RESEARCHREPORT
ANIMAL SCIENCE

Title: Impact of dietary fiber on nutrient utilization by the pig and on the efficacy of the phytase enzyme **NPB #16-016**

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Industry Summary:

Fiber is the carbohydrate portion of the diet which cannot be digested by enzymes secreted by the intestinal tract of the pig. For example, starch is a carbohydrate, but is not considered to be fiber because the pig produces enzymes which breaks down starch [However, to be exact, a small portion of the starch in corn and other grains is resistant to these enzymes, is called "resistant starch" and therefore is considered to be part of the fiber in the diet]. On the other hand, cellulose is considered to be fiber, because the pig cannot digest it. Therefore, fiber is not digested but at least parts of it can be fermented by the microbes that live in the gut of the pig – primarily in the caecum and large intestine. Fiber has been characterized in many ways. One method of characterization is soluble versus insoluble; soluble fiber is that portion of the total fiber which is fermented fairly well in the pig, while insoluble fiber is that portion which is poorly fermented. While fiber is viewed as one, albeit poor source, of energy in the diet, it has other impacts on the pig. Insoluble fiber can affect the gastrointestinal physiology of the pig, especially the digestibility of the diet. In this project two experiments were conducted: In experiment 1, The impact of insoluble dietary fiber (IDF) on diet digestibility was studied under two diet formulation conditions: constant nutrient, meaning as fiber was added to the diet, nutrients were kept constant, or nutrients were allowed to float, meaning that as fiber was added to the diet, nutrient levels changed and ingredients were kept constant. Both approaches are used in research, but there is almost no information on which is the best approach to evaluate the impact of fiber on energy and nutrient digestibility. It is obviously very important to study the effect of fiber on diet digestibility in the best possible manner, e.g., the one that gives us results that can be best used in practical diet formulation. Twenty-one ileal-cannulated growing gilts (33 kg BW) were assigned to 1 of 7 dietary treatments. Diets consisted of a basal corn-soy diet with 0% corn DDGS, or diets containing 15, 30 or 45% of DDGS as a source of IDF. Diets were formulated using either constant nutrient or constant ingredients approaches, as described above. In experiment. 2, the effect of an IDF source on the ability of the phytase enzyme to do its job was evaluated. A total of 480 pigs (6.80 kg BW), housed in 48 pens were fed one of 8 dietary treatments. Diets corresponded to a series of 4 corn-soybean-meal-based diets with 4 levels of added phytase (0, 109, 218 and 327 FTU/kg) to increase the standardized total tract digestible phosphorus (STTD P) and a second series of 4 diets, based on corn, soybean meal and 20% bran (source of IDF) with the same 4 levels of added phytase. All diets were deficient in available phosphorus content. Growth performance, bone ash and total tract digestibility of DM, NDF, and ADF were determined. Results from experiment 1 showed that the addition of IDF (in the form of added DDGS) decreased ileal digestibility of dry matter, energy, starch and the fiber components (except for ADF). Ileal digestibility of fat decreased with the addition of IDF when nutrients were balanced, but increased when nutrients were allowed to float. The addition of IDF decreased total tract digestibility of DM, GE, and the fiber components except for SDF that increased and ADF that was not affected. As for ileal digestibility, total tract digestibility of fat decreased with the addition of IDF when nutrients were balanced, but increased when nutrients were allowed to float. Estimates of ileal digestibility of IDF, TDF and NDF were lower when nutrients were maintained constant compared to when nutrients were allowed to float. Estimates of total tract digestibility of DM, IDF, TDF and NDF were lower when nutrients were maintained constant compared with when nutrients were allowed to float. Results from experiment 2 showed that as expected, phytase increased rate and efficiency of gain as well as the degree of bone mineralization; it did not influence feed intake, or the digestibility of dry matter, neutral detergent fiber or acid detergent fiber. On the other hand, the addition of insoluble fiber (in the form of 20% corn bran) decreased rate and efficiency of gain, the latter in a modest amount. Insoluble fiber did not affect feed intake or bone mineralization. In conclusion, the addition of insoluble dietary fiber decreased the use of most of the diet components along the intestinal tract. However, adding insoluble dietary fiber increased the fermentation of soluble dietary fiber in the large intestine. The effect of IDF on the digestibility of fiber is greater when the constant ingredient approach is used compared to the constant nutrient approach. Additionally, the constant ingredient approach is an inconvenient method when fat digestibility is evaluated since fat level confounded the response to adding IDF. The addition of insoluble fiber did not affect the ability of phytase to improve growth performance and bone mineralization. Therefore, phytase is equally effective at releasing phytate-bound phosphorus in low or higher fiber diets.

Key findings

- Insoluble dietary fiber, such as that found in corn and corn co-products, decreases the digestibility of other dietary components.
- However, in addition to the decrease in digestibility mentioned above, insoluble dietary fiber promoted fermentation of soluble dietary fiber in the large intestine. Thus, the effects of insoluble dietary fiber are not all bad.

- Balancing the level of fat in digestibility studies is recommended; otherwise, fat levels may increase or decrease and this may result in incorrect conclusions about the digestibility of fat.
- Phytase can be used with confidence in both high and low fiber diets, as it works effectively in both cases.

