資料５－３

令和 4 年 10 月 6 日

農林水産省消費・安全局
畜水産安全管理課　御中

AB Agri Limited.
日本における代表者　古川洋史
[redacted]

飼料の公定規格における追加事項の要望について

令和 4 年 7 月 29 日開催の農業資材審議会飼料分科会において審議が終了した *Trichoderma reesei* から産生されるフィターゼ（Quantum Blue）について、添付の解析結果に基づき、飼料の公定規格（昭和 51 年 7 月 24 日、農林省告示第 756 号）中の 1 配合飼料（5）環境負荷低減型配合飼料における aP（フィターゼによる分解の結果生じる非フィチン態りん）の算出方法の表に以下の追加をして頂きたく要望します。

フィターゼの種類	算出方法
Trichoderma reesei から産生されるフィターゼ	飼料 1 kgあたり 164 フィチン酸分解力単位を添加した場合、0.10%

Trichoderma reesei から産生されるフィターゼ（Quantum Blue）
の豚における有効リン（avP）放出量に関するホロ分析による解析

AB Vista

1. 解析に用いたデータ

AB Vista では、2010 年から 2017 年にかけて、Quantum Blue を豚用飼料に添加した場合の効果を評価するために、世界各国で計 57 試験を実施している。これらのうち、体重 10 kg以下の供試豚を用いた試験およびオーバードーズに関する試験を除く、計 29 試験における 201 データを用いて、ホロ分析（特定の要因について利用可能なすべてのデータと利用可能なすべての変数を包括的に解析する統計手法）を実施し、増体日量を指標として、豚用飼料に Quantum Blue を 250、500 および 750 FTU/kg添加した場合の avP 放出量を検討した。

表 1. 使用データの概要

項　目	平均値	最小値	最大値
Quantum Blue 添加量（Log FTU/kg）	2.15	1.70	3.30
飼料中 avP（%）	0.24	0.09	0.49
飼料中可消化リジン（%）	1.11	0.68	1.55
飼料中食塩含量（%）	0.37	0.00	1.03
飼料摂取日量（g/日）	1574	307	3579

資料 2　第 70 回 EAAP Annual Meeting プレゼン資料

表 2-1. 試験区の設定

区	ME (kcal/kg)	可消化リン (%)	カルシウム (%)	フィターゼ (FTU/kg 飼料)
陽性対照区	3300 / 3275	0.28 / 0.26	0.57 / 0.54	0
市販相当区	3300 / 3275	0.16 / 0.11	0.42 / 0.39	645 / 403
陰性対照区 1	3300 / 3275	0.12 / 0.11	0.37 / 0.34	0
陰性対照区 1＋QB				2000 / 2290
陰性対照区 2	3180 / 3156	0.12 / 0.11	0.37 / 0.34	0
陰性対照区 2＋QB				2000 / 2290

注）25～50 kg（4 週間）/50～75 kg（4 週間）

192 頭のオス豚（6 試験区×4 頭/群×8 反復、平均体重 23.5 kg）

体重、飼料摂取量、飼育終了時に各群 1 頭から中足骨を採取して灰分、Ca、P、強度を測定

表 2-2-1. 試験結果（発育成績）

区	日増体量 (g/日)	飼料摂取量 (g/日)	飼料要求率
陽性対照区	853 a	1.65 a	1.94 a
市販相当区	828 a	1.61 a	1.94 a
陰性対照区 1	620 b	1.39 b	2.25 b
陰性対照区 1＋QB	814 a	1.61 a	1.98 a
陰性対照区 2	636 b	1.45 b	2.27 b
陰性対照区 2＋QB	816 a	1.67 a	2.05 a

表 2-2-2. 試験結果（骨成分等）

区	重量 (g)	灰分 (%)	カルシウム (灰分中%)	リン (灰分中%)	強度 (N)
陽性対照区	25.1 a	30.5 a	30.8 ab	19.2 a	753 a
市販相当区	23.7 a	31.1 a	29.0 b	19.1 a	747 a
陰性対照区 1	21.1 b	23.1 b	33.7 a	18.4 b	399 b
陰性対照区 1＋QB	24.4 a	30.0 a	31.3 ab	19.0 a	702 a
陰性対照区 2	22.5 b	22.4 b	32.4 a	18.2 b	355 b
陰性対照区 2＋QB	23.6 a	30.1 a	31.8 ab	19.2 a	700 a

資料 3　第 70 回 EAAP Annual Meeting ポスター発表

表 3-1. 試験区の設定

区	可消化リン (%)	カルシウム (%)	フィターゼ (FTU/kg 飼料)
陽性対照区	0.28	0.70	−
陰性対照区	0.11	0.49	300 / 600 / 1200

180 頭の豚（5 試験区×4 頭（雄、雌各 2 頭）/群×9 反復、平均体重 20 kg）

試験期間：42 日間

体重、飼料摂取量、飼育終了時に各群 1 頭から中足骨を採取して灰分、P、強度を測定

表 3-2. 試験結果

区	陽性対照区	陰性対照区	フィターゼ添加区（FTU/kg 飼料）		
			300	600	1200
日増体量（g/日）	644 a	488 b	634 a	615 a	633 a
飼料要求率	1.99 a	2.09 b	1.99 a	2.04 a	1.99 a
骨灰分（%）	27 a	19 d	22 c	24 b	26 ab
骨リン含量（%）	5.5 a	3.7 d	4.5 c	4.9 bc	5.3 ab
骨強度（N）	548 a	253 c	418 b	411 b	521 a

資料 4　Can. J. Anim.Sci. 94 : 493 - 497 (2014)

表 4-1. 試験区の設定

区	有効リン (%)	カルシウム (%)	フィターゼ (FTU/kg 飼料)
陽性対照区	0.32 / 0.27	0.71 / 0.61	ー
陰性対照区 1	0.22 / 0.17	0.60 / 0.50	250
陰性対照区 2	0.17 / 0.12	0.55 / 0.45	500 / 2000

注）23～38 kg（3 週間）/38～55 kg（3 週間）

360 頭の豚（6 試験区×5 頭/群×6 反復×性、平均体重 23kg）
体重、飼料摂取量、飼育終了時に各群 1 頭から中手骨を採取して灰分、強度を測定

表 4-2-1. 試験結果（発育成績）

区	日増体量 (kg/日)	飼料摂取量 (kg/日)	飼料効率
陽性対照区	0.75 c	1.50	0.501 bc
陰性対照区 1	0.76 bc	1.55	0.493 c
陰性対照区 1＋250	0.78 ab	1.54	0.506 ab
陰性対照区 2	0.74 c	1.51	0.490 c
陰性対照区 2＋500	0.78 ab	1.55	0.509 ab
陰性対照区 2＋2000	0.80 a	1.57	0.513 a

表 4-2-2. 試験結果（骨成分等）

区	強度 (kg)	灰分 (%)	灰分重量 (g)
陽性対照区	77.4 abc	42.3	3.64 a
陰性対照区 1	69.5 bc	39.5	3.17 b
陰性対照区 1＋250	80.9 ab	41.2	3.57 a
陰性対照区 2	65.9 c	39.4	3.17 b
陰性対照区 2＋500	81.2 ab	40.3	3.61 a
陰性対照区 2＋2000	85.9 a	41.3	3.76 a

