14-day treatment period either as a negative control diet without phytase supplementation or with supplementation of IPA Mash Phytase at the levels of 500, 1000 and 2000 units per kg of feed in the period 45th to 58th day of pregnancy. Each treatment (without or with IPA Mash Phytase) consisted of 6 sows and 6 replicates, respectively. For performance data average body weight gain and feed intake were recorded. Scored faecal consistency was monitored by daily inspection. Chromium oxide (Cr_2O_3) at 5g/kg was supplemented as an inert indigestible indicator for apparent faecal digestibility measurements of crude ash, calcium and phosphorus, respectively. The faeces samples were collected by rectal sampling of all pigs at 24-h intervals during the 54th and 58th day of pregnancy. Compared to the unsupplemented control graded levels of IPA Mash Phytase enhanced the apparent digestibility of crude ash, calcium and phosphorus significantly by 33.5, 34.6 and 50.4% respectively. The dose response curves did not allow any overall calculation of the optimal phytase activity in the diets for sows during the faecal collection period at days 54 to 58 of pregnancy. However, based on the fact that the response between dose levels of 1000 and 2000 units per kg feed was not so pronounced than that between 500 and 1000 units per kg of feed it may be concluded that a sufficient dose response was achieved already with 1000 units per kg of feed.

It was concluded that IPA Mash Phytase is effective in improving the apparent digestibility for crude ash, calcium and phosphorus when using diets low in phosphorus supply. The regression equations did not allow any overall calculation of the optimal phytase activity in the diet for sows during the faecal collection period at days 54 to 58 of pregnancy. It was estimated that 500, 1000 and 2000 units of IPA Mash Phytase per kg of feed were equivalent to 1.21, 2.12 and 2.37 g of monocalcium phosphate, respectively.

Efficacy study with IPA Mash Phytase in gestating sows

2 General information

2.1 Study

2.1.1 Trial type: Efficacy study with IPA Mash Phytase at graded levels on apparent digestibility of crude ash, calcium as well as phosphorus in gestating multiparous sows from 31st to 58th day of pregnancy.

2.1.2 Animals: 24 multiparous sows (EUROC line) (Hülsenberger Zuchtschweine GmbH, 33803 Steinhagen).

2.1.3 Main feedstuffs: Maize, optigrain, soybean meal.

2.1.4 Feed additive: IPA Mash Phytase (bacterial 6-Phytase expressed in a genetically modified strain of *Aspergillus oryzae);*

- Batch: PPQ 28684;
- Activities: 59800 U/g;
- Dose levels:
  500 U/kg feed
  1000 U/kg feed
  2000 U/kg feed

2.2 Address of trial facility: [redacted] (b) (4)

2.3 Responsibility: [redacted] (b) (4)

2.4 Time schedule:

Start of experiment: 2009-07-09
End of experiment: 2009-08-12

3 Introduction

The efficiency of the novel IPA Mash Phytase, an enzyme product containing bacterial 6-Phytase, expressed in a genetically modified strain of *Aspergillus oryzae*, as a feed additive in sows during lactation is not known. Therefore, and because of the request for approval of the enzyme as a feed additive, an experiment was carried out to study dose dependent effects of IPA Mash Phytase in sows during the first part of gestation fed diets with high phytin-P but deficient in available phosphorus. Effects on apparent digestibility of crude ash, calcium as well as phosphorus were evaluated by using an indicator method.

4 Experimental procedure

4.1 Animals and housing

A total of 24 multiparous sows in the body weight range 190 to 210 kg were obtained from a pool of fifty sows of the breeder farm, which were of the same genotype (hybrid line Euroc), parity (3rd parity) and reproduction stage (29th to 33rd day of pregnancy). On day 31 of pregnancy (average value) the sows were allocated in eight blocks of three sows per pen in the gestation stable with individual feeding. Environmental temperature was adjusted to about 21°C. The relative humidity was in the range of 65%. Details of daily measured temperatures as well as relative humidity are presented in Figures 1 and 2. The calculated ventilation capacity was in the range of 2.0 changes per hour. Feed was offered in automatic feeders. Fresh drinking water was continuously supplied by drinking bowls.

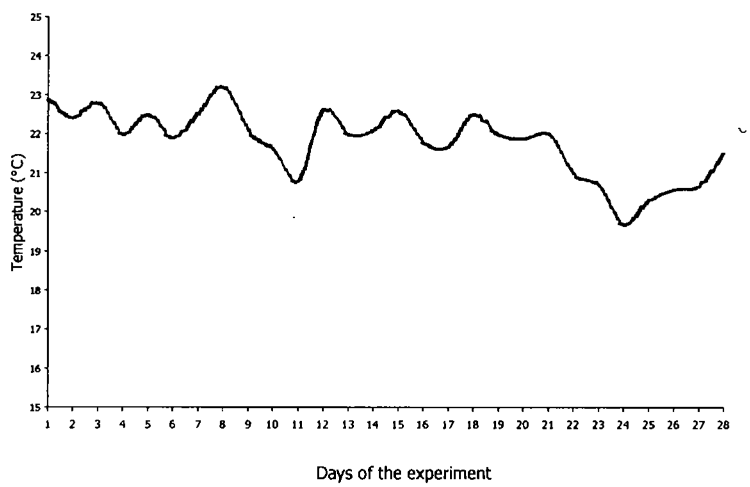

Figure 1. Daily temperatures in the gestation stable during the 28-day-experimental period measured in the animal area

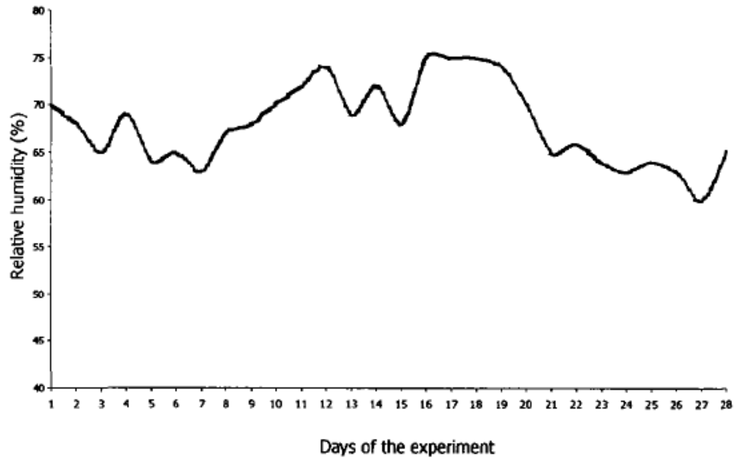

Figure 2. Daily relative humidity in the gestation stable during the 28-day experimental period measured in the animal area

4.2 Experimental design

Four treatments were imposed to the gestating sows from the 45th to 58th day of pregnancy after a 12-day pre-treatment period (31st to 44th day of pregnancy). Six sows per treatment were used. The first treatment (A) was the negative control diet, a low-phosphorus basal diet without IPA Mash Phytase. Treatments B, C and D were identical to the negative control but supplemented with IPA Mash Phytase at levels of 500, 1000 and 2000 U/kg of diet, respectively. Details are presented in Table 1.

Table 1. Overview of the treatments applied to the sows

Treatment		**A**	**B**	**C**	**D**
Sows	n	6	6	6	6
Replicates	n	6	6	6	6
• Pre-treatment period - 31st to 44th day of pregnancy IPA Mash Phytase	U/kg	0	0	0	0
• Treatment period - 45th to 58th day of pregnancy IPA Mash Phytase	U/kg	0	500	1000	2000

4.3 Diets

Prior to the experimental period a basal low phosphorus diet without IPA Mash Phytase was offered from 31st to 44th day of pregnancy. After the pre-treatment period sows received the experimental diets, which were based on the diet fed during the foregoing depletion period, until the end of the experiment. The ingredients and calculated chemical composition of the basal diet are presented in Table 2. Major ingredients were maize, optigrain (heat treated grains: 50% wheat, 25% barley, 25% maize) and soybean meal. The basal ingredients for the diets were provided by the [redacted] (b) (4)). The quantities of all the basal ingredients and the IPA Mash Phytase required for the production of the diets were documented. Except for calcium and phosphorus the diets were calculated in accordance to the recommendations for sows published by GfE (2006). The phosphorus content of the basal diet was estimated to be 3.40 g per kg of feed which was equivalent to

0.88 g available phosphorus per kg of feed. The basal phosphorus deficient diet was manufactured in one batch of 2000 kg and than subdivided for the pre- and treatment period in amounts of 1000 kg, respectively.

Table 2: Composition of the diets and calculated nutrient concentration

		Pre-treatment	Treatment			
Treatment		**A/B/C/D**	**A**	**B**	**C**	**D**
Composition:	*%*					
Maize		34.54	34.04	34.04	34.04	34.04
Optigrain		23.00	23.00	23.00	23.00	23.00
Soyabean meal		12.50	12.50	12.50	12.50	12.50
Wheat		12.00	12.00	12.00	12.00	12.00
Triticale		10.00	10.00	10.00	10.00	10.00
Cellulose		4.50	4.50	4.50	4.50	4.50
Limestone		1.66	1.66	1.66	1.66	1.66
Premix*		1.20	1.20	1.20	1.20	1.20
Soya oil		0.50	0.50	0.50	0.50	0.50
Chromium oxide		0	0.50	0.50	0.50	0.50
Sodium chloride		0.10	0.10	0.10	0.10	0.10
Calculated:						
ME_{BFS}	*MJ/kg*	12.93	12.86	12.86	12.86	12.86
Crude protein	g/kg	144.70	144.42	144.42	144.42	144.42
Lysine	g/kg	6.40	6.40	6.40	6.40	6.40
Methionine	g/kg	2.00	2.00	2.00	2.00	2.00
Cystine	g/kg	2.20	2.20	2.20	2.20	2.20
Threonine	g/kg	5.20	5.20	5.20	5.20	5.20
Tryptophan	g/kg	1.50	1.50	1.50	1.50	1.50
Crude fiber	g/kg	69.10	68.90	68.90	68.90	68.90
Crude fat	g/kg	30.30	30.00	30.00	30.00	30.00
Starch	g/kg	471.90	468.80	468.80	468.80	468.80
Sugars	g/kg	31.10	31.00	31.00	31.00	31.00
Calcium	g/kg	7.00	7.00	7.00	7.00	7.00
Phosphorus	g/kg	3.40	3.40	3.40	3.40	3.40
Sodium	g/kg	2.00	2.00	2.00	2.00	2.00

**Contents per kg Premix: 400000 IE Vit. A; 40000 IE Vit. D_3; 4200 mg Vit. E; 200 mg Vit. K_3; 200 mg Vit. B_1; 250 mg Vit. B_2; 3500 mg niacine; 400 mg Vit. B_6; 3000 μg Vit. B_{12}; 20000 μg biotin; 1500 mg pantothenic acid ; 150 mg folic acid; 80000 mg choline chloride; 5000 mg Zn; 2000 mg Fe; 5000 mg Mn; 1200 mg Cu; 40 mg Co; 35 mg Se; 50 mg J ; 130 g Na; 50 g Mg.*

The basal diet for the experimental period (overall 1000 kg) was subsequently divided into equal parts (250 kg each) without or with IPA Mash Phytase at levels of 500, 1000 and 2000 U/kg feed, respectively. Furthermore Cr_2O_3 was added at 0.5% in all diets fed during the 14-day treatment period. Diets were provided in mash form and produced at the licensed compound feed mill owned by the Institute (administrative allowance number: αDE-BE-100001; administrative registration number: DE-BE-100001). Each batch of the diets was bagged separately in 20 kg bags, which were identified with a label showing the study code number (SL 3/09) and the treatment letters (A, B, C, D). Complete records of diet mixing and test article inventory were maintained. All diets were prepared without addition of other feed additive with the exception of amino acids, vitamins and trace elements.

According to the recommendations and to local feeding schedule multiparous sows in the 3rd parity with an average body weight of 195 kg and a calculated body weight loss during the forgoing lactation period of 5 kg were offered 2.3 kg feed per sow and day, which corresponded to 30 MJ ME per sow and day. The daily estimated intake of available phosphorus was about 2 g, which was 16.7% lower than the recommended available phosphorus intake of 2.4 g per sow and day (GfE 2006).

4.4 Measured parameters

4.4.1 Body weight

All sows were weighed individually in two-weekly intervals at the start of the 14-day pre-treatment and 14-day treatment period, respectively, as well as at the end of the experiment.

4.4.2 Feed intake

During the 14-day pre-treatment and 14-day treatment period daily feed intake and possible feed residues were recorded daily.

4.4.3 Apparent digestibility

Digestibility coefficients of crude ash and the minerals calcium and phosphorus were calculated using Cr_2O_3 at 5g/kg as an inert indigestible indicator. Experimental diets were offered from the 45th to 58th day of pregnancy. The faecal samples were obtained by rectal stimulation of the sows, which was done early in the morning just before feeding and in the afternoon around 15 h at 24-h intervals during the 54th and 58th day of pregnancy. All samples were stored at minus 20°C for subsequent lyophilization. Before blending respective samples collected for each sow were pooled. Calculations for the apparent digestibility of crude ash, calcium and phosphorus were based on the following formula:

$$\text{App. digestibility (\%)} = 100 - \left(\frac{\text{\% indicator in diet}}{\text{\% indicator in faeces}} \times \frac{\text{\% crude ash, Ca, P in faeces}}{\text{\% crude ash, Ca, P in diet}} \right) \times 100$$

Figure 3: Formula for calculating the apparent digestibility for crude ash, calcium and phosphorus

4.5 Health status

Health status of the sows were monitored twice daily throughout the experiment. Additionally the appearance of faeces was ranked daily according to the following categories:

1 = normal (dry matter > 25%)
2 = pasty (dry matter 24 - 18%)
3 = watery (dry matter < 18%)
4 = watery with colour changes (dry matter < 18%)

4.6 Analytical methods

Diets were analysed by the Weender technique, including starch, total sugars, calcium, phosphorus and sodium determination, in accordance to the official VDLUFA methodology (dry matter: VDLUFA 3.1; crude protein: VDLUFA 4.1.2 modified according to macro-N determination (vario Max CN); crude fibre: VDLUFA 6.1.1; crude ash: VDLUFA 8.1; crude fat: VDLUFA 5.1.1; starch: VDLUFA 7.2.5; total sugars: VDLUFA 7.1.1; calcium: VDLUFA 10.3.1 modified according to DIN EN ISO 11885; phosphorus: VDLUFA 10.6.1 modified according to DIN EN ISO 11885; sodium: VDLUFA modified according to DIN EN ISO 11885) by [redacted] (b) (4)). Faeces were analysed for dry matter, crude ash, calcium and phosphorus (dry matter: weighing before and after lyopholization as well as following dry matter examination according to VDLUFA 3.1; crude ash: VDLUFA 8.1; calcium: VDLUFA 10.3.1 modified according to DIN EN ISO 11885; phosphorus: VDLUFA 10.6.1 modified according to DIN EN ISO 11885). Chromium oxide in feed and faecal samples was measured by using the method described by Brisson (1956). Parallel feed samples were analysed for in-feed phytase activity by DSM Biopract before starting and after concluding the trial.

4.7 Statistical analysis

Results are presented as means ± standard deviation. The statistical analyses was performed with the software package SPSS (SPSS, Inc. Chicago, IL). After checking the homogeneity of the variance the means were compared by the usual test procedures (Sheffe test, Tukey test). The significance level was set at $p < 0.05$.

5 Results

The trial was conducted without any technically disturbance. Additionally all sows were healthy and showed no obvious signs of disease. The analyses of the pre- and treatment diets confirmed the calculated values as shown in Table 3.

Table 3: The results of chemical analyses of the diets

		Pre-treatment	**Treatment**			
Experimental group		A/B/C/D	A	B	C	D
Sows	n	24	6	6	6	6
Replicates	n	24	6	6	6	6
IPA Mash Phytase	U/kg	0	0	500	1000	2000
Crude protein	g/kg	142.1	138.5	140.1	141.6	140.3
Crude fibre	g/kg	81.1	80.6.	82.2	79.7	81.2
Crude fat	g/kg	32.6	31.9	31.5	32.4	32.6
Crude ash	g/kg	45.0	45.0	44.6	44.5	50.0
Starch	g/kg	453.0	461.5	467.8	465.0	462.7
Sugars	g/kg	32.0	32.7	32.4	31.9	31.5
Calcium	g/kg	7.4	7.4	7.2	7.4	7.2
Total Phosphorus	g/kg	3.5	3.5	3.6	3.5	3.4
Sodium	g/kg	1.94	1.94	1.94	1.94	1.94
Chromium oxide	g/kg	5.2	5.2	4.8	5.2	5.2

The in-feed phytase activities presented in Table 4 were only slightly higher (diet B and C) than intended, but when considering the native phytase activity of 211 U/kg still within the intended range.

Table 4. Results of in-feed phytase analytics of the diets

		Pre-treatment	**Treatment**			
Experimental group		A/B/C/D	A	B	C	D
IPA Mash Phytase	U/kg					
• intended		0	0	500	1000	2000
• analysed		211 (native)	211 (native)	786	1262	2440

5.1 Weight gain and feed intake

Performance was monitored during the 14-day pre-treatment as well as the 14day- treatment period. The results are summarized in Table 5.

5.1.1 Body weight

During the 14-day pre-treatment period all sows received the basal diet containing 3.5 g total phosphorus per kg diet without phytase supplementation. The overall daily weight gain was 342 g per sow. During the following 14-day treatment period the body weight gain of sows fed the phosphorus deficient diet without supplemental phytase was 375 g per day. With supplementation of the diet deficient in phosphorus but with addition of IPA Mash Phytase at levels of 500, 1000 and 2000 units per kg of feed daily body weight gain increased slightly by 1.9, 9.6 and 11.5% when compared to sows fed without supplemental phytase.

5.1.2 Feed intake

The average daily feed intake during the pre-treatment period was 2.26 kg per sow and day. During the treatment period respective means for daily feed intake of sows fed without or with IPA Mash Phytase were recorded.

5.2 Faecal consistency

The scores for daily faecal consistency (Table 5) were within a range that did not indicate any adverse health effects at any time. The overall average was slightly above 1 thus reflecting only few changes in the physiological faecal consistency (dry matter > 25%). Diarrhoea with liquid faeces was not observed. There was a trend for slightly higher faecal consistency when adding IPA Mash Phytase; however, all means were still above the borderline of abnormal faecal consistency.

Table 5. Indices of performance and faecal consistency of gestating sows during the 14-day pre-treatment and 14-day treatment period for days 31 to 58 of pregnancy

Treatment		A	B	C	D	Oneway Anova
Sows	n	6	6	6	6	
Replicates	n	6	6	6	6	
• **Pre-treatment period**						
Sows	n	6	6	6	6	
Replicates	n	6	6	6	6	
- 31st to 44th day of pregnancy						
IPA Mash Phytase	U/kg	0	0	0	0	
→ Body weight	kg					
- start		192.3 ± 4.9	187.3 ± 6.6	190.2 ± 3.1	190.8 ± 6.4	0.462
- end		197.2 ± 4.1	192.0 ± 6.4	195.0 ± 3.5	195.7 ± 6.2	0.393
→ Body weight gain	kg	4.9 ± 1.0	4.7 ± 0.8	4.8 ± 0.8	4.9 ± 0.7	0.980
→ Feed intake	kg	31.83 ± 0.41	31.67 ± 0.82	31.50 ± 1.22	31.67 ± 0.82	0.930
→ Faecal score		1.25 ± 0.05	1.28 ± 0.04	1.30 ± 0.07	1.25 ± 0.05	0.311
• **Treatment period**						
Sows	n	6	6	6	6	
Replicates	n	6	6	6	6	
- 45th to 58th day of pregnancy						
IPA Mash Phytase	U/kg	0	500	1000	2000	
→ Body weight	kg					
- start		197.2 ± 4.1	192.0 ± 6.4	195.0 ± 3.5	195.7 ± 6.2	0.393
- end		202.4 ± 4.2	197.3 ± 6.1	200.7 ± 3.6	201.5 ± 6.3	0.371
→ Body weight gain	kg	5.2 ± 0.4	5.3 ± 0.5	5.7 ± 1.0	5.8 ± 0.5	0.373
→ Feed intake	kg	31.58 ± 0.49	31.42 ± 0.49	31.83 ± 0.41	31.90 ± 0.30	0.389
→ Faecal score		1.22 ± 0.05	1.28 ± 0.04	1.30 ± 0.07	1.25 ± 0.05	0.193

5.3 Apparent faecal digestibility

The results obtained during the 5-day sampling period are presented in Table 6.

5.3.1 Dry matter concentration

The overall dry matter of the faeces sampled during the 5-day period was on average 34.2% and was reflecting the fact that no abnormalities concerning the faecal consistency were observed during the 14-day treatment period. Sows fed with IPA Mash Phytase at the levels of 500 to 2000 units per kg of feed showed slightly reduced means with increasing levels of IPA Mash Phytase in the range of 1.3 to 4.6% when compared to sows fed without supplemental phytase.

5.3.2 Crude ash

The apparent crude ash digestibility of sows fed without phytase supplementation was 29.4%. With supplementation of IPA Mash Phytase at the levels of 500, 1000 and 2000 units per kg of feed the apparent digestibility increased significantly. The highest response was recorded for the addition of 2000 units per kg of feed.

5.3.3 Calcium

The apparent calcium digestibility of sows fed with IPA Mash Phytase was significantly improved with increasing levels when compared to sows fed without supplemental phytase by 17.5 (500 U/kg), 27.8 (1000 U/kg) and 34.6% (2000 U/kg), respectively. The highest response was shown for sows fed with 2000 units per kg of feed.

5.3.4 Phosphorus

The apparent digestibility of phosphorus in sows fed without supplemental phytase reached 26.5%. In sows fed with IPA Mash Phytase the respective means were significantly improved with inclusion of 1000 and 2000 units per kg of feed by 45.6 and 50.4%, respectively. The addition of IPA Mash Phytase at 500 units per kg of feed was less effective and due to the high standard deviations the difference compared to sows fed without supplemental phytase was not significant at this concentration.

Table 6. Indices of faecal dry matter and apparent digestibility of crude ash, calcium and phosphorus of gestating sows during the 5-day sampling period from days 54 to 58 of pregnancy

Treatment		A	B	C	D	Oneway Anova
Sows	n	6	6	6	6	
Replicates	n	6	6	6	6	
IPA Mash Phytase	U/kg		500	1000	2000	
• Dry matter	%	34.86 ± 0.72	34.42 ± 0.74	34.15 ± 0.99	33.25 ± 0.75	0.017
relative		100	98.7	98.0	95.4	
• Faecal concentrations						
→ Crude ash	g/kg DM	229.3 ± 16.1	218.0 ± 38.6	205.1 ± 23.0	206.3 ± 10.8	0.305
relative	%	100	95.1	89.4	90.0	
→ Calcium	g/kg DM	37.1 ± 2.0[a]	34.3 ± 6.9[ab]	32.4 ± 3.5[ab]	28.8 ± 1.8[b]	0.016
relative	%	100	92.5	87.3	77.6	
→ Phosphorus	g/kg DM	18.6 ± 0.7[a]	17.8 ± 3.5[a]	15.4 ± 2.0[ab]	13.9 ± 1.9[b]	0.007
relative	%	100	95.7	82.8	74.7	
• Apparent digestibility						
→ Crude ash	%	29.44 ± 3.32[a]	33.76 ± 2.18[b]	35.77 ± 2.96[bc]	39.29 ± 1.58[c]	< 0.001
relative	%	100	114.7	121.5	133.5	
→ Calcium	%	30.57 ± 3.18[a]	35.93 ± 0.95[b]	39.08 ± 2.21[bc]	41.14 ± 4.23[c]	< 0.001
relative	%	100	117.5	127.8	134.6	
→ Phosphorus	%	26.51 ± 2.93[a]	33.52 ± 2.97[ab]	38.59 ± 6.04[b]	39.87 ± 5.95[b]	< 0.001
relative	%	100	126.4	145.6	150.4	

[ab] Means with different superscripts within the same line differed significantly

6 Discussion and Conclusions

The analysed total phosphorus content in the phosphorus deficient basal diet was 3.5 g. The monitored feed intake during the pre-treatment and treatment period was 2.26 kg per sow and day which was corresponding to a total phosphorus intake of 7.91 g per day. Therefore the total daily available phosphorus supply (calculated with the digestibility of phosphorus measured for the control group) amounted to 2.10 g available phosphorus intake per sow. This analysed amount was slightly higher than the calculated value. Compared to the official recommendations of 2.4 g available phosphorus per sow and day the supply of sows fed with the phosphorus deficient diet was still 12.5 % lower. From this point of view conditions for testing the efficacy of IPA Mash Phytase were sufficient.

The results of the trial show the potential of IPA Mash Phytase to improve the apparent digestibility of crude ash, calcium and phosphorus in sows. In Figures 4 to 6 the effects of dietary supplementation with IPA Mash Phytase were presented as dose response curves together with the respective regression equations and correlation coefficients.

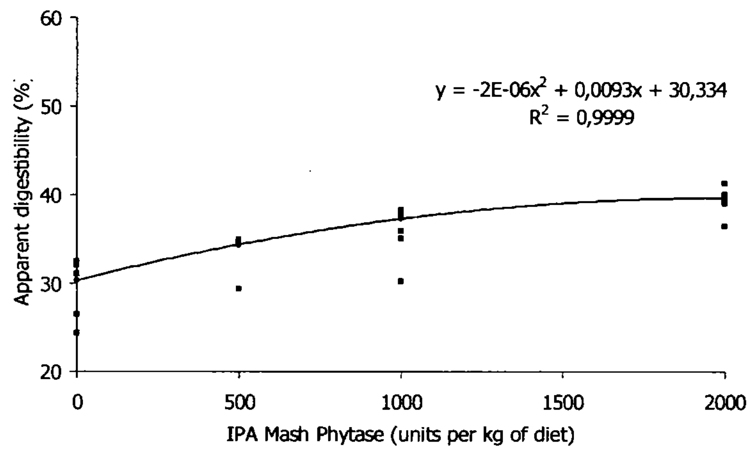

Figure 4. Effect of IPA Mash Phytase supplementation on apparent crude ash digestibility of multiparous sows from days 54 to 58 of pregnancy

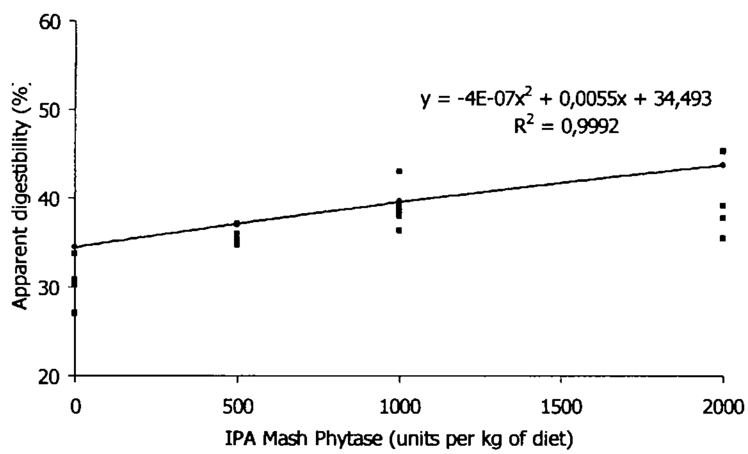

Figure 5. Effect of IPA Mash Phytase supplementation on apparent calcium digestibility of multiparous sows from days 54 to 58 of pregnancy

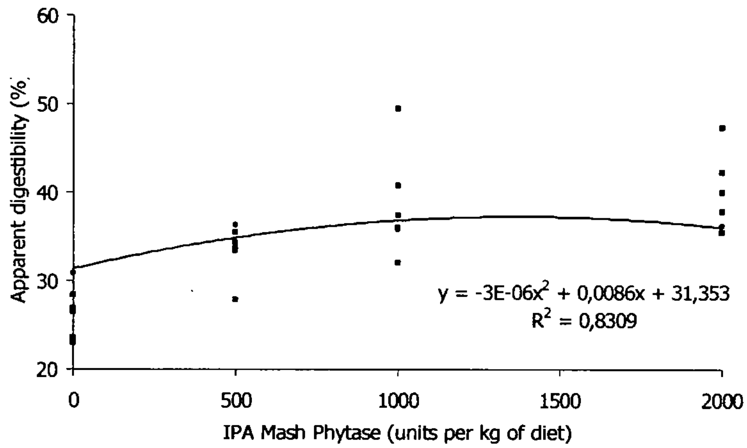

Figure 6. Effect of IPA Mash Phytase supplementation on apparent phosphorus digestibility of multiparous sows from days 54 to 58 of pregnancy

The response curves show that the most pronounced effects for apparent digestibility of crude ash, calcium and phosphorus could be demonstrated in the dose range from 1000 to 2000 U/kg feed. Based on the fact that the response between the dose levels of 1000 and 2000 units per kg feed was not so pronounced as that between 500 and 1000 units per kg feed the conclusion can be made that with regard to the tested dose levels a sufficient dose response was achieved with 1000 units per kg feed.

The daily calcium intake during the experimental period reached 16.5 g per sow. Consequently the intake exceeded the recommended supply according to "GfE" (2006) by 10.1 g per sow and day. Even so the positive effect on apparent digestibility of calcium in sows fed with IPA Mash Phytase was still evident at all phytase levels. Obviously the magnitude of the enzyme response was independent of the amount of calcium concentration in the chyme.

The economic impact of replacing inorganic phosphorus by supplementation with IPA Mash Phytase was evaluated by calculating the replacement of inorganic phosphorus as monocalcium phosphate (MCP) equivalents (MCP: 220 g total phosphorus per kg, 90% digestibility) by using the apparent phosphorus digestibility measurements recorded for sows fed without or with IPA Mash Phytase supplementation (Table 6). Based on these results it was estimated that 500, 1000 and 2000 units of IPA Mash Phytase per kg of feed were equivalent to 1.21, 2.12 and 2.37 g of monocalcium phosphate, respectively.

It can be concluded that IPA Mash Phytase is effective in improving the apparent digestibility for crude ash, calcium and phosphorus when using diets deficient in phosphorus supply. The regression equations did not allow any overall calculation of the optimal Phytase activity in the diet for sows during days 54 to 58 of pregnancy. However, with regard to the smaller differences in the efficiency between 1000 and 2000 units per kg feed than those between 500 and 1000 unite per kg of feed, the dose level of IPA Mash Phytase at 1000 units per kg of feed was obviously sufficient for improving significantly the apparent digestibility of crude ash, calcium and phosphorus in pregnant sows.

[redacted] (b) (4) 2009-10-08

[redacted] (b) (4)

7 Individual data determined for sows

Treatment	BW start	BW start digest	BW end	BW-diff.	BWG/d
A	184.00	190.00	195.50	5.50	0.39
	194.00	198.00	203.00	5.00	0.36
	191.00	196.00	201.00	5.00	0.36
	196.00	200.00	205.00	5.00	0.36
	198.00	202.00	208.00	6.00	0.43
	191.00	197.00	202.00	5.00	0.36
	192.33	**197.17**	**202.42**	**5.25**	**0.38**
	4.93	**4.12**	**4.20**	**0.42**	**0.03**
B	178.00	184.00	189.00	5.00	0.36
	181.00	185.00	191.00	6.00	0.43
	190.00	195.00	200.00	5.00	0.36
	188.00	192.00	198.00	6.00	0.43
	195.00	200.00	205.00	5.00	0.36
	192.00	196.00	200.90	4.90	0.35
	187.33	**192.00**	**197.32**	**5.32**	**0.38**
	6.56	**6.36**	**6.14**	**0.53**	**0.04**
C	194.30	200.50	205.00	4.50	0.32
	193.00	197.50	204.00	6.50	0.46
	190.00	194.00	199.00	5.00	0.36
	190.00	195.00	202.00	7.00	0.50
	186.00	191.00	196.00	5.00	0.36
	188.00	192.00	198.00	6.00	0.43
	190.22	**195.00**	**200.67**	**5.67**	**0.40**
	3.07	**3.54**	**3.56**	**0.98**	**0.07**
D	187.00	191.50	198.00	6.50	0.46
	186.00	191.80	197.00	5.20	0.37
	195.00	199.00	205.00	6.00	0.43
	192.00	196.50	202.00	5.50	0.39
	201.00	206.00	212.00	6.00	0.43
	184.00	189.30	195.00	5.70	0.41
	190.83	**195.68**	**201.50**	**5.82**	**0.42**
	6.43	**6.19**	**6.28**	**0.45**	**0.03**

Treatment	Feed-basis	Feed-dig.	Feed/d	Faecal score	Faecal score-dig.	Faeces DM
A	32.00	32.00	2.29	1.30	1.25	34.58
	32.00	32.00	2.29	1.25	1.20	33.90
	32.00	32.00	2.29	1.20	1.30	35.10
	31.00	32.00	2.29	1.20	1.20	34.30
	32.00	31.50	2.25	1.30	1.15	35.70
	32.00	30.00	2.14	1.22	1.20	35.60
	31.83	**31.58**	**2.26**	**1.25**	**1.22**	**34.86**
	0.41	**0.80**	**0.06**	**0.05**	**0.05**	**0.72**
B	32.00	31.00	2.21	1.32	1.30	33.50
	32.00	31.50	2.25	1.28	1.28	34.14
	32.00	31.00	2.21	1.30	1.30	35.60
	30.00	32.00	2.29	1.20	1.25	34.80
	32.00	31.00	2.21	1.25	1.15	33.90
	32.00	32.00	2.29	1.30	1.25	34.60
	31.67	**31.42**	**2.24**	**1.28**	**1.26**	**34.42**
	0.82	**0.49**	**0.04**	**0.04**	**0.06**	**0.74**
C	32.00	32.00	2.29	1.30	1.30	33.70
	32.00	32.00	2.29	1.25	1.25	34.70
	32.00	32.00	2.29	1.40	1.30	35.77
	29.00	32.00	2.29	1.30	1.20	34.30
	32.00	32.00	2.29	1.35	1.30	33.25
	32.00	31.00	2.21	1.20	1.30	33.20
	31.50	**31.83**	**2.27**	**1.30**	**1.28**	**34.15**
	1.22	**0.41**	**0.03**	**0.07**	**0.04**	**0.99**
D	32.00	32.00	2.29	1.30	1.25	33.20
	30.00	32.00	2.29	1.25	1.30	31.80
	32.00	32.10	2.29	1.20	1.20	33.40
	32.00	32.00	2.29	1.20	1.30	33.69
	32.00	32.00	2.29	1.25	1.30	33.50
	32.00	31.30	2.24	1.32	1.25	33.90
	31.67	**31.90**	**2.28**	**1.25**	**1.27**	**33.25**
	0.82	**0.30**	**0.02**	**0.05**	**0.04**	**0.75**

Treatment	Feaces	ash %	P%	Ca%	Cr%	Feed	ash %	P%	Ca %	Cr %
A		22.65	1.75	3.50	1.85		4.50	0.35	0.74	0.26
		22.45	1.90	3.98	1.89		4.50	0.35	0.74	0.26
		20.42	1.80	3.61	1.71		4.50	0.35	0.74	0.26
		23.86	1.93	3.94	1.97		4.50	0.35	0.74	0.26
		25.28	1.90	3.64	1.90		4.50	0.35	0.74	0.26
		22.90	1.85	3.57	1.77		4.50	0.35	0.74	0.26
		22.93	**1.86**	**3.71**	**1.85**		**4.50**	**0.35**	**0.74**	**0.26**
		1.61	**0.07**	**0.20**	**0.09**		**0.00**	**0.00**	**0.00**	**0.00**
B		25.65	2.01	3.98	2.14		4.46	0.36	0.72	0.24
		23.07	1.89	3.68	1.93		4.46	0.36	0.72	0.24
		22.40	1.79	3.55	1.88		4.46	0.36	0.72	0.24
		14.32	1.09	2.06	1.11		4.46	0.36	0.72	0.24
		23.01	1.87	3.68	1.93		4.46	0.36	0.72	0.24
		22.34	2.00	3.62	1.88		4.46	0.36	0.72	0.24
		21.80	**1.78**	**3.43**	**1.81**		**4.46**	**0.36**	**0.72**	**0.24**
		3.86	**0.35**	**0.69**	**0.36**		**0.00**	**0.00**	**0.00**	**0.00**
C		17.97	1.45	2.88	1.69		4.45	0.35	0.74	0.26
		23.65	1.93	3.64	2.26		4.45	0.35	0.74	0.26
		22.40	1.43	3.65	2.12		4.45	0.35	0.74	0.26
		20.48	1.57	3.11	1.73		4.45	0.35	0.74	0.26
		20.61	1.48	3.25	1.87		4.45	0.35	0.74	0.26
		17.94	1.38	2.89	1.65		4.45	0.35	0.74	0.26
		20.51	**1.54**	**3.24**	**1.89**		**4.45**	**0.35**	**0.74**	**0.26**
		2.30	**0.20**	**0.35**	**0.25**		**0.00**	**0.00**	**0.00**	**0.00**
D		21.13	1.28	2.84	1.80		5.00	0.34	0.72	0.26
		20.66	1.51	3.02	1.77		5.00	0.34	0.72	0.26
		20.11	1.35	2.61	1.70		5.00	0.34	0.72	0.26
		21.95	1.72	2.94	1.92		5.00	0.34	0.72	0.26
		21.11	1.24	3.11	1.78		5.00	0.34	0.72	0.26
		18.79	1.25	2.75	1.52		5.00	0.34	0.72	0.26
		20.63	**1.39**	**2.88**	**1.75**		**5.00**	**0.34**	**0.72**	**0.26**
		1.08	**0.19**	**0.18**	**0.13**		**0.00**	**0.00**	**0.00**	**0.00**

Treatment	Digest. P	Digest. Ca	Digest. ash
A	30.81	34.55	30.35
	26.47	27.15	32.43
	23.01	26.97	32.07
	28.34	30.81	31.10
	26.86	33.72	24.31
	23.55	30.22	26.40
	26.51	**30.57**	**29.44**
	2.93	**3.18**	**3.32**
B	36.34	36.97	34.35
	33.63	35.38	34.53
	35.47	36.01	34.74
	33.44	37.11	29.34
	34.33	35.38	34.70
	27.90	34.75	34.92
	33.52	**35.93**	**33.76**
	2.97	**0.95**	**2.18**
C	35.77	39.66	37.34
	36.07	42.98	38.33
	49.51	39.04	37.73
	32.07	36.35	30.25
	40.75	38.47	35.06
	37.39	37.99	35.93
	38.59	**39.08**	**35.77**
	6.04	**2.21**	**2.96**
D	46.25	43.76	39.69
	35.52	39.18	40.03
	39.97	45.27	39.22
	32.29	45.42	41.26
	47.34	37.72	39.07
	37.84	35.51	36.49
	39.87	**41.14**	**39.29**
	5.95	**4.23**	**1.58**

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA Mash Phytase** | Batch number: **PPQ 28684**

Trial ID: **SL 3/09** | Location: [redacted] (b) (4)

Start date and exact duration of the study: **2009-07-09 to 2009-08-12**

Number of treatment groups (+ control(s)): **4l** | Replicates per group: **6**

Total number of animals: **24** | Animals per replicate: **1**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **0, 500, 1000, 2000 U/kg** | Analysed: **211 (native), 786, 1262, 2440 U/kg**

†

Substances used for comparative purposes:

Intended dose: | Analysed:

Animal species/category: **Gestating sows**

Breed: **Hybrid line Euroc** | Identification procedure:

Sex: **Female** | Age at start: **about 555 days** | Body weight at start: **190 kg**

Physiological stage: **29th to 33rd d of pregnancy** | General health: **normal**

Additional information for field trials:

Location and size of herd or flock: **Swine breeding farm with 500 multiparous sows**

Feeding and rearing conditions: **Individual feeding with a phosporous reduced diet**

Method of feeding: **2. 3 kg per sow and day**

Diets (type(s)): **Complete feed for gestating sows**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize, optigrain, soyabean meal, wheat, triticale, premix**

Nutrient content (relevant nutrients and energy content)

Intended values: **12.93 MJ ME/kg 144 g crude protein/kg, 3.4 g phosphorous /kg**

Analysed values: **140.5 g crude protein/kg; 3.5 g phosphorous/kg**

Date and nature of the examinations performed: **Digestibility trial with chromium oxide**

Method(s) of statistical evaluation used: **Oneway Anova**

Therapeutic/preventive treatments (reason, timing, kind, duration): **no**

Timing and prevalence of any undesirable consequences of treatment: **no**

Date **2009-12-15** | Signature Study Director [redacted] (b) (4)

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

11

Annex 11

Evaluation of the effect of IPA Mash phytase on the nutrient digestibility in gestating sows

REPORT No. 00003286

REPORT No. 00003286
Regulatory Document

DSM

Document Date: 16 December, 2009

Author(s): [redacted] (b) (4)[1] and J. Broz[2]

[1] [redacted] (b) (4)
[2] Animal Nutrition and Health R&D, DSM Nutritional Products Ltd, Basel

Title: **Evaluation of the effect of IPA Mash phytase on the nutrient digestibility in gestating sows**

Project No. 6106

Summary

An experiment involving 24 gestating sows (Large White x Landrace) was conducted in order to evaluate the effects of IPA Mash phytase on the apparent total tract digestibility of organic matter, nitrogen, phosphorus and calcium. The animals were fed a gestation diet containing maize, barley, soybean meal and rapeseed meal as the main ingredients, which was formulated to contain 17.2% crude protein and 0.6% total P. This basal diet was supplemented with IPA Mash phytase at the inclusion levels of 0 (negative control), 500, 1000 and 2000 U/kg, respectively. Each dietary treatment was allocated to 6 animals. After 10 days of a preliminary period (day 98 to 108 of pregnancy), there was a 5-day faeces collection period (day 108 to 113 of pregnancy). Digestibility values were estimated using chromium oxide as a digestibility marker. Dietary supplementation with IPA Mash phytase significantly improved total tract digestibility of P and Ca ($P<0.05$) at all inclusion levels. Phytase inclusion levels at 500, 1000 and 2000 U/kg increased the digestibility of P from 26.7% (control) to 33.6, 39.0 and 37.2 %, respectively. A significantly improved ($P<0.05$) digestibility of nitrogen and organic matter was observed in the diets supplemented with phytase at 1000 and 2000 U/kg. As a result of improved digestibility the concentration of P and Ca in the faeces of sows receiving phytase supplemented diets was significantly reduced.

