EVALUATION OF ASPERGILLUS ORYZAE POSTBIOTIC ON NUTRIENT DIGESTIBILITY, GUT MICROBIOME, AND METABOLOME IN GROWING PIGS FED HIGH FIBER DIETS

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Dedication

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Abstract

Substantial amounts of low-cost, high fiber coproducts produced from various agro-industrial processes are incorporated into swine feed with the aim of reducing the cost and the environmental impacts of food animal production. Because dietary fiber (DF) is not well utilized by pigs, effective strategies are needed to improve the energy and nutrient utilization efficiency of using high fiber coproducts in swine diets. The objective of this thesis was to determine the potential of *Aspergillus oryzae* postbiotic (AOP) for improving nutrient digestibility of high fiber coproducts in growing pig diets and the underlying mechanisms. Results from the first and second experiments showed that the addition of 0.05% AOP to corn distillers dried grains with solubles, rice bran, or wheat middlings and diets containing these high fiber ingredients increased energy and nutrient digestibility *in vivo* and *in vitro*. The magnitude of the AOP response is ingredient- and diet-dependent, which suggested that the effectiveness of fiber-degrading feed additives is associated with DF type and composition of ingredients. Results from the third experiment suggested that dietary addition of AOP significantly altered the diversity and composition of the microbial community and metabolome in the ileal digesta and feces of growing pigs. Dietary addition of AOP alters the abundance of several bacteria that were significantly correlated with nutrient digestibility, including key players of the fiber-degrading bacteria. Overall, findings from this thesis provided the first evidence that AOP is effective in increasing the energy and nutritional value of swine diets containing different types of high fiber ingredients and new insights into mechanisms of action of AOP in pigs.

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List of Abbreviations

ACN	Acetonitrile
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestibility
AOP	*Aspergillus oryzae* postbiotic
ASV	Amplicon sequence variant
AX	Arabinoxylan
ATTD	Apparent total tract digestibility
A:X	Arabinose to xylose ratio
BA	Bile acid
BW	Body weight
CP	Crude protein
DC	Dansyl chloride
DDGS	Distillers dried grains with solubles
DE	Digestible energy
DF	Dietary fiber
DM	Dry matter
EE	Ether extract
FOS	Fructooligosaccharides
GE	Gross energy
GIT	Gastrointestinal tract

GOS	Galactooligosaccharides
G:F	Gain to feed ratio
HQ	2-hydrazinoquinoline
IQR	Interquartile range method
ISA	Indicator species analysis
IVDDM	*In vitro* digestibility of dry matter
IVDGE	*In vitro* digestibility of gross energy
IVFDM	*In vitro* fermentability of dry matter
ME	Metabolizable energy
MOS	Mannanoligosaccharides
NDF	Neutral detergent fiber
NSP	Non-starch polysaccharides
PCA	Principal components analysis
PCoA	Principal coordinates analysis
PLS-DA	Partial least squares-discriminant analysis
PERMANOVA	Permutational multivariate analysis of variance
SCFA	Short-chain fatty acid
SIC	Single-ion count
SID	Standardized ileal digestibility
SmF	Submerged fermentation
SSF	Solid-state fermentation
TIC	Total-ion count
TLR	Toll-like receptors

TOS	Transgalacto-oligosaccharides
RB	Rice bran
WM	Wheat middlings
VIP	Variable importance in projection
XOS	Xylooligosaccharides

Chapter 1. Literature Review

Introduction

With a growing world population and thus, the increasing global demand for food, we are facing one of the greatest challenges to feed the world sustainably (Shurson, 2017). When it comes to food animal production, the main challenges for producers have been to maximize feed efficiency while minimizing production costs and environment impacts to achieve more sustainable production systems (Pomar and Remus, 2019; Buenavista et al., 2021).

To meet these challenges, innovations in technologies are needed to optimize the use of finite feed resources and reduce feed cost in animal production systems. Feed accounts for about 60 to 70% of the total cost of pork production in modern intensive systems (Patience et al., 2015).Traditionally, cereal grains (e.g., corn and wheat) and protein supplements (e.g., soybean meal, canola meal) have served as the primary feedstocks in swine diets to meet energy and nutrient requirements (Agyekum and Nyachoti, 2017). These ingredients are highly valuable sources of energy and nutrients, but not cost-competitive with the emergence of large quantities of lower-cost coproducts of biofuel and food industry, including distillers dried grains with solubles (DDGS) from ethanol production, along with rice bran (RB) and wheat middlings (WM) from cereal grain processing. For instance, during the past two decades, global ethanol production increased from 17 billion liters to 108 billion liters (Iram et al., 2020). In 2020, the U.S. ethanol industry generated 33.1 million tonnes of distillers grains, gluten feed, and gluten meal (RFA, 2021), and about 15% of the total DDGS used in animal feed are consumed by swine (Shurson, 2017). These coproducts are valuable sources of energy, protein, as

well as other essential nutrients for pigs. However, the high concentration of dietary fiber (DF) in these coproducts, especially insoluble fiber, is generally not well utilized by monogastric animals due to the lack of endogenous fiber-degrading enzymes (Agyekum and Nyachoti, 2017) Therefore, effective ways are needed to enhance the digestibility and utilization efficiency of energy and nutrients of high fiber coproducts in pig diets.

Considerable efforts have been devoted to increasing the nutritional value including increasing the digestibility of energy among coproducts. These efforts can be divided into processing the ingredients prior to animal feeding including fermentation with bacteria and fungi microorganisms (Sun 2021). These methods are useful to increase the concentration of essential amino acids and increase the digestibility of nutrients (Barnhast et al., 2021). However, these methods are usually more energy and resource intensive and less applicable in short term. Therefore, feed additives offer a more applicable way to overcome some of the limitations of the current feed industry. Developing and evaluating various feed additives for improving digestibility and utilization efficiency of energy and nutrients of high fiber coproducts is essential immediate method to increase utilization efficiency of coproducts (Kerr and Shurson, 2013; Torres-Pitarch et al., 2017). Among many of these efforts, the development of prebiotic, probiotic (direct fed microbials), and postbiotic feed additives, and their subsequent addition to diets have been shown to improve digestibility of nutrients and performance of animals. One of these commercially available feed additives is *Aspergillus oryzae* postbiotic (AOP), a fermentation extract of a specific *Aspergillus oryzae* strain NRRL458. Intensive research has been conducted to evaluate effects of addition of AOP to ruminant diets, in which the beneficial effects reported include

increased nutrient digestibility (Van Horn et al., 1984; Gomez-Alarcon et al., 1991), stimulation of the proliferation of total and specific bacterial numbers (Newbold et al., 1991; Chang et al., 1999), and increased milk production in cows and body weight gain of heifer calves (Kellems et al., 1990; Beharka et al., 1991; Gomez-Alarcon et al., 1991). Conversely, studies on effects of supplementing AOP to swine diets are very limited, and the potential benefits of AOP for improving digestibility and nutrient utilization of fibrous ingredients and enhancing populations of beneficial gut microbes in pigs remain to be elucidated.

Composition and characteristics of fiber in the fibrous ingredients and diets are associated with *in vitro* degradation and fermentation of these types of fiber and *in vivo* physiological responses of pigs (Bach Knudsen et al., 2016; Zeng et al., 2018; Shurson et al., 2021) The main non-starch polysaccharide (NSP) present in corn DDGS, RB, and WM is arabinoxylan, which consists of a linear β-1,4 linked xylan backbone with the arabinose substitution (Dervilly-Pinel et al., 2004). The arabinose to xylose ratio (A:X) is a direct measure of the extent of substitution and is an important indicator for structural features of arabinoxylan molecules (Courtin and Delcour, 1998). In addition, soluble NSPs are generally more readily fermentable by gut microbes than insoluble NSPs (Bach Knudsen et al., 2013). Therefore, high fiber coproducts (i.e., corn DDGS, RB and WM) that differ in A:X and soluble NSP concentration were selected to evaluate effects of AOP on nutrient digestibility of pigs fed high fiber diets formulated with these coproducts in Chapter 2.

The degradation of fiber in pigs is mainly conducted by microbes in the large intestine, which is generally referred to fiber fermentation. The end-products of fiber

fermentation by microbes in the gut of pigs are short-chain fatty acids (SCFA), which are an important energy source for pigs and colonic epithelium cells. An *in vitro* procedure that simulates the digestive processes of the pig and developed by Boisen and Fernández (1997), allows the measurement of *in vitro* nutrient digestibility and SCFA production during fermentation. In addition, fermentability of fiber depends not only on fiber composition of an individual ingredient, but also the presence of other components in the complete diet (De Vries et al., 2016). Thus, it is unknown if the addition of AOP to complete diets will have an effect similar to that observed when added to individual feed ingredients. Therefore, effects of AOP added to single feed ingredients and complete diets on *in vitro* nutrient digestibility and SCFA production were described in Chapter 3.

The mechanisms of action of AOP are not well understood, especially in pigs. However, the recent development and use of omics technologies (e.g., genomics and metabolomics) enable the acquisition of a comprehensive model of the biological and metabolic processes to provide a better understanding of the mechanisms of AOP responses (Patti et al., 2012; Suravajhala et al., 2016). Therefore, in Chapter 4, metabolome and microbiome analyses were conducted to study effects of the addition of AOP to diets on the modulation of the ileal and fecal metabolome and microbiome in growing pigs.

Dietary fiber and utilization of high fiber ingredients in pigs

This dissertation focuses on evaluation of the potential of AOP in improving nutrient digestibility and utilization efficiency of high fiber ingredients and diets fed to growing pigs and the underlying mechanisms of these responses. Many feed additives such as prebiotics, probiotics and postbiotics that are developed to improve digestibility

of nutrients may fail because there is a lack of understanding of the different characteristics of DF and how these products may affect the digestibility of fiber. Some high fiber feed ingredients may contain fiber types that are more prone to digestibility by the feed additives, while other sources of fiber may have fiber characteristics that make it resistant to digestion. Therefore, it is necessary to review the definitions and characteristics of DF and the processes used to produce high fiber coproducts that may affect digestibility and utilization of fiber.

Definition of dietary fiber

The definition of DF has evolved since the early 1950's when the term "dietary fiber" was first used by Hipsley (1953), who described DF as the sum of non-digestible constituents that were known to include lignin, cellulose, and hemicelluloses. This description did not include soluble components of plant cell walls, but it was an advancement for distinguishing DF with the analytical term "crude fiber", which denotes the portion of plant that escaped solvent, alkali, and acid extractions (Van Soest, 1978). Since then, debates surrounding the definition of DF among experts and organizations have resulted in various proposed versions (Rezende et al., 2021). Trowell expanded the DF definition in 1972 to include all plant cell components that are resistant to digestion by human enzymes (Trowell, 1972), and revised the definition in 1976 as "the plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man" (Trowell et al., 1976). Since then, most people in the scientific community have reached a broad consensus that resistance to the digestion of human digestive enzymes is the basic physiological property of dietary fiber. Therefore, resistance to digestion by gastrointestinal derived enzymes should be a key defining characteristic of DF. As

consumption of DF in human diets increased and its beneficial effects to health gained more attention, it was believed that a physiological basis for the definition of DF was necessary. In 2000, the American Association of Cereal Chemists (AACC, 2000) approved a new definition of DF, that is "the edible parts of plant or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances." This definition emphasized the fermentability of DF in the large intestine, which is partly contributes to its functional and nutritional value to humans and animals. With beneficial effects of fiber to human health being recognized by medical professionals, it became necessary to address this attribute of fiber in its definition. In 2005, the Institute of Medicine developed the new definition of fiber by separating it into DF and functional fiber. Dietary fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants; and functional fiber consists of isolated, non-digestible carbohydrates that have beneficial physiological effects in humans. Total fiber is the sum of DF and functional fiber (Institute of Medicine, 2005). Given that modern definitions of fiber make reference to its functional properties, it is important to understand that characteristics of the different sources of fiber are important to the functional aspects of fiber.

Physicochemical properties and classification of dietary fiber

To understand the benefit of DF to diet energy value or animal health, it is important to understand the physicochemical properties. The physicochemical properties of DF that are of nutritional significance include solubility, viscosity, water binding or

holding capacity, and fermentability. These properties are often the basis for the classification of DF, which is critical for the prediction of the nutrient digestibility, energy contribution, effectiveness of exogenous enzymes, and prebiotic effects of fibrous ingredients or diets (Agyekum and Nyachoti, 2017; Shurson et al., 2021).

Solubility of fiber is usually used as an indicator of its potential function and physiological role in monogastric animals. Therefore, the most commonly used physio-chemical classification of DF is to divide it into soluble and insoluble fiber based on whether it forms a dispersion when mixed with water or not (Williams et al., 2019). The common soluble fractions of plant cell walls include hemicellulose (xyloglucans and galactomannan hemicellulose), pectin, and gum, while insoluble fiber fractions include cellulose, lignin, arabinoxylan hemicellulose and resistant starch (Jiménez-Escrig and Sánchez-Muniz, 2000).

Viscosity is a term used to describe the resistance of a solution to flow (Elleuch et al., 2011). Viscous DF thickens when mixed with fluids, which is strongly dependent on the molecular weight and concentration of DF, and is positively related to its solubility (Capuano, 2017). Gums, pectins, and β-glucans are common viscous fibers in plants (Dikeman and Fahey, 2006). Viscosity of DF plays a critical role in affecting digestive physiology, nutrient absorption, and intestinal morphology (Piel et al., 2005; Hung et al., 2021). Unfortunately, precise evaluation or prediction of dietary DF viscosity on utilization efficiency of high fiber ingredients has not been applied in routine diet formulation because there is a lot of confusion surrounding viscosity measurement due to the various measurement techniques available, inconsistency in instruments used, and the

way in which viscosity data is presented and interpreted (Bourne, 2002; Dikeman and Fahey, 2006).

Fermentability of DF describes its ability to be fermented by the intestinal microflora (Agyekum and Nyachoti, 2017). There has been increasing interest among animal nutritionists to better understand fermentation of fiber in feed ingredients because it is one of the most important mechanisms to make carbohydrates that are resistant to enzymatic digestion available to animals (Van Soest, 1978; Holscher, 2017). In addition, clear evidence has indicated that metabolic products of fiber fermentation (e.g., SCFAs) in the intestine have substantial beneficial effects to the health of animals (Lindberg, 2014; Jha and Berrocoso, 2015). Generally, it has been assumed that soluble fiber is fermented more rapidly than insoluble fiber. Insoluble fibers, such as cellulose, are generally poorly fermented by gut microbes. In contrast, soluble and viscous fiber, such as β-glucan and pectins, are highly fermentable in the hindgut.

Utilization of dietary fiber in pigs

Fractions of DF in cereal grains, such as cellulose, hemicelluloses, and pectic polysaccharides (arabinogalactans, pectins etc.) are predominantly linked by β-glycosidic bonds (Adebowale et al., 2019). Pigs can utilize DF only after microbial fermentation due to the lack of digestive enzymes capable of cleaving these β-glycosidic linkages (Navarro et al., 2019). It is generally accepted that DF escapes enzyme digestion in the small intestine and is fermented by microbes in the large intestine in pigs. However, recent evidence indicated that considerable microbial degradation of DF already occurs before it passes through to the hindgut (Jha and Berrocoso, 2015; Jaworski and Stein, 2017; Abelilla and Stein, 2019). Degradation of DF during passage through the stomach and the

small intestine varies widely from -7 to 40% among different NSP (Jha and Leterme, 2012; Bach Knudsen et al., 2013). There is relatively limited knowledge about microbes present in the upper gastrointestinal tract (GIT) of monogastric animals, but considering the more rapid passage rate and much lower microbial concentration compared with those in the large intestine, it is unlikely to be an important site for microbial fiber degradation (Flint et al., 2012).

Non-digestible carbohydrates may be partially or completely fermented by microbes in the large intestine of pigs. The rate and extent of fermentation of DF depend on its chemical nature, physicochemical properties, and degree of lignification (Jha and Berrocoso, 2015). In general, soluble fiber is more readily fermented than insoluble fiber by gut bacteria. Thus, β-glucans, soluble arabinoxylans, and pectins are fermented faster and to a greater extent in the cecum and proximal colon, while insoluble branched-chain AX from wheat, rye and oat are degraded slower and are less digestible in the distal colon (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993; Bach Knudsen et al., 2012). The ability of pigs to utilize fiber varies with age and body weight. Compared with young pigs, adult pigs or sows have a more developed and larger gastrointestinal tract, lower feed intake per kilogram of body weight, slower digesta passage rate, and higher cellulolytic activity, which leads to a greater ability of sows to digest fibrous components of diets (Bach Knudsen et al., 2013; Lindberg, 2014).

The GIT of poultry and swine are highly diverse and contain over 1,000 bacterial species mainly belonging to predominant phyla *Firmicutes, Bacteroidetes,* and *Proteobacteria* (Zhang et al., 2016). The breakdown of DF is involves a series of enzymes produced by the fibrolytic bacteria, including glycoside hydrolases,

polysaccharide lyases, and carbohydrate esterases (Hamaker and Tuncil, 2014). The end-products of microbial fermentation of DF are SCFAs and gases such as hydrogen, carbon dioxide, and methane (MacFarlane and MacFarlane, 1993; Williams et al., 2001). The primary (≥ 95%) SCFAs produced during microbial fermentation are acetate, propionate, and butyrate, and the relative proportions of SCFAs produced vary depending on the composition of DF. Acetate is the most abundant SCFA and contributes about 60% of the total SCFA produced in the hindgut, followed by propionate and butyrate (Jha and Berrocoso, 2015; Pieper et al., 2015). A high proportion (90 - 99%) of the SCFA produced by microbial fermentation of indigestible carbohydrates in the large intestine of pigs is absorbed by enterocytes or used by microbes (Ruppin et al., 1980; Holscher, 2017). Energy that pigs obtain from SCFA production may contribute up to 15% of maintenance energy requirement of growing pigs and 30% this requirement for sows (Varel, 1987).

Production, chemical composition, and nutrient digestibility of DDGS, rice bran, and wheat middlings

Distillers dried grains with solubles

In the past two decades, global ethanol production has increased from 17 billion liters to 108 billion liters (Iram et al., 2020), which resulted in a dramatic increase in the production of its major coproduct, DDGS. In 2020, the U.S. ethanol industry generated 33.1 million tonnes of distillers grains, gluten feed, and gluten meal (RFA, 2021), and about 15% of the total DDGS used in animal feed were consumed by swine (Shurson, 2017). Wet milling and dry-grind processes are the two major methods for industrial

ethanol production, and DDGS is the coproduct of dry-grind processing. The dry grind process is designed to ferment as much of the carbohydrate in the corn kernel as possible to achieve a high ethanol yield (Iram et al., 2020). A schematic diagram of the dry-grind ethanol process from grains is shown in Figure 1-1. Briefly, whole grains are ground into coarse flour using a hammer mill and then water is added to create a slurry. The raw slurry is cooked, followed by liquefaction, and simultaneous saccharification and fermentation. After that, ethanol is separated and concentrated during distillation. The whole stillage residues are centrifuged to separate wet grains from thin stillage, which are later mixed after some of the oil is separated and moisture is evaporated in thin stillage to produced condensed solubles and subsequently dried to produce DDGS.

Nutrient composition among DDGS sources vary due to many factors including differences in sources, processes (e.g., efficiency of starch fermentation, amount of solubles added back, conditions in dry procedures, and amount of oil removed), and types and varieties of parent grains(Świątkiewicz and Koreleski, 2008; Iram et al., 2020). The nutrient composition of corn DDGS from various published studies over year was summarized by Buenavista et al. (2021) and shown in Table 1-1. The average concentration of gross energy (GE) in DDGS (5245 kcal/kg) is greater than that in corn (4496 kcal/kg). However, the digestibility of energy in DDGS is lower than that in corn. Therefore, the digestible energy (DE) and metabolizable energy (ME) in DDGS (DE = 4140 kcal/kg, ME = 3897 kcal/kg) are similar to those in corn (DE = 4088 kcal/kg, ME = 3989 kcal/kg, Pedersen et al., 2007). The concentration of crude protein (CP), ether extract (EE), fiber, and minerals are greater in DDGS than the parent grains due to the removal of starch during ethanol production (Stein and Shurson, 2009). The CP level in

DDGS ranges from 27.4% to 32.2%, which is approximately four-fold of that in corn and makes it a valuable partial substitute for soybean meal in ruminant and monogastric animal diets. Corn DDGS contains approximately 27 - 35% insoluble and 3 - 6% soluble dietary fiber (Stein and Shurson, 2009; Pedersen et al., 2014; Jaworski et al., 2015). The apparent total tract digestibility (ATTD) of soluble, insoluble, and total DF in corn DDGS are 92.03%, 40.33%, and 49.45% (Table 1-2, Urriola et al., 2010), which resulted in a reduced digestibility of DM and energy compared with corn. The standardized ileal digestibility (SID) of amino acids in DDGS range from 64.90% to 85.44%, with SID of lysine the lowest and SID of leucine the highest. High variability of amino acids digestibility has often been reported in DDGS, and lysine was more variable in digestibility than other indispensable amino acid (Urriola et al,. 2009), which is indicated by the greater coefficient of variation. High variability of nutrient content and SID of amino acids among sources of DDGS limit accuracy of diet formulation and use of DDGS in swine diet, Therefore, several studies developed prediction equation for DE and amino acid content in DDGS for pigs (Pahm et al., 2008; Kim et al., 2012; Urriola et al., 2013; Urriola et al., 2014; Zeng et al., 2017). The capability of pigs to utilize DDGS increases with the increasing age and body weight. It is concluded that DDGS can be included in diets for pigs from 2 to 3 week postweaning with up to 30% of inclusion level, and up to 50% in diets for lactating and gestating sows without negative effects on performance (Stein and Shurson, 2009).

Rice bran

Rice bran is a coproduct of milling process of rice, the third most consumed cereal grain in the world by serving as a source of food for more than 3 million people

(Bodie et al., 2019; Spaggiari et al., 2021). The world's annual production of rice bran has been estimated to be 63- 76 million metric tons (Tran et al., 2017; Bodie et al., 2019). An overview of the milling process of whole rice is shown in Figure 1-2. Briefly, rusk is removed from rice paddy by friction and then the bran layer is separated from the germ, which produces white rice (Bodie et al., 2019). White rice is further polished to create a desired degree of whiteness. Rice bran undergoes stabilization to inactivate the lipase enzymes that cause it to denature through cold storage, heat treatment, control of relative humidity, sun-drying, steaming, and expelling (Rohman et al., 2014). Alternatively, rice bran could be defatted to reduce the risk of oxidation of fat (Stein et al., 2016).

The chemical composition of defatted and full fat rice bran is summarized in Table 1-3**.** The CP content in rice bran ranges from 11% - 17% (Sharif et al., 2014), which along with its low cost and abundant yield makes it a commercially attractive feed source and replacement for cereal grains, such as corn and soybean meal. The lipid concentration in full fat rice bran ranges from 16% to 24.40% and is much lower in defatted rice bran, ranging from 0.5% to 4.10%. The removal of oil significantly decreases energy concentrations in defatted rice bran (GE = 4056 kcal/kg , DE = 2199 kcal/kg, ME = 2081 kcal/kg, and NE = 1553 kcal/kg) compared with full fat rice bran (GE = 4772 kcal/kg , DE = 3100 kcal/kg, ME = 2997 kcal/kg, and NE = 2281 kcal/kg). The neutral detergent fiber (NDF) content in full fat rice bran ranges from 17 - 34% and over 95% of which is insoluble fiber (Casas et al., 2019), which makes the digestibility of fiber is relatively low compared with other feed ingredients. The ATTD of GE and DM, and SID of amino acids are comparable in defatted and full-fat rice bran, but the ATTD of NDF and phosphorus are relatively greater in defatted rice bran than those in full fat

rice bran (Table 1-4). However, these comparisons should be taken with caution because of large variations in both nutrient concentration and their digestibility among sources of rice bran and the small number of samples analyzed in the study (Stein et al., 2016). More research are needed to evaluate the energy and nutritional values of full-fat and defatted rice bran with different sources and oil extraction methods fed to pigs.

Wheat middlings

Wheat middlings are a by-product of wheat milling for flour and consist of fine particles of bran, shorts, germ, flour, and other fractions created during the wheat-milling process (Erickson et al., 1985; Rosenfelder et al., 2013). The schematical process of wheat milling is shown in Figure 1-3. Briefly, the cleaned and conditioned wheat pass through steel break rollers and is broken into coarse particles. Bran and germ are sifted out, and the coarse particles are rolled, sifted, and purified again. In the reduction stage, the coarser particles pass through a series of fine rollers and sieves to be further reduced. The particles are then sieved into flour, wheat germ, shorts, and wheat bran fractions (Hazel and Patel, 2004).

Nutrient content in wheat middlings is highly variable among varieties and growing conditions of wheat grain, processing procedures used, and the proportion of bran and flour (Erickson et al., 1985; Rosenfelder et al., 2013). Wheat middlings that consists of a high proportion of bran and a low proportion of starch is called “light, clean, or branny”, whereas wheat middlings that contain greater amounts of flour attached to the bran are referred as “heavy, starchy, or floury” (Cromwell et al., 1999). Chemical composition of wheat middlings from previous studies are summarized in Table 1-5. The GE content in wheat middlings is between 4500 - 4600 kcal/kg, which is similar to that in

wheat grain (McCann et al., 2006). The DE concentration in wheat middlings varies among studies, ranging from 2610 to 3182 kcal/kg, and ME concentration ranges from 2510 to 3126 kcal/kg. The concentration of CP in the sources of wheat middlings ranges from 16.38 to 20.93%, which is 1.2 - 5.5% and 4.2 - 8.7% higher compared with spring wheat and winter wheat, respectively (Rosenfelder et al., 2013). Wheat middlings has a high NDF content, ranging from 35.67 to 44.77% and a low EE content, ranging from 2.69 to 5.53%. Approximately 97% of the total dietary fiber (TDF) in wheat middlings is insoluble dietary fiber (IDF), and the ATTD of TDF and IDF are 65.5% and 61.60%, respectively (Table 1-6), which is lower than digestibility of other carbohydrates (e.g., starch), lipid, and crude protein. Therefore, there is a negative linear correlation between the concentration of NDF and the concentration of DE in wheat middlings (Huang et al., 2014).

Fiber content and fraction among cereal coproducts

The DF in the cereal coproducts vary substantially in the concentration, composition, and physiochemical properties, which are associated with their degradation and physiological functions in swine (Jha and Berrocoso, 2015; Shurson et al., 2021). Among many analytical methods of DF, total DF method is considered more accurate than crude fiber and van Soest methods because it accounts for both soluble and insoluble fiber components and DF composition characterized by NSP fractions is considered more representative for the physiological functions of DF than other methods (Shurson et al., 2021). Therefore, the total, soluble and insoluble DF concentration and NSP fractions in corn DDGS, full fat rice bran and wheat middlings are summarized in Table 1-7. Wheat middlings has the highest total DF (38.10%), followed by corn DDGS (29.8%) and rice

bran (20.1%). Most of the DF in these coproducts are insoluble DF, which accounts for 89%, 96%, and 97% of total DF in corn DDGS, rice bran, and wheat middlings, respectively. Insoluble DF are generally less readily fermentable in the intestine of pigs compared with soluble dietary fiber (Bach Knudsen et al., 2013). As a result, these coproducts have relatively lower energy digestibility compared with the cereal grains and therefore, strategies are needed to improve utilization efficiency of these coproducts in pigs. Arabinoxylan (AX, sum of arabinose and xylose) is the main NSP present in DDGS (13.1%), full fat rice bran (6.7%), and wheat middlings (18.8%), which accounts for 43.8%, 33.4%, and 49.3% of the total DF in these ingredients, respectively. Insoluble AX comprises 88%, 97%, and 98% of total AX content in corn DDGS, full fat rice bran, and wheat middlings, respectively (Pedersen et al., 2014; Jaworski et al., 2015; Casas et al., 2019), which results in a low digestibility of DF. Arabinoxylan consists of a linear β-1,4 linked xylan backbone with the arabinose substitution through a mixture of either 3-monosubstituted or 2,3-disubstituted α-L-arabinose (Dervilly-Pinel et al., 2004). Some arabinofuranosyl residues are further substituted with ferulic acid, which under certain circumstances can crosslink to other ferulic acid residues, forming diferulate bridges. The distribution of arabinose substituents along the backbone is considered to be of great importance because it affects the conformation and the capacity of arabinoxylans to interact with one another (Sternemalm et al., 2008). The A:X varies depending on the source of AX and is a direct measure for the degree of substitution which is an important indicator for the structural features of AX molecules (Courtin and Delcour, 1998). Rice bran has the greatest A:X (0.99), which indicates a greater degree of substitution and a more complexity structure of the heteroxylans compared with those in corn DDGS (0.77)

and WM (0.62). Wheat DDGS which has a lower A:X (0.68) was more responsive to xylanase degradation compared with corn DDGS which has a higher A:X (0.81, Pedersen et al., 2015; Zeng et al., 2018), indicating that NSPs with higher A:X ratio are less digestible by NSP-degrading enzymes and most likely less fermentable by gut microbes in the intestine of pigs. In addition, the A:X ratio is one of the characteristics that affects solubility of DF in a way that highly substituted arabinoxylans (high A:X) are more soluble compared with low substituted arabinoxylans (Marcotuli et al., 2016). Therefore, the differences in composition and characteristics of NSPs that consist of these coproducts are associated with their physiochemical properties (e.g., solubility, fermentability, viscosity) and physiological functions in pigs.

Aspergillus and application of *Aspergillus oryzae* postbiotic in animal diets

Beneficial effects of addition of AOP to growing pig diets are expected based on results from previous studies in other species. Therefore, a review of the characteristics of the genus *Aspergillus* and the species *Aspergillus oryzae* along with results from dietary supplementation with AOP or its related products to various animal species is useful for understanding its potential benefits when added to swine diets.

The genus Aspergillus

Aspergillus is one of the oldest and widespread fungi, and the genus was first described by Italian botanist Pier A. Micheli (1679-1737) in 1729 (Bennett, 2010). When he was viewing the mode using a microscope, Pier Micheli observed the shape of the fungi was very similar to an aspergillum (holy water sprinkler). Therefore, he named the genus accordingly. Due to their ability to colonize a wide variety of substrates, members of the genus *Aspergillus* are cosmopolitan, and prevalent in different ecosystems in a

wide range of environments and climatic zones, including soil, salterns, animals, stones, water-related, fossil records. As a result, there is a long history for the domestication of *Aspergillus* for applications in food production. The earliest record of use of *Aspergillus* in food fermentation was about 2,000-3,000 years ago in a Chinese book Zhouyi, where rice was fermented with *Aspergillus oryzae* and called "Qu" in Chinese (also known as Koji in Japanese). In other Asian counties, *Aspergillus oryzae*, *Aspergillus sojae* and other related Koji molds have been used in the food industries, such as brewing, baking and traditional food fermentation, for over 1,500 years (Barbesgaard et al., 1992). The genus includes about 250 filamentous fungal species (Geiser et al., 2007), most of which are of high agricultural, economic, and scientific importance because of their important roles in the ecosystem and involvement in the degradation of a wide range of natural organic substrates, particularly plant materials. However, some of the species act as plant and animal pathogens that cause illness. Aspergillosis refers to animal diseases caused by growth of *Aspergillus* in a living host. In contrast, *Aspergilli* are used in biotechnology to produce various products such as organic acids, drugs, proteins and enzymes because of their extraordinary metabolic versatility (Yang et al., 2017). High tolerance to extreme culture conditions makes *Aspergillus* unique compared with bacteria or yeast-derived microbial cells. For example, *Aspergillus* can be cultivated over a wide range of temperatures (10–50°C), pH (2–11), salinity (0–34%), water activity (0.6–1) and under oligo- trophic or nutrient-rich conditions. Hence, *Aspergillus* can be used for solid-state or submerged fermentations, and respective fermentation protocols have been established for large-scale industrial processes. In addition, compared with other eukaryotic

expression systems such as yeast, algae, or insect cells, filamentous fungi have the special advantage of unparalleled secretion ability (Fleißner and Dersch, 2010).

The cell walls of *Aspergillus oryzae* are complex structures (Figure 1-4) composed mainly of polysaccharides, including α-glucans, β-glucans, galactomannan, and chitin (Yoshimi et al., 2016). Glucan is the most important structural polysaccharide of fungal cell walls, accounting for 50-60% of the dry weight of the structure (Garcia-Rubio et al., 2020). In *Aspergillus oryzae*, most polymers of glucan are linked by β -1,3- or α -1,3-glycosidicbonds, although in the central core, there are glucans composed of branched β -1,6-glucan that is crosslinked to chitin. Chitin can comprise 10-20% the dry weight of the *Aspergillus oryzae* and is synthesized by the enzyme chitin synthase. These biologically active components, together with the secondary metabolites from *Aspergillus oryzae*, are jointly termed postbiotics that have been shown beneficial effects to the host, including improved growth and reproduction performance and attenuating heat stress in cows (Kaufman et al., 2021).

The species Aspergillus orazye

Aspergillus oryzae, also known as kōji mold, is a filamentous fungus that is widely used in traditional fermentation and food processing industries to produce soy sauce, soybean paste, and sake brewing (Machida et al., 2008). It has received extensive research interest for the high efficiency in the production of kojic acid and strong capacity to secrete enzymes including proteases, a-amylases, and hydrolases. The long history of application of *Aspergillus oryzae* in food fermentation industries allow it to be listed as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) in the USA, and its safety is also supported by the Word Health Organization (He

et al., 2019). Therefore, protein products from *Aspergillus oryzae* are easier to accept than those produced by non-approved production hosts. Major products and commercial applications of *Aspergillus oryzae* are summarized in Table 1-8.

Aspergillus oryzae produce a diverse array of primary and secondary metabolites. Enzymes are the main compounds produced by *Aspergillus oryzae*, including glucose oxidases, amylases, chymosin, pectinases, catalases, cellulases, proteases, phytases, lipases, and xylanases (Fleißner and Dersch, 2010). The two major fermentation conditions to produce industrial enzymes by *Aspergillus oryzae* are solid-state fermentation (SSF) and submerged fermentation (SmF). In SSF, *Aspergillus oryzae* grow on solid materials in the absence or near absence of free liquid (Singhania et al., 2009). There are a few advantages of SSF over SmF: 1) high yield in a shorter time and relative higher concentration of products (Meini et al., 2021); 2) use of low cost substrates, such as agro-industrial coproduct and wastes; 3) use of a wide variety of matrices varying in composition, size, mechanical resistance, porosity and water-holding capacity, 4) low capital cost, energy expenditure, and less expensive downstream processing. Major secondary metabolites produced by *Aspergillus oryzae* and their biological activities are reviewed by Daba et al., (2021) and summarized in Table 1-9.

Aspergillus orazye postbiotic

The *Aspergillus orazye* postbiotic, is the fermentation extract of a specific strain of *Aspergillus oryzae* (NRRL 458) mixed with wheat bran (4-5%) as a carrier. The fermentation is conducted in a liquid fermenter system in two stages under closed and sterile conditions using standard production media. After the second fermentation, the supernatant, including broth and cell solids, is sprayed on food-grade wheat bran at a

ratio of 4-5% w/w and air-dried at 55°C until the moisture content is less than 10% (average 7.2%). The final commercial product is described as containing about 7×10^6 intact *Aspergillus oryzae* conidiospores, 0.2% citric acid, and 2.3 mg riboflavin, 202.0 mg niacin, 23.2 mg pantothenic acid, 8.5 mg pyridoxine hydrochloride, 0.9 mg folic acid, 0.4 mg biotin kg, 1.2 µg cobalamin per kg, as well as 3.0 IU cellulase, and 40.0 IU amylase per kg activities (European Food Safety Authority, 2006).

Application of *Aspergillus oryzae* and its fermentation extract in animal feed

Intensive research has been conducted *in vivo* and *in vitro* to evaluate effect of *Aspergillus oryzae* and related products in animal feed, including live *Aspergillus oryzae* (direct-fed microbial), AOP, *Aspergillus oryzae* fermented soybean meal, and phytase expressed in *Aspergillus oryzae*. Most of these studies have been focused on use of *Aspergillus oryzae* in ruminant diets, followed by some poultry and pig studies.

Effect of Aspergillus oryzae postbiotic in ruminant diets

More than 60 studies have been published on effects of adding AOP to diets on performance, nutrient digestibility, ruminal bacterial, and SCFA production of ruminants. The results of AOP on fiber degradation, microbial growth, and performance of ruminants are not consistent. Beneficial effects have been reported with dietary addition of AOP, including increased fiber degradation and milk production in cows, and improved growth performance in calves (Table 1-10), while other studies reported no beneficial effects adding AOP to feed for ruminants (Table 1-11). Diets for ruminants usually consist of hay, grass, and grain coproducts that are high in fiber concentration. Thus, it is not surprising that the initial application of AOP in ruminant feed was based on its ability in degrading fiber due its cellulolytic activity. Addition of AOP increased

digestibility of acid detergent fiber (ADF) by 12% in lambs fed a corn-orchard grass hay based diet (Niver et al., 1973). This improvement in digestion of fiber (ADF and/or NDF) was also reported in cows (Van Horn et al., 1984; Wiedmeier et al., 1987; Gomez-Alarcon et al., 1990; Gomez-Alarcon et al., 1991), calves (Di Francia et al., 2008), and in *in vitro* ruminal fermentation (Varel et al., 1993; Hymes-Fecht and Casper, 2021). *Aspergillus oryzae* produces cellulase enzymes (Yang et al., 2017) and the reported minimum cellulase in AOP was 3 IU/kg (European Food Safety Authority, 2006). Therefore, the direct effect of cellulase in AOP maybe minimal in degrading fiber in feedstuffs that contains high amount of cellulose. However, the increased fiber degradation may be partially attributed to the increased cellulolytic bacteria numbers in the rumen. Wiedmeier et al (1987) reported that addition of AOP increased the rumen cellulolytic bacteria numbers by 40%, which was confirmed in other studies in cows (Yoon and Stern, 1996) and sheep (Mathieu et al., 1996). In an *in vitro* fermentation study, the addition of AOP to rumen inoculum increased the total number of bacteria by 70% when hay, barley, and molasses were used as substrates (Newbold et al., 1993).There were other studies that reported no beneficial effects of dietary addition of AOP in ruminant diet. In a study evaluating effects of graded levels of AOP on ruminal nutrient digestibility in cattle, digestibility of crude protein, ADF, and NDF were not affected by addition of any level (1, 2, 4, or 6 g/d) of AOP (Oellermann et al., 1990). The mixed ration used in this study was high in fiber and contained 15% straw, in which the strong lignocellulosic bonds may have impaired the ability of AOP to improve digestibility of fiber. A lack of response to AOP for fiber degradation was also reported in cows (Galloway et al., 1991; Denigan et al., 1992; Sievert and Shaver, 1993a; Sievert

and Shaver, 1993b; Varel and Kreikemeier, 1994; Bertrand and Grimes, 1997), sheep (Jouany et al., 1998) and *in vitro* (Beharka and Nagaraja, 1993). High level of AOP had been shown to decrease digestion of NDF and ADF when 1 g/L of AOP was added to *in vitro* ruminal fermentation (Martin and Nisbet, 1990), which suggested that appropriate dose is necessary for the improvements in digestibility induced by AOP.

Increased performance has been reported in ruminants which were partially a result of improved digestion of nutrients. Gomez-Alarcon et al (1991) reported improved milk yield and efficiency of milk production in lactating cows fed diets supplemented with 3 g of AOP per day during early lactation, which was associated with the increased digestibility of DM and fiber. This beneficial effects of AOP in milk yield of cows were confirmed in several studies (Kellems et al., 1990; Chiou et al., 2002; Tricarico et al., 2005; Sucu et al., 2019), and these researchers concluded that this increased milk yield was, in part, due to enhanced digestibility of nutrients in feed. However, there are several other studies in which improvements in milk production by supplementing AOP were not observed. For instance, supplementation of 3 g/d of AOP did not affect milk yield in Holstein cows (Sievert and Shaver, 1993a; Sievert and Shaver, 1993b; Bertrand and Grimes, 1997). Addition of AOP alone (Higginbotham et al., 1993; Higginbotham et al., 2004) or in combination with *Saccharomyces cerevisiae* culture (Higginbotham et al., 1994) in lactating cow diets had no effects on milk yield or milk composition.

Use of Aspergillus oryzae postbiotic and related fermented product in monogastric animal diets

Compared with the intensive research to evaluate the effects of adding AOP to ruminant diets, studies evaluating the addition of AOP to diets for monogastric animals

are limited. Jackson et al. (2006) studied the effect of adding AOP to lactating sow diets on performance of sows and piglets. Feeding lactating sows 1.3 g/d AOP did not change feed intake and body condition score, nor were there differences observed in litter size, number of piglets weaned, and weaning weight, average daily gain (ADG), and mortality rate of piglets. It is important to note that the sow diet used in the study was a corn-soybean meal-based diet, the fiber content of which was not reported but should be relatively low compared with diets containing high fiber ingredients. The lack of response could be due to that AOP is most effective in degrading DF and therefore, it is most likely to have beneficial effects for diets containing high concentration of DF. In a following study, nursey pigs were fed 0.68 g of AOP per day for 20 days (Jackson et al., 2006). Addition of AOP did not affect ADG, total weight gain, average daily feed intake (ADFI), and gain to feed ratio (G:F) of nursery piglets. Moeller and Hess (2016) evaluated the effect of feeding 5.5 g AOP per day to sows at day 70 of gestation on reproduction performance and piglet performance. Their results showed that addition of AOP to the gestation diet had no effect on number of piglets born alive, litter birth weight, or feed intake during lactation. However, sows fed AOP tended to nurse more piglets after cross-fostering to maintain equal opportunity. These studies were conducted in sows fed corn-soybean meal diets and no high fiber ingredients were used. Therefore, it could be concluded that based on published studies in sows, effect of AOP on reproduction performance was minimal, while the possibilities of AOP to improve nutrient digestibility and performance in a high fiber diet of sows remains to be evaluated.

Besides direct addition of AOP to the diet, *Aspergillus oryzae* is one of the most popular species used in soybean meal fermentation to increase nutritional value and reduce the levels of anti-nutritional factors (Mukherjee et al., 2016). Fermentation of soybean meal with *Aspergillus oryzae* for 48 h has been shown to eliminate trypsin inhibitors and increase the amount of small-size peptides (Hong et al., 2004). Similarly, Feng et al. (2007b) reported that *Aspergillus oryzae* fermentation decreased the level of trypsin inhibitor in soybean meal and greater ATTD of DM, CP, and energy were observed in piglets fed *Aspergillus oryzae* fermented soybean compared with those in piglets fed non-fermented soybean meal. As a result of increased nutrient digestibility and decreased anti-nutritional factors, beneficial effects of feeding soybean meal fermented with *Aspergillus oryzae* on growth performance have been reported, including increased ADG and feed efficiency in neonatal pigs (Zamora and Vecim, 1987), weaned piglets (Feng et al., 2007b), growing pigs (Zamora and Veum, 1979), and broilers (Chah et al., 1975; Feng et al., 2007a).

