2024

Evaluation of Dietary Fiber Technologies in Sow Diets

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Recommended Citation

Shipman, Garrin, "Evaluation of Dietary Fiber Technologies in Sow Diets" (2024). *Electronic Theses and Dissertations*. 954.
https://openprairie.sdstate.edu/etd2/954

EVALUATION OF DIETARY FIBER TECHNOLOGIES IN SOW DIETS

BY

GARRIN SHIPMAN

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Animal Science

South Dakota State University

2024

DISSERTATION ACCEPTANCE PAGE
Garrin Shipman

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Crystal Levesque
Advisor Date

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Director, Graduate School Date

This dissertation is dedicated to Gertha “Sonny” Shipman. Thank you, Paw Paw, for kindling my passion for pigs, horses, and farming.

ACKNOWLEDGEMENTS

First, I will like to thank my advisor, Dr. Crystal Levesque, for mentoring me and dedication to my future. Appreciation is extended to my committee members, Drs. Ryan Samuels, Jeffrey Clapper, and Jon De Jong, for their advice on my research focus. Much appreciation is given to Dr. Jorge Perez-Palencia and Dr. Jinsu Hong for their assistance with trial development, laboratory analysis, and support. Thank you to my fellow swine nutrition graduate students for assistance and unforgettable moments during these past three years. Thank you to all of the ladies (sows) that were instrumental to my dissertation research!

Lastly, thank you to God for giving me this amazing experience.

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ABBREVIATIONS

AA	amino acids
ADF	acid detergent fiber
ADG	average daily gain
AID	apparent ileal digestibility
Ala	alanine
Arg	arginine
Asp	aspartate
ATTD	apparent total tract digestibility
BW	body weight
CF	crude fiber
CON	control
CP	crude protein
Cys	cysteine
d	day
DDGS	corn dried-distillers grains with solubles
DE	digestible energy
DF	dietary fiber
DM	dry matter
EE	ether extract
Eq	equation
GI	Gastrointestinal tract
GE	gross energy
Glu	glutamine
Gly	glycine

h	hour
HF	high fiber
His	histidine
IAA_{end}	ileal endogenous amino acid losses
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
Ile	isoleucine
kcal	kilocalorie
kg	kilograms
LF	low fiber
Leu	leucine
Lys	lysine
ME_{blend}	multienzyme blend
ME	metabolizable energy
Met	methionine
mg	milligram
MJ	megajoule
mL	milliliter
mm	millimeter
MOS	mannan-oligosaccharides
NDF	neutral detergent fiber
NE	net energy
NSP	non-starch polysaccharides
Phe	phenylalanine

post-MSC DDGS	post-protein extraction dried distillers grains
Pro	proline
R^2	coefficient of determination
SEM	standard error of the mean
Ser	serine
SBP	sugar beet pulp
SH	soybean hulls
SID	Standardized ileal digestibility
TDF	Total dietary fiber
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine
VFA	volatile fatty acids
YC	Yeast carbohydrate
χ^2	chi-squared
μg	microgram

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ABSTRACT

EVALUATION OF DIETARY FIBER TECHNOLOGIES IN SOW DIETS

GARRIN SHIPMAN

2024

Various technologies and alternative ingredients are being developed to increase the nutrient value of fibrous feedstuffs, improve animal performance, and promote sustainable swine production. Fiber-degrading enzymes have been extensively studied in growing pigs, with few studies focused on gestating sows. When considering gestating sows have greater energy digestibility of fibrous feedstuffs than grower pigs, the efficacy of fiber-degrading enzymes needs to be determined for implementation in commercial sow diets. As fiber inclusion in gestation diets benefits sow performance, corn dried distiller grains with solubles (**DDGS**) are a candidate fiber source for gestation alternative energy ingredient. Functional fibers, such as yeast carbohydrates (**YC**), also have the potential to benefit both the performance of the sow and her nursing offspring. Thus, the values of these fibrous technologies were evaluated in relation to improving sow feeding strategies.

A multienzyme blend at 0.1% inclusion in complete gestation diets increased total tract digestibility of nutrients and energy for gestating sows by 3 to 10%, depending on the dietary neutral detergent fiber level, but produced no effects on the ileal digestibility of amino acids. Enzyme supplementation did increase the energy content of individual feedstuffs; a greater impact was observed in protein concentrates compared to cereal grains.

The energy content of a post-protein separation DDGS was approximately 28% greater compared to other common fiber sources. Lower gas production and equal

concentrations of volatile fatty acids give value to post-protein separation DDGS as an alternative fibrous ingredient in gestation diets while being environmentally sustainable.

An optimal inclusion level of YC product for sow diets was determined to be 0.2% of dietary intake. This recommendation is based on the observations that sows supplemented with YC at 0.2% had greater colostral immunocrit ratio, weaned more of their light-born offspring, and offspring birthed and suckled from the sows supplemented with the 0.2% YC inclusion level had greater serum concentrations of IgA at the time of weaning.

Overall, these various fibrous technologies can benefit sow feeding by increasing dietary energy, promoting sustainable swine production, and improving performance of suckling offspring.

CHAPTER 1

APPLICATIONS OF DIETARY FIBER IN SOW FEEDING

THE MODERN SOW

General Sow Production

The total number of hogs on feed in the United States was 74.58 million with 6.1 million being breeding females (USDA Reports, 2022). In 2022, the modern sow in the United States had an average 15.4 total pigs per litter, 13.8 pigs born alive per litter, and 11.9 pigs weaned per litter, cumulating in the average number of pigs weaned per mated female per year being 26.3 (PigChamp USA, 2022). This is a vast improvement compared to her predecessor from 18 years earlier who averaged 11.5 total pigs per litter, 10.3 pigs born alive per litter, 9 pigs weaned per litter, and 21.3 pigs weaned per mated female per year (PigChamp USA, 2004). This vast improvement requires intensive nutrient management to feed today's sow to support her litter (see reviews by Kim et al., 2013, Theil et al., 2022, and Langendijk et al., 2023). While the modern sow is the same high producing animal during both gestation and lactation, she is fed differently in each production stage to meet her metabolic demand for production and maintenance.

Feeding the Modern Sow in Gestation

The goal of a gestation feeding program is to maintain an ideal body condition while supplying the pregnant female with adequate nutrients to support maternal maintenance, growth, and fetal development and related conceptus tissue while preparing for a successful lactation. Over-conditioned sows will experience issues at farrowing (*i.e.*, dystocia and agalactia) and low feed intake during lactation (MacPherson et al., 2004). To prevent excessive weight-gain, gestating sows are limit-fed, typically in a single feeding, with feed allowance being based on the body condition of the sow (Meunier-Salaün, et al., 2001; Johnston, 2010). Negative stereotypic behaviors can arise as the restricted feed intake can only satisfy 40-60% of normal voluntary feed intake (Meunier-Salaün, et al., 2001). In group-penned animals, aggressive behavior has been correlated with limited feed intake include vulva biting and skin lesions between pen mates (van Putten and van de Burgwal, 1990; Martin and Edwards, 1994). Sham chewing and bar biting are negative stereotypic behaviors and indicators of hunger displayed by gestating sows housed in stalls (Che et al., 2011).

The inclusion of dietary fiber (**DF**) in gestation diets is a strategic nutritional tool to promote the sensation of satiety, dilute energy intake to limit weight gain, and reduce negative stereotypical behavior (van der Peet-Schwering et al., 2004). Another benefit of DF for the sow is the continual movement of digesta through the gastrointestinal tract (D'eath et al., 2018). Intake of DF around the transition period has shown to reduce constipation in the sow and prevent impaction around the birth canal that may act as a physical barrier and increase the likelihood of rectal prolapses and stillbirths (D'eath et al., 2018). The mechanisms of DF physiochemical properties are discussed later in this chapter.

Regarding gestating sow nutrition, dietary energy intake is based on energy requirements for body maintenance, support for conceptus growth, maternal body protein deposition, and development of uterine and mammary tissue as pregnancy progresses (Ball and Moehn, 2013). These factors can be influenced by parity, bodyweight (**BW**), productivity and environmental conditions. One-hundred kcal/d per kg $BW^{0.75}$ appears to be an effective metabolizable energy (**ME**) intake for maintenance requirements during gestation of sows housed in thermoneutral conditions without affecting the growth of conceptus and maternal tissue. Thus, the targeted effective ME should be 3,300 kcal/kg on an as-fed basis (NRC, 2012).

Feeding the Modern Sow in Lactation

Most production systems feed their lactating females ab *libitum* to maximize their intake without needing to rely on body reserves to meet the high nutrient demand to support milk production and nurse her rapidly growing offspring (Tokach et al., 2019). Although lactation represents only 15% to 20% of the productive cycle of a sow, it is undeniably the most metabolically demanding stage of production. For the neonatal pig, milk acts as a rich source of nutrients (carbohydrates, lipids, proteins, minerals, vitamins, etc.) necessary to stimulate proper growth and immune development. Additional components of milk important for piglet postnatal development include immunoglobulins, microminerals, hormones, and growth factors. The composition of sow milk is unique in the fact that it is 60-70% higher in gross energy (reflective of a 6.8% fat content) and roughly 70% higher in protein (5.5%) compared to the lactating dairy cow (3.7% and 3.2% for fat and protein content, respectively) (Boyd et al., 1995).

Glucose is the primary metabolic precursor for milk synthesis (Tokach et al., 1992). This is reflective of the sow utilizing 65-83% of her net energy (**NE**) requirements to support the production of milk (including the de-novo synthesis of milk fat and secretion of lactose) (Aherne and Williams, 1992; Boyd and Kensinger, 1998). The priority for milk production can result in the sow mobilizing fat and protein body reserves if dietary intake is below requirements (Pettigrew and Moser, 1991). A consequence of excessive utilization of body tissue for milk production is an increased likelihood of being culled from the herd due to compromised subsequent reproduction. This is problematic as genetic selection has produced a leaner sow with low daily feed intake and lower body reserves (Hermesch et al., 2008).

Milk production is proportional to the number of pigs suckling with larger litter sizes, resulting in higher milk yields (Toner et al., 1996). Genetic selection for increased litter size and daily gain has resulted in the sow producing approximately 9.2 kg/d of milk in 2012 compared to 8.2 kg/d in 1985 (see review by Rosero et al., 2016). A study by Harrell et al. (1993) found that artificially reared pigs grew an average of 70% faster (395 g/d) and were 53% heavier (9.8 g/d) than sow-nursed pigs (growth of 232 g/d and 6.4 kg at 21 d of age). This study demonstrated that modern sows cannot produce adequate amounts of milk and nutrient output to nourish and achieve genetic potential of her nursing offspring.

The following equation can be used to predict a practical ME requirement for milk production and is estimated from average daily gain of the litter (ADGL) and litter size (NRC, 2012):

$$\text{Effective ME requirement for milk production (kcal/d)} \\ = 7.03 \times \text{ADGL (g)} - 129 \times \text{Litter size}$$

In practical terms, when using corn and soybean meal-based diets with an effective ME content of 3,300 kcal/kg, a lactating sow with a body weight of 200 kg will require 1.6 kg feed/d for maintenance and 1.7 kg feed/kg/d of litter growth rate.

Current Industry Issues Associated with Sow Nutrition

While DF is beneficial for gestating sows, fibrous ingredients are routinely formulated in commercial sow diets with nutrient specifications attained from grower pigs or prediction equations (Dourmad et al., 2008; Święch, 2017). Gestating sows have a greater utilization of nutrients and energy from fibrous ingredients and higher digestibility of various dietary fiber complexes compared to grower pigs (Lowell et al., 2015; Casa and Stein, 2018). Inaccuracies can lead to over-formulation, increases in feed costs, and inefficiencies (Vonderohe et al., 2022).

Carbohydrase enzymes have been shown to be effective in diets containing fibrous ingredients fed to weaned pigs (Omogbenigun et al., 2004), as well as grower and finishing pigs (Emiola et al., 2009; Velayudhan et al., 2015; Woyengo et al., 2018) for improving the apparent total tract digestibility (**ATTD**) of nutrients and energy. However, there are limited studies investigating the effect of carbohydrase enzymes, either alone or in a combination, in diets fed to gestating sows. Nevertheless, carbohydrases are routinely included in commercial gestating sow diets (McGhee and Stein, 2021). The utilization of exogenous enzymes in commercial swine diets is a strategy to reduce ingredient usage and lower diet costs by increasing the nutrient and energy density of poorly digested ingredients (Olukosi and Adeola, 2013). Considering that gestating sows can more efficiently digest dietary fiber compared to growing pigs (Noblet and Shi, 1993; Stein et al., 2001; Jørgensen et al., 2008; Lowell et al., 2015; Dong et al., 2020), the extent of nutrient and energy improvements in gestating sow diets by carbohydrase enzymes needs to be determined for most effective application of carbohydrase enzymes in commercial sow production.

The United States produced approximately 15.3 billion gallons of ethanol as a form of renewable biofuel in 2022 (Renewable Fuels Association, 2023). Cereal grains (such as corn, wheat, and sorghum) are used as feedstock for ethanol production and are increasingly used as resources for renewable biofuels (Lywood and Pinkney, 2012; de

Corato and Viola, 2023). Cereal grains are also commonly used as the base energy ingredients in livestock feeds; resulting in increased competition for grains between ethanol and food animal production and increase interests in utilizing alternative ingredients for feeding livestock (Shurson, 2017). The starch and fermentable carbohydrate content of cereal grains are isolated during the fermentation process, which represents less than 3% of potential dietary energy in the cereal grain. The leftover nutrients available for energy utilization by the animal (*i.e*., protein, oil, and fiber) concentrate in the co-products. These co-products, such as corn dried distiller grains with solubles (**DDGS**), provide an alternative energy source that are economical to sustain efficient production of pork and other food animal industries (Lywood and Pinkney, 2012; USCG, 2023). The high fiber content of DDGS can reduce performance and efficiency of growing pigs (Shurson et al., 2012) but make it an ideal candidate for inclusion in high fibrous gestation sow diets (Meunier-Salaün and Bolhius, 2015). The impacts of DDGS inclusion in gestation diets on reproductive performance and behavior have been previously investigated, but results have been inconsistent (Wilson et al., 2005; Li et al., 2014). This might be in part because diet formulations for pregnant sows are based on ingredient nutrient specifications from grower pigs.

Other co-products of biofuel production, such as soybean hulls and sugar beet pulp, (de Corato and Viola, 2023) are commonly included in gestation diets to promote satiety and produce energy-efficient volatile fatty acids (**VFA**) from hindgut fermentation (Meunier-Salaün and Bolhius, 2015; Sapkota et al., 2016; Theil et al., 2020; Wisbech et al., 2022). The high fermentable fiber content in some of these biofuel co-products, however, can result in higher gas emissions in gestating sows (Rijnen et al., 2001). While formulating diets accurately to meet nutrient requirements is one way to promote sustainability in the swine industry, feeding ingredients that produce less gas emissions are also a form of environmental stewardship (Vonderohe et al., 2022). This comes at a time when more specialized DDGS and other ethanol by-products are being developed to meet the needs of commercial swine production (Garavito-Duarte et al., 2023; USGC, 2023).Mechanical separation allows the high protein and amino acid (AA) content in the whole stillage to be exctracted to produce high protein DDGS that is better suited for grower swine diets and pet foods (USGC, 2023). The leftover fiber fraction of post-protein separation DDGS (**post-MSC DDGS**) might be a valuable fiber source in gestation diets.

Improvements in sow nutrition are vital to combat the current industry trend of wider variation in piglet birthweights within litters resulting from increased litter sizes, with lower-weight pigs at increased risk of pre-weaning mortality (Feldpausch et al., 2019). This trend in reduced piglet viability has led to the evaluation of functional feed additives in sow diets to promote improved performance of sows and livability of her nursing offspring (Xu et al., 2023). Yeast carbohydrates (**YC**) are hydrolysis products lysed from the cell wall of yeast. These isolated fibers have been linked to immune modulation through prebiotic effects (Patterson et al., 2023). Dietary inclusion of YC and other prebiotic feed additives in sow diets have gained attention in recent years to promote beneficial effects on the offspring (Bass et al., 2019; Duan et al., 2019) as the neonatal pig relies heavily on passive immunity attained from the sow to build a robust immune system needed for survival (Rooke et al., 2003). Supplementation of these products in sow diets has been linked to essential functions related to immune modulation in both sows and piglets, positively impacting their health and performance (Duan et al., 2016; Hasan et al., 2018).

The topics discussed in the current literature (1) the various DF complexes and non-starch polysaccharides (**NSP**), (2) the energy systems implemented in swine nutrition, (3) differences between gestating sows and growing pigs when evaluating dietary energy, (4) the usage of exogenous fiber degrading enzymes on enhanced nutrient digestibility of fibrous ingredients, (5) the value of emerging biofuel co-products in gestation diets, and lastly (6), the health effects of functional fibers for lactating sows and their nursing offspring.

FIBER AND THE NON-STARCH POLYSACCHARIDES

Definition and Classifications of Fiber

The definition of "fiber" is not concise. In the application of animal nutrition, fiber is a collective term to describe the oligosaccharides, resistant starch, and NSP that cannot be hydrolyzed by endogenous enzymes in animals but may have nutritional value following microbial enzyme digestion in the hindgut (Navarro et al., 2019b). In 1859, Henneberg and Stohman designated insoluble organic remnant of feedstuff following hot, dilute sulfuric acid and sodium hydroxide as 'crude fiber' (**CF**) (Choct, 1997). The first proximate analysis to determine CF of feedstuff involved a series of acid and alkaline extractions to attain leftover organic matter and was established by German scientists at Weende Experimental Station during the mid-19th century. This analysis is inaccurate and not applicable to diet formulation. For example, the true fiber content of soybean meal is approximately 25% while the CF analytical procedure will only determine the insoluble polysaccharides, cellulose, and phenolic lignin that makes up about 5% (Choct, 2015).

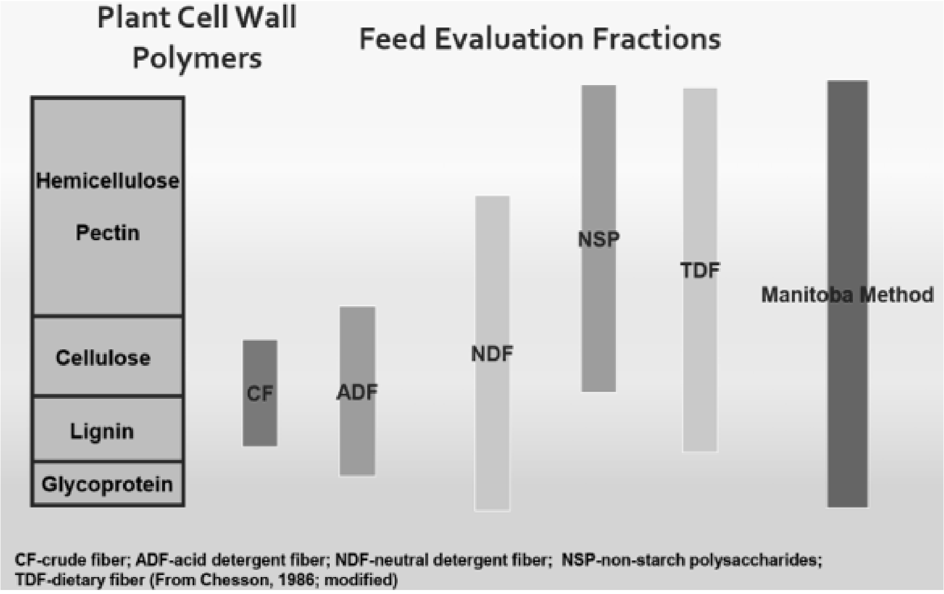

Figure 1-1. Plant cell wall polymers and their corresponding fiber fraction for feed evaluation (modified from Chesson, 1986).

This inconsistent definition of fiber led to the use of classifications to describe fiber content of feedstuff rather than an exact measurement (Figure 1-1). As stated above, CF encompasses the insoluble carbohydrates, cellulose, and phenolic lignin. Development of the detergent fiber fractions (acid-detergent [**ADF**] and neutral-detergent fiber [**NDF**]) begin in the 1960’s by Van Soest and colleagues (van Soest, 1963) at Cornell University to characterize fiber fractions of roughages intended for consumption by ruminants. These two fiber fractions account for lignin and cellulose content but differ in terms of solubility due to the presence of soluble hemicelluloses (arabinoxylans, mixed linked β-glucans, xyloglucans, mannans, galactomannans, galactans, arabinans and any other neutral polysaccharides other than cellulose) in the NDF fraction:

$$\text{ADF} = \text{lignin} + \text{cellulose}$$

$$\text{NDF} = \text{lignin} + \text{cellulose} + \text{hemicellulose}$$

While both fractions do not account for all soluble NSP, they are routinely used for proximate analysis of feedstuffs (Bach Knudsen, 2014; Choct, 2015).

The NSP (cellulose, pectins, and hemicellulose) are discussed in detail further in this chapter but along with lignin are counted as total dietary fiber (**TDF**) (DeVries et al., 1999). Functional fibers, while not considered in diet formulations, comprise the extracts or purified indigestible carbohydrates (*e.g.*, fructo-oligosaccharides, chitin, resistant dextrins, and psyllium) isolated from either the cell wall of plants or animals (*i.e.*, yeast and insects) that have been linked to health benefits (Slavin, 2013; Li and Komarek, 2017).

Fiber can be further classified based on physiochemical properties, including viscosity, solubility, and fermentability (Jha and Zijlstra, 2017). Viscosity is the ability of NSP to form viscous aggregates and thicken the density of the digesta when in the presence of water, which can delay transit time in the small intestine (Dikeman and Fahey, 2007). An increase in the viscosity of digesta while transiting through the gastrointestinal tract can be problematic as endogenous digestive enzymes are unable to penetrate the viscid and sticky substance resulting in a reduction in the digestion and absorption of nutrients (Hooda et al., 2010).

Solubility describes the hydration capacity (also termed as either water-binding or water-holding capacity) for a fiber to incorporate water into the digesta matrix. Starting in the stomach, water is “soaked up” by the soluble fiber and causes the digesta to swell up to result in reduced gastric emptying and an extended retention period in the gastrointestinal (**GI**) tract (Owusu-Asiedu et al., 2006). The reduced gastric emptying can lead to attenuated post-prandial absorption of glucose and hinder the growth performance of grower pigs (Bach Knudsen, 2001). Their physiological function also differs; insoluble fiber decreases retention time, increases feces bulk, makes feces softer, and thus is important in relieving constipation (Wenk, 2001). In contrast, soluble fiber increases the viscosity of gastric contents and thus delays gastric emptying, decreases the rate of glucose absorption, enhances immunity, and lowers serum cholesterol levels (Brennan, 2005).

Peptide YY and glucagon-like peptide-1 (**GLP-1**) suppress the actions of ghrelin, the hormone associated with stimulating the sensation to consume food. The swelling and reduced gastric emptying following the ingestion of soluble NSP stimulate the sensory mechanisms of the gastrointestinal tract smooth muscle to release peptide YY and GLP-1, which then inhibit the release of ghrelin and ghrelin's action to cause the sensation of hunger by the gut-brain axis (Greenway et al., 2007; Zijlstra et al., 2007). The effects of soluble NSP on their release help explain how these NSP reduce appetite and animal feed intake in feed-restricted gestating sows (Bach Knudsen, 2001).

In contrast to soluble NSP, insoluble NSP (e.g., cellulose and hemicellulose) are non-viscid, have negligible effects on gastric emptying, and increase the rate of small intestine transit. There is evidence that insoluble NSP reduce appetite and feed intake at the level of the small intestine. Specifically, insoluble NSP reduce glucose absorption by the small intestine, leading to an increase in its concentration at the terminal ileum and, therefore, the secretion of GLP-1. In addition, insoluble NSP stimulates the secretion of cholecystokinin (Holt et al. 1992). Both GLP-1 and cholecystokinin contribute to satiety and food intake suppression (Flint et al. 1998).

Fermentability is the capability of fiber compounds to undergo microbial fermentation in the GI tract, resulting in the production of VFA (Bach Knudsen, 2001). The hindgut (cecum and colon) are the primary sites of fermentation in pigs (Agyekum and Nyachoti, 2017). While minor in young and growing pigs with less developed GI tracts, microbial fermentation in gestating adult sows can account for 25% of the digestion and fermentation of NSP by the terminal ileum of the small intestine (Jørgenson et al., 2008). Volatile fatty acids can provide 15% and 30% of maintenance energy requirements for grower pigs and adult gestating sows, respectively (Theil et al., 2020).

Non-starch Polysaccharides

In relevance to monogastric nutrition, the NSP are the fibrous fractions of plant materials and feedstuffs that are resistant to endogenous digestive enzyme hydrolysis in both the foregut and midgut but are then subjected to microbial fermentation in the hindgut to result in the production of VFA (Patience et al., 2022). The content of NSP varies among the feedstuffs fed to monogastric species. A low content (10-30%) can be found in corn, wheat, and barley (the three main cereal grains that are used as the energy basis in swine diets), low to moderate levels in the oilseeds and legumes, but moderate to high concentrations in industrial cereal grain and oilseed by-products (Bach-Knudsen, 2014, Choct, 2015). The energy contribution of NSP following microbial fermentation in growing pigs is 30% less compared with enzymatically digested carbohyrates (Newman et al., 2016), and increased ingestion of NSP can result in reduced AID and ATTD of GE and SID of AA (Gutirrez et al., 2014).

Lignin

Lignin is a phenol polymer comprised of coniferyl, *p*-courmaryl, and sinapyl alcohols; thus, is not a carbohydrate (Davin et al., 2008; Figure 1-2). Its covalent linkage to polysaccharides directly or indirectly through sugar residues or esterified ferulic acid, respectively, contributes to the inhibition of chemical digestion of NSP by microbes in the GI tract (Davin et al., 2008; Bach Knudsen, 2014). The anaerobic environment of the GI tract is not an ideal condition for the oxidative breakdown of lignin; however, this might not be the case outside the GI tract. Lignin within the cell wall stiffens as the plant ages, and the potential breakdown of lignin linkages that anchor cellulosic microfibers or other matrix polysaccharides to the cell wall lessens over time (Bach Knudsen, 2001).

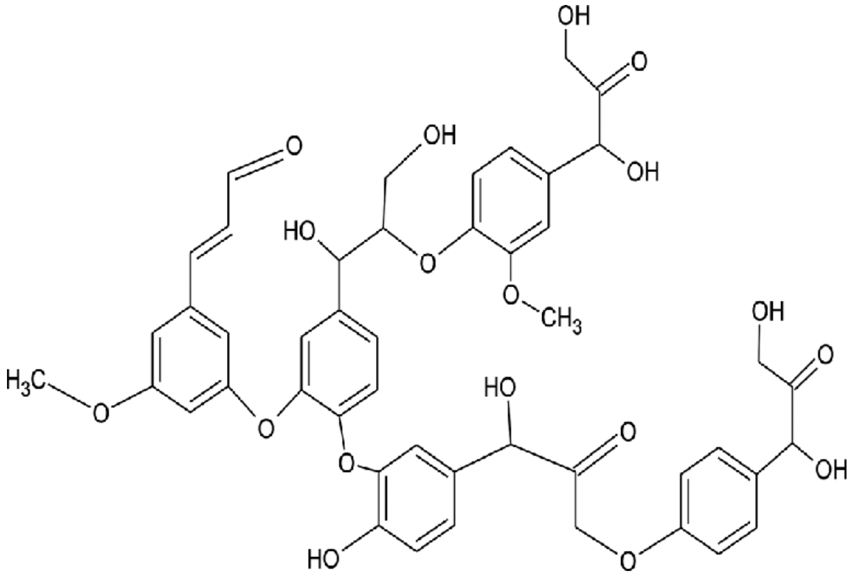

Figure 1-2. The structure of lignin phenylpropane units linked by an irregular 3-dimensional pattern of ether and carbon-carbon bonds in which one of the carbons may be part of the aromatic ring (Davin et al., 2008).

Starch

In cereal grains, high concentrations of tightly compacted glucose polymers in the form of starch granules (amylose and amylopectin) are efficient energy sources for non-ruminant animals. On a DM basis, the starch content of corn, sorghum, and wheat are 620, 690, and 618 g/kg, respectively. Starch will typically be 10x lower in the distiller co-products of these cereal grains as the starch along with the fermentable carbohydrates are extracted to produce ethanol (Jaworski et al., 2015). Barley has a starch content of 587 g/kg on a DM basis. Oats have the lowest starch content of the cereal grains with 468 g/kg on a DM basis (Bach Knudsen, 1997).

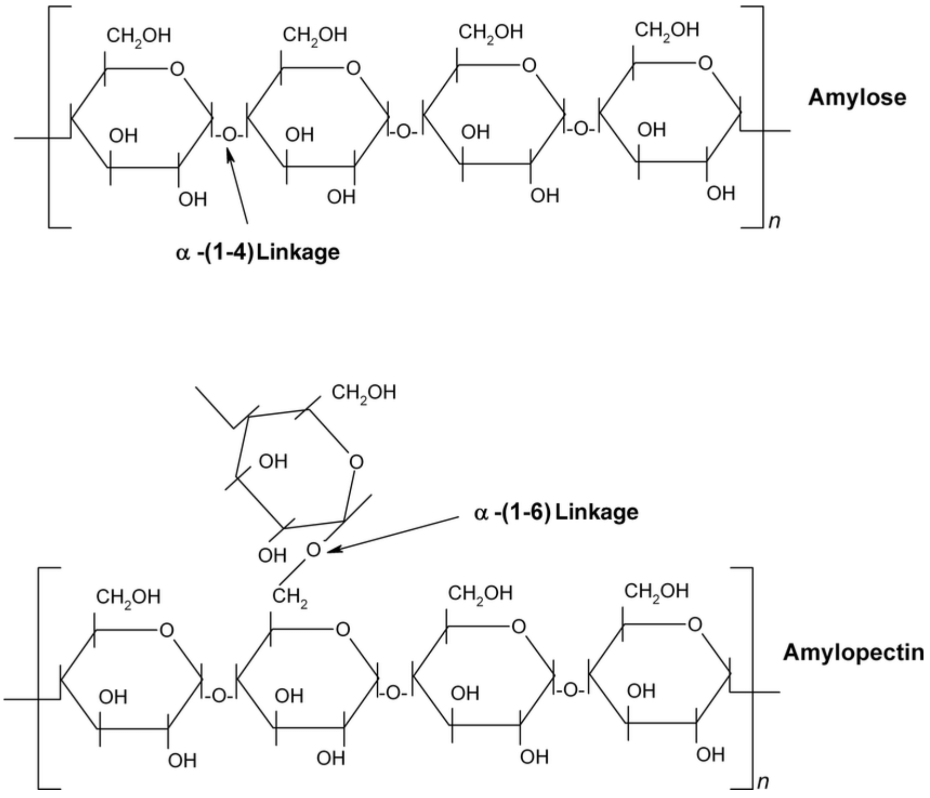

Figure 1-3. Structures of the starch granules, amylose and amylopectin. The α-1,4-linkages of amylose form a singular, uniform line of glucose monomers while the α-1,6-linkages cause branching to occur in amylopectin. While both starch granules are tightly packed to other granules, the branching at the α-1,6-bonds subject amylopectin to more susceptible degradation by endogenous amylase (Matthew et al., 2017).

The granules of amylose (linked by α-1,4 glycosidic bonds) and amylopectin (linked by α-1,6 glycosidic bonds) (Figure 1-3), however, need to be opened for digestion by endogenous amylase for adequate breakdown into smaller oligosaccharides and eventually individual glucose monomers (Cowieson, 2005). Salivary amylase initiates starch digestion in the mouth of swine while pancreatic amylase in both swine and poultry continues digestion of the starch polymers in the small intestine. Dextrins that are the products of starch digestion by amylase, are then hydrolyzed by brush border maltase and isomaltase into the glucose monomers that are capable of being absorbed by intestinal enterocytes (Moran, 1985).

Cellulose

Cellulose is a linear homopolymer comprised of glucose units ranging from 7,000 to 10,000 units that are linked by β-1,4-glycosidic bonds. The cellulose polymer forms a "ribbon like” twofold helix structure tightly packed together with neighboring cellulose units through non-covalent bonds with -OH groups to produce large bundles of macrofibrils (Bach Knudsen, 2014; Chocht, 2015; Figure 1-4). Cellulose is the most abundant organic compound on the planet and comprises 50% of all plant carbon. The largest accumulation of cellulose in plant material can found in the seedcoat (~30%), followed by 25% in the pericarp, 9-12% in the aleurone layer, with the endosperm containing < 1% (Bach Knudsen, 2014).

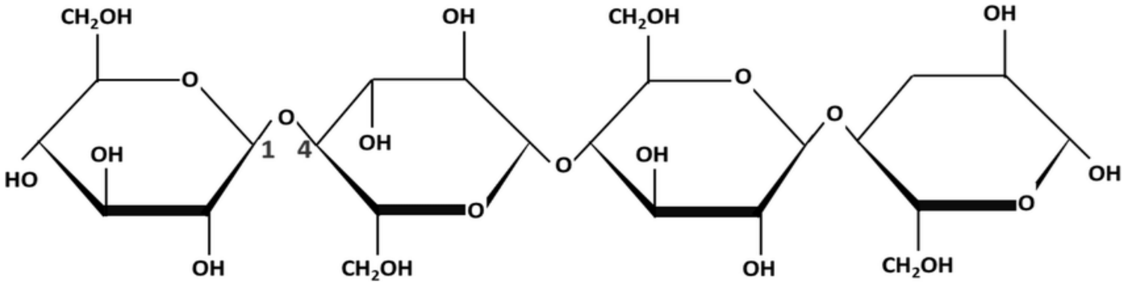

Figure 1-4. Molecular structure of cellulose. Individual cellulose chains lie side by side in bundles, held together by hydrogen bonds between the numerous neighboring -OH groups, to form a "ribbonlike" twofold helix (Choct, 1997).

