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Carbohydrase and Phytase Can Compensate Net Energy, Digestible Amino Acids and Available Phosphorus for High-performing Pigs

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Abstract: Two experiments were conducted to evaluate the efficacy of carbohydrases and phytase to compensate three key nutrients (NE, DAA and avP) for growing-finishing pigs. In Exp. 1, 112 pigs with average live weight 20.7 kg were randomly allocated to 4 dietary treatments, fed on commercial multi-grain diets containing total NSPs 12-15% with added phytase (500 FTU/kg), to examine the effect of two types of carbohydrase enzymes (single xylanase or multi-carbohydrase complex) to compensate NE and DAA: T1, Positive Control (PC); T2, negative control (NC1, PC minus 2% NE and 3% DAA); T3, NC1+ Multi-carbohydrases; and T4, NC1 + Single xylanase. In Exp. 2, 72 pigs averaged 20.7 kg live weight were randomly allocated to 3 dietary treatments: T5, Positive Control (PC, with added phytase); T6, NC2 negative control (PC minus 2.5% NE, 3.2% DAA, and 0.17 pcu avP, without added phytase); T7, NC2 + Multi-enzyme complex (carbohydrases + phytase). The pigs of both studies were reared for 3 phases: Grower 1 20-50 kg, Grower 2 50-75 kg, and Finisher (76-100 kg). The key performance parameters were measured and compared. The results of Epx. 1 showed, the pigs (PC) grew from 20 kg to 100 kg at average daily gain (ADG) close to 1 kg and FCR 2.30, and the reduction of nutrients (NC1) impaired growth rate mostly during the finishing phase (feed intake P=0.07, FCR P=0.09), and supplementation with both NSPase preparations tended to improve FCR over the NC1 with no differences observed between the two types of NSPase. In Exp. 2, the reduction of the three key nutrients clearly suppressed performance, namely lower weight gain (P<0.05) and higher FCR (P<0.05). The supplementation of multi-enzyme preparation partially restored performance with no significant differences from PC and NC2. In conclusion, for high performing pigs, carbohydrases can play a positive role in diets that already contain added phytase, and the contribution of the NSPase and phytase complex appear to be age related, and can be extrapolated as NE 40, 50 and 60 kcal/kg, respectively for Post-weaning, Grower and Finisher phases, and digestible amino acids 2-3% and avP 0.17% are for all phases. The enzyme supplementation showed no impact on feed intake.

Keywords: Carbohydrase, Phytase, Performance, Pig

1. Introduction

In commercial pig diets, starch, and non-starch polysaccharides (NSP) are the largest components of cell wall structure, and their digestibility and utilization have utmost importance for the efficiency of pork production. For monogastric animals, it is commonly understood that exogenous NSP-degrading enzymes (NSPase), or carbohydrases, can hydrolyze the polysaccharide structure in cell wall that renders nutrients more accessible to the endogenous enzyme activities, resulting in increases in digestibility of energy and amino acids (AA). However, the degree of trial consistency varies substantially not only between poultry and swine, but also within species. While exogenous xylanases have been widely proven effective for poultry, evidences seem to be more variable in swine diets [1, 2]. According to Cozannet et al. [3], multi-carbohydrase supplementation can contribute digestible energy ranging from 15 to 86 kcal/kg pig diets. However, the digestibility uplifts are not always translated to improvement in growth performance [4, 5].

Apart from energy and amino acids, phosphorus (P) is the third most costly nutrient. Pigs poorly utilize P bound in myo-inositol-hexaphosphate (IP6) in vegetable ingredients due to lack of relevant digestive enzymes [6]. Adding exogenous phytase to diets improves digestibility of phosphorus and also contributes to better weight gain, which is most likely due to improved nitrogen digestibility [7]. Comparing with NSPase, the benefits of phytase supplementation can be quantitatively measured more easily in terms of P digestibility, bone strength etc. Since the benefits of phytase are consistently proven and it is relatively easy to measure its economical return, today dietary application of exogenous phytase has been widely adopted in swine industry across the globe.

