RESEARCH REPORT

ANIMAL SCIENCE

pork checkoff

Title: Improving nutrient utilization and biological and financial performance through the use of super-dosing of phytase in grow-finish diets – **NPB #13-167** <u>revised</u>

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Industry summary:

The objective of this research was to determine the impact of feeding grow-finish pigs super-dosed levels of phytase on the digestibility of nutrients, and pig growth performance. The enzyme phytase is included in pig diets to improve phosphorus digestibility through the breakdown of phytate, thus decreasing the amount of inorganic phosphorus needed to be added to the pig's diet to meet its phosphorus requirements. This allows producers to decrease ingredient costs as well as improve environmental sustainability, by lessening the amount of phosphorus excreted into the environment. In addition to these benefits, there has been considerable interest in super-dosing phytase, meaning adding phytase to diets at much higher levels than required to meet the phosphorus requirement, with the hope of further enhancing pig performance and nutrient utilization. The logic is that phytate binds more than just phosphorus, and adding very high levels of phytase could release these other nutrients to enhance pig performance. There have been previous studies on this subject, but the results have been highly inconsistent, making it difficult for producers to determine whether or not it is beneficial to include phytase in their diets at super-dosing levels.

In this study, 4 different levels of phytase, 3 of which were considered super-dosed, were used in 2 separate experiments in order to help determine whether it is biologically and financially practical to super-dose phytase. The first experiment was an intensive metabolism study that helped to further understand the mode of action of super-dosing phytase by looking at nutrient digestibility, at both the ileal and total tract levels. The second experiment was a large-scale commercial study to further determine whether there are growth performance benefits that may be gained from super-dosing phytase. This study was done to further evaluate the impact of super-dosing phytase at a commercial production level, and give producers further confidence in the results they can expect at their facilities. The combination of the two different experiments provides a deeper understanding of the mechanism of phytase when super-dosed.

However, at the completion of the two experiments, it was found that under the conditions of these experiments, super-dosing phytase provided no improvement in growth performance to the pig or in nutrient digestibility. Therefore, under the conditions of this study, super-dosing phytase is not recommended for the grow-finish phase of production. However, previous research in our laboratory found a definite response in the starter period.

Key Findings

- Superdosing phytase, up to 2,500 FTU/kg, did not improve either apparent ileal or apparent total tract digestibility of dry matter, energy, crude protein or fat.
- Superdosing phytase, up to 2,500 FTU/kg did not improve apparent ileal digestibility of most essential amino acids. However, there was a trend for phytase to improve ileal digestion of threonine, valine and tryptophan up to 1,750 FTU phytase/kg.
- The results of this experiment confirm that only about 92 to 95% of starch in corn is digested in the small intestine. Thus, about 5% of that starch is treated like fiber and is fermented in the large intestine, meaning it is used with less energy efficiency. Interestingly, larger animals digest starch in the small intestine more effectively than smaller pigs.
- The most noticeable impact of superdosed phytase was its breakdown of phytase into the inositol ring; in other words, it removed phosphorus from phytate in increasing amounts as the concentration of phytase increased.

Keywords: Super-dosing phytase, enzymes, phytase, grow-finish, ingredients

Scientific Abstract:

Two experiments were conducted to determine the effect of super-dosing phytase in pig diets on the digestibility of nutrients and growth performance of grow-finish pigs. In the first experiment, 32 growing gilts were fitted with a T-cannula at the distal ileum and randomly allotted to one of 4 dietary treatments at an average initial body weight of 39.7 ± 0.7 kg. In the second experiment (exp. 2), 2200 pigs with an average initial body weight of 36.6 ± 1.04 kg, were split by sex and randomly allotted to 5 dietary treatments. Diets in exp. 1 consisted of a basal diet containing 250 FTU/kg of Quantum Blue 5000G phytase (QB5G) for a release of 0.08% STTD P, and 3 additional levels of phytase: 1000, 1750, 2500 FTU/kg. In exp. 2 there were 5 dietary treatments: a positive control (PC) with added 1.12% SID lysine, 3% added fat, and 250 FTU/kg QB5G; a negative control (NC) with added 1.00% SID lysine, 2.25% added fat, and 250 FTU/kg QB5G phytase. The remaining 3 diets were the NC with a total of 1000, 1750, and 2500 FTU/kg of QB5G phytase for treatments 3, 4, and 5, respectively. In exp. 1, pigs were fed at 3.2 times maintenance and water was provided *ad libitum*. Feces, urine, and ileal digesta were collected at 40, 60 and 80 kg, respectively. In exp. 2 pigs were weighed and feed weigh backs were determined at day 0, day 14, then every 21 days until market. Feed and water were provided *ad libitum*, and carcass data and pig value was recorded at the finish of the trail. Data were analyzed using the PROC MIXED procedure of SAS (9.4) with treatment and collection weight in exp. 1, and treatment and phase in exp. 2 being fixed effects, and weight block as a random effect in both. In exp. 1 there was a statistically significant effect of the interaction between treatment and weight on the AID of DM and GE. For the AID of DM, pigs on the treatment containing 1000 FTU/kg of phytase at 40 kg had lower AID of DM than pigs on the control diet, and the treatments 1750 and 2500 FTU/kg of phytase at 60 kg, and the control diet and treatments containing 1000 FTU/kg and 1750 FTU/kg of phytase in pigs at 80 kg. For the AID of GE, 40 kg pigs on the 1000 FTU/kg treatment had lower AID of GE than pigs on the control diet at 60 kg, and pigs on the control diet and treatments containing 1000 FTU/kg and 1750 FTU/kg at 80 kg. Phytase level did have an impact on total nitrogen retention, with the treatment containing 1000 FTU/kg retaining greater nitrogen than the treatment containing 1750 FTU/kg of phytase. In exp. 2, there were no differences among different super-dosing phytase levels for any growth performance or carcass parameters; however there was a statistically significant difference between the PC and the NC treatments with pigs on the PC treatment performing better than those on the NC treatment. This is in addition to differences seen between the sexes, with barrows having a higher ADFI and ADG and a lower GF. In conclusion, under the conditions of this study, there may be a differing response to super-dosing phytase on the AID of nutrients, but it is dependent on body weight, and there is no growth performance benefit in a commercial setting.