Aspergillus oryzae has also been used to produce phytase that can hydrolyze phosphomonoester bonds and release phosphorus bound to phytate. Phytase derived from *Aspergillus oryzae* has been reported to be effective in increasing the apparent total tract digestibility of phosphorus in sows (Torrallardona et al., 2012; Nasir et al., 2014), weanling piglets, and growing-finishing pigs (Almeida et al., 2013; Dang and Kim, 2021). Phytate has the capacity to chelate other minerals (e.g., calcium, zinc and iron), which results in reduced availability of these minerals to pigs, and compromises the utilization of other nutrients, such as protein, starch, and lipids (Humer et al., 2015). Hydrolysis of phytate by adding phytase may release the nutrients chelated with it and

therefore, improve the digestibility of these nutrients in pigs (Lei et al., 1993; Mroz et al., 1994; Zeng et al., 2016).

Mode of action of AOP

Several potential mechanisms of responses have been proposed based on *in vitro* and *in vivo* studies in ruminants, where AOP may improve the nutrient digestibility and performance traits mainly through enhanced microbial fermentation of ingested feeds. Increased ruminal degradation of fibrous feed components resulting from the activities of increased numbers of cellulolytic bacteria in rumen digesta was observed in cows, calves, and sheep fed AOP (Wiedmeier et al., 1987; Newbold et al., 1992; Mathieu et al., 1996; Yoon and Stern, 1996; Sun et al., 2017) and also *in vitro* (Frumholtz et al., 1989; Newbold et al., 1991; Newbold et al., 1993; Beharka and Nagaraja, 1998). Another hypothesized mode of action relates to the facilitated degradation of ruminal lactate, which is detrimental to fiber degradation in the rumen because of its strong potential to reduce rumen pH and cellulolytic activity (Frumholtz et al., 1989; Firkins et al., 1990). Lactate uptake by ruminal *Selenomonas ruminantium* and *Megasphaera elsdenii* was increased by AOP (Nisbet and Martin, 1990; Waldrip and Martin, 1993), which indicates that addition of AOP to the diet is beneficial in the stabilization of ruminal pH. *Aspergillus oryzae* are known to produce various polysaccharidase enzymes (e.g., cellulases and hemicellulases) which may facilitate degradation of plant cell walls. Therefore, it is also likely that the fibrolytic enzyme content in AOP contributes to the increased fiber degradation in the rumen and *in vitro* (Beharka and Nagaraja, 1993; Varel et al., 1993). However, the exact mode of action still needs to be elucidated and requires further studies in pigs.

Definition of prebiotic, probiotic, and postbiotic

Aspergillus oryzae fermentation extract has been shown to have prebiotic and postbiotic effects when added to ruminant diets and based on the possible mechanisms described in the previous section, may provide similar effects when added to growing pig diets. The main target organisms of prebiotics are *Bifidobacteria* and *Lactobacilli*, which are commonly used probiotics in animal feed. The use of prebiotics, probiotics, and postbiotics are the three main ways of dietary modulation of gut microbiota in pigs. Therefore, the definition and use of prebiotics, probiotics, and postbiotics in pig diets, and their mechanisms of action are reviewed in the following sections.

A clear definition of pre-, pro-, and postbiotic is important to the scientific community, regulatory agencies, the food industry, consumers, and healthcare professionals (Table 1-12). In the past two decades, there has been continuous debate on the definition of a prebiotic as well as associated criteria to identify compounds of this category. The concept of "prebiotic" was first described by Glenn Gibson and Marcel Roberfroid in 1995 and was defined as "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria already resident in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). Several criteria were associated with this original definition including: 1) resistance to digestion in the upper GIT; 2) selectively stimulate growth of one or a limited number of beneficial bacteria in the colon; 3) be capable of altering colonic microbiota in favor of a heathier composition; and 4) confer beneficial effects to the host. Non-digestible carbohydrates are the only food components that fulfill all of these criteria and are considered to be prebiotics. Although this original definition has

been revised many times, most of the main features have been retained. The definition was updated a decade later because many food components have been claimed to have prebiotic effects but required criteria were not properly considered. The term prebiotic was redefined to be "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Gibson et al., 2004). As per this definition, only three non-digestible oligosaccharides (inulin, transgalacto-oligosaccharides (**TOS**), and lactulose) were among the few candidates reviewed that fit the classification of prebiotic. Due to the limitation of analytical tools in early microbiome research, only species of *Lactobacillus* and *Bifidobacterium* were considered target organisms for prebiotics. In the past decade, advances in analytical tools and methods for the study of the microbiome have allowed identification of additional substances influencing colonization and thus, have improved our knowledge of the composition of microbiota. In 2017, the International Scientific Association for Probiotics and Prebiotics (ISAPP) published a consensus statement on the definition and scope of prebiotics, in which prebiotic is defined to be "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). This definition extends the concept of prebiotics to potentially include non-carbohydrate substances, applications to body parts other than the GIT, and multiple categories other than food. It also clarifies that the target organisms go beyond *Bifidobacteria* and *Lactobacilli*, and health benefits from effects on other beneficial taxa including (but not limited to) *Roseburia, Eubacterium* or *Faecalibacterium spp.* should also be recognized. Today, the commonly accepted substrates that fit the category of prebiotics include non-digestible fructans

(fructooligosaccharides (FOS), oligofructose and inulin) and galactans (galactooligosaccharides (GOS) and TOS), human milk oligosaccharides, and polyphenols.

When the definition of prebiotic was coined, the target organisms of prebiotics were limited to *Bifidobacteria* and *Lactobacilli*, which were commonly used as probiotics, and thus provided a commonality between probiotics and prebiotics. However, there are significant differences between prebiotics and probiotics. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" in 2001 by the Food and Agriculture Organization of the United Nations and the WHO, which was the most widely adopted and accepted definition for probiotic. In 2013, the ISAPP reworded it as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Probiotics should be able to arrive in the gut with a viable state. Mostly used probiotic microorganisms include bacteria belonging to the genus *Bacillus, Lactobacillus*, *Enterococcus*, *Pediococcus*, *Streptococcus*, and fungi and yeast strains such as, *Saccharomyces cerevisiae* and *Kluyveromyces* (Markowiak and Ślizewska, 2018). The combination of prebiotics and probiotics are called "synbiotics", which beneficially affects the host health by improving the survival and colonization of live beneficial microbes in the GIT and stimulating the growth of health-promoting bacteria (Gibson and Roberfroid, 1995). Recently, the potential beneficial impacts of non-viable microorganism cells (with their components) and fermentation compounds have gained increasing attention. To establish a consistent definition for these compounds, the ISAPP defined the term "postbiotic" as "a preparation of inanimate microorganisms and/or their

components that confers a health benefit on the host" (Salminen et al., 2021). Based on the definition, a postbiotic 1) must derive from a living microorganism; 2) must not include live microorganism; 3) can include the cell components of the organisms.

Use of prebiotics, probiotics, and postbiotics in swine feed

Various prebiotics and probiotics have been added and evaluated in swine feed, . Among these feed additives, prebiotics FOS and mannanoligosaccharides (MOS) can be synthesized in *Aspergillus oryzae* (Guío et al., 2009; Amirdahri et al., 2012; Mano et al., 2018) and the probiotics *Bifidobacteria* and *Lactobacilli* are main target of prebiotics and postbiotics, and therefore, are reviewed in this section. Most of the research has focused on the effects of these feed additives for improving performance, intestinal integrity, and establishment of a beneficial gut microbiota of pigs.

Prebiotics

Inulin and FOS are fructans that cannot be hydrolyzed by digestive enzymes in the small intestine of monogastric animals (Roberfroid, 2007; Sabater-Molina et al., 2009). Supplementation of inulin and FOS to diet for weaning piglets has been reported to be useful in alleviating post-weaning disorders by supporting beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*, thereby reducing intestinal pathogens such as *E. coli.* Patterson et al (2010) added inulin with variable chain lengths (short-, long-chain or mixture of both) to weaned piglet diets and their results showed that supplementation of all 3 types of inulin resulted in greater abundance of *Bifidobacterium and Lactobacillus* and less abundance of *Clostridium* in the cecum and colon. Recently, similar results were reported showing that weaned pigs fed diets with 2.5, 5, or 10 g/kg inulin supplementation had increased *Lactobacillus* and decreased *Escherichia coli* populations

in the caecum (Wang et al., 2020). This effect of increased *Lactobacillus* and *Bacteroides* in the GIT was also confirmed in growing pigs fed diets containing 0.5% inulin (He et al., 2021). Supplementation of 2.5 mg/kg FOS in diets for weaned piglets counteracted the negative effects of an *E. coli.* challenge on growth performance and nutrition digestibility by improving intestinal epithelium functions and populations of *Bacillus and Bifidobacterium* (Liu et al., 2020). Weaned piglets may also benefit from improved intestinal morphology, digestion, and absorption capacity by supplementation diets with inulin and FOS. Increased glucose absorption and intestinal permeability in the jejunal mucosa has been reported with 3% inulin supplementation to diets for weaned piglets (Awad et al., 2013). This may be partly due to: 1) elevated villus height in the jejunum and ileum as well as villus height to crypt depth ratio by addition of inulin in diets (W. Wang et al., 2020) and 2) increased activity of protease, trypsin, and amylase in the small intestine by supplementation of FOS (Xu et al., 2002). Wang et al. (2019) reported increased ADG, ADFI, G:F and loin-eye area in growing-finishing pigs fed a diet with 0.5% inulin. Improved Se status and oxidoreductive homeostasis was observed by supplementation of 2% inulin in the diet, but this effect was not accompanied with an improvement in growth performance (Lepczyński et al., 2021).

Mannanoligosaccharides are a glucomannan complex derived from the yeast cell walls of *Saccharomyces cerevisiae* (Spring et al., 2015). Linear mannan contains only mannopyranosyl units linked by β-1,4 linkages, while glucomannan consists of mannopyranosyl and glucopyranosyl units bound by β-1,4 bonds (Mikkelson et al., 2013). The main aim of adding MOS to diets has been to improve health of post-weaning piglets, either by improving the colostrum quality of sows or directly alleviating post-

weaning stress in piglets (Spring et al., 2015). The improvement in gut health is generally associated with increased growth performance of piglets. In a review paper that summarized 54 studies in nursery pigs fed Bio-Mos® (a commercial MOS product), Miguel et al. (2004) concluded that the average improvement of ADG, ADFI, and G:F ratio were 4.12, 2.11, and 2.29%, respectively, compared with pigs fed control diets. The improvement in growth performance may be attributed to the improvements in nutrient digestibility, gut morphology, and a reduction in the inflammation-related factors (Castillo et al., 2008; Agazzi et al., 2020). Greater total tract digestibility of DM and nitrogen was observed in weaning pigs fed diets with 0.1% MOS, which was accompanied by improved ADG, ADFI, and feed efficiency, and reduced diarrhea (Zhao et al., 2012). In another study, increased ileal digestibility of protein, calcium, phosphorus, and indispensable amino acids was reported in weaned piglets fed diets with 1 or 2 g/kg MOS supplementation, but the improvement in digestibility was not reflected in improved growth performance (Nochta et al., 2010). Another main beneficial effect of feeding MOS to piglets is to regulate immune responses and thus enhance the immune system. In a study conducted by Che et al. (2011), nursery pigs challenged with porcine reproductive and respiratory syndrome virus were fed a diet with 0.2% MOS and had increased leukocytes in a short period after infection, and also had decreased inflammatory mediators at the end of the acute phase compared with challenged pigs fed a control diet without MOS supplementation. These collective results indicate that MOS may enhance the immune system of the host at the early stage of infection and help alleviate negative impacts of virus infection.

Improved performance of gestation and lactating sows by adding MOS to the diet would also affect health status and performance of their offspring. In two studies conducted by Duan et al. (2016 and 2019), addition of MOS to either sow diets or piglet diets improved ADG, ADFI, and body weight of piglets at weaning. The authors attributed the improvement to enhancement of beneficial *Lactobacillus* and decreased *E. Coli* in in the small intestine and increased secretory IgA content in the piglets. They observed that dietary addition of MOS to sow or piglet diet increased *Lactobacillus* amount and decreased *E. coli* amount in jejunal digesta. Results from these studies suggest that MOS could be effective in improving the gut health and performance of piglets.

Probiotics

Bacteria in the gut can be beneficial or detrimental to the host health. *Bifidobacteria* and *Lactobacilli* have traditionally been recognized as the primary bacteria that confer positive health benefits to the host and therefore, are the most widely used probiotics found in functional foods and dietary supplements (Gibson and Roberfroid, 1995; Kleerebezem and Vaughan, 2009; O'Callaghan and van Sinderen, 2016). Numerous benefits have been documented in pig with the use of *Bifidobacteria* and *Lactobacilli.* As early as in 1980s, improved ADG and feed efficiency was reported associated with the supplementation of *Lactobacillus* (Pollmann et al., 1980). Similarly, improved ADG and feed conversion was reported in newborn piglets with oral administration of *Bifidobacterium pseudolongum* (Abe et al., 1995). The improved growth performance of pig by these two probiotic were confirmed in many later studies (Huang et al., 2004; Modesto et al., 2009; Zhang et al., 2010; Liao and Nyachoti, 2017).

The beneficial effects of probiotics on performance have been associated with their effects on modulation of gut microbiota, the results of which are the prevention of pathogens and enhancement of balanced gut microbiota and desirable gut environment. The populations of *Lactobacillus* increased in feces of weaned pigs fed a *Lactobacillus brevis* preparation, which was accompanied by improved ADG, ADFI, and feed efficiency (Liu et al., 2015). Similarly, weaned piglets fed probiotic *Lactobacillus plantarum* showed greater microbial diversity and richness and relative abundance of *Firmicutes* and *Ruminococcaceae* in fecal samples (Shin et al., 2019). Zimmermann et al (2016) conducted a meta-analysis of studies on effects of probiotics on growth performance of pigs where they summarized ADG results from 32 studies and feed efficiency results from 29 studies published between 1980 to 2015. The results from the meta-analyzed showed that the average improvement by supplementation of lactic acid bacteria (e.g., *Bifidobacteria* and *Lactobacilli*) was 29.30 g/day for ADG and 96 g feed (dry matter)/ kg body weight for feed efficiency.

Postbiotics

Since postbiotic is a relatively new concept, studies on the use of postbiotics in swine are very limited. A study conducted by Holanda et al (2020) investigated the effects of postbiotics from *Saccharomyces cerevisiae* on growth performance and health of weaned piglets under chronic dietary challenges of low and high dose of mycotoxins (i.e., aflatoxin B1 and deoxynivalenol). The results from their study showed that dietary addition of postbiotic had no effects on ADG, ADFI, but tended to decrease G:F ratio of piglets. Interactions were observed between postbiotic and dose of mycotoxins for apparent ileal digestibility (AID) of DM, GE, and nitrogen, which showed that high dose

of mycotoxins reduced the AID of nutrients, while the addition of AOP attenuated the decrease in AID of nutrients. These results indicate that dietary addition of postbiotics from *Saccharomyces cerevisiae* have beneficial effects on the performance of piglets challenged with mycotoxins. Khafipour et al. (2021) studied the effects of a postbiotic from *Lactobacillus* acidophilus fermentation on gut microbiome of nursery pigs. The results showed that pigs fed diets with the postbiotic and antibiotic exhibited more similar microbial composition compared with the control group and pigs fed postbiotic exhibited clusters with greater abundances of carbohydrate-active-enzymes that was correlated with greater body weight on d 32 compared with other groups, suggesting that postbiotics have the potential to be used as an alternative to antibiotics and growth promoters. However, further studies are required to investigate the effects of this kind of probiotics on performance and gut health of pigs. Another postbiotic from *Lactobacillus* fermentate has been shown to increase beneficial bacteria (e.g., *Lactobacillus salivarius* and *Propionibacterium acnes*) in jejunal mucosa and ameliorate the decreased ADG and BW in *E. coli* challenged pigs (Xu, 2021). With limited data available, it is difficult to draw conclusions on the effects of postbiotics on pig performance and health, because 1) different types of postbiotics were evaluated in these published studies, and therefore, comparisons across studies are not possible; and 2) responses measured in these studies were different, so the most significant and consistent response cannot be identified. However, as interests on the beneficial effects of postbiotic increasing, it can be expected that more studies will be conducted to evaluate the use of various postbiotics in pig production and the potential of postbiotics to improve performance and health of pig will be more clearly elucidated.

Mode of action of prebiotics, probiotics, and postbiotics

Prebiotics

Several mechanisms have been proposed for the action of different types of prebiotics. The most widely accepted mechanism is to enriching the growth and activity of beneficial bacteria (e.g., *Bifidobacteria* and *Lactobacilli*) and therefore, maintain a healthy gut environment (Gibson and Roberfroid, 1995; Kleerebezem and Vaughan, 2009; Liu et al., 2018). Prebiotics, such as inulin and FOS, are selectively fermented by these two beneficial bacteria which allows them to outcompete potential detrimental organisms, such as members of the *Clostridia* class and *E. coli* (Niness, 1999; Xu et al., 2002; Gibson et al., 2017). In addition, MOS can interfere with pathogen adherence by competitively inhibiting the binding of pathogenic bacteria to the host receptors (Chacher et al., 2017; Teng and Kim, 2018). The beneficial effects of prebiotics on the host may also be related to the enhancement of the epithelial barrier, which is a major defense mechanism used to maintain epithelial integrity and to protect the organism from the environment (Wu et al., 2017; Liu et al., 2020; Rose et al., 2021). Another proposed mechanism of action of prebiotics is via modulation of immune system. Emerging evidence suggests that prebiotics exert direct or indirect effects on the stimulation of the host immune response against various pathogens (Leonel and Alvarez-Leite, 2012; Parada Venegas et al., 2019; Pujari and Banerjee, 2021). Prebiotics have also been suggested to contribute to improved intestinal morphology, digestion, and absorption capacity of nutrients in animals. Increased villus height and villus height to crypt depth ratio have been reported by the addition of prebiotics in diets for monogastric animals (Agazzi et al., 2020; Liu et al., 2020; W. Wang et al., 2020). Along with increased

digestive enzyme activities (Xu et al., 2002), improvement in nutrient utilization by prebiotic supplementation contributes to greater feed efficiency and growth performance in monogastric animals, especially young animals that have immature gut system.

Probiotics

Probiotics exert their beneficial effects to the host in multiple ways. First, probiotics modulate the intestinal microbiota and make the gut a balanced and healthier environment by competitive exclusion of pathogenic microorganisms (Ng et al., 2009). The competitive exclusion is mediated by 1) binding to the host epithelial attachment sites and thus preventing the binding of pathogens; 2) competitive depletion of available nutrients that are essential for growth of pathogens; and 3) secretion of antimicrobial substances (Bermudez-Brito et al., 2012). Organic acids, particularly acetic acid and lactic acid produced by probiotics can strongly inhibit a broad range of pathogens, including *E. coli* and *Salmonella* by lowering the lumen pH (Makras et al., 2006; Fukuda et al., 2011; Markowiak-Kopeć and Śliżewska, 2020). Bacteriocins, the antimicrobial peptides produced by probiotics has also been shown to be active against other bacteria, including many Gram-positive pathogens (Cotter et al., 2005; Yang et al., 2014). Second, probiotics have been shown to be involved in maintenance and enhancement of the intestinal epithelial barrier, which is a major defense mechanism to maintain epithelium integrity and separate the body from external environment. Several studies have shown that *Lactobacillus* and *Bifidobacteria* increased expression of genes that encode tight junction proteins, such as ZO-1, ZO-2, and cingulin (Anderson et al., 2010). In addition, the beneficial of probiotics have also been associated with the increased adhesion to intestinal mucosa and modulation of the immune system (Bermudez-Brito et al., 2012).

Postbiotics

Postbiotics have many characteristics in common with probiotics because it is originated from probiotics. Therefore, postbiotics shared some common mechanisms of action with probiotics, including modulation of beneficial microbiota, enhancement of epithelial barrier function and modulation of the immune responses (Salminen et al., 2021). Bioactive compounds present in postbiotics, such as organic acid and bacteriocins could inhibit the growth of pathogens which has been discussed in the last section. Postbiotics that contain adhesins (e.g., fimbriae and lectins) could also directly compete with pathogens for epithelial attachment sites and therefore, preventing these pathogens from colonizing in the gut (Petrova et al., 2016; Tytgat et al., 2016). A postbiotic from *Lactobacillus rhamnosus* GG showed a protective effect on the intestinal barrier by enhancing mucin expression in the intestine and preventing intestinal barrier injury (Gao et al., 2019). Besides the shared mechanisms with probiotics, postbiotics have been shown to attenuates the impact of heat stress in ectothermic and endothermic organisms when postbiotics from *Aspergillus oryzae* was added into the culture medium of *Drosophila melanogaster* and supplemented to diets for lactating dairy cows (Kaufman et al., 2021). These effects of probiotics were attributed to the regulation of expression of genes involved in oxidative stress and immunity (e.g., metallothionein). However, the mechanisms of action of postbiotics are far from fully understood and required further investigation, especially for those that used in animal feed.

Overall summary of the literature and aims of the dissertation

In summary, the use of coproducts from food and biofuel industries provides opportunities to achieve more sustainable food animal production systems while

minimizing production costs and environment impacts. However, innovations in technologies that improve the nutrient utilization efficiency from including these coproducts in swine diets are needed. Dietary fiber in feed ingredients significantly differs in NSP composition and characteristics, which are associated with physiological responses and kinetics of fermentation in pigs. Beneficial effects of AOP addition to ruminant diets have been well documented, but the potential benefits of AOP in pig diets and mechanisms of action remain to be elucidated.

Therefore, the aims of this thesis are to: 1) determine the effects of addition of AOP on nutrient digestibility in growing pigs fed high fiber diets; 2) compare effects of AOP on *in vitro* digestion and fermentation kinetics between single high fiber ingredients and complete diets formulated with these ingredients; 3) measure the production of SCFA of high fiber ingredients and diets when AOP was added; and 4) evaluate the effects of addition of AOP on modulation of microbiome and metabolome in fecal and ileal samples of growing pigs.

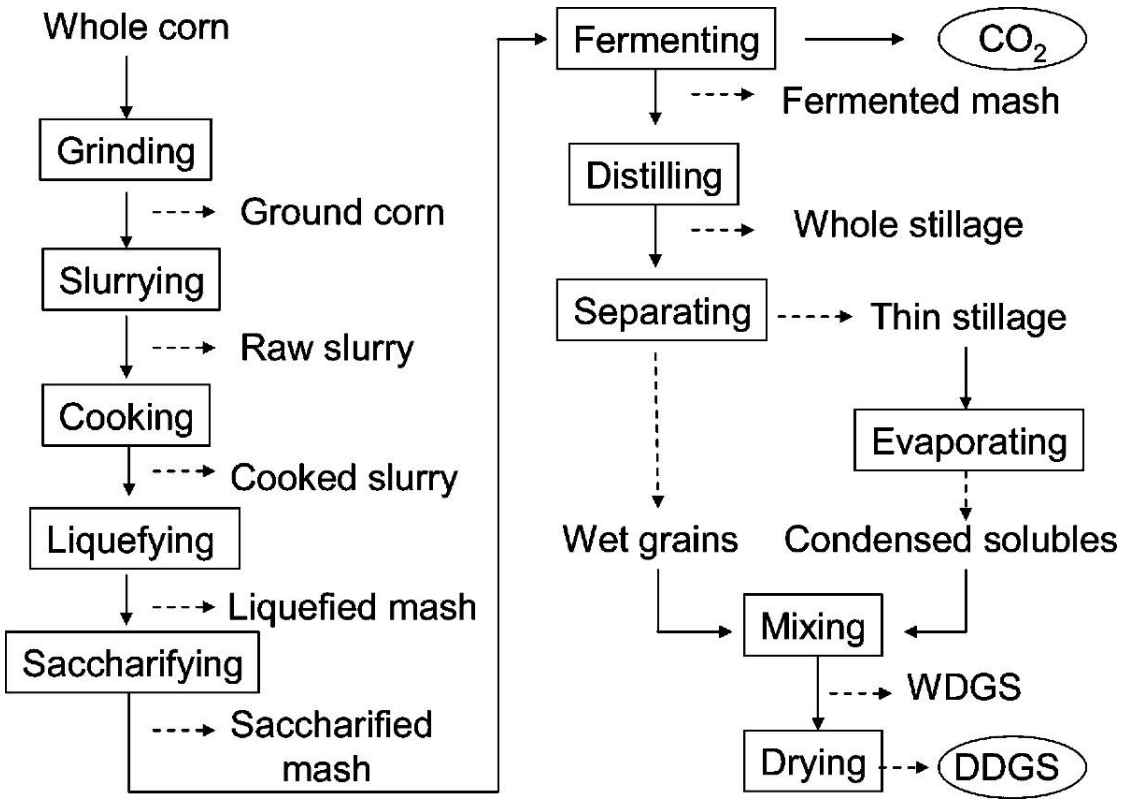

Figure 1-1. Schematic diagram of a conventional dry-grind ethanol production from corn. (Adapted from Liu, 2011)

Table 1-1. Nutrient composition of corn distillers dried grains with solubles and its energy values for pig from published data over years (% DM basis , adapted from Buenavista et al., 2021).

Component	2006	2007	2009	2012	2013	2016	2018	NRC 2012 (6% < oil < 9%)
Gross energy[1]	5,422	5,434	5,593	5,420	4,996	4,805	5,049	4,710
Digestible energy[1]	3,556	4,140	4,072	4,029	3,635	-	-	3,582
Metabolizable energy[1]	-	3,897	-	3,790	3,435	-	-	3,396
Dry matter	88.90	87.60	89.30	89.10	87.00	90.84	-	89.35
Crude protein	31.00	32.20	31.60	31.30	30.60	30.08	29.11	27.36
Ether extract	-	11.70	13.20	11.40	10.60	9.00	11.12	8.90
Starch	7.30	8.20	-	4.30	1.90	-	-	9.63
Ash	-	4.40	-	4.50	4.90	1.95	6.07	4.04
Neutral detergent fiber	45.20	27.60	40.10	40.40	36.60	53.5	37.08	30.46
Acid detergent fiber	12.20	11.60	15.50	12.10	12.40	12.53	13.53	12.02
Hemicellulose	32.00	16.00	24.50	26.80	24.20	-	23.55	-
Crude fiber	-	-	-	7.80	-	9.25	8.84	8.92
Total dietary fiber	-	-	-	36.40	33.70	-	-	-

[1]Kcal/kg dry matter.

Table 1-2. Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of energy and dietary fiber and standardized ileal digestibility (SID) of amino acids in corn distillers dried grains with solubles (DDGS) fed to pigs.

Digestibility, %	Number of samples	Mean	Minimum	Maximum	CV[1]	Reference
AID of SDF[2]	8	64.35	56.40	81.70	12.14	Urriola et al., 2010
AID of IDF[3]	8	20.00	5.90	33.60	45.92	Urriola et al., 2010
AID of TDF[4]	8	28.89	19.60	38.20	21.18	Urriola et al., 2010
AID OF NDF[5]	8	45.85	37.50	52.10	9.63	Urriola et al., 2010
ATTD of GE[6]	10	76.84	73.90	82.80	3.55	Pedersen et al., 2007
ATTD of SDF	8	92.03	89.40	95.30	1.71	Urriola et al., 2010
ATTD of IDF	8	40.33	29.30	51.00	17.33	Urriola et al., 2010
ATTD of TDF	8	49.45	39.40	56.40	11.69	Urriola et al., 2010
ATTD of NDF	8	59.30	51.60	65.80	7.17	Urriola et al., 2010
ATTD of P	10	50.82	44.30	58.60	7.30	Pedersen et al., 2007
SID of crude protein	34	75.37	64.80	84.30	5.70	Pahm et al., 2008; Kim et al., 2012
SID of indispensable amino acids						
Arg	34	84.91	72.90	92.00	4.96	Pahm et al., 2008; Kim et al., 2012
His	34	80.87	70.70	85.90	3.79	Pahm et al., 2008; Kim et al., 2012
Ile	34	78.26	69.20	83.30	3.80	Pahm et al., 2008; Kim et al., 2012
Leu	34	85.44	77.00	89.50	3.03	Pahm et al., 2008; Kim et al., 2012
Lys	34	64.90	45.30	77.90	12.07	Pahm et al., 2008; Kim et al., 2012
Met	34	83.34	73.90	89.20	3.95	Pahm et al., 2008; Kim et al., 2012
Phe	34	82.84	75.30	87.00	3.21	Pahm et al., 2008; Kim et al., 2012
Thr	34	72.12	63.80	78.00	4.85	Pahm et al., 2008; Kim et al., 2012
Trp	34	76.58	59.70	85.80	8.23	Pahm et al., 2008; Kim et al., 2012
Val	34	77.78	68.70	81.90	3.66	Pahm et al., 2008; Kim et al., 2012
SID of dispensable amino acids						
Ala	34	81.16	70.90	84.90	3.67	Pahm et al., 2008; Kim et al., 2012
Asp	34	71.00	61.40	75.90	5.02	Pahm et al., 2008; Kim et al., 2012
Cys	34	75.06	67.50	81.20	4.48	Pahm et al., 2008; Kim et al., 2012

Glu	34	83.14	74.30	87.00	3.45	Pahm et al., 2008; Kim et al., 2012
Gly	34	68.24	53.60	88.60	12.66	Pahm et al., 2008; Kim et al., 2012
Pro	34	79.26	32.10	125.90	27.26	Pahm et al., 2008; Kim et al., 2012
Ser	34	78.58	70.50	82.80	3.82	Pahm et al., 2008; Kim et al., 2012
Tyr	25	82.79	76.20	86.90	2.94	Kim et al., 2012

[1]CV = Coefficient of variation.
[2]SDF = soluble dietary fiber.
[3]IDF = insoluble dietary fiber.
[4]TDF = total dietary fiber.
[5]NDF = neutral detergent fiber.
[6]GE = gross energy.

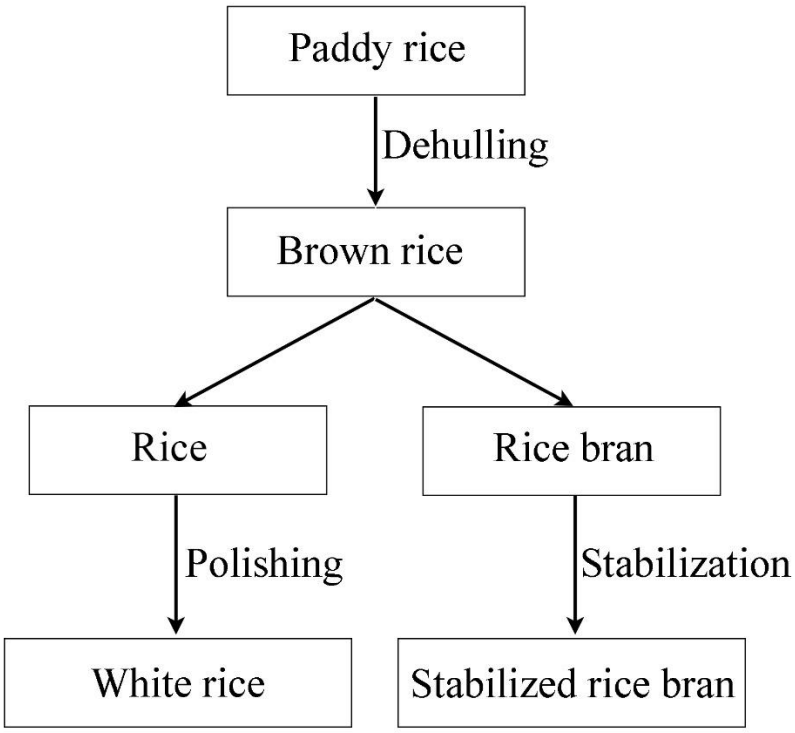

Figure 1-2. Schematic diagram of rice milling process.

Table 1-3. Chemical composition of defatted and full fat rice bran from previous studies, DM basis

Items	Defatted rice bran					Full fat rice bran					
	Study 1[1]	Study 2[2]	Study 3[4]	Study 4[4]	NRC 2012	Study 1[1]	Study 2[5]	Study 3[2]	Study 4[3]	Study 5[6]	NRC 2012
GE, kcal/kg	5019	4132	4348	4228	4056	3776	4926	5044	5234	4465	4772
DE, kcal/kg	-	1864	3054	-	2199	-	3763	3984	-	-	3100
ME, kcal/kg	-	1810	2936	-	2081	-	3580	3856	-	-	2997
NE, kcal/kg	-	1100	-	-	1553	-	2952	-	-	-	2281
Dry matter, %	90.60	-	91.0	89.22	91.35	90.10	89.19	96.20	93.50	88.66	91.60
Organic matter, %	-	-	-	87.81	-	-	-	-	-	88.16	-
Ether extract, %	4.10	1.10	1.11	0.50	3.52	24.40	16.04	19.28	19.58	17.00	13.77
Crude protein, %	21.00	16.70	17.10	18.37	17.30	15.80	15.30	15.30	15.50	13.21	15.11
Ash, %	12.70	11.50	11.97	12.19	11.51	11.00	8.11	8.04	9.10	11.84	14.80
NDF[7], %	26.00	33.50	19.27	25.78	23.56	34.30	17.78	14.13	17.17	20.16	26.28
ADF[8], %	-	20.80	12.00	11.14	1.31	-	7.38	9.09	7.98	8.19	11.87
Lignin, %	-	-	4.34	2.30	-	-	-	3.01	3.26	-	-

[1]Kaufmann et al., 2005.
[2]Lyu et al., 2018.
[3]Casas and Stein, 2016.
[4]Huang et al., 2021.
[5]Li et al., 2018.
[6]Trindade Neto et al., 2021.
[7]NDF = neutral detergent fiber.
[8]ADF = acid detergent fiber.

Table 1-4. Apparent total tract digestibility (ATTD) of energy, dry matter (DM), and fiber, and standardized total tract digestibility (STTD) of phosphorus, and standardized ileal digestibility (SID) of amino acids in rice bran fed to pigs.

Digestibility, %	Defatted rice bran			Full fat rice bran			Reference
	N	Mean	CV[1]	N	Mean	CV[1]	
ATTD of GE[2]	1	79.50	-	1	80.80	-	Casas and Stein, 2016
ATTD of DM	1	79.50	-	1	81.90	-	Casas and Stein, 2016
ATTD of NDF[3]	1	59.00	-	1	44.50	-	Casas and Stein, 2016
ATTD of ADF[4]	1	50.40	-	1	42.70	-	Casas and Stein, 2016
STTD of P	1	33.10	-	6	28.50	39.65	Stein et al., 2016
SID of indispensable amino acids							
Arg	2	88.00	3.75	5	89.00	4.27	Stein et al., 2016
His	2	84.00	1.79	5	86.00	2.56	Stein et al., 2016
Ile	2	75.00	5.60	5	75.00	10.00	Stein et al., 2016
Leu	2	76.00	3.55	5	76.00	8.95	Stein et al., 2016
Lys	2	77.00	8.96	5	79.00	8.61	Stein et al., 2016
Met	2	75.00	6.00	5	80.00	8.88	Stein et al., 2016
Phe	2	75.00	5.60	5	75.00	7.73	Stein et al., 2016
Thr	2	75.00	4.40	5	74.00	8.24	Stein et al., 2016
Trp	2	75.00	9.20	4	77.00	9.61	Stein et al., 2016
Val	2	76.00	5.13	5	76.00	10.26	Stein et al., 2016
SID of dispensable amino acids							
Ala	1	82.00	-	3	80.00	11.00	Stein et al., 2016
Asp	1	77.00	-	3	78.00	8.33	Stein et al., 2016
Cys	2	72.00	5.56	5	72.00	9.86	Stein et al., 2016
Glu	1	82.00	-	3	84.00	4.88	Stein et al., 2016
Gly	1	78.00	-	3	75.00	9.87	Stein et al., 2016
Pro	1	1.35	-	3	1.05	-	Stein et al., 2016
Ser	1	78.00		3	78.00	9.10	Stein et al., 2016
Tyr	1	78.00		6	78.00	4.49	Stein et al., 2016

[1]CV = coefficient of variation.
[2]GE = gross energy.
[3]NDF = neutral detergent fiber.
[4]ADF = acid detergent fiber.

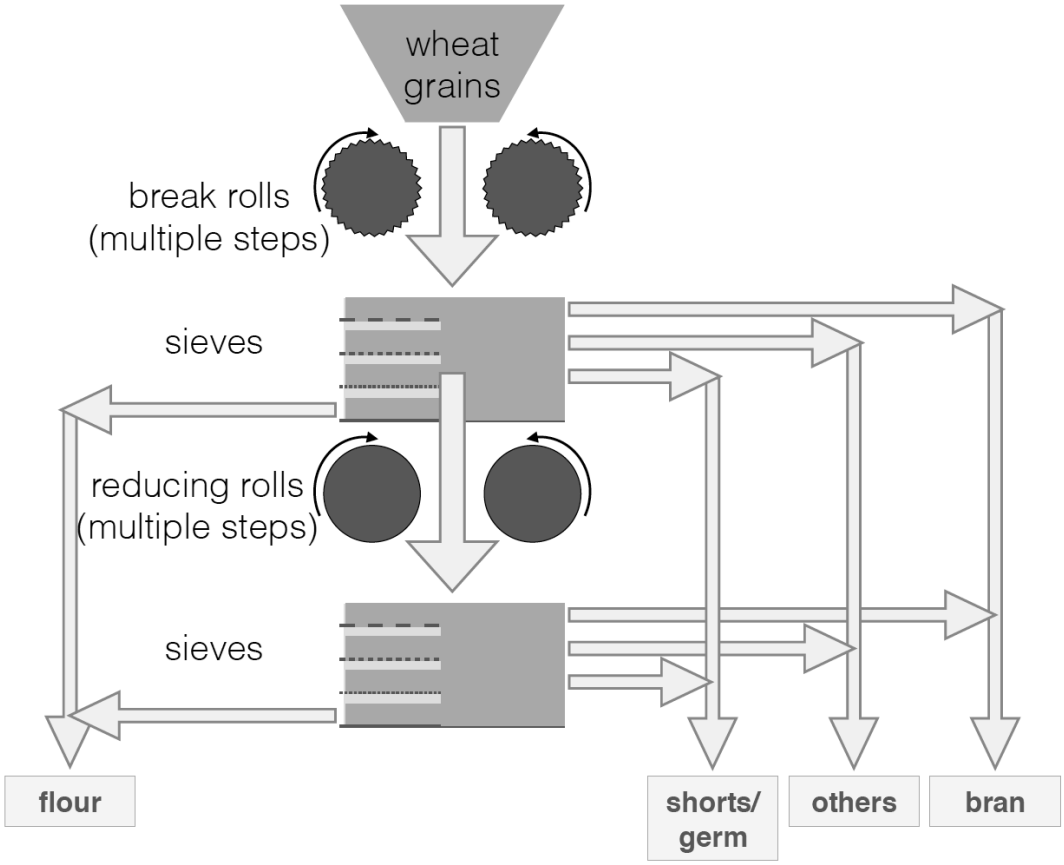

Figure 1-3. Schematic diagram of wheat milling process adapted from (Heinze, 2017).

Table 1-5. Summary of chemical composition of wheat middlings reported from previous studies, DM basis

Item	Salyer 2012	Stewart 2013	Huang 2013	Jaworski 2015	Wu 2016	Casas 2018 n = 10			Rho 2020	NCR 2012
						Mean	Minimum	Maximum		
GE, kcal/kg	-	4553	4562	4446	-	4481	4434	4498	-	3901
DE, kcal/kg	-	-	2610	2697	-	2990	2637	3182	-	3075
ME, kcal/kg	-	-	2510	-	-	2893	2547	3126	-	2968
NE, kcal/kg	-	-	-	-	-	-	-	-	-	2113
Dry matter, %	89.72	89.94	88.00	91.60	88.08	88.80	86.51	89.98	86.60	89.10
Crude protein, %	16.38	19.06	19.89	18.40	17.47	19.90	19.65	20.93	18.90	15.76
Ether extract, %	4.24	4.33	3.52	4.90	3.91	4.58	2.69	5.53	4.61	3.15
NDF[1], %	35.67	-	44.77	-	43.49	39.99	38.24	44.77	40.50	34.97
ADF[2], %	12.71	-	11.14	-	13.18	12.17	10.91	13.58	13.70	5.98
Lignin, %	-	-	-	7.30	-	3.40	2.99	4.80	-	-
Ash, %	-	5.69	5.23	6.10	5.70	6.15	5.64	7.87	4.70	2.05

[1]NDF = neutral detergent fiber.
[2]ADF = acid detergent fiber.

Table 1-6. Apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of energy and fiber in wheat middlings fed to growing pigs.

Digestibility,%	Huang et al., 2013			Jaworski and Stein, 2017	Casas et al., 2018		
	Mean	Minimum	Maximum		Mean	Minimum	Maximum
AID	-	-	-		-	-	-
DM[1]	-	-	-	39.00	-	-	-
GE[2]	-	-	-	42.40	-	-	-
ADF[3]	-	-	-	15.60	-	-	-
NDF[4]	-	-	-	44.90	-	-	-
SDF[5]	-	-	-	28.40	-	-	-
IDF[6]	-	-	-	45.90	-	-	-
TDF[7]	-	-	-	44.60	-	-	-
Cellulose	-	-	-	12.20	-	-	-
ATTD							
DM	61.10	52.70	64.90	68.40	71.20	68.70	73.60
OM[8]	64.88	57.10	68.10	-	72.90	70.10	75.60
GE	57.30	46.70	62.60	66.30	67.20	61.90	70.30
CP[9]	72.46	67.00	76.40	-	-	-	-
ADF	-	-	-	8.20	-	-	-
NDF	-	-	-	60.20	-	-	-
SDF	-	-	-	116.90	-	-	-
IDF	-	-	-	61.60	-	-	-
TDF	-	-	-	65.50	-	-	-
Cellulose	-	-	-	15.90	-	-	-

[1]DM = dry matter; [2]GE = gross energy; [3]ADF = acid detergent fiber; [4]NDF = neutral detergent fiber; [5]SDF = soluble dietary fiber; [6]IDF = insoluble dietary fiber; [7]TDF = total detergent fiber; [8]OM = organic matter; [9]CP = crude protein.