As all feed ingredients of plant origin are complex structure of protein, polysaccharides and others encapsulated in cell wall, it is of practical interest in examining possible interaction between phytase and NSPase in the same diet formulation. In another word, what additional advantages may be conferred by application of NSPase into diets already containing added phytase. Early research in poultry suggested such interaction did exist and the degree of interaction seemed to be related to main ingredients used in the formulation [8]. A study [8] using fast growing broiler reported additional daily weight gain and improved feed conversion ratio following the supplementation with multi-NSPase to the basal diets that already contained high dose phytase (1000 FTU/kg). For weaned pigs, early research reported the addition of an enzyme complex consisting of NSPase, protease and amylase to phytase-supplemented diets can improve growth performance, gut health, and nutrient digestibility of weaned pigs [10-12]. For growing and finishing pigs, quantitative contribution of a multi-enzyme complex containing NSPase and phytase was estimated averaged NE 80 kcal, digestible lysine 0.4 g and digestible P 1.5 g per kg diet [2]. However, very often, the increments observed from digestibility do not always lead to proportional growth performance, as shown by Huang et al. [13].

This study was designed to examine additional benefits of adding NSPase into basal diets that already contained added phytase, and to estimate releases of nutrients following supplementation with an enzyme preparation containing multi-NSPase and high dose phytase, for growing and finishing pigs fed on multi-grain high-performing diets.

2. Materials and Methods

2.1. Experimental Design, Animals, Diets and Enzymes

Two feeding trials were carried out at the Bangkok Animal Research Center (BARC), Samut Prakan, Thailand, from February to July 2021. The objectives of Exp. 1 was to examine and compare the ability of two types of NSPase preparations to compensate reduced net energy (NE) and digestible amino acids (DAA) from basal diets that already contained added phytase (500 FTU/kg). The study employed a total of 112 weaned pigs of averaged live weight 20.7 kg, randomly allocated to 28 pens, with 7 pens for each of the 4 treatments (Table 1). Exp. 2 was designed to examine whether a multi-enzyme complex (multi-NSPase + phytase 1000 FTU/kg) can compensate pre-defined amounts of NE, DAA, Ca and P, using 72 pigs of the same live weight randomly allocated to 3 dietary treatments, 6 pens per treatment and total 18 pens. The trials were arranged as complete randomized block design using 4 piglets per experimental unit. The pigs were reared for 3 phases based on their growing phases: Grower 1 (20-50 kg), Grower 2 (50-75 kg), and Finisher (75-100 kg).

Table 1. *Experimental treatments and tested-enzymes.*

Experiment 1		Experiment 2	
T1	PC, Positive Control, standard diets with phytase[1]	T5	Positive Control, standard diets
T2	NC1, PC - NE and dAA (see Table 2)	T6	Negative Control 2 - NE, dAA, avP (see Table 2)
T3	NC1 + Multi-NSPase Complex[2]	T7	NC2 + Multi-NSPase with phytase[4]
T4	NC1 + Single Xylanase[3]		

[1]Quantum® Blue (AB Vista, UK), providing 500 FTU/kg feed.
[2]Rovabio® Advance T-Flex (Adisseo France), providing ≥1,250 endo-1,4-β-xylanase units, 4,600 α-arabinofuranosidase units, and 860 endo-1,3(4)-β-glucanase units per kg feed.
[3]Dan® Xylanase 40000 (Danisco, UK), providing xylanase 4000 U per kg feed.
[4]Rovabio® Advance Phy T (Adisseo France), providing ≥ 1,250 endo-1,4-β-xylanase units, 4,600 α-arabinofuranosidase units, 860 endo-1,3(4)-β-glucanase units, and 1,000 units of 6-phytase per kg feed.

The choice of ingredients and diet formulation followed commercial diets used in Thailand. Specification of feed ingredients used in this study followed the INRAE/CIRAD/AFZ© 2017-2020 standards and the requirement of energy and nutrients followed the Brazilian Tables (2017), except for amino acids (AA) from Ajinomoto Eurolysine (2016). The Positive Control (PC) diet was formulated according to nutritional specification, supplemented with phytase (500 FTU/kg). This diet was considered adequate in all nutrients that can support expected growth of the pigs from weaning to market weight. The NSP profile of the formulated diets were estimated by multiplying the ingredients inclusion by their NSP contents estimated by Knudsen et al. in both 2011 and 2014 [14-15]. From this PC diet, negative control (NC1) diets were produced by reducing NE by 55 kcal/kg and Dig. Lys by 2.1% for Grower 1, then NE 50 kcal/kg and Dig. Lys by 1.8% for Grower 2, followed by NE 50 kcal/kg and Dig. Lys. by 2.1% for Finisher phase. The reduction of other essential amino acids is listed in Table 2. For Exp. 2, the NC2 diets were formulated by reducing NE by 65, 60 and 60 kcal/kg and Dig. Lys. by 2.2, 2.2 and 2.5%, available P by 0.17 and calcium by 0.15, 0.13 and 0.10 percental units (PCU), respectively for the 3 phases. The scopes of reduction of other essential amino acids are listed in Table 2. The reduction in matrix values, and enzyme doses were derived and adjusted from previous studies [3, 16] that recommended these enzymes may contribute in multi-grain diets for growing and finishing pigs. The composition and level of nutrients are listed in Table 3. The diets were offered in mash form in order to maintain their full enzyme activities throughout the study.