Introduction:

In plant sources, phosphorus is stored as phytic acid, or phytate, where 6 phosphate molecules are bound around a myo-inositol ring. In addition to binding phosphorus in the form of phosphate ions, phytate will also chelate with different cations, including Mg^{2+}, Ca^{2+}, Zn^{2+}, etc (Vitali et al., 2008) making them biologically unavailable to the pig. There has also been evidence that phytate will interact with different positively charged amino acid residues in proteins, making them similarly inaccessible (Yu et al., 2012). By including phytase in pig diets, which is the enzyme that hydrolyzes phytate, the bound phosphate ions are released, increasing phosphorus digestibility, and releasing the different bound cations and protein molecules (Beaulieu et al., 2007; Bikker et al., 2012; Guggenbuhl et al., 2012).

In addition to improving different nutrient digestibilities, research has also demonstrated that there are potential growth performance improvements when super-dosing phytase. Phytase is considered super-dosed when it is included in a diet at levels that exceed what is necessary for full phosphorous release. Walk et al. (2013) observed that super-dosing phytase significantly improved ADG and G:F in nursery pigs. This observation was also seen in grower pigs by Braña and colleagues (2006), who observed an improvement in both ADG and GF in pigs supplemented with super-dosed levels of phytase. However, there is also evidence that there is no improvement gained in performance from super-dosing phytase (I.J. Wellock et al., 2013; Langbein et al., 2013). Therefore the objectives of these experiments were to determine both the mode of action of phytase, through looking at nutrient and energy digestibilities, as well as the impact the enzyme has on growth, carcass, and financial performance in a large-scale commercial setting.

Objectives:

1. To define the response of growing pigs in terms of nutrient availability, to graded super-dose levels of added phytase in the diet.
2. To separate the response to super-dosed phytase between the small intestine, where amino acids and energy sources are used more efficiently, and the large intestine, where amino acids are not made available as such to the pig and where energy sources are used less efficiently due to losses from fermentation.
3. To compare the response in growing pigs at 40 kg, 60 kg and 80 kg.
4. To further test the concept of super-dosing under commercial conditions.
5. To evaluate the net financial impact of using phytase in super doses in commercial production.

Materials and Methods

The protocols for both of the following experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Iowa State University.

Experiment 1

Animals, diets, and experimental design. Thirty-two growing gilts (PIC 337 x C22/C29) in each of 2 replicates (16 pigs per replicate) were selected based on a pre-determined average body weight. Pigs were surgically fitted with a T-cannula in the distal ileum following procedures described by Stein et al. (1998). Following recovery, pigs were weighed (initial BW = 39.7 ± 0.69 kg), blocked by initial body weight, randomly allotted to 1 of 4 dietary treatments, and moved to metabolism crates. Dietary treatments were formulated according to nutrients required by NRC (2012) and were based on a standard corn-soybean meal commercial-type diet (see tables 1 and 2 for ingredient breakdown and nutrient content, respectively). There were 4 dietary treatments, with a basal control diet containing Quantum Blue 5000G Phytase at 250 FTU/kg for a release of 0.08% phosphorous. Three super-dosing treatments were achieved by adding additional phytase at the following levels: 1000, 1750, and 2500 FTU/kg, respectively. Diets also contained chromic oxide at 0.4% (as-fed basis) as an indigestible marker. Diets were fed in 3 dietary phases, coordinating with each collection body weight. Pigs were fed at 3.2 times maintenance and water was available *ad libitum.*

Sample collection. Pigs were given 10 days to adapt to diets. Following this adaption period feces, urine, and ileal digesta were collected, with 3 days of fecal and urine collection, and 3 days of ileal digesta collection.