Cellulose is water insoluble due to heavy rigidness resulting from increased hydrogen-bonding that creates a crystalline structure and is unable to be hydrolyzed by microbial cellulase and can bypass hindgut microbial fermentation. The less rigid amorphic structure of cellulose can be subjected to enzymatic hydrolysis and microbial fermentation (Ciaolacu et al., 2011), but can also reduce the digestibility of energy and nutrients that could potentially be utilized by the animal (Patience et al., 2022). A study by Owusu-Asideu et al. (2006) found the ingestion of purified cellulose by grower pigs resulted in a reduced passage rate by 0.30 %/h at the terminal ileum and lower ileal digestibility of protein and energy by 20 and 22 percentage units, respectively. This ultimately decreased ATTD, plasma glucose concentrations, and poorer growth performance. Interestingly, the intake of cellulose by gestating sows positively correlates with the digestibility of energy (Wishbech et al., 2023).

Arabinoxylan and the Pentosans

In terms of the non-cellulosic polysaccharides (aka the hemicelluloses), arabinoxylan is a xylan composed of the first and second most abundant pentosans, xylose and arabinose. The backbone of arabinoxylan is comprised of xylose (β-1,4-D-xylopyranosyl) residues linked by β-1,4-glycosidic bonds with side chains composed prominently of arabinose (α-l-arabinofuranosyl) residues (Bach Knudsen, 2014; Figure 1-5). Arabinoxylan is predominant NSP in corn (48.6% of total NSP), wheat (61% of total NSP), sorghum (45% of total NSP) and their respective co-products (Jaworski et al., 2015).

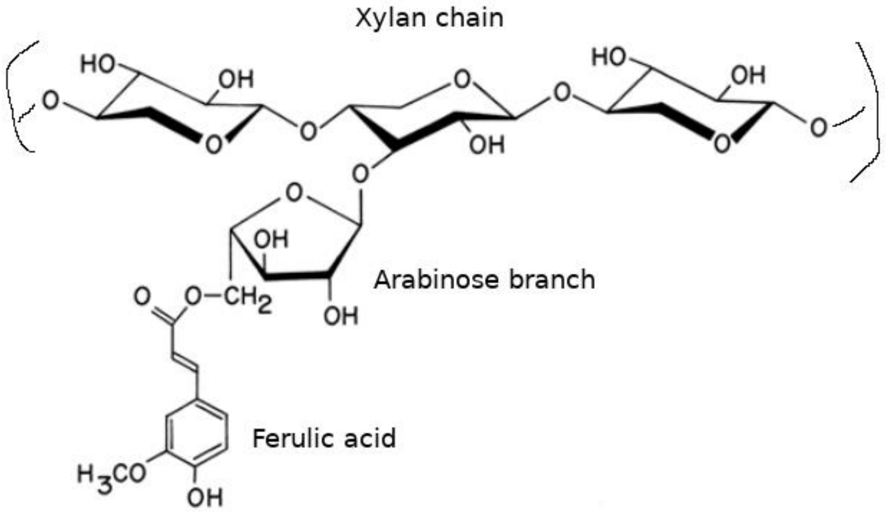

Figure 1-5. Schematic design of arabinoxylan (Choct, 2015).

The presence of oxidative agents can promote free-bound arabinoxylans to produce a highly viscid solution with the capacity to absorb 10x the amount of water (Geissmann and Neukom 1973). Arabinoxylan also has the capability to become insoluble and less fermentable depending on the number and composition of arabinose side chains. The solubility and fermentability of various cereal grains can be predicted based on the arabinose: xylose ratio (A/X). The higher concentration of arabinose side chains relative to xylose are believed to be soluble and fermentable. As the concentration of arabinose side chains decrease (resulting in a lower A/X ratio), the solution will become insoluble (Bach Knudsen, 2014). The distillation process of cereal grains as feedstocks to produce ethanol can cause a reduction of arabinose side chains and result in insoluble co-products (*i.e.*, corn DDGS) compared to the parent grain (Jaworski et al., 2015).

The arabinoxylan and xylose sugar residue (predominantly the insoluble fractions) content in corn and corn co-products strongly correlate with reduced ATTD of energy and the AID of both energy and AA (Gutirrez et al., 2016). The reduced digestibility of energy and AA caused by the insoluble arabinoxylans resulted in reduced growth and poorer growth efficiency in growing pigs (Newman et al., 2016).

β-Glucan

β-glucan shares a similar structure as cellulose where both contain linear homopolymers of d-glucopyranosyl (glucose) molecules. While the glucose monomers in cellulose are linked only by β-1,4-glycosidic bonds, the glucose monomers in β-glucan are linked by a mixture of β-1,4-glycosidic bonds with a β-1,3-glycosidic bond between every second or third glucose molecule (Bach Knudsen, 2014; Choct, 2015; Figure 6). The branching effect caused by the mixed linkages result in β-glucan being less compacted and more soluble compared to cellulose (Choct, 1997). Aside from plant material, β-glucan is a prominent NSP in the cell wall of yeast. The isolation of β-glucan from yeast is of interest due to the potential prebiotic effects through immunomodulation (Patterson et al., 2023). The glucose polymers of yeast soluble β-glucan, however, are linearly linked by β-1,3-glycosidic point with branching occurring at the β-1,6 position (Abuajah et al., 2013).

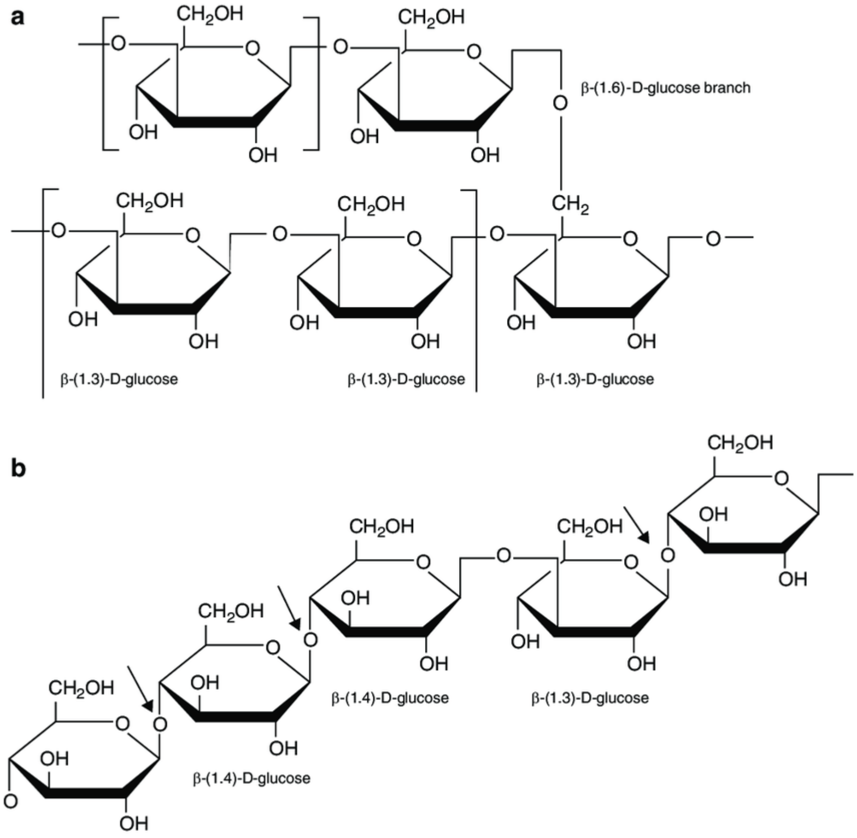

Figure 1-6. The structure of cereal β-glucan (B) in which linear homopolymers of glucose are -linked by β-1,4-glycosidic bonds with a β-1,3-glycosidic bond between the third and fourth d-glucopyranosyl molecule. This differs from β-glucan found in the cell wall of yeast (A) whose glucose polymers are linked by β-1,3-glycosidic bonds with a β-1,6-glycosidic bond between the third and fourth d-glucopyranosyl molecule to cause a branching effect (Abuajah et al., 2013).

Barley and oats contain a significant concentration of β-glucans with a range of 30 to 70 g/kg for oats and 50 to 110 g/kg in barley (DM basis; Skendi et al., 2003), but β-glucans can be found in all cereal grains (Choct, 2015). The cell wall of the endosperm contains the bulk of the β-glucan content with minor concentrations in the aleurone. β-glucan is typically part of a matrix that also contains starch, protein, and lipids (Brennan, 2005). The β-glucan in oat and barley is typically soluble and viscous, but the viscosity and solubility potential of β-glucan can be influenced by molecular weight, particle size, chemical structure and composition, temperature, pH, and the amount of water in contact with the β-glucan (Dikeman and Fahey, 2006).

Following the consumption of β-glucan, viscosity and thickening of digesta begins in the stomach. Protease activity in the stomach is hindered as the enzymes are not capable of penetrating the newly formed gelatinous substance (Schop et al., 2020). While increased water binding capacity brought on by the soluble properties of β-glucan does not occur until entering the distal half of the small intestine, the viscosity of the digesta continues to hinder endogenous enzyme activity on starch and protein digestion along with reduced digesta passage rate in the small intestine. This ultimately results in reduced ileal digestibility of energy and amino acids. Thus, in general, β-glucan reduces the energy content of diets (Hooda et al., 2011; Schop et al., 2020). Fermentation of β-glucan in the small intestine does increase the production of VFA (Hooda et al., 2011). Adult sows appear to have the capacity to ferment β-glucan in their small intestine due to a greater microbial population and can result in greater production of VFA from fermentation (Serena et al., 2008). Interestingly, feeding oat groats to weaned nursery pigs improved growth performance as β-glucan can promote gut health (Choi and Kim, 2023).

Pectins

Pectin is the most structurally complex polysaccharide and is the general name for the family of pectic polymers that include: homogalacturonan, rhamnogalacturonan I, and the substituted galacturonans rhamnogalacturonan II, and xylogalacturonan (Mohnen, 2008; Figure 7). The pectic polymers share a similar backbone of homogalacturonan, which are residues of D-galacturonic acid linked by α-1,4-glycosidic bonds (Mohnen, 2008: Bach Knudsen, 2014). The degrees of methyl and acetyl esterification will vary among the pectic polymers. The backbone of rhamnogalacturonan I contains rhamnose linked by α-1,2-linkages that alternate with the D-galacturonic residues. The side chains of rhamnose linked at the O-4 can be either arabinan, galactan, and arabinogalactans. Rhamnogalacturonan II structurally differs from rhamnogalacturonan I due to the presence of aldehydro- and keto-sugar oligosaccharides at the C-2 or C-3 positions of the D-galacturonic residues. D-xylose residues linked by β-1,3 glycosidic bonds to the homogalacturonan backbone comprise xylogalacturonan (Mohnen, 2008).

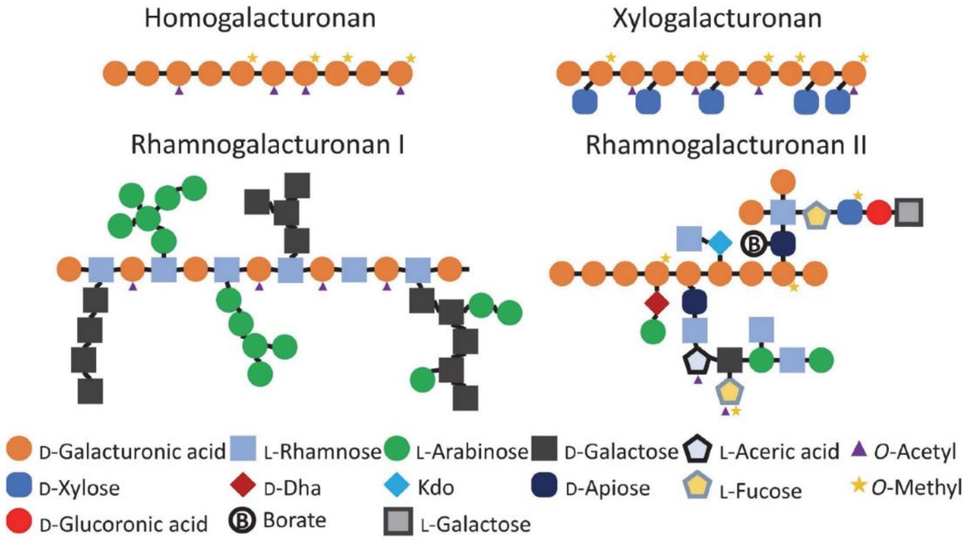

Figure 1-7. Schematic designs of the various pectic polymers (Bai and Gilbert, 2022).

Concentrations of the pectic polymers can be found in the stems and leaves of cereal grain and are minimal among the cereal grains and their co-products. Oilseeds, legumes, and lupines have greater pectin content compared with the cereal grains (Bach Knudsen, 1997; Choct, 1997). Soybeans, for example, have a significant concentration of xylogalacturonan in their cotyledon and reproductive tissues (Choct, 1997; Mohnen, 2008). Thus, the concentrations of pectic polymers will concentrate in the protein and fiber concentrates derived from oilseeds and legumes. Pectin is also the prominent NSP in sugar beet pulp (Bach Knudsen, 1997).

As with β-glucan, pectic polymers also contain viscous and soluble properties that hinder nutrient digestibility. Ingestion of the pectic polymers can result in detrimental effects on the processes of protein digestion and absorption but does not affect the secretion of pancreatic proteolytic enzymes in pigs (Mosenthin et al., 1992). The solubility of pectin allows pectin to be subjected to microbial fermentation in the hindgut and is a sufficient source for VFA. Methane production, however, will subsequently increase due to greater fermentation output from higher intake of pectic polymers (Müller and Kirchgessner, 1985a,b). This can also result in elevated fecal and bacterial N into the environment.

β-Mannan

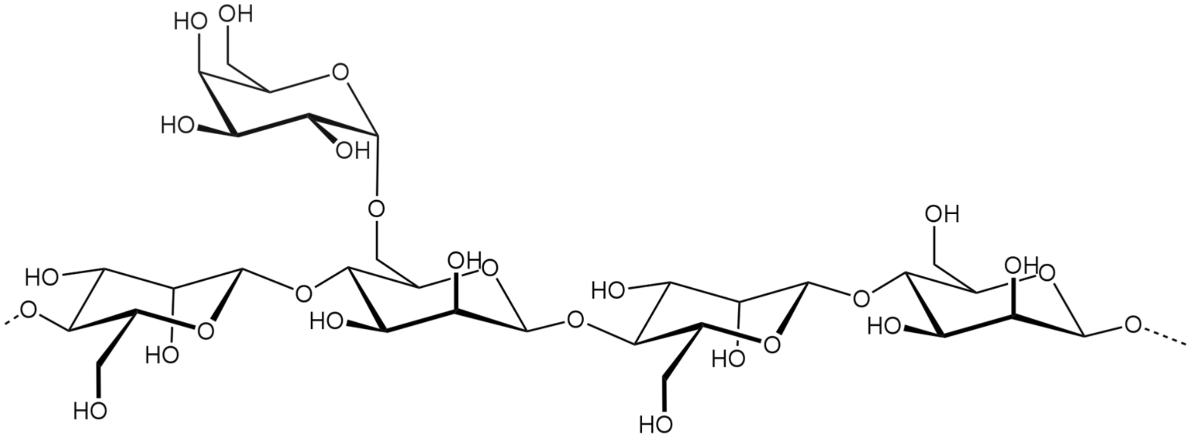

Figure 1-8. The backbone of β-mannan (Carpita and McCann, 2000).

β-mannan, also referred to as β-galactomannan, is a polysaccharide with repeating units of mannose, with galactose or glucose, or both, often found attached to the β-mannan backbone (Carpita and McCann, 2000, Figure 1-8). β-glucomannan, with some glucose replacing mannose in the backbone, also exists. The increase in the number of galactose molecules increases the water-solubility of β-mannan (Carpita and McCann, 2000). Trace amounts of β-mannan can be found in cereal grains, legumes and protein concentrates derived from legumes (*e.g.,* soybean and canola meal) (Bach Knudsen, 1997).

Functional Effects of β-glucan and Mannan oligosaccharide

Functional feeding can be defined as feeding products that contain compounds or live microorganisms that can enhance animal growth or health (de Lange et al., 2010). Yeast carbohydrates are enzyme-treated hydrolysis products (predominantly β-glucan and mannan polysaccharides) lysed from the cell wall of yeast that have been linked to immune modulation through prebiotic effects in livestock feeding (Patterson et al., 2023). Yeast products have been demonstrated to enhance swine performance as both a probiotic or prebiotic with varied outcomes (Soltanian et al., 2009). In human macrophages β-glucan, acting as an immunomodulator, increased synthesis of proinflammatory cytokines (Poutsiaka et al., 1993). The production of cytokines, however, can be suppressed at higher concentrations of β-glucan (Hoffman et al., 1996).

The mannan-oligosaccharides (**MOS**) are short chains of mannose found in relatively high concentrations in yeast cell walls. These compounds have a high affinity for specific sites on certain pathogenic bacteria, which prevents their attachment to the intestinal epithelium. As a result, the potential pathogens flow out of the intestine, and beneficial microorganisms (*e.g., lactobacilli*) are given the opportunity to attach and colonize. This process is commonly called “competitive exclusion” (Spring et al., 2000). Podzorski et al. (1990) demonstrated the presence of MOS in *in vitro* cultures inhibited the proliferation of lymphocytes. A suppression in immune activity is beneficial for animal growth as nutrients that would have been used for immune function are diverted towards bodily gain (Spurlock, 1997).

Supplementation of yeast products comprised of β-glucan and MOS in sow diets has been linked to essential functions related to immune modulation in both sows and piglets, positively impacting their health and performance (Duan et al., 2016; Hasan et al., 2018). The epitheliochorial porcine placenta prevents the transfer of maternal immunoglobulins to the developing fetal pig. Thus, neonatal pigs are born with virtually no circulating humoral immunity and must rely heavily on passive immunity attained from the sow to build a robust immune system needed for survival (Rooke et al., 2003). The predominant immunoglobulin in colostrum is IgG and originates solely from the humoral immune system of the dam, but it declines within the 24 h following the onset of parturition. Immunoglobulin A becomes the main immunoglobulin found in transient (36 – 72 h postpartum) and in mature milk. However, approximately 10-17% of circulating IgA found in the serum of sows contributes to the IgA found in transient and mature milk (Bourne and Curtis, 1973; Theil et al., 2014).

Supplementation of MOS in sow diets increased the colostral IgG and IgA content compared to the colostral composition of sows not supplemented (Czech et al., 2010). Additionally, elevated levels of serum IgG in offspring at birth and weaning were observed when sows were fed supplemental MOS. Hasan et al. (2018) reported reduced farrowing duration and birthing interval in yeast-supplemented sows with increased number of pigs born alive.

Analytical Measurements of Dietary Fiber and Non-Starch Polysaccharides

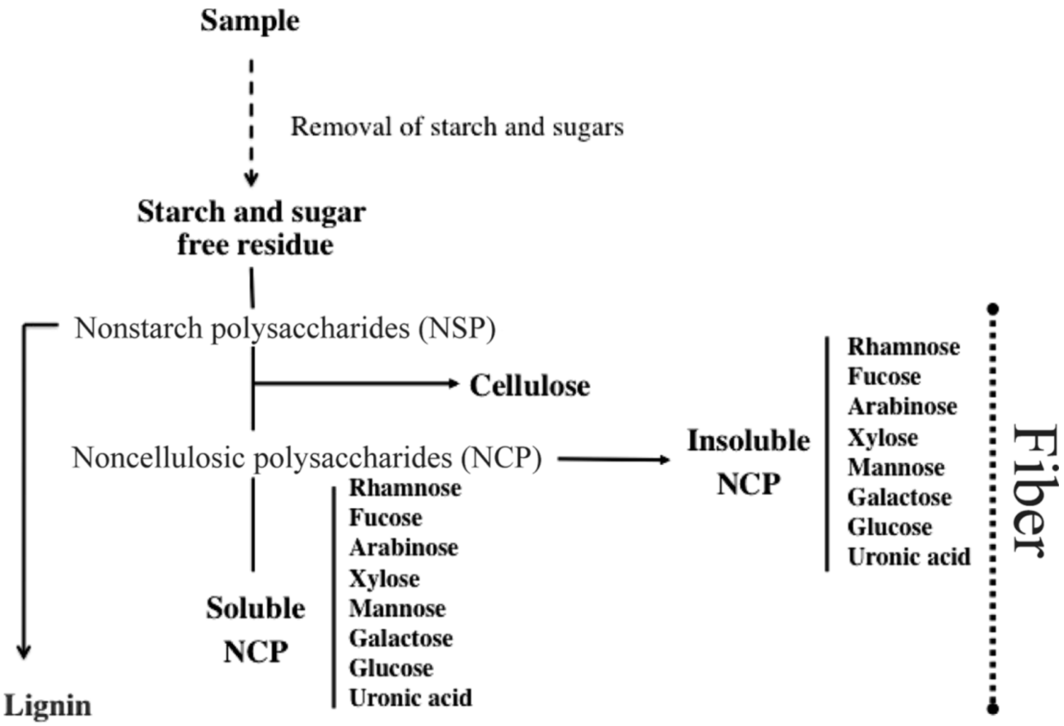

Figure 1-9. Schematic design for the determination of NSP, lignin, and fiber by enzymatic-chemical procedure (from Bach Knudsen, 2014).

A broad variety of analytical methods have been used for the analysis of fiber in feeds. The detergent methods were developed by Van Soest and colleagues (Van Soest, 1963) at Cornell University and were originally developed for the analysis of fiber rich feedstuffs (roughages) but later applied to concentrated feeds. These methods measured the fraction of fiber that is insoluble in neutral detergents (NDF) (hemicellulose, cellulose and lignin) and insoluble in acid detergents (ADF) (cellulose and lignin), allowing estimation of hemicellulose by difference. The flaws of the detergent methods include: (1) underestimation of TDF because pectin and other components are soluble in neutral detergents and are not recovered, (2) contamination of starch and protein residues leftover in NDF of starchy grains, and (3) concentrations of hemicellulose contaminating ADF (Bach Knudsen, 2001).

More recently, the enzymatic-gravimetric AOAC (Association of Official Analytical Chemists) procedures (Prosky et al., 1985) and the enzymatic-chemical Englyst (Englyst et al., 1994) and Uppsala procedures (Theander et al., 1994) for the analysis of fiber (TDF) have been developed. These latter methods were originally developed for foods but have also been used to analyze feedstuffs (Theander et al., 1989; Bach Knudsen, 1997).

In the enzymatic-gravimetric method, starch and protein are removed with the use of enzymes, then precipitation of soluble dietary fiber by aqueous ethanol, isolation, and the weighing of residue. The final step is correction of protein and ash in the residue. The method was modified for insoluble and soluble fiber and simplified using 4 morpholine-ethanesulfonic acid-TRIS buffer (method 991.43; AOAC, 2006). This method involves enzymatic removal of starch, extraction of low molecular weight sugars, and acid hydrolysis of dietary fiber polysaccharides and determination of their monosaccharide residues by gas liquid chromatography, high performance liquid chromatography or calorimetry. Information concerning polymers making up the NCP fraction can be gained from the monomeric sugar residues (*i.e.,* NCP arabinose and xylose are markers for AX and NCP glucose for mixed linkage β-glucan in cereals, uronic acids for the backbone in pectin, and arabinose, galactose, and other markers of the side groups in pectic polysaccharides) (Bach Knudsen, 2014).

The physiochemical properties of NSP and consequential actions resulting from the ingestion NSP have been well studied grow-finishing pigs. There is a lesser amount of studies evaluating the effects of dietary NSP gestating sows; who have a greater digestive and fermentative capacity compared to their growing counterparts.

NUTRIENT AND ENERGY VALUES OF FEEDSTUFFS

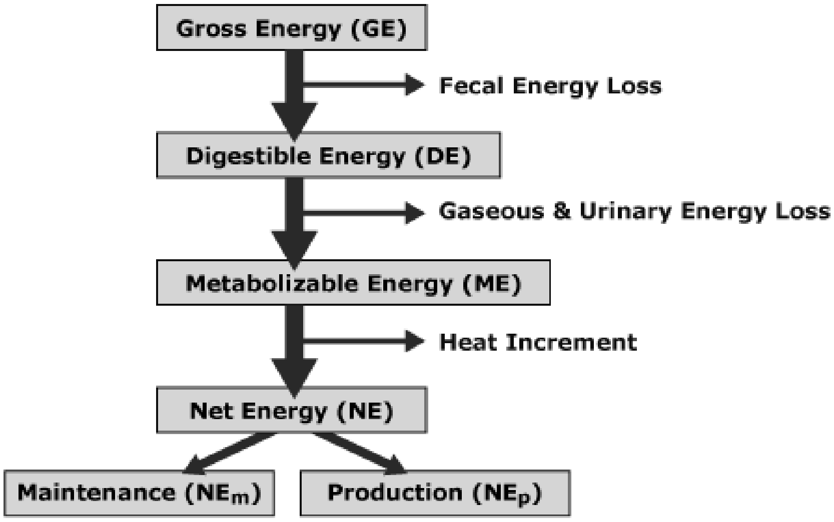

Figure 1-10. Partitioning of dietary energy in swine (NRC, 2012).

Dietary energy can represent > 60% of the total cost of swine production. All dietary energy that is ingested by the animal is not digested or utilized. Thus, the digestible (**DE**), metabolizable (**ME**), and net (**NE**) energy systems were developed to accurately characterize dietary energy content (Figure 1-10) (Noblet and van Milgen, 2013; Kil et al., 2013).

Gross Energy

Gross energy (**GE**) represents the total quantity of energy in feed that is able to be oxidized (Noblet and van Milgen, 2013) and is determined by complete combustion of organic materials through a bomb calorimeter. The ratio of oxygen and hydrogen to carbon present in the feed relates to the degree of oxidation potential of the organic compounds (Noblet and van Milgen, 2013; Kil et al., 2013).

Prediction equations can be utilized to estimate the GE of feed in instances where a bomb calorimeter is not available. The prediction equations rely on the crude protein

(**CP**), ether extract/fat (**EE**), crude fiber (**CF**) and ash content of the feed (on a dry matter basis) (Noblet and van Milgen, 2013):

$$\mathrm{GE} = 17.3 + 0.0617\,\mathrm{CP} + 0.2193\,\mathrm{EE} + 0.0387\,\mathrm{CF} - 0.1867\,\mathrm{ash}$$

Digestible Energy (DE)

The GE content of the diet minus the energy content excreted in feces is defined as DE. It is assumed that the excreted energy content contains organic compounds that have been undigested and that digested compounds have been absorbed and utilized (de Lange and Birkett, 2005). A simplified equation for DE (Adeola, 2001) is:

$$\mathrm{DE} = 100 \times \frac{\mathrm{GE\ intake} - \mathrm{GE\ output}}{\mathrm{GE\ intake}}$$

The DE of a corn-soybean diet fed to grow-finishing pigs is approximately 83% of dietary GE intake, but DE of the diet GE can range between 70 – 90% depending on the feed ingredient of the diet (Kil et al., 2013). The variation in DE relative to GE is dependent on DF levels (Noblet and van Milgen, 2013).

The DE content of a diet (kcal/kg DM or as-fed) can be determined based on the methodology by Adeola (2001). It is recommended that grower pigs be fed 3.5x maintenance energy requirements, or 4% of their body weight, in energy digestibility studies to mimic ab *libitum* feeding in commercial practice (Adeola, 2001). Traditionally, total feces excreted over a five-day period are collected and weighed with a subsample dried and ground for analysis in a bomb calorimeter. The energy content of feces is then calculated relative to diet GE intake to attain a representative DE of the diet.

Thorough and consistent record keeping is required for the total collection method (Kong and Adeola, 2014). An indigestible marker may be included at 0.1-0.5% in the diet for partial fecal collection when total fecal collection is not feasible. Quantification of the indigestible marker in both feed and feces is required in addition to energy determination. Titanium dioxide, chromic oxide, and acid-insoluble ash are commonly used indigestible markers as they: (1) are indigestible or nonabsorbable, (2) nontoxic, (3) relatively uniform in passage in digesta, and (4) easy to quantify (Zhang and Adeola, 2017). The digestibility calculation of energy (DE%) can be calculated as:

$$\mathrm{DE, \%} = 100 - \left[100 \times \left(\frac{\mathrm{IM_{feed}} \times \mathrm{E_{feces}}}{\mathrm{IM_{feces}} \times \mathrm{E_{feed}}}\right)\right]$$

Where $\mathrm{IM_{feed}}$ and $\mathrm{IM_{feces}}$ represent concentrations of the indigestible marker in the feed and feces, respectively; $\mathrm{E_{feed}}$ and $\mathrm{E_{feces}}$ represent concentrations of GE in feed and feces, respectively (Adeola, 2001).

The choice of ingestible marker also needs to be carefully considered as recovery in fecal content between the indigestible markers varies and can impact the outcome of digestibility values (Zhang and Adeola, 2017). In pigs, the recovery and quantification of titanium is greater and less variable compared to chromium (Jagger et al., 1992). Acid-insoluble ash is not recommended as a suitable candidate when feeding diets or feedstuffs containing high DF content due to reduced recovery compared to chromium (Fan and Sauer, 2002). Titanium dioxide appears to be the most consistent indigestible marker whose recovery is not impacted by DF content (Wang et al., 2016).

The two following prediction equations from the NRC (2012; Eq. 1-2 and 1-3; based on Noblet and Perez, 1993) may be utilized to estimate DE (kcal/kg of DM) based on the ash, CP, EE, and neutral-detergent fiber (**NDF**) dietary composition if feces are not able to be collected or in the absence of a bomb calorimeter.

$$\mathrm{DE} = 1{,}161 + (0.749 \times \mathrm{GE}) - (4.3 \times \mathrm{Ash}) - (4.1 \times \mathrm{NDF})$$

$$\mathrm{DE} = 4{,}168 - (9.1 \times \mathrm{Ash}) + (1.9 \times \mathrm{CP}) + (3.9 \times \mathrm{EE}) - (3.6 \times \mathrm{NDF})$$

Factors Affecting Differences in DE Between Gestating Sows and Growing Pigs

Increased intake of DF results in a decrease in DE and the coefficient of digestibility of energy. Aside from DF not being digested by the animal, DF also reduces the digestion of innate dietary starch (polymers of glucose), protein, and lipids that could be used for energy purposes (Noblet and van Milgen, 2004).

An increase in BW as the animal matures results in an increase in the DE and coefficient of DE. The increased coefficient of DE in heavier and older animals is due to greater fermentation capacity in the hindgut as well as slower passage rate. Organ growth in swine follows a sigmoidal curve with respect to time because there is a close relationship between time and BW (Bridges et al., 1988; Brunsgaard, 1997). Consequently, the intestinal tract of an adult sow is larger and more developed than that of a growing pig (Bridges et al., 1986). Adult sows also have a greater intestinal volume (Dierick et al., 1989) and 7x greater concentration of cellulolytic bacteria than growing pigs, indicating a correlation between increased microbial mass and increased intestinal volume (Varel, 1987).

A significant increase in flow of nutrients or rate of passage through the intestinal tract follows a higher intake of DF (Serena et al., 2008). As feed intake is increased, rate of passage increases, and absorption of nutrients is decreased (Cunningham et al., 1962). This indicates that it is possible to overwhelm or decrease the absorptive capacity of pigs by increasing feed intake.

The major feed management difference between the two ages of pigs is restricted feeding for gestating sows and ab *libitum* feeding in grower pigs (McGlone and Pond, 2003). Serotonin, in response to the stretching of esophageal smooth muscle by a food bolus, is secreted to sensory neurons lining the smooth muscles of the GI tract to stimulate the peristaltic propulsion of the digesta through the GI tract (Gershon, 2013). The mean retention time of feed increases along the gastrointestinal tract averaging 1 h from mouth to proximal duodenum, 4 h from the proximal duodenum to the distal ileum, and 35 h from the distal ileum to fecal excretion (Wilfart et al., 2007). Adult animals have reduced feed intake per unit of BW, a slower digestive transit, and, because of their size, greater intestinal volume (Dierick et al., 1989). Casa and Stein (2018) reported that gestating sows fed the same intake as growing gilts at 3.5x their ME requirement for maintenance had 2% greater DE and ME of corn-soybean meal diets. This suggests that digestion is related more to physiological stage or BW than feed intake (Casa and Stein, 2018). Therefore, adult sows have higher DE values than grower pigs.

The greater digestibility in gestating sows to that in grower pigs is particularly pronounced in high fiber ingredients in which DF contributes to energy content in the form of VFA following microbial fermentation (Noblet and van Milgen, 2004). The ATTD of organic matter, CP, and GE is 3, 6, and 4% greater, respectively, in adult sows compared to growing pigs, but the DF (NDF in particular) and content of other nutrients might influence the degree of difference in digestibility between adult sows fed at maintenance and growing pigs fed ab *libitum* (Le Goff and Noblet, 2001). The DE (on a DM basis) of cereal grains (such as corn, wheat, and sorghum, which are low in both protein and NDF) on average is 121 kcal/kg or 5% more for gestating sows than growing pig. The ATTD of GE is also on average 3% greater in gestating sows fed at maintenance in comparison to that of grower pigs (Shi and Noblet, 1993; Lowell et al., 2015; Dong et al., 2020).

Metabolizable Energy (ME)

Metabolizable energy is the energy remaining after urinary energy and gaseous energy (in the form of combustible methane [$\mathbf{CH_4}$] and hydrogen [$\mathbf{H_2}$]) are subtracted from DE (Fig. 1). Because gaseous energy losses are very small in pigs, they are typically ignored in the calculation. In adult sows, where the hindgut is more developed, gas production (primarily methane) can be 4x – 5x greater due to greater fermentative capacity. Increasing fermentable fiber content can also result in greater gas loss (Rijnen et al., 2001; Noblet and van Milgen, 2013). Urinary energy is energy [primarily from the urine nitrogen content (Nuri)] that was digested and not retained by the animal for protein synthesis (Noblet and van Milgen, 2013).

$$\mathrm{ME} = 100 \times \frac{\text{GE intake} - \text{GE output} - \text{GE urine}}{\text{GE intake}}$$

The quantification of ME requires the measurement of energy digestibility (as described above) in addition to quantification of energy loss via urine (Noblet and Van Milgen, 2013). Metabolism crates fitted with a screen floor and a urine tray below suffices to capture total daily urine production, but few research facilities possess metabolism crate large enough to house adult sows (Lowell et al., 2015; Dong et al., 2020; Wang et al., 2022). Two alternative total collections for quantifying urine are with the use of metabolic crates or insertion of urinary ballon catheters into the bladders and attaching to a container. Urinary catheterization is becoming a commonly applied technique in sows as most research facilities do not have adequately sized metabolic cages for adult sows. If total collection is not a possibility, constant infusion of a marker (para-amino-hippuric acid) into blood that is then excreted immediately in urine provides an estimate quantification of hourly urine production (Theil et al., 2020). A concern for housing animals in metabolism crates with screen floors for urine collection is the potential for water from nipple drinkers leaking into the urine tray if the animal is excessively playing with the nipple drinker that can cause an inaccurate volume of urine (Zhang and Adeola, 2017).