2. 解析結果

解析には JMP Pro v 13.0 の段階的回帰モデルを使用し、図に示したとおり Quantum Blue 添加量と日増体量の間の最適予測モデルを得た。

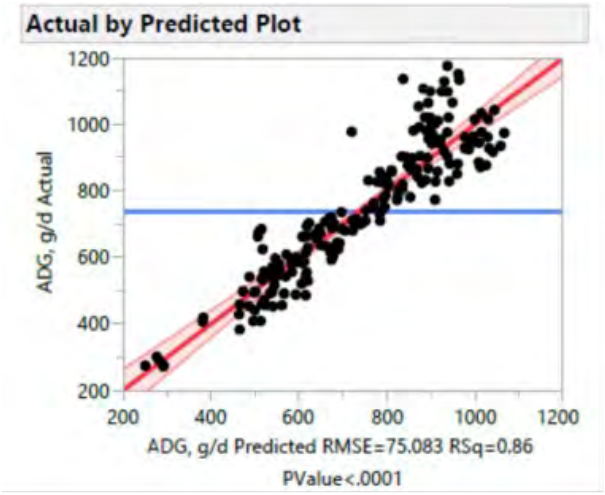


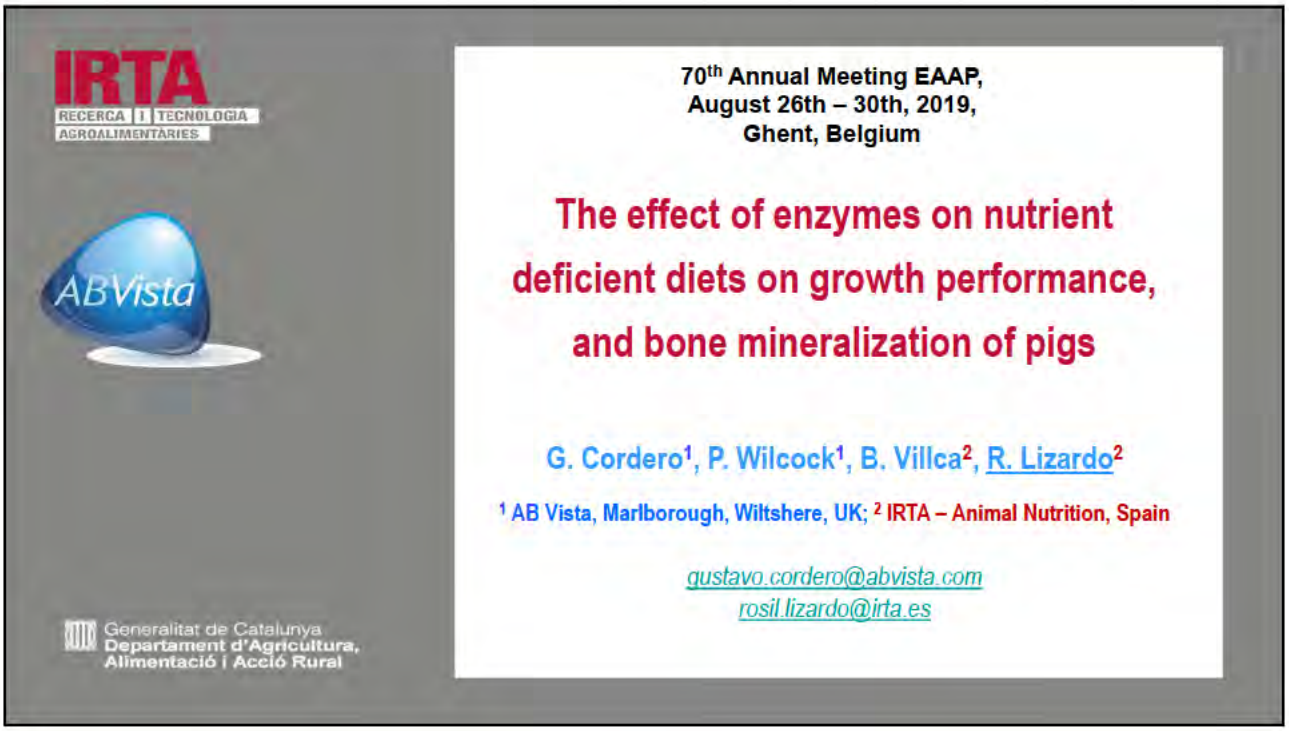
図　Quantum Blue 添加量と日増体量の間で得られた最適予測モデル

次に、この最適予測モデルを用いて、avP を 0.10、0.15 および 0.175%放出させるために必要な Quantum Blue 添加量（FTU/kg飼料）を算出した。その結果は表 5 に示したとおりであり、飼料中の avP を 0.10%増加させるために必要な Quantum Blue 添加量は 164 FTU/kg飼料（85%信頼区間：113～215 FTU/kg）であった。

表 5. Quantum Blue 添加添加による avP 放出量（%）

	avP 放出量(%)		
	0.10	0.15	0.175
Quantum Blue 添加量（FTU/kg飼料）	164 (113～215)	291 (137～445)	390 (140～640)

（　）内は 85%信頼区間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ABVista
Generalitat de Catalunya
Departament d'Agricultura, Alimentació i Acció Rural
70th Annual Meeting EAAP,
August 26th – 30th, 2019,
Ghent, Belgium
The effect of enzymes on nutrient deficient diets on growth performance, and bone mineralization of pigs
G. Cordero[1], P. Wilcock[1], B. Villca[2], R. Lizardo[2]
[1] AB Vista, Marlborough, Wiltshere, UK; [2] IRTA – Animal Nutrition, Spain
gustavo.cordero@abvista.com
rosil.lizardo@irta.es

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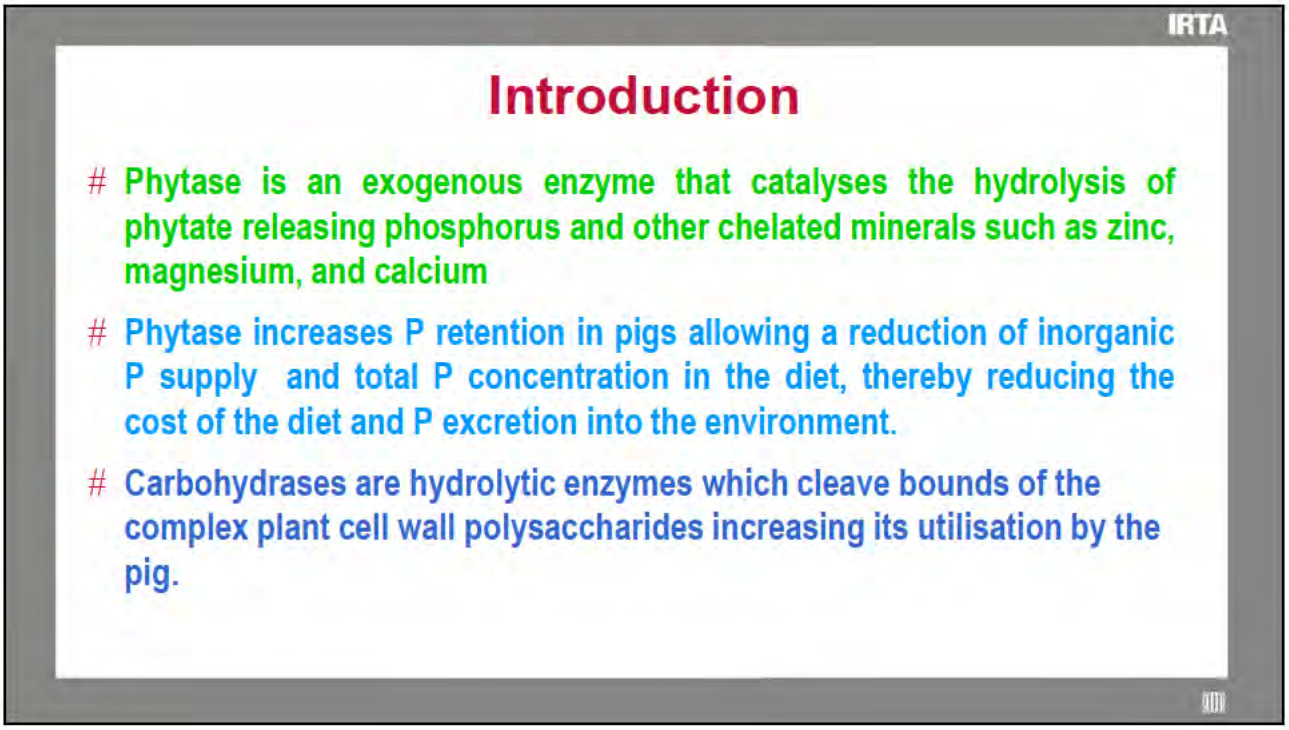
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Introduction
Phytase is an exogenous enzyme that catalyses the hydrolysis of phytate releasing phosphorus and other chelated minerals such as zinc, magnesium, and calcium
Phytase increases P retention in pigs allowing a reduction of inorganic P supply and total P concentration in the diet, thereby reducing the cost of the diet and P excretion into the environment.
Carbohydrases are hydrolytic enzymes which cleave bounds of the complex plant cell wall polysaccharides increasing its utilisation by the pig.

