UTILIZATION OF ENERGY IN HIGH-FIBER DIETS FED TO PIGS

BY

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DISSERTATION

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ABSTRACT: Five experiments were conducted to investigate the utilization of energy in high-fiber diets fed to pigs. Experiment 1 determined the DE, ME, and NE of diets with 0, 15, or 30% wheat bran added to a corn-soybean meal-based diet fed to growing pigs. Indirect calorimetry also was used to determine O_2 consumption and CO_2 and CH_4 production to calculate heat production by pigs. Results indicated that daily O_2 consumption and CO_2 and CH_4 production by pigs fed increasing concentrations of wheat bran linearly decreased ($P \leq 0.05$) resulting in a linear decrease ($P \leq 0.05$) in heat production. The DE (3,454, 3,257, and 3,161 kcal/kg), ME (3,400, 3,209, and 3,091 kcal/kg), and NE (1,808, 1,575, and 1,458 kcal/kg) of diets decreased ($P \leq 0.05$) linearly as wheat bran inclusion increased. Experiments 2 and 3 were conducted to determine effects of dietary fiber concentration and addition of a *Bacillus*-based direct-fed microbial (DFM) on wean-to-finish pigs. Results indicated that nursery pigs fed high-fiber diets had reduced ($P \leq 0.05$) BW at the end of the nursery compared with nursery pigs fed low-fiber diets. This was because nursery pigs fed high-fiber diets had depressed ($P \leq 0.05$) ADFI compared with nursery pigs fed low-fiber diets, indicating that diet bulk may be a hindrance to nursery pig feed intake. However, once pigs entered the grow-finish phase of the experiment (Exp. 3), high-fiber fed pigs experienced compensatory growth and, therefore, BW of high-fiber-fed pigs was not different compared with low-fiber-fed pigs at the end of the finisher. The addition of the *Bacillus*-based DFM to low- or high-fiber diets improved ($P \leq 0.05$) G:F in nursery pigs. We hypothesized DFM addition would increase dietary fiber fermentation, thereby increasing VFA concentration and available energy; however, this was incorrect and we observed no effect of DFM supplementation on VFA concentration in the cecum or feces of nursery pigs. Results also indicated that pigs fed high-fiber diets had decreased ($P \leq 0.05$) dressing percentage because weight of the large intestine was increased ($P \leq 0.05$) compared

with pigs fed low-fiber diets. The objective of Exp. 4 was to determine the effects of dietary fiber, a *Bacillus*-based DFM, and feeding duration on apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrients and energy by growing pigs. Results indicated that AID of starch increased ($P \leq 0.05$) as period (i. e., feeding duration) increased, regardless of diet type, which increased ($P \leq 0.05$) ME as period increased. Contrary to our hypothesis, the ATTD of ADF or NDF was not increased as period increased. Addition of DFM to the low-fiber diet increased ($P \leq 0.05$) the AID of ADF, NDF, Lys, Phe, and Glu. Experiment 5 was conducted to determine the disappearance of energy and dietary fiber fractions in the stomach and small intestine, cecum, and colon of pigs fed a corn-soybean meal basal diet or the basal plus distillers dried grains with solubles (DDGS), wheat middlings, or soybean hulls. The apparent cecal digestibility (ACD) and ATTD of soluble dietary fiber was not different among pigs fed experimental diets. Pigs fed the basal diet, or the basal diet plus wheat middlings, had greater ($P \leq 0.05$) ACD of insoluble dietary fiber compared with pigs fed the basal diet plus DDGS or soybean hulls, whereas pigs fed the basal plus DDGS diet had greater ($P \leq 0.05$) ACD of insoluble dietary fiber compared with pigs fed the basal plus soybean hulls diet. Wheat middlings had greater ($P \leq 0.05$) disappearance of dietary fiber fractions compared with DDGS and soybean hulls. Physical characteristics of dietary fiber in experimental diets were not correlated with digestibility of nutrients and energy by pigs. In conclusion, utilization of energy by pigs fed high-fiber diets, especially diets with a substantial concentration of insoluble dietary fiber and a minimal concentration of soluble dietary fiber, was not improved because of increased dietary fiber digestibility or fermentability, but was improved by increased gastrointestinal tract weight that allowed for increased intake of a high-fiber diet.

Key Words: adaptation, dietary fiber, digestibility, direct-fed microbial, fermentation, pigs

DEDICATION

This dissertation is dedicated in loving memory to my father, William Anthony Jaworski.

When this life I'm in is done,
And at the gates I stand,
My hope is that I answer all
His questions on command.

I doubt He'll ask me of my fame,
Or all the things I knew,
Instead He'll ask of rainbows sent
On rainy days I flew.

The hours logged, the status reached,
The ratings will not matter.
He'll ask me if I saw the rays
And how He made them scatter

Or what about the droplets clear,
I spread across your screen?
And did you see the twinkling eyes,
Of student pilots keen?

How fast, how far, how much, how high?
He'll ask me not of these things
But did I take the time to watch
The moonbeams wash my wings?

And did you see the patchwork fields
And mountains I did mold,
The mirrored lakes and velvet hills,
Of these did I behold?

And when the goals are reached at last,
When all the flying's done,
I'll answer with no regret –
Indeed, I had some fun.

So when these things are asked of me,
And I can reach no higher,
My prayer this day - His hand extends,
To welcome home a Flyer.

Patrick J. Phillips

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CHAPTER 1: INTRODUCTION

Swine production, and agriculture as a whole, must become more sustainable to continue to be profitable and reach the goal of increasing food production by approximately 70% to feed an estimated 9.1 billion people by 2050 (Alexandratos and Bruinsma, 2012). Sustainable swine production effectively minimizes the use of resources, such as feed, to ensure that resources are not depleted so that food production may continue. Today, sustainability of swine production is threatened by the growing competition of feed for fuel and food for human consumption, but this is no different than when Dr. D. E. Becker, a scientist at the University of Illinois at Urbana-Champaign, began the implementation of the "gold standard" corn-soybean meal-based swine diet (Becker et al., 1953). Soybean meal was a co-product of the soybean crushing industry that was un-utilizable for human consumption, and Dr. Becker found a place for this co-product in swine diets. Still today, new industries provide the swine industry with co-products that typically are less expensive and un-utilizable for other purposes. Therefore, swine production must take advantage of co-products to increase the sustainability of pork production.

Co-products are typically less expensive because they mostly contain dietary fiber, which cannot be digested by the pig (Anquita et al., 2006; Jaworski et al., 2015). Pigs may obtain energy from dietary fiber by microbial fermentation in the hindgut, which supplies the pig with volatile fatty acids (**VFA**) used to synthesize energy or adipose tissue (Bach Knudsen, 2001). However, the energy contribution from VFA is not as efficient as the energy contribution obtained from enzymatic hydrolysis in the small intestine, because microbial fermentation of a feedstuff is not complete, and will result in energetic losses through the production of methane and carbon dioxide (Bach Knudsen, 2001). Also, the energy contribution from dietary fiber is not equal among feed ingredients and may have interactions within a mixed diet. When diets contain

fibrous co-products and are formulated to contain similar concentrations of metabolizable energy and standardized ileal digestible amino acids as in a standard corn-soybean meal diet, pig growth performance and efficiency is reduced although, hypothetically, performance should be similar (Gutierrez et al., 2013; Jaworski et al., 2014). Performance was not similar because pigs were unable to consume enough high fiber feed, due to reduced diet bulk density, to meet daily energy requirements. Therefore, the current research was carried out to help advance co-product utilization in swine diets.

The overall goal of this work was to investigate the energy contribution and quantify negative effects of dietary fiber from different co-products added to a corn-soybean meal-based swine diet. A second objective was to test the hypothesis that addition of a direct-fed microbial to high-fiber corn-soybean meal-co-product-based diets fed to pigs would increase dietary fiber fermentation and, subsequently, pig performance would be improved. A third objective was to test the hypothesis that fermentation of dietary fiber will increase with pig BW and age when pigs are fed a high-fiber diet because the gastrointestinal tract of the pig will increase in size and the microbial population in the hindgut will also increase. The fourth objective was to quantify degradation of different dietary fiber fractions in the stomach and small intestine, cecum, and colon of pigs and to determine if dietary fiber degradation is correlated with physicochemical characteristics of dietary fiber present in mixed diets.

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CHAPTER 2: APPROACHES TO INCREASE THE UTILIZATION OF ENERGY IN HIGH-FIBER DIETS FED TO PIGS: REVIEW OF LITERATURE

INTRODUCTION

New agro-industrial industries provide the swine industry with co-products that typically are less expensive because they are often not utilizable for other purposes. Therefore, swine production must take advantage of co-products to increase the sustainability of pork production from an economic and social perspective. However, the sustainability of pork production from an environmental perspecitvie is not always increased when co-product inclusion is increased in swine diets (Mackenzie et al., 2016). For the purposes of this dissertation, therefore, sustainability of pork production from an economic and social perspective was utilized, but environmental considerations warrant further investigation.

Most cereal grain co-products contain a larger proportion of dietary fiber compared to the parent grain (Bach Knudsen, 1997; Jaworski et al., 2015). The pig lacks digestive enzymes to digest dietary fiber and, therefore, dietary fiber must be fermented by the microbes in the intestinal tract of the pig to obtain energy (Anguita et al., 2006). Microbial fermentation provides the pig with VFA that the pig may convert to ATP, which provides the pig with energy. However, fermentation of dietary fiber results in less energy than does starch hydrolysis, which is typically supplied by feeding pigs diets containing a large amount of cereal grains and this is one reason co-products are typically less expensive than cereal grains (Bach Knudsen, 2001). Also, dietary fiber may reduce the digestibility of nutrients and energy supplied by other feed

ingredients included in the diet (Noblet and Le Goff, 2001; Cervantes-Pahm et al., 2014a). These negative effects of dietary fiber reduce pig growth and efficiency (Bindelle et al., 2008). When diets containing fibrous co-products are formulated to contain similar concentrations of ME and standardized ileal digestible AA as a standard corn-soybean meal diet, pig growth performance and efficiency are sometimes reduced although, hypothetically, performance should be similar (Gutierrez et al., 2013; Jaworski et al., 2014). It is, therefore, necessary to further investigate characteristics of dietary fiber that hinder efficient utilization of energy in dietary fiber and to design strategies that may contribute to increased utilization of dietary fiber in pig diets.

CHEMICAL CHARACTERISTICS OF DIETARY FIBER

Dietary fiber is composed of non-starch polysaccharides (**NSP**) and lignin (Bach Knudsen, 1997). Non-starch polysaccharides also are present in the cell wall of plants and the main NSP in cereal grains and grain co-products commonly used in swine diets are arabinoxylans and cellulose (Jaworski et al., 2015). Lignin is composed of polymers of phenylpropanoids and is present in the cell wall of plants and increases in concentration as the plant matures to provide rigidity to the plant (Liyama et al., 1994). Dietary fiber may be soluble or insoluble and this distinction is important when considering the subsequent energy value of fiber fed to pigs (Urriola et al., 2010).

Insoluble Dietary Fiber

Insoluble dietary fiber is composed of lignin, cellulose, and insoluble hemicelluloses. The majority of dietary fiber in ingredients commonly used in swine diets is insoluble (Jaworski et al., 2015). Insoluble dietary fiber increases passage rate, fecal bulk, frequency of laxation, and renders softer feces (Dreher, 2001; Wenk, 2001). Insoluble dietary fiber is less fermentable

compared with soluble dietary fiber. The apparent total tract digestibility (**ATTD**) of insoluble dietary fiber in corn distillers dried grains with solubles (**DDGS**), sorghum DDGS, and a corn-sorghum DDGS blend was 40.3, 41.3, and 28.6%, respectively (Urriola et al., 2010). Therefore, more than 50% of the insoluble dietary fiber in DDGS does not provide energy to the pig. The amount of microbial activity in the large intestine is dependent upon body temperature, presence of fermentable substrates, endogenous secretions, pH, and rate of passage of digesta (Wenk, 2001). A diet composed primarily of wheat bran produced a greater concentration of ATP in the cecum and colon of pigs, whereas a wheat flour diet produced greater concentrations of ATP at the terminal ileum, which is an indication of greater microbial activity in the hindgut of pigs when fed a diet composed primarily of wheat bran (Jørgensen and Just, 1988). This is because wheat flour will be mostly digested by the end of the ileum, leaving little substrate for microbial degradation in the large intestine, whereas wheat bran will not be digested in the small intestine, leaving a large amount of fermentable substrate for the microbial population in the large intestine.

Soluble Dietary Fiber

Soluble dietary fiber is composed mostly of soluble hemicelluloses, pectins, and gums. Most ingredients fed to pigs are low in soluble dietary fiber (Jaworski et al., 2015); however, soybean hulls are a common co-product fed to pigs in the United States and contain approximately 8% soluble dietary fiber (Burkhalter et al., 2001). Soluble dietary fiber results in increased digesta viscosity, decreased gastric emptying, increased satiety, reduced rate of glucose uptake, lower blood cholesterol concentrations, and promotes gut commensal bacterial growth (de Godoy et al., 2013). Soluble dietary fiber negatively impacts small intestinal nutrient absorption through the ability to rapidly hydrate and form a viscous gel (Blaxter et al., 1990).

Soluble dietary fiber also increases water binding capacity (**WBC**) and digesta retention time; therefore, microbes have better access to ferment soluble dietary fiber and that is the major reason the ATTD of soluble dietary fiber by pigs is 92.0% in corn DDGS (Urriola et al., 2010). However, the amount of soluble dietary fiber in corn DDGS is approximately 1.1% and diets containing DDGS contain 1.3%. Therefore, the relative energy contribution of soluble dietary fiber in a typical U.S. pig diet is low, but because of the almost complete fermentation and VFA yield from soluble dietary fiber, it is important to quantify the amount of soluble dietary fiber that is included in the diet.

PHYSICOCHEMICAL CHARACTERISTICS OF DIETARY FIBER

Physicochemical characteristics of dietary fiber include WBC, swelling, viscosity, and bulk density (Eastwood and Morris, 1992). These characteristics are associated with the chemical composition of dietary fiber and, therefore, it may be possible to correlate the physicochemical characteristics of dietary fiber with soluble or insoluble dietary fiber. If feed manufacturers and pig producers are able to quickly analyze diets and ingredients for these physicochemical characteristics and relate them to the amount of soluble and insoluble dietary fiber it may be possible to obtain a better estimate of the amount of energy the diet will provide to the pig.

Water Binding Capacity

Water binding capacity is an estimate of the quantity of water retained in dietary fiber that has been hydrated and after the application of an external force (Robertson et al., 2000). The ability of dietary fiber to hold water is dictated by the composition of NSP, the intermolecular organization of the NSP, and the degree of lignification (Serena and Bach Knudsen, 2007). The method used to measure WBC is quick and easily reproducible and, therefore, may be

advantageous for use in the swine feed industry (Canibe and Bach Knudsen, 2002). Soluble dietary fiber typically has a greater WBC than insoluble dietary fiber, and cellulose and lignin have a low WBC (Auffret et al., 1994; Robertson et al., 2000; Shelton and Lee, 2000). In contrast, arabinose and xylose concentrations are positively correlated with WBC (Holloway and Greig, 1984). The concentration of soluble NSP in brewer's spent grain, pea hull, rye grass, potato pulp, sugar beet pulp, and pectin residue is positively correlated with WBC (Serena and Bach Knudsen, 2007). However, the apparent ileal digestibility (**AID**) of starch may be negatively affected by WBC of diets fed to growing pigs and sows, and dietary ME is reduced if diets have greater WBC due to increased concentrations of dietary fiber (Canibe and Bach Knudsen, 2002; Serena et al., 2008). Increasing WBC also may result in increases in endogenous losses of N, and the AID of CP by pigs fed semi-purified diets is reduced if diets have increased WBC (Leterme et al., 1998; Cervantes-Pahm et al., 2014a). The AID of GE and starch is less in dehulled barley compared with corn, which may be a result of increased WBC in dehulled barley (Cervantes-Pahm et al., 2014b). However, hindgut disappearance of GE is 62.8% greater in pigs fed dehulled barley compared with pigs fed corn, which indicates that WBC is related to the amount of soluble dietary fiber in a feed ingredient and, therefore, the degree of fermentation in the hindgut (Cervantes-Pahm et al., 2014b). Water binding capacity of diets also increased when 5, 10, or 15% copra meal or palm kernel expellers were included in weanling pig diets, and this corresponded with a linear reduction in pig ADG (Jaworski et al., 2014). Thus, it is possible that WBC of ingredients can be used to assess the feeding value of diets and ingredients.

Swelling Capacity

Swelling capacity of dietary fiber is a measure that quantifies the volume occupied by dietary fiber when hydrated (Auffret et al., 1994). The swelling capacity of dietary fiber is

affected by NSP composition and organization, and lignification, and as is the case for WBC, the concentration of soluble NSP in brewer's spent grain, pea hull, rye grass, potato pulp, sugar beet pulp, and pectin residue is positively correlated with soluble NSP (Serena and Bach Knudsen, 2007). The close relationship between swelling and the concentration of soluble NSP is expected because the first step in solubilization of NSP is swelling through the addition of water, which spreads the NSP until they are extended and dispersed. This increases the surface area, which results in greater access for microbial colonization and degradation of the substrate and, therefore, ingredients and diets that have a high swelling capacity are expected to ferment to the greatest extent (Noblet and Le Goff, 2001; Canibe and Bach Knudsen, 2002). Swelling capacity of wheat bran, pea hulls, sugar beet fiber, and citrus fiber was decreased when the particle size of the ingredients was reduced from 900 to 540 to 320 μm (Auffret et al., 1994). A similar effect of grinding was reported by Serena and Bach Knudsen (2007) and it was concluded that after freeze-drying and milling of ingredients, the plant cells within the ingredients are no longer capable of binding water and swelling to the same extent as they are in the original ingredient. As the swelling capacity of dietary fiber in a diet or ingredient increases, the swelling capacity of stomach and small intestinal contents is increased, which may result in slower gastric emptying, increased satiety, and increased bacterial fermentation in the cecum and colon of the pig (Canibe and Bach Knudsen, 2002; Serena et al., 2009).

Viscosity

Viscosity of dietary fiber refers to the ability to thicken or form gels in solution (Dikeman and Fahey, 2006). Water has low viscosity and is free flowing, whereas honey has high viscosity and the flow is much more resistant. Viscosity was first defined by Sir Isaac Newton as the proportional relationship between the flow of a fluid and the force directed on that fluid.

Viscous dietary fibers include most soluble NSP, but gums, pectins, and β-glucans are the NSPs with the greatest viscosity (Dikeman and Fahey, 2006). Insoluble NSP typically are not associated with viscosity; however, they may influence viscosity through the absorption of water (Takahashi et al., 2009). The viscosity of brewer's spent grain, pea hull, rye grass, potato pulp, sugar beet pulp, and pectin residue was not much greater than the viscosity of water, despite a concentration of soluble NSP ranging from 2.1 to 29.0% in these ingredients (Serena and Bach Knudsen, 2007). However, when the ingredients were included in diets formulated for sows, viscosity of diets was positively correlated with the concentration of soluble NSP in diets (Serena et al., 2008). The difference between viscosity of ingredients and diets may imply that viscosity may not only be affected by soluble NSP, but also starch, protein, and lipid that are present in mixed diets.

The quantity of dietary fiber also affects the viscosity, but in a non-linear fashion indicating that there is a critical concentration at which point physical entrapment occurs and molecular movement is impaired (Oakenfull, 2001; Dikeman and Fahey, 2006). Viscosity also displays positive correlation with the molecular weight of dietary fiber (Tosh et al., 2004; Lan-Pidhainy et al., 2007; Le Gall et al., 2009).

Once NSP are ingested and mixed with gastrointestinal fluids, they may thicken and become viscous by forming physical entanglements, overlapping and interpenetrating one another within the fluid. Due to this physical entanglement, the digestion and absorption of nutrients and energy in the small intestine may be negatively affected (Eastwood and Morris, 1992). Two mechanisms of action may explain how increased viscosity may reduce small intestinal digestion and absorption of nutrients and energy. First, viscosity may reduce the ability of digestive enzymes to reach their substrates, therefore reducing the digestion of nutrients and

energy. The second mechanism is that viscosity may impair peristalsis and mixing of digesta in the lumen of the small intestine; therefore, diffusion and transport of nutrients across the unstirred water layer may be restricted, reducing the absorption of nutrients and energy (Eastwood and Morris, 1992). Pigs fed rye bread had an increased ileal digesta viscosity compared with pigs fed wheat bread and the increased viscosity may have been the reason AID of starch and fat by pigs fed rye bread was less than that of pigs fed wheat bread (Le Gall et al., 2009). A diet containing 11.0 and 30.2% soluble and insoluble dietary fiber, respectively, had increased diet viscosity compared with a diet containing 7.3 and 36.8% soluble and insoluble dietary fiber, respectively, but small intestinal digesta viscosity in sows fed these diets was unaffected (Serena et al., 2008). However, sows had greater nutrient digestibility and energy utilization when fed the diet containing more soluble dietary fiber with greater viscosity. It was concluded that viscosity of ileal digesta of sows may not impair nutrient and energy digestibility and absorption (Serena et al., 2008). A semi-purified diet that was low in soluble dietary fiber and contained synthetic cellulose had a much lower viscosity and produced a lower ileal digesta viscosity than a semi-purified diet containing more soluble dietary fiber and carboxymethyl cellulose (Hooda et al., 2011). When both diets were fed to growing pigs, the AID and ATTD of nutrients and energy were reduced in pigs fed the low viscosity diet, which was due to decreased digesta passage rate in pigs fed the high viscosity diet compared with the low viscosity diet (Hooda et al., 2011). This observation is in agreement with data indicating that soluble dietary fiber in guar gum increased ileal digesta viscosity, but had no effect on the AID of nutrients and energy because passage rate to the ileum was reduced and total tract retention time was increased (Owusu-Asiedu et al., 2006). In theory, high viscosity in diet and digesta should impair nutrient digestibility, but it appears that increased viscosity in the small intestine slows gastrointestinal

transit time and, therefore, allows more time for enzymatic digestion and microbial fermentation, which negates possible negative effects of increased viscosity. However, it is possible that synthetic fiber sources have different effects on diet and digesta viscosity compared with fiber sources that are typically included in swine diets. Compared with corn, Nutridense corn, dehulled barley, dehulled oats, polished white rice, sorghum, and wheat, feeding of rye increased ileal digesta viscosity in pigs and, therefore, AID and ATTD of nutrients and energy in rye were less than for the other cereal grains (Cervantes-Pahm et al., 2014b).

Bulk Density

Bulk density is a measure of the weight of a feed ingredient or diet when placed in a container with a known volume (Giger-Reverdin, 2000). Bulk density is measured by placing a feed ingredient or diet in a graduated cylinder with a known volume and the weight is recorded (Cromwell et al., 2000). Bulk density of 24 different feed ingredients with a wide range of dietary fiber composition had a strong negative correlation with the NDF concentration in the ingredients and, therefore, bulk density may give a good approximation of the quantity of insoluble dietary fiber within a feed ingredient (Giger-Reverdin, 2000). Bulk density of diets also decreased due to addition of wheat bran and dried grass meal, but bulk density of diets was increased by dried citrus pulp, indicating bulk density decreased in diets due to increased concentrations of insoluble dietary fiber, whereas bulk density is unaffected or may be increased in diets due to increased concentrations of soluble dietary fiber (Kyriazakis and Emmans, 1995). Addition of 5, 10, or 15% copra meal, palm kernel expellers, or palm kernel meal to diets also reduced bulk density of diets, further indicating that increased NDF results in reduced bulk density (Jaworski et al., 2014). Bulk density also may provide an indication of feed intake of pigs because when a bulky, less digestible fibrous feed ingredient is added to a diet, the pig will

increase feed intake to maintain a constant intake of DE, which will maintain growth. However, there is a point at which the pig is unable to consume enough of the bulky, fibrous feed ingredient to maintain growth and this effect is referred to as gut fill (Kyriazakis and Emmans, 1995). Also, as bulk density of diets decreased, weight of the gastrointestinal tract of pigs was increased, thereby increasing the energy required by the pig for maintenance (Kyriazakis and Emmans, 1995). Therefore, bulk density of diets may provide an indication of feed intake and gastrointestinal tract weight, both of which affect energy utilization of pigs.

UTILIZATION OF DIETARY FIBER

Dietary fiber must be fermented by microbes in the gastrointestinal tract of the pig to obtain energy because the pig lacks digestive enzymes capable of dietary fiber digestion (Anguita et al., 2006). Fermentation is defined as an enzymatically controlled anaerobic breakdown of an energy containing compound which, in the case of the pig, is typically dietary fiber because most other nutrients are digested and absorbed by the end of the small intestine. Total viable counts of anaerobic bacteria increase from 10^7 viable counts in the pig stomach to 10^9 viable counts in the distal ileum to 10^{12} viable counts in pig feces (Jensen and Jørgensen, 1994). Microbial populations increase from the stomach to the large intestine in pigs because the large intestine has a low oxygen concentration, a low flow rate, and a high moisture content, which are all favorable conditions for microbial growth (Bach Knudsen et al., 2013).

Most fermentation occurs in the hindgut of the pig (i. e., cecum and large intestine); however, the AID of NSP by pigs ranges from -7 to 40%, indicating that some fermentation can occur prior to the hindgut of the pig (Bach Knudsen et al., 2013). Fermentation is a symbiotic

advantage for the pig and the microbial population in the gastrointestinal tract because the microbes enzymatically break down dietary fiber into products that the microbes may use as an energy source, but also the microbes break down dietary fiber into smaller energy-containing end-products that can be further oxidized by the pig to obtain energy. Dietary fiber is fermented into smaller polysaccharides, and monomers are absorbed by the microbes and metabolized to ATP (White, 2000). Through this process, intermediates (i. e., by-products) such as ethanol, lactate, and succinate are produced by the microbes and are excreted (Flint et al., 2008). Other microbes then can use these intermediates as a substrate and excrete a second product (Urriola, 2010). The final end-products of microbial fermentation of dietary fiber are acetate, propionate, butyrate, ammonia, hydrogen, methane, and carbon dioxide (Jensen and Jørgensen, 1994).

The gases produced are a loss of energy because the pig is unable to absorb and metabolize the gases and approximately 25% of dietary energy is lost in these gases (Jørgensen, 2007; Bach Knudsen et al., 2013). The gases must be excreted through flatus, and most of the ammonia and methane are excreted this way. However, only small amounts of hydrogen are excreted as flatus by the pig and a number of different pathways have been suggested (Jensen and Jørgensen, 1994). In ruminants, hydrogen is used by methanogens to produce methane that is eructated, but the amount of methanogens in the hindgut of pigs is fairly low so the hydrogen must be eliminated through other routes such as the saturation of unsaturated fatty acids, reduction of nitrate to ammonia, reduction of sulfate to sulfide, reduction of carbon dioxide to methane, and reduction of carbon dioxide to acetate (Jensen and Jørgensen, 1994).

The common ratio of short-chain fatty acids found in feces is 60:20:20 acetate, propionate, and butyrate, respectively (Flint et al., 2012). However, different sources of dietary fiber may affect this ratio producing ranges from 60-90, 10-30, and 1-20 for acetate, propionate,

and butyrate, respectively (Titgemeyer et al., 1991). The fermentation of insoluble dietary fiber in the form of wheat bran yields a greater amount of propionate, whereas the fermentation of soluble dietary fiber in the form of sugar beet fiber yields a greater amount of acetate (Michel and Rerat, 1998). The fermentation of resistant starch yields greater acetate production, but also the molar ratio of butyrate to acetate and propionate is increased (van der Meulen et al., 1997; Topping and Clifton, 2001; Guiberti et al., 2015).

Short-chain fatty acids are absorbed through passive diffusion, carrier-mediated, or by transporters. Passive diffusion requires the protonated form, and only 1% of total short-chain fatty acids in the intestinal lumen is protonated, but hydrogens are exchanged at the apical epithelium where the pH is lower compared with the center of the lumen and, therefore, almost 50% of short-chain fatty acids are protonated by the time they are present at the apical epithelium (Cook and Sellin, 1998). It has been indicated that up to 60% of short-chain fatty acids are absorbed this way.

The carrier-mediated mechanism exchanges bicarbonate for the short-chain fatty acid at the intestinal epithelium (Cook and Sellin, 1998). More recent discoveries have indicated that short-chain fatty acid transporters exist throughout the human body, but abundance of transporters corresponds with short-chain fatty acid production (Gill et al., 2005). These transporters are known as monocarboxylate transporters (***MCT***) and *MCT1* is the transporter that is present in the pig intestine (Welter and Claus, 2008). A second transporter in the colon of humans is the sodium monocarboxylate transporters (***SMCT***), which is a sodium-coupled electrogenic transporter with a high affinity for butyrate (Thangaraju et al., 2008). The *SLC5A8* form of 1 *SMCT* was first identified due to its ability to aid in butyrate transport and caused growth arrest and apoptosis in human colon cancer cells (Ganapathy et al., 2013). Little research

has been conducted on *MCT* in pigs because it has been assumed that absorption of short-chain fatty acids occurs mainly through passive diffusion and maximum absorption is always reached. This assumption is a result of infusion studies that indicate less than 1% of short-chain fatty acids infused in the cecum are recovered in the feces of pigs (Jørgensen et al., 1997). However, many experiments utilize the concentration of short-chain fatty acids in ileal and cecal digesta and feces of pigs as an indication of the amount of fermentation that occurred (Urriola and Stein, 2010; Jaworski et al., 2014; Rojas, 2015). Short-chain fatty acids recovered in feces have not been absorbed by the pig and, therefore, absorption of short-chain fatty acids is not maximized by the pig and this is a loss of energy from the fermentation of dietary fiber. More research is necessary on the absorption of short-chain fatty acids by pigs to increase the amount of energy obtained from the fermentation of dietary fiber.

ENERGY SYSTEMS

Gross Energy

Gross energy, also known as the heat of combustion ($\mathbf{\Delta H_c}$), is defined as the amount of energy released as heat when a compound is oxidized completely. This energy value is typically expressed as calories per gram or as Joules per gram. Joule is the international unit for expressing energy (1 calorie = 4.184 J). Gross energy in animal nutrition experiments is determined directly through the use of a bomb calorimeter. Classical bomb calorimetry, known as adiabatic bomb calorimetry, was first proposed by Holman (1895). In adiabatic calorimetry, there is no exchange of heat between the calorimeter and the surroundings, hence the term adiabatic (McLean and Tobin, 1987). Today, most calorimeters are isoperibol, which allows for

heat exchange between the calorimeter and the environment and a microprocessor in the calorimeter measures the effect of any heat leak (Zumdahl and DeCoste, 2010). Gross energy also may be estimated from the chemical composition of a compound where carbohydrates contain a range from 3.7 kcal / kg (glucose and simple sugars) to 4.2 kcal / kg (starch and cellulose), protein contains 5.6 kcal / kg, lignin contains 6.9 kcal / kg, and fat contains 9.4 kcal / kg (Atwater and Bryant, 1900; Jung et al., 1999). The GE in a molecule increases as the carbon chain in that molecule increases (Pond et al., 2005). The amount of ether extract (**EE**), CP, and ash that a feed ingredient or diet may contain also can be used to predict the GE in that ingredient or diet using Eq. [1] (Ewan, 1989):

$$GE = 4{,}143 + (56 \times \%\ EE) + (15 \times \%\ CP) - (44 \times \%\ Ash). \quad [1]$$

However, the GE of feed ingredients is a measurement of potential energy, which is not an appropriate measure to determine which ingredients will provide the most energy to the pig (NRC, 2012; Table 2.1).

Digestible Energy

The amount of energy that is digested throughout the entire gastrointestinal tract is defined as DE. The DE of a feed ingredient or diet may be calculated by subtracting the amount of GE in feces from the amount of GE in the ingredient or diet (NRC, 2012). However, this is an apparent measurement because feces contains endogenous losses of cells, microbes, enzymes, and by-products from microbial fermentation (Pond et al., 2005). This becomes more important pertaining to fibrous ingredients and diets because endogenous losses of energy containing components are expected to increase as dietary fiber concentration increases and, therefore, DE of a fibrous ingredient or diet will be underestimated. On the other hand, endogenous losses of

energy containing components are a loss of energy to the pig and, therefore, DE values may be appropriate because endogenous losses of energy are included in the maintenance energy requirement of swine.

Metabolizable Energy

Metabolizable energy is equal to GE minus DE minus energy lost in urine and fermentative gases (NRC, 2012). The energy lost as fermentative gases is relatively low in growing pigs and has been reported to range from 0.008 to 0.10% of dietary DE in 120 to 150 kg pigs (Noblet and Le Goff, 2001). Therefore, most ME values are calculated by subtracting GE in urine from DE values. Urinary energy losses are mostly influenced by the AA balance in the diet. However, if a greater amount of fibrous co-products are fed to growing swine, the potential for increasing the production of fermentative gases is greater. Also, urinary N excretion is decreased if the concentration of dietary fiber is increased in diets fed to growing pigs because of the increased microbial mass in the large intestine. Microbes utilize N bound for urinary excretion as a substrate for growth (Zervas and Zijlstra, 2002). Therefore, ME values may overestimate the available energy in fibrous co-products due to the increased loss of fermentative gases and the shift from urinary N excretion to fecal N excretion.

Net Energy

In the United States, the energy content of pig feed ingredients has generally been evaluated using DE and ME systems. However, the energy value of high-fiber or high-protein ingredients may be overestimated, whereas the energy value of high-fat or high-starch ingredients may be underestimated using DE and ME systems (Noblet and Henry, 1993). Therefore, a more accurate estimate of the energy content of pig feed ingredients may be

obtained using a NE system because NE accounts for the heat increment associated with the metabolic utilization of ME and with the energy cost of ingestion, digestion, and physical activity (Noblet and van Milgen, 2004). Also, the NE system expresses diet energy and the energy requirement of the pig on the same basis, making it a more accurate system (Noblet and van Milgen, 2004).

The heat increment is equal to the metabolic rate, which is the heat production (**HP**) per unit of time and is expressed relative to body surface area. Based on research that determined the surface areas of two bodies of similar shape and density, but of different size, it was determined that surface area can be calculated as the two-third power of their weights ($BW^{0.67}$; Kleiber, 1975; van Milgen and Noblet, 2003). Expressing energy values relative to metabolic BW ensures that differences in BW are not the sole cause of changes in heat increment (van Milgen and Noblet, 2003). By measuring the NE of feed ingredients, it may be possible to more accurately predict the energy contribution of the feed ingredient to the diet, especially with regards to ingredients containing high concentrations of dietary fiber. Therefore, it is hypothesized that high-fiber co-products may be more efficiently utilized in pig diets through a more accurate prediction and correlation of the NE contribution in a diet from the physicochemical characteristics of the dietary fiber within feed ingredients.

Net energy is divided into energy used for maintenance (**NE_m**) and energy used for production (**NE_p**). Maintenance energy is measured directly in a fasted state or estimated by regressing energy retention of animals consuming graded levels of ME on energy intake (Noblet and van Milgen, 2013). Energy used for maintenance corresponds to the energy needed for resting heart rate, organ function, and thermogenesis.

Dietary fiber may affect the NE_m in different ways. The most widely accepted cause is that as growing pigs are fed increasing concentrations of dietary fiber, the gastrointestinal tract increases in size and length (Kyriazakis and Emmans, 1995; Jørgensen et al., 1996). Baldwin (1995) reported that the gastrointestinal tract and liver account for 30% of the maintenance energy requirement; therefore, as the gastrointestinal tract becomes larger, the more energy is required to maintain this metabolically expensive organ. The NE_p corresponds to the energy required for productive functions, which include body growth, reproduction, milk production in lactating sows, and fetus growth in gestating sows. Dietary fiber affects the NE_p mainly because the digestibility of energy in dietary fiber is low, but dietary fiber also may limit the digestibility of protein and lipids in the mixed diet, thereby decreasing the amount of energy available for NE_p (Noblet and Le Goff, 2001). The heat increment and NE_p are difficult to separate from total HP, and therefore, NE is usually calculated as the sum of NE_m and retained energy (Noblet, 2007).

Retained energy can be determined using the comparative slaughter method, direct calorimetry, or indirect calorimetry (Kil et al., 2013). The comparative slaughter method involves slaughtering an initial subset of pigs at the beginning of the experiment and then slaughtering all or a subset of animals at the end of the experiment. The total quantity of energy, protein, and lipids in each animal then are calculated from the sum of the energy, protein, and lipids in blood, viscera, and carcass and retained energy is calculated as the difference between final quantity of energy, protein, and lipids, and the initial quantity of energy, protein, and lipids (Kil et al., 2011; Ayoade et al., 2012). When the comparative slaughter method is used, the NE_m must be calculated by multiplying the $BW^{0.6}$ by 197 kcal (NRC, 2012), which is the estimated maintanence energy requirement of growing swine. The advantages of this method are that a

better estimate of body composition gain is obtained, the feeding, housing, and management systems usually resemble those similar to commercially raised pigs, and this method is less expensive because no respiration chambers are necessary (Kil et al., 2013). The disadvantages of the comparative slaughter method are that the energy concentration of the initial group of slaughtered pigs may not appropriately account for the large variation typically found in the energy concentration of the pigs fed the experimental diets. Therefore, in order to account for this error, large numbers of animals and long experimental periods must be utilized (Quiniou et al., 1995; Boisen and Verstegen, 2000; Kil et al., 2013).

Calorimetry has come a long way since the first direct calorimeter was invented in 1782 by Lavoisier, which used a guinea pig inside a chamber surrounded with ice and the heat that the guinea pig produced melted the ice, allowing for the quantification of HP (Lavoisier, 1789). Direct calorimetry measures total heat loss from the animal, whereas indirect calorimetry measures total energy production (Ferrannini, 1988). Temperature in the calorimeter must be constant when using direct calorimetry and, therefore, effects of temperature and humidty on HP may not be determined when using direct calorimetry. Armsby determined that HP measurements determined using direct or indirect calorimetry are not different and, therefore, direct calorimetry is no longer used because it cannot account for differences in temperature (cited by Brody, 1945).

Calorimetry is founded on the first two laws of thermodynamics that state: 1) energy can neither be created nor destroyed, and 2) any change in the total energy content of a system results in a change in both the free energy and the entropy of the system (Kleiber, 1975). Animals consume nutrients that must be oxidized to free chemical energy in order to produce a high-energy compound that can be utilized by the animal (e. g., ATP); however, this process is not

100% efficient and heat is lost during the reaction (Ferrannini, 1988). Therefore, the theory of indirect calorimetry rests on the assumption that all consumed oxygen is utilized to oxidize nutrients to free chemical energy and through this process, carbon dioxide is produced. As a consequence, it is possible to calculate the total amount of heat produced and the amount of energy retained (Ferrannini, 1988). This is possible because HP in indirect calorimetery is calculated from the amount of oxygen consumed and the quantities of carbon dioxide, methane, and urinary N produced during metabolism (McLean and Tobin, 1987). This was developed by Brouwer (1965) using a multiple regression equation based on constants derived from the oxidation of mixed carbohydrates, lipids, and proteins according to Eq. [2] (Brouwer, 1965):

$$\text{HP (kcal)} = 3.866 \times O_2\,(\text{L}) + 1.200 \times CO_2\,(\text{L}) - 0.518 \times CH_4\,(\text{L}) - 1.431 \times \text{urinary N (g)} \qquad [2]$$

where O_2 represents the amount of oxygen consumed by the animal and CO_2, CH_4, and urinary N represent the amount of carbon dioxide, methane, and urinary N, respectively, excreted by the animal. Therefore, an indirect calorimeter unit must be able to measure these parameters to calculate HP. Most indirect calorimetry units place the test subject inside a sealed chamber with a single air inlet and outlet. The chamber has fresh outside air being continually introduced and this is called an open-circuit respiration chamber (Young et al., 1975). One type of open-circuit respiration chamber uses a fan placed at the air outlet to pull fresh air into the chamber and, hence, this system is termed a pull ventilation system and results in a negatively pressurized chamber. A second type is called a push ventilation system that uses a fan to push air through the opening inlet and is a positively pressurized chamber (Brown et al., 1984). A pull system relies on the chamber being completely sealed, whereas a push system relies on the air exiting the chamber through the outlet (Ramirez, 2014). Gas consumption and production then are calculated by the difference in fresh incoming gas minus gas exiting the chamber. Chamber

volume, pressure, temperature, humidity, and the velocity of gas flow also must be measured to complete the calculations (Young et al., 1975). Indirect calorimetry is a much more complex and expensive system used to measure retained energy compared with the comparative slaughter method. However, it is advantageous because the retained energy of pigs may be measured over a short period of time, effects of temperature and humidity on metabolism can be determined, and a more complete picture of metabolism is determined (Kil et al., 2013).