Table 2. *Reduction of energy (ME, NE), digestible amino acids, and available P of the Negative Control diets.*

	Experiment 1 (NC1)*			Experiment 2 (NC2)*		
	20-50 kg	50-75 kg	75-100 kg	20-50 kg	50-75 kg	75-100 kg
ME, kcal/kg	70	65	60	79	74	69
NE, kcal/kg	55	50	50	65	60	60
Crude Protein, %	3.6	3.2	1.6	3.6	3.2	1.6
Dig. Lys, %	2.1	1.8	2.1	2.2	2.2	2.5
Dig. Met, %	1.6	1.3	2.1	1.6	1.0	2.1
Dig. Cys, %	2.6	2.7	2.1	2.6	3.5	2.5
Dig. Met+Cys, %	2.0	1.8	2.1	2.0	2.0	2.3
Dig. Thr, %	2.0	1.8	2.0	2.1	1.9	2.2
Dig. Trp, %	1.9	1.6	1.9	2.8	1.6	1.9
Dig. Arg, %	5.4	5.2	2.7	5.7	5.6	2.7
Dig. Val, %	2.2	4.2	3.1	2.9	4.4	3.5
Dig. Iso, %	4.8	5.3	4.5	4.9	6.9	5.2
Dig. Leu, %	3.0	4.0	4.9	2.8	6.0	5.9
Dig. His, %	3.9	4.0	2.6	3.9	4.5	2.6
Dig. Phe, %	4.2	4.5	3.8	4.4	5.8	4.1
Dig. Phe+Tyr, %	4.1	4.6	4.0	4.2	5.9	4.7
Available P, pcu				0.17	0.17	0.17
Calcium, pcu				0.15	0.13	0.10

*NE, DAA, Ca and avP reductions are based on recommendation for Rovabio® Advance T-Flex and Rovabio® Advance Phy T, respectively.

Table 3. *Formulations and nutrients of the experimental diets.*

Ingredient, %	Grower 1 (20-50 kg)			Grower 2 (50-75 kg)			Finisher (75-100 kg)		
	PC	NC1	NC2	PC	NC1	NC2	PC	NC1	NC2
Corn	37.16	39.31	40.02	36.33	35.88	32.38	29.29	24.09	22.39
Wheat	12.00	12.00	12.00	15.00	15.00	15.00	18.00	18.00	18.00
Barley	6.00	6.00	6.00	7.00	7.00	7.00	8.00	8.00	8.00
Cassava	8.00	9.00	10.00	10.00	11.00	13.00	12.00	14.00	16.00
Rapeseed meal	3.00	3.00	3.00	4.00	4.00	4.00	5.00	5.00	5.00
DDGS	4.00	4.00	4.00	5.00	5.00	5.00	6.00	6.00	6.00
SBM	19.59	17.67	17.50	14.04	12.09	11.55	6.93	5.59	5.47
Wheat bran	4.00	4.00	4.00	5.00	7.09	9.95	11.88	16.49	17.35
Soybean oil	2.51	1.21	0.56	0.75			0.40	0.33	
MCP	1.07	1.09	0.36	0.70	0.70	0.05	0.57	0.47	
Limestone 39.4%	0.97	0.98	0.83	0.76	0.76	0.58	0.55	0.65	0.40
Swine premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.28	0.28	0.28	0.22	0.22	0.21	0.21	0.21	0.21
L-Lysine HCl	0.49	0.52	0.52	0.44	0.47	0.47	0.44	0.45	0.45
L-Threonine	0.21	0.22	0.22	0.16	0.18	0.18	0.15	0.15	0.15
DL-Methionine	0.13	0.13	0.13	0.07	0.07	0.08	0.03	0.03	0.03
L-Tryptophan	0.05	0.06	0.06	0.04	0.05	0.05	0.04	0.04	0.04
L-Valine	0.02	0.04	0.03						
Antimold	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Mycotoxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[1]	+	+	-	+	+	-	+	+	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrition value, g/kg									
ME, kcal/kg	3,200	3,130	3,121	3,100	3,035	3,026	3,000	2,940	2,931
NE, kcal/kg	2,414	2,359	2,349	2,339	2,289	2,279	2,272	2,222	2,212
Crude protein	170.1	164.0	164.0	156.4	151.4	151.4	141.4	139.2	139.2
Crude fat	48.6	36.1	29.9	31.9	24.7	24.6	28.9	27.9	24.4
Linoleic acid	23.9	17.7	14.6	15.4	11.8	11.6	13.6	12.9	11.1