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Objective

Validate the Matrix Nutrient Concept of phytase and xylanase for growing pigs fed diets limited in Ca, digP, AA and energy

Growth performance, feed efficacy, bone mineralisation and bone strength are used as response criteria.

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Experimental design

Treatment	Basal diet	Mineral matrix	AA matrix	ME matrix kcal/kg	QB FTU/kg	EXT BXU/kg
1	Positive control (PC)	According to NRC (2012) for 25-50 and 50-75kg pigs				
2	Industry control (IC)	0.15% digP 0.165% Ca 0.035% Na	-	-	500	-
3	Negative control (NC 1)	0.20% digP 0.22% Ca 0.045% Na	Lys 0.050% Thr 0.050% M+C 0.050% Val 0.040% Trp 0.020% Ile 0.036% Arg 0.026%			
4	NC1 + QB phytase + Econase XT				2,000	9,600
5	Negative control (NC 2)	0.20% digP 0.22% Ca 0.045% Na	Lys 0.050% Thr 0.050% M+C 0.050% Val 0.040% Trp 0.020% Ile 0.036% Arg 0.026%	120		
6	NC2 + QB phytase + Econase XT				2,000	9,600

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Experimental design

Treatment	Basal diet	Mineral matrix	AA matrix	ME matrix kcal/kg	QB FTU/kg	EcoXT BXU/kg
1	Positive control (PC)	According to NRC (2012) for 25-50 and 50-75kg pigs				
2	Industry control (IC)	0.15% digP 0.165% Ca 0.035% Na	-	-	500	-
3	Negative control (NC 1)	0.20% digP 0.22% Ca 0.045% Na	Lys 0.050% Thr 0.050% M+C 0.050% Val 0.040% Trp 0.020% Ile 0.036%	-	-	-
4	NC1 + QB phytase + Econase XT		-	-	2,000	9,600
5	Negative control (NC 2)	0.20% digP 0.22% Ca 0.045% Na	Lys 0.050% Thr 0.050% M+C 0.050% Val 0.040% Trp 0.020% Ile 0.036%	120	-	-
6	NC2 + QB phytase + Econase XT		-	-	2,000	9,600

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Ingredient composition of diets

Feeding Phase	Grower 1 (0-4 weeks)						Grower 2 (4-8 weeks)					
Ingredient, %	PC	IC	NC1	NC1+	NC2	NC2+	PC	IC	NC1	NC1+	NC2	NC2+
Q Blue Phytase	-	0.01	-	0.04	-	0.04	-	0.01	-	0.04	-	0.04
Econase XT	-	-	-	0.006	-	0.006	-	-	-	0.006	-	0.006
Barley	30.0	30.0	30.0		35.0		40.0	40.0	32.5		35.0	
Wheat	21.5	23.3	26.2		24.1		24.4	25.7	28.5		28.1	
Corn	20.0	20.0	20.0		20.0		13.4	14.0	21.5		22.4	
Soybean meal	20.4	20.0	17.6		17.0		14.4	14.0	12.1		11.5	
Fat	4.79	4.18	4.00		1.70		4.76	4.13	3.42		1.00	
Mono Phos	0.95	0.29	0.09		0.06		0.88	0.22	0.03		0	
Ca Carbonate	0.91	0.79	0.74		0.76		0.71	0.58	0.55		0.56	
Salt	0.41	0.30	0.30		0.30		0.44	0.32	0.33		0.33	
Lysine HCl	0.41	0.41	0.42		0.42		0.43	0.44	0.44		0.44	
Threonine	0.12	0.12	0.10		0.10		0.12	0.10	0.10		0.10	
Methionine	0.13	0.12	0.09		0.09		0.08	0.07	0.07		0.07	
Tryptophan	0.01	0.01	0		0		0.01	0.01	0		0	
Vit-Min premix	0.42	0.42	0.42		0.42		0.42	0.42	0.42		0.42	

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Nutrient content of diets

Feeding Phase	Grower 1 (0-4 weeks)						Grower 2 (4-8 weeks)					
Nutrient	PC	IC[1]	NC1	NC1+	NC2	NC2+	PC	IC[1]	NC1	NC1+	NC2	NC2+
Phytase, FTU/kg	-	645	-	2000	-	2000	-	403	-	2290	-	2290
Xylanase, BXU/kg	-	-	-	10400	-	10400	-	-	-	10400	-	10400
ME (kcal/kg)	3300	3300	3300		3180		3275	3275	3275		3156	
NE (kcal/kg)	2510	2510	2518		2405		2504	2504	2524		2400	
CP (%)	16.50	16.60	15.97		15.90		14.70	14.70	14.09		14.00	
sid Lys (%)	1.01	1.01	0.96		0.96		0.90	0.90	0.85		0.85	
sid Met (%)	0.35	0.35	0.31		0.30		0.28	0.28	0.26		0.26	
sid M+C (%)	0.61	0.61	0.56		0.56		0.52	0.52	0.49		0.49	
sid Thr (%)	0.61	0.61	0.56		0.56		0.54	0.54	0.49		0.49	
sid Trp (%)	0.18	0.18	0.16		0.16		0.16	0.16	0.14		0.14	
sid Ile (%)	0.58	0.58	0.55		0.55		0.50	0.50	0.46		0.46	
sid Val (%)	0.67	0.67	0.63		0.64		0.59	0.59	0.55		0.55	
Total P (%)	0.57	0.42	0.37		0.37		0.54	0.39	0.34		0.34	
Digestible P (%)	0.28	0.16	0.12		0.12		0.26	0.14	0.11		0.11	
Ca (%)	0.70	0.54	0.48		0.48		0.60	0.44	0.38		0.38	
Na (%)	0.17	0.13	0.13		0.13		0.18	0.14	0.14		0.14	

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Material & Methods

6 treatments (PC, IC, NC1, NC1+, NC2, NC2+)
192 male pigs (Pi * (LW*LR); 23.50±3.50kg)
48 pens (4 pigs/pen): 8 replicates / treatment
2-phase feeding program (25-50, 50-75kg)
Pellet diets, offered ad libitum
Wheat, barley pre-conditioned at >85ºC to destroy phytases
Performance after 8 weeks trial (2 phases: 0-4, 4-8 wk)
1 pig/pen euthanized and metatarsal bones collected for analysis
Bone ash, minerals (Ca, P) and bone strength measurements

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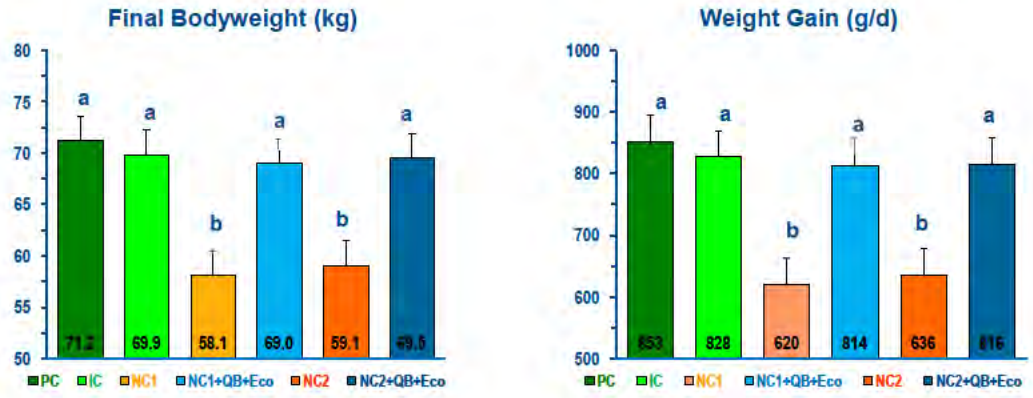
Growth performance