Keywords:

Swine, insoluble dietary fiber, exogenous enzyme, corn bran, corn DDGS

Scientific Abstract

A set of experiments were designed to test two hypotheses; first, that adding insoluble dietary fiber (IDF) would decrease the digestibility of various fiber components in the gastrointestinal tract, where the response to IDF was assessed to determine differences under constant nutrient or floating nutrient conditions; and second, that fiber would affect the efficacy of the phytase enzyme. In experiment 1, a total of 21 ileal-cannulated gilts (33 ± 0.4 kg BW) were randomly allocated to 1 of 7 treatments over 3 sample collection periods. Treatments consisted of a 0% corn DDGS basal diet, plus diets containing 15, 30, or 45% of DDGS as a source of IDF. Diets were formulated using 1 of 2 different approaches: constant nutrient (CN) where nutrients were held equal to the basal diet, or where DDGS were added at the expense of corn and all other ingredients remained constant, so nutrient levels were allowed to "float" (CI). Chromic oxide was added to the diets at 0.5% as an indigestible marker. The MIXED procedure of SAS was used to test linear estimates with pig and collection as random effects. In experiment 1, all of the apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) variables tested were affected linearly with the addition of IDF ($P < 0.05$), except the ATTD of acid detergent fiber (ADF) which was not affected ($P = 0.753$). The addition of IDF decreased the AID of DM, GE, starch, IDF, soluble dietary fiber (SDF), total dietary fiber (TDF) and neutral detergent fiber (NDF), but increased AID of acid detergent fiber (ADF). The AID of acid hydrolyzed ether extract (AEE) decreased with the addition of IDF when nutrients were balanced, but increased when nutrients were allowed to float. The addition of IDF decreased the ATTD of DM, GE, IDF, TDF, and NDF. In contrast, the ATTD of SDF increased with the addition of IDF. The ATTD of AEE decreased with the addition of IDF when nutrients were balanced, but increased when nutrients were allowed to float. Estimates of the effect of IDF on the AID of DM, GE, starch, SDF, and ADF were similar between CN and CI. In contrast, estimates of the AID of IDF, TDF and NDF were smaller for CN than CI ($P < 0.050$). Estimates of the effect of IDF on the ATTD of GE and SDF were similar between CN and CI. In contrast, the estimates of the effect of IDF on of the ATTD of DM, IDF and TDF were greater for CI than CN ($P < 0.050$) and tended to be greater for CI than CN for the ATTD of NDF ($P = 0.053$). In experiment 2, a total of 480 weaned pigs (6.80 ± 0.18 kg BW) were allotted to 48 pens. At 14 days post-weaning, for the next 21 days, pigs received 8 dietary treatments: a series of 4 corn-soybean meal based diets with 4 levels of added phytase (0, 109, 218, and 327 FTU/kg; Quantum Blue 5 G, AB Vista, Wiltshire, UK;) and a second series of 4 diets, based on corn, soybean meal and 20% bran with the same 4 levels of added phytase (0, 109, 218, and 327 FTU/kg). Titanium dioxide was added to the diet at 0.4% as an indigestible marker. Pigs were blocked by BW and allotted to pens, with 6 pens per treatment. On day 21, one pig representing the average BW for each pen was euthanized, and fibulas were collected and analyzed for bone ash. Fecal samples were collected from each pen on days 18-20. Adding phytase increased ADG ($P < 0.001$), while insoluble fiber decreased ADG ($P = 0.033$). There were no effects of phytase or insoluble fiber on ADFI ($P = 0.381$ and $P = 0.632$, respectively). Phytase improved G:F ratio ($P < 0.001$), while insoluble fiber tended to decrease G:F ratio ($P = 0.097$). Phytase increased bone ash ($P = 0.005$), but there was no effect of insoluble fiber ($P = 0.949$). Phytase did not affect the ATTD of DM, NDF, or ADF ($P > 0.050$) while insoluble fiber decreased the ATTD of DM ($P < 0.001$), NDF ($P < 0.001$) and ADF ($P < 0.001$). There were no interactions between fiber and phytase in any of the variables evaluated ($P > 0.050$). In conclusion, the addition of IDF decreased the use of most of the dietary components. However, adding IDF increased fermentation of SDF in the large intestine. Letting nutrients float can result in greater estimates for digestibility of fiber components related to insoluble fiber. Additionally, letting nutrients float is an inconvenient method when fat digestibility is evaluated since fat level confounds the response to IDF. The addition of insoluble fiber did not affect the ability of phytase to improve growth performance and bone mineralization.

Introduction

High fiber ingredients like corn co-products are added to the diet because of their cost, availability and nutrient profile. Insoluble dietary fiber (IDF) comprises the majority of the fiber present in these ingredients and is characterized for being poorly fermented in the gastrointestinal tract of pigs. Furthermore, the addition of IDF to swine diets results in a decrease in the digestibility of other dietary components of nutritional importance such as energy, dry matter, amino acids (Gutierrez et al., 2016; Urriola and Stein, 2010) and minerals (Nortey et al., 2007). Thus, although there is a reasonable idea of what IDF does, there is still a need for reliable estimations that would help to develop strategies to use fiber most effectively.

Experiments that evaluate high fiber ingredients generally formulate diets in which the ingredient of interest is added in place of corn, with most of the other ingredients being held constant. In other words, nutrients are allowed to float – they are not held constant across all diets. This approach may have a potential flaw because changes in nutrient levels may confound the response of the pigs to the dietary treatments. Certainly, in commercial practice, diets are formulated to constant energy and nutrient levels. Therefore, it can be claimed that these experiments should balance for nutrient composition as much as possible. On the other hand, formulating to constant energy and nutrient levels will result in changes in other ingredients in the diets, and this could confound the response of the pigs to the experimental treatments; inadvertent affects occurring as a result of changing ingredient levels could affect outcomes. Stated another way, we can formulate diets to constant nutrient levels, or to constant ingredient levels; both approaches have merit, but both also have limitations. Consequently, it is useful to determine if the response to dietary treatment – in this case, fiber levels - is different under constant ingredient compared to a constant nutrient scenario.