Urine production and collection determine urinary energy excretion. As urine N is the main energy yield component excreted in urine, N-balance to measure N-retention can be calculated to estimate urinary energy (Theil et al., 2020). Urine samples can be freeze-dried, and energy analyzed on a bomb calorimeter similar to feed and fecal energy (Kim et al., 2009).

As stated previously, energy loss due to gaseous production can be significantly greater in adult sows compared to grower pigs when fed fibrous ingredients. Quantification of the production of combustible CH_4 and H_2 requires animals to be housed in respiration chambers (Theil et al., 2020). Recent studies that compared energy digestibility between gestating sows and grower pigs ignore gas production (Noblet and Shi, 1993; Lowell et al., 2015; Casa and Stein, 2018; Dong et al., 2020). Urinary energy depends heavily on urine excretion of N, and the metabolic efficiency of DE (ME: DE ratio) linearly correlates with CP content of the diet (Noblet and van Milgen, 2004). The AA balance and protein will influence the protein retained by the animal and amount converted to urinary N (NRC, 2012). Adult sows, however, excrete a higher proportion of urinary N compared to grower pigs (Le Goff and Noblet, 2001). Thus, the ME: DE ratio will be lower for adult sows than grower pigs.

The two following prediction equations from the NRC (2012; Eq. 1-5 and 1-6; based on Noblet and Perez, 1993) may be utilized to estimate DE (kcal/kg of DM) based on the ash, CP, EE, and NDF dietary composition if fecal or urine samples are not able to be collected or in the absence of a bomb calorimeter.

$$\mathrm{ME} = 4{,}194 - (9.2 \times \mathrm{Ash}) + (1.0 \times \mathrm{CP}) + (4.1 \times \mathrm{EE}) - (3.5 \times \mathrm{NDF})$$

$$\mathrm{ME} = (1.00 \times \mathrm{DE}) - (0.68 \times \mathrm{CP})$$

Net Energy (NE)

The NE content of feed is defined as ME minus heat increment which is associated with energy cost of metabolic utilization, ingestion, digestion, and some physical activities (Noblet and van Milgen, 2004). The NE system was originally developed to account for the energy efficiency of retention in growing pigs by considering heat production resulting from maintenance. Maintenance energy requirements are those needed to perform basic physiological function (*e.g.*, blood flows, respiration, muscle tone, ion balance, immune responses tissue response, and homeostasis) for the animal while the animal is under minimal physical activity (Kil et al., 2013). This is important for growing pigs as their energy utilization for maintenance will be prioritized over production which can be referred to as growth of protein and lipid tissue (Theil et al., 2020). Regarding the ingestion and digestion of nutrients, increased heat production can result from the digestion of lesser digestible components (DF and protein).

The energy for production in gestating sows predominantly corresponds to growth of the fetus, placenta, uterus, and mammary tissue, which only undergo considerable growth during the last trimester. In this regard, maintenance energy is prioritized such as seen in the grower pig but can be influenced by metabolic processes associated with compositional gain of these reproductive tissues (Noblet et al., 1990). The priority for maintenance requirements (primarily associated with thermoregulation and activity) become minimal compared to the high metabolic stress of milk production which can result in the sow depleting her energy reserves (fat tissues and muscle) to nurse her offspring (Theil et al., 2020).

Few studies have determined the NE value of feedstuffs for reproductive sows due to the complexity of the methodology to determine NE (Theil et al., 2020). The two methodologies applied to determine body energy retention and total heat production to calculate NE for pigs are comparative slaughter and indirect calorimetry. Comparative slaughter is the preferred method because it considers body compositional gain while housing animals under practical conditions (which simulate thermal conditions and physical activity). The disadvantage to the comparative slaughter method is predicting energy retention (*i.e.,* whole body energy gain) based off the pigs slaughtered at the initiation of the trial. Indirect calorimetry allows measurement of gaseous exchange during a shorter time period and can be completed in the same animal(s) multiple times but housing the animals in respiration chambers can influence thermoregulation by minimizing physical activity (Kil et al., 2013; Theil et al., 2020).

Due to the limitations of the methods to determine NE, prediction equations have been developed for feedstuffs for both growing and reproducing sows (Noblet et al., 1993; Noblet et al., 1994). The NRC (2012; Eq. 1-7 and 1-8) compiled Noblet and co-workers' prediction equations based on chemical composition of the ingredient:

$$\begin{aligned}\mathrm{NE} &= (0.7 \times \mathrm{DE}) + (1.61 \times \mathrm{EE}) \\ &\quad + (0.48 \times \mathrm{Starch}) - (0.91 \times \mathrm{CP}) - (0.87 \times \mathrm{ADF})\end{aligned}$$

$$\begin{aligned}\text{NE} = {} & (0.726 \times \text{ME}) + (1.33 \times \text{EE}) \\ & + (0.39 \times \text{Starch}) - (0.62 \times \text{CP}) - (0.83 \times \text{ADF})\end{aligned}$$

These equations for predicting NE value of complete diets and feedstuffs are not always accurate as the energy value of high-protein and high-fat are overestimated and underestimated on a DE and ME basis, respectively. The inaccuracy of the prediction can be seen when pigs are fed a low-protein diet formulated on a DE basis produce a higher carcass fat content as the DE system underestimates dietary energy content thus animals are ingesting a higher energy content (Noblet and van Milgen, 2004).

Impact of Dietary Fiber on Ileal Digestibility of Nutrients

Digestibility is one factor that defines the protein quality of a feedstuff (Nørgaard et al., 2020). The DF fraction is the largest component of the dietary intake that is undigested by endogenous enzymes secreted by the GI tract. As stated previously, increased consumption of DF can hinder digestion of the various macronutrients that can be utilized for energy to meet maintenance, growth, and reproduction demands by encapsulating the energy yielding macronutrients (*e.g.*, starch, protein, and lipids) (Theil et al., 2020). The ileal digestibility of protein and AA are key factors when formulating balanced diets because AA that are absorbed in the hindgut provide no value to metabolic processes nor contributes to meeting the animal’s essential AA requirements (Nørgaard et al., 2020). Microbial fermentation of DF will result in the production of microbial protein and is not representative of the ingested protein and AA (Stein et al., 2007). Ingestion of DF, in general, reduces the AID of both energy, CP, and AA (Owusu-Asiedu et al., 2006).

Amino acid bioavailability and ileal digestibility are two common *in vivo* procedures to evaluate the protein and AA quality of ingredients (Stein et al., 2007). Amino acid bioavailability represents the proportion of ingested AA that is rendered for absorption, metabolism, and synthesized into protein for deposition or other biological functions. The slope-ratio assay method is applied to determine the AA bioavailability using a slope regression with the AA or protein source being evaluated included in the diet to replace the basal diet at increasing rates. Variables of interests (*e.g.,* growth) are compared to a standard curve that is based on the basal diet with increasing levels of the first limiting nutrient (*e.g.,* Lys) typically from a source considered 100% bioavailable (*e.g.* crystalline AA). Bioavailability is defined as the slope of the test ingredient relative to the standard curve. Some limitations of the slope-ratio assay are: all diets need to contain the test AA at a level below the estimated test AA requirement to produce a linear response in the dependent variable and only being able to evaluate one AA at a time (Stein et al., 2007; Nørgaard et al., 2020).

Ileal digestibility quantifies the amount of AA that has been subjected to small intestine microbial fermentation, underwent enzyme hydrolysis, was digested, and absorbed by the time the digesta has reached the terminal ileum relative to AA that have been ingested or are of endogenous origin (Stein et al., 2007). This method allows for multiple AA to be evaluated at once (Norgaard et al., 2020). Ileal digestibility of AA can be measured either using the slaughter method (total collection) or by using cannulated pigs (used for random sampling with the use of an indigestible marker). Apparent ileal digestibility is the ileal AA outflow in relation to ingested AA and AA of endogenous origin. Standardized ileal digestibility (**SID**) measures the difference between AID and basal ileal endogenous AA losses (IAA_{end}; protein synthesized endogenously and secreted into the gut lumen that have been digested and reabsorbed before reaching the distal ileum). Basal IAA_{end} are independent of the diet but can reduce as the intake of DM increases (Stein et al., 1999). Specific IAA_{end} are diet dependent and can be altered based on the composition of the diet (Stein et al., 2007). For example, a high intake of DF can program cell death of intestinal epithelia and can result in an increase of specific IAA_{end}. The differences between AID and specific IAA_{end} is referred to as the true ileal digestibility, but the SID of AA is typically applied for diet formulation due to limitations of determining specific IAA_{end}. Endogenous basal losses can be determined by feeding a N-free or protein-free diet (Stein et al., 2007).

Apparent ileal digestibility values are calculated from the difference between the dietary intake of nutrients and the composition of nutrients in the digesta present in the distal ileum of the sows according to the equation (Stein et al., 2007):

$$\mathrm{AID},\% \;= \left[1 - \left(\frac{\mathrm{C_{dig}} \times \mathrm{Ti_d}}{\mathrm{C_d} \times \mathrm{Ti_{dig}}}\right)\right] \times\ 100$$

where C_{dig} = concentration of the component in digesta; C_d = concentration of the component in the diet; Ti_d = concentration of titanium in the diet and Ti_{dig} = concentration of titanium in digesta (Stein et al., 2007).

Standardized ileal digestibility values of AA and CP are then calculated by the difference between the amount of AA and CP ingested, the amount of AA from the digesta in the ileum, and the endogenous loss of AA, according to the equation (Stein et al., 2007):

$$\text{Basal AA}_{\text{end}} = \text{AA}_{\text{dig}} \times \frac{\text{Ti}_{\text{d}}}{\text{Ti}_{\text{dig}}}$$

$$\text{SID, \%} = \left\{\frac{[\text{AA intake} - (\text{ileal AA outflow} - \text{basal AA}_{\text{end}})]}{\text{AA intake}}\right\} \times 100$$

The use of T-cannulas for digesta collection is a limitation as surgical modification of animals is required. There have been few studies determining nutrient digestibility using ileal cannulated adult sows (Stein et al., 2001; Serena et al., 2008; Jørgensen et al., 2008; Velayudhan et al., 2019). The SID of CP and AA on average are 10% greater in gestating sows fed at maintenance compared to ab *libitum* growing pigs and lactating sows (Stein et al., 2001). The ingestion of DF can reduce the ileal digestibility of AA and GE in adult sows (Serena et al., 2008), but the microbes in the more developed small intestine of the adult sows can better digest the various fiber complexes by the end of the terminal ileum compared to growing pigs (Jørgensen et al., 2008).

The ingestion of DF and NSP can be an antinutritional factor by hindering the digestibility and limiting the nutrient availability of feedstuffs fed to growing pigs. Gestating sows, for various reasons, appear to have greater digestive capacity and ability to extract dietary energy from fibrous feedstuffs. Potential strategies to increase energy and nutrient availability of feedstuffs could potentially differ between grower pigs and gestating sows.

FIBER DEGRADING ENEZYMES

Overview and Application in Swine Diets

Exogenous feed enzymes are extensively utilized in modern livestock production to increase nutrient digestibility and feed efficiency of poor-quality feedstuffs (Torres-Pitarch et al., 2019). The feed enzyme market is worth approximately $1 billion USD with the two main enzymes groups being phytase and the class of fiber-degrading enzymes (which may be referred to as NSP-degrading enzymes, carbohydrases, NSPases, or as their individual components) (Vehmaanperä, 2022). Xylanase, β-glucanase, β -mannanase, α-galactosidase, cellulase, pectinase, and amylase are the common and most studied carbohydrases as a single activity or in combination (Torres-Pitarch et al., 2019). The three established philosophies regarding modes of action that explain the increase in nutrient digestibility and improved animal performance are: (1) reduction in viscosity (by acting on β-glucan and other viscous fibers) that would hinder endogenous enzyme activity, (2) depolymerization and breakdown of the cell wall of ingested plant material to release starch and protein that would have been excreted, and (3) cleavage of VFA precursors that will be fermented by gut microbiota and act as a prebiotic (Bedford, 2018).

Xylanase is one of the predominant fiber degrading enzymes and hydrolyzes the β-1,4-arabinoxylan-glycosidic bonds of arabinoxylan found in corn, wheat, and cereal DDGS based diets (Ndou et al., 2015). In grower pigs, xylanase supplemented into a high fiber corn-fiber based diets increased the DE and ME by 5 and 4%, respectively (Petry et al., 2020). Xylanase cleaves the various fiber complexes and dietary arabinoxylan components while in the small intestine, reducing the insoluble components (Petry et al., 2019), and resulting in increased AID of GE and CP (Moran et al., 2016), and greater average daily gain and improved feed efficiency (Ndou et al., 2015). Viscosity is not influenced with the supplementation of xylanase in corn-based diets, but solubility might be increased from the cleavage of the insoluble arabinoxylans and produce a greater water binding substance during transit through the GI tract (Stein, 2019; Petry et al., 2024). The improvement in nutrient digestibility and growth performance is most likely due to the release of encapsulated starch and protein that can be absorbed by the end of the ileum and be utilized for energy and protein deposition (Petry and Patience, 2020). Pigs fed wheat-based diets supplemented with xylanase showed improved feed efficiency and AID and ATTD of GE and CP (Ndou et al., 2015). The concentrations of arabinose and xylose component sugars also appeared in higher concentration in ileal samples collected from pigs fed diets containing wheat as the only AA source and supplemented with xylanase (Lærke et al., 2015), suggesting that the action of xylanase is occurring mostly in the small intestine and breaking down the cell wall arabinoxylan to release starch, lipids, and protein that would have escaped the animal. Rye, however, appears to not be impacted by xylanase inclusion (Lærke et al., 2015).

The similar structure between cellulose and β-glucan allows for the series of cellulases or β-glucanases to act on and cleave either polymer (Sadhu and Maiti, 2013). Cellulases and β-glucanases rose to prominence in the 1950’s due to their benefits with reducing the frequency of wet litter from poultry fed high viscous feedstuffs such as barley, rye, and some wheat varieties. A reduction in wet and sticky litter improved waste management in commercial poultry operations and increased growth performance of the birds due to better digestibility of a less glutinous digesta (Bedford and Schulze, 1998). The response of β-glucanase supplementation in barley-based diets for pigs is inconsistent. Li et al. (1996) reported that β-glucanase supplementation to a hulless barley–soybean meal diet increased the AID of GE, CP, and AA by approximately 6 percentage units. The supplementation of β -glucanase to barley diets to grower pigs, however, produced no effect on digestibility (Kong and Adeola, 2012).

Mannanases, also called β-mannanases, cleave the β-1,4-mannosyl or a-1,6-galactosyl to reduce the mannose and galactose side chains of mannan. The mode of action by β-mannanase results in a less viscid digesta that is susceptible to endogenous enzyme activity, inhibiting the colonization of pathogenic microbes in the GI tract, and suppressed immune responses to intact galactomannan (Lee and Brown, 2022). The ultimate outcome has shown to be improved growth performance of the animal. The ATTD of GE and NDF increased by 3-6% along with improved feed efficiency for grower pigs when fed corn-soybean meal based diets supplemented with β-mannanase at 0.02% of dietary intake (Lv et al., 2013). The extent of the response to β-mannanase might depend on ingredients. β-mannanase appears to increase the ileal digestibility of AA by 5% effectively and the ATTD of energy on average by 5% (Mok et al., 2013; Kim et al., 2017). While suppressed immune responses have been found in many trials, the primary action of β-mannanase, similar to that of the xylanase, has been breaking and releasing encapsulated nutrients (Lee and Brown, 2022).

The mode of action of α-galactosidases is to catalyze the hydrolysis of terminal, non-reducing α-D-galactose residues at the α-1,6-glycosidic bonds in α-D-galactosides, which is comprised of the raffinose family of oligosaccharides such as raffinose, verbascose, and stachyose (Kote et al., 2020). Inclusion of α-galactosidases has been researched extensively in soybean meal diets to reduce the antinutritional effects of raffinose, stachyose, and verbascose (Llama-Moya et al., 2022). α-Galactosidases supplemented to weaned pig diets increased the digestibility of energy on the total tract level by 3% and reduced the incidents of diarrhea while improving gut morphology (Shang et al., 2018). The fermentative capacity increases as pigs mature (Longland et al., 1994) and may limit the response of α-galactosidases as the older animal is capable of digesting and fermenting raffinose (Baucells et al., 2000; Pan et al., 2002).

Plants that contain significant concentrations of raffinose oligosaccharides also contain high concentrations of pectic polymers, thus pectinase might be employed in conjunction with α-galactosidases (Choct, 2010). Pectinase targets, depolymerizes, and hydrolyzes the various galactouronic units that comprise the pectic heteropolymers (Shrestha et al., 2021). The cleavage of pectin reduces its water-holding, viscosity, and turbidity (Shrestha et al., 2021). The little work that has investigated the response of pectinase supplementation in swine included pectinase as an enzyme mixture rather than pectinase as a single active component (Thacker and Haq, 2008; Hong et al., 2019). This is also the case in poultry where most studies investigated pectinase in combination with other enzymes (Lee and Brown, 2022).

Usage of Multienzyme Blends

Most enzyme sources used in animal feeds contain more than a single enzyme activity (Bedford and Classen, 1993) and most microorganisms used for enzyme production can produce multiple activities (Bhat and Hazlewood, 2001). For example, an enzyme isolated for xylanase activity has the potential to produce side activities, such as a mannanase, but the secondary activity is difficult to quantify relative to the primary activity (Sheehan, 2022). As all the NSP are present to some extent in the plant feedstuffs commonly fed to livestock, multienzyme blends (also referred to as enzyme cocktails or simply multienzymes) have been made and are comprised of a mixture of single isolated enzymes with known activities. In an *in vitro* degradation trial, increasing the complexity of an enzyme blend degraded on average 25% more of the total NSP in the cell walls of wheat, canola meal, and soybean meal in comparison to incubating a single component enzyme. The supplementation of a more complex enzyme blend in diets to fed to roosters not only saw increased digestibility of the NSP sugar component but also resulted in improved degradation of starch granules and reduced digesta viscosity leading to better animal performances (Meng et al., 2005).

Multienzyme blends have been shown to be effective in diets containing fibrous ingredients fed to weaned pigs (Omogbenigun et al., 2004), as well as grower and finishing pigs (Emiola et al., 2009; Velayudhan et al., 2015; Woyengo et al., 2018) for improving the apparent ATTD of nutrients and energy. In finisher diets containing 30% of wheat DDGS, a multienzyme blend supplementation resulted in a 3% increase in AID of Arg and 11% increase in AID of Thr when fed to 80.8 kg finisher pigs (Emiola et al., 2009).

Application of Exogenous Enzymes in Sow Diets

There are limited studies investigating the effect of carbohydrase enzymes, either alone or in a combination, in diets fed to gestating sows. Nevertheless, carbohydrases are routinely included in commercial gestating sow diets, with inclusion levels determined based on values obtained from grower pig studies (McGhee and Stein, 2021). There have been a fair number of investigations into enzyme supplementation during the lactation period as nutrient digestibility and ab *libitum* feeding is similar between growing pigs and lactating sows (Stein et al., 2001). de Souza et al. (2007) observed the supplementation of a single xylanase in corn-soybean meal diets fed to lactating sows produced a 2% greater ileal digestion of protein. Xylanase inclusion in lactation diets containing wheat, barley, and oats resulted in a greater ATTD of GE and total NSP by 2 and 5%, respectively (Zhou et al., 2018). Some of the performance parameters that have improved with enzyme supplementation include lower loss in BW or body condition and increased litter survival (Walsh et al., 2012, Zhou et al., 2018; Llamas-Moya et al., 2022).

The supplementation of xylanase in corn-soybean meal gestation diets had no influence on ileal CP digestibility (de Souza et al., 2007). Velayudhan et al. (2019) reported no effect of a carbohydrase blend at 0.1% inclusion (comprised of cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, protease, and phytase activities) on AID or SID of AA or CP when canola meal was fed to sows in mid- or late-gestation. The lack of an enzyme effect on ileal CP and AA digestibility in gestating sows may be attributed to feed restriction practices, which allow sows to have a greater SID of CP and AA than grower pigs, where ad-libitum feeding reduces digestibility coefficients (Stein et al., 2001).

The extent to which fiber-degrading enzymes increase dietary energy content in gestating sows might not be extrapolated based on data in grower pigs when considering the greater nutrient and energy digestibility of fibrous feedstuffs in gestating sows. Thus, the effects of enzyme supplementation for gestating sows need to be determined to be applied in commercial feeding.

RATIONALE AND OBJECTIVES

Rationale

Gestating sows fed at maintenance have demonstrated greater digestive capacity and energy utilization than ab *libitum* fed grower pigs. The extent of technologies to increase the nutrient value of poorly digested feedstuffs, such as fiber-degrading enzymes, might be minimal for gestating sows compared to grower pigs. As the NSP varies among commonly fed feedstuffs, the efficacy of enzymes on feedstuff that differ in NSP will differ when fed to gestating sows. Lastly, fibrous components, such as β-glucan and mannan, that are more digestible in gestating sows are shown to benefit both sow and litter performance. The optimal inclusion of these functional feed additives might differ between grower pigs and adult sows. The breeding herd is estimated to consume approximately 20% of the total feed required within the swine production system (Ball and Moehn, 2013). Factors that influence nutritional value of sow diets have the potential to impact economic viability of swine production systems and meet the genetic potential of the modern hyperprolific sow.

Specific Hypothesis

1. The efficacy of multienzyme supplementation in a high-fiber gestation diet will differ between gestating sows fed at maintenance and grower pigs fed ab *libitum*.
2. The optimal inclusion level of a multienzyme when supplemented in gestation will be lower than that reported for grower pig diets.
3. The extent of nutrient and energy digestibility by multienzyme supplementation, when fed to gestating sows, will differ between feedstuffs.

4. The fermentability of energy of fibrous biofuel co-products will differ when fed to gestating sows.
5. The optimal inclusion level of a YC comprised of β-glucan and mannan on sow and litter performance will be lower in sow diets than reported for grower pigs.

Objectives

The objective of Chapter 2 was to investigate the effect of multienzyme inclusion level on ATTD of energy and nutrients, as well as ileal digestibility of CP and AA in gestation diets differing in fiber levels to gestation sows. For comparison, growing pigs were fed the same higher fiber diets to directly compare ATTD values with the gestating sows. The objective of Chapter 3 was to determine ATTD of energy and fiber of post-MSC DDGS provided to gestating sows in comparison with other commonly used fiber sources. A simultaneous study was conducted with fecal inoculum collected from sows fed different fiber sources to characterize hindgut gas production following in-vitro fermentation. Lastly, Chapter 4 aimed to determine the efficacy and optimal inclusion level of a novel YC product in sow diets for improving sow performance and litter survivability through immune modulation.

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CHAPTER 2

EVALUATION OF MULTIENZYME SUPPLEMENTATION AND FIBER LEVELS ON NUTRIENT AND ENERGY DIGESTIBILITY OF DIETS FED TO GESTATING SOWS AND GROWING PIGS[1]

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[1] Published in *Journal of Animal Science* (2023) https://doi.org/10.1093/jas/skad375.

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LAY SUMMARY

Fiber-degrading enzymes have been extensively studied in growing pigs with minimal studies focusing on gestating sows; however, commercial gestating sow diets often contain more fiber than grower pig diets to stimulate the sensation of satiety without influencing weight gain. A challenge with dietary fiber is its hindrance on digestibility of nutrients. Supplementation of multienzyme blends increases nutrient digestibility of fibrous diets in grower pigs but there is little data characterizing the effects of fiber degrading enzymes in gestation diets for pregnant sows. In this study, inclusion of a multienzyme comprised of various carbohydrases and a protease in gestation diets increased apparent total tract digestibility of nutrients and energy for both gestating sows and growing pigs; however, digestibility of non-starch polysaccharides was only improved in growing pigs. Enzyme supplementation to gestating sow diets had limited impact on the ileal digestibility of nutrients, but ileal digestibility of amino acids and crude protein was reduced when gestating sows were fed diets higher in neutral detergent fiber. When formulating high-fiber diets for gestating sows and growing pigs using similar ingredients, it is critical to consider the differences in digestibility of fibrous components, particularly regarding ileal digestibility of amino acids.

TEASER TEXT

A multienzyme blend improved total tract digestibility of fiber and dietary energy content in gestating sows but the magnitude of effect was greater in low- than high-fiber diets; however, the effect on ileal digestibility was limited.

ABSTRACT

The objective was to investigate the effect of a multienzyme blend (**$\mathbf{ME}_{\mathbf{blend}}$**) and inclusion level on apparent total tract digestibility (**ATTD**) of energy and nutrients, as well as ileal digestibility of crude protein (**CP**) and amino acids (**AA**) in gestation diets with low (**LF**) or high dietary fiber (**HF**) fed to gestation sows. For comparison, growing pigs were fed the same HF diets to directly compare ATTD values with the gestating sows. In Exp.1, 45 gestating sows (parity 0 to 5; 187 ± 28 kg bodyweight; **BW**) were blocked by parity in a 2 x 3 factorial arrangement and fed 2.2 kg/d of the HF (17.5% neutral detergent fiber; **NDF**) or LF (13% NDF) diet and one of 3 levels of ME_{blend} (0.0, 0.08, and 0.1%) to determine impacts of ME_{blend} on ATTD. Twenty-seven growing pigs (initial 35.7 ± 3.32 kg BW) were fed the same HF diet (5% of BW) and one of 3 ME_{blend} inclusions. The ME_{blend} at both 0.08% and 0.1% increased ATTD of energy, NDF, and acid detergent fiber (ADF) ($P < 0.05$) in gestating sows but ATTD of total non-starch polysaccharides (**NSP**) and its residues were not affected. Sows fed HF, regardless of ME_{blend}, had greater ATTD of NDF, xylose, and total NSP ($P < 0.05$) in comparison to grower pigs. In Exp. 2, ileal cannulas were placed in 12 gestating sows (parity 0 to 2; BW 159 ± 12 kg) to determine apparent and standardized ileal digestibility (**AID** and **SID**) of AA and NSP. In a cross-over design, sows were fed the same six diets, as in Exp. 1, and a nitrogen-free diet during 5 periods of 7 days each to achieve 8 replicates per diet. There was no interaction between diet fiber level and ME_{blend} inclusion. Supplementation of ME_{blend} to gestating sow diets did not impact SID of CP and AA regardless of dietary fiber level. The SID of His, Ile, Lys, Phe, Thr, Trp, and Val were 3 to 6% lower ($P < 0.09$) in HF than LF independent of ME_{blend}. Supplementation of ME_{blend} did not impact AID of NSP components, but sows fed HF had higher AID of arabinose (LF: 26.5% vs.

HF: 40.6%), xylose (LF: 3.5% vs. HF: 40.9%), and total NSP (LF: 25.9% vs. HF: 40.0%) compared to sows fed LF ($P < 0.05$). Dietary supplementation of ME_{blend} increased ATTD of nutrients, NSP, and energy in diets fed to gestating sows regardless of inclusion level, with ME_{blend} having a greater incremental increase in diets with lower NDF levels. Inclusion of ME_{blend} impacted neither SID of AA nor AID of NSP in low- or high-fiber gestation diets, but high-fiber diet, negatively affected SID of AA.

KEY WORDS

Fiber, gestating sow, grower pig, multienzyme, non-starch polysaccharides, nutrient digestibility.

LIST OF ABBREVIATIONS

AA, amino acids; ADF, acid detergent fiber; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; BW; body weight; CF, crude fiber; CP, crude protein; DDGS, dried-distillers grains with solubles; DE, digestible energy; DM, dry matter; EE, ether extract; GE, gross energy; HF, high fiber; LF, low fiber; ME_{blend}, multienzyme blend; ME, metabolizable energy; NDF, neutral detergent fiber; NE, net energy; NSP, non-starch polysaccharides; SID, standardized ileal digestibility.

INTRODUCTION

Breeding sows are fed a restricted diet during gestation to prevent extreme weight gain during pregnancy, which can result in issues in farrowing and lactation performance (van der Peet-Schwering et al., 2004). However, limiting the feed intake of sows has been associated with stereotypic behaviors indicating constant hunger (Meunier-Salaün et al., 2001). Inclusion of dietary fiber in gestation diets increases the bulk density of feed, which can result in feedback from stretch receptors in the gastrointestinal tract, signaling satiety (Bach Knudsen, 1997; Bach Knudsen and Jørgensen, 2001). However, the lack of endogenous enzymes capable of hydrolyzing fibrous complexes leads to reduced dietary energy and negatively affects the digestibility of other nutrients that are vital for sow productivity (Le Goff et al., 2002; Holt et al., 2006; Renteria-Flores et al., 2008).

Multienzyme blends (**$\mathbf{ME_{blend}}$**) comprised of various carbohydrases have been shown to be effective in diets containing fibrous ingredients fed to weaned pigs (Omogbenigun et al., 2004), as well as grower and finishing pigs (Emiola et al., 2009; Velayudhan et al., 2015; Woyengo et al., 2018) for improving the apparent total tract digestibility (**ATTD**) of nutrients and energy. However, there are limited studies investigating the effect of carbohydrase enzymes, either alone or in a combination, in diets fed to gestating sows. Nevertheless, carbohydrases are routinely included in commercial gestating sow diets, with inclusion levels determined based on values obtained from grower pig studies (McGhee and Stein, 2021). It is estimated that the breeding herd consumes approximately 20% of the total feed required within the swine production system (Ball and Moehn, 2013). As such, factors that influence nutritional value of sow diets have the potential to impact economic viability of swine production systems. The utilization of exogenous enzymes in commercial swine diets is a strategy to reduce ingredient usage and lower diet costs by increasing the nutrient and energy density of poorly digested ingredients (Olukosi and Adeola, 2013). Considering that gestating sows can more efficiently digest dietary fiber compared to growing pigs (Noblet and Shi, 1993; Stein et al., 2001; Jørgensen et al., 2007; Lowell et al., 2015; Dong et al., 2020), the extent of nutrient and energy improvements in gestating sow diets by ME_{blend} inclusion needs to be determined for most effective application of ME_{blend} in commercial sow production. For example, a similar enhancement of fiber degradation and thus dietary energy uplift may be achieved with a lower inclusion level of non-starch polysaccharide (**NSP**) degrading enzymes when added in gestating sows diets.

The objective of Exp. 1 was to determine the effect of two inclusion levels of a $\mathrm{ME_{blend}}$ on ATTD of nutrients and fiber fractions, and energy content in gestation diets with low or high dietary fiber content, as fed to pregnant sows. Subsequently, grower pigs were fed the same high fiber gestation diet either with or without $\mathrm{ME_{blend}}$ supplementation enabling a comparison of results with those attained in high fiber-fed gestating sows. The objective of Exp. 2 was to evaluate the effect of $\mathrm{ME_{blend}}$ and dietary fiber level on the standardized ileal digestibility (**SID**) of protein and amino acids (**AA**), as well as the apparent ileal digestibility (**AID**) of NSP in gestation diets fed to pregnant sows.

MATERIALS AND METHODS

Experimental procedures were conducted at the South Dakota State University Swine Education and Research Facility, in Brookings, SD, following approval by the Institutional Animal Care and Use Committee (IACUC #18-013A).

Two corn-based diets were formulated to meet or exceed NRC (2012) nutrient recommendations for gestating sows (Table 2-1). Low (**LF**) and high (**HF**) diets were formulated to contain neutral detergent fiber (**NDF**) at <10% and >17%, respectively. Dried-distillers grains with solubles (DDGS; Dakota Gold, Sioux Falls, SD) and soybean hulls were utilized as the fiber sources and best representative of commercially available fiber ingredients fed in the midwestern United States. The diets were formulated to contain equivalent metabolizable energy (**ME**) content (3,326 and 3,328 kcal/kg, as-fed, in LF and HF diet, respectively) calculated from nutrient composition based on NRC (2012) ingredient composition values assigned to grower pigs and fed in mash form. The ME_{blend} was supplemented to LF and HF diets at 0 (acting as the control), 0.08 and 0.10% based on recommended application for growing pigs (0.10 %) and 20 % below recommendation. Because feed restriction will result in slower passage rate (Dierick et al., 1989) and greater digestibility of nutrients in sows than growing pigs (Casas and Stein, 2017; Dong et al., 2020), it was hypothesized that a lower level of ME_{blend} supplementation may be sufficient to improve energy digestibility. The ME_{blend} supplied 1,300, 150, 800, 12,000, 6,000, 700, and 900 units of xylanase, glucanase, cellulase, amylase, protease, invertase and pectinase, respectively, per kilogram of diet (CBS Bio-Platforms Inc., Calgary, AB, Canada) at 0.10 % inclusion and designed to target dietary NSP specific to these complete diets. Titanium dioxide was included at 0.30 % of the diet as an indigestible marker to determine nutrient digestibility (Zhang and Adeola, 2016). A nitrogen-free diet was formulated based on nitrogen-free diets used in grower pig ileal digestibility studies (Stein et al., 2007) fed in Exp. 2 to account for endogenous losses when calculating SID values. Mineral and vitamin inclusion in the nitrogen-free diet were adjusted to meet mineral and vitamin NRC (2012) recommendations for gestating sows. Sows allotted to the nitrogen-free diet received the same amount of feed (2.2 kg/d) as the sows fed the treatment diets in a single feeding.