This report consists of Pages I – II and 1 – 20 & Annex C

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Principal Scientist / Competence Mgr Dr. J. Broz, NRD/CA	signed by J. Broz	16.12.2009
Research Center Head Dr. A.-M. Klünter, NRD/CA	signed by A.-M. Klünter	17.12.2009
Project Manager Dr. F. Fru, NRD/PA	signed by F. Fru	17.12.2009

Nomenclature

IPA phytase (M), enzyme product containing bacterial 6-phytase (b) (4)), produced by (b) (4) fermentation of a genetically modified *Aspergillus oryzae* strain. Lot PPQ 28656 was used in this study, manufactured by Novozymes A/S, (b) (4).

[redacted] (b) (4)

Evaluation of the effect of IPA Mash phytase on the nutrient digestibility in gestating sows

Final report

Principal investigator: [redacted] (b) (4)
Investigators:

November 200

Summary

A digestibility experiment using 24 gestating sows (initial BW 239.8 kg) was carried out to study the effect of a novel microbial phytase (IPA Mash phytase) on total tract digestibility of organic matter (OM), nitrogen (N), phosphorus (P) and calcium (Ca). The enzyme was added to the diets for gestating sows at four inclusion levels (0, 500, 1000 and 2000 U/kg). The sows were in the range from the 3rd to the 5th parity and in the last third of pregnancy, between day 98 and 113 of pregnancy. There were four dietary treatments in the experiment and each dietary treatment was allocated to 6 animals. On the 95th day of pregnancy the sows were housed in individual pens and during following 3 days were fed with a commercial diet for gestating sows. After 10 days of preliminary period (from day 98 to 108 of pregnancy) in which were the animals fed with the experimental diets, there was a 5-day collection period (from day 108 to 113 of pregnancy) during which the faeces were collected. Digestibility values were estimated using Cr_2O_3 as a digestibility marker.

Dietary supplementation with IPA Mash phytase significantly improved total tract digestibility of P and Ca ($P<0.05$) at all inclusion levels. The strongest improvement was observed at the level of 1000 U/kg. Phytase supplementation resulted in a relative improvement of P digestibility by 25.8, 46.27 and 39.27 % in the diets supplemented with IPA Mash phytase at 500, 1000 and 2000 U/kg, respectively. The digestibility of Ca was higher in compare with the control diet by 17.6, 34.5 and 24.23 % in respectively experimental diets with different phytase level. A significantly improved ($P<0.05$) digestibility of N and OM was observed in the diets with phytase addition at 1000 and 2000 U/kg. As a result of improved digestibility the concentration of P and Ca in faeces of sows receiving phytase supplemented diets was significantly reduced when compared to the control diet ($P<0.05$).

1. Introduction

Microbial phytase is now an accepted feed additive that is used extensively in commercial diets for both pigs and poultry. It effectively improves the availability of plant phosphorus (P) as well as some other minerals and decreases P excretion, thus reducing environmental pollution. The efficiency of the novel IPA Mash phytase, an enzyme product containing bacterial 6-phytase expressed in a genetically modified strain of *Aspergillus oryzae,* as a feed additive in gestating sows is not yet known. Therefore the purpose of the present study was to evaluate the effect of this phytase preparation (IPA Mash phytase) on the availability of organic matter, nitrogen, P and Ca, when using a practical-type, P deficient diet for gestating sows during the last third of pregnancy.

2. Materials and methods

2.1. Animals and experimental design

A total of 24 sows in the mean body weight of 239.8 ± 3.8 kg were selected for this study in a commercial breeding farm. The sows were of the same genotype (Large White x Landrace), in the range from 3rd to 5th parity (Table 13, Appendix) and in the last third of pregnancy, between days 98 and 113 of pregnancy.
Four dietary experimental treatments (F0, F1, F2, F3) were involved in this study, to which the sows were allocated according to their body weight and parity. Six sows per dietary treatment were used. The first treatment (F0) was the negative control receiving a low-phosphorus basal diet without IPA Mash phytase. Treatments F1, F2, F3 were identical to the negative control but supplemented with IPA Mash phytase at the levels of 500, 1000 and 2000 U/kg feed, respectively.
On day 95 of pregnancy the sows were housed in 24 individual pens allowing individual feeding. Drinking water was continuously supplied by drinking bowls. From day 95 to 98 of pregnancy there was an adaptation period in which the sows were fed with a commercial diet for gestating sows. After 10 days of preliminary period (from day 98 to 108 of pregnancy) in which the animals received the respective experimental diets, followed a 5-day collection period (from day 108 to 113 of pregnancy) during which the faeces were collected daily. The experiment was finished after 15 days.
Health status of the sows was monitored every day of experiment.
The experiment was carried out in June 2009. All experimental procedures were reviewed and approved by the Ethical Committee of the [redacted] (b) (4).

2.2. Diets and feeding

The basal diet was formulated to contain maize, barley, soybean meal and rapeseed meal as the main ingredients. The basal, phosphorus deficient diet was mixed in one batch of 2000 kg. After that the basal diet was subsequently divided into equal parts (500 kg each), which were further mixed without or with IPA Mash phytase at the levels of 500, 1000 and 2000 U/kg feed, respectively. Each experimental diet was bagged separately in 40 kg bags which were identified with the label showing the respective treatment (F0, F1, F2, F3). All diets were prepared without addition of other feed additives with the exception of amino acids, vitamins and trace elements. The nutrient composition of diets was according to the requirements for gestating sows (NRC 1998). Bacterial 6-phytase expressed in a genetically modified strain of *Aspergillus oryzae* (IPA Mash phytase, Lot No. PPQ28656, DSM Nutritional Products Ltd, Switzerland) was added to the basal diet via premix at three levels equivalent to 500, 1000 and 2000 U/kg feed, thus forming three experimental diets (F1, F2, F3). Chromic oxide and cellite were included in the diets as indigestible markers. The ingredient composition and calculated contents of nutrients of the basal diet are given in Table 1.

Table 1: Composition of basal diet and calculated nutrient concentration (g/ kg air dry basis)

Maize	470.50
Barley	200.00
Soybean meal (46 % CP)	118.00
Rapeseed meal (33 % CP)	80.00
Premix[1]	29.50
Cellite	10.00
Chromium oxide	3.00
Wheat flour T 39000	10.00
Sunflower meal (27 % CP)	64.00
Sunflower oil	15.00
Calculated values:	
Metabolizable energy (MJ/kg)	12.7
Crude protein	171.7
Fibre	55.0
Lysine	9.6
Threonine	6.7
Methionine + Cystine	6.3
Tryptophan	1.9
Calcium	8.0
Phosphorus total	6.0
Phosphorus digestible	2.4
Sodium	2.0

[1]Content per kg premix: lysine 6 %; methionine 1 %; threonine 1 %; Ca 21 %; P 4.50 %; Na 6 %; Cu 650 mg; Fe 3500 mg; Zn 4500 mg; Mn 2500 mg; Co 40 mg; I 100 mg; Vit. A 370000 IU; Vit. D3 60000 IU; Vit. E (α-tocopherol) 2500 mg; Vit. K3 100 mg; Vit. B1 70 mg; Vit B2 150 mg; Vit. B6 100 mg; Vit. B12 1000 mcg; biotin 6 mg; niacin amid 900 mg; folic acid 40 mg; calcium pantothenate 600 mg; choline chloride 12500 mg; ethoxyquin (E321) 242 mg; citric acid (E330) 42 mg; silicic acid (E551a) 515 mg; propyl galate (E310) 33 mg; Pigortek 3350 mg.

The diets were fed twice daily at 6:00 and 16:00 hours, in two equal meals at a daily amount 3.6 kg/day. Water was offered *ad libitum*.

2.3. Experimental procedure

The experiment consisted of a 10-day preliminary period, followed by a 5-day collection period, during which faeces were collected.

The experimental schedule was as follows:

Day	-3 - 0	Adaptation period
Day	1 - 10	Preliminary period
Day	11 - 15	Collection of faeces

Samples of faeces were stored at 4°C, homogenized and consequently analysed. For each sow 5 daily samples of faeces were analysed. The sows were weighed at the beginning of experimental period.

2.4. Chemical analyses

Air-dried samples of faeces and samples of diets were finely ground to pass through a 1-mm screen prior to the chemical analyses.
Analyses of diets and faeces for DM, N, P, Ca, ash and acid insoluble ash (AIA) were performed in accordance with standard methods of AOAC (1990).
Chromium oxide was analysed by atomic absorption spectrometry as described by Williams et al. (1962).
The phytase activity in diets was analysed by BIOPRACT GmbH, Berlin, Germany, on behalf of DSM Nutritional Products Ltd.

2.5. Calculations

Coefficients of total tract digestibility of nutrients were calculated using the following formula:

$$\text{Digestibility (\%)} = 100 \times [1-(N_i \times M_d)/(N_d \times M_i)]$$

where N_d = dietary concentration of the nutrient under study, M_d = dietary concentration of marker, N_i = concentration of the nutrient in faeces and M_i = concentration of marker in faeces (all values in $g.kg^{-1}$ dry matter).

2.6. Statistical analysis

Data were subjected to ANOVA using Statgraphic Plus 3.1. package. When a significant value for treatment effect ($P<0.05$) was obtained, the differences between means were assessed using Fisher's LSD procedure. Regression analysis was used to evaluate the relationship between supplemental phytase level and nutrient digestibility.

3. Results and discussion

The experimental sows were in good health throughout the whole experiment and consumed the offered feed.

Analysed phytase activity in control and experimental diets was as follows: F0 – 124 (native activity), F1 – 531, F2 – 898 and F3 1890 U/kg. Considering the fact that the results obtained with chromium oxide as an indigestible marker were less variable than in case of acid insoluble ash, the digestibility coefficients were calculated only using chromium oxide. The primary data for individual sows from which the mean values were calculated are given in Appendix, Tables 9 – 12.

The mean values of total tract digestibility of DM, OM, N, Ca and P are given in Table 2. The analysis of variance showed that the graded levels of phytase supplementation had a significant effect on DM digestibility. The lowest digestibility was observed in diet F0. Digestibility of DM was improved relatively by 1.88 % and 1.30 % in diets F2 and F3, when compared to the negative control and those differences were significant. Similar results were obtained for digestibility of OM and significant relative improvements by 1.65 and 0.96 % were observed for diets supplemented with phytase at 1000 and 2000 U/kg, respectively.
The sows that received diets F2 and F3 had significantly higher digestibility of N in comparison with the sows that consumed diets F0 and F1. The digestibility in diets F2 and F3 was relatively higher by 4.16 % and 2.62 %, when compared to the control diet.
The digestibility of both P and Ca was significantly increased as a result of phytase supplementation. Again, the strongest effect was observed in diet F2. The total tract digestibility of P increased relatively by 25.81, 46.27 and 39.27 % in diets F1, F2 and F3, respectively, when compared to the control.
Furthermore, the digestibility of Ca was significantly increased in the diets supplemented with phytase at 500, 1000 and 2000 U/kg by 17.06, 34.52 and 24.23 %, respectively, when compared to the control diet.

Table 2: The effect of IPA Mash phytase on nutrient digestibility (%)

Phytase addition (U/kg diet)	DM	OM	N	Ca	P
0	83.1^{a}	86.7^{a}	82.9^{a}	35.5^{a}	26.7^{a}
500	83.7^{ab}	87.1^{ab}	83.5^{a}	41.6^{b}	33.6^{b}
1000	84.7^{c}	88.1^{c}	86.3^{b}	47.8^{c}	39.0^{c}
2000	84.2^{bc}	87.5^{bc}	85.1^{b}	44.1^{b}	37.2^{bc}
Pooled SEM	0.1	0.1	0.2	0.5	0.7

[abc] Means within a column followed by the different superscript are significantly different ($P<0.05$)

The effects of dietary supplementation with IPA Mash phytase are presented in Figures 1 and 2 as dose-response curves together with the respective regression equations and correlation coefficients. The curves show that the most pronounced effects on total tract digestibility of P and Ca were demonstrated in the dose range of IPA Mash Phytase from 1000 to 2000 U/kg feed.

A positive linear relationship between the digestibility of P (x) and Ca (y) was observed, which was described by the equation y = 0.87x + 12.562 (see Figure 3). Based on the correlation coefficient value, the relationship between the variables can be characterized as moderately strong in both cases.

Figure 1: Relationship between digestibility of P and phytase supplementation in sows

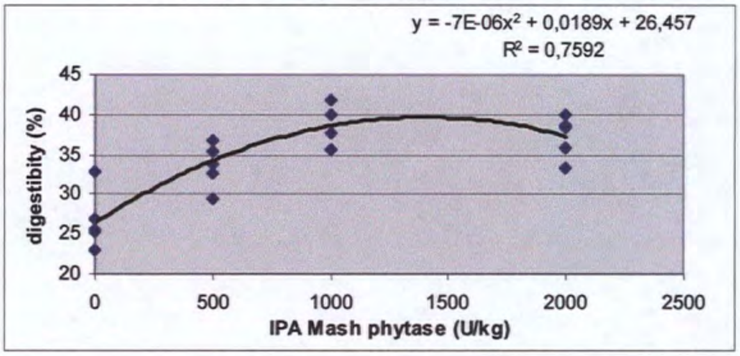


Figure 2: Relationship between digestibility of Ca and phytase supplementation in sows

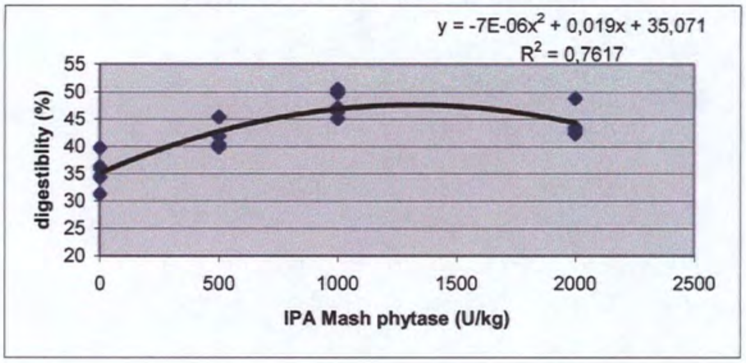
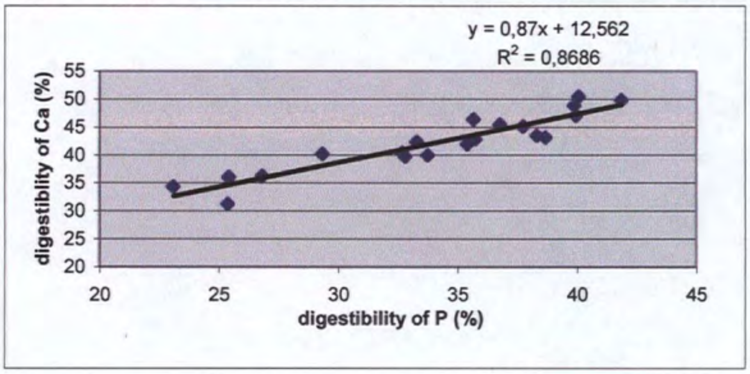


Figure 3: Relationship between digestibility of Ca and P



As a result of improved nutrient digestibility due to the phytase supplementation, concentration of nutrients in faeces was reduced (see Table 3). In comparison with the control diet, the reduction of P and Ca concentration ranged from 6.51 to 7.9% in case of P and from 7.0 to 8.6 % in case of Ca.

Table 3: The effect of phytase supplementation on the content of nutrients in faeces (g/kg DM)

Phytase addition (U/kg diet)	OM	N	Ca	P
0	723.3[a]	30.1[ab]	35.2[a]	27.9[a]
500	733.4[b]	31.4[b]	32.3[b]	26.1[b]
1000	726.3[ab]	28.9[a]	32.2[b]	26.1[b]
2000	725.9[ab]	29.4[ab]	32.7[b]	25.7[b]
Pooled SEM	1.4	0.4	0.3	0.2

[ab] Means within a column followed by the different superscript are significantly different ($P<0.05$)

Conclusions

As shown in Table 4, summarizing the effect of IPA Mash phytase supplementation in diets for gestating sows, the added phytase significantly increased total tract digestibility of P and Ca when compared to the negative control. The strongest increase of digestibility for both P and Ca was observed in the diet supplemented phytase at 1000 U/kg. Similarly the digestibility of N was significantly higher in diets with added phytase at 1000 and 2000 U/kg, respectively. The response of P and Ca digestibility was linear until the phytase level of 1000 U/kg.
It can be concluded that the IPA Mash phytase efficiently increased the total tract digestibility of phosphorus and calcium and reduced the concentration of P in faeces.

Table 4: Summary of effects of IPA Mash phytase on the experimental parameters (expressed as % change relative to NC)

Parameter	Phytase level (U/kg)		
	500	1000	2000
Total tract digestibility (%):			
DM	0.67	1.88*	1.30*
OM	0.45	1.65*	0.96*
N	0.68	4.16*	2.62*
Ca	17.06*	34.52*	24.23*
P	25.81*	46.27*	39.27*
Concentration of P in faeces (g/kg DM)	-6.51*	-6.56*	-7.99*

* Denotes significant effect ($P<0.05$)

5. References

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Appendix

Tables of primary data used for the calculation of total tract digestibilities

Table 5: Nutrient digestibility in diet F0 (%)

Period	DM	OM	N	Ca	P
I.	83.7^{a}	87.2^{a}	81.2^{a}	36.2^{ab}	26.8^{ab}
II.	83.5^{a}	86.8^{a}	82.4^{a}	39.7^{b}	32.8^{b}
III.	82.5^{a}	86.1^{a}	82.2^{a}	36.0^{ab}	25.4^{ab}
IV.	82.4^{a}	86.1^{a}	83.2^{ab}	31.3^{a}	25.4^{ab}
V.	83.5^{a}	87.0^{a}	85.5^{b}	34.4^{ab}	23.1^{a}
Pooled SEM	0.2	0.2	0.4	0.8	1.1

Table 6: Nutrient digestibility in diet F1 (%)

Period	DM	OM	N	Ca	P
I.	83.8^{a}	87.0^{a}	82.2^{a}	42.0^{a}	35.4^{a}
II.	84.1^{a}	87.3^{a}	82.8^{a}	45.5^{a}	36.8^{a}
III.	83.4^{a}	86.9^{a}	83.7^{a}	40.4^{a}	32.7^{a}
IV.	83.3^{a}	86.8^{a}	83.9^{a}	39.9^{a}	33.7^{a}
V.	83.9^{a}	87.3^{a}	84.6^{a}	40.2^{a}	29.3^{a}
Pooled SEM	0.2	0.2	0.4	1.4	1.5

Table 7: Nutrient digestibility in diet F2 (%)

Period	DM	OM	N	Ca	P
I.	84.3^{a}	87.4^{a}	84.0^{a}	46.4^{ab}	35.7^{a}
II.	85.1^{a}	88.1^{a}	85.5^{ab}	49.8^{b}	41.8^{a}
III.	85.7^{a}	88.7^{a}	86.7^{bc}	50.5^{b}	40.1^{a}
IV.	85.2^{a}	88.4^{a}	88.6^{c}	47.1^{ab}	39.9^{a}
V.	84.6^{a}	87.9^{a}	86.9^{bc}	45.1^{a}	37.7^{a}
Pooled SEM	0.3	0.3	0.4	0.7	1.5

Table 8: Nutrient digestibility in diet F3 (%)

Period	DM	OM	N	Ca	P
I.	85.3^{b}	88.4^{a}	85.0^{ab}	48.8^{a}	39.9^{b}
II.	83.8^{ab}	87.1^{a}	85.0^{ab}	42.8^{a}	35.7^{ab}
III.	84.2^{ab}	87.5^{a}	83.9^{a}	43.2^{a}	38.7^{ab}
IV.	84.2^{ab}	87.6^{a}	86.6^{b}	42.3^{a}	33.3^{a}
V.	83.6^{a}	86.8^{a}	85.0^{ab}	43.5^{a}	38.3^{ab}
Pooled SEM	0.2	0.2	0.4	1.0	0,9

Table 9: Individual data for calculation of nutrient digestibility in diet F0 (g/kg DM)

Diet F0						
	Cr	DM	OM	N	Ca	P
	2.05	1000.00	915.18	29.70	9.20	6.42
Diet F0 faeces						
Animal No.	Cr	DM	OM	N	Ca	P
Collection 1						
177	11.79	1000.00	730.75	36.18	37.66	29.94
176	13.59	1000.00	716.70	26.93	36.56	29.95
235	12.45	1000.00	724.60	37.39	35.22	29.69
249	14.01	1000.00	698.62	32.57	37.53	27.29
289	12.03	1000.00	718.99	36.39	35.07	30.26
250	11.88	1000.00	730.58	35.01	34.11	25.70
Collection 2						
177	11.46	1000.00	744.73	36.14	31.57	23.69
176	13.12	1000.00	733.53	27.89	32.75	27.16
235	13.57	1000.00	715.17	27.33	37.59	29.13
249	12.25	1000.00	721.91	39.02	33.06	27.15
289	12.13	1000.00	748.13	28.88	31.64	24.60
250	12.26	1000.00	719.91	30.33	35.47	25.73
Collection 3						
177	10.96	1000.00	740.31	27.43	32.48	27.07
176	12.32	1000.00	727.40	31.82	33.17	24.62
235	10.90	1000.00	746.14	30.07	31.53	27.35
249	12.59	1000.00	726.49	33.72	31.86	27.12
289	11.80	1000.00	717.07	28.81	35.95	28.96
250	11.96	1000.00	702.49	30.08	37.14	29.09
Collection 4						
177	10.74	1000.00	730.01	25.90	34.17	27.19
176	13.10	1000.00	705.53	31.25	36.94	27.77
235	10.71	1000.00	726.39	29.93	36.00	28.87
249	12.54	1000.00	721.69	29.79	32.96	26.05
289	11.84	1000.00	714.75	29.18	38.07	29.25
250	11.33	1000.00	720.03	24.76	37.38	24.16
Collection 5						
177	11.76	1000.00	740.05	28.95	34.01	29.81
176	12.45	1000.00	726.04	27.47	35.96	30.77
235	11.84	1000.00	714.47	26.88	37.10	30.86
249	13.51	1000.00	705.26	21.54	37.00	30.63
289	13.23	1000.00	703.33	27.99	38.82	29.30
250	12.16	1000.00	728.31	23.60	37.48	28.63

Nutrient digestibility in diet F0 (%)

Diet F0					
Animal No.	DM	OM	N	Ca	P
Collection 1					
177	82.61	86.11	78.81	28.82	18.90
176	84.92	88.19	86.32	40.06	29.63
235	83.54	86.97	79.27	36.98	23.87
249	85.37	88.83	83.96	40.33	37.82
289	82.96	86.61	79.12	35.04	19.68
250	82.75	86.23	79.66	36.05	30.95
Collection 2					
177	82.10	85.44	78.22	38.59	33.96
176	84.37	87.47	85.32	44.37	33.88
235	84.89	88.20	86.10	38.28	31.46
249	83.26	86.80	78.01	39.86	29.22
289	83.10	86.18	83.57	41.87	35.24
250	83.28	86.84	82.92	35.52	32.97
Collection 3					
177	81.30	84.87	82.72	33.97	21.14
176	83.36	86.78	82.17	40.01	36.19
235	81.19	84.67	80.95	35.55	19.88
249	83.72	87.07	81.51	43.61	31.21
289	82.63	86.39	83.15	32.13	21.65
250	82.86	86.85	82.64	30.82	22.35
Collection 4					
177	80.91	84.78	83.36	29.11	19.17
176	84.35	87.94	83.53	37.16	32.31
235	80.86	84.81	80.71	25.10	13.93
249	83.65	87.11	83.60	41.43	33.67
289	82.69	86.48	82.99	28.36	21.12
250	81.91	85.77	84.92	26.52	31.94
Collection 5					
177	82.56	85.90	83.00	35.54	19.04
176	83.53	86.93	84.76	35.62	21.06
235	82.68	86.48	84.33	30.17	16.76
249	84.82	88.30	88.99	38.96	27.58
289	84.50	88.09	85.39	34.61	29.28
250	83.14	86.58	86.60	31.30	24.80

Table 10: Individual data for calculation of nutrient digestibility in diet F1 (g/kg DM)

Diet F1						
Animal No.	Cr	DM	OM	N	Ca	P
	2.05	1000.00	924.35	30.91	9.2	6.42
Diet F1 faeces						
Animal No.	Cr	DM	OM	N	Ca	P
Collection 1						
165	14.19	1000.00	733.77	34.47	34.98	24.21
212	13.45	1000.00	725.73	33.05	37.69	29.64
282	10.05	1000.00	740.96	32.92	37.56	28.18
285	12.47	1000.00	747.28	34.07	27.86	23.27
242	12.85	1000.00	743.66	37.53	27.84	22.55
9470	13.72	1000.00	744.40	31.39	30.54	24.77
Collection 2						
165	13.20	1000.00	753.02	37.25	28.17	23.39
212	13.81	1000.00	731.74	34.51	34.34	27.63
282	13.15	1000.00	730.22	32.26	33.53	26.50
285	12.94	1000.00	738.53	38.52	29.02	24.31
242	11.34	1000.00	755.96	29.83	28.22	24.40
9470	13.06	1000.00	723.81	28.66	36.36	26.82
Collection 3						
165	12.74	1000.00	722.97	35.84	31.06	25.54
212	11.88	1000.00	735.72	32.79	34.87	28.87
282	12.02	1000.00	739.75	33.53	32.86	26.88
285	13.21	1000.00	720.16	29.54	31.55	22.21
242	11.84	1000.00	746.58	21.44	31.49	25.83
9470	12.56	1000.00	722.04	28.90	36.40	26.60
Collection 4						
165	13.12	1000.00	722.76	29.51	32.88	26.34
212	12.61	1000.00	726.09	31.72	35.55	27.75
282	12.43	1000.00	734.85	30.75	31.97	25.21
285	12.80	1000.00	726.59	33.58	30.50	21.20
242	11.42	1000.00	751.01	26.28	29.97	24.25
9470	11.44	1000.00	722.21	27.35	37.51	27.86
Collection 5						
165	13.08	1000.00	716.22	25.05	34.74	31.08
212	12.97	1000.00	722.17	31.12	36.43	30.36
282	11.71	1000.00	722.53	27.71	36.03	29.01
285	12.79	1000.00	740.34	35.28	30.19	24.66
242	12.17	1000.00	738.63	30.20	31.48	26.68
9470	13.86	1000.00	722.94	27.93	36.11	27.23

Nutrient digestibility in diet F1 (%)

Diet F1					
Animal No.	DM	OM	N	Ca	P
Collection 1					
165	85.55	88.53	83.89	45.06	45.51
212	84.75	88.03	83.70	37.54	29.61
282	79.60	83.65	78.28	16.72	10.47
285	83.57	86.71	81.89	50.24	40.44
242	84.05	87.17	80.64	51.74	43.98
9470	85.06	87.97	84.83	50.41	42.36
Collection 2					
165	84.47	87.35	81.28	52.44	43.41
212	85.16	88.25	83.43	44.60	36.12
282	84.41	87.68	83.72	43.17	35.39
285	84.15	87.34	80.25	50.01	39.99
242	81.92	85.21	82.55	44.54	31.28
9470	84.30	87.71	85.45	37.96	34.42
Collection 3					
165	83.90	87.41	81.34	45.66	35.97
212	82.75	86.27	81.70	34.62	22.43
282	82.95	86.35	81.50	39.10	28.61
285	84.49	87.91	85.17	46.80	46.33
242	82.68	86.01	87.99	40.72	30.32
9470	83.68	87.26	84.75	35.45	32.40
Collection 4					
165	84.38	87.79	85.09	44.18	35.92
212	83.74	87.23	83.31	37.17	29.71
282	83.51	86.89	83.60	42.71	35.26
285	83.98	87.41	82.59	46.89	47.10
242	82.05	85.42	84.74	41.53	32.20
9470	82.08	86.00	84.15	26.94	22.24
Collection 5					
165	84.32	87.85	87.29	40.80	24.10
212	84.20	87.66	84.09	37.43	25.28
282	82.49	86.32	84.31	31.44	20.90
285	83.98	87.17	81.71	47.42	38.46
242	83.16	86.54	83.55	42.38	30.02
9470	85.21	88.43	86.63	41.94	37.26

Table 11: Individual data for calculation of nutrient digestibility in diet F2 (g/kg DM)

Diet F2						
	Cr	DM	OM	N	Ca	P
	2.05	1000.00	917.71	31.60	9.2	6.42
Animal No.	Cr	DM	OM	N	Ca	P
Collection 1						
179	15.19	1000.00	755.37	39.84	33.88	26.94
167	13.29	1000.00	723.22	25.89	31.45	29.44
287	11.88	1000.00	754.83	31.20	29.98	26.11
217	12.30	1000.00	733.28	25.35	33.12	23.54
152	13.64	1000.00	743.46	35.29	27.50	24.20
5445	12.68	1000.00	716.50	37.46	33.04	28.12
Collection 2						
179	13.70	1000.00	754.07	34.99	28.16	23.69
167	13.87	1000.00	719.65	32.23	30.23	27.95
287	11.05	1000.00	766.11	33.55	25.44	23.14
217	13.59	1000.00	712.69	25.89	34.11	27.60
152	15.26	1000.00	708.24	29.08	34.57	23.66
5445	16.03	1000.00	706.34	27.04	35.17	24.16
Collection 3						
179	13.03	1000.00	746.63	30.40	29.68	29.68
167	15.73	1000.00	702.01	27.69	34.00	31.94
287	13.65	1000.00	751.24	33.46	28.42	25.50
217	14.37	1000.00	724.22	29.27	31.94	26.88
152	14.74	1000.00	723.30	26.22	31.82	23.16
5445	14.71	1000.00	697.95	28.56	35.80	24.13
Collection 4						
179	13.42	1000.00	735.30	24.30	28.66	22.06
167	14.63	1000.00	719.10	20.14	32.84	30.10
287	12.25	1000.00	728.99	21.26	30.57	24.10
217	13.38	1000.00	720.94	23.35	33.24	28.93
152	14.71	1000.00	711.32	25.13	34.85	24.68
5445	15.04	1000.00	698.08	26.43	37.95	26.73
Collection 5						
179	12.90	1000.00	734.36	31.64	30.78	25.38
167	13.58	1000.00	720.13	26.10	32.40	31.42
287	10.80	1000.00	754.53	32.72	28.40	22.60
217	13.47	1000.00	701.19	29.57	35.47	30.76
152	15.92	1000.00	710.64	24.42	35.13	23.59
5445	14.51	1000.00	713.89	29.22	36.77	22.65

Nutrient digestibility in diet F2 (%)

Diet F2					
Animal No.	DM	OM	N	Ca	P
Collection 1					
179	86.51	88.89	82.99	50.32	43.39
167	84.57	87.84	87.36	47.27	29.26
287	82.74	85.80	82.95	43.75	29.80
217	83.34	86.69	86.63	40.02	38.91
152	84.97	87.83	83.22	55.09	43.36
5445	83.84	87.38	80.84	41.95	29.20
Collection 2					
179	85.03	87.70	83.43	54.19	44.78
167	85.22	88.41	84.92	51.42	35.64
287	81.44	84.51	80.30	48.69	33.12
217	84.92	88.29	87.64	44.08	35.16
152	86.57	89.64	87.64	49.54	50.51
5445	87.21	90.16	89.06	51.12	51.88
Collection 3					
179	84.27	87.20	84.87	49.26	27.29
167	86.97	90.03	88.58	51.84	35.16
287	84.98	87.71	84.10	53.61	40.35
217	85.68	88.68	86.68	50.48	40.05
152	86.09	89.04	88.46	51.89	49.82
5445	86.07	89.40	87.40	45.78	47.63
Collection 4					
179	84.72	87.76	88.25	52.41	47.50
167	85.98	89.02	88.64	49.97	34.29
287	83.27	86.71	88.74	44.42	37.24
217	84.68	87.97	88.68	44.66	30.98
152	86.06	89.20	88.91	47.19	46.41
5445	86.37	89.63	88.59	43.76	43.23
Collection 5					
179	84.11	87.29	84.09	46.85	37.20
167	84.90	88.15	87.53	46.82	26.10
287	81.02	84.39	86.87	41.41	33.19
217	84.78	88.37	85.76	41.31	27.06
152	87.13	90.03	90.05	50.84	52.69
5445	85.87	89.01	86.93	43.52	50.15

Table 12: Individual data for calculation of nutrient digestibility in diet F3 (g/kg DM)