Ingredient, %	Grower 1 (20-50 kg)			Grower 2 (50-75 kg)			Finisher (75-100 kg)		
	PC	NC1	NC2	PC	NC1	NC2	PC	NC1	NC2
Crude fiber	40.9	40.6	41.0	42.3	43.3	45.5	46.7	49.7	50.8
Dig. Lys	10.69	10.47	10.45	9.27	9.10	9.07	8.05	7.88	7.85
Dig. Met	3.73	3.67	3.67	2.99	2.95	2.96	2.42	2.37	2.37
Dig. Cys	2.67	2.60	2.60	2.57	2.50	2.48	2.40	2.35	2.34
Dig. M+C	6.41	6.28	6.28	5.56	5.46	5.45	4.83	4.73	4.72
Dig. Thr	7.16	7.02	7.01	6.21	6.10	6.09	5.39	5.28	5.27
Dig. Trp	2.14	2.10	2.08	1.85	1.82	1.82	1.61	1.58	1.58
Dig. Arg	9.20	8.70	8.68	8.07	7.65	7.62	6.75	6.57	6.57
Dig. Val	6.95	6.80	6.75	6.17	5.91	5.90	5.41	5.24	5.22
Dig. Ile	5.86	5.58	5.57	5.24	4.96	4.88	4.40	4.20	4.17
Dig. Leu	12.43	12.06	12.08	11.61	11.15	10.91	10.20	9.70	9.60
Dig. His	3.86	3.71	3.71	3.54	3.40	3.38	3.11	3.03	3.03
Dig. Phe	7.07	6.77	6.76	6.43	6.14	6.06	5.55	5.34	5.32
Dig. Phe	12.27	11.77	11.76	11.18	10.67	10.52	9.65	9.26	9.20
Calcium	7.22	7.22	5.57	5.75	5.75	4.10	4.97	4.97	3.32
P, avail.	3.57	3.57	2.07	2.81	2.81	1.50	2.42	2.42	1.47
Potassium	8.35	8.11	8.17	7.88	7.78	8.07	7.58	7.85	8.05
Sodium	1.20	1.20	1.20	1.00	1.00	1.00	1.00	1.00	1.00
Chloride	3.15	3.15	3.20	2.71	2.77	2.78	2.74	2.75	2.73
Salt	2.84	2.82	2.81	2.21	2.19	2.17	2.14	2.11	2.09
DEB, mEq/kg	177	171	171	169	165	172	160	167	172

[1]Quantum® Blue at 100 g/mt, providing phytase 500 FTU/kg feed.

The phytase used in Exp. 1 was Quantum® Blue (AB Vista, at 500 FTU/kg feed). One unit of phytase is defined as the amount of enzyme that liberates one micromole of inorganic orthophosphate from phytic acid per minute at pH 5.5 and 37°C. The two enzyme preparations of carbohydrase were 1) Rovabio® Advance T-Flex (Adisseo France, providing ≥1250 viscos units of endo-1,4-β-xylanase, 4600 units of α-arabinofuranosidase, and 860 units of endo-1,3(4)-β-glucanase per kg feed); 2) Dan Xylanase 40000 (endo-1,4-ß-xylanase produced by Trichoderma reesei, Danisco Animal Nutrition, UK); In Exp. 2, Rovabio® Advance Phy T was tested (Adisseo France, providing ≥1800 units of endo-1,4-β-xylanase, 1244 units of endo-1,3(4)-β-glucanase, 6600 units of α-arabinofuranosidase, and 1000 units of 6-phytase per kg diet), respectively. The units of these enzyme activities were defined according to Jlali et al. [17]. Specifically, one viscos-unit of endo-1,4- β-xylanase activity is defined as the amount of enzyme reducing the viscosity of the solution, to give a change in relative fluidity of 1 dimension less unit per minute per milliliter (or per gram) under the conditions of the assay (pH 5.5 and 30°C). One exo-β-glucanase unit of endo-1,3(4)-β-glucanase activity is defined as the amount of enzyme releasing oligomers, which are soluble in ethanol, to give an absorbance of 0.820 units at 590 nm under the conditions of the assay (20 min at pH 4.6 and 30°C). One unit of arabinofuranosidase refers to the amount of enzyme that releases 1 nmol of arabinose per min from the hydrolysis of wheat arabinoxylan in defined assay conditions (pH 4, 50°C). The analyses of xylanase and phytase activities are described by European Food Safety Authority (EFSA) (2014) and ISO standard 30024 (2009).