Experiment 1: Apparent Total Tract Digestibility

Forty-five gestating sows (Camborough L1050, PIC, Hendersonville, TN; parity 0 to 5; body weight [**BW**] 187 ± 28 kg) in two groups were utilized in a 2 x 3 factorial arrangement to determine the impact of dietary fiber level and ME_{blend} supplementation on ATTD of nutrients in gestating sows. Sows were blocked by parity for allotment to either the HF or the LF diet and one of 3 levels of ME_{blend} supplementation levels.

Sows were housed individually in gestation stalls (0.68 m × 1.98 m) which were equipped with a nipple drinker and a dry feeder. Sows were fed experimental treatments from d 50 to 64 of gestation and limit-fed 2.2 kg/d in a single feeding. Diets were fed for 14 d with the d0 - 10 considered an adaptation period followed by 4 d of total collection of urine and grab fecal samples (d11 - 14). On d 10, urinary catheters (Lubricath, 2-way; Bard Medical Division, Covington, GA; 18 Fr x 30 mL, gilts and parity 1; 28 Fr x 75 mL, parity 2+) were inserted and connected to closed containers in the same manner as described by Miller et al., 2016. Sample collection commenced on d 11 and continued until d 14. Sulfuric acid (10 – 20 mL/24 h) was added to the collection containers to stabilize pH; containers were weighed and 10% of each 24 h collected urine by weight was stored at -20°C. Urine samples were thawed following the conclusion of the experiment to be pooled within sow and collection period, subsampled, and stored at -20°C until further analysis. Fecal samples were collected once daily by rectal palpation, pooled within sow and collection period, and stored at -20°C until analysis. Rectal palpation was deemed sufficient to induce a complete defecation from which fecal samples were collected.

Twenty-seven growing pigs (Camborough L1050-PIC x Duroc; initial BW 35.7 ± 3.32 kg) in three groups of 9 pigs each were randomly allotted to one of 3 dietary treatments consisting of the HF diet supplemented at 0, 0.08 and 0.10%. Sufficient diet was mixed in a single batch for both gestating sows and growing pigs.

Grower pigs were housed in pens located in the swine metabolism room of the Animal Science Complex at South Dakota State University. Each pen was equipped with one nipple drinker and one dry feeder and experimental diets were provided for 14 d with adaptation and fecal collections the same as for sows. Feeding levels were based on pig BW (5% BW/d) in 2 equal feedings at 0800 h and 1500 h. Pigs were moved to metabolic crates on d 8 of receiving treatment to allow for adaption to the crates prior to 4 d of fecal collections from d 11 - 14. Urine samples were not collected on the grower pigs. Fecal samples were obtained by rectal palpation, pooled within pig and period and processed (homogenized, dried, and ground).

Experiment 2: Ileal Digestibility

Twelve gestating sows (Camborough L1050, PIC, Hendersonville, TN; parity 0 to 2; BW 159 ± 12 kg) at approximately 30 d of gestation (after confirmation of pregnancy) were surgically fitted with a simple T-cannula at the distal ileum following the procedure as described by Stein et al. (1998). Sows were given a minimum of 7 d of recovery from surgery before observations began. In a cross-over design, sows were fed the same six diets, as in Exp. 1, and a nitrogen-free diet during 5 periods to achieve 8 replicates per diet. Sows were restrictively fed (2.2 ± 0.4 kg/d) once per day (0800 h) with water provided ad libitum and housed in farrowing crates (1.83 m x 2.43 m) for the duration of the experiment. Each collection period lasted 7 d, the initial 5 d was considered a diet adaptation period followed by 12 h of continuous ileal digesta collection on d 6 and 7 (0800 h to 2000 h). Digesta samples were stored at -20°C after collection. At the conclusion of the collection, ileal digesta samples were thawed and pooled for each sow and period and a subsample was taken for chemical analysis.

Chemical Analyses

Prior to analysis, feces and ileal digesta samples were freeze dried (Dura-Dry, Fits Systems, Kinetics Thermal Systems) and finely ground (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany). The dry matter (**DM**) content of the diets, feces, and digesta samples were determined by drying samples at 102°C for 24 hours using a drying oven (Myers et al., 2004). Nutrient analyses of feed and fecal samples were conducted at the Agricultural Experiment Chemical Laboratories, University of Missouri (Columbia, MO). Feed samples were analyzed for crude protein (**CP**; AOAC Official Method 990.03, 2006), ether extract (**EE**; AOAC Official Method 920.39 (A); 2006), ash (AOAC Official Method 942.05, 2006), crude fiber (**CF**; AOAC Official Method 978.10, 2006), NDF (JAOAC 56, 1352-1356, 1973), acid-detergent fiber (**ADF**; AOAC Official Method 973.18, 2006), and a complete AA profile (AOAC Official Method 982.30 E (a,b,c)). Freeze dried feces were similarly analyzed for CP, EE, ash, CF, NDF, ADF while ileal digesta samples were submitted for CP and AA to a commercial laboratory (Mizzou Agricultural Experiment Station Chemical Laboratories, Columbia, MO).

Non-starch polysaccharides of feed ingredients (corn, soybean meal, DDGS, and soybean hulls), experimental diets, feces, and digesta samples from the control and 0.1% ME_{blend} groups were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with modifications (Slominski et al., 1994). Due to time constraints for this analysis, the level of ME_{blend} inclusion that represented current manufacturer recommended level (0.1%) was analyzed along with control (0% inclusion). In brief, a 100 mg sample was treated with dimethylsulphoxide ($(CH_3)_2SO$)

and incubated overnight at 45°C with a solution of starch-degrading enzymes composed of amylase, pullulonase and amyloglucosidase (Sigma, St. Louis, MO). Ethanol was then added, the mixture left for 1 h, centrifuged and the supernatant discarded. The dry residue was dissolved in 1 mL of 12M sulfuric acid (H_2SO_4) and incubated for 1 h at 35°C; 6 mL of water and 5 mL of myo-inositol (internal standard) solution were then added, and the mixture was boiled for 2 h. A 1 mL volume of the hydrolysate was then taken and neutralized with 12 M ammonium hydroxide (NH_4OH), reduced with sodium borohydride ($NaBH_4$), and acetylated with acetic anhydride ($(CH_3CO)_2O$) in the presence of 1(N)-methylimidazole. Component neutral sugars were separated using an SP-2340 column and a Varian CP3380 gas chromatograph (Varian Inc., Palo Alto, CA, USA) and uronic acids were measured using a Biochrom Ultrospec 50 (Biochrom Ltd., Cambridge, UK).

The gross energy (**GE**) content of feed, feces, and urine was analyzed by bomb calorimetry (Parr 6300 calorimeter, Parr Instruments Co., Moline, IL). For fecal samples 1 g of ground feces were pressed into a pellet using a pellet press (Parr Instruments Co., Moline IL), and placed into the bomb calorimeter. The sample was analyzed in duplicate and repeated if the difference between the two values was more than 2 %. Analysis of urine GE followed the procedure as described by Kim et al. (2009).

Quantification of titanium in feed, fecal, and digesta samples was based on Myers et al. (2004) with modifications. Briefly, DM was determined by weighing out 5.0 g of feed, 0.3 g of feces, or 0.5 g of digesta into a pyrex tube. The tube containing the sample was placed in a drying oven (105 °C) for 24 h. Samples were then ashed in a muffle furnace at 525°C for 10 h and difference in tube weight after ashing was used to calculate

DM content. A 0.05 g subsample of the ashed feed samples were weighed out before continuing with the quantification. A total of 0.8 g of anhydrous sodium sulphate (Na_2SO_4) and 5 mL of concentrated sulphuric acid (H_2SO_4) was added to each ashed sample. Samples were heated for 20 h at 120°C and filtered; 5 mL of the filtered sample solution was transferred to plastic tubes to be combined with 0.2 mL of 30% hydrogen peroxide (H2O3). A total of 280 µL of the standards and samples were transferred into a 96 well plated in duplicate. The absorbance was measured on SpectraMAX 190 plate reader (Corning Inc., Corning, NY, USA) at 408 nm.

Total starch content in the diets was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland) following manufacturer's procedures.

Calculations

The ATTD of energy and nutrients was calculated according to the indirect evaluation method using the marker approach. The following formula was used to calculate ATTD of nutrients:

$$\mathrm{ATTD}, \% = 100 - \left[100 \times \left(\frac{\mathrm{Ti_d} \times \mathrm{C_f}}{\mathrm{Ti_f} \times \mathrm{C_d}}\right)\right]$$

where Ti_d = concentration of titanium in the diet; Ti_f = concentration of titanium in feces; C_f = concentration of the component in feces and C_d = concentration of the component in the diet (Zhang and Adeola, 2017).

Digestible energy (**DE**) and ME of diets (kcal/kg of DM and as fed) were calculated following the procedures of Adeola (2001).

$$\mathrm{DE} = 100\ \times \frac{\text{GE intake} - \text{GE output}}{\text{GE intake}}$$

$$\mathrm{ME} = 100\ \times \frac{\text{GE intake} - \text{GE output} - \text{GE urine}}{\text{GE intake}}$$

DE was only calculated for the grower pigs because urine samples were not collected from the grower pigs. Concentrations of net energy (**NE**) (kcal/kg of DM) were calculated using equation 1 from NRC (2012):

$$\begin{aligned}\mathrm{NE}\ &= (0.726\ \times\ \mathrm{ME})\ +\ (1.33\ \times\ \mathrm{EE}) \\ &\quad +\ (0.39\ \times\ \text{Starch}) - (0.62\ \times\ \mathrm{CP}) - (0.83\ \times\ \mathrm{ADF})\end{aligned}$$

where all nutrient and digestible nutrient contents are expressed as g/kg DM.

AID values were calculated from the difference between the dietary intake of nutrients and the composition of nutrients in the digesta present in the distal ileum of the sows according to the equation (Stein et al., 2007):

$$\mathrm{AID}, \%\ = \left[1 - \left(\frac{\mathrm{C_{dig}}\ \times \mathrm{Ti_d}}{\mathrm{C_d}\ \times \mathrm{Ti_{dig}}}\right)\right]\ \times\ 100$$

where C_{dig} = concentration of the component in digesta; C_d = concentration of the component in the diet; Ti_d = concentration of titanium in the diet and Ti_{dig} = concentration of titanium in digesta (Stein et al., 2007).

SID values of AA and CP were calculated by the difference between the amount of AA and CP ingested, the amount of AA from the digesta in the ileum, and the endogenous loss of AA, according to the equation (Stein et al., 2007):

$$\text{Basal AA}_{\text{end}} = \text{AA}_{\text{dig}} \times \frac{\text{Ti}_{\text{d}}}{\text{Ti}_{\text{dig}}}$$

$$\text{SID, \%} = \left\{\frac{[\text{AA intake} - (\text{ileal AA outflow} - \text{basal AA}_{\text{end}})]}{\text{AA intake}}\right\} \times 100$$

Statistical Analyses

The UNIVARIATE procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) was used to confirm the homogeneity of variance and to analyze for outliers. Data were analyzed with the MIXED procedure of SAS. Energy and nutrient digestibility and ileal digestibility data from the sows was analyzed as a 2 × 3 factorial design to test the impact of dietary fiber level and enzyme supplementation in gestating sows. The statistical model contained the fixed effects of dietary fiber level (LF or HF), ME_{blend} supplementation level (0, 0.08 and 0.1%), and their interactions. For energy and nutrient digestibility group of sows was the blocking factor. For ileal digestibility, period and sow were random effects. To test ATTD of nutrients and NSP between gestating sows and growing pigs fed the HF supplemented at 0, 0.08, and 0.1% ME_{blend} the statistical model included dietary enzyme supplementation, animal category (grower or sow) and their interaction as main effects. Following review of the data, analysis of variance was deemed the most effective comparison for the effect of ME_{blend} inclusion due the small differences in most response variables between 0.08% and 0.1% inclusion levels for both sows and growing pigs. Tukey’s adjusted means test was used to detect differences among treatments where $P \leq 0.05$ was considered significant. Values are presented as lsmeans and standard error of the mean (SEM).

RESULTS

Analyzed proximate analysis of experimental diets are listed in Table 2-1. In particular CF, NDF, and ADF were 2.63x, 1.77x, and 2.58x, respectively, higher in HF diet compared to the LF diet.

The ingredients with the highest concentration of total NSP were soybean hulls followed by DDGS, SBM, and corn (Table 2-2). Based on the component sugar profile of corn and DDGS NSP, arabinoxylans would predominate, although significant amounts of β-glucans and cellulose were also present. The relatively high concentration of uronic acid along with glucose residues indicates that pectic-type substances and cellulose are the major cell wall constituents of soybean meal. The arabinose and galactose not associated with pectic substances derived from arabinan and arabinogalactan. Glucose was the predominant NSP component sugar in soybean hulls, indicating the presence of the cellulosic polysaccharides. Xylose in the feed ingredients indicates the presence of xylans and xyloglucan. The HF diet was approximately twofold higher in NSP in comparison to the LF diet.

Nutrient ATTD in Gestating Sows

Overall, energy digestibility of gestation diets fed to pregnant sows was significantly affected by ME_{blend} inclusion (Table 2-3). Where significant interactions between dietary fiber level and vinclusion occurred, the interaction alone is described; otherwise, main effects are described. The ME kcal/kg (DM and as-fed) and NE kcal/kg (DM and as-fed) were greater ($P = 0.05$) in the LF diet at both ME_{blend} supplementation levels and greater in the HF-fed sows at 0.1% inclusion only. Similarly, both 0.08% and 0.1% ME_{blend} inclusion levels increased ($P < 0.05$) metabolizable GE (ME%) and NDF digestibility in LF diet; whereas only 0.10% ME_{blend} inclusion increased GE% and NDF digestibility in HF diets. There were no interactions between ME_{blend} and fiber level on the ATTD of GE, CF, ADF and NSP as well as the DE content of the diets.

The ATTD of GE, NDF, and ADF increased in gestating sows supplemented with ME_{blend} ($P < 0.05$; Table 2-3). There was also a tendency for increased digestibility of CF ($P = 0.06$) with ME_{blend} supplementation. The ATTD of CF, NDF, ADF were greater for the HF than LF diets in gestating sows ($P < 0.01$). Multienzyme supplementation increased the DE, ME, and NE content (kcal/kg DM and kcal/kg as-fed) of the gestating sow diets.

The supplementation of ME_{blend} had no effect on the ATTD of NSP (Table 2-3). The ATTD of xylose, glucose, uronic acids, and total NSP was greater in gestating sows fed the HF diet compared to the LF diet ($P < 0.05$), while the ATTD of galactose tended to be greater in the HF diet ($P = 0.08$).

Comparison of ATTD between Gestating Sows and Growing Pigs

There was no interaction between ME_{blend} inclusion and pig category (Table 2-4). Across both animal groups, ME_{blend} inclusion improved ATTD of CF, ADF, total NSP, xylose, galactose, uronic acids, and GE ($P < 0.05$). There were tendencies for increases in the ATTD of arabinose and glucose ($P < 0.10$) by ME_{blend} inclusion. Sows expressed higher digestibility of NDF ($P = 0.01$) and showed a tendency towards higher ATTD of ADF ($P = 0.06$). Additionally, sows demonstrated greater digestibility of total NSP and NSP xylose and glucose ($P < 0.05$) with a tendency towards higher NSP arabinose digestibility ($P = 0.10$) when compared to growing pigs.

SID and AID in Gestating Sows

There was no difference in AID or SID of DM, AA, or CP with ME_{blend} supplementation and no interaction between ME_{blend} inclusion and fiber level (Table 2-5 and 2-6). Sows fed the HF diet had reduced AID of DM ($P < 0.01$) and all AA ($P < 0.05$) except for Arg and Pro. There was no effect of ME_{blend} inclusion on AID of NSP. The HF-fed sows had greater AID of total NSP, and NSP arabinose and xylose when compared to LF-fed sows ($P < 0.01$). The HF-fed sows had reduced SID of His, Ile, Lys, Phe, Trp, Asp, Cys, Glu, Gly, Ser, and Tyr ($P < 0.05$) with tendencies for Thr ($P = 0.07$) and Val ($P = 0.09$) when compared to LF-fed sows.

DISCUSSION

Numerous studies (Omogbenigun et al., 2004; Emiola et al., 2009; Velayudhan et al., 2015; Woyengo et al., 2018) have shown that NSP degrading enzyme blends can enhance nutrient digestibility in high-fiber diets for weaned and growing pigs. However, it is important to note that the digestibility values obtained from studies in grower pigs may not be directly applicable to commercial gestation diets. In part, because gestating sows have greater nutrient digestibility compared to growing pigs (Noblet and Shi, 1993; Casas and Stein, 2017; Dong et al., 2020). The overall objective of this work was to investigate the effect of ME_{blend} supplementation and inclusion level on the ATTD of energy and nutrients, as well as the ileal digestibility of CP and AA in gestation diets with low or high dietary fiber fed to gestation sows. For comparison, growing pigs were fed the same HF diet to directly compare the ATTD values with those of gestating sows.

In this study, the HF diet fed to gestating sows were higher in DE, ME, and NE contents compared to the LF diet, both on DM and as-fed basis, without enzyme inclusion. Specifically, DE, ME, and NE content of HF diet exceeded that of LF diet by 222, 247, and 174 kcal/kg, respectively. Diets were formulated to be isoenergetic thus differences in determined energy value may be related to true differences in digestibility. Typically, the energy density of a diet tends to decrease as the concentration of dietary NSP increases (Grieshop et al., 2001); in this case, oil was added to maintain available energy. Li et al., (2018) reported that a 1% inclusion of soy oil in diets can increase the DE, ME, and NE of diets by 60 kcal/kg DM. The HF diet contained 2.2% more oil compared to the LF diet, which can, in part, account for the observed higher energy content compared to LF diet. Further, Renteria-Flores et al. (2008) report that a high-fiber diet, rich in its water-soluble fraction from oat hulls, fed to gestating sows resulted in a DE and ME content approximately 100 kcal/kg greater than a lower fiber diet. This suggests that the relationship between fiber and energy density is not always straightforward. However, based on analyzed GE of the mixed diets the HF diet had approximately 250 kcal greater GE. Thus differences in determined DE, ME, and NE may be related to nutrient loadings values in the formulation program that underestimated energy content of the fiber ingredients resulting in a greater formulated energy level than expected.

For the gestating sows, supplementation of ME_{blend} at both 0.08% and 0.10% inclusion resulted in an average increase of 6% in the ATTD of nutrients in the complete diets. When the gestating sows were fed diets with enzyme supplementation, the DE contents of the complete diets increased by 174 kcal/kg on DM basis and 156 kcal/kg as-fed basis. Similar increases were observed for ME (+ 220 kcal/kg DM and 195 kcal/kg as-fed) and NE (+ 161 kcal/kg DM and 142 kcal/kg as-fed). It should be noted that the

3.9 % increase in ATTD of GE observed in this study due to ME_{blend} supplementation is greater than the 1.5 % increase reported by Zhou et al. (2018), where xylanase was added to a wheat-barley-based diet fed to lactating sows. These differences could likely be related to variation in factors such as daily feed intake, enzyme type and dietary fiber level and structure. Within the current study, 0.10% ME_{blend} supplementation improved the dietary energy value (DE, ME, and NE) by 10% in the LF diet and 3% in the HF diet when fed to gestating sows. The limited effect of ME_{blend} in the HF diet may be attributed to the rate of digesta passage. While feed restriction between both groups of sows would have reduced the rate of passage through the gastrointestinal tract (Parker and Clawson, 1967), the increased concentration of dietary NDF in the HF diet may have counteracted the feed restriction effect and resulted in an increased passage rate through the gastrointestinal tract (Le Goff et al., 2002). The reduced time, in turn, limited the timeframe for which the enzymes could effectively hydrolyze the substrates present in the digests. Another factor that might have hindered the efficacy of the ME_{blend} in the HF diet is the higher inclusion of soy oil. Danicke et al. (1997) reported that when xylanase was supplemented to rye-based diets fed to broilers, its efficacy on nutrient digestibility was lower when soy oil was added to the diet compared to when tallow was added. Therefore, the combined effects of increased passage rate due to higher dietary fiber and the presence of soy oil in the HF diet could explain the lower impact of ME_{blend} supplementation in this diet. Further, efficacy of enzymes is in part related to a balance between substrate availability and enzyme concentration (Olukosi and Adeola, 2013). It is possible that substrate availability in the HF diet overwhelmed potential enzyme activity at the 0.08% inclusion (Emiola et al., 2009).

Supplementation of ME_{blend}, regardless of the inclusion level, resulted in 4 to 17% improvement in ATTD of nutrients in HF gestation diets fed to grower pigs, which agrees with findings from other studies (Omogbenigun et al., 2004, Emiola et al., 2009). The inclusion of enzymes increased DE content by 216 kcal/kg on a DM basis and 193 kcal/kg on as-fed basis. Despite the lack of significant ME_{blend} x category interaction, absolute differences in enhanced digestion with ME_{blend} inclusion by pig category is worth noting. Similar to NDF, ADF and GE, ME_{blend} supplementation in gestation diets fed to growing pigs improved ATTD of total NSP and its constituents by 6 to 12 %. Compared to gestating sows, the improvement of DE content was two times greater in growing pigs (6 vs 3%) fed the HF diet. The relatively smaller enhancement in DE, NDF, ADF, and NSP digestibility with enzyme inclusion in diets fed to gestating sows, compared to growing pigs, may be attributed to the overall greater capacity of sows to digest nutrients compared to growing pigs, irrespective of enzyme inclusion (Lowell et al., 2015). Dong et al. (2020) similarly reported that mature sows had greater ATTD of NDF and ADF in ingredients containing higher fiber content compared to their growing pig counterparts. This can be attributed to the more developed gastrointestinal tract of adult sows, which allows for greater digestibility of dietary fiber and a higher fermentative capacity to degrade fiber compared to growing pigs (Dierick et al., 1989). The growing pigs were fed at 4% of BW in 2 feedings which means each feeding represented 2% of BW which is somewhat similar to the sows (2.2 kg/187 kg x 100 = 1.1%). Similarly, Casas and Stein (2018) found that 2nd to 6th parity gestating sows fed the same level of intake as 51 kg growing gilts (*i.e.*, 3.5x maintenance ME requirement) had greater ATTD of GE and dietary DE and ME. Therefore, differences in digestive capacity likely contributed more to differences between sows and growing pigs than feed intake.

In this trial, gestating sows received their entire meal in a single feeding while the meal for the grower pigs was divided among two feedings. This feeding frequency is not the same at the comparative ATTD trials between gestating sows and grower pigs conducted by Lowell et al. (2015), Casa and Stein (2017), and Dong et al. (2020). Limit-fed gestating sows in the mentioned studies received the entirety of their meal in two feedings and had the same feeding frequency as that of grower pig on digestibility trials to represent ab-libitum feeding. The two-feeding technique commonly applied in swine digestibility trials does not mimic the voluntary frequent feedings of ab-lib fed grower pigs (Chassé et al., 2022) or limit-fed single meal of gestating sows (Holt et al., 2006) in commercial production. However, feeding gestating sows either a single meal or divided between two equal meals does not affect the ATTD of DM, GE or nitrogen for pregnant females (Holt et al., 2006). Nutrient and energy ATTD or supplemental fiber-degrading enzyme activity on digestibility is also not affected by meal frequency in grower pigs receiving equal intake of high fiber diets (Chassé et al., 2022).

Multienzyme supplementation did not impact the ileal digestibility of CP, AA, and NSP. At the time of this work, there were limited investigations on the impacts of ME_{blend} supplementation on the AID and SID of AA in gestating sows. Similar findings were reported by Velayudhan et al. (2019) who reported no effect of a carbohydrase blend comprised of cellulase, pectinase, mannase, galactanase, xylanase, glucanase, amylase, protease, and phytase activities, (at 0.1% inclusion) on AID or SID of AA or CP when canola meal was fed to sows in mid- or late-gestation. In finisher diets containing

30% of wheat DDGS, ME_{blend} supplementation resulted in a 3% increase in AID of Arg and 11% increase in AID of Thr when fed to 80.8 kg finisher pigs (Emiola et al., 2009). However, the lack of effect of ME_{blend} supplementation on ileal CP and AA digestibility in gestating sows may be attributed to feed restriction practices, which allow sows to have a greater SID of CP and AA than grower pigs, where ad-libitum feeding reduces digestibility coefficients (Stein et al., 2001). Moreover, gestating sows can digest 25% of their dietary NSP intake in the terminal ileum (Jørgensen et al., 2007), which could explain the lack of effect of ME_{blend} supplementation on the AID of NSP in the current study. It is worth noting that the HF diet-fed sows had higher AID values of the total NSP and NSP components, i.e., arabinose, xylose, and total NSP by 13.1, 14.2, 37.4 %, respectively, than the LF diet-fed sows. The 3-6% reduction in the SID of His, Ile, Lys, Phe, Thr, Trp, and Val in the HF diet-fed sows compared to LF diet-fed sows is consistent with prior studies conducted with the grower pig, where the SID for AA and CP decreases as dietary NDF increases (Kahindi et al., 2014).

In conclusion, the inclusion of a multienzyme blend in gestation diets at 0.08% or 0.1% inclusion levels can increase ATTD of nutrients and energy for gestating sows by approximately 5 to 10%, depending on the dietary NDF level. Although the multienzyme supplementation did not impact the ileal digestibility of amino acids, higher dietary fiber reduced standardized ileal digestibility of amino acids in gestating sows by approximately 3%. This should be considered when formulating high fiber diets for gestating sows that contain similar ingredients. The response to the carbohydrase enzyme complexes observed in growing pigs does not necessarily reflect the same response in gestating sows. Therefore, extrapolating the effects of enzyme supplementation from growing pigs to gestating sows could be taken with caution.

ACKNOWLEDGEMENT

This study was supported by funding from the SDSU Agricultural Experiment Station Hatch Funds and USDA National Institute of Food and Agriculture (Hatch project: SD00H666-18) and CBS Bio-Platforms Inc. (partial funding; provision of the multienzyme blend). The authors thank the swine research group at South Dakota State University.

DISCLOUSURE

Rob Patterson is the Vice President of Innovation and Commercialization for CBS Bio-Platforms.

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Table 2-1. Diet formulation and analyzed composition of the Low and High Fiber gestation diets (Exp.1 and Exp. 2) and the N-free diet (Exp. 2) (as-fed basis)[1, 2].

Items	Low Fiber	High Fiber	Nitrogen-free
Ingredient, %			
Corn	76.80	54.04	-
Sugar	-	-	44.00
Cornstarch	-	-	43.55
Soybean meal	15.00	12.00	-
Solka floc	-	-	5.00
Dakota Gold, DDGS[3]	3.00	18.00	-
Soybean hulls	1.00	10.00	-
Soybean oil	0.80	3.00	2.00
Calcium carbonate	1.08	1.10	0.70
Potassium carbonate	-	-	0.70
Salt	0.32	0.26	0.65
Monocalcium phosphate	0.80	0.40	2.90
Vitamin premix[4]	0.05	0.05	0.05
Mineral premix[5]	0.15	0.15	0.15
Titanium dioxide	0.30	0.30	0.30
Analyzed Composition			
GE, Kcal/kg	3,878	4,163	-
DM, %	87.76	88.73	95.41
CP (N x 6.25), %	12.45	15.98	0.84
EE, %	2.43	4.84	1.34
Ash, %	4.09	4.28	4.06
CF, %	2.49	6.55	2.90
NDF, %	10.36	18.30	-
ADF, %	4.02	10.38	-
Indispensable AA, %			
Arg	0.72	0.81	0.00
His	0.35	0.41	0.00
Ile	0.53	0.60	0.00
Leu	1.24	1.46	0.03
Lys	0.63	0.72	0.02
Met	0.22	0.26	0.00
Phe	0.64	0.71	0.02
Thr	0.47	0.57	0.00
Trp	0.13	0.12	<0.02
Val	0.60	0.70	0.01
Dispensable AA, %			
Ala	0.74	0.88	0.01
Asp	1.15	1.28	0.01
Cys	0.26	0.32	0.00
Glu	2.37	2.57	0.02
Gly	0.52	0.66	0.00
Pro	0.87	1.02	0.02

Ser	0.57	0.67	0.00
Tyr	0.44	0.51	0.02
Total AA, %	12.79	14.61	0.43

[1] Abbreviations: DDGS, dried-distillers grain with solubles; GE, gross energy; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; AA, amino acids.

[2] Multienzyme blend was included at 0, 0.08, and 0.1% at expense of corn to create 3 different dietary treatments.

[3] POET, LLC, 4506 N Lewis Ave, Sioux Falls, SD 57104, USA.

[4] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin 9.9 mg, Thiamine 3.3 mg.

[5] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Copper 20 mg, Manganese 40 mg, Selenium 0.3 mg, Zinc 170 mg.

Table 2-2. Concentrations of the total non-starch polysaccharides (NSP) and NSP component sugars of the feed ingredients and experimental diets (as-fed basis)[1].

	Ingredient				Diets	
Item, %	Corn	Soybean meal	DDGS	Soybean hulls	Low Fiber	High Fiber
Total NSP	6.2	14.2	31.4	61.1	6.2	13.9
NSP component sugars						
Rhamnose	ND	ND	ND	0.4	ND	ND
Arabinose	1.3	2.1	6.9	3.8	1.2	2.1
Xylose	1.9	0.9	9.5	6.4	1.4	2.8
Mannose	ND	0.6	1.3	4.3	0.1	0.3
Galactose	0.3	4.3	1.5	2.3	0.4	0.9
Glucose	2.1	3.1	9.9	30.9	2.2	5.5
Uronic acids	0.6	3.2	2.5	12.9	1.0	2.2

[1] Abbreviations: DDGS, dried-distillers grains with solubles; ND, not detected.

Table 2-3. Interactive effects between fiber level and ME$_{blend}$ inclusion on energy content and apparent total tract digestibility (ATTD) of nutrients in gestation diets fed to gestating sows[1].-

	Low Fiber			High Fiber				*P*-value		
ME$_{blend}$ inclusion, %	0	0.08	0.10	0	0.08	0.10	SEM	ME$_{blend}$	Diet	ME$_{blend}$ x Diet
ATTD of GE, %	77.9	84.3	82.5	79.8	81.0	83.3	1.37	0.015	0.852	0.194
DE, kcal/kg										
DM basis	3,405	3,683	3,607	3,713	3,768	3,877	123	0.018	<0.001	0.222
As-fed basis	3,016	3,263	3,195	3,316	3,364	3,462	109	0.018	<0.001	0.222
Metabolizable GE, %	74.7^{b}	83.0^{a}	81.3^{a}	78.5^{b}	79.4^{b}	82.3^{a}	2.93	0.001	0.720	0.042
ME, kcal/kg										
DM basis	3,266^{b}	3,633^{a}	3,558^{a}	3,660^{ab}	3,702^{ab}	3,838^{a}	135	0.002	<0.001	0.053
As-fed basis	2,893^{b}	3,218^{a}	3,152^{a}	3,268^{b}	3,306^{b}	3,427^{a}	121	0.002	<0.001	0.053
NE, kcal/kg[2]										
DM basis	2,353^{b}	2,619^{a}	2,567^{a}	2,634^{b}	2,665^{ab}	2,763^{a}	98	0.002	<0.001	0.052
As-fed basis	2,084^{b}	2,320^{a}	2,274^{a}	2,351^{b}	2,379^{ab}	2,467^{a}	87	0.002	<0.001	0.052
ATTD of CF, %	34.0	40.7	37.9	57.4	67.5	68.3	3.49	0.064	<0.001	0.640
ATTD of NDF, %	50.9^{c}	62.1^{b}	61.7^{b}	68.5^{a}	67.5^{ab}	70.5^{a}	2.52	0.012	<0.001	0.024
ATTD of ADF, %	43.5	52.8	52.9	67.4	69.8	74.2	3.61	0.012	<0.001	0.406
ATTD of NSP, %										
Arabinose	61.9	-	70.0	69.7	-	75.5	4.81	0.164	0.186	0.816
Xylose	36.6	-	46.9	52.6	-	62.4	7.03	0.194	0.049	0.978
Galactose	66.9	-	67.2	71.6	-	79.2	4.18	0.391	0.076	0.424
Uronic acid	79.3	-	84.8	74.1	-	75.1	3.15	0.355	0.039	0.516
Total	56.1	-	61.9	67.7	-	74.9	4.80	0.219	0.026	0.888

[1] Abbreviations: ME$_{blend}$, multienzyme blend; SEM, standard error of the mean; GE, gross energy; DE, digestible energy; DM, dry matter; ME, metabolizable energy; NE, net energy; CF, crude fiber; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; NSP, non-starch polysaccharides.

[2] NE (kcal/kg) = (0.726 x ME) + (1.33 x EE) + (0.39 x Starch) – (0.62 × CP) – (0.83 × ADF); (Equation 1; NRC, 2012).

[a,b] Means within a row with different superscripts are significant at $P \leq 0.05$.

Table 2-4. Interactive effects of ME_{blend} in High Fiber (HF) gestation diet fed to growing pigs and gestating sows on apparent total tract digestibility (ATTD) of fiber and GE[1].

	Growing pigs			Gestating sows				*P*-value		
ME_{blend} inclusion, %	0	0.08	0.10	0	0.08	0.10	SEM	ME_{blend}	Category	ME_{blend} x Category
ATTD of CF, %	56.32	65.77	64.63	57.36	67.46	68.29	3.152	<0.001	0.425	0.916
ATTD of NDF, %	58.94	66.82	65.53	68.47	67.55	70.47	2.342	0.180	0.014	0.202
ATTD of ADF, %	61.68	69.14	68.69	67.43	69.79	74.18	2.476	0.029	0.064	0.535
ATTD of NSP, %										
Total NSP, %	56.61	-	64.42	67.96	-	75.01	3.441	0.049	<0.001	0.917
NSP component sugars										
Arabinose, %	63.78	-	70.55	69.91	-	75.56	3.082	0.065	0.096	0.863
Xylose, %	40.67	-	52.52	53.02	-	62.60	4.863	0.045	0.036	0.826
Galactose, %	72.46	-	78.23	71.76	-	79.24	2.722	0.028	0.957	0.766
Glucose, %	51.04	-	58.82	70.08	-	76.89	3.834	0.080	<0.001	0.905
Uronic acids, %	77.32	-	81.68	79.35	-	84.76	2.201	0.043	0.278	0.822
ATTD of GE, %	79.84	84.33	84.63	79.64	81.0	83.16	1.300	0.011	0.120	0.462
DE, kcal/kg										
DM basis	3,836	3,917	3,847	3,714	3,768	3,878	88	0.111	0.225	0.226
As-fed basis	3,425	3,497	3,449	3,316	3,364	3,463	78	0.111	0.225	0.226

[1] Abbreviations: ME_{blend}, multienzyme blend ; SEM, standard error of the mean; CF, crude fiber; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; NSP, non-starch polysaccharides; GE, gross energy.