Diet F3						
	Cr	DM	OM	N	Ca	P
	2.05	1000.00	918.03	31.15	9.20	6.42
Diet F3 faeces						
Animal No.	Cr	DM	OM	N	Ca	P
Collection 1						
175	11.76	1000.00	745.75	30.26	31.61	25.48
275	13.75	1000.00	716.21	32.13	34.73	27.34
208	13.63	1000.00	729.91	32.61	25.45	25.45
178	16.80	1000.00	678.37	31.52	41.58	30.16
254	14.42	1000.00	732.79	37.61	30.74	25.09
255	14.39	1000.00	731.62	27.12	30.36	25.04
Collection 2						
175	10.52	1000.00	746.98	29.84	27.59	21.18
275	13.85	1000.00	721.73	25.59	31.54	26.47
208	12.47	1000.00	740.35	37.40	30.77	26.58
178	13.38	1000.00	700.90	23.04	39.58	28.16
254	13.25	1000.00	723.13	32.06	33.46	25.99
255	12.89	1000.00	728.98	24.26	33.11	25.19
Collection 3						
175	12.24	1000.00	748.29	30.42	30.57	23.26
275	13.73	1000.00	725.36	29.39	31.30	23.30
208	13.37	1000.00	718.75	35.20	31.76	28.36
178	13.23	1000.00	692.13	29.78	39.58	26.42
254	13.04	1000.00	722.75	30.78	32.75	23.41
255	12.32	1000.00	731.99	34.47	32.27	24.79
Collection 4						
175	12.62	1000.00	750.53	27.45	31.53	23.99
275	13.41	1000.00	697.40	24.83	34.67	28.05
208	11.58	1000.00	738.05	26.75	29.80	25.65
178	14.15	1000.00	684.49	25.81	40.40	33.09
254	12.75	1000.00	737.78	26.71	33.20	27.19
255	13.62	1000.00	718.51	26.14	32.58	25.33
Collection 5						
175	11.62	1000.00	747.18	28.77	31.20	23.67
275	11.03	1000.00	766.09	26.20	25.16	20.00
208	13.00	1000.00	736.09	30.73	28.91	22.85
178	13.19	1000.00	701.28	25.67	41.07	27.89
254	12.88	1000.00	745.55	34.69	30.28	22.05
255	13.63	1000.00	716.60	25.37	34.54	29.43

Nutrient digestibility in diet F3 (%)

Diet F3					
Animal No.	DM	OM	N	Ca	P
Collection 1					
175	82.56	85.84	83.06	40.09	30.79
275	85.09	88.37	84.62	43.71	36.50
208	84.96	88.04	84.25	58.40	40.38
178	87.80	90.98	87.65	44.85	42.67
254	85.78	88.65	82.83	52.50	44.44
255	85.75	88.64	87.59	52.98	44.42
Collection 2					
175	80.52	84.15	81.33	41.57	35.73
275	85.20	88.36	87.84	49.25	38.97
208	83.56	86.75	80.26	45.03	31.96
178	84.67	88.30	88.66	34.07	32.78
254	84.53	87.81	84.08	43.74	37.38
255	84.09	87.37	87.61	42.74	37.57
Collection 3					
175	83.25	86.34	83.64	44.33	39.30
275	85.07	88.20	85.91	49.20	45.81
208	84.67	88.00	82.67	47.08	32.28
178	84.50	88.32	85.18	33.32	36.22
254	84.28	87.62	84.46	44.02	42.66
255	83.36	86.73	81.58	41.62	35.73
Collection 4					
175	83.76	86.72	85.68	44.33	39.30
275	84.72	88.39	87.82	42.41	33.28
208	82.30	85.77	84.80	42.68	29.30
178	85.51	89.19	87.99	36.36	25.30
254	83.92	87.07	86.21	41.96	31.88
255	84.95	88.22	87.37	46.70	40.62
Collection 5					
175	82.36	85.65	83.71	40.19	34.98
275	81.41	84.48	84.36	49.15	42.08
208	84.24	87.36	84.44	50.47	43.90
178	84.46	88.13	87.19	30.63	32.49
254	84.09	87.08	82.28	47.62	45.34
255	84.96	88.26	87.75	43.53	31.04

Table 13: Characteristics of gestating sows involved in the digestibility study

Dietary treatment	Animal No.	Parity	Sow weight (kg)
F0	177	5	250
	176	5	255
	235	4	255
	249	4	255
	289	3	235
	250	3	245
	mean	**4**	**249.17**
F1	165	5	253
	212	4	243
	282	3	215
	285	3	235
	242	4	220
	9470	3	225
	mean	**3.67**	**231.83**
F2	179	4	243
	167	5	265
	287	3	218
	217	4	265
	152	5	245
	5445	3	215
	mean	**4**	**241.83**
F3	175	5	262
	275	3	235
	208	4	202
	178	5	230
	254	4	235
	255	4	255
	mean	**4.17**	**236.50**

Table 14: Feed consumption during experiment (kg)

Dietary treatment	Feed consumption (kg)	Num. of animals in period	Feed consumption kg/animal
F0	320	6	53.3
F1	325	6	54.2
F2	327	6	54.5
F3	328	6	54.7

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ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA Mash Phytase** Batch number: **Lot PPQ 28656**

Trial ID: **GF 024** Location: [redacted] (b) (4)

Start date and exact duration of the study: **June 2009, 18 days**

Number of treatment groups (+ control(s)): **4** Replicates per group: **6**

Total number of animals: **24** Animals per replicate: **1**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **500, 1000 and 2000 U/kg** Analysed: **531, 898 and 1890 U/kg**

†

Substances used for comparative purposes: -

Intended dose: Analysed:

Animal species/category: **Gestating sows**

Breed: **Large White x Landrace** Identification procedure: **ear numbers**

Sex: **female** Age at start: **2.5 - 4 years (3rd to 5th parity)** Body weight at start: **initial BW 239.8 kg**

Physiological stage: **pregnant** General health: **very good**

Additional information for field trials:

Location and size of herd or flock: [redacted] (b) (4)

Feeding and rearing conditions: **individual housing and feeding**

Method of feeding: **2 times a day**

Diets (type(s)): **Diet for gestating sows**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize, barley, soybean meal, rapeseed meal**

Nutrient content (relevant nutrients and energy content)

Intended values: **Crude protein - 171.7 g/kg; Ca - 8.0 g/kg; P total - 6.0 g/kg**

Analysed values: **Crude protein - 192.7 g/kg DM, Ca - 9.2 g/kg DM, P total - 6.42 g/kg DM**

Date and nature of the examinations performed: **Collection of faeces (day 10 to 15)**

Method(s) of statistical evaluation used: **ANOVA, Fisher's LSD procedure**

Therapeutic/preventive treatments (reason, timing, kind, duration): -

Timing and prevalence of any undesirable consequences of treatment: -

Date **November 2009**	Signature Study Director [redacted] (b) (4)

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

[1] Please submit this form using a common word processing format (e.g. MS Word).

TAB

12

Annex 12

Assessment of the effects of phytase (Ronozyme® HiPhos) to improve nutrient digestibility in lactating sows

REPORT No. 00015939

REPORT No. 00015939
Regulatory Document

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

Document Date: 6 December 2012

Author(s): R.T. Zijlstra[1], Z. Nasir[1] and J. Broz[2]
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[2] Nutrition Innovation Center, R&D, Scientific Networks ANH, Basel

Title: **Assessment of the effects of phytase (Ronozyme® HiPhos) to improve nutrient digestibility in lactating sows**

Project No. 6562

Summary
A digestibility study was conducted to determine the effects of Ronozyme® HiPhos phytase at the inclusion level of 500 U/kg diet on nutrient digestibility and various blood variables in lactating sows. A total of 45 gestating sows (Large White x Landrace) were used in the present study. The sows were individually kept and allocated one of the three experimental diets (15 sows per diet). The dietary treatments were as follows: 1) positive control fed a regular lactation diet containing 0.77% total P, with added monocalcium phosphate (PC); 2) negative control (NC) fed a diet with reduced available P (0.45% total P), without addition of monocalcium phosphate; 3) NC diet plus phytase at 500 U/kg (NC + P). Feacal samples were collected three times from each sow. First faecal sample was collected after moving the sows to farrowing pens, before switching them to experimental lactation diets. Second sample was collected on day 8 of lactation and third sample was collected on day 15 of lactation. Blood samples were collected from jugular vein from each sow both at farrowing and at the end of study (day 15). The apparent total tract digestibility (ATTD) of dry matter, crude protein, energy, calcium and phosphorus were calculated using titanium dioxide as an indigestible marker. Phytase supplementation to the low-P diet significantly increased ($P<0.05$) the ATTD of phosphorus on days 8 and 15 post-farrowing, when compared to the negative control. Specifically, supplementation of Ronozyme® HiPhos increased the ATTD of phosphorus from 36.0 to 42.1% on day 8, and from 33.9 to 46.0% on day 15. Digestibility values of crude protein, Ca, energy and organic matter were not affected significantly.

This report consists of 35 pages & Annex C

Distribution

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Main Author Dr. J. Broz, NIC-RD/NA	Signed by J. Broz	07.12.2012
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R&D Director Dr. G. Kau, NIC-RD	Signed by G. Kau	11.12.2012
Innovation Project Manager Dr. F. Fru, NIC-IPM	Signed by F. Fru	14.12.2012

Study Report

Assessment of the effects of phytase (Ronozyme® HiPhos) to improve nutrient digestibility in lactating sows

Experiment code: RTZ/ZN-2011-Phytase

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Report submitted to: **Dr. Jiri Broz**
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Study Title:	**Assessment of the effects of phytase (Ronozyme® HiPhos) to improve nutrient digestibility in lactating sows**	
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Sponsor(s):	DSM Nutritional Products, Ltd CH- 4002- Kaiseaugst, Switzerland	
Test Article(s):	Ronozyme® HiPhos Lot ELN-09FIBo0031-2GT Minimum phytase activity: 10,000 U/g	
Control Article(s):	Positive control diet (commercial)	
Initiation Date:	Date the protocol is signed by the Principal Investigator:	___________
Start Date:	First date the test articles are applied:	October 18, 2011
Termination date:	Last day data are collected:	December 31, 2011
Completion date:	Date final report is signed:	_____________

1. Objectives

The objectives of the present study were to determine the effects of phytase (Ronozyme® HiPhos) at the inclusion level of 500 U/kg in diets of lactating sows on nutrient digestibility, performance and various blood variables.

2. Materials and methods

2.1 Test and control article

There were three diets: 1) positive control (the regular sow diet containing mono calcium phosphate), 2) negative control (NC) diet with reduced available P (without added mono calcium phosphate), and 3) NC diet plus 500 U of phytase/kg diet. Feed was obtained from a commercial source (Viterra Feeds, Sherwood Park, Alberta, Canada). Indigestible marker and phytase were added at the University of Alberta Feed Mill, Edmonton, Canada.

2.2 Animals and housing

The animal procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock, and followed principles established by the Canadian Council on Animal Care (CCAC, 2009) and were conducted at the "Swine Research and Technology Centre (SRTC)". In total, 45 gestating sows (Large White x Landrace) were used for the present trial. The sows were individually kept and allocated one of the three experimental diets (15 sows per diet).

The sows were kept in five rooms (11.3 m long × 7.62 m wide), each having nine sows at the same stage of farrowing and lactation. Each room is equipped with two parallel rows of five farrowing pens separated by a center alley (Figure 1). The individual farrowing pens measured 1.83 m wide x 2.20 m long x 0.50 m high. All four pen sides were made of solid plastic planking. Each pen was longitudinally divided into three compartments with central farrowing crate and two pens for piglets.

Each farrowing crate (0.55 m wide x 2.20 m long x 1.0 m high) was equipped with a stainless steel, dry self-feeder attached to the front of the pen. The feeder was 0.35 m wide x 0.6 m high, 0.15 m off the floor. A single drinker was also attached to the right side wall of farrowing crate (0.76 m above pen floor) near the feeder, and a drinker closer to the pen floor for piglet access. Each pen was equipped with a heating pad and sprinkler for extreme temperature conditions. There were four gutters (0.56 m wide x 0.61 m deep) in each room, one located under the front and one running under the back of each row of pens. The slurry was removed by gravity to a holding tank.

The room uses negative pressure to ventilate, created by a single exhaust fan chimney located in the centre of the ceiling. Outside air flowed into the barn attic from below the barn side gutters. Air was pre-warmed in the attic and then drawn into the room through ten air inlets located along sidewalls of the room. Incoming air was further heated by contact flow with a re-circulating hot water pipe suspended under the air baffles. All components of the ventilation system including the water heating and ceiling fresh air inlets were integrated and controlled by one environmental controller. Artificial light was provided using three light fixtures equipped with two 40-watt fluorescent light tubes. Room lights were automatically controlled for 12 hours of light and 12 hours of dark.

2.3 Experiment design and diets

During this experiment, effects of phytase were studied in a randomized complete block design, where sows are either designated by parity (first, second, third and higher), and are randomized within each set of three sows. During the experiment sows were offered lactation diet obtained from a commercial source (Viterra Feeds Sherwood Park, Alberta, Canada) in crumbles form, which were identical except for the amount of total phosphorus as:

1. PC: Positive control (regular lactation diet containing 0.77% total P)
2. NC: Negative control diet (containing 0.45 % total P)
3. NC+P: Negative control diet plus 500 units of phytase/kg diet (Ronozyme® HiPhos)

Diets were fortified to meet vitamin and mineral requirements (NRC, 1998); except for being marginally limiting in P. The indigestible marker titanium dioxide (TiO_2) was included in diets as well. Sows were the sampling and experimental unit for all measurements. The test diets were formulated to provide approximately 21% CP and exceeded NRC requirements for most nutrients. At the University of Alberta Feed Mill, feeds were mixed with marker (TiO_2) and phytase (Ronozyme® HiPhos). Prior to making the final batch for each diet, the phytase enzyme and marker were mixed with the same diet as carrier. Mixing time of diet were six minutes (based on the results obtained from Pre-experimental Mixer Efficiency Test). After mixing, representative samples (~ 1000 g) were collected from each test diet for nutrient analysis. The test diets were properly labelled and delivered in plastic, rodent-proof feed bins to SRTC. On arrival at the SRTC, the test diets were stored in the feed bins.

One grab sample (~100 g) of each test diet was collected at the barn at the start of each experimental phase (total ~ 500 g). These grab samples were thoroughly mixed using a beater mixer (Met Unit). A sub-sample (~250 g) of each composite test diet was used for laboratory analyses at the end of the study. The remaining sub-samples (~250g each) were stored in a freezer (approx. -20°C) at the SRTC for future analyses as may be required.

The null hypothesis is that the phytase will not affect the nutrient digestibility. If the probability is 5% or less than 5%, the null hypothesis will be rejected and the alternative hypothesis (that the phytase will affect the nutrient digestibility) will be accepted.

2.4 Experiment management

Acclimation to housing period:

Pregnant sows were weighed and moved to farrowing pens in the test room 5-7 days prior to farrowing. Sows were continued to be offered gestation diet (SRTC diet specification) on the first day in farrowing pens, which was changed to the specific lactation diet on the 2^{nd} day. Before changing gestation diet to lactation diet, faeces were collected from individual sows.

Throughout the entire experiment, sows had free access to water from a pen drinker at all times. The temperature of the room was maintained at 21 ± 2.0°C. Supplemental heat for piglets was provided through heated floor pads present in each farrowing pen.

Diet adaptation:

In farrowing pens the sows were switched from the gestation diet to the experimental lactation diet (Table 1). Once on the assigned test diet, sows were offered it for a minimum of 5 d adaptation prior to farrowing. Prior to farrowing, sows were fed 3.0 kg of diet per day (SRTC SOP). The test diets were offered once daily.

Collection of faecal samples:

Faecal samples were collected three times from each sow as:

First faeces sample: collected after moving the sows to farrowing pens, while fed gestation diet. After collecting first faecal samples, diets of sows were switched to lactation diet.

Second faeces sample: collected on 8^{th} day of lactation.

Third faeces sample: collected on 15^{th} day of lactation.

Faecal samples were collected soon after excreted. The individual sow faecal collections were weighed (warm) and kept in bag in the freezer(s) at –20 °C. Each bag was labelled with the experiment number, sow ID and date of collection.

Collection of plasma samples:

Blood samples (15-20 ml) were collected from jugular vein (SRTC SOP # S-77) from each sow both at farrowing (day 1 of lactation) and at the end of experiment (day 15) using an anticoagulant (heparin). Immediately after collection, blood samples were centrifuged (3000 rpm, 10 minutes, 4°C) and plasma was separated. Plasma was stored at -80°C until further analysis.

2.5 Measurements and observations

Sows were individually weighed using standardized scales at the beginning of experiment, on the day 1 and 15 of lactation. Back fat measurements of the sows were performed on day 1 and 15 using ultrasonic instrument. All piglets in a litter were weighed together using standardized scales on 1, 8 and 15 day of age. Weight gain of piglets was used to estimate sow milk production.

During diet adaptation phase and specimen collection phase, the amount and consumption of each diet were confirmed and recorded once daily. After the daily health checks, room temperature and humidity were measured using a digital thermometer and hygrometer and recorded

2.6 Specimen processing, storage and analysis

Once the specimen collection was completed, the collected faecal samples were thawed and weighed. The faeces were thoroughly homogenized using a beater (SRTC Engineering Lab). Faeces were sub-sampled into shallow aluminium dishes in the barn. Two subsamples were carefully weighed, frozen again and kept at approximately –20°C. Both the subsamples' container and lid were labelled with the experiment number, sow ID and period.

Frozen faecal samples were freeze-dried, equilibrated to room temperature and moisture, and subsequently ground through a 1 mm screen using a Retsch mill. Dried and ground faecal samples and subsamples of each of the test diets were packaged for lab analyses.

Analysis of gross energy, dry matter, crude protein (LECO), ash, marker (Titanium dioxide) and phosphorus in the diet and faeces were conducted at the Department of Agricultural, Food and Nutritional Science at the University of Alberta. Analysis of calcium, zinc, copper and magnesium in diet and faeces were conducted at the lab of department of Renewable Resources, University of Alberta, Edmonton, Canada. Plasma samples were analysed at Prairie Diagnostic Services, Saskatoon, Saskatchewan. Activity of phytase in diets was determined by DSM Biopract GmbH (Dr. J. König), Magnusstrasse 11, D-12489 Berlin, Germany.

2.7 Calculations and statistical analysis

The results of the diet and faecal laboratory analyses were transcribed checked and verified twice prior to initiating calculations. All digestibility calculations were made using the indicator method. The apparent total tract digestibility (ATTD) of dry matter (DM), crude protein, energy, calcium and phosphorus were calculated as follows:

Apparent digestibility, % = 100 – [100 x (concentration of TiO_2 in feed x concentration of nutrient in faeces) / (concentration of TiO_2 in faeces x concentration of nutrient in feed)]

Data were analysed using the PROC MIXED procedure of SAS.

3. Results

Supplementation of phytase to low P diet increased ($P < 0.05$) ATTD of P on d 8 and 15 post farrowing (Table 3). Specifically, supplementation of Ronozyme® HiPhos to the NC diet increased ($P<0.05$) the ATTD of P by 17% on d 8 and by 35% on d 15 as compared with NC diet. Supplementation of phytase did not affect ($P>0.05$) ATTD of CP, Ca, GE and organic matter.

On d 1 and d 15 post-farrowing, plasma P was reduced ($P<0.05$) in sows fed the NC instead of the PC diet by 0.66 and 0.30 mmol/L, respectively (Table 4). On d 1 but not d 15, phytase supplementation increased ($P<0.05$) plasma P by 0.40 mmol/L in sows fed the NC diet. Phytase supplementation did not affect ($P>0.05$) plasma Ca and total protein or other plasma variables like Ca, Na, K, Cl, Mg, Mn, Fe, Co, Cu, Zn, Se, Mb, urea, creatinine, glucose, total protein, albumin, globulin and AG ratio on d 1 and d 15. Compared with NC diet, the sows fed phytase-supplemented diet had lower plasma bicarbonate (P<0.05) on d 15 and higher anion gap on day 1. However, all variables were in the normal physiological range.

Feeding the three test diets did not affect ($P>0.05$) total feed consumption, milk production, back fat thickness and BW changes of sows during the lactation and liter weight gain of piglets (Table 5). There was a trend (P = 0.067) of 10% reduced daily feed consumption in sows fed on phytase supplemented diets, while keeping all other parameters similar.

4. Conclusion

In conclusion, supplementation of Ronozyme® HiPhos phytase at 500 U/kg increased P digestibility with the potential to reduce P excretion in lactating sows. Thus, even supplementation of low doses of microbial phytase in the sow diets can reduce the environmental footprint of pig production.

Table 1. Composition (as-fed basis) of experimental diets[1]

	Gestation diet	Lactation diets		
Ingredients, %		PC	NC	NC+P
Wheat	25.0	51.7	53.5	53.5
Barley	50.4	-	-	-
Soybean meal	-	13.9	13.3	13.3
Field peas	10.0	10.0	10.0	10.0
Corn DDGS	10.0	10.0	10.0	10.0
Meat and bone meal	2.20	-	-	-
Fat, downstream	-	2.50	2.50	2.50
Canola meal	-	6.50	6.50	6.50
Limestone / glass rock	1.40	1.86	2.50	2.50
Dicalcium phosphate (21%)	-	1.54	-	-
Fat, blend, tallow	-	0.437	-	-
Salt	0.42	0.435	0.437	0.437
L-Lysine HCL	0.02	0.401	0.412	0.412
Premix (UF Fort 510S-03)	0.25	0.250	0.250	0.250
L-Threonine	-	0.124	0.127	0.127
Biotin	0.150	-	-	-
Choline, Liq 70%	0.065	-	-	-
MHA (Alimet) [2]	-	0.112	0.110	0.110
Xylanase[3]	0.040	-	-	-
Selplex 2000 [4]	0.010	-	-	-
Ethoxyquin (66%)	0.020	0.017	0.017	0.017
Folic acid (1.0%)	0.010	-	-	-
Marker (TiO_2)	-	0.300	0.300	0.300
Phytase (%)	0.01	-	-	0.005
Calculated composition, %				
Dry matter, %	89.9	89.6	89.4	89.4
Crude fat, %	3.27	5.03	4.62	4.62

Crude fibre, %	6.23	4.02	4.04	4.04
Crude protein, %	14.9	20.5	20.5	20.5
DE (swine), Kcal	3102	3467	3479	3479
ME (swine), Kcal	2906	3285	3294	3294
NE (swine, sow), Kcal	2167	2424	2426	2426
SID Lysine, %	0.498	1.07	1.07	1.07
SID Methionine, %	0.178	0.36	0.36	0.36
Calcium, %	0.956	1.08	1.08	1.08
Total Phosphorus, %	0.720	0.770	0.450	0.450
Available Phosphorus, %	0.426	0.517	0.202	0.202
Analyzed composition				
Dry matter, %	90.9	90.1	90.2	89.8
Crude protein, %	18.4	23.3	23.2	23.2
Gross energy, Kcal/kg	3887	4051	4056	4038
Ash, %	7.00	9.05	7.54	7.54
Acid Insoluble Ash, %	0.558	0.600	0.631	0.631
Calcium, %	0.883	1.296	1.166	1.161
Phosphorus, %	0.690	0.860	0.590	0.590
Magnesium, %	0.252	0.209	0.218	0.228
Zinc, mg/kg	167	165	147	165
Copper, mg/kg	203	240	313	311

[1]PC, positive control; NC, negative control; NC+P, negative control plus phytase.

[2]MHA (Alimet), Methionine Hydroxy-Analogue (2-Hydroxy-4-(Methylthio) Butanoic Acid, HMTBA), Novus International, Inc., USA

[3]Porzyme 9300, endo-1, 4-β-xylanase, minimum activity 4000 U/g, Danisco Animal Nutrition

[4]Selplex 2000, Selenium yeast feed additive, contains 0.3 ppm Selenium (Alltech, USA)

Table 2. Enzyme (Phytase) activity (U/kg) in experimental diets

Test Diets	Batch 1	Batch 2
Inclusion level (mg/kg)	50	50
U/kg	500	500
Positive control (PC)	LOQ	LOQ
Negative Control (NC)	124	129
NC + Phytase	758*	771*

LOQ, below limit of quantification

* Includes intrinsic phytase activity

Table 3. Effects of phytase on nutrient digestibility (%) and sow performance[1]

Item[2]	PC	NC	NC+P	SEM	P value
P digestibility, %					
d-5	42.0	41.6	40.5	1.672	0.779
d 8	34.9[b]	36.0[b]	42.1[a]	2.756	0.044
d15	29.7[b]	33.9[b]	46.0[a]	2.375	<.0001
Ca digestibility, %					
d-5	29.1	23.1	23.1	2.810	0.178
d 8	31.7	23.6	24.3	3.206	0.074
d 15	31.0	24.9	28.7	2.540	0.414
CP digestibility, %					
d-5	82.3	81.8	82.3	0.388	0.524
d 8	82.8	83.2	83.3	0.775	0.534
d15	81.0[b]	82.6[a]	82.6[a]	0.708	0.008
GE digestibility, %					
d-5	80.1	80.0	80.1	0.251	0.844
d 8	83.1	82.9	82.9	0.638	0.758
d15	81.2[b]	82.0[ab]	82.6[a]	0.648	0.007
DM digestibility, %					
d-5	80.4	80.5	80.8	0.257	0.331
d 8	81.0	81.6	82.1	0.723	0.104
d15	78.8 [c]	80.5 [b]	81.8[a]	0.699	<.0001
Ash digestibility, %					
d-5	54.9	53.5	53.6	1.024	0.565
d 8	52.0[a]	44.7[b]	46.4[b]	2.067	0.002
d15	46.6[a]	41.8[b]	45.7[a]	1.976	0.010
OM digestibility, %					
d-5	84.0	83.7	83.8	0.186	0.491
d 8	85.3	85.3	85.9	0.590	0.385
d15	83.7[b]	84.6[ab]	85.3[a]	0.591	0.007

[1]PC, positive control; NC, negative control; NC+P, negative control plus phytase.
[2]d –5, 5 d before farrowing; d 8, 8 d post farrowing; d 15, 15 d post farrowing; CP, crude protein; DM, dry matter; OM, organic matter.

Table 4. Effects of phytase on plasma variables[1]

Item[2]		PC [1]	NC [2]	NC+P [3]	SEM	P value
P, mmol/l						
	d 1	2.33[a]	1.67[b]	2.07[a]	0.106	<0.001
	d 15	2.03[a]	1.72[b]	1.76[b]	0.092	0.032
Ca, mmol/l						
	d 1	2.66	2.69	2.66	0.046	0.836
	d 15	2.73[b]	2.88[a]	2.79[ab]	0.036	0.014
Na, mmol/l						
	d 1	147	147	148	0.935	0.555
	d 15	146	144	144	0.991	0.234
K, mmol/l						
	d 1	4.29	4.26	4.36	0.131	0.928
	d 15	4.36	4.19	4.32	0.102	0.496
Chloride, mmol/l						
	d 1	104	104	104	0.669	0.982
	d 15	105[a]	102[b]	103[b]	0.535	<0.001
Bicarbonate, mmol/l						
	d 1	27.2	29.7	27.9	1.198	0.086
	d 15	26.9[c]	30.1[a]	28.6[b]	0.843	0.001
Anion Gap, mmol/l						
	d 1	19.7[a]	16.7[b]	20.0[a]	1.615	0.049
	d 15	18.6	16.4	17.8	1.397	0.146
Mg, mmol/l						
	d 1	0.837	0.792	0.834	0.019	0.131
	d 15	0.936	0.911	0.938	0.017	0.451
Urea, mmol/l						
	d 1	5.38	5.63	5.49	0.267	0.779
	d 15	7.53	8.43	8.16	0.363	0.185
Creatinine, mmol/l						
	d 1	204	201	203	7.10	0.948
	d 15	151	138	153	6.84	0.238
Glucose, mmol/l						
	d 1	4.75	4.71	4.93	0.171	0.521
	d 15	4.83	4.83	4.76	0.183	0.985

Total Protein, G/l						
	d 1	76.9	74.9	73.8	1.445	0.212
	d 15	79.3	80.3	78.9	1.227	0.627
Albumin, G/l						
	d 1	47.3	46.1	46.1	0.756	0.493
	d 15	46.3	46.2	46.8	0.655	0.794
Globulin, G/l						
	d 1	29.6	28.9	27.7	1.448	0.452
	d 15	33.0	34.1	33.1	1.213	0.447
AG ratio						
	d 1	1.65	1.66	1.78	0.110	0.511
	d 15	1.44	1.39	1.48	0.057	0.501
Mn, ppb						
	d 1	4.17	4.00	4.47	0.30	0.492
	d 15	4.53	4.50	5.30	0.50	0.591
Fe, ppm						
	d 1	2.20	2.22	2.08	0.213	0.861
	d 15	1.98	1.59	1.91	0.125	0.062
Co, ppb						
	d 1	0.635	0.808	0.649	0.101	0.289
	d 15	1.033	0.805	0.772	0.142	0.370
Cu, ppm						
	d 1	2.27	2.34	2.20	0.119	0.527
	d 15	1.72[b]	1.89[a]	1.77[ab]	0.064	0.054
Zn, ppm						
	d 1	0.559	0.520	0.525	0.032	0.635
	d 15	0.814	0.807	0.814	0.053	0.997
Se, ppm						
	d 1	0.213	0.216	0.205	0.009	0.702
	d 15	0.240	0.262	0.253	0.014	0.175
Mb, ppm						
	d 1	0.011	0.013	0.012	0.001	0.431
	d 15	0.013	0.012	0.013	0.008	0.339

[1]PC, positive control; NC, negative control; NC+P, negative control plus phytase

[2]d 1, 1 d post farrowing; d 15, 15 d post farrowing.

Table 5. Effects of phytase supplementation on sows and piglet performance and other variables[1]

	PC	NC	NC+P	SEM	P value
Feed consumed (total), kg	109.4	114.5	102.4	4.504	0.117
Feed consumed (daily), kg	5.17	5.55	4.97	0.193	0.067
Sow's weight, kg					
d -5	252.1	256.9	239.5	9.021	0.382
d 1	241.1	246.4	234.8	9.213	0.673
d 15	238.5	242.1	230.5	8.992	0.651
d 19	233.6	238.7	222.5	9.156	0.448
Milk production, kg					
Total (d 1 to d 15)	144.2	135.2	127.0	14.01	0.416
daily	10.4	9.73	9.37	0.918	0.481
Weight loss, kg					
d 1 - (d-5)	-10.93 [b]	-10.71 [b]	-4.786 [a]	1.971	0.055
d 15 - (d-5)	-13.86	-15.00	-9.00	2.346	0.172
d 19 - (d-5)	-18.57	-18.36	-17.14	3.733	0.958
d 19 - d1	-7.54	-7.86	-12.49	3.892	0.600
Back fat measurements, mm					
d -5	18.4	18.8	19.4	0.833	0.700

d 1	17.9	18.1	18.9	0.812	0.660
d 15	17.1	17.4	18.2	0.808	0.633
d 19	16.3	16.6	16.7	0.831	0.939
Litter weight, kg					
d 1	11.1	13.7	13.3	0.958	0.128
d 8	41.8	30.4	32.2	7.777	0.542
d 15	47.8	50.0	51.9	2.943	0.624
d 19	56.0	61.6	60.0	3.783	0.402
Weight gain, kg					
Litter	33.6	31.5	29.6	3.220	0.419
Piglet	3.44	3.13	3.00	0.305	0.447

[1]PC, positive control; NC, negative control; NC+P, negative control plus phytase

[2]d –5, 5 d before farrowing; d 1, 1 d post farrowing; d 8, 8 d post farrowing; d 15, 15 d post farrowing, d 19, 19 d post farrowing (weaning day)

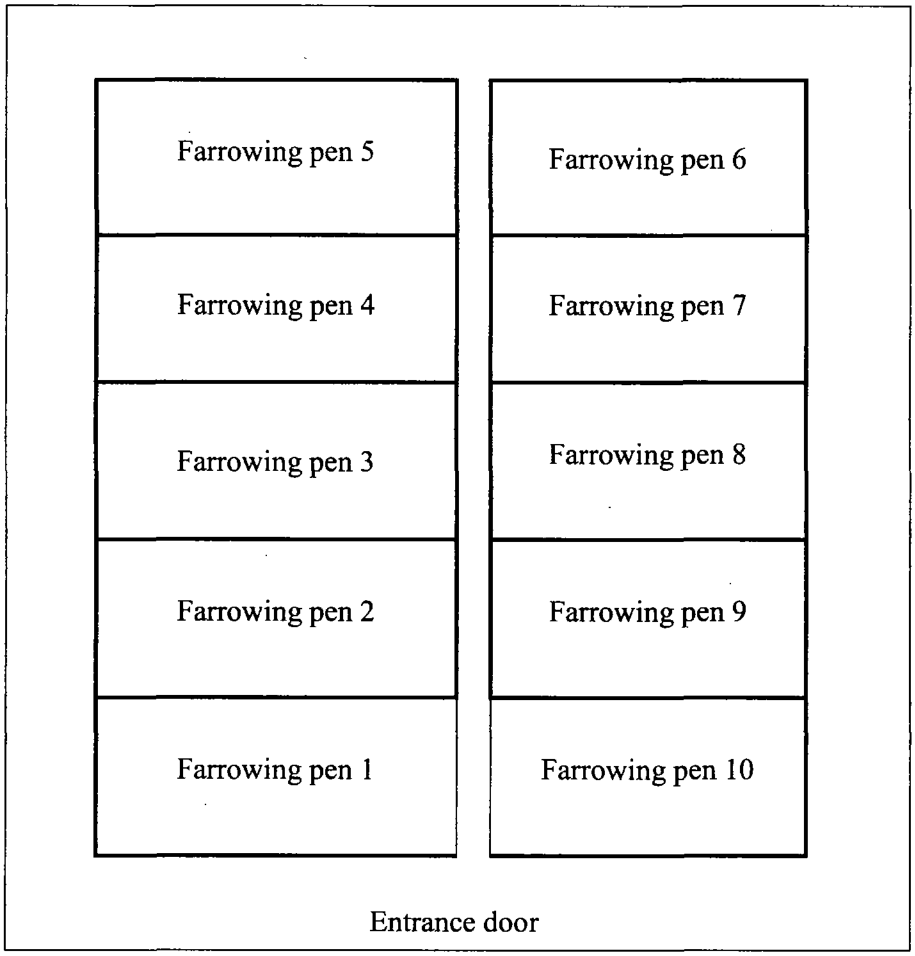

Figure 1. Layout of the farrowing rooms showing the location of the sows involved in the study. *(Image not to scale)*

Annex 1- Poster presented at Digestive Physiology of Pigs, (May 29 – June 1, 2012 Keystone, Colorado, USA)

The effects of supplementation of a novel bacterial 6-phytase on mineral digestibility and plasma minerals in lactating sows

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Introduction

- Approximately two-thirds of P in plant feedstuffs is phytate-P (Nortey et al., 2007).
- Lack of endogenous phytase in pigs results in excretion of large amounts of unutilized P in feces, thus increasing environmental footprint of pig production.
- Supplemental phytase increased P and Ca digestibility in piglets and grower-finisher pigs (Jongblood et al., 2004).
- Effects of adding a novel bacterial 6-phytase expressed in *Aspergillus oryzae* (Ronozyme HiPhos) in lactating sows on nutrient digestibility have not been assessed.

Objective

- To determine the effects of supplementation of a novel bacterial 6-phytase expressed in *Aspergillus oryzae* (Ronozyme HiPhos, DSM Nutritional Products, Ltd) at the rate of 500 FTU/kg diet on nutrient digestibility, various plasma variables and sow condition.

Methodology

Animals: 45 gestating sows (Large White x Landrace), individually housed, divided into 3 groups, fed 3 diets

Major feedstuffs: wheat (50%), soybean meal (13%), field pea (10%) and corn DDGS (10%).

TiO_2 as indigestible marker.

Feeding period: from 5 d before farrowing (d -5) to 15 d post farrowing

Experimental diets		Amount of Phosphorus/Phytase
PC	Positive control	0.52% available P, contains 1.54% dicalcium phosphate as source of inorganic P
NC	Negative control	0.20% available P without inorganic P
NC+P	NC + Phytase	NC + 500 Phytase units(Ronozyme HiPhos)/kg diet

Sample and data collection:

- Sow's body weight and backfat on d -5, d 1 and d 15
- Faeces: d -5, d 8 and 15 post farrowing
- Blood plasma: d 1 and 15 post farrowing

Statistical analysis: MIXED procedure of SAS.

Results

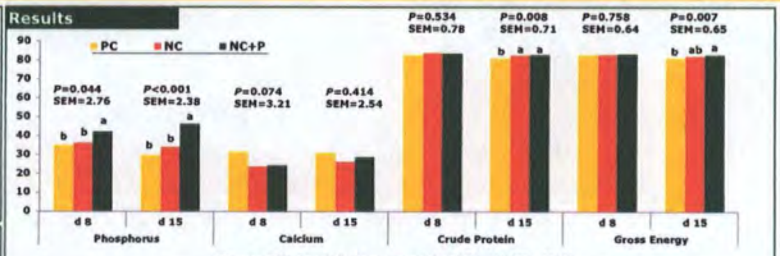

Figure. Effects of phytase on nutrient digestibility (%)[1]

Table 1. Effects of phytase on sow performance[1]

Item	PC	NC	NC+P	SEM	*P*-value
ADFI, kg	5.17	5.55	4.97	0.19	0.067
Sow BW, kg					
d -5	252	257	240	9.02	0.382
d 1	241	246	235	9.21	0.673
d 15	238	242	230	9.00	0.651

Table 2. Effect of phytase on plasma variables[1]

Item		PC	NC	NC+P	SEM	*P*-value
P, mmol/L	d 1	2.33^{a}	1.67^{b}	2.07^{a}	0.11	<0.001
	d 15	2.02^{a}	1.72^{b}	1.72^{b}	0.09	0.032
Ca, mmol/L	d 1	2.66	2.69	2.65	0.05	0.836
	d 15	2.73^{b}	2.88^{a}	2.80^{ab}	0.04	0.014
Na, mmol/L	d 1	147	147	148	0.94	0.555
	d 15	146	144	145	0.99	0.234
K, mmol/L	d 1	4.29	4.26	4.33	0.13	0.928
	d 15	4.36	4.19	4.32	0.10	0.496

[1]PC, positive control; NC, negative control; NC+P, negative control plus phytase, d -5, 5 d before farrowing; d 1, 1 d post farrowing; d 8, 8 d post farrowing; d 15, 15 d post farrowing
[a,b] mean values on a specific day differ significantly at $P<0.05$.