Table 1-7. Monosaccharide composition of soluble and insoluble non-starch polysaccharides and total dietary fiber content in corn distillers dried grains with solubles (DDGS), full fat rice bran, and wheat middlings, % DM basis

Fiber fraction	Corn DDGS		Full fat rice bran	Wheat middlings
	Pedersen 2014	Jaworski 2015	Casas 2019	Jaworski 2015
S-NCP[1]	3.00	3.40	0.87	1.20
Arabinose	0.70	0.90	0.20	0.20
Xylose	0.60	0.90	0.01	0.20
Mannose	0.60	0.70	0.05	-
Galactose	0.30	0.20	0.22	0.20
Glucose	0.30	0.30	0.17	0.20
Uronic acids	0.50	0.40	0.22	0.30
I-NCP[2]	18.50	15.80	8.98	22.70
Arabinose	5.50	4.30	3.13	7.00
Xylose	7.10	6.20	3.37	11.40
Mannose	1.10	1.20	0.28	0.30
Galactose	1.20	1.10	0.80	0.50
Glucose	2.50	1.80	0.60	2.30
Uronic acids	1.10	1.20	0.80	1.20
Cellulose	6.70	5.80	4.39	6.70
Total NSP[3]	28.30	25.00	14.24	30.70
Lignin	2.50	3.90	5.84	7.30
SDF[4]	3.10	3.40	0.87	1.20
IDF[5]	27.70	25.50	19.21	36.90
TDF[6]	30.80	28.90	20.08	38.10
A/X ratio[7]	0.81	0.73	0.99	0.62
UA/X ratio[8]	0.21	0.23	0.30	0.13
IDF/TDF[9]	0.90	0.88	0.96	0.97

[1]S-NCP = soluble non-cellulosic polysaccharides; [2]I-NCP = insoluble non-cellulosic polysaccharides; [3]Total NSP = total non-starch; polysaccharides; [4]SDF = soluble dietary fiber; [5]IDF = insoluble dietary fiber; [6]TDF = total dietary fiber, the sum of total NSP and lignin; [7]A/X ratio = arabinose to xylose ratio; [8]UA/X ratio = uronic acids to xylose ratio; [9]IDF/TDF = insoluble dietary fiber to total dietary fiber ratio.

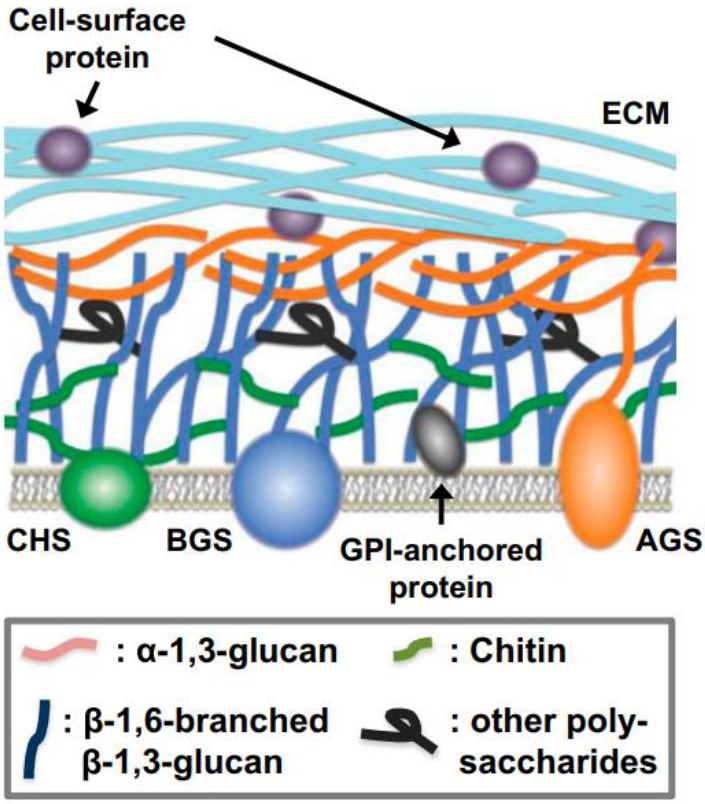

Figure 1-4. Schematic illustration of cell wall architecture in *Aspergillus* species (adapted from Yoshimi et al., 2016). AGS = α-1,3-glucan synthase; BGS = β-1,3-glucan synthase; CHS = chitin synthase; and ECM = extra cellular matrix.

Table 1-8. Products and commercial applications of *Aspergillus oryzae* (modified and summarized from Daba et al., 2021)

Product	Application	References
Dry lyophilized powder of *A. oryzae*	Probiotic; functional feed additive	Lee et al., 2006; Zamanizadeh et al., 2021
A. oryzae fermentation extract	Postbiotic; functional feed additive for ruminants and monogastric animals	Wiedmeier et al., 1987; Kellems et al., 1990; Nickles and Relling, 2017; Zhu et al., 2020
A. oryzae fermented soybean meal	Feed ingredient for pigs with improved nutritional quality	Feng et al., 2007b; Liu et al., 2007
Phytase	Feed additive to increase phosphorus digestibility	Torrallardona et al., 2012; Almeida et al., 2013; Nasir et al., 2014
A. oryzae mycelia	Fermented foods industry (miso, shoyu, tane-koji, douche, bean curd curd seasoning, vinegar)	Taylor and Richardson, 1979; Yasui et al., 2020
Amylases (α-amylases, β-amylases, and glucoamylases)	Food industry (Produced glucose during the initial stage of starch hydrolysis)	Balakrishnan et al., 2021
Lipase	Laundry detergent	Christensen et al., 1988; Machida et al., 2008
Cellulases	Pollution treatment; animal feed, food industry; textile	Bhat, 2000
Pectinases	Juice and beverage processing, vegetable oil extraction and other food industries	Pinheiro et al., 2017
β-galactosidase	Food and dairy industries	Furlan et al., 2000
Kojic acid	Antioxidant, whitening agent in cosmetics	Lobato et al., 2020

Table 1-9. Major secondary metabolites produced by *Aspergillus oryzae* (adapted from Daba et al., 2021)

Metabolite	Chemical class	Bioactivity and uses
Kojic acid	Carboxylic acid	Antimicrobial, pain killer; antioxidant; flavor enhancer, insecticide activator; has melanoma genes inhibitory activity; used in skin whitening, and UV protecting products; used as iron chelator
Glutamic acid	Carboxylic acid	Protein synthesis; food additive and flavor enhancer; anticancer agent
L-Malate	Carboxylic acid	Used in food and beverage industries
Penicillin	β-lactam antibiotics	Antimicrobial agent
Isocoumarins	Lactones	Anticancer activities
3-Nitropropionic acid	Carboxylic acid	Neurotoxic; mitochondrial inhibitor
Pyridoxine (vitamin B6)	Vitamin	Treat or prevent vitamin B6 deficiency; treat anemia; prevent or treat a certain nerve disorder
Cyanocobalamine	Vitamin	Prevent and treat vitamin B12 deficiency
Aspergillic acid	Carboxylic acid	Antimicrobial and antihypertensive agent
Mutaaspergillic acid	Carboxylic acid	Growth inhibitor against hiochi-bacteria
Aspergillomarasmine A	Polyamino acid	Metallo-β-lactamase Inhibitor
Aspirochlorin	Halogenated spiro compound	Antifungal and antibacterial activities
Asperfuran	Dihydrobenzofuran	Antifungal and anticancer activities
Tocopherols	Phenol	Antioxidant
Sporogen AO1	Sesquiterpene	Antifungal, antimalarial activities
Phomenone	Sesquiterpene	Stimulate pro-inflammatory responses in murine cells

Table 1-10. Studies that reported beneficial effects of *Aspergillus oryzae* postbiotic in ruminant diets

Studies	Species	Dose	Diet	Response
Niver et al., 1973	lamb	900 mg/kg	Corn, orchard grass	Increased apparent digestibility of acid detergent fiber by 12%
Wiedmeier et al., 1987	cow	2.63 g/d	Alfalfa hay, barley, straw, wheat bran	Increased digestibility of dry matter, crude protein, hemicellulose; Increased cellulolytic bacteria by 27%
Judkins and Stobart, 1988	lamb	35 g/d	Alfalfa hay, corn	Increased digestibility of neutral detergent fiber
Fondevila et al., 1990	sheep	2 g/d	Barley straw	Increased the initial rate of straw degradation; Increased total culturable bacteria
Kellems et al., 1990	lactating cow	3 g/d	Alfalfa silage, corn, barley, cottonseed	Increased milk flow and fat-corrected milk production during the latter stages of the trial
Gomez-Alarcon et al., 1990	cow	3 g/d	Alfalfa, cottonseed hull, soybean meal	Increased rumen and total tract digestibility of dry matter and neutral detergent fiber; Increased rate of rumen fermentation of alfalfa but not of milo or wheat straw
Beharka et al., 1991	calf	0.5, 1, or 3 g/d	Corn, soybeans, soybean hull, oat	Increased total short-chain fatty acids, propionate, and acetate in the rumen; increased hemicellulolytic and pectinolytic bacterial
Gomez-Alarcon et al., 1991	lactating cow	3 g/d	40% forage, 60% concentrate	Increased digestibility of dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber; Increased milk yield
Newbold et al., 1992	sheep	2 g/d	Hay, barley, molasses	Increased total and cellulolytic bacterial numbers in rumen by 34% and 90%, respectively
Caton et al., 1993	steer	2 g/d	Pasture	Increased forage intake
Mathieu et al., 1996	sheep	3 g/d	Hay, barley, soybean meal	Increased total bacteria number

Yoon and Stern, 1996	cow	3 g/d	Corn silage, alfalfa hay, corn grain, soybean meal	Increased proteolytic and cellulolytic bacterial number
Chiou et al., 2000	cow	3 g/d	Hay, soybean meal, corn, wheat bran	Increased digestibility of rice distillers grain and some beancurd pomance
Chiou et al., 2002	lactating cow	3 g/d	Alfalfa hay, corn silage, oat hay, soybean	Increased dry matter intake and milk
Di Francia et al., 2008	calf	6 g/kg	Maize gran, soybean meal, wheat middlings, molasses	Increased average daily gain and total tract digestibility of fiber
Zerby et al., 2011	lamb and steer	1 g/d	Corn, soybean hull, soybean meal, alfalfa	Improved gain to feed ratio
Sun et al., 2017	cow	5 g/d	Corn silage, grass hay, alfalfa hay, DDGS	Increased body weight gain and total short-chain fatty acids
Nickles and Relling, 2017	cattle	1.5 or 3 g/d	Corn silage, DDGS, whole shelled corn	Linearly improved body weight, average daily gain, and gain to feed ratio on the first week of feeding
Sucu et al., 2019	cow	15 g/d	Alfalfa hay, cotton seed	Increased milk production

Table 1-11. Studies that reported no beneficial effects of *Aspergillus oryzae* postbiotic in ruminant diets

Studies	Species	Dose	Diet	Response
Oellermann et al., 1990	cattle	1, 2, 4, 6 g/d	Alfalfa hay, barley, straw, wheat bran	No effects on digestibility of crude protein and acid detergent fiber; No differences in the number of aerobic and anaerobic fungi
Firkins et al., 1990	heifer	7.5 g/head/d	Orchard grass hay, corn, soybean hull	No effects in digestion of fiber component
Galloway et al., 1991	steer	0.08%	Bermudagrass hay and corn supplement	No effects in dry matter and neutral detergent fiber intake and digestion
Denigan et al., 1992	lactating cow	0, 1.5, 3, 6 g/d	Alfalfa hay, cottonseed, concentrate	No effects in digestibility of crude protein, neutral detergent fiber, and dry matter
Newbold et al., 1992	sheep	2 g/d	Grass hay and barley	No differences in numbers of cellulolytic bacteria and ciliate protozoa were unchanged
Sievert and Shaver, 1993a	lactating cow	3 g/d	Alfalfa silage, corn, dried beet pulp, soy hulls	No effects in dry matter intake, milk yield, or nutrient digestion
Sievert and Shaver, 1993b	lactating cow	3 g/d	Alfalfa silage, corn, soy hull, soybean meal	No effects in dry matter intake, milk production, short-chain fatty acids or total tract apparent digestibility of nutrient
Higginbotham et al., 1993	lactating cow	3 g/d	Alfalfa silage, hay, corn silage, barley	No differences in actual milk, 3.5% fat-corrected milk production or short-chain fatty acid production
Higginbotham et al., 1994	lactating cow	3 g/d	Alfalfa silage, hay, corn silage, barley	No differences in actual milk, 3.5% fat-corrected milk, or percentages of milk fat or protein

Varel and Kreikemeier, 1994	cattle	3, 9, or 27 g/d	Bromegrass hay	No effects in degradation of cell walls, cellulose, or hemicellulose; No differences in total ruminal anaerobic or cellulolytic bacteria
Bertrand and Grimes, 1997	cow	3 g/d	Corn silage, alfalfa hay, corn grain, soybean meal	No effects in fiber digestion or milk production
Jouany et al., 1998	sheep	3 g/d	Grass hay, barley, soybean meal	No effects on digestion of organic matter, neutral detergent fiber, or acid detergent fiber
Chiou et al., 2000	cow	3 g/d	Hay, soybean meal, corn, wheat bran	No effects on soybean meal for digestibility of dry matter, organic matter, acid detergent fiber and neutral detergent fiber
Higginbotham et al., 2004	lactating cow	5 g/d	Alfalfa hay, silage, corn silage	No effects on lactation performance and rumen parameters (milk yield, rumen pH, rumen concentration of short-chain fatty acid)
Yohe et al., 2015	calf	2 g/d	Milk replacer	No effects on calf growth, intake, and ruminal or health measurements
Zicarelli et al., 2016	goat	20 g/d	Pasture	No effects on milk yield

Table 1-12. Definition of probiotic, prebiotic, and postbiotic by the International Scientific Association for Probiotics and Prebiotics (ISAPP)

Category	Reference	Definition	Type (component)
Probiotic	Hill et al., 2014	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.	Bacillus, lactic acid-producing bacteria, and yeast
Prebiotic	Gibson et al., 2017	A substrate that is selectively utilized by host microorganisms conferring a health benefit.	Non-digestible fructans (fructooligosaccharides, oligofructose and inulin) and galactans (galactooligosaccharides and transgalacto-oligosaccharides), human milk oligosaccharides, and polyphenols
Postbiotic	Salminen et al., 2021	A preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.	Inanimate microbial cells, cell fragments, and metabolites (e.g., organic acid, peptides, enzymes, secreted proteins, bacteriocins)

Chapter 2. Effects of *Aspergillus oryzae* Postbiotic on Dietary Energy and Nutrient Digestibility of Growing Pigs

Summary

The objective of this study was to determine the effects of *Aspergillus oryzae* postbiotic (AOP) on nutrient digestibility in growing pigs fed high fiber diets. Eighteen growing barrows (initial body weight = 50.6 ± 4.9 kg) were surgically equipped with a T-cannula at the distal ileum. Corn and soybean meal-based diets were formulated with fiber from cereal grain coproducts corn (distillers dried grains with solubles, DDGS), rice (rice bran, RB) or wheat (wheat middlings, WM) to meet or exceed all nutrient requirements for 50 to 75 kg growing pigs. Three additional diets were formulated to contain 0.05% AOP supplemented at the expense of corn in the DDGS diet (DDGS+AOP), RB diet (RB+AOP), and WM diet (WM+AOP). All diets contained 0.5% of titanium dioxide as an indigestible marker. Pigs were allotted randomly to a triplicated 6 × 2 Youden square design with six diets and two successive periods. Ileal digesta and fecal samples were collected for 2-d after a 21-d adaptation period, and dry matter (DM), gross energy (GE), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and ash were analyzed to calculate apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD). Standardized ileal digestibility (SID) of amino acids (AA) was calculated by correcting AID with basal endogenous AA losses from the same set of pigs. Pigs fed the DDGS+AOP diet had greater ($P < 0.05$) AID of EE compared with those fed the DDGS diet. However, supplementation of AOP did not ($P > 0.05$) affect AID of GE, DM, CP, NDF, ash or SID of AA of any high fiber diet. Supplementation of 0.05% AOP increased ($P < 0.05$) ATTD of DM, GE, CP, NDF, and ash in DDGS, RB, and WM diets.

Diet digestible energy was 35 kcal/kg greater ($P < 0.05$) in pigs fed AOP supplemented diets compared with those fed diets without AOP. In conclusion, supplementation of AOP increased ATTD of nutrients and energy value in high fiber diets containing DDGS, RB, or WM.

Introduction

Large quantities of coproducts of biofuel and food industry, such as distillers dried grains with solubles (DDGS), wheat middlings (WM), and rice bran (RB) are available to be used in swine feeds to reduce feed cost (Cromwell et al., 1999; Stein and Shurson, 2009; G. A. Casas and Stein, 2016) and increase upcycle of nutrients to produce high value protein (Shurson, 2020). However, dietary fiber, especially from DDGS, WM, and RB, is generally not well utilized by monogastric animals due to the lack of fiber-degrading enzymes (Agyekum and Nyachoti, 2017; Zeng et al., 2018). Therefore, effective ways are needed to enhance fundamental elements of gastrointestinal function such as the digestibility and utilization efficiency of energy and nutrients of high fiber coproducts in pigs.

Pigs fed *Lactobacillus* based postbiotic had improved growth performance during an *Escherichia coli* challenge from improvement in gut health (Xu, 2021). Fundamental elements of gut health are better defined by the functions of the gastrointestinal tract that include nutrient digestion and absorption, absence of illness, normal microbiota, effective immune system, and effective sensing and signaling (Bischoff, 2011). Some of these functions are modulated by postbiotics which can contain fungi cell-wall carbohydrates, enzymes, and metabolites in the fermentation media (Amirdahri et al., 2012; Mano et al., 2018). These constituents of *Aspergillus oryzae* and other postbiotic products may

enhance diverse aspects of gastrointestinal functions by a variety of mechanism that are currently under investigation but include modulation of resident microbiota, enhancement of epithelial functions, modulation of immune responses, and modulation of signaling via nervous system (Gibson et al., 2017; Salminen et al., 2021).

Aspergillus oryzae postbiotic (AOP) has been used as a feed additive in ruminant diets to increase nutrient digestibility through enhanced fiber degradation (Newbold et al., 1991; Chang et al., 1999; Newbold, 2007). Conversely, studies on the potential benefits of supplementing AOP in swine diets are very limited. No beneficial effects on performance of lactating sows fed a corn soybean-meal diets supplemented with AOP were observed by Jackson et al. (2006), but Moeller and Hess (2016) reported that sows fed AOP supplemented diets weaned more piglets during lactation. Both studies in sows did not test effects of AOP fed high fiber ingredients and no studies have been conducted to evaluate the effect of AOP supplementation on energy and nutrient digestibility in growing pigs fed high fiber diets.

Corn, wheat, and rice are the common cereal grains globally produced and their coproducts contain high levels of neutral detergent fiber (NDF): corn DDGS (30.46%), WM (34.97%), and full fat RB (26.28%). All three coproducts have similar arabinose to xylose ratio (A:X) but the soluble arabinoxylan content in corn-DDGS, WM and RB are different (4.80%, 45.22%, and 3.13% respectively). The efficacy of non-starch polysaccharide degrading enzymes was less in corn DDGS that in WM when fed to growing pigs (Zeng et al., 2018). Therefore, we hypothesized that supplementing AOP in growing pigs fed diets with fibrous ingredients will increase energy and nutrient digestibility and the magnitude of the response to AOP may depend on the fiber source.

Therefore, the objective of this study was to determine the effects of supplementing AOP to growing pigs fed diets containing cereal grain based fibrous ingredients that vary in composition and characteristics of fiber on energy and nutrient digestibility.

Materials and Methods

The animal use protocol (#1805-35983A) was reviewed and approved by the Institution Animal Care and Use Committee at University of Minnesota.

Animals and diets

Eighteen growing barrows (initial body weight = 50.6 ± 4.9 kg) were surgically equipped with a T-cannula at the distal ileum (Stein et al., 1998). Pigs were housed individually in metabolism cages (198 × 84 × 71 cm) at the Southern Research and Outreach Center of University of Minnesota in Waseca, MN. Corn DDGS, WM, and RB were obtained, and samples were collected and analyzed for chemical composition (Table 2-1). Three corn and soybean meal-based diets were formulated to contain 29.65% corn-DDGS, 36.65% full fat RB or 24.59% WM and meet or exceed all nutrient requirements for 50 to 75 kg growing pigs (NRC, 2012). Three additional diets were formulated to contain 0.05% AOP (the fermentation extract of a specific strain *Aspergillus oryzae* NRRL458 in a sub-merged fermentation, Amaferm®, BioZyme Inc., St. Joseph, MO) following the manufacturer's recommendation, which was supplemented at the expense of corn in the DDGS diet (DDGS+AOP), RB diet (RB+AOP), and WM diet (WM+AOP, Table 2-2). Titanium dioxide (Sachtleben Chemie, Duisburg, Germany) was added at 0.5% to each diet as an indigestible marker. Pigs were allotted randomly to a triplicated 6 × 2 Youden square design with 6 diets and 2 successive periods. Within each period, 3 pigs received 1 of the 6 diets for a total of 6 observations per diet for the two periods.

Pigs received a daily feed allowance equivalent to 3% initial BW (Table 2-3) and were fed twice daily in two equal meals at 0800 h and 1700 h. Pigs had free access to water from nipple drinkers throughout the experiment.

Sample collection

The entire experiment was conducted using two 25-d periods for a total of 50 d. To obtain stable measurements of digestibility when feeding high fiber diets in pigs, at least 21-d are recommended to adapt pigs and their gut microflora to diets (Longland et al., 1993a; Huang et al., 2018). Therefore, a 21-d adaptation period was applied for each of the two periods. After adaptation, representative fecal samples were collected twice daily from each pig on d 22 to d 23 (period 1), and d 47 to 48 (period 2). Fecal samples from the 2-d collection in each period were pooled within pig and stored in a -20 °C freezer until further analysis. Ileal digesta samples were collected for 8 h starting at 0800 h until 1600 h on d 24 to 25 (period 1) and d 49 to 50 (period 2) using a 207 mL sealed plastic bag (Whirl-pak®, Nasco, Fort Atkinson, WI) attached to the cannula barrel. Bags were removed every 30 min or whenever full. Ileal digesta samples were pooled into 1-L wide-mouth high density polyethylene bottles (Fisher Scientific Company, Ottawa, ON) and stored at –20°C immediately to prevent bacterial fermentation until further analysis. One pig fed the DDGS+AOP diet became ill when samples were collected in period 2 and consequently was removed from the experiment.

Chemical analyses

At the conclusion of the experiment, frozen ileal digesta samples were thawed at room temperature and then mixed thoroughly, sub-sampled and lyophilized. Frozen feces were thawed and dried in a forced-air oven at 65°C for 72 h. Ingredients, diets, dried ileal

digesta and feces were ground to pass through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Samples were analyzed using AOAC (2007) methods for dry matter (DM, method 934.01), crude protein (CP, method 990.03), ether exact using Soxhlet apparatus and petroleum ether (EE, method 920.39), and ash (method 942.05). Crude protein was analyzed using Kjeldahl method with a LECO FP-528 analyzer (St. Joseph, MI, USA). Neutral detergent fiber was analyzed using filter bags and ANKOM^{200} fiber analyzer (Ankom Technology, Macedon, NY) following the procedure described by Van Soest et al. (1991) with minor modifications. The concentration of NDF was analyzed using heat stable α-amylase and sodium sulfite without correction for insoluble ash. Amino acid concentrations in ingredients, diets and ileal digesta were analyzed with a Hitachi Amino Acid Analyzer (Model L8800; Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for post-column derivatization and norleucine as the internal standard (Method 982.30 E [a, b, c]; AOAC 2006). The gross energy (GE) content in all samples was determined using an isoperibol bomb calorimeter (model 6400; Parr Instrument Co., Moline, IL). Benzoic acid (6,318 kcal GE/kg) was used as the standard for calibration. The concentration of titanium dioxide in diets, ileal digesta and feces was determined photometrically according to the technical procedure described by Myers et al (2004).

Calculations and statistical analysis

The apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of GE, DM, CP, EE, ash, and NDF were calculated based on the following equation (Stein et al., 2007):

$$\text{AID or ATTD, \%} = [1 - (N_{\text{digesta or feces}}/N_{\text{diet}}) \times (M_{\text{diet or feces}}/M_{\text{digesta}})] \times 100,$$

where $N_{digesta\ or\ feces}$ and N_{diet} are the nutrient concentrations (g/kg) in digesta/feces and diet DM, respectively, and M_{diet} and $M_{digesta/feces}$ are the titanium dioxide concentrations (g/kg) in diet and digesta/feces DM, respectively.

The standardized ileal digestibility (SID) of AA was calculated using the following equation (Stein et al., 2007). The basal endogenous AA losses were determined with the same set of pigs by feeding a N-free diet and described previously (Fung et al., 2019).

SID (%) = [AA intake − (ileal AA outflow−basal IAA_{end})/AA intake] × 100%,

where basal IAA_{end} is the basal endogenous loss of an AA (g/kg DM intake).

The hindgut fermentation of nutrients was calculated using the following equation (Chen et al., 2013):

Hindgut fermentation (%) = ATTD – AID.

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS institute Inc., Cary, NC) with AOP, diet, AOP × diet, and period as fixed effects. Pig was considered as the random effect. The outliers were identified using interquartile range method (IQR). Values that are more than 1.5 IQR below the first quartile or more than 1.5 IQR above the third quartile are considered outliers and were therefore removed. Two negative values of ATTD of EE in WM group and one extremely low value of ATTD of EE in WM+AOP group were removed from the calculation. The experimental unit was individual pig. The LSMEANS procedure was used to calculate means and PDIFF option was used to separate least squares means with a Tukey-Kramer adjustment. A value of $P < 0.05$ was considered statistically significant, and $P < 0.1$ was considered a trend.

Results and Discussion

Nutrient content in ingredients and diets

The NDF content in corn DDGS (Table 2-1) was 28.35%, which is in agreement with previously reported ranges of 20.1- 32.9% by Stein and Shurson (2009) and 17.95 - 43.66% by Zeng et al., (2017) , but was less than the value (33.75%) from NRC (2012). The NDF content of WM was 28.86% and in agreement with the values reported by Jha et al. (2012), but less than values reported by Jong et al. (2014) and Zeng et al. (2018). The RB used in the present study had a NDF content of 10.21%, which is less than values reported in previous studies in China and North America which ranges between 17.17% and 33.90% (Li et al., 2018; Casas et al., 2019). These differences were expected because chemical composition of high fiber coproducts varies widely among parent grain varieties and regions, and processing methods could also contribute to variation in chemical composition (Shi et al., 2015). Consistent with the NDF content in the ingredients, DDGS diets had the greatest NDF followed by WM diets, and RB diets had the lowest NDF content (Table 2-2). The EE and ash content in RB were the highest followed by those of corn-DDGS and WM, which was also reflected in the diets.

Ileal digestibility of nutrients

The AID of GE and DE value were greater ($P < 0.05$) in diets with RB compared with corn-DDGS and WM. However, the AID of GE in diets with AOP were not different from those without AOP. There were no differences in the AID of DM or CP among diets with DDGS, RB, or WM or among diets with and without AOP, but there was a significant interaction ($P < 0.01$) between diet type and AOP treatment for AID of EE. Pigs fed DDGS+AOP diet had greater ($P < 0.05$) AID of EE compared with those

fed DDGS diet, there were no differences observed when RB or WB were compared with their corresponding AOP supplemented groups (Table 2-4).

The SID of AA was calculated by correcting AID with basal endogenous AA losses from the same set of pigs (Table 2-5). The SID of Arg, His, Ile, Lys, Asp, and Cys was greater ($P < 0.05$) in pigs fed RB or WM compared with those fed DDGS. The SID of Trp in pigs fed RB was greater ($P < 0.05$) compared with those fed DDGS or WM but did not differ between DDGS and WM diets. None of the SID of AA were affected ($P > 0.05$) by supplementing AOP in any of the high fiber diets.

Apparent total tract digestibility of nutrients

The ATTD of GE, DM, CP, NDF and ash were greater ($P < 0.05$) in pigs fed diets with AOP compared with those fed diets without AOP (Table 2-4). The greatest improvement of ATTD was observed for ash in WM+AOP diet, which was 12.29% greater compared with the WM diet. The magnitude of improvement in ATTD of GE and DM tended to be greatest (AOP × diet interaction, $P < 0.10$) when AOP was supplemented in WM than in RB or DDGS diets. The digestible energy content of the diets increased by 63, 32, and 10 kcal/kg in pigs fed DDGS+AOP, RB+AOP, and WM+AOP compared with those fed the corresponding diets without AOP, respectively. It should be noted that GE in WM was 98 kcal/kg less than WM+AOP, which indicates that the actual improvement of DE in pigs fed WM+AOP should be larger than observed if diets were adjusted to the same GE level. The increase in ATTD of GE and DM in WM was mainly a result of an increase in ATTD of CP, in which the magnitude of improvement was greatest (AOP × Diet interaction, $P < 0.10$) in diets with WM

compared with RB or DDGS diets. The ATTD of EE ($P > 0.05$) was not affected by including AOP in the diets.

These findings suggest that AOP may be effective for improving the nutritional value of fibrous ingredients fed to swine. With feed cost representing the greatest expense of pork production, coproducts of biofuel and food industry are attractive for decreasing diet cost (Kerr and Shurson, 2013). However, most of these coproducts have relatively high concentrations of fiber, which monogastric animals have very limited ability to utilize energy from these fibers (Bach Knudsen et al., 2016). Therefore, effective feed additives that enhance the utilization efficiency of fiber and other nutrients of high-fiber ingredients in pigs are needed.

In the present study, a greater magnitude of improvement in nutrient digestibility by supplementing AOP was observed in WM diets compared with those in DDGS or RB diets. These results are in agreement with study by Zeng et al. (2018) where they observed that supplementing exogenous enzymes significantly increased *in vitro* digestibility of DM and GE in WM but the effects of those enzymes on corn-DDGS were negligible. Differences in the compositional complexity among these coproducts and their corresponding parent grains are the most likely reason for the different responses to AOP treatment. Despite similar arabinoxylan content and arabinose to xylose ratio, corn-DDGS contains a greater fraction of insoluble arabinoxylan compared with WM (Pedersen et al., 2014). In addition, the concentration of diferulates in the insoluble dietary fiber fraction in corn is approximately 5-7 times greater than found in wheat (Bunzel et al., 2001). Compared with WM, rice coproducts have a much greater arabinose to xylose ratio and insoluble arabinoxylan fraction (Annison et al., 1995; Casas

et al., 2019). In addition, the greater proportion of cellulose in fiber of corn-DDGS and RB compared with WM could also impair digestibility of fiber and other nutrients, because the apparent total tract digestibility of cellulose is much less than other components of dietary fiber in pigs (Pedersen et al., 2015). Therefore, the differential responses from adding AOP to growing pig diets are attributed mainly to composition and type (characteristic) of fiber in the corresponding ingredients.

Interestingly, supplementation of AOP increased AID of EE by 28.5% in DDGS but no similar effects were observed in RB and WM. However, caution is needed when interpreting these results because relatively large CV of AID of EE was observed in diets with relative low content of EE. The CV of AID of EE in DDGS and DDGS+AOP were 50.67% and 21.33%, respectively. Similarly, a significant increase in ATTD of minerals and numerical improvement of AID of minerals was observed in pigs fed RB diets supplemented with AOP. The reason for this increase in mineral digestibility in unknown but we speculate that it could be due to 1) breakdown of fiber matrix which release mineral from the matrix. When the fiber matrix is broken down, nutrients, including minerals, embedded in that matrix may be released and available for absorption. The breakdown of fiber matrix is indicated by greater ATTD of NDF in pigs fed AOP compared with those fed diets without AOP observed in the present study. 2) the increased solubility of minerals resulting from more acidic digesta pH. Absorption of calcium and other minerals increase with increased solubility of minerals and decreased pH in the lumen increase solubility of minerals (e.g. Ca, P, and Mg) by preventing the formation insoluble Ca-Mg-phosphate complexes (Greenwald et al., 1940); 3) increase in gut epithelial nutrient absorption from improved intestinal barrier integrity. The intestinal

barrier plays important roles in not only facilitating the absorption of nutrients, electrolytes, and water but also limiting the transport of potentially harmful antigens and microorganisms (Vancamelbeke and Vermeire, 2017). Postbiotics can enhance epithelial barrier integrity via mechanisms mediated by secreted proteins (Yan et al., 2013; Gao et al., 2019), exopolysaccharides(Schiavi et al., 2016), and short-chain fatty acids (SCFAs; Ohata et al., 2005; Feng et al., 2018). Short-chain fatty acids are the end-production of fiber in hindgut of pigs, including lactate, acetate, propionate, and butyrate (Lindberg, 2014). An increase in total SCFAs was reported by Newbold et al. (1991) when they tested the effects of AOP on the rumen fermentation *in vitro*. To the best of our knowledge, no previous experiments have evaluated effects of AOP on digestibility of fibrous ingredients in pigs. However, it has been observed before that supplementation of prebiotics is capable to modify the ability of microbes to degrade fiber in the hindgut of pigs (Mountzouris et al., 2006).

To the best of our knowledge, this is the first report to investigate the impact of adding AOP on nutrient digestibility in swine and it is not the objective of the present study to investigate the mechanism of action of AOP in swine. In fact, the mechanisms of actions of postbiotics are far from well understood compared with those of prebiotics and probiotics (Bermudez-Brito et al., 2012; Davani-Davari et al., 2019; Salminen et al., 2021). Therefore, it is still difficult to know the specific mechanism of action of AOP in pigs. Our data in the present study provided possible clues to the mode of action of AOP and several postulated mechanisms of probiotics may also apply for AOP that maybe useful for further investigation. Firstly, AOP may modulate the gut microbiota which results in enrichment of beneficial microbes such as *Lactobacillus and Bifidobacterium*.

Several oligosaccharides, including fructooligosaccharides and mannoligosaccharides can be synthesized in *Aspergillus oryzae* fermentation (Guío et al., 2009; Amirdahri et al., 2012; Mano et al., 2018). These oligosaccharides can selectively stimulate the growth of beneficial bacteria, including *Lactobacillus and Bifidobacterium* in pigs (Duan et al., 2016; Liu et al., 2020), and therefore exert beneficial effects on gut health of pigs. Secondly, postbiotics can enhance epithelial barrier integrity via activities mediated by secreted proteins (Yan et al., 2013; Gao et al., 2019), exopolysaccharides (Schiavi et al., 2016), and SCFAs (Ohata et al., 2005; Feng et al., 2018). Xu et al (2021) reported enhanced intestinal health and improved growth performance in nursery pigs fed diets with a postbiotic from *Lactobacillus* fermentate.

Conclusions

In conclusion, this experiment is the first to demonstrate the postbiotics improve aspects of gastrointestinal functions such as enhanced nutrient digestibility and absorption. The magnitude of the impact of AOP is ingredient- and diet-dependent. The mode of action of AOP is not fully understood, and further studies are needed to elucidate the mechanisms by which AOP increases nutrient digestibility in pigs.

Table 2-1. Analyzed chemical composition of test feed ingredients (as-fed basis).

Items	Corn	Soybean meal	DDGS[1]	Wheat middlings	Full fat rice bran
Gross energy, kcal/kg	3,943	4,234	4,492	4,053	4,621
Dry matter, %	88.51	89.73	89.56	90.20	94.35
Crude protein, %	6.92	46.45	27.00	16.49	13.52
Ether extract, %	1.26	1.82	5.03	3.24	16.94
Crude fiber, %	1.65	3.80	8.00	6.80	5.71
NDF[2], %	8.29	7.72	28.35	28.86	10.21
Ash, %	1.18	6.65	5.18	7.11	12.96
Indispensable AA					
Arg	0.34	3.47	1.21	1.02	1.03
His	0.21	1.23	0.81	0.44	0.39
Ile	0.25	2.30	1.07	0.55	0.48
Leu	0.80	3.68	2.97	1.03	0.92
Lys	0.26	3.07	1.04	0.69	0.68
Met	0.14	0.64	0.53	0.24	0.26
Phe	0.34	2.45	1.23	0.67	0.58
Thr	0.25	1.85	1.06	0.52	0.49
Trp	0.04	0.56	0.17	0.20	0.16
Val					
Dispensable AA					
Ala	0.50	2.08	1.96	0.76	0.80
Asp	0.48	5.38	1.79	1.11	1.21
Cys	0.16	0.69	0.61	0.34	0.31
Glu	1.21	8.57	4.13	3.32	1.80
Gly	0.28	2.01	1.15	0.82	0.73
Pro	0.55	2.36	2.10	1.10	0.56
Ser	0.31	2.18	1.24	0.63	0.55
Tyr	0.21	1.70	0.95	0.44	0.43
Total AA	6.91	47.13	26.18	14.96	12.45

1DDGS = corn distillers dried grains with solubles.

[2]NDF = neutral detergent fiber.

Table 2-2. Composition and nutrient levels of experimental diets formulated with corn distillers dried grains with solubles (DDGS), wheat middlings, or full-fat rice bran with or without the supplementation of 0.05% *Aspergillus oryzae* postbiotic (AOP) (as-fed basis)

Item	DDGS		Rice bran		Wheat middlings	
	-AOP	+AOP	-AOP	+AOP	-AOP	+AOP
Ingredient, %						
Corn, yellow dent	44.91	44.86	38.86	38.81	48.52	48.47
Soybean meal	22.94	22.94	22.27	22.27	24.13	24.13
Corn DDGS	29.65	29.65	-	-	-	-
Wheat middlings	-	-	-	-	24.59	24.59
Rice bran	-	-	36.65	36.65	-	-
Monocalcium phosphate	0.30	0.30	0.17	0.17	0.56	0.56
Limestone	1.20	1.20	1.05	1.05	1.20	1.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50
AOP	-	0.05	-	0.05	-	0.05
Calculated nutrient content, %						
DE, kcal/kg	3,442	3,440	3,283	3,281	3,303	3301
ME, kcal/kg	3,287	3,285	3,150	3,148	3,170	3168
NE, kcal/kg	2,374	2,372	2,339	2,337	2,319	2319
Standardized ileal digestible amino acid, %						
Lys	0.85	0.85	0.85	0.85	0.85	0.85
Met	0.34	0.34	0.27	0.27	0.27	0.27
Met + Cys	0.64	0.64	0.53	0.53	0.55	0.55
Thr	0.67	0.67	0.58	0.58	0.58	0.58
Trp	0.20	0.20	0.20	0.20	0.21	0.21
Analyzed nutrient content, %						
Gross energy, kcal/kg	4,061	4,118	4,116	4,152	3974	3876
Dry matter	88.98	89.90	90.99	91.51	89.76	88.66
Crude protein	21.60	21.29	18.51	18.70	19.08	18.78
Ether extract	2.09	2.41	5.95	6.26	1.41	1.11
Neutral detergent fiber	14.50	13.73	9.79	10.47	13.28	13.33
Ash	6.15	6.02	8.47	8.69	6.37	6.74

[1]The premix provided the following per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 30 IU; vitamin K3, 3 mg; vitamin B12, 0.012 mg; riboflavin, 4 mg; niacin, 40 mg; pantothenic acid, 15 mg; choline chloride, 400 mg; folic acid, 0.7 mg; thiamin, 1.5 mg; pyridoxine, 3 mg; biotin, 0.1 mg; Zn, 105 mg; Mn, 22 mg; Fe, 84 mg; Cu, 10 mg; I, 0.50 mg; Se, 0.35 mg.

Table 2-3. Initial body weight of pigs and daily feed allowance in each phase[1,2]

Pig ID	Phase 1			Phase 2		
	Diet	Initial body weight, kg	Daily feed allowance, kg	Diet	Initial body weight, kg	Daily feed allowance, kg
1	Rice bran+AOP	49.44	1.48	Rice bran	68.04	2.04
2	Wheat middlings	52.16	1.56	DDGS	74.84	2.25
3	Wheat middlings	49.44	1.48	DDGS + AOP	69.85	2.10
4	DDGS+AOP	58.51	1.76	DDGS	83.91	2.52
5	Wheat middlings+AOP	44.00	1.32	Rice bran	64.41	1.93
6	DDGS+AOP	45.36	1.36	Wheat middlings	64.41	1.93
7	Rice bran	45.36	1.36	DDGS+AOP	59.87	1.80
8	Rice bran	54.43	1.63	Rice bran+AOP	73.94	2.22
9	Rice bran+AOP	52.62	1.58	Wheat middlings	73.94	2.22
10	Rice bran	59.87	1.80	Wheat middlings+AOP	77.56	2.33
11	DDGS	49.44	1.48	Rice bran+AOP	70.76	2.12
12	Rice bran+AOP	44.45	1.33	DDGS	63.96	1.92
13	Wheat middlings	57.15	1.71	Rice bran	81.65	2.45
14	Wheat middlings+AOP	54.43	1.63	Wheat middlings	74.84	2.25
15	Wheat middlings+AOP	51.26	1.54	Rice bran+AOP	73.94	2.22
16	DDGS	49.90	1.50	DDGS+AOP	71.21	2.14
17	DDGS+AOP	48.99	1.47	Wheat middlings+AOP	65.77	1.97
18	DDGS	44.00	1.32	Wheat middlings+AOP	63.50	1.91

[1]Pigs were allotted randomly to a triplicated 6 × 2 Youden square design with 6 diets and 2 successive periods and received 1of 6 diets, including corn distillers dried grains with solubles (DDGS), DDGS+ *Aspergillus oryzae* postbiotic (AOP), rice bran, rice bran+AOP, wheat middlings, and wheat middlings+AOP.

[2]The daily feed allowance was calculated as 3% initial body weight (kg) at the beginning of each phase.