Table 2-5. Effect of ME$_{blend}$ and fiber level on apparent ileal digestibility (AID) of dry matter (DM), amino acids (AA), and non-starch polysaccharides (NSP) in diets fed to gestating sows (Exp. 2)$^{1, 2}$.

	ME$_{blend}$, %				Diet			*P*-value		
Item, %	0.0	0.08	0.10	SEM	Low fiber	High fiber	SEM	ME$_{blend}$	Diet	ME$_{blend}$ x Diet
CP (N x 6.25)	70.2	69.7	69.4	1.19	70.6	68.9	1.03	0.867	0.173	0.589
DM	70.7	70.6	70.3	0.87	76.1	65.0	0.74	0.954	<0.001	0.994
Indispensable AA										
Arg	82.7	81.8	81.5	0.71	82.6	81.4	0.61	0.393	0.106	0.369
His	79.0	78.6	78.5	0.83	80.7	76.7	0.73	0.873	<0.001	0.463
Ile	75.2	74.8	73.8	1.04	76.5	72.7	0.92	0.513	0.001	0.328
Leu	82.1	82.2	81.7	0.67	82.8	81.2	0.57	0.830	0.042	0.695
Lys	71.1	71.7	70.5	1.13	73.7	68.5	0.98	0.683	<0.001	0.477
Met	81.8	82.0	81.0	0.89	82.4	80.8	0.80	0.576	0.052	0.831
Phe	79.1	78.4	77.9	0.95	80.0	76.9	0.86	0.512	0.001	0.409
Thr	66.2	65.7	65.1	1.52	67.5	63.9	1.38	0.806	0.011	0.436
Trp	73.6	73.6	73.6	1.50	76.5	70.7	1.39	1.000	<0.001	0.125
Val	72.0	71.7	70.8	1.12	73.0	70.0	1.00	0.663	0.010	0.247
Mean	76.3	76.1	75.4	1.03	77.6	74.3	0.92	0.685	0.032	0.438
Dispensable AA										
Ala	75.8	75.7	74.8	1.11	76.7	74.2	0.97	0.757	0.033	0.336
Asp	72.7	71.8	71.4	1.05	75.0	68.9	0.91	0.618	<0.001	0.531
Cys	70.5	69.8	69.3	1.12	72.6	67.1	0.95	0.724	<0.001	0.670
Glu	82.7	81.9	81.9	0.83	84.1	80.3	0.74	0.642	<0.001	0.402
Gly	56.6	58.6	59.0	1.77	61.7	54.4	1.47	0.578	0.001	0.108
Pro	70.1	72.0	69.4	3.23	72.2	68.8	3.09	0.637	0.124	0.817
Ser	74.2	73.6	73.5	1.09	76.2	71.3	0.97	0.803	<0.001	0.639
Tyr	78.5	77.8	77.5	1.00	79.6	76.3	0.90	0.694	0.001	0.526
Mean	72.6	72.6	72.1	1.40	74.8	70.2	1.25	0.681	0.032	0.504
Total AA	75.9	75.4	75.1	0.99	77.6	73.3	0.84	0.827	<0.001	0.554
NSP										
Arabinose	34.2	-	32.8	2.98	26.4	40.6	2.96	0.674	<0.001	0.231
Xylose	23.2	-	21.3	4.31	3.5	40.9	4.25	0.671	<0.001	0.328
Glucose	44.1	-	46.5	3.04	45.2	45.4	3.01	0.255	0.948	0.896

Uronic acids	45.5	-	44.7	2.12	45.4	44.7	2.07	0.694	0.748	0.869
Total NSP	32.7	-	32.2	3.21	25.9	39.0	3.18	0.255	0.001	0.216

[1] Abbreviations: ME_{blend}; multienzyme blend; SEM, standard error of the mean.

[2] Multienzyme blend contained 1,300, 150, 800, 12,000, 6,000, 700, and 900 units of xylanase, glucanase, cellulase, amylase protease, invertase and pectinase, respectively, per gram of diet (CBS Bio-platforms Inc., Calgary, AB, Canada) at 0.10 % inclusion. Diets were formulated to contain either 10 or 18% neutral detergent fiber (NDF). Corn dried distillers grain and soyhulls were used to adjust diet NDF level. The High fiber diet included 3% soybean oil to ensure similar formulated metabolizable energy content (3326 and 3328 kcal/kg in Low and High fiber diet, respectively). Diets were formulated to meet or exceed NRC (2012) nutrient requirements for gestating sows.

Table 2-6. Effect of ME_{blend} and fiber level[1] on standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in diets fed to gestating sows (Exp. 2).

	ME_{blend} inclusion, %				Diet			*P*-value		
Item, %	0.0	0.08	0.10	SEM	Low Fiber	High Fiber	SEM	ME_{blend}	Diet	ME_{blend} x Diet
CP (N x 6.25)	80.4	79.9	79.4	1.39	80.3	79.4	1.22	0.844	0.519	0.435
Indispensable AA										
Arg	91.1	89.9	89.4	0.87	89.9	90.4	0.77	0.304	0.531	0.216
His	84.6	84.2	84.0	0.93	86.1	82.4	0.82	0.886	0.001	0.349
Ile	82.7	81.7	80.4	1.19	82.8	79.9	1.06	0.458	0.015	0.216
Leu	88.0	88.1	87.4	0.77	88.2	87.4	0.66	0.770	0.357	0.571
Lys	76.5	77.3	75.9	1.25	78.9	74.2	1.09	0.658	0.007	0.410
Met	87.4	87.8	86.5	1.06	87.7	86.8	0.95	0.556	0.372	0.728
Phe	85.0	84.3	83.5	1.08	85.4	83.2	0.99	0.446	0.027	0.280
Thr	74.8	74.3	73.6	1.71	75.6	72.9	1.56	0.805	0.071	0.307
Trp	83.0	83.3	83.1	1.89	84.8	81.5	1.78	0.978	0.028	0.070
Val	78.2	78.0	76.9	1.25	78.7	76.7	1.13	0.658	0.090	0.168
Mean	83.0	82.9	82.1	1.20	83.8	81.5	1.08	0.652	0.149	0.331
Dispensable AA										
Ala	82.8	82.9	81.8	1.31	83.3	81.7	1.15	0.738	0.219	0.240
Asp	79.0	78.0	77.5	1.16	80.9	75.3	1.01	0.570	<0.001	0.421
Cys	75.8	75.0	74.4	1.22	77.8	72.4	1.05	0.689	<0.001	0.597
Glu	88.5	88.2	87.8	0.89	89.5	86.8	0.80	0.822	0.005	0.462
Gly	71.5	74.2	74.7	2.20	77.1	69.8	1.84	0.517	0.005	0.073
Pro	112.2	115.3	110.2	6.54	112.3	112.8	6.50	0.529	0.907	0.839
Ser	82.7	82.1	81.8	1.24	84.4	79.9	1.12	0.796	0.001	0.501
Tyr	85.7	85.0	84.5	1.17	86.4	83.8	1.06	0.661	0.016	0.378
Mean	84.8	85.1	84.1	1.97	86.5	82.8	1.82	0.665	0.192	0.439
Total AA	85.7	85.3	84.7	1.20	86.9	83.6	1.03	0.796	0.009	0.390

[1] Abbreviations: ME_{blend}; multienzyme blend; SEM, standard error of the mean.

[2] Multienzyme blend contained 1,300, 150, 800, 12,000, 6,000, 700, and 900 units of xylanase, glucanase, cellulase, amylase protease, invertase and pectinase, respectively, per gram of diet (CBS Bio-platforms Inc., Calgary, AB, Canada) at 0.10 % inclusion. Diets were formulated to contain either 10 or 18% neutral detergent fiber (NDF). Corn dried distillers grain and soyhulls were used to adjust diet NDF level. The High fiber diet

included 3% soybean oil to ensure similar formulated metabolizable energy content (3326 and 3328 kcal/kg in Low and High fiber diet, respectively). Diets were formulated to meet or exceed NRC (2012) nutrient requirements for gestating sows.

CHAPTER 3

EFFECTS OF MULTIENZYME BLEND SUPPLEMENTATION ON TOTAL TRACT DIGESTIBILITY OF ENERGY AND NUTRIENTS IN COMMON SWINE FEEDSTUFFS FED TO GESTATING SOWS[1]

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[1] Formatted in preparation for submission to *Journal of Animal Science*

ABSTRACT

The utilization of exogenous fiber-degrading enzymes in commercial swine diets is a strategy to reduce ingredient usage and lower diet costs by increasing the nutrient and energy density of poorly digested ingredients. In a prior set of trials, dietary multienzyme blend (**ME_{blend}**) supplementation increased apparent total tract digestibility (**ATTD**) of nutrients, non-stach polysaccharides, and energy in complete high-fibrous gestation diets by 6% when fed to gestating sows. This study aimed to determine the effects of ME_{blend} supplementation at 0.1% inclusion on ATTD of energy and nutrients of individual feedstuffs commonly included in gestating sow diets in the main pork-producing areas of the globe that differ in fibrous components. Twenty pregnant gilts (initial bodyweight 176 ± 6.6 kg) in a single group were allocated to one of 7 diets with or without ME_{blend} in a crossover design with four periods to determine ATTD of energy and neutral detergent fiber (**NDF**). Three diets contained corn, wheat, and sorghum as the sole source of energy; soybean meal (**SBM**), field peas (**FP**), canola meal (**CM**), and sugar beet pulp (**SBP**) each replaced 25% of corn in the corn diet to determine energy value of individual feedstuffs. Data was analyzed using a student's t-test to determine the effect of enzyme supplementation on feedstuffs. Multienzyme blend (xylanase, glucanase, cellulase, amylase, protease, and invertase) supplemented at 0.1% of the diet increased metabolizable and net energy of corn and wheat by 2% and 3% ($P \leq 0.05$). The energy content of sorghum was not impacted by ME_{blend}. A 6%, 4%, and 10% uplift was observed in metabolizable and net energy of SBM, FP, and CM, respectively ($P \leq 0.05$). Sugar beet pulp was not affected by ME_{blend} supplementation. In conclusion, a multienzyme blend in diets at 0.1% increased the energy content ofcorn, wheat, soybean meal, field peas, and canola meal fed to gestating sows by approximately 5 to 10%, depending on the feedstuffs. Multienzyme supplementation did not impact the energy of sorghum and sugar beet pulp. This should be considered when formulating fibrous diets for gestating sows with different ingredients to reduce diet costs.

KEY WORDS

Fiber, feedstuffs, gestating sow, multienzyme, non-starch polysaccharides, nutrient digestibility

LIST OF ABBREVIATIONS

ADF, acid detergent fiber; ATTD, apparent total tract digestibility; CM, canola meal; CP, crude protein; DE, digestible energy; DM, dry matter; EE, ether extract; FP, field peas; GE, gross energy; ME_{blend}, multienzyme blend; ME, metabolizable energy; NDF, neutral detergent fiber; NE, net energy; NSP, non-starch polysaccharides; SBM, soybean meal; SBP; sugar beet pulp; TDF; total dietary fiber.

INTRODUCTION

Breeding sows consume approximately 20% of the total feed in production systems (Ball and Moehn, 2013). Regional availability of feedstuffs is considered when formulating least-cost rations, and the use of feedstuffs will vary between the main pork-producing regions of the globe (*e.g.*, Canada, United States, European Union, and Brazil) due to agronomic conditions (Bikker and Jansman, 2023). Adequate growing conditions in the U.S. corn belt allow corn to be the economically favored cereal grain to provide dietary energy in U.S. swine diets. Swine-producing areas with shorter crop-growing periods will incorporate wheat instead, but climates with a greater likelihood of drought conditions will utilize sorghum as the base cereal grain (Sauber and Owens, 2001). Canola meal (**CM**) is a protein concentrate predominantly fed in Western Canada and Europe; while the increase in global acreage for soybeans allows soybean meal (**SBM**) to be utilized in many geographical regions as the primary protein source. The significant starch and protein content of field peas (**FP**) provides caloric and protein density to diets (Woyengo and Zjilstra, 2021). Sugar beet pulp (**SBP**) is a valued fiber source included in gestation diets that increases the bulk density of feed, which can result in feedback from stretch receptors in the gastrointestinal tract and signaling satiety in limit-fed gestating sows (Bach Knudsen and Jørgensen, 2001). These feedstuffs, however, vary in dietary fiber and non-starch polysaccharides (**NSP**) that hinder nutrient digestibility and energy availability (Bach Knudsen, 1997; Bach Knudsen, 2014).

The utilization of exogenous NSP-degrading enzymes in commercial swine diets is a strategy to reduce ingredient usage and lower diet costs by increasing the nutrient and energy density of poorly digested ingredients (Pitchard-Torres et al., 2019). In a prior set of trials, dietary multienzyme blend (**ME_{blend}**) supplementation increased apparent total tract digestibility (**ATTD**) of nutrients, NSP, and energy in high fibrous gestation diets by 6% when fed to gestating sows (Shipman et al., 2023). The effects of ME_{blend} on the digestibility of common feedstuffs, rather than complete diets, have been evaluated in grower pigs (Torres-Pitarch et al., 2019). Little to no data is available on the effects of ME_{blend} on feedstuffs when fed to gestating sows. Thus, this study aimed to determine the effects of ME_{blend} supplementation on ATTD of energy and nutrients and energy content of feedstuffs commonly included in gestating sow diets in the main pork-producing areas of the globe that differ in fibrous components.

MATERIAL AND METHODS

Experimental procedures were conducted at the South Dakota State University Swine Education and Research Facility in Brookings, SD, following approval by the Institutional Animal Care and Use Committee (2310-003A).

Feedstuffs and Dietary Treatments

All ingredients originated from sources in the upper Midwestern United States. Three diets were formulated to contain corn, wheat, and sorghum as the sole energy source (Table 3-2). The SBM, FP, CM, and SBP were added to the corn diet replacing 25% of the corn to calculate energy content using the differential procedure (Zhang and Adeola, 2017). Mineral and vitamin inclusion in the diets were adjusted to meet mineral and vitamin NRC (2012) recommendations for gestating sows. The ME_{blend} (Superzyme®; CBS Bio-Platforms Inc., Calgary, AB, Canada) was included at 0.0% or 0.1% in the diets at the expense of the base cereal grain. The ME_{blend} was prepared to target a range of NSP and supplied 3,000, 500, 2,000, 12,000, 6,000, and 700 units of xylanase, glucanase, cellulase, amylase, protease, and invertase, respectively, per kg of diet at 0.1 % inclusion. Titanium dioxide was included at 0.3 % of the diet as an indigestible marker to determine nutrient digestibility (Zhang and Adeola, 2017). Seven diets with 2 ME_{blend} inclusion levels resulted in 14 dietary treatments.

Animals, Experimental Design, and Sample Collection

Following confirmation of pregnancy, 27 market gilts (Camborough L1050-PIC x Duroc; initial bodyweight 176 ± 6.6 kg) in a single group were allocated to one of the 14 dietary treatments in a crossover design with four periods (periods 1, 2, 3, 4 from d 41 to 55, 56 to 70, 71 to 85, 86 to 100 of gestation, respectively) to determine ATTD of energy and neutral detergent fiber (**NDF**). This allowed for a minimum of 7 observations for each dietary treatment. Sows were housed individually in gestation stalls (0.68 m × 1.98 m) equipped with a nipple drinker and a dry feeder. Sows were limit-fed 2.2 kg/d of their assigned experimental diet in a single feeding (0800 h). In each period, diets were fed for 14 d with d0 - 9 considered an adaptation period followed by 5 d of total collection of urine and grab fecal samples (d11 - 14).

Urinary catheters (Lubricath, 2-way; Bard Medical Division, Covington, GA, USA; 18 Fr x 30 mL) were inserted on d 9 of each period and connected to closed containers in the same manner described by Miller et al. (2016). Urine sample collection commenced on d10 and continued until 3 successful 24 h collections of urine were achieved. Three days was deemed adequate for a representative sample and to avoid potential contamination from urinary tract infections (Levesque, unpublished data). A 24 h flush period was allowed if a sow removed her urinary catheter and displayed signs of urinary tract infection before reinserting catheters. Sulfuric acid (10–20 mL/24 h) was added to the collection containers to stabilize pH; containers were weighed and 10% of each 24 h collected urine by weight was stored at -20°C. Urine samples were thawed following the conclusion of the experiment to be pooled within sow and collection period, subsampled, and stored at -20°C until further analysis. Fecal samples were collected once daily by rectal palpation, pooled within sow and collection period, and stored at -20°C until analysis. Rectal palpation was sufficient to induce a complete defecation from which fecal samples were collected.

Chemical Analyses

Prior to analysis, fecal samples were freeze-dried (Dura-Dry, Fits Systems, Kinetics Thermal Systems, Pennsauken, NJ, USA). Feedstuffs, diets, and fecal samples were finely grounded (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany). The dry matter (**DM**) content of the feedstuffs, diets, and fecal samples was determined by drying samples at 102°C for 24 hours using a drying oven (Myers et al., 2004). Neutral detergent fiber was measured on the feedstuffs, diets, and fecal samples according to AOAC procedure 2002.04 by an Ankom fiber analyzer (Ankom Technology, Macedon, NY, USA).

Samples of the feedstuffs and diets were analyzed for crude protein (**CP**) (method 968.06), ether extract (**EE**) (method 920.39), ash (method 923.03) according to AOAC (2005). Acid detergent fiber (**ADF**) in diets and feedstuffs was analyzed using an Ankom 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA) according to the method of Van Soest et al. (1991). Sugars, total dietary fiber (**TDF**), NSP, and water-soluble NSP were analyzed as described by Slominski and Campbell (1990) and Slominski et al. (1994). Total starch content in the diets and feedstuffs was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland) following the manufacturer’s procedures.

The feed, feces, and urine gross energy (**GE**) content was analyzed by bomb calorimetry (Parr 6300 calorimeter, Parr Instruments Co., Moline, IL). For fecal samples 1 g of ground feces were pressed into a pellet using a pellet press (Parr Instruments Co., Moline IL), and placed into the bomb calorimeter. The sample was analyzed in duplicate and repeated if the difference between the two values was more than 2 %. Analysis of urine GE followed the procedure as described by Kim et al. (2009).

Quantification of titanium in the diet and feed samples was based on Myers et al. (2004) with modifications. Briefly, DM was determined by weighing out 5.0 g of feed, 0.3 g of feces, or 0.5 g of digesta into a pyrex tube. The tube containing the sample was placed in a drying oven (105 °C) for 24 h. Samples were then ashed in a muffle furnace at 525°C for 10 h and difference in tube weight after ashing was used to calculate DM content. A 0.05 g subsample of the ashed feed samples were weighed out before continuing with the quantification. A total of 0.8 g of anhydrous sodium sulphate (Na_2SO_4) and 5 mL of concentrated sulphuric acid (H_2SO_4) was added to each ashed sample. Samples were heated for 20 h at 120°C and filtered; 5 mL of the filtered sample solution was transferred to plastic tubes to be combined with 0.2 mL of 30% hydrogen peroxide (H_2O_3). A total of 280 µL of the standards and samples were transferred into a 96 well plated in duplicate. The absorbance was measured on SpectraMAX 190 plate reader (Corning Inc., Corning, NY, USA) at 408 nm.

Calculations

The ATTD of energy and nutrients was calculated according to the indirect evaluation method using the marker approach. The following formula was used to calculate the ATTD of nutrients:

$$\mathrm{ATTD,\%} = 100 - \left[100 \times \left(\frac{\mathrm{Ti_d} \times \mathrm{C_f}}{\mathrm{Ti_f} \times \mathrm{C_d}}\right)\right]$$

where Ti_d = concentration of titanium in the diet; Ti_f = concentration of titanium in feces; C_f = concentration of the component in feces and C_d = concentration of the component in the diet (Zhang and Adeola, 2017).

Digestible energy and ME of diets (kcal/kg of DM and as-fed) were calculated following the procedure of Adeola (2001).

$$\mathrm{DE} = 100 \times \frac{\mathrm{GE\ intake} - \mathrm{GE\ output}}{\mathrm{GE\ intake}}$$

$$\mathrm{ME} = 100 \times \frac{\mathrm{GE\ intake} - \mathrm{GE\ output} - \mathrm{GE\ urine}}{\mathrm{GE\ intake}}$$

Energy content (DE and ME) and digestibility of DM, NDF, and GE of SBM, FP, CM and SBP were calculated using the differential procedure (Kong and Adeola, 2014):

$$\mathrm{D_{ti}} = \frac{[\mathrm{D_{td}} - (\mathrm{D_{td}} \times \mathrm{D_{bd}})]}{\mathrm{P_{ti}}}$$

in which D_{bd}, D_{td}, and D_{ti} are the digestibility (%) of the component in the basal diet, test diets, and test ingredient, respectively, and P_{bd} and P_{ti} are the proportional contribution of the component by the basal diet and test ingredient to the test diet, respectively.

The NE values of the feedstuffs were also calculated from the ME and analyzed macronutrient content using prediction equation 1-7 from NRC (2012):

$$\mathrm{NE} = (0.726 \times \mathrm{ME}) + (1.33 \times \mathrm{EE}) + (0.39 \times \mathrm{Starch}) - (0.62 \times \mathrm{CP}) - (0.83 \times \mathrm{ADF})$$

where all nutrient and digestible nutrient contents are expressed as g/kg DM, the calculated NE on a DM basis was also recalculated to be expressed on an as-fed basis.

Statistical Analyses

The UNIVARIATE procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) was used to confirm the homogeneity of variance and to analyze for outliers. Data were analyzed using the MIXED procedure of SAS. Supplementation of ME_{blend} (with or without) was the fixed effect, and the collection period was used as the random effect. A student's t-test was determined to be the most sufficient model to evaluate the effect of enzyme supplementation on feedstuffs. Tukey's adjusted means test was used to detect differences among treatments where $P \leq 0.05$ was considered significant. Values are presented as lsmeans and standard error of the mean (SEM).

RESULTS

General Observations

One gilt aborted during the first period and was removed from the trial. A second gilt was found deceased during the fourth collection period. Causes for abortion and death were determined by a veterinary pathologist to be not related to dietary treatments. Samples collected from these gilts during the collection period of their incidence were discarded and not evaluated for further analysis.

Chemical Analysis of Feedstuffs

The chemical composition of the feedstuffs evaluated in this study is listed in Table 3-1. Wheat had the greatest CP and starch content of the cereal grains. Neutral detergent fiber and ADF were the greatest in sorghum among the cereal grains. Corn had the greatest content of EE out of all feedstuffs. Of the protein concentrates, FP contained the greatest starch concentrations but was lowest in CP. Canola meal was 1.7x, 2.4x, and

3.3x greater in EE, NDF, and ADF concentrations, respectively, compared to SBM and FP.

The feedstuff with the highest concentration of total NSP was SBP (Table 3-1). Arabinoxylans were predominant, with significant amounts of β-glucans and cellulose in the cereal grains. Mannose was detected only in SBM. Canola meal and SBP contained the greatest amount of pectic polymers, as indicated by the relatively high concentrations of uronic acids and glucose. The arabinose and galactose not associated with pectic substances were derived from arabinan and arabinogalactan. In the concentrates, glucose was indicative of cellulosic polysaccharides. Xylose in the feed ingredients indicates the presence of xylans and xyloglucan. Total dietary fiber and water-insoluble NSP were greatest in the SBP followed by CM.

Apparent Total Tract Digestibility in Cereal Grains

Tendencies were observed for ME_{blend} supplementation to increase metabolizability of GE, ME (kcal/kg DM and as-fed), and predicted NE (kcal/kg DM and as-fed) ($P = 0.1$) of corn, but no other effects on corn were observed with ME_{blend} supplementation (Table 3-3). Multienzyme blend supplementation increased the ATTD of DM, NDF, and GE, as well as the DE and ME content (kcal/kg DM and kcal/kg as-fed) of wheat ($P < 0.01$). The predicted NE of wheat increased with ME_{blend} ($P \leq 0.01$). The digestibility and energy of sorghum were unaffected by ME_{blend} supplementation.

Apparent Total Tract Digestibility of Protein and Fiber Concentrates

The ATTD of GE was increased in SBM and CM diets with ME_{blend} supplementation ($P < 0.05$) with no significant effects on ATTD of DM and NDF (Table

3-4). Digestible energy (kcal/kg DM and as-fed), metabolizable GE, and ME (kcal/kg DM and as-fed) increased in SBM and CM diets with ME_{blend} supplementation ($P < 0.05$). The metabolizable GE in SBM diet tended to increase with ME_{blend} supplementation ($P = 0.08$). Multienzyme complex supplementation increased NE (kcal/kg DM and as-fed) of CM diet ($P = 0.02$) but did not affect NE content of SBM diet. Multienzyme blend supplementation impacted neither the digestibility nor energy content of the FP and SBP diets.

Apparent total tract digestibility of DM and GE increased in SBM with ME_{blend} ($P \leq 0.05$) (Table 3-5). The DE, ME, and NE (kcal/kg DM and as-fed) of SBM were increased with ME_{blend} ($P \leq 0.02$). The metabolizable GE, ME (kcal/kg DM and as-fed), and predicted NE (kcal/kg DM and as-fed) in FP tended to increase with ME_{blend} supplementation ($P = 0.01$). No other effects were observed for FP. Multienzyme blend increased the ATTD of GE, DE (kcal/kg DM and as-fed), ME (kcal/kg DM and as-fed), and predicted NE (kcal/kg DM and as-fed) of CM ($P = 0.01$). Sugar beet pulp was not influenced by ME_{blend} supplementation.

DISCUSSION

Exogenous feed enzymes are extensively utilized in modern livestock production to increase nutrient digestibility and feed efficiency of poor-quality feedstuffs (Torres-Pitarch et al., 2019). Considering that adult gestating sows have greater energy digestibility of feedstuffs compared to growing pigs (Lowell et al., 2015), ME_{blend} supplementation increased the ATTD of nutrients, NSP, and energy in high-fibrous gestation diets by 6% when fed to gestating sows (Shipman et al., 2023). The effects of

ME_{blend} on the digestibility of individual common feedstuffs, rather than complete diets, have been evaluated in grower pigs (Torres-Pitarch et al., 2019). Thus, the objective of this current study was to evaluate the effect of ME_{blend} supplementation on the energy digestibility of various feedstuffs representative of global swine-producing areas when fed to gestating sows.

Metabolizable energy (both DM and as-fed) for the corn, wheat, and sorghum without ME_{blend} supplementation were similar to values for gestating sows reported by Lowell et al. (2015). Wang et al. (2022) recently reported that the DE and ME of SBM fed to gestating sows were 4271 and 4039 kcal/kg, respectively, on a DM basis. The DE and ME observed in the study by Wang et al. (2022) are 10% greater than the DE and ME for SBM without ME_{blend} supplementation in the current trial. The lesser energy values for SBM observed in the current study might be due to the use of young pregnant gilts, while Wang et al. (2020) conducted their trial with older pregnant sows. Regardless of feed intake, the energy value of feed can be 10% greater in older adult sows than in younger gilts (Casa and Stein, 2017).

The low NSP and fiber content in corn, wheat, and sorghum allow these cereal grains to be primary energy sources in swine diets (Jaworski et al., 2015; Lowell et al., 2015). The arabinoxylan content found in these cereal grains can reduce potential energy availability due to the insoluble fraction which can reduce gastric transit time and limit nutrient digestibility in the gastrointestinal tract (Wenk, 2001; Jaworski et al., 2015; Petry et al., 2020). In this trial, the supplementation of a ME_{blend} increased the ME and NE of corn by 2% and produced a 3% uplift in the ME and DE of wheat when fed to the gestating gilts. Xylanase hydrolyzes the β-1,4-arabinoxylan-glycosidic bonds of arabinoxylan. The action of xylanase cleaves the fiber complexes and dietary arabinoxylan components while reducing the insoluble components in the small intestine (Petry et al., 2019). In grower pigs, single-component xylanase supplemented into high corn-fiber diets increased the DE and ME by 5 and 4%, respectively (Petry et al., 2020). This mode of action also occurs when xylanase is supplemented into wheat when fed to grower pigs (Lærke et al., 2015). Emiola et al. (2009) observed that the supplementation of a ME_{blend} (containing units of xylanase, glucanase, and cellulase) increased the total tract digestibility of energy, but not NDF, in corn-wheat dried-distiller grains with solubles based diet fed to grower pigs. This aligns with the observation in the current study in which the energy content of the corn was improved with ME_{blend} supplementation with no effects on the ATTD of NDF or DM.

Interestingly, the effect of ME_{blend} on energy uplift in wheat was 1% greater than seen with corn. No studies have been published that directly compared the effects of ME_{blend} in corn against wheat. Abelilla and Stein (2019), however, saw the supplementation of a single component xylanase in wheat-based diets fed to growing pigs was effective in increasing the ATTD of GE and DE and ME by 2%, but no impacts were seen with xylanase supplementation in corn-based diets. Xylanase supplementation also increased the ATTD of water-insoluble fibers of the wheat-based diets but no effects on the water-insoluble fraction in the corn-based diets. These authors (Abelilla and Stein, 2019) speculated the lack of a response by the specific xylanase used in their experiment were not effective in hydrolyzing the glycosidic and ester bonds in the arabinoxylans in corn as the structure of arabinoxylan in corn differs from wheat (Vehmaanperä, 2022). The structure and functionality of xylanases and other carbohydrases will differ based on the microbial organism the enzymes are produced by (Vehmaanperä, 2022). The efficacy of enzymes is in part related to a balance between substrate availability and enzyme concentration (Olukosi and Adeola, 2013). With there being a greater concentration of xylose and arabinose in the wheat than corn, the greater substrate availability in the wheat may allow for the exogenous xylanase in the ME_{blend} to be more effective compared to the corn. Furthermore, based on *in vitro* total tract digestibility (Jaworski et al., 2015), the total tract digestibility of the NSP component sugars in wheat is 20% greater than in corn. Therefore, greater ATTD of the NSP component sugars in wheat and ME_{blend} explain the greater uplift in energy for the wheat.

The effects of enzyme supplementation in sorghum-based diets have attracted less attention because sorghum has less soluble NSP than wheat and corn, and the viscosity is likely irrelevant in this grain (Bach Knudsen, 2014). Xylanase supplementation over a 42d period improved the performance of animals fed sorghum-based diets justifying that sorghum has sufficient arabinoxylan content to elicit a response to xylanase. (González-Ortiz et al., 2020). The studies that have evaluated NSP degrading enzymes in sorghum-based pig diets have been inconclusive because combinations or enzyme cocktails were used in most of these studies, so that the beneficial effects, if any, could not be distinguished. Park et al. (2003) found that inclusion of amylase and cellulase in finisher pig diets containing sorghum-soybean tended to increase average daily weight gain but did not affect feed efficiency and N digestibility and carcass characteristics. Plus, the majority of the NSP in the sorghum were insoluble. Insoluble NSP, through various mechanisms, decrease gastric transit time and hinder enzyme activity (Wenk, 2001). If the insoluble NSP decreased gastric transit time, then it is possible that the ME_{blend} did not have enough time to act on its respective substrates.

As all the NSP are present in plant feedstuffs to some extent, ME_{blend} that contain a wider range of the NSP-degrading enzymes allow for greater hydrolysis, cleavage, and digestibility of the NSP substrates compared to single-component carbohydrases (Meng et al., 2005). The extent of ME_{blend} effect was more prominent in the protein concentrates (SBM, FP, and CM) than in wheat and corn. Legumes, oil seeds, and their respective co-products contain a higher concentration of cellulose compared to cereal grains (Bach Knudsen, 1997). The similar structure between cellulose and β-glucan allows for the series of cellulases or glucanases to act on and cleave either polymer (Sadhu and Maiti, 2013). Greater nutrient digestion and energy uplift in the protein concentrates most likely came from the combined activities of cellulase and glucanase supplied in the ME_{blend}.

Soybean meal is a rich protein source with a highly digestible AA compared to other protein concentrates (Liu et al., 2016). In a study by Velayudhan et al. (2015), supplementing SBM with a ME_{blend} comprised of cellulase, pectinase, mannanase, galactanase, xylanase, glucanase, amylase, and protease increased the energy content of SBM by 6% when fed to growing pigs. This is in agreement with the current 6% energy uplift in SBM fed to pregnant gilts supplemented with ME_{blend}. The ME_{blend} used by Velayudhan et al. (2015) contained pectinase, mannanase, and galactanase that were not included in the ME_{blend} utilized in the current trial. Soybean meal contains a significant concentration of pectin polymers, as indicated by NSP uronic acids and galactose. The ATTD of NSP uronic acids and galactose, however, are, on average, 2 percentage units greater in gestating sows fed restrictively compared to ab *libitum* fed growing pigs (Shipman et al., 2023).