In conclusion, indirect calorimetry typically results in greater estimates of energy retention compared with the comparative slaughter method and this directly effects NE values (Quiniou et al., 1995; van Milgen and Noblet, 2003; Kil et al., 2013). However, Ayoade et al. (2012) recently reported that the NE of diets containing 0, 15, or 30% wheat-corn DDGS was not different when determined with the comparative slaughter or indirect calorimetry method, but retained energy was greater if measured with the comparative slaughter method. Therefore, further research is necessary to elucidate effects of methodology on retained energy and NE of diets.

Factors Affecting Dietary Net Energy

(1) Fasting heat production. The NE_m is equal to fasting heat production (**FHP**) plus energy allocated for physical activity (van Milgen et al., 2001), whereas the NE_m is equal to FHP plus heat increment associated with maintenance (NRC, 2012). Therefore, it is understood that FHP is the best estimate of NE_m. The NE_m for growing swine has been suggested to be 197 kcal/kg $BW^{0.6}$ (Birkett and de Lange, 2001; NRC, 2012). This is typically measured by using indirect calorimetry through fasting the pig and the value obtained is referred to as FHP. This value can range from 191 to 216 kcal/kg $BW^{0.6}$ in growing pigs, and is influenced by the length of fasting period, feeding level, diet composition prior to fasting, physical activity of pigs,

genotype, and sex (Koong et al., 1982; van Milgen et al., 1998; de Lange et al., 2002; Labussière et al., 2011; NRC, 2012). Fasting heat production also can be estimated using linear regression by extrapolating HP measured at different feeding levels to 0 ME intake (**FHP_r**; Noblet and van Milgen, 2013). However, FHP determined directly by fasting the pig is greater compared with FHP_r (de Lange et al., 2006; Noblet and van Milgen, 2013; Liu et al., 2014) and is because previous diet and feeding level affects FHP (Labussière et al., 2011; Zhang et al., 2014). Therefore, it may be more accurate to estimate FHP directly by fasting the pig immediately after a period of feeding (Noblet and van Milgen, 2013). However, when determing FHP_r, Noblet et al. (1994) and Labussière et al. (2011) assumed that the relationship between HP and ME intake below maintenance in pigs was linear. This was because de Lange et al. (2006) determined the relationship between HP and ME intake above maintenance in pigs was linear. However, Kleiber (1975) reported that the partial efficiency of energy utilization for maintenance was greater than it was for production and, therefore, the relationship between HP and ME intake below maintenance in pigs may not be linear. Indeed, the relationship between HP and ME intake below maintenance in pigs is not linear, but exponential (Zhang et al., 2014). Therefore, a more accurate estimate of the NE_m is attained from a wide range of ME intakes both below and above the requirement and estimated using exponential regression between HP and ME intake (Zhang et al., 2014). The NE_m estimated from this approach is 181 and 175 kcal/kg $BW^{0.6}$/d for growing and finishing pigs, respectively (Zhang et al., 2014).

Dietary fiber fed to pigs influences the estimation of FHP due to an increased gastrointestinal size and influences protein and lipid deposition of pigs, which will also affect FHP. Depending on diet formulation and energy digestibility, addition of dietary fiber may limit dietary ME or dietary ME intake. Therefore, lipid deposition will be limited and the subsequently

measured FHP will be less because it is energetically expensive to deposit fat over lean (Quiniou and Noblet, 1995; van Milgen et al., 1998; van Milgen et al., 2001). Addition of dietary fiber to swine diets may reduce HP due to reduced physical activity of pigs; therefore, pigs previously fed high-fiber diets may have reduced physical activity when measuring FHP, which may affect the accuracy of FHP estimates because approximately 8% of ME intake may be used for physical activity in growing pigs (Schrama and Bakker, 1999; van Milgen and Noblet, 2000).

(2) Heat production. Heat production is represented as the amount of energy required by the pig for conversion of feed energy to body energy and the energy cost of physical activity. Heat production is a measure of the conversion of feed to body protein and lipid. Oxidation of organic compounds in diets produces energy that is available to the pig, but through the oxidation process, some energy is lost as heat and, hence, the term HP. Heat production is estimated from gas exchanges and urinary losses of N according to Brouwer (1965) using Eq. [2].

Dietary fiber has a 60% efficiency of ME utilization compared with 60, 82, and 90% for CP, starch, and lipid, respectively (Schiemann et al., 1972; Just et al., 1983; Noblet et al., 1994; van Milgen et al., 2001). Therefore, HP is expected to increase with increased inclusion of dietary fiber in pig diets due to the low efficiency of ME utilization, but also because of increased feed intake, increased size of the gastrointestinal tract in relation to BW, and increased hindgut fermentation resulting in energetic losses of methane (Jørgensen et al., 1996). The HP of gestating sows increased from 6,267 to 6,422 to 6,475 kcal/d when fed a control corn-wheat-barley-soybean meal-based diet (8.6% NDF), the control diet plus 22.2% alfalfa (15.2% NDF), or the control diet plus 22.2% straw (21.4% NDF), respectively (Noblet et al., 1989). Sows fed the control diet and the straw-containing diet lost less than 1% of DE as methane, whereas sows

fed the alfalfa containing diet lost 2.8 to 3.0% of DE (Noblet et al., 1989). Pectin is present in alfalfa and fermentation of pectin results in a greater production of methane, whereas pectin is not present in straw, but straw does contain cellulose and fermentation of cellulose does not result in a large production of methane because it is less fermentable (Müller and Kirchgessner, 1985; 1986). However, methane production is not always indicative of the amount of hindgut fermentation (Noblet et al., 1989). Methane energy loss as a percentage of DE increased from 0.2% when pigs were fed a low-fiber diet to 1.2% when pigs were fed a high-soluble fiber diet (Jørgensen et al., 1996). However, HP was not different between pigs fed the low-fiber (2,149 kcal ME/kg DM) versus the high-soluble fiber diet (2,087 kcal ME/kg DM; Jørgensen et al., 1996). Methane production and HP of group-housed pigs were not different between pigs fed a high-starch diet containing 13.34% tapioca meal compared with pigs fed a high-soluble dietary fiber diet containing 16.66% sugar beet pulp silage formulated to have similar calculated NE concentrations (Schrama et al., 1996). However, pigs fed the sugar beet pulp silage diet were less active compared with pigs fed the high-starch diet; therefore, pigs fed the sugar beet pulp silage diet had a greater amount of HP related to inactivity, which can be inferred to be from the thermic effects of feeding (Schrama et al., 1996).

The thermic effects of feeding are HP related to feed intake and the two components are; 1) short-term due to the ingestion and digestion of feed, and 2) long-term due to the metabolism associated with nutrient deposition (van Milgen and Noblet, 2000; Labussière et al., 2013). In a follow up experiment, Schrama et al. (1998) fed group-housed growing pigs diets containing 0, 5, 10, or 15% sugar beet pulp silage and, again, HP was not different among the diets, but HP related to activity was decreased as the concentration of sugar beet pulp silage increased in the diets. Also, daily methane production increased from 1.17 to 2.29 kcal/kg as sugar beet pulp

silage increased from 0 to 15% in the diets, indicating that a greater amount of fermentation occurs with increased sugar beet pulp silage (i. e., soluble dietary fiber) in the diet (Schrama et al., 1998). Therefore, pigs become less active to compensate for their greater HP associated with the thermic effects of feeding, which in this case is hypothesized to be associated with the increased fermentation due to increased dietary fiber in the diet. Indeed, this is the case because Schrama and Bakker (1999) determined that the HP related to activity of group-housed growing pigs was decreased due to the substitution of gelatinized corn starch (almost completely digested before the cecum in pigs) with raw potato starch (resistant to enzymatic digestion and is fermented in the hindgut of pigs) in the diets. Results of a more recent experiment indicated that the HP related to activity of group-housed growing pigs also was decreased due to the substitution of pregelatinized potato starch with raw potato starch in the diets (Bolhuis et al., 2008), confirming the results of Schrama and Bakker (1999).

Total HP increased in gestating sows fed high-fiber diets compared with sows fed low-fiber diets, and this was mainly caused by the thermic effect of feeding, which was 11.7 and 8.2% of ME intake in gestating sows fed a high-fiber diet or a low-fiber diet, respectively (Ramonet et al., 2000). Gestating sows fed the high-fiber diet also had less HP due to activity because they compensated for the increased HP due to the thermic effect of feeding by being less active (Ramonet et al., 2000). In contrast, HP of group-housed gestating sows was not different when sows were fed diets containing 0, 10, 20, or 30% sugar beet pulp silage, although daily methane production increased from 0.88 to 1.89 kcal/$BW^{0.75}$ as sugar beet pulp silage inclusion increased from 0 to 30% (Rijnen et al., 2001). The HP of sows was not different when fed a corn bran, wheat bran, or sugar beet pulp supplemented diet compared with sows fed a wheat-based control diet (Le Goff et al., 2002). However, the HP associated with the thermic effect of feeding

was greater in the sows fed the wheat bran-supplemented diet, which is in contrast to the conclusion by Schrama and Bakker (1999) that HP and its association with the thermic effect of feeding was due to fermentation of dietary fiber and not due to the bulkiness of fiber. Heat production increased in group-housed growing pigs fed 10 diets with increasing concentrations of copra meal or soybean hulls; however, HP related to activity and resting was not different among diets (Rijnen et al., 2003). Also, HP of pigs fed soybean hulls was slightly greater compared with pigs fed copra meal (Rijnen et al., 2003). Finally, the HP of growing pigs fed diets with 0, 15, or 30% wheat-corn DDGS was not different (Ayoade et al., 2012).

In conclusion, the HP of pigs and sows is influenced by the amount and type of dietary fiber that is fed. The HP contributed by the thermic effects of feeding is increased as dietary fiber increases in the diets and the pig compensates for this energy loss by reducing energy spent on activity. However, results are inconclusive because results of some experiments report increases in HP due to increasing concentrations of dietary fiber, whereas other experiments report no change in HP. It is, therefore, likely that specific properties of dietary fibers may result in different activities of pigs and differences in HP. Therefore, further investigation into the effects of dietary fiber on HP are warranted.

PREBIOTICS

"A non-digestible feed ingredient that alters the compositon, or metabolism, of the gut microbiota in a beneficial matter" is a prebiotic (de Lange et al., 2010). Most prebiotics are oligasschardes that are highly fermentable and include manna oligosaccharides (**MOS**), fructooligosaccharides (**FOS**), galactooligosaccharides, and chitooligosaccharides (Cromwell,

2013). Also, several novel fibers and fermentable carbohydrates exist that elicit a prebiotic response because they increase VFA production thus, reducing intestinal pH and, therefore, positively manipulate microbial populations (Beloshapka, 2011).

Yeast cell walls contain large concentrations of MOS and may be supplemented to pig diets to promote growth (Miguel et al., 2004). It has been indicated that the elicited growth response may be due to the ability of MOS to inhibit attachment of pathogens with type I fimbriae to the intestinal wall of pigs (Che et al., 2012). Price et al. (2010) reported that the addition of MOS (Original XPC; Diamond V, Cedar Rapids, IA) to diets fed to weanling pigs inoculated with *Salmonella* did not increase BW or ADG, but reduced fecal shedding of *Salmonella*. Post-infection results indicated that weanling pigs fed diets supplemented with MOS had greater compensatory BW gain compared with pigs fed diets without MOS and this was attributed to an increase in the beneficial bacteria *Bacteroidetes* and *Lactobacillus* (Price et al., 2000). Further research is necessary to determine the concentration of MOS in co-products, especially with regard to DDGS. This is because corn is fermented by yeast to produce ethanol and DDGS and, therefore, MOS may be present in DDGS.

Short and medium chains of fructose with a terminal glucose unit are FOS. Natural sources of FOS are Jerusalem artichoke, chicory root, onion, asparagus, wheat, rye, and garlic (Clevenger et al., 1988; Cromwell, 2013). Fructooligosaccharides are not digested in the stomach and small intestine of pigs, but serve as a fermentative substrate for some bacteria in the large intestine, which promotes select bacteria such as *Bifidobacterium* spp. and *Lactobacilli* spp. to proliferate at the expense of others (Willard et al., 2000; Swanson et al., 2002). Nursery pigs fed diets supplemented with FOS had increased villus height and villus-to-crypt ratio and this may

be attributed to an increased VFA production because FOS is highly fermentable (Spencer et al., 1997).

In conclusion, most research pertaining to prebiotics has focused on improving health status of weanling pigs. However, due to the high fermentability of prebiotics, a greater research emphasis is necessary on VFA production and absorption and, subsequently, the energetic value prebiotics may have when supplemented to pig diets.

DIRECT-FED MICROBIALS

Direct-fed microbials (**DFM**), which may be more commonly known as probiotics, are defined as, "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001)." Since 1989, the FDA has required that the term probiotic only be used when referring to human microbial products; therefore, the term "DFM" is used in the U.S. feed industry, whereas "probiotic" is used interchangeably with human and animal feed worldwide (Kremer, 2006). Direct-fed microbials are categorized into three main groups: *Bacillus*, lactic acid-producing bacteria, and yeast (NRC, 2012). *Bacillus*-based DFM are spore-forming, which makes them thermostable and able to survive at low pH. Also, *Bacillus*-based DFM may secrete fiber-degrading enzymes (Schreier, 1993). Lactic-acid producing bacteria are not spore-forming and survival during feed processing is of concern (de Lange et al., 2010). Lactic-acid producing bacteria dominate the gastrointestinal tract of the nursing pig (Li et al., 2003; Richards et al., 2005), which helps reduce the pH in the gut by producing lactic acid through fermentation, inhibiting enteric pathogens (Vandenbergh, 1993), and improving host immunity (Niers et al., 2005; de Lange et al., 2010). However, after weaning of pigs, populations

of lactic-acid bacteria diminish; therefore, supplementation of weaned pig diets with lactic-acid producing DFM may be beneficial (Stein and Kil, 2006). Yeast cultures may produce enzymes and vitamins along with other nutrients, which have been reported to produce a variety of responses when fed to swine (Kornegay et al., 1995).

Addition of DFM to swine diets may improve gut health by modifying the microflora, which may help control pathogens (Prescott et al., 2005), enhance immune regulation and response (Galdeano and Perdigon, 2006), increase nutrient digestibility (Giang et al., 2011), improve health status, and improve pig performance (Kenny et al., 2011; Cromwell, 2013). The use of DFM in swine diets is expected to increase due to the recent restrictions on the use of antibiotic growth promoters. Continued use of fibrous co-products also may increase the use of DFM because it has been suggested that combining DFM and prebiotics (i.e., symbiotics) may increase the efficacy of DFM (de Lange et al., 2010).

Mode of Action

As the name suggests, DFM are added to the diet where they must survive processing technologies such as extrusion and pelleting. Once consumed by the pig, DFM enter the stomach where they are subjected to a low pH and pepsin. *Bacillus* DFM are metabolically inactive spores that are thermostable and survive at a low pH and, therefore, survive feed processing and digestion in the stomach. The pH in the small intestine is 6 to 7, which is optimal for the spores to germinate, grow, and produce enzymes. The DFM continue to survive due to their ability to produce enzymes that degrade the feed and produce VFA as a by-product of fermentation. The VFA produced are utilized by the pig as an energy source, and the increased VFA concentration reduces the pH in the gastrointestinal tract, which may inhibit growth of pathogenic bacteria. The DFM also may degrade NSP to reducing sugars that may serve as an energy source for the pig

(Nielsen et al., 2013). Direct-fed microbials are suggested to improve gastrointestinal health by promoting the growth of beneficial bacteria such as lactobacilli and bifidobacteria, thereby decreasing the growth of deleterious bacteria from the large family of Gram-negative Enterobacteriaceae. The decrease in pathogenic bacteria and increase in gastrointestinal health may correspond to an increase in the ability of the pig to digest and ferment nutrients, enhance their utilization of feed and energy, decrease the maintenance energy requirement associated with immune system stimulation, and thereby increase growth performance (Kenny et al., 2011).

Efficacy of Direct-Fed Microbials

Previous reviews have concluded that the efficacy of DFM added to swine diets is inconclusive because variable results have been observed (Pollmann, 1986; 1992; Nosiainen and Setala, 1993; Stavric and Kornegay, 1995). However, a recent review has stated that results of 30 out of 31 nursery pig trials indicated an increased ADG and G:F due to DFM supplementation (Kremer, 2006). Therefore, reports prior to 2000 may not be appropriate today because the development of DFM and the technology associated with DFM has improved, which may lead to the increased efficacy.

Addition of 0, 5.0×10^4, 6.7×10^6, or 7.5×10^8 cfu/d of *Bifidobacterium globosum A* (lactic-acid producing DFM) to weanling pig corn-soybean meal-based diets quadratically improved ADG and ADFI, but did not affect G:F, immune response, or pH of intestinal contents (Apgar et al., 1993). This same feeding regimen was maintained through the growing-finishing phase and pig performance and carcass characteristics were not affected by DFM addition (Apgar et al., 1993). Kornegay et al. (1995) investigated the ability of a yeast culture containing *Saccharomyces cervisiae* to increase nutrient digestibility by pigs because yeast culture supplementation increased cellulolytic bacteria in the rumen of cows (Dawson et al., 1990) and

was suggested to enhance dietary fiber fermentation in the horse (Godbee, 1983). Addition of 0, 8, or 16% peanut hulls, added at the expense of corn, to diets fed to pigs linearly reduced the ATTD of DM, ADF, and NDF, and DFM addition did not ameliorate the reduced digestibility (Kornegay et al., 1995). Kornegay and Risley (1996) observed no difference in the ATTD of DM, NDF, and ADF by 60 kg pigs fed either a corn-soybean meal diet without or with a DFM containing *Bacillus subtilis* and *Bacillus licheniformis*, or with a DFM containing *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus pumilus*.

A more recent study utilizing 270 wean-to-finish pigs tested the dose of DFM (0, 0.64×10^6, 1.28×10^6, 1.92×10^6 viable spores of BioPlus 2B, which contained *Bacillus licheniformis* and *Bacillus subtilis* in a 1:1 ratio; Chr. Hansen, Hørsholm, Denmark) and duration of DFM addition (weaning only or wean-to-finish) to diets, and results indicated that ADG, G:F, and carcass quality were improved with increased dose and duration of DFM addition (Alexopoulos et al., 2004). Lee et al. (2014) produced a *Bacillus subtilis* DFM grown on citrus-juice waste and included this DFM at 0, 1.5, 3.0, or 4.5 g/kg in phase 1 and phase 2 corn-soybean meal based nursery pig diets. Linear improvements were observed in pig growth performance, ATTD of nutrients and energy, serum immunoglobulins, and small intestinal morphology (Lee et al., 2014). Lee et al. (2014) concluded that the observed improvements were mostly caused by producing the *Bacillus subtilis* DFM using solid substrate fermentation (Lee et al., 2014).

Improved ADG and G:F, and decreased time required to wash manure off of mats was observed by addition of 0.05% DFM comprised of two strains of *Bacillus lichenformis* and one strain of *Bacillus subtilis* (Davis et al., 2008). The authors hypothesized that performance and pen cleaning were improved with DFM addition because of increased dietary fiber degradation by enzymes secreted by the DFM. Therefore, further research is necessary to determine the effect

of *Bacillus*-based DFM on dietary fiber fermentation. The ATTD of N and energy by pigs fed a corn-soybean meal-based diet were improved by addition of a DFM composed of *Bacillus subtilis* and *Clostridium butyricum* and, subsequently, pig ADG and G:F were improved (Meng et al., 2010). Pigs challenged with *Salmonella enterica* had reduced ADG and G:F and increased bacterial shedding scores compared with non-challenged pigs, but addition of a *Lactobacillus plantarum* DFM did not influence recovery from the challenge (Gebru et al., 2010). Weanling pigs fed a *Lactobacillus reuteri* and *Lactobacillus plantarum* DFM for 28 d had improved overall ADG and ATTD of N and GE compared with pigs fed no DFM. Results for pigs fed the DFM were similar to results for pigs fed a diet containing 0.01% apramycin, indicating that the *Lactobacillus reuteri* and *Lactobacillus plantarum* DFM may minimize antibiotic use in weanling pig diets (Zhao and Kim, 2015). However, DFM cannot replace antibiotics in terms of preventing or treating of sickness or disease, but seem to be a viable alternative to antibiotics used as growth promoters.

A diet containing corn, soybean meal, and DDGS supplemented with 500 g/MT *Bacillus* spp. DFM and fed to nursery pigs had a 100 kcal/kg increase in DE due to a 9.2% increase in the ATTD of NDF compared with the control diet with no DFM (Owusu-Asiedu et al., 2014). Growing-finishing pigs fed high-fiber diets based on corn, soybean meal, DDGS, wheat middlings, corn germ, and soybean hulls supplemented with a *Bacillus* spp. DFM had increased fecal VFA concentrations and, subsequently, greater available dietary energy, which corresponded with improved ADG and G:F, and a greater loin eye area and fat-free lean percentage compared with pigs fed no DFM (Jaworski et al., 2014).

In conclusion, DFM supplementation to swine diets has produced more beneficial results in the past decade compared with earlier reports, indicating an improvement in the development

and use of DFM. Lactic acid-producing bacteria DFM appear to be more beneficial for weanling pigs to help stabilize the gastrointestinal tract after weaning, whereas *Bacillus*-based DFM may be more beneficial for growing-finishing pigs to increase the digestibility of energy and nutrients in high-fiber diets and, subsequently, increase performance and carcass characteristics.

ADAPTATION TO HIGH FIBER DIETS

The fermentability and, subsequently the available energy from a high-fiber diet may be influenced by the length of time the pig has been fed that diet. Adaptation to a high-fiber diet may reflect long-term adaptations of the gastrointestinal tract by hypertrophy of the gastrointestinal tract, slower digesta passage rate, and adaptations of the microbial population in the gastrointestinal tract (Martinez-Puig et al., 2003). Feeding of high-fiber diets will result in increased size of the large intestine and increased microbial population (Kyriazakis and Emmans, 1995; Jørgensen et al., 1996) This will allow the pig to increase fermentation of dietary fiber. However, these changes take time to occur, and it is, therefore, possible that the pig, more specifically the microbial population in the hindgut, may require a certain period of time to adapt to a high-fiber diet to maximize fermentation.

The ATTD of GE and NSP in pigs fed wheat-soybean meal plus solka-floc or sugar beet pulp diets were not different if pigs had been adapted to the diets for 2, 4, or 6 weeks (Longland et al., 1993). Pigs do not need more than 7 d to adapt to fermentation of insoluble (solka-floc) compared with soluble (sugar beet pulp) dietary fiber added to a wheat-soybean meal based diet (Longland et al., 1993). The ATTD of OM and CP increased in pigs fed a barley-soybean meal-based diet plus corn starch or raw potato starch from d 9 to d 38 of feeding (Martinez-Puig et al.,

2003). However, the ATTD of OM and CP stabilized after pigs were fed the barley-soybean meal-based diet plus corn starch after 16 d of feeding, whereas the ATTD of OM and CP never stabilized and increased from 77.8 to 84.1% and 71.3 to 78.4%, respectively, for pigs fed the barley-soybean meal-based diet plus raw potato starch (Martinez-Puig et al., 2003). These conflicting reports indicate that further research is necessary to elucidate the effect of adaptation time on the ATTD of energy and nutrients in high-fiber diets fed to swine. However, the AID of CP and AA by pigs fed a corn-soybean meal based diet was not different over 6 weeks, indicating that a 5 d diet adaptation period is sufficient when determining the AID of CP and AA in a low-fiber diet (Stewart et al., 2010).

The ATTD of GE is greater in sows compared with growing pigs and this is attributed to the greater intestinal capacity in sows than in growing pigs (Noblet et al., 1994; Le Goff et al., 2002; Lowell et al., 2015). The ATTD of GE increases by 0.003 to 0.0045% for every 10 kg BW from 30 to 100 kg (Noblet, 2001). However, the ATTD of NDF was not different between growing pigs and sows indicating that increased BW may not play a role in the fermentation capacity of pigs (Lowell et al., 2015). Kim et al. (2007) demonstrated that the ATTD of DM was not correlated with BW for weanling, growing, or finishing pigs, but longer retention times of digesta improved the ATTD of DM regardless of the physiological stage of the pigs, but diets greater in dietary fiber decreased retention time and the ATTD of DM was decreased (Ravindran et al., 1984). These results indicate that BW may not play a role in energy digestibility; however, dietary fiber influences passage rate, which is correlated with DM digestibility and this may be a more likely reason for the difference between sows and pigs. However, most comparative data with values for both sows and growing pigs are confounded by level of feed intake, and it is,

therefore, not possible to determine if nutrient and energy digestibility is greater in sows per se or if the observed differences are a result of differences in feed intake.

CONCLUSION

Physicochemical characteristics of feed ingredients and diets may aid in accurate predictions of energy supply from feed ingredients or diets fed to pigs because they are related to the concentration of soluble and insoluble dietary fiber within feed ingredients and diets. Three major factors are necessary to better understand the energy supply and utilization by pigs fed high-fiber diets: 1) the quantity of soluble dietary in a feed ingredient or diet because soluble dietary fiber is fermented to a greater extent compared with insoluble dietary fiber; 2) quantification of VFA and methane production from dietary fiber fermentation and subsequent VFA absorption and energy loss from methane; and 3) total HP, activity HP, and HP associated with the thermic effects of feeding pigs diets containing commonly fed fibrous co-products. Two promising strategies to increase energy supply by increased dietary fiber fermentation are DFM supplementation and the adaptation of the gastrointestinal tract of the pig to diets containing greater concentrations of dietary fiber.

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TABLES

Table 2.1. Gross energy, digestible energy, metabolizable energy, and net energy of 4 ingredients varying in chemical composition used in pig diets, as-fed basis[1]

	Corn	Soybean meal	DDGS[2]	Wheat bran
Type of ingredient	High-starch	High-protein	High-fat	High-fiber
GE, kcal / kg	3,933	4,256	4,849	4,010
DE, kcal / kg	3,451	3,619	3,620	2,420
ME, kcal / kg	3,395	3,294	3,434	2,318
NE, kcal / kg	2,672	2,087	2,384	1,646
NE:GE	0.68	0.49	0.49	0.41
NE:DE	0.77	0.58	0.66	0.68
NE:ME	0.79	0.63	0.69	0.71

[1]Values obtained from NRC, 2012.

[2]DDGS = distillers dried grains with solubles, > 10% oil.

CHAPTER 3: DIGESTIBLE, METABOLIZABLE, AND NET ENERGY IN DIETS CONTAINING 0, 15, OR 30% WHEAT BRAN FED TO GROWING PIGS

ABSTRACT: An experiment was conducted to determine the DE, ME, and NE in diets with 0, 15, or 30% wheat bran added to a corn-soybean meal-based diet fed to growing pigs. A second objective was to test the hypothesis that the DE, ME, and NE in wheat bran can be determined using the difference procedure with the same efficacy as with a regression method. Eighteen barrows (initial BW: 54.4 ± 4.3 kg) were individually housed in metabolism cages. The experiment had 3 periods and 6 replicate pigs per diet. The control diet contained corn, soybean meal, and no wheat bran, and 2 additional diets were formulated by mixing 15 or 30% wheat bran with 85 or 70% of the control diet, respectively. Each period lasted 15 d. During the initial 7 d, pigs were adapted to their experimental diets and housed in metabolism crates in an environmentally controlled room and fed 573 kcal ME / kg $BW^{0.6}$ per d. On d 8, metabolism crates with pigs were moved into open-circuit respiration chambers for measurement of O_2 consumption and CO_2 and CH_4 production. The feeding level was the same as in the adaptation period and feces and urine also were collected during this period. On d 13 and 14, pigs were fed 225 kcal ME / kg $BW^{0.6}$ per d, and pigs then were fasted for 24 h to obtain fasting heat production. The apparent total tract digestibility of DM, GE, crude fiber, ADF, and NDF linearly decreased ($P \leq 0.05$) as wheat bran inclusion increased in the diets. The daily O_2 consumption and CO_2 and CH_4 production by pigs fed increasing concentrations of wheat bran linearly decreased ($P \leq 0.05$) resulting in a linear decrease ($P \leq 0.05$) in heat production. The DE (3,454, 3,257, and 3,161 kcal/kg), ME (3,400, 3,209, and 3,091 kcal/kg), and NE (1,808, 1,575, and 1,458 kcal/kg) of diets linearly decreased ($P \leq 0.05$) as wheat bran inclusion increased. The DE,

ME, and NE in wheat bran determined using the difference procedure was 2,168, 2,117, and 896 kcal/kg and these values were within the 95% confidence interval of the DE (2,285 kcal/kg), ME (2,217 kcal/kg), and NE (961 kcal/kg) estimated by linear regression. In conclusion, increasing inclusion of wheat bran decreased nutrient digestibility and heat production as well as DE, ME, and NE in diets. Finally, in agreement with our hypothesis, the DE, ME, and NE values for wheat bran determined using the difference procedure were similar compared to estimates using linear regression.

Key words: dietary fiber, energy concentration, heat production, pig, wheat bran

INTRODUCTION

In the United States, the energy content of diets fed to pigs is most often evaluated using DE and ME systems (Whitney, 2005). However, DE and ME systems overestimate the energy value of protein and fibrous feedstuffs and underestimate the energy value of fat- and starch-containing feedstuffs; therefore, it is possible that a more accurate estimate of the energy content of pig diets may be obtained using a NE system (Noblet and van Milgen, 2013).

An increased use of dietary fiber in pig diets because of increased inclusion of co-products, such as wheat bran, has been observed in pig production for the past decade and is believed to continue in the future (Woyengo et al., 2014). Therefore, there is a need to determine the energy contribution from dietary fiber in co-products, but the effect of dietary fiber on heat production (**HP**) and NE of the diet remains unclear (Noblet and van Milgen, 2004). Biologically, HP is expected to increase with the inclusion of dietary fiber in pig diets due to increased feed intake, increased size of the gastrointestinal tract in relation to body weight, and

increased hindgut fermentation resulting in energetic losses of methane (Jørgensen et al., 1996). However, the physical activity and overall metabolism may be modified by the addition of dietary fiber causing a decrease or no change in HP (Schrama et al., 1998). Therefore, the first objective of this experiment was to test the hypothesis that increased dietary fiber in the form of wheat bran added to a corn-soybean meal diet will increase HP and reduce calculated values for DE, ME, and NE when fed to growing pigs. Also, the difference procedure may not be appropriate to measure NE in feed ingredients because the nutrient content of the test diet may vary substantially compared with the basal diet and this will impact HP of pigs fed the diets, thus making the difference method inappropriate for determining the NE of feed ingredients. However, this theory has not been experimentally verified. Therefore, the second objective was to test the hypothesis that NE in wheat bran can be determined using the difference procedure with the same efficacy as with a regression method.

MATERIALS AND METHODS

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee at China Agricultural University and the experiment was conducted in the Open-Circuit Respiration Laboratory at the Swine Nutrition Research Centre of the National Feed Engineering Technology Research Center (Chengde, Hebei Province, China).

Indirect Calorimetry Equipment. Six open-circuit respiration chambers with a volume of approximately 7.8 m^3 were used based on a design similar to that of van Milgen et al. (1997). Gas was extracted continuously from the respiration chamber by a vacuum pump. The respiration chamber was maintained at a constant temperature and humidity using an air

conditioner and a heater. Temperature and atmospheric pressure in the chamber were measured and used to calculate the standard temperature and pressure (STP; 0°C, 101 kPa) extraction rate. Oxygen inside and outside the chamber was measured with a paramagnetic differential analyzer (Oxymat 6E, Siemens, Munich, Germany), whereas CO_2, CH_4, and NH_3 were measured with infrared analyzers (Ultramat 6E, Siemens, Munich, Germany). The analyzers had a range of measurement of 19.5 to 21.0% for O_2, 0 to 1% for CO_2, 0 to 0.1% for CH_4, and 0 to 0.1% for NH_3 with a sensitivity of 0.2% within the measurement range. The gas extraction rate was measured by a mass flow meter (Alicat, Tucson, USA). Two respiration chambers shared one gas analyzer. Gas concentrations in each chamber were measured at 5-min intervals.

Animals, Housing, Experimental Design, and Diets. Eighteen Duroc × (Landrace × Large White) barrows with an initial BW of 54.4 ± 4.3 kg were used. Six open-circuit respiration chambers were available and, therefore, the experiment had 3 diets, 3 periods, and a total of 6 replicate pigs per diet. All pigs were housed in metabolism cages for the duration of the experiment (adaptation plus experimental period). The metabolism cages were equipped with a feeder and a water trough that prevented contamination of feces and urine with feed and water. Pigs stood on fully slatted floors with a screen underneath for fecal collection and a urine tray underneath the fecal screen, which allowed for the total, but separate, collection of urine and feces from each pig. Prior to each experimental period, pigs were adapted to their experimental diet for 8 d. Each experimental period lasted a total of 8 d, which consisted of a 5 d energy balance, a 2 d pre-fasting period, and a final 24 h fast. Pigs were weighed at the beginning of the collection period and at the beginning and end of the fasting period. During the experimental period, pigs were housed individually in metabolism cages that were placed inside open-circuit respiration chambers. The chamber temperature was maintained at 22°C during the 5 d energy

balance, 23°C during the 2 d pre-fasting period, and 24°C during the final 24 h fast. The relative humidity in the chambers was maintained at 70% and the air velocity was 0.1 m/s.

Three experimental diets were formulated (Table 3.1). The basal diet contained corn, soybean meal, and no wheat bran. Two additional diets were formulated by mixing 15 or 30% wheat bran with 85 or 70% of the basal diet, respectively. The basal diet was over-formulated compared with the expected requirement (NRC, 2012) to ensure that the diet containing 30% wheat bran had 0.85% standardized ileal digestible (**SID**) Lys and met current requirement estimates for SID indispensable AA, standardized total tract digestible (**STTD**) P, vitamins, and minerals (NRC, 2012). The quantity of feed provided per pig daily during the 5 d energy balance was calculated as 573 kcal ME / kg $BW^{0.6}$ and divided into two equal meals that were provided at 0700 and 1600 h, at which time the respiration chamber doors were opened and gas measurements during these times were disregarded from the final calculations. The quantity of feed provided was based on previous research that determined the ad libitum ME intake to be 573 kcal ME / kg $BW^{0.6}$ and the feeding level was based on each pigs' individual BW (Zhang et al., 2014). The quantity of feed provided per pig daily during the 2 d pre-fasting period was calculated as 225 kcal ME / kg $BW^{0.6}$ and only urine was collected during this time. Pigs were fasted for the final 24 h in the respiration chambers and only urine was collected during this time. Feces were not collected during the pre-fasting and the fasting period because only urine is necessary for the calculation of fasting heat production (**FHP**). The pre-fasting period was utilized to adapt pigs to a lower feed intake in preparation for the 24 h fast based on unpublished data from our laboratory that determined the noise in gas measurements during the 24 h fast was much less when a pre-fasting period is utilized, therefore, giving a better measurement of FHP. Water was available on an ad libitum basis throughout the experiment.

During the 5 d energy balance, total, but separate, collection of feces and urine was conducted. Feces were collected each day when the chamber doors were opened for approximately 1 h to feed the pigs (i.e., at 0700 and 1600 h) and immediately stored at -20°C. Urine was collected each morning at 0700 h over a preservative of 50 mL of 6*N* HCl. Each day, the total urine volume produced by each pig was measured and a 5% aliquot was filtered through cheesecloth, transferred into a plastic bottle, and stored at -20°C. At the end of the collection period, urine samples were thawed, thoroughly mixed, and 50 mL of urine from each pig was collected into screw-cap tubes and this sample was used for analysis. At the conclusion of the 5 d energy balance period, feces were thawed, mixed, weighed, and duplicate subsamples of approximately 350 g were dried for 72 h in a 65°C drying oven. The subsamples then were weighed, ground through a 1-mm screen, and used for analysis.

Sample Analysis and Calculations. Diet, ingredient, and fecal samples were analyzed for DM (Method 930.15; AOAC Int., 2007), ash (Method 942.05; AOAC Int., 2007), crude fiber (Method 978.10; AOAC Int., 2007), ADF (Method 973.18; AOAC Int., 2007), and NDF (Holst, 1973). All diets, ingredients, and fecal samples were analyzed for CP using the combustion procedure (Method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. Diets and ingredients were analyzed for AA on a Hitachi AA Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc, Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [Method 982.30 E(a); AOAC Int., 2007]. Urinary N was determined as Kjeldahl N (Thiex et al., 2002). Acid hydrolyzed ether extract (**AEE**) was determined in all diet and ingredient samples by acid

hydrolysis using 3*N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Diets and ingredients also were analyzed for total dietary fiber, insoluble dietary fiber, and soluble dietary fiber according to Prosky et al. (1992). Monosaccharides and oligosaccharides in the ingredients were analyzed as described by Cervantes-Pahm and Stein (2010). Total starch was analyzed in all diets and ingredients by the glucoamylase procedure (Method 979.10; AOAC Int., 2007). Diet, ingredient, fecal, and urine samples were analyzed in duplicate for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL), and the ATTD of GE in each diet was calculated (Adeola, 2001).

The energy lost in feces and urine was calculated and quantities of DE and ME in each of the three diets were calculated (Adeola, 2001). Although CH_4 production by pigs was measured, it was not included in the calculation of ME because most ME values disregard energy losses of CH_4 even though energy losses of CH_4 can range from 0.1 to 3.0% of DE (Shi and Noblet, 1993). The DE and ME in the basal diet then was multiplied by 85 or 70% to calculate the contribution from the basal diet to the DE and ME in diets containing 15 or 30% wheat bran, respectively. The DE and ME in wheat bran then was calculated by difference (Stewart et al., 2013).

During each of the 8 d experimental periods, O_2, CO_2, and CH_4 concentrations were measured in each respiratory chamber and outside of each respiration chamber at 5 min intervals. These concentrations then were used to calculate O_2 consumption and CO_2 and CH_4 production during each 5 min interval by pigs and these were summed over a 24 h period. Heat production then was calculated from gas exchanges and urinary losses of N according to Brouwer (1965) using Eq. [1]:

$$HP\ (kcal) = 3.866 \times O_2\ (L) + 1.200 \times CO_2\ (L) - 0.518 \times CH_4\ (L) - 1.431 \times \text{urinary N (g)}. \quad [1]$$

Fasting heat production was calculated using the same equation, but using gas exchanges and urinary losses of N during the 24 h fasting period.

Retention of dietary energy (**RE**) was calculated according to Ayoade et al. (2012) using Eq. [2]:

$$RE\ (kcal) = ME\ intake\ (kcal) - HP\ (kcal). \quad [2]$$

Retention of energy as protein ($\mathbf{RE_P}$) was calculated according to Ewan (2001) as N retention (g) × 6.25 × 5.68 (kcal/g). Retention of energy as lipid ($\mathbf{RE_L}$) was calculated as the difference between RE and RE_P (Labussière et al., 2009).

