

The effect of supplemental phytase on iron bioavailability to weanling pigs in a wheat-based diet

Honors Thesis

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Abstract

Two experiments were conducted to investigate the effect of supplemental dietary phytase on intrinsic Fe bioavailability in a wheat-based diet fed to anemic weanling pigs. Previous work has shown that supplementing phytase in corn-soybean based diets improves iron bioavailability in the pig model. In Experiment 1, pigs were either fed an iron deficient basal diet or the basal diet supplemented with 2,000 phytase U/kg of feed. In this experiment, weanling pigs had access to a commercial creep feed prior to being fed the experimental diets, and the pigs were initially less anemic than pigs in Experiment 2. Supplemental phytase had no apparent effect on intrinsic iron bioavailability in Experiment 1. In Experiment 2 pigs were fed an iron deficient creep feed and weaning diet prior to being fed one of three experimental diets. In Experiment 2, pigs fed the iron deficient basal diet had lower growth performance, packed cell volume, and blood hemoglobin concentrations than pigs fed the basal diet supplemented with 2,500 phytase U/kg of feed, or the basal diet supplemented with inorganic iron at 40 mg/kg of feed. Phytase supplementation in a wheat-based diet appears to improve intrinsic iron bioavailability, and appears to correct iron deficiency as effectively as supplementing a source of inorganic iron in swine diets. These results highlight the necessity for using sensitive models when investigating nutrient bioavailability. The ability for phytase supplementation to provide an alternative means for preventing and correcting iron deficiency warrants further research.

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Introduction

The World Health Organization (2009) lists iron deficiency as the most common and widespread nutritional disorder in the world. An estimated two billion people are anemic world wide, and it is estimated that 50% of the cases result from iron deficiency (WHO, 2004). With so many people affected, methods for improving iron nutrition on a long term and wide scale basis are needed. One method for improving iron nutrition would be by improving iron bioavailability in food products commonly consumed by humans.

According to Hurrell (1997), iron bioavailability is the amount of iron in the diet that is absorbed and used for normal metabolism. There are many factors that can affect iron absorption; proteins, calcium, polyphenols and phytate can all reduce iron bioavailability (Hurrell, 1997). To overcome the antagonistic effects that these compounds have on iron bioavailability, researchers have investigated how supplementing diets with various ingredients may improve iron bioavailability. One such supplement that has been investigated is phytase.

Phytate is the primary form of phosphorous storage in cereals, and is indigestible by simple stomached animals (Kies *et al.*, 2006). The negative charge on phytate also binds cationic metals, including iron, making them unavailable for digestion (Kies *et al.*, 2006). By cleaving the ortho-phosphate groups in phytate, the phytase enzyme not only releases phosphorous making it available for absorption, but it also frees phytate-bound cationic metals (Kies *et al.*, 2005 and 2006).

Several studies have shown that supplementing animal diets with phytase improves mineral bioavailability (Simons *et al.*, 1990; Lei *et al.*, 1993; Adeola, 1995;

Adeola *et al.*, 1995; Stahl *et al.*, 1999; Augspurger and Baker, 2004; Shelton *et al.*, 2005). Stahl *et al.* (1999) found that supplementing phytase in a corn-soy based diet improved hemoglobin synthesis in young pigs. They concluded that the phytase supplemented in the diets was able to degrade the phytate in the diets, thereby releasing phytate-bound iron that would otherwise not be absorbable. In an *in vitro* study conducted by Porres *et al.* (2001) they found that phytase improved iron dialyzability in whole-wheat bread.

The appropriateness of using pigs as a model for human nutrition has been reviewed by Miller and Ullrey (1987), who believe that the pig is one of the best models to study human nutrition issues. Many researches have investigated the effect of phytase supplementation on nutrient availability in pigs (Lei *et al.*, 1993; Adeola, 1995; Adeola *et al.*, 1995; Stahl *et al.*, 1999; Kies *et al.*, 2005; Shelton *et al.*, 2005; Willaims *et al.*, 2005; Veum *et al.*, 2006). These previous studies also used corn-soybean based diets. With the ultimate goal of applying nutrient research to humans, phytase supplementation should be tested for food products that are commonly consumed by humans.

Staple crops are consumed in high quantities by humans and not normally considered an important source of trace minerals in the diet (Gregorio, 2002). For this reason, we investigated the effect of phytase supplementation on iron bioavailability to weanling pigs in a wheat-based diet. Our objective was to determine if supplementing phytase in a wheat-based diet could improve intrinsic iron bioavailability to anemic weanling pigs, using similar experimental parameters as in previous studies that used corn-soybean based diets.