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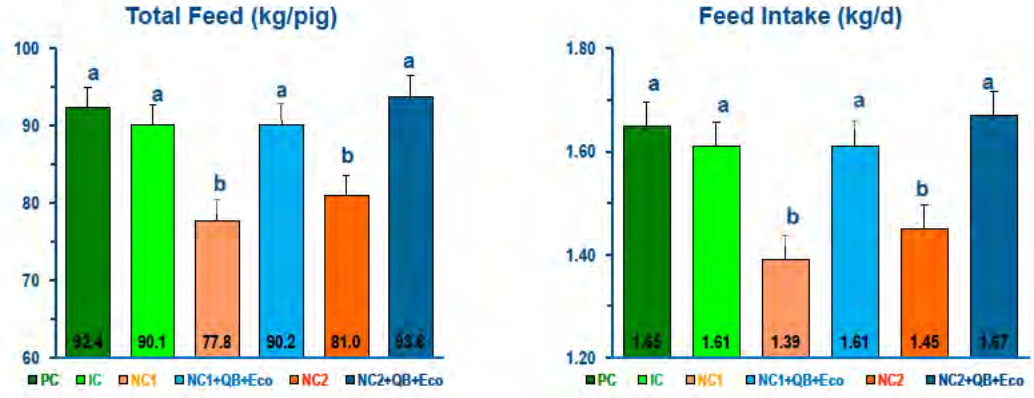
Feed intake



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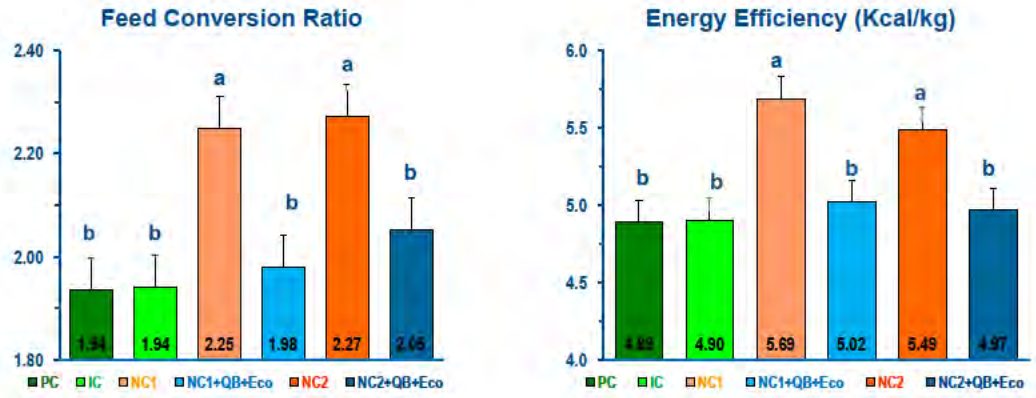
Feed efficacy



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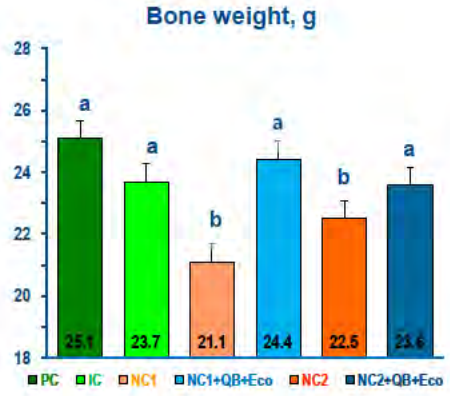
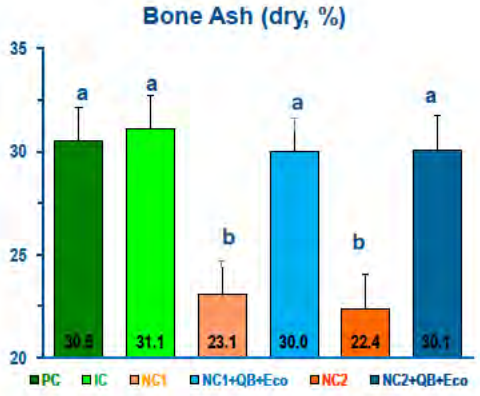
Bone mineralisation





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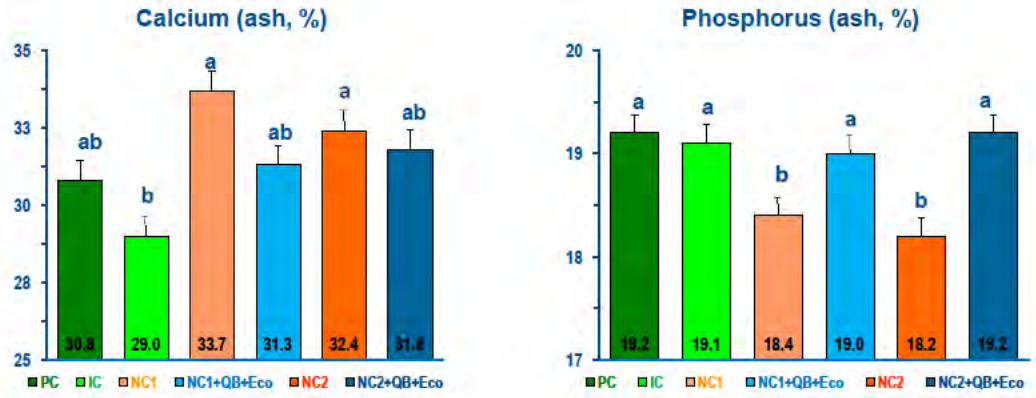
Bone mineralisation



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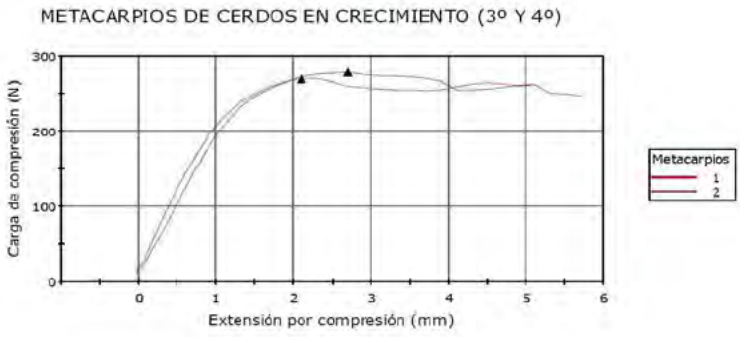
Bone strength



	Máximo Carga de compresión (N)	Extensión por compresión al Máximo Carga de compresión (mm)	Módulo (Automática) (MPa)	EnergíaalMáximo Carga de compresión (J)
1	269,68765	> 2,69791	232,45840	0,39293
2	278,16443	> 2,69812	212,01874	0,53840
Media	273,92604	2,39802	222,23857	0,46567