Net energy of each diet was calculated according to Noblet et al. (1994) using Eq. [3]:

$$NE\ (kcal/kg\ DM) = [RE\ (kcal) + FHP\ (kcal)] / DMI\ (kg). \quad [3]$$

After the NE of each diet was calculated, the NE of wheat bran also was calculated by difference as described previously for the calculation of DE and ME in wheat bran (Stewart et al., 2013). The respiration quotient (**RQ**) was calculated as the ratio between CO_2 production and O_2 consumption (Noblet et al., 2001).

Statistical Analysis. Homogeneity of variances was confirmed using the UNIVERIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were determined as any value that deviated from the treatment mean by ± 2 standard deviations and three were removed from the data set. A pig fed the 30% wheat bran diet died on d 4 of the 5 d experimental period and was not included in the calculations. Data were analyzed using the MIXED procedure. The model included diet as the fixed effect and pig and period as random effects. Least squares means were calculated for

each independent variable. Orthogonal polynomials were used to determine linear and quadratic effects of diet. Regression equations to estimate the DE, ME, and NE of wheat bran were developed using the REG procedure in SAS following methods of Young et al. (1977) and Noblet et al. (1993). The DE, ME, and NE of wheat bran then were estimated by solving the prediction equations when wheat bran inclusion was equal to 100%. The CLB statement in SAS was used to determine the 95% confidence levels for the regression coefficients used for estimating DE, ME, and NE of wheat bran. The DE, ME, and NE of wheat bran obtained using the difference procedure was considered not different than the DE, ME, and NE of wheat bran estimated using linear regression if the values fell within the 95% confidence interval for the DE, ME, and NE of wheat bran estimated using linear regression. The pig was the experimental unit and a probability of $P \leq 0.05$ was considered significant and $0.05 < P \leq 0.10$ was considered a trend.

RESULTS AND DISCUSSION

The wheat bran used in this experiment contained 11.84, 13.77, and 44.76% crude fiber, ADF, and NDF, respectively, compared with average values of 7.77, 11.00, and 32.28%, respectively (NRC, 2012; Table 3.2). The concentration of soluble, insoluble, and total dietary fiber in wheat bran used in this experiment was 2.9, 48.0, and 50.9%, respectively, whereas Jaworski et al. (2015) reported the concentration of soluble, insoluble, and total dietary fiber in wheat bran to be 3.5, 34.9, and 38.4%, respectively. Also, the concentration of starch in wheat bran used in the current experiment was 11.26% whereas NRC (2012) and Jaworski et al. (2015) reported the starch concentration of wheat bran to be 22.56 and 15.67%, respectively. These differences indicate that the source of wheat bran used in this experiment was produced from a

flour mill that was more efficient in extracting the starch from the wheat compared with those used to produce the wheat bran included in NRC (2012) and Jaworski et al. (2015). The soybean meal used in this experiment contained 4.97% crude fiber, 8.86% ADF, 10.31% NDF, 5.92% sucrose, 1.52% raffinose, and 4.16% stachyose, which is within the range of values previously reported (Baker and Stein, 2009; Cervantes-Pahm and Stein, 2010; NRC, 2012). The concentrations of CP and AA in the soybean meal used in this experiment were comparable with NRC (2012). The nutrient composition of the corn used in this experiment also was in agreement with previous values (NRC, 2012; Rojas et al., 2013). The analyzed nutrient and energy concentrations in experimental diets were not different from calculated values (Table 3.1). The concentration of GE and insoluble dietary fiber increased and the concentration of starch and soluble dietary fiber decreased as wheat bran inclusion increased in the diets.

Final BW of pigs linearly decreased ($P \leq 0.05$) as the concentration of wheat bran increased in the diet (Table 3.3). These results are in agreement with data for growing pigs fed diets containing 30% soybean hulls or wheat middlings compared with pigs fed a corn-soybean meal-based diet (Stewart et al., 2013). Daily feed intake of pigs tended to linearly decrease ($P < 0.10$) as wheat bran inclusion increased in diets. Feeding level does not impact energy digestibility and, therefore, feed intake did not influence energy digestibility in this experiment (Moter and Stein, 2004). The ATTD of DM, GE, CP, crude fiber, ADF, and NDF decreased linearly ($P \leq 0.05$) as wheat bran inclusion increased in the diets (Table 3.4). There was also a tendency for a quadratic decrease ($P \leq 0.10$) in the ATTD of ADF by pigs as wheat bran inclusion increased in the diets. There was a linear increase ($P \leq 0.05$) in fecal output, GE in the feces, and fecal GE output as wheat bran inclusion in the diets increased. Therefore, the DE in the diets decreased linearly ($P \leq 0.05$) from 3,454 to 3,257 and 3,161 kcal/kg as wheat bran

inclusion increased in the diets. Urine output by pigs tended to decrease linearly ($P \leq 0.10$) as wheat bran inclusion increased in the diets, but GE in the urine of pigs fed experimental diets was not different and urine GE output was not different among diets. The ME in diets decreased linearly ($P \leq 0.05$) from 3,400 to 3,209 and 3,091 kcal/kg as wheat bran inclusion increased.

Total HP and daily HP by pigs decreased linearly ($P \leq 0.05$) as wheat bran inclusion increased in diets, and this observation contradicts our hypothesis. Previous research has observed no differences in HP in 50 kg pigs fed a high-starch diet versus a high-fiber diet (Schrama et al., 1996), in group-housed growing pigs fed diets with concentrations of 0, 5, 10, or 15% sugar beet pulp silage (Schrama et al., 1998), in group-housed growing pigs fed a corn-based diet versus a diet containing corn plus 15% wheat straw (Schrama and Bakker, 1999), and in 18.5 kg pigs fed diets with 0, 15, or 30% wheat-corn distillers dried grains with solubles (Ayoade et al., 2012). It is possible that as dietary fiber concentrations increase in diets fed to growing pigs, the HP related to physical activity decreases, resulting in no change or potentially a decrease in HP (Schrama and Bakker, 1999). However, physical activity was not measured in the current experiment and we are, therefore, not able to verify the hypothesis by Schrama and Bakker (1999).

The concentration of N in the urine decreased linearly ($P \leq 0.05$) by pigs fed diets containing increasing concentrations of wheat bran, but urinary N output was not different. There was a linear decrease ($P \leq 0.05$) in O_2 consumption from 663.71 to 659.82 and 636.38 L/d as wheat bran inclusion in diets increased. Carbon dioxide and CH_4 production by pigs also decreased linearly ($P \leq 0.05$) from 700.42 to 678.27 and 656.19 L/d and from 4.83 to 3.21 and 1.51 L/d, respectively, as wheat bran inclusion increased in the diets. The CH_4 excretion of growing pigs in the current experiment is in agreement with values previously reported for CH_4

excretion by growing pigs (Christensen and Thorbek, 1987). The RQ of pigs fed experimental diets decreased linearly ($P \leq 0.05$) from 1.06 to 1.03 and 1.03 as wheat bran inclusion in the diets increased, which may be indicative of the diets becoming limited in energy supply.

Chinese Latang gilts fed a corn-soybean meal-based diet with 21% wheat bran produced 3.9 L of CH_4 per day (Cao et al., 2013), which is in agreement with the current study where pigs fed 15% wheat bran produced 3.21 L of CH_4 per day. Diets containing greater quantities of insoluble dietary fiber promote gut fill, increase frequency of laxation, and decrease transit time to increase feed intake to compensate for reduced dietary energy obtained from the consumption of dietary fiber (Kyriazakis and Emmans, 1995). The decrease in transit time may have reduced the amount of time the microbial population in the hindgut of the pig had access to ferment the dietary fiber in wheat bran, which may be the reason for the reduction in the fermentation end-product (i. e., CH_4) that was observed in the current experiment as wheat bran inclusion increased. In vitro total tract digestibility of DM and non-starch polysaccharides in wheat bran is 63.6 and 20.6%, respectively (Jaworski et al., 2015), indicating that the dietary fiber in wheat bran has a low fermentability, which may have contributed to the reduction in CH_4 excretion that was observed in the current experiment as inclusion of wheat bran increased.

Pigs fed diets containing greater amounts of dietary fiber in the form of wheat bran have an increased empty weight of the gastrointestinal tract compared with pigs fed a wheat-based diet lower in dietary fiber (Kyriazakis and Emmans, 1995). The gastrointestinal tract of animals may consume as much as 30% of FHP and when the size of the tract is increased, the energy required to maintain the tract increases. Thus, the FHP or NE required for maintenance is increased (Baldwin, 1995). However, the FHP and fasting RQ of pigs were not different among pigs that were previously fed different experimental diets. The relatively short duration of feeding the

experimental diets limited the expansion of the gastrointestinal tract, which is the reason FHP was not different among treatments. The FHP obtained in this experiment is within the range of FHP values obtained in similar experiments conducted in the same facility (Liu et al., 2014; Zhang et al., 2014), but the FHP obtained in this experiment was only slightly greater than the FHP suggested by Noblet et al. (1994) and by NRC (2012). The fasting RQ of pigs previously fed different experimental diets was not different and was close to the level for fasting metabolism where the RQ becomes equivalent to the catabolism of fat (NRC, 1981).

Daily retained energy by pigs decreased linearly ($P \leq 0.05$) from 266.87 to 223.85 and 216.28 kcal/kg $BW^{0.6}$ as pigs were fed diets containing increasing amounts of wheat bran. The daily retained energy by pigs fed the basal diet or the 30% wheat bran diet were greater compared with previous work (Stewart et al., 2013). However, the results obtained by Stewart et al. (2013) were determined using the comparative slaughter method, and it has been suggested that the comparative slaughter method may underestimate the energy retention of pigs compared with indirect calorimetry (Quiniou et al., 1995; van Milgen and Noblet, 2003; Kil et al., 2011, 2013a, 2013b). Retained protein did not differ among pigs fed the experimental diets, which was most likely due to the fact that all diets were formulated to meet or exceed NRC (2012) requirements for standardized ileal digestible indispensable AA. Therefore, protein synthesis was not limited in this experiment. However, retained lipid decreased linearly ($P \leq 0.05$) when pigs were fed increasing amounts of wheat bran and this was due to the decreased ATTD of nutrients and energy and the reduced DE, ME, and NE in the diets as wheat bran inclusion increased.

The NE in the experimental diets decreased linearly ($P \leq 0.05$) from 1,808 to 1,575 and 1,458 kcal/kg as wheat bran inclusion increased in the diets. A diet containing 30% wheat middlings and fed to growing pigs was determined to contain 1,759 kcal/kg (Stewart et al.,

2013), which is slightly greater than the NE in the 30% wheat bran diet in the current experiment, but wheat bran also contains less DE, ME, and NE than wheat middlings. The NE in the corn-soybean meal basal diet used in this experiment (1,808 kcal/kg) is in agreement with a recent estimate (1,870 kcal/kg) for a similar diet (Kil et al., 2013a). Net energy of the diets with 0, 15, and 30% added wheat bran was calculated according to Noblet et al. (1994) and was 2,927, 2,750, and 2,647 kcal/kg DM, respectively, and these values are greater than the experimentally determined NE of the diets. In the development of the NE prediction equations, Noblet et al. (1994) had a maximum inclusion level of 28% corn, whereas the inclusion of corn ranged from 55.6 to 79.5% in the current experiment. Also, Noblet et al. (1994) had a maximum pig BW of 46.7 kg, whereas the initial pig BW used in the current experiment was 54.4 kg. These differences may be the cause of the discrepancy between values obtained experimentally in the current experiment versus calculated values according to Noblet et al. (1994).

Linear regression analyses were used to examine the relationship between energy and dietary wheat bran according to Young et al. (1977) and Noblet et al. (1993). The dependent variable in the three prediction equations was dietary DE, ME, and NE in kcal/kg (as-fed basis), respectively, and the independent variable was dietary wheat bran inclusion in percent (as-fed basis; Table 3.5). The prediction equation for dietary DE had an intercept equal to 3,457.7 ($P \leq 0.05$) and a slope estimate of -11.725 ($P \leq 0.05$) with 90% of the variation in dietary DE explained by the model. The prediction equation for dietary ME had an intercept equal to 3,389.6 ($P \leq 0.05$) and a slope estimate of -11.725 ($P \leq 0.05$) with 92% of the variation in dietary ME explained by the model. The prediction equation for dietary NE had an intercept equal to 1,788.1 ($P \leq 0.05$) and a slope estimate of -8.273 ($P \leq 0.05$) with 35% of the variation in dietary NE explained by the model. The poor prediction of NE was because only three diets were utilized in

the regression model and a large SEM was attributed to the NE of the diets. The y-intercept of the three prediction equations is equal to the DE, ME, and NE in the basal diet (kcal/kg as-fed basis). The slope of the prediction equations for DE and ME were the same, which indicates that the percent change in wheat bran inclusion produces the same decrease in DE as ME. The DE, ME, and NE of wheat bran estimated using the prediction equations were 2,285, 2,217, and 961 kcal/kg as-fed basis, respectively (Table 3.6). The DE, ME, and NE of wheat bran determined using the difference method were 2,168, 2,117, and 896 kcal/kg as-fed basis, respectively. In agreement with our hypothesis and Bolarinwa and Adeola (2012), the DE, ME, and NE of wheat bran obtained using the difference procedure were within the 95% confidence intervals obtained for the DE, ME, and NE of wheat bran estimated using linear regression indicating that both procedures may be used to estimate values for DE, ME, and NE. The NE of wheat middlings was recently reported at 987 kcal/kg (Stewart et al., 2013) and is slightly greater than the NE of wheat bran obtained using either method in the current experiment, which was expected because wheat bran has a greater amount of dietary fiber compared with wheat middlings. Values for DE, ME, and NE of wheat bran obtained in this experiment using either method are less than the values reported by NRC (2012; 3,151, 2,902, and 1,847 kcal/kg, respectively) and the NE value obtained when calculated according to Noblet et al. (1994; 1,338 kcal/kg DM), which is likely a result of the greater concentration of dietary fiber, in particular insoluble dietary fiber, in the wheat bran used in this experiment compared with previously reported values.

CONCLUSION

Inclusion of 0, 15, or 30% wheat bran in diets fed to growing pigs resulted in a decreased ATTD of nutrients and energy as wheat bran inclusion increased, which led to a decrease in dietary DE, ME, and NE as dietary wheat bran inclusion increased. The HP of pigs decreased

linearly as dietary wheat bran inclusion increased which was in contrast to our hypothesis. However, the FHP of pigs was unaffected by inclusion of wheat bran in the diets. The excretion of CH_4 decreased as wheat bran inclusion increased in the experimental diets, indicating that the fermentation of wheat bran is low due to the large concentrations of insoluble dietary fiber. The DE, ME, and NE in wheat bran determined using the difference procedure were in good agreement with the DE, ME, and NE estimated using linear regression indicating that both procedures may be used to estimate energy values in feed ingredients. However, caution must be used when applying this research to other ingredients because wheat bran was the sole ingredient tested and, in addition, prediction equations were developed using linear regression with only three different inclusion levels of wheat bran. Therefore, further research is necessary to validate the conclusions drawn using different ingredients and more diets for the development of prediction equations.

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TABLES

Table 3.1. Ingredient, calculated, and analyzed composition of experimental diets (as-fed basis)

	Diet		
Item	Basal	15% Wheat bran	30% Wheat bran
Ingredients, %			
Corn	79.47	67.55	55.63
Soybean meal (48% CP)	16.00	13.60	11.20
Wheat bran	0.00	15.00	30.00
Limestone	1.40	1.19	0.98
Dicalcium phosphate	1.00	0.85	0.70
L-Lys HCl	0.62	0.53	0.43
DL-Met	0.06	0.05	0.04
L-Thr	0.15	0.13	0.11
L-Trp	0.03	0.03	0.02
Salt	0.57	0.48	0.40
Vitamin-mineral premix[1]	0.70	0.60	0.50
Total	100.00	100.00	100.00
Calculated composition[2]			
ME, kcal/kg	3,225	3,089	2,953
NE[3], kcal/kg DM	2,927	2,750	2,647
CP, %	13.68	13.89	14.10
SID[4] Lys, %	1.05	0.95	0.85
STTD[5] P, %	0.28	0.32	0.36
Analyzed composition			
GE, kcal/kg	3,775	3,797	3,846
DM, %	87.10	86.95	86.87
CP (N × 6.25), %	15.05	15.30	15.43
AEE[6], %	2.59	2.85	2.95
Ash, %	5.10	4.72	4.87
Crude fiber, %	3.12	4.17	5.17
ADF, %	5.07	5.83	6.96
NDF, %	9.24	14.96	20.55
Insoluble dietary fiber, %	15.02	20.45	25.51
Soluble dietary fiber, %	3.95	3.61	2.11
Total dietary fiber, %	18.97	24.06	27.62
Starch, %	56.42	53.23	50.11
Indispensable AA, %			
Arg	0.88	0.92	0.94
His	0.39	0.40	0.40
Ile	0.60	0.58	0.55
Leu	1.40	1.34	1.25
Lys	1.03	1.02	0.97
Met	0.27	0.29	0.27

Table 3.1. (cont.)

Phe	0.72	0.70	0.67
Thr	0.71	0.62	0.61
Trp	0.18	0.20	0.20
Val	0.69	0.71	0.71
Dispensable AA, %			
Ala	0.83	0.82	0.80
Asp	1.38	1.31	1.26
Cys	0.25	0.25	0.27
Glu	2.60	2.63	2.61
Gly	0.60	0.64	0.67
Pro	0.94	0.95	0.92
Ser	0.68	0.66	0.64
Tyr	0.52	0.49	0.47
Total AA, %	14.94	14.79	14.46

[1]The vitamin-micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A, 5,512 IU; vitamin D_3, 2,200 IU; vitamin E, 30 IU; vitamin K_3, 2.2 mg; vitamin B_{12}, 27.6 μg; 2.2 mg; thiamine 1.5 mg; riboflavin, 4.0 mg; pantothenic acid, 14 mg; niacin, 30 mg; choline chloride, 400 mg; folacin, 0.7 mg; pyridoxine, 3 mg; biotin, 44 μg; Fe, 120 mg; Cu, 100 mg; Zn, 75 mg; Mn, 40 mg; I, 0.3 mg; Se, 0.3 mg.

[2]Calculated from NRC (2012) values.

[3]Calculated NE according to Noblet et al. (1994) and NRC (2012). NE (kcal/kg DM) = [0.700 × DE (kcal/kg DM)] + [1.61 × EE (g/kg DM)] + [0.48 × Starch (g/kg DM)] – [0.91 × CP (g/kg DM)] – [0.87 × ADF (g/kg DM)].

[4]SID = standardized ileal digestible.

[5]STTD = standardized total tract digestible.

[6]AEE = acid hydrolyzed ether extract.

Table 3.2. Analyzed nutrient composition of ingredients (as-fed basis)

	Ingredient		
Item	Corn	Soybean meal	Wheat bran
GE, kcal/kg	3,867	4,192	3,969
DM, %	86.87	86.37	87.50
Ash, %	1.21	6.63	5.15
AEE[1], %	3.78	1.76	4.09
CP (N × 6.25), %	8.05	46.89	17.28
Crude fiber, %	2.15	4.97	11.84
ADF, %	3.76	8.86	13.77
NDF, %	8.41	10.31	44.76
Insoluble dietary fiber, %	10.78	17.67	48.00
Soluble dietary fiber, %	1.71	0.91	2.90
Total dietary fiber, %	12.49	18.58	50.90
Starch, %	67.28	2.31	11.26
Fructose, %	0.22	1.12	0.75
Glucose, %	1.13	3.55	1.61
Sucrose, %	0.63	5.92	0.65
Maltose, %	0.16	0.11	0.10
Raffinose, %	0.13	1.52	1.12
Stachyose, %	ND[2]	4.16	0.09
Verbascose, %	ND	0.37	ND
Indispensable AA, %			
Arg	0.35	3.46	1.16
His	0.23	1.22	0.46
Ile	0.28	2.21	0.52
Leu	0.98	3.73	1.01
Lys	0.25	2.97	0.68
Met	0.18	0.64	0.23
Phe	0.38	2.45	0.63
Thr	0.29	1.86	0.53
Trp	0.06	0.73	0.19
Val	0.38	2.28	0.78
Dispensable AA, %			
Ala	0.59	2.09	0.80
Asp	0.54	5.48	1.20
Cys	0.18	0.64	0.33
Glu	1.45	8.42	2.84
Gly	0.30	2.03	0.90
Pro	0.69	2.34	0.95
Ser	0.38	2.28	0.65
Tyr	0.25	1.77	0.40
Total AA, %	7.94	46.94	14.45

[1]AEE = acid hydrolyzed ether extract.

[2]ND = not detectable.

Table 3.3. Energy balance and gas consumption and production by growing pigs fed experimental diets

Item	Diet			Pooled SEM	*P*-value	
	Basal	15% Wheat bran	30% Wheat bran		Linear	Quadratic
Initial BW, kg	54.37	54.33	54.53	2.63	0.77	0.81
Final BW, kg	59.07	58.57	57.87	2.66	0.02	0.82
Daily feed intake, kg	1.89	1.84	1.78	0.05	0.07	0.87
Total feed intake, kg	9.43	9.19	8.90	0.28	0.20	0.93
GE intake, kcal	35,578	34,898	32,974	1,539	0.25	0.75
N intake, g	226.91	224.90	227.19	8.09	0.97	0.75
Dry feces output, kg	0.76	1.19	1.56	0.05	< 0.01	0.58
GE in feces, kcal/kg	3,999	4,183	4,225	28.41	< 0.01	0.06
Fecal GE output, kcal	3,035	4,957	6,599	163.12	< 0.01	0.43
N in feces, %	2.78	2.32	2.32	0.07	< 0.01	0.01
Fecal N output, g	20.92	27.55	36.17	1.20	< 0.01	0.47
DE in diet, kcal/kg	3,454	3,257	3,161	33.10	< 0.01	0.18
DE in diet, kcal/kg DM	3,966	3,746	3,639	38.10	< 0.01	0.20
DE in diet, kcal/kg $BW^{0.6}$	306.20	290.96	282.32	8.33	< 0.01	0.53
Urine output, kg	16.63	13.22	11.53	2.23	0.05	0.66
Daily urine output, kg	3.13	2.45	2.04	0.29	< 0.01	0.42
GE in urine, kcal/kg	43.80	50.93	61.67	11.59	0.16	0.86
Urinary GE output, kcal/d	128.83	116.65	135.35	22.62	0.51	0.08
ME in diet, kcal/kg	3,400	3,209	3,091	31.63	< 0.01	0.34
ME in diet, kcal/kg DM	3,904	3,690	3,558	36.40	< 0.01	0.36
ME in diet, kcal/kg $BW^{0.6}$	302.22	285.18	276.10	8.59	< 0.01	0.52
Efficiency of ME						
ME/DE	0.98	0.98	0.98	< 0.01	0.30	0.54
5 d total HP[1], kcal	16,997	16,832	15,085	614.43	0.04	0.31
5 d total HP, kcal/kg $BW^{0.6}$	1,509	1,457	1,349	54.67	0.03	0.64
Daily HP, kcal	3,391	3,347	3,229	111.45	0.02	0.50
Daily HP, kcal/kg $BW^{0.6}$	300.79	297.11	287.54	5.93	0.02	0.55
HP, kcal/kg FI	1,797	1,826	1,756	40.89	0.32	0.16

Table 3.3. (cont.)

Urinary N, %	0.42	0.50	0.64	0.06	< 0.01	0.56
Urinary N output, g/d	13.08	12.10	11.82	1.56	0.21	0.68
O_2 consumption, L/d	663.75	659.83	634.31	24.49	0.02	0.32
CO_2 production, L/d	700.27	678.05	653.70	19.25	< 0.01	0.92
CH_4 production, L/d	4.83	3.21	1.48	0.42	< 0.01	0.85
RQ	1.06	1.03	1.03	0.01	0.01	0.22
FHP, kcal	2,065	1,972	2,194	142.01	0.33	0.18
FHP, kcal/kg $BW^{0.6}$	192.75	177.40	198.08	13.22	0.74	0.22
Fasting RQ	0.74	0.74	0.73	0.02	0.90	0.66
5 d total ME intake, kcal	32,041	29,380	27,238	912.03	< 0.01	0.82
ME intake, kcal/d	6,408	5,876	5,645	178.64	< 0.01	0.04
5 d total RE, kcal	15,044	12,548	12,153	761.19	0.01	0.24
Daily RE, kcal	3,010	2,617	2,400	103.57	< 0.01	0.24
Daily RE, kcal/kg $BW^{0.6}$	266.87	223.85	216.28	12.34	0.01	0.26
5 d total RE, kcal/kg	1,595	1,363	1,360	60.05	0.01	0.14
Daily RE, kcal/kg	1,611	1,372	1,293	42.71	< 0.01	0.02
Retained protein, g/d	179.24	166.56	164.43	10.12	0.20	0.59
RE_P, kcal/d	1,018	946.05	933.95	57.47	0.20	0.59
RE_P, kcal/d/kg $BW^{0.6}$	90.33	83.89	83.46	4.09	0.22	0.53
Retained lipid, g/d	221.19	173.73	166.29	16.68	0.03	0.30
RE_L, kcal/d	1,991	1,564	1,497	150.15	0.03	0.30
RE_L, kcal/d/kg $BW^{0.6}$	176.54	139.96	132.83	12.98	0.03	0.36
NE, kcal/kg	1,808	1,575	1,462	236.89	< 0.01	0.17
NE, kcal/kg DM	2,076	1,812	1,683	272.4	< 0.01	0.17
NE, kcal/kg $BW^{0.6}$	160.97	140.46	129.86	21.35	< 0.01	0.21
Efficiencies of NE						
NE/DE	0.53	0.49	0.49	0.02	0.24	0.34
NE/ME	0.54	0.49	0.51	0.02	0.23	0.25

[1]HP = heat production.

Table 3.4. Apparent total tract digestibility (ATTD) of nutrients and energy by growing pigs fed experimental diets (as-fed basis)

ATTD, %	Diet			Pooled SEM	*P*-value	
	Basal	15% Wheat bran	30% Wheat bran		Linear	Quadratic
DM	91.74	86.09	81.62	0.60	< 0.01	0.40
CP	91.20	87.73	83.87	0.65	< 0.01	0.80
GE	91.92	85.78	81.13	0.55	< 0.01	0.25
Crude fiber	69.54	54.93	39.32	6.43	< 0.01	0.93
ADF	79.60	61.30	52.22	2.55	< 0.01	0.05
NDF	74.17	65.42	64.71	2.29	< 0.01	0.10

Table 3.5. Regression coefficients used for estimating DE, ME, and NE in wheat bran (as-fed basis)[1]

Dependent variable	Prediction equation	SE		*P*-value		R^2	RMSE
		Intercept	Estimate	Intercept	Estimate		
Dietary DE, kcal/kg	3457.7-11.725 × (wheat bran inclusion, %)	18.27	0.98	< 0.001	< 0.001	0.90	48.87
Dietary ME, kcal/kg	3389.6-11.725 × (wheat bran inclusion, %)	17.09	0.92	< 0.001	< 0.001	0.92	45.70
Dietary NE, kcal/kg	1788.1-8.273 × (wheat bran inclusion, %)	54.55	2.94	< 0.001	0.013	0.35	145.89

[1]Data were subjected to linear regression analysis with the percent inclusion of wheat bran as the independent variable and the kcal/kg DE, ME, or NE of the diet as the dependent variable. The regression coefficients indicate the change in the DE, ME, or NE of the diets for each percentage point change of wheat bran included in the diet: thus, the coefficient multiplied by 100 is equal to the DE, ME, or NE of wheat bran.

Table 3.6. Energy concentration of wheat bran determined using the difference procedure or estimated from prediction equations

Item	Method		95% Confidence interval
	Difference procedure[1]	Prediction equations	
As-fed basis			
DE, kcal/kg	2,168	2,285	2,036 – 2,534
ME, kcal/kg	2,117	2,217	1,984 – 2,450
NE, kcal/kg	896	961	218 – 1,704
DM basis			
DE, kcal/kg	2,478	2,611	2,327 – 2,896
ME, kcal/kg	2,419	2,534	2,267 – 2,800
NE, kcal/kg	1,024	1,098	249 – 1,947

[1]The values presented are the mean DE, ME, and NE of wheat bran calculated using the difference procedure for the two diets containing 15 or 30% wheat bran.

CHAPTER 4: EFFECT OF A 3-STRAIN *BACILLUS*-BASED DIRECT-FED MICROBIAL ON GROWTH PERFORMANCE AND INTESTINAL CONCENTRATIONS OF VOLATILE FATTY ACIDS IN NURSERY PIGS FED LOW- OR HIGH-FIBER DIETS

ABSTRACT: The effect of a *Bacillus*-based direct-fed microbial (DFM) on growth performance, plasma tumor necrosis factor alpha (TNF-α), relative gene expression, and intestinal VFA concentrations in weanling pigs fed low- or high-fiber diets was evaluated. Two hundred pigs (initial BW: 6.31 ± 0.73 kg) were allotted to 1 of 4 dietary treatments (5 pigs per pen and 10 pens per treatment). Treatments were arranged in a 2 × 2 factorial arrangement with 2 diet types [low-fiber (LF) or high-fiber (HF)] and 2 concentrations of DFM (0 or 60 g DFM / t of feed). The DFM contained 1.5×10^5 cfu / g and was obtained from Danisco Animal Nutrition-DuPont Industrial Biosciences (Marlborough, UK). Phase 1 diets were fed for 2 weeks post-weaning and phase 2 diets for the following 29 d. The LF diets contained corn and soybean meal as main ingredients and HF diets contained corn, soybean meal, corn distillers dried grains with solubles (7.5 and 15.0% in phase 1 and 2, respectively), and wheat middlings (10.0%). The NE in phase 1 LF and HF diets was 2,525 and 2,463 kcal / kg, respectively, and the NE in phase 2 LF and HF diets was 2,483 and 2,414 kcal / kg, respectively. Pigs and feed were weighed at the start and at the end of each phase, and ADG, ADFI, and G:F calculated. At the conclusion of phase 2, blood was collected from 1 pig per pen and 1 pig per pen was sacrificed. Cecum and rectum contents were analyzed for VFA, while tissue samples were collected from the ileum, cecum, rectum, and liver to determine gene expression. Results indicated that feeding HF diets resulted in a reduction ($P \leq 0.05$) in ADFI and ADG of pigs compared with feeding LF diets.

Pigs fed DFM diets had improved ($P \leq 0.05$) G:F compared with pigs fed non-DFM diets. Pigs fed LF diets had a greater ($P \leq 0.05$) BW at the end of phase 2 compared with pigs fed HF diets. The concentration of VFA in rectum contents was greater ($P \leq 0.05$) in pigs fed LF diets than pigs fed HF diets. The expression of a VFA transporter in the rectum of pigs fed HF diets was increased ($P \leq 0.05$), while pigs fed DFM-containing diets had an increased ($P \leq 0.05$) expression of glucagon-like peptide-2 receptor in the liver. Pigs fed HF had greater ($P \leq 0.05$) PUN compared with LF fed pigs. Dietary fiber and DFM had no effect on the plasma concentration of TNF-α. In conclusion, the *Bacillus*–based DFM improved overall G:F, but contrary to our hypothesis, this was not caused by increased fermentation and subsequent VFA yield.

Key words: dietary fiber, direct-fed microbial, growth performance, swine, volatile fatty acids

INTRODUCTION

Direct-fed microbials (**DFM**), which may be more commonly known as probiotics, are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2001)." The addition of DFM to swine diets may improve gut health by modifying the microbiota, which may enhance immune regulation, health status, and improve pig performance (Kenny et al., 2011; Cromwell, 2013). Addition of DFM to nursery diets may reduce diarrhea (Eigel, 1989), but improved growth performance has not been consistently observed (Stavric and Kornegay, 1995).

Bacillus-based DFM are spore-forming bacteria, which allows them to survive high temperatures and low pH, but when *Bacillus*-based DFM germinate in the intestine of the pig, they also produce a large amount and a wide variety of fiber-degrading enzymes (Schreier, 1993;

Kenny et al., 2011). Therefore, the addition of a *Bacillus*-based DFM may enhance the fermentation of dietary fiber in swine diets and, subsequently, increase the available dietary energy in the form of VFA (Davis et al., 2008). A high-fiber concentration in the diet reduced ADFI and G:F, as well as the digestibility of nutrients and energy by nursery pigs (Bindelle et al., 2008). However, dietary fiber in nursery pig diets may act as a prebiotic and stimulate beneficial gut microbiota and, therefore, reduce post-weaning diarrhea (Smith and Halls, 1968). Combining DFM and dietary fiber may increase the efficacy of DFM, but data to confirm this hypothesis has not been reported (de Lange et al., 2010). Therefore, it may be beneficial to supplement diets containing high-fiber ingredients such as distillers dried grains with solubles (**DDGS**) and wheat middlings with a *Bacillus*-based DFM that has the ability to secrete fiber-degrading enzymes. The objective of this experiment, therefore, was to test the hypothesis that addition of a *Bacillus*-based DFM would increase fermentation and maintain growth performance of nursery pigs fed high-fiber diets relative to pigs fed low-fiber diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for these experiments.

Animals, Diets, and Experimental Design. A total of 200 weanling pigs (initial BW: 6.31 ± 0.73 kg) that were the offspring of G-Performer boars mated to F-25 females (Genetiporc, Alexandria, MN) were used in this experiment in 2 separate blocks of 100 pigs each. Pigs were randomly allotted in a completely randomized design to 4 dietary treatments. There were 5 pigs per pen and 10 replicate pens per treatment. Pigs were housed in environmentally controlled nursery barns in 1.4 × 1.4 m pens with fully slatted floors. A feeder and a nipple drinker were

provided in each pen and feed and water were provided on an ad libitum basis throughout the experiment.

Treatments were arranged in a 2 × 2 factorial arrangement with 2 diet types [low-fiber (**LF)** or high-fiber (**HF**)] and 2 concentrations of DFM (0 or 60 g DFM / t of feed; Tables 4.1 and 4.2). The *Bacillus*-based DFM contained 1.5×10^5 cfu / g and was obtained from Danisco Animal Nutrition-DuPont Industrial Biosciences (Marlborough, UK). Phase 1 diets were fed for 2 weeks post-weaning and phase 2 diets for the following 29 d. The LF diets contained corn and soybean meal as main ingredients and HF diets contained corn, soybean meal, corn DDGS (7.5 and 15.0% in phase 1 and 2, respectively) and wheat middlings (10.0%). Phase 1 diets contained no microbial phytase, whereas phase 2 diets contained 500 units of microbial phytase (Axtra® PHY; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI) per kg of complete diet. No diets contained antibiotic growth promoters. Diets were not formulated to be isocaloric or isonitrogenous and, therefore, the HF diets contained less NE and more CP than the LF diets. However, all diets were formulated to meet or exceed requirements for standardized ileal digestible AA, standardized total tract digestible P, and vitamins and minerals according to NRC (2012).

Individual pig weights were recorded at weaning and at the conclusion of each phase. Daily allotments of feed were recorded and feed remaining in the feeder at the end of each phase was recorded and feed intake calculated. Data were summarized and ADG, ADFI, and G:F were calculated. The G:F also was calculated as kg gain/Mcal NE intake because LF and HF diets were not formulated to be isocaloric.

Sample Collection. Blood samples (10 mL; 1 pig per pen) were collected from the same pig per pen at weaning and at the conclusion of phase 1 and phase 2. Blood samples were

analyzed for plasma urea nitrogen (**PUN**) and plasma concentrations of tumor necrosis factor alpha (**TNF-α**) in duplicate using a porcine sandwich ELISA kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Intra-assay CV was 5.5% for TNF-α.

At the conclusion of phase 2, 1 pig per pen was sacrificed using captive bolt penetration. These pigs were selected as the pig that was closest to the average BW of pigs in the pen and 5 gilts and 5 barrows were sacrificed from each treatment. Ileal and cecal digesta and rectal contents were collected. The pH of each of the samples was measured immediately after collection using a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA). After the pH was measured, cecal and rectal samples were mixed with 2*N* HCl in a 1:1 ratio and stored at -20°C until analyzed for concentrations of VFA. The remaining cecal digesta and rectal contents were stored at -20°C for further analysis.

A 5-cm tissue sample was collected from the ileum 10 cm cranial to the ileo-cecal sphincter, from the tail of the cecum, from the rectum 10 cm cranial to the internal anal sphincter, and from the left lateral lobe of the liver. After collection, tissue samples, with the exception of liver tissue, were opened at the mesentery, rinsed with ice-cold PBS, snap-frozen in liquid nitrogen, and stored at -80°C.

RNA Extraction and Quantitative Reverse Transcription-PCR. Total RNA was isolated from 100 mg of frozen tissue samples according to the PureLink® RNA Mini Kit (Life Technologies, Grand Island, NY) manufacturer's instructions. Total RNA was quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), and purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All RNA samples had 260/280 nm ratios greater than 1.9 and less than 2.1. The RNA quality was checked using a 2100 Bioanalyzer (Agilent Technologies, Santa

Clara, CA) and all RNA samples used for reverse transcription had an RNA integrity number greater than 8.

Total RNA (100 ng/µl) was reverse transcribed by means of a SuperScript® III First-Strand Synthesis SuperMix kit (Life Technologies, Grand Island, NY) to synthesize the double-stranded cDNA. Double-stranded cDNA was diluted and used for quantitative reverse transcription (**qRT-PCR**). Each 10 µL reaction consisted of 5 µL SYBR Green (Applied Biosystems, Foster City, CA), 4 µL diluted cDNA sample, 0.4 µL of 10 µM forward and reverse primer, and 0.2 µL DNase/RNase free water. The reactions were performed in an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. An additional dissociation stage was added to verify the presence of a single PCR product. All reactions were run in triplicate. Data were analyzed using the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, Foster City, CA).

Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) and hydroxymethylbilane synthase (***HMBS***), were used to normalize the expression of tested genes (Vigors et al., 2014). The tested genes included mucin 2 (***MUC2***), monocarboxylate transporter 1 (***MCT1***), basigin (***CD147***), phosphoenolpyruvate carboxykinase 1 (***PCK1***), and glucagon-like peptide – 2 receptor (***GLP-2R***). Mucin 2 is responsible for the production of mucin and was selected because previous research has indicated that high-fiber diets may increase mucin production (de Lange et al., 1989). Monocarboxylate transporter 1 is a proton-coupled transporter of VFA and *CD147* is responsible for translocation and function of *MCT1* (König et al., 2010) and, therefore, these 2 genes were selected to aid in the explanation of intestinal concentrations of VFA. Phosphoenolpyruvate carboxykinase 1 is the rate-controlling enzyme of

gluconeogenesis (Shulman and Petersen, 2012) and was selected because we hypothesized that HF fed pigs would have less dietary glucose and, therefore, have increased gluconeogenesis. Glucagon-like peptide - 2 receptor is a G-protein-coupled, transmembrane receptor for the peptide glucagon-like peptide - 2, which has been indicated to control gastrointestinal growth and function (Guan et al., 2006). Ileum, cecum, and rectum tissue were tested for mRNA expression of *MUC2*, *MCT1*, *CD147*, and *GLP-2R*, whereas liver tissue was tested for *MCT1*, *CD147*, *GLP-2R*, and *PCK1*. Primers used for amplification of target genes are provided in Table 4.3. To obtain the relative gene expression, the average quantity of triplicate samples was calculated and divided by the geometric mean of the 2 internal control genes.