Review of the Literature

Phytate and Iron

Phytate can account for up to 5% of the weight in cereal grains and legumes (Maga, 1982). It accounts for 0.62–1.35% of dry weight in wheat, 0.84–1.01% of dry weight in oats, and 0.97–1.08% of dry weight in barley (Maga, 1982). More than 80% of the total phosphorous in corn is in the form of phytate phosphorous (O'Dell *et al.*, 1972). It has long been recognized for its antagonistic effect on nutrient availability. In particular, the effect of phytate on iron absorption has been under investigation since the 1940s (Sharpe *et al.*, 1950). As pointed out by Cowan *et al.* (1966), many of the early studies in this area produced inconsistent or controversial results. For example, Sharpe *et al.* (1950) found no correlation between the content of phytate in foods and a reduction in iron absorption in humans. But Hussain and Patwardhan (1959) found that phytate did reduce iron absorption in human diets.

Several studies conducted with rats, produced no strong evidence to support the notion that phytate had an inhibitory affect on iron absorption. Cowan *et al.* (1966) demonstrated that replacing 45 or 75% of the phosphorous in rat diets with phytate phosphorous did not significantly influence Hb repletion, when compared to rats fed the control diet that contained no phytate. Hunter (1981) found that supplementing sodium phytate into the diets of iron-adequate and iron-deficient rats had no differential effect on iron bioavailability. Morris and Ellis (1976) demonstrated that monoferric phytate from wheat bran has a high biological availability to the rat.

A couple of considerations must be kept in mind with these rat studies. The first consideration is that many of these studies were concerned with supplementing phytate

into the diet, and not directly investigating the effects of intrinsic phytate on iron bioavailability. The second consideration is that the rat's physiology may not be optimal for studying the effects of phytate on iron bioavailability in general, and how it relates to human nutrition. Morris and Ellis (1976) point out that the apparent non-inhibitory effect of phytate on iron bioavailability in the rat may be attributed to the presence of an intestinal phytase. Furthermore, in a study conducted with mice, Graf and Eaton (1984) conceded that their results demonstrating that phytate had no effect on iron absorption should not be extrapolated directly to situations in animals or humans where complex diets are consumed.

Despite studies that have used rats to investigate the role phytate plays in iron bioavailability, more recent research clearly demonstrates phytate's antagonistic role in iron nutrition. Hurrell (1997) cites phytate as being the major factor contributing to low iron bioavailability in cereal grains. In a study with humans (Hurrell *et al.*, 1992), phytate in a soy protein isolate was reduced to <0.01 mg of phytate/g of protein isolate. Reducing phytate to this level increased iron absorption four- to fivefold (Hurrell *et al.*, 1992). Hallberg *et al.* (1987) investigated the effects of bran phytate on iron absorption in humans. They found that they could restore the inhibition on iron absorption by reconstituting phytate level in the bran. They concluded that phytates present in the bran were the main inhibitor of iron absorption. In another study using humans, Brune *et al.* (1992) tested the effects of phytate and fiber content on iron absorption. They found that reducing phytate increased the bioavailability of iron despite increasing the levels of fiber content. From this they concluded that the inhibitory effect of bran on iron bioavailability was a result of phytate content and not fiber.

The research discussed so far has mostly only investigated the effect of phytate on iron bioavailability in isolation. While this has produced some seemingly inconsistent and controversial results, the next section discusses research that has involved phytase. This will further highlight that phytate inhibits nutrient availability in foodstuffs.

Phytase

Phytase is an enzyme that catalyzes the breakdown of phytate through hydrolysis (Reddy *et al.*, 1989). Numerous studies have been undertaken to investigate the role of phytase and its effect on nutrient availability. *In vitro* studies have shown that phytase can improve nutrient bioavailability. Porres *et al.* (2001) found that they could increase iron dialyzability of whole wheat bread by 15-fold when the dough was supplemented with phytase and citric acid. Sandberg and Svanberg (1991) were able to increase iron solubility simulated under physiological conditions from 3 to 53% in wheat bran, when endogenous phytate was entirely hydrolyzed by phytase.

An experiment performed by Sandberg and Andersson (1988) was instrumental in demonstrating the digestibility of phytate by humans. In their experiment, subjects were either fed wheat bran that was phytase-deactivated (phytase activity was removed by sending the bran through an enzyme deactivation steamer) or wheat bran that was untreated. For subjects that had consumed the phytase-deactivated wheat bran, the researchers recovered 95% of the ingested phytate; whereas 40% of the ingested phytate was recovered from subjects that consumed the untreated wheat bran. From this they concluded that iron precipitation methods, which indirectly measure phytate content based on the determination of the residual iron in solution after the precipitation of ferric

phytate from a known concentration of ferric salt in acid solution (Maga, 1982), were not appropriate.

The Pig as a Model For Iron Nutrition

There is a significant amount of literature concerning iron nutrition of not only humans and pigs, but how they relate to one another as well. As one of several examples, Bothwell (1995) outlined the mechanism of iron regulation in humans, and how the body's iron balance can be affected by iron deficiency. A comparable amount of knowledge exists for iron regulation and pigs (Miller *et al.*, 1961; Furugouri, 1971 and 1972).

In a review of the appropriateness of pigs as a model for human nutrition, Miller and Ullrey (1987) discussed the similarities and differences between humans and pigs with respect to iron metabolism. They pointed out that the pig serves as an excellent model for iron deficiency because anemia develops rapidly, and they are sensitive to factors that influence iron metabolism and iron bioavailability. They concluded by noting that the similarity between humans and pigs is so great, that the pig is one of the best models to study human nutrition issues.