Table 2-4. Effect of *Aspergillus oryzae* postbiotic (AOP) on apparent ileal digestibility (AID), hindgut disappearance (HGD), and apparent total tract digestibility (ATTD) of energy (kcal/kg) and nutrients (%) in diets with corn distillers dried grains with solubles (DDGS), rice bran, and wheat middlings[1,2]

Items	DDGS		Rice bran		Wheat middlings		SEM	*P* value		
	-AOP	+AOP	-AOP	+AOP	-AOP	+AOP		AOP	Diet	AOP × Diet
AID										
GE[3]	63.44	64.21	66.82	67.41	63.40	66.59	1.07	0.12	0.04	0.46
DE[4], kcal/kg	2,577	2,645	2,750	2,799	2,519	2,581	43.29	0.14	< 0.01	0.97
Dry matter	60.74	61.41	62.89	62.88	60.96	64.72	1.24	0.15	0.26	0.28
CP[5]	71.96	73.10	74.11	72.91	73.43	74.54	1.35	0.74	0.58	0.62
NDF[6]	25.25^{a}	17.80^{a}	-7.07^{b}	2.05^{b}	22.79^{a}	30.07^{a}	3.18	0.30	< 0.01	0.09
Ether exact	28.20^{b}	56.70^{a}	72.43^{a}	76.72^{a}	33.41^{b}	23.20^{b}	4.29	0.07	< 0.01	0.007
Ash	22.25	22.57	24.60	22.51	22.69	29.69	2.63	0.44	0.45	0.29
HGD										
GE[3]	18.01	17.56	13.85	13.59	17.21	15.95	1.25	0.54	0.03	0.92
DE[4], kcal/kg	731.74	723.43	569.93	563.89	683.86	618.41	51.03	0.54	0.04	0.82
Dry matter	21.39	21.00	15.49	15.86	20.41	18.85	1.26	0.63	< 0.01	0.76
CP[5]	12.06	11.25	8.58	8.98	11.71	13.25	1.35	0.73	0.05	0.69
NDF[6]	33.81	46.61	52.32	51.14	28.09	24.71	4.77	0.48	< 0.01	0.23
Ether exact	-4.67	-25.54	-5.44	-6.23	-15.06	-8.89	5.30	0.31	0.29	0.12
Ash	34.07	30.31	8.15	12.76	28.83	27.21	2.68	0.90	< 0.01	0.29
ATTD										
GE[3]	81.37	81.78	80.73	80.79	80.79	83.10	0.52	0.04	0.10	0.10
DE[4], kcal/kg	3,305	3,368	3,323	3,355	3,211	3,221	20.91	0.05	< 0.01	0.44
Dry matter	82.00^{b}	82.32^{b}	78.39^{c}	78.55^{c}	81.61^{b}	84.08^{a}	0.49	0.02	< 0.01	0.05
CP[5]	84.07^{bc}	84.40^{bc}	82.65^{cd}	81.72^{d}	85.10^{b}	88.10^{a}	0.48	0.04	< 0.01	0.003
NDF[6]	56.49^{a}	60.98^{a}	47.61^{b}	54.31^{ab}	50.43^{ab}	54.39^{ab}	2.23	< 0.01	< 0.01	0.52
Ether exact	23.53^{b}	31.09^{b}	70.00^{a}	70.50^{a}	19.34^{b}	18.48^{b}	3.32	0.28	< 0.01	0.55
Ash	56.23^{a}	53.65^{ab}	32.95^{c}	36.47^{c}	44.80^{bc}	57.09^{a}	1.94	0.02	< 0.01	0.01

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). Means in a row with different letters significantly differ ($P < 0.05$).
[2]N = 5 for DDGS+AOP; otherwise, n = 6.
[3]GE = gross energy.
[4]DE = digestible energy.
[5]CP = crude protein.
[6]NDF = neutral detergent fiber.

Table 2-5. Effects of *Aspergillus oryzae* postbiotic (AOP) on standardized ileal digestibility of amino acids[1,2,3] (%)

Items	DDGS[4]		Rice bran		Wheat middlings		SEM	*P*-value		
	-AOP	+AOP	-AOP	+AOP	-AOP	+AOP		AOP	Diet	AOP × Diet
Indispensable AA										
Arg	86.61	88.01	91.13	88.64	89.40	90.11	1.02	0.86	0.05	0.15
His	81.31	82.39	86.28	85.50	85.40	85.29	1.19	0.95	0.02	0.73
Ile	77.80	79.56	83.05	81.83	81.38	80.64	1.32	0.95	0.05	0.49
Leu	81.79	84.11	82.88	81.97	82.47	82.28	1.22	0.66	0.88	0.39
Lys	77.62^{b}	79.58^{ab}	84.75^{a}	83.65^{a}	82.66^{a}	82.44^{a}	1.07	0.80	< 0.01	0.37
Met	83.46	86.29	84.70	84.57	85.20	85.03	1.13	0.34	0.91	0.33
Phe	80.24	82.15	83.44	82.14	82.39	82.51	1.22	0.79	0.43	0.43
Thr	74.04^{b}	76.05^{ab}	81.10^{a}	80.06^{ab}	78.83^{ab}	79.96^{ab}	1.35	0.52	< 0.01	0.53
Trp	84.95	84.38	89.16	88.28	85.02	84.98	1.41	0.65	0.03	0.96
Val	75.40	77.24	79.13	77.95	77.46	77.62	1.34	0.80	0.31	0.55
Dispensable AA										
Ala	79.04	81.88	80.74	79.79	78.88	78.79	1.60	0.65	0.58	0.49
Asp	74.77^{c}	76.09^{bc}	82.93^{a}	81.33^{ab}	80.13^{abc}	80.38^{abc}	1.32	0.99	< 0.01	0.54
Cys	73.53^{b}	75.25^{ab}	79.03^{ab}	78.66^{ab}	77.52^{ab}	80.04^{a}	1.36	0.20	0.01	0.53
Glu	82.09	83.82	86.04	85.18	85.45	85.81	1.24	0.63	0.08	0.54
Gly	77.29	78.46	83.46	81.06	81.75	83.69	2.48	0.90	0.16	0.66
Pro	109.01	107.01	120.14	106.45	116.94	119.31	5.74	0.35	0.28	0.40
Ser	79.33	80.90	83.07	81.50	81.19	84.26	1.19	0.28	0.12	0.19
Tyr	82.68	84.57	86.09	83.90	83.64	84.93	1.12	0.69	0.50	0.18
Total AA	81.29	83.03	86.08	84.11	84.60	85.24	1.36	0.89	0.11	0.39

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). Means in a row with different letters significantly differ ($P < 0.05$). [2]N = 5 for DDGS with AOP; otherwise, n = 6.[3]Standardized ileal digestibility of amino acids were calculated using the following basal endogenous AA losses (g/kg of dry matter intake): Arg, 0.44; His, 0.18; Ile, 0.41; Leu, 0.65; Lys, 0.46; Met, 0.12; Phe, 0.41; Thr, 0.67; Trp, 0.13; Val, 0.58; Ala, 0.71; Asp, 0.92; Cys, 0.23; Glu, 1.1; Gly, 1.6; Pro, 5.08; Ser, 0.57; Tyr, 0.32; and total AA, 14.78. [4]DDGS = distillers dried grains with solubles.

Chapter 3. Effects of *Aspergillus oryzae* postbiotic on *in vitro* digestibility and fermentability of high fiber feed ingredients and diets

Summary

Improving the sustainability of pork production requires the development and use of nutritional technologies that improve the utilization of high fiber feed ingredients in swine diets. The objective of this study was to determine the effects of an *Aspergillus oryzae* postbiotic (AOP, Amaferm®) on digestibility of high fiber feed ingredients for swine diets using a 3-step in vitro enzymatic digestion and fermentation system. Corn DDGS (DDGS), rice bran (RB), and wheat middlings (WM) were selected to represent three major high fiber cereal grain coproducts that vary in fiber concentration and composition and were evaluated along with three corn-soybean meal-based diets containing these ingredients. During in vitro hydrolysis and fermentation, 0.05% AOP was added to each diet and ingredient (DDGS+AOP, RB+AOP, and WM+AOP). Each 2 g sample was hydrolyzed for 2 h with pepsin and for a subsequent 4 h with pancreatin. Hydrolyzed residues were filtered, dried, weighed, and pooled within the same sample, and fermented using swine fecal inoculum. Volume of gas produced was recorded at 11 time points during the 72 h of incubation and concentrations of short-chain fatty acids (SCFA) were determined. Supplementation of AOP to diets increased ($P < 0.05$) *in vitro* digestibility of gross energy by 3.25, 0.86, 0.59 percentage units, and dry matter by 3.55, 0.76, and 1.16 percentage units for DDGS+AOP, RB+AOP, and WM+AOP, respectively, compared with unsupplemented diets. Supplementation of AOP during fermentation did not affect maximum gas production in ingredients or diets. Addition of AOP decreased ($P = 0.04$) the half-time to asymptote (T/2) in the ingredients and increased ($P = 0.03$) the

fractional rate of degradation at T/2 in the diets. The amounts of propionic acid, butyric acid, and total SCFA increased ($P < 0.05$) when AOP was added to diets compared with those without AOP, while the production of acetic acid and branch-chain fatty acids was not affected. Adding AOP had no effect on the production of SCFA when added to the coproducts. Similarly, energy production from SCFAs increased ($P < 0.01$) by AOP addition to the diets but not in the coproducts. In conclusion, the results of this study demonstrate the potential of AOP to increase the in vitro digestibility of gross energy and dry matter, as well as the fermentability of high fiber diets. The magnitude of these effects appears to be ingredient- and diet- dependent with the greater effects observed in corn DDGS.

Introduction

There is an increasing need to improve the utilization of coproducts from the food and biofuel industries into animal feed to decrease the environmental footprint of animal production (Woyengo et al., 2014), but inherently, these coproducts contain relatively high concentrations of dietary fiber. Dietary fiber, especially insoluble fiber, is generally not well utilized by pigs due to the lack of fiber-degrading enzymes in their gastrointestinal tracts (Agyekum and Nyachoti, 2017). Therefore, feeding diets containing high fiber ingredients to pig results in suboptimal caloric and nutritional efficiency unless effective feed processing or feed additives can be used to enhance their nutritional value. Considerable efforts have been devoted to developing and evaluating various exogenous enzymes for improving nutritional efficiency of diets containing high fiber ingredients in pigs (Kerr and Shurson, 2013; Torres-Pitarch et al., 2017). In addition, several commercially available prebiotic, probiotic (direct-fed microbials) and

postbiotic feed additives have been shown to improve microbial fermentation of fiber in the lower gut of swine (Liao and Nyachoti, 2017; Barba-Vidal et al., 2018; Liu et al., 2018). One of these commercially available feed additives is *Aspergillus oryzae* postbiotics (AOP), a fermentation extract of a specific *Aspergillus oryzae* strain. Previous studies have reported beneficial effects of AOP in ruminant diets, including increased milk production (Kellems et al., 1990; Gomez-Alarcon et al., 1991), which was attributed to greater digestibility of nutrients in lactating cows (Van Horn et al., 1984; Gomez-Alarcon et al., 1991), and improved daily weight gain of heifer calves (Beharka et al., 1991). Our previous research showed that supplementing 0.05% AOP increased apparent total tract digestibility (ATTD) of energy and neutral detergent fiber (NDF) in diets containing high fiber ingredients including corn distillers dried grains with solubles (DDGS), rice bran (RB), and wheat middlings (WM) for growing pigs (Zhu et al., 2020b). These coproducts contain greater concentrations of NDF (Table 3-1) and total dietary fiber (28.90, 20.08, and 38.10% in DDGS, RB, and WM, respectively; Table 3-2) than corn and soybean meal, but differ in the proportion of soluble and insoluble fiber (Pedersen et al., 2014; Jaworski et al., 2015; Casas et al., 2019). Corn DDGS has greater soluble dietary fiber concentration (3.4%) than RB (0.87%) and WM (1.20%). The arabinose to xylose ratio (A:X) is a direct measure for the degree of substitution and an important indicator for structural feature of arabinoxylan molecules, and RB has greater A:X (0.99) than corn DDGS (0.73) and WM (0.62).

Dietary fiber is partially (insoluble nonstarch polysaccharides such as cellulose and hemicellulose) or completely (most soluble nonstarch polysaccharides such as β-glucans and pectins) degraded by microbial fermentation in the large intestine of pigs,

and the principal end-product of the fermentation are short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate (Bach Knudsen et al., 2013). Most SCFAs that are not metabolized by microbes are absorbed by enterocytes and contribute to the energy supply of pigs (Dierick et al., 1989; Wenk, 2001; Holscher, 2017). However, there are no data on the amount of SCFA produced from fiber fermentation or the amount of energy derived from SCFA in high fiber diets with the addition of AOP.

The energy contributions of individual feed ingredients are assumed to be additive in contributing to the total energy value of complete diets. However, fermentability of fiber depends on not only fiber composition of an individual ingredient, but also the presence of other components in the complete diet. De Vries et al. (2016) reported that the addition of β-glucan to complete diet increased the ATTD of non-starch polysaccharides (NSP) from rapeseed meal, and addition of resistant starch decreased ATTD of NSP from rapeseed meal and DDGS. This associative interacting effect of fiber components is less described in pigs than in ruminants. Therefore, it is unknown if the addition of AOP to complete diets will have an effect similar to when added to individual feed ingredients.

An *in vitro* procedure that simulates the digestive processes of the pig was developed by Boisen and Fernández (1997), and results using this procedure indicate that *in vitro* ileal and total tract dry matter (DM) and organic matter (OM) digestibility values are well correlated with values obtained using *in vivo* digestibility methods (Boisen and Fernández, 1997; Noblet and Jaguelin-Peyraud, 2007). This modified 3-step *in vitro* procedure allows the measurement of fermentation kinetics and production of SCFA during fermentation in single ingredients and diets (Huang et al., 2018; Zeng et al., 2018).

Therefore, we hypothesized that AOP will increase enzymatic digestion and modify fermentation kinetics and end-products of high fiber ingredients and/or diets based on results from using the modified 3-step *in vitro* procedure. The objectives of this *in vitro* study were to 1) compare effects of AOP on *in vitro* digestion and fermentation kinetics between single high fiber ingredients and complete diets formulated with these ingredients; and 2) measure the production of SCFA of high fiber ingredients and diets when AOP was added.

Materials and Methods

Feed ingredients, diets, and Aspergillus orazye postbiotic

Three commonly used high fiber ingredients including corn-DDGS, RB, and WM were obtained from commercial sources. The ingredients were selected because they represent major coproducts obtained from cereal grain processing used in commercial swine diets. They also have different NDF concentrations (Table 3-1), dietary fiber composition (Table 3-2), and unknown effects of AOP in these different dietary fiber matrices. Three diets containing these 3 ingredients that were used in a previous *in vivo* trial (Table 2-2) were also used for *in vitro* hydrolysis and fermentation in the current study. These diets were formulated to contain equivalent amount of NDF. Samples of ingredients and diets were ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1 mm mesh screen prior to enzymatic hydrolysis. The AOP (Amaferm®) was provided by BioZyme Inc. (St. Joseph, Missouri). The product contains about 7 x 10^6 non-viable *Aspergillus oryzae* conidiospores and 0.2% citric acid, 2.3 mg riboflavin /kg, 202.0 mg niacin /kg, 23.2 mg pantothenic acid /kg, 8.5 mg pyridoxine hydrochloride /kg, 0.9 mg folic acid /kg, 0.4 mg biotin /kg, 1.2 µg cobalamin /kg, as well

as 3.0 IU amylase /kg and 40.0 IU cellulase /kg activities (European Food Safety Authority, 2006).

Enzymatic hydrolysis

The *in vitro* hydrolysis was conducted with pepsin and pancreatin following the first 2-step protocol as described by (Zeng et al., 2018). Briefly, about 2 g (± 0.01) samples were weighed into 500 mL conical flasks. One hundred mL of phosphate buffer solution (0.1 M, pH 6.0) and 40 mL HCl solution (0.2 M) were added into each flask. Solution pH was adjusted to 2.0 with 1 M HCl or 1 M NaOH. In addition, 2 mL of chloramphenicol (Sigma C-0378, Sheboygan Falls, WI) solution (0.5 g 100 mL/L ethanol) were added to inhibit microbial activity. One mL of the prepared AOP solution or blank phosphate buffer solution was pipetted into the 500 mL conical flask. Fresh porcine pepsin solution (4 mL, 25 g /L, P-7000, Sigma Aldrich, St. Louis, MO) was added, and flasks were placed in a water-bath at 39 ± 0.5°C for 2 h under gentle agitation after being closed with a rubber stopper. Subsequently, 40 mL phosphate buffer (0.2 M, pH 6.8) and 20 mL of 0.6 M NaOH were added to the flask. Then pH was adjusted to 6.8 with 1 M HCl or 1 M NaOH. Fresh pancreatin solution (2 mL, 100 g/L pancreatin, P-1750, Sigma) was added and flasks were incubated in water bath for 4 h. After hydrolysis, the residues were collected by filtration through a nylon bag (R510, 50 μm porosity, Ankom Technology, Macedon, NY), washed with ethanol (2 × 25 mL 95% ethanol) and acetone (2 × 25 mL 99.5% acetone), dried for 48 h at 60°C, and weighed. A portion of the filtrates from the nylon bags was collected before washing with ethanol and acetone, and centrifuged at 10,000 × *g* for 10 min. The supernatant from each sample was stored at −80°C for later analysis. The enzymatic hydrolysis was repeated 16 times under

the conditions described previously, to obtain sufficient residue for multiple analyses. Hydrolyzed residues from the different replicates and batches of same treatments (n = 8) were pooled for subsequent *in vitro* fermentation. The remaining 8 replicates were stored individually for GE determination using an isoperibol bomb calorimeter (Parr 6400, Parr Instrument Company, Moline, IL).

In vitro fermentation

The amount of gas produced during fermentation of the hydrolyzed substrates was measured using the *in vitro* cumulative gas-production technique (Bindelle et al., 2007). Briefly, 200 mg of pooled enzymatic hydrolytic residues for each treatment was added to 125 mL-glass bottles with 30 mL buffer solution containing macro- and micro-minerals (Menke and Stengass, 1988), along with fecal inoculum (0.05 g/mL), and incubated at 39°C. Fecal inocula were prepared by collecting feces from 6 finishing pigs (about 90 kg body weight) at a local farm. The pigs were fed a corn-soybean meal diet free of antibiotics. Fecal samples were collected directly from the rectum, immediately placed in airtight plastic syringes, and kept in a water bath at 39°C until incubation. The inoculum was diluted to 0.05 g of wet feces per mL of the buffer solution. The prepared inoculum was filtered through a 250 μm screen and transferred into the bottle with fermentation substrates. Bottles were sealed with a rubber stopper and placed in the water bath for incubation. An anaerobic environment was maintained throughout the experiment, from the time of inoculum preparation until the incubation step, by adding CO_2 gas to bags and bottles. The gas generated from fermentation was manually recorded at 0, 2, 5, 8, 12, 18, 24, 36, 48 and 72 h by using inverted burette assembly for gas measurement. The bottles were vented to release gas that accumulated after every measurement. Fermentation was

terminated at 72 h of incubation by quenching the bottles in ice cold water. At the end of the fermentation period, the supernatant (randomly selected 4 replicates per treatment) from each bottle was collected and frozen until analysis for SCFA (e.g., acetic, propionic, butyric, and branched chain fatty acids). The unfermented residues were collected by filtration (R510, 50 μm porosity, Ankom Technology), washed, dried, and weighed following the same procedures described for the hydrolyzed residues. Samples of the fecal inoculum prior to fermentation were also analyzed for SCFA concentrations.

Chemical analyses

All samples and feed ingredients were ground to pass through a 1-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) prior to chemical analysis. Samples were analyzed according to AOAC (2007) with specific methods as follows: DM (method 934.01), crude protein (CP, method 990.03), ether exact (EE, method 920.39), and ash (method 942.05). The gross energy (GE) was determined by bomb calorimeter (Parr 6400; Parr Instrument Company, Moline, IL) and benzoic acid was used as standard. Neutral detergent fiber was determined using fiber bags and fiber analyzer (Ankom Technology, Macedon, NY, USA) following an adaptation of the procedure described by Van Soest et al. (1991). The SCFAs were determined via gas chromatography (Agilent 6890 System, Böblingen, Germany) after extraction with diethyl ether (Greenberg et al., 1992). Briefly, 2 mL of the samples were transferred into the centrifuge tube, and 0.5 mL of sulfuric acid (1/1), 0.4 g sodium chloride, 0.4 mL internal standard, and 2 mL of diethyl ether were added. The samples were mixed for 2 min and centrifuged at 3000 × *g* for 3 min. Subsequently, the ether layer was transferred to vials and loaded on the gas chromatography analyzer (Agilent 6890 System). The

concentration of branched-chain fatty acids was calculated as the sum of iso-butyric and iso-valeric acids.

Calculations and statistical analysis

In vitro digestibility of gross energy (IVDGE) and dry matter (IVDDM) during the pepsin and pancreatin hydrolysis were calculated as follows:

IVDDM (GE) = (dry weight (GE) of the sample before hydrolysis − dry weight (GE) of the residue) /dry weight (GE) of the sample before hydrolysis

In vitro fermentability of DM (IVFDM) during feces fermentation was calculated as follows:

IVFDM = (dry weight of the hydrolyzed residue – dry weight of the residue after fermentation) / dry weight of the hydrolyzed residue

Gas accumulation curves recorded during the 72 h of fermentation were modeled according to France *et al.* (1993):

G (mL g/DM) = 0, if $0 < t < L$

G (mL g/DM) = $Gf (1- \exp (- [b (t - L) + c (\sqrt{t} - \sqrt{L})]))$, if $t \geq L$

where G denotes the gas accumulation at a specific time (t), Gf (ml/g DM) was the maximum gas volume for $t = \infty$, and L (h) represents the lag time before the fermentation began. In the present study, gas accumulation rapidly reached one-fourth of the maximum accumulation in 2 h, and the parameter L was very close to 0, which resulted in the model fails to converge. Therefore, L(h) data were removed from the final

model. The constants b (h−1) and c (h−1/2) determine the fractional rate of degradation of the substrate μ (h−1), which is postulated to vary with time as follows:

$$\mu = b + c/(2\ t\ ),\ \text{if}\ t \geq L$$

Kinetics parameters (Gf, t = T/2, and μ at T/2) were compared in the statistical analysis, with T/2 representing the time to half-asymptote when G = Gf/2.

The total energy derived from SCFA was calculated following the equation below (Christensen et al., 1999):

Total energy production from SCFA (kcal/g DM of hydrolyzed substrate) = [acetate (mmol)] × 209.6 cal/mmol + [propionate (mmol)] × 366.4 cal/mmol + [butyrate (mmol)] × 522.2 cal/mmol

where 209.6, 366.4, and 522.2 cal/mmol represent the amount of energy produced from acetate, propionate, and butyrate catabolism, respectively.

Total energy production from SCFA (kcal/g DM of original ingredient/diet) = total energy production from SCFA of hydrolyzed substrate × (1 - IVDDM)

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS institute Inc., Cary, NC) with AOP, ingredient (diet), AOP × ingredient (diet), and batch as fixed effects. The UNIVARIATE procedure was used to identify outliers. The experimental unit was each bag. The LSMENAS procedure was used to calculate means and PDIFF option was used to separate means with a Tukey-Kramer adjustment. A value of $P < 0.05$ was considered statistically significant, and $P < 0.1$ was considered a trend.

Results and Discussion

Fiber concentration in ingredients and diets

The NDF concentration in corn DDGS and WM (Table 3-1) is comparable to previously reported NDF concentration ranges of 17.95- 43.66% for corn DDGS (Stein and Shurson, 2009; Zeng et al., 2017) and 26.6-32.4% for WM (Jha et al., 2012; De Jong et al., 2014). The RB used in the present study had a NDF concentration of 10.21%, which is less than values reported in previous studies in China and North America, which ranged between 17.17% and 33.90% (Li et al., 2018; Casas et al., 2019). These differences were expected because chemical composition of high fiber coproducts varies widely among parent grain varieties, geographic regions, and processing methods (Shi et al., 2015).

These ingredients were selected based on 1) the production of large quantities from industries and high availability in North America and 2) their relatively high fiber concentrations and different fiber characteristics. The specific fiber fractions of each coproduct were obtained from published studies and are summarized in Table 3-2. Arabinoxylan is the main fiber present in these coproducts, which consists of a linear β-1,4 linked xylan backbone with the arabinose substitution through a mixture of either 3-monosubstituted or 2,3-disubstituted α-L-arabinose (Dervilly-Pinel et al., 2004). The distribution of arabinose substituents along the backbone is considered of great importance because it affects the conformation and the capacity of arabinoxylans to interact with one another (Sternemalm et al., 2008). The A:X ratio is a direct measure for the degree of substitution and an important indicator for structural feature of arabinoxylan molecules (Courtin and Delcour, 1998). The A:X ratio is the greatest in RB (0.99), which

indicates a greater degree of substitution and a more complexity structure of the heteroxylans compared to those in corn DDGS (0.73) and WM (0.62). Insoluble arabinoxylan comprises 88, 97, and 98% of total arabinoxylan content in corn DDGS, RB, and WM, respectively. Composition and characteristics of fiber in the ingredients and diets are associated with *in vitro* degradation and fermentation of these types of fiber and *in vivo* physiological responses of pigs (Bach Knudsen et al., 2016; Zeng et al., 2018; Shurson et al., 2021). In the diets formulated with these ingredients, the NDF concentrations were comparable with or without the addition of AOP (14.50% for DDGS vs. 13.73% for DDGS+AOP; 9.79% for RB vs. 10.47% for RB+AOP; 13.28% for WM vs. 13.33% for WM+AOP; Table 2-2).

In vitro hydrolysis

The addition of AOP had no effects on IVDDM of the ingredients but significantly increased ($P < 0.001$) IVDDM of the diets (Table 3-3). There was also an interaction ($P < 0.001$) between AOP and diet on IVDDM of the diets. Specifically, the magnitude of the percentage improvement in IVDDM of DDGS (4.92%) diet was greater than those in RB (1.02%) and WM (1.64%) diets. The smaller A:X ratio in corn DDGS compared with RB and greater soluble arabinoxylan concentration compared with RB and WM are likely the reasons for greater response to AOP (Williams et al., 2011; De Vries et al., 2013). The addition of AOP increased IVDGE of ingredients ($P = 0.02$) and diets ($P < 0.01$). An interaction ($P < 0.001$) was also detected between AOP and ingredient/diet. To the best of our knowledge, there are no studies showing improved *in vitro* enzymatic digestibility of high fiber ingredients from the addition of AOP in a monogastric digestion model. Based on information provided by the manufacturer, the

final product of AOP is stated to contain about 7 x 10^6 non-viable *A. oryzae* conidiospores and 0.2% citric acid, 2.3 mg riboflavin /kg, 202.0 mg niacin /kg, 23.2 mg pantothenic acid /kg, 8.5 mg pyridoxine hydrochloride /kg, 0.9 mg folic acid /kg, 0.4 mg biotin /kg, 1.2 μg cobalamin /kg, as well as 3.0 IU amylase /kg and 40.0 IU cellulase /kg activities (European Food Safety Authority, 2006). Other components of AOP are *A. oryzae* cell wall polysaccharides, which are secondary metabolites that accumulate during *A. oryzae* growth, and enzymes remaining in the *A. oryzae* cultivation broth. The existence of *Aspergillus oryzae* amylase and cellulase activity remaining in the AOP product could contribute to the increased digestibility observed, but the amount is minimal compared with that derived from pancreatin hydrolysis or addition of exogenous enzymes in commercial swine diets (Zeng et al., 2018). Therefore, the mechanism of AOP enhancing digestibility during hydrolysis is still unknown.

Interestingly, we observed that the magnitude of increase in *in vitro* enzymatic digestibility was greater in diets with corn DDGS than RB or WM. In our previous *in vivo* study (Zhu et al., 2020b), we observed an increase in the apparent ileal digestibility of ether extract in pigs fed diets with corn DDGS, RB, and WM and supplemented with AOP. This greater apparent ileal digestibility of ether extract agreed with increased apparent ileal digestibility of energy in diets with AOP in corn DDGS (2.64%), RB (1.78%), and WM (2.46%). These findings indicate the potential of addition of AOP to improve energy value of DDGS and increase energy supply to pigs. However, the mechanism of this effect is unknown.

In vitro fermentation and kinetics of gas production

The magnitude of increase in IVFDM was greatest (interaction $P < 0.05$) when AOP was added to corn DDGS than RB or WM (Table 3-4). Likewise, the magnitude of decrease in T/2 was greatest (interaction $P < 0.05$) when AOP was added to corn DDGS than RB or WM. Addition of AOP to corn DDGS, RB, or WM did not affect any of the other fermentation kinetic parameters tested. Together with increased digestibility of DDGS during hydrolysis, our results indicate that addition of AOP has greater impact on *in vitro* digestibility and fermentability of DDGS compared with that of WM and RB. Differences in the compositional complexity among these ingredients are the most likely reason for the different responses to AOP treatment. A lower A:X in corn DDGS compared with RB indicates a lower degree of substitution of arabinose on the xylose backbone and less complexity of heteroxylans in corn DDGS. Decreased A:X ratio by maleic acid pretreatment in corn-DDGS was reported to associated with improvement of *in vitro* DM digestibility from the addition of NSP-degrading enzymes (De Vries et al., 2013). In addition, corn DDGS contains more soluble arabinoxylan compared with RB and WM, which are more readily fermented (Williams et al., 2011; Jaworski et al., 2015; Casas et al., 2019; Williams et al., 2019).

Addition of AOP to complete diets with corn DDGS, RB, and WM tended to increase ($P = 0.06$) the IVFDM, and this increase was associated with an increase ($P < 0.05$) in the fractional rate of degradation (μ). The greatest increase (interaction $P < 0.05$) in maximum gas volume was observed when AOP was added to diets containing corn DDGS than when added to RB or WM diets. These observations suggest that the fermentable OM in DDGS was depleted within a shorter period of incubation time

(Zangaro et al., 2019), and it can be speculated that supplementation of AOP to pig diets with DDGS may decrease the time required for microbiota to ferment fiber in the hindgut.

Short-chain fatty acid profile and energy contributions during in vitro fermentation

In pigs, end-products of fiber fermentation in hindgut by microbiota are SCFAs (e.g., acetate, propionate, and butyrate) that are well absorbed and utilized in intermediate metabolism for energy production or lipid synthesis (Bach Knudsen et al., 2012). Addition of AOP to single ingredients did not affect the amount of any of the SCFAs measured after fermentation. However, the concentration of propionic acid, butyric acid, and total SCFA was greater ($P < 0.05$) in the three types of diets when AOP was added compared with those in the diets without AOP, but addition of AOP did not affect the production of acetic acid and branch-chain fatty acids (Table 3-5). The inconsistent responses to AOP addition between individual ingredients and complete diets could be due to the interactive effects of fiber components from the coproducts (e.g., corn DDGS, RB, and WM) and fiber from other ingredients in the diets (e.g., corn and soybean meal). The presence and characteristics of specific types of fiber may affect digestibility and fermentability of the complete diet. For example, De Vries et al. (2016) reported that the addition of β-glucan to complete diet increased the ATTD of NSP from rapeseed meal, and addition of resistant starch decreased ATTD of NSP from rapeseed meal and DDGS.

In pigs, the energy provided from SCFA produced during the fermentation of dietary fiber in the large intestine can be up to 15% of the maintenance energy of growing pigs (Noblet and Le Goff, 2001) and energy from SCFA was calculated based on previous equations. For the ingredients, addition of AOP did not affect energy production

(kcal/kg DM) from SCFA for hydrolyzed substrate or ingredients. For the diets, the addition of AOP increased ($P < 0.01$) the calculated energy from SCFA production per gram of fermented hydrolyzed residue. This effect tended to be of greater magnitude in the diet with corn DDGS (interaction $P = 0.07$) than in the diets with RB and WM. These findings are in agreement with our previous observations that supplementation of AOP increased ATTD of GE of the complete diets formulated with DDGS, RM, and WM in growing pigs (Zhu et al., 2020b).

There are a few limitations of the present study that may need to be addressed in future research. First, feces used for inoculum were collected from pigs not consuming AOP. Therefore, there was no time for microbes in the feces to adapt to AOP. In *in vivo* trials, it is generally recommended at least 7 to 21-d for animals and the microflora in their intestine to adapt to diets when high fiber ingredients are tested in *in vivo* studies (Longland et al., 1993; Huang et al., 2018). During the adaptation period, microbiota have time to adapt to diets and additives by changing species richness and profiles, increase in the population of fibrolytic species, or increase in the ability of the fibrolytic species to degrade fiber from the diet. Therefore, it is possible that the magnitude of improvement in SCFA production may be greater if pigs are adapted to the test ingredient than what was observed in this study. Second, fecal inoculum used in the study was collected from the same group of pigs fed diets without AOP. The donor animal diet may cause differences in fermentation measurement due to microbial activity already occurred in the hindgut (Rymer et al., 2005). Third, the dose of AOP used in the present study followed that of our *in vivo* study and different doses of AOP were not tested. Beharka and Nagaraja (1993) found differences *in vitro* fiber degradation with AOP where

addition of AOP at 0.8 g/L and 1.2 g/L increased NDF and ADF degradations but not at 0.4 g/L.

Conclusions

In conclusion, the results of this study demonstrated that postbiotics derived from the fermentation extract of *Aspergillus oryzae* can increase the digestibility of energy and DM of fiber. This effect of AOP was of greater magnitude on *in vitro* digestion and fermentation of corn DDGS diets, indicating a high potential of AOP to degrade DDGS fiber and release nutrients *in vitro*. It appears that AOP could increase energy value of ingredients and diets by increasing the production of SCFA during fermentation and by altered fermentation kinetics. However, it is unknown if these effects are due to changes in microbial population or their ability to degrade fiber.

Table 3-1. Analyzed chemical composition of fiber containing ingredients (as-fed basis)

Items	Corn	Soybean meal	Corn DDGS[1]	Wheat middlings	Rice bran
Gross energy, kcal/kg	3,943	4,234	4,492	4,053	4,621
Dry matter, %	88.51	89.73	89.56	90.20	94.35
Crude protein, %	6.92	46.45	27.00	16.49	13.52
Crude fat, %	1.26	1.82	5.03	3.24	16.94
Crude fiber, %	1.65	3.80	8.00	6.80	5.71
Neutral detergent fiber, %	8.29	7.72	28.35	28.86	10.21
Ash, %	1.18	6.65	5.18	7.11	12.96

[1]DDGS = corn distillers dried grains with solubles.

Table 3-2. Fiber composition of corn distillers dried grains with solubles (DDGS), rice bran, and wheat middlings from published studies (%, DM basis)

Fiber fraction	DDGS[1]	Rice bran[2]	Wheat middlings[1]
Soluble non-cellulosic polysaccharides	3.40	0.87	1.20
Arabinose	0.90	0.20	0.20
Xylose	0.90	0.01	0.20
Mannose	0.70	0.05	-
Galactose	0.20	0.22	0.20
Glucose	0.30	0.17	0.20
Uronic acid	0.40	0.22	0.30
Insoluble non-cellulosic polysaccharides	15.80	8.98	22.70
Arabinose	4.30	3.13	7.00
Xylose	6.20	3.37	11.40
Mannose	1.20	0.28	0.30
Galactose	1.10	0.80	0.50
Glucose	1.80	0.60	2.30
Uronic acid	1.20	0.80	1.20
Cellulose	5.80	4.39	6.70
Total non-starch polysaccharides[3]	25.00	14.24	30.70
Lignin	3.90	5.84	7.30
Soluble dietary fiber[4]	3.40	0.87	1.20
Insoluble dietary fiber[5]	25.50	19.21	36.90
Dietary fiber[6]	28.90	20.08	38.10
Arabinose to xylose ratio	0.73	0.99	0.62
Uronic acids to xylose ratio	0.23	0.30	0.13
SDF/IDF[7]	0.13	0.05	0.03

[1]Data source: Jaworski et al., 2015.
[2]Data source: Casas et al., 2019.
[3]Total non-starch polysaccharides = Soluble non-cellulosic polysaccharides + Insoluble non-cellulosic polysaccharides + cellulose.
[4]Soluble dietary fiber = soluble non-cellulosic polysaccharides.
[5]Insoluble dietary fiber = insoluble non-cellulosic polysaccharides + cellulose + lignin.
[6]Dietary fiber = soluble dietary fiber + insoluble dietary fiber.
[7]SDF/IDF = soluble dietary fiber to insoluble dietary fiber ratio.

Table 3-3. Effect of *Aspergillus oryzae* postbiotic (AOP) on *in vitro* digestibility[1] of dry matter (IVDDM) and gross energy (IVDGE), %[2]

Items	DDGS[3]		Rice bran		Wheat middlings		SEM	*P*-value		
	- AOP	+ AOP	- AOP	+ AOP	- AOP	+ AOP		AOP	Ingredient (Diet)	AOP × Ingredient (Diet)
Ingredient										
IVDDM	66.49^{a}	66.76^{a}	64.56^{b}	65.79^{ab}	61.27^{c}	61.82^{c}	0.56	0.12	<0.001	0.63
IVDGE	68.64^{a}	68.55^{a}	67.77^{b}	67.47^{b}	59.85^{d}	60.72^{c}	0.07	0.02	<0.001	<0.001
Diet										
IVDDM	72.15^{b}	75.70^{a}	74.70^{a}	75.46^{a}	70.55^{b}	71.71^{b}	0.63	<0.001	<0.001	0.04
IVDGE	73.62^{c}	76.87^{b}	76.65^{b}	77.51^{a}	71.80^{e}	72.39^{d}	0.07	<0.001	<0.001	<0.001

[1]The *in vitro* enzymatic digestion was conducted with porcine pepsin (Sigma P7000) hydrolysis for 2 h and pancreatin hydrolysis (Sigma P1750) for 4 h in water bath.
[2]Data are shown as LS-means with pooled standard error of the mean (SEM). Means in a row with different letters differ. N = 20.
[3] DDGS = corn distillers dried grains with solubles.

Table 3-4. Effect of *Aspergillus oryzae* postbiotic (AOP) on *in vitro* fermentability of dry matter (IVFDM, %) and gas production kinetics[1]

Items	Corn DDGS[2]		Rice bran		Wheat middlings		SEM	*P*-value		
	- AOP	+ AOP	- AOP	+ AOP	- AOP	+ AOP		AOP	Ingredient (Diet)	AOP × Ingredient (Diet)
Ingredient										
G_f[3], mL/g	296.30^{a}	311.75^{a}	153.51^{b}	139.16^{b}	173.46^{b}	176.56^{b}	40.53	0.58	<0.001	0.85
T/2[4], h	63.71^{a}	27.81^{b}	7.85^{b}	5.37^{b}	5.84^{b}	5.61^{b}	6.43	0.04	<0.001	0.04
μ[5]	0.030^{b}	0.033^{b}	0.142^{a}	0.148^{a}	0.106^{ab}	0.122^{a}	0.035	0.60	<0.001	0.92
IVFDM, %	61.13^{b}	65.24^{a}	52.83^{b}	50.56^{b}	50.49^{b}	49.69^{b}	3.17	0.65	<0.001	0.002
Diet										
G_f[3], mL/g	247.9^{b}	280.56^{a}	263.80^{ab}	257.51^{ab}	272.51^{ab}	269.07^{ab}	20.98	0.23	0.42	0.02
T/2[4], h	10.18	10.15	7.49	7.12	6.79	6.83	0.53	0.70	<0.001	0.86
μ[5]	0.068^{c}	0.066^{c}	0.079^{b}	0.090^{a}	0.084^{ab}	0.089^{a}	0.003	0.03	<0.001	0.03
IVFDM, %	80.42^{a}	81.38^{a}	79.31^{a}	81.23^{a}	76.19^{b}	75.88^{b}	1.54	0.06	<0.001	0.14

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). Means in a row with different letters differ. N=16.
[2]DDGS = corn distillers dried grains with solubles.
[3]Maximum gas volume, mL per g DM inoculated.
[4]Half-time to asymptote (h).
[5]Fractional rate of degradation (h^{-1}) at t=T/2.

Table 3-5. Effect of *Aspergillus oryzae* postbiotic (AOP) on short-chain fatty acids (SCFA) production during fermentation, mmol/g dry matter[1]

Items	Corn DDGS[2]		Rice bran		Wheat middlings		SEM	*P*-value		
	- AOP	+ AOP	- AOP	+ AOP	- AOP	+ AOP		AOP	Ingredient (Diet)	AOP × Ingredient (Diet)
Ingredient										
Acetic acid	2.44^{ab}	2.62^{a}	1.47^{c}	1.13^{c}	2.14^{b}	2.32^{ab}	0.11	0.99	<0.01	0.03
Propionic acid	1.20^{a}	1.30^{a}	0.73^{b}	0.58^{b}	1.13^{a}	1.19^{a}	0.05	0.94	<0.01	0.03
Butyric acid	0.44^{a}	0.50^{a}	0.27^{b}	0.16^{b}	0.42^{a}	0.41^{a}	0.03	0.32	<0.01	0.02
BCFA[3]	0.05^{ab}	0.04^{b}	0.09^{a}	0.05^{ab}	0.09^{a}	0.10^{a}	0.01	0.21	<0.01	0.16
Total SCFA	4.16^{a}	4.48^{a}	2.61^{b}	1.95^{b}	3.83^{a}	4.07^{a}	0.17	0.81	<0.01	<0.01
Energy production, kcal/g DM of hydrolyzed substrate[4]	1.18^{a}	1.29^{a}	0.72^{b}	0.53^{b}	1.09^{a}	1.13^{ab}	0.05	0.77	<0.01	<0.01
Energy production from ingredient, kcal/kg DM[5]	0.40^{a}	0.43^{a}	0.25^{b}	0.18^{b}	0.42^{a}	0.43^{a}	0.02	0.48	<0.01	<0.01
Diet										
Acetic acid	3.00^{a}	3.29^{a}	2.55^{b}	2.52^{b}	2.56^{b}	2.80^{ab}	0.12	0.10	<0.01	0.38
Propionic acid	1.50^{bc}	1.75^{a}	1.44^{c}	1.48^{bc}	1.53^{bc}	1.64^{ab}	0.04	<0.01	<0.01	0.05
Butyric acid	0.63^{b}	0.80^{a}	0.72^{ab}	0.74^{ab}	0.71^{ab}	0.75^{ab}	0.03	<0.01	0.79	0.04
BCFA	0.13^{ab}	0.15^{a}	0.06^{b}	0.05^{b}	0.08^{ab}	0.11^{ab}	0.02	0.73	<0.01	0.50
Total SCFA	5.34^{b}	6.08^{a}	4.80^{b}	4.81^{b}	4.92^{b}	5.32^{b}	0.18	0.01	<0.01	0.14
Energy production, kcal/g DM of hydrolyzed residue	1.51^{b}	1.75^{a}	1.44^{b}	1.46^{b}	1.47^{b}	1.58^{ab}	0.05	<0.01	<0.01	0.07
Energy production from diet, kcal/g DM	0.42^{a}	0.42^{a}	0.36^{b}	0.36^{b}	0.43^{a}	0.45^{a}	0.001	0.70	<0.01	0.70

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). Means in a row with different letters differ; n=8.
[2]DDGS = corn distillers dried grains with solubles.

[3]BCFA = Branched-chain fatty acids (sum of so-butyric and iso-valeric acids).
[4]Calculated as total energy production from SCFA (kcal/g DM of hydrolyzed substrate) = [acetate (mmol)] × 209.6 cal/mmol + [propionate (mmol)] × 366.4 cal/mmol + [butyrate (mmol)] × 522.2 cal/mmol
[5]Calculated as total energy production from SCFA (kcal/g DM of original ingredient/diet) = total energy production from SCFA of hydrolyzed substrate × (1 - IVDDM).