Chemical Analyses. Prior to analysis, ileal and cecal digesta and rectal contents were freeze-dried and ground through a 1-mm screen in a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ). All main ingredients used in the diets and all diets, cecal, and rectal samples were analyzed for DM (Method 930.15; AOAC Int., 2007; Table 4.4). All diets and main ingredients were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom^{2000} Fiber Analyzer, Ankom Technology, Macedon, NY), while insoluble and soluble dietary fiber was analyzed according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Total dietary fiber was then calculated as the sum of insoluble and soluble dietary fiber. All diets and main ingredients also were analyzed for ash (Method 942.05; AOAC Int., 2007) and GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL). All cecal digesta samples and rectal samples that were stabilized in 2*N* HCl were analyzed for concentrations of VFA by gas chromatography according to Erwin et al. (1961) using a gas chromatograph (Hewlett-Packard 5890A Series II, Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4

on 80/100 + mesh Chomosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. The physicochemical characteristics of the main ingredients and diets were determined by measuring the water binding capacity (Robertson et al., 2000; Cervantes-Pahm et al., 2014) and bulk density (Cromwell et al., 2000; Table 4.5).

Statistical Analysis. Normality of residuals were determined by the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Outliers were determined using the BOXPLOT procedure of SAS (SAS Inst. Inc., Cary, NC) and any value that deviated from the treatment mean by 1.5 times the interquartile range was removed; 6 outliers were identified and removed. Gene expression data were log-10 transformed to align measures to a normal distribution. Growth performance, intestinal concentrations of VFA, and log-scale relative gene expression data were analyzed as a 2 × 2 factorial arrangement of treatments with dietary fiber concentration and DFM as the 2 factors and block as the random effect using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). An interaction between dietary fiber concentration and DFM was observed for initial BW of pigs and, therefore, initial BW was used as a covariate for growth performance. Relative gene expression data presented were back-transformed using antilog. The pen was the experimental unit for the growth performance data, but the pig was the experimental unit for the VFA and gene expression data.

A repeated measures analysis was conducted for TNF-α and PUN data and each individual pig was considered an experimental unit (Littell et al., 1998). Appropriate covariance structures were chosen based on the Akaike information criterion. Data were subjected to a 3-way ANOVA that included dietary fiber concentration, DFM, and d, as well as the interactions among these factors using PROC MIXED. Block was considered the random effect. The SLICE

option of PROC MIXED was used to evaluate the main effects and interaction of dietary fiber concentration and DFM at each d. For all outcomes, a *P*-value ≤ 0.05 was used to determine significance among dietary treatments and a *P*-value > 0.05, but < 0.10 was considered a tendency.

RESULTS

Ingredient and Diet Analysis

Phase 1 LF diets contained 1.3 and 10.1% soluble and insoluble dietary fiber, respectively, and phase 1 HF diets contained 0.4 and 15.1% soluble and insoluble dietary fiber, respectively. Phase 2 LF diets contained 0.5 and 13.5% soluble and insoluble dietary fiber, respectively, and phase 2 HF diets contained 3.0 and 17.0% soluble and insoluble dietary fiber, respectively. Corn, soybean meal, DDGS, and wheat middlings contained 0.6, 1.4, 1.9, and 6.3% soluble dietary fiber, respectively, and 12.1, 15.0, 29.0, and 37.1% insoluble dietary fiber, respectively (Table 4.4). The water binding capacity was 0.97, 2.69, 1.74, and 3.11 g/g for corn, soybean meal, DDGS, and wheat middlings, respectively, and the bulk density was 683.0, 807.3, 601.0, and 363.7 g/L, respectively (Table 4.5). The water binding capacity was 0.99 and 1.23 g/g and the bulk density was 757.5 and 689.4 g/L in phase 1 LF and HF diets, respectively. The water binding capacity was 1.20 and 1.34 g/g and the bulk density was 759.3 and 681.9 g/L in phase 2 LF and HF diets, respectively.

Growth Response

Initial BW was greater ($P \leq 0.05$) for pigs fed diets without DFM and an interaction ($P \leq 0.05$) was observed between dietary fiber and DFM (Table 4.6). Phase 1 ADG and BW at the conclusion of phase 1 were not affected by dietary fiber concentration or DFM. Phase 1 ADFI

was greater ($P \leq 0.05$) for pigs fed LF diets compared with pigs fed HF diets. Pigs fed diets containing DFM had a greater ($P \leq 0.05$) G:F in phase 1 compared with pigs fed diets without DFM. During phase 2, ADFI and G:F of pigs were unaffected by dietary fiber concentration or DFM, but ADG and BW of pigs fed LF diets was greater ($P \leq 0.05$) compared with pigs fed HF diets. Overall, pigs fed LF diets had a greater ($P \leq 0.05$) ADG, ADFI, and final BW compared with pigs fed HF diets and pigs fed diets containing DFM had a greater ($P \leq 0.05$) G:F than pigs fed no DFM.

Intestinal Concentrations of VFA and pH

The pH of ileal digesta was not affected by dietary fiber concentration or DFM, but cecal digesta pH tended to be greater ($P < 0.10$) in pigs fed LF diets compared with pigs fed HF diets (Table 4.7). Rectal content pH tended to be greater ($P < 0.10$) in pigs fed HF diets compared with pigs fed LF diets. The concentrations of all VFA in cecal digesta were not affected by dietary fiber concentration or DFM. The concentrations of acetate, propionate, and isovalerate were greater ($P \leq 0.05$) and the concentration of isobutyrate tended to be greater ($P < 0.10$) in rectal contents of pigs fed LF diets compared with pigs fed HF diets. Total short-chain fatty acid concentration in rectal contents of pigs fed LF diets was greater ($P \leq 0.05$) compared with pigs fed HF diets and the concentration of total branched-chain fatty acids tended to be greater ($P < 0.10$) in the rectal contents of pigs fed LF diets.

TNF-α and PUN

No effects of dietary fiber concentration or DFM addition on plasma concentrations of TNF-α were observed (Table 4.8). However, the effect of d impacted the concentration of TNF-α in that on d 0, the concentration of TNF-α was the lowest ($P \leq 0.05$) and on d 14 greatest ($P \leq 0.05$), while the concentration of TNF-α on d 43 was between d 0 and d 14 concentrations of

TNF-α. The PUN of pigs fed DFM-containing diets was less ($P \leq 0.05$) on d 0 compared with pigs fed diets without DFM. Also on d 0, a tendency for an interaction between dietary fiber concentration and DFM was observed ($P < 0.10$) because HF-fed pigs tended to have a reduced ($P < 0.10$) PUN compared with LF-fed pigs. On d 14, the PUN was not different among pigs fed experimental diets. The HF diets increased ($P \leq 0.05$) PUN of pigs on d 43, but an interaction ($P \leq 0.05$) between dietary fiber concentration and DFM also was observed on d 43 because DFM addition to LF diets increased PUN of pigs, whereas DFM addition to HF diets decreased PUN. Finally, as the experiment progressed from d 0, 14, and d 43, the PUN of pigs increased over time ($P \leq 0.05$).

Gene Expression

The expression of internal control genes were confirmed to be unaffected by dietary treatment. The expression of *MCT1* was decreased ($P \leq 0.05$) in the ileum of pigs due to DFM addition to the diets (Table 4.9). The expression of tested genes from cecum tissue was not affected by dietary fiber concentration or DFM addition. Pigs fed HF diets had increased ($P \leq 0.05$) *MCT1* expression in the rectum. Pigs fed DFM-containing diets had increased ($P \leq 0.05$) *CD147* and *GLP-2R* expression and tended to have increased ($P < 0.10$) *MCT1* expression in the liver.

DISCUSSION

Results of previous research indicated that nursery pig growth performance is not reduced if 7.5 and 15% DDGS is included in phase 1 and 2 diets, respectively (Spencer et al., 2007). Likewise, inclusion of 20% DDGS in diets of nursery pigs may not impact pig growth performance, but wheat middlings included at 0, 5, 10, 15, or 20% in a corn-soybean meal

nursery diet linearly reduced ADFI and ADG (De Jong et al., 2014). It is, therefore, likely that the inclusion of wheat middlings in the diets used in this experiment resulted in the reduction in ADG observed for the pigs fed the HF diets.

Jaworski et al. (2014b) reported increased water binding capacity and decreased bulk density as inclusion of 0, 5, 10, or 15% copra meal, palm kernel meal, or palm kernel expellers was included in nursery pig diets, and similar results were observed in the current experiment when DDGS and wheat middlings were added to diets. A decrease in nursery pig ADFI and ADG was observed as water binding capacity increased and bulk density decreased in diets, and this is in agreement with Jaworski et al. (2014b). Together, these results indicate that nursery pigs may not be able to overcome the gut fill effect attributed to HF diets, particularly those that result in a greater water binding capacity and decreased bulk density.

Addition of a *Bacillus*-based DFM to a corn-soybean meal diet fed to growing-finishing pigs may improve ADG (Davis et al., 2008). It has also been reported that addition of 8% soybean hulls or 8% peanut hulls to a corn-soybean meal diet fed to weanling pigs reduced G:F, but when a yeast culture DFM was added to those diets, G:F was maintained compared with a corn-soybean meal control diet (Kornegay et al., 1995). The observation that addition of a *Bacillus*-based DFM to LF and HF diets in this experiment improved overall G:F of nursery pigs is in agreement with data of Kornegay et al. (1995) and Davis et al. (2008). However, in a review of the literature, Pollmann (1986) reported that addition of *Bacillus*-based DFM to nursery pig diets did not consistently improve growth performance, whereas a more recent review indicated that DFM addition to swine diets was beneficial in 30 of 31 research trials (Kremer, 2006). The inconsistencies reported in the literature regarding *Bacillus*-based DFM added to nursery pig diets may be a result of differences in ingredient composition of diets or health status of pigs, but

it is also possible that improvements have been made in the development and implementation of DFM. Finally, inconsistency in a DFM response also may arise from differences in the functionality of the strains being assessed and whether single strains are being compared to a combination of strains. Therefore, it is possible that the *Bacillus*-based DFM used in this experiment may have been more efficient in terms of stimulating microbial enzyme synthesis compared with the DFM used in previous experiments.

Bacillus-based DFM may secrete enzymes capable of degrading DM in swine manure (Schreier, 1993; Davis et al., 2008). Swine manure DM is mostly composed of dietary fiber due to the indigestible nature of dietary fiber fed to pigs. Therefore, we hypothesized that a *Bacillus*-based DFM added to swine diets may be capable of fermenting dietary fiber, which may increase the amount of energy available to the pig in the form of VFA. It was expected that DFM would increase dietary fiber fermentation, resulting in a lower pH and greater VFA concentration in cecal and rectal contents of pigs fed diets containing DFM. Supplementation of diets of feedlot cattle and horses with DFM has been unsuccessful in increasing dietary fiber fermentation (Beauchemin et al., 2003; Swyers et al., 2008), but shifted fermentation and microbial populations in the rumen of feedlot steers from lactate to acetate production, aiding in the prevention of acidosis (Ghorbani et al., 2002). Addition of a *Bacillus*-based DFM to growing pig diets containing multiple sources of dietary fiber increased the concentration of VFA in the feces and, therefore, enhanced fermentation and available ME, which resulted in increased ADG and G:F (Jaworski et al., 2014a). However, in the current experiment, DFM had no effect on pH or VFA concentrations in ileal, cecal, or rectal contents.

Also, contradictory to our hypothesis, the concentrations of acetate, propionate, isovalerate, total short-chain fatty acids, and total branched-chain fatty acids in rectal contents of

pigs fed LF diets were greater compared with HF diets. Three reasons may explain this difference, but none of them have been experimentally verified: 1) increased dietary fiber results in increased rate of digesta passage, which decreased the amount of time the microbes have to ferment dietary fiber (Chesson, 2006); 2) absorption of VFA was decreased in LF fed pigs compared with HF fed pigs because of a lack of VFA transporters; and 3) dietary fiber present in corn and soybean meal are more fermentable compared with dietary fiber present in DDGS and wheat middlings. Along these lines, the substrate concentration after the terminal ileum in LF fed pigs is expected to be much less, enabling microbes greater access to a comparatively lesser amount of dietary fiber than HF fed pigs. This is due to the lower apparent ileal digestibility of DM in diets containing a greater concentration of dietary fiber.

Jørgensen et al. (1997) infused VFA into the cecum of growing pigs and less than 1% of the infused VFA were excreted in the feces and this observation contrasts the second reason listed above. Therefore, it appears that VFA absorption is quite efficient (Barcroft et al., 1944). Also, the expression of the VFA transporter *MCT1* in the rectum of HF-fed pigs was greater compared with LF-fed pigs, which is in contrast with the concentrations of VFA in the rectum of pigs. These results indicate that the increased VFA concentration in rectal contents of LF-fed pigs did not correspond with an increase in *MCT1* expression. Metzler-Zebeli et al. (2012) determined that *MCT1* expression in the cecum and colon of weaned pigs was positively correlated with butyrate concentration ($R = 0.99$; $P < 0.001$) and propionate concentration ($R = 0.84$; $P < 0.001$), respectively. Butyrate concentration in cecal digesta of pigs was not different due to dietary treatments; therefore, *MCT1* expression in the cecum was not affected by dietary treatments in the current study.

On the other hand, lower VFA concentrations in rectum contents of HF-fed pigs may be due to greater VFA absorption because HF fed pigs had increased relative gene expression of *MCT1* in rectum tissue. However, it has been indicated that *CD147* is required for *MCT1* translocation to the plasma membrane as well as for *MCT1* transporter function and, in the current experiment, *CD147* expression in the rectum was unaffected by dietary fiber concentration (Kirk et al., 2000; Wilson et al., 2005). Taking all of this information into account, further research is warranted on VFA production, absorption, and utilization by pigs. The VFA molar proportions in cecal and rectal contents observed in this experiment are in agreement with the VFA molar proportion (i. e., 65:25:10, acetate:propionate:butyrate) usually observed in pigs (Robertson, 2007).

The third reason listed above is in agreement with previous research that indicates the in vitro total tract digestibility of non-starch polysaccharides in corn is greater than in DDGS and wheat middlings (Jaworski et al., 2015). On the other hand, Urriola et al. (2010) determined the apparent total tract digestibility (**ATTD**) of total dietary fiber by growing pigs to be 23.1% in corn, but 44.5% in corn DDGS. Yet, Lowell et al. (2015) reported that the ATTD of NDF by growing pigs was 72.94% in a corn-soybean meal diet, 50.58% in a corn-corn DDGS diet, and 62.51% in a corn-wheat middlings diet. The difference between Urriola et al. (2010) and Lowell et al. (2015) is not only that Urriola et al. (2010) determined the ATTD of total dietary fiber, but also the test ingredient was the sole source of total dietary fiber in the diet, whereas Lowell et al. (2015) determined the ATTD of NDF by growing pigs fed a mixture of corn and soybean meal. This digestibility was much greater than the other mixtures of corn and co-product, indicating that the dietary fiber present in a corn and soybean meal diet, such as the LF diet fed in the current study, may be more fermentable, which would lead to greater VFA concentrations in the

rectal contents of pigs, which was observed in the current experiment. Therefore, it is concluded that a LF corn-soybean meal diet is more fermentable on a μmol/g basis compared with a HF diet.

The fact that nursery pig G:F, expressed as kg/Mcal NE, was not different between pigs fed LF or HF diets is quite remarkable, indicating that nursery pigs are just as efficient converting dietary NE from LF or HF diets to BW gain, but cannot overcome the gut fill effect associated with HF diets and, therefore, nursery pig ADFI is reduced, thus, ADG is reduced. Interestingly, nursery pig overall G:F, expressed as kg/Mcal NE, was improved 1.45 and 5.07% in LF and HF diets, respectively, due to DFM addition. Therefore, the addition of the 3-strain *Bacillus*-based DFM to LF and HF diets fed to nursery pigs increased the amount of energy the pigs received from the diets or decreased the pigs' maintenance energy requirement. Unfortunately, the increased available energy from the diet was not attributed to increased hindgut fermentation associated with an increased VFA concentration in cecal digesta or rectal contents of pigs fed DFM-containing diets and, therefore, our hypothesis was incorrect and we are unable to experimentally verify if the DFM increased the amount of energy the pigs received from the diets.

The addition of DFM to diets fed to nursery pigs may reduce the maintenance energy requirement of the pig by multiple modes of action such as reduced immune stress, reduced endogenous secretions, and improved gastrointestinal integrity. The plasma concentration of TNF-α was not different in nursery pigs fed experimental diets; therefore, it is concluded that pig pro-inflammatory immune cell regulation at the systemic level was not affected by DFM addition and was not the cause for the reduced maintenance energy requirement (Elsasser et al., 2008). However, changes in pro-inflammatory immune response are possible at the mucosal

level, but this was not experimentally verified in the current experiment and, therefore, it cannot be ruled out that pig pro-inflammatory immune cell regulation was not affected by DFM addition.

The expression of *GLP-2R* in the liver of nursery pigs fed DFM-containing diets was increased, indicating that the presence of *GLP-2* was increased in the liver as well (Connor et al., 2015). Glucagon-like peptide-2 increased expression of maltase-glucoamylase and sucrose-isomaltase digestive enzymes (Petersen et al., 2001, 2002), decreased gastric emptying, gastric acid secretion, and gut motility (Wøjdemann et al., 1999; Guan et al., 2012), and increased intestinal cell proliferation and reduced apoptosis in weanling pigs (Burrin et al., 2005, 2007). It may, therefore, be speculated that the greater *GLP-2R* expression in the liver was a result of increased *GLP-2* being synthesized, and therefore, the improved G:F of pigs fed the DFM diets could partly be a result of a reduced maintenance energy requirement due to reduced gastric emptying, reduced gastric acid secretions, and less gut motility. However, this hypothesis needs to be experimentally verified.

CONCLUSION

Inclusion of 7.5 and 15% DDGS in phase 1 and 2 nursery diets, respectively, and 10% wheat middlings decreased phase 1 ADFI and overall ADG and ADFI, resulting in a decreased BW at the conclusion of phase 2. However, G:F was unaffected by dietary fiber concentration, indicating that nursery pigs are just as efficient converting dietary NE from LF or HF diets to BW gain. However, they cannot overcome the gut fill effect associated with HF diets and, therefore, nursery pig ADFI was reduced and, thus, ADG was reduced. Addition of a 3-strain *Bacillus*-based DFM to LF or HF diets improved overall G:F but had no effect on VFA

concentration in cecum or rectal contents. Pigs fed LF diets had greater concentrations of VFA in rectal contents of pigs compared with pigs fed HF diets. Therefore, it is concluded that a *Bacillus*-based DFM may be added to LF or HF nursery diets to increase G:F; however, G:F appears not to have been increased due to increased fermentation and more available energy in the form of VFA. However, it is concluded that the *Bacillus*-based DFM used in this experiment was effective in increasing energy utilization of diets, which may have been a result of increased synthesis of *GLP-2* because the relative expression of *GLP-2R* in the liver increased in pigs fed diets fortified with DFM. Further research is necessary to relate fermentation of dietary fiber to cecal and large bowel VFA production, absorption, and utilization by pigs, and to quantify the role DFM may play in stimulating *GLP-2* secretion aiding in enhanced gastrointestinal health, thereby decreasing the maintenance energy requirement.

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TABLES

Table 4.1. Ingredient, calculated, and analyzed composition of phase 1 diets (as-fed basis)

Dietary fiber concentration	Low		High	
Direct-fed microbial	-	+	-	+
Ingredient, %				
Corn	51.44	51.38	37.09	37.03
Soybean meal, 48% CP	20.00	20.00	17.00	17.00
Whey, dried	15.00	15.00	15.00	15.00
DDGS[1]	-	-	7.50	7.50
Wheat middlings	-	-	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00
Blood plasma	4.00	4.00	4.00	4.00
Soybean oil	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.25	1.25
Dicalcium phosphate	0.40	0.40	-	-
L-Lys HCl	0.30	0.30	0.33	0.33
DL-Met	0.10	0.10	0.08	0.08
L-Thr	0.06	0.06	0.05	0.05
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
DFM mixture[3]	-	0.06	-	0.06
Calculated composition[4]				
NE, kcal/kg	2,525	2,525	2,463	2,463
CP, %	21.80	21.80	22.81	22.81
SID[5] Lys, %	1.43	1.43	1.43	1.43
Ca, %	0.85	0.85	0.85	0.85
STTD[6] P, %	0.46	0.46	0.46	0.46
Analyzed composition				
GE, kcal/kg	3,986	3,947	4,067	4,103
DM, %	87.1	87.1	86.7	86.2
Ash, %	6.3	5.6	6.0	6.0
ADF, %	4.3	4.5	5.5	4.5
NDF, %	7.1	8.7	12.6	12.3
Insoluble dietary fiber, %	9.5	10.7	15.4	14.7
Soluble dietary fiber, %	1.1	1.4	0.2	0.6
Total dietary fiber, %	10.6	12.1	15.6	15.3

[1]DDGS = distillers dried grains with solubles.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial mixture consists of 30 g of DFM mixed with 270 g of corn.

[4]Calculated from NRC (2012) values.

[5]SID = standardized ileal digestible.

[6]STTD = standardized total tract digestible.

Table 4.2. Ingredient, calculated, and analyzed composition of phase 2 diets (as-fed basis)

Dietary fiber concentration	Low		High	
Direct-fed microbial	-	+	-	+
Ingredient, %				
Corn	54.10	54.04	35.24	35.18
Soybean meal, 48% CP	27.00	27.00	21.00	21.00
Whey, dried	10.00	10.00	10.00	10.00
DDGS[1]	-	-	15.00	15.00
Wheat middlings	-	-	10.00	10.00
Fish meal	4.00	4.00	4.00	4.00
Soybean oil	2.00	2.00	2.00	2.00
Limestone	1.07	1.07	1.42	1.42
Dicalcium phosphate	0.50	0.50	-	-
L-Lys HCl	0.40	0.40	0.45	0.45
DL-Met	0.12	0.12	0.09	0.09
L-Thr	0.10	0.10	0.09	0.09
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
DFM mixture[3]	-	0.06	-	0.06
Calculated composition[4]				
NE, kcal/kg	2,483	2,483	2,414	2,414
CP, %	20.95	20.95	22.29	22.29
SID[5] Lys, %	1.36	1.36	1.35	1.35
Ca, %	0.85	0.85	0.85	0.85
STTD[6] P, %	0.39	0.39	0.39	0.39
Analyzed composition				
GE, kcal/kg	3,963	3,949	4,045	4,077
DM, %	87.8	87.5	87.8	87.9
Ash, %	5.0	5.2	5.4	5.7
ADF, %	4.7	4.0	6.1	5.8
NDF, %	11.4	10.5	14.0	14.1
Insoluble dietary fiber, %	13.5	13.5	17.0	17.0
Soluble dietary fiber, %	0.4	0.5	3.0	3.0
Total dietary fiber, %	13.9	14.0	20.0	20.0

[1]DDGS = distillers dried grains with solubles.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial mixture consists of 30 g of DFM mixed with 270 g of corn.

[4]Calculated from NRC (2012) values.

[5]SID = standardized ileal digestible.

[6]STTD = standardized total tract digestible.

Table 4.3. Gene-specific primer sequences

Gene	Acc. No	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
MUC2[1]	AK231524	CAACGGCCTCTCCTTCTCTGT	GCCACACTGGCCCTTTGT	Leonard et al. (2011)
MCT1[2]	AM286425	GGTGGAGGTCCTATCAGCAG	TGAAGGCAAGCCCAAGAC	Metzler-Zebeli et al. (2012)
CD147[3]	NM_001123086	CCTCGGAGACCAAGACAGAG	TCATTCACGTGGTGTCCACT	König et al. (2010)
PCK1[4]	NM_001123158.1	CCCTGCCTTTGAAAAAGCCC	GGAGATGATTTCTCGGCGGT	Qu et al. (2015)
GLP-2R[5]	NM_001246266.1	TGTCCTACGTGTCGGAGATGTC	TAATTGGCGCCCACGAA	Guan et al. (2006)

[1]*MUC2* = mucin 2.
[2]*MCT1* = monocarboxylate transporter 1.
[3]*CD147* = basigin.
[4]*PCK1* = phosphoenolpyruvate carboxykinase 1.
[5]*GLP-2R* = glucagon-like peptide-2 receptor.

Table 4.4. Analyzed energy and nutrient composition of main ingredients (as-fed basis)

	Ingredient			
Item	Corn	Soybean meal	DDGS[1]	Wheat middlings
GE, kcal/kg	3,773	4,175	4,421	4,027
DM, %	84.5	88.4	86.9	86.7
Ash, %	1.1	6.1	4.5	4.8
ADF, %	3.8	6.1	11.6	10.8
NDF, %	12.2	7.0	24.1	38.4
Insoluble dietary fiber, %	12.1	15.0	29.0	37.1
Soluble dietary fiber, %	0.6	1.4	1.9	6.3
Total dietary fiber, %	12.7	16.4	30.9	43.4

[1]DDGS = distillers dried grains with solubles.

Table 4.5. Physicochemical characteristics of diets and ingredients

Item	Water binding capacity, g/g	Bulk density, g/L
Ingredients		
Corn	0.97	683.0
Soybean meal	2.69	807.3
DDGS[1]	1.74	601.0
Wheat middlings	3.11	363.7
Phase 1 diets		
Low fiber	0.93	754.3
Low fiber + DFM[2]	1.04	760.7
High fiber	1.23	683.7
High fiber + DFM	1.22	695.0
Phase 2 diets		
Low fiber	1.21	760.3
Low fiber + DFM	1.19	758.3
High fiber	1.35	680.7
High fiber + DFM	1.32	683.0

[1]DDGS = distillers dried grains with solubles.

[2]DFM = direct-fed microbial.

Table 4.6. Growth performance of nursery pigs fed low- or high-fiber diets without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Phase 1 (d 0 – 14)								
Initial BW, kg	6.336	6.276	6.298	6.309	0.268	0.802	0.048	0.006
ADG, g/d	189	187	178	168	12.35	0.178	0.613	0.744
ADFI, g/d	240	219	206	172	23.30	0.033	0.138	0.726
G:F, g/g	0.802	0.923	0.830	1.048	0.084	0.350	0.043	0.555
G:F, kg/Mcal NE	0.317	0.366	0.337	0.426	0.032	0.227	0.041	0.536
Final BW, kg	8.988	8.900	8.785	8.664	0.350	0.176	0.514	0.918
Phase 2 (d 14 – 43)								
ADG, g/d	619	629	598	599	38.31	0.025	0.600	0.694
ADFI, g/d	922	936	924	875	71.55	0.127	0.335	0.099
G:F, g/g	0.672	0.676	0.649	0.678	0.015	0.298	0.118	0.240
G:F, kg/Mcal NE	0.271	0.272	0.269	0.281	0.006	0.436	0.113	0.228
Final BW, kg	26.929	27.127	26.117	26.037	1.202	0.024	0.883	0.730
d 0 – 43								
ADG, g/d	479	485	461	459	24.24	0.025	0.835	0.665
ADFI, g/d	700	702	691	646	43.94	0.048	0.192	0.153
G:F, g/g	0.685	0.695	0.667	0.702	0.013	0.592	0.022	0.192
G:F, kg/Mcal NE	0.275	0.279	0.276	0.290	0.005	0.138	0.020	0.179

[1]DFM = direct-fed microbial.

Table 4.7. pH and VFA concentrations, expressed as µmol/g DM basis, in cecal and rectal contents of pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ileal digesta pH	6.78	6.77	6.83	7.04	0.18	0.216	0.444	0.413
Cecal digesta								
pH	6.01	6.01	5.84	5.89	0.10	0.053	0.696	0.767
Acetate	100.73	100.17	102.38	102.67	4.59	0.470	0.962	0.883
Propionate	27.02	28.24	29.59	29.75	1.99	0.156	0.629	0.709
Butyrate	14.30	12.88	16.64	13.37	1.66	0.343	0.121	0.536
Total SCFA[2]	142.06	141.29	149.51	145.79	7.85	0.233	0.650	0.765
Valerate	2.08	1.76	2.15	1.93	0.36	0.715	0.397	0.878
Isovalerate	0.48	0.50	0.35	0.61	0.09	0.913	0.101	0.180
Isobutyrate	0.52	0.60	0.58	0.71	0.08	0.322	0.206	0.747
Total BCFA[3]	3.08	2.87	3.07	3.25	0.39	0.618	0.961	0.612
SCFA:BCFA	53.58	55.47	48.98	49.84	5.21	0.312	0.785	0.919
Rectal contents								
pH	6.56	6.53	6.77	6.63	0.09	0.093	0.373	0.561
Acetate	112.99	111.44	100.78	93.95	5.59	0.006	0.416	0.608
Propionate	25.94	24.43	21.21	19.43	2.00	0.021	0.418	0.949
Butyrate	12.94	11.54	10.95	11.56	1.08	0.367	0.716	0.355
Total SCFA	151.71	147.41	141.19	129.79	7.20	0.027	0.204	0.563
Valerate	2.10	2.35	2.03	2.01	0.20	0.321	0.582	0.507
Isovalerate	2.79	2.71	2.18	2.21	0.39	0.043	0.920	0.825
Isobutyrate	2.29	2.06	1.81	1.71	0.23	0.059	0.445	0.749
Total BCFA	6.86	6.89	5.62	5.90	0.60	0.065	0.787	0.834
SCFA:BCFA	21.68	21.91	23.91	21.84	3.13	0.616	0.669	0.590

[1]DFM = direct-fed microbial.
[2]SCFA = short chair fatty acids.
[3]BCFA = branched-chain fatty acids.

Table 4.8. Plasma urea nitrogen (PUN) and plasma concentration of tumor necrosis factor-alpha (TNF-α) of pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance[1]		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[2]	Dietary fiber × DFM
d 0								
PUN, mg/dL	6.6	4.7	6.0	3.8	0.8	0.370	0.015	0.081
TNF-α, pg/mL	29.6	17.9	17.6	16.7	6.0	0.299	0.319	0.586
d 14								
PUN, mg/dL	8.0	6.8	8.2	7.7	0.8	0.511	0.310	0.649
TNF-α, pg/mL	87.9	109.2	103.5	98.9	15.0	0.861	0.583	0.782
d 43								
PUN, mg/dL	9.5	11.9	13.9	13.0	0.8	0.001	0.370	0.002
TNF-α, pg/mL	68.2	81.6	86.2	65.0	18.0	0.968	0.829	0.810

[1]Effect of d ($P \leq 0.05$) was observed.
[2]DFM = direct-fed microbial.

Table 4.9. Relative mRNA expression of genes from ileum, cecum, rectum, and liver tissue from pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ileum								
MCT1[1]	2.14	1.48	1.98	1.70	1.13	0.777	0.017	0.300
CD147[1]	0.97	1.18	1.12	1.11	1.10	0.687	0.318	0.276
MUC2[1]	0.82	0.70	1.09	0.82	1.29	0.265	0.268	0.762
GLP-2R[1]	0.85	0.84	0.76	0.65	1.24	0.378	0.710	0.741
Cecum								
MCT1	0.66	0.58	0.64	0.55	1.19	0.801	0.424	0.969
CD147	3.40	2.46	2.52	2.66	1.12	0.344	0.245	0.110
MUC2	0.42	0.34	0.36	0.34	1.12	0.467	0.241	0.520
GLP-2R	0.29	0.32	0.26	0.27	1.21	0.387	0.718	0.767
Rectum								
MCT1	0.85	1.00	1.59	1.65	1.22	< 0.001	0.477	0.634
CD147	1.16	1.12	1.15	1.45	1.16	0.147	0.265	0.136
MUC2	1.06	0.94	0.92	1.22	1.19	0.629	0.496	0.084
GLP-2R	0.59	0.80	0.77	0.89	1.26	0.238	0.146	0.599
Liver								
MCT1	0.74	0.97	0.81	0.88	1.11	0.999	0.090	0.351
CD147	1.03	1.25	1.11	1.16	1.06	0.990	0.038	0.180
GLP-2R	0.94	1.20	0.88	1.33	1.17	0.899	0.011	0.492
PCK1[1]	0.81	0.54	0.84	1.00	1.30	0.161	0.626	0.228

[1]DFM = direct-fed microbial, *MCT1* = monocarboxylate transporter 1, *CD147* = basigin, *MUC2* = mucin 2, *GLP-2R* = glucagon-like peptide-2 receptor, *PCK1* = phosphoenolpyruvate carboxykinase 1.

CHAPTER 5: EFFECT OF A 3-STRAIN *BACILLUS*-BASED DIRECT-FED MICROBIAL ON GROWTH PERFORMANCE, INTESTINAL CONCENTRATIONS OF VOLATILE FATTY ACIDS, CARCASS CHARACTERISTICS, AND GASTROINTESTINAL TRACT WEIGHTS IN GROWING-FINISHING PIGS FED LOW- OR HIGH-FIBER DIETS

ABSTRACT: The effect of a *Bacillus*-based direct-fed microbial (DFM) on growth performance, intestinal VFA concentration, carcass characteristics, and gastrointestinal tract weights in growing-finishing pigs was evaluated. A total of 160 pigs (initial BW: 26.61 ± 2.17 kg) were randomly allotted to a 2 × 2 factorial arrangement with 2 diet types [low-fiber (LF) or high-fiber (HF)] and 2 concentrations of DFM [0 or 60 g DFM (1.5×10^5 cfu / g) / t of feed] and 4 pigs per pen and 10 pens per treatment. Grower, early-finisher, and late-finisher diets were fed for 35, 35, and 24 d, respectively. Pigs were previously fed their respective treatment diets since weaning at d 21 of age. The LF diets contained corn and soybean meal as main ingredients and HF diets contained corn, soybean meal, corn distillers dried grains with solubles (30%), and wheat middlings (10%). Pig weights were recorded at the beginning of the experiment and conclusion of each phase. Daily feed allotments also were recorded and feed left in the feeders was recorded on the same days as pig weights were obtained. One pig per pen was harvested at the conclusion of the experiment. Cecum and rectum contents were analyzed for VFA. Carcass characteristics and gastrointestinal tract weights were measured. Results indicated that for the overall growing-finishing period, there was no difference in ADG or G:F, expressed as kg gain/Mcal NE, between pigs fed LF and HF diets, but pigs fed HF diets had greater ($P \leq 0.05$) ADFI, reduced ($P \leq 0.05$) backfat thickness, reduced ($P \leq 0.05$) dressing percentage, and

increased ($P \leq 0.05$) weight of the large intestine as a percent of BW compared with pigs fed LF diets. Pigs fed LF diets had greater ($P \leq 0.05$) concentrations of acetate and propionate in cecum contents and greater ($P \leq 0.05$) concentrations of all VFA in rectal contents compared with pigs fed HF diets. Pigs fed DFM-containing diets had decreased ($P \leq 0.05$) concentrations of total VFA in cecal contents, but increased ($P \leq 0.05$) concentrations of total VFA in rectal contents. Pigs fed diets supplemented with DFM had greater ($P \leq 0.05$) HCW and backfat thickness, but fat-free lean percentage was reduced ($P < 0.05$) compared with pigs fed diets with no DFM. In conclusion, pigs fed HF diets had similar ADG and G:F and a lower dressing percentage than pigs fed LF diets. The addition of this DFM to LF or HF diets had no effect on growth performance, but increased backfat thickness and reduced fat-free lean percentage.

Key words: carcass, dietary fiber, direct-fed microbials, growth performance, pigs

INTRODUCTION

Addition of direct-fed microbials (**DFM**) to swine diets may improve gut health by modifying the microbiota, which may enhance immune regulation and health status of the pigs (Kenny et al., 2011; Cromwell, 2013). Addition of DFM to growing-finishing pig diets also may increase ADG, G:F, and carcass quality (Alexopoulos et al., 2004; Davis et al., 2008).

Bacillus-based DFM are thermo-stable spore-forming bacteria and they produce a large amount and a wide variety of manure-degrading enzymes (Schreier, 1993; Kenny et al., 2011). Pig manure is mostly composed of dietary fiber and, therefore, the addition of a *Bacillus*-based DFM may enhance the fermentation of dietary fiber in swine diets (Davis et al., 2008). High-fiber diets based on fibrous co-products fed to growing-finishing pigs may reduce growth performance and carcass characteristics compared with pigs fed low-fiber diets based on corn

and soybean meal (Bindelle et al., 2008). However, when growing-finishing pigs were fed high-fiber diets based on corn, soybean meal, DDGS, wheat middlings, corn germ, and soybean hulls supplemented with a *Bacillus* spp. DFM, it was observed that pigs had increased fecal VFA concentrations, improved ADG and G:F, and a greater loin eye area and fat-free lean percentage compared with pigs fed no DFM (Jaworski et al., 2014). Therefore, it may be beneficial to supplement high-fiber diets containing distillers dried grains with solubles (**DDGS**) and wheat middlings with a *Bacillus*-based DFM that has been selected on the basis of the ability to secrete fiber-degrading enzymes. The objective of this experiment, therefore, was to test the hypothesis that addition of a 3-strain *Bacillus*-based DFM will increase dietary fiber fermentation, which will increase growth performance and carcass characteristics of growing-finishing pigs fed high-fiber diets compared with pigs fed low-fiber diets.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for these experiments.

Animals, Diets, and Experimental Design. A total of 160 pigs (initial BW: 26.61 ± 2.17 kg) that were the offspring of G-Performer boars mated to F-25 females (Genetiporc, Alexandria, MN) were used in this experiment in 2 separate blocks of 80 pigs each. Pigs were randomly allotted in a completely randomized block design to 4 dietary treatments. There were 4 pigs per pen and 10 replicate pens per treatment. Pigs were housed in pens equipped with a feeder, a nipple drinker, and partly slatted concrete floors in an environmentally controlled building. Ad libitum access to feed and water was allowed throughout the experiment.

Treatments were arranged in a 2 × 2 factorial arrangement with 2 diet types [low-fiber (**LF)** or high-fiber (**HF**)] and 2 concentrations of DFM (0 or 60 g DFM / t of feed; Tables 5.1, 5.2, and 5.3). The 3-strain *Bacillus*-based DFM contained 1.5×10^5 CFU / g and was obtained from Danisco Animal Nutrition-DuPont Industrial Biosciences (Marlborough, UK). Pigs were previously fed similar diets so that treatment contrasts (i. e., dietary fiber concentration and DFM addition) had been fed since weaning. Grower diets were fed for 5 weeks. Pigs then were fed early-finisher diets for 5 weeks and late-finisher diets were fed during the final 24 d. The LF diets contained corn and soybean meal as main ingredients and HF diets contained corn, soybean meal, DDGS (30.0%) and wheat middlings (10.0%). All diets contained 500 units of microbial phytase (Axtra® PHY; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI) per kg of complete diet. Diets were not formulated to be isocaloric or isonitrogenous and, therefore, the HF diets contained less NE and more CP than the LF diets. However, all diets were formulated to meet or exceed requirements for standardized ileal digestible (**SID**) AA, standardized total tract digestible (**STTD**) P, and vitamins and minerals according to NRC (2012).

Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each phase. Daily allotments of feed were recorded and feed left in the feeder at the end of each phase was recorded and feed intake calculated. Data were summarized and ADG, ADFI, and G:F calculated. The G:F also was calculated as kg gain / Mcal NE because LF and HF diets were not formulated to be isocaloric.

Sample Collection. Blood samples (10 mL; 1 pig per pen) were collected from 1 pig per pen at the start of the experiment and blood was collected from the same pig per pen at the conclusion of the experiment. Blood samples were analyzed for plasma urea nitrogen (**PUN**) and

plasma concentrations of tumor necrosis factor alpha (**TNF-α**) in duplicate using a porcine sandwich ELISA kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Intra-assay CV was 6.2% for TNF-α.

At the conclusion of the late-finisher phase, 1 pig per pen was harvested and 5 gilts and 5 barrows were harvested from each treatment. The weight of the full intestinal tract was recorded and ileal and cecal digesta and rectal contents collected. The pH of each of the samples was measured immediately after collection using a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA). After the pH was measured, cecal and rectal samples were mixed with 2*N* HCl in a 1:1 ratio and stored at -20°C until analyzed for concentrations of VFA. The remaining cecal digesta and rectal contents were stored at -20°C for further analysis.