Discussion

- Supplementation of Ronozyme HiPhos to low available P, wheat-based diet increased the ATTD of P, thereby confirming the liberation of P from phytate by phytase.
- Increase (35%) in P digestibility could be because new generation bacterial phytases resist proteolytic digestion more than fungal phytases and are more active in hydrolyzing phytate; thus liberating more P from phytate present in plant based feedstuffs.
- On d 1 post farrowing, plasma P was reduced for the NC compared to the PC diet. This reduction could be due to sudden reduction in availability of dietary P, which was partly compensated by supplemental phytase. Phytase supplementation could reduce incidences of post-partum hypophosphataemia (Liesegang et al., 2005).

Conclusion

- Ronozyme HiPhos (DSM Nutritional Products, Ltd) supplemented at the rate of 500 FTU/kg diet increased P digestibility with the potential to reduce P excretion in lactating sows.
- Thus, even supplementation of low doses of microbial phytase in the sow diets can reduce the environmental footprint of pig production.

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Liesegang, A., L. Loch, E. Buergi, and J. Risteli. 2005. J. Anim. Physiol. Anim. Nutr. 89:120–128.

Nortey, T. N., J. F. Patience, P. H. Simmins, N. L. Trottier, and R. T. Zijlstra. 2007. J. Anim. Sci. 85:1432–1443.

Acknowledgement

DSM

Best Copy Available

Annex II- Raw data

Assessment of the effects of phytase (Ronozyme® HiPhos) to improve nutrient digestibility in lactating sows

Experiment code: RTZ/ZN-2011-Phytase

Table 1. Effects of Phytase (Ronozyme® HiPhos) on Nutrient Digestibility (%)[1]

Sow ID	Diet	Block	Day	P	Ca	CP	GE	DM	Ash	OM
65005	1	1	d -5	40.6	27.1	82.2	80.7	80.4	50.4	84.5
			d 8	44.1	38.0	85.9	85.8	83.9	56.2	87.9
			d 15	30.5	22.2	83.5	83.3	80.7	43.6	86.1
74202	2	1	d -5	42.8	28.3	82.0	79.7	79.7	52.4	83.6
			d 8	48.0	27.1	87.2	85.8	84.2	54.4	88.0
			d 15	48.1	33.0	85.3	84.6	82.9	51.9	86.8
56404	3	1	d -5	42.4	31.7	83.7	81.2	81.2	56.3	84.8
			d 8	50.3	24.1	86.9	85.7	84.8	56.9	88.4
			d 15	56.7	33.0	86.7	86.5	86.0	57.7	88.7
77102	1	1	d -5	46.6	39.1	82.1	79.8	80.4	58.7	83.7
			d 8	46.8	48.8	86.2	85.6	84.5	62.9	87.9
			d 15	43.5	35.7	83.6	83.8	81.3	53.1	85.8
75205	2	1	d -5	44.4	21.5	79.8	79.2	80.1	55.8	83.2
			d 8	36.8	23.0	81.5	81.9	81.1	50.5	84.7
			d 15	37.8	33.1	83.3	82.9	81.4	52.2	84.8
74705	3	1	d -5	29.0	20.2	81.3	78.7	78.7	48.3	82.8
			d 8	57.2	25.2	87.2	86.3	86.1	57.1	88.9
			d 15	53.1	41.1	85.1	85.0	84.5	54.5	87.9
74101	1	1	d -5	45.5	30.6	81.5	79.5	79.6	53.5	83.5
			d 8	50.9	36.7	86.1	86.9	85.1	60.4	87.8
			d 15	43.8	37.4	83.2	84.3	81.1	56.2	86.1
65202	2	1	d -5	36.0	30.0	81.6	80.1	79.8	46.3	83.8
			d 8	48.3	32.3	87.7	87.1	85.4	52.3	89.2
			d 15	40.7	23.3	84.1	83.6	81.9	43.4	86.4
69903	3	1	d -5	41.1	30.0	80.4	79.3	80.6	52.5	83.1
			d 8	53.0	29.6	84.5	84.4	84.2	55.3	87.4
			d 15	37.5	27.7	80.7	81.5	80.8	43.0	85.3
56903	1	2	d -5	37.3	28.2	81.6	80.5	80.4	53.1	84.0
			d 8	40.9	36.9	85.7	83.9	82.1	58.4	85.9
			d 15	41.8	44.1	86.8	82.5	83.6	58.0	87.1
57903	2	2	d -5	31.7	7.8	80.7	79.3	79.9	53.1	83.3
			d 8	46.8	26.8	86.4	85.3	84.4	52.4	87.7
			d 15	37.0	27.8	85.3	84.1	82.7	49.1	86.7
74001	3	2	d -5	37.7	26.6	81.1	79.4	79.9	55.2	83.1
			d 8	48.7	38.2	83.9	83.9	84.4	53.6	87.0
			d 15	50.5	37.7	83.5	82.7	82.0	50.3	84.5
71302	1	2	d -5	40.3	14.1	81.1	79.7	79.9	48.8	83.9
			d 8	39.5	39.3	83.6	83.2	81.9	59.0	85.6
			d 15	35.8	42.9	82.2	82.6	81.1	52.4	85.5
74510	2	2	d -5	51.3	28.3	83.0	80.4	81.1	58.6	83.9

			d 8	45.8	36.2	84.5	84.2	83.2	51.3	86.2
			d 15	37.8	16.6	82.5	82.5	80.8	46.0	84.9
57203	3	2	d -5	39.6	18.8	82.4	80.5	80.9	53.4	84.2
			d 8	28.8	.	81.1	81.8	80.5	41.1	85.1
			d 15	43.1	29.4	81.5	82.7	82.1	47.4	85.2
74003	1	2	d -5	43.5	29.2	84.4	80.9	81.1	57.1	84.3
			d 8	45.9	52.2	85.9	85.8	83.5	58.7	87.3
			d 15	34.9	38.4	81.7	82.9	79.7	52.8	84.0
72904	2	2	d -5	24.9	.	79.4	77.4	77.6	44.6	81.8
			d 8	41.3	24.4	86.0	86.2	84.9	53.3	88.2
			d 15	34.2	28.9	84.1	84.2	83.3	49.6	87.2
77702	3	2	d -5	42.3	28.5	82.6	80.1	80.5	56.5	83.7
			d 8	39.1	16.5	82.9	82.1	81.0	46.2	85.1
			d 15	54.1	26.1	86.0	84.6	83.3	49.7	87.1
59203	1	3	d -5	39.6	16.5	82.5	80.2	80.2	53.9	83.8
			d 8	46.6	35.3	88.8	87.4	86.0	62.3	89.4
			d 15	40.2	43.1	83.6	82.7	81.3	55.7	83.3
74901	2	3	d -5	39.7	12.0	79.9	79.2	80.5	48.8	83.1
			d 8	33.7	26.8	82.5	81.8	80.2	45.7	83.5
			d 15	45.2	.	86.1	85.0	83.1	45.3	87.3
77601	3	3	d -5	44.6	25.9	85.1	80.9	80.9	56.9	84.2
			d 8	30.6	.	85.0	83.2	83.4	43.2	86.3
			d 15	60.8	30.9	86.1	84.6	84.0	52.4	87.8
68307	1	3	d -5	41.6	22.9	84.7	81.5	81.5	54.5	85.1
			d 8	33.8	33.7	83.7	84.0	81.3	53.1	85.6
			d 15	31.8	40.7	83.7	83.2	82.1	52.3	86.5
58802	2	3	d -5	37.4	13.7	82.9	79.7	80.0	52.3	83.6
			d 8	39.7	24.9	86.1	84.9	84.4	50.1	85.3
			d 15	32.2	25.5	85.7	85.1	83.0	50.4	85.7
68501	3	3	d -5	42.8	13.1	82.5	80.6	81.0	54.4	84.3
			d 8	36.8	10.2	80.9	81.6	80.2	44.0	83.7
			d 15	48.0	19.2	83.3	83.1	82.3	49.5	86.1
75304	1	3	d -5	26.8	20.6	80.8	79.8	80.2	57.1	83.5
			d 8	19.1	16.6	75.7	79.2	76.9	45.8	82.2
			d 15	24.3	21.5	77.6	81.5	78.1	43.2	84.1
78204	2	3	d -5	52.0	42.8	82.4	80.9	82.0	60.9	84.2
			d 8	34.9	27.4	79.7	78.3	78.2	45.8	81.2
			d 15	24.2	27.1	79.8	79.6	77.5	35.8	82.3
75704	3	3	d -5	44.8	.	80.4	78.7	80.8	50.7	82.7
			d 8	45.5	27.1	84.8	83.6	82.7	50.3	86.3
			d 15	58.0	20.4	85.1	85.0	84.0	53.5	87.4
60001	1	4	d -5	43.0	25.0	81.4	79.8	79.9	52.3	83.8
			d 8	15.6	31.0	79.3	79.9	76.3	38.3	81.5

			d 15	22.6	20.6	77.5	79.6	75.2	36.9	81.2
58401	2	4	d -5	35.1	15.6	83.0	81.0	81.5	50.4	83.8
			d 8	3.7	20.0	81.3	81.1	77.1	22.7	82.5
			d 15	26.0	21.1	80.5	80.8	78.5	34.6	82.4
75001	3	4	d -5	39.2	10.9	82.9	81.5	82.0	49.8	84.5
			d 8	39.0	20.4	81.5	81.6	79.3	42.0	84.1
			d 15	41.0	25.7	79.4	81.0	79.6	37.2	82.6
75302	1	4	d -5	39.2	22.3	83.0	80.8	81.0	54.5	84.2
			d 8	38.2	39.2	82.4	82.6	80.6	48.2	84.7
			d 15	17.4	44.2	77.4	79.3	75.7	41.7	81.1
75301	2	4	d -5	35.0	14.0	78.9	78.8	79.3	52.6	82.2
			d 8	30.2	20.0	80.7	81.2	79.3	41.9	83.5
			d 15	25.8	17.7	81.1	80.9	77.5	37.0	81.9
58301	3	4	d -5	39.2	14.3	83.3	81.2	83.0	54.0	84.7
			d 8	30.0	10.5	81.7	81.2	79.9	38.9	83.6
			d 15	26.8	25.5	80.2	79.9	78.4	33.1	80.6
75303	1	4	d -5	41.1	27.5	81.9	79.8	80.1	55.7	83.7
			d 8	16.3	22.5	78.0	79.4	75.3	39.2	81.1
			d 15	15.6	13.7	81.0	80.1	75.6	34.3	81.1
73104	2	4	d -5	42.6	13.2	80.8	79.7	81.2	49.8	83.7
			d 8	35.1	14.4	81.9	82.5	80.3	36.3	85.2
			d 15	34.3	.	81.6	80.4	79.1	31.0	84.1
78205	3	4	d -5	39.9	19.1	81.3	79.9	80.4	54.8	83.6
			d 8	39.1	22.5	80.2	80.7	79.8	34.9	85.7
			d 15	34.0	17.5	79.5	81.4	79.2	34.3	82.6
75505	1	5	d -5	54.4	41.0	85.2	81.6	81.9	63.1	84.9
			d 8	31.6	16.3	80.3	80.4	78.9	48.0	83.8
			d 15	26.3	16.4	78.2	77.5	76.2	40.9	81.2
73002	2	5	d -5	50.1	38.6	84.2	82.2	82.2	59.2	85.4
			d 8	28.4	15.5	80.2	81.0	79.4	37.6	84.6
			d 15	25.0	17.7	79.5	78.1	77.1	30.6	82.5
74802	3	5	d -5	33.0	15.5	82.0	80.4	82.1	48.6	84.3
			d 8	48.8	27.9	84.4	84.4	83.9	48.9	86.7
			d 15	50.4	29.5	83.6	83.6	82.3	43.4	85.7
64101	1	5	d -5	40.8	52.1	81.3	78.2	79.5	52.0	83.3
			d 8	29.5	18.8	82.5	82.1	80.0	44.6	85.3
			d 15	21.7	31.3	79.1	78.5	76.4	39.6	82.1
72602	2	5	d -5	46.1	38.7	85.1	81.6	81.5	58.5	84.8
			d 8	30.9	.	81.3	81.4	80.1	37.4	85.1
			d 15	31.7	35.5	79.6	79.1	79.1	35.4	83.2
69801	3	5	d -5	42.7	33.7	81.4	80.0	79.9	53.6	83.6
			d 8	40.4	34.0	83.4	82.5	81.2	43.1	85.9

			d 15	42.9	35.9	79.9	79.7	79.9	42.9	83.8
77501	1	5	d -5	49.2	40.1	81.2	79.4	79.8	59.2	83.2
			d 8	24.4	10.0	78.5	80.1	78.5	44.4	83.8
			d 15	15.8	13.3	76.2	76.6	74.1	38.9	80.7
75005	2	5	d -5	54.4	19.5	83.3	80.7	81.7	59.4	84.4
			d 8	35.9	11.4	81.4	80.7	81.5	39.2	84.2
			d 15	28.6	16.2	79.8	79.4	79.4	34.9	83.3
73201	3	5	d -5	49.3	34.3	83.6	79.9	80.2	59.4	83.4
			d 8	44.3	29.3	81.0	80.2	79.4	42.2	84.2
			d 15	33.1	31.0	78.7	77.7	78.4	37.2	83.7

[1]d –5, 5 d before farrowing; d 1, 1 d post farrowing; d 8, 8 d post farrowing; d 15, 15 d post farrowing, d 19, 19 d post farrowing (weaning day)

P, Phosphorus; Ca, Calcium; CP, Crude protein, GE, Gross energy; DM, Dry matter; OM, organic matter

Table 2. Effects of Ronozyme® HiPhos supplementation on sows and piglet performance and other variables[1]

	Sow ID	Block	Diet	Parity	Sow's weight, kg				Feed consumed (kg, daily)	Sow's Back fat, mm								Piglets					
					d -5	d 1	d 15	d 19		d -5		d 1		d 15		d 19		d 1		d 15		d 19	
										L	R	L	R	L	R	L	R	No.	wt.	No.	wt	No.	wt.
1	65005	1	1	4	284	270	268	267	5.32	18	17	18	18	16	16	16	17	14	16.4	8	36	8	46
2	74202	1	2	2	252	235	232	235	6.07	18	18	17	17	18	17	15	16	17	21.2	11	51	11	76
3	56404	1	3	6	291	290	273	270	4.56	24	25	23	23	23	21	19	20	8	12.1	11	53	11	73
4	77102	1	1	1	228	211	210	206	4.34	16	17	17	18	18	19	17	17	18	19.7	12	44	12	61
5	75205	1	2	2	231	218	210	209	5.14	22	22	22	22	19	18	18	17	14	21.5	10	48	10	66
6	74705	1	3	2	239	235	230	208	4.89	16	15	15	15	14	14	13	13	9	10.5	12	56	12	70
7	74101	1	1	2	244	224	212	209	4.17	19	19	19	18	15	15	14	15	16	21	12	62	12	80
8	65202	1	2	4	274	268	264	260	4.99	19	19	18	18	18	18	18	18	11	14.6	8	36	8	52
9	69903	1	3	3	265	255	254	250	5.89	18	19	18	18	17	17	16	16	12	20.3	10	57	10	76
10	56903	2	1	6	272	274	258	242	4.12	24	23	22	23	20	21	20	20	11	13.5	8	40	8	50
11	57903	2	2	6	294	289	283	279	5.92	23	25	24	24	22	22	20	21	11	16.3	10	49	10	67
12	74001	2	3	2	235	221	215	191	4.23	19	18	20	18	17	16	13	13	12	16.4	11	55	11	68
13	71302	2	1	3	252	253	237	199	6.48	21	22	20	18	18	17	16	16	11	15.1	9	55	9	67
14	74510	2	2	2	252	242	240	232	5.42	18	18	18	17	18	18	16	16	13	17.4	10	41	10	56
15	57203	2	3	6	280	276	271	236	5.41	26	26	26	26	26	26	25	25	15	17.5	10	43	10	58
16	74003	2	1	2	231	234	229	223	5.46	20	21	20	20	19	18	18	18	12	13.2	10	48	10	62
17	72904	2	2	3	265	254	251	251	4.87	19	19	19	19	17	18	16	17	14	18.2	10	42	10	58
18	77702	2	3	1	192	189	180	176	4.33	15	16	16	16	15	16	14	14	18	17.5	11	41	11	56
19	59203	3	1	6	301	283	288	288	4.13	23	22	22	22	22	21	20	20	18	21.1	9	36	9	55
20	74901	3	2	2	227	205	203	206	4.86	14	13	13	12	12	13	11	11	11	15.1	9	45	9	61
21	77601	3	3	1	210	198	186	182	3.28	18	19	17	17	16	17	16	15	12	14.5	10	42	10	53
22	68307	3	1	4	254	232	223	224	4.13	16	15	14	13	13	12	10	11	15	22.1	10	50	10	66
23	58802	3	2	6	298	297	294	300	5.85	24	23	23	23	23	22	23	22	10	12.3	10	38	10	52
24	68501	3	3	4	269	266	251	238	5.42	21	21	20	20	18	19	17	17	10	15.2	10	54	10	72
25	75304	3	1	2	236	228	228	228	5.27	18	19	19	19	19	18	17	17	14	16.9	10	44	10	53
26	78204	3	2	1	184	176	175	172	5.02	15	16	16	16	14	15	15	15	14	17.2	10	39	10	50

27	75704	3	3	2	238	232	216	220	4.60	20	20	20	20	18	18	16	16	13	17.9	10	48	10	66
28	60001	4	1	5	285	266	270	272	4.57	16	15	15	15	15	14	15	14	13	17.4	12	47	10	58
29	58401	4	2	6	286	270	266	266	5.02	22	21	21	22	21	21	19	19	18	19.1	8	36	8	45
30	75001	4	3	2	214	213	231	235	5.75	18	17	17	16	17	17	17	17	5	6	8	44	8	55
31	75302	4	1	2	219	197	206	205	6.27	15	14	16	15	16	15	15	15	15	22.2	8	44	8	53
32	75301	4	2	2	217	207	190	184	6.29	21	20	20	20	18	17	16	16	9	14.2	12	61	12	75
33	58301	4	3	6	266	265	271	268	5.69	23	22	22	22	23	22	21	21	20	17.6	10	39	10	54
34	75303	4	1	2	230	205	213	215	6.22	14	13	13	13	13	13	11	11	16	20	11	51	11	70
35	73104	4	2	3	289	277	268	257	6.31	18	17	16	16	13	14	13	14	12	21.8	10	65	10	81
36	78205	4	3	1	192	184	192	189	4.65	20	19	20	19	20	19	18	17	10	12.9	10	39	10	56
37	75505	5	1	2	234	230	230	226	5.14	17	17	17	18	16	17	18	18	12	17.2	8	53	10	28
38	73002	5	2	3	265	257	258	250	6.01	18	19	17	17	18	18	19	19	10	16.8	9	53	9	63
39	74802	5	3	2	201	208	202	208	5.36	20	19	19	17	18	18	16	16	8	12.7	9	53	9	64
40	64101	5	1	4	308	298	290	290	6.02	22	22	20	21	19	19	19	19	15	17.8	12	58	12	79
41	72602	5	2	3	275	267	262	258	5.85	19	19	17	17	17	18	18	18	16	18.5	12	55	12	74
42	69801	5	3	4	258	246	249	244	5.80	17	16	17	16	17	15	16	14	14	20.3	10	53	10	73
43	77501	5	1	1	194	195	188	184	96.0	19	18	18	18	17	18	16	16	12	12	11	46	11	58
44	75005	5	2	2	216	206	202	190	5.47	16	16	16	15	14	14	13	12	12	19.9	12	65	12	83
45	73201	5	3	3	264	264	258	248	5.80	16	16	17	16	14	15	15	14	9	17	12	73	12	96

[1]d –5, 5 d before farrowing; d 1, 1 d post farrowing; d 15, 15 d post farrowing, d 19, 19 d post farrowing (weaning day)

L, left; R, right; No, number; wt, weight (kg)

Table 3. Effects of Ronozyme® HiPhos supplementation on plasma variables[1]

	Sow ID	Diet	Block	Day	P	Ca	Mg	Mn	Fe	Co	Cu	Zn	Se	Mb	Na	K	Cl
					mmol/l	mmol/l	mmol/l	ppm	ppm	ppb	ppm	ppm	ppm	ppm	mmol/l	mmol/l	mmol/l
1	65005	1	1	d 1	2.36	2.67	0.81	0.005	2.040	0.770	2.140	0.670	0.200	0.012	151.0	5.20	108.0
2	65005			d15	1.96	2.67	0.92	0.010	1.800	4.300	2.170	1.120	0.320	0.008	147.0	4.80	105.0
3	74202	2	1	d 1	1.49	2.87	0.91	0.005	0.950	0.790	2.380	0.580	0.240	0.012	149.0	4.50	104.0
4	74202			d 15	1.43	3.04	0.89	0.005	1.570	1.090	1.870	0.890	0.320	0.012	148.0	3.90	104.0
5	56404	3	1	d 1	2.02	2.81	0.80	0.004	3.600	0.530	1.780	0.550	0.220	0.012	150.0	4.60	107.0
6	56404			d 15	1.47	2.91	0.86	0.004	2.350	0.960	1.830	1.100	0.340	0.009	149.0	3.90	106.0
7	77102	1	1	d 1	2.79	2.72	1.02	0.004	2.680	0.450	2.140	0.770	0.220	0.007	152.0	4.40	105.0
8	77102			d15	2.19	2.66	0.99	0.004	2.270	0.530	1.910	0.510	0.220	0.013	148.0	4.50	106.0
9	75205	2	1	d 1	1.73	3.00	0.78	0.004	4.310	1.590	2.440	0.890	0.270	0.009	150.0	4.40	108.0
10	75205			d 15	1.17	3.16	0.94	0.003	2.800	0.810	1.820	1.120	0.310	0.016	153.0	4.00	104.0
11	74705	3	1	d 1	2.59	2.71	0.96	0.005	1.880	0.670	2.270	0.720	0.190	0.008	154.0	4.60	104.0
12	74705			d 15	1.44	2.91	0.94	0.005	2.500	0.660	1.880	0.740	0.320	0.016	151.0	4.70	106.0
13	74101	1	1	d 1	2.79	2.50	0.82	0.003	1.250	0.360	1.920	0.700	0.180	0.002	150.0	3.90	100.0
14	74101			d15	2.44	2.74	1.04	0.004	2.920	1.350	1.830	0.910	0.290	0.014	151.0	4.50	107.0
15	65202	2	1	d 1	2.06	2.56	0.80	0.003	1.460	0.780	2.450	0.480	0.250	0.012	153.0	4.40	108.0
16	65202			d 15	1.49	2.82	0.83	0.002	1.160	0.450	2.120	0.610	0.280	0.013	149.0	4.10	105.0
17	69903	3	1	d 1	2.47	2.62	0.93	0.006	1.530	0.590	1.510	0.440	0.180	0.009	152.0	4.70	105.0
18	69903			d 15	2.11	2.90	1.04	0.004	2.020	0.720	1.670	0.730	0.300	0.012	152.0	3.70	105.0
19	56903	1	2	d 1	2.40	3.07	0.90	0.004	3.380	0.680	2.040	0.660	0.310	0.009	153.0	3.90	107.0
20	56903			d15	1.96	2.69	0.94	0.004	1.780	0.540	1.640	0.710	0.290	0.005	150.0	3.90	106.0
21	57903	2	2	d 1	1.23	3.01	0.81	0.004	3.970	0.760	2.410	0.530	0.320	0.016	149.0	4.10	106.0
22	57903			d 15	1.23	3.20	1.00	0.005	2.260	1.240	2.290	0.780	0.390	0.014	148.0	4.20	100.0
23	74001	3	2	d 1	2.62	2.53	0.72	0.004	1.480	0.630	2.300	0.380	0.230	0.004	149.0	4.20	102.0
24	74001			d 15	1.63	2.96	0.97	0.004	1.580	1.030	2.030	0.870	0.320	0.012	146.0	4.20	101.0
25	71302	1	2	d 1	2.32	2.79	0.87	0.004	0.930	0.630	2.140	0.580	0.240	0.012	152.0	4.40	111.0
26	71302			d15	2.06	2.87	0.89	0.003	2.150	0.860	1.810	0.750	0.340	0.018	153.0	5.30	109.0

27	74510	2	2	d 1	1.81	2.44	0.86	0.003	1.370	0.685	2.205	0.380	0.174	0.016	142.0	5.40	103.0
28	74510			d 15	1.98	2.66	0.90	0.004	1.205	0.865	1.910	0.855	0.242	0.010	141.0	4.50	100.0
29	57203	3	2	d 1	1.91	2.65	0.79	0.005	1.780	2.570	2.260	0.540	0.270	0.013	150.0	4.00	109.0
30	57203			d 15	1.81	2.75	1.09	0.003	2.150	0.600	1.970	0.790	0.350	0.012	149.0	4.10	105.0
31	74003	1	2	d 1	2.83	3.06	0.85	0.006	4.270	0.990	2.780	0.720	0.260	0.016	154.0	4.50	108.0
32	74003			d15	2.66	2.84	0.98	0.002	3.090	0.910	2.150	0.770	0.250	0.020	151.0	4.90	105.0
33	72904	2	2	d 1	1.46	2.55	0.71	0.003	1.395	0.465	2.045	0.300	0.188	0.013	146.0	4.20	104.0
34	72904			d 15	1.81	2.80	0.80	0.004	1.745	1.055	1.570	0.715	0.279	0.012	142.0	4.20	101.0
35	77702	3	2	d 1	2.49	2.78	0.89	0.003	2.230	0.275	2.170	0.585	0.235	0.015	146.0	5.00	102.0
36	77702			d 15	1.92	2.85	0.94	0.003	2.090	0.765	1.520	0.725	0.233	0.016	141.0	5.00	100.0
37	59203	1	3	d 1	2.11	2.57	0.85	0.003	3.095	0.865	1.485	0.505	0.176	0.015	140.0	5.80	102.0
38	59203			d15	1.74	2.80	1.01	0.003	1.820	0.720	1.440	0.775	0.230	0.011	143.0	4.60	105.0
39	74901	2	3	d 1	2.76	2.39	0.72	0.006	2.140	0.705	2.030	0.510	0.181	0.005	147.0	4.40	105.0
40	74901			d 15	1.94	2.68	1.00	0.003	1.390	0.995	1.620	0.785	0.300	0.009	142.0	3.90	101.0
41	77601	3	3	d 1	2.46	2.62	0.97	0.009	1.695	0.385	2.420	0.660	0.202	0.011	144.0	4.90	101.0
42	77601			d 15	1.69	2.89	0.83	0.009	1.700	0.885	2.185	0.830	0.178	0.015	140.0	4.20	98.0
43	68307	1	3	d 1	1.88	2.36	0.85	0.002	1.715	0.350	2.205	0.445	0.166	0.009	142.0	4.10	103.0
44	68307			d15	1.91	2.61	0.99	0.003	1.670	1.175	1.850	0.775	0.236	0.008	143.0	4.10	103.0
45	58802	2	3	d 1	1.24	2.89	0.93	0.003	2.130	0.395	1.615	0.450	0.175	0.020	147.0	4.00	101.0
46	58802			d 15	1.65	2.83	0.91	0.006	2.020	0.800	1.765	0.790	0.218	0.009	143.0	3.90	103.0
47	68501	3	3	d 1	1.47	2.66	0.80	0.003	1.925	0.405	2.255	0.450	0.163	0.019	146.0	3.90	105.0
48	68501			d 15	1.50	2.53	0.87	0.004	1.780	0.630	1.640	0.830	0.233	0.015	141.0	4.30	103.0
49	75304	1	3	d 1	2.07	2.80	0.78	0.003	2.485	0.365	1.945	0.465	0.180	0.016	149.0	4.00	108.0
50	75304			d15	1.84	2.82	0.85	0.003	1.865	1.015	1.535	0.765	0.187	0.015	145.0	3.90	104.0
51	78204	2	3	d 1	2.02	2.90	0.85	0.005	2.345	1.440	2.600	0.665	0.204	0.014	150.0	4.70	106.0
52	78204			d 15	1.90	3.00	0.99	0.005	1.435	0.595	2.240	0.925	0.230	0.011	145.0	4.60	101.0
53	75704	3	3	d 1	1.98	2.87	0.86	0.005	2.450	0.390	1.740	0.605	0.195	0.018	148.0	4.10	103.0
54	75704			d 15	1.76	2.86	0.83	0.004	1.920	0.710	1.535	0.925	0.247	0.012	143.0	4.00	100.0
55	60001	1	4	d 1	2.24	2.56	0.80	0.008	1.185	1.055	2.385	0.480	0.225	0.013	144.0	4.20	103.0

56	60001			d15	1.67	2.69	0.98	0.006	1.545	0.710	1.600	0.590	0.196	0.015	141.0	4.50	104.0
57	58401	2	4	d 1	1.73	2.53	0.68	0.006	1.610	0.885	1.870	0.260	0.229	0.011	145.0	4.20	105.0
58	58401			d 15	2.94	2.67	0.86	0.004	0.325	0.880	2.015	0.450	0.260	0.006	141.0	4.80	98.0
59	75001	3	4	d 1	2.41	2.58	0.79	0.005	2.210	0.535	2.640	0.435	0.204	0.009	145.0	4.10	104.0
60	75001			d 15	2.37	2.80	1.01	0.006	1.815	0.730	1.570	0.665	0.236	0.013	140.0	4.90	101.0
61	75302	1	4	d 1	1.81	2.41	0.73	0.003	1.880	0.635	2.855	0.605	0.215	0.005	143.0	3.50	101.0
62	75302			d15	2.04	2.76	0.86	0.008	2.315	0.910	1.520	0.895	0.201	0.013	143.0	3.80	101.0
63	75301	2	4	d 1	1.44	2.64	0.78	0.003	2.535	0.800	1.925	0.585	0.186	0.014	146.0	3.60	102.0
64	75301			d 15	1.32	3.08	0.85	0.006	1.985	0.710	1.430	0.695	0.220	0.012	143.0	3.90	104.0
65	58301	3	4	d 1	1.38	2.42	0.89	0.005	2.195	0.445	1.640	0.505	0.183	0.016	142.0	3.80	103.0
66	58301			d 15	1.60	2.77	0.93	0.006	2.455	0.690	1.405	0.670	0.192	0.015	141.0	4.20	102.0
67	75303	1	4	d 1	2.56	2.58	0.76	0.004	1.430	0.470	1.800	0.350	0.159	0.011	144.0	4.30	102.0
68	75303			d15	1.93	2.76	0.84	0.004	1.615	0.635	1.305	1.570	0.214	0.013	142.0	4.00	102.0
69	73104	2	4	d 1	1.33	2.62	0.76	0.005	1.625	0.765	2.840	0.505	0.200	0.015	147.0	4.40	105.0
70	73104			d 15	1.24	2.61	0.88	0.007	1.490	0.795	1.875	1.300	0.234	0.012	141.0	4.40	101.0
71	78205	3	4	d 1	2.45	2.70	0.87	0.004	2.315	0.275	2.165	0.535	0.201	0.012	145.0	5.40	102.0
72	78205			d 15	2.38	2.81	0.90	0.014	1.360	1.080	1.820	1.100	0.221	0.010	145.0	4.80	102.0
73	75505	1	5	d 1	2.24	2.54	0.83	0.008	1.980	0.615	3.285	0.470	0.195	0.011	147.0	4.30	104.0
74	75505			d15	2.05	2.69	0.89	0.006	1.790	0.650	1.875	0.730	0.203	0.011	143.0	3.90	103.0
75	73002	2	5	d 1	1.25	2.87	0.78	0.004	3.610	0.945	1.990	0.600	0.207	0.016	145.0	3.90	103.0
76	73002			d 15	1.51	2.73	0.96	0.006	1.695	0.590	2.330	0.910	0.193	0.009	145.0	3.70	104.0
77	74802	3	5	d 1	1.59	2.70	0.74	0.003	2.150	0.820	1.925	0.455	0.190	0.012	148.0	3.60	107.0
78	74802			d 15	1.51	2.68	0.97	0.004	1.880	0.770	1.905	0.955	0.203	0.012	142.0	4.50	102.0
79	64101	1	5	d 1	2.22	2.67	0.81	0.004	2.790	0.585	3.060	0.485	0.235	0.016	145.0	3.90	102.0
80	64101			d15	1.96	2.70	0.96	0.006	1.650	0.640	1.615	0.720	0.200	0.015	142.0	4.20	104.0
81	72602	2	5	d 1	1.12	2.65	0.79	0.004	2.350	0.610	3.065	0.565	0.210	0.011	147.0	3.70	104.0
82	72602			d 15	2.18	2.93	0.93	0.005	1.815	0.630	1.940	0.775	0.234	0.013	143.0	3.80	100.0
83	69801	3	5	d 1	2.00	2.72	0.80	0.005	2.175	0.615	2.845	0.495	0.200	0.014	149.0	4.80	104.0
84	69801			d 15	1.69	2.53	0.90	0.006	1.780	0.700	1.490	0.635	0.200	0.012	143.0	4.10	103.0

85	77501	1	5	d 1	2.38	2.65	0.87	0.003	1.840	0.710	1.900	0.485	0.230	0.013	142.0	4.00	101.0
86	77501			d15	1.97	2.62	0.90	0.004	1.400	0.555	1.525	0.615	0.233	0.016	142.0	4.50	104.0
87	75005	2	5	d 1	2.40	2.46	0.71	0.003	1.570	0.500	3.230	0.505	0.207	0.010	142.0	4.00	101.0
88	75005			d 15	2.07	2.95	0.92	0.004	0.905	0.565	1.590	0.500	0.225	0.018	140.0	5.00	101.0
89	73201	3	5	d 1	1.31	2.53	0.74	0.004	1.565	0.595	3.115	0.535	0.207	0.011	147.0	3.70	107.0
90	73201			d 15	1.50	2.71	0.99	0.005	1.230	0.655	1.790	0.735	0.226	0.018	142.0	4.20	103.0

	Sow ID	Diet	Block	Day	Urea	Creatinine	Glucose	Total protein	Albumin	Globulin	Albumin/Globulin ratio
					mmol/l	mmol/l	mmol/l	G/L	G/L	G/L	
1	65005	1	1	d 1	4.20	217.0	5.60	83.0	43.0	40.0	1.08
2	65005			d15	7.50	142.0	4.50	91.0	43.0	48.0	0.90
3	74202	2	1	d 1	5.00	161.0	4.20	88.0	48.0	40.0	1.20
4	74202			d 15	8.70	128.0	5.30	85.0	46.0	39.0	1.18
5	56404	3	1	d 1	4.70	207.0	5.00	77.0	50.0	27.0	1.85
6	56404			d 15	5.50	165.0	5.10	85.0	50.0	35.0	1.42
7	77102	1	1	d 1	6.80	242.0	5.80	82.0	48.0	34.0	1.41
8	77102			d15	9.10	158.0	5.10	81.0	51.0	30.0	1.70
9	75205	2	1	d 1	4.40	164.0	6.00	75.0	51.0	24.0	2.13
10	75205			d 15	8.50	133.0	4.10	81.0	53.0	28.0	1.89
11	74705	3	1	d 1	7.50	216.0	4.30	78.0	42.0	36.0	1.17
12	74705			d 15	8.20	168.0	4.70	85.0	47.0	38.0	1.24
13	74101	1	1	d 1	5.70	221.0	4.50	81.0	52.0	29.0	1.79
14	74101			d15	5.20	186.0	6.70	80.0	50.0	30.0	1.67
15	65202	2	1	d 1	6.70	234.0	4.80	80.0	47.0	33.0	1.42
16	65202			d 15	7.70	164.0	5.20	86.0	46.0	40.0	1.15
17	69903	3	1	d 1	5.40	228.0	4.50	74.0	43.0	31.0	1.39
18	69903			d 15	9.20	167.0	5.00	82.0	46.0	36.0	1.28
19	56903	1	2	d 1	4.30	170.0	5.20	80.0	49.0	31.0	1.58
20	56903			d15	5.80	152.0	4.20	79.0	47.0	32.0	1.47
21	57903	2	2	d 1	4.90	217.0	5.30	82.0	45.0	37.0	1.22
22	57903			d 15	11.50	160.0	6.10	87.0	47.0	40.0	1.17
23	74001	3	2	d 1	4.80	233.0	3.40	77.0	47.0	30.0	1.57
24	74001			d 15	7.60	169.0	4.40	83.0	48.0	35.0	1.37
25	71302	1	2	d 1	5.40	161.0	5.00	70.0	46.0	24.0	1.92
26	71302			d15	8.00	148.0	5.10	78.0	48.0	30.0	1.60