Chapter 4. *Aspergillus oryzae* postbiotic modulates gut microbiome and metabolome associated with nutrient digestibility in growing pigs fed high fiber diets

Summary

Inclusion of high fiber and low-cost coproducts from biofuel and food industries in diets of food animal can reduce environmental impacts of food production by reducing the competition for grains and oilseeds between use in human diets, biofuels production, and livestock diets. The *Aspergillus oryzae* postbiotic (AOP) has been shown to increase nutrient digestibility when added to diets fed to growing pigs but the mechanism underlying these beneficial effects are not fully understood. In a two-period feeding trial, ileal digesta and feces were collected from 36 growing pigs fed diets containing corn distillers dried grains with solubles (DDGS), rice bran (RB) or wheat middlings (WM). Three additional diets were formulated to contain 0.05% AOP in DDGS (DDGS+AOP), RB (RB+AOP), or WM (WM+AOP) diet. Data from the ileal and fecal microbiome and metabolome analyses were correlated with nutrient digestibility data. Results showed that the addition of AOP significantly altered diversity ($P < 0.05$) and composition (PERMANOVA $P < 0.05$) of the microbial community between pigs fed AOP and those fed control diet in the ileal digesta and feces. We identified amplicon sequence variants (ASVs) belonging to genera *Clostridium*, *Bifidobacterium*, *Lactobacillus*, *Streptococcus* in the ileum and ASVs belongings to family *Veillonellaceae*, *S24-7*, *Fibrobacteraceae*, *Ruminococcaceae*, and *Bifidobacteriaceae* that distinguished pigs fed AOP from the control groups. Dietary addition of AOP altered the ileal and fecal metabolome as well as the concentrations ($P < 0.05$) of Asp, taurine, taurocholic acid, C12:0 and C17:0 in a diet-

dependent manner. Mantel analysis revealed significant correlations between microbiome and nutrient digestibility (r = 0.38, $P = 0.01$) and between microbiome and metabolome (r = 0.54, $P = 0.001$). Several ASVs were correlated with nutrient digestibility, including key fiber-degrading bacteria belonging to order *Bacteroidale* and family *S24-7* and *Ruminococcaceae*, which were significantly enriched ($P < 0.05$) in WM+AOP. Our results indicated that AOP can play an important role in modulating the ileal and fecal microbiome and metabolome which are associated with improved nutrient digestibility.

Introduction

Today, one of the major challenges facing food animal production is to produce abundant, safe, wholesome, and affordable food products while minimizing the environment impact of production to achieve a more sustainable production system (Shurson, 2017; Pomar and Remus, 2019). Uses of biofuels and food processing coproducts is an effective way to decrease the opportunity cost of food animal production by upcycling nutrients and decreasing the environmental impact of animal production (Van Zanten et al., 2018). Coproducts including distillers dried grains with solubles (DDGS) from biofuel production along with rice bran (RB) and wheat middlings (WM) from cereal grain processing represent low-opportunity cost biomass suitable for upcycling in a sustainable food system. However, these coproducts contain relatively high amounts of recalcitrant fiber that are generally not well utilized by monogastric animals due to the lack of fiber-degrading enzymes in the intestine (Agyekum and Nyachoti, 2017). Ammonia fiber expansion processing of DDGS has been shown to decrease fiber crystallinity and increases the energy that pigs may obtain from these high fiber coproducts (Zeng et al., 2021). In addition, fungal fermentation of soybean hull and

wet distillers grains with solubles by *Trichoderma reesei* and *Aspergillus oryzae* in solid-state fermentation has been shown to improve feeding values of these coproducts for monogastric animals (Sun et al., 2021). However, currently these techniques are not available at a large commercial scale and requires significant investment of resources. Therefore, it is necessary to develop less resource intense strategies such as feed additives that are capable of enhancing the digestibility and utilization efficiency of energy and nutrients of high fiber coproducts in pig diets.

One of these commercially available feed additives is *Aspergillus oryzae* postbiotic (AOP), a fermentation extract of a specific *Aspergillus oryzae* strain NRRL 458. This feed additive has been used in ruminant diet for a long time, and several studies have shown beneficial effects of AOP in enhancing nutrient digestibility and growth performance (Wiedmeier et al., 1987; Gomez-Alarcon et al., 1990; Sucu et al., 2019), while other studies reported no effect (Firkins et al., 1990; Oellermann et al., 1990; C J Newbold et al., 1992; Chiou et al., 2000). In pigs, results from our previous research showed that the addition of AOP to diets containing high fiber ingredients increased apparent total tract digestibility (ATTD) of energy and nutrients in growing pigs (Zhu et al., 2020b). However, the mechanism underlying these beneficial effects are not fully understood, making it difficult for nutritionists and producers to identify specific types of diets or ingredients that are most likely to benefit from the addition of this feed additive.

The porcine intestinal microbiota plays a significant role in nutrient digestion and utilization, especially for dietary fiber (Varel and Yen, 1997; Krajmalnik-Brown et al., 2012; Reyer et al., 2020). Indeed, energy derived from microbial fermentation of dietary fiber in the intestinal tract may account for up to 30% of the maintenance energy required

by growing pigs (Varel and Yen, 1997; Bach Knudsen et al., 2012). The microbial communities residing in the gastrointestinal tract (GIT) have been reported to be influenced by many factors, including diet type and composition, nutrient concentrations, energy source, and the addition of feed additives, such as antibiotics, prebiotics, probiotics, and postbiotics (Wang et al., 2020). Nutrient digestion and metabolism in pigs is impacted by the complex interplay between microbes residing in the gut, their metabolites, and enterocyte function (Rowland et al., 2018; Reyer et al., 2020). A better understanding of this multi-directional interaction is a key step to understanding the mechanisms of action of AOP underlying the increased nutrient digestibility in pigs. Therefore, research is needed to integrate data from responses involving changes in the microbiome and metabolome induced by addition of AOP with nutrient digestibility.

Results from previous study showed that feeding *Aspergillus oryzae* culture increased the number of *Lactobacillus* spp. while reduced the number of *E. coli* in excreta of laying hens, indicating that the fungus could alter microbial composition in the digestive system of poultry (Han et al., 1999). Additionally, results from an *in vitro* study demonstrated that the addition of AOP to ruminal fluid increased the growth rates of certain bacteria that digest fiber (Beharka and Nagaraja, 1998). However, no data are available on the modulation effects of AOP on gut microbiome and metabolome in pigs. In this study, we hypothesized that the addition of AOP to diets containing high fiber ingredients may modulate the gut microbiome and metabolome of growing pigs which is associated with its effects in enhancing nutrient digestibility. Therefore, the objective of this study was to evaluate the effect adding AOP to DDGS, RB and WM diets on the

modulation of the gut microbiome and metabolome and associations with nutrient digestibility in growing pigs.

Materials and Methods

The animal use protocol (#1805-35983A) was reviewed and approved by the Institution Animal Care and Use Committee at University of Minnesota.

Animals, diets, and experimental design

A total of 18 growing barrows with an initial body weight (BW) of 50.6 ± 4.9 kg (mean ± SEM) were surgically equipped with a T-cannula at the distal ileum (Stein et al., 1998). Pigs were housed individually in metabolism cages (198 × 84 × 71 cm) at the Southern Research and Outreach Center of University of Minnesota in Waseca, MN. Three corn and soybean meal-based diets were formulated to contain 29.65% corn-DDGS, 36.65% full fat RB or 24.59% WM and meet or exceed all nutrient requirements for 50 to 75 kg growing pigs (Table 2-2). Three additional diets were formulated to contain 0.05% AOP (Amaferm®, BioZyme Inc., St. Joseph, MO), which was supplemented at the expense of corn in the DDGS diet (DDGS+AOP), RB diet (RB+AOP), and WM diet (WM+AOP). Pigs were allotted randomly to a triplicated 6 × 2 Youden square design with 6 diets and 2 successive periods. Within each period, 3 pigs were fed 1 of the 6 experimental diets for a total of 6 observations per diet for the two periods. Pigs received a daily feed allowance equivalent to 3% initial BW and were fed twice daily in two equal meals at 0800 h and 1700 h. Pigs had free access to water from nipple drinkers throughout the experiment. Pigs consumed their allotted diets for 21-d in each period.

Sample collection

On d 22 to d 23, approximately 2 g fresh feces were collected in the morning using 2 mL tubes (RNase/DNase-free tubes) and were immediately snap frozen in liquid nitrogen and stored at -80 °C until further analysis. Ileal digesta samples were collected for 8 h starting at 0800 h until 1600 h on d 24 to 25 using a 207 mL Whirl-pak® plastic bag (Nasco, Fort Atkinson, WI) attached to the cannula barrel. Bags were removed every 30 min or whenever full. Ileal digesta samples were pooled into 1 L wide-mouth high density polyethylene bottles (Fisher Scientific Company, Ottawa, ON) and stored at -20°C immediately to prevent bacterial fermentation until analysis. After the completion of 2-d collection, ileal samples were mixed, and two 1.5 mL aliquot were collected using 2 mL tubes (RNase/DNase-free tubes), immediately snap frozen in liquid nitrogen and stored at -80 °C until analysis.

LC-MS analysis

LC-MS-grade water, acetonitrile (ACN) and formic acid were purchased from Fisher Scientific (Houston, TX, USA). LC-MS analysis was conducted using an ultra-performance liquid chromatography (UPLC) system (Acquity, Waters, Milford, MA, USA) equipped with a BEH C18 column (Waters) and a Xevo-G2-S quadrupole time-of-flight mass spectrometer (QTOFMS, Waters). Approximately 50 mg fecal and ileal digesta samples were prepared by mixing the samples with 500 µL (10 ×, v/w) of 50% aqueous ACN. Samples were then extracted by vertexing and sonication for 10 min. After sonication, supernatants were obtained by centrifuging at 18,000 for 10 min. For free amino acid analysis, samples were derivatized with dansyl chloride (DC) prior to LC-MS analysis. 5 µL of sample or standard was mixed with 5 µL of 100 µM p-

chlorophenylalanine (internal standard), 50 µL of 10 mM sodium carbonate, and 100 µL of DC (3 mg/mL in acetone). The mixture was incubated at 60°C for 15 min and centrifuged at 18,000 × *g* for 10 min. The supernatant was transferred into a HPLC vial for LC-MS analysis. For fatty acid analysis, samples were mixed with 200 µM 2D4-acetic acid as an internal standard and were subjected to 2-hydrazinoquinoline (HQ) derivatization by mixing samples with a 100 µL of ACN solution containing 1 mM 2,2'-dipyridyl disulfide (DPDS), 1 mM triphenylphosphine (TPP) and 1 mM HQ derivatized by 2-hydrazinequinoline (HQ). The reaction mixture was incubated at 60°C for 30 min, chilled on ice, and then mixed with 100 µL of ice-cold deionized water. After centrifugation at 18,000 × *g* for 10 min, the supernatant was transferred into a vial for analysis.

Conditions of LC-MS analysis

A 5 µL sample aliquot was injected into an Acquity ultraperformance liquid chromatography system (Waters, Milford, MA, USA). For amino acid analysis, samples were separated in a BEH C18 column (Waters, Milford, MA, USA) using a mobile phase gradient containing 0.1% formic acid (A) and ACN containing 0.1% formic acid (B). For fatty acid analysis, the mobile phase gradient used contained 2mM ammonium acetate in water with 0.05% acetic acid (A), and 2mM ammonium acetate in 95% ACN and 5% H_2O with 0.05% acetic acid (B). For bile acid (BA) analysis, 10 mM ammonium acetate in water (pH = 9) (A) and 10 mM ammonium acetate in 95% ACN and 5% water (pH = 9) (B) were used as mobile phase.

The LC eluant was directly introduced into a Xevo-G2-S quadrupole time-of-flight mass spectrometer (QTOFMS, Waters, Milford, MA, USA) for accurate mass

measurement and ion counting. Capillary voltage and cone voltage for electrospray ionization were maintained at 3 kV and 30 V for positive-mode detection, respectively and 0.1 kV and 5 V for negative-mode detection, respectively. Source temperature and desolvation temperature were set at 120 and 350 °C, respectively. Nitrogen was used as both cone gas (50 L/h) and desolvation gas (600 L/h) and argon as collision gas. For accurate mass measurement, the mass spectrometer was calibrated with sodium formate solution with a mass-to-charge ratio (m/z) of 50−1000 and monitored by intermittent injection of the lock mass leucine enkephalin ($[M + H]^+$ = *m/z* 556.2771 or $[M + H]^-$ = *m/z* 554.2615) in real time. Mass chromatograms and mass spectral data were acquired and processed by MassLynx software (Waters, Milford, MA, USA) in centroided format. The relative abundance of individual compounds was determined by calculating the ratio between the peak area of compound and the peak area of internal standard and fitting with a standard curve using QuanLynx software (Waters, Milford, MA, USA).

Multivariate analysis and data visualization

The chromatographic and spectral data of samples were deconvoluted and analyzed using MarkerLynx software (Waters, Milford, MA, USA). A multivariate data matrix containing information on sample identity, ion identity (retention time and *m/z*), and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The intensity of each ion was calculated by normalizing the single-ion counts (SIC) vs. the total-ion counts (TIC) in the whole chromatogram. The data matrix and sample list were exported into SIMCA® software (version 14.1, Umetrics, Kinnelon, NJ, USA) and transformed by Pareto scaling, and then characterized by multivariate data analysis. Both unsupervised principal components analysis (PCA)

models and supervised models from partial least squares-discriminant analysis (PLS-DA) were constructed to delineate the relationship among sample groups, as well as the contribution of each MS signal to the principal components of the multivariate model.

Bacterial DNA extraction and 16S rRNA gene sequencing

Ileal digesta and fecal samples were thawed to room temperature and microbial DNA was extracted using a DNeasy PowerSoil kit (Qiagen, Duesseldorf, Germany) following the manufacturer's instructions. The quantification and quality check of the extracted DNA were performed with a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, DE, USA). The 16S sequencing procedure was performed at the University of Minnesota Genomic Center as previously described (Gohl et al., 2016). Briefly, ileal and fecal DNA samples (25 ng) were used as templates for PCR amplification of the V4 variable region of the 16S rRNA gene on the MiSeq sequencing platform and the primer used were V4_515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and V4_806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reactions were performed using KAPA HiFidelity Hot Start Polymerase (Kapa Biosystems, Woburn, MA) under the following cycling conditions: 5 min at 95°C, 35 cycles of 20 s at 98°C, 15 s at 55°C, and 1 min at 72°C; and, finally, 5 min at 72°C followed by holding at 4°C. Genomic DNA sequencing was performed using Illumina MiSeq Next Generation platform with a targeted average sequencing depth of 100,000 reads per sample as previously described (Wang et al., 2018). Raw sequences were denoised and quality filtered using fastx_toolkit and trimmomatic, and high-quality reads were processed with Dada2, mafft, and fasttree plugins in Qiime2 to obtain unique amplicon sequence variants (ASVs) and were then

compared against the Greengenes 13.8 database for taxonomic assignment (DeSantis et al., 2006; Gordon and Hannon, 2010; Bolyen et al., 2019).

Data analysis and statistics

Statistical analysis was performed using SAS 9.4 (SAS institute Inc., Cary, NC) and R programming. The amino acid, fatty acid, and BA data were analyzed using the MIXED procedure of SAS with AOP, diet, AOP × diet as fixed effects and individual pig was considered as the random effect. The outliers were identified using interquartile range method (IQR). Values that were more than 1.5 IQR below the first quartile or more than 1.5 IQR above the third quartile were considered outliers and removed. The LSMEANS procedure was used to calculate means and PDIFF option was used to separate least squares means with a Tukey-Kramer adjustment. A value of $P < 0.05$ was considered statistically significant, and $0.05 < P < 0.1$ was considered a trend. Correlations between metabolite concentration and nutrient digestibility were analyzed by Spearman's correlation using the function *corr.test* in R package *psych* with Benjamini-Hochberg correction. The correlation coefficients were visualized in heatmaps using R package *Pheatmap*. For untargeted metabolomics, data were analyzed and visualized with SIMCA software (version 14.1, Umetrics, Kinnelon, NJ, USA). The metabolites with a threshold of variable importance in projection (VIP) values > 1 and *P* value < 0.05 were considered differentially abundant in each treatment. The α-diversity was calculated using Shannon and Simpson diversity indices and comparisons between AOP group and the control group were made with Kruskal-Wallis tests with Benjamini-Hochberg correction. Principal coordinates analysis (PCoA) was performed on weighted Bray-Curtis distance calculated using the *vegan* package in R. Permutational multivariate

analysis of variance (PERMANOVA) and analysis of similarity (ANOSIM) using the *adnois* and *anosim* function in vegan package of R were performed. To identify specific taxa that were responsible for bacterial diversity variation between AOP and control, indicator species analysis was conducted using the *indval* function in R package *labdsv* (Dufrêne and Legendre, 1997). Taxa with an indicator value of > 0.5 and $P < 0.05$ were considered indicator species. Correlations between indicator ASVs, differential metabolites, and ATTD of nutrients were analyzed using the mantel test and Procrustes analyses also within the *vegan* package in R. The *envfit* function in R package *vegan* was used to fit microbial vectors to ordination plot with nutrient digestibility variables or metabolomic variables.

Results and Discussion

Diet is one of the main factors that determines the composition of ileal and fecal microbiome

After quality filtering and assembly, a total of 4,324,427 and 3,904,077 16S rRNA gene sequences were obtained from 35 ileal and 35 fecal samples (average of 123,555 sequences/ileal sample and 111,545 sequences/fecal sample). In the present study, we selected 3 common basal diets to represent different fiber type and composition, including DDGS, RB, and WM. Diet is a key factor that determines the composition of the gastrointestinal microbiome in human and animals (Frese et al., 2015; Singh et al., 2017). Our results are consistent with this concept and showed that diet type and composition is one of the main factors that contribute to the variation in the gut microbiome of growing pigs indicated by the distinct clustering of diet groups in PCoA analysis (Figure 4-1). PERMANOVA analyses confirmed the differences in microbial

communities for both ileal (R^2 = 0.15, P = 0.003) and fecal samples (R^2 = 0.25, P = 0.001) among the 3 dietary treatments. Specifically, evidence suggests that different types of fiber cause differential changes to the gut microbiota and metabolic status in human and pigs (Flis et al., 2017; Cronin et al., 2021). The three basal diets in our study consisted of different fibers with different solubility, viscosity, and fermentability characteristics. Therefore, it is likely that the variation in microbial composition among the dietary treatment groups can be attributed to the differences in fiber type and composition.

Aspergillus oryzae postbiotic differentially influenced α-diversity of microbial community in ileal digesta but not in feces

Considering that diet type contributes to major variations in microbiome, comparisons between pigs fed diets with AOP and without AOP were made separately within each basal diet. Compared with the large intestine, the pig small intestine harbors less abundant and less diverse microbes, which actively participate in the digestion and metabolism of nutrients (i.e., carbohydrates, lipids, and amino acids) (Dai et al., 2010; Martinez-Guryn et al., 2018). In pigs, even though the large intestine is the major location in the GIT where fiber fermentation occurs, there is also a substantial microbial fermentation of fiber (20-25%) in the small intestine (Bach Knudsen et al., 2013). In the ileal digesta, addition of AOP decreased the α-diversity in pigs fed RB diet as determined by observed number of ASVs (P < 0.05, Figure 4-2B) and Shannon index (P < 0.05, Figure 4-3B). In contrast, addition of AOP increased the α-diversity in pigs fed WM diet as determined by Shannon (P < 0.01, Figure 4-3C) and Simpson (P < 0.05, Figure 4-4C) indices. In pigs fed the DDGS diet, addition of AOP had no effects (P > 0.05) on the α-

diversity measured by observed ASVs (Figure 4-2A), Shannon (Figure 4-3A) or Simpson indices (Figure 4-4A). These results indicate that AOP influenced the microbial richness and diversity in the ileal digesta in a diet-dependent manner, which can be attributed to the combination of the differences in the fiber fractions among the three high fiber ingredients and the selective promotion of bacteria growth by AOP. Arabinoxylan (AX, sum of arabinose and xylose) is the main fiber type present in DDGS (13.10%), full fat RB (6.71%), and WM (18.80%), and accounts for 43.85, 33.42, and 49.34% of the total dietary fiber in these ingredients, respectively. The arabinose to xylose ratio (A:X) varies depending on the source of AX and is a direct measure for the degree of substitution which is an important indicator for structural feature of AX molecules (Courtin and Delcour, 1998). The A:X ratio was greatest in RB and lowest in WM, with an intermediate ratio in DDGS (Pedersen et al., 2014; Jaworski et al., 2015; Casas et al., 2019).

Interestingly, we found no differences ($P > 0.05$) in α-diversity of the microbial community in the feces measured by observed ASVs (Figure 4-2D, E, and F), Shannon (Figure 4-3D, E, and F) or Simpson indices (Figure 4-4D, E, and F) between AOP group and control group. This may be attributed to the much greater microbial abundance and diversity in the large intestine relative to the small intestine (Adhikari et al., 2019; Martinez-Guryn et al., 2019). Therefore the microbiome is more stable and less prone to be affected by the addition of microbial feed additives (Crespo-Piazuelo et al., 2018).

Addition of Aspergillus oryzae postbiotic changed β-diversity of microbial community in both ileal digesta and feces

To analyze the differences in microbial composition across AOP and control groups (without addition of AOP), the β-diversity of ileal and fecal microbiota was visualized with PCoA analysis and statistically analyzed with PERMANOVA using Bray-Curtis distance. The PCoA plots showed similar clustering patterns in ileal and fecal samples where AOP groups had clusters distinctly different from the control groups indicating that addition of AOP contributed to the variation of microbiota in ileal digesta and feces of pigs (Figure 4-5). Results of PERMANOVA confirmed the distinct clustering with *P* values for all comparisons < 0.05. Our results show for the first time that addition of AOP to high fiber diets significantly changed microbial composition in both ileal digesta and feces in growing pigs. Recently results from an *in vitro* study indicated that addition of a co-cultivation extract of *Aspergillus oryzae* and *Aspergillus niger* significantly changed the β-diversity of rumen fluid fermented with several types of substrates, including corn silage, oat hay, alfalfa hay, and a total mixed ratio (Kong et al., 2021). Probiotics have been used for many years as a feed additive to modulate gut microbiota associated with beneficial impacts on immune response, feed efficiency, and gut health of pigs (Siggers et al., 2008; Liao and Nyachoti, 2017; Shin et al., 2019). Our data suggest that AOP, biologically active compounds including cell-wall polysaccharides, and metabolites of probiotic *Aspergillus oryzae* could also effectively alter microbial richness and composition in the GIT of pigs which is associated with improved nutrient digestibility.

Identification of differentially abundant microbes

The results of β-diversity and PERMANOVA indicated significant dissimilarity in bacterial communities between AOP and control groups. Therefore, we conducted the indicator species analysis (ISA) to identify microbial taxa that could discriminate between the AOP and control group in the ileal digesta and feces. Indicator species are defined as those microbial species that are significantly more abundant and present in most samples belonging to one group, and also absent or with low abundance in other groups. Higher indicator values suggest better performances in the microbial signature of the assigned taxa. At the species level, there were 31, 9, and 39 ASVs enriched for DDGS+AOP (Figure 4-6A), RB+AOP (Figure 4-6B), and WM+AOP (Figure 4-6C), respectively, and 6, 15, and 10 ASVs for DDGS+AOP, RB+AOP, and WM+AOP, respectively were depleted in ileal digesta. There were more ASVs that were depleted than those were enriched in RB+AOP, which may explain the decrease in α-diversity we observed in the ileal digesta of RB+AOP. The identified indicator species with the highest indicator values for DDGS+AOP were ASV35, ASV107, ASV605, ASV1086, and ASV4, belonging to the genus *Clostridium* and *Streptococcus* (Table 4-1). The indicator species with the highest indicator values for RB+AOP were ASV648, ASV43, ASV27, ASV122, and ASV26, which belong to the genus *Epulopiscium*, *Bifidobacterium*, *Veillonella*, and *Actinobacillus* (Table 4-2). The indicator species with the highest indicator values for WM+AOP were ASV188, ASV1419, ASV683, ASV770, and ASV628, belonging to the genus *Acidaminococcus*, *Butyrivibrio*, *Atopobium*, and *Prevotella* (Table 4-3). Additionally, among the indicator species, ASV2 (*Bacteria*; *Firmicutes*; *Bacilli*; *Lactobacillales*; *Lactobacillaceae*; *Lactobacillus*) was the most

abundant with an indicator value of 0.74. *Lactobacillus* is considered one of the beneficial bacteria and the most commonly used probiotic in pigs (Parvez et al., 2006; Vieco-Saiz et al., 2019). The increased abundance of *Lactobacillus* observed from adding AOP to the WM diet suggests the potential of AOP for promoting growth and enhancing the health status of pigs. At the phylum level, the indicator taxa for DDGS+AOP were *Chloroflexi*, *Euryarchaeota*, *Proteobacteria*, and *Synergistetes* (Figure 4-7A). For RB+AOP, the indicator taxon was *Proteobacteria* (Figure 4-7B), and for WM+AOP, the indicator taxa were *Actinobacteria* and *Bacteroidetes* (Figure 4-7C).Changes of these taxa in the ileum by adding AOP did not cause differences in apparent ileal digestibility (AID) of dry matter (DM), gross energy (GE), crude protein (CP), neutral detergent fiber (NDF), and ash (Zhu et al., 2020b), indicating that microbial digestion of these nutrients in the small intestine are minimal relative to the digestion conducted by endogenous enzymes and microbial degradation in the large intestine of pigs.

In feces, there were 105, 50 and 70 ASVs that were enriched for DDGS+AOP (Figure 4-6D), RB+AOP (Figure 4-6E), and WM+AOP (Figure 4-6F), respectively, and 27, 68, and 37 ASVs that were depleted for DDGS+AOP, RB+AOP, and WM+AOP, respectively. The identified indicator species with the highest indicator values for DDGS+AOP were ASV248, ASV352, ASV481, ASV525, and ASV594, belonging to the family *Veillonellaceae*, *S24-7*, *Fibrobacteraceae*, *Erysipelotrichaceae*, and *Christensenellaceae* (Table 4-4). The indicator species with the highest indicator values for RB+AOP were ASV262, ASV363, ASV700, ASV1309, and ASV43 which belong to the family *Prevotellaceae*, *Ruminococcaceae*, *Bifidobacteriaceae*, and *S24-7* (Table 4-5). The indicator species with the highest indicator values for WM+AOP were ASV173,

ASV 327, ASV373, ASV455, and ASV208, belonging to the family *RF16*, *S24-7*, and *Clostridiaceae* (Table 4-6). The *RF16* is an unclassified family of the *Bacteroidales*, and the metabolism mechanism of which is not yet clear (Liu et al., 2019), Results from a recent study on intestinal microbiota associated feed efficiency in pigs showed that the *RF16* family was 20 times more abundant in the feces of pigs with a high feed efficiency than in the feces of pigs with a low feed efficiency (McCormack et al., 2022). Our findings in the present study, and results from a previous *in vivo* study showed an increase in nutrient digestibility in pigs fed WM+AOP, which suggested an association of family *RF1*6 with increased nutrient digestibility induced by the dietary addition of AOP (Zhu et al., 2020b). Furthermore, pigs fed diets containing AOP consistently had enriched family *S24-7* in the feces regardless of diet type. The *Bacteroidales S24-7* plays an important role in maintain gut health by inhibiting inflammation and was reported to be positively correlated with body weight gain of piglets (Han et al., 2019; Yang et al., 2021). The family *Veillonellaceae* is directly involved in the metabolic functions related to proteins and enzymes essential to the host (Zhang et al., 2018). Indeed, results from previous study showed that the addition of a probiotic increased relative abundance of *Veillonellaceae* in the feces of weaned piglets (Vieira et al., 2021). At the phylum level, the indicator taxa for DDGS+AOP were *Cyanobacteria*, *Elusimicrobia*, and *WPS.2* (Figure 4-7D). For RB+AOP, the indicator taxa were *Firmicutes* and *TM7* (Figure 4-7E), and for WM+AOP, the indicator taxon was *Actinobacteria* (Figure 4-7F). Many members of the phyla *Firmicutes*, *Actinobacteria*, and *Elusimicrobia* are known to be able to degrade fiber (Ikeda-Ohtsubo et al., 2010; Simpson and Campbell, 2015; Makki et al.,

2018). These results indicate that addition of dietary AOP may enrich certain types of fiber-degrading species.

Addition of Aspergillus oryzae postbiotic induced changes in ileal and fecal metabolome

To examined the effect of the dietary addition of AOP on ileal and fecal metabolites, targeted and untargeted metabolome analyses for ileal digesta and feces were conducted using LC-MS. Quantitative analysis of targeted metabolites showed that there was an interaction ($P = 0.04$) of AOP and dietary fiber source on the free Asn concentration in ileal digesta because Asn concentration in DDGS+AOP was greater than that in DDGS but did not differ in RB+AOP or WM+AOP when compared with the control treatments (Table 4-7). Moreover, dietary addition of AOP significantly decreased ($P < 0.05$) free taurine concentration in DDGS+AOP compared with DDGS in the ileal digesta. To assess the correlation of metabolite concentration and nutrient digestibility, we conducted Spearman correlation analysis using digestibility data from our previous *in vivo* study (Zhu et al., 2020b). Except for taurine, no significant correlations ($P > 0.05$) were observed between ileal concentrations of free amino acid concentration and apparent AID of DM, GE, CP, NDF, ether extract (EE), and ash (Figure 4-8A). The lack of correlations is not unexpected because the free amino acids measured in this study do not include those that constitute proteins in the undigested feed. Interestingly, we observed a significant negative correlation ($P = 0.04$, r = -0.61) between taurine and AID of EE. Bile acids are conjugated to taurine or glycine before being excreted into the intestine, where a major portion of conjugated BAs are absorbed in the terminal ileum and about 5% of total bile salt are metabolized by intestinal bacteria via

promoting deconjugation, dehydrogenation, and dihydroxylation of primary BAs (Wahlström et al., 2016; Staley et al., 2017). Therefore, the decrease in taurine concentration may suggest a depression in microbial metabolism of BAs in the intestine. Indeed, we observed an increase ($P < 0.05$) in total taurine-conjugated BAs in DDGS+AOP compared with DDGS in the ileal digesta (Table 4-8). Bile acids are essential for facilitating digestion and absorption of lipids in the small intestine (Lefebvre et al., 2009). Our previous study found that the addition of AOP significantly increased AID of EE in DDGS+AOP compared that in AOP (Zhu et al., 2020b). These results suggest that AOP may enhance lipid digestibility via modulation of bacteria that metabolize taurine-conjugated BAs.

In the feces, there was an interaction ($P < 0.01$) between AOP and dietary fiber source on concentration of Phe, which increased in WM+AOP compared with WM, but did not differ in DDGS+AOP or RB+AOP compared with control groups. Spearman correlation analysis indicated a positive correlation between fecal Phe and ATTD of CP (Figure 4-8D). Proteins in the feed are mainly digested in the small intestine of pigs, while undigested proteins can be utilized by bacteria in the large intestine (Pieper et al., 2016). Therefore, it is likely that AOP enhanced the bacterial breakdown of undigested protein in the large intestine of pigs.

Addition of AOP to diets significantly decreased ($P < 0.01$) the concentration of lauric acid (C12:0) in ileal digesta but had no effects on short-chain fatty acids, (SCFAs, i.e., acetic acid, butyric acid, propionic acid, and valeric acid) or long-chain fatty acids (Table 4-9). Dietary addition of AOP significantly decreased ($P < 0.05$) the concentration of C17:0 in the feces but did not affect the concentration of SCFAs and other fatty acids.

In the present study, the lack of effect of AOP on SCFA concentration is inconsistent with our previous findings observed in an *in vitro* study where the addition of AOP increased the amounts of butyric acid, propionic acid and total SCFA produced during *in vitro* fermentation with DDGS, RB, and WM as substrates (Zhu et al., 2020a). However, this inconsistency in responses could be explained by the fact that 90% of SCFAs produced are absorbed via passive diffusion in the caecum and proximal colon and therefore, SCFAs measured in the feces only accounts for less than 10% of the total SCFA produced (Imoto and Namioka, 1978; Jørgensen et al., 1997).

Identification of differentially abundant metabolites

The metabolome of ileal digesta and feces were compared among the three basal diets (i.e., DDGS, RB, and WM) and between the two treatment groups (i.e., AOP and control groups) by untargeted metabolomic analysis. In an unsupervised PCA model, the distribution of metabolites in ileal digesta (Figure 4-9A) and feces (Figure 4-10A) in the scores plots show clear separation among the three basal diets (i.e., DDGS, RB and WM) but there was no distinct clustering between AOP and control groups (Figure 4-11A and B), indicating that major metabolic changes were induced by different dietary fiber types. Therefore, we analyzed the effects of dietary addition of AOP on ileal and fecal metabolome separately within each basal diet in a supervised partial least squares-discriminant analysis (PLS-DA) model. Subtle changes in ileal (Figure 4-9B, C, and D) and fecal (Figure 4-10B, C, and D) metabolome were observed based on the separation of samples from pigs fed diets with AOP and those fed diets without AOP as indicated by the clustering patterns between AOP and control groups.

We further identified the metabolites that were differentially abundant between groups of pigs fed diets with and without AOP based on *P* value and VIP value. Compared with the control group, the relative abundance of 16 metabolites increased (VIP > 1; $P < 0.05$) in ileal digesta of pigs fed DDGS+AOP (Table 4-10), 11 in RB+AOP (Table 4-11), and 10 in WM+AOP (Table 4-12). Relative abundance of 3α-hydroxy-6-oxo-5β-cholan-24-oic acid, chaksine, 3-oxochol-4-en-24-oic acid, pangamic acid, 5β-chola-3,8(14),11-trien-24-oic acid, sphinganine, sphingofungin-B were greater in pigs fed DDGS+AOP compared with those fed DDGS. Oxo-bile acids (3α-hydroxy-6-oxo-5β-cholan-24-oic acid, 3-oxochol-4-en-24-oic acid) are metabolic derivatives of BAs (Porru et al., 2022), and the high abundance of these metabolites is consistent with the effects of dietary AOP on concentrations of taurine and taurine-conjugated BAs we observed in the ileal digesta of pigs fed DDGS+AOP. There is very limited information on the metabolic pathways and role of these metabolites, which makes it difficult to interpret the effects of AOP on these oxo-BAs and require further investigation. However, our data provide useful insights that the effect of AOP on AID of EE may be associated with its effects on gut microbes and their metabolism of BAs. We observed a decrease in relative abundance of chrysin, which is a natural occurring flavonoid found in diets possessing beneficial pharmacological functions (Samarghandian et al., 2017), in the ileum of pigs fed RB+AOP and WM+AOP. Pervious research has shown that chrysin is not metabolized by the pig intestinal microflora (Labib et al., 2004), and therefore, it is likely that the decrease in chrysin concentration observed in the current study was due to an increase in the absorption of chrysin by the small intestine. The underlying mechanisms of this AOP effect are not clear and these proposed explanations should be taken with caution because

the identification of metabolites was based on the search and match of the molecular mass of metabolites with those in the database in the present study and were not confirmed with tandem MS fragmentation and standards.

In feces, abundance of 23, 7 and 18 metabolites were increased, and 19, 15 and 17 metabolites were decreased by the dietary addition of AOP in pigs fed DDGS+AOP (Table 4-13), RB+AOP (Table 4-14), and WM+AOP (Table 4-15), respectively, compared with those fed the control diets. Interestingly, we observed greater abundance of phosphatidylcholine (PC) in feces of pigs fed AOP diets compared with those fed control diets, including PC (0:0/16:0), PC(18:1(6Z)/0:0), PC(O-16:0/2:0), PC(18:2(2E,4E)/0:0), PC(15:0/0:0), and PC(P-14:0/0:0). The PC (lecithin) is the major dietary source of choline, which is a semi-essential nutrient that is part of the B-complex vitamin family (Tang et al., 2013). Choline is converted to trimethylamine by gut microflora, of which are the known genera *Olsenell*a and *Desulfovibrio* (Martínez-del Campo et al., 2015). The increase in fecal PC in pigs fed DDGS+AOP suggests that AOP may inhibit trimethylamine-producing bacteria. Therefore, we compared the relative abundance of genera *Olsenella* and *Desulfovibri* in feces of pigs fed DDGS+AOP and DDGS. We did not find *Olsenella* in any of the 6 fecal samples from the DDGS group, and 2 of 6 samples in DDGS+AOP group were found to contain *Olsenella* with relative abundance averaging only 0.005%. Surprisingly, the relative abundance of *Desulfovibri* in feces of pigs fed DDGS+AOP was greater (P = 0.05, data no shown) than that for pigs fed DDGS. This inconsistency could be due to 1) other unidentified bacteria that may metabolize choline; and 2) other unidentified metabolites that are PC. The metabolites with the highest VIP values in WM+AOP were 13-deoxytedanolide, unidentified

MTB998 (4.219_391.2823_neg), MTB285 (1.4232_120.0818_Pos), MEB967 (4.0827_389.267_neg), and sulfadoxine. There is little information on the metabolism of these metabolites and therefore, the interpretation of these results requires further investigation.

Changes in fecal microbiome are associated with total tract digestibility of nutrient and fecal metabolome

In previous *in vivo* study, we found that dietary addition of AOP significantly increased ATTD of nutrients (i.e., DM, GE, CP, NDF, EE, and ash) in growing pigs fed DDGS, RB or WM diets, and the magnitude of improvement in ATTD was greater in pigs fed WM diet compared with DDGS or RB (Zhu et al., 2020b). Therefore, to identify differential ASVs that were associated with the ATTD of nutrients and metabolites, correlation analysis was conducted between ATTD of nutrient and different microbes and metabolites induced by feeding AOP. Mantel test showed a positive correlation (r = 0.38, *P* = 0.01) between ATTD of nutrients and different ASVs in feces of pigs fed WM and WM+AOP diets, which was further confirmed by the Procrustes analysis based on the Spearman method (correlation in a symmetric Procrustes rotation = 0.68, *P* = 0.02). The comparison of results by *envfit* analysis between WM+AOP and WM groups further revealed 14 different ASVs, showing significant correlations with ATTD of nutrients (Figure 4-12A, detailed in Table 4-16). The fecal metabolome matrix was also positively correlated with the microbiome composition, as indicated by the Mantel test (r = 0.54, *P* = 0.001) and Procrustes analysis (correlation in a symmetric Procrustes rotation = 0.83, *P* = 0.001). *Envfit* analysis revealed 14 different ASVs that were significantly correlated with the different fecal metabolites induced in pigs fed WM and WM+AOP diets (Figure

4-12B). Among the explanatory variables (ASVs) for ATTD of nutrients, there were 6 ASVs (i.e., ASV36, ASV77, ASV164, ASV208, ASV373, and ASV609) belonging to the order *Bacteroidales*, and two of them were identified members of the family *Muribaculaceae*. These ASVs were more abundant ($P < 0.05$) in feces of pigs fed WM+AOP compared with those fed AOP (Figure 4-12C). *Bacteroides* are the major fiber-degrading organisms in the gut and the abundance of *Bacteroides* has been shown to be correlated with the digested amount of xylose in growing pigs (Varel et al., 1987; Dicksved et al., 2015; G. Liu et al., 2021). The family *S24-7*, which was recently named *Muribaculaceae*, are cable of producing enzymes that degrade complex polysaccharides and has been identified as a key fiber degrader in mice (Lagkouvardos et al., 2019; H. Liu et al., 2021). Another explanatory variable for ATTD of nutrient was ASV447 (*Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae*) which belongs to the family *Ruminococcaceae* and is a well-known fiber-degrading bacteria with cellulolytic and hemicellulolytic activity that is dominant in the rumen as well as the large intestine of pigs (Perea et al., 2017; Quan et al., 2018). Therefore, the correlations we observed in the present study indicated that the addition of AOP to corn DDGS, RB and WM diets enriched certain fiber-degrading bacteria which were associated with the increased ATTD of nutrients in growing pigs.

Conclusions

In conclusion, we show for the first time that the addition of AOP to high fiber diets containing corn DDGS, RB, and or WM causes changes in the microbiome composition and metabolic profiles in the ileal digesta and feces of growing pigs. Altered diversity and composition of microbiome and metabolome from including AOP in these

high fiber diets was associated with improved nutrient digestibility in growing pigs. We further identified microbes and metabolites that were changed by adding AOP to high fiber diets and determined their associations with nutrient digestibility. These findings provide new insights into mechanisms of action of AOP and demonstrated the feasibility of dietary addition of AOP in improving feeding values of high fiber coproducts via modulation of microbiome and metabolome in pigs.

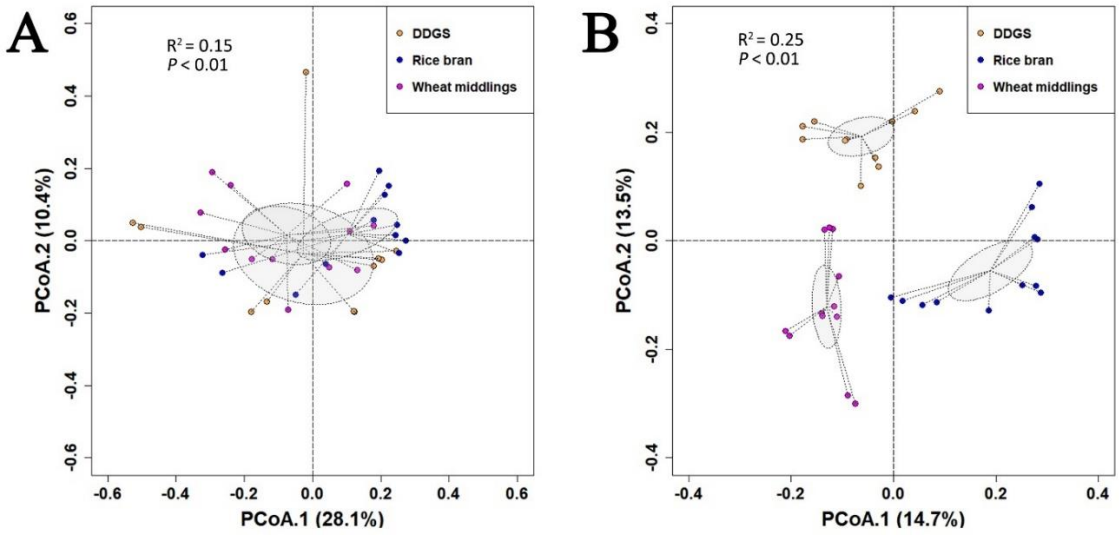

Figure 4-1. The β-diversity of the ileal (A) and fecal (B) microbiota among three basal diet groups. Principal coordinates analysis of community composition was conducted based on weighted Bray-Curtis distance. DDGS = distillers dried grains with solubles; RB= rice bran; WM = wheat middlings.