A 5-cm tissue sample was collected from the ileum 10 cm cranial to the ileo-cecal sphincter, from the tail of the cecum, from the rectum 10 cm cranial to the internal anal sphincter, and from the left lateral lobe of the liver. After collection, tissue samples, with the exception of liver tissue, were opened at the mesentery, rinsed with ice-cold PBS, snap-frozen in liquid nitrogen, and stored at -80°C.

RNA Extraction and Quantitative Reverse Transcription-PCR. Total RNA was isolated from 100 mg of frozen tissue samples according to the PureLink® RNA Mini Kit (Life Technologies, Grand Island, NY) manufacturer's instructions. Total RNA was quantified by measuring the absorbance at 260 nm using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), and purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All RNA samples had 260/280 nm ratios greater than 1.9 and less than 2.1. The RNA quality was checked using a 2100 Bioanalyzer (Agilent Technologies, Santa

Clara, CA) and all RNA samples used for reverse transcription had an RNA integrity number greater than 8.

Total RNA (100 ng/µl) was reverse transcribed by means of a SuperScript® III First-Strand Synthesis SuperMix kit (Life Technologies, Grand Island, NY) to synthesize the double-stranded cDNA. Double-stranded cDNA was diluted and used for quantitative reverse transcription (**qRT-PCR**). Each 10 µL reaction consisted of 5 µL SYBR Green (Applied Biosystems, Foster City, CA), 4 µL diluted cDNA sample, 0.4 µL of 10 µM forward and reverse primer, and 0.2 µL DNase/RNase free water. The reactions were performed in an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. An additional dissociation stage was added to verify the presence of a single PCR product. All reactions were run in triplicate. Data were analyzed using the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems, Foster City, CA).

Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (***GAPDH***) and hydroxymethylbilane synthase (***HMBS***) were used to normalize the expression of tested genes (Vigors et al., 2014). The tested genes included mucin 2 (***MUC2***), monocarboxylate transporter 1 (***MCT1***), basigin (***CD147***), phosphoenolpyruvate carboxykinase 1 (***PCK1***), and glucagon-like peptide - 2 receptor (***GLP-2R***). Mucin 2 is responsible for the production of mucin and was selected because previous research has indicated that high-fiber diets may increase mucin production (de Lange et al., 1989). Monocarboxylate transporter 1 is a proton-coupled transporter of VFA and *CD147* is responsible for translocation and function of *MCT1* (König et al., 2010) and, therefore, these two genes were selected to aid in the explanation of intestinal concentrations of VFA. Phosphoenolpyruvate carboxykinase 1 is the rate-controlling enzyme of

gluconeogenesis (Shulman and Petersen, 2012) and was selected because we hypothesized that HF fed pigs would have less dietary glucose and, therefore, have increased gluconeogenesis. Glucagon-like peptide - 2 receptor is a G-protein-coupled, transmembrane receptor for the peptide glucagon-like peptide - 2, which has been indicated to control gastrointestinal growth and function (Guan et al., 2006). Ileum, cecum, and rectum tissue were tested for *MUC2*, *MCT1*, *CD147*, and *GLP-2R*, whereas liver tissue was tested for *MCT1*, *CD147*, *GLP-2R*, and *PCK1*. Primers used for amplification of target genes are provided in Table 5.4. To obtain the relative gene expression, the average quantity of triplicate samples was calculated and divided by the geometric mean of the two internal control genes.

Ultrasound Measurements and Carcass Characteristics. At the conclusion of the late-finishing phase, all pigs were ultrasonically scanned at the time of pig weighing using an Aloka Model 500V B-mode ultrasound scanner fitted with an Aloka 5011 probe (Corometrics Medical Systems, Wallingford, CT). A transverse image was taken over the middle of the Longissimus muscle (**LM**) at the 10th rib, and backfat thickness, Longissimus muscle depth (**LD**), and Longissimus muscle area (**LMA**) were measured on the image. One pig per pen then was selected based on sex, keeping the sex selection the same within a replicate, and then BW. Next, pigs were tattooed and transported to the Meat Science Laboratory at the University of Illinois (Urbana, IL) and held overnight in lairage. Pigs were provided ad libitum access to water during this time, but had no access to feed. Pigs were weighed immediately prior to slaughter to determine ending live weight. Pigs were slaughtered under the supervision of the Food Safety and Inspection Service branch of the United States Department of Agriculture using head-to-heart electrical immobilization and exsanguination. Intestinal weights were collected as described by Boler et al. (2014). Initially, the full intact intestinal tract was weighed. The large

intestine was separated from the small intestine at the ileocecal junction. The small intestine was separated from the stomach between the pylorus of the stomach and the duodenum of the small intestine. The stomach was removed from the esophagus where the esophagus empties into the cardia of the stomach. Each section of the intestinal tract was rinsed with water to remove all digesta and fecal material. Mesenteric tissue that surrounds the intestinal tract was removed and weighed separately. Gut fill was calculated as the difference in the weight of the full intestinal tract and the sum of the empty sections.

Carcasses were weighed approximately 45 min postmortem to determine HCW. Carcass dressing percentage was calculated by dividing HCW by ending live weight. Carcasses then were allowed to chill at 4^{o}C for approximately 24 h. Fresh meat quality was determined on the left side of the carcass at approximately 24 h postmortem. The left side of each chilled carcass was cut between the 10th and 11th rib interface to expose the LM. The surfaces of the LM were allowed to bloom for at least 20 min before quality evaluations were conducted. Ultimate pH was determined using a MPI hand-held pH meter (MPI pH-Meter, Topeka, KS; 2 point calibration: pH 4 and 7). Subjective color, marbling, and firmness scores were conducted by a single individual according to standards established by the National Pork Producers Council (NPPC, 1991; 1999). Objective L*, a*, and b* values were collected with a Minolta CR-400 utilizing a D65 light source, a 0^{o} observer, and an aperture size of 8 mm. Tenth rib backfat was measured at ¾ the distance of the LM from the dorsal process of the vertebral column. The LMA was measured by tracing the surface of the LM on double matted acetate paper. Longissimus muscle tracings were measured in duplicate using a digitizer tablet (Wacom, Vancouver, WA) and Adobe Photoshop CS6 and the average of the two measurements were reported. A section of the

LM, posterior to the 10th rib, was excised and cut into one 1.25 cm chop and three 2.54 cm thick chops to determine 48 h drip loss.

Chemical Analyses. Prior to analysis, cecal digesta and rectal contents were freeze-dried and ground through a 1-mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ). All main ingredients used in the diets and all diets, cecal, and rectal samples were analyzed for DM (Method 930.15; AOAC Int., 2007). All diets and main ingredients were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom2000 Fiber Analyzer, Ankom Technology, Macedon, NY), while insoluble and soluble dietary fiber were analyzed according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). All diets and main ingredients were analyzed in duplicate for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL). All cecal digesta samples and rectal samples that were stabilized in 2*N* HCl were analyzed for concentrations of VFA by gas chromatography according to Erwin et al. (1961), using a gas chromatograph (Hewlett-Packard 5890A Series II, Palo Alto, CA) and a glass column (180 cm × 4 mm i.d.) packed with 10% SP-1200/1% H_3PO_4 on 80/100 + mesh Chomosorb WAW (Supelco Inc., Bellefonte, PA). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125, 175, and 180°C, respectively. The physicochemical characteristics of the main ingredients and diets were determined by measuring the water binding capacity (Urriola and Stein, 2010) and bulk density (Cromwell et al., 2000).

Statistical Analysis. Normality of residuals were determined by the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Outliers were determined using the BOXPLOT procedure of SAS (SAS Inst. Inc., Cary, NC) and any value that deviated the treatment mean by 1.5 times the interquartile range was removed. Four outliers were identified and removed. Gene

expression data were log-10 transformed to align measures to a normal distribution. All data, except for PUN and TNF-α, were analyzed as a 2 × 2 factorial arrangement of treatments with dietary fiber concentration and DFM as the two factors and block as the random effect using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Relative gene expression data presented were back-transformed using antilog.

A repeated measures analysis was conducted for PUN and TNF-α data and each individual pig was considered an experimental unit (Littell et al., 1998). Appropriate covariance structures were chosen based on the Akaike information criterion. Data were subjected to a 3-way ANOVA that included dietary fiber, DFM, and d, as well as the interactions among these factors using PROC MIXED. The SLICE option was used to evaluate the main effects and interaction of dietary fiber and DFM at each d. The pen was the experimental unit for growth performance and carcass characteristics determined using ultrasound portions of the study, whereas the pig was the experimental unit for sample pH, PUN, TNF-α, cecal and rectal content VFA concentrations, carcass characteristics, gastrointestinal tract weights, and gene expression data. For all outcomes, a P-value ≤ 0.05 was used to determine significance among dietary treatments and a P-value > 0.05, but < 0.10 was considered a tendency.

RESULTS

Ingredient and Diet Analysis

Grower LF diets contained 1.0 and 17.2% soluble and insoluble dietary fiber, respectively, and grower HF diets contained 1.5 and 23.0% soluble and insoluble dietary fiber, respectively. Early-finisher LF diets contained 1.0 and 14.4% soluble and insoluble dietary fiber, respectively, and early-finisher HF diets contained 1.0 and 22.3% soluble and insoluble dietary

fiber, respectively. Late-finisher LF diets contained 2.0 and 16.9% soluble and insoluble dietary fiber, respectively, and late-finisher HF diets contained 2.0 and 20.8% soluble and insoluble dietary fiber, respectively. Corn, soybean meal, DDGS, and wheat middlings contained 0.6, 1.4, 1.9, and 6.3% soluble dietary fiber, respectively, and 12.1, 15.0, 29.0, and 37.1% insoluble dietary fiber, respectively (Table 5.5). The water binding capacity was 0.97, 2.69, 1.74, and 3.11 g/g in corn, soybean meal, DDGS, and wheat middlings, respectively, while the bulk density was 683.0, 807.3, 601.0, and 363.7 g/L, respectively (Table 5.6). The water binding capacity was 1.28 and 1.56 g/g and the bulk density was 728.5 and 646.7 g/ L in grower LF and HF diets, respectively. The water binding capacity was 1.28 and 1.54 g/g and the bulk density was 692.0 and 579.0 g/L in early-finisher LF and HF diets, respectively. The water binding capacity was 1.18 and 1.44 g/g and the bulk density was 727.8 and 645.5 g/L in late-finisher diets, respectively.

Growth Response

Initial BW, grower G:F, and d 35 BW were not affected by dietary fiber concentration or DFM addition, but ADG and ADFI were increased ($P \leq 0.05$) in grower pigs fed HF diets compared with pigs fed LF diets (Table 5.7). Also, G:F, expressed as kg/Mcal NE, was greater ($P \leq 0.05$) in HF-fed pigs compared with LF-fed pigs. During the early-finisher phase, pigs fed HF diets had increased ($P \leq 0.05$) ADFI compared with pigs fed LF diets, but dietary fiber concentration and DFM addition did not affect early-finisher pig ADG, G:F, or d 70 BW. Late-finisher pigs fed HF diets had decreased ($P \leq 0.05$) G:F compared with pigs fed LF diets. Dietary fiber concentration and DFM addition did not affect late-finisher pig ADG, ADFI, or d 94 BW. Overall, pigs fed HF diets had increased ($P \leq 0.05$) ADFI and decreased ($P \leq 0.05$) G:F compared with pigs fed LF diets. However, G:F, expressed as kg/Mcal NE, was not different

between pigs fed HF diets and pigs fed LF diets. The addition of DFM to LF or HF diets did not affect ADG, ADFI, or G:F.

Carcass Characteristics and Gastrointestinal Tract Weights

Backfat thickness, measured using ultrasound on all pigs, was greater ($P \leq 0.05$) for pigs fed LF diets compared with pigs fed HF diets (Table 5.8). Dietary fiber concentration and DFM addition did not affect ultrasonically measured pig LD, LMA, or predicted carcass lean weight. Calculated fat-free lean percentage from ultrasound measurements tended to be decreased ($P < 0.10$) in pigs fed diets containing DFM. The slaughter weight tended to be greater ($P < 0.10$) and HCW of pigs fed diets containing DFM was greater ($P \leq 0.05$) compared with pigs fed diets without DFM. The dressing percentage of pigs fed LF diets was greater ($P \leq 0.05$) compared with pigs fed HF diets. Backfat at the 10^{th} rib was greater ($P \leq 0.05$) for pigs fed diets supplemented with DFM compared with pigs fed diets without DFM. The LMA and calculated fat-free lean (kg) was unaffected by dietary fiber concentration or DFM addition. However, the calculated fat-free lean percentage of pigs fed diets containing DFM was reduced ($P \leq 0.05$) compared with pigs fed diets with no DFM. The LM marbling was greater ($P \leq 0.05$) for pigs fed LF diets compared with HF diets. An interaction ($P \leq 0.05$) between dietary fiber concentration and DFM addition was observed for LM firmness because HF fed pigs tended to have a reduced ($P < 0.10$) LM firmness compared with LF-fed pigs, while DFM addition to the HF diet increased LM firmness. Pigs fed HF diets had a greater ($P \leq 0.05$) LM 24-h pH compared with pigs fed LF diets. Pigs fed LF diets and diets supplemented with DFM had a greater ($P \leq 0.05$) LM L* compared with pigs fed HF diets or diets without DFM, respectively. Pigs fed LF diets had greater ($P \leq 0.05$) LM a* and b* compared with pigs fed HF diets, but an interaction ($P \leq 0.05$) between dietary fiber and DFM was observed for both LM a* and b*. The L* of backfat

from pigs fed LF diets was greater ($P \leq 0.05$) compared with pigs fed HF diets, and there was a tendency for pigs fed HF diets to have greater ($P < 0.10$) backfat a* compared with pigs fed LF diets.

Dietary fiber concentration or DFM did not affect the weights of the full intestinal tract, esophagus, stomach, or small intestine, but the weight of the large intestine tended to be greater ($P < 0.10$) for pigs fed HF diets (Table 5.9). Pigs fed LF diets had a greater ($P \leq 0.05$) weight of mesenteric fat compared with pigs fed HF diets. Pigs fed HF diets had a greater ($P \leq 0.05$) gut fill compared with pigs fed LF diets. When gastrointestinal tract weight was expressed as a percentage of pig BW, the weight of the large intestine and gut fill were greater ($P \leq 0.05$) for pigs fed HF diets compared with pigs fed LF diets. The empty weight of the intestinal tract was not affected by dietary fiber concentration or DFM

Intestinal Concentrations of VFA and pH

An interaction between dietary fiber concentration and DFM was observed ($P \leq 0.05$) for pH of ileal digesta because DFM addition to the HF diet increased pH, but decreased ileal digesta pH when added to the LF diet (Table 5.10). An interaction also was observed ($P \leq 0.05$) for pH of cecal digesta because pigs fed LF diets had reduced cecal digesta pH compared with HF fed pigs, but DFM addition increased ($P \leq 0.05$) cecal digesta pH. The concentration of acetate and propionate in cecal digesta was greater ($P \leq 0.05$) and total short-chain fatty concentration tended to be greater ($P < 0.10$) in pigs fed LF diets, while DFM addition to diets decreased ($P \leq 0.05$) acetate, propionate, and total short-chain fatty acid concentrations in cecal digesta. The ratio of short- to branched-chain fatty acids was greater ($P \leq 0.05$) in pigs fed LF diets compared with pigs fed HF diets.

The pH of rectal contents was not affected by dietary fiber concentration or DFM. The concentration of all short- and branched-chain fatty acids in rectal contents of pigs fed LF diets were greater ($P \leq 0.05$) compared with pigs fed HF diets. Pigs fed diets containing DFM had increased ($P \leq 0.05$) concentration of acetate and total short-chain fatty acids in rectal contents.

TNF-α and PUN

No effect of dietary fiber concentration or DFM addition was observed on plasma concentrations of TNF-α on d 0, but an interaction tended to be observed ($P < 0.10$) between dietary fiber concentration and DFM on d 94 (Table 5.11). The interaction was because DFM addition to the LF diet substantially increased TNF-α, but reduced TNF-α in pigs fed HF diets. The plasma concentration of TNF-α in pigs was greater ($P \leq 0.05$) at d 0 compared with the plasma concentration of TNF-α in pigs at d 94. The d 0 and d 94 PUN of pigs fed HF diets was greater ($P \leq 0.05$) compared with pigs fed LF diets; however, an interaction ($P \leq 0.05$) between dietary fiber and DFM was observed because addition of DFM to LF diets increased d 0 and d 94 PUN, but DFM addition to HF diets decreased d 0 and d 94 PUN.

Gene Expression

The expression of internal control genes was confirmed to be unaffected by dietary treatment. No effect of dietary fiber concentration or DFM was observed for expression of selected genes in ileal tissue of pigs (Table 5.12). Pigs fed HF diets had increased ($P \leq 0.05$) expression of *MCT1* and *CD147* in cecum tissue. Pigs fed diets containing DFM had increased ($P \leq 0.05$) cecum *MUC2* expression and tended to have increased ($P < 0.10$) rectum *MUC2* expression. An interaction ($P \leq 0.05$) between dietary fiber and DFM was observed for expression of *MCT1* in the liver of pigs. This interaction was due to greater *MCT1* expression in pigs fed DFM over those fed no DFM in the HF diet group, with a decreased *MCT1* expression

in the liver of pigs fed LF diets containing DFM. Expression of *GLP-2R* in liver of pigs fed HF diets was increased ($P \leq 0.05$) compared with pigs fed LF diets.

DISCUSSION

Pigs fed HF diets had increased ADG, ADFI, and G:F (expressed as kg/Mcal NE) compared with pigs fed LF diets during the grower phase. This may be attributed to a compensatory gain effect because pigs were fed similar diets since weaning, and HF-fed pigs had reduced ADFI and ADG compared with LF-fed pigs as they finished the nursery phase. Also in this experiment, HF-fed pigs increased ADFI to maintain ADG and final BW equal to their LF-fed counterparts, and this was because the HF diets contained less NE. Although ADFI was increased, HF-fed pigs were able to maintain G:F (expressed as kg/Mcal NE) during the grower, early-finisher, and overall experiment, but not during the late-finisher phase, equal to LF-fed pigs. Taken together, the results indicate that growing-finishing pigs are capable of handling the gut fill effect of HF diets and, therefore, can consume enough feed to maintain growth, albeit the feed is not as digestible as the LF diet. A review on DDGS inclusion in swine diets is also in agreement with the results of this experiment and, again, it was confirmed that up to 30% DDGS may be included in growing-finishing swine diets with no negative impact on growth performance (Stein and Shurson, 2009).

The addition of DFM to LF or HF diets fed to pigs did not affect growth performance in this experiment. This response is consistent with results of previous experiments that indicated addition of DFM to growing-finishing pig diets did not affect growth performance (Apgar et al., 1993; Stavric and Kornegay, 1995). A reason for a lack of a consistent response in growing-finishing pigs fed diets containing DFM may be that the growing-finishing pig has a better health status and development of the gastrointestinal tract at this age compared with a nursery pig.

The slaughter weight and, therefore, HCW tended to be greater for pigs fed diets with DFM compared with pigs fed diets without DFM. However, this is an artifact of the experiment because ending BW was not different among pigs fed experimental diets. The artifact was caused by our criteria used to select the pigs to slaughter at the end of the experiment. The first selection criteria was based on sex within replicate in order to account for any effect of sex, and the second selection criteria was the pig per pen with the closest BW to the pen mean. Due to mortality, which was 3.75%, some pens within a replicate had only one pig of the correct sex to select; therefore, BW was not always as close to the pen mean, which influenced slaughter weight and HCW. However, dressing percentage of pigs fed diets with DFM was not affected and, therefore, suggest the tendency for increased slaughter weight and HCW were an artifact of the slaughter selection criteria.

The dressing percentage of pigs fed HF diets was decreased compared with pigs fed LF diets because pigs fed HF diets had a greater weight of the large intestine and a greater gut fill. Previous research also indicated that pigs fed diets higher in concentration of dietary fiber have increased gastrointestinal tract weights and greater gut fill (Kyriazakis and Emmans, 1995; Jørgensen et al., 1996). It was expected that the increased weight of the large intestine would correspond with a greater amount of *GLP-2R* in rectum tissue, but this was not observed. Glucago-like peptide – 2 receptor is increased in the presence of increased *GLP-2*, which is a pleiotropic peptide that has been shown to increase secretion with increased carbohydrate intake and restore growth of intestinal mucosa by increasing cell proliferation and decreasing apoptosis (Burrin et al., 2003; Barrett, 2012). Relative expression of *GLP-2R* was increased in liver tissue of pigs fed HF diets, and we speculate that portal blood may contain *GLP-2*, which the liver may

use to signal the gastrointestinal tract to increase in size, but further research is necessary to determine the role between HF diets, gastrointestinal tract weight, and *GLP-2*.

The backfat thickness and LM marbling of pigs fed LF diets was greater compared with pigs fed HF diets, indicating an increased amount of available energy that was deposited as lipid. The increased energy may be attributed to the greater amount of NE in LF diets compared with HF diets. However, fat-free lean, expressed as both kg and as a percentage of BW, was not affected by dietary fiber concentration, indicating that protein deposition was not limited in HF diets. The diets were not isonitrogenous; however, they were formulated to meet or exceed the requirements at each phase of pig growth performance for SID indispensable AA. Taken together, the results indicate that the NRC (2012) values used to formulate the diets based on NE and SID indispensable AA of ingredients were adequate.

The addition of DFM to both LF and HF diets fed to pigs did not affect fat-free lean, expressed in kg, but decreased the fat-free lean, expressed as a percentage of BW. This is an indication that DFM addition did not reduce protein deposition, but increased lipid deposition. Therefore, the addition of DFM made more energy available to the pig to be deposited as lipid.

We hypothesized that the addition of the 3-strain *Bacillus*-based DFM to diets, especially HF diets, would increase hindgut fermentation, thereby increasing the quantity of VFA and energy available to the pig. Total short-chain fatty acid concentration was decreased in cecal digesta of pigs fed diets containing DFM; however, the concentration of total short-chain fatty acids was increased in rectal contents of pigs fed diets containing DFM, indicating that the DFM may have a more beneficial effect in the colon of the pig rather than the cecum. Therefore, this may be the reason for the decreased fat-free lean percentage of DFM-fed pigs.

Another interesting finding was that LF-fed pigs had increased concentrations of VFA in both cecal digesta and rectal contents compared with HF fed pigs, indicating that the corn-soybean meal-based diet is more fermentable compared with the corn-soybean meal-DDGS-wheat middlings based HF diet. However, the data could also suggest that absorption of VFA was greater in the cecum of pigs fed HF diets compared with LF diets. In corroboration of the previous statement, the expression of *MCT1* and *CD147* in cecal tissue of HF-fed pigs was increased compared with LF-fed pigs. Previous research indicated that the abundance of *MCT1* and *CD147* correspond with increased concentrations of VFA in order to increase the absorption of VFA (Metzler-Zebeli et al., 2012). These results indicate that LF-fed pigs had more available NE, not only because the corn-soybean meal-based diet was more digestible, but also because it was more fermentable and VFA was deposited as lipid, yielding a greater backfat thickness and marbling.

CONCLUSION

In conclusion, pigs fed HF diets were able to maintain overall ADG, G:F (expressed as kg/Mcal NE), and final BW compared with their LF-fed counterparts due to their ability to increase ADFI and were not affected by gut fill. Pigs fed LF diets had a greater amount of energy available, both from a more digestible and fermentable diet and, therefore, had increased lipid deposition as indicated by increased backfat thickness and marbling. Pigs fed HF diets had a reduced dressing percentage compared with LF-fed pigs due to their increased weight of the large intestine and increased gut fill. The addition of DFM to both LF and HF diets tended to increase total VFA concentration in rectal contents, which increased available energy to the pig leading to a decreased fat-free lean percentage. Therefore, it is recommended that a 3-strain

Bacillus-based DFM be added to growing-finishing pig diets in order to decrease fat-free lean percentage.

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TABLES

Table 5.1. Ingredient, calculated, and analyzed composition of grower diets (as-fed basis)

Dietary fiber concentration	Low		High	
Direct-fed microbial	-	+	-	+
Ingredient, %				
Corn	74.08	74.02	40.40	40.34
Soybean meal, 48% CP	22.00	22.00	16.00	16.00
DDGS[1]	-	-	30.00	30.00
Wheat middlings	-	-	10.00	10.00
Choice white grease	1.00	1.00	1.00	1.00
Limestone	1.15	1.15	1.55	1.55
Dicalcium P	0.60	0.60	-	-
Lys HCl	0.34	0.34	0.34	0.34
DL-Met	0.04	0.04	-	-
Thr	0.08	0.08	-	-
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Phytase	0.01	0.01	0.01	0.01
DFM mixture[3]	-	0.06	-	0.06
Calculated composition[4]				
NE, kcal/kg	2,492	2,491	2,381	2,380
CP, %	16.6	16.6	20.7	20.7
SID[5] Lys, %	0.98	0.98	0.98	0.98
Ca, %	0.65	0.65	0.66	0.66
STTD[6] P, %	0.23	0.23	0.26	0.26
Analyzed composition				
GE, kcal/kg	3,817	3,882	4,096	4,089
DM, %	87.8	87.6	87.6	87.5
Ash, %	5.3	5.2	5.0	5.5
ADF, %	5.0	6.0	6.4	8.0
NDF, %	14.4	14.2	17.8	17.5
Insoluble dietary fiber, %	16.8	17.5	24.4	21.6
Soluble dietary fiber, %	1.0	1.0	1.5	1.5
Total dietary fiber, %	17.8	18.5	25.9	23.1

[1]DDGS = distillers dried grains with solubles.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial mixture consists of 30 g of DFM mixed with 270 g of corn.

[4]Calculated from NRC (2012) values.

[5]SID = standardized ileal digestible.

[6]STTD = standardized total tract digestible.

Table 5.2. Ingredient, calculated, and analyzed composition of early-finisher diets (as-fed basis)

Dietary fiber concentration	Low		High	
Direct-fed microbial	-	+	-	+
Ingredient, %				
Corn	80.00	79.94	46.70	46.64
Soybean meal, 48% CP	16.50	16.50	10.00	10.00
DDGS[1]	-	-	30.00	30.00
Wheat middlings	-	-	10.00	10.00
Choice white grease	1.00	1.00	1.00	1.00
Limestone	1.10	1.10	1.30	1.30
Dicalcium P	0.35	0.35	-	-
Lys HCl	0.27	0.27	0.29	0.29
DL-Met	0.01	0.01	-	-
Thr	0.06	0.06	-	-
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Phyase	0.01	0.01	0.01	0.01
DFM mixture[3]	-	0.06	-	0.06
Calculated composition[4]				
NE, kcal/kg	2,536	2,534	2,424	2,423
CP, %	14.5	14.5	18.4	18.4
SID[5] Lys, %	0.79	0.79	0.79	0.79
Ca, %	0.56	0.56	0.55	0.55
STTD[6] P, %	0.18	0.18	0.25	0.25
Analyzed composition				
GE, kcal/kg	3,990	3,906	3,844	3,920
DM, %	87.4	87.3	86.3	86.3
Ash, %	3.6	3.8	4.7	4.7
ADF, %	5.3	5.3	9.5	8.7
NDF, %	11.7	10.6	20.7	20.3
Insoluble dietary fiber, %	13.9	14.9	22.2	22.3
Soluble dietary fiber, %	1.0	1.0	1.0	1.0
Total dietary fiber, %	14.9	15.9	23.2	23.3

[1]DDGS = distillers dried grains with solubles.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial mixture consists of 30 g of DFM mixed with 270 g of corn.

[4]Calculated from NRC (2012) values.

[5]SID = standardized ileal digestible.

[6]STTD = standardized total tract digestible.

Table 5.3. Ingredient, calculated, and analyzed composition of late-finisher diets (as-fed basis)

Dietary fiber concentration	Low		High	
Direct-fed microbial	-	+	-	+
Ingredient, %				
Corn	82.81	82.75	50.83	50.77
Soybean meal, 48% CP	14.00	14.00	6.00	6.00
DDGS[1]	-	-	30.00	30.00
Wheat middlings	-	-	10.00	10.00
Choice white grease	1.00	1.00	1.00	1.00
Limestone	1.00	1.00	1.20	1.20
Dicalcium P	0.25	0.25	-	-
Lys HCl	0.20	0.20	0.26	0.26
Thr	0.03	0.03	-	-
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Phytase	0.01	0.01	0.01	0.01
DFM mixture[3]	-	0.06	-	0.06
Calculated composition[4]				
NE, kcal/kg	2,558	2,557	2,451	2,450
CP, %	13.5	13.5	16.8	16.8
SID[5] Lys, %	0.68	0.68	0.67	0.67
Ca, %	0.49	0.49	0.50	0.50
STTD[6] P, %	0.16	0.16	0.24	0.24
Analyzed composition				
GE, kcal/kg	3,848	3,988	4,141	4,107
DM, %	86.5	87.2	87.5	88.8
Ash, %	3.4	3.6	4.5	5.0
ADF, %	3.8	4.0	6.5	6.6
NDF, %	12.0	10.7	17.3	16.1
Insoluble dietary fiber, %	16.6	17.2	20.9	20.7
Soluble dietary fiber, %	1.0	2.9	1.6	2.3
Total dietary fiber, %	17.6	20.1	22.5	23.1

[1]DDGS = distillers dried grains with solubles.

[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial mixture consists of 30 g of DFM mixed with 270 g of corn.

[4]Calculated from NRC (2012) values.

[5]SID = standardized ileal digestible.

[6]STTD = standardized total tract digestible.

Table 5.4. Gene-specific primer sequences

Gene	Acc. No	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
MUC2[1]	AK231524	CAACGGCCTCTCCTTCTCTGT	GCCACACTGGCCCTTTGT	Leonard et al. (2011)
MCT1[2]	AM286425	GGTGGAGGTCCTATCAGCAG	TGAAGGCAAGCCCAAGAC	Metzler-Zebeli et al. (2012)
CD147[3]	NM_001123086	CCTCGGAGACCAAGACAGAG	TCATTCACGTGGTGTCCACT	König et al. (2010)
PCK1[4]	NM_001123158.1	CCCTGCCTTTGAAAAAGCCC	GGAGATGATTTCTCGGCGGT	Qu et al. (2015)
GLP-2R[5]	NM_001246266.1	TGTCCTACGTGTCGGAGATGTC	TAATTGGCGCCCACGAA	Guan et al. (2006)

[1]*MUC2* = mucin 2.
[2]*MCT1* = monocarboxylate transporter 1.
[3]*CD147* = basigin.
[4]*PCK1* = phosphoenolpyruvate carboxykinase 1.
[5]*GLP-2R* = glucagon-like peptide-2 receptor.

Table 5.5. Analyzed energy and nutrient composition of main ingredients (as-fed basis)

	Ingredient			
Item	Corn	Soybean meal	DDGS[1]	Wheat middlings
GE, kcal/kg	3,773	4,175	4,421	4,027
DM, %	84.5	88.4	86.9	86.7
Ash, %	1.1	6.1	4.5	4.8
ADF, %	3.8	6.1	11.6	10.8
NDF, %	12.2	7.0	24.1	38.4
Insoluble dietary fiber, %	12.1	15.0	29.0	37.1
Soluble dietary fiber, %	0.6	1.4	1.9	6.3
Total dietary fiber, %	12.7	17.1	30.9	43.4

[1]DDGS = distillers dried grains with solubles.

Table 5.6. Physicochemical characteristics of diets and ingredients

Item	Water binding capacity, g/g	Bulk density, g/L
Ingredients		
Corn	0.97	683.0
Soybean meal	2.69	807.3
DDGS[1]	1.74	601.0
Wheat middlings	3.11	363.7
Grower diets		
Low fiber	1.26	729.3
Low fiber + DFM[2]	1.29	727.7
High fiber	1.58	639.3
High fiber + DFM	1.54	654.0
Early-finisher diets		
Low fiber	1.30	687.7
Low fiber + DFM	1.27	696.3
High fiber	1.55	578.7
High fiber + DFM	1.53	580.0
Late-finisher diets		
Low fiber	1.16	738.3
Low fiber + DFM	1.20	717.3
High fiber	1.46	638.7
High fiber + DFM	1.41	652.3

[1]DDGS = distillers dried grains with solubles.

[2]DFM = direct-fed microbial.

Table 5.7. Performance of grow-finish pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Grower (d 0 – 35)								
Initial BW, kg	26.73	27.15	26.25	26.32	0.985	0.111	0.545	0.664
ADG, kg/d	0.733	0.758	0.786	0.796	0.016	0.002	0.216	0.576
ADFI, kg/d	1.706	1.750	1.812	1.794	0.044	0.012	0.641	0.264
G:F, kg/kg	0.430	0.434	0.436	0.444	0.010	0.254	0.385	0.723
G:F, kg/Mcal NE	0.173	0.174	0.183	0.187	0.004	< 0.001	0.363	0.713
Final BW, kg	52.58	53.61	53.76	54.16	1.154	0.198	0.287	0.636
Early-finisher (d 35 – 70)								
ADG, kg/d	0.907	0.900	0.941	0.948	0.031	0.127	0.999	0.796
ADFI, kg/d	2.544	2.516	2.711	2.657	0.084	0.022	0.521	0.838
G:F, kg/kg	0.358	0.359	0.348	0.357	0.009	0.508	0.560	0.624
G:F, kg/Mcal NE	0.141	0.142	0.143	0.147	0.003	0.240	0.533	0.621
Final BW, kg	84.90	85.43	86.71	87.35	2.214	0.145	0.642	0.967
Late-finisher (d 70 – 94)								
ADG, kg/d	0.993	1.017	0.912	0.904	0.070	0.115	0.894	0.789
ADFI, kg/d	2.923	3.162	3.168	3.141	0.110	0.274	0.299	0.197
G:F, kg/kg	0.340	0.318	0.288	0.289	0.014	0.003	0.386	0.357
G:F, kg/Mcal NE	0.133	0.124	0.118	0.118	0.005	0.034	0.393	0.366
Final BW, kg	109.02	108.75	108.61	109.05	3.126	0.973	0.955	0.809
d 0 – 94								
ADG, kg/d	0.864	0.877	0.876	0.880	0.024	0.615	0.597	0.783
ADFI, kg/d	2.329	2.396	2.493	2.459	0.064	0.007	0.671	0.206
G:F, kg/kg	0.371	0.366	0.352	0.358	0.005	0.006	0.872	0.240
G:F, kg/Mcal NE	0.147	0.145	0.145	0.148	0.002	0.610	0.824	0.230

[1]DFM = direct-fed microbial.

Table 5.8. Carcass characteristics of finishing pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ultrasound[2]								
Backfat thickness, cm	1.56	1.58	1.43	1.46	0.08	0.022	0.611	0.985
Longissimus muscle depth, cm	4.87	5.03	5.05	5.05	0.09	0.296	0.420	0.408
Longissimus muscle area, sq. cm	40.69	41.49	40.66	41.08	1.46	0.779	0.434	0.812
Predicted carcass lean weight[3], kg	45.58	46.09	45.93	46.17	1.26	0.746	0.561	0.831
Fat-free lean[4], %	54.20	52.30	54.65	52.16	1.10	0.891	0.055	0.793
Meat science laboratory[5]								
Slaughter wt, kg	108.48	112.58	108.30	114.69	3.64	0.713	0.053	0.662
Carcass composition								
HCW, kg	84.57	88.36	84.12	88.70	2.87	0.978	0.046	0.844
Dressing, %	77.99	78.49	77.68	77.36	0.27	0.005	0.693	0.095
10th rib backfat, cm	1.60	1.93	1.45	1.87	0.16	0.480	0.018	0.771
Longissimus muscle area, sq. cm	48.29	49.72	48.85	48.02	2.08	0.712	0.846	0.466
Fat-free lean, kg	47.17	47.94	47.67	47.99	1.28	0.761	0.548	0.801
Fat-free lean[6], %	56.02	54.40	56.67	54.18	0.89	0.810	0.028	0.631
Muscle quality								
Subjective color[7]	2.5	2.4	2.6	2.5	0.17	0.501	0.501	1.00
Marbling[8]	1.4	1.4	1.1	1.0	0.13	0.008	0.692	0.692
Firmness[9]	2.5	2.2	1.5	2.4	0.23	0.090	0.200	0.013
24-h pH, Longissimus muscle	5.54	5.55	5.57	5.65	0.05	0.023	0.125	0.222
48-h drip loss, %	5.45	6.76	5.42	5.18	1.00	0.233	0.426	0.250
Longissimus muscle color, L*[10]	50.07	52.33	48.92	49.51	1.41	0.007	0.046	0.230
Longissimus muscle color, a*[10]	7.71	8.50	8.02	6.49	0.32	0.010	0.242	0.001
Longissimus muscle color, b*[10]	3.00	4.50	3.12	2.61	0.29	0.004	0.100	0.002
Backfat color								
Fat color, L*[10]	74.91	75.16	73.59	74.38	0.57	0.032	0.269	0.565
Fat color, a*[10]	3.86	4.01	5.10	4.10	0.36	0.067	0.239	0.112

Table 5.8. (cont.)

Fat color, b*[10]	4.40	4.08	4.58	4.81	0.31	0.151	0.880	0.374

[1]DFM = direct-fed microbial.

[2]On d 94, all pigs were ultrasonically scanned at the time of pig weighing using an Aloka Model 500V B-mode ultrasound scanner fitted with an Aloka 5011 probe (Corometrics Medical Systems, Wallingford, CT). A transverse image was taken over the middle of the *Longissimus* muscle at the tenth rib, and backfat thickness, *Longissimus* muscle depth, and *Longissimus* muscle area were measured on the image.

[3]Predicted carcass lean weight, kg = 0.63 + 0.324 * BW (kg) – 0.640 * 10th rib backfat (cm) + 0.271 * *Longissimus* muscle area (cm^2) [Schinckel et al., 2001].

[4]Fat-free lean, % = predicted carcass lean weight (kg) / HCW (kg) obtained from Meat Science Laboratory.

[5]On d 95, 10 pigs per diet were harvested at the University of Illinois Meat Science Laboratory where slaughter weight, carcass composition, muscle quality, and backfat color were measured.

[6]Fat-free lean, % = calculated from NPPC (1999): pounds fat-free lean = 8.588 – 21.896 * 10th rib backfat (in.) + 0.465 * HCW (lbs.) + 3.005 * *Longissimus* muscle area (in.^{2}), (pounds fat-free lean / HCW) * 100 = % fat-free lean.

[7]NPPC (1991) color scale (1 to 6): 1 = pale pinkish gray to white and 6 = dark purplish red.

[8]NPPC (1991) marbling scale (1 to 10): 1 = devoid and 10 = abundant.

[9]NPPC (1991) firmness scale (1 to 5): 1 = very soft and 5 = very firm and dry.

[10]L* = lightness; a* = redness; b* = yellowness.