The ME and NE in FP were uplifted by 4% with ME_{blend} supplementation. Field peas contain high concentrations of starch and moderate concentration of aminos and fiber. There is also a high ratio of amylose: amylopectin in the starch granules (Woyengo and Zijlistra, 2021). To the author's knowledge, no studies have been conducted to evaluate the direct effects of ME_{blend} on energy digestibility in swine. A ME_{blend} (comprised of glucanase, phytase, protease, amylase, cellulase, and pectinase) reduced the excretion of P and N in growing pigs when fed diets containing FP (Nyachoti et al., 2006). A broiler study conducted by Meng and Slominski (2005) found the apparent metabolizable energy of a corn-pea diet was increased by + 140 kcal/kg (as-fed basis) when including a ME_{blend} comprised xylanase, glucanase, pectinase, cellulase, mannanase, and galactanase. The granules of amylose (linked by α-1,4 glycosidic bonds) and amylopectin (linked by α-1,6 glycosidic bonds), however, need to be opened for digestion by endogenous amylase for adequate breakdown into smaller oligosaccharides and eventually individual glucose monomers (Cowieson, 2005). Supplementation of exogenous amylase can act on the amylose and amylopectin that escape endogenous amylase activity and increase feedstuffs' energy and starch digestibility (Rupolo et al., 2023).

Canola meal contains a lower protein but a fiber concentration 3x greater than SBM (Liu et al., 2016; Woyengo and Zijlstra, 2021). As such, the inclusion of CM in gestation diets cannot be as high as SBM when formulating dietary energy (Liu et al., 2018). Utilizing a porcine *in vitro* digestion model, including an ME_{blend} increased the *in* *vitro* digestibility of the NSP component sugars of CM (Lee et al., 2018). Reducing the NSP content and breaking the caging effect of fiber allows for feedstuff to be more available in energy and fermentability, which will contribute to energy. Enzymatic action on breaking fiber encapsulation potentially explains the 10% uplift in energy of the CM when fed to gestating sows with ME_{blend} supplementation. Supplementation of ME_{blend} in CM or diets containing CM has been studied before in adult pregnant sows. Multienzyme complex inclusion in lactation diets containing CM had no effect on the ATTD of GE (Velayudhan et al., 2018). Velayudhan et al. (2019) reported no effect of a carbohydrase blend comprised of cellulase, pectinase, mannase, galactanase, xylanase, glucanase, amylase, protease, and phytase activities, (at 0.1% inclusion) on the ileal digestibility of amino acid or crude when CM was fed to sows in mid- or late-gestation.

Multienzyme supplementation did not influence the ATTD or energy content of the SBP. Crome et al. (2023) reported a multienzyme supplemented at 0.05% in complete corn-SBM based diets that contained a 20% inclusion of SBP increased the ATTD of GE, DM, and NDF by approximately 5% in gestating sows. The authors also observed a 4% uplift in the DE and ME of the corn-SBM-SBP diet with multienzyme, which included pectinase activity (Crome et al., 2023). Pectins (which include rhamnogalacturonan with associated side chains consisting of arabinose, galactose, and xylose residues) are the primary NSP in FP (Meng and Slominski, 2005). Exogenous pectinase can cleave the galacturonic polymer and de-esterifying the methyl esters of the pectic backbone (Sharma et al., 2013). This is most likely explains a lack of effect in the current trial as pectinase was not included in the ME_{blend}.

In summary, the inclusion of a multienzyme complex in diets at 0.1% inclusion levels can increase ATTD of nutrients and energy for gestating sows fed corn, wheat, soybean meal, field peas, and canola meal by approximately 5 to 10%, depending on the feedstuffs. The multienzyme blend had a greater effect on protein feedstuffs compared to the cereal grains. Multienzyme supplementation did not impact the energy or digestibility of sorghum and sugar beet pulp. From a practical standpoint, exogenous multienzyme blends can be used to increase the energy content of feedstuffs fed to gestating sows while also reducinging diet costs , but they are not recommended when feeding sorghum or sugar beet pulp.

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Table 3-1. Analyzed chemical composition of feedstuffs (%, as-fed basis).

Items	Corn	Wheat	Sorghum	Soybean meal	Field peas	Canola meal	Sugar beet pulp
Gross energy (kcal/kg)	3903	3843	3880	4123	3976	4330	3762
Dry matter	87.97	89.04	88.06	88.46	88.38	90.03	91.73
Crude protein (N x 6.25)	8.69	15.25	9.08	47.90	23.80	40.30	9.23
Ether extract	3.39	2.33	3.08	1.99	1.71	3.08	1.36
Starch	64.79	66.81	64.01	0.55	43.54	3.45	0.90
Ash	1.07	1.81	1.75	5.84	2.59	6.26	7.31
Neutral detergent fiber	8.36	12.89	15.63	8.62	15.70	26.96	37.57
Acid detergent fiber	2.59	4.24	7.08	5.69	7.01	20.59	22.40
Total NSP	6.63	8.85	8.54	14.13	13.12	17.78	54.97
Arabinose	1.07	2.12	1.30	1.99	2.27	3.78	17.44
Xylose	1.49	3.37	2.41	0.97	1.16	1.37	0.84
Mannose	nd	nd	nd	0.65	Nd	nd	0.70
Galactose	0.30	0.00	0.00	4.20	0.70	1.44	3.66
Glucose	3.22	2.88	4.29	3.41	6.45	6.12	16.35
Uronic acids	0.56	0.48	0.55	2.90	2.54	5.07	15.99
Water-soluble NSP	1.54	1.53	1.02	1.75	1.56	0.78	5.76
Water-insoluble NSP	5.09	7.32	7.53	12.37	11.56	16.99	49.21
Total dietary fiber	10.91	14.50	17.42	16.34	21.17	33.72	67.19

Abbreviations: NSP = non-starch polysaccharides; nd = not detected.

Table 3-2. Ingredient and analyzed chemical composition of the experimental diets (%, as-fed basis)[1].

Item	Corn	Wheat	Sorghum	Soybean meal	Field peas	Canola meal	Sugar beet pulp
Ingredient composition							
Corn	97.35	-	-	72.8	72.5	72.65	72.75
Wheat	-	97.77	-	-	-	-	-
Sorghum	-	-	97.5	-	-	-	-
Soybean meal	-	-	-	25.0	-	-	-
Field peas	-	-	-	-	25.0	-	-
Canola meal	-	-	-	-	-	25.0	-
Sugar beet pulp	-	-	-	-	-	-	25.0
Limestone	0.9	1.08	0.98	0.8	0.9	1.1	0.25
Dicalcium phosphate	1.1	0.5	0.9	0.9	0.95	0.75	1.35
Salt	0.15	0.15	0.12	-	0.15	-	0.15
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
TiO_2	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Analyzed composition							
Gross energy (kcal/kg)	3810	3797	3783	3809	3776	3858	3718
Dry matter	87.17	88.50	87.69	88.27	88.51	88.16	89.58
Crude protein (N x 6.25)	8.65	14.75	8.63	20.4	12.6	16.5	8.55
Ether extract	3.06	1.55	3.02	2.72	2.40	2.91	2.19
Starch	63.03	65.25	62.58	47.19	57.91	47.96	47.41
Ash	3.70	3.40	3.38	4.07	3.56	4.51	4.91
Neutral detergent fiber	8.28	12.03	11.82	8.52	12.03	13.95	16.22
Acid detergent fiber	2.36	3.65	5.46	3.79	4.02	6.86	7.53
Total NSP	7.10	7.82	7.33	8.53	9.34	8.86	18.91
Arabinose	1.07	1.67	1.06	1.31	1.42	1.63	4.43
Xylose	1.48	2.84	1.75	1.42	1.40	1.41	1.27
Mannose	nd	nd	nd	2.37	nd	nd	nd
Galactose	0.25	0.29	0.00	1.22	0.36	0.55	1.23
Glucose	3.78	2.64	4.05	3.12	5.07	3.55	6.48
Uronic acids	0.53	0.38	0.47	1.23	1.09	1.72	5.49
Total dietary fiber	10.83	13.80	13.94	12.52	16.35	16.87	25.55

Abbreviations: NSP = non-starch polysaccharides; nd = not detected.

[1] Multienzyme blend was included at 0 and 0.1% at expense of corn to create 2 different dietary treatments.

[2] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin 9.9 mg, Thiamine 3.3 mg.

[3] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Copper 20 mg, Manganese 40 mg, Selenium 0.3 mg, Zinc 170 mg.

Table 3-3. Apparent total tract digestibility (ATTD) of nutrients and energy in cereal grains fed to gestating sows with (+) or without multienzyme blend (ME_{blend}) by ingredient.

Feedstuff	Corn				Wheat				Sorghum			
Item	-	+	SEM	*P*-value	-	+	SEM	*P*-value	-	+	SEM	*P*-value
n	7	7			8	8			7	7		
ATTD, %												
DM	86.7	86.7	0.73	0.958	88.3	91.1	0.54	<0.001	84.2	85.1	0.55	0.265
NDF	50.1	54.7	4.79	0.374	63.9	73.1	1.27	<0.001	61.3	62.9	2.13	0.600
GE	88.2	88.7	0.76	0.538	89.3	91.9	0.46	<0.001	84.1	85.8	0.66	0.117
DE, kcal/kg												
DM	3794	3813	33.0	0.538	3810	3921	18.2	<0.001	3575	3643	27.9	0.117
As-fed	3338	3355	29.0	0.538	3392	3491	16.2	<0.001	3183	3244	2.1	0.117
ME: GE, %	86.2	88.0	1.11	0.099	88.8	91.2	0.45	<0.001	83.5	84.7	0.86	0.374
ME, kcal/kg												
DM	3708	3787	47.6	0.099	3786	3888	19.1	<0.001	3549	3597	36.4	0.374
As-fed	3262	3331	33.0	0.099	3371	3462	17.0	<0.001	3160	3203	27.9	0.374
NE, kcal/kg[1]												0.374
DM	2945	3002	34.6	0.099	2930	3004	13.9	<0.001	2773	2808	26.5	0.374
As-fed	2590	2641	30.4	0.099	2609	2675	12.3	<0.001	2469	2501	23.6	0.374

Abbreviations: SEM, standard error of the mean; GE, gross energy; DE, digestible energy; DM, dry matter; ME, metabolizable energy; NDF, neutral-detergent fiber.

[1] NE (kcal/kg) = (0.726 x ME) + (1.33 x EE) + (0.39 x Starch) – (0.62 × CP) – (0.83 × ADF); (Equation 1; NRC, 2012).

Table 3-4. Apparent total tract digestibility (ATTD) of nutrients and energy in test diets containing protein and fiber feedstuff fed to gestating sows with (+) or without multienzyme blend (ME_{blend}) by ingredient.

Feedstuff	SBM				FP				CM				SBP			
Item	-	+	SEM	*P*-value	-	+	SEM	*P*-value	-	+	SEM	*P*-value	-	+	SEM	*P*-value
n	8	8			7	7			8	8			7	7		
ATTD, %																
DM	86.3	86.9	0.76	0.159	87.4	47.9	0.75	0.884	78.5	79.7	1.11	0.345	80.7	80.6	0.54	0.902
NDF	53.2	54.0	3.27	0.772	70.1	72.2	2.60	0.503	47.4	50.3	3.65	0.555	65.6	63.3	3.28	0.442
GE	87.2	88.3	0.65	0.015	88.0	88.6	0.61	0.416	80.4	83.7	1.08	0.008	83.1	83.3	0.48	0.793
DE, kcal/kg																
DM	3842	3893	28.4	0.015	3757	3782	26.0	0.416	3578	3704	47.8	0.008	3505	3513	20.1	0.793
As-fed	3391	3436	25.1	0.015	3325	3348	23.0	0.416	3154	3265	42.2	0.008	3139	3147	18.0	0.793
ME: GE, %	86.1	87.0	0.78	0.075	87.4	87.8	0.61	0.341	79.8	82.9	1.23	0.018	82.4	82.5	0.62	0.944
ME, kcal/kg																
DM	3795	3834	33.0	0.748	3727	3745	25.8	0.341	3531	3669	54.4	0.018	3477	3480	26.2	0.944
As-fed	3350	3385	29.1	0.748	3299	3315	22.8	0.341	3113	3234	48.0	0.018	3115	3117	23.5	0.944

Abbreviations: SBM = soybean meal; FP = field peas; CM = canola meal; SBP = sugar beet pulp; SEM, standard error of the mean; GE, gross energy; DE, digestible energy; DM, dry matter; ME, metabolizable energy; NDF, neutral-detergent fiber.

Table 3-5. Apparent total tract digestibility (ATTD) of nutrients and energy in protein and fiber feedstuff fed to gestating sows with (+) or without multienzyme blend (ME_{blend}) by ingredient.

Feedstuff	SBM				FP				CM				SBP			
Item	-	+	SEM	*P*-value	-	+	SEM	*P*-value	-	+	SEM	*P*-value	-	+	SEM	*P*-value
n	8	8			7	7			8	8			7	7		
ATTD, %																
DM	87.8	92.4	2.58	0.015	90.2	91.9	2.01	0.358	53.0	57.8	4.46	0.347	62.0	62.3	2.35	0.889
NDF	51.8	52.6	10.83	0.931	76.3	78.1	4.58	0.788	50.6	54.3	7.74	0.730	73.7	79.8	6.80	0.270
GE	83.4	86.9	2.58	0.050	86.9	88.2	2.44	0.655	58.1	68.4	4.41	0.014	66.7	67.0	1.90	0.906
DE, kcal/kg																
DM	3980	4138	113.5	0.049	3685	3740	104.1	0.656	2860	3308	187.4	0.014	2735	2742	81.9	0.951
As-fed	3521	3660	100.4	0.049	3256	3305	92.0	0.656	2574	2977	168.6	0.014	2508	2514	75.1	0.951
ME: GE, %	81.4	86.2	2.82	0.020	79.3	82.3	2.30	0.093	55.7	68.2	4.43	0.011	64.2	65.8	2.37	0.677
ME, kcal/kg																
DM	3794	4016	131.6	0.020	3567	3704	103.4	0.093	2678	3280	213.2	0.011	2635	2698	97.2	0.677
As-fed	3356	3552	116.4	0.020	3152	3273	91.4	0.093	2410	2952	191.9	0.011	2417	2474	89.1	0.677
NE, kcal/kg[1]																
DM	2398	2559	95.5	0.020	2574	2673	75.1	0.093	1537	1974	154.8	0.011	1672	1717	70.6	0.677
As-fed	2121	2263	84.5	0.020	2275	2363	66.4	0.093	1384	1777	139.3	0.011	1533	1575	64.7	0.677

Abbreviations: SBM = soybean meal; FP = field peas; CM = canola meal; SBP = sugar beet pulp; SEM, standard error of the mean; GE, gross energy; DE, digestible energy; DM, dry matter; ME, metabolizable energy; NDF, neutral-detergent fiber.

[1] NE (kcal/kg) = (0.726 x ME) + (1.33 x EE) + (0.39 x Starch) – (0.62 × CP) – (0.83 × ADF); (Equation 1; NRC, 2012).

CHAPTER 4

EFFECTS OF POST-MSC DDGS INCLUSION IN GESTATION DIETS ON TOTAL TRACT NUTRIENT DIGESTIBILITY AND IN VITRO VOLATILE FATTY ACID PRODUCTION

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ABSTRACT

The objectives of this study were 1) to determine apparent total tract digestibility (**ATTD**) of energy and nutrients of post-protein separation (**post-MSC**) DDGS provided to gestating sows in comparison with commonly used fiber sources and 2) to characterize hindgut gas production following in-vitro fermentation using fecal inoculum collected from the sows fed different fiber sources. Twenty-two pregnant sows (parity 0 to 1; bodyweight 190 ± 10 kg) were allocated to 1 of 6 dietary treatments in a crossover design with two collection periods (period 1 from day 40-59 of gestation and period 2 from day 64-83 of gestation). Two of the 6 diets were corn-soybean meal based complete diets formulated to meet nutrient requirements for gestating sows with either soybean hulls (**SH**) or post-MSC DDGS to determine the ATTD of energy and nutrients of the diets. The remaining 4 diets included a corn-basal diet and the basal diet plus 300 g/kg inclusion of the post-MSC DDGS, SH or sugar beet pulp (**SBP**) to determine the respective energy digestibility of each fiber source. Sows were fed experimental diets in two 20-day collection periods where the initial 14 days were considered a diet adaptation period followed by 5 days for a total collection of urine and grab fecal samples. For *in vitro* fermentation, fresh fecal samples were collected on day 16 of each collection period, prepared as inoculum, and subjected to the gas production measurement technique through *in vitro* fermentation. Data for the two practical diets were analyzed using a t-test while an analysis of variance was conducted on the data of the fiber sources. The ATTD coefficient of ether extract of the post-MSC DDGS complete diet was 13% greater than the complete SH diet ($P < 0.01$). Total gas production of the post-MSC DDGS practical diet was 132 ml/g lower than the complete SH diet ($P = 0.03$). The

ATTD coefficient of gross energy of post-MSC DDGS was 16% greater than SH but 4% lower than SBP (*P* < 0.01). This resulted in 5.1 and 4.9 MJ/kg greater digestible energy and metabolizable energy content, respectively on an as-fed basis in post-MSC DDGS compared to soyhulls (*P* < 0.01). Total gas production was 140 mg/l less for post-MSC DDGS than SH (*P* < 0.01). In conclusion, post-MSC DDGS is suitable for inclusion in gestating sow diets and can contribute to sustainable swine production by lowering total gas production.

KEYWORDS

Energy digestibility, gestating sows, *in vitro* fermentation, post-MSC DDGS, volatile fatty acids

ABBREVIATIONS

AA, amino acids; ADF, acid detergent fiber; ATTD, apparent total tract digestibility; CF, crude fiber; DDGS, corn dried distillers grains with solubles; DE, digestible energy; DM, dry matter; EE, ether extract; GE, gross energy; ME, metabolizable energy; NDF, neutral detergent fiber; post-MSC DDGS, post-protein extraction dried distillers grains; SBP, sugar beet pulp; SH, soybean hulls; SID, standardized ileal digestibility; VFA; volatile fatty acids.

INTRODUCTION

The United States produced approximately 15.3 billion gallons of ethanol as a renewable biofuel in 2022 (Renewable Fuels Association, 2023). Cereal grains (such as corn, wheat, and sorghum) are used as feedstock for ethanol production and are increasingly used as resources for renewable biofuels (Lywood and Pinkney, 2012; de Corato and Viola, 2023). This is problematic as these cereal grains are also commonly used as the base energy ingredients in livestock feeds, resulting in increased competition for grains between ethanol and food animal production, thus there is interest in utilizing alternative ingredients for feeding livestock (Shurson, 2017). Cereal grain starch and fermentable carbohydrate content are isolated during the fermentation process. This is only a 1% of 2.5% loss in energy when converting the cereal grain to ethanol. The leftover nutrients available for energy utilization by the animal (*i.e.*, protein, oil, and fiber) concentrate in the co-products. These co-products, such as corn-dried distiller grains with solubles (**DDGS**), provide an economical alternative energy source to sustain efficient pork and other food animal production (Lywood and Pinkney, 2012; USCG, 2023).

The high fiber content of DDGS can reduce the performance and efficiency of growing pigs (Shurson et al., 2012) but make it an ideal candidate for inclusion in high-fibrous gestation sow diets (Meunier-Salaün and Bolhius, 2015). The impacts of DDGS inclusion in gestation diets on reproductive performance and behavior have been previously investigated, but results have been inconsistent (Wilson et al., 2005; Li et al., 2014). This might be partly due to diet formulations for pregnant sows being based on ingredient nutrient specifications from grower pigs; in particular fibrous ingredients are routinely formulated in commercial sow diets with nutrient specifications attained from grower pigs or prediction equations (Dourmad et al., 2008; NRC, 2012). Gestating sows have greater utilization of nutrients and energy from fibrous ingredients and higher digestibility of various dietary fiber complexes., compared to grower pigs (Shipman et al., 2023). This inaccuracy can lead to over-formulation, increases in feed costs, and inefficiencies (Vonderohe et al., 2022).

Soybean hulls (**SH**) and sugar beet pulp (**SBP**) are other co-products of biofuel production (de Corato and Viola, 2023) that are commonly fed as a fiber source in gestation diets to promote satiety and sources for fermentation in the hindgut to produce energy-efficient volatile fatty acids (**VFA**) (Meunier-Salaün and Bolhius, 2015; Theil et al., 2020; Wisbech et al., 2022). Sugar beet pulp is a rich source of soluble and fermentable NSP (Bach Knudsen, 1997), but a higher intake of fermentable fiber can result in higher methane and cumulative gas emissions by gestating sows (Rijnen et al., 2001; Phillippe et al., 2015). Corn co-products and SH have a lower content of soluble NSP and greater insoluble properties that reduce the potential of microbial fermentation, which may result in higher gas emissions (Bach Knudsen, 2014; Jaworski et al., 2015).

While formulating diets accurately is one way to promote sustainability in the swine industry, feeding ingredients that produce less gas emissions is also a form of environmental stewardship (Stinn et al., 2014). This comes at a time when more specialized DDGS and other ethanol by-products are being developed to meet the needs of commercial swine production (Garavito-Duarte et al., 2023; USGC, 2023).Mechanical separation allows the high protein and amino acid (**AA**) content in the whole stillage to be exctracted to produce high protein DDGS that is better suited for grower swine diets and pet foods (USGC, 2023). The leftover fiber fraction of post-protein separation DDGS (**post-MSC DDGS**) might be a valuable fiber source in gestation diets. Thus, the objective of this study was to determine apparent total tract digestibility (**ATTD**) of energy and fiber of post-MSC DDGS provided to gestating sows in comparison with commonly used fiber sources. A simultaneous study was conducted with fecal inoculum collected from sows fed different fiber sources to characterize hindgut gas production using an *in-vitro* fermentation model**.**

MATERIALS AND METHODS

Experimental procedures were conducted at the South Dakota State University Swine Education and Research Facility, in Brookings, SD, USA, following approval by the Institutional Animal Care and Use Committee (IACUC #2310-003A).

Feedstuffs and Dietary Treatments

The three fiber feedstuffs evaluated in this study were post-MSC DDGS (POET Bioproducts, Sioux Falls, SD 57104, USA), SH, and SBP. All ingredients originated from sources in the upper Midwestern United States.

There was a total of six diets fed during this experiment. Two of the six diets were practical corn-soybean meal-based gestation diets with either SH (inclusion of 175.0 g/kg SH) or post-MSC DDGS (inclusion of 150.0 g/kg post-MSC DDGS) and formulated to a similar neutral detergent fiber (**NDF**) level (*i.e*., 175.0 g/kg), 13.8 MJ metabolizable energy (**ME**, MJ/kg), and standardized ileal digestible AA content (Table 4-2). The post-MSC DGGS and SH were submitted for proximate analysis, NDF analysis, and complete AA profiles (Agricultural Experiment Chemical Laboratories, University of Missouri, Columbia, MO, USA) before diet formulations. Digestible energy (**DE**), ME, net energy (**NE**) and AA standardized ileal digestibility content of the post-MSC DDGS used to formulate and meet gestating energy and AA requirements were attained from an energy and ileal digestibility trial conducted in grower pigs (POET Bioproducts; unpublished).

Energy and standardized ileal digestible AA content of the SH and other ingredients included in the complete practical diets were based on the ingredient nutrient composition tables in NRC (2012) which are based on data in growing pigs. The complete post-MSC DDGS diet contained 85.0 g/kg SH to limit any potential negative impacts of branched-chain AA antagonism resulting from the high Leu content in the post-MSC DDGS (Cemin et al., 2019; Holen et al., 2022).

The remaining four diets were corn-basal diets and the basal diet plus 300 g/kg post-MSC DDGS, SH, or SBP to determine ATTD of energy of each added fiber source. Mineral and vitamin inclusion met mineral and vitamin NRC (2012) recommendations for gestating sows (Table 4-2). Synthetic AA were included in the energy-corn-basal diets to meet 75% of AA requirements for gestating sows (NRC, 2012) to limit any negative impacts of lower AA intake from the energy-corn-basal diets in the proceeding farrowing and lactation period.

All six experimental diets were fed in mash form and contained 3.0 g/kg titanium dioxide (TiO_2) as an indigestible marker to determine nutrient digestibility (Zhang and Adeola, 2016).

Sows, Experimental Design, and Sample Collection

Twenty-two pregnant sows (Camborough L1050, PIC, Hendersonville, TN, USA; parity 0 and 1; bodyweight 190 ± 10 kg) were allocated to 1 of the 6 dietary treatments in a crossover design with two periods (period 1 from days 40-59 of gestation and period 2 from days 64-83 of gestation) to determine ATTD of energy and fiber. This allowed for 7 observations per diet except for the post-MSC and SH test diets, which had 8 observations each. Across the two periods, all sows received one practical diet and one corn-basal diet. Sows were housed individually in gestation stalls (0.68 m × 1.98 m) equipped with a nipple drinker and a dry feeder. Sows were limit-fed 2.2 kg/day of their assigned experimental diet in a single feeding. Diets were fed for 19 days, with days 0-14 considered an adaptation period followed by 5 days for total collection of urine and grab focal samples (days 15 – 19). There was a 4-day flush period between periods in which sows were fed the swine facility standard gestation diet that met or exceeded NRC (2012) nutrient requirement estimates.

Urinary catheters (Lubricath, 2-way; Bard Medical Division, Covington, GA, USA; 18 Fr x 30 mL) were inserted on day 14 of each period and connected to closed containers in the same manner as described by Miller et al. (2016). Sample collection commenced on day 15 and continued until day 19. Sulfuric acid (10–20 mL/24 h) was added to the collection containers to stabilize pH; containers were weighed and 10% of each 24 h collected urine by weight was stored at -20°C. Urine samples were thawed following the conclusion of the experiment, pooled within sow and collection period, subsampled, and stored at -20°C until further analysis. Fecal samples were collected once daily by rectal palpation, pooled within sow and collection period, and stored at -20°C until analysis. Rectal palpation was deemed sufficient to induce a complete defecation from which fecal samples were collected. Prior to analysis, fecal samples were freeze dried (Dura-Dry, Fits Systems, Kinetics Thermal Systems, Pennsauken, NJ, USA) and finely grounded (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany).

In-vitro Fermentation and Cumulative Gas Production

Prior to *in-vitro* fermentation to measure cumulative gas production, samples were subjected to *in vitro* enzymatic hydrolysis as modified by Bindelle et al. (2007). Briefly, 4 g of the ground samples were weighed into conical flasks (500 mL) and mixed thoroughly with 200 mL of 0.1 M phosphate buffer solution (pH 6.0) and 80 mL of 0.2 M HCl solution. Two milliliters of chloramphenicol solution (0.5 g/100 mL ethanol) were added to each flask to inhibit bacterial growth during enzymatic hydrolysis. Eight milliliters of a freshly prepared pepsin solution (20 g/l porcine pepsin; P-7012, Sigma-Aldrich Corp., St. Louis, MO, USA) was added to each flask, and the mixture was incubated in a shaking water bath (50 rpm) at 39 ◦C for 2 h. After pepsin hydrolysis, 80 mL of 0.2 M phosphate buffer solution (pH 6.8) and 40 mL of 0.6 M NaOH were added to each flask and mixed. Eight milliliters of freshly prepared pancreatin solution (100 g/l pancreatin; P-1750, Sigma-Aldrich Corp., St. Louis, MO, USA) was added to each flask, and incubated in a water bath for 4 h under the same conditions. At the end of the enzymatic hydrolysis, the undigested residue was collected by filtration using nylon clothes (50 μm) and thoroughly washed with 96% ethanol, and 99.5% acetone. The washed residue was dried at 60 ◦C for 16 h. The dried residue was accurately weighed to determine the coefficient of in vitro disappearance of dry matter. Each treatment's undigested residue from the batches was pooled for further *in-vitro* fermentation.

Microbial fermentation rate and extent of experimental diets were determined using a cumulative gas-production technique as described in Bindelle et al. (2007) and Jha et al. (2011) with modifications. Fresh fecal samples from each sow were collected on days 16 and 17 of each collection period and prepared as inoculum for their respective dietary treatments. This ensured that the *in vitro* fermentation was conducted under similar hindgut conditions representative of the sow. The collected feces were immediately placed in plastic syringes to avoid exposure to aerobic conditions, diluted 2 times in the buffer solution, filtered through a 250 μm-screen sieve, and dispersed amongst 4 bottles containing their respective dietary treatment. The bottles were completely sealed with rubber stoppers and immediately placed in the water bath for incubation (39 ◦C). During the preparation of inoculum and its transfer into bottles, anaerobic conditions were maintained by flushing with CO_2 gas. Briefly, 200 mg samples of *in-vitro* digested residue were incubated for 72 h at 39 ◦C in 125 mL-glass bottles with 30 mL of the freshly prepared fecal inoculums in a water bath shaking at 50 rpm. Using a pressure transducer, gas production was recorded at 0, 2, 5, 8, 12, 18, 24, 36, 48 and 72 h during the incubation. At the conclusion of the incubation, the glass bottles were immediately immersed in ice to terminate microbial fermentation. Bottle contents were transferred into 50 mL centrifuge tubes and stored at -20◦C for further analysis. The experimental scheme was: {[(6 treatments × 7 or 8 replicates × 4 bottles) + 6 blanks] × 2 batches} with bottle and animals serving as the replicates for dietary treatments.

Chemical Analyses

The dry matter (DM) content of the feedstuffs, diets, and fecal samples was determined by drying samples at 102℃ for 24 h using a drying oven (Myers et al., 2004). Nutrient analyses of feedstuffs, diets, and fecal samples were conducted at the Agricultural Experiment Chemical Laboratories, University of Missouri (Columbia, MO, USA). Samples were analyzed for moisture (AOAC Official Method 934.01, 2006), crude protein (**CP**; AOAC Official Method 990.03, 2006), ether extract (**EE**; AOAC Official Method 920.39 (A); 2006), ash (AOAC Official Method 942.05, 2006), NDF "assayed with a heat stable amylase and expressed inclusive of residual ash” (**aNDF**; van Soest et al., 1991), acid-detergent fiber (**ADF**; AOAC Official Method 973.18, 2006), and a complete AA profile (AOAC Official Method 982.30 E (a,b,c)).

The gross energy (**GE**) content of feedstuffs, diets, feces, and urine was analyzed by bomb calorimetry (Parr 6300 calorimeter, Parr Instruments Co., Moline, IL, USA). For feedstuffs, diets, and feces samples, 1 g of grounded samples were pressed into a pellet using a pellet press (Parr Instruments Co., Moline IL, USA) and placed into the bomb calorimeter. The sample was analyzed in duplicate and repeated if the difference between the two values was more than 2 %. Urine GE analysis followed the procedure described by Kim et al. (2009).

Quantification of titanium in feed, fecal, and digesta samples was based on Myers et al., (2004) with modifications. Briefly, dry matter was determined by weighing out 5.0 g of feed or 0.3 g of feces into a pyrex tube. The tube containing the sample was placed in a drying oven (105 °C) for 24 h. Samples were then ashed in a muffle furnace at 525°C for 10 h, and the difference in tube weight after ashing was used to calculate DM content. A 0.05 g subsample of the ashed feed samples were weighed out before continuing with the quantification. A total of 0.8 g of anhydrous sodium sulphate (Na_2SO_4) and 5 mL of concentrated sulphuric acid (H_2SO_4) was added to each ashed sample. Samples were heated for 20 h at 120°C and filtered; 5 mL of the filtered sample solution was transferred to plastic tubes to be combined with 0.2 mL of 30% hydrogen peroxide (H_2O_3). A total of 280 μL of the standards and samples were transferred into a 96 well plated duplicate. The absorbance was measured on SpectraMAX 190 plate reader (Corning Inc., Corning, NY, USA) at 408 nm.

The total starch content of the corn, post-MSC DDGS, SH, SBP, and two complete practical diets was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd., Wicklow, Ireland) following the manufacturer's procedures.

Fermentation supernatants were analyzed at the University of Kentucky. Volatile fatty analysis by gas chromatography (Hewlett-Packard 6890 Plus GC; Agilent Technologies Inc., Santa Clara, CA, USA) following the procedures by Erwin et al. (1961) and Ottenstein et al. (1971). Total VFA concentration was considered as the sum of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate concentrations. Molar VFA proportions were calculated as the individual VFA concentration divided by the total VFA concentration and multiplied by 100.

Calculations

The ATTD coefficients of energy and nutrients were calculated according to the indirect evaluation method using the marker approach. The following formula was used to calculate the ATTD coefficient of nutrients:

$$\text{ATTD coefficient} = 1 - [(\text{Ti}_\text{d} \times \text{C}_\text{f})/(\text{Ti}_\text{f} \times \text{C}_\text{d})]$$

where Ti_d = concentration of titanium in the diet; Ti_f = concentration of titanium in feces; C_f = concentration of the component in feces and C_d = concentration of the component in the diet (Zhang and Adeola, 2017).

Digestible energy and ME of diets (MJ/kg of DM and as-fed) were calculated following the procedures of Adeola (2001).

$$\text{DE} = 100 \times [(\text{GE intake} - \text{GE output})/\text{GE intake}]$$

$$\mathrm{ME} = 100 \times [(\mathrm{GE\ intake} - \mathrm{GE\ output} - \mathrm{GE\ urine})/\mathrm{GE\ intake}]$$

Energy content (DE and ME) and digestibility of fiber of post-MSC DDGS, SH, and SBP were using the differential procedure (Kong and Adeola, 2014):

$$\mathrm{Dti} = [\mathrm{D_{td}} - (\mathrm{D_{td}} \times \mathrm{D_{bd}})]/\mathrm{P_{ti}}$$

in which D_{bd}, D_{td}, and D_{ti} are the digestibility (%) of the component in the basal diet, test diets, and test ingredient, respectively, and P_{bd} and P_{ti} are the proportional contribution of the component by the basal diet and test ingredient to the test diet, respectively.

The NE values of the feedstuffs and two practical diets were also calculated from the ME and analyzed macronutrient content using prediction equation 1-7 from NRC (2012):

$$\mathrm{NE} = (0.726 \times \mathrm{ME}) + (1.33 \times \mathrm{EE}) + (0.39 \times \mathrm{Starch}) – (0.62 \times \mathrm{CP}) – (0.83 \times \mathrm{ADF})$$

where all nutrient and digestible nutrient contents are expressed as g/kg DM. The calculated NE on a DM basis was also recalculated to be expressed on an as-fed basis. The DE, ME, and predicted NE were originally given in kcal/kg and were converted to MJ/kg using the conversion factor of 0.004184.