Pigs have served as a useful model to study the effects of supplemental phytase on nutrient availability. Shelton *et al.* (2005) investigated the general effects of phytase supplementation on growth performance and bone mineral content of pigs. Pigs that were fed diets lacking a trace mineral premix (TMP) suffered from poor growth performance and decreased bone mineral content. When pigs were fed the same TMP-free diet, but phytase was supplemented, it prevented the decrease in growth performance and had variable effects on tissue mineral concentrations. Veum *et al.* (2006) also

reported a significant increase in growth performance for pigs fed diets with varying amounts of supplemental phytase.

Supplementing with phytase into swine diets has also proven effective in improving zinc bioavailability (Lei *et al.*, 1993; Adeola *et al.*, 1995). Both Lei *et al.* (1993) and Adeola *et al.* (1995) demonstrated that supplementing with phytase increased the bioavailability of intrinsic dietary zinc. However, zinc bioavailability was not increased when both phytase and zinc were supplemented in the diet. Improving intrinsic dietary mineral bioavailability with supplemental phytase was also shown for iron in three experiments performed by Stahl *et al.* (1999). In their first experiment (*in vitro*), they investigate what effect phytase had on dietary phytate-bound iron. They found that iron concentrations increased when phytase was supplemented at 1,200 U/kg. In their second and third experiments using pigs, they showed that supplementing phytase into diets increased hemoglobin repletion and packed cell volume over the unsupplemented diet.

Phytase Studies Using Pigs and Wheat-Based Diets

The majority of the aforementioned research involving pigs and phytase has been done using corn-soybean based diets. There has been much less work done using wheat-based experimental diets. However, the studies that have been conducted using wheat-based diets have shown that phytase has a positive effect on nutrient availability. Moehn *et al.* (2007) found that supplemental phytase in a wheat-based diet increased daily gain and dietary metabolizable energy. Additionally, three separate studies have confirmed that phytase added to a wheat-based diet can improve dietary phosphorous utilizations by pigs (Selle *et al.*, 2003; Liao *et al.*, 2006; Woyengo *et al.*, 2008).

Material and Methods

Inulin and Phytase

OptiPhos® (Phytex, LLC., Sheridan, IN) was used as the source of supplemental phytase in Experiments 1 and 2.

Animals, Management

Cornell University's Institutional Animal Care and Use Committee approved all experimental protocols. Experiments were conducted at Cornell's Swine Research Farm. Yorkshire × Hampshire × Landrace weanling pigs were randomly selected from the Cornell University Swine Farm for both experiments. At birth, all pigs received only half (50 mg) of a normal iron dose injection. In Experiment 1 all pigs were individually housed in concrete floor pens measuring 0.88 m x 2.35 m. In Experiment 2 all pigs were group housed according to treatment group in slat floor pens. Temperature and lighting were controlled throughout both experiments. Pigs had free access to water and were fed ad libitum. Feed intake was monitored in both experiments to measure growth performance. General health was also monitored daily.

Experimental Design, Experiment 1

Pigs were allotted into treatment groups based on body weight (BW), sex, and litter. A total of 16 weanling pigs (mean BW = 11.93 kg) were allotted into two groups (n = 8). Each group was randomly assigned to one of the experimental diets (see Appendix for diet composition). Diet 1 (Table 1) was the basal diet (BD). The BD was made according to nutrient requirements for 5 to 10 kg growing pigs (NRC, 1998), however, an inorganic iron source was not included in the trace mineral premix. Diet 2 was the BD supplemented with 2,000 phytase U/kg of feed. Before beginning the experiment, all pigs

were fed the BD for a transition period of 10 days. All diets were made from the same sources of wheat, soy bean meal, vitamins, and minerals.

Experimental Design, Experiment 2

Pigs were allotted into treatment groups based on body weight (BW), sex, litter and initial blood Hb concentration. 24 weanling pigs (mean BW = 9.21 kg) were allotted into three groups (n = 8). Each group was randomly assigned to one of four diets (see Appendix for diet composition). Diet 1 (Table 2) was the basal diet (BD). The BD was made according to nutrient requirements for 5 to 10 kg growing pigs (NRC, 1998), however, an inorganic iron source was not included in the trace mineral premix. Diet 2 was the BD with inorganic iron included in the trace mineral premix. Diet 3 was the BD supplemented with 2,500 phytase U/kg of feed. Before beginning the experiment, all pigs were fed the BD as a creep feed through weaning until the start of the experiment. All diets were made from the same sources of wheat, soybean meal, corn, vitamins, and minerals.

Sample Collection

Measurements and samples were collected every two weeks for six weeks from the start of the each experiment (Experiment 1: at weeks 0, 2, 4 and 6; Experiment 2: at weeks 0, 2, and 4). Pigs were fasted for 8 hours before samples were collected. After fasting, each pig was weighed, and a blood sample was taken from the anterior vena cava using a 20 gauge needle and a heparinized vacutainer collection tube. All blood samples were kept on ice until analysis was done in the lab on the same day.