Feces were collected through fresh grab sampling, and both feces and urine were collected twice daily and stored at -20℃. Ileal digesta was collected over an 8-hour period and stored at -20℃.

Sample processing and data analysis. Prior to analysis, fecal samples were dried in a convection oven at 65ºC, and ileal samples were lyophilized and stored for further processing. Diet, fecal, and ileal samples were ground in a Wiley Mill through a 1-mm screen. Diet, fecal, ileal, and urine samples were analyzed for N (Leco Corporation, St. Joseph, MI) using EDTA (9.56% nitrogen; Leco Corporation, St. Joseph, MI) for calibration. Feed, fecal, and ileal samples were analyzed for DM (Method 930.15; AOAC Int., 2007), and chromium (Method 990.08; AOAC Int., 2006, ICP method). Gross energy was also determined by bomb calorimetry (Parr 6200 calorimeter, Parr Instruments Co., Moline, IL) using benzoic acid as a standard (6,318 kcal GE/kg; Parr Instrument Co., Moline, IL). Diet and ileal digesta samples were also analyzed for total amino acids (Method 994.12; AOAC Int., 2015), free amino acids (Method 999.13; AOAC Int., 2015), tryptophan (ISO 13904:2005 E), and free tryptophan (Method 999.13, AOAC Int., 2015).

For each dietary treatment, the AID of AA and the AID and ATTD were calculated for GE, DM, and N according to the procedures of Oresanya et al (2008). The amounts of dietary components reaching the terminal ileum and excreted in feces were calculated relative to the indigestible marker (Cr_2O_3).

Data were checked for normality using the PROC UNIVARIATE procedures in SAS 9.4 (SAS Inst., Cary, NC). All statistical analyses were performed with the PROC MIXED procedure using repeated measures, with pig as the experimental unit, treatment as a fixed effect, block as a random effect, and pig over body weight as the repeated measure. Differences were considered significant if $P < 0.05$ and trends if $P \geq 0.05$ and $P < 0.10$. Interactions between period and treatment were included in the model.

Experiment 2

Diets, animals, and experimental design. This experiment was conducted at the Hanor Co. Research Facility in White Hall, IL. In a complete barn fill, 2200 pigs were received and sorted into 100 pens and were separated by sex, allowing for 50 pens of barrows and 50 pens of gilts. There were approximately 19 to 24 pigs in each pen, and pigs were blocked by initial body weight (36.6 ± 1.04 kg). Pens were randomly allotted to 1 of 5 dietary treatments within block, and treatment assignment was adjusted to ensure equal body weights across treatments. Pens were weighed on d 0, 14, 35, 56, 77, and 98, the latter being the day of the first cut. Feed intake was recorded in conjunction with pen weights to enable calculation of feed consumption for each pen. Feed and water were both available *ad libitum*.

Pens were fed using the Howeema Feed System, and feed was dropped twice a day as needed, once in the morning and once in the evening. There were 5 treatments: a positive control (PC) with adequate SID lysine, 3% added fat, and 250 FTU/kg Quantum Blue 5000G phytase (QB5G); a negative control (NC) with 11% less SID lysine, 2.25% added fat, and 250 FTU/kg QB5G phytase (see tables 3 and 4 for ingredient breakdown and nutrient content, respectively); and the highest level of super-dosing was the NC diet with 2500 FTU/kg of phytase. The remaining 2 treatments, or treatments 3 (1000 FTU/kg of phytase) and 4 (1750 FTU/kg of phytase), were created by blending the NC treatment and treatment 5, at 67% and 33% respectively, for treatment 3, and the reverse for treatment 4. Treatments were fed in 5 phases, which were coordinated with each weigh day, with the 5th phase starting after the first cut.

Sample analysis and data calculation. Samples from each test diet within phase were collected, homogenized, and ground through a 1-mm screen. Diets were then analyzed for N (Leco Corporation, St. Joseph, MI) using EDTA (9.56% nitrogen; Leco Corporation, St. Joseph, MI) for calibration. In addition to the sample analysis, the following information was calculated from each pen: average daily gain (live weight and carcass basis [overall only]), average daily feed intake, GF (live weight and carcass basis [overall only]), feed cost per pig placed, feed cost per pig sold, return over feed cost per pig and per pig place (overall trial only). Daily nutrient intake was determined for ME, NE, lysine, methionine, TSAA, threonine, tryptophan, calcium, and total phosphorus.