The ideal gas law was used to convert gas pressure measurements during 72 h of microbial fermentation into gas volume (*G*, g^{-1} DM), using an atmospheric pressure of 101,325 Pa and a temperature of 312.15 K. Accumulative gas curves during microbial fermentation were modeled using an equation described in France et al. (1993):

$$\mathrm{G}\ (\mathrm{ml\ g} - 1\ \mathrm{DM}) = 0, \mathrm{if}\ 0 < \mathrm{t} < \mathrm{L}$$

$$\mathrm{G}\ (\mathrm{ml\ g^{-1}\ DM}) = \mathrm{G_f}\left(1 - \exp\left\{-\langle b(t - L) + c\left(\sqrt{t} - \sqrt{L}\right)\rangle\right\}\right), \text{if } t \geq L$$

where G denotes the gas accumulation to time, Gf (mlg^{-1} DM) denotes the maximum gas volume for t = ∞ and L (h) denotes the lag time before the fermentation starts. The constants (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:

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

Kinetics parameters (G_f, L, $\mu_{t=T/2}$ and $T/2$) were compared in the statistical analysis. The $T/2$ is the time to half-asymptote when $G = G_f/2$.

Statistical Analyses

Data for the two practical diets were compared with a student’s t-test while energy diets were analyzed using an analysis of variance and fixed to a mixed linear model using treatment as the main effect and collection period or batch as a random variable. The UNIVARIATE procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) was used to confirm the homogeneity of variance and to analyze for outliers. Data were analyzed as the MIXED procedure of SAS with collection period as the blocking factor. Tukey’s adjusted means test was used to detect differences among treatments where $P \leq 0.05$ was considered significant. Values are presented as lsmeans and standard error of the mean (SEM).

RESULTS

Chemical Composition of Feedstuffs and Experimental Diets

The GE content of the post-MSC DDGS was higher compared to the corn, SH, and SBP (Table 4-1). The content of CP and EE was higher in the post-MSC DDGS than in the other feedstuffs. Ash content was similar between the post-MSC DDGS, SH, and SBP but greater in the fibrous feedstuffs than of corn. Sugar beet pulp was lower in starch compared to the post-MSC DDGS and SH, but corn contained approximately 800 g/kg of starch more on average than the fibrous feedstuffs. Crude fiber, aNDF, and ADF were greater in the SH compared to corn, post-MSC DDGS, and SBP.

The dietary aNDF content was similar for the post-MSC DDGS and SH practical dietary at an average of 160.0 g NDF/kg of diet (Table 4-2). Gross energy content was higher in the post-MSC DDGS practical diet. Dietary CP and EE were also greater in the practical post-MSC DDGS diet. Ash content was similar between both practical diets. The practical SH diet had higher CF, ADF, and starch concentrations than the practical post-MSC DDGS diet.

Regarding the corn-energy diets (Table 4-2), the basal + post-MSC DDGS diet contained a greater GE content compared to the corn-basal, basal + SH, and basal + SBP diets. Crude protein and EE were also greater in the basal + post-MSC DDGS diet. The basal + SH diet had the greatest CF, aNDF, and ADF concentrations than the other corn-basal diets.

Complete Diets

The coefficients for ATTD of DM, aNDF, or ADF did not differ between the practical diets (Table 4-3). The ATTD coefficient of EE in the practical post-MSC DDGS diet was greater than the practical SH diet ($P < 0.01$). The SH practical diet had a greater ATTD of CF ($P = 0.05$).

While the coefficient ATTD of GE between the two diets did not differ, the practical diet containing the post-MSC DDGS had greater DE content compared to the SH practical diet (both on an as-fed and DM basis; $P < 0.01$) (Table 4-3). The ME content of the post-MSC DDGS complete diet was also greater than that of the SH practical diet (both on an as-fed and DM basis; $P < 0.01$) without differences in the metabolizable GE content (ME/GE). The predicted NE was higher in the practical post-MSC DDGS diet (both on an as-fed and DM basis; $P < 0.01$).

Lag time (time required to break down organic matter into gases and initiate fermentation), rate of degradation (rate of degradation of undigested residues), or half-time (period when gas volume was half of the cumulative gas volume) for the *in-vitro* fermentation did not differ between the two practical diets. Total gas production (Table 4-3) of the practical diet containing post-MSC DDGS diet was lower ($P = 0.03$) in comparison to the practical SH diet (Figure 4-1).

The quantitative concentrations (mM mol/100mol) of total VFA, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate did not differ between the two practical diets (Table 4-4). The proportion of isovalerate relative to the total quantitative concentration of VFA (% of total VFA mM mol/100mol) tended to be greater in the practical post-MSC DDGS diet ($P = 0.09$). Proportions of acetate, propionate, isobutyrate, butyrate, isovalerate and valerate relative to the total concentration of VFA did not differ between either practical diet.

Test Diets and Ingredients

The corn-basal diet had the highest ATTD coefficient for DM, EE, and aNDF (*P* < 0.01) (Table 4-5). The post-MSC DDGS diet was greatest in the ATTD coefficient of ash (P < 0.01) but tended to be lowest in the ATTD coefficient for ADF (*P* = 0.08). Lag time did not differ between the corn-basal diets. The rate of degradation was highest in the corn-basal and SBP diets (*P* < 0.01). Half-time was highest in the post-MSC DDGS diet and lowest in the corn-basal diet (*P* = 0.01). The post-MSC DDGS diet had the lowest total gas production (*P* < 0.01; Fig 2).

There were no differences in the quantitative concentrations of acetate, isobutyrate, butyrate, isovalerate, or total VFA among the corn-basal diets (Table 4-6). The corn-basal diet had the highest quantitative concentrations of propionate and valerate (*P* < 0.05). Relative to the total concentrations of VFA, the post-MSC DDGS diet had the highest concentrations of butyrate and isovalerate (*P* < 0.03) but lowest proportion of propionate (*P* = 0.01). The corn-basal diet tended to have the greatest relative proportion of valerate (*P* = 0.06). A tendency was also detected for the relative concentration of acetate being greatest in the SH diet (*P* = 0.07).

The ATTD coefficient for GE of post-MSC DDGS was greater than SH and SBP (*P* < 0.01). This resulted in a greater DE and ME content, respectively on an as-fed basis in post-MSC DDGS compared to soyhulls and sugar beet pulp (*P* < 0.01). The post-MSC DDGS also had a NE value (as-fed basis) greater than SH and SPB (*P* < 0.01).

The concentrations of butyrate and isovalerate (relative to total VFA) were greater for the post-MSC DDGS than in the SH or SBP fermentation residues (*P* < 0.05). Concentrations of other VFA relative to total VFA did not differ between fiber sources.

DISCUSSION

The leftover fiber fraction in post-MSC DDGS might be a valuable fiber source in gestation diets as the addition of fiber is a strategic nutritional tool to promote the sensation of satiety, dilute energy intake to limit weight gain, and reduce negative stereotypical behavior associated with being limit-fed (Che et al., 2011). Fibrous ingredients are routinely formulated in commercial sow diets with nutrient specifications attained from grower pigs or prediction equations (Dourmad et al., 2008; NRC, 2012). Gestating sows, due to various factors, have approximately 7 and 11 percentage units greater ATTD of the dietary fiber fractions (NDF and ADF) and the NSP sugar components, respectively, compared to grower pigs (Shipman et al., 2023). This greater capacity to digest fiber results in the energy content of fibrous ingredients being greater when fed to gestating sows (Noblet and Shi, 1993). Lowell et al. (2015) found that gestating sows had 6% greater DE of diet containing either a conventional DDGS or low fiber-DDGS with a 19% greater total tract digestibility of NDF compared to growing pigs. Thus, energy content of alternative feed ingredients needs to be evaluated for more precise diet formulation and reduce the likelihood of overformulation.

While the two practical diets that included either the post-MSC DDGS or SH were formulated to a similar ME and aNDF content, both the DE and ME were 1.0 MJ/kg greater in the practical post-MSC DDGS compared the practical SH diet. The greater energy value in the practical post-MSC DDGS diet may reflect differences in the analyzed DE and ME of the post-MSC DGGS than the SH with the energy content being 1.5x greater in the post-MSC DDGS. The ATTD of GE is 4% greater in adult sows fed at maintenance compared to growing pigs (Le Goff and Noblet, 2001). The energy derived from fiber fermentation and the production of VFA can meet an estimated 15% of the maintenance requirement for energy for growing pigs and 30% for gestating sows (Jha and Berrocoso, 2016; Theil et al., 2020). Dietary oil is a highly digestible energy source for pigs and a 1% inclusion of soy oil can increase the DE, ME, and NE of diets by 0.25 MJ/kg (Li et al., 2018). The post-MSC DDGS also had a 5x greater EE content compared to that of the SH.

In adult sows, where the hindgut is more developed, gas production (primarily in the form of methane) can be 4x – 5x greater due to greater fermentative capacity. Increasing fermentable fiber content can also result in greater gas loss (Rijnen et al., 2001; Noblet and van Milgen, 2004). The greater cumulative gas production can explain the lowered energy ME and predicted NE of the SBP compared to the post-MSC DDGS and corn. The greater energy content of the post-MSC DDGS compared to the other two fiber sources is due to the significant EE and CP content that would have concentrated following the distillation process and removal of starch from the corn (Noblet and van Milgen, 2004; Shurson, 2017).

Interestingly, the ATTD coefficients for aNDF and ADF in the fibrous ingredients increased as aNDF and ADF intake increased. This aligns with a previous study conducted in our lab (Shipman et al., 2023), which found the ATTD of NDF increased when the intake of NDF increased in sows fed a 13.5% NDF diet and a 17.5% NDF diet. The digestion of fiber by microbial fermentation in the small intestine in grower pigs with a less developed gastrointestinal tract is minor. Adult gestating sows, however, can digest and ferment 25% of ingested dietary fiber by the terminal ileum (Jørgensen et al., 2007). The bulk of fiber fermentation occurs in the cecum and proximal and distal colon (Nahm, 2003). While minor and with little contribution to energy in growing pigs, gestating sows can digest fiber in their more developed small intestine (Jørgensen et al., 2007). Microbial carbohydrate degrading enzymes need to first hydrolyze, cleave, and depolymerize the fiber substrate to begin the fermentation process (Macfarlane and Macfarlane, 2003; Flint et al., 2012; Giuberti et al., 2015). The greater microbe density in the small intestine of adult sows and the availability of fiber substrates interact to result in extensive fiber digestion and energy utilization for gestating sows.

While not measured in this trial, the differences in gas production among the fiber diets might be attributed to the differences in non-starch polysaccharide and solubility properties among the test feedstuffs. Sugar beet pulp is a rich source of pectin (Bach Knudsen, 1997). Pectin is a favorable fermentable NSP that can result in the production of VFA. Soybean hulls share a similar pectin content as SBP but differ in solubility. The pectin and other NSPs comprising SBP are predominantly soluble and capable of incorporating water into the digesta matrix, resulting in swelling and increased bulkiness. This increased bulkiness slows down transit time in the gastrointestinal tract and gives the gut microbes extended time to ferment the fiber sourced by SBP. Soybean hulls, on the other hand, contain a significant amount of insoluble NSP that have a lower water-binding and swelling capacity (Bach Knudsen, 2014; Bachmann et al., 2021). The greater soluble fiber properties of the SBP allow it to be more rapidly degraded and fermented by microbes in the hindgut and culminates in greater gas production (Bachmann et al., 2021). This is evident in this trial as the *in* vitro gas production and rate of degradation was 24 and 7% greater, respectively, in the SBP diet compared to the SH diet.

The predominant non-starch polysaccharide found in corn and corn co-products are insoluble arabinose and xylose that serve as the building blocks for the polymer arabinoxylan, and the content of NSP in corn co-products can be 3x the amount seen in the parent grain (Jaworski et al., 2015). In this trial, the corn-basal diet and SBP diet shared a similar *in vitro* rate of degradation and cumulative gas production. The similarities of the *in vitro* kinetics, when corn has minimal soluble properties, can be attributed to the high starch content in the corn. Starch is also a fermentable polysaccharide that can be subjected to and favored for microbial fermentation. This observation agrees with Bachmann et al. (2021), who observed that *in vitro* kinetics were similar between SBP and corn starch. Corn DDGS is lower in soluble NSP compared to SH (Bach Knudsen, 2014; Jaworski et al., 2015). Huang et al. (2017) conducted a similar *in vitro* gas production study and found the gas production of corn DDGS was lesser than that of SH.

The most abundant VFA from fiber fermentation is acetate, propionate, and butyrate. Other acids include formate, valerate, caproate, in addition to biogases that are produced such as combustible methane and carbon-dioxide (Williams et al., 2001). In this trial, the quantitative and qualitative concentration of the VFA and branched-fatty acids (isobutyrate and isovaerate) were similar in the fermentation residues of the practical post-MSC DDGS and practical SH diets. Starch from the corn and the potential pectin from the SBP are favorable precursors to produce propionate during microbial fermentation (Giuberti et al., 2013; Larsen et al., 2018). This supports the observations in this trial of propionate being in similar quantitative and qualitative concentrations amongst the corn-basal, SH, and SBP diets and lower in the post-MSC DDGS diet. Concentrations of the other VFA and branched-chain fatty acids were similar amongst the fiber sources. This suggests that all fibrous feedstuffs evaluated are sufficient precursors for microbial fermentation to produce VFA that can be used for energy.

Increasing dietary fiber intake can divert the fermentation of protein and reduce the production of ammonia, and it can have a positive environmental impact on farms (Rijnen et al., 2001). However, the production of the biogas, methane, will increase with a greater intake of fermentable carbohydrates (Rijnen et al., 2001; Philippe et al., 2015). While methane and the other combustible gases were not measured in this trial, the lower cumulative gas production of the post-MSC DDGS diet might be suggestive that the post-MSC DDGS is a viable alternative fiber source that can produce similar concentrations of microbial fermented VFA without resulting in greater gas emissions.

Conclusion

Based on the results of this study, post-MSC DDGS is suitable for inclusion in gestating sow diets as both a fiber and energy source. When taking into consideration the societal pressure on the swine industry to minimize gas emission, ingredients such as post-MSC DDGS, that lower gas emission, can be important tools to ensure sustainable swine production. In conclusion, post-MSC DDGS has the potential to contribute to sustainable swine production when fed to gestating by lowering total gas production and being a source of volatile fatty acids.

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Table 4-1. Chemical composition of corn, post-MSC DDGS, soybean hulls, and sugar beet pulp (g/kg, as-fed basis).

Item	Corn	Post-MSC DDGS	Soybean hulls	Sugar beet pulp
GE, MJ/kg	15.6	18.0	16.3	16.0
DM	876.4	913.1	945.9	917.3
CP	79.3	253.2	123.5	92.1
EE	39.2	73.0	13.7	14.7
Ash	12.0	48.9	47.1	46.8
Starch	836.7	43.5	60.3	10.9
aNDF	105.3	384.4	578	451.1
ADF	22.9	137.2	439.9	236.9
Indispensable AA				
Arg	3.5	11.7	5.4	3.4
His	2.2	6.8	3.0	2.8
Ile	2.6	8.9	4.4	3.4
Leu	9.1	29.2	7.3	5.3
Lys	2.4	7.0	7.5	5.1
Met	1.6	5.1	1.3	1.4
Phe	3.6	22.2	5.8	3.3
Thr	2.7	9.3	4.0	4.1
Trp	0.6	1.4	0.6	1.0
Val	3.6	12.2	5.1	5.4
Dispensable AA				
Ala	5.6	18.9	4.8	4.2
Asp	5.2	15.7	10.6	6.5
Cys	1.7	5.2	2.0	1.0
Glu	13.9	40.6	13.1	9.2
Gly	3.0	10.6	8.2	3.9
Pro	6.6	22.2	5.8	4.1
Ser	3.7	11.3	5.6	4.4
Tyr	2.8	7.7	3.6	4.1
Total AA	73.3	239.9	103.4	69.2

Abbreviations: Post-MSC DDGS, post-protein extraction dried distiller grains with solubles (POET Bioproducts, Sioux Falls, SD, USA); GE, gross energy; DM, dry matter; CP, crude protein (N × 6.25); EE, ether extract; aNDF; neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber; AA, amino acid.

Table 4-2. Ingredient and analyzed composition of experimental diets (g/kg as-fed).

Item	Practical diets		Corn basal diets			
	Post-MSC DDGS[a]	Soybean hulls	Basal	Post-MSC DDGS	Soybean hulls	Sugar beet pulp
Ingredient						
Corn	659.0	704.0	964.0	675.0	671.0	670.0
Soybean meal	50.0	75.0	-	-	-	-
Soybean hulls	85.0	175.0	-	-	300.0	-
Post-MSC DDGS	150.0		-	300.0	-	-
Sugar beet pulp	-	-	-	-	-	300.0
Soy oil	30.0	18.0	-	-	-	-
Lysine-HCl	2.3	1.5	2.8	2.0	2.0	2.5
Threonine	0.6	0.8	0.8	-	0.8	1.3
Methionine	-	-	-	-	0.1	0.5
L-Tryptophan	-	-	0.3	0.1	0.3	0.3
L-Isoleucine	-	-	0.2	-	-	0.5
L-Valine	-	-	-	-	-	0.3
Limestone	11.0	8.0	10.5	15.0	6.0	3.5
Monocalcium-phosphate	6.5	11.5	12.5	15.0	14.0	15.0
Salt	3.4	3.5	4.3	3.5	4.0	2.7
Mineral premix[1]	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Titanium dioxide	3.0	3.0	3.0	3.0	3.0	3.0
Analyzed composition						
GE, MJ/kg	17.4	16.2	15.6	18.3	15.5	15.5
DM	885.3	881.6	869.2	8813	884.1	884.5
CP	133.5	113.4	79.3	128.7	92.8	82.1
EE	61.8	38.9	30.4	43.9	25.8	19.5
Ash	44.4	42.0	36.6	42.5	44.5	52.3
Starch	86.8	99.3	na	na	na	na
aNDF	162.9	157.0	75.7	158.8	211	208.6
ADF	86.8	99.3	29.5	58.3	140.9	115.4
Indispensable AA						
Arg	6.7	5.9	3.5	5.7	4.3	3.3

His	3.5	2.9	2.1	3.4	2.5	2.3
Ile	4.9	4.3	2.9	4.4	3.4	3.3
Leu	13.2	10.1	8.8	14.1	8.5	7.2
Lys	6.7	6.9	4.6	5.0	6.3	4.5
Met	2.2	1.6	1.5	2.3	1.6	2.0
Phe	6.1	5.1	3.6	5.9	3.9	3.4
Thr	4.9	4.9	3.4	4.4	4.0	3.5
Trp	0.7	0.8	0.9	1.0	0.9	0.8
Val	6.1	5.1	3.7	5.9	4.2	4.5
Dispensable AA						
Ala	8.2	6.1	5.4	9.0	5.3	4.8
Asp	10.0	9.6	5.4	8.0	7.4	5.6
Cys	2.4	1.9	1.6	2.5	1.8	1.4
Glu	22.1	18.3	13.9	21.7	14.1	11.1
Gly	5.4	5.0	2.9	4.9	4.5	3.2
Pro	9.6	7.5	6.6	10.6	6.4	5.5
Ser	5.6	4.9	3.3	5.2	4.0	3.4
Tyr	4.4	3.8	2.7	4.2	3.0	2.9
Total AA	125.7	108.2	79.4	120.7	89.9	77.3

Abbreviations: Post-MSC DDGS, post-protein extraction dried distiller grains with solubles (POET Bioproducts, Sioux Falls, SD, USA); GE, gross energy; DM, dry matter; CP, crude protein (N × 6.25); EE, ether extract; aNDF; neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber; AA, amino acids.

[1] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin 9.9 mg, Thiamine 3.3 mg.

[2] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Copper 20 mg, Manganese 40 mg, Selenium 0.3 mg, Zinc 170 mg.

Table 4-3. The apparent total tract digestibility (ATTD) coefficients of nutrients, digestible energy, metabolizable energy, net energy, and gas kinetics of practical diets containing either post-MSC DDGS or soybean hulls fed to gestating sows.

Item	Post-MSC DDGS	Soybean hulls	SEM	*P*-value
ATTD coefficients				
DM	0.85	0.84	0.007	0.333
EE	0.87	0.75	0.017	<0.001
Ash	0.40	0.30	0.037	0.050
aNDF	0.73	0.78	0.020	0.102
ADF	0.72	0.80	0.031	0.131
GE	0.85	0.84	0.007	0.333
Digestible energy, MJ/kg				
As-fed basis	15.0	14.1	0.10	<0.001
Dry matter basis	16.9	15.9	0.13	<0.001
ME/GE	0.85	0.84	0.006	0.103
Metabolizable energy, MJ/kg				
As-fed basis	14.9	13.9	0.11	<0.001
Dry matter basis	16.8	15.8	0.13	<0.001
Net energy, MJ/kg[1]				
As-fed basis	11.3	10.5	0.08	<0.001
Dry matter basis	12.8	12.0	0.09	<0.001
Kinetic parameters				
Lag time, h	3.52	4.01	1.047	0.458
Rate of degradation[2]	0.039	0.040	0.001	0.137
Half-time, h[3]	27.5	27.6	1.21	0.859
Total gas[4]	800	932	39.2	0.032

Abbreviations: Post-MSC DDGS, post-protein extraction dried distiller grains with solubles (POET Bioproducts, Sioux Falls, SD, USA); GE, gross energy; DM, dry matter; CP, crude protein (N × 6.25); EE, ether extract; aNDF; neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber.

[1] NE (kcal/kg) = (0.726 x ME) + (1.33 x EE) + (0.39 x Starch) – (0.62 × CP) – (0.83 × ADF); (Equation 1; NRC, 2012).

[2] Fractional rate of degradation (h-1) at t = T/2.

[3] Half-time to asymptote (h; T/2).

[4] Cumulative gas production recorded during microbial incubation (ml/g of sample).

Figure 4-1. Gas production kinetics of the undigested residue of the practical diets containing soybean hulls (SH) or the post-protein separation DDGS (post-MSC DDGS) during a 72 h of microbial fermentation.

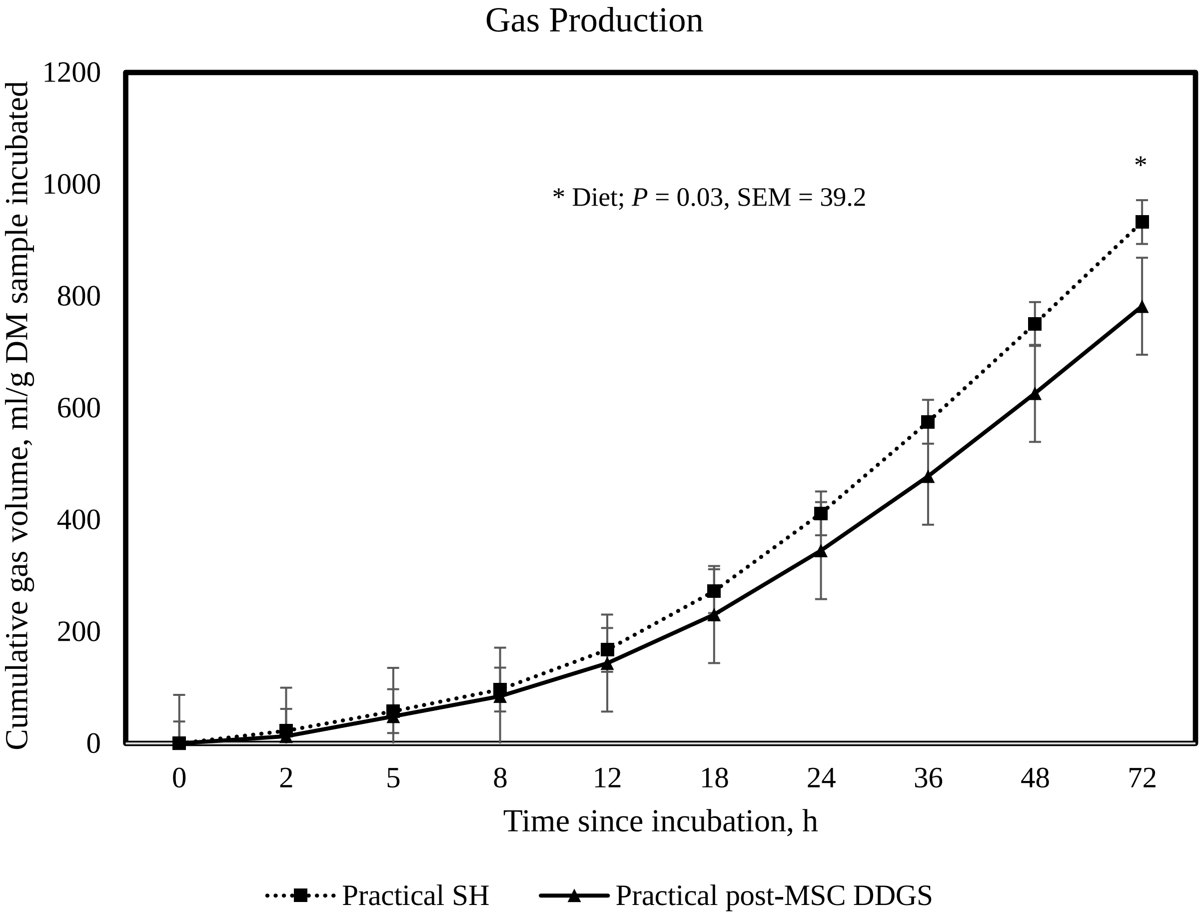

Table 4-4. Analyzed volatile fatty acid (VFA) concentrations following *in vitro* fermentation of practical diets containing either post-MSC DDGS or soybean hulls fed to gestating sows.

Item	Post-MSC DDGS	Soybean hulls	SEM	*P*-value
mM mol/100 mol				
Acetate	29.5	29.4	0.87	0.983
Propionate	11.8	11.6	0.48	0.773
Isobutyrate	0.48	0.46	0.026	0.641
Butyrate	4.1	4.4	0.16	0.142
Isovalerate	0.89	0.77	0.055	0.151
Valerate	0.76	0.77	0.094	0.928
Total	47.5	47.5	1.1	0.993
VFA (% of Total)				
Acetate	62.0	61.9	0.8	0.958
Propionate	24.8	24.4	0.8	0.697
Isobutyrate	1.01	0.97	0.042	0.503
Butyrate	8.5	9.5	0.37	0.157
Isovalerate	1.87	1.61	0.098	0.090
Valerate	1.6	1.6	0.2	0.896

Table 4-5. Apparent total tract digestibility (ATTD) coefficients of nutrients and gas kinetic parameters of corn-basal diets containing post-MSC DDGS, soybean hulls or sugar beet pulp fed to gestating sows.

	Basal	Post-MSC DDGS	Soybean hulls	Sugar beet pulp	SEM	*P*-value
ATTD coefficients						
DM	0.86^{a}	0.74^{b}	0.60^{c}	0.75^{b}	0.018	<0.001
EE	0.70^{a}	0.59^{b}	0.34^{c}	0.18^{d}	0.022	<0.001
Ash	0.16^{b}	0.24^{a}	0.08^{c}	0.12^{bc}	0.016	<0.001
aNDF	0.63^{a}	0.49^{bc}	0.43^{c}	0.57^{ab}	0.032	0.002
ADF	0.44^{x}	0.32^{y}	0.42^{x}	0.50^{x}	0.061	0.080
Kinetic parameters						
Lag time, h	2.55	3.08	1.85	2.30	0.521	0.220
Rate of degradation[1]	0.041^{a}	0.039^{b}	0.038^{b}	0.042^{a}	0.0005	<0.001
Half-time, h[2]	22.1^{b}	26.8^{a}	24.7^{ab}	24.0^{ab}	1.06	0.011
Total gas[3]	1039^{a}	705^{c}	845^{b}	1080^{a}	39.5	<0.001

Abbreviations: DM, dry matter; EE, ether extract; aNDF; neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber.

[a,b,c] Means within a row with different superscripts are significantly different ($P \leq 0.05$)

[1] Fractional rate of degradation (h-1) at t = T/2.

[2] Half-time to asymptote (h; T/2).

[3] Cumulative gas production recorded during microbial incubation (ml/g of sample).

Figured 4-2. Gas production kinetics of the undigested residue of the corn-basal diets containing either post-protein separation (post-MSC) DDGS, soybean hulls (SH) or sugar beet pulp (SBP) during a 72 h of microbial fermentation.

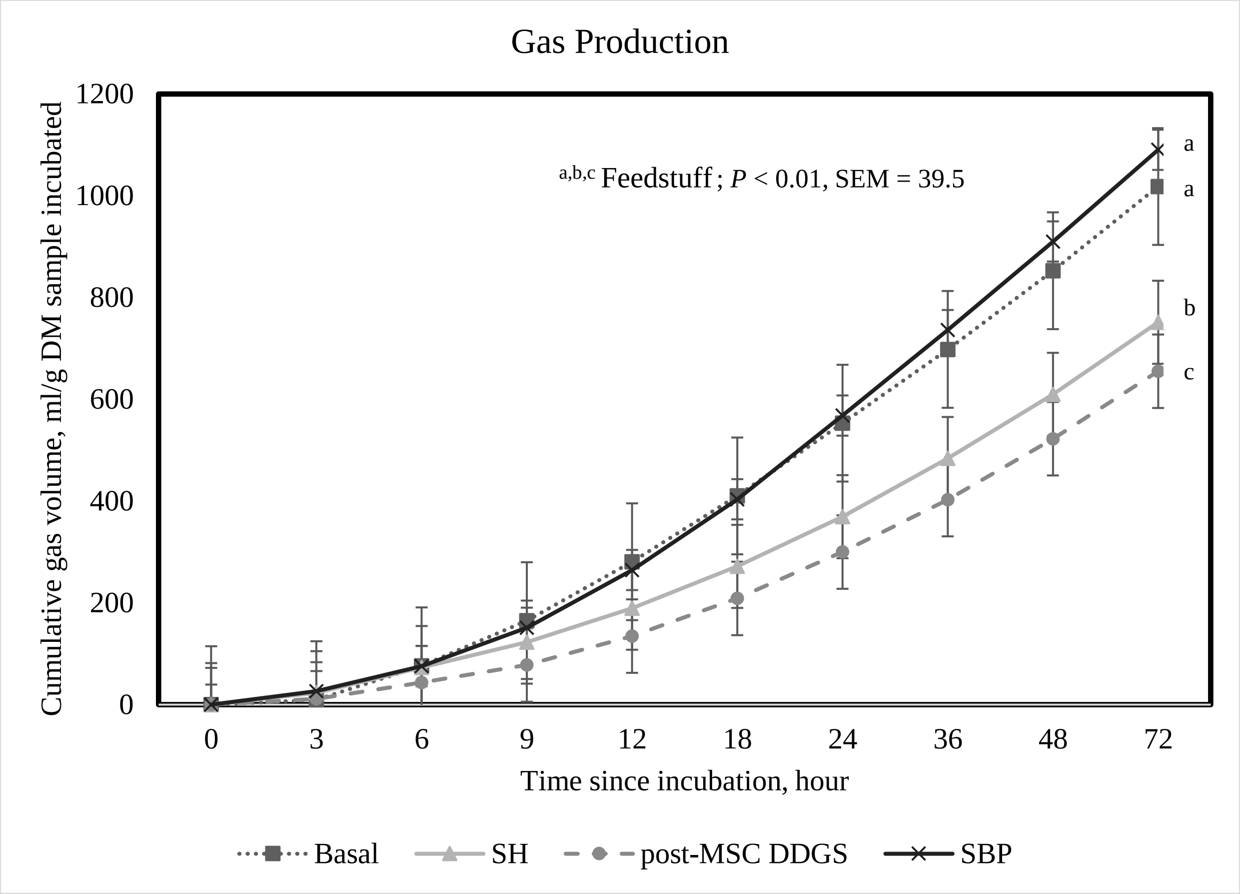

Table 4-6. Analyzed volatile fatty acid (VFA) concentrations following *in vitro* fermentation of corn-basal diets containing post-MSC-DDGS, soybean hulls or sugar beet pulp fed to gestating sows.

Item	Basal	Post-MSC DDGS	Soybean hulls	Sugar beet pulp	SEM	*P*-value
VFA (mM mol/100 mol)						
Acetate	29.2	27.1	29.4	28.9	1.39	0.641
Propionate	14.5^{a}	10.7^{b}	11.8^{ab}	12.1^{ab}	0.81	0.028
Isobutyrate	0.42	0.44	0.45	0.45	0.031	0.970
Butyrate	3.82	4.28	3.64	4.33	0.302	0.330
Isovalerate	0.70	0.82	0.70	0.67	0.049	0.115
Valerate	1.35^{a}	0.84^{ab}	0.87^{ab}	0.67^{b}	0.152	0.038
Total	50.0	44.2	47.0	47.2	2.15	0.350
VFA (% of Total)						
Acetate	58.5^{y}	61.2^{xy}	62.6^{x}	61.2^{xy}	0.98	0.065
Propionate	28.8^{x}	24.4^{y}	25.4^{xy}	25.8^{xy}	1.18	0.010
Isobutyrate	0.85	1.00	0.95	0.94	0.049	0.122
Butyrate	7.7^{b}	9.7^{a}	7.7^{b}	9.2^{ab}	0.52	0.027
Isovalerate	1.4^{b}	1.8^{a}	1.5^{b}	1.4^{b}	0.07	< 0.001
Valerate	2.8^{x}	1.9^{xy}	1.8^{xy}	1.4^{y}	0.31	0.055

[a,b] Means within a row with different superscripts are significantly different ($P \le 0.05$).

[x,y] Means within a row with different superscripts tended to differ ($P \le 0.10$).

Table 4-7. Apparent total tract digestibility (ATTD) coefficients of nutrients and energy, digestible energy, metabolizable energy, and net energy of corn, post-MSC DDGS, or soybean hulls or sugar beet pulp fed to gestating sows.