In both experiments, samples of each diet were also taken at the time of mixing for future analysis of mineral content and phytase activity (results not included in this thesis).

Sample Analysis

After all samples had been collected they were returned to the lab for analyses of blood Hb concentration and packed cell volume (PCV). A cyanomethemoglobin method was used to determine blood Hb concentration. In this method, whole blood is reacted with Drabkin's Reagent (Sigma, St. Louis, MO) to produce a level of color in the solution directly proportional to the amount of Hb present in the blood. Absorbancies of the samples are then measured at 540 nm in a spectrophotometer against a known standard to determine blood Hb concentration. PCV volume was measured by centrifuging blood samples in 75 mm heparinized microcapillary tubes (Fisher Scientific Co., Pitsburg, PA) for two minutes at 11,500 rpm in a microcapillary centrifuge (International Equipment Co., model MB). After centrifugation, PCV was measured by reading the microcapillary tubes against a hematocrit reader (Fisher Scientific Co., Pitsburg, PA).

In the event that blood Hb assays would need to be rerun, 1 to 2 ml of whole blood from each pig was saved in labeled microcentrifuge tubes and stored in a -14 C° freezer. Whole blood samples can be kept frozen for up to two years after collection before conducting blood Hb concentration analysis (Schoen and Solomon, 1962). After completing blood Hb concentration and PCV analysis, the remaining whole blood samples were centrifuged for ten minutes at 4 C° and speed of 2,000 rpm. After centrifugation the plasma was removed and stored in labeled microcentrifuge tubes at -14 C° for future

analysis of plasma inorganic phosphorous and alkaline phosphatase (results not included in this thesis).

Statistical Analysis

Statistical analysis was done using the software JMP 7 Statistical Discovery From SAS. Main effect was analyzed using one-way ANOVA. The Student's t test was used to determine the difference between means of groups for growth performance, PCV, and blood Hb concentration.

Table 1. Composition of diets in Experiment 1

Ingredient	% of Diet
Wheat	63.4
Soybean meal, 48% CP	25.00
Corn Oil	1.00
Corn Starch	4.00
Sodium Phosphate	1.20
Calcium Carbonate	1.85
Sodium Chloride	0.25
Vitamin/Mineral Premix ¹	0.50
Plasma Spray	2.00
L-Lysine	0.20
L-Threonine	0.05
DL-Methionine	0.05
Tylan 10®	0.50
Total	100.00
Phytase ²	2,000 U/kg

¹Vitamin and mineral premix supplies per kilogram of diet: 2,200 IU of vitamin A, 220 IU of vitamin D, 16 IU of vitamin E, 0.5 mg of vitamin K, 0.05 mg of Biotin, 0.5 g of Choline, 0.3 mg of Folicin, 15 mg of Niacin, 10 mg of Panthothenic, 3.5 mg of Riboflavin, 1mg of Thiamin, 1.5 mg of Vitamin B₆, 17.5 ug of Vitamin B₁₂, 1.7 mg of Cu, 0.14 mg of I, 4 mg of Mn, 0.3 mg of Se, 52 mg of Zn.

²Phytase was not supplemented in the basal diet.

Table 2. Composition of diets in Experiment 2

Ingredient	% of Diet
Wheat	60.00
Soybean meal, 48% CP	25.00
Corn Oil	1.00
Corn	7.40
Sodium Phosphate	1.00
Calcium Carbonate	1.80
Sodium Chloride	0.20
Vitamin/Mineral Premix ^{1,2}	0.50
Magnesium Oxide	0.30
Plasma Spray	2.00
L-Lysine	0.20
L-Threonine	0.05
DL-Methionine	0.05
Tylan 10®	0.50
Total	100.00
Phytase ³	2,500 U/kg

¹Vitamin and mineral premix supplies per kilogram of diet: 2,200 IU of vitamin A, 220 IU of vitamin D, 16 IU of vitamin E, 0.5 mg of vitamin K, 0.05 mg of Biotin, 0.5 g of Choline, 0.3 mg of Folicin, 15 mg of Niacin, 10 mg of Panthothenic, 3.5 mg of Riboflavin, 1mg of Thiamin, 1.5 mg of Vitamin B₆, 17.5 ug of Vitamin B₁₂, 6 mg of Cu, 0.14 mg of I, 4 mg of Mn, 0.3 mg of Se, 80 mg of Zn.

²Fe was included in the trace mineral premix at 40 mg/kg of diet for the “BD + Fe” diet.

³Phytase was not supplemented in the basal diet or the diet with Fe in the trace mineral premix.

Results

Growth Performance

All pigs showed normal growth. There was no significant difference ($P < 0.05$) between growth performance of pigs fed the BD and pigs fed the BD supplemented with phytase in Experiment 1 (Table 3). In Experiment 2, pigs that were fed the BD supplemented with phytase had a higher average daily gain ($P < 0.10$) than pigs that were fed the basal diet (Table 3). Pigs fed the BD with an Fe supplemented trace mineral premix (TMP) had an ADG that was not different ($P < 0.10$) than pigs fed the BD and the BD + phytase.