Data were checked for normality using the PROC UNIVARIATE procedures in SAS 9.4 (SAS Inst., Cary, NC). All statistical analyses were performed using PROC MIXED procedure with repeated measures, with pen as the experimental unit, treatment as a fixed effect, and pen over phase as the repeated measure. Contrasts were applied in order to compare the PC diet to the NC diet, and to compare the 3 different levels of phytase to the NC. Differences were considered significant if $P < 0.05$ and trends if $P \geq 0.05$ and $P < 0.10$. Interactions between period and treatment were included in the model.

Results: Experiment 1

<u>Objective:</u>

1. *To define the response of growing pigs in terms of nutrient availability, to graded super-dose levels of added phytase in the diet.*

2. *To separate the response to super-dosed phytase between the small intestine, where amino acids and energy sources are used more efficiently, and the large intestine, where amino acids are not made available as such to the pig and where energy sources are used less efficiently due to losses from fermentation.*

3. *To compare the response in growing pigs at 40 kg, 60 kg and 80 kg.*

All results for experiment 1 are summarized in tables 5 and 6. The main effect of phytase level was not statistically significant for apparent ileal digestibility (AID) of DM, GE, N, or amino acids. There was, however, a statistically significant interaction between phytase level and body weight on the AID of both DM ($P < 0.01$), and GE ($P < 0.01$). At 40 kg, AID of DM for the treatment containing 1000 FTU/kg of phytase was 5% less than the control and the treatments containing 1750 and 2500 FTU/kg of phytase at 60 kg, and 7% less than the control and the treatments containing 1000, and 1750 FTU/kg at 80 kg. The control diet containing 250 FTU/kg of phytase was also 5% less than the control and the 1000 and 1750 FTU/kg treatments at 80 kg. For the AID of GE, at 40 kg, 1000 FTU/kg treatment was 5% less than the control diet at 60 kg, and 6% less than the control diet and treatments containing 1000 and 1750 FTU/kg at 80 kg.

There were no interactions for the ATTD of DM ($P = 0.11$), GE ($P = 0.15$), or N ($P = 0.24$). There were also no statistically significant differences among treatments on the ATTD of DM ($P = 0.15$), GE ($P = 0.13$), or N ($P = 0.62$). There was, however, a statistically significant difference among body weights on the ATTD of DM with pigs at 60 kg having a lower ATTD of DM than at 80 kg ($P = 0.04$), as well as ATTD of N with pigs at 40 kg having a lower ATTD of N than pigs at 60 kg ($P = 0.01$). There were also statistically significant differences among body weight on the ATTD of GE ($P = 0.04$), however this difference is no longer evident among the means when the Tukey-Kramer adjustment is made, indicating a model effect.

There was no effect of treatment on the total nitrogen excretion ($P = 0.63$), but there was an effect of treatment on total nitrogen retention ($P = 0.03$), with pigs on the treatment containing 1000 FTU/kg of phytase retaining more nitrogen than pigs on the treatment containing 1750 FTU/kg of phytase. There was a linear effect of body weight ($P < 0.01$) on both nitrogen retention and excretion, with pigs retaining and excreting more nitrogen with increasing body weights.

<u>Experiment 2</u>

4. *To further test the concept of super-dosing under commercial conditions.*

5. *To evaluate the net financial impact of using phytase in super doses in commercial production.*

All growth performance and carcass results for experiment 2 are summarized in table 7. This trial finished with pigs having an average final body weight of 123.6 kg for barrows, and 120.6 kg for gilts and an overall ADG of 1.06 kg/d for barrows and 0.89 kg/d for gilts. Treatment was significant for final body weight, but this was due to the difference between the PC and the treatments containing the NC as the basal diet ($P < 0.01$) versus differing phytase levels ($P = 0.29$). Treatment affected overall ADG ($P < 0.01$), and GF ($P < 0.01$), however it did not affect ADFI ($P = 0.38$). However, after further analysis, the differences seen among the treatments were due to the difference between the PC and the remaining 4 treatments containing the NC for ADG ($P < 0.01$) and GF ($P < 0.01$), rather than the different super-dosing phytase levels ($P = 0.38$, $P = 0.08$, respectively).

Within each phase, ADG was affected by treatment ($P < 0.01$), sex ($P < 0.01$), and phase ($P < 0.01$), and there was an interaction between sex and phase ($P < 0.01$). Also, similar to the total ADG, upon further analysis, the differences for ADG among phases that were seen across treatments were due to the differences between the PC and NC diets, rather than the 4 different levels of phytase. There was a quadratic affect of phase ($P < 0.01$), with the ADG increasing from phase 1 to phase 3, and decreasing from phase 3 to phase 5.