2.2. Experimental Facilities and Procedures

The animals were reared in closed-house with tunnel ventilation and evaporative cooling system, with solid-concrete-floor pens. Pen dimensions were 2.0 m x 2.4 m, equipped with 1 feeder and 1 nipple drinker. Housing and management systems used were representative of standards practiced by large swine integrators in Thailand. Feed was supplied *ad libitum* along the entire experimental period. At the end of each growing phase, the feed remaining in the feeder of each pen was weighed and recorded and the pigs were individually weighed to calculate pen average LW, and pen Feed Intake (FI). Sick or dead pigs were monitored and recorded daily.

2.3. Data and Statistical Analysis

Live weight gain (LWG), average daily gain (ADG) and daily FI (DFI), group uniformity and livability were calculated for each of the 3 feeding phases, and for the entire growth cycle. Feed efficiency was calculated as Feed Conversion Ratio (FCR) from FI and LWG. Herd uniformity was calculated by subtracting the coefficient of variation for each pen from 100% uniformity, and expressed as average per treatment. The data were subjected to analysis of variance using the GLM procedure of the Statistical Analysis Software (SAS Institute, NC). Differences among treatment means were detected by Duncan's new Multiple Range Test, with dietary treatments as the main effect. Statistical significance was considered at $P<0.05$ level.

3. Results

3.1. Experimental Diets and NSP Profile

The basal diets in this study were consisted of corn, wheat, barley, cassava and byproducts such as rapeseed meal, DDGS, wheat bran, with a significant and mixed profile of NSPs due to their complex composition. The 3-phases PC diets contained total NSPs 12.4-12.5% for Grower 1, 12.9-14.0%

for Grower 2, and 14.4-15.8% for Finisher diets. In the total NSPs, arabinoxylans counted on 52.6%, celluloses 25.4%, β-glucans 4.8%, pectins and others 17.1%. The level and composition of the NSPs in the diets provided considerable substrates for NSPase to hydrolyze.

3.2. Growth Performance, Feed Efficiency and Mortality

Exp. 1. The performance results are listed in Table 4. All pigs reached 100 kg live weight at growth rate very close to 1 kg/day at feed conversion ratio 2.30 for the Positive Control (PC), no morbidity nor mortality was recorded throughout this study. These results suggest the pig herd achieved high level of performance as expected. For the phase of Grower 1, when comparing between PC and NC1, the reduction of NE 55 kcal/kg and dig. AA by approximately 2-3% caused noticeable impact by slightly reduced feed intake and daily weight gain at higher FCR, though not statistically significant (P>0.05). The addition of the two types of NSPase preparations (T3 and T4) did not improve these parameters of NC1. The results of Grower 2 indicated adaptation of the pigs in NC1 to the lower density of nutrients as they increased their feed intake in order to obtain extra nutrients to gain the same live weight as of the PC, which resulted in higher FCR despite the impact remained statistically insignificant (P>0.05). The supplementation of the two NSPase preparations showed similar tendency of improving FCR although the effect remained statistically small (P>0.05). For Finisher phase, as expected, the dietary treatment effect had accumulated and revealed, namely the reduction of nutrients (NC1) led to 5% more feed intake (P=0.07), and 5.7% higher FCR or a loss of 16 FCR points (P=0.09), yet the pigs managed to gain the similar weight as of the PC group. The two enzyme treatments produced a tendency of improving feed efficiency comparing with the NC1, but did not fully restore feed intake and FCR to the level of the PC group. For the entire growth cycle, the accumulated impact of reduction of nutrients was 1 kg lower final live weight and 3.62% higher FCR, comparing with the PC group (P=0.09). The supplementation of the two NSPase preparations tended to improve feed efficiency but did not fully restore their FCR to the level of the PC group, with no differences observed between the two types of NSP-degrading enzymes.