Table 3. Effect of dietary supplemental phytase on pig growth performance.

	Initial BW ¹ , kg	Final BW ¹ , kg	ADG ² , kg	ADFI ³ , kg	Gain:Feed ⁴
Experiment 1					
BD ⁵	12.02 ± 0.34	40.25 ± 1.23	0.67 ± 0.03	1.4 ± 0.08	0.53 ± 0.01
BD + Phytase	11.84 ± 0.35	38.30 ± 2.23	0.63 ± 0.04	1.3 ± 0.08	0.53 ± 0.02
Experiment 2					
BD	9.28 ± 0.32	21.00 ± 1.51	0.42 ^a ± 0.03	0.7 ± 0.06	0.62 ± 0.02
BD + Fe	9.17 ± 0.32	22.21 ± 2.24	0.47 ^{ab} ± 0.03	0.8 ± 0.06	0.62 ± 0.03
BD + Phytase	9.18 ± 0.36	22.51 ± 2.47	0.48 ^b ± 0.04	0.8 ± 0.08	0.62 ± 0.07

¹ Body weight values are group means ($n = 8$) ± SEM.

² Average daily gain values are group means ($n = 8$) ± SEM over the entire experimental period.

³ Average daily feed intake values are group means ($n = 8$) ± SEM over the entire experimental period.

⁴ Gain-to-feed ratios are group means ($n = 8$) ± SEM over the entire experimental period.

⁵ Basal diet.

^{a,b} Values not sharing the same letter in their superscripts differ significantly ($P < 0.10$)

Packed Cell Volume

The change in packed cell volume (PCV) over the course of Experiment 1 is shown in Figure 1. In general PCV increased over the period of the experiment, however, the percent change in PCV decreased for the BD fed group from weeks 2 to 4, and the PCV at week 4 was lower than the PCV at week 2 for the group fed the BD supplemented with phytase. The group fed the BD supplemented with phytase tended to have a higher PCV than the BD fed group throughout the experiment, but this was not statistically significant ($P > 0.05$).

The change in PCV over the course of Experiment 2 is shown in Figure 2. At week 2, the mean PCV for the group fed the BD supplemented with phytase and the group fed the BD with the Fe supplemented TMP were significantly higher than the PCV for the group fed only the BD ($P < 0.05$). At week 4, the group fed the BD with the TMP supplemented with Fe had a significantly higher PCV than the group fed the BD ($P < 0.05$). At week 4 the group fed the BD supplemented with phytase did not differ significantly from the other two groups, but its PCV was still higher than the unsupplemented BD group ($P < 0.05$).

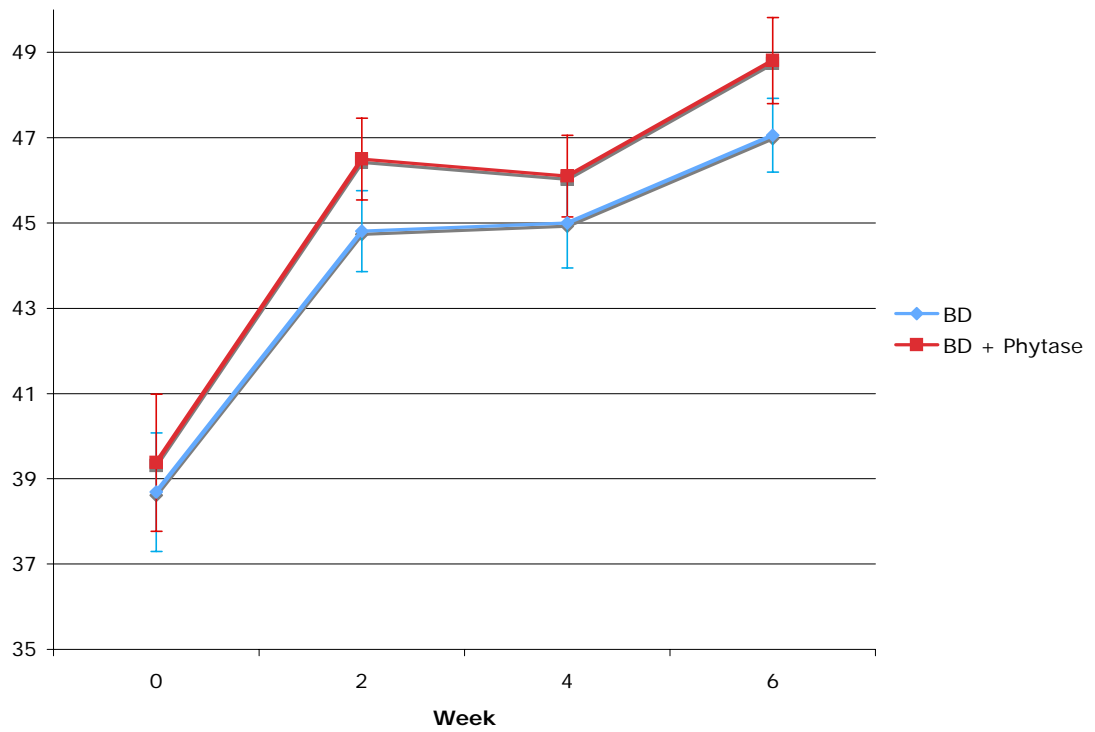


Figure 1. The effect of phytase supplementation on packed cell volume in Experiment 1.