There was no effect of treatment on ADFI; however there was an interaction between phase and sex ($P < 0.01$), with barrows consuming more than gilts, and pigs in phase 5 consuming more than pigs in phase 1. Treatment also had an effect on GF between each phase change ($P < 0.01$) but it was again due to the effect of the PC versus the NC diets ($P < 0.01$), with the PC having improved feed efficiency over the treatments containing the NC. There was also an interaction between phase and sex, with gilts, on average, being more efficient than barrows, but this varies depending on body weight.

There was a statistically significant difference among treatments on energy conversion ($P < 0.01$) as well as a statistically significant interaction between treatment and sex ($P < 0.01$). Gilts on the PC were the most efficient at converting energy to gain, relative to other treatments and sexes.

Treatment and sex both impacted average market weight ($P < 0.01$, $P < 0.01$, respectively) and hot carcass weight ($P < 0.01$, $P = 0.02$, respectively), but there was no interaction between treatment and sex for either. The differences in treatment can again be explained by the PC versus the treatments containing the NC, with the PC finishing with a higher average market and hot carcass weight than the other 4 treatments. Barrows also finished with a higher average market and hot carcass weight than gilts.

There was a statistically significant impact of both treatment and sex on carcass gain ($P < 0.01$, and $P < 0.01$, respectively). The former is due to the pigs on the PC treatment versus the treatments containing the NC ($P < 0.01$), and the latter, with barrows having, on average, a higher carcass gain than gilts. This same result occurred in carcass ADG, with there being a statistically significant difference among treatments, but it being due to the PC versus the treatments containing the NC ($P < 0.01$), rather than the phytase levels ($P = 0.26$), and barrows having a higher carcass ADG than gilts ($P < 0.01$). There was a statistically significant effect of treatment and sex on carcass GF ($P < 0.01$), as well as an interaction of treatment and sex ($P < 0.01$). Gilts on the PC treatment were more efficient than gilts on the diets containing NC, and were more efficient than barrows on all treatments. Both treatment and sex were statistically significant on carcass energy conversion ($P < 0.01$), and the interaction was also statistically significant ($P < 0.01$). Gilts on the PC diet were more efficient at utilizing energy for carcass gain than barrows, however barrows on all treatments were more efficient at utilizing energy for carcass gain than gilts on the NC and 3 super-dosing treatments.

There were also no statistically significant differences among treatments ($P = 0.67$) or between sexes ($P = 0.33$) on the ability to achieve a full value product, with the average being 96.45% for barrows and 97.32% for gilts.

The economic analysis is summarized in table 8. The PC had higher feed costs per pig than the NC or the 3 super-dosed treatments. However, the pigs on the PC also had a higher final weight and therefore provided greater income per pig, compared to the NC or the 3 super-dosed treatments. This allowed greater return over feed cost per pig. Among the super-dosed treatments, the treatment containing the highest level of phytase, 2500 FTU/kg, was the most expensive, and although it had the highest income per pig place, it had the lowest income per pig, due to the pigs on this treatment taking longer to reach market weight causing fewer turns per year. The treatment containing 1750 FTU/kg of phytase had the lowest average feed cost per pig, and the highest income per pig place, and of the 3 super-dosed treatments the highest return over feed cost per pig. The treatment containing 1750 FTU/kg also had the highest return over feed cost per pig place across all treatments, as the performance of the pigs on this treatment will allow for greater turns per year and thus greater return per pig place.

Discussion

Under the conditions of these experiments, super-dosing phytase did impact the ileal digestibility of DM and GE, but this was dependent upon body weight. However, the impact that super-dosing had on ileal digestibility was not reflected on a total tract basis, with there being no effect of super-dosing on the ATTD of DM or GE. It was also seen that pigs consuming a properly balanced diet performed better than those on a diet limiting in energy and amino acids, which is expected. It was also observed that there was no improvement in growth performance when phytase was super-dosed in grow-finish pigs. This is contradictory to data presented by Walk et al. (2013) who found super-dosing phytase to cause improvements in ADG and GF. This may be explained by the stage of growth that the pigs were in, as Walk et al. looked at nursery pig performance rather than grow-finish pigs. Unpublished data by Holloway et al. (2014) supports the findings of Walk et al. having observed growth performance improvements in nursery pigs.

However, Braña and company (2006) did observe performance improvements in grow-finish pigs that were super-dosed, which is contradictory to the results of these experiments. But, in that experiment, the basal diet was limiting in phosphorus, which also created an unfavorable calcium:available phosphorus ratio. This may mean the performance benefits gained in that study were impacted by the fact that the diets were initially deficient in phosphorus, or had an undesirable calcium:available phosphorus ratio. This is in addition to having a much higher level of super-dosing phytase at 10,000 FTU/kg compared to 2500 FTU/kg being the highest level in the 2 experiments summarized in this report. However, the conclusion can be drawn that the performance benefits observed in these two experiments, were not due to additional improvement in energy, dry matter, or amino acid digestibility.