27	74510	2	2	d 1	5.80	164.0	4.00	72.0	40.0	32.0	1.25
28	74510			d 15	7.50	131.0	4.50	76.0	41.0	35.0	1.17
29	57203	3	2	d 1	5.50	239.0	5.70	74.0	45.0	29.0	1.55
30	57203			d 15	9.00	172.0	4.20	88.0	51.0	37.0	1.38
31	74003	1	2	d 1	6.60	178.0	4.40	81.0	52.0	29.0	1.79
32	74003			d15	8.90	145.0	4.90	78.0	47.0	31.0	1.52
33	72904	2	2	d 1	4.60	191.0	4.50	73.0	44.0	29.0	1.52
34	72904			d 15	8.00	166.0	5.40	81.0	47.0	34.0	1.38
35	77702	3	2	d 1	7.60	185.0	5.20	81.0	50.0	31.0	1.61
36	77702			d 15	10.70	167.0	5.10	80.0	48.0	32.0	1.50
37	59203	1	3	d 1	4.80	217.0	4.90	77.0	44.0	33.0	1.33
38	59203			d15	6.00	157.0	5.30	83.0	49.0	34.0	1.44
39	74901	2	3	d 1	6.10	225.0	3.30	76.0	48.0	28.0	1.71
40	74901			d 15	7.70	1.9	4.80	83.0	46.0	37.0	1.24
41	77601	3	3	d 1	6.50	207.0	5.00	78.0	50.0	28.0	1.79
42	77601			d 15	6.90	167.0	4.30	74.0	46.0	28.0	1.64
43	68307	1	3	d 1	4.20	194.0	4.30	75.0	49.0	26.0	1.88
44	68307			d15	5.60	145.0	4.20	84.0	45.0	39.0	1.15
45	58802	2	3	d 1	5.40	200.0	5.30	75.0	44.0	31.0	1.42
46	58802			d 15	7.40	143.0	3.60	82.0	48.0	34.0	1.41
47	68501	3	3	d 1	6.20	171.0	5.80	67.0	47.0	20.0	2.35
48	68501			d 15	9.30	133.0	4.80	70.0	44.0	26.0	1.69
49	75304	1	3	d 1	5.80	165.0	4.50	77.0	52.0	25.0	2.08
50	75304			d15	8.90	131.0	5.40	75.0	47.0	28.0	1.68
51	78204	2	3	d 1	5.20	195.0	4.00	77.0	47.0	30.0	1.57
52	78204			d 15	8.30	137.0	4.60	78.0	48.0	30.0	1.60
53	75704	3	3	d 1	5.40	177.0	5.30	77.0	47.0	30.0	1.57
54	75704			d 15	6.50	138.0	4.60	78.0	46.0	32.0	1.44
55	60001	1	4	d 1	6.70	272.0	4.70	69.0	46.0	23.0	2.00

56	60001			d15	9.80	164.0	4.40	80.0	46.0	34.0	1.35
57	58401	2	4	d 1	4.60	235.0	4.90	75.0	45.0	30.0	1.50
58	58401			d 15	6.00	137.0	3.20	89.0	45.0	44.0	1.02
59	75001	3	4	d 1	4.00	189.0	4.80	61.0	46.0	15.0	3.07
60	75001			d 15	9.60	128.0	5.20	72.0	46.0	26.0	1.77
61	75302	1	4	d 1	4.80	221.0	3.10	77.0	48.0	29.0	1.66
62	75302			d15	7.60	133.0	4.60	77.0	47.0	30.0	1.57
63	75301	2	4	d 1	7.40	191.0	4.50	76.0	47.0	29.0	1.62
64	75301			d 15	7.90	155.0	7.00	76.0	44.0	32.0	1.38
65	58301	3	4	d 1	6.00	202.0	4.90	76.0	42.0	34.0	1.24
66	58301			d 15	8.10	127.0	4.80	80.0	44.0	36.0	1.22
67	75303	1	4	d 1	6.10	203.0	4.40	70.0	41.0	29.0	1.41
68	75303			d15	8.50	154.0	4.40	75.0	40.0	35.0	1.14
69	73104	2	4	d 1	4.60	231.0	4.50	76.0	50.0	26.0	1.92
70	73104			d 15	8.70	167.0	4.20	77.0	47.0	30.0	1.57
71	78205	3	4	d 1	5.40	173.0	5.20	77.0	46.0	31.0	1.48
72	78205			d 15	7.70	135.0	5.10	76.0	47.0	29.0	1.62
73	75505	1	5	d 1	3.80	192.0	5.30	74.0	44.0	30.0	1.47
74	75505			d15	7.70	139.0	4.20	73.0	43.0	30.0	1.43
75	73002	2	5	d 1	7.10	209.0	5.00	69.0	44.0	25.0	1.76
76	73002			d 15	7.80	150.0	4.70	75.0	42.0	33.0	1.27
77	74802	3	5	d 1	3.80	178.0	5.20	63.0	46.0	17.0	2.71
78	74802			d 15	5.80	126.0	4.80	74.0	47.0	27.0	1.74
79	64101	1	5	d 1	4.70	244.0	5.20	72.0	49.0	23.0	2.13
80	64101			d15	7.90	164.0	4.70	77.0	48.0	29.0	1.66
81	72602	2	5	d 1	5.90	214.0	4.60	69.0	47.0	22.0	2.14
82	72602			d 15	10.20	161.0	4.70	74.0	48.0	26.0	1.85
83	69801	3	5	d 1	5.00	220.0	5.60	74.0	48.0	26.0	1.85
84	69801			d 15	9.10	155.0	4.80	78.0	47.0	31.0	1.52

85	77501	1	5	d 1	6.80	160.0	4.30	85.0	46.0	39.0	1.18
86	77501			d15	6.50	147.0	4.70	79.0	44.0	35.0	1.26
87	75005	2	5	d 1	6.70	178.0	5.70	61.0	44.0	17.0	2.59
88	75005			d 15	10.60	129.0	5.00	74.0	45.0	29.0	1.55
89	73201	3	5	d 1	4.50	231.0	4.00	73.0	43.0	30.0	1.43
90	73201			d 15	9.20	183.0	4.50	78.0	45.0	33.0	1.36

[1]d 1, 1 d post farrowing; d 15, 15 d post farrowing,

P, Phosphorus; Ca, Calcium; Mg, Magnesium; Mn, Manganese; Fe, Iron; Co, Cobalt; Zn, Zinc; Mb, Molybdenum; Na, Sodium; K, Potassium; Cl, Chloride;

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ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **Ronozyme® HiPhos**	Batch number: **Lot ELN-09FIBo0031-2GT**
Trial ID: **RTZ/ZN-2011-Phytase**	Location: **Swine Research & Technology Centre F-62, University of Alberta, Edmonton, AB, Canada**
Start date and exact duration of the study: **October 18, 2011 (5 months, 12 days)**	
Number of treatment groups (+ control(s)): **3**	Replicates per group: **15**
Total number of animals: **45**	Animals per replicate: **1**
Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg⁻¹ complete feed/L⁻¹ water)	
Intended: **500 units of Phytase/kg diet**	Analysed: **765 U/kg, incl. intrinsic Phytase activity**
†	
Substances used for comparative purposes:	
Intended dose:	Analysed:
Animal species/category: **Swine (lactating sows)**	
Breed: **F1 sows (Large White x Landrace)**	Identification procedure: **Ear tag and Pen number**
Sex: **Female** Age at start:	Body weight at start: **248 kg**
Physiological stage: **Lactating**	General health: **Good**
Additional information for field trials:	
Location and size of herd or flock: **Swine Research & Technology Centre, F-62, Edmonton Research Station, University of Alberta, Edmonton, AB, Canada T6H 2V8**	
Feeding and rearing conditions: **Individual feeding**	
Method of feeding: **Manual**	
Diets (type(s)): **Lactating sow diet**	
Presentation of the diet: Mash ☐ Pellet ☐ Extruded ☐	Other **Crumbles**
Composition (main feedingstuffs): **Wheat, Soybean meal**	
Nutrient content (relevant nutrients and energy content)	
Intended values: **CP: 20.45%; ME:3290 Kcal, Total Phosphorus:0.45%,**	
Analysed values: **CP: 23.2%; Gross Energy: 4048 Kcal, Total Phosphorus:0.59%,**	
Date and nature of the examinations performed: **Nutrient digestibility, plasma variables, perfromance**	
Method(s) of statistical evaluation used: **Proc- Mixed Modeal (SAS)**	
Therapeutic/preventive treatments (reason, timing, kind, duration): **No**	
Timing and prevalence of any undesirable consequences of treatment: **No**	
Date **2012 Jul 25**	Signature Study Director [signature]

[1] Please submit this form using a common word processing format (e.g. MS Word).

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

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TAB

13

<u>Annex 13</u>

Excerpt of

Comparative effects of RONOZYME® HiPhos (M), Phyzyme® XP 4000 TPT and OptiPhos® 2000 PF on the zootechnical performance and the mineral utilization in the growing pig.

REPORT No. 00013767

EXCERPT OF:

REPORT No. 00013767
Research Project Document

Document Date: 14-Mar-2012
Excerpt Date: 27-June-2013

Author(s): Guggenbuhl P, Waché Y, Simões Nunes C, Portier C, Kurtz N, Lehmann A and Bilger M

Title: Comparative effects of RONOZYME® HiPhos (M), Phyzyme® XP 4000 TPT and OptiPhos® 2000 PF on the zootechnical performance and the mineral utilization in the growing pig.

Excerpt Contents: Bone Properties

Project No. 6106
Compound No.
Summary

1. INTRODUCTION

The aim of the present study (S10-2010) was to evaluate the effects of the microbial 6-phytase, RONOZYME® HiPhos (M) comparatively to the phytases Phyzyme® XP 4000 TPT and OptiPhos® 2000 PF on the zootechnical performance, mineral blood concentrations and the utilisation of phosphorus (P) and calcium (Ca) in the growing pig. The experiment was performed during June-September 2010 in the facilities of the Centre de Recherche en Nutrition Animale (CRNA), DSM Nutritional Products France, BP 170, 68305 Saint-Louis cedex, France. It has been performed according to the French legal regulations on experiments with live animals.

2. MATERIAL AND METHODS

2.1. Test compounds

The used RONOZYME® HiPhos (M) phytase (batch PPQ 30595) was expressed in *Aspergillus oryzae,* had an activity at pH 5.5 of 63046 U/g and was in mash micro-granulated form. Phyzyme® XP 4000 TPT (batch 1248168) and OptiPhos® 2000 PF (batch 12248A01) had an activity at pH 5.5 of 5421 U/g and 5568 U/g, respectively. NIC-RD/A measured the phytase activity in the enzyme preparations and in the feed. One unit of phytase is defined as the quantity of enzyme which sets free 1 µmole of inorganic phosphate per minute from 0.005 moles per litre sodium phytate at pH 5.5 and at 37°C. Dicalcium phosphate (DCP), batch S1784 6212, was supplied by TIMAC Industries and had a P concentration of 18.2 % and a Ca concentration of 24 %.

2.2. Animal trial

Sixty four Large-White ( ) x Redon ( ) growing pigs having an initial body weight of 16.66 ± 1.54 kg were used. The animals were allocated to 8 equal groups of 8 animals each and housed in floor-pen cages in two sub-groups of 4 animals each in an environmentally controlled room. Each pen had a plastic-coated welded wire floor and was equipped with two water nipples and four stainless-steel individualized feeders. Room temperature was 21-22° C and humidity percentage was 50 %. The piglets were fed, throughout a 71-day observation period, a basal diet without addition of mineral P (group A: control(-)) or the diet A supplemented with 12 g/kg of DCP (group B: control(+)) or with RONOZYME® HiPhos (M) at the levels of 1000 U/kg (group C) and 1500 U/kg (group D), Phyzyme® XP 4000 TPT at the levels of 500 U/kg (group E) and 750 U/kg (group F) and OptiPhos® 2000 PF at the levels of 500 U/kg (group G) and 750 U/kg (group H). The basal diet A (Table 1) was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the digestible P supply, the animals' requirements according to Henry *et al.* (1989) and NRC (1998). The total P content was of 0.38 % and of 0.81 % in control(-) diet and control(+) diet, respectively. According to the NRC tables the theoretical digestible P in the control(-) diet was 0.11 g/kg and 0.28 g/kg in the control(+) diet.

An indigestible marker, chromium oxide (Cr), was added at a concentration of 0.4 % to all the diets allowing calculation of the digestibility of P and Ca. The feed was distributed *ad libitum* in mash form, under pen feed consumption control, and the animals had free access to drinking water. The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water in the region is 80 mg/L. Performance was evaluated after 29 days of experimentation and at the end of the 71 days of the trial duration. Blood was collected by jugular puncture from all the animals at the 28th day and 70th day of the experiment for the

determination of the P, Ca and alkaline phosphatase concentrations. Faecal P, Ca and Cr concentrations were measured at the 29th day and the 71st day of the second period. Faeces were sampled individually, in approximately the same amount at the same time of the day, during the last 3 days preceding that date. All minerals were determined according to standard Association of Official Analytical Chemists (1990) methods using a Vista-MPX ICP-OES spectrometer (Varian Australia Pty Ltd, Mulgrave Victoria, 3170 Australia). The apparent digestibility (% of the intake) of the minerals was calculated for the mentioned 3-day period. At the end of the evaluation the animals were slaughtered after tranquilization followed by electronarcosis for bone collection. The collected bones were the right external metacarpal and metatarsal. Samples were prepared from each of the collected bones immediately after slaughter. After careful dissection and removal of the soft tissue, a diaphysis section was obtained by sawing each bone. The obtained sections of about 3.5-cm long were immediately subjected to compression in order to determine the force in Newton necessary to break them (maximal-breaking force at the fracture point). The measurements were performed with a LR10K compression machine, using a XLC/10K/A1 force captor and a compression device TH23-196/AL (Lloyd Instruments, Fareham, UK).
The broken bones were then used for the determination of ash content, which was measured after 48-h incineration at 550°C.

2.3. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu + Ai + Bij + Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Snedecor and Cochran, 1989). These calculations were performed using StatGraphics Plus 5.1 (Manugistics, Rockville, U.S.A. 2001).

2.4. Effects on bone parameters

The results of the external metacarpal and external metatarsal bone resistance and mineralization are presented in Table 3. The bone resistance of the external metacarpal was dose dependently and significantly increased in all phytase supplemented groups as well as in that receiving DCP comparatively to the control(-). The DCP supplemented animals had an external metacarpal bone breaking force significantly higher than those of the control(-) and all phytases groups. In counter-part the external metacarpal bone mineralization was only significantly improved in the RONOZYME® HiPhos 1500 U/kg and control(+) fed pigs comparatively to the control(-) group. The differences to the control (-) of the external metatarsal bone resistance were dose dependently increased with the three tested phytases. The breaking force was significantly improved in all phytase supplemented groups with the exception of OptiPhos® 2000 PF 500 U/kg. The highest bone strength was observed for the animals receiving the control(+) diet and was significantly higher than those of the control(-) and all phytases groups. The external metatarsal bone mineralization of the RONOZYME® HiPhos 1000 U/kg and OptiPhos® 2000 PF 500 U/kg groups was similar to that of the control(-) fed animals. It was significantly improved in all other phytase supplemented groups. In the control(+) supplemented pigs the external metatarsal bone mineralization was significantly higher than in all other groups.

The mean breaking force of both bones, comparatively to the control (-) fed pigs (80 N), was dose dependently and significantly increased in the RONOZYME® HiPhos 1000 U/kg (144 N) and 1500 U/kg (169 N), Phyzyme® XP 4000 TPT 500 U/kg (136 N) and 750 U/kg (152 N) and OptiPhos® 2000 PF 500 U/kg (112 N) and 750 U/kg (132 N) by 81, 112, 70, 90, 40 and 66 %, respectively. That of the control (+) group (235 N) was significantly higher than the breaking force of all other groups.

Table 1 - Composition (%) of the control (-) diet and of control (+) diet supplemented with DCP.

INGREDIENTS	Control(-) diet without mineral P (%)	Control(+) diet with DCP (%)
Maize	58.0	58.0
Soybean meal	17.85	17.85
Rapeseed meal	14.15	14.15
Oat meal	6.0	6.0
Soya oil	1.0	1.0
Dicalcium phosphate	-	1.2
Minerals(1), vitamins and synthetic aa	3.0	1.8
Analyzed Crude Protein - %	*18.28*	*18.43*
Analyzed Crude Ash - %	*4.93*	*6.21*
Analyzed Lysine - %	*1.13*	*1.10*
Analyzed Threonine - %	*0.54*	*0.56*
Analyzed Methionine + cysteine - %	*0.64*	*0.63*
Analyzed total Ca - %	*0.86*	*1.42*
Analyzed total P - %	*0.38*	*0.81*
Analyzed Phytic P - %	*0.11*	*0.11*
Theoretically digestible P - %	*0.11*(2)	*0.28*(3)
Estimated digestible energy - MJ/kg	*15.28*	*15.42*

(1) Mixture without mineral P.
(2) Digestible P estimated from the NRC tables.
(3) Sum of the digestible P estimated from the NRC tables and 80 % of added mineral P as generally accepted.

Table 2 - Phytase activity (U(a)/kg) and % of the target in the different diets.

Treatment groups	Control (-)	DCP 12 g/kg	RONOZYME® HiPhos (M)		Phyzyme®XP 4000 TPT		OptiPhos® 2000 PF	
Programmed phytase addition (U/kg)	0	0	1000	1500	500	750	500	750
Measured phytase activity (U/kg)(1)	<LOQ	<LOQ	1068 ±32	1568 ±30	520 ±41	753 ±22	377 ±173	702 ±27
Actually added phytase (U/kg)	-	-	1068	1568	520	753	377	702
% of target	-	-	107	105	104	100	75	94

(a) Quantity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 3.2 and at 37°C.
(1) Mean ± standard deviation of 4 determinations.

Table 3 - Bone strength and mineralization in the growing pig fed a diet without or with DCP or different phytases.
(means per group ± standard deviation, *% of variation from group Control (-)*).
(1) n = 7 animals

Treatment groups (n = 8 animals)	Control (-)	DCP 12 g/kg	RONOZYME®HiPhos (M)		Phyzyme®XP 4000 TPT		OptiPhos® 2000 PF	
	A	B	C	D	E(1)	F	G	H
Planned phytase addition (U/kg)	**0**	**0**	**1000**	**1500**	**500**	**750**	**500**	**750**
Breaking force for the external metacarpal bone (N)	83a ± 31	246d ± 44 *+197*	148bc ± 23 *+79*	182c ± 46 *+119*	149bc ± 32 *+80*	177c ± 24 *+113*	129b ± 29 *+55*	138b ± 35 *+66*
External metacarpal bone mineralization (% of ash in DM)	57.80a ± 2.07	62.53b ± 0.85 *+ 8.2*	58.66a ± 1.80 *+ 1.5*	61.27b ± 0.85 *+ 6.0*	58.68a ± 0.55 *+ 1.5*	58.45a ± 1.62 *+ 1.1*	57.43a ± 3.32 *- 0.6*	59.12a ± 1.53 *+ 2.3*
Breaking force for the external metatarsal bone (N)	77a ± 32	223d ± 53 *+191*	140bc ± 38 *+82*	155c ± 51 *+102*	122bc ± 34 *+59*	129bc ± 26 *+68*	96ab ± 34 *+25*	125bc ± 30 *+62*
External metatarsal bone mineralization (% of ash in DM)	56.96a ± 3.19	62.47d ± 0.82 + *9.7*	57.68ab ± 2.38 + *1.3*	60.53cd ± 1.47 + *6.3*	59.26bc ± 1.45 + *4.0*	59.61bc ± 1.56 + *4.7*	57.99ab ± 2.29 + *1.8*	59.70bc ± 1.63 + *4.8*
Mean breaking force for both bones (N)	80a ± 31	235e ± 48 *+195*	144cd ± 31 *+81*	169d ± 49 *+112*	136bc ± 35 *+70*	152cd ± 35 *+90*	112b ± 35 *+40*	132bc ± 32 *+66*

(1) n = 7 animals
a, b, c, d, e Means within the same row without a common letter are significantly different (*P*<0.05)

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T-2

DSM
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DSM Nutritional Products

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Food and Drug Administration
Division of Animal Feeds (HFV-224)
Office of Surveillance and Compliance
Center for Veterinary Medicine
7519 Standish Place
Rockville, Maryland 20855

April 7, 2014

Revision for GRAS Notification Of RONOZYME HiPhos® for Swine Feed
Submitted by DSM Nutritional Products

Dear Mr. Wong,

Enclosed is the revision of page 5 of our GRAS Notice, AGRN 15 as discussed on April 4, 2014.
In the applicable condition of use section the pH was incorrectly noted as pH 6.5 rather than the correct pH 5.5.
I have also included revised Annexes 3, 6 and 13 where the footnote to a table in each annex noted an incorrect pH for the enzyme activity; page 10, page 8 and page 5 respectively. We discussed adding these documents to the Notice as supplements.

Sincerely DSM Nutritional Products,

James La Marta, Ph.D.

James La Marta, Ph.D., CFS
Senior Manager Regulatory Affairs

RECEIVED
APR 09 2014
By

1.3 Name and Address of the Exclusive Distributor

DSM Nutritional Products
45 Waterview Blvd.
Parsippany, New Jersey, 07054, USA
Tel: 973-257-8294

1.4 Common or Usual Name of the Substance

DSM's phytase enzyme preparation is obtained from a Genetically Engineered strain of *Aspergillus oryzae* produced by [redacted] (b) (4) fermentation. The common or usual name of the substance is "phytase". It is produced and sold in three forms; a liquid, a micro-granulate and a thermo-tolerant granulate. The trade name of the enzyme is RONOZYME® HiPhos.

1.5 Applicable Condition of Use

RONOZYME® HiPhos will be included in swine feed for the nutritional purpose of increasing the digestibility of phytate. The recommended use level of RONOZYME® HiPhos is 500 FYT to 4000 FYT/Kg of swine feed; where one FYT is the amount of enzyme that releases 1 µmol of inorganic phosphorous from phytate per minute at 37°C and pH 5.5.

1.6 AAFCO Definition O.P. 2011

Phytase derived from *Aspergillus niger* variants and *Aspergillus oryzae* variants are permissible as feed ingredients in swine and poultry diets. See reference 3.

Table 30.1 Enzymes/Source Organisms Acceptable for Use in Animal Feeds

Phytase	*Aspergillus niger,* var. *Aspergillus oryzae,* var.	Corn, soybean meal, sunflower meal, hominy, tapioca, plant by-products	Hydrolyzes phytate	Increases the digestibility of phytin-bound phosphorus in swine and poultry diets

1.7 Description of ingredient

Three product forms of RONOZYME® HiPhos will be available, two dry forms and a liquid form. RONOZYME® HiPhos (GT) is a granulated thermo-tolerant form with a minimum enzyme activity of 10,000 FYT/gram. RONOZYME® HiPhos (M) is a micro granulated form with a minimum enzyme activity of 50,000 FYT/gram. RONOZYME® HiPhos (L) is an aqueous liquid with a minimum enzyme activity of 20,000 FYT/g. Additional forms may be manufactured with ingredients suitable for feed use if there are additional market needs.

Revised Annex 13
EXCERPT OF REPORT No. 00013767
Correction made on page 5

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EXCERPT OF:

REPORT No. 00013767
Research Project Document

Document Date: 14-Mar-2012
Excerpt Date: 27-June-2013

Author(s): Guggenbuhl P, Waché Y, Simões Nunes C, Portier C, Kurtz N, Lehmann A and Bilger M

Title: **Comparative effects of RONOZYME® HiPhos (M), Phyzyme® XP 4000 TPT and OptiPhos® 2000 PF on the zootechnical performance and the mineral utilization in the growing pig.**

Excerpt Contents: **Bone Properties**

Project No. 6106
Compound No.
Summary

1. INTRODUCTION

The aim of the present study (S10-2010) was to evaluate the effects of the microbial 6-phytase, RONOZYME® HiPhos (M) comparatively to the phytases Phyzyme® XP 4000 TPT and OptiPhos® 2000 PF on the zootechnical performance, mineral blood concentrations and the utilisation of phosphorus (P) and calcium (Ca) in the growing pig. The experiment was performed during June-September 2010 in the facilities of the Centre de Recherche en Nutrition Animale (CRNA), DSM Nutritional Products France, BP 170, 68305 Saint-Louis cedex, France. It has been performed according to the French legal regulations on experiments with live animals.

2. MATERIAL AND METHODS

2.1. Test compounds

The used RONOZYME® HiPhos (M) phytase (batch PPQ 30595) was expressed in *Aspergillus oryzae,* had an activity at pH 5.5 of 63046 U/g and was in mash micro-granulated form. Phyzyme® XP 4000 TPT (batch 1248168) and OptiPhos® 2000 PF (batch 12248A01) had an activity at pH 5.5 of 5421 U/g and 5568 U/g, respectively. NIC-RD/A measured the phytase activity in the enzyme preparations and in the feed. One unit of phytase is defined as the quantity of enzyme which sets free 1 µmole of inorganic phosphate per minute from 0.005 moles per litre sodium phytate at pH 5.5 and at 37°C. Dicalcium phosphate (DCP), batch S1784 6212, was supplied by TIMAC Industries and had a P concentration of 18.2 % and a Ca concentration of 24 %.

2.2. Animal trial

Sixty four Large-White ( ) x Redon ( ) growing pigs having an initial body weight of 16.66 ± 1.54 kg were used. The animals were allocated to 8 equal groups of 8 animals each and housed in floor-pen cages in two sub-groups of 4 animals each in an environmentally controlled room. Each pen had a plastic-coated welded wire floor and was equipped with two water nipples and four stainless-steel individualized feeders. Room temperature was 21-22° C and humidity percentage was 50 %. The piglets were fed, throughout a 71-day observation period, a basal diet without addition of mineral P (group A: control(-)) or the diet A supplemented with 12 g/kg of DCP (group B: control(+)) or with RONOZYME® HiPhos (M) at the levels of 1000 U/kg (group C) and 1500 U/kg (group D), Phyzyme® XP 4000 TPT at the levels of 500 U/kg (group E) and 750 U/kg (group F) and OptiPhos® 2000 PF at the levels of 500 U/kg (group G) and 750 U/kg (group H). The basal diet A (Table 1) was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the digestible P supply, the animals' requirements according to Henry *et al.* (1989) and NRC (1998). The total P content was of 0.38 % and of 0.81 % in control(-) diet and control(+) diet, respectively. According to the NRC tables the theoretical digestible P in the control(-) diet was 0.11 g/kg and 0.28 g/kg in the control(+) diet.

An indigestible marker, chromium oxide (Cr), was added at a concentration of 0.4 % to all the diets allowing calculation of the digestibility of P and Ca. The feed was distributed *ad libitum* in mash form, under pen feed consumption control, and the animals had free access to drinking water. The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water in the region is 80 mg/L. Performance was evaluated after 29 days of experimentation and at the end of the 71 days of the trial duration. Blood was collected by jugular puncture from all the animals at the 28th day and 70th day of the experiment for the

determination of the P, Ca and alkaline phosphatase concentrations. Faecal P, Ca and Cr concentrations were measured at the 29th day and the 71st day of the second period. Faeces were sampled individually, in approximately the same amount at the same time of the day, during the last 3 days preceding that date. All minerals were determined according to standard Association of Official Analytical Chemists (1990) methods using a Vista-MPX ICP-OES spectrometer (Varian Australia Pty Ltd, Mulgrave Victoria, 3170 Australia). The apparent digestibility (% of the intake) of the minerals was calculated for the mentioned 3-day period. At the end of the evaluation the animals were slaughtered after tranquilization followed by electronarcosis for bone collection. The collected bones were the right external metacarpal and metatarsal. Samples were prepared from each of the collected bones immediately after slaughter. After careful dissection and removal of the soft tissue, a diaphysis section was obtained by sawing each bone. The obtained sections of about 3.5-cm long were immediately subjected to compression in order to determine the force in Newton necessary to break them (maximal-breaking force at the fracture point). The measurements were performed with a LR10K compression machine, using a XLC/10K/A1 force captor and a compression device TH23-196/AL (Lloyd Instruments, Fareham, UK).
The broken bones were then used for the determination of ash content, which was measured after 48-h incineration at 550°C.

2.3. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu + Ai + Bij + Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Snedecor and Cochran, 1989). These calculations were performed using StatGraphics Plus 5.1 (Manugistics, Rockville, U.S.A. 2001).

2.4. Effects on bone parameters

The results of the external metacarpal and external metatarsal bone resistance and mineralization are presented in Table 3. The bone resistance of the external metacarpal was dose dependently and significantly increased in all phytase supplemented groups as well as in that receiving DCP comparatively to the control(-). The DCP supplemented animals had an external metacarpal bone breaking force significantly higher than those of the control(-) and all phytases groups. In counter-part the external metacarpal bone mineralization was only significantly improved in the RONOZYME® HiPhos 1500 U/kg and control(+) fed pigs comparatively to the control(-) group. The differences to the control (-) of the external metatarsal bone resistance were dose dependently increased with the three tested phytases. The breaking force was significantly improved in all phytase supplemented groups with the exception of OptiPhos® 2000 PF 500 U/kg. The highest bone strength was observed for the animals receiving the control(+) diet and was significantly higher than those of the control(-) and all phytases groups. The external metatarsal bone mineralization of the RONOZYME® HiPhos 1000 U/kg and OptiPhos® 2000 PF 500 U/kg groups was similar to that of the control(-) fed animals. It was significantly improved in all other phytase supplemented groups. In the control(+) supplemented pigs the external metatarsal bone mineralization was significantly higher than in all other groups.

The mean breaking force of both bones, comparatively to the control (-) fed pigs (80 N), was dose dependently and significantly increased in the RONOZYME® HiPhos 1000 U/kg (144 N) and 1500 U/kg (169 N), Phyzyme® XP 4000 TPT 500 U/kg (136 N) and 750 U/kg (152 N) and OptiPhos® 2000 PF 500 U/kg (112 N) and 750 U/kg (132 N) by 81, 112, 70, 90, 40 and 66 %, respectively. That of the control (+) group (235 N) was significantly higher than the breaking force of all other groups.

Table 1 - Composition (%) of the control (-) diet and of control (+) diet supplemented with DCP.

INGREDIENTS	Control(-) diet without mineral P (%)	Control(+) diet with DCP (%)
Maize	58.0	58.0
Soybean meal	17.85	17.85
Rapeseed meal	14.15	14.15
Oat meal	6.0	6.0
Soya oil	1.0	1.0
Dicalcium phosphate	-	1.2
Minerals(1), vitamins and synthetic aa	3.0	1.8
Analyzed Crude Protein - %	*18.28*	*18.43*
Analyzed Crude Ash - %	*4.93*	*6.21*
Analyzed Lysine - %	*1.13*	*1.10*
Analyzed Threonine - %	*0.54*	*0.56*
Analyzed Methionine + cysteine - %	*0.64*	*0.63*
Analyzed total Ca - %	*0.86*	*1.42*
Analyzed total P - %	*0.38*	*0.81*
Analyzed Phytic P - %	*0.11*	*0.11*
Theoretically digestible P - %	*0.11*(2)	*0.28*(3)
Estimated digestible energy - MJ/kg	*15.28*	*15.42*

(1) Mixture without mineral P.
(2) Digestible P estimated from the NRC tables.
(3) Sum of the digestible P estimated from the NRC tables and 80 % of added mineral P as generally accepted.

Table 2 - Phytase activity (U(a)/kg) and % of the target in the different diets.

Treatment groups	Control (-)	DCP 12 g/kg	RONOZYME® HiPhos (M)		Phyzyme®XP 4000 TPT		OptiPhos® 2000 PF	
Programmed phytase addition (U/kg)	0	0	1000	1500	500	750	500	750
Measured phytase activity (U/kg)(1)	<LOQ	<LOQ	1068 ±32	1568 ±30	520 ±41	753 ±22	377 ±173	702 ±27
Actually added phytase (U/kg)	-	-	1068	1568	520	753	377	702
% of target	-	-	107	105	104	100	75	94

(a) Quantity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 3.2 and at 37°C.

(1) Mean ± standard deviation of 4 determinations.

Erratum - footnote (a) should read 'Qualtity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 5.5 and at 37 °C.'
This change was made on 7 April 2014 by James La Marta, Sr. Mgr. Regulatory Affairs after consultation with the lead author Dr. Peter Guggenbuhl.

Table 3 - Bone strength and mineralization in the growing pig fed a diet without or with DCP or different phytases.

(means per group ± standard deviation, *% of variation from group Control (-)*).

(1) n = 7 animals

Treatment groups (n = 8 animals)	Control (-)	DCP 12 g/kg	RONOZYME®HiPhos (M)		Phyzyme®XP 4000 TPT		OptiPhos® 2000 PF	
	A	B	C	D	E(1)	F	G	H
Planned phytase addition (U/kg)	**0**	**0**	**1000**	**1500**	**500**	**750**	**500**	**750**
Breaking force for the external metacarpal bone (N)	83a ± 31	246d ± 44 *+197*	148bc ± 23 *+79*	182c ± 46 *+119*	149bc ± 32 *+80*	177c ± 24 *+113*	129b ± 29 *+55*	138b ± 35 *+66*
External metacarpal bone mineralization (% of ash in DM)	57.80a ± 2.07	62.53b ± 0.85 *+ 8.2*	58.66a ± 1.80 *+ 1.5*	61.27b ± 0.85 *+ 6.0*	58.68a ± 0.55 *+ 1.5*	58.45a ± 1.62 *+ 1.1*	57.43a ± 3.32 *- 0.6*	59.12a ± 1.53 *+ 2.3*
Breaking force for the external metatarsal bone (N)	77a ± 32	223d ± 53 *+191*	140bc ± 38 *+82*	155c ± 51 *+102*	122bc ± 34 +59	129bc ± 26 *+68*	96ab ± 34 *+25*	125bc ± 30 *+62*
External metatarsal bone mineralization (% of ash in DM)	56.96a ± 3.19	62.47d ± 0.82 + *9.7*	57.68ab ± 2.38 + *1.3*	60.53cd ± 1.47 + *6.3*	59.26bc ± 1.45 + *4.0*	59.61bc ± 1.56 + *4.7*	57.99ab ± 2.29 + *1.8*	59.70bc ± 1.63 + *4.8*
Mean breaking force for both bones (N)	80a ± 31	235e ± 48 *+195*	144cd ± 31 *+81*	169d ± 49 *+112*	136bc ± 35 *+70*	152cd ± 35 *+90*	112b ± 35 *+40*	132bc ± 32 *+66*

(1) n = 7 animals

a, b, c, d, e Means within the same row without a common letter are significantly different (*P*<0.05)

Revised Annex 3
Internal Report 2500761
Correction made on page 10

REPORT No. 2500761
Regulatory Document

DSM

Document Date:	11-Jun-2009
Author(s):	P. Guggenbuhl, C. Simões Nunes, A. Piñón Quintana, C. Portier, N. Kurtz and A. Lehmann NRD/CA
	DSM Nutritional Products France, BP 170, 68305 Saint Louis, France
Title:	Evaluation of the effects of graded amounts of a microbial phytase in the weaner piglet.
Project No.	6106

Compound No.

Summary

The aim of the present study (S12-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the zootechnical performance, mineral blood concentrations, digestibility of phosphorus (P) and calcium (Ca) and bone mineralisation and resistance in the weaned piglet. The basal diet, without addition of mineral P, was based on maize, soybean meal and rapeseed meal. IPA phytase was included in the diet at the levels of 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg. A dietary treatment was based in the very slightly modified control diet containing the recommended available P by addition of dicalcium phosphate (diCaP).Supplementation with graded amounts of IPA phytase in piglets induced an increased performance in a dose dependant manner. Inclusion levels over 1000 U/kg were more efficient than the diCaP supplementation. IPA phytase restored dose dependently phosphataemia, calcaemia and phosphatasaemia to physiological levels comparatively to the controls. The mean P faecal concentration of the enzyme supplemented animals was significantly lower than that observed for the animals ingesting the control diet. All the phytase inclusion levels increased the bioavailability of P and accordingly reduced the piglet quantitative faecal excretion of P comparatively to the basal diet. The P digestibility was dose dependant and highly significantly improved with the exception of the lowest phytase inclusion level. The increases represented in comparison to the control group 12, 66, 77, 110, 132, 129, 156 and 149 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively. The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control, of 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg of the phytase were 0.13, 0.73, 0.84, 1.22, 1.50, 1.39, 1.75 and 1.62 g of full available P/kg feed respectively. Ca digestibility was improved by all the inclusion levels of the phytase. IPA phytase supplements improved bone mineralisation and bone resistance comparatively to the non-supplemented animals. In conclusion the IPA phytase improved the digestibility and the apparent absorption of P and Ca, reduced the P faecal excretion, restored phosphataemia, calcaemia and phosphatasaemia to physiologic values, increased bone mineralisation and resistance and improved the zootechnical performance in the weaned piglet fed on a diet containing P exclusively from vegetable origin.

Distribution

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Name	Signature	Date
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Research Center Head Dr. A-M Klünter, NRD/CA	[signature]	11.06.09
Project Manager Dr. F. Fru, NRD/PA	[signature]	15.06.09

Nomenclature and Structural Formula (if available)

Liquid form IPA phytase expressed in *Aspergillus oryzae,* batch PPQ28432, activity at pH 5.5 of 26500 U/g.

1. INTRODUCTION

The aim of the present study (S12-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the zootechnical performance, mineral blood concentrations, digestibility of phosphorus (P) and calcium (Ca) and bone mineralisation and resistance in the weaned piglet.
The experiment was performed in July-August 2008 in the facilities of the Centre de Recherche en Nutrition Animale (CRNA), DSM Nutritional Products France, BP 170, 68305 Saint-Louis cedex, France. It has been performed according to the French legal regulations on experiments with live animals.

2. MATERIAL AND METHODS

2.1. Test compounds

The used IPA phytase was expressed in *Aspergillus oryzae,* batch PPQ28432, had an activity at pH 5.5 of 26500 U/g and was in a liquid form.

NRD/CM measured the phytase activity in the enzyme preparation and in the feed. One unit of phytase is defined as the quantity of enzyme which sets free 1 μmole of inorganic phosphate per minute from 0.005 moles per litre sodium phytate at pH 5.5 and at 37°C.

Di-calcium phosphate (diCaP), batch S1784 6212, was supplied by TIMAC Industries and had a P concentration of 18.2 % and a Ca concentration of 24 %.