Individual points are group means ($n = 8$) \pm SEM. There is no significant difference between group means for a given week ($P > 0.05$).

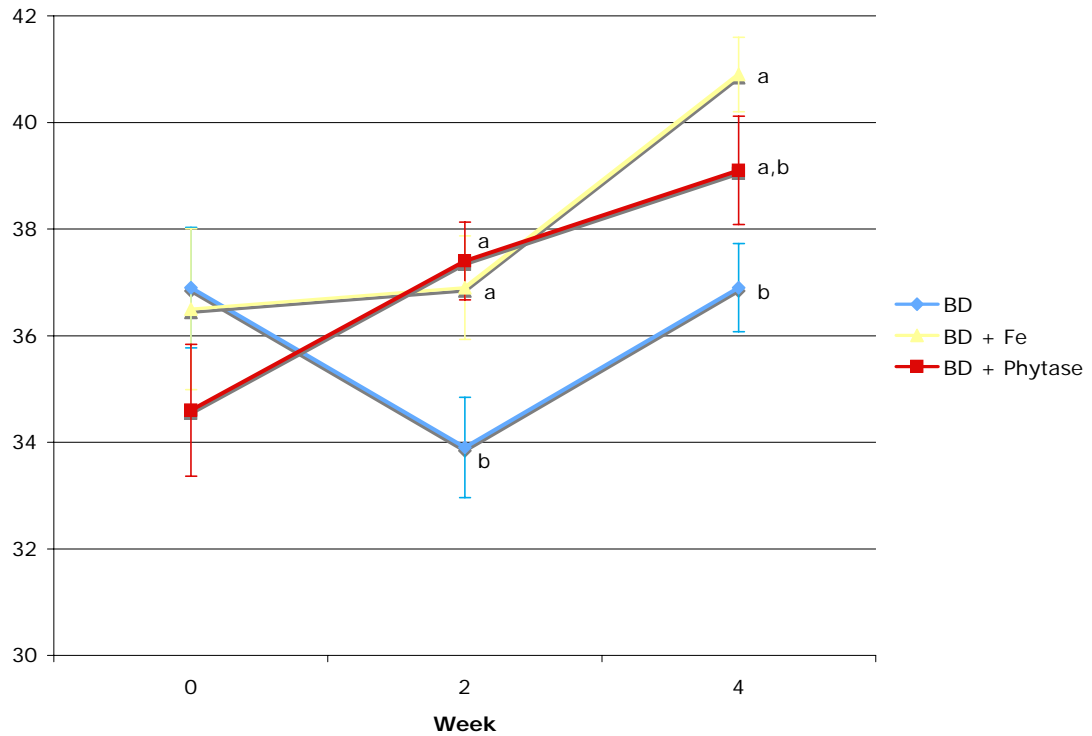


Figure 2. The effect of phytase supplementation on packed cell volume in Experiment 2. Individual points are group means ($n = 8$) \pm SEM. For a given week, points that do not share the same letter differ significantly ($P < 0.05$).

Hemoglobin Concentration

The change in blood Hb concentration over the course of Experiment 1 is shown in Figure 3. Blood Hb concentration rose from week 0 to 2 and decreased from weeks 2 to 6 for both groups. There was no difference in blood Hb concentration between the group fed the BD and the group fed the BD supplemented with phytase ($P < 0.05$).

Change in blood Hb concentration over the course of Experiment 2 is shown in Figure 4. Both the group fed the BD supplemented with phytase and the group fed the BD with the Fe supplemented TMP showed a continual increase in blood Hb concentration. However, the group fed only the BD showed a decrease in blood Hb

Concentration from week 0 to 2, before rising from week 2 to 4. At week 2, the group fed the phytase supplemented BD had a higher blood Hb concentration than that of the BD group ($P < 0.05$), but did not differ from the blood Hb concentration of the BD with supplemental Fe. At week 4, both the phytase supplemented BD group and the Fe supplemented TMP group had significantly higher blood Hb than the group fed only the BD ($P < 0.05$). At week 4, the Fe supplemented group had a higher blood Hb concentration than the phytase supplemented group, however, this was not statistically significant ($P < 0.05$).