In conclusion, under the conditions of these 2 experiments, the digestibility of nutrients was affected by both the location of digestion, and the body weight of the pig, and there was no growth performance benefit. Furthermore, no financial benefit accrued from the use of super dosed phytase.

Table 1. Ingredient composition of diets: experiment 1 (as-fed basis).

	Body weights*		
Ingredient, %	40	60	80
Corn	67.91	73.84	78.47
SBM	28.50	22.75	18.30
Soybean oil	0.50	0.50	0.50
Vit Premix	0.200	0.200	0.200
Mineral Premix	0.200	0.200	0.200
Monocal	0.400	0.310	0.260
Limestone	1.240	1.150	1.030
Salt	0.500	0.500	0.500
Lysine, HCL	0.135	0.150	0.135
Chromic Oxide	0.400	0.400	0.400
Methionine	0.015	-	-
Quantum Blue 5G, Phytase	0.005	0.005	0.005

* Phytase was added at the expense of corn with each super-dosing treatment (1000, 1750, 2500 FTU/kg of phytase).

Table 2. Nutrient composition of diets in experiment 1 (as-fed basis)

	Body weights*		
Nutrient	40	60	80
GE	3.85	3.78	3.80
Lysine, %	0.98	0.85	0.73
Threonine, %	0.60	0.52	0.46
Tryptophan, %	0.20	0.17	0.15
SAA, %	0.54	0.49	0.45
STTD P,%	0.30	0.27	0.39
Ca, %	0.66	0.59	0.52

*Based on including 250 FTU/kg phytase for 0.08% P release.

Table 3. Ingredient composition diets experiment 2 (as-fed basis).

Phase	1		2		3		4		5	
Ingredient, %	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
Corn	56.70	57.69	63.02	63.94	67.44	68.37	67.44	68.37	68.73	69.62
Wheat Midds	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
SBM	22.50	22.50	16.25	16.25	15.00	15.00	12.00	12.00	10.75	10.75
CWG	3.00	2.25	3.00	2.25	3.00	2.25	3.00	2.25	3.00	2.25
Vit Premix	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Monocal	0.580	0.580	0.560	0.560	0.570	0.560	0.580	0.580	0.590	0.590
Limestone	0.730	0.730	0.760	0.760	0.770	0.770	0.790	0.790	0.800	0.800
Salt	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
L-Lysine	0.300	0.100	0.280	0.110	0.230	0.070	0.150	0.020	0.110	-
L-Threonine	0.090	-	0.080	-	0.060	-	0.040	-	0.030	-
L-Tryptophan	0.030	-	0.030	-	0.020	-	0.020	-	-	-
DL-Methionine	0.015	-	0.050	-	0.020	-	-	-	-	-
Quantum Blue 5G, Phytase	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005

*Super-dosing levels treatments were achieved by including Quantum Blue 5000G at 0.05% for the highest level of phytase (2500 FTU/kg) to the NC, and blending the high diet with the low diet at 33% and 67% respectively for the diet with 1000 FTU/kg phytase, and the reverse for the diet containing 1750 FTU/kg phytase.

Table 4. Nutrient contents of diets experiment 2 (as fed basis)

Phase	1		2		3		4		5	
Ingredient, %	PC	NC	PC	NC	PC	NC	PC	NC	PC	NC
ME	3.40	3.36	3.40	3.36	3.39	3.36	3.39	3.36	3.39	3.35
NE	2.57	2.53	2.40	2.56	2.60	2.57	2.62	2.58	2.62	2.59
Calcium	0.52	0.52	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total P	0.56	0.57	0.53	0.53	0.53	0.53	0.52	0.52	0.52	0.52
SID AA										
Lysine	1.05	0.89	0.87	0.74	0.80	0.68	0.66	0.56	0.60	0.51
Methionine	0.32	0.24	0.26	0.21	0.22	0.21	0.19	0.19	0.19	0.19
TSAA	0.59	0.51	0.49	0.45	0.45	0.44	0.41	0.41	0.39	0.40
Threonine	0.64	0.55	0.54	0.47	0.51	0.45	0.44	0.41	0.42	0.39
Tryptophan	0.21	0.19	0.18	0.15	0.16	0.15	0.14	0.13	0.12	0.12