2.2. Animal trial

One hundred and twenty Large White × Landrace weaner piglets having an initial body weight of 8.03 ± 1.09 kg were used. The animals were allocated to 10 equal groups of 12 animals each and housed in floor-pen cages in two sub-groups (1 of 7 animals and 1 of 5 animals) in an environmentally controlled room. Each pen had a plastic-coated welded wire floor and was equipped with four water nipples and four stainless-steel individualised feeders. Room temperature was initially 27°C and was lowered weekly by about 2°C until 21-22° C and humidity percentage was 50 %.

The piglets were fed, throughout a 32 days observation period, a basal diet without addition of mineral P (group A) or the diet A supplemented with 16 g/kg of diCaP (group B) or with IPA phytase at the levels of 250 U/kg (group C), 500 U/kg (group D), 1000 U/kg (group E), 1500 U/kg (group F), 2000 U/kg (group G), 3000 U/kg (group H), 4000 U/kg (group I) and 8000 U/kg (group J).

The basal diet A was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the available P supply, the animals' requirements according to Henry *et al.* (1989) and NRC (1998). The basal diet A (table 1) had a theoretical P content of 0.41 % and an analysed content of 0.45 %. The theoretical available P in the diet was 1.20 g/kg and the observed availability of 1.09 g/kg.

An indigestible tracer (chromium oxide) was added at a concentration of 0.4 % to all the diets allowing calculation of the digestibility of P and Ca. The feed was distributed *ad libitum* in mash form, under pen feed consumption control, and the animals had free access to drinking water.

The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water in the region is 120 mg/L.

Performance was evaluated for the 32 days of the trial duration. Blood was collected by jugular puncture from all the animals at the 31st day of the experiment for the determination of the P, Ca, alkaline phosphatase and zinc (Zn) concentrations.

Faecal P, Ca and Cr concentrations were measured at the 32nd day of the second period. Faeces were sampled per pen, in approximately the same amount at the same time of the day, during the last 3 days preceding that date. Thus, for each dietary treatment and for each criterion a total of 6 individual determinations have been performed. All minerals were determined according to standard Association of Official Analytical Chemists (1990) methods using a Vista-MPX ICP-OES spectrometer (Varian Australia Pty Ltd, Mulgrave Victoria, 3170 Australia). The apparent digestibility (% of the intake) of the minerals was calculated for the mentioned 3 day period.

At the end of the evaluation all animals were slaughtered after tranquilization and stunning for the right femur collection. Samples of the collected bones were prepared immediately after slaughter. After careful dissection and removal of the soft tissue, a diaphysis section was obtained by sawing each bone. The obtained sections of about 3.5-cm long were immediately subjected to compression in order to determine the force in Newton necessary to break them (maximal-breaking force at the fracture point). The measurements were performed with a LR10K compression machine, using a XLC/10K/A1 force captor and a compression device TH23-196/AL (Lloyd Instruments, Fareham, UK). The broken bones were then used for the determination of the ash content, which was measured after 72-h incineration at 550°C.

2.3. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu + Ai + Bij + Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Snedecor and Cochran, 1989). These calculations were performed using StatGraphics Plus 5.1 (Manugistics, Rockville, U.S.A. 2001).

3. RESULTS AND DISCUSION

3.1. Phytase and animals

The observed phytase activity in the supplemented feed used was in general excellent agreement with the programmed inclusion levels (table 2). The basal diet without addition of mineral P (group A) and with diCaP (group B) had an endogenous phytase activity of 108 ± 34 U/kg.

The animals grew normally during the observation period to reach a final mean body weight of 16.45 ± 2.85 kg. Three animals, one in the control group, one in the 2000 U/kg and one in the 3000 U/kg phytase supplemented groups had to be euthanized during the early stage of the trial after leg injuries. No mortality was observed during the rest of the experiment.
All the groups ingesting phytase supplements and the group supplemented with 16 g/kg of diCaP had higher daily weight gain (DWG) and lower feed conversion ratio (FCR) than those observed for the control group (table 3). The highest DWG and the best FCR were observed for the group ingesting 3000 U/kg. The performances of the group supplemented with diCaP were equivalent to those of the group receiving 1000 U/kg of phytase.

Supplementation with graded amounts of IPA phytase in piglets induced an increased performance in a dose dependant manner. Inclusion levels over 1000 U/kg were more efficient than the diCaP supplementation.

3.2. Effects on plasma mineral and alkaline phosphatase concentrations

Phosphataemia was increased dose dependently in the phytase supplemented groups in comparison to the control group (table 4). The increases were highly significant with the exception of the lowest inclusion level. The group supplemented with diCaP presented also a high significant increase of the phosphataemia but at a lower level than the 4000 and 8000 U/kg phytase supplemented groups. The consumption of phytate rich diets like the control one induced hypophosphataemia. IPA phytase restored the physiological P blood level confirming the sensitiveness of phosphataemia to the dietary available P.

Comparatively to the control group, calcaemia was decreased in all the phytase supplemented animals (table 4). The effects of the phytase were dose dependant although the curve levelled off from the 2000 U/kg inclusion and highly significant with the exception of the lowest inclusion level. As observed in the control group, hypophosphataemia is generally associated with hypercalcaemia in swine. In the present study, calcaemia in the animals ingesting the basal diet supplemented with diCaP or with phytase was within the normal piglet values.

Zincaemia was not significantly influenced by the supplementation of phytase or diCaP, although these treatment groups presented higher mean concentrations than the control group (table 4). Zn is well known to bind to phytate and generally its digestibility is improved by phytases in growing pigs. Nevertheless, it seems that in piglet the blood Zn concentration is not altered by the dietary treatments used in the present experiment.

Compared to the control group, phosphatasaemia was decreased dose dependently in the phytase supplemented groups (table 5). The decreases were only significant at the 500 and 8000 U/kg inclusion levels. The group supplemented with diCaP presented also a decrease of the phosphatasaemia at a level similar to that observed with 1500 U/kg phytase supplemented group. Alkaline phosphatase plays an important role in bone metabolism. As observed in the control non-supplemented group, hypophosphataemia induces osteoblasts heperphosphatasaemia in response to an increased activity of osteoclasts in bone. In the present study, phosphatasaemia of the phytase supplemented animals was systematically lower than that of the control clearly indicating restored normal bone function.

3.3. Effects on phosphorus digestion

The mean P faecal concentration of the enzyme supplemented animals was very significantly lower than that measured in the animals ingesting the control diet (table 6). There was a decrease of the P faecal concentration with the increasing allowance of IPA phytase. The lowest P faecal concentration was observed in the animals ingesting phytase at 8000 U/kg and represented the half of that of the control group.

The P digestibility was dose dependant and highly significantly improved with the exception of the lowest phytase inclusion level. The increases represented in comparison to the control group 12, 66, 77, 110, 132, 129, 156 and 149 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively (table 7, figure 1). The digestibility of P in the diCaP supplemented diet was also significantly higher than that of the control by 69 % and very similar to the enzyme supplementation at 500 U/kg.

The faecal excretion of P was significantly reduced by 4, 20, 25, 34, 41, 41, 49 and 48 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively. It was increased by 35 % with the diCa-P supplemented group (table 8, figure 2).

The apparent absorbed P was 1.22, 1.82, 1.93, 2.31, 2.59, 2.48, 2.84 and 2.71 g/kg feed in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively and 3.18 g/kg feed in the diCaP supplemented group (table 9). It was significantly increased in all the supplemented groups with the exception of the lowest phytase inclusion level in comparison to the control diet (1.09 g/kg). The highest inclusion levels of IPA phytase were in accordance with the recommended requirements of 2.80 g of digestible P per kg feed for piglets.

The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control, of 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase were 0.13, 0.73, 0.84, 1.22, 1.50, 1.39, 1.75 and 1.62 g of full available P/kg feed respectively (table 10, figure 3). In comparison the P equivalency of diCaP supplemented diet was 2.10 g of full available P/kg feed.
In the present study, using the equation of the tendency curve the calculated inclusion level to reach 1.5 g of full available P/kg feed was 3109 U/kg feed of IPA phytase ($y = 167.21e^{1.9486x}$, $R^2 = 0.8897$) but was reached experimentally with the 2000 U/kg inclusion level.

In general on all the P parameters, IPA phytase showed high dose dependant potency.

3.3. Effects on calcium digestion

The Ca faecal concentration of the animals ingesting the non-supplemented diet was higher than that of the animals ingesting the phytase, with the exception of the lowest inclusion level (table 11). The observed differences were statistically significant for the enzyme supplemented groups excepted for the 250 and 1000 U/kg inclusion levels. The highest Ca faecal concentration was observed in the diCaP supplemented group.

The Ca digestibility was improved in the supplemented groups with the exception of the diCaP group and the 250 U/kg phytase group (table 12). The variations were -9, 7, 5, 16, 26, 12, 25 and 17 % in the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively and significant for the five highest concentrations. The Ca digestibility of the diCaP supplemented diet was decreased by 16 % comparatively to the control group.

The faecal excretion of Ca was reduced by 8, 7, 22, 37, 20, 40, and 26 % with the IPA phytase in the 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg supplemented groups respectively and significantly with the five highest concentrations. It was significantly increased by 13 % and 87 % with the 250 U/kg phytase and diCaP groups respectively (table 13).

The apparent absorbed Ca was 4.38, 5.28, 5.08, 5.61, 5.94, 5.20, 5.63 and 5.42 g/kg feed with the 250, 500, 1000, 1500, 2000, 3000, 4000 and 8000 U/kg phytase supplemented groups respectively and 6.10 g/kg feed in the diCa-P group (table 14). It was significantly increased in all the 1500, 2000, 4000 and 8000 U/kg phytase and diCaP supplemented groups and significantly decreased in the 250 U/kg phytase inclusion level in comparison to the Ca apparent absorption in the control diet (4.79 g/kg).

3.4. Bone resistance and bone ash

The phytase supplements strongly influenced the bone strength (table 15). For the IPA phytase inclusion level of 8000 U/kg the increase of the femur resistance was similar to that of diCaP. It represented 121 % and 126 % respectively of that observed for the animals ingesting the basal diet. The increases were significant in all supplemented groups excepted for the 250 U/kg and 1000 U/kg phytase inclusion levels.
The ash content of the femur was increased in a significant way by the phytase excepted for the lowest dosage and by the diCaP (table 15). Nevertheless, the addition of graded amounts of IPA phytase resulted in a non-linear increase of the ash content of the femur.

In the present study IPA phytase supplements in young pigs confirmed the positive effects of phytases on the improvement of bone resistance and the positive but moderate effect on bone mineralisation of animals fed diets containing P exclusively from vegetable origin.
The bone mineralisation data were in agreement with the improvements in P digestibility and with P and Ca blood concentrations.

4. CONCLUSION

It can be concluded that the IPA phytase improved the digestibility and the apparent absorption of P and Ca, reduced the P faecal excretion, restored phosphataemia, calcaemia and phosphatasaemia to physiologic values, increased bone mineralisation and resistance and improved the zootechnical performance in the weaned piglet fed on a diet containing P exclusively from vegetable origin. There was a dose dependant effect of the IPA phytase on the availability of the dietary P.

Table 1 - Composition (%) of the basal diet (A) and of that supplemented with diCa-P (B)

INGREDIENTS	Basal diet without P (%)	Basal diet with diCA-P (%)
Maize	68.52	68.125
Soybean meal	15.1	15.1
Rapeseed meal	12.5	12.5
Salt	0.55	0.55
Soya oil	1.0	1.0
Calcium carbonate	1.56	0.355
Di-calcium phosphorus	-	1.6
Minerals[1] , vitamins and synthetic aa	0.77	0.77
Crude Protein – N x 6.25	15.5	15.5
Lysine - %	0.96	0.96
Methionine + cystine - %	0.54	0.54
Ca – analyzed - % in DM	0.82	1.24
P – analyzed - % in DM	0.45	0.78
Theoritically available P - %	0.12 [2]	0.35 [3]
Observed available P - %	0.11	0.32
Phytic P – calculated - %	0.28	0.54
Estimated digestible energy – MJ/kg	*13.31*	*13.31*
Phytase activity – U [4]/kg	*108 ± 34*	*108 ± 34*

[1] Mixture without mineral P;
[2] Estimated from the mean P digestibility of the previous realized trials
[3] Sum of the theoretically available P and 80 % of added mineral P as generally accepted
[4] Quantity of enzyme that sets free 1 μmole of inorganic phosphate per minute from 0.005 mole per litre sodium phytate at pH 5.5 and at 37°C.

Table 2 - Phytase activity (U[(a)]/kg) and % of the target in the different diets.

Treatment groups	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A	B	C	D	E	F	G	H	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Measured phytase addition (U/kg) [(1)]	108 ± 34	108 ± 34	374 ± 111	601 ± 25	1097 ± 21	1611 ± 41	2225 ± 45	3098 ± 104	4030 ± 208	8238 ± 283
Actually added phytase (U/kg)	-	-	266	493	989	1503	2117	2990	3922	8130
% of target	-	-	106	99	99	100	106	100	98	102

[(a)] Quantity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 3.2 and at 37°C.
[(1)] Mean ± standard deviation of 4 determinations.

Erratum - footnote (a) should read 'Qualtity of enzyme that sets free 1 µmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 5.5 and at 37 °C.'
This change was made on 7 April 2014 by James La Marta, Sr. Mgr. Regulatory Affairs after consultation with the lead author Dr. Peter Guggenbuhl.

Table 3 – **Effects on the zootechnical performances in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A	B	C	D	E	F	G	H	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Initial Body Weight (kg)	8.03 ± 1.13	8.02 ± 1.13	8.02 ± 0.84	8.03 ± 1.27	8.03 ± 1.52	8.03 ± 1.08	8.03 ± 0.86	8.02 ± 1.04	8.03 ± 1.19	8.02 ± 1.16
Final Body Weight (kg)	15.20 (1) ± 2.10 *(100)*	16.24 ± 3.11 *(107)*	16.14 ± 1.99 *(106)*	15.98 ± 4.13 *(105)*	16.01 ± 3.58 *(105)*	16.69 ± 3.14 *(110)*	17.44 (1) ± 2.86 *(115)*	17.48 (1) ± 2.29 *(115)*	16.79 ± 2.51 *(110)*	16.58 ± 2.38 *(109)*
Total Weight Gain (kg)	7.04 (1) ± 2.32 *(100)*	8.22 ± 2.25 *(117)*	8.12 ± 1.64 *(115)*	7.96 ± 3.09 *(113)*	7.98 ± 2.71 *(113)*	8.66 ± 2.30 *(123)*	9.46 (1) ± 2.24 *(134)*	9.61 (1) ± 1.86 *(137)*	8.77 ± 1.94 *(125)*	8.56 ± 2.03 *(122)*
Daily Weight Gain (g)	220 (1) ± 73 *(100)*	257 ± 70 *(117)*	254 ± 51 *(115)*	249 ± 97 *(113)*	249 ± 85 *(113)*	271 ± 72 *(123)*	296 (1) ± 70 *(134)*	300 (1) ± 58 *(137)*	274 ± 61 *(125)*	268 ± 63 *(122)*
Feed intake (g/day) (2)	468 (1) ± 16 *(100)*	484 ± 55 *(103)*	499 ± 40 *(107)*	475 ± 136 *(101)*	478 ± 34 *(102)*	489 ± 99 *(104)*	510 (1) ± 42 *(109)*	523 (1) ± 57 *(112)*	497 ± 36 *(106)*	491 ± 55 *(105)*
Feed Conversion Ratio (kg/kg) (2)	2.448 (1) ± 0.220 *(100)*	1.914 ± 0.014 *(78)*	1.981 ± 0.032 *(81)*	1.985 ± 0.048 *(81)*	1.931 ± 0.002 *(79)*	1.835 ± 0.068 *(75)*	1.819 (1) ± 0.064 *(74)*	1.793 (1) ± 0.013 *(73)*	1.834 ± 0.021 *(75)*	1.865 ± 0.023 *(76)*
Mortality	1	0	0	0	0	0	1	1	0	0

(1) n = 11 animals ; (2) n = 2 pens

Table 4 – Effects on plasma mineral concentrations in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P plasma levels (mg/dl)	5.11 ± 1.15 *(100)*	7.69 ± 1.03 *(151)*	5.53 ± 0.86 *(108)*	6.23 ± 0.82 *(122)*	6.59 ± 1.00 *(129)*	7.07 ± 1.03 *(138)*	7.23 ± 1.32 *(141)*	7.33 ± 0.86 *(144)*	8.10 ± 0.91 *(159)*	7.97 ± 0.90 *(156)*
Statistical analysis										
	A -	P<0.001	NS	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	NS	NS	NS	NS	NS	NS
			C -	NS	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	NS	NS	P<0.001	P<0.001
					E -	NS	NS	NS	P<0.001	P<0.001
						F -	NS	NS	P<0.05	P<0.05
							G -	NS	P<0.05	P<0.05
								H -	NS	NS
									I -	NS
Ca plasma levels (mg/dl)	13.34 ± 0.69 *(100)*	11.06 ± 0.21 *(83)*	13.17 ± 0.87 *(99)*	12.18 ± 0.60 *(91)*	12.05 ± 0.85 *(90)*	11.67 ± 0.48 *(88)*	11.76 ± 0.47 *(88)*	11.61 ± 0.55 *(87)*	11.62 ± 0.86 *(87)*	11.56 ± 0.41 *(87)*
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	NS	NS	NS	NS	NS
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS
Zn plasma levels (µg/dl)	65.99 ± 12.65 *(100)*	70.93 ± 14.72 *(108)*	78.28 ± 7.03 *(119)*	71.99 ± 14.94 *(109)*	70.50 ± 12.65 *(107)*	75.27 ± 9.52 *(114)*	69.82 ± 11.27 *(106)*	70.50 ± 10.62 *(107)*	68.20 ± 11.62 *(103)*	71.01 ± 10.78 *(108)*
	No statistical differences									

[(1)] n = 11 animals NS : non significant

Table 5 – **Effects on plasma alkaline phosphatase concentrations in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A(1)	B	C	D	E	F	G(1)	H(1)	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
ALP plasma levels (U/L)	325.1 ± 96.1	239.4 ± 50.4	285.9 ± 46.7	223.4 ± 48.0	280.3 ± 78.4	237.4 ± 90.7	255.5 ± 104.8	255.7 ± 85.8	251.5 ± 57.5	202.3 ± 47.5
Variation from A (%)	100.0	73.7	87.9	68.7	86.2	73.0	78.6	78.6	77.4	62.2
Statistical analysis										
	A -	NS	NS	P<0.05	NS	NS	NS	NS	NS	P<0.001
		B -	NS	NS	NS	NS	NS	NS	NS	NS
			C -	NS	NS	NS	NS	NS	NS	NS
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 6 - Effects on the faecal concentration of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A(1)	B	C	D	E	F	G(1)	H(1)	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal concentration (mg/g DM)	22.4 ± 0.6	24.6 ± 2.6	20.8 ± 1.3	16.3 ± 1.1	16.4 ± 0.6	14.1 ± 1.0	12.8 ± 0.6	12.5 ± 1.1	11.5 ± 1.2	11.2 ± 0.7
Variation from A (%)	100.0	110.0	93.2	73.1	73.5	63.0	57.1	55.8	51.6	49.9
Statistical analysis										
	A -	P<0.001	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.001
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 7 - Effects on the total apparent digestibility of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[1]	B	C	D	E	F	G[1]	H[1]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal apparent digestibility (%)	24.1 ± 1.5	40.8 ± 5.1	26.9 ± 3.5	40.0 ± 3.6	42.7 ± 2.4	50.7 ± 5.4	56.0 ± 1.9	55.1 ± 3.6	61.8 ± 3.5	60.1 ± 4.8
Variation from A (%)	100	169	112	166	177	210	232	229	256	249
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	NS	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.001
							G -	NS	P<0.05	NS
								H -	P<0.05	NS
									I -	NS

[1] n = 11 animals NS : non significant

Table 8 - Effects on the faecal excretion of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A(1)	B	C	D	E	F	G(1)	H(1)	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal excretion (mg/g)	3.43 ± 0.07	4.62 ± 0.40	3.30 ± 0.16	2.73 ± 0.17	2.58 ± 0.11	2.24 ± 0.25	2.03 ± 0.09	2.02 ± 0.16	1.76 ± 0.16	1.80 ± 0.22
Variation from A (%)	100	135	96	80	75	66	59	59	51	52
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.001
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 9 - Effects on the faecal apparent absorption of phosphorus in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P fecal apparent absorption (mg/g)	1.09 ± 0.07	3.18 ± 0.40	1.22 ± 0.16	1.82 ± 0.17	1.93 ± 0.11	2.31 ± 0.25	2.59 ± 0.09	2.48 ± 0.16	2.84 ± 0.16	2.71 ± 0.22
Variation from A (%)	100	292	112	168	177	212	238	228	261	249
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.05
							G -	NS	NS	NS
								H -	P<0.05	NS
									I -	NS

(1) n = 11 animals NS : non significant

Figure 1

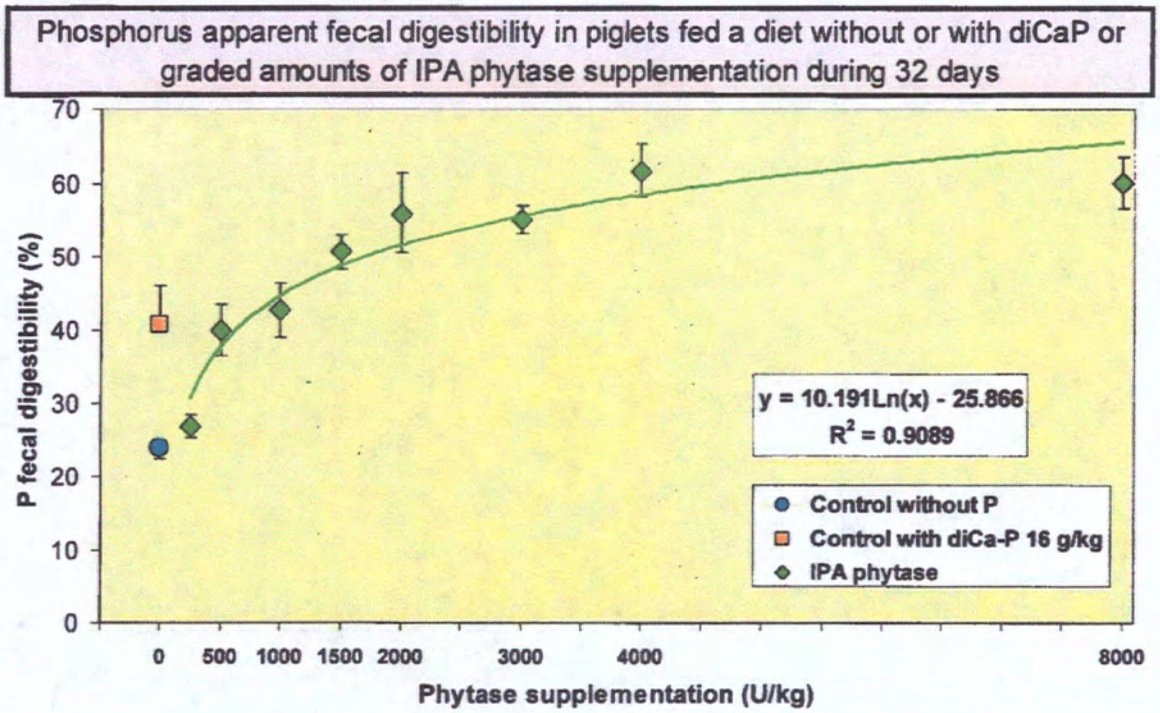

Figure 2

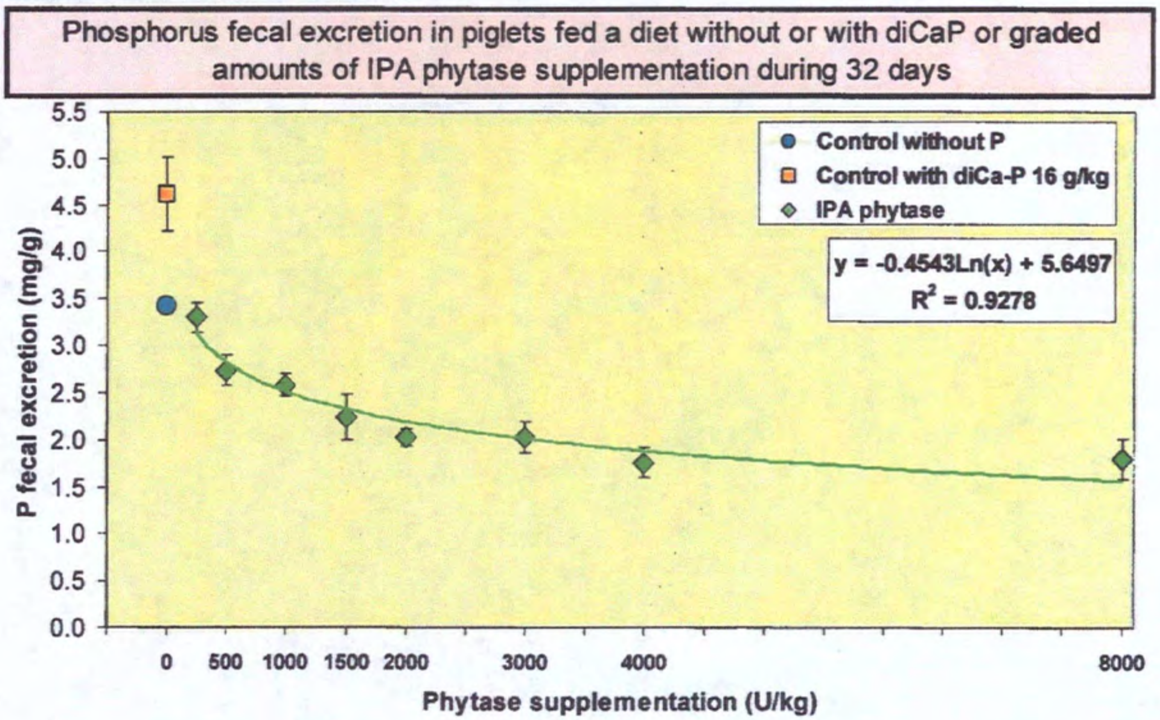

Table 10 - **Phosphorus equivalencies (g of full available supplemental P per kg of feed comparatively to the non-supplemented control) in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A(1)	B	C	D	E	F	G(1)	H(1)	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
P equivalence (mg/g)	0.00 ± 0.07	2.10 ± 0.40	0.13 ± 0.16	0.73 ± 0.17	0.84 ± 0.11	1.22 ± 0.25	1.50 ± 0.09	1.39 ± 0.16	1.75 ± 0.16	1.62 ± 0.22
Variation from C (%)	-	-	100	577	659	957	1177	1096	1378	1272
Statistical analysis										
	A -	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
					E -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
						F -	NS	NS	P<0.001	P<0.05
							G -	NS	NS	NS
								H -	P<0.05	NS
									I -	NS

(1) n = 11 animals NS : non significant

Figure 3

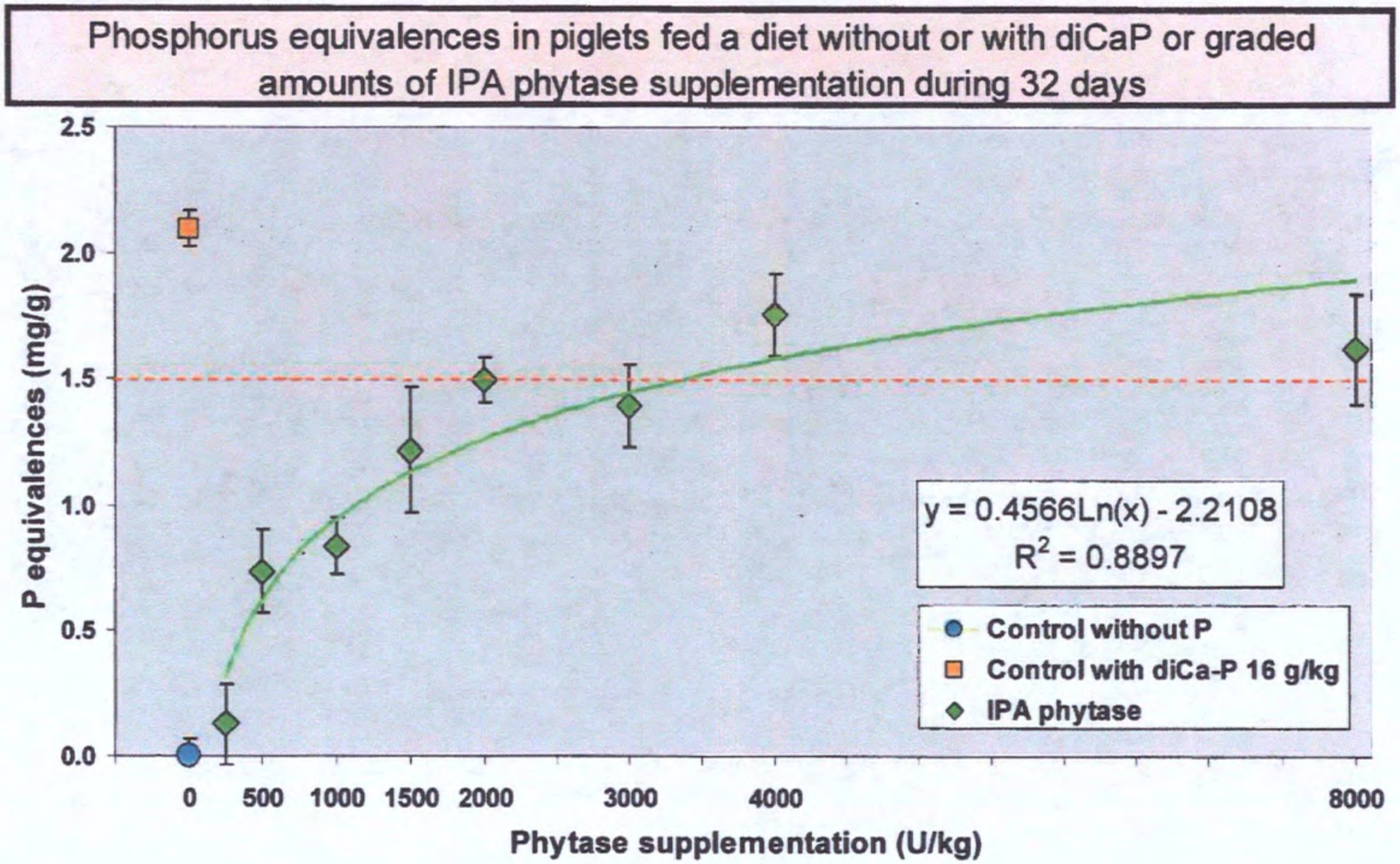
Phosphorus equivalences in piglets fed a diet without or with diCaP or graded amounts of IPA phytase supplementation during 32 days
P equivalences (mg/g)
2.5
2.0
1.5
1.0
0.5
0.0
0
500
1000
1500
2000
3000
4000
8000
Phytase supplementation (U/kg)
y = 0.4566Ln(x) - 2.2108
R2 = 0.8897
Control without P
Control with diCa-P 16 g/kg
IPA phytase

Table 11 - Effects on the faecal concentration of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal concentration (mg/g DM)	22.1 ± 1.3	33.6 ± 3.3	24.0 ± 1.6	18.6 ± 2.1	20.0 ± 0.8	16.5 ± 1.4	13.4 ± 0.7	16.6 ± 1.4	13.3 ± 1.2	15.6 ± 2.2
Variation from A (%)	100.0	152.4	108.7	84.4	90.5	74.8	60.5	75.3	60.4	70.5
Statistical analysis										
	A -	P<0.001	NS	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	P<0.001	P<0.05
					E -	P<0.05	P<0.001	P<0.05	P<0.001	P<0.001
						F -	P<0.05	NS	P<0.05	NS
							G -	P<0.05	NS	NS
								H -	P<0.05	NS
									I -	NS

[(1)] n = 11 animals NS : non significant

Table 12 - **Effects on the total apparent digestibility of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal apparent digestibility (%)	58.7 ± 1.2	49.2 ± 3.7	53.5 ± 3.5	62.9 ± 4.3	61.8 ± 3.6	68.1 ± 3.6	73.6 ± 2.6	65.7 ± 4.5	73.4 ± 3.2	68.4 ± 5.1
Variation from A (%)	100	84	91	107	105	116	126	112	125	117
Statistical analysis										
	A -	P<0.001	P<0.05	NS	NS	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001
		B -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	P<0.001	NS
					E -	P<0.05	P<0.001	NS	P<0.001	P<0.05
						F -	NS	NS	NS	NS
							G -	P<0.001	NS	NS
								H -	P<0.001	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 13 - **Effects on the faecal excretion of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).**

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[1]	B	C	D	E	F	G[1]	H[1]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal excretion (mg/g)	3.38 ± 0.10	6.31 ± 0.46	3.81 ± 0.29	3.12 ± 0.36	3.14 ± 0.13	2.63 ± 0.29	2.13 ± 0.21	2.71 ± 0.36	2.04 ± 0.25	2.50 ± 0.41
Variation from A (%)	100	187	113	92	93	78	63	80	60	74
Statistical analysis										
	A -	P<0.001	P<0.05	NS	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	P<0.001	P<0.05
					E -	NS	P<0.001	NS	P<0.001	P<0.05
						F -	P<0.05	NS	P<0.05	NS
							G -	P<0.05	NS	NS
								H -	P<0.001	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 14 - Effects on the faecal apparent absorption of calcium in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation, *% of variation from group A*).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A(1)	B	C	D	E	F	G(1)	H(1)	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Ca fecal apparent absorption (mg/g)	4.79 ± 0.10	6.10 ± 0.46	4.38 ± 0.29	5.28 ± 0.36	5.08 ± 0.13	5.61 ± 0.29	5.94 ± 0.21	5.20 ± 0.36	5.63 ± 0.25	5.42 ± 0.41
Variation from A (%)	100	127	92	110	106	117	124	109	109	113
Statistical analysis										
	A -	P<0.001	P<0.05	NS	NS	P<0.001	P<0.001	NS	P<0.001	P<0.05
		B -	P<0.001	P<0.001	P<0.001	NS	NS	P<0.001	NS	P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	P<0.001	NS	NS	NS
					E -	NS	P<0.001	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	P<0.001	NS	P<0.05
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

Table 15 – Effects on bone ash and bone resistance in the weaning pig fed a diet without or with diCaP or graded amounts of IPA phytase (means per group ± standard deviation).