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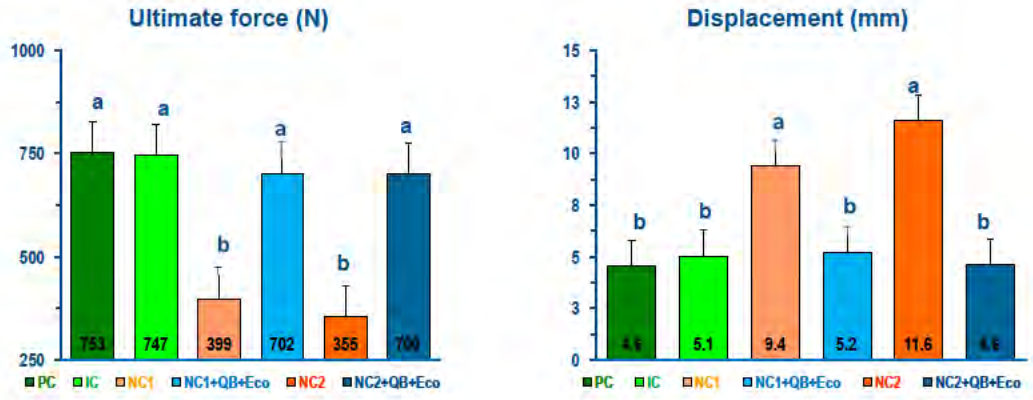
Bone strength



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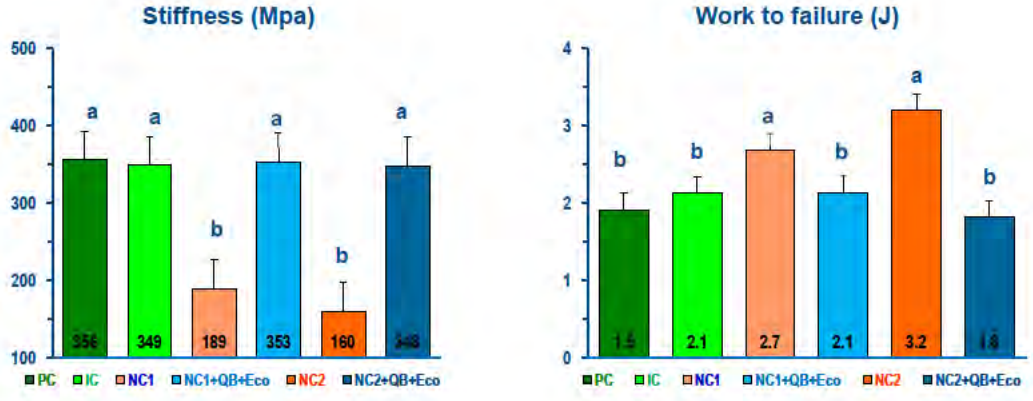
Bone strength



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Conclusions

Inclusion of QB (500 FTU/kg) in Ca/P limited diets improved performance and bone characteristics (PC similar to IC)

Inclusion of phytase (2000FTU/kg) and xylanase (9600BXU/kg) improved performance, feed efficacy, bone ash and strength validating the mineral and AA matrix (NC1 *vs* NC1+) and energy utilisation (NC2 *vs* NC2+)

These results confirm that supplementation with high doses of phytase and xylanase in diets limited in Ca, digP, AA and Energy can result in performance, bone ash and bone strength equivalent to that of pigs fed more expensive nutrient adequate diets.

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EFFECT OF PHYTASE ON PERFORMANCE AND BONE MINERALIZATION IN GROWING PIGS FED A DEFICIENT P DIET

Cordero, G[1]., Villca, B[2]., Wilcock, P[1]., Lizardo, R[2]

[1]AB Vista, United Kingdom [2] IRTA – Animal Nutrition, Spain

INTRODUCTION

Phytase is an exogenous enzyme that catalyses the hydrolysis of phytate releasing phosphorus (P) and other chelated minerals such as zinc, magnesium or calcium for use by the animal (Rutherfurd et al. 2012). In pigs, phytase increases P digestibility and absorption (Selle et al. 2003). This allows for a reduction of dietary P, thereby reducing the cost of the formula and P excretion into the environment. The amount of P released by phytase is dependent on the level of dietary phytate in the diet.

OBJECTIVE

To determine if phytase can be added to replace 0.17% digestible P in a diet containing 0.23% level of dietary phytate-P, and its influence on growth performance, and bone characteristics in growing pigs.

MATERIAL & METHODS

Phytase: Quantum Blue (*E.coli* derived, intrinsic thermo-tolerant 6-phytase produced by *Trichoderma reesei*).
Diets: Corn (40%), pre-treated wheat (35%) and soybean meal (18%) based diets, fed *ad libitum* in pelleted form.
Experimental design: 5 treatments, 9 replicates each, 4 pigs per pen.
Treatments:

T1- Positive control (PC)
T2- Negative control (NC)
T3- NC + 300 FTU/kg of phytase (NC+300)
T4- NC+600 FTU/kg of phytase (NC+600)
T5- NC+1200 FTU/kg of phytase (NC+1200)

Table 1. Dietary P and Ca level (%) of the experimental diets

Diet	P total	P digestible	Ca
Positive Control (PC)	0.58	0.28	0.70
Negative Control (NC)*	0.35	0.11	0.49

*added with phytase at 300 (NC+300), 600 (NC+600) or 1200 FTU/kg feed (NC+1200)

Animals: 180 male and female Pietrain*(Landrace*Large White) pigs of 20 kg liveweight.
Parameters: Growth performance, and feed efficiency after 42 days of trial.
At the end, 1 pig / pen was euthanized and metatarsal bones collected for bone strength, and bone ash and mineral analysis.
Statistical evaluation: GLM procedure of SAS, and means were compared using a Student-Newman-Keuls test.

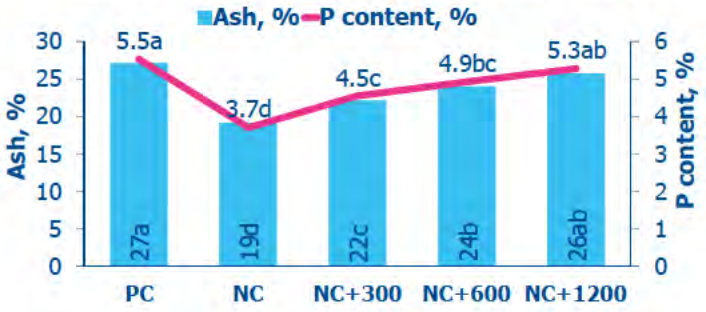
RESULTS & DISCUSSION

Table 2. Growth performance and bone strength of growing pigs.

Diet	BW, kg	ADG, g/d	FCR	Strength, N
T1-PC	47.2a	644a	1.99b	548a
T2-NC	40.6b	488b	2.09a	253c
T3-QB300	46.9a	634a	1.99b	418b
T4-QB600	45.9a	615a	2.04b	411b
T5-QB1200	46.7a	633a	1.99b	521a
SEM	*0.69*	*16.3*	*0.03*	*24.2*

a-c Means followed by different superscripts within columns are statistically different (P≤0.05)

Figure 1. Bone ash and P content (%) of growing pigs.



- Regardless the dose used, phytase inclusion improved weight gain, feed efficiency, and bone strength (P<0.01) relatively to the NC diet.
- No differences were observed between the PC diet and those containing phytase.
- Metatarsals of pigs fed PC and NC diets showed the highest and lowest bone strength (P<0.001), respectively.

- Bone ash and P contents were reduced (P<0.01) on pigs fed the NC relatively to PC diet.
- Bone ash and P content respond proportionally to increasing levels of phytase supplementation.
- The highest phytase dosage was able to restore bone ash and P content to similar levels as the PC.

CONCLUSION

- ❑ Reduction of dietary P and Ca impaired performance and bone characteristics of growing pigs.
- ❑ Phytase supplementation, regardless of dose level, restored performance to similar levels of PC fed pigs.
- ❑ Bone mineralization and bone strength were improved in a linear fashion by phytase addition while the highest phytase dosage was able to restore bone parameters to similar levels as PC fed pigs.
- ❑ These results highlight the ability of the tested phytase to breakdown phytate-P increasing the availability of dietary P.