Table 5.9. Gastrointestinal tract weights of finishing pigs fed low- or high-fiber diets without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Full intestinal tract, kg	7.18	7.32	7.17	7.83	0.24	0.396	0.184	0.372
Esophagus, kg	0.07	0.08	0.07	0.08	0.01	0.803	0.258	0.418
Stomach, kg	0.59	0.60	0.58	0.62	0.03	0.873	0.305	0.580
Small intestine, kg	1.48	1.41	1.37	1.51	0.04	0.937	0.628	0.097
Large intestine, kg	1.71	1.68	1.74	1.97	0.13	0.071	0.266	0.138
Mesenteric fat, kg	1.58	1.69	1.36	1.45	0.15	0.005	0.205	0.836
Empty intestinal tract, kg	5.42	5.46	5.13	5.61	0.25	0.711	0.173	0.235
Gut fill, kg	1.76	1.86	2.04	2.22	0.17	0.045	0.375	0.800
Full intestinal tract, % BW	6.59	6.50	6.62	6.81	0.17	0.285	0.748	0.382
Esophagus, % BW	0.07	0.07	0.07	0.07	< 0.01	0.992	0.714	0.445
Stomach, % BW	0.55	0.54	0.54	0.54	0.04	0.712	0.638	0.747
Small intestine, % BW	1.37	1.25	1.27	1.32	0.07	0.687	0.500	0.104
Large intestine, % BW	1.56	1.49	1.60	1.71	0.07	0.021	0.780	0.121
Mesenteric fat, % BW	1.45	1.51	1.25	1.25	0.10	0.001	0.597	0.602
Empty intestinal tract, % BW	4.98	4.86	4.73	4.88	0.09	0.244	0.900	0.169
Gut fill, % BW	1.60	1.64	1.89	1.93	0.15	0.019	0.738	0.960

[1]DFM = direct-fed microbial.

Table 5.10. pH and short- and branched-chain fatty acid concentrations, expressed as µmol/g DM basis, in cecal and rectal contents of pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ileal digesta pH	7.56	7.32	7.25	7.48	0.45	0.296	0.964	0.006
Cecal digesta								
pH	5.93	6.72	6.46	6.52	0.12	0.167	0.001	0.004
Acetate	88.81	74.37	74.78	71.47	4.56	0.046	0.037	0.183
Propionate	25.99	20.35	21.14	19.39	1.19	0.006	0.001	0.056
Butyrate	14.80	10.54	12.62	11.70	1.66	0.761	0.128	0.322
Total SCFA[2]	129.59	103.53	108.55	102.56	7.63	0.087	0.016	0.118
Valerate	2.32	2.10	2.48	2.26	0.16	0.323	0.197	0.987
Isovalerate	2.46	1.95	2.33	2.52	0.23	0.336	0.496	0.140
Isobutyrate	1.73	1.38	1.67	1.89	0.18	0.218	0.708	0.115
Total BCFA[3]	6.27	5.30	6.48	6.68	0.50	0.128	0.451	0.255
SCFA:BCFA	22.56	20.44	18.10	14.92	1.93	0.014	0.183	0.792
Rectal contents								
pH	6.25	6.23	6.36	6.28	0.18	0.349	0.538	0.725
Acetate	75.06	91.08	62.78	67.52	7.02	0.001	0.035	0.241
Propionate	20.67	24.44	16.83	17.45	1.83	0.005	0.237	0.394
Butyrate	14.78	19.46	12.16	10.75	1.96	0.007	0.411	0.130
Total SCFA	104.45	134.98	91.77	95.25	8.36	0.003	0.047	0.110
Valerate	2.65	3.26	2.14	1.91	0.32	0.007	0.561	0.201
Isovalerate	3.37	4.16	2.64	2.72	0.30	0.001	0.156	0.248
Isobutyrate	2.25	2.80	1.87	1.94	0.22	0.007	0.158	0.262
Total BCFA	8.13	10.22	6.65	6.57	0.80	0.003	0.218	0.184
SCFA:BCFA	14.30	13.54	14.18	14.79	1.01	0.511	0.930	0.427

[1]DFM = direct-fed microbial.
[2]SCFA = short-chain fatty acids.
[3]BCFA = branched-chain fatty acids.

Table 5.11. Plasma urea nitrogen (PUN) and plasma concentration of tumor necrosis factor-alpha (TNF-α) of pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance[1]		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[2]	Dietary fiber × DFM
d 0								
PUN, mg/dL	9.5	11.9	13.9	13.0	0.8	0.001	0.370	0.002
TNF-α, pg/mL	68.2	81.6	86.2	65.0	18.0	0.968	0.829	0.810
d 94								
PUN, mg/dL	10.0	9.4	13.2	12.2	1.1	0.007	0.479	0.042
TNF-α, pg/mL	8.9	23.5	27.2	12.4	5.3	0.511	0.983	0.098

[1]Effect of d ($P \leq 0.05$) was observed.
[2]DFM = direct-fed microbial.

Table 5.12. Relative mRNA expression of genes from ileum, cecum, rectum, and liver tissue from pigs fed either a low- or high-fiber diet without or with a *Bacillus*-based direct-fed microbial

Dietary fiber concentration	Low		High			Significance		
Direct-fed microbial	-	+	-	+	SEM	Dietary fiber	DFM[1]	Dietary fiber × DFM
Ileum								
MCT1[1]	0.95	0.89	0.95	1.21	1.31	0.343	0.530	0.415
CD147[1]	0.87	0.95	0.88	0.87	1.13	0.867	0.931	0.811
MUC2[1]	1.09	1.01	1.30	1.03	1.19	0.552	0.337	0.618
GLP-2R[1]	0.99	1.27	1.01	1.02	1.32	0.719	0.632	0.669
Cecum								
MCT1	0.38	0.27	0.50	0.74	1.32	0.008	0.884	0.121
CD147	1.75	1.59	2.68	3.02	1.19	0.003	0.942	0.523
MUC2	0.68	0.84	0.54	0.82	1.18	0.361	0.033	0.475
GLP-2R	0.62	0.80	0.58	0.73	1.38	0.664	0.157	0.926
Rectum								
MCT1	0.59	0.77	0.73	0.67	1.23	0.773	0.492	0.222
CD147	0.70	0.82	0.86	0.85	1.10	0.114	0.365	0.268
MUC2	1.02	0.83	1.06	1.08	1.16	0.079	0.283	0.214
GLP-2R	1.25	1.29	1.26	1.50	1.19	0.498	0.367	0.523
Liver								
MCT1	0.74	0.46	0.53	0.71	1.11	0.610	0.422	0.001
CD147	0.86	0.84	0.77	0.76	1.12	0.319	0.862	0.979
GLP-2R	0.63	0.60	0.78	0.87	1.47	0.048	0.816	0.594
PCK1[1]	0.95	1.02	1.27	1.01	1.15	0.248	0.523	0.222

[1]DFM = direct-fed microbial, *MCT1* = monocarboxylate transporter 1, *CD147* = basigin, *MUC2* = mucin 2, *GLP-2R* = glucagon-like peptide-2 receptor, *PCK1* = phosphoenolpyruvate carboxykinase 1.

CHAPTER 6: EFFECT OF DIET ADAPTATION TIME ON APPARENT ILEAL AND APPARENT TOTAL TRACT DIGESTIBILITY OF ENERGY AND NUTRIENTS BY GROWING PIGS FED DIETS WITH DIFFERENT CONCENTRATIONS OF DIETARY FIBER WITHOUT OR WITH A *BACILLUS*-BASED DIRECT-FED MICROBIAL

ABSTRACT: Effects of dietary fiber, a *Bacillus*-based direct-fed microbial (DFM), and feeding duration on the apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrients and energy by growing pigs were determined. Twenty-four barrows (initial BW: 31.5 ± 1.0 kg) were surgically equipped with a T-cannula in the distal ileum and used in the experiment. Pigs were randomly allotted to 4 treatments with 6 pigs per treatment during a 12-wk experiment with six 2-week periods. Dietary treatments were arranged in a 2 × 2 factorial arrangement with 2 diet types (low- or high-fiber) and 2 levels of DFM (0 or 60 g DFM/t of feed). The *Bacillus*-based DFM contained 1.5×10^5 CFU/g (Danisco Animal Nutrition-DuPont Industrial Biosciences, Marlborough, UK). Pigs were fed their respective treatment diets during periods 2, 3, and 4, but during periods 1, 5, and 6, all pigs were fed the low-fiber diet without DFM. Each period involved a 5 d adaptation period, total collection of feces and urine from d 6 to 11, and ileal digesta collection on d 13 and 14. Results indicated that DE and ME increased ($P \leq 0.05$) over time because AID of starch increased ($P \leq 0.05$) and ATTD of GE and ADF tended to increase ($P < 0.10$) from period 1 to period 6, regardless of diet type. High-fiber diets in periods 2, 3, and 4 had reduced ($P \leq 0.05$) AID of most AA, reduced ($P \leq 0.05$) ATTD of GE, ADF, and NDF, and reduced ($P \leq 0.05$) ME compared with low-fiber diets. Addition of DFM to the high-fiber diet did not ameliorate the negative effects of dietary fiber on digestibility, but addition of DFM to the low-fiber diet increased ($P \leq 0.05$) AID of ADF, NDF, Lys, Phe, and Glu. When

DFM was withdrawn from the low-fiber diet, digestibility values were reduced, indicating that the *Bacillus*-based DFM must be fed continuously to exert beneficial effects and that no carry-over effects can be expected. In conclusion, the AID of starch and the ATTD of GE and ADF increased as pig BW increased, but digestibility values of energy and nutrients were reduced by increased dietary fiber although the AID of some nutrients were improved by DFM.

Key words: adaptation, dietary fiber, digestibility, direct-fed microbials, pigs

INTRODUCTION

Addition of direct-fed microbials (**DFM**) to swine diets has recently gained more attention due to mandated reduced use of antibiotics in the United States. Direct-fed microbials included in swine diets may improve immune regulation (Weese et al., 2008), enhance beneficial gastrointestinal microbiota (Baker et al., 2013), increase nutrient digestibility (Lee et al., 2014), and increase pig performance and carcass characteristics (Alexopoulos et al., 2004). *Bacillus*-based DFM may increase dietary fiber fermentation in swine diets due to their ability to secrete fiber-degrading enzymes (Schreier, 1993) and, in turn, increase the production of VFA, which may be utilized as an energy source by the pig (Jaworski et al., 2014).

The concentration of fiber in swine diets often is increased when diets are formulated to contain grain co-products, and this typically is done to reduce diet cost (Jaworski et al., 2015). However, increased dietary fiber may reduce apparent ileal digestibility (**AID**) and apparent total tract digestibility (**ATTD**) of energy and nutrients (Bindelle et al., 2008), but it is possible that negative effects of dietary fiber may be ameliorated by addition of a *Bacillus*-based DFM to the diet. Feeding of high-fiber diets also result in increased size of the large intestine and increased

microbial population (Kyriazakis and Emmans, 1995; Jørgensen et al., 1996), which may allow the pig to increase fermentation of dietary fiber. However, these changes take time to occur, and it is, therefore, possible that a certain period of time is required for the microbiota to adapt to a diet high in fiber to maximize fermentation. Thus, the objective of this experiment was to test the hypothesis that addition of a *Bacillus*-based DFM to low- or high-fiber diets results in an increase in AID and ATTD of nutrients and energy. A second objective was to determine if the AID and ATTD of nutrients and energy change over time by growing pigs fed a low- or high-fiber diet without or with DFM.

MATERIALS AND METHODS

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. Pigs were the offspring of G-Performer boars mated to F-25 females (Genetiporc, Alexandria, MN).

Animals, Housing, Diets, and Experimental Design. Twenty-four barrows (initial BW: 31.5 ± 1.0 kg) were surgically fitted with a T-cannula in the distal ileum (Stein et al., 1998). After surgery, pigs were housed individually in metabolism crates that were equipped with a feeder and a nipple drinker, a fully slatted floor, a fecal collection screen, and a urine tray that allowed for total, but separate, collection of urine and feces from each pig. Water was available at all times. Pigs were allowed to recover from surgery for 7 d before experimental diets were fed.

Dietary treatments were arranged in a 2 × 2 factorial arrangement with 2 diet types (low- or high-fiber) and 2 levels of DFM (0 or 60 g DFM/t of feed; Table 6.1). The *Bacillus*-based DFM contained 1.5×10^5 CFU/g and was obtained from Danisco Animal Nutrition-DuPont

Industrial Biosciences (Marlborough, UK). All diets also contained 500 units of microbial phytase (Axtra® PHY; Danisco Animal Nutrition-DuPont Industrial Biosciences, Waukesha, WI) per kg of complete diet and titanium dioxide (0.4%) was included in all diets as an indigestible marker.

Pigs were randomly allotted to 4 treatment groups with 6 pigs per treatment in a completely randomized design. The experiment was conducted during six 14-d periods. All pigs were fed the low-fiber diet without DFM during the first 14 d period (period 1). During the following three 14-d periods (periods 2, 3, and 4), pigs were fed 1 of the 4 experimental diets, but all pigs were fed the low-fiber diet without DFM during the last two 14-d periods (periods 5 and 6). The experimental design is illustrated in Table 6.2. Feed was provided to each pig in quantities equivalent to 3 times the estimated requirement for maintenance energy (i.e., 197 kcal ME/$kg^{0.6}$; NRC, 2012). Daily feed allotments were divided into two equal meals and fed at 0800 and 1600 h, respectively. The BW of each pig was recorded at the beginning of the experiment and at the end of each period. The initial 5 d of each period was considered an adaptation to the experimental diets. Feces and urine were quantitatively collected for 5 d from day 6 to day 11 using the marker to marker approach (Adeola, 2001). Feces were collected twice daily and stored at -20°C immediately after collection. Urine was collected once daily in a preservative of 50 mL of 6*N* HCl and 20% of daily urine collected was stored at -20°C immediately after collection. Ileal digesta were collected for 8 h on d 13 and 14 by attaching a 225-mL plastic bag to the cannula barrel using a cable tie (Stein et al., 1999). Bags were removed every 30 min or whenever full and replaced with a new bag. Digesta were stored at -20°C immediately after collection.

Physicochemical Characteristics, Chemical Analysis, and Calculations. The physicochemical characteristics of all diets were determined by measuring the water binding capacity (Robertson et al., 2000; Cervantes-Pahm et al., 2014) and bulk density (Cromwell et al., 2000). All diets, freeze-dried ileal digesta, and oven-dried fecal samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. All diets were analyzed for ash (Method 942.05; AOAC Int., 2007). All diets, freeze-dried ileal digesta, and fecal samples were analyzed for DM (Method 930.15; AOAC Int., 2007), and ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom^{2000} Fiber Analyzer, Ankom Technology, Macedon, NY). All diets were analyzed for insoluble and soluble dietary fiber according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Diets and freeze-dried ileal digesta samples were analyzed for AA on a Hitachi AA Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [Method 982.30 E(a); AOAC Int., 2007]. Total starch was analyzed in all diets and freeze-dried ileal samples by the glucoamylase procedure (Method 979.10; AOAC Int., 2007). The concentration of titanium in diets and freeze-dried ileal digesta was measured following the procedure of Myers et al. (2004). Diets, fecal samples, and urine samples were analyzed in duplicate for GE using bomb calorimetry (Model 6300; Parr Instruments, Moline, IL) and the ATTD of GE in each diet was calculated (Adeola, 2001). The energy lost in feces and urine was calculated and the quantities of DE and ME in each of the diets was calculated (Adeola, 2001). The AID of AA, ADF, NDF, and total starch as well as ATTD of ADF and NDF was calculated for each diet according to Stein et al. (2007).

Statistical Analysis. Data for each treatment group were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included period as the independent variable and AID, ATTD, DE, and ME values as response variables within dietary treatment groups. Single degree of freedom contrasts were used to compare effects of dietary fiber concentration and DFM inclusion within each treatment group (e.g., period 1 vs. periods 2, 3, and 4; periods 2, 3, and 4 vs. periods 5 and 6; and period 1 vs. periods 5 and 6; Stewart et al., 2010). Results for all treatment groups for periods 2, 3, and 4 were analyzed as repeated measures using the MIXED procedure (Littell et al., 1998; Stewart et al., 2010). Fixed effects included period, dietary fiber concentration, DFM addition, and the interaction between period, dietary fiber concentration, and DFM addition. Appropriate covariance structures were chosen based on the Akaike information criterion. The pig was the experimental unit for all analyses. For all outcomes, a *P*-value ≤ 0.05 was used to determine significance among treatments and a *P*-value > 0.05 but < 0.10 was considered a tendency.

RESULTS AND DISCUSSION

One pig allotted to treatment 2 died at the end of period 2 and another pig allotted to treatment 4 was euthanized at the conclusion of period 4 due to a broken hip; therefore, treatment 2 had only 5 observations in periods 3 through 6 and treatment 4 had only 5 observations in periods 5 and 6. The ADG of the pigs during the experiment was 0.84 kg/d and the final BW was 102.2 ± 5.8 kg.

The experimental design allowed for the investigation of the AID and ATTD of energy and nutrients by growing pigs over six 14-d periods (84 d in total). Period represents both age and BW of pigs. The AID of starch (Table 6.3) and the ATTD of DM, GE, ADF, NDF, and DE

and ME (Table 6.4) were greater ($P \leq 0.05$) during periods 5 and 6 compared with period 1 if pigs were fed the low-fiber diet without DFM for the entire experiment. These results are in agreement with results that demonstrated the digestibility of energy is greater in heavier or more mature pigs than in lighter or less mature pigs (Graham et al., 1986; Noblet et al., 1994). Also, Noblet et al. (2001) indicated that the ATTD of GE increased by 0.003 to 0.0045% for every 10 kg BW from 30 to 100 kg and, in agreement, results from this experiment indicate that the ATTD of GE increased by approximately 0.38% for every 10 kg BW from 31.1 to 102.2 kg. Along with increased energy digestibility, the AID of His and Pro by pigs fed the low-fiber diet without DFM was greater ($P \leq 0.05$) during periods 2, 3, and 4 and periods 5 and 6 compared with period 1. This observation is in contrast with data that indicated the AID of AA by growing pigs fed a corn-soybean meal diet was not influenced by the BW of growing pigs from 35 to 67 kg over a period of 6 wks (Stewart et al., 2010). The differences may be attributed to longer experimental periods and a greater BW range used in the current experiment whereas the diets used, experimental design, and location were similar.

The AID of Lys, Phe, and Glu was increased ($P \leq 0.05$) when the *Bacillus*-based DFM was added to the low-fiber diet in periods 2, 3, and 4 compared with the low-fiber diet without DFM fed in period 1 and periods 5 and 6 (Table 6.5). To our knowledge, this is the first reported instance of increased AID of AA by swine fed a DFM-containing diet. The *Bacillus*-based DFM added to the low-fiber diet also increased ($P \leq 0.05$) AID of ADF and NDF, which may indicate that as the DFM degrades ADF and NDF, a greater amount of Lys, Phe, and Glu are made available for digestion and absorption. The AID of starch was greater ($P \leq 0.05$) in periods 2, 3, and 4 and periods 5 and 6 compared with period 1, but was not different during periods 2, 3, and 4 compared with periods 5 and 6. The AID of starch in the low-fiber diet without DFM was

increased as pig BW increased and this data is in agreement, but also indicates that the DFM is not responsible for increased AID of starch. The observation that ATTD of NDF was decreased ($P \leq 0.05$) when the *Bacillus*-based DFM was added to the low-fiber diet indicates that the DFM may have a more beneficial effect on dietary fiber fermentation in the upper-tract rather than the lower-tract (Table 6.6). The ATTD of GE and the concentration of DE and ME in the low-fiber diet without DFM fed to pigs in periods 5 and 6 were greater ($P \leq 0.05$) compared with the low-fiber diet fed in period 1 and the low-fiber diet plus DFM fed in periods 2, 3, and 4. This indicates that the DFM does not increase DE or ME in low-fiber diets, rather, DE and ME increases in low-fiber diets as pig BW and age advances. When the DFM was removed from the low-fiber diet, AID of Lys, Phe, Glu, ADF, and NDF decreased to a level similar to the diet without DFM, indicating that the *Bacillus*-based DFM fed over a period of 6 consecutive weeks did not colonize the pigs' gastrointestinal tract. As a consequence, it appears that DFM must be continuously fed to pigs to achieve improvements in nutrient digestibility.

For pigs fed the high-fiber diet without DFM during periods 2, 3, and 4 and the low-fiber diet without DFM during periods 1, 5, and 6, AID of starch was greater ($P \leq 0.05$) in periods 2, 3, and 4 compared with period 1 (Table 6.7). The AID of starch was not reduced when the high-fiber diet was fed because the contribution of starch from DDGS and wheat middlings was low and, therefore, the majority of starch digestion reflected digestion of starch in corn. However, the AID of most AA was reduced ($P \leq 0.05$) in periods 2, 3, and 4 compared with period 1 and periods 5 and 6. This was expected and is in agreement with previous research because DDGS and wheat middlings contribute a significant proportion of AA to the high-fiber diet and the digestibility of AA in DDGS and wheat middlings are less than in corn and soybean meal (Lin et al., 1987; Urriola and Stein, 2010).

The ATTD of DM, GE, ADF, NDF, and concentrations of DE and ME of diets were reduced ($P \leq 0.05$) in periods 2, 3, and 4 when pigs were fed the high-fiber diet without DFM compared with period 1 and periods 5 and 6 when pigs were fed low-fiber diet without DFM (Table 6.8). This was expected because the digestibility of DM, GE, ADF, and NDF are less in DDGS and wheat middlings compared with corn and soybean meal (Lin et al., 1987; Urriola and Stein, 2010).

For pigs fed the high-fiber diet with DFM during periods 2, 3, and 4 and the low-fiber diet without DFM during periods 1, 5, and 6, AID of starch was greater ($P \leq 0.05$) in periods 2, 3, and 4 compared with period 1 (Table 6.9). However, the AID of starch also was greater ($P \leq 0.05$) in periods 5 and 6 compared with period 1, which is an indication that the AID of starch by pigs increased as pig BW and age advanced, regardless of diet type or DFM addition. The AID of most AA was greater in period 1 and periods 5 and 6 when pigs were fed the low-fiber diet without DFM compared with periods 2, 3, and 4 when pigs were fed the high-fiber diet plus DFM, indicating that DFM addition to the high-fiber diet did not ameliorate the negative effect of the high-fiber diet on AA digestibility. The ATTD of DM, GE, ADF, NDF, and concentrations of DE and ME were reduced ($P \leq 0.05$) in periods 2, 3, and 4 when pigs were fed the high-fiber diet with DFM compared with period 1 and periods 5 and 6 when pigs were fed low-fiber diet without DFM (Table 6.10). Again, this is an indication that the addition of the *Bacillus*-based DFM did not ameliorate the negative effect that the high-fiber diet had on ATTD of energy and nutrients.

When data for periods 2, 3, and 4 were pooled within each treatment group and effects of period, fiber level, and DFM addition were determined, the AID of starch increased ($P \leq 0.05$) from 93.4% in period 2 to 96.8% in period 3 to 97.7% in period 4 (Table 6.11). High-fiber diets

reduced ($P \leq 0.05$) the AID of NDF and most AA compared with low-fiber diets and this is in agreement with previous research (Schulze et al., 1994; Urriola and Stein, 2010). The AID of Ser was increased ($P \leq 0.05$) due to DFM addition to diets, but this was the only effect DFM addition had on the AID of nutrients by pigs.

The DE and ME of experimental diets increased ($P \leq 0.05$) as period advanced (Table 6.12). It was hypothesized that the DE and ME of experimental diets would increase as period advanced because the digestibility of fiber would increase. It was also speculated that fiber digestibility would increase over time because the size of the gastrointestinal tract of pigs would increase as well as the size of the microbial population (Kyriazakis and Emmans, 1995; Jørgensen et al., 1996). However, period had no effect on ATTD of ADF or NDF and, therefore, a 5 d adaptation period to low- or high-fiber diets is sufficient to determine the ATTD of ADF and NDF. High-fiber diets reduced ($P \leq 0.05$) ATTD of GE, DM, ADF, NDF and concentrations of DE and ME compared with low-fiber diets. This observation was expected because the high-fiber diets were formulated to contain less DE and ME compared with low-fiber diets. The addition of DFM to diets increased ($P \leq 0.05$) GE in dry feces compared with diets without DFM, but this was the only effect DFM addition had on the ATTD of energy and nutrients by pigs. The increase in GE in dry feces may be attributed to a greater amount of microbial energy excreted by pigs fed DFM-containing diets, but this hypothesis was not experimentally verified.

CONCLUSION

As age and BW of pigs advanced, concentrations of DE and ME increased independent of diet type. In contrast to our hypothesis, the increased DE and ME was not due to increased ATTD of ADF or NDF, rather it was because the AID of starch increased as period advanced.

Pigs fed high-fiber diets had reduced AID of most AA, ATTD of GE, ADF, and NDF, and DE and ME compared with pigs fed low-fiber diets. The addition of a *Bacillus*-based DFM to high-fiber diets fed for 6 consecutive weeks did not ameliorate the negative effects of high-fiber diets on digestibility. However, addition of the *Bacillus*-based DFM to low-fiber diets fed for 6 consecutive wks improved AID of ADF and NDF, which may be the reason for improved AID of Lys, Phe, and Glu by pigs fed the low-fiber diet with DFM. Also, when the DFM was withdrawn from the low-fiber diet, digestibility values were reduced to the levels they had in diets without DFM, indicating that the *Bacillus*-based DFM must be fed continuously to exert beneficial effects. The ATTD of ADF and NDF was not different in pigs adapted to the diet for 5 d compared with pigs adapted for 19 or 33 d indicating that a 5 d adaptation period to experimental diets is sufficient when determining the ATTD of ADF and NDF by growing pigs.

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TABLES

Table 6.1. Ingredient composition and, calculated, and analyzed composition of experimental diets (as-fed basis)

	Low fiber		High fiber	
Item	- DFM[1]	+ DFM	- DFM	+ DFM
Ingredient				
Corn	73.68	73.62	40.00	39.94
Soybean meal, 48% CP	22.00	22.00	16.00	16.00
DDGS[1]	-	-	30.00	30.00
Wheat middlings	-	-	10.00	10.00
Choice white grease	1.00	1.00	1.00	1.00
Limestone	1.15	1.15	1.55	1.55
Dicalcium P	0.60	0.60	-	-
L-Lys HCl	0.34	0.34	0.34	0.34
DL-Met	0.04	0.04	-	-
L-Thr	0.08	0.08	-	-
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Titanium dioxide	0.40	0.40	0.40	0.40
DFM[3]	-	0.06	-	0.06
Phytase	0.01	0.01	0.01	0.01
Calculated values				
DE, kcal/kg	3,426	3,424	3,429	3,427
ME, kcal/kg	3,310	3,308	3,285	3,283
NE, kcal/kg	2,481	2,480	2,371	2,369
CP, %	16.57	16.57	20.72	20.71
Ca, %	0.65	0.65	0.66	0.66
P[4], %	0.31	0.31	0.33	0.33
Amino acids[5], %				
Arg	0.95	0.95	1.05	1.05
His	0.40	0.40	0.47	0.47
Ile	0.59	0.59	0.68	0.68
Leu	1.32	1.32	1.74	1.74

Table 6.1. (cont.)

Lys	0.98	0.98	0.98	0.98
Met	0.28	0.28	0.32	0.32
Met + Cys	0.56	0.56	0.61	0.61
Phe	0.71	0.71	0.86	0.86
Thr	0.59	0.59	0.59	0.59
Trp	0.17	0.17	0.17	0.17
Val	0.66	0.66	0.81	0.81
Analyzed values				
GE, kcal/kg	3,858	3,869	4,134	4,128
DM, %	86.2	86.3	86.7	86.5
Ash, %	5.0	4.4	5.3	4.6
Starch, %	50.9	50.5	32.2	33.0
ADF, %	5.0	4.7	8.8	8.8
NDF%	11.6	11.1	19.8	21.6
Insoluble dietary fiber, %	14.5	13.0	20.9	22.6
Soluble dietary fiber, %	1.0	0.8	3.3	1.3
Total dietary fiber, %	15.5	13.9	24.2	24.0
Water binding capacity, g/g	1.3	1.3	1.7	1.8
Bulk density, g/L	736	732	621	626

[1]DDGS = distillers dried grains with solubles; DFM = direct-fed microbial.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[3]DFM = direct-fed microbial (60 g/t, Danisco Animal Nutrition-DuPont Industrial Biosciences, Marlborough, UK).

[4]Standardized total tract digestible P.

[5]Amino acids are indicated as standardized ileal digestible AA.

Table 6.2. Dietary treatments during the 84 d experimental period

	Period					
Item	1	2	3	4	5	6
Treatment 1	Low-fiber	Low-fiber	Low-fiber	Low-fiber	Low-fiber	Low-fiber
Treatment 2	Low-fiber	Low-fiber + DFM[1]	Low-fiber + DFM	Low-fiber + DFM	Low-fiber	Low-fiber
Treatment 3	Low-fiber	High-fiber	High-fiber	High-fiber	Low-fiber	Low-fiber
Treatment 4	Low-fiber	High-fiber + DFM	High-fiber + DFM	High-fiber + DFM	Low-fiber	Low-fiber

[1]DFM = direct-fed microbial (60 g/MT, Danisco Animal Nutrition-DuPont Industrial Biosciences, Marlborough, UK).

Table 6.3. Effect of feeding period on AID[1] of ADF, NDF, starch, and AA in the low-fiber diet without DFM[1] fed to pigs for 6 periods (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
ADF, %	39.5	44.6	48.0	37.5	40.1	32.4	3.9	0.41	0.03	0.50
NDF, %	44.3	45.3	53.0	45.6	43.1	32.7	4.0	0.48	0.01	0.23
Starch, %	93.4	92.0	97.0	97.0	95.6	97.2	0.9	0.07	0.13	0.01
Indispensable AA, %										
Arg	89.4	89.1	91.2	90.3	89.8	90.4	0.5	0.14	0.81	0.21
His	83.9	84.8	87.1	86.7	87.5	85.5	0.8	0.03	0.67	0.02
Ile	81.9	81.8	83.6	80.4	81.5	79.9	1.1	0.94	0.16	0.39
Leu	83.2	84.1	85.9	83.4	84.8	82.3	1.0	0.27	0.26	0.74
Lys	86.0	86.0	88.0	86.2	86.5	84.6	0.9	0.52	0.11	0.66
Met	85.4	87.3	88.5	86.5	87.5	85.3	1.0	0.08	0.17	0.39
Phe	82.1	82.6	84.5	81.9	82.8	80.6	1.0	0.41	0.08	0.72
Thr	76.4	76.0	78.2	75.6	77.7	75.0	1.4	0.88	0.82	0.99
Trp	82.5	83.4	82.6	81.6	85.3	82.2	1.4	0.97	0.29	0.47
Val	78.2	78.6	80.1	77.0	78.9	76.3	1.4	0.81	0.36	0.71
Dispensable AA, %										
Ala	77.7	78.8	76.9	72.4	75.6	73.3	1.9	0.43	0.26	0.15
Asp	80.5	80.4	82.4	79.7	80.9	79.9	0.9	0.77	0.55	0.91
Cys	73.2	72.2	74.5	73.3	76.7	72.9	1.7	0.92	0.20	0.36
Glu	86.2	86.5	87.1	85.3	86.3	85.5	0.8	0.94	0.53	0.74
Gly	67.9	68.9	72.6	70.2	72.7	69.0	1.7	0.12	0.81	0.10
Pro	74.4	76.2	81.9	79.5	80.0	79.2	2.0	< 0.01	0.68	< 0.01
Ser	81.0	80.6	81.7	79.7	81.6	81.4	0.9	0.81	0.29	0.64
Tyr	82.9	82.4	84.2	82.4	83.7	82.7	1.0	0.94	0.77	0.79

[1]DFM = direct-fed microbial; AID = apparent ileal digestibility.

[2]Date are means of 6 observations per period.

Table 6.4. Effect of feeding period on ATTD[1] and concentrations of DE and ME in the low-fiber diet without DFM[1] fed to pigs for 6 periods (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
Total feed intake, kg/5 d	6.96	8.39	9.72	10.78	12.09	13.36	0.12	< 0.01	< 0.01	< 0.01
GE intake, kcal/5 d	26,835	32,351	37,494	41,573	46,652	51,541	447	< 0.01	< 0.01	< 0.01
Dry feces output, kg/5 d	0.714	0.874	0.936	1.041	1.167	1.195	0.037	< 0.01	< 0.01	< 0.01
GE in dry feces, kcal/kg	4,719	4,693	4,607	4,391	4,454	4,527	47	< 0.01	0.08	< 0.01
Fecal GE output, kcal/5 d	3,361	4,100	4,307	4,569	5,196	5,407	158	< 0.01	< 0.01	< 0.01
ATTD[1] of GE, %	87.5	87.3	88.5	89.0	88.9	89.5	0.35	0.03	< 0.01	< 0.01
ATTD of DM, %	88.8	88.5	89.3	89.3	89.4	90.1	0.33	0.45	0.01	0.01
ATTD of ADF, %	65.0	67.8	70.2	73.8	74.0	72.1	2.90	0.10	0.34	0.03
ATTD of NDF, %	69.4	69.3	70.2	70.4	70.3	75.6	0.94	0.59	< 0.01	< 0.01
DE in diet, kcal/kg	3,375	3,369	3,415	3,433	3,428	3,452	13	0.03	< 0.01	< 0.01
Urine output, kg/5 d	40.75	34.00	49.04	54.05	52.94	55.98	9.90	0.49	0.13	0.08
GE in urine, kcal/kg	51.65	62.28	59.49	38.98	31.40	27.97	9.62	0.83	0.01	0.04
Urinary GE output, kcal/5 d	1,724	1,739	2,378	1,768	1,658	1,392	270	0.33	0.06	0.48
ME in diet, kcal/kg	3,128	3,161	3,171	3,270	3,286	3,349	28	0.02	< 0.01	< 0.01

[1]DFM = direct-fed microbial; ATTD = apparent total tract digestibility.

[2]Date are means of 6 observations per period.

Table 6.5. Effects of feeding period and addition of a *Bacillus*-based DFM[1] on AID[1] of ADF, NDF, starch, and AA in the low-fiber diet plus DFM fed to pigs in periods 2, 3, and 4 and the low-fiber diet without DFM in periods 1, 5, and 6 (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
ADF, %	41.4	46.8	46.6	39.6	37.7	29.9	3.9	0.55	0.01	0.15
NDF, %	46.2	54.1	53.0	48.1	40.7	34.6	3.6	0.23	< 0.01	0.08
Starch, %	93.0	94.6	96.6	96.6	95.8	95.8	0.8	< 0.01	0.79	0.01
Indispensable AA, %										
Arg	89.2	90.2	91.5	91.3	89.7	91.1	0.7	0.04	0.30	0.17
His	83.1	85.2	87.3	87.6	87.9	86.3	1.0	0.01	0.62	< 0.01
Ile	81.4	82.3	83.6	82.9	82.2	80.4	0.9	0.13	0.03	0.91
Leu	82.8	84.5	85.8	85.8	85.7	83.3	0.9	0.02	0.24	0.12
Lys	85.3	86.8	87.8	87.4	86.8	84.7	0.8	0.05	0.04	0.62
Met	85.1	87.0	88.2	87.5	88.4	85.7	0.9	0.03	0.51	0.10
Phe	81.6	83.7	84.3	84.8	83.8	81.3	0.8	0.01	0.02	0.36
Thr	74.9	77.2	78.9	78.3	77.9	75.1	1.3	0.07	0.18	0.37
Trp	79.4	81.8	82.0	81.8	86.6	84.5	1.2	0.09	< 0.01	< 0.01
Val	77.3	78.8	80.1	80.1	80.1	77.1	1.1	0.08	0.25	0.34
Dispensable AA, %										
Ala	77.0	78.7	79.0	78.0	76.7	74.7	1.4	0.32	0.02	0.42
Asp	80.1	80.8	82.2	81.5	81.5	81.1	1.0	0.22	0.82	0.31
Cys	69.5	73.4	76.0	77.2	77.1	73.3	1.7	0.01	0.82	0.02
Glu	85.5	87.4	88.3	87.9	86.6	85.9	0.8	0.01	0.02	0.43
Gly	65.0	68.3	72.8	72.4	71.4	67.2	2.5	0.01	0.23	0.08
Pro	71.3	76.3	83.0	80.5	76.0	78.0	3.5	0.02	0.23	0.11
Ser	80.4	81.8	83.4	83.2	82.0	82.1	1.0	0.06	0.41	0.21
Tyr	82.8	83.3	85.0	85.0	83.8	81.4	1.0	0.18	0.05	0.91

[1]DFM = direct-fed microbial; AID = apparent ileal digestibility.

[2]Date are means of 6 observations for periods 1 and 2 and 5 observations during periods 3 through 6.

Table 6.6. Effects of feeding period and addition of a *Bacillus*-based DFM[1] on ATTD[1] and concentrations of DE and ME in the low-fiber diet with DFM fed to pigs in periods 2, 3, and 4 and the low-fiber diet without DFM in periods 1, 5, and 6 (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
Total feed intake, kg/5 d	7.04	8.42	9.41	9.89	10.72	12.37	0.06	< 0.01	< 0.01	< 0.01
GE intake, kcal/5 d	27,166	32,575	36,401	38,270	41,373	47,735	199	< 0.01	< 0.01	< 0.01
Dry feces output, kg/5 d	0.672	0.760	0.888	1.069	1.074	1.067	0.04	< 0.01	< 0.01	< 0.01
GE in dry feces, kcal/kg	4,815	4,772	4,627	4,358	4,501	4,590	40	< 0.01	0.26	< 0.01
Fecal GE output, kcal/5 d	3,231	3,626	4,103	4,558	4,670	4,881	182	< 0.01	< 0.01	< 0.01
ATTD[1] of GE, %	88.1	88.9	88.7	88.1	88.7	89.4	0.48	0.31	0.22	0.07
ATTD of DM, %	89.6	90.0	89.4	88.1	89.1	90.2	0.43	0.30	0.14	0.81
ATTD of ADF, %	69.0	64.1	69.4	73.0	77.7	69.0	2.89	0.95	0.10	0.21
ATTD of NDF, %	72.4	70.0	69.7	67.5	69.8	76.6	1.10	< 0.01	< 0.01	0.43
DE in diet, kcal/kg	3,398	3,438	3,432	3,407	3,421	3,447	19	0.12	0.57	0.07
Urine output, kg/5 d	27.40	27.05	39.38	53.03	63.29	59.56	9.21	0.15	0.01	< 0.01
GE in urine, kcal/kg	55.05	64.18	73.71	54.97	26.76	30.71	11.56	0.43	< 0.01	0.05
Urinary GE output, kcal/5 d	1,310	1,452	2,400	2,298	1,477	1,474	196	< 0.01	< 0.01	0.46
ME in diet, kcal/kg	3,213	3,266	3,175	3,173	3,273	3,318	30	0.78	< 0.01	0.01

[1]DFM = direct-fed microbial; ATTD = apparent total tract digestibility.

[2]Date are means of 6 observations per periods 1 and 2 and 5 observations per periods 3 through 6.