Table 4. *Effect of multi- or single NSPase on performance of pigs (Exp. 1)*.*

Initial LW, kg	**PC**	**NC1**	**M-NSPase[1]**	**S-NSPase[2]**	**CV**	**P value**
	20.71	**20.71**	**20.71**	**20.71**		
Grower 1 (20-50 kg)						
LWG, kg	30.38	29.48	29.70	29.56	3.22	0.3092
ADG, kg/day	0.921	0.893	0.900	0.896	3.21	0.3052
DFI, kg	1.709	1.679	1.681	1.683	3.78	0.7989
FCR, kg/kg	1.861	1.886	1.870	1.884	3.87	0.8966
Grower 2 (50-75 kg)						
LWG, kg	25.52	25.54	25.76	26.03	4.34	0.8138
ADG, kg/day	0.912	0.912	0.920	0.930	4.33	0.8122
DFI, kg	2.191	2.268	2.234	2.236	3.77	0.4139
FCR, kg/kg	2.404	2.489	2.431	2.408	4.42	0.4489
Finisher (75-100 kg)						
LWG, kg	24.61	24.47	24.56	24.77	4.11	0.9549
ADG, kg/day	1.230	1.223	1.228	1.238	4.12	0.9571
DFI, kg	3.391	3.560	3.458	3.510	3.27	0.0684
FCR, kg/kg	2.755	2.913	2.815	2.839	3.84	0.0897
Overall (20-100 kg)						
Final LW, kg	101.23	100.21	100.73	101.36	1.59	0.5368
LWG, kg	80.51	79.49	80.02	80.64	2.01	0.5422
ADG, kg/day	0.994	0.981	0.988	0.996	2.02	0.5478
DFI, kg	2.291	2.347	2.311	2.341	2.14	0.1554
FCR, kg/kg	2.305	2.393	2.339	2.352	2.59	0.0908

*Means in the same row not bearing the same superscript differ significantly (P<0.05), [1]Multi enzymatic complex (Rovabio® Advance T-Flex, 50 g/mt feed), [2]Single xylanase (Danisco xylanase 40000, 100 g/mt feed)

Exp. 2. The results are described in Table 5. The pigs in PC group reached the expected live weight of 100 kg within 80 days, growth rate was near 1 kg/day at FCR 2.3, suggesting excellent performance. The dietary treatment responses were clearer than those of Exp. 1. For Grower 1, comparing between PC and NC2 groups, the reduction of the three key nutrients (NE 65 kcal/kg, Dig. Lys. 2.2% and avP 0.17 pcu) resulted in lower weight gain (P<0.05), mainly attributable to their lower feed intake with poorer FCR (P=0.10). The supplementation with the multi-enzyme preparation did not reverse weight gain but appeared to have improved FCR. For Grower 2, interestingly, the pigs of NC2 group significantly increased their feed intake by 5% (P<0.05), and gained the same weight as of the PC group at higher FCR (P>0.05). Similar to Grower 1, the supplementation with the combined enzyme did not restore daily gain but tended to benefit feed conversion. This trend continued till the end of the Finisher phase, NC2 group consumed more feed to gain similar weight as of the PC group but at higher FCR (P=0.06). Again, the supplementation with the combined enzyme improved feed conversion. The treatment effect was accumulated and pronounced for the entire growth cycle. The reduction of the three key nutrients led to loss of weight gain by 2.47 kg (P<0.05), FCR by 6% (P<0.05). No significant difference was observed in feed intake due to a reverse from lower intake in Grower 1 towards higher intake during the remaining 2 phases. The benefits of the enzyme preparation can be observed as alleviating the negative impact of the NC2, by partially restoring weight gain and FCR that brought performance of NC2 group to the middle between the PC and NC2 groups.

Table 5. *Effect of Multi-NSPase + Phytase complex on performance of pigs (Exp. 2)[1].*

Initial LW, kg	PC	NC2	NC2 + (NSPase+Phytase)[2]	CV	P value
	20.71	20.71	20.71		
Grower 1 (20-50 kg)					
LWG, kg	30.41	28.28	28.50	3.68	0.0116
ADG, kg/day	0.921	0.857	0.864	3.67	0.0113
DFI, kg	1.717	1.667	1.661	4.11	0.3438
FCR, kg/kg	1.869	1.950	1.925	3.15	0.1058
Grower 2 (50-75 kg)					
LWG, kg	25.46	25.56	25.90	4.89	0.8235
ADG, kg/day	0.909	0.913	0.925	4.90	0.8205
DFI, kg	2.175	2.285	2.279	2.48	0.0107
FCR, kg/kg	2.394	2.507	2.470	5.09	0.3212
LWG, kg	24.41	23.97	24.43	3.14	0.5225
ADG, kg/day	1.220	1.198	1.222	3.15	0.5224
DFI, kg	3.351	3.533	3.523	4.74	0.1496
FCR, kg/kg	2.745	2.949	2.884	4.70	0.0659
Overall (20-100 kg)					
Final LW, kg	100.99	98.52	99.54	1.29	0.0238
LWG, kg	80.27	77.81	78.82	1.63	0.0241
ADG, kg/day	0.991	0.961	0.973	1.64	0.0254
DFI, kg	2.279	2.342	2.335	2.89	0.2534
FCR, kg/kg	2.301	2.439	2.400	2.84	0.0141