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資料3

Performance and bone characteristics of growing pigs fed diets marginally deficient in available phosphorus and a novel microbial phytase

T. T. Santos[1], C. L. Walk[1], P. Wilcock[1], G. Cordero[1], and J. Chewning[2]

[1]AB Vista Feed Ingredients, 3 Woodstock Court, Marlborough, Wiltshire, United Kingdom, SN8 4AN (e-mail: tiago.santos@abvista.com); and [2]Swine Research Services, Inc., P.O. Box 6908, Springdale, Arkansas, United States 72766.

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Santos, T. T., Walk, C. L., Wilcock, P., Cordero, G. and Chewning, J. 2014. **Performance and bone characteristics of growing pigs fed diets marginally deficient in available phosphorus and a novel microbial phytase**. Can. J. Anim. Sci. **94**: 493–497. The efficacy of a novel microbial 6-phytase on pig performance, bone breaking strength, and bone ash was evaluated. Pigs were allotted to one of six diets with 12 replicate pens/treatment and five pigs/pen. Diets consisted of a positive control (PC), negative control (NC) 1 with Ca and available phosphorus (avP) reduction by 0.11 and 0.10%, NC 1 plus 250 FTU kg^{-1} phytase, NC 2 with Ca and avP reduction by 0.16 and 0.15%, NC 2 plus 500 and 2,000 FTU kg^{-1} phytase. On day 43, one pig per pen was euthanized and the 4th metacarpal was obtained to determine bone parameters. Average daily gain (ADG) was higher ($P < 0.05$) in pigs fed NC 2 plus 500 or 2000 FTU kg^{-1} compared with PC. NC 2 with 2000 FTU kg^{-1} improved ($P < 0.05$) G:F compared with PC. NC 1 or NC 2 had reduced ($P < 0.05$) bone ash weight and NC 2 had reduced ($P < 0.05$) bone breaking strength compared with PC. Phytase supplementation in NC 1 or NC 2 improved bone ash weight compared with PC. Bone breaking strength or bone ash weight was more sensitive to low dietary Ca and avP than bone ash percent. Phytase supplementation at 2000 FTU kg^{-1} improved G:F beyond that of the PC.

Key words: Bone breaking strength, bone ash, performance, phytase, pigs

Santos, T. T., Walk, C. L., Wilcock, P., Cordero, G. et Chewning, J. 2014. **Performance de croissance et caractéristiques des os des porcs nourrisd'aliments légèrement carencés en phosphore assimilable et incluant une nouvelle phytase microbienne**. Can. J. Anim. Sci. **94**: 493–497. L'efficacité d'une nouvelle 6-phytase microbienne a été évaluée selon les performances de croissance des porcs, de résistance des os à la rupture (BBS – « bone breaking strength »), et de la teneur en cendre d'os chez les porcs. Les porcs ont été attribués à 1 de 6 diètes (traitements), avec 12 enclos/traitement et 5 porcs/enclos. Les traitements étaient un témoin positif (PC – « positive control »), un témoin négatif 1 (NC 1 – « negative control 1 ») ayant un taux de Ca et de P assimilable réduits de 0,11 et 0,10 % respectivement, NC 1 plus 250 FTU kg^{-1} de phytase, NC 2 ayant un taux de Ca et de P assimilable réduits de 0,16 et 0,15 % respectivement, NC 2 plus 500 ou 2000 FTU kg^{-1} de phytase. Au jour 43, 1 porc/enclos a été euthanasié et le quatrième métacarpe prélevé pour déterminer les paramètres osseux. Le gain moyen quotidien (ADG – « average daily gain ») était plus élevé ($P < 0{,}05$) chez les porcs nourris à la diète NC 2 plus 500 ou 2000 FTU kg^{-1} par rapport à la diète PC. La diète NC 2 plus 2000 FTU kg^{-1} a permis d'améliorer ($P < 0{,}05$) le ratio G:F par rapport à la diète PC. Les diètes NC 1 et NC 2 ont réduit ($P < 0{,}05$) le poids de la cendre d'os et la diète NC 2 a réduit ($P < 0{,}05$) la BBS par rapport à la diète PC. L'ajout de phytase dans les diètes NC 1 ou NC 2 améliore le poids de la cendre d'os par rapport à la diète PC. La BBS et le poids de la cendre d'os étaient plus sensibles aux faibles teneurs en Ca alimentaire et en P assimilable que le pourcentage de cendre d'os. L'ajout de la phytase, à raison de 2000 FTU kg^{-1}, améliore le ratio G:F au-delà de celui de la diète PC.

Mots clés: Résistance des os à la rupture, cendre d'os, performance, phytase, porcs

Phytate (salt of myo-inositol hexaphosphate) is the main source of P in vegetable ingredients used in animal feeds (Ravindran et al. 1994) and is poorly digested by monogastric animals. Phytase catalyses the hydrolysis of phytate and increases P digestibility and absorption in pigs (Selle et al. 2003). This allows for a reduction in the total P concentration in the diet, thereby reducing the cost of the diet and P excretion into the environment. In addition to being a low available source of P, phytate is a significant anti-nutrient in pig diets. Phytate reduced mineral (Schlegel et al. 2010), protein, and energy digestibility (Liao et al. 2005) and significantly reduced young pig growth performance (Woyengo et al. 2012).

The effect of superdoses of a novel microbial phytase to reduce the anti-nutritional effects of phytate has been previously reported in young piglets fed nutritionally adequate diets (Walk et al. 2013). In addition, previous authors have reported improvements in growth

Abbreviations: **ADG**, average daily gain; **avP**, available phosphorus; **BW**, body weight; **FTU**, phytase unit; **G:F**, gain to feed ratio; **NC**, negative control; **PC**, positive control

performance and Ca and P digestibility of growing pigs fed 250 and 500 FTU kg^{-1} of the same novel microbial phytase in reduced Ca and available P (avP) diets (Kuhn and Manner, 2012). Therefore, the objective of the present trial was to evaluate the efficacy of the novel microbial phytase on growth performance and bone parameters using two levels of avP and Ca and three doses of phytase (250, 500 and 2000 FTU kg^{-1}) in growing pigs between 23 and 55 kg.

MATERIALS AND METHODS

The trial was conducted according to the US government principles for the utilization and care of vertebrate animals used in testing, research, and training at Swine Research Services, Inc. in Springdale, Arkansas.

Approximately 6 wk post-weaning, 360 castrated male and female PIC pigs (22.7±8 kg) were randomly distributed into six dietary treatments according to body weight (BW) and gender. Each treatment contained six pens of castrated males and six pens of females to total 12 replicate pens per treatment with five pigs per pen. Pigs had ad libitum access to pelleted feed and water for the duration of the trial. Experimental diets were fed in a two-phase feeding program. Phase 1 was fed from 23 to 38 kg (day 0 to day 21) and phase 2 was fed from 38 to 55 kg (day 22 to day 43). Treatments in each phase consisted of a positive control (PC) diet formulated to meet or exceed nutritional requirements for each feeding phase (National Research Council 1998), a negative control (NC) 1 diet with a 0.11 and 0.10% reduction of Ca and avP, respectively, from the PC, NC 1 plus 250 FTU kg^{-1} phytase, a NC 2 diet with a 0.16 and 0.15% reduction of Ca and avP, respectively, from the PC, NC 2 plus 500 FTU kg^{-1} phytase, and NC 2 plus 2000 FTU kg^{-1} phytase. The phytase used was a novel, intrinsically thermostable, *Escherichia* coli 6-phytase expressed in *Trichoderma reesei* and contained a declared activity of 5000 FTU g^{-1} (Quantum Blue, AB Vista Feed Ingredients, Marlborough, UK). Feed samples were collected and analysed for phytase recovery according to methods of Engelen et al. (2001), where 1 phytase unit is defined as the amount of enzyme required to release 1 μmol of inorganic P min^{-1} from sodium phytate at pH 5.5 and 60°C. The ingredient composition and nutrient analyses of the experimental diets for phase 1 and phytase 2 are presented in Tables 1 and 2, respectively. Prior to the start of the experiment, a sample of each diet was collected and analyzed for CP, ether extract, Ca and total P (Association of Official Analytical Chemists 1990).