More effective use of fiber also implies the validation of its effects on feed additives such as the use of exogenous enzymes. Phytase is an exogenous enzyme that breaks down phytate (a source of phosphorus from plant origin) in pig diets. However, its effectiveness may be affected by the addition of fiber. Although proven mechanisms of fiber and phytase interaction have not been established, we suggest that there are at least two potential effects. First, insoluble fiber is known to increase passage rate and stomach emptying, reducing the time for enzyme-substrate interaction (Wenk, 2001); this, in turn, could reduce the effectiveness of the enzyme and thus lower the quantity of phosphorus released by the enzyme and absorbed by the small intestine. Second, by physically isolating or trapping the substrate, fiber can delay the enzyme-substrate interaction decreasing the efficacy of phytate degradation. However, as a first step, it is important to determine if the phytase-fiber interaction impacts parameters that swine producers can detect.

Therefore, two experiments were conducted to evaluate the addition of IDF under a constant nutrient or constant ingredient nutrient scenarios, as well as to assess the effects of fiber on the efficacy of the phytase enzyme.

Objectives from the original proposal

1. To determine the impact of increasing levels of insoluble (corn) fiber on the fractional availability of energy and nutrients in corn and soybean meal.

2. To determine the impact of increasing quantities of dietary fiber on the efficacy of phytase in releasing phosphorus from phytate.

Specific objectives

1.1 To determine if the impact of added insoluble fiber is different when diets are adjusted by nutrient or by ingredient composition.

1.2 To determine if phytase affects the digestibility of DM and fiber components.

Materials and Methods

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research and were approved by the Iowa State University Institutional Animal Care and Use Committee (number 8-17-8584-S and 12-17-8657-S for objective 1 and objective 2, respectively).

Objective 1

Animal, Diets, and Experimental Design

Twenty-one crossbreed growing gilts (PIC; Hendersonville, TN) were surgically fitted with a T-cannula at the distal ileum following the procedures described by Stein et al. (1998) with minor modifications. After recovery from surgery, pigs were weighed (33.1 ± 0.4 kg initial BW) and randomly allocated to 1 of 7 dietary treatment groups in a 3-period incomplete Latin square design, resulting in 9 observations per treatment. Animals were housed in individual pens (1.2 × 1.5 m) in an environmentally-controlled facility with a 12-h light-dark cycle. Each pen was equipped with a feeder, a nipple waterer, and a half slated concrete floor. Treatments consisted of a 0% corn DDGS basal diet, plus diets containing 15, 30, or 45% DDGS as a source of IDF. Diets were formulated using 1 of 2 different approaches **(Table 1)**: constant nutrient

(CN) where the nutrient levels were maintained equal to those of the basal diet, or constant ingredients (CI) where DDGS were added at the expense of corn and all other ingredients remained fixed and the nutrients allowed to float. Amino acids, vitamins, and minerals were added to all diets **(Table 2)** to meet or exceed estimated requirement (NRC, 2012). Chromic oxide was added at 0.5% as an indigestible marker.

Pigs were reassigned to dietary treatment at the end of each collection period and were not allowed to repeat dietary treatments across periods. Each collection period involved 9 days of adaptation to dietary treatments followed by 2 days of feces subsample collection and 3 days of ileal digesta subsample collection.

Sample collection and chemical analyses

Several diet subsamples were collected at the feed mill at the time of mixing and then pooled in a blended subsample. Fecal subsamples were obtained via grab sampling. Ileal subsamples were collected by attaching a 207-mL plastic bag (Whirl-Pak; Nasco, Fort Atkinson, WI) to the opened cannula with a cable tie. Bags were removed once they were filled with digesta or at least every 30 minutes for 8 hours per collection day. All subsamples were stored at –20°C to avoid bacterial degradation.

Prior to being assayed, fecal subsamples were thawed and oven-dried in a convection oven at 65°C until subsamples reached constant weight (Jacobs et al., 2011); ileal subsamples were lyophilized. Diets and dried ileal and fecal subsamples were ground in a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ) through a 1 mm screen and stored in desiccators to maintain a constant percentage of DM.

Chemical analysis of diets, feces, and ileal digesta subsamples was performed at the Monogastric and Comparative Nutrition Laboratory (Iowa State University-Ames, IA) and the Monogastric Nutrition Laboratory (University of Illinois-Champaign, IL). Assays included the concentration of DM using a drying oven (method 930.15; AOAC, 2007). Acid hydrolyzed ether extract was assayed using a SoxCap hydrolyzer (model SC 247) and a Soxtec fat extractor (model 255; Foss, Eden Prairie, MN; method 968; AOAC, 2007). Starch (only analyzed for diets and ileal samples) was analyzed using a Megazyme total starch assay kit analysis (Wicklow, Ireland; modified method 996.11, AOAC 1996). Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were determined using the AnkomTDF Dietary Fiber Analyzer (AOAC 991.43, AOAC Int., 2007; Ankom Technology, Macedon, NY). The total dietary fiber was determined as the sum of IDF and SDF. Acid and neutral detergent fiber (ADF and NDF, respectively) were determined using an Ankom automated fiber analyzer (model 2000, Macedon, NY) according to Van Soest and Robertson (1979). Gross energy was determined using a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6,318 kcal/kg; Parr instruments, Moline, IL) was used as the standard for calibration and was determined to contain 6319± 2.2 kcal/kg. Chromium was determined feces using the method of Fenton and Fenton (1979); absorption was measured at 440 nm using a spectrophotometer (Synergy 4; BioTek Instruments Inc., Winooski,VT).

The AID and the ATTD were determined using the following equation (Oresanya et al., 2008):

ATTD or AID, % = [100 – [100 * (% chromic oxide in feed / % chromic oxide in feces or ileal digesta) x (concentration of component in feces / concentration of component in feed)]]

Statistical analysis

The ROBUSTREG procedure of SAS (SAS Inst., Inc., Cary, NC) was used to analyze for outliers. Data were analyzed using PROC MIXED of SAS according to the following model: type of approach and added IDF as fixed effects, and pig and collection period as random effects. Linear and quadratic effects were tested. The CONTRAST statement was used to compare the CN and CI estimates. Effects were considered statistically significant with $P \leq 0.05$. The pig was the experimental unit for all analyzes.