Table 5. AID and ATTD of DM, GE, N, and N retention and excretion

BW	**40**				**60**				**80**				
PHY	**250***	**1000**	**1750**	**2500**	**250***	**1000**	**1750**	**2500**	**250***	**1000**	**1750**	**2500**	***P***[♦]
AID													
DM	73.07^{bc}	71.13^{c}	73.39^{abc}	74.77^{abc}	76.87^{ab}	74.50^{abc}	76.78^{ab}	76.43^{ab}	77.81^{a}	78.46^{a}	79.25^{a}	74.86^{abc}	<0.001
GE	74.89^{ab}	72.44^{b}	76.24^{ab}	75.93^{ab}	77.66^{a}	75.33^{ab}	77.28^{ab}	76.76^{ab}	78.28^{a}	78.65^{a}	79.57^{a}	75.10^{ab}	<0.001
N	80.36	80.45	82.07	82.24	81.98	81.72	82.81	81.72	81.63	82.08	82.94	78.81	0.09
ATTD													
DM	90.47	88.97	90.36	90.86	89.44	89.33	90.31	90.58	90.51	90.62	90.84	90.31	0.11
GE	89.56	87.64	89.22	89.65	88.36	87.87	88.90	89.11	89.22	89.14	89.56	88.84	0.15
N	90.25	89.32	90.45	91.10	88.94	88.15	89.58	89.66	89.24	89.76	89.62	88.45	0.24

[a,b,c] Means within a row lacking a common superscript are different ($P < 0.05$)

*Control diet

Table 6: Apparent Ileal Digestion of AA as affected by superdosed phytase, %

BW	40				60				80				
PHY	**250***	**1000**	**1750**	**2500**	**250***	**1000**	**1750**	**2500**	**250***	**1000**	**1750**	**2500**	***P***
AID													
Indispensible AA													
Arg	90.05	90.26	91.12	90.71	90.5	90.37	90.96	90.29	89.82	89.87	90.07	88.4	0.41
Ile	84.33	84.44	87.17	85.37	83.96	84.81	85.93	84.58	84.83	84.53	85.58	82.19	0.16
His	85.271	84.12	86.70	84.577	85.795	83.98	86.66	85.36	85.50	84.38	85.33	82.49	0.21
Lue†	85.11	85.14	87.70	86.19	86.33	86.02	86.95	86.00	86.94	87.17	87.49	84.41	0.04
Lys	86.15	86.63	87.31	87.19	86.92	86.50	87.02	87.20	85.70	85.63	86.01	83.44	0.17
Met	87.57	88.10	89.84	88.41	87.93	87.98	88.82	87.79	87.88	88.61	88.70	86.00	0.19
Phe	84.11	84.28	36.38	85.14	85.11	84.53	85.61	85.19	84.78	85.47	85.86	83.19	0.11
Thr	77.74	78.20	80.78	78.99	78.94	77.83	79.85	78.70	78.23	78.81	79.23	75.10	0.08
Tyr	85.06	85.93	86.95	86.69	85.13	86.57	86.62	86.83	86.48	86.98	87.01	83.92	0.07
Val	80.04	80.31	83.32	81.27	80.58	80.00	82.65	81.34	80.79	81.02	81.31	78.04	0.06
Trp	78.85	80.02	82.48	81.38	79.43	79.38	82.65	80.18	78.75	79.55	80.62	76.65	0.49
Dispensable AA													
Asp A	83.40	83.52	84.97	84.12	84.37	83.63	84.98	84.24	84.09	84.55	84.82	82.17	0.29
Cys	70.93	68.60	71.95	69.86	74.18	71.62	74.93	73.71	76.32	76.79	76.56	71.92	0.69
Glu A	83.64	83.93	86.20	84.54	88.02	86.38	88.34	86.56	88.34	88.76	88.47	87.32	0.44
Gly	73.60	71.65	76.90	72.90	75.00	71.61	73.66	72.87	72.85	71.95	72.14	68.15	0.43
Ala	80.84	81.37	83.57	82.45	82.27	81.99	82.6	81.89	82.55	83.24	83.54	79.92	0.14
SAA	79.48	78.65	81.11	79.48	81.05	79.81	81.77	80.75	82.00	82.56	82.55	78.86	0.53
Pro	83.22	83.44	85.36	83.22	83.10	83.73	83.05	82.09	83.22	83.94	83.42	80.90	0.75
Ser	82.35	83.21	85.24	83.52	83.64	82.95	84.39	83.42	83.23	83.95	84.16	81.04	0.07