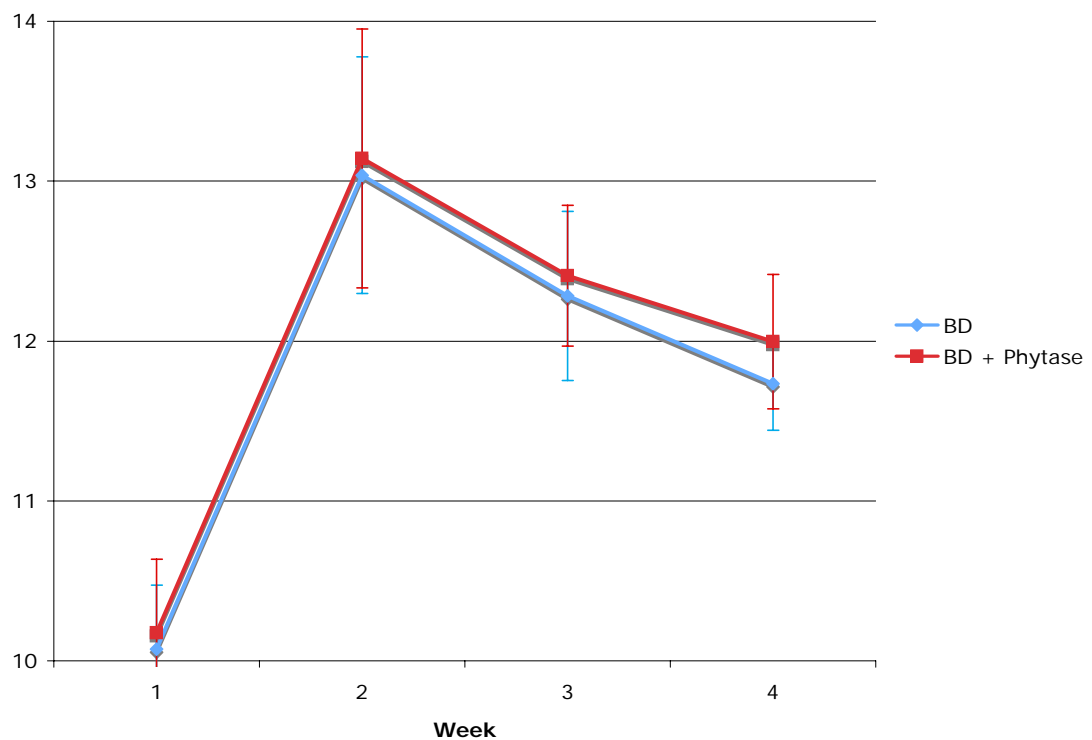


Figure 3. The effect of phytase supplementation on hemoglobin concentration in Experiment 1. Individual points are group means ($n = 8$) \pm SEM. There is no significant difference between group means for a given week ($P < 0.05$).

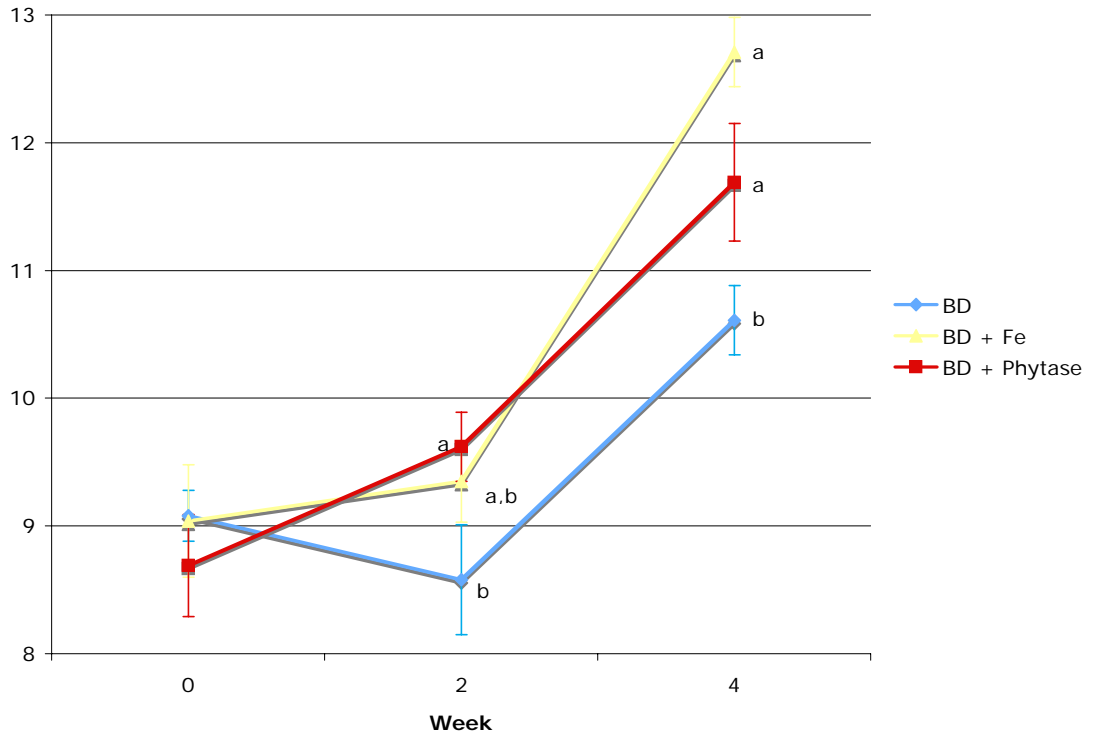


Figure 4. The effect of phytase supplementation on hemoglobin concentration in Experiment 2. Individual points are group means ($n = 8$) \pm SEM. For a given week, points that do not share the same letter differ significantly ($P < 0.05$).