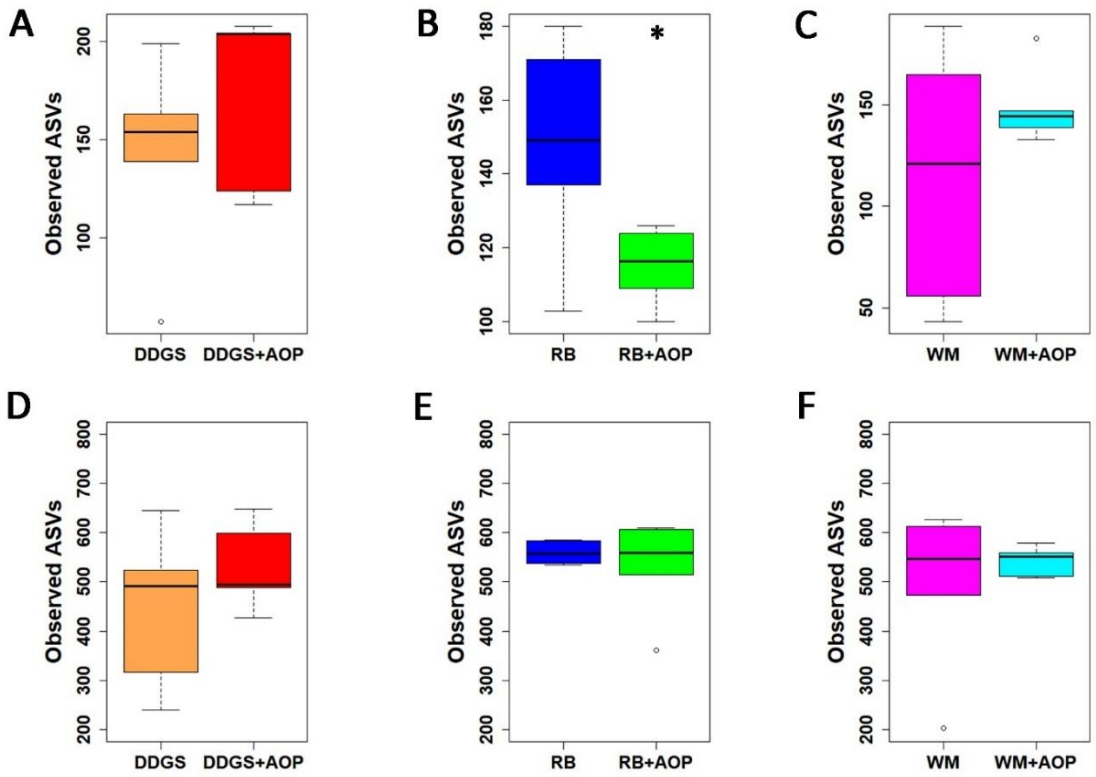

Figure 4-2. Boxplot of α-diversity measured by number of observed amplicon sequence variants (ASVs) of microbial communities in the ileal digesta of growing pigs fed diets formulated with corn distillers dried grains with solubles (DDGS), rice bran (RB) or wheat middlings (WM) with or without the addition of *Aspergillus oryzae* postbiotic (AOP). (A-C) Comparisons of observed ASVs between AOP and control group in ileal digesta. (D-F) Comparisons of observed ASVs between AOP and control in feces. * and ** represent $P < 0.05$ and $P < 0.01$ in a Wilcoxon test.

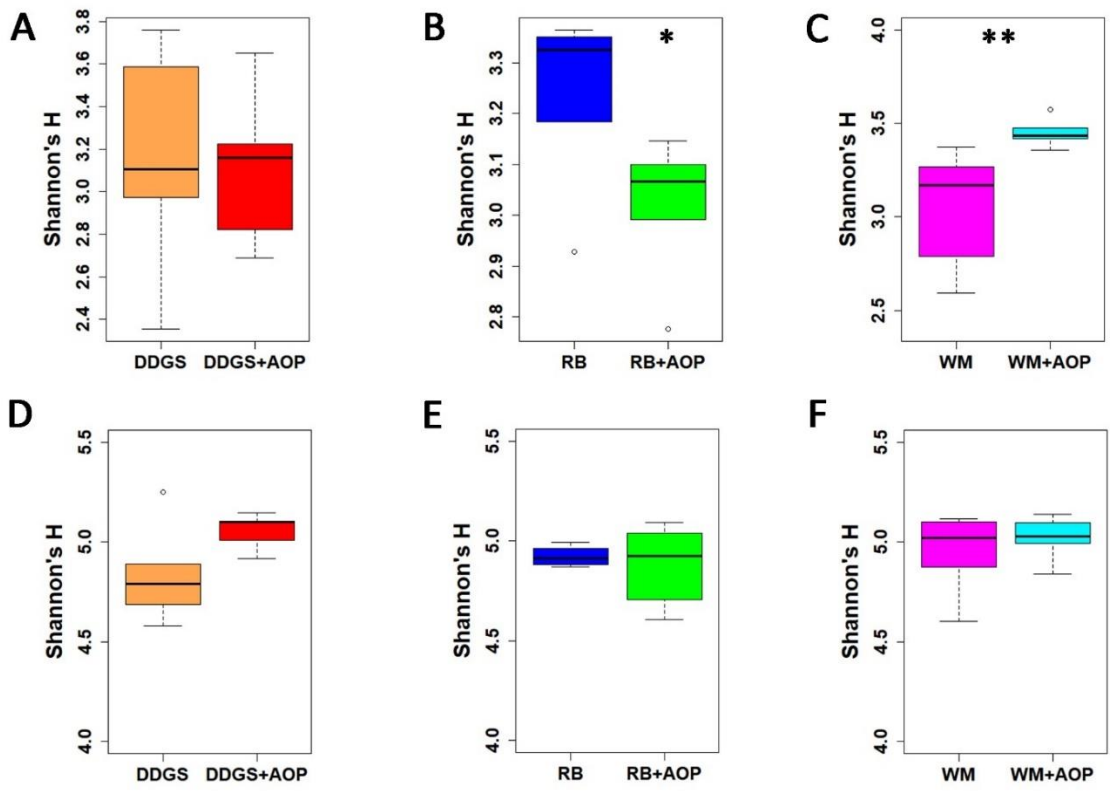

Figure 4-3. Boxplot of α-diversity measured by Shannon'H index of microbial communities in the ileal digesta of growing pigs fed diets formulated with corn distillers dried grains with solubles (DDGS), rice bran (RB) or wheat middlings (WM) with or without the addition of *Aspergillus oryzae* postbiotic (AOP). (A-C) Comparisons of Shannon'H index between AOP and control group in ileal digesta. (D-F) Comparisons of Shannon'H index between AOP and control in feces. * and ** represent $P < 0.05$ and $P < 0.01$ in a Wilcoxon test.

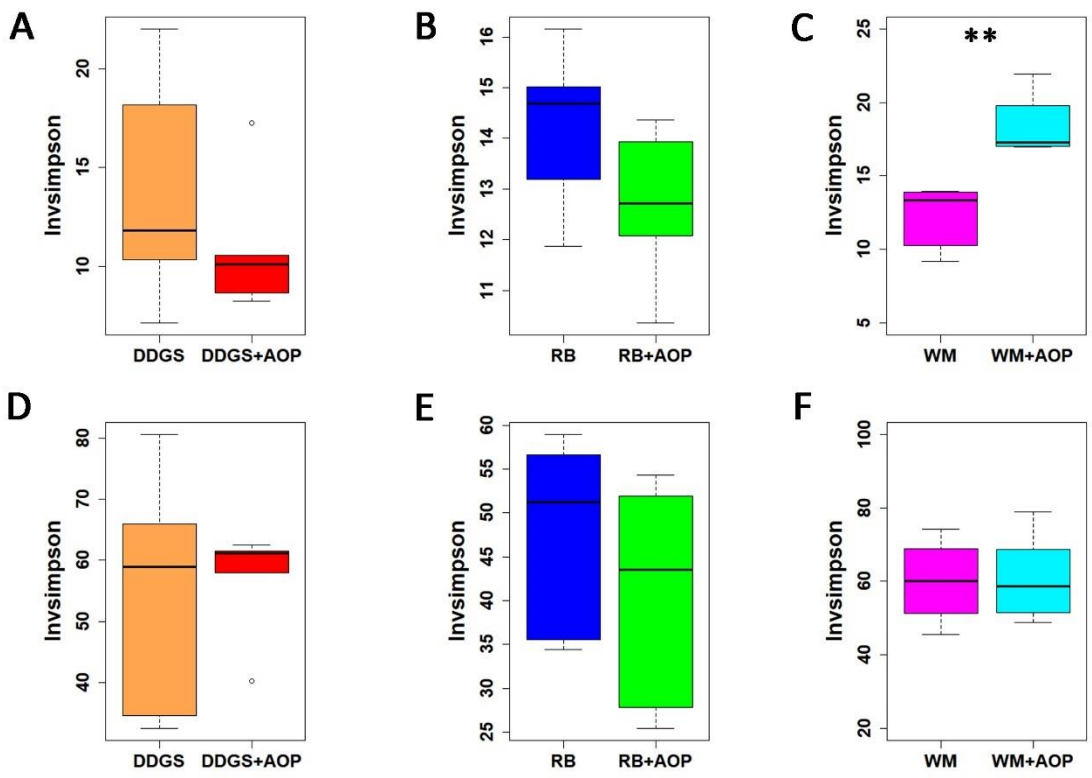

Figure 4-4. Boxplot of α-diversity measured by Simson index of microbial community in the ileal digesta of growing pigs fed diets formulated with corn distillers dried grains with solubles (DDGS), rice bran (RB) or wheat middlings (WM) with or without the addition of *Aspergillus oryzae* postbiotic (AOP). (A-C) Comparisons of Simson index between AOP and control group in ileal digesta. (D-F) Comparisons of Simson index between AOP and control in feces. * and ** represent $P < 0.05$ and $P < 0.01$ in a Wilcoxon test.

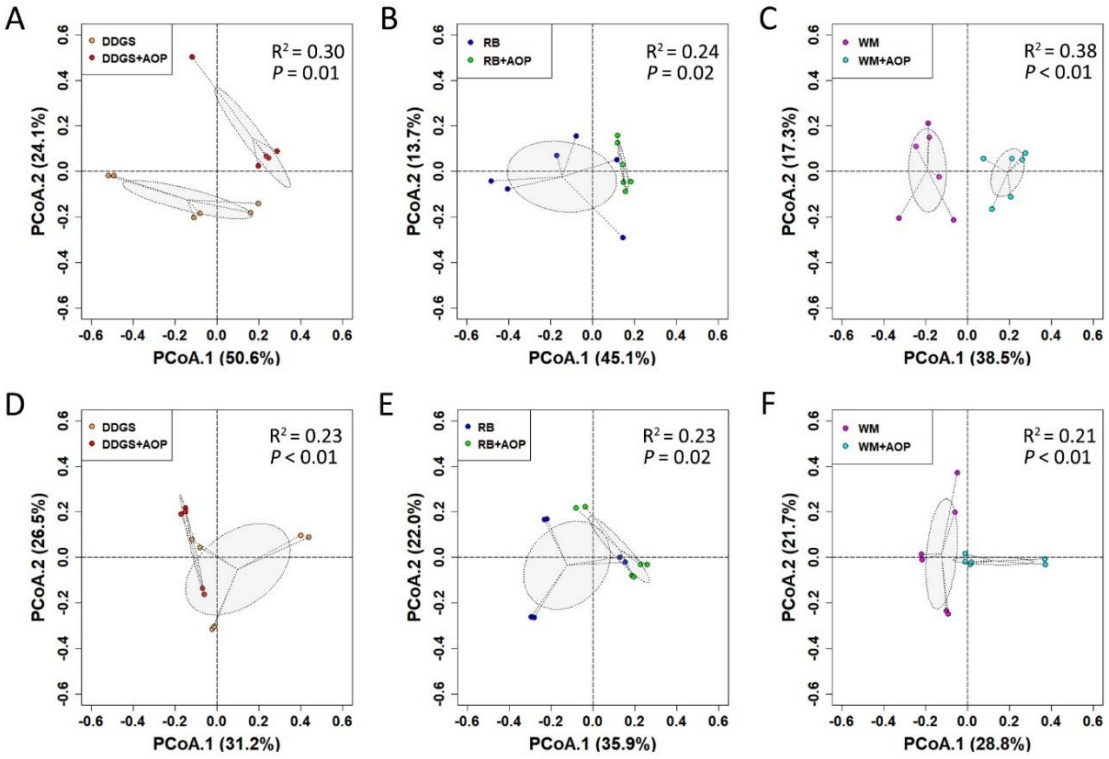

Figure 4-5. The β-diversity of the ileal (A-C) and fecal (D-F) microbiota between pigs fed diets with and without addition of *Aspergillus oryzae* postbiotic (AOP). Comparisons were made within the same basal diet, e.g., corn distillers dried grains with solubles (DDGS), rice bran (RB) or wheat middlings (WM). Principal coordinates analysis of microbial community composition was conducted based on weighted Bray-Curtis distance. R square and *P* value in a Permutational multivariate analysis of variance (PERMANOVA) were shown on each plot.

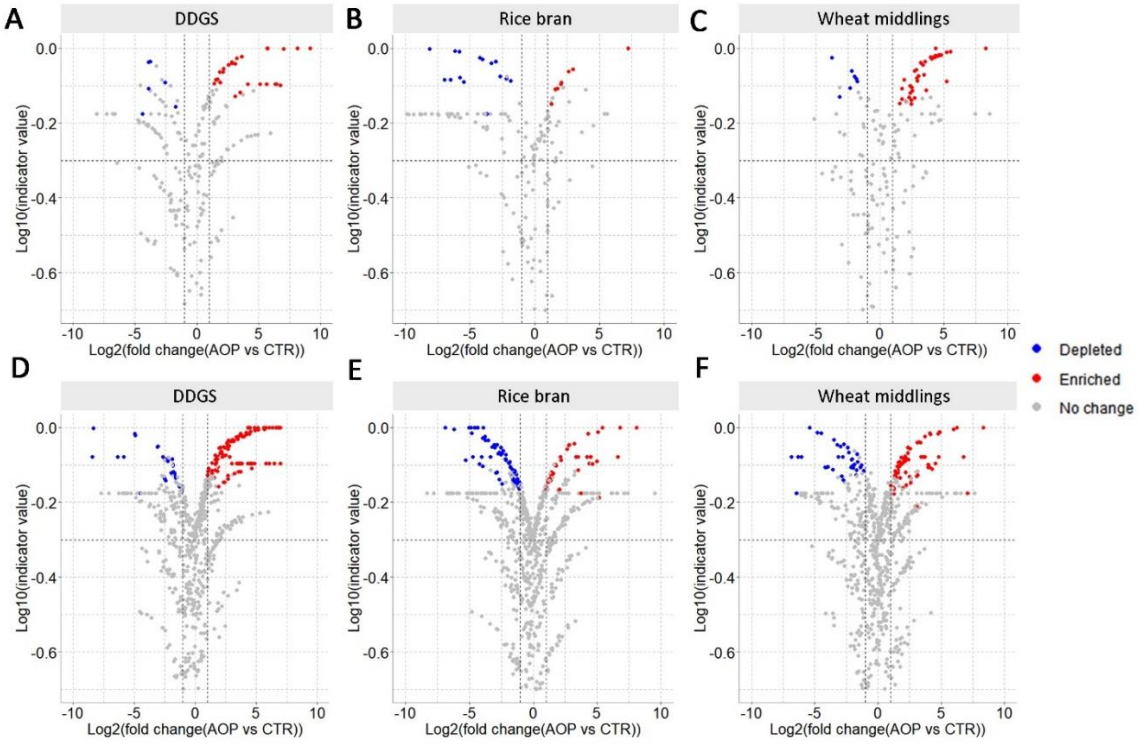

Figure 4-6. Addition of *Aspergillus oryzae* postbiotic (AOP) enriched or depleted certain amplicon sequence variants (ASVs) in pigs fed high fiber diets. (A - C) Enrichment and depletion of ASVs in ileal digesta from AOP groups compared with controls as determined by indicator species analysis (ISA). (D - F) Enrichment and depletion of ASVs in feces from AOP groups compared with controls as determined by ISA. Each point represents an individual ASV, and the position along the x axis represents the abundance fold changes in AOP groups compared with the control groups, and position along the y axis represents the indicator value. Points in red or blue represent ASVs that have an indicator value >= 0.5, *P* value < 0.05, and absolute fold change >= 2.

Table 4-1. Differentially abundant amplicon sequence variant (ASV) in the ileal digesta between pigs fed DDGS diet with (DDGS+AOP) and without AOP (DDGS) identified with the indicator species analysis[1]

ASV_ID	Taxonomic annotation	Group[2]	Indicator value[3]	*P* value[4]	DDGS	DDGS+AOP
ASV55	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	DDGS	0.92	0.04	0.80	0.07
ASV2	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	DDGS	0.92	0.01	14.37	1.28
ASV24	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__	DDGS	0.81	< 0.01	1.01	0.24
ASV683	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS	0.78	0.05	0.02	0.00
ASV25	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__mucosae	DDGS	0.70	0.02	1.39	0.61
ASV92	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	DDGS	0.67	0.05	0.15	0.00
ASV35	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium	DDGS+AOP	1.00	< 0.01	0.00	0.37
ASV107	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae	DDGS+AOP	1.00	< 0.01	0.00	0.72
ASV605	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__agalactiae	DDGS+AOP	1.00	< 0.01	0.00	0.06
ASV1086	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__	DDGS+AOP	1.00	< 0.01	0.00	0.07
ASV4	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__alactolyticus	DDGS+AOP	0.99	0.01	0.04	7.69
ASV250	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__D168	DDGS+AOP	0.95	0.01	0.02	0.31
ASV1134	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__[Eubacterium]; s__	DDGS+AOP	0.94	0.01	0.00	0.05
ASV293	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	DDGS+AOP	0.92	0.02	0.02	0.18

ASV98	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__; s__	DDGS+AOP	0.91	0.01	0.07	0.69
ASV26	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae; g__Actinobacillus; s__porcinus	DDGS+AOP	0.91	0.01	0.17	1.64
ASV378	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Clostridium; s__ramosum	DDGS+AOP	0.90	0.01	0.04	0.32
ASV276	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__	DDGS+AOP	0.88	0.01	0.01	0.05
ASV502	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	DDGS+AOP	0.86	0.01	0.01	0.05
ASV562	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__Atopobium; s__	DDGS+AOP	0.86	0.01	0.01	0.05
ASV1193	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfobacterales; f__Desulfobulbaceae; g__Desulfobulbus; s__	DDGS+AOP	0.83	0.03	0.00	0.02
ASV527	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Actinomycetaceae; g__Actinomyces; s__	DDGS+AOP	0.82	0.01	0.03	0.14
ASV27	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Veillonella; s__parvula	DDGS+AOP	0.82	0.02	0.40	1.81
ASV48	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria	DDGS+AOP	0.81	0.02	0.46	1.93
ASV99	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__; s__	DDGS+AOP	0.80	0.03	0.13	0.51
ASV314	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__	DDGS+AOP	0.80	0.01	0.00	0.10
ASV808	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Shuttleworthia; s__	DDGS+AOP	0.80	0.02	0.00	0.06
ASV839	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Neisseriales; f__Neisseriaceae; g__Neisseria; s__canis	DDGS+AOP	0.80	0.02	0.00	0.11
ASV925	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Actinomycetaceae; g__Actinomyces; s__	DDGS+AOP	0.80	0.01	0.00	0.04
ASV1717	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus	DDGS+AOP	0.80	0.01	0.00	0.02
ASV660	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Leuconostocaceae; g__Weissella; s__viridescens	DDGS+AOP	0.80	0.04	0.00	0.21

ASV1877	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria; g__; s__	DDGS+AOP	0.76	0.03	0.00	0.02
ASV37	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Pirellulales; f__Pirellulaceae; g__; s__	DDGS+AOP	0.74	0.02	0.00	0.01
ASV227	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Phascolarctobacterium; s__	DDGS+AOP	0.74	0.04	0.10	0.28
ASV105	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Fusobacterium; s__	DDGS+AOP	0.73	0.04	0.13	0.36
ASV8	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Streptophyta; f__; g__; s__	DDGS+AOP	0.72	0.04	3.83	9.66
ASV38	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Fusobacterium; s__	DDGS+AOP	0.69	0.04	0.38	0.86

[1]Indicator species were identified based on the criteria that 1) with an indicator value greater than 0.5, and 2) a *P* value less than 0.05. These species were significantly more abundant and present in most samples belonging to one group, and also absent or with low abundance in the other group.

[2]The group each ASV has maximum indicator value for.

[3]The indicator value for each species to its maximum group ranging from 0 to 1. Higher indicator values suggested better performances in the microbial signature of the assigned taxa.

[4]The probability of obtaining as high an indicator values as observed over the specified iterations.

Table 4-2. Differentially abundant amplicon sequence variant (ASV) in the ileal digesta between pigs fed rice bran diet with (RB+AOP) and without AOP (RB) identified with the indicator species analysis[1]

ASV_ID	Taxonomic annotation	Group[2]	Indicator value[3]	*P* value[4]	Rice bran	Rice bran +AOP
ASV24	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__	Rice bran	1.00	0.01	1.24	0.01
ASV15	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Megasphaera; s__	Rice bran	0.98	0.01	2.76	0.05
ASV45	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Sharpea; s__azabuensis	Rice bran	0.98	< 0.01	0.99	0.02
ASV9	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__delbrueckii	Rice bran	0.94	0.01	0.79	0.05
ASV2	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	Rice bran	0.93	0.05	9.80	0.72
ASV731	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Rice bran	0.92	0.02	0.03	0.00
ASV749	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__uniformis	Rice bran	0.91	0.01	0.05	0.01
ASV329	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Dialister; s__	Rice bran	0.83	0.02	0.05	0.00
ASV18	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53; s__	Rice bran	0.83	0.03	3.17	0.66
ASV115	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Mitsuokella	Rice bran	0.82	0.02	0.33	0.00
ASV310	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	Rice bran	0.82	0.03	0.16	0.00
ASV565	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Epulopiscium; s__	Rice bran	0.82	0.01	0.03	0.01
ASV135	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	Rice bran	0.81	0.02	0.06	0.00
ASV120	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__mucosae	Rice bran	0.67	0.05	0.23	0.00

ASV199	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae	Rice bran	0.67	0.05	0.10	0.00
ASV648	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Epulopiscium; s__	Rice bran+AOP	1.00	< 0.01	0.00	0.14
ASV43	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__pseudolongum	Rice bran+AOP	0.88	0.02	0.13	0.94
ASV27	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Veillonella; s__parvula	Rice bran+AOP	0.87	< 0.01	0.21	1.37
ASV122	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae; g__Actinobacillus; s__porcinus	Rice bran+AOP	0.81	0.01	0.15	0.66
ASV26	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae; g__Actinobacillus; s__porcinus	Rice bran+AOP	0.80	< 0.01	0.80	3.20
ASV12	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__celatum	Rice bran+AOP	0.78	0.01	1.28	4.50
ASV298	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae	Rice bran+AOP	0.78	0.02	0.05	0.16
ASV111	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__; s__	Rice bran+AOP	0.71	0.02	0.24	0.59
ASV72	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__; s__	Rice bran+AOP	0.65	0.01	0.23	0.43

[1]Indicator species were identified based on the criteria that 1) with an indicator value greater than 0.5, and 2) a *P* value less than 0.05. These species were significantly more abundant and present in most samples belonging to one group, and also absent or with low abundance in the other group.

[2]The group each ASV has maximum indicator value for.

[3]The indicator value for each species to its maximum group ranging from 0 to 1. Higher indicator values suggested better performances in the microbial signature of the assigned taxa.

[4]The probability of obtaining as high an indicator values as observed over the specified iterations.

Table 4-3. Differentially abundant amplicon sequence variant (ASV) in the ileal digesta between pigs fed wheat middlings diet with (WM+AOP) and without AOP (WM) identified with the indicator species analysis[1]

ASV_ID	Taxonomic annotation	Group	Indicator value[2]	*P* value[3]	Wheat middlings[4]	Wheat middlings+AOP
ASV99	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__; s__	Wheat middlings	0.94	0.01	0.79	0.05
ASV53	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings	0.87	0.01	0.76	0.11
ASV227	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Phascolarctobacterium; s__	Wheat middlings	0.84	<0.01	0.31	0.06
ASV38	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Fusobacterium; s__	Wheat middlings	0.83	0.01	1.26	0.26
ASV23	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Fusobacteriaceae; g__Fusobacterium; s__	Wheat middlings	0.82	0.02	2.74	0.62
ASV310	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	Wheat middlings	0.74	0.04	0.12	0.02
ASV153	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings	0.73	0.05	0.27	0.10
ASV7	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae; g__; s__	Wheat middlings	0.68	0.03	4.96	2.32
ASV3	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53; s__	Wheat middlings	0.68	0.01	14.14	6.69
ASV188	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Acidaminococcus; s__	Wheat middlings+AOP	1.00	0.01	0.00	0.28
ASV1419	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Butyrivibrio; s__	Wheat middlings+AOP	1.00	<0.01	0.00	0.02

ASV683	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	Wheat middlings+AOP	0.98	0.01	0.00	0.08
ASV770	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__Atopobium; s__	Wheat middlings+AOP	0.97	0.01	0.00	0.06
ASV628	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.96	0.01	0.00	0.08
ASV189	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.96	<0.01	0.03	0.70
ASV274	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Bulleidia; s__p-1630-c5	Wheat middlings+AOP	0.96	<0.01	0.01	0.32
ASV60	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.96	<0.01	0.09	2.07
ASV92	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.95	0.01	0.10	1.87
ASV167	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Mitsuokella	Wheat middlings+AOP	0.95	0.01	0.02	0.35
ASV907	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__	Wheat middlings+AOP	0.95	0.01	0.00	0.04
ASV90	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Acidaminococcus; s__	Wheat middlings+AOP	0.95	<0.01	0.09	1.52
ASV41	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.95	0.01	0.22	3.83
ASV137	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.94	0.01	0.02	0.34
ASV67	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Mitsuokella; s__	Wheat middlings+AOP	0.94	<0.01	0.05	0.79
ASV848	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Bulleidia; s__	Wheat middlings+AOP	0.92	<0.01	0.00	0.04

ASV666	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Megasphaera; s__	Wheat middlings+AOP	0.91	0.01	0.00	0.04
ASV135	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	Wheat middlings+AOP	0.90	<0.01	0.09	0.79
ASV74	k__Bacteria; p__Proteobacteria; c__Epsilonproteobacteria; o__Campylobacterales; f__Campylobacteraceae; g__Campylobacter; s__	Wheat middlings+AOP	0.87	0.02	0.03	0.20
ASV55	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	Wheat middlings+AOP	0.87	0.01	0.22	1.46
ASV329	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Dialister; s__	Wheat middlings+AOP	0.85	0.02	0.02	0.13
ASV68	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	Wheat middlings+AOP	0.84	<0.01	0.14	0.76
ASV45	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Sharpea; s__azabuensis	Wheat middlings+AOP	0.83	<0.01	0.21	1.06
ASV225	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Porphyromonas; s__	Wheat middlings+AOP	0.82	0.02	0.06	0.28
ASV382	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	Wheat middlings+AOP	0.82	0.01	0.03	0.15
ASV261	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__agilis	Wheat middlings+AOP	0.81	0.02	0.00	0.10
ASV825	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Eubacteriaceae; g__Pseudoramibacter_Eubacterium; s__	Wheat middlings+AOP	0.79	0.01	0.01	0.04
ASV316	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]	Wheat middlings+AOP	0.79	0.01	0.05	0.17
ASV680	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Succinivibrionaceae	Wheat middlings+AOP	0.78	0.03	0.01	0.04

ASV1423	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	Wheat middlings+AOP	0.77	0.03	0.00	0.02
ASV9	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__delbrueckii	Wheat middlings+AOP	0.77	0.03	2.08	6.94
ASV30	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__; s__	Wheat middlings+AOP	0.76	0.01	1.08	3.38
ASV2	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	Wheat middlings+AOP	0.74	0.03	3.12	8.82
ASV289	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	Wheat middlings+AOP	0.74	0.03	0.04	0.10
ASV242	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	Wheat middlings+AOP	0.73	0.03	0.08	0.22
ASV119	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Dialister; s__	Wheat middlings+AOP	0.73	0.03	0.18	0.48
ASV15	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Megasphaera; s__	Wheat middlings+AOP	0.72	0.01	1.07	2.74
ASV18	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53; s__	Wheat middlings+AOP	0.71	0.03	2.20	5.41
ASV1214	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__Bulleidia; s__	Wheat middlings+AOP	0.71	0.04	0.00	0.02
ASV570	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__delbrueckii	Wheat middlings+AOP	0.67	0.05	0.00	0.16

[1]Indicator species were identified based on the criteria that 1) with an indicator value greater than 0.5, and 2) a *P* value less than 0.05. These species were significantly more abundant and present in most samples belonging to one group, and also absent or with low abundance in the other group.

[2]The group each ASV has maximum indicator value for.

[3]The indicator value for each species to its maximum group ranging from 0 to 1. Higher indicator values suggested better performances in the microbial signature of the assigned taxa.

[4]The probability of obtaining as high an indicator values as observed over the specified iterations.

Table 4-4. Differentially abundant amplicon sequence variant (ASV) in the feces between pigs fed DDGS diet with (DDGS+AOP) and without AOP (DDGS) identified with the indicator species analysis[1]

ASV_ID	Taxonomic annotation	Group[2]	Indicator value[3]	*P* value[4]	DDGS	DDGS+AOP
ASV85	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	DDGS	1.00	<0.01	1.07	0.00
ASV514	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Z20; f__R4-45B; g__; s__	DDGS	0.96	<0.01	0.20	0.01
ASV444	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	DDGS	0.95	0.01	0.17	0.01
ASV240	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS	0.89	<0.01	0.14	0.02
ASV193	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	DDGS	0.89	0.02	0.69	0.09
ASV186	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	DDGS	0.83	0.03	0.40	0.08
ASV259	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	DDGS	0.83	0.02	0.10	0.00
ASV367	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS	0.83	0.02	0.50	0.00
ASV475	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	DDGS	0.83	0.02	0.08	0.00
ASV1059	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS	0.83	0.02	0.07	0.00
ASV641	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS	0.82	0.01	0.03	0.01
ASV75	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	DDGS	0.82	<0.01	1.37	0.29
ASV285	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS	0.81	<0.01	0.20	0.05
ASV19	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__p-2534-18B5; g__; s__	DDGS	0.81	0.04	3.40	0.79
ASV171	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS	0.80	0.03	0.17	0.04
ASV42	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	DDGS	0.79	<0.01	2.05	0.55

ASV215	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS	0.76	<0.01	0.27	0.08
ASV936	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Eubacteriaceae; g__Anaerofustis; s__	DDGS	0.76	0.03	0.08	0.02
ASV391	k__Archaea; p__Euryarchaeota; c__Thermoplasmata; o__E2; f__[Methanomassiliicoccaceae]; g__; s__	DDGS	0.76	0.02	0.11	0.04
ASV59	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Selenomonas; s__bovis	DDGS	0.75	0.02	0.41	0.14
ASV224	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	DDGS	0.73	<0.01	0.36	0.13
ASV308	k__Bacteria; p__Fibrobacteres; c__Fibrobacteria; o__Fibrobacterales; f__Fibrobacteraceae; g__Fibrobacter; s__	DDGS	0.73	0.04	0.08	0.01
ASV967	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	DDGS	0.72	0.04	0.02	0.00
ASV219	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS	0.70	0.01	0.16	0.07
ASV199	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae	DDGS	0.68	0.04	0.10	0.02
ASV2	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	DDGS	0.67	0.01	3.35	1.63
ASV135	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	DDGS	0.67	0.05	0.01	0.00
ASV155	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	DDGS	0.67	0.05	0.05	0.00
ASV556	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS	0.67	0.05	0.27	0.00
ASV248	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Anaerovibrio; s__	DDGS+AOP	1.00	0.01	0.00	0.19
ASV352	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.18
ASV481	k__Bacteria; p__Fibrobacteres; c__Fibrobacteria; o__Fibrobacterales; f__Fibrobacteraceae; g__Fibrobacter; s__	DDGS+AOP	1.00	<0.01	0.00	0.10
ASV525	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__RFN20; s__	DDGS+AOP	1.00	<0.01	0.00	0.13

ASV594	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.04
ASV696	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.08
ASV769	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	DDGS+AOP	1.00	0.01	0.00	0.05
ASV814	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.17
ASV837	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.16
ASV879	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	DDGS+AOP	1.00	<0.01	0.00	0.12
ASV942	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	DDGS+AOP	1.00	<0.01	0.00	0.07
ASV1066	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	DDGS+AOP	1.00	<0.01	0.00	0.04
ASV1129	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.09
ASV1292	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.03
ASV1342	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.03
ASV1353	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.04
ASV1450	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__	DDGS+AOP	1.00	<0.01	0.00	0.03
ASV1548	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Mitsuokella; s__multacida	DDGS+AOP	1.00	0.01	0.00	0.04
ASV1808	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	1.00	<0.01	0.00	0.03
ASV690	k__Bacteria; p__Elusimicrobia; c__Elusimicrobia; o__Elusimicrobiales; f__Elusimicrobiaceae; g__; s__	DDGS+AOP	0.99	0.01	0.00	0.12
ASV338	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Tremblayales; f__; g__; s__	DDGS+AOP	0.98	<0.01	0.00	0.26

ASV35	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium	DDGS+AOP	0.98	<0.01	0.00	0.10
ASV1314	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	DDGS+AOP	0.98	0.01	0.00	0.05
ASV608	k__Bacteria; p__Tenericutes; c__RF3; o__ML615J-28; f__; g__; s__	DDGS+AOP	0.97	0.01	0.01	0.17
ASV146	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	DDGS+AOP	0.97	0.01	0.02	0.55
ASV544	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__; s__	DDGS+AOP	0.96	0.01	0.01	0.22
ASV1151	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.96	<0.01	0.00	0.04
ASV985	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; s__	DDGS+AOP	0.96	0.01	0.00	0.11
ASV202	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__YS2; f__; g__; s__	DDGS+AOP	0.96	0.01	0.02	0.49
ASV735	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Lachnospira; s__	DDGS+AOP	0.95	0.01	0.01	0.14
ASV559	k__Bacteria	DDGS+AOP	0.95	0.01	0.02	0.30
ASV87	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__	DDGS+AOP	0.95	0.01	0.04	0.62
ASV772	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.94	0.01	0.00	0.06
ASV729	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.93	<0.01	0.00	0.04
ASV784	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Succinivibrionaceae; g__Succinivibrio; s__	DDGS+AOP	0.93	0.01	0.01	0.06
ASV638	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Anaeroplasmatales; f__Anaeroplasmataceae; g__Anaeroplasma; s__	DDGS+AOP	0.93	0.01	0.01	0.14
ASV812	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	DDGS+AOP	0.92	<0.01	0.01	0.14
ASV606	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	0.92	0.02	0.01	0.06
ASV101	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	DDGS+AOP	0.92	0.01	0.05	0.58

ASV646	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii	DDGS+AOP	0.92	0.04	0.01	0.14
ASV377	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.91	0.01	0.01	0.13
ASV9	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__delbrueckii	DDGS+AOP	0.90	0.01	0.02	0.15
ASV276	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__	DDGS+AOP	0.89	0.02	0.03	0.28
ASV218	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	DDGS+AOP	0.89	0.01	0.05	0.44
ASV817	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__callidus	DDGS+AOP	0.89	0.01	0.01	0.06
ASV537	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.89	0.01	0.01	0.08
ASV619	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	DDGS+AOP	0.88	0.01	0.01	0.07
ASV1015	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	DDGS+AOP	0.88	0.01	0.01	0.06
ASV237	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.87	0.01	0.04	0.28
ASV987	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	0.86	0.03	0.01	0.07
ASV809	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.85	0.01	0.01	0.05
ASV417	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__; s__	DDGS+AOP	0.85	0.02	0.02	0.13
ASV533	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__; s__	DDGS+AOP	0.84	0.01	0.01	0.07
ASV803	k__Bacteria; p__WPS-2; c__; o__; f__; g__; s__	DDGS+AOP	0.84	0.01	0.01	0.06
ASV362	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	DDGS+AOP	0.84	0.02	0.06	0.32
ASV33	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__	DDGS+AOP	0.84	0.02	0.03	0.16
ASV833	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	DDGS+AOP	0.83	0.02	0.02	0.08

ASV536	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	DDGS+AOP	0.83	0.02	0.04	0.18
ASV1244	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.82	0.02	0.01	0.04
ASV96	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	DDGS+AOP	0.82	0.02	0.13	0.62
ASV133	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__	DDGS+AOP	0.82	0.01	0.04	0.19
ASV1172	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.82	0.02	0.00	0.02
ASV82	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	DDGS+AOP	0.80	<0.01	0.13	0.53
ASV1011	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.80	0.02	0.01	0.03
ASV363	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	DDGS+AOP	0.80	0.01	0.00	0.04
ASV495	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.80	0.02	0.00	0.02
ASV737	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.80	0.02	0.00	0.02
ASV750	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	DDGS+AOP	0.80	0.02	0.00	0.03
ASV764	k__Bacteria; p__Verrucomicrobia; c__Verruco-5; o__WCHB1-41; f__RFP12; g__; s__	DDGS+AOP	0.80	0.01	0.00	0.01
ASV794	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	DDGS+AOP	0.80	0.02	0.00	0.09
ASV818	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__	DDGS+AOP	0.80	0.02	0.00	0.16
ASV822	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.80	0.02	0.00	0.18
ASV951	k__Bacteria; p__Elusimicrobia; c__Elusimicrobia; o__Elusimicrobiales; f__Elusimicrobiaceae; g__; s__	DDGS+AOP	0.80	0.01	0.00	0.12
ASV1234	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__stercorea	DDGS+AOP	0.80	0.02	0.00	0.09

ASV1283	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__; f__; g__; s__	DDGS+AOP	0.80	0.01	0.00	0.03
ASV1319	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__; g__; s__	DDGS+AOP	0.80	0.01	0.00	0.02
ASV1347	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__callidus	DDGS+AOP	0.80	0.02	0.00	0.04
ASV1413	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.80	0.01	0.00	0.04
ASV1601	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.80	0.02	0.00	0.02
ASV1685	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__YS2; f__; g__; s__	DDGS+AOP	0.80	0.02	0.00	0.03
ASV2012	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	DDGS+AOP	0.80	0.02	0.00	0.02
ASV332	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri	DDGS+AOP	0.80	0.01	0.10	0.41
ASV4	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__alactolyticus	DDGS+AOP	0.79	0.01	1.02	3.72
ASV450	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__	DDGS+AOP	0.78	0.03	0.00	0.11
ASV314	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__Desulfovibrio; s__	DDGS+AOP	0.78	0.04	0.05	0.16
ASV21	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Phascolarctobacterium; s__	DDGS+AOP	0.77	0.03	0.70	2.31
ASV317	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	DDGS+AOP	0.77	0.05	0.09	0.29
ASV645	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	DDGS+AOP	0.76	0.04	0.02	0.07
ASV615	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptococcaceae; g__rc4-4; s__	DDGS+AOP	0.76	0.01	0.01	0.03
ASV1322	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.76	0.04	0.00	0.01
ASV1069	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	DDGS+AOP	0.76	0.03	0.00	0.02

ASV578	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	DDGS+AOP	0.75	0.04	0.02	0.05
ASV521	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Clostridium; s__lavalense	DDGS+AOP	0.75	0.03	0.00	0.04
ASV370	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__flavefaciens	DDGS+AOP	0.75	0.03	0.02	0.06
ASV999	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.74	0.03	0.00	0.02
ASV25	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__mucosae	DDGS+AOP	0.73	0.02	0.13	0.36
ASV1057	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__; s__	DDGS+AOP	0.73	0.03	0.00	0.04
ASV256	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	DDGS+AOP	0.72	0.02	0.04	0.11
ASV604	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	DDGS+AOP	0.71	0.05	0.00	0.04
ASV49	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	DDGS+AOP	0.71	0.01	0.38	0.92
ASV51	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri	DDGS+AOP	0.70	0.03	0.67	1.56
ASV188	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__Acidaminococcus; s__	DDGS+AOP	0.70	0.05	0.00	0.03
ASV322	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	DDGS+AOP	0.70	0.03	0.01	0.04
ASV207	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	DDGS+AOP	0.67	0.02	0.09	0.18
ASV270	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	DDGS+AOP	0.62	0.05	0.06	0.10

[1]Indicator species were identified based on the criteria that 1) with an indicator value greater than 0.5, and 2) a *P* value less than 0.05. These species were significantly more abundant and present in most samples belonging to one group, and also absent or with low abundance in the other group.

[2]The group each ASV has maximum indicator value for.

[3]The indicator value for each species to its maximum group ranging from 0 to 1. Higher indicator values suggested better performances in the microbial signature of the assigned taxa.
[4]The probability of obtaining as high an indicator values as observed over the specified iteration.