*Control diet

† Model effect

Table 7. Impact of superdosed phytase on growth performanc3from 35 to 125 kg

	Barrows					Gilts					Linear *P*-Values	
	PC*	NC*	1000	1750	2500	PC*	NC*	1000	1750	2500	1-2	2-5
BW, kg												
Initial[1]	36.09	35.79	35.78	35.84	35.79	37.45	37.34	37.35	37.17	37.20		
Final	126.32	121.53	123.12	123.82	123.39	124.20	118.70	120.59	118.96	120.70	<0.01	0.29
ADG, kg/d											<0.01	0.34
14	0.96	0.91	0.92	0.90	0.93	0.89	0.83	0.83	0.82	0.82		
35	1.13	1.11	1.11	1.09	1.11	1.00	0.93	0.92	0.94	0.93		
56	1.19	1.14	1.16	1.14	1.15	0.99	0.95	0.95	0.94	1.01		
77	1.02	0.96	1.00	0.99	1.00	0.86	0.79	0.83	0.79	0.76		
98	0.90	0.84	0.95	0.95	0.84	0.81	0.70	0.77	0.83	0.77		
1-98	1.08	1.04	1.06	1.04	1.06	0.94	0.88	0.89	0.88	0.89	<0.001	0.38
ADFI, kg/d											0.31	0.82
14	2.34	2.30	2.33	2.25	2.28	2.02	2.05	2.03	2.01	2.03		
35	2.93	2.92	2.89	2.84	2.90	2.54	2.53	2.49	2.50	2.53		
56	3.40	3.34	3.42	3.30	3.33	2.81	2.82	2.88	2.83	2.85		
77	3.65	3.59	3.72	3.59	3.63	2.98	3.00	2.95	3.01	3.02		
98	4.07	3.88	3.96	3.84	3.97	2.96	2.96	3.01	2.96	2.96		
1-98	3.12	3.08	3.14	3.04	3.08	2.61	2.63	2.62	2.61	2.64	0.58	0.66
Gain:feed											<0.01	0.10
14	0.41	0.40	0.40	0.40	0.41	0.44	0.41	0.41	0.41	0.41		
35	0.39	0.38	0.39	0.39	0.38	0.39	0.37	0.37	0.38	0.37		
56	0.34	0.34	0.34	0.35	0.35	0.35	0.34	0.33	0.33	0.35		
77	0.28	0.27	0.27	0.27	0.28	0.29	0.26	0.28	0.27	0.25		
98	0.22	0.22	0.24	0.25	0.21	0.27	0.24	0.26	0.28	0.26		
1-98	0.35	0.34	0.34	0.34	0.34	0.36	0.34	0.34	0.34	0.34	<0.01	0.08
Energy Conversion											0.01	0.47
14	8.33	8.47	8.50	8.37	8.27	7.75	8.27	8.25	8.24	8.30		

35	8.80	8.86	8.73	8.73	8.78	8.63	9.18	9.09	8.92	9.16		
56	9.68	9.86	9.89	9.69	9.74	9.58	9.95	10.33	10.07	9.57		
77	12.19	12.62	12.50	12.24	12.24	11.73	12.73	12.07	12.75	13.81		
98	15.51	15.65	14.72	13.96	15.93	12.88	14.59	13.53	13.28	13.30		
1-98	9.81	9.95	9.93	9.80	9.77	9.45	10.03	9.93	9.98	10.01	<0.01	0.08
Market weight, kg	125.59	121.69	123.17	123.20	123.95	123.85	118.76	119.40	119.51	125.59	<0.01	0.15
Hot Carcass weight, kg	93.57	90.52	92.00	91.68	91.77	92.36	87.51	88.05	87.95	88.02	<0.01	0.03
Carcass Gain, kg	66.86	64.03	65.52	65.16	65.28	64.66	59.88	60.41	60.44	60.49	<0.01	0.12
Carcass ADG	0.79	0.76	0.78	0.77	0.77	0.68	0.63	0.64	0.64	0.64	<0.01	0.28
Carcass GF	0.26	0.26	0.26	0.26	0.26	0.27	0.25	0.26	0.26	0.25	<0.01	0.08
Carcass Energy Conversion	13.39	13.63	13.70	13.45	13.53	13.00	14.00	14.00	13.99	14.13	<0.01	0.79
Full Value Pig, %	96.71	96.14	97.71	96.34	95.35	95.99	98.66	97.77	96.86	97.35	0.26	0.38

*Contain 250 FTU/kg phytase

[1]There were no statistically significant differences among treatments on initial body weight, $P = 0.33$.

Table 8. Economic analysis[1]

Treatment[2]	**PC**	**NC**	**1,000**	**1,750**	**2,500**
Days to 129.28 kg[3]	92.36	97.17	96.16	97.17	96.16
Turns per year[4]	3.22	3.09	3.12	3.09	3.12
Income per pig, $[5]	$184.87	$184.87	$184.87	$184.87	$184.87
Income per pig place, $	$595.27	$571.04	$575.92	$571.04	$575.92
Average feed cost per pig, $	$64.59	$64.82	$64.74	$63.94	$64.68
Average feed cost per pig place, $	$207.98	$200.22	$201.69	$197.50	$201.50
Return over feed cost per pig, $	$120.28	$120.05	$120.13	$120.93	$120.19
Return over feed cost per pig place, $	$387.29	$370.82	$374.23	$373.54	$374.42

[1]Costs based on average market pricing in May 2015
[2]PC: positive control, NC: negative control
[3]Based on a start weight of 36 kg
[4]Based on treatment total ADG, and includes 21 days to account for barn cleaning and miscellaneous down time
[5]Based on $65/cwt