Objective 2

Animal, Diets, and Experimental Design

A total of 480 crossbreed pigs (PIC; Hendersonville, TN; 5.48 ± 0.14 kg BW) at approximately 21 days of age were blocked by initial BW (in 6 blocks) and allotted to 48 pens. Pens were randomly assigned to treatments within the block. Genders were not separated, but in pens within a block, there were equal numbers of barrows and gilts. Nine days after arrival, all pigs were fed a common corn-soybean meal diet containing 0.16% standardized total tract digestible (STTD) phosphorus (half of the pigs receiving a corn soy diet, and the other half a corn-soybean meal-corn bran diet) for 5 days to acclimatize pigs to a phosphorus-deficient diet. From day 14-35 (post-arrival), pigs received 1 of 8 dietary treatments.

The 8 different dietary treatments corresponded to a series of 4 corn-soybean-meal based diets with 4 levels of added phytase (0, 109, 218 and 327 FTU/kg; Quantum Blue 5 G, AB Vista, Wiltshire, UK; **Table 3**) and a second series of 4 diets, based on corn and soybean meal and 20% bran with the same 4 levels of added phytase. All diets were deficient in STTD phosphorus (NRC, 2012). The 4 levels of the STTD phosphorus in the corn-soybean meal diets (0.161, 0.211, 0.261 and 0.311%) were formulated to meet 46, 60, 75, and 88% (for the 0, 109, 218 and 327 FTU/kg, respectively) of the requirement. The 4 levels of STTD phosphorus in the diets with corn bran (0.142, 0.192, 0.242 and 0.292%) were formulated to meet 41, 55, 69 and 83% (for the 0, 109, 218 and 327 FTU/kg respectively) of requirement.

Sample collection handling and chemical analyses

At day 18-20 (of the testing period), fresh fecal subsamples were collected via grab sampling from each pen. Prior to being assayed, fecal subsamples were thawed and oven-dried in a convection oven at 65°C until subsamples reached a constant weight (Jacobs et al., 2011). Diets and dried fecal subsamples were ground in a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ) through a 1 mm screen and stored in desiccators to maintain a constant percentage of DM. At day 21, one pig with a BW close to the pen average was euthanized to remove the fibula bone of the right leg.

Ingredients and feed samples were assayed at Mid-west labs (Omaha, NE) for total calcium and phosphorus. Feed and fecal samples were assayed at the Monogastric Nutrition Laboratory (Iowa State University-Ames, IA). Assays included the concentration of DM using a drying oven (method 930.15; AOAC, 2007). Acid and neutral detergent fiber (ADF and NDF were determined using Ankom automated fiber analyzer (model 2000, Macedon, NY; according to Van Soest and Robertson, 1979). Titanium dioxide was determined using a spectrophotometer (model Synergy 4, BioTek, Winooski, VT; according to the method of Leone, 1973).

The AID and the ATTD were determined using the following equation (Oresanya et al., 2008):

ATTD, % = [100 – [100 * (% titanium dioxide in feed / % titanium dioxide in feces or ileal digesta) x (concentration of component in feces / concentration of component in feed)]]

Fibula bones were assayed for bone ash (600 °C for 16 hours) preceded by autoclaving, tissue removal, and oven dried (103 °C for 36 hours) before and after being soaked in fresh hexane until clear. Fibula ash content was expressed as a percentage of the dry bone weight.

Statistical Analysis

The ROBUSTREG procedure of SAS (SAS Inst., Inc., Cary, NC) was used to analyze for outliers. Data were analyzed using PROC MIXED of SAS according to the following model: main (fixed) effects of block, phytase, fiber and the interaction between phytase and fiber. Effects were considered statistically significant with $P \leq 0.05$. Pen was the experimental unit in all analyses, except for bone ash in which pig was the experimental unit.

Results and Discussion

Objective 1

All animals successfully recovered from surgery, remained healthy, and fully consumed their diets during the entire experimental period. The AID and the ATTD of all variables tested were linearly affected by the addition of IDF ($P < 0.050$; **Table 4**), excluding the ATTD of ADF ($P = 0.753$). None of the AID and ATTD variables tested resulted in a significant quadratic effect ($P > 0.050$).

Comparison of the AID linear slope estimations between CN and CI (**Table 5**) indicate that each 1% of additional IDF decreased the AID of DM (-2.10%, CN; $P < 0.001$ and -2.23%, CI; $P < 0.001$), and the AID of GE (-1.65%, CN; $P < 0.001$ and -1.69%, CI; $P < 0.001$). There were no differences between CN and CI estimates for the AID of DM and GE. Each 1% of added IDF decreased the AID of AEE (-0.62%; $P < 0.001$) for CN, but the AID of AEE was increased for CI (0.36%; $P < 0.001$). The slope estimates of CN and CI of the AID of AEE were different ($P < 0.001$). Each 1% of IDF decreased the AID of starch (-0.17%, CN; $P = 0.045$ and -0.25%, CI; $P = 0.012$). There were no differences between CN and CI slope estimates for the AID of starch.

Each 1% of added IDF decreased the AID of IDF (-1.98%, CN; $P < 0.001$ and -2.78, CI; $P < 0.001$). Slope estimate of CN was different than the estimate of CI ($P = 0.012$). Likewise, each 1% of added IDF decreased the AID of SDF (-3.26, CN; *P* < 0.001 and -2.26, CI *P* = 0.008). However, there were not differences between CN and CI slope estimates for the AID of SDF. Each 1% of added IDF decreased AID of TDF and NDF (-1.25%, CN; *P* < 0.001 and -1.87%, CI; *P* < 0.001 for the AID of TDF and -1.68%, CN; *P* < 0.001 and -2.06%, CI; *P* < 0.001 for the AID of NDF). The AID of TDF and NDF slopes CN and CI estimates were significantly different (*P* = 0.034 and *P* = 0.006 for CN and CI respectively). Each 1% of IDF increased the AID of ADF for CN (1.00; *P* = 0.006) and tended to increase the of AID of ADF for CI (0.76; *P* = 0.054). The AID of ADF CN and CI estimate slopes did not differ.