Pig BW and feed disappearance were measured at the beginning of the experiment (day 0), at the end of phase 1 (day 21), and at the end of the experiment (day 43) to calculate average daily gain (ADG), average daily feed intake, and gain to feed ratio (G:F). On day 43, one pig of average BW/pen was selected and euthanized for determination of bone breaking strength and ash percent. The right front foot was collected and the 4th metacarpal was dissected, placed into labeled 50-mL Falcon tubes, and stored at −25°C until further analyses. Bone breaking strength was determined after thawing using a three-point bend rig with a load cell capacity of 250 kg and cross-head speed of 100 mm min^{-1} (HD 250 Texture Machine, Scarsdale, NY). After determining breaking strength, any pieces from the individual bones were collected, wrapped in cheese cloth, and dried in an oven at 100°C for 24 h. Fat was extracted from the metacarpals using a 48 h Soxhlet extraction in ethyl alcohol followed by a 48-h extraction with diethyl ether. The bones were then dried at 110°C in an oven for 24 h and weighed. To determine bone ash percent, the dry, de-fatted metacarpals were ashed in a muffle furnace at 560°C for 48 h.

Table 1. Nutrient and chemical composition of experimental diets (as-fed basis; phase 1)

Item	Positive control	Negative control 1[z]	Negative control 2[y]
Ingredient (%)			
Corn	65.78	66.93	67.38
Soybean meal	28.40	28.30	28.30
Pork fat	2.25	1.80	1.60
Salt	0.40	0.40	0.40
L-Lys HCl	0.30	0.30	0.30
DL-Met	0.12	0.12	0.12
L-Thr	0.10	0.10	0.10
Dicalcium phosphate	1.10	0.60	0.40
Limestone	1.05	0.95	0.90
Vitamin-mineral premix[x]	0.50	0.50	0.50
Calculated analysis			
AME (kcal kg^{-1})	3,300	3,300	3,300
CP (%)	18.50	18.50	18.50
Digestible lysine (%)	1.10	1.10	1.10
Ca (%)	0.71	0.60	0.55
Total P (%)	0.60	0.50	0.45
Available P (%)	0.32	0.22	0.17
Analysed composition			
DM (%)	87.50	87.00	87.90
Ether extract (%)	4.08	3.83	3.63
CP (%)	18.30	19.30	18.90
Ca (%)	0.78	0.66	0.62
Total P (%)	0.59	0.48	0.46

[z]An additional diet identical to negative control 1 was formulated to contain phytase at the expense of corn at 0.005%. This level of phytase was equivalent to 250 FTU kg^{-1} of the diet. The phytase used was Quantum Blue (AB Vista Feed Ingredients, Marlborough, UK) and had an expected activity of 5000 FTU kg^{-1}.

[y]Two additional diets identical to negative control 2 were formulated to contain phytase at the expense of corn at 0.010 or 0.040%. This level of phytase was equivalent to 500 or 2000 FTU kg^{-1} of the diet, respectively. The phytase used was Quantum Blue (AB Vista Feed Ingredients, Marlborough, UK) and had an expected activity of 5000 FTU kg^{-1}.

[x]Supplied per kilogram of diet: vitamin A (retinyl acetate), 2500 IU; vitamin D (cholecalciferol), 600 IU; vitamin E (α-tocopherol acetate), 11 IU; vitamin K (menadione dimethylpiridinol bisulfate), 11 mg; riboflavin, 3 mg; pantothenic acid, 7 mg; niacin, 9 mg; thiamine, 12 mg; pyridoxine, 20 mg; vitamin B_{12}, 0.10 μg; Zn (ZnO), 120 mg; Fe ($FeSO_4.H_2O$), 100 mg; Mn (MnO), 20 mg; Cu ($CuSO_4.5H_2O$), 30 mg; I (KI), 1 mg and Se (Na_2SeO_3) 40 μg.

Table 2. Nutrient and chemical composition of experimental diets (as-fed basis; phase 2)

Item	Positive control	Negative control 1[z]	Negative control 2[y]
Ingredient (%)			
Corn	72.04	73.24	73.69
Soybean meal	23.10	22.95	22.90
Pork fat	1.70	1.25	1.05
Salt	0.40	0.40	0.40
L-Lys HCl	0.30	0.30	0.30
DL-Met	0.08	0.08	0.08
L-Thr	0.08	0.08	0.08
Dicalcium phosphate	0.90	0.40	0.20
Limestone	0.90	0.80	0.80
Vitamin-mineral premix[x]	0.50	0.50	0.50
Calculated analysis			
AME (kcal kg^{-1})	3,100	3,100	3,100
CP (%)	16.50	16.50	17.00
Digestible lysine (%)	0.96	0.96	0.96
Ca (%)	0.61	0.50	0.45
Total P (%)	0.54	0.44	0.39
Available P (%)	0.27	0.17	0.12
Analysed composition			
DM (%)	87.10	87.80	87.80
Ether extract (%)	4.27	3.56	3.34
CP (%)	16.00	16.70	17.60
Ca (%)	0.61	0.57	0.36
Total P (%)	0.59	0.46	0.41

[z]An additional diet identical to negative control 1 was formulated to contain phytase at the expense of corn at 0.005%. This level of phytase was equivalent to 250 FTU kg^{-1} of the diet. The phytase used was Quantum Blue (AB Vista Feed Ingredients, Marlborough, UK) and had an expected activity of 5000 FTU kg^{-1}.
[y]Two additional diets identical to negative control 2 were formulated to contain phytase at the expense of corn at 0.010 or 0.040%. This level of phytase was equivalent to 500 or 2000 FTU kg^{-1} of the diet. The phytase used was Quantum Blue (AB Vista Feed Ingredients, Marlborough, UK) and had an expected activity of 5000 FTU kg^{-1}.
[x]Supplied per kilogram of diet: vitamin A (retinyl acetate), 2500 IU; vitamin D (cholecalciferol), 600 IU; vitamin E (α-tocopherol acetate), 11 IU; vitamin K (menadione dimethylpiridinol bisulfate), 11 mg; riboflavin, 3 mg; pantothenic acid, 7 mg; niacin, 9 mg; thiamine, 12 mg; pyridoxine, 20 mg; vitamin B_{12}, 0.10 μg; Zn (ZnO), 120 mg; Fe ($FeSO_4.H_2O$), 100 mg; Mn (MnO), 20 mg; Cu ($CuSO_4.5H_2O$), 30 mg; I (KI), 1 mg and Se (Na_2SeO_3) 40 μg.

Data were analysed using the least square means procedure in Minitab (v. 14–13th edition, Minitab Ltd. 2004, Coventry, UK). Pen served as the experimental unit for performance and pig was the experiment until for bone breaking strength and metacarpal ash. The model included block and treatment. Significance was accepted at $P < 0.05$. Significant means were separated using Tukey's Highly Significant Difference test.