Discussion

Supplemental phytase improved intrinsic iron bioavailability to anemic weanling pigs in Experiment 2 but not in Experiment 1. In Experiment 1 there was no significant difference between the growth performances, PCV, or blood Hb concentrations of pigs fed the BD and pigs fed the BD supplemented with phytase. In this initial experiment it was interesting that all pigs experienced a sharp increase in blood Hb concentrations from weeks 0 to 2 then a decrease in blood Hb concentrations from weeks 2 to 6. Similarly, the PCV increased sharply in the first two weeks of the experiment for both groups. From weeks 2 to 4 the BD group's PCV increased less than 0.5%, and the phytase group's PCV decreased by approximately 0.5%. From weeks 4-6 both groups' PCV increased, though not as sharply as in the first two weeks.

Miller *et al.* (1961) demonstrated that the PCV and blood Hb concentrations profiles followed similar patterns from birth to maturity. They also demonstrated that increases and decreases in PCV and blood Hb concentrations are characteristic of pigs during the first nine weeks of life. In a previous experiment (Stahl *et al.*, 1999) similar to the present study, some groups of pigs fed a corn-soybean based diet showed a decrease from initial blood Hb concentrations during the first two weeks of the experiment.

The design of Experiment 2 was similar to Experiment 1 with a few exceptions. In this experiment, an additional group was added for further comparison. This was the group fed the BD that had an iron supplemented the trace mineral premix. This diet was formulated to meet all nutritional requirements for growing pigs, unlike the other two diets, which were iron deficient and contained no supplemental inorganic iron. This allowed us not only to compare the response of the pigs fed the BD supplemented with

phytase to pigs fed only the BD, but also compare it to pigs fed a nutritionally adequate diet.

In Experiment 2, pigs fed the BD and pigs fed the BD + iron performed as expected. The BD group had lower growth performance, PCV, and blood Hb concentrations compared to the iron supplemented group. The phytase supplemented group performed equally as well as the iron supplemented group. By week 2, the phytase group had a higher PCV and blood Hb concentrations than the BD group, and remained that way through the rest of the experiment. The phytase group also had a higher ADG than the BD group ($P < 0.10$). These results demonstrate that supplemental dietary phytase is as effective as supplementing inorganic iron into the diet to correct iron deficiency anemia in weanling pigs.

The reason that the pigs did not respond to phytase treatment in Experiment 1 as they did in Experiment 2 is likely attributed to several factors. The first factor is the difference in initial body weights between pigs in Experiment 1 and Experiment 2. In Experiment 1 the average initial weight of the pigs used was 11.93 kg, and the average initial weight of pigs in Experiment 2 was 9.21 kg. Pigs in Experiment 1 were older and bigger at the start of the experiment. Stahl *et al.* (1999) showed similar positive results with phytase supplementation with pigs that had an average initial body weight of 8.01 kg. Two other factors that likely explain the difference in results was the initial blood Hb concentrations of pigs in Experiment 1 and the method used to deplete the pigs' body iron. In Experiment 1 the pigs were given a commercial creep feed before weaning and being put on the transition BD. This creep feed did contain inorganic iron, which is highly bioavailable for Hb synthesis. In Experiment 2 pigs were fed the same BD that

contained no supplemental iron as a creep feed, through weaning and until the start of the experiment. The average initial blood Hb concentrations for pigs in Experiment 1 was approximately 10.0 g/dL, whereas in Experiment 2 the average initial blood Hb concentrations was approximately 9.0 g/dL. In the studies performed by Stahl *et al.* (1999) the average initial blood Hb concentrations was between 7-8 g/dL. It is likely that the pigs used in Experiment 1 were already past the most sensitive period to show the effects of phytase on iron bioavailability.

Conclusion

Though we did not have the same results in Experiments 1 and 2, this can be attributed to the difference in sensitivity of pigs to phytase supplementation. Results from Experiment 2 indicate that phytase can improve intrinsic iron bioavailability in a wheat-based diet. The initial body weight and blood Hb concentrations of pigs in Experiment 2 were closer to that of other experiments where a positive effect of phytase supplementation on iron bioavailability was seen using a corn-soybean based diet. This research further demonstrates the importance of having an appropriate and sensitive enough model when investigating bioavailability of nutrients. Future directions for research should include studying if there is a dose dependency on phytase supplementation for adequate release of phytate-bound iron in wheat. Future research is also necessary to determine if phytase may provide a means for improving human iron nutrition, and alleviating or preventing problems associated with inadequate dietary iron intake, such as iron deficiency anemia.

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