Comparison of the ATTD linear slope estimations between CN and CI indicate that each 1% of IDF decreased the ATTD of DM (-1.48%, CN; *P* < 0.001 and -1.67%, CI; *P* < 0.001). The ATTD of DM Slope estimate of CN was different than the estimate of CI (*P* = 0.008). Likewise, each 1% of IDF decreased the ATTD of GE (-1.28%, CN; *P* < 0.001 and -1.35%, CI, *P* < 0.001). However, the ATTD of GE CN and CI slope estimates were not different. Each 1% of IDF decreased the ATTD of AEE for CN (-0.29%, *P* = 0.047). In contrast, each 1% of IDF increased the ATTD of AEE for CI (0.75%, *P* < 0.001). Slope estimates of the ATTD of AEE between CN and CI were different (*P* < 0.001). Each 1% of IDF decreased the ATTD of IDF (-1.57%, CN; *P* < 0.001 and -2.25%, CI; *P* < 0.001). Slope ATTD of IDF estimates between CN and CI were different (*P* = 0.002). Each 1% increased the ATTD of SDF (1.53%, CN; *P* < 0.001 and 1.14%, CI; *P* = 0.017). Slope estimates between CN and CI for ATTD of SDF were similar. Each 1% of IDF decreased the ATTD of TDF and NDF (-1.25%, CN; *P* < 0.001 and -1.87%, CI; *P* < 0.001 for the ATTD of TDF and -1.68% CN, *P* < 0.001 and -2.06%, CI; *P* < 0.001 for the ATTD of NDF). The ATTD of TDF slopes CN and CI estimates were significantly different (*P* = 0.002), while the ATTD of NDF CN and CI slopes only tended to differ (*P* = 0.053).

This experiment corroborates the influence of IDF on digestibility of the different dietary components (Urriola and Stein, 2010; Gutierrez et al., 2013). The linear relationship suggests that addition of corn DDGS (the source of IDF) has a proportional effect on the digestibility estimates. Navarro et al., 2018 testing two levels (15 and 30%) of various high fiber ingredients concluded that the inclusion of high-fiber ingredients has a negative effect on the concentration of DE and ME in diets fed to pigs. However, the inclusion rate did not affect calculated values for DE and ME in high fiber ingredients. Therefore, the addition of IDF did not change the effect (slope) on digestibility of energy, which supports the results obtained in the current experiment.

Added IDF decreased the AID of SDF in the small intestine, but increased the ATTD of SDF at the total tract level which suggests that SDF is used (fermented) in the large intestine. This is important since fiber plays a major role in the large intestine because it serves as a substrate for microorganisms as well as may shift the profile of the microbiota (Chen et al., 2014). For example, fermentable fiber can be used as an energy source shifting the use of other fermentable products such as amino acids that induce the production of toxic products in the large intestine of pigs (Williams et al., 2016). Thus, addition of IDF may have beneficial effects modulating the fermentation in the large intestine of pigs.

Although it is known that starch is well digested by pigs, the nutritional composition, profile particularly the level of fiber, seems to play a role in starch digestibility (Rosenfelder-

Kuon et al. 2017). Our results confirm both principles, the average AID of starch was 95% for the basal diet, and fiber moderately decreased starch digestibility (-0.21% / 1% of IDF).

This experiment also tested if formulating to constant nutrients or keeping ingredients constant would result in different slope estimates. Results showed that the constant ingredients approach resulted in a more pronounced decrease in digestibility responses to IDF. This response can be explained by the excess of nutrients such as ether extract, minerals, and amino acids observed in the constant ingredient approach. An excess of nutrients may overwhelm the digestive capacity of the gastrointestinal tract, increasing the non-digestible fraction of the diet.

Despite these estimate differences between the two formulation strategies, interpretation of the results did not change drastically using either approach. The only exception was the digestibility of AEE. Using the same level of AEE resulted in a lower apparent digestibility of AEE (this is what was expected for an increased IDF); however, when AEE was allowed to float (increased level of fat) apparent digestibility increased. This discrepancy can be explained by the fact that fat level is a greater confounding factor when evaluating apparent digestibility of AEE (Jørgensen et al., 1993; Gutierrez et al., 2016). Therefore, when comparing different levels of fat, apparent digestibility needs to be corrected for endogenous secretions.

Objective 2

No interactions between phytase and fiber addition were observed for final BW, growth performance, bone ash, and nutrient digestibility. From day 0-7 the addition of phytase did not affect ADG, ADFI or G:F **(Table 6)**. Although the increased addition of insoluble fiber did not affect ADG, pigs fed more insoluble fiber tended to increase ADFI (P = 0.055), and decreased G:F (P = 0.022). From day 8-14, pigs fed phytase increased ADG (P = 0.035), had no effect on ADFI and tended to increase G:F (P = 0.051). On the other hand, pigs fed more insoluble fiber decreased ADG (P = 0.005) and G:F (P < 0.001), while ADFI was not affected. From day 15-21, pigs fed phytase increased ADG (P < 0.001) and G:F (P < 0.001), but had no impact on ADFI. Pigs fed insoluble fiber had similar ADG and G:F. However, pigs fed insoluble fiber tended to decrease ADFI (P = 0.053).

By design, initial BW was not affected by the addition of phytase or insoluble fiber. However, final BW increased with the addition of phytase (P = 0.001; **Table 7**), and decreased with the addition of insoluble fiber (P = 0.007). For the growth performance of the overall period, pigs fed phytase increased ADG and G:F (P < 0.001 and P < 0.001 respectively), while pigs fed more insoluble fiber decreased ADG (P = 0.033) and tended to decreased G:F (P = 0.097). The addition of phytase or insoluble fiber did not affect ADFI. Pigs fed phytase improved bone ash percentage (P = 0.005), while insoluble fiber did not affect bone ash percentage.