[1]Means in the same row not bearing the same superscript differ significantly (P<0.05) [2]Multi-enzymatic complex with phytase (Rovabio® Advance Phy T, 100 g/t feed)

4. Discussion

4.1. The Effect of NSPase in Diets with Added Phytase

Today the swine industry has adopted phytase extensively. This is because approximately two thirds of phosphorus (P) in common grains and byproducts are in the form of phytate, for which the pigs lack of significant endogenous phytase activity. The addition of exogenous phytase can greatly improve the P digestion and utilization, and produce measurable economic return, such as reduced use of mineral P sources and P excretion through manure [6]. In contrast, the industry adoption of NSPase has been slow and often limited to use in the weaning diets, due mainly to inconsistent trial findings, especially during the growing and finishing phases. This study attempted to measure the value of NSPase supplemented to diets already containing regular level of phytase. The basal diets are complex including several viscous grains plus several by-products in the formulation, providing approximately 12-15% total NSPs for the two NSP-degrading enzymes to show their effect.

The results of Exp. 1 showed young pigs (Grower 1) responded the reduction of NE and DAA by slightly reduced feed intake, resulting in noticeable declines in weight gain. The results indicated young pigs have limited capacity to adjust feed intake based on the density of nutrients, and the supplementation of the two NSPase preparations did not reverse the trend. As the pigs grew bigger, their digestive capacity evolves to cope with low density diets (NC1) as they clearly increased their feed consumption during the Grower 2 throughout the Finisher phases, such increases in feed intake allowed the animals to grow as fast as that of the PC group, but at the expense of FCR. The supplementation with the NSPase preparations resulted in a small but noticeable effect in Grower 2 that lasted till the end of the growth cycle, resulting in 500-1000 g extra final live weight comparing with that of NC1 group, and FCR standing between NC1 and PC, suggesting the two enzyme preparations played a positive role, particularly in FCR and weight gain. These results are well in line with previous findings by Fang et al. [18] who reported supplementation with both single and multi-carbohydrase significantly improved feed efficiency of growing pigs. In terms of quantitative release, Cozannet et al. [2] estimated the supplementation with multi-NSPase would generate a wide range of digestible energy stretching from 63 to 359 kJ (15-86 kcal)/kg for growing and finishing pigs. The authors explained such extensive variation may be attributable to the choice of major ingredients, energy level, age of the animals, their density as well as sanitary conditions.

It must be pointed out that most of NSPase efficacy studies are based on control diets without supplemental phytase, in order to focus on the responses of NSPase only. The presence of both phytase and NSPase in the gastro-intestinal tract, as of Exp. 1, would mean multiple interactions between the substrates and enzymes by nature. For poultry, Cowieson et al. [7] speculated phytase and xylanase have sub-additive or additive benefits in corn soy-based diets as the enzymes may act independently on different substrates. However, their effect on nutrients such as amino acids overlap and largely depend on the digestible fraction or the nutritional value of the control diet to which the enzymes are added. When the basal diet already contains added phytase, it can be assumed that both phytase and NSPase will interact with the same cell wall structure whenever accessible, the sequence and degree of hydrolyses within limited transit time in the small intestines will depend on multiple factors and will be difficult to predict [8]. For pigs, the interaction among substrates and different enzymes will be even more sophisticated due to larger digestive capacity and longer transit time in comparison with poultry, which further complicates measurement of nutrients released by NSPase in the presence of phytase. Overall, the results of Exp 1 reveals that, despite the effect of NSPase being small during the early stage of growth, the responses do exist and are accumulative, and become clear by the end of their growth cycle, as shown by extra weight gain of 500 -1000 g/pig with improved FCR. It can be anticipated that, for growing and finishing pigs, measuring independent value of NSPase remains a true challenge due to multiple factors such as diet composition, density of nutrients, age of the animal and whether the diets carry added phytase or not.

As of no differences in the enzyme responses between the single xylanase and multi-NSPase, our findings are well in line with the results of Fang et al. [18] who compared responses between xylanase (Porzyme® 9300) and multi-NSPase (Rovabio® Excel), and reported both enzyme preparations significantly improved weight gain and FCR of growing pigs, with no differences observed between the two enzyme preparations.