Treatment groups (n = 12 animals)	Basal Diet	Basal Diet + diCa-P	IPA phytase							
	A[(1)]	B	C	D	E	F	G[(1)]	H[(1)]	I	J
Programmed phytase addition (U/kg)	0	0	250	500	1000	1500	2000	3000	4000	8000
Bone resistance maximal strength (N)	272.8 ± 88.7	615.5 ± 179.1	334.6 ± 76.0	476.1 ± 124.4	384.1 ± 77.2	500.3 ± 118.7	523.5 ± 157.7	476.5 ± 97.1	542.2 ± 108.7	604.1 ± 119.2
Variation from A (%)	100	226	123	175	141	183	192	175	199	221
Statistical analysis										
	A -	P<0.001	NS	P<0.001	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.001	NS	P<0.001	NS	NS	NS	NS	NS
			C -	NS	NS	NS	P<0.05	NS	P<0.05	P<0.001
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	P<0.001
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS
Bone ash (%)	62.17 ± 1.91	63.70 ± 1.58	62.38 ± 1.88	65.19 ± 0.94	65.67 ± 0.95	65.80 ± 1.70	64.85 ± 1.55	65.70 ± 1.06	66.36 ± 1.21	65.24 ± 1.78
Variation from A (%)	100	103	100	105	106	106	104	106	107	105
Statistical analysis										
	A -	P<0.05	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
		B -	P<0.05	NS	P<0.05	P<0.05	NS	P<0.05	P<0.001	NS
			C -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
				D -	NS	NS	NS	NS	NS	NS
					E -	NS	NS	NS	NS	NS
						F -	NS	NS	NS	NS
							G -	NS	NS	NS
								H -	NS	NS
									I -	NS

(1) n = 11 animals NS : non significant

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Groupe A	Porcelet	Poids	GP	GMQ	Cons. Moy. Ind./Jour	IC/ Lot
A 1	551	17.057	7.778	0.243		
	529	13.074	3.877	0.121		
	524	17.914	8.995	0.281		
	525	11.010	2.293	0.072		
	534	18.103	9.446	0.295		
	526	14.292	5.668	0.177		
	535	15.153	6.567	0.205	0.457	2.292
A 11	527	15.007	7.400	0.231		
	531	14.508	7.063	0.221		
	569	16.285	9.348	0.292		
	528					
	530	14.818	8.971	0.280	0.479	2.604
B 2	586	20.933	10.750	0.336		
	543	21.077	11.513	0.360		
	545	18.604	10.052	0.314		
	538	16.933	8.534	0.267		
	595	17.028	8.670	0.271		
	547	13.936	5.800	0.181		
	542	14.257	6.237	0.195	0.523	1.904
B 12	537	17.251	9.350	0.292		
	539	17.085	9.924	0.310		
	566	12.808	5.748	0.180		
	541	11.147	4.466	0.140		
	540	13.816	7.536	0.236	0.445	1.923
C 3	557	15.389	6.179	0.193		
	556	17.656	8.503	0.266		
	550	19.748	10.630	0.332		
	555	15.734	7.238	0.226		
	532	17.917	9.705	0.303		
	549	15.760	7.682	0.240		
	553	16.841	9.044	0.283	0.528	2.004
C 13	552	17.498	9.807	0.306		
	559	14.828	7.321	0.229		
	554	12.005	4.825	0.151		
	558	15.858	8.807	0.275		
	548	14.474	7.691	0.240	0.471	1.958
D 4	561	23.364	13.012	0.407		
	567	17.659	8.465	0.265		
	642	19.957	10.926	0.341		
	570	17.668	8.869	0.277		
	571	18.034	9.422	0.294		
	565	15.279	6.842	0.214		
	568	16.134	7.951	0.248	0.571	1.952
D 14	563	13.474	6.224	0.195		
	564	14.196	7.297	0.228		
	560	17.515	10.737	0.336		
	533	10.798	4.180	0.131		
	544	7.723	1.573	0.049	0.379	2.019
E 5	588	20.147	9.765	0.305		
	621	20.443	10.803	0.338		
	577	18.385	9.193	0.287		
	579	11.647	2.466	0.077		
	581	21.223	12.341	0.386		
	576	15.762	7.260	0.227		
	573	14.136	6.349	0.198	0.502	1.932
E 15	572	16.857	9.367	0.293		
	578	17.255	9.821	0.307		
	575	12.013	5.850	0.183		
	580	12.414	6.560	0.205		
	583	11.843	6.028	0.188	0.454	1.930
F 6	590	22.327	12.850	0.402		
	593	19.510	10.119	0.316		
	594	19.230	10.379	0.324		
	536	16.214	7.503	0.234		
	584	15.576	6.934	0.217		
	591	17.981	9.691	0.303		
	631	16.986	8.969	0.280	0.558	1.883
F 16	574	15.707	7.937	0.248		
	589	18.000	10.428	0.326		
	585	15.848	9.018	0.282		
	592	11.884	5.356	0.167		
	587	10.991	4.760	0.149	0.419	1.787
G 7	604	20.936	11.509	0.360		
	600	21.090	11.869	0.371		
	605	22.392	13.664	0.427		
	601	17.931	9.229	0.288		
	607					
	598	14.229	6.410	0.200		
	599	15.830	8.080	0.253	0.540	1.865
G 17	606	15.489	7.951	0.248		
	596	16.169	8.679	0.271		
	597	14.179	6.960	0.218		
	639	17.811	10.817	0.338		
	603	15.816	8.889	0.278	0.480	1.774
H 8	612					
	611	21.615	12.470	0.390		
	609	19.252	10.612	0.332		
	619	18.103	9.706	0.303		
	618	20.422	12.061	0.377		

	617	17.545	9.242	0.289		
	608	16.738	8.763	0.274	0.563	1.783
H 18	**614**	15.200	7.250	0.227		
	582	14.012	6.099	0.191		
	615	17.438	10.320	0.323		
	610	16.611	9.599	0.300		
	613	15.302	9.567	0.299	0.483	1.802
I 9	**630**	20.113	10.500	0.328		
	625	18.614	9.404	0.294		
	562	16.777	7.691	0.240		
	626	17.032	7.950	0.248		
	616	17.977	9.300	0.291		
	546	19.335	11.183	0.349		
	629	16.133	8.332	0.260	0.523	1.819
I 19	**627**	18.284	10.562	0.330		
	628	16.478	8.822	0.276		
	620	11.662	4.370	0.137		
	623	16.508	10.411	0.325		
	637	12.589	6.662	0.208	0.472	2.868
J 10	**622**	19.107	9.177	0.287		
	640	16.330	6.986	0.218		
	624	14.946	5.777	0.181		
	602	18.971	10.115	0.316		
	643	19.117	10.756	0.336		
	634	17.568	9.535	0.298		
	638	19.740	11.901	0.372	0.530	1.849
J 20	**635**	13.779	6.172	0.193		
	633	17.025	9.816	0.307		
	632	13.374	6.170	0.193		
	641	15.744	9.169	0.287		
	636	13.290	7.156	0.224	0.453	1.881

BILAN

Traitement	ALP (U/L)	PHOSPHORE mg/dl	CALCIUM (mg/dl)	Zinc (mg/dL)
A	197.10	6.05	12.88	0.06403
A	406.58	6.55	12.43	0.06767
A	232.39	6.65	13.32	0.08153
A	328.08	5.03	13.68	0.06679
A	287.52	5.87	13.88	0.09008
A	301.20	4.90	13.61	0.06588
A	202.01	5.77	12.33	0.07292
A	349.51	4.52	13.36	0.06328
A	472.13	3.70	13.13	0.04488
A	328.41	3.60	13.36	0.05312
A				
A	471.18	3.59	14.80	0.06572
B	201.91	8.12	11.24	0.07588
B	174.50	9.54	10.78	0.07316
B	203.48	7.86	10.89	0.10568
B	234.97	7.93	11.02	0.07648
B	209.87	8.55	10.75	0.07624
B	338.96	7.20	10.93	0.06288
B	311.92	8.13	11.00	0.06564
B	192.74	8.09	11.32	0.05928
B	237.69	7.90	11.34	0.07764
B	289.05	6.26	11.36	0.06452
B	252.83	5.61	11.00	0.04312
B	225.36	7.12	11.11	0.07068
C	247.85	5.40	13.75	0.07152
C	246.38	6.54	11.49	0.08032
C	317.77	6.17	12.55	0.08116
C	315.11	6.42	12.65	0.08532
C	300.62	5.97	13.84	0.09372
C	217.59	6.03	12.08	0.07468
C	330.44	6.06	13.28	0.08404
C	307.47	4.75	14.03	0.07664
C	315.89	5.65	14.01	0.07584
C	194.15	5.33	12.58	0.06784
C	331.96	4.18	13.97	0.07412
C	305.57	3.91	13.78	0.07416
D	186.44	7.40	12.26	0.08232
D	281.86	6.15	11.80	0.06784
D	209.97	7.42	12.10	0.09392
D	236.47	5.98	12.94	0.08532
D	181.51	6.79	11.87	0.08368
D	144.16	6.02	11.77	0.06028
D	223.03	7.14	12.51	0.07832
D	254.58	5.51	12.78	0.07644
D	267.09	6.16	12.35	0.07208
D	268.59	5.40	12.69	0.06440
D	273.13	5.96	12.46	0.08144
D	154.01	4.81	10.71	0.03780
E	205.74	7.89	11.59	0.07992
E	339.14	7.87	11.02	0.08692
E	174.16	5.91	12.27	0.06376
E	246.97	5.46	10.90	0.03904

E	316.12	8.01	11.64	0.08448
E	336.17	6.09	12.92	0.07248
E	232.89	4.92	12.24	0.06892
E	317.19	6.81	12.18	0.06908
E	455.35	6.45	14.00	0.07404
E	280.24	6.84	12.15	0.07176
E	194.77	7.03	11.42	0.07600
E	264.86	5.78	12.34	0.05964
F	255.08	6.64	11.94	0.07844
F	194.47	7.72	11.50	0.07516
F	180.88	8.18	11.63	0.08888
F	219.67	5.54	11.48	0.07336
F	260.24	5.91	11.02	0.06496
F	512.17	7.62	11.59	0.07828
F	205.23	6.56	12.66	0.08768
F	225.21	7.77	12.14	0.07984
F	213.06	8.09	10.91	0.07464
F	190.60	8.55	11.43	0.08096
F	169.27	6.12	11.89	0.06500
F	222.57	6.14	11.92	0.05604
G	497.55	6.35	11.33	0.08268
G	185.40	7.83	12.57	0.07824
G	233.11	6.87	12.26	0.08420
G	412.84	6.09	11.27	0.06508
G				
G	277.09	5.17	12.44	0.07100
G	176.53	8.83	11.81	0.06024
G	198.81	9.52	11.75	0.07620
G	189.82	7.65	11.60	0.06284
G	221.58	5.76	11.67	0.04900
G	233.53	7.66	11.18	0.05956
G	184.06	7.78	11.51	0.07896
H				
H	444.61	8.08	11.70	0.05916
H	171.77	8.53	11.25	0.05456
H	346.47	7.06	11.59	0.06900
H	204.17	7.47	12.25	0.07204
H	273.47	8.01	10.68	0.08304
H	257.50	5.83	11.36	0.06040
H	168.62	8.05	11.28	0.08764
H	255.67	7.33	11.61	0.07050
H	267.03	7.01	12.60	0.07548
H	192.62	6.27	11.48	0.06700
H	230.48	7.05	11.93	0.07664
I	281.58	8.59	11.44	0.08556
I	231.98	8.45	11.23	0.08108
I	209.13	8.22	11.05	0.06108
I	312.47	8.16	10.62	0.06856
I	252.58	8.52	11.91	0.07836
I	289.83	7.44	11.15	0.06980
I	356.04	7.84	10.55	0.06700
I	256.24	8.26	12.38	0.07604
I	196.20	7.72	12.38	0.06276
I	139.10	6.86	11.02	0.04148
I	219.94	10.31	13.33	0.06532

I	273.48	6.88	12.46	0.06136
J	166.06	7.63	11.31	0.07812
J	210.52	7.03	11.66	0.07236
J	202.04	6.09	12.01	0.06408
J	215.30	8.42	11.42	0.07412
J	199.44	7.55	11.59	0.07628
J	198.55	9.20	11.94	0.07548
J	197.00	8.28	11.10	0.08844
J	325.61	8.15	11.48	0.07616
J	147.96	9.13	10.79	0.05056
J	220.65	8.87	11.29	0.07712
J	134.12	7.67	12.21	0.05348
J	210.67	7.60	11.92	0.06588

Diet	Treatment	FECES (mg/g DM)	DIGESTIBILITY (%)	Absorbed (mg/g)	Excreted (mg/g)
A1	Control without P	23.522	57.5	4.700	3.472
A1		20.524	59.8	4.889	3.283
A1		22.861	58.5	4.778	3.394
A11		23.152	56.8	4.641	3.532
A11		20.083	59.9	4.894	3.279
A11		22.335	59.4	4.857	3.315
B12	Control with diCa-P 16 g/kg	38.592	44.7	5.552	6.864
B12		35.725	46.4	5.758	6.659
B12		31.685	50.2	6.237	6.179
B2					
B2		29.055	55.4	6.881	5.535
B2		33.152	49.1	6.091	6.325
C13	IPA Phytase 250 U/kg	25.321	49.1	4.021	4.171
C13		24.771	51.9	4.248	3.944
C13		24.661	49.8	4.076	4.116
C3		24.971	55.8	4.568	3.624
C3		23.502	55.8	4.572	3.620
C3		20.718	58.9	4.823	3.369
D14	IPA Phytase 500 U/kg	18.704	63.3	5.313	3.080
D14		19.429	59.2	4.970	3.424
D14		17.398	64.2	5.387	3.007
D4		22.444	55.7	4.673	3.721
D4		15.429	67.8	5.691	2.703
D4		18.351	67.1	5.629	2.765
E15	IPA Phytase 1000 U/kg	19.319	61.3	5.043	3.177
E15		19.615	63.2	5.193	3.028
E15		20.150	60.8	5.000	3.221
E5		21.621	59.2	4.864	3.356
E5		19.730	63.3	5.207	3.014
E5		19.426	63.1	5.191	3.029
F16	IPA Phytase 1500 U/kg	18.203	65.7	5.411	2.825
F16		14.611	74.2	6.110	2.126
F16		17.973	67.5	5.556	2.680
F6		15.786	69.3	5.706	2.530
F6		17.277	62.5	5.151	3.085
F6		15.209	69.3	5.704	2.531
G17	IPA Phytase 2000 U/kg	13.105	75.5	6.097	1.976
G17		12.035	77.7	6.274	1.799
G17		13.778	74.7	6.033	2.040
G7		14.162	70.2	5.665	2.407
G7		13.369	72.7	5.866	2.206
G7		13.671	71.0	5.731	2.341
H18	IPA Phytase 3000 U/kg	17.467	64.2	5.083	2.835
H18		13.509	74.5	5.901	2.017
H18		17.903	66.9	5.299	2.619
H8		16.764	60.5	4.794	3.124
H8		17.082	61.8	4.896	3.021
H8		17.090	66.3	5.246	2.672
I19	IPA Phytase 4000 U/kg	14.068	74.7	5.721	1.941
I19		10.763	78.6	6.023	1.639
I19		14.697	70.8	5.425	2.237
I9		13.691	70.0	5.364	2.298
I9		13.555	70.5	5.403	2.259
I9		13.277	75.9	5.818	1.844
J10	IPA Phytase 8000 U/kg	18.050	59.8	4.734	3.181
J10		14.826	67.6	5.349	2.566
J10		15.392	71.2	5.635	2.281
J20		11.471	77.0	6.093	1.822
J20		15.504	67.7	5.361	2.554

J20		18.140	67.3	5.327	2.589

Diet	Treatment	P in FECES (mg/g DM)	DIGESTIBILITY (%)	Absorbed (mg/g)	Excreted (mg/g)	equivalence (mg/g)
A1	Control without P	23.143	24.3	1.099	3.416	0.010
A1		22.165	21.5	0.969	3.546	-0.119
A1		22.730	25.3	1.140	3.375	0.052
A11		22.637	23.5	1.062	3.453	-0.027
A11		21.114	23.7	1.068	3.447	-0.021
A11		22.370	26.5	1.194	3.321	0.106
B12	Control with diCa-P 16 g/kg	28.366	35.3	2.756	5.045	1.667
B12		26.519	36.6	2.859	4.943	1.770
B12		24.149	39.6	3.092	4.709	2.003
B2						
B2		20.690	49.5	3.860	3.942	2.771
B2		23.304	43.0	3.355	4.446	2.267
C13	*IPA Phytase 250 U/kg*	20.864	24.0	1.083	3.437	-0.006
C13		21.555	24.1	1.088	3.432	-0.001
C13		20.470	24.4	1.103	3.416	0.014
C3		22.466	27.9	1.259	3.260	0.170
C3		21.356	27.2	1.230	3.289	0.141
C3		18.360	33.9	1.534	2.986	0.445
D14	*IPA Phytase 500 U/kg*	15.963	42.3	1.929	2.629	0.840
D14		16.233	37.2	1.697	2.861	0.608
D14		14.475	45.1	2.056	2.502	0.967
D4		17.895	34.9	1.591	2.967	0.502
D4		16.252	37.5	1.710	2.847	0.621
D4		17.258	42.9	1.957	2.600	0.869
E15	*IPA Phytase 1000 U/kg*	16.450	40.0	1.803	2.705	0.714
E15		16.356	44.0	1.983	2.525	0.894
E15		15.592	44.7	2.016	2.492	0.927
E5		17.542	39.6	1.785	2.723	0.696
E5		15.861	46.3	2.086	2.423	0.997
E5		16.787	41.9	1.891	2.618	0.802
F16	*IPA Phytase 1500 U/kg*	15.252	48.0	2.184	2.367	1.095
F16		12.924	58.7	2.671	1.880	1.582
F16		12.861	57.9	2.633	1.918	1.544
F6		14.904	47.5	2.162	2.388	1.073
F6		13.580	46.7	2.126	2.425	1.037
F6		14.933	45.4	2.065	2.485	0.976
G17	*IPA Phytase 2000 U/kg*	12.771	58.3	2.690	1.925	1.601
G17		12.705	58.9	2.716	1.899	1.628
G17		13.975	55.2	2.546	2.069	1.458
G7		12.138	55.3	2.552	2.063	1.463
G7		12.501	55.3	2.552	2.063	1.463
G7		12.573	53.3	2.462	2.153	1.373
H18	*IPA Phytase 3000 U/kg*	14.327	48.4	2.181	2.325	1.092
H18		12.242	59.4	2.679	1.828	1.590
H18		13.358	56.6	2.552	1.954	1.463
H8		11.460	52.6	2.371	2.136	1.282
H8		11.210	56.0	2.524	1.983	1.435
H8		12.234	57.6	2.593	1.913	1.505
I19	*IPA Phytase 4000 U/kg*	13.426	59.7	2.745	1.853	1.656
I19		9.403	68.9	3.166	1.432	2.077
I19		12.051	60.1	2.763	1.834	1.674
I9		11.329	58.6	2.696	1.902	1.607
I9		11.020	60.1	2.761	1.837	1.672
I9		12.058	63.6	2.922	1.675	1.834
J10	*IPA hytase 00 U/kg*	12.463	51.2	2.306	2.196	1.217
J10		10.780	58.6	2.636	1.866	1.547
J10		10.812	64.4	2.900	1.602	1.811
J20		10.231	63.9	2.877	1.625	1.788

J20	P 80	11.476	58.0	2.612	1.891	1.523
J20		11.146	64.7	2.912	1.590	1.823

Matières sèches
Cendres

Box Traitement	N° Porc	N° Creuset	% Cendres 100% MS
A	530	1	60.85
	527	2	61.14
	531	3	62.39
	569	4	58.03
	524	5	62.41
	534	6	63.20
	551	8	65.18
	525	11	64.52
	526	12	62.62
	529	13	61.33
	535	14	62.18
B	539	15	63.33
	541	16	66.00
	540	17	63.14
	566	18	60.89
	537	19	63.01
	586	20	62.92
	595	21	63.14
	543	22	63.82
	542	23	66.26
	547	25	62.02
	545	26	65.06
	538	27	64.82
C	552	28	64.15
	548	29	64.05
	559	30	60.78
	558	31	61.32
	554	32	65.66
	550	33	64.14
	549	35	59.85
	556	36	60.93
	532	38	62.49
	555	39	60.27
	553	42	61.36
	557	44	63.60
D	564	45	65.63
	570	47	65.87
	563	48	63.56
	560	49	63.99
	533	50	65.08
	567	51	65.48
	561	52	66.53
	565	53	65.64
	544	54	65.35
	568	46	63.83
	642	55	65.08
	571	56	66.19
E	578	57	65.33
	583	58	66.36
	575	59	65.92
	580	60	64.07

Traitement	Force maximale (N)	
535 Tr:A	256.7426249	
529 Tr:A	340.1596004	
526 Tr:A	258.093239	
525 Tr:A	186.7291519	
551 Tr:A	530.3761638	
534 Tr:A	384.5513903	
524 Tr:A	323.8680103	
569 Tr:A	115.666211	
531 Tr:A	266.7461561	
527 Tr:A	164.2895492	
530 Tr:A	173.7323481	
		272.81404
538 Tr:B	540.0311813	
545 Tr:B	834.7405529	
547 Tr:B	427.9822428	
542 Tr:B	591.1987166	
543 Tr:B	921.8980087	
595 Tr:B	778.6862945	
586 Tr:B	539.8671732	
537 Tr:B	439.0113013	
566 Tr:B	334.4434488	
540 Tr:B	845.074253	
541 Tr:B	361.5372753	
539 Tr:B	771.7388637	615.517443
557 Tr:C	402.801209	
553 Tr:C	344.8880304	
555 Tr:C	470.3058998	
532 Tr:C	312.6604302	
556 Tr:C	297.0565641	
549 Tr:C	263.1132889	
550 Tr:C	528.383459	
554 Tr:C	235.6361231	
558 Tr:C	354.6941817	
559 Tr:C	362.5725794	
548 Tr:C	185.7726641	
552 Tr:C	257.5002348	334.615389
565 Tr:D	363.321649	
561 Tr:D	688.0720657	
567 Tr:D	532.6939528	
571 Tr:D	648.9839943	
642 Tr:D	341.1408643	
568 Tr:D	487.1100546	
570 Tr:D	663.1119311	
564 Tr:D	537.1049144	
563 Tr:D	257.0583716	
560 Tr:D	475.6459217	
533 Tr:D	521.6086111	
544 Tr:D	197.1909669	
621 Tr:E	481.3788921	
581 Tr:E	456.2503067	
577 Tr:E	400.4909892	
579 Tr:E	497.5923086	

	572	61	64.41	588 Tr:E	491.1624503
	576	62	65.03	573 Tr:E	279.9124038
	573	63	66.28	576 Tr:E	332.075501
	588	65	65.89	572 Tr:E	339.4116251
	579	66	67.40	580 Tr:E	225.6324171
	577	67	65.86	575 Tr:E	323.5987187
	581	68	66.56	583 Tr:E	340.8468524
	621	69	64.89	578 Tr:E	441.0039327
F	594	70	66.23	590 Tr:F	733.3963922
	593	74	67.11	631 Tr:F	732.8905364
	536	73	65.67	584 Tr:F	381.1834395
	584	76	66.58	536 Tr:F	441.4039541
	631	78	67.75	593 Tr:F	575.2005417
	590	79	66.44	594 Tr:F	480.4039022
	589	80	66.22	591 Tr:F	366.3464737
	574	81	65.38	587 Tr:F	355.7700955
	585	82	63.74	592 Tr:F	442.8848729
	592	96	66.22	585 Tr:F	471.6250791
	587	5	66.85	574 Tr:F	350.7080104
	591	20	61.42	589 Tr:F	672.300791
G	639	25	66.03	605 Tr:G	677.2170074
	603	29	64.90	600 Tr:G	674.3742296
	596	38	65.40	601 Tr:G	346.47432
	606	75	63.87	599 Tr:G	579.8156437
	604	79	65.48	598 Tr:G	771.2172716
	598	80	66.78	604 Tr:G	419.0117661
	597	82	65.19	606 Tr:G	340.948089
	599	86	60.91	596 Tr:G	437.8630664
	601	88	64.20	597 Tr:G	366.4934948
	600	94	65.93	603 Tr:G	363.1637289
	605	103	64.68	639 Tr:G	782.2427814
H	613	107	64.57	618 Tr:H	651.9685548
	610	109	65.77	611 Tr:H	474.3866234
	615	110	66.69	609 Tr:H	456.6356886
	582	111	63.84	619 Tr:H	278.1951272
	614	113	65.98	608 Tr:H	382.0124437
	617	117	66.06	617 Tr:H	478.0334909
	618	119	65.10	614 Tr:H	832.5413146
	611	148	66.14	582 Tr:H	413.9346984
	608	155	65.77	615 Tr:H	346.0081756
	619	175	65.00	610 Tr:H	450.7266936
	609	197	67.77	613 Tr:H	477.3702603
I	629	199	65.49	629 Tr:I	386.0671629
	562	221	67.45	562 Tr:I	737.3985065
	546	280	66.35	546 Tr:I	713.7054483
	628	304	67.12	630 Tr:I	544.540216
	627	308	66.84	618 Tr:I	649.0371071
	630	331	69.05	625 Tr:I	525.0902763
	616	336	66.68	626 Tr:I	588.7252952
	625	338	65.67	637 Tr:I	448.852972
	637	339	65.56	620 Tr:I	317.7936126
	626	342	66.47	623 Tr:I	454.9275191

	620	343	64.71	627 Tr:I	468.0993745
	623	352	64.90	628 Tr:I	672.3203289
J	638	355	65.55	643 Tr:J	811.7108032
	624	357	63.06	640 Tr:J	623.5242283
	602	362	62.64	622 Tr:J	630.5928393
	633	365	67.79	634 Tr:J	649.8534942
	640	390	67.05	624 Tr:J	481.5921059
	643	25	67.37	638 Tr:J	670.7008961
	622	52	64.99	602 Tr:J	410.3670993
	634	55	65.44	633 Tr:J	927.3685516
	632	68	63.47	636 Tr:J	488.385717
	641	70	63.68	635 Tr:J	506.7045849
	636	80	64.88	632 Tr:J	418.3187809
	635	58	66.97	641 Tr:J	629.8908135

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA phytase**

Batch number: **PPQ28432**

Trial ID: **S 12-08 VN**

Location:
DSM Nutritional Products France
Centre de Recherche en Nutrition Animale
BP 170
68305 Saint-Louis cedex, France

Start date and exact duration of the study: **July 3rd 2008 - 32 days**

Number of treatment groups (+ control(s)): **8 + (2)**

Replicates per group: **2**

Total number of animals: **120**

Animals per replicate: **7 + 5 = 12**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **0 / 250 / 500 / 1000 / 1500 / 2000 / 3000 / 4000 / 8000 U/kg**

Analysed: **108 (endogenous activity) / 374 / 601 / 1097 / 1611 / 2225 / 3098 / 4030 / 8238 U/kg**

†

Substances used for comparative purposes: **Dicalcium phosphate**

Intended dose: **16 g per kg of feed. Equivalent to 3.2 g of additional P per kg of feed in a dry matter basis**

Analysed: **3.3 g of additional P per kg of feed in a dry matter basis**

Animal species/category: **Swine / weaners**

Breed: **Large White x Landrace**

Identification procedure: **Pen and individual earring**

Sex: **Males**

Age at start: **28 days**

Body weight at start: **8.03 ± 1.09 kg**

Physiological stage: **Weaned piglets**

General health: **Three animals presented leg injuries and were euthanized. No clinical sings were observed in the rest of the animals**

Additional information for field trials:

Location and size of herd or flock:

Feeding and rearing conditions:

Method of feeding:

Diets (type(s)): **Basal diet formulated to provide P exclusively from vegetable origin and according to the NRC**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize - 68.52%, soybean meal - 15.1% and rapeseed meal - 12.5%**

Nutrient content (relevant nutrients and energy content)

Intended values: **Crude protein - 15.5%, lysine - 0.96%, methionine + cystine - 0.54%, P - 0.41% in D.M. and digestible energy - 13.31 MJ/kg**

Analysed values: **Ca - 0.82% in D.M. and P - 0.45% in D.M.**

[1] Please submit this form using a common word processing format (e.g. MS Word).

Date and nature of the examinations performed: **July 3rd and August 4th - weight measurement** **July 29th, 30th and 31st - faecal sampling per pen** **July 31st - individual blood sampling** Method(s) of statistical evaluation used: **Two-factor analysis of variance (diet and diet + animal or pen) followed by a Duncan multiple range test** Therapeutic/preventive treatments (reason, timing, kind, duration): **No therapeutic / preventive treatments were used** Timing and prevalence of any undesirable consequences of treatment: **Nothing to report**	
Date **22.02.2010**	Signature Study Director **Dr P. GUGGENBUHL** [signature]

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.

Revised Annex 6
Internal Report 2500672
Change made to page 8 on 7 April 2014

REPORT No. 2500672
Regulatory Document

DSM

Document Date:	10-Jun-2009
Author(s):	Guggenbuhl P, Simões Nunes C, Piñón Quintana A, Portier C, Kurtz N and Lehmann A
Title:	Evaluation of graded amounts of a microbial phytase on the faecal digestibility and excretion of phosphorus, calcium and zinc in growing pigs.
Project No.	6106
Compound No.	

Summary

The aim of the present study (S 05-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the digestibility of phosphorus (P), calcium (Ca) and zinc (Zn) in the growing pig.

The basal diet, without addition of mineral P, was based on soybean meal, maize and barley. IPA phytase was included in the diet at the levels of 500 U/kg, 1000 U/kg, 1500 U/kg, 1750 U/kg, 2000 U/kg, 2500 U/kg and 3000 U/kg. A dietary treatment was based in the very slightly modified control diet containing the recommended available P by addition of dicalcium phosphate (diCa-P).

The mean P faecal concentration of the enzyme supplemented animals was significantly lower than that observed for the animals ingesting the control diet.

All the phytase inclusion levels increased the bioavailability of P and accordingly reduced the growing pig quantitative faecal excretion of P comparatively to the basal diet.

The P digestibility was dose dependant and highly significantly improved by 21.1, 28.5, 30.5, 32.0, 32.2, 37.3 and 38.7 % in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. The digestibility of P in the diCa-P supplemented diet was also significantly higher than that of the control.

The faecal excretion of P was significantly reduced by 29.3, 40.1, 42.8, 45.8, 45.6, 53.0, and 55.2 % with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. It was increased by 10.1 % with the diCa-P supplemented group.

The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control of 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg were 0.91, 1.22, 1.30, 1.32, 1.36, 1.56 and 1.60 g of full available P/kg feed respectively. In comparison the P equivalency of the diCa-P supplemented diet was 1.70 g of full available P/kg feed.

Ca and Zn digestibilities were significantly improved by all the inclusion levels of the phytase.

It can be concluded that the IPA phytase improved the digestibility and the apparent absorption of P, Ca and Zn, and reduced the P faecal excretion in the pig fed on a diet containing P exclusively from vegetable origin.

There was a dose dependant increase of the effects of the enzyme on the availability of the dietary P.

This report consists of 22 pages

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Nomenclature and Structural Formula (if available)

Liquid form IPA phytase expressed in *Aspergillus oryzae*, batch PPQ27987, activity at pH 5.5 of 24850 U/g.

1. INTRODUCTION

The aim of the present study (S 05-08 VN) was to evaluate the effects of graded amounts of a microbial phytase (IPA) on the digestibility of phosphorus (P), calcium (Ca) and zinc (Zn) in the growing pig. The basal diet, without addition of mineral P, was based on soybean meal, maize and barley. IPA phytase was included in the diet at the levels of 500 U/kg, 1000 U/kg, 1500 U/kg, 1750 U/kg, 2000 U/kg, 2500 U/kg and 3000 U/kg. A dietary treatment was based in the very slightly modified control diet containing the recommended available P by addition of dicalcium phosphate (diCa-P).

The experiment was performed in March-April 2008 in the facilities of the Centre de Recherche en Nutrition Animale (CRNA), DSM Nutritional Products France, BP 170, 68305 Saint-Louis cedex, France. It has been performed according to the French legal regulations on experiments with live animals.

2. MATERIAL AND METHODS

2.1. Test enzymes

The used IPA phytase was expressed in *Aspergillus oryzae*, batch PPQ27987, had an activity at pH 5.5 of 24850 U/g and was in a liquid form.

NRD/CM measured the phytase activity in the enzyme preparation and in the feed. One unit of phytase is defined as the quantity of enzyme which sets free 1 µmole of inorganic phosphate per minute from 0.005 moles per litre sodium phytate at pH 5.5 and at 37°C.

2.2. Animal trial

Thirty six Large White × Landrace pigs having an initial body weight of 19.06 ± 1.82 kg were used. The animals were housed in floor-pen cages in 9 groups of 4 animals each in an environmentally controlled room. Each pen had a plastic-coated welded wire floor and was equipped with two water nipples and four stainless-steel individualised feeders. Room temperature was 21-22° C and humidity percentage was 50 %.
The pigs were fed a basal diet without addition of mineral P (diet A) during an adaptive period of 16 days. After that period they were allocated into 9 equal groups and fed for 12 days the basal diet (group A) or the diet A supplemented with 12 g/kg of dicalcium phosphate (group B) or with IPA phytase at the levels of 500 U/kg (group C), 1000 U/kg (group D), 1500 U/kg (group E), 1750 U/kg (group F), 2000 U/kg (group G), 2500 U/kg (group H), 3000 U/kg (group I).
The basal diet A was formulated to provide P exclusively from vegetable origin and to meet, with the exception of the available P supply, the animals' requirements according to Henry *et al.* (1989) and NRC (1998). The basal diet A (table 1) had a theoretical P content of 0.41 % and an analysed content of 0.42 %. The theoretical available P in the diet was 1.20 g/kg and the observed availability of 1.24 g/kg.

An indigestible tracer (chromium oxide) was added at a concentration of 0.4 % to all the diets allowing calculation of the digestibility of P, Ca and Zn. The feed was distributed *ad libitum* in mash form, under pen feed consumption control, and the animals had free access to drinking water. The digestibility of Ca was not corrected for Ca intake with the drinking water. Mean Ca content of the drinking water in the region is 120 mg/L.

Faecal P, Ca, Zn and Cr concentrations were measured at the 12[th] day of the second period. Faeces were sampled individually, in approximately the same amount at the same time of the day, during the last 3 days preceding that date. Thus, for each dietary treatment and for each criterion a total of 12 individual determinations have been performed. All minerals were determined according to standard Association of Official Analytical Chemists (1990) methods using a Vista-MPX ICP-OES spectrometer (Varian Australia Pty Ltd, Mulgrave Victoria, 3170 Australia). The apparent digestibility (% of the intake) of the minerals was calculated for the mentioned 3 day period.

2.3. Statistical analysis

Statistical treatment of the results involved the calculation of the mean and of the standard deviation of the mean as well as a two-factor hierarchical analysis of variance. The mathematical model was:

$$Yijk = \mu+Ai+Bij+Zijk,$$

where μ is the mean, Ai is the diet effect, Bij is the combined effect of the diet and animal or pen and Zijk is the residual term. The analysis of variance was followed by a Duncan multiple range test when a significant Ai effect without Bij effect was observed (Snedecor and Cochran, 1989). These calculations were performed using StatGraphics Plus 5.1 (Manugistics, Rockville, U.S.A. 2001).

3. RESULTS

3.1. Phytase and animals

The observed IPA phytase activity in the supplemented feed used was in general in excellent agreement with the programmed inclusion levels (table 2).

The animals grew normally during the observation period to reach a final mean body weight of 44.84 ± 3.37 kg. Their daily weight gain was of 679 ± 5 g. No mortality was observed during the experiment.
Two animals from group H, receiving the diet supplemented with IPA phytase at 2500 U/kg presented diarrhoea during the sampling period, so that no faeces could be collected from them. No statistical analysis was performed for this group as the total amount of faeces samples was only the half (n = 6) of the other groups (n = 12).

3.2. Effects on phosphorus

The mean P faecal concentration of the enzyme supplemented animals was very significantly lower than that measured in the animals ingesting the control diet (table 3). There was a decrease of the P faecal concentration with the increasing allowance of IPA phytase. The lowest P faecal concentration was observed in the animals ingesting IPA phytase at 3000 U/kg feed.

The P digestibility was dose dependant and highly significantly improved by 21.1, 28.5, 30.5, 32.0, 32.2, 37.3 and 38.7 percentage units in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg IPA phytase supplemented groups respectively (table 4, figure 2).
The digestibility of P in the diCa-P supplemented diet was also significantly higher than that of the control by 17.9 percentage units and very similar to the enzyme supplementation at 500 U/kg.

The faecal excretion of P was significantly reduced by 29.3, 40.1, 42.8, 45.8, 45.6, 53.0, and 55.2 % with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. It was increased by 10.1 % with the diCa-P supplemented group (table 5, figure 2).

The apparent absorbed P was 2.15, 2.45, 2.54, 2.56, 2.60, 2.80 and 2.84 g/kg feed with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively and 2.93 g/kg feed in the diCa-P supplemented group. It was significantly increased in all the supplemented groups in comparison to the control diet (1.24 g/kg). With the exception of the IPA phytase 500 U/kg inclusion level, all other supplemented groups were over the recommended requirements of 2.25 g of digestible P per kg feed (Ernandoréna *et al.*, 2008).

The P equivalencies, considered as supplemental P digested comparatively to the non-supplemented control, of 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg of IPA phytase were 0.91, 1.22, 1.30, 1.32, 1.36, 1.56 and 1.60 g of full available P/kg feed respectively (table 6, figure 3). In comparison the P equivalency of the diCa-P supplemented diet was 1.70 g of full available P/kg feed.
In the present study, using the equation of the tendency curve the calculated inclusion level to reach 1.5 g of full available P/kg feed was 2412 U/kg feed of IPA phytase ($y = 48.982e^{2.5978x}$, $R^2 = 0.9597$).

3.3. Effects on calcium

The Ca faecal concentration of the animals ingesting the basal diet supplemented or not with diCa-P was systematically higher than that of the animals ingesting the diets supplemented with the phytase (table 7). The observed differences were statistically significant for all the enzyme supplemented groups.

The Ca digestibility was significantly improved by the phytase and by all the inclusion levels of IPA phytase (table 8, figure 4). The improvements were 8.6, 12.8, 12.6, 15.5, 15.1, 26.5 and 21.6 percentage units in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg IPA phytase supplemented groups respectively.
The Ca digestibility of the IPA phytase supplemented diets was more or less dose dependant.

The faecal excretion of Ca was significantly reduced by 23.9, 34.6, 36.8, 42.3, 41.7, 67.9, and 57.0 % with IPA phytase in the 500, 1000, 1500, 1750, 2000, 2500 and 3000 U/kg supplemented groups respectively. It was increased by 12.1 % with the diCa-P supplemented group (table 9, figure 5).

3.3. Effects on zinc

The Zn faecal concentration of the animals ingesting the non-supplemented control diet was systematically higher than that of the animals ingesting the diets supplemented with phytase with the exception of the two highest dosage of IPA phytase (table 10). The observed differences were not statistically significant for all the supplemented groups.

The Zn digestibility was significantly improved by the phytase for all inclusion levels in comparison to the basal diet (table 11). The Zn digestibility of the IPA phytase supplemented diets presented high biological variations from one group to the others giving no regularity in the dose curve.

The faecal excretion of Zn was significantly reduced in the phytase supplemented groups (table 12). IPA phytase presented inconsistency in the faecal Zn excretion reduction in regard to the increasing inclusion levels.

4. CONCLUSION

It can be concluded that the IPA phytase improved the digestibility and the apparent absorption of P, Ca and Zn, and reduced the P faecal excretion in the pig fed on a diet containing P exclusively from vegetable origin. There was a dose dependant effect of the IPA phytase on the availability of the dietary P.

Table 1 - Composition (%) of the basal diet (A) and of that supplemented with diCa-P (B)

INGREDIENTS	Basal diet A without P (%)	Basal diet B with diCa-P (%)
Maize	53	53
Soybean meal	18	18
Barley	13.9	13
Oat meal	6	6
Wheat bran	5.4	5.4
Soya oil	1	1
diCa-P	-	1.2
Minerals [1], vitamins and synthetic aa	2.7	2.4
Crude protein - N x 6.25 - %	15.5	15.5
Lysine - %	0.96	0.96
Methionine + cystine - %	0.54	0.54
Ca - calculated - % in DM	0.66	0.86
Ca - analysed in - % in DM	0.70	0.80
P - calculated - % in DM	0.41	0.65
P - analysed - % in DM	0.42	0.62
Theoretically available P - %	0.12 [2]	1.86 [3]
Phytic-P - calculated - %	0.28	0.28
Estimated digestible energy - MJ / kg	*13.31*	*13.31*
Phytase activity - U[4]/ kg	*225 ± 4*	*219 ± 4*

[1] Mixture without mineral P;
[2] Estimated from the mean P digestibility of the previous realized trials
[3] Sum of the theoretically available P and 80 % of added mineral P as generally accepted
[4] Quantity of enzyme that sets free 1 μmole of inorganic phosphate per minute from 0.005 mole per litre sodium phytate at pH 5.5 and at 37°C.