Addition of phytase did not influenced the ATTD of DM (**Table 8**; P = 0.670), the ATTD of NDF (P = 0.187) and the ATTD of ADF (P = 0.343). However, pigs fed more insoluble fiber decreased the ATTD of DM (P < 0.001), the ATTD of NDF (P < 0.001), and the ATTD of ADF (P < 0.001).

Phytase has been shown to effectively improve the availability of phosphorus from phytate (Zeng et al., 2016; Jones et al., 2010; Veum et al., 2006). A key to guaranteeing this effectiveness is to maximize the degradation of phytate in the proximal gastrointestinal tract (Blaabjerg et al., 2011). Insoluble fiber has the potential to reduce digesta passage rate, thereby allowing less time for enzyme-substrate interaction (Wenk, 2001), thus decreasing the efficacy of phytate degradation. Additionally, the insoluble fiber matrix can isolate or trap feed components (Bedford and Schulze, 1998) including phytate. Thus, there is a rational foundation to suggest an interaction between insoluble fiber and phytase.

Results of this experiment showed that these interaction mechanisms are absent or not enough to significantly affect production outcomes and bone mineralization. It appears that phytase action takes place before or at the gastrointestinal tract locations in which phosphorus can be absorbed regardless of the increased passage rate resulting from the increased insoluble fiber. Also, the insoluble fiber matrix that may work as a barrier for phytase is not affecting the enzyme-substrate relation. A possible explanation is that most of the phytate in corn is concentrated in the germ (Hídvégi and Lásztity, 2003) and not directly associated with the fiber matrix. Additionally, a great proportion of the phytate belongs to other ingredients than the one with the higher fiber. For instance, in this experiment, phytate was likely being trapped in the fiber matrix of corn bran.

Results also suggest a strong effect of insoluble fiber on digestibility of DM, NDF, and ADF, while no effect of phytase was observed. As mentioned before, our results of fiber's effect on nutrient digestibility are substantially supported in the literature. On the other hand, phytase has not shown consistent results. Zeng et al., (2014) reported an increase in the AID of DM (using 1,000 to 20,000 FTU/kg), but Zeng et al. (using 0 to 20,000 FTU/ kg; 2016) and She et al. (using 0 to 4,000 FTU/ kg 2018) did not find this same effect. All of these experiments used much higher levels of phytase than those used in the current study (0-327 FTU/kg). Generally, the most reported mechanism for a positive effect on digestibility is when phytase is superdosed (Zeng et al., 2014). When superdosed, phytase is believed to decrease the antinutritional effects of phytate and phytate derivates, generally improving mineral and amino acid digestibility.

Conclusions

- The addition of IDF linearly decreased the use of most of the dietary components along the intestinal tract. However, adding IDF increased fermentation of SDF in the large intestine.
- The effect of IDF on the digestibility of fiber is greater when the constant ingredient approach is used compared to the constant nutrient approach. Additionally, the constant ingredient approach is an inconvenient method when fat digestibility is evaluated since fat level confounded the response to adding IDF.
- The addition of fiber did not affect the ability of phytase to improve growth performance and bone mineralization.

Implications

- Insoluble dietary fiber decreases digestibility of dietary components in a linear fashion; therefore, the inclusion of a high fiber ingredient affects nutrient digestibility in proportion to its inclusion.
- In addition to decrease digestibility of most of dietary components, insoluble dietary fiber may exert functional effects such as promoting fermentation of fermentable fiber in the large intestine.
- Balancing the level of fat in digestibility studies is recommended; otherwise, fat levels may increase or decrease and this will be a confounding factor for apparent fat digestibility.
- Phytase can be used with confidence in high and low fiber diets, as it works effectively in both cases.

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Table 1 Ingredient composition of the experimental diets, experiment 1.

	Basal	Constant nutrients			Constant ingredients		
		Added IDF, %			Added IDF, %		
Ingredient, %	**0**	**3.3**	**5.3**	**8.4**	**2.9**	**4.7**	**7.4**
Corn	82.860	68.558	54.253	39.951	67.861	52.858	37.859
Corn DDGS-RO	0.000	14.999	30.002	45.000	14.999	30.002	45.000
HP300	5.891	5.891	5.891	5.891	5.891	5.891	5.891
Bovine casein	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Plasma proteins	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Soybean oil	1.200	0.800	0.400	0.000	1.200	1.200	1.200
Limestone	1.286	1.366	1.445	1.525	1.286	1.286	1.286
Monocalcium phosphate	0.714	0.476	0.238	0.000	0.714	0.714	0.714
L-Lys HCl	0.391	0.320	0.249	0.178	0.391	0.391	0.391
DL-Met	0.061	0.041	0.020	0.000	0.061	0.061	0.061
Thr	0.111	0.074	0.037	0.000	0.111	0.111	0.111
Trp	0.032	0.021	0.011	0.000	0.032	0.032	0.032
Salt	0.600	0.600	0.600	0.600	0.600	0.600	0.600
Vitamin premix	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Trace mineral premix	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Chromic oxide	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Phytase[1]	0.005	0.005	0.005	0.005	0.005	0.005	0.005

[1]Quantum Blue 5G Phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK) was added to provide 500 FTU/kg to release 0.15% standardized total tract digestible P to all dietary treatments.