RESULTS AND DISCUSSION

The analysed dietary CP, ether extract, Ca and total P are presented in Tables 1 and 2 and confirm target levels. Phytase activities recovered in the diets were similar to formulated values when assay and sampling variation are considered (Table 3). The NC diets were

Table 3. Recovered phytase activity of feed samples[zy]

Diet	Expected phytase activity (FTU kg^{-1})[x]	Recovered phytase activity (FTU kg^{-1})
Phase 1		
Positive control	0	<50
Negative control (NC) 1	0	<50
NC 1+250 (FTU kg^{-1})	250	260
NC 2	0	<50
NC 2+500 (FTU kg^{-1})	500	619
NC 2+2000 (FTU kg^{-1})	2,000	2,070
Phase 2		
Positive control	0	<50
NC 1	0	<50
NC 1+250 (FTU kg^{-1})	250	233
NC 2	0	<50
NC 2+500 (FTU kg^{-1})	500	446
NC 2+2,000 (FTU kg^{-1})	2,000	2,360

[z]Means represent the average of triplicate analyses per sample.
[y]Phytase recovered in the diets was analysed as described by Engelen et al. (2001).
[x]One phytase unit (FTU) is defined as the amount of enzyme required to release one μM of inorganic P per minute from sodium phytate at 37°C and pH 5.5.

formulated with reductions in Ca and avP. However, overall (day 0 to day 43) ADG or G:F were not different between pigs fed the PC, NC 1 or NC 2 diets and these results were not expected (Table 4). Average daily gain and G:F were significantly reduced in growing pigs fed diets formulated with 0.21% reductions in avP and only 0.12% reductions in Ca from 25 to 120 kg (Kuhn and Manner 2012). Body weight gain of 45 kg pigs was more influenced by dietary P levels rather than dietary Ca levels and the rate of gain increased as total P supplementation increased from 0.2 to 0.6%, regardless of the level of Ca in the diet (Chapman et al. 1962). More recently, there was no influence on growth performance of PIC337 or PIC280 growing pigs fed diets deficient in total P by 20% compared with pigs fed adequate P diets (Alexander et al. 2008). In the current trial, avP was reduced in the NC diets approximately 18 and 22% and thus this may not have been enough to elicit a significant depression in growth from day 0 to day 43.

Average daily gain in the younger growing pigs (23 to 37 kg) and bone breaking strength or bone ash weight in the 55-kg pigs appeared to be more susceptible to the low Ca and avP levels in the NC 2 diet than growth performance in the older pigs or bone ash percent. Low dietary Ca and P levels did not influence ADG in pigs at any age from 28 to 192 d post-weaning (Crenshaw et al. 1981) and the effect was negated in 37- to 55-kg pigs in the current trial. In addition, Crenshaw et al. (1981)

Table 4. Influence of diet on growth performance and metacarpal ash of pigs from 23 to 55 kg[z]

Item	Positive control[y]	NC 1[x]	NC 1+250 (FTU kg^{-1})	NC 2[w]	NC 2+500 (FTU kg^{-1})	NC 2+2000 (FTU kg^{-1})	SEM	*P* value
Body weight (kg)								
Initial (day 0)	22.8	22.9	22.9	22.9	22.9	22.9	0.15	0.98
Final (day 43)	55.1*c*	55.7*bc*	56.2*ab*	54.7*c*	56.7*ab*	57.3*a*	0.10	***
Phase 1 (day 0 to day 21)								
ADFI[v] (kg)	1.30	1.32	1.32	1.31	1.34	1.34	0.11	0.52
ADG[v] (kg)	0.68*b*	0.68*b*	0.68*b*	0.63*c*	0.72*a*	0.71*ab*	0.11	***
G:F[v] (kg kg^{-1})	0.527*ab*	0.518*b*	0.516*b*	0.479*c*	0.535*a*	0.532*ab*	0.06	***
Phase 2 (day 22 to day 43)								
ADFI (kg)	1.70	1.76	1.75	1.71	1.75	1.80	0.10	0.24
ADG (kg)	0.82*c*	0.83*bc*	0.87*ab*	0.85*abc*	0.85*abc*	0.89*a*	0.09	*
G:F (kg kg^{-1})	0.482*bc*	0.475*c*	0.498*a*	0.498*ab*	0.490*abc*	0.499*a*	0.05	*
Overall (day 0 to day 43)								
ADFI (kg)	1.50	1.55	1.54	1.51	1.55	1.57	0.10	0.22
ADG (kg)	0.75*c*	0.76*bc*	0.78*ab*	0.74*c*	0.78*ab*	0.80*a*	0.08	***
G:F (kg kg^{-1})	0.501*bc*	0.493*c*	0.506*ab*	0.490*c*	0.509*ab*	0.513*a*	0.04	***
Metacarpal measurements (55 kg)								
Breaking strength (kg)	77.4*abc*	69.5*bc*	80.9*ab*	65.9*c*	81.2*ab*	85.9*a*	0.06	*
Ash (%)	42.3	39.5	41.2	39.4	40.3	41.3	0.08	0.23
Ash weight (g)	3.64*a*	3.17*b*	3.57*a*	3.17*b*	3.61*a*	3.76*a*	0.03	***

[z]Data are means of eight replicates and six pigs per replicate pen.
[y]The positive control was formulated to be adequate in all nutrients.
[x]NC, negative control 1 was formulated with a reduction in Ca and available P from the PC by 0.11 and 0.10%, respectively.
[w]NC, negative control 2 was formulated with a reduction in Ca and available P from the PC by 0.16 and 0.15%, respectively.
[v]ADFI, average daily feed intake, ADG, average daily gain; G:F, gain to feed ratio.
a–c Means within rows with different letters are different ($P < 0.05$).
*, *** $P < 0.01$ and $P < 0.05$, respectively.

determined there was a non-significant relationship between the percentage of bone ash and bone strength parameters and any relationship, albeit poor, is dependent on the bone evaluated, the age of the pig, and the Ca and P level of the diet. Therefore, in the current trial, the lack of a significant effect of diet on metacarpal ash percentage may indicate bone ash percentage is not as sensitive a measure of mineral concentration as breaking strength or ash weight, particularly in the absence of growth responses to reduced dietary Ca and avP.

Phytase supplementation at 500 or 2000 FTU kg^{-1} in NC 2 improved ($P < 0.05$) ADG and G:F compared with pigs fed NC 2 from day 0 to day 21 and overall (day 0 to day 43). This has been previously reported in growing pigs fed avP and Ca deficient diets with 500 FTU kg^{-1} phytase (Kuhn and Manner 2012) or young pigs fed nutritionally adequate diets and 2500 FTU kg^{-1} phytase (Walk et al. 2013). In addition, phytase supplementation at 500 FTU kg^{-1} in the NC 2 diet improved ($P < 0.05$) metacarpal ash weight and bone breaking strength comparable to pigs fed the PC, and there was no additional benefit on bone ash to feeding 2000 FTU kg^{-1} phytase (Table 4). These results indicate the diets were deficient enough in Ca and avP to create a reduction in metacarpal ash concentration or breaking strength. Phytase supplementation at 500 FTU kg^{-1} improved bone ash comparable to the PC indicating this novel phytase was efficacious at hydrolysing phytate and providing a source of avP and Ca. This has been previously reported in growing pigs fed P deficient diets with *E. coli* or fungal phytases (Jendza et al. 2005; Brana et al. 2006). Phytate is present in vegetable ingredients (Ravindran et al. 1994), is poorly digested by monogastric animals, and reduced mineral availability (Schlegel et al. 2010) and protein and energy digestibility (Liao et al. 2005) in growing pigs. In the current trial, supplementation of phytase at 2000 FTU kg^{-1} in pigs fed the NC 2 diet improved ($P < 0.05$) overall G:F compared with the PC. The current results and previously published results in younger pigs (Walk et al. 2013) indicate supplementation of superdoses of phytase, above 500 FTU kg^{-1}, may improve growth performance or feed efficiency due to hydrolysis of phytate and improvements in overall nutrient utilisation or efficiency, rather than through the provision of P. In conclusion, reducing dietary avP and Ca in the current diet did not negatively influence growth performance. However, bone breaking strength or bone ash weight were reduced and may be more sensitive to reduced dietary Ca and P. Phytase supplementation at 500 FTU kg^{-1} improved bone ash weight and breaking strength comparable to the PC. Phytase supplementation at 2000 FTU kg^{-1} improved G:F compared with the PC, but had no further impact on metacarpal parameters indicating further improvements in performance may be associated with phytate destruction rather than P provision.

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