Table 6.7. Effects of feeding period and a high-fiber diet on AID[1] of ADF, NDF, starch, and AA in a high-fiber diet without DFM[1] fed to pigs in periods 2, 3, and 4 and the low-fiber diet without DFM in periods 1, 5, and 6 (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
ADF, %	50.4	49.2	50.6	43.3	40.1	36.9	3.1	0.48	< 0.01	< 0.01
NDF, %	55.8	42.5	46.2	37.0	44.5	40.9	3.2	< 0.01	0.75	< 0.01
Starch, %	94.7	94.3	97.1	98.8	94.1	97.7	0.6	< 0.01	0.07	0.08
Indispensable AA, %										
Arg	90.1	86.9	88.5	89.3	89.2	90.9	0.6	< 0.01	< 0.01	0.98
His	85.7	81.8	83.3	83.7	86.9	85.3	0.7	< 0.01	< 0.01	0.69
Ile	83.5	81.2	82.5	81.4	82.5	81.0	0.7	0.04	0.93	0.05
Leu	85.1	85.5	86.4	85.8	85.2	83.8	0.7	0.34	0.03	0.50
Lys	86.9	82.6	83.2	82.7	87.1	85.3	0.7	< 0.01	< 0.01	0.36
Met	87.3	84.6	84.6	84.7	88.4	86.6	0.6	< 0.01	< 0.01	0.87
Phe	83.8	83.9	84.7	84.3	83.5	81.8	0.8	0.60	0.02	0.23
Thr	78.7	72.6	73.9	73.5	78.8	76.8	0.9	< 0.01	< 0.01	0.44
Trp	83.6	78.8	81.0	80.6	85.5	82.2	0.9	< 0.01	< 0.01	0.81
Val	80.2	78.5	80.0	78.6	79.8	77.5	0.9	0.23	0.75	0.17
Dispensable AA, %										
Ala	80.1	79.1	78.2	76.1	78.5	76.1	1.0	0.07	0.56	0.03
Asp	81.9	76.3	77.7	78.1	81.3	81.2	0.8	< 0.01	< 0.01	0.48
Cys	75.5	70.6	73.7	75.1	76.7	74.7	1.2	0.13	0.03	0.90
Glu	87.6	85.6	85.0	85.6	86.9	86.9	0.7	0.01	0.01	0.37
Gly	70.8	68.1	72.4	70.9	71.8	70.5	1.4	0.81	0.52	0.84
Pro	73.7	70.0	77.5	79.6	80.2	78.7	2.4	0.36	0.03	0.02
Ser	82.4	79.4	80.4	80.9	82.6	82.7	0.8	0.02	< 0.01	0.84
Tyr	84.7	83.9	85.6	85.7	84.2	84.0	0.8	0.67	0.16	0.55

[1]DFM = direct-fed microbial; AID = apparent ileal digestibility.

[2]Date are means of 6 observations per period.

Table 6.8. Effects of feeding period and a high-fiber diet on ATTD[1] and concentrations of DE and ME in a high-fiber diet without DFM[1] fed to pigs in periods 2, 3, and 4 and the low-fiber diet without DFM fed to pigs in periods 1, 5, and 6 (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
Total feed intake, kg/5 d	6.97	8.20	9.45	10.14	11.73	12.99	0.28	< 0.01	< 0.01	< 0.01
GE intake, kcal/5 d	26,874	33,887	39,070	41,915	45,251	50,096	1,137	< 0.01	< 0.01	< 0.01
Dry feces output, kg/5 d	0.687	1.409	1.604	1.732	1.179	1.246	0.050	< 0.01	< 0.01	< 0.01
GE in dry feces, kcal/kg	4,832	4,707	4,729	4,752	4,433	4,397	50	0.08	< 0.01	< 0.01
Fecal GE output, kcal/5 d	3,318	6,632	7,582	8,191	5,019	5,467	211	< 0.01	< 0.01	< 0.01
ATTD[1] of GE, %	87.6	80.4	80.6	81.2	88.9	89.1	0.39	< 0.01	< 0.01	< 0.01
ATTD of DM, %	89.1	81.1	81.2	81.8	89.2	89.5	0.36	< 0.01	< 0.01	0.55
ATTD of ADF, %	68.4	61.2	61.6	62.7	72.1	69.4	2.22	0.01	< 0.01	0.35
ATTD of NDF, %	70.3	59.3	59.4	59.3	68.5	72.0	0.87	< 0.01	< 0.01	0.97
DE in diet, kcal/kg	3,381	3,325	3,332	3,358	3,430	3,437	16	0.01	< 0.01	< 0.01
Urine output, kg/5 d	32.58	41.75	42.00	45.52	44.90	61.93	9.66	0.17	0.09	0.01
GE in urine, kcal/kg	50.24	61.58	62.49	39.81	48.67	26.38	9.15	0.58	0.02	0.15
Urinary GE output, kcal/5 d	1,579	2,158	2,181	1,628	1,525	1,334	200	0.08	0.01	0.55
ME in diet, kcal/kg	3,155	3,059	3,102	3,198	3,296	3,334	31	0.31	< 0.01	< 0.01

[1]DFM = direct-fed microbial; ATTD = apparent total tract digestibility.

[2]Date are means of 6 observations per period.

Table 6.9. Effects of feeding period and a high-fiber diet with a *Bacillus*-based DFM[1] on AID[1] of ADF, NDF, starch, and AA in a high-fiber diet with DFM in periods 2, 3, and 4 and the low-fiber diet without DFM in periods 1, 5, and 6 fed to pigs (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
ADF, %	39.8	42.6	44.7	39.3	37.4	36.7	3.0	0.50	0.07	0.49
NDF, %	41.3	38.6	44.9	40.0	40.6	39.2	2.9	0.96	0.64	0.71
Starch, %	93.1	92.3	96.5	98.3	97.6	96.0	1.0	0.03	0.23	0.01
Indispensable AA, %										
Arg	89.0	86.6	89.6	89.7	91.0	90.7	0.5	0.54	< 0.01	0.02
His	83.2	79.9	82.9	83.3	87.9	85.5	0.7	0.11	< 0.01	< 0.01
Ile	81.6	81.4	82.5	81.1	82.6	80.3	0.7	0.93	0.73	0.87
Leu	83.1	84.5	85.8	85.6	86.2	83.0	0.7	0.01	0.21	0.08
Lys	85.1	81.6	84.4	83.7	87.1	84.9	0.5	< 0.01	< 0.01	0.14
Met	85.0	83.0	84.6	83.9	88.0	85.6	0.7	0.13	< 0.01	0.04
Phe	81.7	83.0	84.2	83.7	84.2	81.1	0.6	0.01	0.10	0.22
Thr	74.4	71.6	73.9	72.7	78.0	74.7	0.9	0.13	< 0.01	0.11
Trp	79.1	77.6	80.2	79.3	84.9	82.1	1.0	0.97	< 0.01	< 0.01
Val	77.0	78.0	79.6	78.7	79.9	76.3	0.8	0.05	0.35	0.23
Dispensable AA, %										
Ala	77.2	78.6	77.8	76.2	77.2	74.0	0.8	0.70	0.01	0.11
Asp	79.7	75.5	79.5	78.2	81.7	79.8	0.7	0.03	< 0.01	0.25
Cys	69.7	67.5	70.8	71.5	75.6	71.4	1.2	0.88	< 0.01	0.02
Glu	85.6	84.2	85.7	85.5	87.6	86.2	0.6	0.47	< 0.01	0.08
Gly	62.6	66.5	70.6	70.1	71.4	66.5	1.7	< 0.01	0.93	< 0.01
Pro	75.0	77.0	81.3	81.2	82.6	79.9	2.7	0.09	0.52	0.05
Ser	80.1	79.8	82.3	81.7	83.2	81.4	0.7	0.22	0.12	0.03
Tyr	82.2	82.1	84.8	83.7	83.6	82.9	0.7	0.11	0.70	0.22

[1]DFM = direct-fed microbial; AID = apparent ileal digestibility.

[2] Date are means of 6 observations for periods 1 through 4 and 5 observations during periods 5 and 6.

Table 6.10. Effects of feeding period and a high-fiber diet plus a *Bacillus*-based DFM[1] on ATTD[1] and concentrations of DE and ME in a high-fiber diet with DFM[1] in periods 2, 3, and 4 and the low-fiber diet without DFM in periods 1, 5, and 6 fed to pigs (as-fed basis)[2]

	Period							Contrast *P*-value		
Item	1	2	3	4	5	6	SEM	Period 1 vs. period 2, 3, 4	Period 2, 3, 4 vs. period 5, 6	Period 1 vs. period 5, 6
Total feed intake, kg/5 d	7.01	8.37	9.63	10.74	12.03	13.08	0.13	< 0.01	< 0.01	< 0.01
GE intake, kcal/5 d	27,048	34,535	39,769	44,332	46,437	50,455	498	< 0.01	< 0.01	< 0.01
Dry feces output, kg/5 d	0.669	1.452	1.637	1.764	1.081	1.076	0.045	< 0.01	< 0.01	< 0.01
GE in dry feces, kcal/kg	4,796	4,789	4,813	4,877	4,587	4,650	57	0.64	< 0.01	0.02
Fecal GE output, kcal/5 d	3,210	6,953	7,881	8,602	4,942	5,008	218	< 0.01	< 0.01	< 0.01
ATTD[1] of GE, %	88.1	79.9	80.2	80.6	89.4	89.5	0.35	< 0.01	< 0.01	0.01
ATTD of DM, %	89.5	80.9	81.3	81.6	90.0	90.6	0.24	< 0.01	< 0.01	0.01
ATTD of ADF, %	69.5	60.2	61.2	63.6	71.0	72.0	2.37	0.02	< 0.01	0.53
ATTD of NDF, %	70.7	60.7	61.1	61.5	69.8	75.2	1.05	< 0.01	< 0.01	0.18
DE in diet, kcal/kg	3,400	3,297	3,310	3,327	3,448	3,454	14	< 0.01	< 0.01	0.01
Urine output, kg/5 d	30.31	34.71	50.07	64.09	35.79	44.70	8.09	0.01	0.11	0.18
GE in urine, kcal/kg	60.76	71.53	51.63	47.22	51.83	32.09	11.63	0.66	0.07	0.07
Urinary GE output, kcal/5 d	1,567	2,126	2,482	2,068	1,514	1,245	221	0.01	< 0.01	0.48
ME in diet, kcal/kg	3,176	3,043	3,052	3,133	3,321	3,382	36	0.02	< 0.01	< 0.01

[1]DFM = direct-fed microbial; ATTD = apparent total tract digestibility.

[2]Date are means of 6 observations per periods 1 through 4 and 5 observations per periods 5 and 6.

Table 6.11. Effects of period, fiber level, and addition of a *Bacillus*-based direct-fed microbial on the AID[1] of ADF, NDF, starch, and AA in diets fed to pigs during periods 2, 3, and 4 (as-fed basis)

	Period 2				Period 3				Period 4							
Fiber concentration	Low		High		Low		High		Low		High			Main effects		
DFM[1]	-	+	-	+	-	+	-	+	-	+	-	+	SEM	Period	Fiber	DFM
ADF, %	47.0	45.9	48.8	42.6	48.0	46.6	50.6	44.7	37.5	39.6	43.3	39.3	3.3	0.01	0.64	0.13
NDF, %	44.9	54.1	42.4	38.6	53.0	53.0	46.2	44.9	45.6	48.1	37.0	40.0	3.3	0.03	< 0.01	0.41
Starch[2], %	92.0	94.8	94.3	92.3	97.0	96.6	97.1	96.5	97.0	96.6	98.8	98.3	0.7	< 0.01	0.29	0.69
Indispensable AA, %																
Arg[3]	89.1	90.4	86.8	86.6	91.2	91.5	88.5	89.6	90.3	91.2	89.3	89.7	0.6	< 0.01	< 0.01	0.16
His	84.9	85.5	81.4	79.9	87.1	87.2	83.3	82.9	86.7	87.5	83.7	83.3	0.8	< 0.01	< 0.01	0.78
Ile	82.3	82.6	80.9	81.4	83.6	83.6	82.5	82.5	80.4	82.9	81.4	81.1	0.8	0.01	0.11	0.40
Leu	84.4	84.8	85.3	84.5	85.9	85.7	86.4	85.8	83.4	85.7	85.8	85.6	0.8	0.04	0.26	0.76
Lys	86.1	86.9	82.2	81.6	88.0	87.8	83.2	84.4	86.2	87.4	82.7	83.7	0.7	< 0.01	< 0.01	0.20
Met	87.5	87.3	84.6	83.0	88.5	88.1	84.6	84.6	86.5	87.4	84.7	83.9	0.8	0.18	< 0.01	0.48
Phe	82.9	84.0	83.5	83.0	84.5	84.3	84.7	84.2	81.9	84.8	84.3	83.7	0.8	0.06	0.78	0.52
Thr	76.5	77.3	72.4	71.6	78.2	78.9	73.9	73.9	75.6	78.3	73.5	72.7	1.1	0.05	< 0.01	0.54
Trp	83.7	82.1	78.6	77.6	82.6	82.0	81.0	80.2	81.6	81.8	80.6	79.3	1.1	0.39	< 0.01	0.28
Val	79.1	79.2	78.3	78.0	80.1	80.0	79.6	79.6	77.0	79.9	78.6	78.7	1.0	0.08	0.54	0.50
Dispensable AA, %																
Ala	79.3	79.3	78.8	78.6	76.9	78.8	78.2	77.8	72.4	77.8	76.1	76.2	1.2	< 0.01	0.81	0.18
Asp[3]	80.5	81.1	76.0	75.5	82.4	82.0	77.7	79.5	79.7	81.3	78.1	78.2	0.8	< 0.01	< 0.01	0.27
Cys[4]	72.9	73.5	70.5	67.5	74.5	76.0	73.7	70.8	73.3	77.2	75.1	71.5	1.4	0.01	< 0.01	0.53
Glu	86.7	87.8	85.2	84.2	87.1	88.2	85.0	85.7	85.3	85.5	85.6	85.5	0.7	0.39	< 0.01	0.14
Gly	69.5	70.3	67.1	66.5	72.6	72.0	72.4	70.6	70.2	71.6	70.9	70.1	1.7	< 0.01	0.28	0.83
Pro	76.7	76.4	65.9	77.0	81.9	82.1	77.5	81.3	79.5	79.6	79.6	81.2	2.7	< 0.01	0.36	0.27
Ser	80.9	81.7	79.1	79.8	81.7	83.4	80.4	82.3	79.7	83.2	80.9	81.7	0.7	0.01	0.01	< 0.01
Tyr[4]	82.7	83.4	83.6	82.1	84.2	84.9	85.6	84.8	82.4	84.9	85.7	83.7	0.7	< 0.01	0.28	0.89

[1]DFM = direct-fed microbial; AID = apparent ileal digestibility.
[2]Interaction of fiber and DFM ($P < 0.10$) was observed.
[3]Interaction of period and fiber ($P < 0.10$) was observed.
[4]Interaction of fiber and DFM ($P \leq 0.05$) was observed.

Table 6.12. Effects of period, dietary fiber concentration, and addition of a *Bacillus*-based direct-fed microbial on ATTD[1] and concentrations of DE and ME in diets fed to pigs during periods 2, 3, and 4 (as-fed basis)

	Period 2				Period 3				Period 4							
Fiber concentration	Low		High		Low		High		Low		High			Main effects		
DFM[1]	-	+	-	+	-	+	-	+	-	+	-	+	SEM	Period	Fiber	DFM
Total feed intake[2], kg/5 d	8.39	8.42	8.20	8.37	9.72	9.41	9.45	9.63	10.78	9.89	10.14	10.74	0.17	< 0.01	0.93	0.82
GE intake[3], kcal/5 d	32,351	32,575	33,887	34,535	37,494	36,412	39,070	39,769	41,573	38,275	41,915	44,332	715	< 0.01	< 0.01	0.91
Dry feces output[4], kg/5 d	0.874	0.760	1.409	1.452	0.936	0.881	1.604	1.637	1.041	1.063	1.733	1.764	0.039	< 0.01	< 0.01	0.82
GE in dry feces[5], kcal/kg	4,693	4,772	4,707	4,789	4,607	4,631	4,729	4,813	4,391	4,365	4,752	4,877	38	< 0.01	< 0.01	< 0.01
Fecal GE output[6], kcal/5 d	4,100	3,626	6,632	6,953	4,307	4,083	7,582	7,881	4,569	4,541	8,201	8,602	186	< 0.01	< 0.01	0.73
ATTD[1] of GE, %	87.3	88.9	80.4	79.9	88.5	88.8	80.6	80.2	89.0	88.1	81.2	80.6	0.40	0.09	< 0.01	0.68
ATTD of DM[7], %	88.5	90.0	81.1	80.9	89.3	89.5	81.2	81.3	89.3	88.2	81.8	81.6	0.35	0.57	< 0.01	0.88
ATTD of ADF, %	67.8	64.1	61.2	60.2	70.2	69.5	61.6	61.2	73.8	73.1	61.7	63.6	2.40	0.06	< 0.01	0.45
ATTD of NDF[8], %	69.3	70.0	59.3	60.7	70.2	69.9	59.4	61.1	70.4	67.4	59.3	61.3	0.89	0.63	< 0.01	0.46
DE in diet[9], kcal/kg	3,369	3,438	3,325	3,297	3,415	3,435	3,332	3,310	3,433	3,410	3,361	3,327	16	0.04	< 0.01	0.80
Urine output, kg/5 d	34.00	27.05	41.75	34.71	49.04	39.04	42.00	50.07	54.05	52.08	45.52	64.09	9.11	< 0.01	0.63	0.99
GE in urine, kcal/kg	62.28	64.18	61.58	71.53	59.49	73.29	62.49	53.36	38.98	55.43	39.81	43.30	11.19	0.01	0.69	0.50
Urinary GE output[10], kcal/5 d	1,739	1,452	2,158	2,216	2,378	2,408	2,181	2,482	1,768	2,305	1,628	2,031	226	< 0.01	0.57	0.33
ME in diet[11], kcal/kg	3,161	3,266	3,059	3,043	3,171	3,179	3,102	3,052	3,270	3,177	3,208	3,139	30	< 0.01	< 0.01	0.39

[1]DFM = direct-fed microbial; ATTD = apparent total tract digestibility.
[2]Interactions of period and DFM, fiber and DFM, and period, fiber, and DFM ($P < 0.05$) were observed.
[3]Interactions of period and fiber, period and DFM, fiber and DFM, and period, fiber, and DFM ($P < 0.05$) were observed.
[9]Interaction of period and fiber ($P < 0.10$) was observed.
[5]Interactions of period and fiber ($P < 0.05$) were observed.
[6]Interactions of period and fiber and fiber and DFM ($P < 0.05$) were observed.
[7]Interactions of period and fiber, period and DFM, and period and fiber and DFM ($P < 0.05$) were observed.
[8]Interaction of fiber and DFM ($P < 0.05$) was observed.
[9]Interactions of period and DFM, fiber and DFM, and period, fiber, and DFM ($P < 0.10$) were observed.
[10]Interactions of period and fiber ($P < 0.05$) and period and DFM ($P < 0.10$) were observed.
[11]Interactions of period and fiber and period and DFM ($P < 0.05$) were observed.

CHAPTER 7: DISAPPEARANCE OF NUTRIENTS AND ENERGY IN THE STOMACH AND SMALL INTESTINE, CECUM, AND COLON OF PIGS FED CORN-SOYBEAN MEAL DIETS CONTAINING DISTILLERS DRIED GRAINS WITH SOLUBLES, WHEAT MIDDLINGS, OR SOYBEAN HULLS

ABSTRACT: Disappearance of nutrients and energy in the stomach and small intestine, cecum, and colon of pigs fed diets containing distillers dried grains with solubles (DDGS), wheat middlings, or soybean hulls was determined. A second objective was to test the hypothesis that physical characteristics of dietary fiber in diets are correlated with the digestibility of nutrients and energy by pigs fed experimental diets. Eight barrows (initial BW = 37.3 ± 1.0 kg) were surgically equipped with a T-cannula in the distal ileum and a T-cannula in the colon approximately 10 cm distal to the cecocolic junction. Pigs were randomly allotted to a replicated 4 × 4 Latin square design with 4 diets and 4 periods in each square. The basal diet was a corn-soybean meal diet and 3 additional diets were formulated by substituting 30% of the nutrients and energy from corn, soybean meal, and L-Lys HCl with DDGS, wheat middlings, or soybean hulls. Titanium dioxide was included as an indigestible marker. Each period lasted 14 d. The initial 8 d were considered an adaptation to the diet. On d 9 and 10, fecal samples were collected. Colon digesta were collected for 8 h on d 11 and 12, whereas ileal digesta were collected for 8 h on d 13 and 14. Values for apparent ileal digestibility (AID), apparent cecal digestibility (ACD), and apparent total tract digestibility (ATTD) of nutrients and energy by pigs fed experimental diets were calculated. Nutrient and energy flow along the gastrointestinal tract was calculated, and disappearance of nutrients and energy was calculated using digestibility values and flow. Results indicated that ACD and ATTD of soluble dietary fiber by pigs fed experimental diets

was not different. Pigs fed basal or wheat middlings diets had greater ($P \leq 0.05$) ACD of insoluble dietary fiber compared with pigs fed diets containing DDGS or soybean hulls. Insoluble dietary fiber disappearance in the colon of pigs fed the soybean hulls diet was greater ($P \leq 0.05$) compared with other diets. Wheat middlings had greater ($P \leq 0.05$) disappearance of dietary fiber fractions compared with DDGS and soybean hulls. Water binding capacity, bulk density, and viscosity of dietary fiber in experimental diets were not correlated with digestibility of nutrients and energy by pigs. In conclusion, disappearance in the colon of most dietary fiber fractions and energy was greater in diets containing soybean hulls or DDGS compared with basal or wheat middlings diets.

Key words: cecum, co-products, dietary fiber, digestibility, pigs

INTRODUCTION

The U.S. swine feed industry has increased interest in co-product utilization because of the potential to reduce diet costs by inclusion of less expensive ingredients in the diets. Distillers dried grains with solubles (**DDGS**), wheat middlings, and soybean hulls are cost-effective co-products that contain more dietary fiber and less starch compared with corn (Burkhalter et al., 2001; Urriola et al., 2010; Jaworski et al., 2015). Feeding diets containing more dietary fiber results in pigs obtaining a greater proportion of dietary energy from VFA produced via microbial fermentation of dietary fiber compared with pigs fed high-starch and low-fiber diets (Bach Knudsen, 2011). Microbial fermentation of dietary fiber varies among sources of dietary fiber and, therefore, VFA absorption and utilization also varies (Urriola et al., 2010).

It is believed that the majority of microbial fermentation of dietary fiber occurs in the

cecum of pigs; however, the extent and degradation of specific dietary fiber fractions fermented in the cecum and large intestine are unknown. Analyzed dietary fiber fractions, as well as the physicochemical characteristics of diets, may be related to the amount of dietary fiber degraded in specific sites of the gastrointestinal tract of pigs. Therefore, an experiment was conducted to test the hypothesis that dietary physical characteristics of dietary fiber are correlated with the digestibility of dietary fiber fractions and energy and may be used to predict the disappearance of dietary fiber fractions and energy along the gastrointestinal tract of the pig. The first objective of this experiment, therefore, was to quantify the disappearance of dietary fiber fractions in the stomach and small intestine, cecum, and colon of pigs. The second objective was to determine the correlation coefficients between physical dietary characteristics and the disappearance of dietary fiber fractions along the gastrointestinal tract of the pig.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

Animals, Housing, and Diets. Eight barrows (initial BW = 37.3 ± 1.0 kg) that were the offspring of PIC359 boars and F-46 sows (Pig Improvement Company, Hendersonville, TN) were surgically equipped with two T-cannulas. One cannula was placed in the distal ileum according to Stein et al. (1998) and a second cannula was placed in the proximal colon approximately 10 cm distal to the cecocolic junction. After surgery, pigs were housed in individual pens and allowed to recover for 8 d. Each pen had a fully slatted tri-bar floor and was equipped with a feeder and a nipple drinker. Cannulated pigs (initial BW = 41.0 ± 1.5 kg) were

randomly allotted to a replicated 4 × 4 Latin square design with 4 diets and 4 periods in each square.

The DDGS was procured from One Earth Energy, Gibson City, IL (Table 7.1). Wheat middlings were procured from Siemers Milling, Teutopolis, IL. Soybean hulls were procured from Archer Daniels Midland Company, Decatur, IL.

Four experimental diets were prepared. The basal diet was a corn-soybean meal diet (Table 7.2). Three additional diets were formulated by substituting 30% of the nutrients and energy from corn, soybean meal, and L-Lys HCl with DDGS, wheat middlings, or soybean hulls. Vitamins and minerals were included in all diets at 0.2% to meet current requirements (NRC, 2012) and titanium dioxide was included in all diets at 0.4% as an indigestible marker.

Feeding and Sample Collection. Pigs were provided feed in an amount equivalent to 3 times the estimated requirement for maintenance energy (i.e., 197 kcal ME / $kg^{0.60}$; NRC, 2012) and daily feed allotments were divided into two daily meals that were provided at 0700 and 1600 h. Water was available at all times. The BW of each pig was recorded at the beginning of the experiment and at the end of each period. Each diet was fed during one 14-d period. The initial 8 d were considered the diet adaptation period. On d 9 and 10, fecal samples were collected and stored at – 20°C immediately after collection. Colon digesta were collected for 8 h on d 11 and 12, whereas ileal digesta were collected for 8 h on d 13 and 14. Digesta were stored at -20°C immediately after collection. The final BW of pigs was 84.7 ± 6.4 kg.

Chemical Analysis. Diets, ingredients, freeze-dried samples of ileal and colon digesta, and feces dried at 65°C were ground through a 1 mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ). All samples were analyzed for DM (Method 930.15; AOAC Int., 2007). Diets and ingredients were analyzed for ash (Method 942.05; AOAC Int., 2007) and acid

hydrolyzed ether extract (**AEE**) was determined by acid hydrolysis using 3*N* HCl (Ankom^{HCl}, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY). The concentration of GE in all samples was determined using an isoperibol bomb calorimeter (model 6300, Parr Instruments, Moline, IL). Benzoic acid was the standard for calibration. All diets and ingredients were analyzed for AA on a Hitachi AA Analyzer, Model No. L8800 (Hitachi High Technologies America, Inc, Pleasanton, CA) using ninhydrin for postcolum derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [Method 982.30 E(a); AOAC Int., 2007]. Titanium concentration in all diets, ileal digesta samples, colon digesta samples, and fecal samples were determined using an ICP procedure (Method 990.08; AOAC Int., 2007). Samples were prepared using nitric acid-perchloric acid (Method 968.08 D(b); AOAC Int., 2007). Total starch was analyzed in all diets and ingredients by the glucoamylase procedure (Method 979.10; AOAC Int., 2007). All samples were analyzed for ADF and NDF using Ankom Technology method 12 and 13, respectively (Ankom^{2000} Fiber Analyzer, Ankom Technology, Macedon, NY), and ADL was analyzed in ingredients and diets using Ankom Technology method 9 (Ankom Daisy^{II} Incubator, Ankom Technology, Macedon, NY). Insoluble and soluble dietary fiber was analyzed in all samples according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY).

Physicochemical Analysis. All samples of ingredients and diets were analyzed for water binding capacity (Robertson et al., 2000) and bulk density (Cromwell et al., 2000). Values for water binding capacity were expressed as the amount of water retained by the pellet (g / g; Urriola and Stein, 2010). Viscosity was measured in ileal and colon digesta that was not freeze dried using a Brookfield LV-DV-II+ Viscometer (Brookfield Eng. Lab. Inc., Middleboro, MA)

as described by Dikeman and Fahey (2006) using V-72, V-73, and V-75 spindles over a range of speeds (0.5 to 6 rpm).

Calculations and Statistical Analysis. The concentration of total dietary fiber (insoluble dietary fiber + soluble dietary fiber), cellulose (ADF – ADL), insoluble hemicelluloses (NDF – ADF), non-starch polysaccharides (**NSP**; total dietary fiber – ADL), insoluble NSP (NSP – soluble NSP), and non-cellulosic NSP (NSP – cellulose) were calculated for all samples. Total nutrient concentration, on an as-fed basis, was calculated as the sum of ash, AEE, total AA, starch, sugars, oligosaccharides, and total dietary fiber. Values for apparent ileal digestibility (**AID**), apparent cecal digestibility (**ACD**), and apparent total tract digestibility (**ATTD**) of nutrients and energy by pigs fed experimental diets were calculated according to Stein et al. (2007). Values for AID, ACD, and ATTD of nutrients and energy in DDGS, wheat middlings, and soybean hulls were calculated by multiplying the AID, ACD, or ATTD of nutrients and energy in the corn-basal diet by 70.9% to calculate the contribution from the basal diet to the AID, ACD, or ATTD of nutrients and energy in the DDGS, wheat middlings, or soybean hulls test diets.

The ileal, cecal, and total tract flow of nutrients and energy (g or kcal / kg DMI) by pigs fed experimental diets was calculated according to Urriola and Stein (2010). The disappearance of nutrients and energy (g or kcal / kg DMI) in the stomach and small intestine of pigs was calculated by subtracting the flow of nutrients and energy at the ileum from the nutrients and energy in the experimental diets. Cecum disappearance of nutrients and energy was calculated by subtracting the flow of nutrients and energy at the cecum from the flow of nutrients or energy at the ileum. Disappearance of nutrients and energy by pigs in the colon was calculated by subtracting the flow of nutrients and energy at the total tract from the flow of nutrients and

energy at the cecum. The disappearance of nutrients and energy in the stomach, small intestine, cecum, and colon from DDGS, wheat middlings, and soybean hulls was calculated as the difference between the flow of nutrients and energy from 70.9% of the basal corn-soybean meal diet and the 3 diets containing DDGS, wheat middlings, or soybean hulls.

Viscosity of ileal and cecal digesta was calculated using the Rheocalc software (Brookfield Eng. Lab. Inc., Middleboro, MA). The NLREG statistical software (NLREG, Brentwood, TN) was used to report viscosity measurements in terms of the power law equation (Cervantes-Pahm et al., 2013).

Homogeneity of the variance among treatments was confirmed using the UNIVARIATE procedure of SAS. The BOXPLOT procedure of SAS (SAS Inst. Inc., Cary, NC) was used to check for outliers. However, no outliers were identified. Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) using pig and period as the random effects and diet or ingredient as the fixed effect. Means were calculated using the LSMEANS statement in SAS. Differences were evaluated using the PDIFF option. Correlation coefficients among physicochemical characteristics of diets and the AID, ACD, and ATTD of nutrients and energy by pigs fed experimental diets were determined using the CORR procedure (SAS Inst. Inc., Cary, NC). The pig was the experimental unit for all analyses, except that dietary treatment was the experimental unit for correlation analysis. A P-value ≤ 0.05 was used to determine significance among dietary treatments for all outcomes.

RESULTS

All pigs were successfully cannulated at the distal ileum and in the proximal colon at approximately 10 cm distal to the cecocolic junction. Pigs recovered from surgery without

complications and digesta were successfully collected from the cannula in the ileum and in the colon. One pig fed the corn-soybean meal plus soybean hulls diet died in the middle of the adaptation of period 3. Therefore, there were only 7 observations for the corn-soybean meal plus soybean hulls diet.

Apparent Ileal, Cecal, and Total Tract Digestibility

The AID of DM and GE was least ($P \leq 0.05$) in the diet containing soybean hulls and greatest ($P \leq 0.05$) in the basal corn-soybean meal diet, and the diet containing wheat middlings had greater ($P \leq 0.05$) AID of DM and GE than the DDGS diet (Table 7.3). The AID of ADF was greater ($P \leq 0.05$) in the basal diet and the diet containing wheat middlings compared with the diet containing soybean hulls. The AID of NDF was least ($P \leq 0.05$) in the diet containing soybean hulls and greatest ($P \leq 0.05$) in the wheat middlings diet. The AID of soluble dietary fiber was greater ($P \leq 0.05$) in the basal diet compared with diets containing soybean hulls or DDGS. The AID of insoluble dietary fiber, total dietary fiber, NSP, insoluble NSP, and non-cellulosic NSP was greater ($P \leq 0.05$) in basal and wheat middlings diets compared with DDGS and soybean hulls diets, but the AID of cellulose was less ($P \leq 0.05$) in the soybean hulls diet compared with the basal diet. The diet containing wheat middlings had the highest ($P \leq 0.05$) AID of insoluble hemicelluloses compared with the other 3 dietary treatments.

The ACD of DM and GE was least ($P \leq 0.05$) in the diet containing soybean hulls and greatest ($P \leq 0.05$) in the basal corn-soybean meal diet, but the diet containing wheat middlings had greater ($P \leq 0.05$) ACD of DM and GE than the DDGS diet. The ACD of ADF in the basal diet was greater ($P \leq 0.05$) compared with diets containing DDGS or soybean hulls, and the ACD of ADF in the wheat middlings diet was greater ($P \leq 0.05$) compared with the diet containing soybean hulls. The ACD of NDF was greater ($P \leq 0.05$) in the wheat middlings diet

than in all other diets, but the soybean hull diet had less ($P \leq 0.05$) ACD of NDF than all other diets. The basal diet and the wheat middlings diet had the greatest ($P \leq 0.05$) ACD of insoluble dietary fiber, total dietary fiber, NSP, and insoluble NSP, followed by the diet containing DDGS, whereas the soybean hulls diet had the least ($P \leq 0.05$) ACD of these fractions. The basal diet had greater ($P \leq 0.05$) ACD of cellulose than diets containing DDGS or wheat middlings, whereas the ACD of cellulose in the soybean hulls diet was less ($P \leq 0.05$) than in all other diets.

The basal corn-soybean meal diet had the greatest ($P \leq 0.05$) ATTD of DM and GE, and diets containing DDGS or wheat middlings had greater ATTD of DM and GE ($P \leq 0.05$) than the soybean hull diet. With the exception of insoluble hemicelluloses and cellulose, the basal diet had greater ($P \leq 0.05$) ATTD of all dietary fiber components than the other diets but, with a few exceptions, no differences among the other diets were observed. The DE was different ($P \leq 0.05$) among diets and was 3,430, 3,299, 3,218, and 2,948 kcal/kg in the basal diet, the DDGS diet, the wheat middlings diet, and the soybean hull diet, respectively.

Wheat middlings had the greatest ($P \leq 0.05$) AID of DM and GE followed by DDGS and soybean hulls (Table 7.4). The AID of NDF, insoluble dietary fiber, total dietary fiber, insoluble hemicelluloses, NSP, insoluble NSP, and non-cellulosic NSP also was greater ($P \leq 0.05$) in wheat middlings compared with DDGS and soybean hulls.

Wheat middlings also had the greatest ($P \leq 0.05$) ACD of DM, GE, NDF, insoluble dietary fiber, and total dietary fiber, and soybean hulls had the least ($P \leq 0.05$) ACD of these components. The ACD of ADF was greater ($P \leq 0.05$) in DDGS compared with soybean hulls, and the ACD of soluble dietary fiber, insoluble hemicelluloses, NSP, insoluble NSP, and non-cellulosic NSP were greater ($P \leq 0.05$) in wheat middlings compared with DDGS and soybean hulls.

The ATTD of DM and GE were greater ($P \leq 0.05$) in DDGS and wheat middlings compared with soybean hulls, but wheat middlings had the least ($P \leq 0.05$) ATTD of ADF and cellulose compared with DDGS and soybean hulls. The ATTD of soluble dietary fiber was greater ($P \leq 0.05$) in wheat middlings than in soybean hulls, but DDGS had the least ATTD of soluble dietary fiber. Wheat middlings had the greatest ($P \leq 0.05$) ATTD of total dietary fiber, NSP, insoluble NSP, and non-cellulosic NSP compared with DDGS and soybean hulls. The DE was different ($P \leq 0.05$) among ingredients and was 2,975, 2,697, and 1,763 kcal/kg in DDGS, wheat middlings, and soybean hulls, respectively.

Disappearance of Nutrients and Energy in the Stomach and Small intestine, Cecum, and Colon

Disappearance of GE and DM before the end of the ileum was greater ($P \leq 0.05$) in pigs fed the corn-soybean meal basal diet than in pigs fed the other diets, and pigs fed the soybean hull diet had the least ($P \leq 0.05$) disappearance of GE and DM in the stomach and small intestine (Table 7.5). Disappearance of dietary fiber components before the end of the ileum was greater ($P \leq 0.05$) in pigs fed the diet containing wheat middlings, whereas the basal diet had less disappearance of dietary fiber components in the stomach and small intestine compared with the diets containing DDGS or soybean hulls.

The disappearance of soluble dietary fiber in the cecum was greater ($P \leq 0.05$) in the diet containing soybean hulls compared with the basal and the wheat middlings diets, but for all other measured components, no differences in cecal disappearance among diets were observed. The degradation of DM and most dietary fiber components in the colon was greater ($P \leq 0.05$) in the diet containing soybean hulls compared with the other diets, with the exception that pigs fed the diet containing DDGS had the greatest ($P \leq 0.05$) degradation of insoluble hemicelluloses. The

degradation of GE in the large intestine of pigs fed diets containing DDGS or soybean hulls was greater ($P \leq 0.05$) compared with the degradation in the basal diet and the diet containing wheat middlings.

The disappearance of DM and all dietary fiber components before the end of the ileum was greater ($P \leq 0.05$) from wheat middlings compared with DDGS and soybean hulls (Table 7.6). Disappearance of GE in the stomach and small intestine was greater for wheat middlings compared with soybean hulls.

There were no differences among DDGS, wheat middlings, or soybean hulls in the disappearance of DM, GE, or dietary fiber components in the cecum of pigs. However, disappearance of DM and most dietary fiber components in the colon was greater ($P \leq 0.05$) from soybean hulls than from DDGS and wheat middlings, and wheat middlings had the least ($P \leq 0.05$) disappearance of dietary fiber components in the colon. The disappearance of GE in the large intestine of pigs was also less ($P \leq 0.05$) for wheat middlings compared with DDGS and soybean hulls.

Physical Characteristics of Ileal and Cecal Digesta and Feces

The water binding capacity of ileal digesta from pigs fed the diet containing soybean hulls was greater ($P \leq 0.05$) compared with the other 3 diets (Table 7.7). Ileal digesta viscosity was less ($P \leq 0.05$) in pigs fed the diet containing wheat middlings than in digesta from pigs fed diets containing DDGS or soybean hulls. The water binding capacity of cecal digesta from pigs fed the diet containing soybean hulls was greater ($P \leq 0.05$) than in digesta from all other diets, and water binding capacity of cecal digesta from pigs fed the wheat middlings or DDGS diets was greater ($P \leq 0.05$) than in digesta from pigs fed the basal diet. The water binding capacity of feces from pigs fed the wheat middlings diet was greater ($P \leq 0.05$) than that of all other diets,

but pigs fed the basal diet or the soybean hull diets had water binding capacity in feces that was less ($P \leq 0.05$) than in the other diets.

Correlations between Physical Characteristics and Digestibility

A positive correlation between bulk density of experimental diets and ACD of GE (r = 0.88; $P \leq 0.05$) was observed; however, no other correlations between physical characteristics of experimental diets and digestibility were significant. Therefore, only the correlation coefficients between physical characteristics of diets and ACD of nutrients and energy are presented in Table 7.8.

DISCUSSION

Ingredients used in this experiment contained similar concentrations of nutrients and energy as reported by NRC (2012). Oil was not removed from DDGS used in this experiment because DDGS contained 9.89% AEE which is approximately 3 times greater compared with corn (3.27%). Corn contained 13.41% total dietary fiber and DDGS contained 38.72% total dietary fiber, once again, approximately 3 times greater compared with corn. Soybean meal, wheat middlings, and soybean hulls contained 18.80, 37.11, and 67.46% total dietary fiber, respectively.

The ATTD of DM and GE in the corn-soybean meal basal diet and the diet containing DDGS used in the current experiment are in agreement with previous research that used similar corn-soybean meal diets (Urriola and Stein, 2010). The ATTD of DM, GE, insoluble dietary fiber, total dietary fiber, and insoluble NSP for the corn-soybean meal basal diet compared with the other 3 diets is likely the reason for the greater DE that was observed in the corn-soybean meal basal diet compared with the other 3 diets. The DE obtained for experimental diets in the current experiment are in agreement with calculated values (NRC, 2012). The ATTD of soluble

dietary fiber in experimental diets was, on average, 86.5% and this was in agreement with Urriola and Stein (2010). The average ATTD of soluble dietary fiber was 20 percentage units greater compared with the ATTD of insoluble dietary fiber among experimental diets, thus confirming results indicating that soluble dietary fiber is more fermentable by pigs compared with insoluble dietary fiber (Urriola et al., 2010). Due to the differentiation of components of dietary fiber, it was possible to distinguish the digestibility of the different dietary fiber fractions. The ATTD of cellulose by pigs fed the basal diet or the DDGS diet was greater compared with the ATTD of insoluble hemicelluloses, whereas diets containing wheat middlings and soybean hulls had greater ATTD of insoluble hemicelluloses, NSP, insoluble NSP, and non-cellulosic NSP compared with cellulose. It may be speculated that cellulolytic enzymes and bacteria are utilized in ethanol production, and this may render the cellulose in DDGS more susceptible for fermentation in the pig.