Table 4-5. Differentially abundant amplicon sequence variant (ASV) in the feces between pigs fed rice bran diet with (RB+AOP) and without AOP (RB) identified with the indicator species analysis[1]

ASV_ID	Taxonomic annotation	Group[2]	Indicator value[3]	P value[4]	Rice bran	Rice bran +AOP
ASV732	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	1.00	0.00	0.13	0.00
ASV789	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Anaeroplasmatales; f__Anaeroplasmataceae; g__Anaeroplasma; s__	RB	1.00	0.00	0.02	0.00
ASV832	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	RB	1.00	0.00	0.03	0.00
ASV1098	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Clostridium; s__lavalense	RB	1.00	0.00	0.02	0.00
ASV1150	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	1.00	0.00	0.03	0.00
ASV1274	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	RB	1.00	0.00	0.04	0.00
ASV1633	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	RB	1.00	0.01	0.02	0.00
ASV165	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.99	0.01	0.40	0.00
ASV1140	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__mitochondria; g__; s__	RB	0.97	0.00	0.02	0.00
ASV481	k__Bacteria; p__Fibrobacteres; c__Fibrobacteria; o__Fibrobacterales; f__Fibrobacteraceae; g__Fibrobacter; s__	RB	0.95	0.00	0.13	0.01
ASV518	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Victivallales; f__Victivallaceae; g__; s__	RB	0.94	0.01	0.08	0.01
ASV197	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.92	0.01	0.87	0.07
ASV379	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.92	0.00	0.21	0.02
ASV619	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	RB	0.90	0.00	0.04	0.00

ASV76	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	RB	0.90	0.00	0.79	0.09
ASV112	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	RB	0.89	0.03	1.00	0.13
ASV245	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Sphaerochaetales; f__Sphaerochaetaceae; g__Sphaerochaeta; s__	RB	0.89	0.01	0.16	0.02
ASV1203	k__Bacteria; p__Tenericutes	RB	0.88	0.01	0.02	0.00
ASV9	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__delbrueckii	RB	0.88	0.03	0.09	0.01
ASV1374	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.87	0.01	0.02	0.00
ASV411	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB	0.86	0.01	0.18	0.03
ASV902	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Victivallales; f__Victivallaceae; g__; s__	RB	0.85	0.02	0.03	0.01
ASV664	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	RB	0.85	0.01	0.02	0.00
ASV688	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	RB	0.85	0.01	0.04	0.01
ASV1241	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.85	0.01	0.03	0.01
ASV455	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae	RB	0.83	0.02	0.03	0.00
ASV1440	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.83	0.02	0.01	0.00
ASV1582	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Blautia; s__	RB	0.83	0.01	0.02	0.00
ASV651	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.82	0.00	0.06	0.01
ASV75	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	RB	0.82	0.00	0.43	0.09
ASV1599	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus	RB	0.82	0.02	0.02	0.00

ASV429	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.82	0.01	0.04	0.00
ASV697	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	RB	0.81	0.02	0.02	0.00
ASV32	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB	0.80	0.01	1.26	0.31
ASV1050	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Faecalibacterium; s__prausnitzii	RB	0.80	0.02	0.03	0.01
ASV1161	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	RB	0.80	0.02	0.05	0.00
ASV1294	k__Bacteria; p__Deferribacteres; c__Deferribacteres; o__Deferribacterales; f__Deferribacteraceae; g__Mucispirillum; s__schaedleri	RB	0.79	0.02	0.02	0.00
ASV1437	k__Bacteria	RB	0.78	0.02	0.02	0.01
ASV938	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	RB	0.78	0.03	0.03	0.01
ASV1506	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	RB	0.78	0.02	0.02	0.01
ASV689	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.77	0.04	0.08	0.01
ASV241	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Z20; f__R4-45B; g__; s__	RB	0.77	0.04	0.33	0.10
ASV1462	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	RB	0.76	0.03	0.01	0.00
ASV887	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__RFN20; s__	RB	0.76	0.04	0.01	0.00
ASV1508	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	RB	0.76	0.03	0.02	0.00
ASV393	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	RB	0.75	0.04	0.06	0.02
ASV630	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.74	0.02	0.10	0.03
ASV272	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__L7A_E11; s__	RB	0.73	0.04	0.11	0.04

ASV344	k__Bacteria; p__Verrucomicrobia; c__Verruco-5; o__WCHB1-41; f__RFP12; g__; s__	RB	0.73	0.01	0.05	0.02
ASV631	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__	RB	0.73	0.01	0.07	0.01
ASV707	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.72	0.04	0.04	0.02
ASV13	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Succinivibrionaceae; g__Succinivibrio; s__	RB	0.72	0.03	4.41	1.68
ASV606	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.72	0.03	0.10	0.04
ASV159	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	RB	0.71	0.01	0.18	0.07
ASV476	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.71	0.05	0.06	0.02
ASV711	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	RB	0.71	0.05	0.02	0.00
ASV581	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.71	0.03	0.04	0.02
ASV1479	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	RB	0.71	0.03	0.02	0.00
ASV117	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.71	0.04	0.45	0.19
ASV50	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	RB	0.69	0.02	1.06	0.47
ASV615	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptococcaceae; g__rc4-4; s__	RB	0.69	0.04	0.04	0.02
ASV440	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.68	0.02	0.16	0.07
ASV567	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	RB	0.67	0.05	0.04	0.00
ASV1094	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__YS2; f__; g__; s__	RB	0.67	0.05	0.04	0.00
ASV1690	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.67	0.05	0.02	0.00

ASV293	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	RB	0.66	0.04	0.15	0.08
ASV267	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	RB	0.64	0.01	0.27	0.15
ASV161	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__RF16; g__; s__	RB	0.63	0.01	0.39	0.23
ASV240	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB	0.62	0.04	0.22	0.13
ASV291	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__; s__	RB	0.60	0.03	0.10	0.07
ASV36	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	RB	0.59	0.00	1.12	0.77
ASV348	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	RB	0.57	0.02	0.17	0.13
ASV262	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB+AOP	1.00	0.00	0.00	0.34
ASV363	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	RB+AOP	1.00	0.00	0.00	0.06
ASV700	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	RB+AOP	1.00	0.00	0.00	0.14
ASV1309	k__Bacteria; p__; c__; o__; f__; g__; s__	RB+AOP	0.97	0.00	0.00	0.02
ASV43	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Bifidobacteriales; f__Bifidobacteriaceae; g__Bifidobacterium; s__pseudolongum	RB+AOP	0.96	0.00	0.06	1.42
ASV208	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	RB+AOP	0.90	0.02	0.01	0.11
ASV457	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB+AOP	0.90	0.02	0.03	0.30
ASV269	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	RB+AOP	0.89	0.02	0.02	0.18
ASV416	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__bromii	RB+AOP	0.88	0.00	0.01	0.04

ASV144	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB+AOP	0.83	0.00	0.06	0.32
ASV205	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	RB+AOP	0.83	0.01	0.00	0.03
ASV217	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	RB+AOP	0.83	0.01	0.00	0.11
ASV394	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	RB+AOP	0.83	0.02	0.00	0.02
ASV965	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	RB+AOP	0.83	0.01	0.00	0.02
ASV1280	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Epulopiscium; s__	RB+AOP	0.83	0.02	0.00	0.03
ASV1766	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__	RB+AOP	0.83	0.01	0.00	0.01
ASV678	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__YS2; f__; g__; s__	RB+AOP	0.82	0.00	0.02	0.09
ASV372	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus; s__ruminis	RB+AOP	0.81	0.03	0.00	0.12
ASV125	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	RB+AOP	0.80	0.02	0.00	0.04
ASV1232	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	RB+AOP	0.80	0.04	0.00	0.03
ASV246	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	RB+AOP	0.80	0.01	0.06	0.24
ASV29	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae	RB+AOP	0.79	0.00	0.06	0.21
ASV211	k__Bacteria; p__Verrucomicrobia; c__Verruco-5; o__WCHB1-41; f__RFP12; g__; s__	RB+AOP	0.79	0.01	0.04	0.16
ASV96	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	RB+AOP	0.79	0.04	0.04	0.15
ASV179	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Veillonellaceae; g__; s__	RB+AOP	0.77	0.03	0.02	0.25
ASV341	k__Bacteria; p__Tenericutes; c__RF3; o__ML615J-28; f__; g__; s__	RB+AOP	0.76	0.04	0.00	0.05
ASV52	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	RB+AOP	0.76	0.01	0.59	1.86

ASV114	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB+AOP	0.76	0.01	0.34	1.08
ASV127	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB+AOP	0.75	0.01	0.19	0.59
ASV4	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__alactolyticus	RB+AOP	0.75	0.01	3.92	11.62
ASV244	k__Bacteria; p__TM7; c__TM7-3; o__CW040; f__F16; g__; s__	RB+AOP	0.74	0.02	0.09	0.26
ASV141	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__CF231; s__	RB+AOP	0.73	0.02	0.07	0.18
ASV403	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	RB+AOP	0.73	0.02	0.02	0.05
ASV446	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	RB+AOP	0.73	0.05	0.01	0.03
ASV260	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB+AOP	0.73	0.04	0.02	0.04
ASV545	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__Mogibacterium; s__	RB+AOP	0.73	0.04	0.00	0.03
ASV386	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB+AOP	0.72	0.01	0.01	0.03
ASV12	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__celatum	RB+AOP	0.72	0.01	0.14	0.37
ASV406	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__[Ruminococcus]; s__gnavus	RB+AOP	0.72	0.01	0.04	0.11
ASV104	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	RB+AOP	0.71	0.03	0.07	0.17
ASV1284	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__YS2; f__; g__; s__	RB+AOP	0.70	0.05	0.01	0.02
ASV175	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	RB+AOP	0.69	0.05	0.21	0.48
ASV889	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	RB+AOP	0.68	0.04	0.01	0.04
ASV283	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__; s__	RB+AOP	0.68	0.02	0.08	0.17

ASV264	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri	RB+AOP	0.67	0.05	0.00	0.08
ASV31	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__butyricum	RB+AOP	0.65	0.01	0.15	0.28
ASV451	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	RB+AOP	0.64	0.00	0.03	0.05
ASV207	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	RB+AOP	0.61	0.03	0.24	0.38

[1]Indicator species were identified based on the criteria that 1) with an indicator value greater than 0.5, and 2) a *P* value less than 0.05. These species were significantly more abundant and present in most samples belonging to one group, and also absent or with low abundance in the other group.

[2]The group each ASV has maximum indicator value for.

[3]The indicator value for each species to its maximum group ranging from 0 to 1. Higher indicator values suggested better performances in the microbial signature of the assigned taxa.

[4]The probability of obtaining as high an indicator values as observed over the specified iterations.

Table 4-6. Differentially abundant amplicon sequence variant (ASV) in the feces between pigs fed wheat middlings diet with (WM+AOP) or without AOP (WM) identified with the indicator species analysis[1]

ASV_ID	Taxonomic annotation	Group[2]	Indicator value[3]	P value[4]	Wheat middlings	Wheat middlings +AOP
ASV927	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	WM	1.00	0.00	0.06	0.00
ASV247	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__GMD14H09; f__; g__; s__	WM	0.97	0.00	0.33	0.01
ASV76	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM	0.97	0.01	0.99	0.03
ASV70	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__GMD14H09; f__; g__; s__	WM	0.93	0.01	1.32	0.10
ASV730	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Acholeplasmatales; f__; g__; s__	WM	0.90	0.03	0.11	0.01
ASV51	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__copri	WM	0.90	0.00	0.52	0.06
ASV252	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM	0.89	0.00	0.19	0.02
ASV389	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	WM	0.88	0.00	0.11	0.01
ASV136	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM	0.86	0.01	0.23	0.04
ASV835	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM	0.85	0.02	0.04	0.01
ASV244	k__Bacteria; p__TM7; c__TM7-3; o__CW040; f__F16; g__; s__	WM	0.83	0.01	0.08	0.00
ASV268	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae	WM	0.83	0.01	0.12	0.00
ASV597	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	WM	0.83	0.02	0.11	0.00
ASV1558	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM	0.83	0.01	0.02	0.00
ASV1914	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Victivallales; f__Victivallaceae; g__; s__	WM	0.83	0.02	0.01	0.00

ASV106	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	WM	0.82	0.00	0.91	0.20
ASV4	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus; s__alactolyticus	WM	0.82	0.03	2.94	0.64
ASV399	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	WM	0.81	0.01	0.22	0.05
ASV95	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	WM	0.79	0.04	0.93	0.24
ASV162	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__02d06; s__	WM	0.79	0.03	0.31	0.08
ASV417	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__; s__	WM	0.79	0.01	0.14	0.04
ASV87	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__	WM	0.78	0.03	0.28	0.02
ASV85	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM	0.78	0.03	0.05	0.00
ASV97	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Succinivibrionaceae; g__Succinivibrio; s__	WM	0.78	0.02	0.12	0.01
ASV345	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__[Eubacterium]	WM	0.78	0.03	0.06	0.02
ASV32	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM	0.78	0.01	1.48	0.41
ASV89	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM	0.78	0.04	1.08	0.30
ASV340	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	WM	0.77	0.01	0.09	0.01
ASV37	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Pirellulales; f__Pirellulaceae; g__; s__	WM	0.77	0.02	1.54	0.47
ASV166	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	WM	0.77	0.00	0.22	0.07

ASV61	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM	0.75	0.01	0.39	0.13
ASV635	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Coprococcus; s__	WM	0.74	0.04	0.07	0.01
ASV420	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM	0.73	0.05	0.06	0.02
ASV693	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM	0.72	0.02	0.03	0.00
ASV451	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	WM	0.70	0.03	0.07	0.03
ASV580	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM	0.67	0.05	0.10	0.00
ASV416	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__bromii	WM	0.67	0.00	0.05	0.02
ASV173	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__RF16; g__; s__	WM+AOP	1.00	0.00	0.00	0.35
ASV327	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM+AOP	1.00	0.00	0.00	0.09
ASV373	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	WM+AOP	0.99	0.00	0.01	0.42
ASV455	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae	WM+AOP	0.97	0.01	0.00	0.15
ASV208	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	WM+AOP	0.97	0.01	0.02	0.52
ASV885	k__Bacteria	WM+AOP	0.97	0.00	0.00	0.02
ASV135	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	WM+AOP	0.96	0.00	0.00	0.10
ASV421	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	WM+AOP	0.96	0.03	0.01	0.24
ASV68	k__Bacteria; p__Actinobacteria; c__Coriobacteriia; o__Coriobacteriales; f__Coriobacteriaceae; g__; s__	WM+AOP	0.95	0.00	0.00	0.08
ASV126	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	WM+AOP	0.91	0.03	0.04	0.38

ASV658	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM+AOP	0.91	0.01	0.01	0.13
ASV182	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	WM+AOP	0.91	0.01	0.02	0.25
ASV489	k__Bacteria; p__Elusimicrobia; c__Elusimicrobia; o__Elusimicrobiales; f__Elusimicrobiaceae; g__; s__	WM+AOP	0.88	0.01	0.01	0.07
ASV802	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	WM+AOP	0.88	0.01	0.00	0.03
ASV36	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM+AOP	0.85	0.00	0.26	1.55
ASV431	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	WM+AOP	0.84	0.02	0.02	0.09
ASV1171	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Dorea; s__	WM+AOP	0.84	0.01	0.01	0.03
ASV29	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae	WM+AOP	0.83	0.02	0.00	0.03
ASV242	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	WM+AOP	0.83	0.01	0.00	0.01
ASV286	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	WM+AOP	0.83	0.01	0.00	0.11
ASV403	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	WM+AOP	0.83	0.01	0.00	0.02
ASV1643	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__; s__	WM+AOP	0.83	0.02	0.00	0.01
ASV267	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	WM+AOP	0.83	0.02	0.06	0.28
ASV77	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM+AOP	0.83	0.04	0.20	1.00
ASV668	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__; g__; s__	WM+AOP	0.83	0.02	0.01	0.05
ASV164	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	WM+AOP	0.83	0.03	0.08	0.36
ASV609	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	WM+AOP	0.82	0.02	0.03	0.13

ASV619	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	WM+AOP	0.82	0.00	0.01	0.04
ASV357	k__Bacteria; p__Verrucomicrobia; c__Verruco-5; o__WCHB1-41; f__RFP12; g__; s__	WM+AOP	0.81	0.02	0.07	0.32
ASV34	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae	WM+AOP	0.81	0.03	0.07	0.28
ASV662	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	WM+AOP	0.81	0.01	0.02	0.09
ASV447	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM+AOP	0.81	0.01	0.03	0.11
ASV245	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Sphaerochaetales; f__Sphaerochaetaceae; g__Sphaerochaeta; s__	WM+AOP	0.81	0.03	0.05	0.21
ASV948	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__p-75-a5; s__	WM+AOP	0.80	0.03	0.00	0.04
ASV257	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae	WM+AOP	0.80	0.00	0.01	0.06
ASV103	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	WM+AOP	0.80	0.01	0.28	1.11
ASV756	k__Bacteria; p__Tenericutes; c__Mollicutes; o__RF39; f__; g__; s__	WM+AOP	0.80	0.01	0.00	0.02
ASV1061	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae	WM+AOP	0.79	0.00	0.01	0.03
ASV359	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__CF231; s__	WM+AOP	0.79	0.01	0.04	0.15
ASV18	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53; s__	WM+AOP	0.79	0.01	0.10	0.35
ASV499	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	WM+AOP	0.78	0.02	0.01	0.16
ASV840	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM+AOP	0.78	0.04	0.00	0.06
ASV868	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Victivallales; f__Victivallaceae; g__; s__	WM+AOP	0.77	0.03	0.01	0.10

ASV79	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM+AOP	0.77	0.00	0.32	1.08
ASV604	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	WM+AOP	0.76	0.03	0.01	0.03
ASV12	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__Clostridium; s__celatum	WM+AOP	0.76	0.01	0.14	0.43
ASV533	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__; s__	WM+AOP	0.76	0.01	0.03	0.09
ASV558	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	WM+AOP	0.75	0.02	0.00	0.02
ASV320	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	WM+AOP	0.75	0.04	0.09	0.27
ASV198	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Porphyromonadaceae; g__Parabacteroides; s__	WM+AOP	0.74	0.01	0.08	0.24
ASV139	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	WM+AOP	0.74	0.04	0.19	0.55
ASV875	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Ruminococcus; s__	WM+AOP	0.74	0.02	0.01	0.05
ASV2	k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Lactobacillaceae; g__Lactobacillus	WM+AOP	0.74	0.03	0.94	2.66
ASV175	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM+AOP	0.73	0.02	0.03	0.09
ASV1203	k__Bacteria; p__Tenericutes	WM+AOP	0.73	0.04	0.01	0.03
ASV353	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM+AOP	0.71	0.05	0.02	0.09
ASV1709	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM+AOP	0.70	0.03	0.00	0.01
ASV288	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__Oscillospira; s__	WM+AOP	0.70	0.05	0.05	0.12
ASV42	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	WM+AOP	0.69	0.01	0.38	0.85

ASV387	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	WM+AOP	0.69	0.03	0.01	0.06
ASV291	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfovibrionaceae; g__; s__	WM+AOP	0.69	0.02	0.08	0.18
ASV144	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Prevotellaceae; g__Prevotella; s__	WM+AOP	0.69	0.00	0.09	0.20
ASV685	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Aeromonadales; f__Succinivibrionaceae; g__Ruminobacter; s__	WM+AOP	0.67	0.04	0.00	0.15
ASV276	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Roseburia; s__	WM+AOP	0.67	0.04	0.04	0.08
ASV7	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae; g__; s__	WM+AOP	0.66	0.05	0.44	0.88
ASV266	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	WM+AOP	0.66	0.00	0.11	0.22
ASV517	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	WM+AOP	0.65	0.04	0.02	0.05
ASV600	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__	WM+AOP	0.63	0.03	0.03	0.04
ASV1535	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales	WM+AOP	0.61	0.05	0.00	0.01

[1]Indicator species were identified based on the criteria that 1) with an indicator value greater than 0.5, and 2) a *P* value less than 0.05. These species were significantly more abundant and present in all samples belonging to one group, and also absent or with low abundance in the other group.

[2]The group each ASV has maximum indicator value for.

[3]The indicator value for each species to its maximum group ranging from 0 to 1. Higher indicator values suggested better performances in the microbial signature of the assigned taxa.

[4]The probability of obtaining as high an indicator values as observed over the specified iteration.

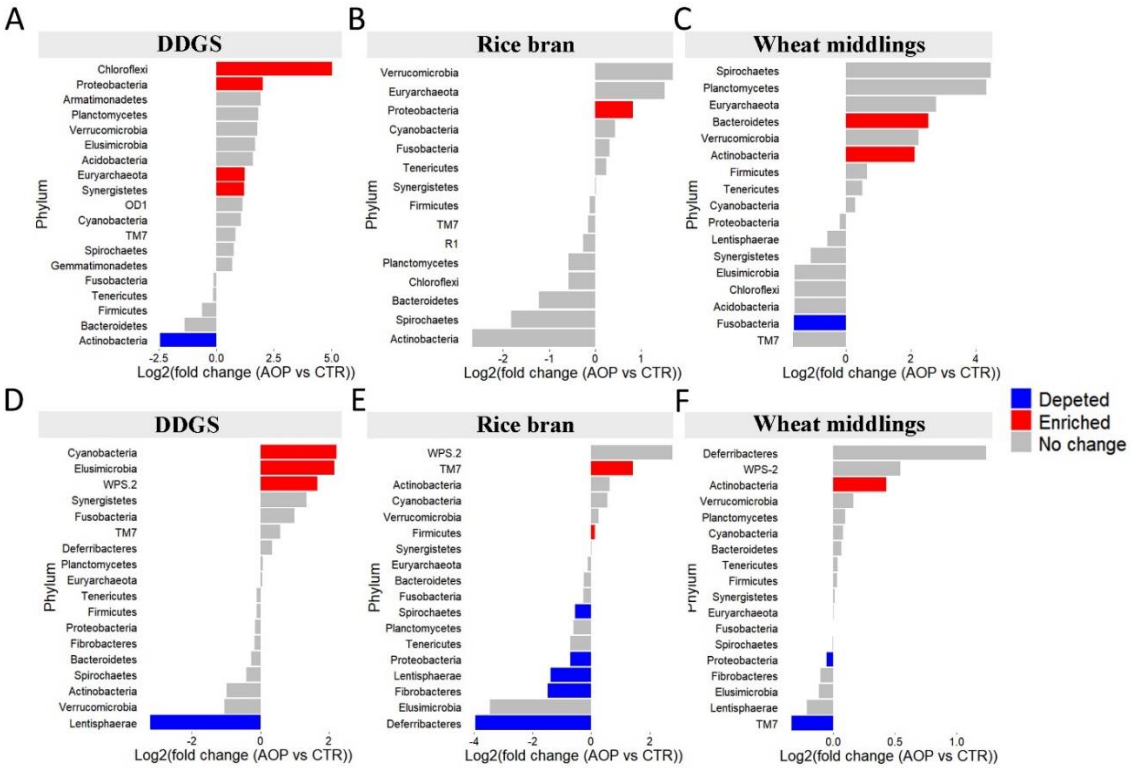

Figure 4-7. Bar plots show that dietary addition of *Aspergillus oryzae* postbiotic (AOP) changed the microbial composition at the phylum level in ileal digesta (A-C) and feces (D-F) of pigs fed diets formulated with corn distillers dried grains with solubles (DDGS), rice bran (RB) or wheat middlings (WM) compared with those fed the control diet (CTR). Differential phyla were determined by indicator species analysis with the threshold of an indicator value >= 0.5, and *P* value < 0.05, and phyla with a *P* value >0.05 or an indicator value < 0.5 were considered no changes.

Table 4-7. Effect of *Aspergillus oryzae* postbiotic (AOP) on ileal and fecal free amino acids, µg/g

Amino acid	DDGS		Rice bran		Wheat middlings		SEM	*P*-value		
	-AOP	+AOP	-AOP	+AOP	-AOP	+AOP		AOP	Diet	AOP×diet
Ileal sample										
Alanine	83.71	93.72	46.64	51.09	50.25	60.15	15.9	0.55	0.06	0.98
Arginine	19.77	30.89	3.26	4.26	7.96	6.07	4.73	0.42	< 0.01	0.42
Asparagine	3.24[b]	9.25[a]	0.73[b]	1.21[b]	2.42[b]	1.54[b]	1.24	0.09	< 0.01	0.04
Aspartic acid	7.23	37.28	1.50	5.79	19.68	4.02	9.56	0.43	0.18	0.09
Citrulline	0.70	0.16	0.14	0.99	1.26	0.70	0.55	0.85	0.57	0.35
Glutamic acid	139.68	435.62	10.62	58.19	629.89	46.34	155.51	0.54	0.15	0.04
Glycine	174.60	193.76	184.67	68.07	141.52	58.30	43.82	0.11	0.17	0.28
Histidine	7.06	10.93	1.60	0.96	2.28	0.68	1.67	0.71	< 0.01	0.29
Isoleucine	86.60	173.12	38.26	41.47	41.95	42.55	23.48	0.15	< 0.01	0.17
Leucine	86.60	173.12	38.26	41.47	41.95	42.55	23.48	0.15	< 0.01	0.17
Lysine	54.60	76.43	7.50	7.16	17.03	8.57	10.82	0.65	< 0.01	0.41
Methionine	1.96	2.72	0.42	0.70	0.39	1.04	0.43	0.15	< 0.01	0.86
Ornithine	0.00	0.00	0.04	0.04	0.00	0.00	0.01	1.00	0.03	1.00
Phenylalanine	115.03	202.93	47.61	50.19	55.57	46.55	28.27	0.24	< 0.01	0.18
Proline	25.14	27.30	1.65	2.45	7.98	1.55	9.78	0.89	0.06	0.90
Serine	2.19	5.25	0.72	1.30	0.73	1.08	1.46	0.31	0.15	0.62
Taurine	4.46[a]	0.46[b]	0.77[b]	0.38[b]	1.40[ab]	0.81[b]	0.88	0.04	0.13	0.11
Threonine	8.60	13.95	1.00	1.07	2.36	2.00	3.2	0.54	0.01	0.63
Tryptophan	0.34	0.34	0.13	0.16	0.18	0.16	0.07	0.99	0.04	0.94
Tyrosine	404.62	782.17	183.37	226.86	288.14	178.45	100.74	0.24	< 0.01	0.09
Valine	68.01	125.38	31.40	31.73	48.40	44.49	17.95	0.26	< 0.01	0.23
Fecal sample										
Alanine	40.39	51.47	16.08	13.84	25.06	8.00	10.50	0.76	0.02	0.46
Asparagine	0.09	0.00	0.09	0.04	0.00	0.07	0.04	0.56	0.75	0.25
Aspartic acid	9.58	10.30	59.39	394.98	31.17	1.66	113.14	0.29	0.12	0.23

Citrulline	0.32	0.35	0.96	0.41	0.56	0.61	0.20	0.35	0.23	0.25
Glutamic acid	58.13	144.47	75.53	320.20	37.32	25.33	88.94	0.16	0.20	0.37
Glycine	1.93	2.19	0.48	0.41	0.56	0.34	0.52	0.98	0.01	0.89
Histidine	0.10	0.09	0.05	0.08	0.00	0.05	0.03	0.49	0.19	0.67
Isoleucine	12.66	0.85	1.25	0.37	0.63	0.92	4.85	0.32	0.40	0.42
Leucine	12.66	0.85	1.25	0.37	0.63	0.92	4.85	0.32	0.40	0.42
Lysine	2.88	5.80	2.22	2.34	1.00	0.34	0.91	0.30	< 0.01	0.16
Methionine	0.62	0.57	0.54	0.10	0.92	0.60	0.22	0.17	0.18	0.70
Ornithine	0.09	0.00	0.09	0.08	0.00	0.00	0.03	0.17	0.02	0.24
Phenylalanine	0.41^{b}	0.85^{b}	0.58^{b}	0.25^{b}	0.77^{b}	4.24^{a}	0.27	< 0.01	< 0.01	< 0.01
Proline	1.30	7.26	1.92	0.35	4.26	7.20	1.47	0.06	0.02	0.06
Serine	0.54	0.90	0.35	0.37	0.42	0.23	0.17	0.65	0.06	0.29
Taurine	0.04	0.05	0.00	0.04	0.00	0.00	0.02	0.37	0.18	0.69
Threonine	0.85	0.77	0.36	0.24	0.26	0.24	0.25	0.73	0.07	0.98
Tyrosine	2.68	7.39	1.16	0.72	4.86	21.23	4.27	0.08	0.04	0.18
Valine	20.79	3.36	1.78	0.59	1.46	1.31	7.79	0.34	0.31	0.49

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). Means in a row with different superscript letters differ ($P < 0.05$). n=6.
[2]DDGS = distillers dried grains with soluble.

Table 4-8. Effect of *Aspergillus oryzae* postbiotic (AOP) on ileal and fecal bile acids, µg/g

Bile acid	DDGS		Rice bran		Wheat middlings		SEM	*P* value		
	-AOP	+AOP	-AOP	+AOP	-AOP	+AOP		AOP	Diet	AOP×diet
Ileal sample										
Chenodeoxycholic acid	9.82	0.08	0.31	0.09	0.27	1.63	3.97	0.39	0.46	0.35
Deoxycholic acid	384.91	359.79	331.40	115.80	225.57	321.09	105.65	0.60	0.43	0.37
Glycochenodeoxycholic acid	72.03	128.17	69.50	7.29	38.60	41.93	33.33	0.97	0.18	0.27
Glycocholic acid	15.80	87.21	122.95	10.59	71.64	7.38	46.80	0.37	0.84	0.16
Glycodeoxycholic acid	20.95	37.27	20.23	2.13	11.24	12.20	9.70	0.97	0.18	0.27
Hyodeoxycholic acid	1093.88	673.69	1625.27	1322.89	780.35	880.54	361.57	0.49	0.17	0.76
Lithocholic acid	274.64	256.73	236.47	82.62	160.96	229.11	75.38	0.60	0.43	0.37
Taurochenodeoxycholic acid	0.81	10.85	0.34	0.02	0.43	0.07	4.16	0.37	0.33	0.38
Taurocholic acid	40.34	12.70	49.45	0.67	14.26	1.68	16.11	0.04	0.49	0.56
Taurodeoxycholic acid	38.11	152.32	108.85	2.98	52.78	9.72	38.92	0.73	0.30	0.04
Total taurine-conjugated bile acid	71.13	175.18	158.64	3.67	65.49	10.64	46.65	0.37	0.22	0.04
Fecal sample										
Chenodeoxycholic acid	0.05	0.03	0.01	0.03	0.02	0.02	0.01	0.94	0.04	0.17
Deoxycholic acid	0.03	0.03	0.00	0.01	0.02	0.02	0.01	0.62	0.24	0.81
Glycochenodeoxycholic acid	0.02	0.00	0.01	0.01	0.01	0.02	0.00	0.36	0.91	0.05
Glycodeoxycholic acid	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.25	0.47	0.39
Hyodeoxycholic acid	332.70	406.82	293.53	441.43	291.19	488.29	100.64	0.11	0.97	0.83
Lithocholic acid	399.95	442.25	306.66	336.79	468.94	501.26	112.60	0.71	0.37	0.99
Taurocholic acid	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.27	0.33	0.15
Taurodeoxycholic acid	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.72	0.04	0.19

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). n=6.
[2]DDGS = distillers dried grains with solubles.

Table 4-9. Effect of *Aspergillus oryzae* postbiotic (AOP) on ileal and fecal fatty acids, mg/g

Fatty acid	DDGS		Rice bran		Wheat middlings		SEM	P value		
	-AOP	+AOP	-AOP	+AOP	-AOP	+AOP		AOP	Diet	AOP×diet
Ileal sample										
Acetic acid	0.97	0.46	0.81	1.25	0.93	2.08	0.40	0.29	0.18	0.16
Butyric acid	0.30	0.06	0.14	0.36	0.19	0.80	0.22	0.30	0.37	0.20
Propionic acid	0.85	0.11	0.13	0.17	0.17	1.04	0.41	0.87	0.54	0.18
Valeric acid	0.06	0.01	0.04	0.01	0.00	0.10	0.04	0.57	0.61	0.23
C12:0	0.004	0.001	0.002	0.001	0.002	0.001	0.001	0.01	0.58	0.43
C14:0	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.89	0.44	0.65
C16:0	1.18	1.25	1.17	1.19	1.08	1.10	0.29	0.90	0.91	1.00
C17:0	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.70	0.88	0.61
C18:0	0.91	0.90	0.97	0.95	0.64	0.92	0.24	0.67	0.74	0.79
C18:1	40.73	46.04	29.83	25.10	16.88	36.35	9.96	0.43	0.21	0.49
C18:2	6.98	7.98	5.11	4.30	2.89	6.23	1.71	0.43	0.21	0.49
Fecal sample										
Acetic acid	9.79	8.23	9.70	14.04	6.06	2.49	2.30	0.89	0.01	0.23
Butyric acid	7.10	5.94	6.56	9.79	4.46	1.61	1.69	0.85	0.02	0.21
Propionic acid	9.23	7.20	7.99	11.87	5.78	2.04	2.11	0.72	0.03	0.20
Valeric acid	3.36	2.73	4.27	5.24	2.84	1.09	0.94	0.55	0.03	0.37
C6:0	0.06	0.02	0.00	0.02	0.28	0.00	0.06	0.05	0.10	0.07
C12:0	0.009	0.008	0.020	0.009	0.050	0.040	0.010	0.43	0.02	0.88
C14:0	0.27	0.26	0.48	0.37	1.07	0.74	0.19	0.37	0.01	0.73
C15:0	0.41	0.42	0.67	0.60	1.20	0.76	0.21	0.36	0.05	0.54
C16:0	2.52	2.31	4.85	4.17	7.87	6.38	1.79	0.69	0.18	0.90
C16:1	0.000	0.004	0.001	0.002	0.003	0.002	0.001	0.25	0.42	0.12
C17:0	0.44	0.09	0.20	0.16	0.17	0.11	0.07	0.01	0.21	0.05
C18:0	2.17	2.16	3.52	2.46	4.93	5.20	1.57	0.85	0.22	0.91
C18:1	109.28	211.48	177.72	235.87	282.00	235.54	84.02	0.60	0.55	0.69

C18:2	18.73	36.26	30.46	40.43	48.34	40.38	14.40	0.60	0.55	0.69

[1]Data are shown as LS-means with pooled standard error of the mean (SEM). n=6.
[2]DDGS = distillers dried grains with solubles.

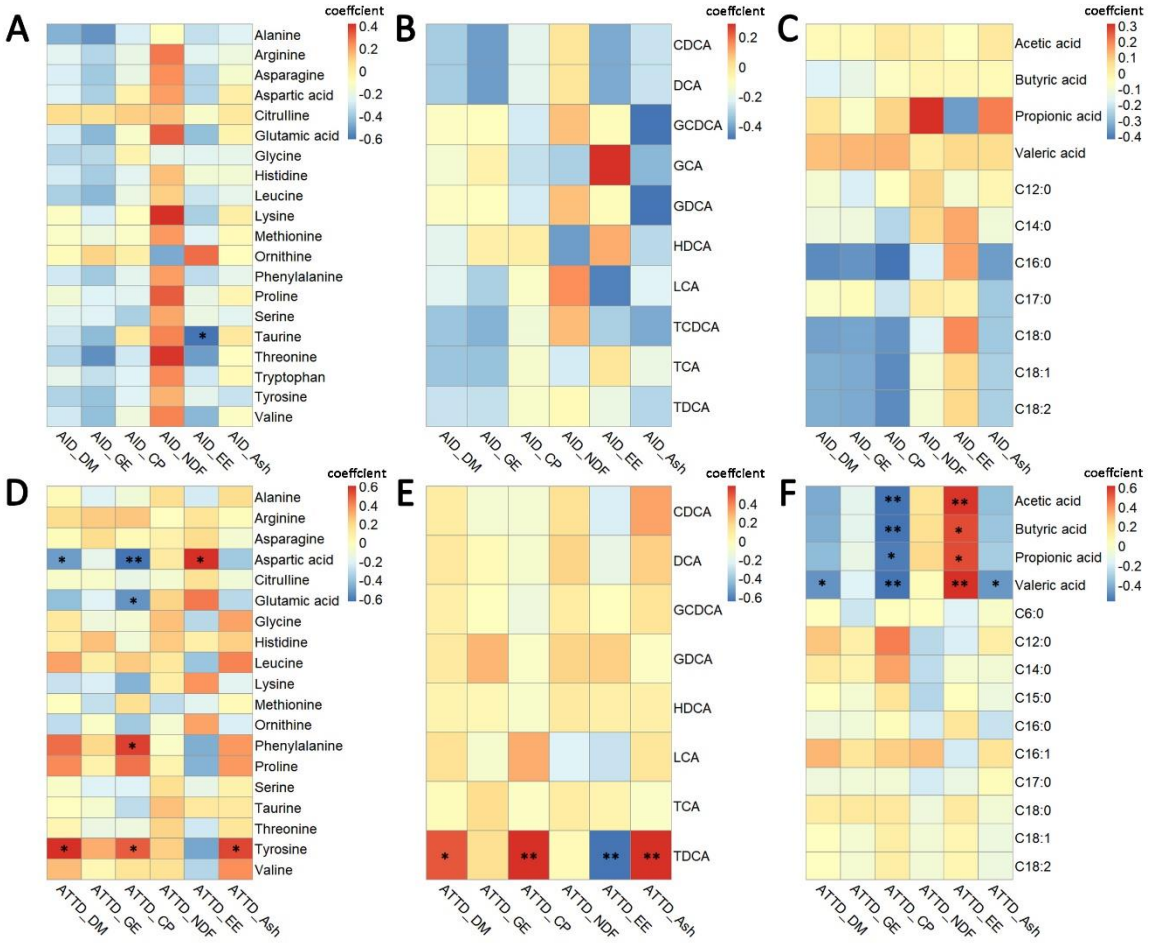

Figure 4-8. Spearman correlation analysis between metabolite concentration and nutrient digestibility. (A) Correlations between apparent ileal digestibility (AID) of nutrients and amino acid concentration in ileal digesta. (B) Correlations between AID of nutrients and bile acid concentration in ileal digesta. (C) Correlations between AID of nutrients and fatty acid concentration in ileal digesta. (D) Correlations between apparent total tract digestibility (ATTD) of nutrients and amino acid concentration in feces. (E) Correlations between ATTD of nutrients and bile acid concentration in feces. (F) Correlations between ATTD of nutrients and fatty acid concentration in feces. Spearman's rank correlation coefficient is indicated using a color gradient: red indicates postive correlation; blue, negative correlation; * and ** represent $P < 0.05$ and $P < 0.01$ in a correlation significance test with Benjamini-Hochberg correction, respective. CDCA = chenodeoxycholic acid; CP = crude protein; DCA = deoxycholic acid; DM = dry matter; EE = ether extract; GCA = glycocholic acid; GCDCA = glycochenodeoxycholic acid; GDCA = glycodeoxycholic acid; GE = gross energy; HDCA = hyodeoxycholic acid; LCA = lithochollic acid; NDF = neutral detergent fiber; TCA = taurocholic acid; TCDCA = taurochenodeoxychollic acid; TDCA = taurodeoxycholic acid.

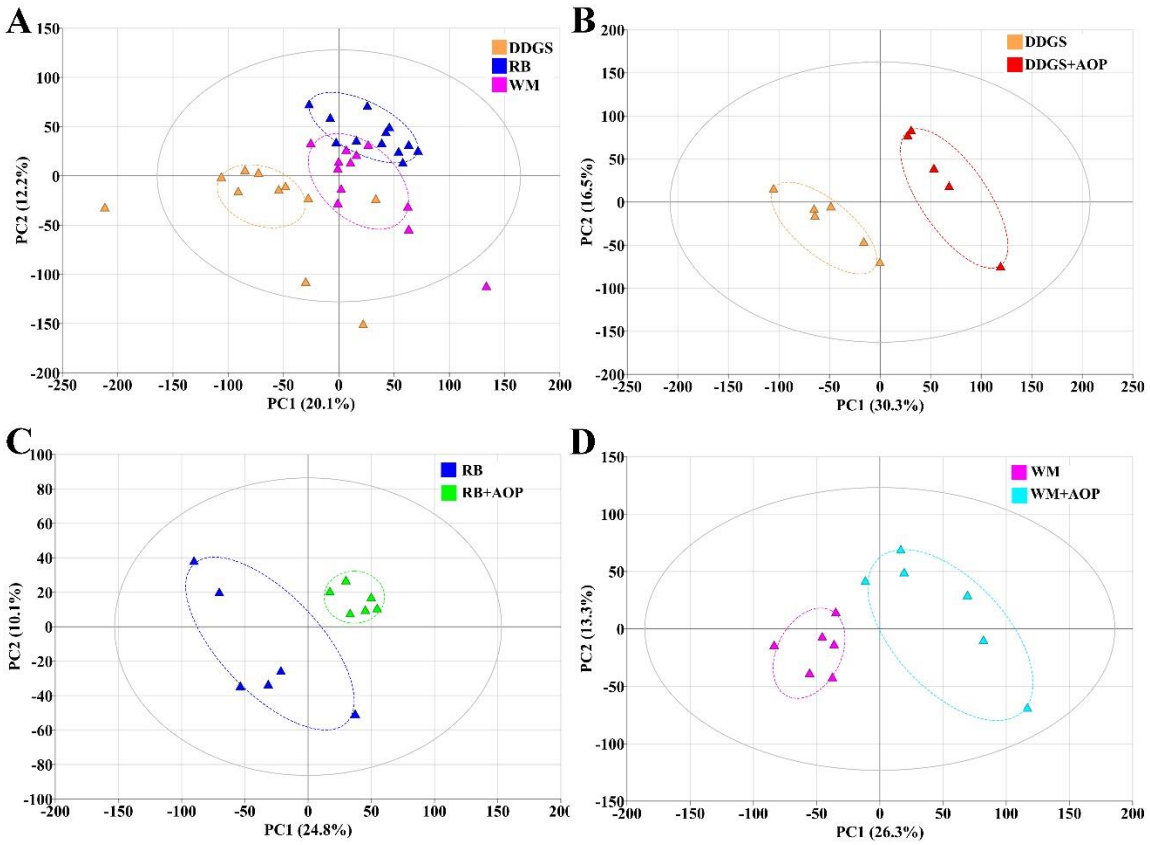

Figure 4-9. Effects of dietary addition of *Aspergillus oryzae* postbiotic (AOP) on the ileal digesta metabolome of growing pigs. (A) Scores plot from a principal components analysis (PCA) comparing ileal digesta samples form pigs fed different basal diets. (B) Scores plot from a supervised partial least squares-discriminant analysis (PLS-DA) model comparing samples of ileal digesta from pigs fed the corn distillers dried grains with solubles (DDGS) diet and those fed the DDGS diet with the addition of AOP (DDGS+AOP). (C) Scores plot from a PLS-DA model comparing samples of ileal digesta from pigs fed the rice bran diet (RB) and those fed the RB diet with the addition of AOP (RB+AOP). (D) Scores plot from a PLS-DA model comparing samples of ileal digesta from pigs fed the wheat middlings (WM) and those fed the WM diet with the addition of AOP (WM+AOP).