Table 2. Chemical composition of the experimental diets, experiment1.[1]

Item	Basal	Constant nutrients			Constant ingredients		
	Added IDF, %;						
	0	3.3	5.3	8.4	2.9	4.7	7.4
DM, %	88.3	88.6	88.8	89.3	88.8	89.0	89.5
GE, Mcal/kg	3.94	4.02	4.07	4.15	4.03	4.11	4.19
CP, %	15.0	17.9	20.9	23.8	17.9	20.8	23.7
Standardized ileal digestibility of AA, %							
Lys	0.98	0.98	0.98	0.98	1.04	1.09	1.15
Met	0.32	0.35	0.37	0.40	0.37	0.41	0.46
Thr	0.59	0.63	0.67	0.71	0.66	0.74	0.81
Acid hydrolyzed ether extract, %	4.8	4.7	4.6	4.7	5.2	5.6	6.1
Starch, %	49.6	43.0	34.0	24.8	39.7	31.3	26.3
Insoluble dietary fiber, %	9.2	12.5	14.5	17.6	12.1	13.8	16.7
Soluble dietary fiber, %	0.9	1.3	1.7	2.0	1.4	1.7	1.9
Total dietary fiber, %	10.1	13.8	16.2	19.6	13.5	15.6	18.6
Neutral detergent fiber, %	7.9	10.7	12.6	15.9	10.5	12.4	15.3
Acid detergent fiber, %	1.8	2.6	3.3	4.6	2.6	3.4	4.4
Calcium, %	0.66	0.66	0.66	0.66	0.67	0.68	0.69
STTD phosphorus, %	0.38	0.38	0.38	0.38	0.42	0.47	0.51

[1]CP, standardized ileal digestibility (SID) Lys, SID Met, SID Thr, calcium, and phosphorus are calculated values; while dry matter, gross energy, insoluble dietary fiber, soluble dietary fiber, total dietary fiber, neutral detergent fiber, acid detergent fiber, and acid detergent lignin are assayed values

Table 3. Ingredient and chemical composition of the experimental diets, experiment 2.[1]

	Corn bran	
Ingredient, %	-	+
Corn	61.51	42.10
Soybean meal	32.68	32.08
Corn bran	-	20.00
Soybean oil	3.00	3.00
Limestone	0.72	0.73
L-Lysine HCl	0.45	0.45
DL-Methionine	0.16	0.16
D-Threonine	0.13	0.13
Salt	0.35	0.35
Vitamin premix	0.15	0.15
Trace mineral premix	0.25	0.25
Titanium dioxide	0.40	0.40
Zinc oxide	0.20	0.20
Chemical composition		
DM, %	88.1	88.5
NE, Mcal/kg	2.56	2.43
ME, Mcal/kg	3.44	3.27
AEE, %	5.7	6.7
ADF, %	2.8	3.8
NDF, %	7.5	15.2
CP, %	20.7	20.7
SID AA, %		
Lys	1.33	1.33
Met	0.44	0.45
Met+Cys	0.73	0.73
Thr	0.78	0.78
Trp	0.23	0.23
SID AA:Lys		
Lys	1.00	1.00
Met	0.34	0.34
TSAA	0.55	0.55
Thr	0.59	0.59
Trp	0.17	0.17
Total P, %	0.39	0.35
STTD P, %	0.16	0.14
Ca, %	0.50	0.50

[1]Quantum Blue 5G Phytase (AB Vista Feed Ingredients; Marlborough, Wiltshire, UK) were added to D1 and D5 at 0.00148% to create the 109 FTU/kg (D2 and D6) dietary treatments, added at 0.00297% to create the 218 FTU/kg (D3 and D7) dietary treatments, and added at 0.004445% to create the 327 FTU/kg (D4 and D8) dietary treatments.

Table 4. Impact of adding insoluble dietary fiber (IDF) on the apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) of different dietary components, experiment 2.[1]

	Basal	Constant nutrients			Constant ingredients				*P*-value	
	Added IDF, %;									
	0	3.3	5.3	8.4	2.9	4.7	7.4	SEM[2]	Linear	Quadratic
AID, %										
DM	77.3	71.7	67.2	59.5	72.0	66.2	61.7	0.9	<0.001	0.344
GE	78.5	73.5	70.7	64.4	74.1	69.7	66.7	0.9	<0.001	0.474
AEE[3]	73.4	70.4	69.2	68.2	73.6	74.7	75.7	1.0	<0.001	0.623
Starch	95.0	94.5	93.6	93.3	94.1	93.1	93.2	0.8	0.036	0.668
IDF	39.6	32.5	26.9	22.4	32.5	20.8	20.5	2.2	<0.001	0.289
SDF[4]	30.6	28.0	12.2	9.1	39.4	16.7	18.5	4.0	<0.001	0.785
TDF[5]	38.8	32.2	25.6	20.9	33.0	20.1	20.3	2.0	<0.001	0.346
NDF	37.9	32.1	27.5	23.2	31.6	21.9	20.7	2.4	<0.001	0.417
ADF	2.3	3.6	4.3	10.6	3.2	3.8	7.2	2.8	0.020	0.451
ATTD, %										
DM	85.7	82.8	78.6	73.7	82.4	78.0	74.3	0.4	<0.001	0.052
GE	84.8	82.3	78.6	74.4	82.1	78.4	75.6	0.5	<0.001	0.108
AEE	61.9	61.7	60.8	60.1	65.4	66.2	67.5	1.0	<0.001	0.510
IDF	53.7	54.5	45.8	41.3	50.3	41.8	40.2	1.1	<0.001	0.051
SDF	73.6	81.6	83.9	87.8	80.1	82.6	82.3	2.5	0.002	0.406
TDF	55.5	57.1	49.9	45.9	53.3	46.3	44.5	1.1	<0.001	0.052
NDF	53.3	50.5	42.7	39.5	48.8	40.8	39.9	1.2	<0.001	0.312
ADF	29.8	32.8	29.4	34.5	32.2	32.3	33.4	1.7	0.753	0.773

[1]9 pigs per treatment.

[2]SEM = pooled SEM.