The AID, ACD, and ATTD of DM and GE are less in DDGS, wheat middlings, and soybean hulls than in the experimental diets because these ingredients contain more dietary fiber and less starch. The ATTD of total dietary fiber from DDGS was 54.69% in the current experiment, which is in agreement with the average ATTD of total dietary fiber from 8 DDGS sources (49.5%) obtained by Urriola et al. (2010). The ATTD of most dietary fiber fractions were greater in wheat middlings compared with DDGS and soybean hulls; however, the ATTD of GE was not different between wheat middlings and DDGS. This may be explained by the greater concentration of fat in DDGS compared with wheat middlings.

The AID of dietary fiber fractions in diets and ingredients are relatively low and in agreement with data from Bach Knudsen et al. (2013), indicating that the AID of NSP by pigs range from -7 to 40%. The ACD of soluble dietary fiber in diets and ingredients was greater than

the AID of soluble dietary fiber, whereas values for the ACD of insoluble dietary fiber were close to values observed for the AID of insoluble dietary fiber. This observation indicates that mainly soluble dietary fiber is fermented in the cecum. However, the ACD of GE was close to the AID of GE in diets and ingredients, which indicates that fermentation of soluble dietary fiber in the cecum has a low energy contribution. This is likely mostly because the concentration of soluble dietary fiber is low in the diets and ingredients used in the current experiment.

The colon of pigs is the main site for insoluble dietary fiber fermentation as indicated by the greater disappearance of insoluble dietary fiber fractions in the colon compared with the stomach and small intestine, and the cecum. To our knowledge, this is the first time dietary fiber fermentation has been estimated separately in the cecum and in the colon of pigs. The structure of insoluble dietary fiber fractions is much more hydrophobic and crystalline and, therefore, microbial fermentation of insoluble dietary fiber fractions occurs more slowly and requires longer retention time in the colon of pigs compared with soluble dietary fiber (Bach Knudsen and Hansen, 1991; Wilfart et al., 2007). Differences in size and microbial populations of the cecum and the colon also may influence dietary fiber fermentation. The cecum and colon have been reported to be 0.3 and 1.75% of the empty BW of pigs, respectively, and this difference in size indicates the importance of the colon to dietary fiber fermentation (Agyekum et al., 2012). Total viable counts of anaerobic bacteria increase from 10^9 viable counts in the distal ileum to 10^{12} viable counts in pig feces and it is expected that viable counts in the cecum is between the values in the ileum and the colon (Jensen and Jørgensen, 1994).

Antithetical to our hypothesis, water binding capacity and bulk density of experimental diets were not correlated with ileal, cecal, or total tract digestibility of nutrients and energy, with the exception that bulk density was positively correlated with ACD of GE. Serena et al. (2008)

also were unable to correlate physicochemical properties of dietary fiber and digestibility of energy in sows.

Overall, ATTD of insoluble dietary fiber in wheat middlings was greater than in DDGS and soybean hulls, but the ATTD of cellulose was less in wheat middlings. However, the energy contribution from cellulose fermentation in wheat middlings is relatively low because wheat middlings has a low concentration of cellulose. Soybean hulls had the greatest concentration of total dietary fiber and the least concentrations of starch and fat and, as a result, fermentation of dietary fiber contributes the majority of the DE in soybean hulls. The energy contribution from dietary fiber fermentation is much less compared with the energy contribution from enzymatic digestion of starch and fat, which is the reason soybean hulls had the least DE compared with DDGS and wheat middlings (Nelson and Cox, 2008). The ATTD of soluble dietary fiber in DDGS in the current experiment was less compared with the ATTD of soluble dietary fiber in 8 sources of DDGS determined by Urriola et al. (2010). The ATTD of soluble dietary fiber was also less than the ATTD of insoluble dietary fiber in DDGS and these differences may be attributed to the low concentration of soluble dietary fiber in DDGS as well as differences in ethanol production today compared with several years ago. A greater variety of cellulolytic enzymes and bacteria are utilized in ethanol plants today and it is also likely that the efficacy of cellulose degradation by ethanol plants has been improved; therefore, the dietary fiber fractions remaining in DDGS may be different.

CONCLUSION

In contrast to our hypothesis, the physical characteristics of dietary fiber in experimental diets were not correlated with the digestibility of energy or dietary fiber fractions in experimental diets. Soluble dietary fiber is mostly fermented in the cecum of pigs, but this does not contribute

a great amount of energy supply to the pig due to the low concentration of soluble dietary fiber in most swine diets. Insoluble dietary fiber is mostly fermented in the colon of pigs and contributes a significant energy supply to pigs fed diets containing DDGS, wheat middlings, or soybean hulls because the concentration of insoluble dietary fiber is greater when these co-products are added to a corn-soybean meal diet. Dietary fiber fractions in wheat middlings are more fermentable compared with the dietary fiber fractions in DDGS and soybean hulls; however, the DE in DDGS is similar to that of wheat middlings because of the greater concentration of fat in DDGS compared with wheat middlings. The DE in soybean hulls is mostly attributed to insoluble dietary fiber fermentation in the colon, and this is the reason the DE in soybean hulls is less than in DDGS or wheat middlings.

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TABLES

Table 7.1. Chemical and physical composition of feed ingredients

Item	Corn	Soybean meal	DDGS[1]	Wheat middlings	Soybean hulls
GE, kcal/kg	3,822	4,204	4,518	4,034	3,692
DM, %	85.89	88.76	85.18	87.38	87.68
Ash, %	1.06	6.54	5.13	4.81	4.18
AEE[1], %	3.27	1.75	9.89	4.24	1.87
Indispensable AA, %					
Arg	0.34	3.47	1.21	1.08	0.37
His	0.21	1.23	0.71	0.44	0.23
Ile	0.27	2.32	1.08	0.55	0.34
Leu	0.86	3.69	2.94	1.02	0.58
Lys	0.27	3.01	0.92	0.71	0.62
Met	0.15	0.66	0.49	0.23	0.10
Phe	0.35	2.40	1.28	0.66	0.32
Thr	0.25	1.74	0.98	0.49	0.29
Trp	0.05	0.74	0.23	0.20	0.06
Val	0.35	2.43	1.37	0.79	0.41
Dispensable AA, %					
Ala	0.53	2.04	1.76	0.75	0.39
Asp	0.48	5.24	1.65	1.10	0.74
Cys	0.15	0.61	0.45	0.32	0.15
Glu	1.29	8.45	3.29	3.08	0.93
Gly	0.30	2.05	1.17	0.85	0.79
Pro	0.58	2.41	1.92	1.04	0.50
Ser	0.31	1.88	1.08	0.56	0.42
Tyr	0.20	1.68	0.95	0.39	0.32
Total AA, %	7.05	46.18	23.62	14.39	8.24
Carbohydrates, %					
Fructose	0.16	0.10	0.08	0.67	0.24
Glucose	0.36	0.08	0.39	0.91	0.26
Sucrose	1.09	6.33	0.04	1.38	0.28
Maltose	0.31	0.01	0.30	0.11	0.07
Raffinose	0.13	0.94	0.03	1.06	0.08
Stachyose	0.01	4.10	0.02	0.02	0.23
Verbascose	N.D.[2]	0.12	N.D.	N.D.	0.01
Starch	53.93	2.01	2.74	22.20	7.49
ADF	2.53	7.38	17.78	9.76	40.28
NDF	8.07	7.51	36.99	33.16	55.37
ADL	0.47	0.39	4.83	3.14	1.94
Soluble dietary fiber	1.57	1.83	1.74	2.64	5.31
Insoluble dietary fiber	11.84	16.97	36.98	34.47	62.15

Table 7.1. (cont.)

Total dietary fiber[3]	13.41	18.80	38.72	37.11	67.46
Cellulose[4]	2.06	6.99	12.95	6.62	38.34
Insoluble hemicelluloses[5]	5.54	0.13	19.21	23.40	15.09
Non-starch polysaccharides[6]	12.94	18.41	33.89	33.97	65.52
Insoluble non-starch polysaccharides[7]	11.37	16.58	32.15	31.33	60.21
Non-cellulosic non-starch polysaccharides[8]	10.88	11.42	20.94	27.35	27.18
Total[9], %	80.78	86.96	80.96	86.90	90.41
DE[10], kcal/kg	3,484	3,590	2,635	2,470	1,334
Bulk density, g/L	559.75	644.93	442.65	356.57	435.63
Water binding capacity, g/g	1.07	2.81	2.02	2.99	4.22

[1]DDGS = distillers dried grains with solublesAEE = acid hydrolyzed ether extract.

[2]N.D. = not detectable.

[3]Total dietary fiber = soluble dietary fiber + insoluble dietary fiber.

[4]Cellulose = ADF – ADL.

[5]Insoluble hemicelluloses = NDF – ADF.

[6]Non-starch polysaccharides = total dietary fiber – ADL.

[7]Insoluble non-starch polysaccharides = non-starch polysaccharides – soluble dietary fiber.

[8]Non-cellulosic non-starch polysaccharides = non-starch polysaccharides – cellulose.

[9]Total = ash + AEE + total AA + starch + sugars + oligosaccharides + total dietary fiber.

[10]DE (kcal / kg of DM) = 1,161 + (0.749 × GE) – (4.3 × ash) – (4.1 × NDF) (Noblet and Perez, 1993).

Table 7.2. Ingredient composition, analyzed nutrients and energy, and physical characteristics of experimental diets

Item	Basal	Basal + DDGS[1]	Basal + wheat middlings	Basal + soybean hulls
Ingredient, %				
Corn	64.50	45.15	45.15	45.15
Soybean meal	32.25	22.58	22.58	22.58
DDGS	-	29.10	-	-
Wheat middlings	-	-	29.10	-
Soybean hulls	-	-	-	29.10
Limestone	0.85	0.85	0.85	0.85
Dicalcium P	1.15	1.15	1.15	1.15
Lysine HCl	0.25	0.18	0.18	0.18
Titanium dioxide	0.40	0.40	0.40	0.40
Salt	0.40	0.40	0.40	0.40
Vitamin-mineral premix[2]	0.20	0.20	0.20	0.20
Analyzed composition				
GE, kcal/kg	3,831	3,968	3,862	3,745
DM, %	87.22	87.04	87.44	87.59
Ash, %	5.76	6.01	6.07	6.12
AEE[3], %	3.15	4.97	3.33	2.52
Indispensable AA, %				
Arg	1.38	1.24	1.24	1.01
His	0.55	0.57	0.50	0.43
Ile	0.94	0.91	0.80	0.73
Leu	1.81	2.05	1.54	1.39
Lys	1.37	1.15	1.21	1.13
Met	0.29	0.33	0.27	0.22
Phe	1.03	1.06	0.89	0.78
Thr	0.76	0.78	0.65	0.58
Trp	0.28	0.26	0.25	0.21
Val	1.04	1.07	0.93	0.81
Dispensable AA, %				
Ala	1.02	1.20	0.93	0.81
Asp	2.09	1.87	1.74	1.61
Cys	0.30	0.33	0.29	0.25
Glu	3.67	3.50	3.45	2.77
Gly	0.86	0.90	0.84	0.83
Pro	1.23	1.38	1.13	0.98
Ser	0.85	0.88	0.74	0.69
Tyr	0.68	0.71	0.57	0.54
Total AA, %	20.33	20.34	18.14	16.11
Carbohydrates, %				

Table 7.2. (cont.)

Fructose	0.20	0.12	0.37	0.35
Glucose	0.26	0.37	0.60	0.40
Sucrose	2.69	1.80	2.22	1.78
Maltose	0.16	0.18	0.23	0.33
Raffinose	0.39	0.26	0.59	0.28
Stachyose	1.29	0.77	0.91	0.90
Verbascose	0.03	0.02	0.02	0.02
Starch	35.09	27.64	32.51	28.83
ADF	3.93	6.43	5.18	15.00
NDF	7.68	16.30	15.01	21.72
ADL	0.45	1.11	1.21	0.88
Soluble dietary fiber	1.49	1.37	2.02	2.21
Insoluble dietary fiber	12.14	19.00	19.06	26.35
Total dietary fiber[4]	13.63	20.37	21.08	28.56
Cellulose[5]	3.48	5.32	3.97	14.12
Insoluble hemicelluloses[6]	3.75	9.87	9.83	6.72
Non-starch polysaccharides[7]	13.18	19.26	19.87	27.68
Insoluble non-starch polysaccharides[8]	11.69	17.89	18.66	26.80
Non-cellulosic non-starch polysaccharides[9]	9.70	13.94	15.90	13.56
Total[10], %	82.98	82.85	86.07	86.20
DE[11], kcal/kg	3,393	3,429	3,270	2,959
Bulk density, g/L	638.68	584.13	533.40	574.07
Water binding capacity, g/g	1.47	1.58	1.84	2.21

[1]DDGS = distillers dried grains with solubles.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 126 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.25 mg as sodium selenite and selenium yeast; and Zn, 124.9 mg as zinc sulfate.

[3]AEE = acid hydrolyzed ether extract.

[4]Total dietary fiber = soluble dietary fiber + insoluble dietary fiber.

[5]Cellulose = ADF – ADL.

[6]Insoluble hemicelluloses = NDF – ADF.

[7]Non-starch polysaccharides = total dietary fiber – ADL.

[8]Insoluble non-starch polysaccharides = non-starch polysaccharides – soluble dietary fiber.

[9]Non-cellulosic non-starch polysaccharides = non-starch polysaccharides – cellulose.

[10]Total = ash + AEE + total AA + starch + sugars + oligosaccharides + total dietary fiber.

[11]DE calculated from NRC (2012).

Table 7.3. Apparent ileal, cecal, and total tract digestibility of dry matter, energy, and nutrients in experimental diets

Item	Basal	Basal + DDGS	Basal + wheat middlings	Basal + soybean hulls	SEM	*P*-value
Apparent ileal digestibility, %						
DM	72.6[a]	56.0[c]	62.8[b]	48.9[d]	1.4	< 0.001
GE	74.6[a]	60.8[c]	65.5[b]	54.7[d]	1.3	< 0.001
ADF	29.5[a]	20.6[ab]	24.5[a]	10.7[b]	4.9	0.014
NDF	26.0[b]	24.1[b]	38.5[a]	15.0[c]	4.2	< 0.001
Soluble dietary fiber	43.9[a]	5.3[c]	33.7[ab]	17.8[bc]	7.6	0.002
Insoluble dietary fiber	41.2[a]	23.3[b]	43.0[a]	17.6[b]	3.6	< 0.001
Total dietary fiber	41.5[a]	22.1[b]	42.0[a]	17.6[b]	3.5	< 0.001
Cellulose	30.2[a]	17.4[bc]	24.9[ab]	9.9[c]	5.1	0.009
Insoluble hemicelluloses	22.5[b]	26.4[b]	46.0[a]	25.4[b]	4.0	< 0.001
Non-starch polysaccharides	42.0[a]	21.4[b]	43.2[a]	17.5[b]	3.6	< 0.001
Insoluble non-starch polysaccharides	41.9[a]	22.6[b]	44.3[a]	17.4[b]	3.7	< 0.001
Non-cellulosic non-starch polysaccharides	46.5[a]	22.8[b]	47.9[a]	24.9[b]	4.0	< 0.001
Apparent cecal digestibility, %						
DM	75.7[a]	61.3[c]	68.0[b]	53.3[d]	1.4	< 0.001
GE	74.6[a]	61.2[c]	67.6[b]	55.7[d]	1.5	< 0.001
ADF	27.0[a]	21.9[ab]	19.3[b]	8.6[c]	3.1	< 0.001
NDF	26.6[b]	23.3[b]	37.6[a]	16.0[c]	2.7	< 0.001
Soluble dietary fiber	67.6	62.1	66.7	67.5	4.5	0.637
Insoluble dietary fiber	48.7[a]	31.9[b]	47.6[a]	22.7[c]	2.2	< 0.001
Total dietary fiber	50.7[a]	33.9[b]	49.4[a]	26.1[c]	2.3	< 0.001
Cellulose	30.4[a]	20.0[b]	21.5[b]	7.8[c]	3.2	< 0.001
Insoluble hemicelluloses	26.1[bc]	24.3[c]	47.4[a]	32.2[b]	2.9	< 0.001
Non-starch polysaccharides	52.5[a]	34.1[b]	51.7[a]	26.4[c]	2.3	< 0.001
Insoluble non-starch polysaccharides	50.6[a]	32.0[b]	50.1[a]	22.9[c]	2.2	< 0.001
Non-cellulosic non-starch polysaccharides	60.4[a]	39.5[c]	59.4[a]	45.3[b]	2.5	< 0.001
Apparent total tract digestibility, %						
DM	89.1[a]	82.8[b]	82.9[b]	78.3[c]	0.7	< 0.001
GE	89.5[a]	83.1[b]	83.3[b]	78.7[c]	0.7	< 0.001
ADF	66.3[a]	67.2[a]	40.3[c]	56.9[b]	3.7	< 0.001
NDF	63.9	66.2	61.9	63.5	2.1	0.345
Soluble dietary fiber	86.6	84.1	90.1	85.4	3.8	0.122
Insoluble dietary fiber	71.2[a]	64.1[b]	64.7[b]	64.0[b]	1.9	0.001

Table 7.3. (cont.)

Total dietary fiber	72.9[a]	65.5[b]	67.1[b]	65.7[b]	1.8	< 0.001
Cellulose	72.2[a]	69.8[a]	52.7[c]	60.1[b]	3.3	< 0.001
Insoluble hemicelluloses	61.3[b]	65.6[b]	73.3[a]	78.0[a]	2.1	< 0.001
Non-starch polysaccharides	74.7[a]	66.1[c]	71.3[ab]	67.6[bc]	1.9	< 0.001
Insoluble non-starch polysaccharides	73.1[a]	64.7[c]	69.1[b]	66.0[bc]	1.9	0.001
Non-cellulosic non-starch polysaccharides	75.5[a]	64.7[b]	75.9[a]	75.3[a]	1.7	< 0.001
DE, kcal/kg	3,430[a]	3,299[b]	3,218[c]	2,948[d]	27	< 0.001

[a-d]Values within a row lacking a common superscript letter are different ($P < 0.05$).

Table 7.4. Apparent ileal, cecal, and total tract digestibility of dry matter, energy, and nutrients in distillers dried grains with solubles (**DDGS**), wheat middlings, and soybean hulls

Item	DDGS	Wheat middlings	Soybean hulls	SEM	*P*-value
Apparent ileal digestibility, %					
DM	15.7[b]	39.0[a]	-8.1[c]	5.4	< 0.001
GE	29.2[b]	42.4[a]	1.3[c]	4.7	< 0.001
ADF	9.3	15.6	6.7	8.7	0.657
NDF	22.7[b]	44.9[a]	11.0[b]	7.1	0.001
Soluble dietary fiber	-74.3[b]	28.4[a]	-3.1[a]	18.8	0.001
Insoluble dietary fiber	7.7[b]	45.9[a]	5.3[b]	7.0	< 0.001
Total dietary fiber	4.1[b]	44.6[a]	4.5[b]	7.0	< 0.001
Cellulose	4.5	12.2	5.5	10.3	0.752
Insoluble hemicelluloses	35.1[b]	57.2[a]	24.7[b]	7.1	0.001
Non-starch polysaccharides	1.5[b]	46.6[a]	3.8[b]	7.4	< 0.001
Insoluble non-starch polysaccharides	5.5[b]	48.2[a]	4.5[b]	7.4	< 0.001
Non-cellulosic non-starch polysaccharides	-0.5[b]	55.3[a]	2.1[b]	8.3	< 0.001
Apparent cecal digestibility, %					
DM	24.5[b]	47.7[a]	-2.3[c]	5.1	< 0.001
GE	28.0[b]	47.1[a]	2.1[c]	5.4	< 0.001
ADF	11.7[a]	7.2[ab]	3.2[b]	4.2	0.023
NDF	21.1[b]	42.4[a]	11.3[c]	3.3	< 0.001
Soluble dietary fiber	26.8[b]	81.8[a]	50.7[b]	10.7	0.001
Insoluble dietary fiber	17.1[b]	47.8[a]	9.3[c]	3.4	< 0.001
Total dietary fiber	17.6[b]	50.2[a]	13.0[b]	3.5	< 0.001
Cellulose	7.2	4.1	2.1	4.9	0.433
Insoluble hemicelluloses	29.9[b]	57.8[a]	32.5[b]	3.9	< 0.001
Non-starch polysaccharides	16.7[b]	53.5[a]	12.6[b]	3.6	< 0.001
Insoluble non-starch polysaccharides	16.0[b]	51.2[a]	9.0[b]	3.6	< 0.001
Non-cellulosic non-starch polysaccharides	22.0[b]	66.0[a]	24.9[b]	4.9	< 0.001
Apparent total tract digestibility, %					
DM	68.6[a]	68.4[a]	52.7[b]	2.8	< 0.001
GE	64.8[a]	66.3[a]	46.9[b]	3.0	< 0.001
ADF	46.7[a]	8.2[b]	56.6[a]	5.8	< 0.001
NDF	66.6	60.2	63.9	3.5	0.209
Soluble dietary fiber	46.4[c]	116.9[a]	63.5[b]	9.4	< 0.001
Insoluble dietary fiber	55.1	61.6	59.1	3.7	0.069
Total dietary fiber	54.7[b]	65.5[a]	59.4[b]	3.6	0.002
Cellulose	50.1[a]	15.9[b]	59.6[a]	5.7	< 0.001
Insoluble hemicelluloses	84.9	81.9	82.3	2.5	0.535

Table 7.4. (cont.)

Non-starch polysaccharides	57.2[b]	72.4[a]	61.4[b]	3.7	0.001
Insoluble non-starch polysaccharides	57.7[b]	68.7[a]	61.2[b]	3.9	0.010
Non-cellulosic non-starch polysaccharides	61.6[b]	86.1[a]	63.3[b]	3.6	< 0.001
DE, kcal/kg	2,975[a]	2,697[b]	1,763[c]	116	< 0.001

[a-c]Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

Table 7.5. Disappearance of dietary dry matter, energy, and nutrients (g or kcal/kg of DMI) in the stomach and small intestine, cecum, and colon of pigs fed experimental diets

Item	Basal	Basal + DDGS[1]	Basal + wheat middlings	Basal + soybean hulls	SEM	*P*-value
Stomach and small intestine						
DM	633.4[a]	487.6[c]	548.8[b]	428.0[d]	11.9	< 0.001
GE	3,276[a]	2,769[b]	2,894[b]	2,337[c]	57	< 0.001
ADF	13.1	15.2	14.4	19.4	3.2	0.328
NDF	22.1[c]	45.2[b]	66.0[a]	38.0[b]	7.2	< 0.001
Soluble dietary fiber	7.5[a]	0.9[b]	7.7[a]	4.5[ab]	1.5	0.002
Insoluble dietary fiber	57.2[b]	50.9[b]	93.9[a]	54.0[b]	7.7	< 0.001
Total dietary fiber	64.6[b]	51.8[b]	101.6[a]	58.4[b]	8.2	< 0.001
Cellulose	11.9	10.7	11.2	17.2	2.7	0.153
Insoluble hemicelluloses	9.2[d]	23.0[b]	51.8[a]	18.9[c]	4.2	< 0.001
Non-starch polysaccharides	63.4[b]	47.3[b]	98.3[a]	56.0[b]	7.9	< 0.001
Insoluble non-starch polysaccharides	55.9[b]	46.4[b]	90.6[a]	51.6[b]	7.4	< 0.001
Non-cellulosic non-starch polysaccharides	51.6[b]	36.5[c]	87.3[a]	38.7[bc]	6.2	< 0.001
Cecum						
DM	30.8	45.8	49.5	43.6	15.9	0.690
GE	26.4	18.5	116.5	72.2	73.8	0.548
ADF	-1.0	1.0	-3.0	-2.9	3.1	0.685
NDF	0.3	-1.3	-2.1	1.8	5.8	0.953
Soluble dietary fiber	4.0[c]	8.9[ab]	7.6[bc]	12.7[a]	2.0	0.012
Insoluble dietary fiber	10.6	18.9	10.3	16.4	8.0	0.720
Total dietary fiber	14.6	27.8	17.9	29.0	8.3	0.389
Cellulose	0.3	1.6	-1.3	-2.5	2.7	0.646
Insoluble hemicelluloses	1.5	-2.4	1.1	4.7	3.2	0.385
Non-starch polysaccharides	15.8	28.4	19.5	29.4	8.7	0.420
Insoluble non-starch polysaccharides	11.9	19.5	11.9	16.8	7.8	0.769
Non-cellulosic non-starch polysaccharides	15.2	26.9	20.5	31.8	6.9	0.095
Colon						
DM	114.8[c]	187.6[b]	128.8[c]	217.3[a]	11.4	< 0.001
GE	647[b]	999[a]	687[b]	977[a]	61	< 0.001
ADF	17.5[c]	33.5[b]	12.1[c]	80.7[a]	4.5	< 0.001
NDF	33.1[c]	80.2[b]	41.6[c]	116.6[a]	6.3	< 0.001
Soluble dietary fiber	3.3	3.5	5.4	4.5	1.0	0.106
Insoluble dietary fiber	31.3[c]	70.3[b]	36.9[c]	123.2[a]	6.7	< 0.001
Total dietary fiber	34.7[c]	73.9[b]	42.4[c]	128.0[a]	7.3	< 0.001
Cellulose	16.4[c]	30.4[b]	13.8[c]	81.9[a]	4.3	< 0.001

Table 7.5. (cont.)

Insoluble hemicelluloses	15.3[c]	46.6[a]	29.3[b]	35.5[b]	3.1	< 0.001
Non-starch polysaccharides	33.6[c]	70.8[b]	44.1[c]	129.2[a]	7.1	< 0.001
Insoluble non-starch polysaccharides	30.2[c]	67.3[b]	38.6[c]	124.4[a]	6.6	< 0.001
Non-cellulosic non-starch polysaccharides	17.0[c]	40.3[a]	30.1[b]	46.8[a]	4.7	< 0.001

[1]DDGS = distillers dried grains with solubles.

[a-d]Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

Table 7.6. Disappearance of dry matter, energy, and nutrients (g or kcal/kg of DMI) from distillers dried grains with solubles (**DDGS**), wheat middlings, and soybean hulls in the stomach and small intestine, cecum, and colon of pigs

Item	DDGS	Wheat middlings	Soybean hulls	SEM	*P*-value
Stomach and small intestine					
DM	430.8[b]	549.3[a]	428.0[b]	32.1	0.015
GE	2,472[ab]	2,896[a]	2,333[b]	164	0.050
ADF	14.0	14.4	19.6	3.3	0.289
NDF	43.2[b]	66.2[a]	38.3[b]	7.7	0.006
Soluble dietary fiber	0.3[b]	7.8[a]	4.6[a]	1.5	0.002
Insoluble dietary fiber	45.8[b]	94.2[a]	54.3[b]	7.9	< 0.001
Total dietary fiber	46.0[b]	101.9[a]	58.7[b]	8.3	< 0.001
Cellulose	9.6	11.2	17.3	2.8	0.071
Insoluble hemicelluloses	29.2[b]	51.9[a]	19.0[c]	4.7	< 0.001
Non-starch polysaccharides	41.6[b]	98.6[a]	56.4[b]	8.0	< 0.001
Insoluble non-starch polysaccharides	41.3[b]	90.9[a]	51.9[b]	7.5	< 0.001
Non-cellulosic non-starch polysaccharides	32.0[b]	87.5[a]	39.1[b]	6.5	< 0.001
Cecum					
DM	21.7	26.1	19.5	17.8	0.942
GE	-15.6	87.5	38.9	83.3	0.523
ADF	1.7	-2.2	-2.1	3.4	0.549
NDF	-1.4	-2.3	1.8	6.5	0.871
Soluble dietary fiber	6.0	4.7	9.7	2.1	0.141
Insoluble dietary fiber	11.7	3.0	9.1	9.0	0.699
Total dietary fiber	17.7	7.7	18.9	9.9	0.576
Cellulose	1.4	-1.5	-2.5	3.6	0.533
Insoluble hemicelluloses	-3.4	0.3	4.0	3.7	0.241
Non-starch polysaccharides	17.4	8.3	18.3	9.8	0.623
Insoluble non-starch polysaccharides	11.4	3.7	8.6	8.8	0.746
Non-cellulosic non-starch polysaccharides	16.1	9.6	20.9	7.7	0.361
Colon					
DM	108.5[b]	50.4[c]	137.0[a]	11.8	< 0.001
GE	563[a]	248[b]	530[a]	62	< 0.001
ADF	21.3[b]	-0.1[c]	68.3[a]	4.9	< 0.001
NDF	57.2[b]	18.4[c]	93.2[a]	6.9	< 0.001
Soluble dietary fiber	1.2	3.1	2.1	1.0	0.219
Insoluble dietary fiber	48.4[b]	15.0[c]	101.0[a]	7.4	< 0.001
Total dietary fiber	49.5[b]	18.1[c]	103.3[a]	8.0	< 0.001

Table 7.6. (cont.)

Cellulose	19.1[b]	2.4[c]	70.4[a]	5.0	< 0.001
Insoluble hemicelluloses	36.0[a]	18.4[b]	24.6[b]	3.5	0.002
Non-starch polysaccharides	47.3[b]	20.6[c]	105.4[a]	8.0	< 0.001
Insoluble non-starch polysaccharides	46.1[b]	17.4[c]	103.0[a]	7.4	< 0.001
Non-cellulosic non-starch polysaccharides	27.9[a]	18.5[b]	35.0[a]	5.5	0.003

[a-c]Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

Table 7.7. Viscosity of ileal and cecal digesta and water binding capacity of ileal and cecal digesta and feces from pigs fed experimental diets

Item	Basal	Basal + DDGS[1]	Basal + wheat middlings	Basal + soybean hulls	SEM	*P*-value
Ileal digesta						
Water binding capacity, g/g	2.95[b]	3.12[b]	2.81[b]	3.82[a]	0.32	< 0.001
Viscosity						
Constant, cP	15,675[ab]	19,164[a]	6,361[b]	20,516[a]	4,218	0.044
Exponent	-1.21	-1.38	-1.01	-1.40	0.14	0.125
R^2	0.92	0.99	0.91	0.96	-	-
Cecal digesta						
Water binding capacity, g/g	1.71[c]	2.03[b]	2.23[b]	2.73[a]	0.11	< 0.001
Viscosity						
Constant, cP	7,362	8,203	4,735	14,822	3,405	0.134
Exponent	-0.91	-0.98	-0.92	-1.19	0.14	0.232
R^2	0.96	0.98	0.96	0.99	-	-
Feces						
Water binding capacity, g/g	2.09[c]	2.65[b]	3.07[a]	2.21[c]	0.06	< 0.001

[a-c]Values within a row lacking a common superscript letter are different ($P \leq 0.05$).

Table 7.8. Correlation coefficients[1] between physical characteristics of experimental diets and apparent cecal digestibility (**ACD**) of dry matter, energy, and dietary fiber fractions and physical characteristics of cecal digesta from pigs fed experimental diets

	Correlation coefficient							
Cecal digesta measurement	ACD of DM, %	ACD of GE, %	ACD of soluble dietary fiber, %	ACD of insoluble dietary fiber, %	ACD of total dietary fiber, %	ACD of non-starch polysaccharides, %	Water binding capacity, g/g	Viscosity, cP
Physical characteristic								
Water binding capacity	-0.64	-0.61	-0.31	-0.70	-0.68	-0.66	0.37	0.38
Bulk density	0.87	0.88*	0.86	0.61	0.65	0.65	-0.86	0.48

[1]Correlation coefficients were determined between all variables, but the table has been reduced for brevity.
*$P \leq 0.050$ ** $P < 0.01$.

CHAPTER 8: CONCLUDING REMARKS

The overall focus of this dissertation was to evaluate effects of feeding diets with greater concentrations of dietary fiber to pigs. Distillers dried grains with solubles (**DDGS**), wheat middlings, wheat bran, and soybean hulls were the fibrous co-products that were added to corn-soybean meal-based pig diets because these co-products are readily available and typically included in U.S. swine diets. Three major factors necessary to better understand the energy supply and utilization by pigs fed high fiber diets were previously outlined and identified, and these were focused on throughout the dissertation to achieve the goal of developing strategies to increase dietary fiber fermentation and, subsequently, energy supply to pigs.

High fiber diets, created by addition of DDGS and wheat middlings at the expense of corn and soybean meal, have decreased concentrations of NE. Swine nutritionists have two options: 1) increase NE by adding fat to the diet, or 2) rely on the pig to increase feed intake to meet its energy requirement. Option 2 was utilized in this dissertation, and it was determined that ADFI of weanling pigs fed high fiber diets decreased by about 5%, which led to a 3.6% reduction in BW at the end of the nursery period compared with weanling pigs fed low fiber diets. Once pigs entered the grower phase however, ADFI of high fiber-fed pigs increased 4.3% compared with low fiber-fed pigs, which led to a 6% increase in ADG of high fiber-fed pigs. This observation of compensatory gain is remarkable and exciting and leads to the conclusion that the weanling pig is not capable of compensating for lower dietary NE through increased feed intake due to gut fill associated with the lower bulk density of the high fiber diet. Rather, the growing-finishing pig that has been adapted to the high fiber diet since weaning is capable of compensating for lower dietary NE through increased feed intake, and gut fill is not a hindrance. Therefore, it is concluded that pigs require a certain period of time to allow their gastrointestinal

tract to adapt to the bulk of high fiber diets and this certainly will increase co-product utilization in swine diets, leading to the overall goal of increasing the sustainability of swine production.

On the other hand, high fiber-fed pigs adapted by increasing the weight of the large intestine, and this reduced dressing percentage of the carcass. Most pork producers are paid on a carcass basis and, therefore, the price paid to producers for high fiber-fed pigs will be less. Producers must account for this plus the increased feed intake that will occur when they reduce diet costs by increasing inclusion of fibrous co-products. Further research is, therefore, necessary to determine a time period of withdrawal of the high fiber diet prior to harvest to decrease the weight of the large intestine in order to maintain dressing percentage.

Intestinal concentrations of VFA were greater in low fiber-fed pigs compared with high fiber fed pigs. These results, however, may be interpreted multiple ways. Taken as presented in this dissertation, it is concluded that a corn-soybean meal-based diet is more fermentable compared with a corn-soybean meal-DDGS-wheat middlings based diet. This conclusion is further strengthened by the fact that apparent total tract digestibility (**ATTD**) of ADF and NDF were both greater in low fiber diets compared with high fiber diets. However, the intestinal concentrations of VFA also may be interpreted such that high fiber-fed pigs had greater intestinal VFA absorption compared with low fiber-fed pigs. This interpretation was addressed by analyzing tissue samples for relative gene expression of monocarboxylate transporter – 1 (***MCT1***) and basigin (***CD147***) because these are VFA transporters, and it has been indicated that increased intestinal concentrations of VFA correspond with increased abundance of VFA transporters. Finishing pigs fed high fiber diets did, indeed, have increased relative expression of *MCT1* and *CD147* in the cecum, and this may be the reason for the lower concentration of VFA in the cecum of finishing pigs fed high fiber diets. To further complicate interpretation, feed

intake and diet digestibility can be used to calculate fecal output and, therefore, express VFA concentration in feces on a g per d basis. Results from this calculation indicate that high fiber-fed pigs have a much greater concentration of VFA in feces per d compared with low fiber-fed pigs because high fiber-fed pigs had a greater feed intake and lower digestibility and, therefore, more fermentable substrate. Also, antithetical with the interpretation is that increased dietary fiber will decrease passage rate. Therefore, caution is warranted when analyzing and interpreting VFA data. It is suggested that future experiments concerning VFA may be best presented as a proportion of the indigestible marker included in the diet. This will remove the confounding effects that feed intake, diet digestibility, and passage rate on intestinal VFA concentration.

Heat production (**HP**) of pigs fed increasing concentrations of wheat bran decreased. This was not expected because it was understood that increased dietary fiber would lead to increased fermentation and, therefore, result in greater HP. However, from what we have learned, the fermentability of wheat bran is low and increased insoluble dietary fiber decreases passage rate, which decreases the amount of time microbes have to ferment dietary fiber. The ATTD of nutrients and energy also decreased, which decreased overall metabolism, thereby decreased HP of pigs fed increasing concentrations of wheat bran was observed.

As pigs increased in age and BW, along with time fed a high fiber diet, high fiber-fed pigs were just as efficient as low fiber-fed pigs. We speculated that perhaps high fiber-fed pigs increased energy supply through increased dietary fiber fermentation as pigs adapted over time. However, this was not the case because the pigs fed low or high fiber diets both increased apparent ileal digestibility (**AID**) of starch, which led to slight increases in DE and ME over a period of 12 wks. Therefore, it is concluded that pigs merely adapt to a high fiber diet by increased size of the gastrointestinal tract to enable the pig to increase feed intake of a bulky diet.

This research also indicates that a 5 d adaptation period is sufficient to determine concentrations of DE and ME in high fiber diets fed to pigs.

Addition of a *Bacillus*-based direct-fed microbial (**DFM**) to low or high fiber diets improved G:F of nursery pigs. In contrast with our hypothesis, this was not caused by increased intestinal concentrations of VFA. Relative gene expression of glucagon-like peptide-2 receptor (***GLP-2R***) increased in liver tissue of nursery pigs fed DFM-containing diets and, therefore, it is speculated that DFM increased the amount of *GLP-2* in circulation, which may lead to improved intestinal growth and permeability. However, further research is necessary to elucidate the mode of action by which DFM improved nursery pig G:F.

The cecum of pigs is sometimes thought of like a rumen in that it is the fermentation chamber of pigs. This thought was confirmed because about 25 g of total dietary fiber per kg DMI was degraded in the cecum compared with 81 g total dietary fiber degraded per kg DMI in the colon of pigs fed high fiber diets containing DDGS, wheat middlings, or soybean hulls. The cecum of pigs is only about 0.3% of empty BW of pigs compared with the colon, which is 1.75% of empty BW. It is quite remarkable that 25 g of total dietary fiber may be degraded in a much smaller organ compared with the larger colon.

Finally, it was determined that water binding capacity and bulk density of diets containing corn, soybean meal, DDGS, wheat middlings, and soybean hulls were not correlated with the digestibility of nutrients and energy by pigs. Therefore, it appears that physicochemical characteristics of dietary fiber may not be good predictors of dietary fiber fermentation although the physicochemical characteristics of dietary fiber may still be useful to characterize dietary fiber as well as serve as a potential aid in determining the feed intake capacity in nursery pigs fed high-fiber diets.

Collectively, this dissertation has substantially increased the understanding of energy utilization in high and low fiber diets fed to pigs. Importance must be placed on 3 key findings: 1) the pigs ability to adapt to a high fiber diet by increasing the size of the gastrointestinal tract, enabling greater feed intake to meet its energy requirement is remarkable, but 2) unfortunately the pig is unable to increase dietary fiber fermentation through adaptation; and 3) intestinal concentrations of VFA were greater in pigs fed low-fiber diets compared with high-fiber diets, but caution is warranted as regards data interpretation. From this work, pork production can become more sustainable by increasing inclusion of fibrous co-products in swine diets. It is recommended that inclusion of fibrous co-products begin at weaning and continuously fed up to a certain period of time prior to harvest. A withdrawal period from the high-fiber diet will prevent the producer from being penalized by the packer for a lower dressing percentage. Co-product inclusion in swine diets also may be increased through use of feed additive technologies such as DFM; however, further research and strategic implementation are necessary to obtain consistent beneficial results.