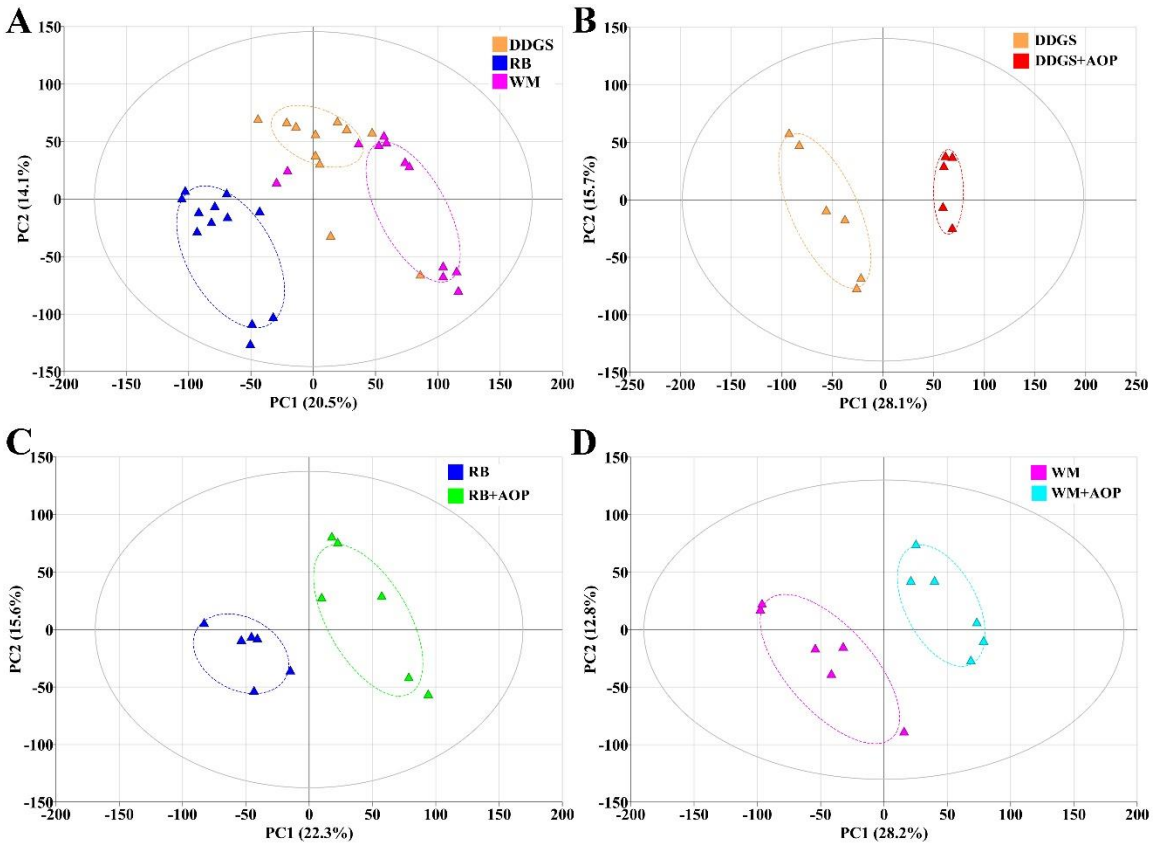

Figure 4-10. Addition of *Aspergillus oryzae* postbiotic (AOP) on the fecal metabolome of growing pigs. (A) Scores plot from a principal components analysis (PCA) comparing fecal samples form pigs fed different basal diets. (B) Scores plot from a supervised partial least squares-discriminant analysis (PLS-DA) model comparing samples of feces from pigs fed the corn distillers dried grains with solubles (DDGS) diet and those fed the DDGS diet with the addition of AOP (DDGS+AOP). (C) Scores plot from a PLS-DA model comparing samples of feces from pigs fed the rice bran diet (RB) and those fed the RB diet with the addition of AOP (RB+AOP). (D) Scores plot from a PLS-DA model comparing samples of feces from pigs fed the wheat middlings (WM) and those fed the WM diet with the addition of AOP (WM+AOP).

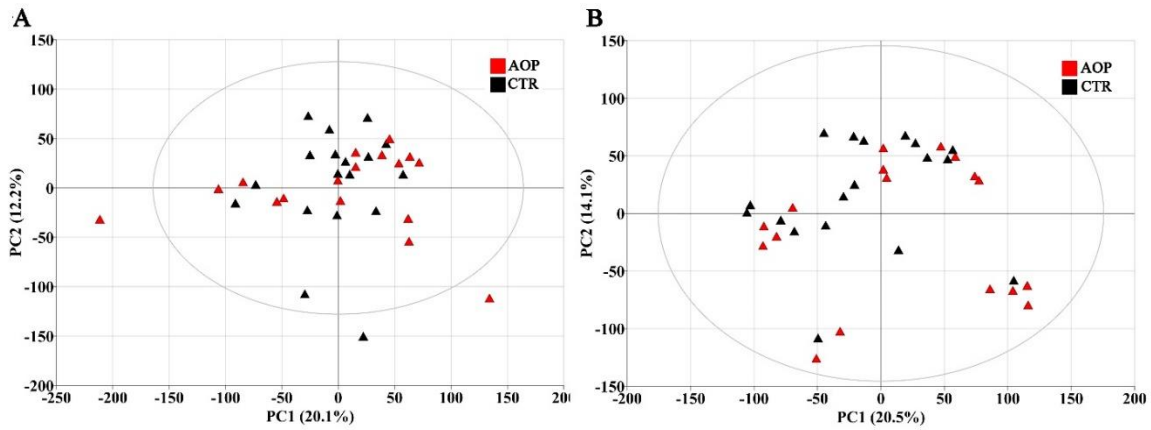

Figure 4-11. Addition of *Aspergillus oryzae* postbiotic (AOP) on the ileal and fecal metabolome. (A) Scores plot of ileal digesta showed distribution patterns of samples between AOP and control (CTR) group in a principal components analysis (PCA) model. (B) Scores plot of feces showed distribution patterns of samples between AOP and control (CTR) group in a PCA model.

Table 4-10. Significantly different metabolites in ileal digesta of pigs fed corn distillers dried grains with soluble (DDGS) or wheat middlings diet with addition of AOP (DDGS+AOP)[1]

Metabolite ID	Metabolite	Identity	*P* value	DDGS	DDGS+AOP	Fold change	VIP value
MTB1437	4.2691_389.2665_Neg	3α-Hydroxy-6-oxo-5β-cholan-24-oic Acid	0.01	0.68	2.46	3.65	7.06
MTB1442	4.2701_449.2886_Neg	Chaksine	0.01	0.12	0.96	7.82	4.86
MTB1680	6.0505_391.2849_Pos	3α-Hydroxy-6-oxo-5β-cholan-24-oic Acid	0.03	0.25	0.55	2.25	2.66
MTB1673	6.0502_373.2744_Pos	3-Oxochol-4-en-24-oic Acid	0.02	0.16	0.40	2.47	2.35
MTB1431	4.2687_435.2726_Neg	Pangamic acid	0.00	0.00	0.18	43.25	2.30
MTB1261	7.299_422.3159_HQ	-	0.03	0.69	0.90	1.33	2.10
MTB1636	6.0485_355.2639_Pos	5β-Chola-3,8(14),11-trien-24-oic Acid	0.02	0.08	0.24	3.13	1.99
MTB1571	6.9019_302.306_Pos	Sphinganine	0.04	0.15	0.25	1.70	1.68
MTB1356	5.9443_365.1531_DC	-	0.04	0.23	0.39	1.69	1.66
MTB1260	7.4381_424.3318_HQ	-	0.04	0.27	0.37	1.39	1.37
MTB1207	6.456_257.1067_HQ	-	0.03	0.20	0.27	1.37	1.19
MTB1277	6.249_532.3535_HQ	Sphingofungin B	0.01	0.00	0.06	11.54	1.18
MTB1367	5.6013_351.1374_DC	-	0.04	0.13	0.21	1.61	1.15
MTB1896	4.664_512.3436_Pos	-	0.02	0.04	0.11	2.44	1.10
MTB1778	3.5688_442.2706_Pos	Methanandamide Phosphate	0.04	0.47	0.01	0.01	2.83
MTB1136	2.0623_166.0317_HQ	-	0.01	0.53	0.19	0.37	2.66
MTB1770	4.4433_445.2338_Pos	Dimetacrine tartrate	0.04	0.40	0.00	0.00	2.63
MTB1888	6.9694_496.3401_Pos	1-Palmitoyl-rac-glycero-3-phosphocholine	0.03	1.18	0.88	0.75	2.60
MTB1458	4.0961_815.5711_Neg	-	0.03	0.29	0.02	0.08	2.56
MTB1190	2.1615_232.1074_HQ	-	0.02	0.31	0.13	0.43	1.95
MTB1686	6.147_386.2908_Pos	N-palmitoyl glutamic acid	0.00	0.17	0.03	0.16	1.79
MTB1368	5.3056_351.1373_DC	-	0.02	0.21	0.09	0.46	1.46
MTB1462	4.2634_779.5484_Neg	-	0.01	0.00	0.05		1.13
MTB1455	6.453_521.2925_Neg	-	0.03	0.05	0.00	0.08	1.10
MTB1212	6.0813_295.0699_HQ	-	0.02	0.32	0.28	0.86	1.10
MTB1412	6.6931_555.2096_DC	6'-Dehydro-6'-oxoparomamine	0.04	0.07	0.00	0.00	1.08

[1]The metabolites with a threshold of variable importance projection (VIP) values > 1and *P* value < 0.05 were considered differential metabolites.
[2]Metabolites are listed as retention time_mass_method of derivatization used [either negative mode (neg), positive mode (pos), dansyl chloride (DC), or 2-hydrazinoquinoline (HQ)].
[3]Fold change of metabolites in feces of pigs fed DDGS+AOP compared with those fed DDGS.

Table 4-11. Significantly different metabolites in ileal digesta of pigs fed rice bran (RB) or rice bran diet with addition of AOP (RB+AOP)[1]

Metabolite ID	Metabolite	Identity	*P* value	RB	RB+AOP	Fold change	VIP value
MTB1191	2.5846_230.1281_HQ	-	0.01	0.41	0.91	2.20	4.05
MTB1177	2.0331_202.097_HQ	-	0.02	1.36	1.72	1.25	3.41
MTB1760	3.8689_438.239_Pos	Lunarine	0.04	0.56	0.83	1.47	2.92
MTB1214	2.2537_287.1495_HQ	-	0.00	0.02	0.06	3.94	1.41
MTB1195	2.0341_224.0788_HQ	-	0.01	0.04	0.08	2.16	1.22
MTB1157	3.4975_186.0658_HQ	-	0.01	0.02	0.06	2.62	1.20
MTB1122	3.0121_158.0709_HQ	-	0.04	0.03	0.05	1.93	1.17
MTB1844	4.0074_468.2496_Pos	Malyngamide T	0.05	0.05	0.09	1.76	1.10
MTB1127	2.5772_143.06_HQ	-	0.01	0.02	0.05	2.73	1.08
MTB1977	3.7343_581.8084_Pos	-	0.02	0.03	0.07	2.20	1.06
MTB1896	4.664_512.3436_Pos	-	0.04	0.02	0.06	2.78	1.03
MTB1446	4.3632_446.2885_Neg	-	0.02	0.77	0.06	0.08	4.86
MTB1424	3.2961_253.0468_Neg	Gly-Ala-OH	0.01	1.22	0.84	0.68	4.03
MTB1447	4.4021_498.2875_Neg	Taurochenodeoxycholic acid	0.00	0.33	0.01	0.04	3.58
MTB1454	4.1832_464.2996_Neg	Glycocholic Acid	0.02	0.41	0.09	0.22	3.21
MTB1534	4.7433_255.066_Pos	Chrysin	0.02	0.62	0.34	0.55	2.96
MTB1561	4.8614_285.0765_Pos	Calycosin	0.00	0.36	0.17	0.47	2.64
MTB1792	5.6024_448.306_Pos	-	0.03	0.17	0.02	0.10	2.24
MTB1349	4.7016_309.0902_DC	-	0.04	0.28	0.12	0.43	2.13
MTB1551	5.1559_271.0607_Pos	Apigenin	0.04	0.64	0.48	0.75	2.13
MTB1748	5.6029_430.2954_Pos	GANT 61	0.04	0.12	0.01	0.06	1.91
MTB1456	4.27_514.2826_Neg	Taurocholic acid	0.05	0.10	0.00	0.00	1.86
MTB1710	5.798_414.3218_Pos	N-stearoyl glutamic acid	0.02	0.12	0.07	0.64	1.56
MTB1443	4.1195_448.3043_Neg	-	0.04	0.08	0.00	0.00	1.53
MTB1749	5.3962_430.2954_Pos	GANT 61	0.02	0.06	0.01	0.23	1.16
MTB2037	5.8831_633.2557_Pos	Bacteriochlorophyllid	0.05	0.05	0.01	0.12	1.09

MTB1825	5.2269_464.2836_Pos	PA(19:3)	0.03	0.04	0.00	0.00	1.06

[1]The metabolites with a threshold of variable importance projection (VIP) values > 1 and *P* value < 0.05 were considered differential metabolites.
[2]Metabolites are listed as retention time_mass_method of derivatization used [either negative mode (neg), positive mode (pos), dansyl chloride (DC), or 2-hydrazinoquinoline (HQ)].
[3]Fold change of metabolites in feces of pigs fed RB+AOP compared with those fed RB.

Table 4-12. Significantly different metabolites in ileal digesta of pigs fed wheat middlings diet (WM) or wheat middlings diet with addition of AOP (WM+AOP)[1]

Metabolite ID	Metabolite	Identity	P-value	WM	WM+AOP	Fold change	VIP value
MTB1770	4.4433_445.2338_Pos	Dimetacrine tartrate	0.03	0.00	0.10	39.06	1.43
MTB1779	7.1591_441.3728_Pos	26,27-Dihomo-1α-hydroxyvitamin D2	0.02	0.00	0.05	22.48	1.04
MTB1412	6.6931_555.2096_DC	6'-Dehydro-6'-oxoparomamine	0.03	0.01	0.12	12.65	1.50
MTB1410	6.8259_569.2252_DC	Almotriptan	0.01	0.02	0.18	9.07	1.94
MTB1162	2.3683_184.0422_HQ	-	0.01	0.02	0.08	3.76	1.13
MTB1739	1.4707_434.1896_Pos	-	0.03	0.06	0.16	2.70	1.50
MTB1195	2.0341_224.0788_HQ	-	0.03	0.04	0.09	2.23	1.01
MTB1411	7.0195_567.2098_DC	-	0.02	0.05	0.09	1.89	1.06
MTB1327	6.372_280.1003_DC	-	0.02	0.37	0.43	1.16	1.16
MTB1423	3.5274_269.0418_Neg	Gly-Ser-OH	0.04	2.90	1.45	0.50	5.41
MTB1534	4.7433_255.066_Pos	Chrysin	0.01	0.84	0.31	0.37	3.56
MTB1168	3.9257_221.0196_HQ	-	0.05	6.65	6.19	0.92	3.20
MTB1551	5.1559_271.0607_Pos	Apigenin	0.01	0.91	0.51	0.56	3.10
MTB1267	7.4049_398.3157_HQ	-	0.01	0.89	0.70	0.77	2.28
MTB1268	5.3818_579.1615_HQ	-	0.03	1.95	1.77	0.90	2.02
MTB1349	4.7016_309.0902_DC	-	0.04	0.28	0.08	0.29	1.99
MTB1259	7.6303_426.3473_HQ	-	0.04	0.49	0.41	0.83	1.31
MTB1780	5.807_441.332_Pos	-	0.00	0.05	0.01	0.18	1.13
MTB1781	4.8872_441.2023_Pos	Diferuloylputrescine	0.01	0.07	0.01	0.19	1.12
MTB2128	5.2285_839.5596_Pos	-	0.05	0.00	0.04		1.08
MTB1152	2.0931_188.0813_HQ	-	0.05	0.07	0.04	0.53	1.05

[1]The metabolites with a threshold of variable importance projection (VIP) values > 1 and *P* value < 0.05 were considered differential metabolites.
[2]Metabolites are listed as retention time_mass_method of derivatization used [either negative mode (neg), positive mode (pos), dansyl chloride (DC), or 2-hydrazinoquinoline (HQ)].
[3]Fold change of metabolites in feces of pigs fed WM+AOP compared with those fed WM.

Table 4-13. Significantly different metabolites in feces of pigs fed corn distillers dried grains with soluble (DDGS) or DDGS diet with addition of AOP (DDGS+AOP)[1]

Metabolite ID	Metabolite	Identity	P-value	DDGS	DDGS+AOP	Fold change	VIP value
MTB926	6.2852_299.2557_neg	-	0.01	1.14	2.75	2.44	5.76
MTB930	6.4055_297.2401_neg	-	0.02	0.80	2.26	2.86	5.33
MTB733	6.9603_496.3402_Pos	PC(0:0/16:0)	0.00	0.47	1.05	2.26	3.75
MTB968	4.2453_389.2669_neg	-	0.03	0.97	1.53	1.60	3.25
MTB774	7.1061_522.3561_Pos	PC(18:1(6Z)/0:0)	0.00	0.13	0.55	4.41	3.20
MTB773	7.4932_524.3715_Pos	PC(O-16:0/2:0)	0.01	0.43	0.84	1.98	2.89
MTB776	6.7851_520.34_Pos	PC(18:2(2E,4E)/0:0)	0.01	0.23	0.53	2.34	2.63
MTB885	3.1986_162.052_neg	-	0.05	0.06	0.36	6.12	2.20
MTB315	2.921_162.056_Pos	-	0.04	0.17	0.43	2.55	2.14
MTB967	4.0827_389.267_neg	-	0.00	0.04	0.23	6.64	2.04
MTB1017	6.8323_453.2604_neg	-	0.01	0.33	0.53	1.60	1.96
MTB722	6.7102_482.3246_Pos	PC(15:0/0:0)	0.00	0.02	0.12	5.47	1.55
MTB1035	7.1108_467.2757_neg	PG(P-16:0/0:0)	0.00	0.08	0.19	2.37	1.50
MTB299	3.3509_146.0611_Pos	-	0.05	0.09	0.21	2.27	1.43
MTB683	5.3872_452.312_Pos	PC(P-14:0/0:0)	0.00	0.03	0.10	3.28	1.27
MTB954	4.0325_359.1872_neg	Aldosterone	0.01	0.03	0.10	3.12	1.20
MTB797	7.9617_603.3673_Pos	Garcinol	0.00	0.00	0.05	35.38	1.16
MTB1056	7.0011_483.2707_neg	PG(16:0/0:0)	0.00	0.01	0.07	5.70	1.14
MTB1083	4.0175_593.3343_neg	L-Urobilin	0.01	0.04	0.09	2.68	1.10
MTB285	1.4232_120.0818_Pos	-	0.01	0.05	0.10	2.28	1.07
MTB1048	7.2276_493.2921_neg	-	0.00	0.00	0.05	25.23	1.06
MTB906	6.693_241.2136_neg	-	0.00	4.47	1.75	0.40	8.18
MTB931	6.2454_297.2401_neg	-	0.05	1.34	0.10	0.07	4.46
MTB898	7.4107_269.2449_neg	-	0.00	0.85	0.17	0.20	4.11
MTB925	6.1229_299.256_neg	-	0.02	0.68	0.20	0.30	2.96
MTB455	2.9778_325.1765_Pos	Dihydroxycarteolol M1	0.01	1.03	0.75	0.73	2.36

MTB431	7.2901_321.2408_Pos	-	0.01	0.25	0.00	0.00	2.28
MTB133	7.2513_384.3003_HQ	-	0.03	0.46	0.27	0.59	1.96
MTB391	7.3208_281.2484_Pos	Linoleic acid	0.00	0.24	0.10	0.43	1.77
MTB956	6.7312_351.2182_neg	Prostaglandin I2	0.04	0.16	0.00	0.00	1.73
MTB1009	8.5003_431.3508_neg	-	0.05	0.31	0.13	0.41	1.72
MTB346	2.2562_240.1239_Pos	N-benzyl-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine	0.00	0.21	0.09	0.44	1.66
MTB1071	6.2821_507.3132_neg	-	0.02	0.16	0.04	0.24	1.62
MTB652	5.5893_452.3118_Pos	PC(P-14:0/0:0)	0.00	0.11	0.03	0.33	1.34
MTB793	5.3995_549.3435_Pos	-	0.03	0.18	0.09	0.47	1.33
MTB406	2.5903_295.166_Pos	Tyrosyl-Leucine	0.01	0.41	0.32	0.79	1.28
MTB624	5.8983_437.3013_Pos	-	0.00	0.13	0.06	0.46	1.27
MTB623	7.4405_437.3361_Pos	-	0.03	0.00	0.09		1.27
MTB107	7.1459_370.2847_HQ	-	0.03	0.17	0.08	0.51	1.20
MTB757	7.228_506.3612_Pos	PC(P-18:1(9Z)/0:0)	0.00	0.00	0.05		1.18
MTB907	6.0318_213.1819_neg	-	0.00	0.07	0.00	0.03	1.16
MTB379	6.938_266.2848_Pos	8-butyl-5-hexyloctahydroindolizine	0.00	0.07	0.02	0.28	1.13
MTB676	4.8857_455.2655_Pos	18-acetoxy-PGF2α-11-acetate	0.03	0.05	0.00	0.00	1.06
MTB981	7.0458_365.2339_neg	Tetrahydrocortisol	0.03	0.05	0.00	0.00	1.00

[1]The metabolites with a threshold of variable importance projection (VIP) values > 1 and *P* value < 0.05 were considered differential metabolites.
[2]Metabolites are listed as retention time_mass_method of derivatization used [either negative mode (neg), positive mode (pos), dansyl chloride (DC), or 2-hydrazinoquinoline (HQ)].
[3]Fold change of metabolites in feces of pigs fed DDGS+AOP compared with those fed DDGS.

Table 4-14. Significantly different metabolites in feces of pigs fed rice bran (RB) or rice bran diet with addition of AOP (RB+AOP)[1]

Metabolite_ID	Metabolite	Identity	P-value	RB	RB+AOP	Fold change	VIP value
MTB320	2.0047_162.0558_Pos	-	0.02	0.05	1.24	22.74	5.89
MTB930	6.4055_297.2401_neg	-	0.01	0.46	1.18	2.52	4.49
MTB303	2.3236_144.0818_Pos	-	0.00	0.32	0.62	1.93	3.15
MTB232	6.0997_395.1061_DC	-	0.04	0.01	0.16	17.88	2.05
MTB516	3.9834_360.2285_Pos	-	0.03	0.53	0.61	1.13	1.28
MTB149	6.8903_440.3267_HQ	-	0.02	0.06	0.13	2.16	1.27
MTB200	6.2587_305.1319_DC	-	0.01	0.03	0.08	2.96	1.15
MTB799	5.0897_595.3481_Pos	13-Deoxytedanolide	0.02	5.83	4.17	0.70	6.55
MTB1027	6.866_441.2992_neg	(22S)-1α,22,25-trihydroxy-23,24-tetradehydro-24a-homo-20-epivitamin D3	0.04	1.06	0.50	0.47	3.60
MTB703	4.9236_470.2653_Pos	-	0.00	0.24	0.02	0.09	2.72
MTB528	6.0772_376.2598_Pos	-	0.04	0.56	0.37	0.66	2.28
MTB422	4.6274_305.1867_Pos	-	0.00	0.41	0.28	0.68	2.02
MTB327	1.9959_192.0965_Pos	-	0.01	0.53	0.41	0.76	2.00
MTB956	6.7312_351.2182_neg	Prostaglandin I2	0.02	0.19	0.05	0.27	1.83
MTB1036	4.3837_465.3037_neg	cholesterol sulfate	0.03	0.40	0.27	0.67	1.76
MTB981	7.0458_365.2339_neg	Tetrahydrocortisol	0.00	0.13	0.04	0.30	1.65
MTB823	4.9159_611.3437_Pos	-	0.02	0.17	0.06	0.35	1.63
MTB250	5.553_457.1062_DC	4-(2-Amino-3-hydroxyphenyl)-2,4-dioxobutanoic acid	0.00	0.07	0.01	0.15	1.32
MTB801	5.0369_593.3333_Pos	Mesobilirubinogen	0.04	0.16	0.08	0.52	1.32
MTB802	5.2632_593.333_Pos	Mesobilirubinogen	0.03	0.06	0.01	0.08	1.18
MTB330	1.9957_191.0902_Pos	-	0.01	0.12	0.08	0.65	1.13
MTB291	1.9959_150.086_Pos	-	0.02	0.15	0.11	0.74	1.08

[1]The metabolites with a threshold of variable importance projection (VIP) values > 1 and *P* value < 0.05 were considered differential metabolites.

[2]Metabolites are listed as retention time_mass_method of derivatization used [either negative mode (neg), positive mode (pos), dansyl chloride (DC), or 2-hydrazinoquinoline (HQ)].
[3]Fold change of metabolites in feces of pigs fed RB+AOP compared with those fed RB.

Table 4-15. Significantly different metabolites in feces of pigs fed wheat middlings diet (WM) or WM diet with addition of AOP (WM+AOP)[1]

Metabolite ID	Metabolite[2]	Identity	P-value	WM	WM+AOP	Fold change[3]	VIP value
MTB799	5.0897_595.3481_Pos	13-Deoxytedanolide	0.00	4.64	5.83	1.26	5.11
MTB998	4.219_391.2823_neg	-	0.01	1.37	1.91	1.41	3.69
MTB285	1.4232_120.0818_Pos	-	0.00	0.08	0.37	4.73	2.79
MTB967	4.0827_389.267_neg	-	0.02	0.13	0.49	3.73	2.79
MTB415	4.712_311.0813_Pos	Sulfadoxine	0.04	1.91	2.25	1.19	2.60
MTB1116	4.2086_783.5801_neg	-	0.01	0.05	0.23	4.28	2.17
MTB50	3.9296_221.0195_HQ	-	0.01	4.75	4.93	1.04	2.12
MTB123	7.4656_424.3315_HQ	-	0.05	0.60	0.86	1.44	2.08
MTB909	7.8449_295.2606_neg	-	0.04	0.06	0.25	4.23	2.02
MTB522	5.9307_357.2791_Pos	tetracosahexaenoic acid	0.00	0.42	0.59	1.41	1.99
MTB122	7.6289_426.3472_HQ	-	0.00	0.25	0.40	1.58	1.90
MTB136	6.3058_534.3688_HQ	-	0.00	0.07	0.20	3.05	1.85
MTB130	7.4051_398.3157_HQ	-	0.03	0.61	0.78	1.28	1.75
MTB704	5.0515_470.2651_Pos	-	0.00	0.01	0.07	5.89	1.22
MTB1020	4.2152_451.3044_neg	4,4-difluoro-1α,25-dihydroxyvitamin D3 / 4,4-difluoro-1α,25-dihydroxycholecalciferol	0.04	0.30	0.37	1.24	1.20
MTB1001	4.2168_437.2886_neg	-	0.01	0.09	0.14	1.55	1.13
MTB322	7.8018_218.1547_Pos	4'-Methyl-alpha-pyrrolidinopropiophenone	0.05	0.12	0.19	1.62	1.09
MTB35	2.9339_186.0658_HQ	-	0.01	0.08	0.13	1.74	1.07
MTB197	4.036_251.0849_DC	-	0.03	2.12	1.19	0.57	4.05
MTB935	4.5802_297.1099_neg	-	0.00	0.79	0.42	0.54	3.11
MTB303	2.3236_144.0818_Pos	-	0.01	0.86	0.46	0.54	3.11
MTB941	4.1828_313.2352_neg	-	0.01	0.64	0.26	0.41	2.78
MTB75	2.5856_230.1281_HQ	-	0.03	2.08	1.75	0.85	2.38

MTB88	4.3014_258.1594_HQ	-	0.02	0.22	0.02	0.08	2.05
MTB1058	6.6789_480.3101_neg	PC(15:0/0:0)	0.00	0.11	0.01	0.11	1.81
MTB299	3.3509_146.0611_Pos	-	0.03	0.17	0.01	0.03	1.76
MTB1018	7.1865_452.2761_neg	PC(13:0/0:0)	0.04	0.12	0.05	0.38	1.53
MTB641	7.0095_441.3015_Pos	Suillin	0.01	0.12	0.00	0.00	1.53
MTB703	4.9236_470.2653_Pos	-	0.01	0.11	0.00	0.00	1.51
MTB995	3.6733_393.1928_neg	Nigakilactone M	0.03	0.14	0.04	0.28	1.30
MTB917	3.6041_285.11_neg	-	0.00	0.12	0.05	0.40	1.27
MTB1003	5.9001_435.3095_neg	24,24-difluoro-1α-hydroxyvitamin D3 / 24,24-difluoro-1α-hydroxycholecalciferol	0.03	0.38	0.32	0.84	1.26
MTB652	5.5893_452.3118_Pos	PC(P-14:0/0:0)	0.03	0.18	0.11	0.59	1.24
MTB1057	6.5664_482.326_neg	-	0.01	0.05	0.01	0.18	1.10
MTB406	2.5903_295.166_Pos	Tyrosyl-Leucine	0.02	0.05	0.01	0.18	1.01

[1]The metabolites with a threshold of variable importance projection (VIP) values > 1 and *P* value < 0.05 were considered differential metabolites.
[2]Metabolites are listed as retention time_mass_method of derivatization used [either negative mode (neg), positive mode (pos), dansyl chloride (DC), or 2-hydrazinoquinoline (HQ)].
[3]Fold change of metabolites in feces of pigs fed WM+AOP compared with those fed WM.

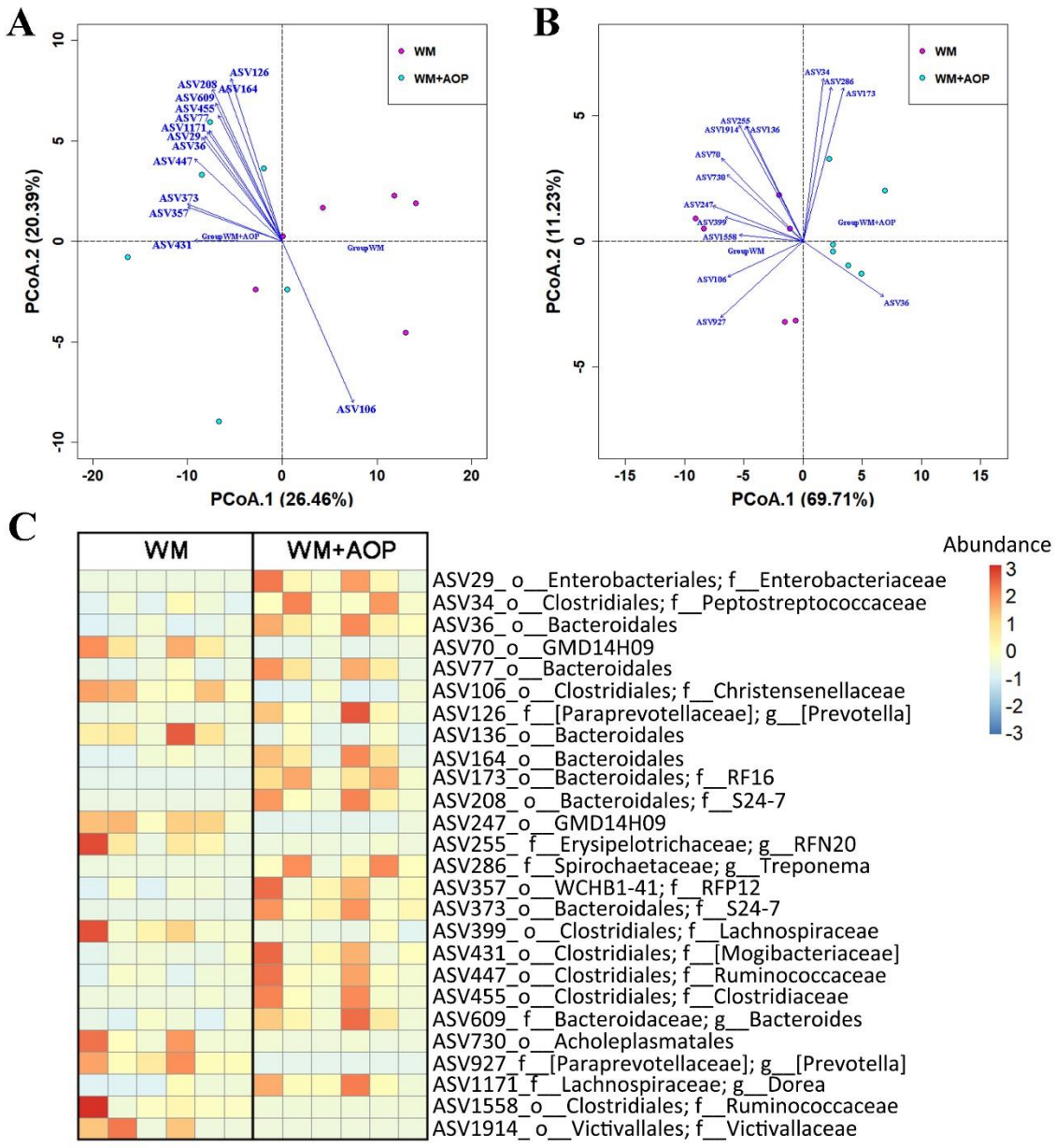

Figure 4-12. *Envfit* analysis revealed (A) the correlation between apparent total tract digestibility (ATTD) of nutrients (dry matter, gross energy, crude protein, neutral detergent fiber and ash) and differential amplicon sequence variants (ASVs) in the feces induced by the addtion of *Aspergillus oryzae* postbiotic (AOP) in growing pigs fed diets containing wheat middlings (WM) and (B) the correlation between fecal differential metabolites and fecal differential ASVs induced by the addtion of AOP (Taxonomy of ASVs were listed in Table S13). (C) Heatmap shows the relative abundance (Z score transformation) of ASVs that were significantly correlated with ATTD of nutrients and differencial metabolites in the feces of pigs fed WM+AOP or WM diets.

Table 4-16. Relative abundance of differential amplicon sequence variants (ASVs) that were significantly correlated with apparent total tract digestibility of nutrients and differential metabolites in growing pigs fed wheat middlings (WM) diet or wheat middlings diet with the addition of *Aspergillus oryzae* postbiotic (WM+AOP)

ASV ID	Taxonomy	WM	WM+AOP	*P* value
ASV29	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae	0.00	0.03	0.02
ASV34	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Peptostreptococcaceae	0.07	0.28	0.04
ASV36	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	0.26	1.55	0.02
ASV70	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__GMD14H09; f__; g__; s__	1.32	0.10	0.02
ASV77	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	0.20	1.00	0.07
ASV106	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Christensenellaceae; g__; s__	0.91	0.20	0.02
ASV126	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	0.04	0.38	0.04
ASV136	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	0.23	0.04	0.04
ASV164	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__	0.08	0.36	0.13
ASV173	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__RF16; g__; s__	0.00	0.35	0.02
ASV208	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	0.02	0.52	0.02
ASV247	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__GMD14H09; f__; g__; s__	0.33	0.01	0.02
ASV255	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae; g__RFN20; s__	0.48	0.07	0.39
ASV286	k__Bacteria; p__Spirochaetes; c__Spirochaetes; o__Spirochaetales; f__Spirochaetaceae; g__Treponema; s__	0.00	0.11	0.02
ASV357	k__Bacteria; p__Verrucomicrobia; c__Verruco-5; o__WCHB1-41; f__RFP12; g__; s__	0.07	0.32	0.03
ASV373	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__S24-7; g__; s__	0.01	0.42	0.02
ASV399	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__; s__	0.22	0.05	0.02
ASV431	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__[Mogibacteriaceae]; g__; s__	0.02	0.09	0.02
ASV447	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	0.03	0.11	0.02
ASV455	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae	0.00	0.15	0.02
ASV609	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides; s__	0.03	0.13	0.04
ASV730	k__Bacteria; p__Tenericutes; c__Mollicutes; o__Acholeplasmatales; f__; g__; s__	0.11	0.01	0.07

ASV927	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__[Paraprevotellaceae]; g__[Prevotella]; s__	0.06	0.00	0.02
ASV1171	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Lachnospiraceae; g__Dorea; s__	0.01	0.03	0.02
ASV1558	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Ruminococcaceae; g__; s__	0.02	0.00	0.02
ASV1914	k__Bacteria; p__Lentisphaerae; c__[Lentisphaeria]; o__Victivallales; f__Victivallaceae; g__; s__	0.01	0.00	0.02

Chapter 5. Overall Summary, Limitations, and Implications

Global food demand will keep increasing in the next decades to meet the needs of the increasing population (Fukase and Martin, 2020), and demand for animal-derived food products is likely to increase at a faster rate than the demand for plant-derived foods. At the same time, we are facing the challenges of producing sufficient, safe, and affordable food while minimizing the environmental impacts of its production (Shurson, 2017). Some economic models suggest that this issue may be resolved by changing the current perception of the food system as a linear series of human activities with the sole goal of producing food. Circular economic models suggest that food production is a series of activities that are interrelated and offer the potential for synergistic opportunities for increasing the value and decreasing the environmental footprint of food production (Hamam et al., 2021). In circular economic models, substantial amounts of low-cost, high fiber coproducts produced from various agro-industrial processes are incorporated into animal feed with the aim of enhancing sustainable use of feed resources and reducing the cost and the environmental impacts of food animal production.

The high concentration of dietary fiber (DF) in low-cost, high fiber coproducts is generally not well utilized by pigs due to the lack of endogenous fiber-degrading enzymes (Agyekum and Nyachoti, 2017), which results in reduced energy and nutrient digestibility along with increased nutrient excretion and gas emissions from increased animal manure production. Several feed technologies have been developed for improving the nutritional value of high fiber coproducts for swine, including various processing techniques, exogenous fiber-degrading enzymes, ammonia fiber expansion, and solid-state fungal fermentation pre-treatment (Kerr and Shurson, 2013; Sun et al., 2021; Zeng

et al., 2018). However, these technologies have several drawbacks including: 1) inconsistency in the frequency and magnitude of responses due to differences in ingredient composition, substrate type, or enzyme characteristics; 2) digestibility improvements do not result in growth performance improvement; 3) technologies are not available at a large commercial scale; and 4) relatively high cost or a large investment of resources for processing. Furthermore, due to the wide differences among analytical methods for DF measurement in studies (e.g., crude fiber, acid detergent fiber, neutral detergent fiber, and total DF), nutritionists often do not use appropriate measures that accurately relate to the physiochemical characteristics of DF, which is essential for determining diets and feeding conditions where various types of feed additives may be most effective in degrading specific types of DF. Therefore, effective, inexpensive, and widely applicable nutritional strategies are greatly needed to improve the energy and nutrient utilization efficiency of using high fiber feedstuffs in swine diets.

Aspergillus oryzae postbiotic (AOP) has been shown to be effective in improving nutrient digestibility in ruminants. However, because studies on the use of AOP in pig diets are very limited, there is a need to examine the potential of AOP for improving nutrient digestibility of high fiber feedstuffs in growing pig diets and the underlying mechanisms. The results from chapter 2 and chapter 3 showed that the addition of 0.05% AOP to corn distillers dried grains with solubles (DDGS), rice bran (RB), or wheat middlings (WM) and diets containing these high fiber ingredients increased energy and nutrient digestibility *in vivo* and *in vitro*. Corn DDGS is one of the major coproducts produced in large quantities from bioethanol industry, but the DF characteristics have shown to be generally resistant to degradation by adding exogenous carbohydrase and

protease enzymes to diets. Results from chapter 2 suggested that AOP has the potential to substantially increase apparent ileal digestibility of ether extract of DDGS diets, and in chapter 3, the digestibility response of AOP for DDGS was greater compared with RB and WM. Further studies are required to confirm this potential of AOP to increase the feeding value of DDGS in swine. Additionally, we found that the magnitude of the AOP response is ingredient- and diet-dependent, which confirms that the effectiveness of fiber-degrading feed additives is associated with DF type and composition of ingredients. Therefore, it is important to understand the mechanisms of action of various types of feed additives and determine which types of feed additives are effective in degrading specific types of fiber. With this knowledge, it may be possible to develop feeding strategies that involve combinations of additives capable of degrading various types of dietary fiber in feedstuffs without causing antagonistic effects, and therefore, improve the effectiveness and strategic use of feed additives in the future.

Results from chapter 4 suggested that dietary addition of AOP significantly altered diversity and composition of the microbial community and metabolome in the ileal digesta and feces of growing pigs. We identified differentially abundant bacteria and metabolites that are involved in changes in the metabolism of fiber, amino acids, and fatty acids from feeding AOP diets. Further, we confirmed that AOP alters the abundance of several bacteria that were significantly correlated with nutrient digestibility, some of which are key players among the fiber-degrading bacteria (e.g., order *Bacteroidale*, family *S24-7* and *Ruminococcaceae*). These findings may partially explain the improvements observed in digestibility of fiber and other nutrients by adding AOP to diets, and thus assist in the design of strategies for effective nutrient utilization in pigs fed

high fiber diets. With the associations between degradation and enzymatic digestion or microbial fermentation of DF well documented, it is only logical that future development of feed additives should aim to improve DF utilization by focusing on enhancing enzymatic digestion of DF and/or enriching key fiber-degrading bacteria in the gastrointestinal tract of pigs.

A few limitations of the studies described in this dissertation need to be addressed in future research. First, DF content affects digestibility of other nutrients, as well as gut microbiome diversity and composition. Therefore, diets should be formulated to contain equivalent DF concentrations to compare nutrient digestibility and microbiome changes among dietary groups fed AOP. Due to the variation in DF content among sources within each ingredient type, RB used in our studies had relatively low DF concentration compared with the concentration reported in NRC (2012), which resulted in lower DF content in RB diets compared with DDGS and WM diets evaluated. Secondly, DF measured as non-starch polysaccharide (NSP) composition based on individual soluble and insoluble components is more accurate and representative of its complex physiological effects. Moreover, because many feed additives are effective for specific types of DF found in various ingredients, it is necessary to determine the effects of feed additives in degrading specific fractions of DF. Evaluation of effects of AOP on DF degradation based on NSP composition may be a more accurate and comprehensive approach and requires further investigation. Lastly, many different types of abundant bacteria were not identified at the species level in digesta and feces, and the identities of many differential metabolites were not confirmed in chapter 4 due to the limitations in analytical methods. Further studies are needed using metagenomic and LC-MS/MS to

identify these differential bacteria and metabolites and determine their roles related to digestion of DF and other nutrients in pigs fed various types of high fiber diets containing AOP.

The findings in the thesis have important implications for nutritionists and producers in the swine industry for achieving sustainable swine production and reducing feed costs when using low-cost coproducts in swine diets. First, the addition of AOP to swine diets is effective for improving energy and nutrient digestibility of diets containing corn DDGS, RB, or WM, and is associated with its effects on modulating the microbiome and metabolome. Feed accounts for about 60 to 70% of the total cost of pork production in modern intensive systems. Therefore, improvements in energy and nutrient utilization of high fiber coproducts by the addition of AOP to diets containing them contributes to reducing the cost of swine production, especially under conditions of high feed ingredient prices and limited supplies are available. Our results showed that the improvement in digestible energy by addition of AOP to these high fiber coproducts averaged 90 kcal/kg dry matter, which could reduce the feed cost by $6.57/metric ton feed and $1.97 per pig calculated based on corn price of $7.42/bushel, and by $18.45/metric ton feed and $5.54 per pig calculated based on soybean oil price of $1.76/kg. Secondly, efficacy of feed additives that aim to increase fiber digestion in monogastric animals depends on the fiber type and composition of the ingredients, which implies that the use of combinations of different feed additives capable of degrading different types of fiber could be more effective and widely applicable than the use of a single type of feed additive. Lastly, integration of multi-omics techniques with traditional growth performance evaluations is a promising approach to reveal mechanisms underlying beneficial impacts of dietary

interventions in pigs. A better understanding of the mechanisms of nutritional responses is essential for the proper use and application of feed additives to more consistently achieve the desired response, because only by understanding the mechanisms can we then strategically use feed additives that are appropriate for the commercial production conditions where the greatest benefits and most consistent response will be observed. Innovations are needed to develop strategies that overcome limitations of current technologies that aim to improve the feeding value of high fiber coproducts so that we can improve our ability to feed the world more sustainably with an increasing global population in the coming decades.

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