[3]AEE = acid hydrolyzed ether extract

[4]SDF = soluble dietary fiber

[5]TDF = total dietary fiber

Table 5. Added Insoluble dietary fiber (IDF) linear slope estimates, Experiment 2

		Constant nutrients			Constant ingredients			Constant nutrient vs. Constant ingredients
Item	**Intercept**	**IDF slope Estimate**	**SE**	***P*-value**	**IDF slope Estimate**	**SE**	***P*-value**	***P*-value**
AID, %								
DM	77.8	-2.10	0.13	<0.001	-2.23	0.15	<0.001	0.298
GE	78.7	-1.65	0.12	<0.001	-1.69	0.13	<0.001	0.700
AEE[1]	72.9	-0.62	0.15	<0.001	0.36	0.17	0.044	<0.001
Starch	94.8	-0.17	0.08	0.045	-0.25	0.09	0.012	0.322
IDF	38.6	-1.98	0.33	<0.001	-2.78	0.37	<0.001	0.012
SDF[2]	34.8	-3.26	0.69	<0.001	-2.26	0.80	0.008	0.139
TDF[3]	38.2	-2.10	0.32	<0.001	-2.74	0.36	<0.001	0.034
NDF	37.2	-1.67	0.30	<0.001	-2.47	0.33	<0.001	0.006
ADF	0.93	1.00	0.34	0.006	0.76	0.38	0.054	0.439
ATTD, %								
DM	86.5	-1.48	0.07	<0.001	-1.67	0.08	<0.001	0.008
GE	85.5	-1.28	0.07	<0.001	-1.35	0.08	<0.001	0.264
AEE	62.4	-0.29	0.14	0.047	0.75	0.16	<0.001	<0.001
IDF	55.4	-1.57	0.21	<0.001	-2.25	0.24	<0.001	0.002
SDF	75.6	1.53	0.39	<0.001	1.14	0.45	0.017	0.308
TDF	57.4	-1.25	0.19	<0.001	-1.87	0.22	<0.001	0.002
NDF	53.5	-1.68	0.20	<0.001	-2.06	0.23	<0.001	0.053
ADF	29.9	0.43	0.25	0.094	0.51	0.29	0.084	0.753

[1]AEE = acid hydrolyzed ether extract

[2]SDF = soluble dietary fiber

[3]TDF = total dietary fiber

Table 6. Impact of phytase, fiber and their interaction on growth performance of nursery pigs

	0% corn bran				20% corn bran					*P*- value		
	Phytase FTU/kg											Phytase ×
Item	**0**	**109**	**217**	**327**	**0**	**109**	**217**	**327**	**SEM**	**Phytase**	**Fiber**	**fiber**
Day 0-7												
ADG, kg	0.215	0.219	0.239	0.235	0.220	0.217	0.209	0.242	0.010	0.171	0.493	0.261
ADFI, kg	0.384	0.372	0.382	0.382	0.400	0.378	0.387	0.408	0.009	0.163	0.055	0.658
G:F	0.558	0.582	0.627	0.613	0.551	0.571	0.540	0.591	0.018	0.111	0.022	0.133
Day 8-14												
ADG, kg	0.292	0.316	0.323	0.331	0.281	0.295	0.297	0.302	0.010	0.035	0.005	0.814
ADFI, kg	0.516	0.525	0.537	0.532	0.535	0.532	0.534	0.588	0.012	0.350	0.148	0.636
G:F	0.568	0.603	0.599	0.624	0.524	0.553	0.557	0.545	0.015	0.051	<0.001	0.554
Day 15-21												
ADG, kg	0.302	0.342	0.346	0.361	0.308	0.318	0.345	0.385	0.014	<0.001	0.890	0.369
ADFI, kg	0.658	0.678	0.670	0.683	0.626	0.632	0.640	0.632	0.028	0.944	0.053	0.979
G:F	0.462	0.514	0.515	0.540	0.497	0.502	0.548	0.609	0.031	0.028	0.161	0.638

Table 7. Impact of phytase, fiber and their interaction on BW overall growth performance and bone ash of nursery pigs

	0% corn bran				20% corn bran					*P*- value		
	Phytase FTU/kg											Phytase ×
Item	**0**	**109**	**217**	**327**	**0**	**109**	**217**	**327**	**SEM**	**Phytase**	**Fiber**	**fiber**
Initial BW, kg	7.09	6.64	6.85	6.75	6.76	6.70	6.69	6.85	0.11	0.140	0.295	0.185
Final BW, kg	12.50	12.56	12.86	12.92	12.17	12.20	12.30	12.96	0.15	0.001	0.007	0.263
Day 0-21												
ADG, kg	0.268	0.289	0.300	0.306	0.268	0.274	0.281	0.306	0.006	<.0001	0.033	0.194
ADFI, kg	0.513	0.516	0.523	0.525	0.515	0.507	0.514	0.528	0.009	0.381	0.632	0.840
G:F	0.521	0.559	0.574	0.582	0.519	0.538	0.544	0.578	0.012	<0.001	0.097	0.641
Bone ash, %	44.6	45.8	47.1	47.4	44.6	45.8	47.1	47.5	0.8	0.005	0.949	0.999

Table 8. Impact of phytase, fiber, and their interaction on apparent total tract (ATTD) of DM and fiber components[1], experiment 2

	0% corn bran				20% corn bran					*P*- value		
	Phytase, FTU/kg											Phytase
Item	**0**	**109**	**217**	**327**	**0**	**109**	**217**	**327**	**SEM**	**Phytase**	**Fiber**	**× fiber**
ATTD, %												
DM	85.1	85.1	85.1	85.1	75.4	74.8	74.6	74.3	0.4	0.670	<0.001	0.688
NDF	55.4	54.3	55.6	53.4	16.3	10.6	8.2	10.6	2.0	0.187	<0.001	0.190
ADF	56.2	54.0	55.8	51.8	7.3	4.9	2.0	3.6	2.2	0.343	<0.001	0.588

[1]Neutral detergent fiber = NDF; acid detergent fiber = ADF.