4.2. The Effect of Multi-NSPase and High Dose Phytase

Since the industry has a keen interest in simplicity of the enzyme application, by using only one enzyme preparation for all commercial formulations, we designed Exp. 2 to validate a set of recommended nutrition matrix values of a combined enzyme complex containing multi-NSPase and high dose of phytase, for fast growing pigs. Namely, NE 65 kcal/kg, Dig. Lys 2.2% and other DAA from 1.6% to 5.4%, and avP 0.17 pcu for Grower 1, and NE 60 kcal/kg, Dig. Lys 2.2% and other DAA for 1 to 6%, avP 0.17 pcu for the remaining phases (Table 2). These values were obtained through a series of studies on digestibility [16, 19]; and performance [2] of growing and finishing pigs using the same enzyme preparation. The results of Grower 1 confirmed the reduction of these nutrients caused lower weight gain and poorer FCR. Similar to the Exp. 1, the decline of feed intake during Grower 1 was reversed during Grower 2 and Finisher phases, the pigs in NC2 slowly adapted to the lower density diets by consuming more feed and gained the same weight as of the PC group. For the entire growth cycle, comparing with PC group, the NC2 group lost live weight by 2.47 kg or 2.44% ($P<0.05$) and FCR by 6% ($P<0.05$). The addition of the enzyme preparation tended to reverse the negative impact and brought these parameters to the middle between PC and NC2 groups, and increased final live weight by 1.02 kg or 1.04% and reduced FCR by 3.9 points or 1.6% compared to NC2. These results are in line with the observation by Huang et al. [13] who investigated the same enzyme preparation for growing and finishing pigs, to validate the enzyme contribution NE 74 kcal/kg, digestible amino acids (dig. AA) 7.0%, digestible P (dig. P) 0.134 pcu, and Ca 0.119 pcu. They found the enzyme addition improved total tract digestibility of gross energy (80.0 *vs.* 81.8%), protein 71.5 *vs.* 76.5%), fat (61.0 *vs.* 77.1%) and P (48.4 *vs.* 65.3%), also improved final weight gain and FCR during the later growing phases over the negative control, but the enzyme did not fully restore final live weight and FCR to the level of the positive control.

In practical swine formulation, it is desirable to define a set of matrix value for an enzyme preparation for the convenience in application. Unfortunately research by far has proven it is of great difficulty to achieve such objective due to the complicity of diet formulation, expected growth rate and the actual efficacy of various enzymes involved. Several studies have attempted to define nutritional contribution of multi-NSPase with phytase. Based on multiple studies of similar design, Cozannet et al. [2] suggested that the same enzyme cocktail as used in the current study, should contribute on average ADG 20 g/d ($P<0.001$) and FCR -0.06 points or 2.3%, with no impact on feed intake. However, their enzyme responses vary according to the test diets: ADG from -2.2 to + 4.2% and FCR from +0.4 to -6.3%, depending on the level of release of nutrients by the enzyme. Based on equations established from control diets, they concluded the enzyme effect can be converted to the release of 334 kJ or 80 kcal NE, 0.4 g dig Lys and 1.5 g dig P per kg feed. Nonetheless, the effect of these releases of nutrients on performance varies due to the density of digestible nutrients and actual growth rate. The present study observed the enzyme contribution as ADG 12.75 g and FCR 0.04 points or 1.6%, which is in line with the above estimation, despite both density of nutrients and growth rate of the pigs in the present study are higher.

5. Conclusion

The present study examined the effect of two types of NSPase supplemented to nutrients reduced diets, for growing and finishing pigs. The diets contained 12-14% of total NSPs and added phytase. The pigs of the positive control reached 100 kg with ADG about 1 kg at FCR 2.305. The reduction of nutrients affected mainly FCR which is mostly attributable to the reduced NE in the NC diet and the NSPase preparations tended to improve FCR especially during the finishing phase. No differences were observed between multi-NSPase and single xylanases. The second study checked whether a pre-assumed releases of NE, dig. AA and avP, can be compensated by addition of a multi-NSPase and phytase. The results showed the reduction of the nutrients caused significant loss of FCR ($P<0.05$) and the supplementation with the enzyme partially restored performance, which can be translated to a contribution of ADG 12.75 g and FCR 0.039 points or 1.6%. Clearly, more research is required to define contribution of NE, Dig AA and avP in high-performing diets for growing and finishing pigs.

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