Table 2 - Phytase activity (U[(a)]/kg) and % of the target in the different diets.

Treatment groups	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Measured phytase addition (U/kg) [(1)]	225 ± 4	219 ± 4	678 ± 6	1179 ± 24	1723 ± 13	1985 ± 8	2232 ± 34	2798 ± 35	3329 ± 54
Actually added phytase (U/kg)	-	-	453	954	1498	1760	2007	2573	3104
% of target	-	-	91	94	100	101	100	103	103

[(a)] Quantity of enzyme that sets free 1 μmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 3.2 and at 37°C.
[(1)] Mean ± standard deviation of 2 determinations.

Erratum - footnote (a) should read 'Qualtity of enzyme that sets free 1 μmole of inorganic phosphate per minute from 5 mM sodium phytate at pH 5.5 and at 37 °C.'
This change was made by James La Marta, Sr. Mgr. regulatory Affairs, on 7 April 2014 after consultation with the lead author Dr. Peter Guggenbuhl.

Table 3 - Effects of the IPA phytase on the faecal concentration of phosphorus in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal P concentration (%of DM) (1)	1.59 ± 0.25	1.67 ± 0.37	1.19 ± 0.13	1.08 ± 0.10	0.97 ± 0.10	0.99 ± 0.17	0.94 ± 0.14	0.86(2) ± 0.18	0.83 ± 0.18
Variation from A (%)	100	105.6	74.8	68.2	61.4	62.2	59.2	54.5	52.6
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	NS	P<0.05		P<0.001
				D -	NS	NS	NS		P<0.05
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

<u>Table 4</u> - **Effects of the IPA phytase on the total apparent digestibility of phosphorus in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal P digestibility (%) (1)	29.3 ± 5.5	47.2 ± 7.9	50.4 ± 5.6	57.8 ± 3.9	59.8 ± 3.5	61.3 ± 3.9	61.5 ± 3.3	66.6(2) ± 4.2	68.0 ± 6.2
Variation from A (%)	-	61.0	72.2	97.5	104.1	109.2	110.0	127.2	132.0
Statistical analysis									
	A -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	NS	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.001
						F -	NS		P<0.05
							G -		P<0.05
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

<u>Table 5</u> - **Effects of the IPA phytase on the faecal excretion of phosphorus in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal P excretion (mg/g DM) [1]	2.99 ± 0.23	3.29 ± 0.49	2.11 ± 0.24	1.79 ± 0.16	1.71 ± 0.15	1.62 ± 0.16	1.63 ± 0.14	1.40[2] ± 0.18	1.34 ± 0.26
Variation from A (%)	100	110.1	70.7	59.9	57.2	54.2	54.4	47.0	44.8
Statistical analysis									
	A -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.001
						F -	NS		P<0.05
							G -		P<0.05
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
[1] Mean ± standard deviation of the mean of 12 determinations. [2] Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Figure 1

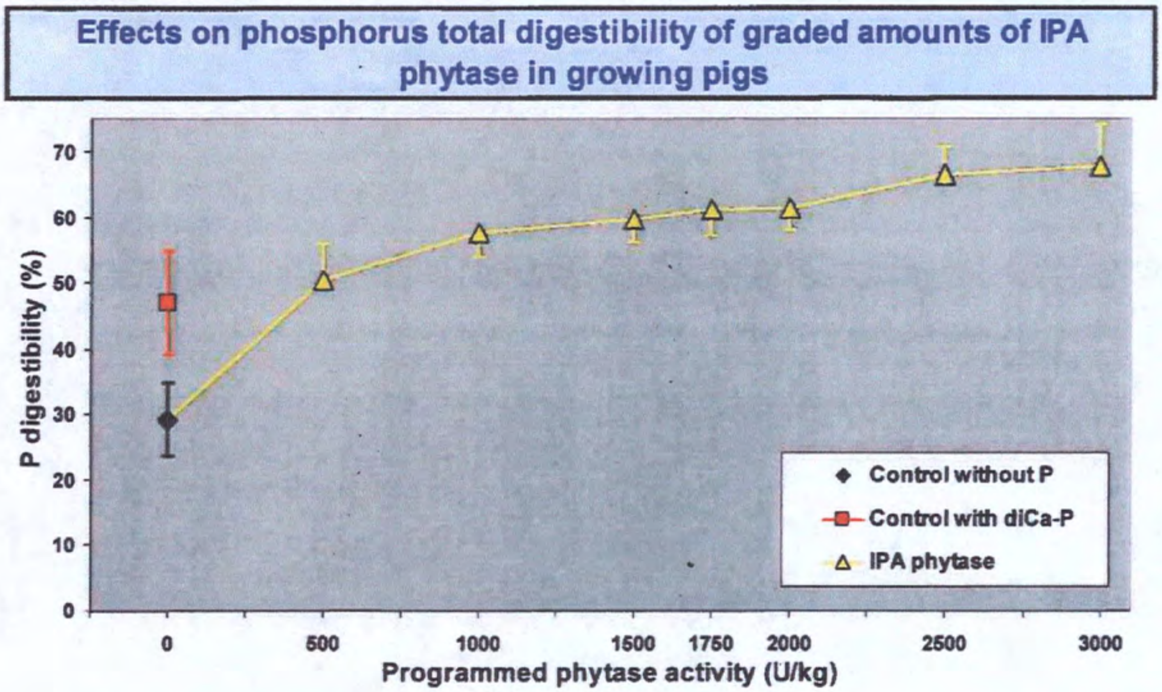

Figure 2

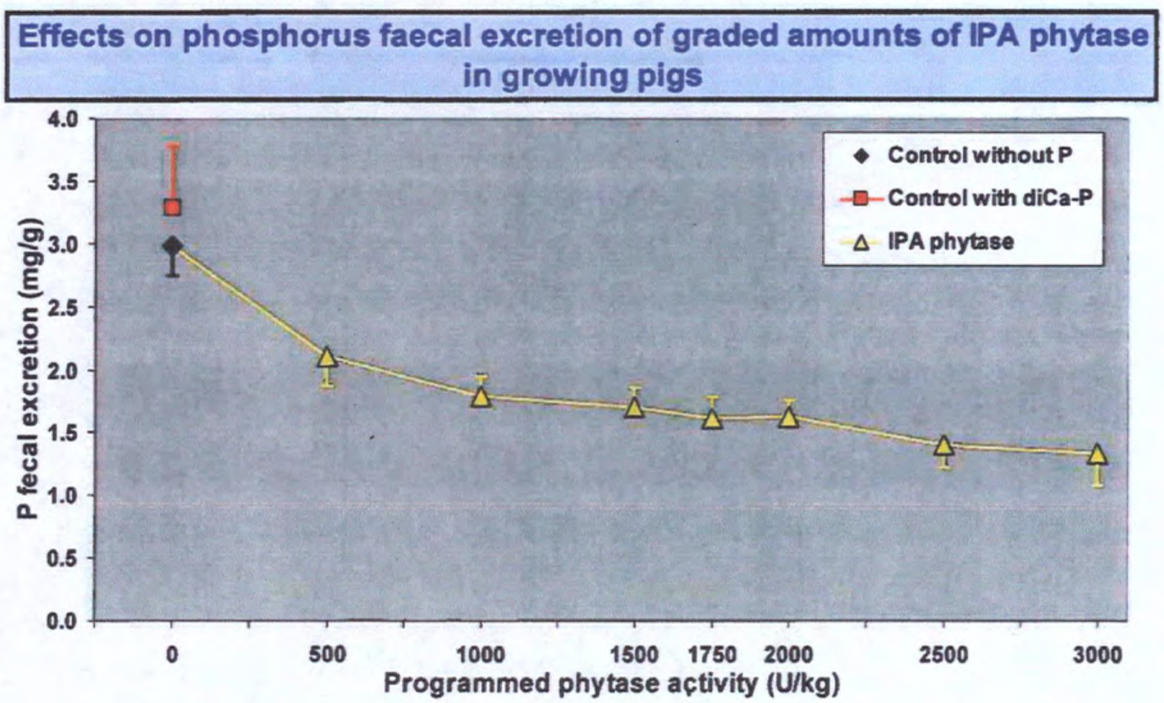

Table 6 - Phosphorus equivalencies (g of full available supplemental P per kg of feed comparatively to the non-supplemented control) of the IPA phytase in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
P equivalence (g/kg feed)	0.00 ± 0.23	1.70 ± 0.49	0.91 ± 0.24	1.22 ± 0.16	1.30 ± 0.15	1.32 ± 0.16	1.36 ± 0.14	1.56[(2)] ± 0.18	1.60 ± 0.26
P eq. variation from C (%)	-	-	100	133.5	143.1	145.2	149.3	171.0	175.7
Statistical analysis									
	A -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		NS
			C -	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.05
						F -	NS		P<0.05
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Figure 3

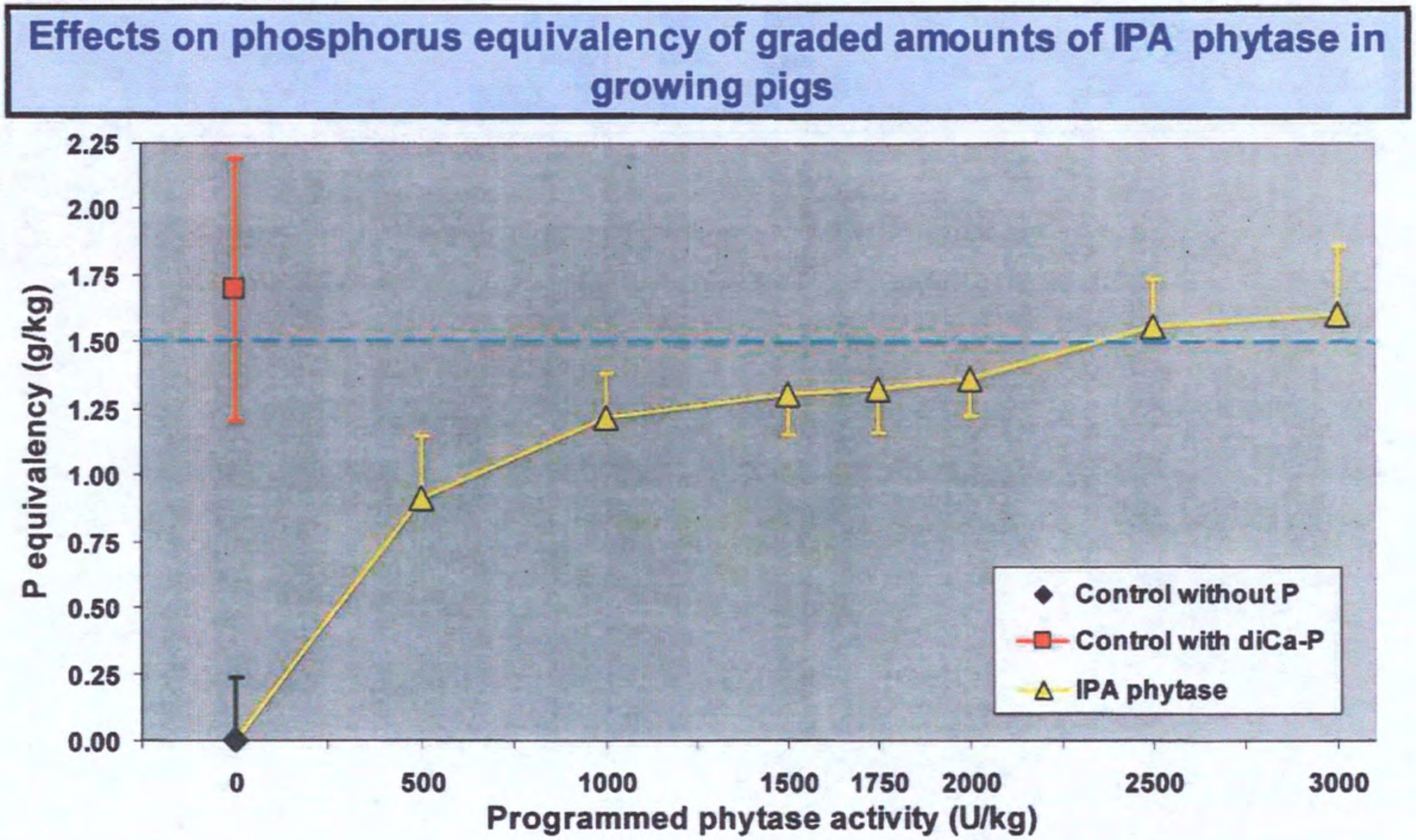
Effects on phosphorus equivalency of graded amounts of IPA phytase in growing pigs
P equivalency (g/kg)
2.25
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
500
1000
1500
1750
2000
2500
3000
Programmed phytase activity (U/kg)
Control without P
Control with diCa-P
IPA phytase

Table 7 - **Effects of the IPA phytase on the faecal concentration of calcium in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Ca concentration (% of DM) (1)	1.48 ± 0.30	1.58 ± 0.38	1.18 ± 0.20	1.09 ± 0.21	1.00 ± 0.22	0.97 ± 0.16	0.93 ± 0.15	0.54(2) ± 0.17	0.74 ± 0.30
Variation from A (%)	100	106.9	79.8	73.7	67.6	65.5	63.0	36.7	49.8
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	NS	NS		P<0.001
				D -	NS	NS	NS		P<0.05
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 8 - Effects of the IPA phytase on the total apparent digestibility of calcium in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Ca digestibility (%) (1)	60.2 ± 3.7	61.1 ± 7.1	68.8 ± 5.9	73.0 ± 5.8	72.8 ± 5.5	75.7 ± 3.6	75.3 ± 2.7	86.7(2) ± 4.2	81.8 ± 7.9
Variation from A (%)	100	101.5	114.3	121.2	120.9	125.7	125.1	143.9	135.8
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	P<0.05	P<0.05		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.001
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 9 - **Effects of the IPA phytase on the faecal excretion of calcium in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Ca excretion (mg/g DM) [(1)]	2.77 ± 0.26	3.11 ± 0.57	2.11 ± 0.40	1.81 ± 0.39	1.75 ± 0.35	1.60 ± 0.23	1.62 ± 0.18	0.89[(2)] ± 0.28	1.19 ± 0.52
Variation from A (%)	100	112.1	76.1	65.4	63.2	57.7	58.3	32.1	43.0
Statistical analysis									
	A -	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
			C -	NS	NS	P<0.05	P<0.05		P<0.001
				D -	NS	NS	NS		P<0.001
					E -	NS	NS		P<0.05
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Figure 4

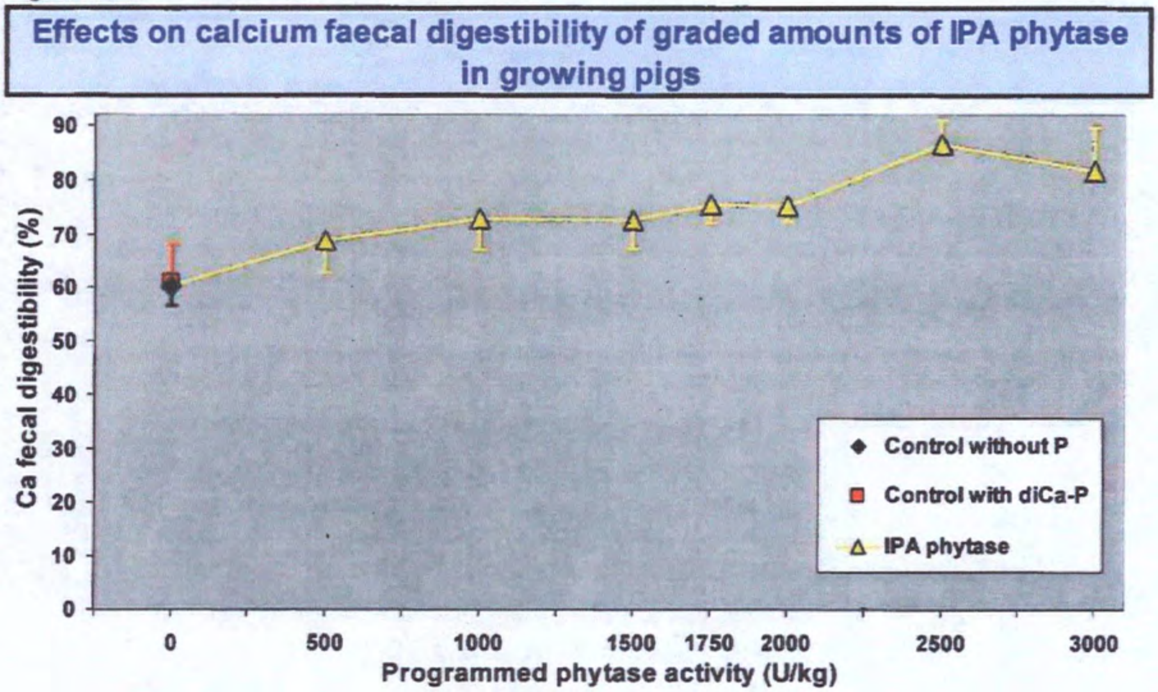

Figure 5

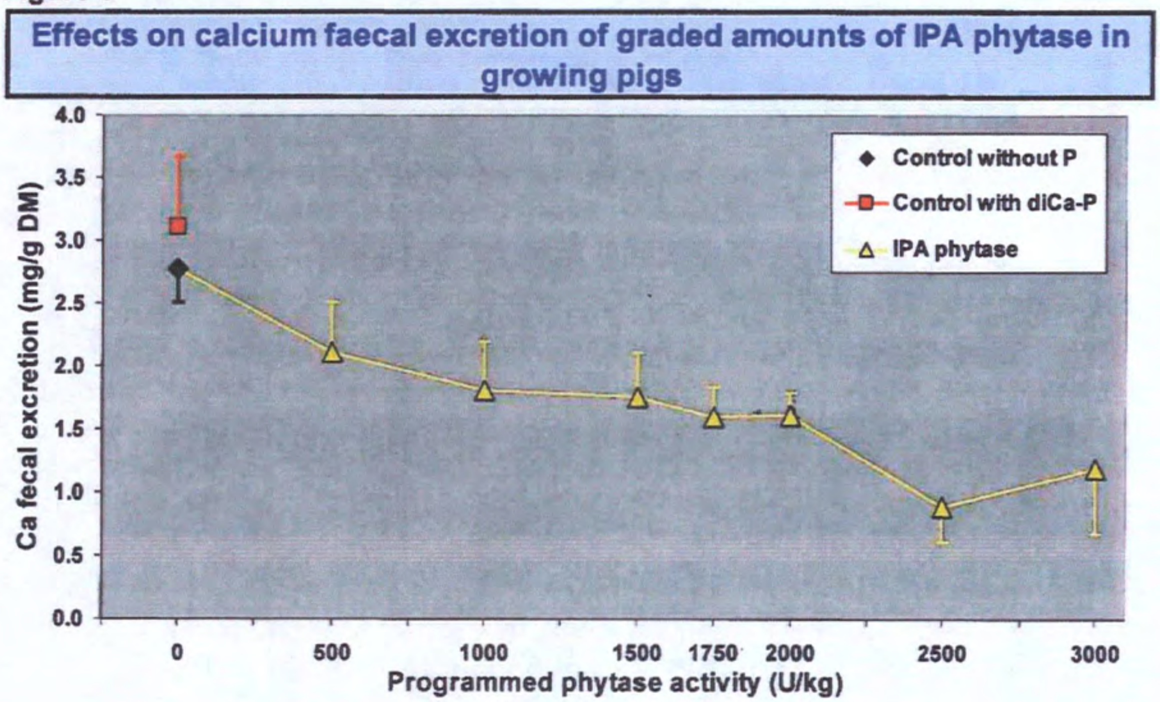

Table 10 - Effects of the IPA phytase on the faecal concentration of zinc in the growing pig.

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Zn concentration (% of DM) (1)	0.48 ± 0.09	0.46 ± 0.11	0.40 ± 0.06	0.45 ± 0.04	0.42 ± 0.06	0.42 ± 0.06	0.44 ± 0.06	0.49(2) ± 0.06	0.49 ± 0.08
Variation from A (%)	100	96.0	83.4	93.5	89.0	89.2	92.1	103.1	102.9
Statistical analysis									
	No significant differences between the groups								

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 11 - **Effects of the IPA phytase on the total apparent digestibility of zinc in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	B	C	D	E	F	G	H	I
Programmed phytase addition (U/kg)	0	0	500	1000	1500	1750	2000	2500	3000
Faecal Zn digestibility (%) [1]	11.4 ± 5.6	16.7 ± 9.4	25.5 ± 7.0	21.4 ± 6.5	17.3 ± 8.7	25.0 ± 6.2	21.6 ± 4.8	17.5[2] ± 3.7	18.1 ± 9.7
Variation from A (%)	100	146.8	223.6	187.8	151.5	119.1	189.3	153.5	158.4
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001		P<0.05
		B -	P<0.05	NS	NS	P<0.05	NS		NS
			C -	NS	NS	NS	NS		NS
				D -	NS	NS	NS		NS
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
(1) Mean ± standard deviation of the mean of 12 determinations. (2) Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

Table 12 - **Effects of the IPA phytase on the faecal excretion of zinc in the growing pig.**

Treatment groups (n = 12)	Basal Diet	Basal Diet + diCa-P	IPA phytase						
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**
Programmed phytase addition (U/kg)	**0**	**0**	**500**	**1000**	**1500**	**1750**	**2000**	**2500**	**3000**
Faecal Zn excretion (mg/g DM) [(1)]	**0.086 ± 0.005**	**0.083 ± 0.009**	**0.070 ± 0.007**	**0.074 ± 0.006**	**0.074 ± 0.008**	**0.070 ± 0.006**	**0.076 ± 0.005**	**0.080**[(2)] **± 0.004**	**0.078 ± 0.009**
Variation from A (%)	**100**	**97.1**	**81.6**	**85.6**	**86.3**	**81.1**	**88.2**	**93.1**	**91.2**
Statistical analysis									
	A -	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.001
		B -	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001		P<0.05
			C -	NS	NS	NS	NS		NS
				D -	NS	NS	NS		NS
					E -	NS	NS		NS
						F -	NS		NS
							G -		NS
								H -	

Animals: growing pigs of an initial body weight of 19.06 ± 1.82 kg; diet based on soybean meal, maize and barley.
[(1)] Mean ± standard deviation of the mean of 12 determinations. [(2)] Mean ± standard deviation of the mean of 6 determinations.
NS : non significant

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Diet	Treatment	Pig	P conc feces	DIGESTIBILITY	EXCRETION	EQUIV
A1	Control without P	178	19.187	31.7	2.886	0.102
A1		241	15.024	25.0	3.168	-0.181
A1		236	16.071	31.0	2.916	0.072
A1		223	13.467	27.4	3.066	-0.079
A1		178	16.404	31.1	2.912	0.075
A1		241	15.654	30.0	2.957	0.030
A1		236	17.975	24.2	3.202	-0.214
A1		223	16.308	24.6	3.185	-0.198
A1		178	16.587	34.6	2.761	0.226
A1		241	11.013	39.2	2.570	0.417
A1		236	12.479	34.8	2.756	0.231
A1		223	20.102	17.9	3.470	-0.482
B2	Control with di-CaP	176	13.837	54.5	2.833	2.153
B2		234	18.579	43.4	3.523	1.462
B2		175	13.603	58.0	2.613	2.373
B2		189	15.302	47.8	3.251	1.734
B2		176	18.533	43.6	3.510	1.476
B2		234	17.179	46.5	3.328	1.658
B2		175	15.308	54.3	2.844	2.142
B2		189	13.966	51.2	3.036	1.950
B2		176	29.555			
B2		234	17.068	45.7	3.377	1.609
B2		175	26.962	26.5	4.571	0.415
B2		189	13.884	47.1	3.291	1.695
C3	IPA phytase 500 U/kg	225	9.399	55.9	1.878	1.144
C3		217	12.208	54.1	1.954	1.068
C3		197	11.358	51.5	2.065	0.958
C3		235	13.257	38.0	2.642	0.381
C3		225	12.841	43.7	2.398	0.625
C3		217	11.169	56.5	1.851	1.171
C3		197	10.128	55.0	1.916	1.106
C3		235	11.057	53.4	1.987	1.035
C3		225	14.088	47.8	2.222	0.800
C3		217	12.227	54.5	1.938	1.085
C3		197	12.354	50.4	2.114	0.908
C3		235	12.179	44.4	2.369	0.654
D4	IPA phytase 1000 U/k	212	8.947	66.0	1.442	1.563
D4		232	11.004	56.4	1.849	1.156
D4		237	12.150	56.0	1.866	1.139
D4		208	10.965	54.4	1.936	1.069
D4		212	11.029	54.1	1.947	1.058
D4		232	10.821	63.0	1.568	1.437
D4		237	9.794	59.0	1.738	1.268
D4		208	12.390	54.9	1.914	1.091
D4		212	9.734	59.7	1.711	1.295
D4		232	11.989	51.9	2.041	0.965
D4		237	11.100	58.6	1.758	1.247
D4		208	9.861	60.1	1.691	1.315
E5	IPA phytase 1500 U/k	198	9.496	61.5	1.638	1.375
E5		192	8.921	60.9	1.662	1.351
E5		195	9.277	58.5	1.764	1.249
E5		227	9.381	58.2	1.777	1.236
E5		198	11.231	56.1	1.868	1.145
E5		192	8.647	61.0	1.658	1.355
E5		195	10.438	56.4	1.854	1.159
E5		227	12.158	52.4	2.025	0.988
E5		198	9.383	60.8	1.667	1.346
E5		192	8.864	63.0	1.574	1.439
E5		195	9.039	65.1	1.485	1.528
E5		227	10.026	63.8	1.539	1.474
F6	750 U/k	233	9.341	57.1	1.792	1.149
F6		218	13.480	56.1	1.835	1.107
F6		210	9.751	57.0	1.795	1.146
F6		202	10.848	58.2	1.747	1.194

F6	IPA phytase 1	233	9.422	60.0	1.671	1.270
F6		218	12.510	58.6	1.729	1.212
F6		210	9.288	64.5	1.482	1.459
F6		202	8.302	67.7	1.349	1.593
F6		233	7.052	67.8	1.348	1.594
F6		218	9.947	63.5	1.525	1.417
F6		210	9.289	62.3	1.574	1.367
F6		202	9.204	62.3	1.577	1.365
G7	IPA phytase 2000 U/k	182	10.983	60.7	1.659	1.327
G7		186	7.560	59.7	1.700	1.286
G7		201	8.009	63.4	1.547	1.439
G7		238	10.804	55.5	1.881	1.105
G7		182	11.341	59.1	1.725	1.260
G7		186	9.281	62.3	1.591	1.394
G7		201	9.497	60.0	1.689	1.297
G7		238	9.942	59.4	1.714	1.272
G7		182	10.900	63.2	1.555	1.431
G7		186	8.860	60.2	1.682	1.304
G7		201	7.358	68.4	1.333	1.653
G7		238	8.150	66.2	1.427	1.559
I9	IPA phytase 3000 U/k	203	7.513	68.4	1.319	1.621
I9		191	10.379	58.1	1.752	1.188
I9		216	8.043	69.0	1.297	1.643
I9		228	6.691	75.1	1.040	1.900
I9		203	11.186	63.4	1.530	1.410
I9		191	7.644	73.8	1.096	1.844
I9		216	12.326	56.6	1.813	1.127
I9		228	7.707	72.9	1.132	1.808
I9		203	6.726	70.5	1.231	1.709
I9		191	7.081	66.3	1.407	1.533
I9		216	8.003	64.6	1.478	1.462
I9		228	6.736	76.8	0.970	1.970

Diet	Treatment	Pig	Zn conc feces
A1	Control without P	178	0.617
A1		241	0.389
A1		236	0.435
A1		223	0.358
A1		178	0.526
A1		241	0.522
A1		236	0.558
A1		223	0.481
A1		178	0.498
A1		241	0.360
A1		236	0.382
A1		223	0.591
B2	Control with di-CaP	176	0.416
B2		234	0.472
B2		175	0.382
B2		189	0.454
B2		176	0.511
B2		234	0.455
B2		175	0.394
B2		189	0.324
B2		176	0.636
B2		234	0.445
B2		175	0.691
B2		189	0.311
C3	IPA phytase 500 U/kg	225	0.297
C3		217	0.511
C3		197	0.432
C3		235	0.322
C3		225	0.396
C3		217	0.433
C3		197	0.350
C3		235	0.361
C3		225	0.496
C3		217	0.429
C3		197	0.411
C3		235	0.332
D4	IPA phytase 1000 U/k	212	0.412
D4		232	0.474
D4		237	0.532
D4		208	0.432
D4		212	0.449
D4		232	0.434
D4		237	0.389
D4		208	0.509
D4		212	0.437
D4		232	0.407
D4		237	0.423
D4		208	0.448
E5	IPA phytase 1500 U/k	198	0.370
E5		192	0.354
E5		195	0.429
E5		227	0.439
E5		198	0.495
E5		192	0.311
E5		195	0.462
E5		227	0.444
E5		198	0.444
E5		192	0.381
E5		195	0.453
E5		227	0.505
F6	750 U/k	233	0.338
F6		218	0.531
F6		210	0.355
F6		202	0.495

F6	IPA phytase 1	233	0.383
F6		218	0.493
F6		210	0.375
F6		202	0.453
F6		233	0.345
F6		218	0.456
F6		210	0.403
F6		202	0.470
G7	IPA phytase 2000 U/k	182	0.520
G7		186	0.356
G7		201	0.356
G7		238	0.476
G7		182	0.504
G7		186	0.440
G7		201	0.367
G7		238	0.449
G7		182	0.553
G7		186	0.406
G7		201	0.407
G7		238	0.434
I9	IPA phytase 3000 U/k	203	0.410
I9		191	0.539
I9		216	0.360
I9		228	0.525
I9		203	0.615
I9		191	0.508
I9		216	0.585
I9		228	0.487
I9		203	0.477
I9		191	0.347
I9		216	0.465
I9		228	0.569

Diet	Treatment	Pig	Ca conc feces	DIGESTIBILITY	EXCRETION
A1	Control without P	178	21.336	54.0	3.209
A1		241	12.427	62.4	2.621
A1		236	17.477	54.5	3.171
A1		223	11.727	61.7	2.670
A1		178	16.467	58.1	2.924
A1		241	13.977	62.1	2.640
A1		236	15.386	60.7	2.740
A1		223	14.497	59.4	2.831
A1		178	17.083	59.2	2.843
A1		241	9.301	68.9	2.170
A1		236	12.362	60.8	2.730
A1		223	15.809	60.9	2.729
B2	Control with di-CaP	176	13.976	64.2	2.862
B2		234	18.635	55.8	3.534
B2		175	10.803	74.0	2.075
B2		189	13.075	65.2	2.778
B2		176	19.201	54.5	3.636
B2		234	18.229	55.8	3.531
B2		175	14.121	67.2	2.623
B2		189	11.664	68.3	2.535
B2		176	27.817		
B2		234	18.470	54.3	3.655
B2		175	23.651	49.8	4.010
B2		189	12.441	63.1	2.949
C3	IPA phytase 500 U/kg	225	7.638	77.5	1.526
C3		217	9.837	76.7	1.574
C3		197	14.526	61.0	2.641
C3		235	12.320	63.7	2.455
C3		225	12.918	64.4	2.412
C3		217	10.172	75.1	1.686
C3		197	10.570	70.5	2.000
C3		235	11.404	69.7	2.049
C3		225	13.207	69.2	2.083
C3		217	10.847	74.6	1.719
C3		197	14.809	62.6	2.534
C3		235	13.639	60.8	2.653
D4	IPA phytase 1000 U/k	212	8.469	79.6	1.365
D4		232	7.761	80.6	1.304
D4		237	10.888	75.1	1.672
D4		208	13.246	65.1	2.339
D4		212	13.582	64.2	2.398
D4		232	9.169	80.2	1.329
D4		237	8.329	78.0	1.478
D4		208	14.545	66.5	2.247
D4		212	11.571	69.7	2.034
D4		232	12.311	68.8	2.095
D4		237	9.823	76.8	1.556
D4		208	11.296	71.1	1.937
E5	IPA phytase 1500 U/k	198	12.281	67.1	2.118
E5		192	7.312	78.9	1.362
E5		195	9.466	72.1	1.800
E5		227	9.605	71.8	1.819
E5		198	13.378	65.5	2.225
E5		192	5.460	83.8	1.047
E5		195	12.292	66.1	2.184
E5		227	10.661	72.4	1.776
E5		198	11.869	67.3	2.108
E5		192	8.501	76.6	1.509
E5		195	10.140	74.2	1.666
E5		227	9.186	78.1	1.410
F6	750 U/k	233	9.202	73.2	1.766
F6		218	11.122	77.0	1.514
F6		210	9.710	72.9	1.788
F6		202	12.653	69.1	2.038

F6	IPA phytase 1	233	9.337	74.9	1.656
F6		218	11.409	76.1	1.577
F6		210	7.560	81.7	1.206
F6		202	10.317	74.6	1.676
F6		233	7.299	78.8	1.395
F6		218	8.234	80.9	1.262
F6		210	8.739	77.5	1.481
F6		202	10.832	71.9	1.856
G7	IPA phytase 2000 U/k	182	9.346	78.4	1.411
G7		186	7.755	73.4	1.744
G7		201	7.522	77.8	1.452
G7		238	10.873	71.1	1.893
G7		182	11.464	73.4	1.744
G7		186	9.151	76.0	1.569
G7		201	10.123	72.5	1.800
G7		238	10.358	72.7	1.785
G7		182	11.731	74.4	1.673
G7		186	8.378	75.7	1.591
G7		201	7.316	79.8	1.325
G7		238	8.021	78.5	1.405
I9	IPA phytase 3000 U/k	203	5.349	85.6	0.939
I9		191	15.007	61.3	2.533
I9		216	6.003	85.2	0.968
I9		228	4.263	89.9	0.663
I9		203	9.177	80.8	1.255
I9		191	5.547	87.8	0.795
I9		216	10.626	76.1	1.563
I9		228	5.887	86.8	0.864
I9		203	3.795	89.4	0.695
I9		191	8.966	72.8	1.781
I9		216	6.298	82.2	1.163
I9		228	7.616	83.2	1.097

European Food Safety Authority

FEEDAP UNIT

ANNEX C [1]

TRIAL PROTOCOL DATA SHEET: FOR TERRESTRIAL ANIMALS

Identification of the additive: **IPA phytase**

Batch number: **PPQ27987**

Trial ID: **S 05-08 VN**

Location:
DSM Nutritional Products France
Centre de Recherche en Nutrition Animale
BP 170
68305 Saint-Louis cedex, France

Start date and exact duration of the study: **March 10th 2008 - 38 days**

Number of treatment groups (+ control(s)): 7 + **(2)**

Replicates per group: **1**

Total number of animals: **36**

Animals per replicate: **4**

Dose(s) of the additive/active substance(s)/agent(s) (mg/Units of activity/CFU kg^{-1} complete feed/L^{-1} water)

Intended: **0 / 500 / 1000 / 1500 / 1750 / 2000 / 2500 / 3000 U/kg**

Analysed: **225 (endogenous activity) / 678 / 1179 / 1723 / 1985 / 2232 / 2798 / 3329 U/kg**

†

Substances used for comparative purposes: **Dicalcium phosphate**

Intended dose: **12 g per kg of feed. Equivalent to 2.4 g of additional P per kg of feed in a dry matter basis**

Analysed: **2.0 g of additional P per kg of feed in a dry matter basis**

Animal species/category: **Swine / growers**

Breed: **Large White x Landrace**

Identification procedure: **Pen and individual earring**

Sex: **Males**

Age at start: **90 days**

Body weight at start: **19.06 ± 1.82 kg**

Physiological stage: **Growing pigs**

General health: **Normal - no clinical signs were observed**

Additional information for field trials:

Location and size of herd or flock:

Feeding and rearing conditions:

Method of feeding:

Diets (type(s)): **Basal diet formulated to provide P exclusively from vegetable origin and according to the NRC**

Presentation of the diet: Mash ☒ Pellet ☐ Extruded ☐ Other

Composition (main feedingstuffs): **Maize - 53%, soybean meal - 18% and barley - 13.9%**

Nutrient content (relevant nutrients and energy content)

Intended values: **Crude protein - 15.5%, lysine - 0.96%, methionine + cystine - 0.54%, Ca - 0.66% in D.M., P - 0.41% in D.M. and digestible energy - 13.31 MJ/kg**

Analysed values: **Ca - 0.70% in D.M. and P - 0.42% in D.M.**

Date and nature of the examinations performed:

[1] Please submit this form using a common word processing format (e.g. MS Word).

March 10th and April 17th - weight measurement **March 10th and March 19th - acclimatation period** **March 20th and April 4th - 1st period** **April 5th and April 17th - 2nd period** **April 15th, 16th and 17th - individual faecal sampling**	
Method(s) of statistical evaluation used: **Two-factor analysis of variance (diet and diet + animal or pen) followed by a Duncan multiple range test**	
Therapeutic/preventive treatments (reason, timing, kind, duration): **No therapeutic / preventive treatments were used**	
Timing and prevalence of any undesirable consequences of treatment: **Nothing to report**	
Date **22.02.2010**	Signature Study Director **Dr P. GUGGENBUHL**

† In case the concentration of the additive in complete feed/water may reflect insufficient accuracy, the dose of the additive can be given per animal day^{-1} or mg kg^{-1} body weight or as concentration in complementary feed.