Item	Corn	Post-MSC DDGS	Soybean hulls	Sugar beet pulp	SEM	*P*-value
ATTD coefficients						
aNDF	0.66^{b}	0.63^{b}	0.73^{b}	0.95^{a}	0.041	<0.001
ADF	0.38^{c}	0.43^{c}	0.73^{b}	0.98^{a}	0.092	<0.001
GE	0.91^{a}	0.79^{b}	0.64^{c}	0.82^{b}	0.017	<0.001
Digestible energy, MJ/kg						
As-fed basis	14.9^{a}	13.8^{a}	8.8^{c}	12.3^{b}	0.29	<0.001
DM basis	16.2^{a}	15.4^{b}	13.3^{d}	14.8^{c}	0.10	<0.001
ME/GE	0.91^{a}	0.78^{b}	0.63^{c}	0.81^{b}	0.017	<0.001
Metabolizable energy, MJ/kg						
As-fed basis	14.8^{a}	13.5^{a}	8.6^{c}	12.2^{b}	0.31	<0.001
DM basis	16.9^{a}	14.8^{b}	9.1^{d}	13.3^{c}	0.33	<0.001
Net energy, MJ/kg[1]						
As-fed basis	11.9^{a}	9.2^{b}	4.6^{d}	7.9^{c}	0.22	<0.001
DM basis	13.5^{a}	10.0^{b}	4.9^{d}	8.6^{c}	0.24	<0.001

Abbreviations: aNDF; neutral detergent fiber assayed with heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber; GE, gross energy; DM, dry matter.

[a,b,c,d] Means within a row with different superscripts are significantly different ($P \leq 0.05$).

[1] NE (kcal/kg) = (0.726 x ME) + (1.33 x EE) + (0.39 x Starch) – (0.62 × CP) – (0.83 × ADF); (Equation 1; NRC, 2012).

Table 4-8. Analyzed volatile fatty acid (VFA) concentrations following *in vitro* fermentation of corn, post-MSC DDGS, soybean hulls or sugar beet pulp fed to gestating sows.

Item	Corn	Post-MSC DDGS	Soybean hulls	Sugar beet pulp	SEM	*P*-value
VFA (mM mol/100 mol)						
Acetate	30.6^{ab}	22.2^{b}	33.3^{a}	28.1^{ab}	2.18	0.013
Propionate	15.7^{a}	0.6^{b}	6.8^{b}	5.7^{b}	1.53	< 0.001
Isobutyrate	0.44	0.49	0.60	0.50	0.083	0.577
Butyrate	3.8	5.8	4.6	5.9	0.84	0.309
Isovalerate	0.74^{ab}	1.14^{a}	0.84^{ab}	0.58^{b}	0.113	0.018
Valerate	1.18^{a}	0.19^{b}	0.60^{b}	-	0.354	0.027
Total	52.5^{a}	30.4^{b}	50.3^{a}	40.9^{b}	3.79	0.002
VFA (% of Total)						
Acetate	58.5^{b}	71.8^{a}	74.1^{a}	70.9^{ab}	3.51	0.016
Propionate	29.9^{a}	3.7^{c}	12.9^{b}	12.4^{b}	3.24	< 0.001
Isobutyrate	0.8^{b}	1.6^{a}	1.2^{ab}	1.1^{ab}	0.24	0.017
Butyrate	7.3^{b}	18.9^{a}	9.2^{b}	15.0^{ab}	2.09	0.003
Isovalerate	1.4^{b}	3.8^{a}	1.7^{b}	1.3^{a}	0.26	< 0.001
Valerate	2.24^{x}	0.63^{y}	1.05^{y}	nd	0.789	0.092

^{a,b,c} Means within a row with different superscripts are significantly different ($P \leq 0.05$).

^{x,y} Means within a row with different superscripts tended to differ ($P \leq 0.10$)

CHAPTER 5

YEAST CARBOHYDRATE SUPPLEMENTATION IN SOW DIETS IMPROVES SURVIVABILITY OF LOW-BIRTH WEIGHT PIGS[1]

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[1] Formatted as submitted to *Animal – The International Journal of Animal Biosciences* (2024)

ABSTRACT

This study aimed to determine the optimal inclusion level of a yeast carbohydrate (**YC**) product in sow diets based on sow and suckling piglet performance. Forty-nine gestating sows (parity 0 to 5; 217 ± 30 kg body weight [**BW**]) were randomly assigned to 1 of 3 YC dietary inclusion levels (0.0, 0.1, and 0.2%) beginning at day 105 and through lactation. Yeast carbohydrate was top-dressed in a corn-carrier to achieve daily active ingredient intake of 3 and 6 g/day in gestation (0.1 and 0.2% YC, respectively) and 7 and 15 g/day in lactation (0.1 and 0.2% YC, respectively) at the 0730 and 0800 h feed time in gestation and lactation, respectively. Variables measured included sow BW, backfat, feed intake, litter characteristics at birth, piglet BW, piglet weight distribution at birth and weaning, and serum, colostrum, and milk immune markers. Data was analyzed using a mixed linear model with supplementation as the main effect, sow (block) as a random variable. Variables that displayed quadratic responses were analyzed using non-linear regression to determine the optimal inclusion level. Piglet weight distributions were examined using Chi-distribution test. Sows in both YC groups had fewer stillborns per litter than control group (YC effect; $P = 0.04$). The 0.2% YC sows weaned more pigs per litter (quadratic effect; $P = 0.05$) than control and 0.1% YC groups. Colostral immunocrit ratio tended to be higher in 0.2% YC sows than control sows (YC effect; $P = 0.09$). Control litters had a greater proportion of lightweight pigs at birth (< 1.20 kg), and both YC groups had a greater proportion of average-weight pigs (1.20 to 1.65 kg; $\chi2 < 0.01$). Pig BW at 2 days before weaning and overall average daily gain were greater in control group ($P \geq 0.05$). The 0.2% YC group had a higher frequency ($\chi2 < 0.01$) of weaning lightweight (< 5.00 kg) and average weight (5.00 to 6.40 kg) pigs. There was lower pre-weaning mortality of light-born pigs in 0.2% YC group compared to control and 0.1% YC groups (χ2 = 0.03). Suckling pigs from 0.2% YC sows had higher (quadratic effect; *P* = 0.05) concentrations of serum IgA at weaning. The optimal YC inclusion was deemed to be 0.18%. In conclusion, YC supplementation in sow diets benefited both sow and litter performance from the perspective of weaning more light-born pigs and a higher colostral immunocrit ratio and offspring IgA levels at weaning.

KEY WORDS

Immunity, lactation, sow, yeast carbohydrate

IMPLICATIONS

Supplementation of a yeast carbohydrate at 0.2% dietary inclusion in sow diets benefited both sow and litter performance based on weaning more light born pigs as well as a higher colostral immunocrit ratio and offspring IgA levels at weaning. Yeast supplementation in sow diets may provide a means to enhance performance of lightweight, higher risk piglets.

INTRODUCTION

For the neonatal pig, milk acts as a rich source of nutrients (carbohydrates, lipids, proteins, minerals, vitamins, etc.) necessary to stimulate proper growth and immune development but potentially at the cost of the dam undergoing metabolic stress (Theil et al., 2014). The priority for milk production can result in the sow entering a catabolic state (Pettigrew and Moser, 1991). This is problematic as genetic selection has produced a leaner sow with low daily feed intake and lower body reserves (Tokach et al., 2019). Milk production is highly proportional to the number of pigs suckling with larger litter sizes resulting in higher milk yield (Pluske et al., 1998). Due to genetic selection for increased litter size and daily gain, it can be estimated modern hyperprolific sows nursing litters growing at 2.35 kg/d need to produce 9.2 kg/d of milk in 2012 compared to 8.2 kg/d in 1985 assuming 3 pigs more born and weaned per litter in 2012 (Rosero et al., 2016). While the sow is in a catabolic state and depleting her own nutrient reserves, she is still not able to produce adequate amounts of milk to maximize growth of her offspring (Tokach et al., 2019).

Improvements in sow nutrition are vital to combat the current industry trend of wider variation in piglet birthweights within litters resulting from increased litter sizes, with lower-weight pigs at increased risk of pre-weaning mortality (Feldpausch et al., 2019). This has led to the evaluation of functional feed additives in sow diets to improve performance of sows and livability of her nursing offspring (Xu et al., 2023). Yeast carbohydrates (**YC**) are enzyme-treated hydrolysis products (predominantly β-glucan and mannan polysaccharides) lysed from the cell wall of yeast that have been linked to immune modulation through prebiotic effects (Patterson et al., 2023). Dietary inclusion of YC and other yeast derived feed additives in sow diets have gained attention in recent years to promote beneficial effects on the offspring (Bass et al., 2019; Duan et al., 2019) as the neonatal pig relies heavily on passive immunity attained from the sow to build a robust immune system needed for survival (Rooke et al., 2003). Supplementation of these products in sow diets has been linked to essential functions related to immune modulation in both sows and piglets, positively impacting their health and performance (Duan et al., 2016; Hasan et al., 2018).

This study aimed to determine the efficacy and optimal inclusion level of a novel YC product in sow diets for improving sow performance and litter survivability through immune modulation.

MATERIALS AND METHODS

Animals and Management

The study was conducted in the sow barn at the South Dakota State University Swine Education and Research Facility (Brookings, SD, USA). A total of 49 sows and gilts (Camborough L1050, PIC, Hendersonville, TN; parity 0 to 5; 217 ± 30 kg body weight [**BW**]) in two blocks, were used in a randomized incomplete block arrangement to test three supplementation levels of YC (control – 0%, 0.1%, and 0.2%) during late gestation and lactation (day 105 of gestation until weaning). Females were moved at day 105 of gestation from group pens to individual gestation stalls (0.68 x 1.98 m; equipped with a nipple drinker and dry feeder) to initiate dietary treatments. Animals were hand-fed gestation diet (2.20 ± 0.25 kg/d) based on BW and body condition (target 3.0) once daily at 0730 h. Sows and gilts were moved into the farrowing room at day 110 of gestation and housed in individual farrowing crates (1.83 m x 2.43 m) equipped with an electronic feeding system (Gestal 3G; Jyga Technologies, Greeley, KS, USA) where daily feed allotment was provided in 6 meals at 3 h intervals beginning at 0500 h daily. The feed system allowed daily intake up to 20% above the set daily allotment. Lactation feed intake followed a step-up program based on parity and barn historical data for ad libitum intake within 4 days following farrowing. In lactation, if a female was discovered to be a poor eater (defined as ≤ 50% of daily allotment) or consumed the entire allotment plus the 20% overage on 2 consecutive days, the daily allotment dispensed was decreased or increased accordingly. Any feed ‘refusals’ were removed prior to the first AM feeding, dried, weighed, and recorded. Water was provided ad libitum during both gestation and lactation phases. Farrowing was monitored daily by an assigned graduate research assistant from 0500 h to 2200 h. Cameras (Reolink Argus Eco, Wilmington, DE, USA) were placed above farrowing crates to record and monitor farrowing sows after 2200 h. A 1 mL intravulval injection of Dinoprost tromethamine (Lutalyse, Zoetis, Pasippany, NJ, USA) was administered at day 116 of gestation to females that had yet to farrow. Cross-fostering of piglets was kept to a minimum, but if required, piglets were cross-fostered immediately after 24 h postpartum. This was done not only to allow for colostrum intake from the dams of the piglets but also to ensure that all piglets could be nourished. Cross-fostering only occurred within dietary treatment, and litter sizes were limited to a maximum of 14 pigs per litter. Fallbacks were defined as small or thin piglets with an average daily gain (ADG) of ≤ 30 g from birth to the time of weighing. At day 3 of lactation, animals were processed (tail docked, tattooed, and castrated), administered a 1mL intramuscular injection of iron dextran (Uniferon 200, Pharmacosmos, Watchung, NJ, USA), and orally vaccinated with K88 Escherichia Coli vaccine (1 mL, Entero-vac, ARKO Laboratories, Jewell, IA, USA). Individual animals or whole litters identified with scours after day 3 were treated with a 1 mL oral dose of Spectinomycin (Spectogard Scour-chek, Bimeda, Oakbrook Terrace, IL, USA) twice daily for three days. At weaning, all animals were administered a 2 mL intramuscular injection of Circumvent PCV-M G2 (Merck Animal Health, Madison, NJ). Piglets were weaned at 20.1 ± 1.2 days of age. Animals removed from the trial due to poor health, death, or euthanized were recorded with date and weight at removal.

Experimental Design and Dietary Treatments

Females were randomly allotted to one of the three experimental diets (n=16-17 sows/treatment), balanced by BW and parity as possible. The control diets were standard gestation and lactation diets formulated to meet or exceed nutrient recommendations for sows according to NRC (2012; Table 5-1). Yeast carbohydrate (Maxi-Nutrio; CBS Bio-Platforms Inc., Calgary, AB, Canada) was top dressed using ground corn as the carrier to achieve dietary inclusion of 0.1 and 0.2 % YC. The top dress was placed on top of the 0730 and 0800 h feeding in gestation and lactation, respectively. In gestation, sows were hand-fed gestation diet (2.20 ± 0.23 kg/d; based on BW and body condition to maintain 3.0 condition score) once daily with the average YC provided in gestation being 3 and 6 g/day (0.1 and 0.2% YC, respectively). Sow lactation daily feed allocation followed the standard South Dakota State University feed curve based on sow parity. Daily top dress amount was adjusted based on each sow's prior day intake to achieve the targeted YC supplementation levels. Thus, the average YC for the 0.1 and 0.2% YC groups were 7 and 15 g/day, respectively.

Data Collection and Chemical Analyses

Sow BW was recorded at day 105 of gestation, time of entering farrowing room (approximately day 110 of gestation), day 2 of lactation, and weaning. Back fat (**BF**) at the last rib was measured at day 105 of gestation, day 2 of lactation, and weaning using an ultrasound (Ibex pro, E.I. Medical Imaging, Loveland, CO, USA). Blood samples were collected via jugular venipuncture into a nonheparinized blood collection tube (B.D. Vacutainer, Franklin Lakes, NJ, USA) at day 105 of gestation and weaning. Blood samples were kept on ice at collection, stored overnight (24 h) at 5°C, centrifuged at

5,000 x g for 10 minutes, and serum was transferred to 1.5 mL microcentrifuge tubes (Thermo Fisher Scientific, Waltham, MA, USA) to be stored at -20°C for later analysis.

Litter characteristics (total born, born alive, stillborn, mummies) were recorded within 24 h following parturition. Farrowing duration was monitored using cameras (Reolink Argus Eco, Wilmington, DE, USA) placed above the back of the farrowing crates. The onset of parturition was defined as when the first pig passed, and the end of farrowing followed the expulsion of the last pig. Piglets were weighed within 24 h of farrowing, at day 7 post-farrowing, and 2 days before weaning. At each weigh period for the suckling pigs, BW of the population across both blocks was compiled and used to establish three weight categories: light, average, and heavy based on quartiles.

Where possible, colostrum was collected following the birth of the first piglet and before suckling using gentle stripping from all teats for a total volume of 40 mL in sterile conical tubes (Fisher Scientific, Pittsburgh, PA, USA). At weaning, a milk sample was collected; piglets were removed, then 2 mL of oxytocic principle (Oxytocin, Aspen Veterinary Resources, Liberty, MO, USA) was administered (2.54 cm x 20 ga needle) intravaginally one hour following the removal of the piglets and the same technique and total volume previously noted for colostrum was utilized. Colostrum and milk were stored at -20°C until further use. On day 2 of age, a 1 mL blood sample from three piglets in each litter (the lightest, heaviest, and the pig closest to average litter BW) was collected from the mammary vein based on birth weight category. Samples were centrifuged at 5,000 x g for 10 minutes, transferred to 2.0 mL microcentrifuge tubes (Thermo Fisher Scientific, Waltham, MA, USA), and stored at -80°C for later analysis.

Blood was collected from the average-sized birthweight pig selected at birth in each litter 2 days before weaning using jugular venipuncture as described for the sows.

Serum and colostrum immunocrit ratios were based on the method of Vallet et al. (2013). Briefly, serum was diluted in a 1:1 ratio with 40 % ammonium sulfate in distilled water. The newly diluted sample was loaded into a microcapillary tube, placed into a hematocrit centrifuge (MX12 PCV Centrifuge, L.W. Scientific, Lawrenceville, GA, USA), and centrifuged at 12,000 x g for 10 min. Immunocrit ratio was determined as the ratio of the precipitate length divided by the total length of diluted serum.

In conjunction with piglet sera samples, immunocrit was evaluated in colostrum. A modified methodology from Vallet and Miles (2017) was used. Briefly, colostrum samples were diluted in a 1:1 ratio with 1 % bovine serum albumin (1 mL: 9mL Saline; Fisher BP6751) in 0.9 % saline. In duplicate, diluted colostrum samples were combined with 40 % (wt/vol) ammonium sulfate in distilled water to precipitate immunoglobulins and then loaded into a hematocrit centrifuge and centrifuged at 12,000 x g for 10 minutes. Immunocrit ratio was determined as the ratio of the precipitate length divided by the total length of diluted colostrum, then doubled to account for prior colostrum dilution.

Colostrum and milk samples from the two extreme groups (0.0% and 0.2% YC inclusion), along with serum samples from all groups (0.0%, 0.1%, and 0.2% YC inclusion), were analyzed for immunoglobulin A (IgA) and immunoglobulin G (IgG) with commercially available ELISA kits per manufacturer's instructions (Pig ELISA Quantitation Set, Bethyl Laboratories, Inc., Montgomery, TX, USA). All samples were diluted (50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20) as necessary to fall within the standard curve, which ranged from 15.6 to 1,000 ng/mL for IgA and 7.8 to

1,000 ng/ mL for IgG. The resulting data were multiplied by the dilution factor to obtain the reported concentrations for each immunoglobulin.

Colostrum and milk samples were submitted to the Division of Regulatory Services at the University of Kentucky (Lexington, KY, USA) to be analyzed for protein, lactose, total solids, and fat using fourier transform infrared spectroscopy on a specialized milk analysis instrument (MilkoScan™ FT+, Foss, Denmark). Samples were analyzed in duplicate, warmed in a water bath (40°C), then inverted several times to mix well before being presented to the FTIR analyzer pipette. The instrument was calibrated using raw low-fat non-fortified bovine milk (Eurofins DQCI, MN, USA).

Statistical Analysis

The UNIVARIATE procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC, USA) was used to confirm the homogeneity of variance and to analyze for outliers. Data was analyzed using the MIXED procedure of SAS considering the effect of dietary supplementation. The sow was the experimental unit, and sow (block) was the random effect. Pig age at 2 days before weaning was used as a covariate for piglet final BW and overall ADG. Orthogonal contrasts were applied to determine linear and quadratic responses of sow reproductive performance, sow serum Ig concentrations, and piglet serum and performance to increasing YC levels. Significant differences were reported at $P < 0.05$ and tendencies for significance at $0.05 \leq P \leq 0.10$ A broken-line regression analysis was performed using the NLIN procedure of SAS on variables where a quadratic response was observed (*i.e.*, stillborns per litter, number of pigs weaned per litter, and serum concentration of IgG at weaning) to determine optimal inclusion level (Robbins et al., 2006). Weight categories were analyzed using the Freq procedure in SAS. The effect of sow BW and parity on piglet performance and sow serum metabolites were tested and found to be not significant; therefore, were not included in the final model. Values are presented as lsmeans and standard error of the mean (SEM).

RESULTS

Sow Performance

One sow from the 0.2% YC group was removed from the study due to agalactia. Data and litter performance from this sow were not used in further analysis. Parity (1.6 ± 1.6) was equal among all sow treatment groups. Sow feed intake during gestation day 105-110 (2.20 ± 0.23 kg/d) and lactation (6.16 ± 1.37 kg/d) periods was not different across groups (Table 5-2).

The BW of sows in the 0.2% YC treatment group were 24.4 kg, 21.8 kg, and 23.3 kg heavier at the time of placement, upon entering farrowing (day 110 gestation), and day 2 of lactation, respectively, compared to the control group and 0.1% YC groups ($P < 0.05$). There was no difference in sow BW at weaning between treatment groups and a similar numerical difference existed between 0.2% supplemented sows and control group (22.6 kg) as at trial start. Backfat thickness did not differ between treatment groups at any point of collection (Table 5-2).

The average number of total born pigs (16.2 ± 3.9), pigs born alive (14.4 ± 3.2), and mummies (0.3 ± 0.5) per litter did not differ among treatment groups. There was a lower average number of stillborn pigs per litter in the YC supplemented groups compared to control (YC effect; $P = 0.04$). Average number of stillborns per litter also decreased as YC supplementation level increased (linear effect; $P = 0.02$) with the 0.2% YC supplemented sows having the lowest average number of stillborns per litter (quadratic effect; $P = 0.01$). Farrowing duration, however, did not differ between treatment groups. Sows in both YC supplementation groups tended to wean more pigs per litter than the control group (YC effect; $P = 0.09$). The average number of pigs weaned per litter linearly decreased as YC supplementation level increased (linear effect; P = 0.03). The average number of pigs weaned per litter was greatest in the 0.2% YC group (quadratic effect; $P = 0.05$) (Table 5-2). The results of the break-point analysis indicated the optimal inclusion for number of stillborns and pigs weaned per litter was 0.18% ($R^2 = -0.60$) and 0.14% ($R^2 = 0.99$), respectively.

Sow Serum Immunoglobulins and Composition of Colostrum and Milk

Sow serum concentration of IgG at day 105 of gestation and IgA at weaning did not differ among treatment groups (Table 5-3). The composition of colostrum (% of fat, protein, lactose, total solids, and solids not fat) was not impacted by the inclusion of YC at 0.2% compared to the control group. Colostral IgG concentration did not differ between the control and 0.2% YC treatment groups. The 0.2% YC treatment group tended to have a higher colostral immunocrit ratio than the control group ($P = 0.09$). Milk composition and IgA concentration at weaning did not differ between the control and 0.2% YC treatment groups.

Litter Performance, Pigs' Immunological Parameters, and Weight Distributions

Piglets born from sows in both YC treatment groups had greater average BW within 24 h post-farrowing compared to pigs from sows in the control (YC effect; $P = 0.04$) (Table 5-4). Neither day 7 post-farrowing BW nor week 1 ADG for suckling pigs differed between treatment groups. Age of pigs in the control group was greater prior to weaning ($P < 0.01$). Using pig age as a covariate, pigs in the control group had greater

BW at 2 days prior to weaning (YC effect; *P* = 0.04) and overall lactation ADG (*P* = 0.01). Pig weights at 2 days before weaning tended to linearly decrease as YC supplementation level increased (linear effect; *P* = 0.08). Pigs in the 0.1% YC supplementation group tended to be heavier than the pigs in the 0.2% YC supplementation group (quadratic effect; *P* = 0.09).

Day 2 immunocrit ratio was not different between treatment groups (Table 5-4). Serum IgG concentration at weaning tended to be higher in the control group compared to the two YC supplemental groups (YC effect; *P* = 0.07). Suckling pigs from sows in the 0.2% YC group had elevated serum IgA concentration at weaning compared to the control and 0.1% YC treatment groups (quadratic effect; *P* = 0.05). The breakpoint for pig serum IgA concentration at weaning based on the break-point analysis was determined to be 0.18% ($R^2 = 0.06$).

Weight categories for birth weight distribution were < 1.2 kg, 1.2-1.65 kg, and > 1.65 kg for light, average, and heavy birthweight pigs, respectively (Table 5-5). The control group had a higher frequency of light-born pigs with lower proportions of average and heavyweight born pigs compared to the two YC inclusion groups ($\chi 2 < 0.01$). At weaning, the 0.2%YC group weaned more lightweight (< 5.0 kg) and average weight (5.0-6.4 kg) pigs with less heavyweight (> 6.4 kg) pigs in comparison to the control and 01%YC treatment groups ($\chi 2 < 0.01$).

Regarding pre-weaning mortality, there were no significant differences for overall pre-weaning mortality, mortality of average-born pigs, or mortality of heavy-born pigs between treatment groups. The 0.2%YC treatment group, however, had a lower pre-weaning mortality of light-born pigs ($\chi 2 = 0.03$). There weas no significant difference between treatment groups on the frequency of suckling pig medical treatment (χ2 = 0.55).

DISCUSSION

This study aimed to determine the optimal inclusion level of a novel YC product in sow diets based on the immunoglobulin content in colostrum, milk, and serum and the performance of sows and suckling piglets. The supplementation of a YC product at 0.2% of daily inclusion in sow diets benefited both sow and litter performance with the capacity to wean more light born pigs and a higher colostral immunocrit ratio and offspring IgA levels at weaning.

While not intended, heavier sows were allocated to the 0.2% YC treatment group compared to the control and 0.1% YC group; however, similar differences in sow BW remained through to weaning suggesting that differences to piglet growth and health observed in this study were not due to differences in sow BW or BW gains or losses. Further, backfat thickness remained similar throughout the trial. Declerk et al. (2015) and Piñeiro et al. (2019) note that sow parity is expected to influence humoral immunity more than BW. The humoral immune status of the sows, as indicated by the concentration of serum IgG, was similar across treatment groups further supporting the hypothesis that sow BW did not influence sow response to YC supplementation. In this study, there was a lower average number of stillborns per litter for sows supplemented with 0.2% YC. This observation is consistent with Peng et al. (2020), where sows supplemented with a live yeast starting at day 90 of gestation had a lower proportion of stillborn piglets. Czech et al. (2010) also observed a reduction in the number of stillborns per litter and an increased number of pigs born alive per litter for sows supplemented with a mannanoligosaccharide prebiotic four weeks prepartum. Studies by Hasan et al. (2018)

and Bass et al. (2019) reported yeast product supplementation in gestation increased the number of pigs born alive but did not affect the number of stillborn pigs. While litter size is determined during early gestation (Edwards et al., 2012), longer farrowing duration and birthing intervals have been associated with increased stillbirths (Vanderhaeghe et al., 2013). Hasan et al. (2018) reported reduced farrowing duration and birthing interval in yeast-supplemented sows with increased number of pigs born alive; although no difference in farrowing duration was detected herein. The lack of difference in litter size and farrowing duration between sow treatment groups suggests the lower stillborns with 0.2% YC supplementation may be due to an improvement in offspring vigor.

The lack of difference in sow serum concentration of IgG at day 105 of gestation suggests a similar humoral status of sows at trial start. Studies investigating yeast additives in gestating sow diets have focused on suckling pig serum immunoglobulins rather than the dam. In the few studies that reported sow serum immunoglobulin levels, dietary yeast additive also did not affect sow serum concentration of IgG or IgA at either the prepartum or weaning periods (Scharek et al., 2007; Bass et al., 2019). Similarly, in this study YC supplementation did not impact serum concentration of IgA in sows at weaning. The predominant immunoglobulin in colostrum is IgG and originates solely from the humoral immune system of the dam, but it declines within the 24 h following the onset of parturition. Immunoglobulin A becomes the main immunoglobulin found in transient (36 – 72 h postpartum) and in mature milk. However, approximately 10-17% of circulating IgA found in the serum of sows contributes to the IgA found in transient and mature milk (Bourne and Curtis, 1973; Theil et al., 2014). Supplementation of an enzymatic treated Saccharomyces cerevisiae yeast product was reported to stimulate the production of jejunal IgA in pigs (Christensen et al., 2022). Secretory immunoglobulins synthesized in the jejunum can enter blood circulation through active transepithelial transport. Gut secretory IgA, however, contributes to less than 2% of IgA circulating throughout the body (Jonard et al., 1984).

Yeast carbohydrate supplementation did not alter the composition of colostrum or milk collected at weaning. Supplementation of yeast products to gestating and lactating sows has been previously shown to increase the concentration of colostral protein, lactose, solids-not-fat (Peng et al., 2020), and fat (Hasan et al., 2018). Graugnard et al. (2014) reported that mannan-rich fraction derived from the cell wall of yeast increased the concentration of milk protein and solids-not-fat when supplemented to sow during the entirety of gestation. Colostral lactose and fat synthesis begins at day 105 (Hartman et al., 1984; Kensinger et al., 1986), the same day diet treatments were started in this trial, thus the lack of a difference in colostral nutrient composition is likely due to timing of supplementation relative to the onset of colostral nutrient synthesis.

Yeast carbohydrate did not alter IgG concentration in colostrum or IgA concentration in milk. The higher colostral immunocrit ratio of the 0.2%YC sows maybe related to greater concentration of other immunoglobulins than IgG because colostral IgG concentrations have a stronger correlation with colostral protein than colostral immunocrit (Vallet and Miles, 2017) and there was no difference in colostral protein percentage between sow groups. The lack of difference in immunocrit ratio in the serum of 2-day old pigs could be explained by a reduction of both IgG and immunocrit ratio in colostrum within 24 hours after farrowing (Vallet and Miles, 2017).

Average piglet birthweight within 24 hours post-farrowing was heavier in both YC supplementation groups than the control group when considering YC groups had reduced stillborn pigs per litter. This observation is in agreement with Gourley et al. (2020) who observed increased mean pig birth weight being associated with a reduced stillborn rate. The YC supplementation groups also had fewer light born (< 1.2 kg) pigs. However, considering the short supplementation period prior to farrowing (9 – 12 days) sow diet supplementation may partially explain the difference in pig weight and litter characteristics at birth as fetal growth is rapid between day 105 of gestation up until farrowing (Noblet et al., 1985).

It should be noted that both YC supplementation groups had a 3.9% greater frequency of pigs in lower weight categories at the time of weaning and YC sows suckled more piglets which can in part explain the lower piglet BW at 2 days prior to weaning and lesser overall daily gain in YC-supplemented litters. Aside from being older at 2 days prior to weaning, the heavier average pig weight and greater concentration of serum IgG in the average pig from control sows might also be associated with a more mature humoral immune system by the time of weaning. Humoral IgG synthesis begins at approximately 7 days of age in pigs, but sera concentration might not reach a sufficient threshold by the time of weaning (Rooke et al., 2003). The greater IgG concentrations in the control pigs at weaning could be explained by greater milk intake. Milk intake has a strong positive correlation with pig BW and BW gain. Milk intake and BW gain in suckling piglets can also be increased with smaller litter sizes as there is less competition for the dam’s milk (Hojgaard et al., 2020) While IgG concentrations in the mature milk were not influenced by dietary treatment, the smaller litter sizes in the control group resulted in greater IgG intake and BW in the suckling piglets by the weaning due to greater milk intake.

Sows in the 0.2%YC supplementation group lost 15.7% less light born pigs by the time of weaning. Notably, the distribution of pigs receiving medical treatment was equal between the treatment group, and the selected average weight piglet in the 0.2%YC supplementation had elevated concentrations of circulating IgA. Secretory IgA synthesis by the gut lumen begins closer to weaning but is associated with a better intact and functioning gastrointestinal tract (Xun et al., 2018). There is a potential that pigs nursing sows in the 0.2%YC supplementation group attained greater gut function by the time of weaning that resulted in elevated secretory IgA synthesis and contributed to weaning more lightweight born pigs.

In conclusion, an inclusion level of yeast carbohydrate product for sow diets is 0.2% of dietary intake. This recommendation is based on the observations that sows supplemented with yeast carbohydrate at 0.2% had greater colostral immunocrit ratio, weaned more of their light-born offspring, and offspring birthed and suckled from the sows supplemented with the 0.2% yeast carbohydrate inclusion level had greater serum concentrations of IgA at the time of weaning.

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Table 5-1. Ingredients and calculated nutrient composition (as-fed) of gestation and lactation diets fed to sows in this experiment.

Item	Gestation	Lactation
Ingredient (g/kg)		
Corn	817.0	664.0
Soybean meal, 46.5% CP	145.0	299.0
Monocalcium phosphate	18.0	18.0
Limestone ($CaCO_3$)	13.0	12.0
Salt (NaCl)	5.0	5.0
Vitamin premix[1]	0.5	0.5
Mineral premix[2]	1.5	1.5
Calculated composition[3]		
Metabolizable energy (MJ/kg)	13.7	13.7
Dry Matter (g/kg)	888.3	889.4
Crude protein (*N x 6.25*) (g/kg)	135.0	194.0
SID Lys (g/kg)	55.0	97.0
Available phosphorus (g/kg)	44.0	44.0
Calcium (g/kg)	89.0	89.0
SID Met: Lys, %	38.0	29.0
SID Thr: Lys, %	76.0	64.0
SID Trp: Lys, %	22.0	21.0
SID Ile: Lys, %	85.0	74.0
SID Val: Lys, %	102.0	82.0

Abbreviations: SID = standardized ileal digestibility.

[1] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin 9.9 mg, Thiamine 3.3 mg.

[2] J & R Distributing Inc., 518 Main Ave, Lake Norden, SD 57248, USA. Minimum provided the following per kg of diet: Copper 20 mg, Manganese 40 mg, Selenium 0.3 mg, Zinc 170 mg.

[3] Calculated according to the NRC (2012) ingredient composition values.

Table 5-2. Reproductive performance of sows supplemented with yeast carbohydrate (YC) from day 105 of gestation to weaning.

	YC inclusion, %				*P*-value		
Item	0.0	0.1	0.2	SEM	YC[1]	Linear	Quadratic
Sows, *n*	16	16	17				
Parity	1.63	1.34	1.40	0.704	0.816	0.626	0.532
Feed intake, kg							
Day 105-110 gestation	2.25	2.20	2.14	0.065	0.434	0.200	0.281
Lactation	6.36	6.01	6.25	0.672	0.658	0.783	0.502
Sow BW, kg							
Day 105 Gestation	200.1	217.3	233.1	6.69	0.625	0.609	0.005
Day 110 Gestation	211.1	225.8	240.2	5.84	0.671	0.496	0.005
Day 2 Lactation	194.2	208.1	224.4	6.23	0.353	0.320	0.006
Weaning	190.1	206.1	212.6	10.35	0.825	0.842	0.905
Backfat, mm							
Day 105 Gestation	15.1	15.4	16.0	0.77	0.680	0.387	0.486
Day 2 Lactation	15.3	14.5	16.1	0.78	0.364	0.445	0.953
Weaning	14.0	12.8	13.5	0.65	0.418	0.571	0.278
Pigs per litter, n							
Total Born	16.7	16.3	15.2	1.50	0.459	0.229	0.374
Born Alive	14.0	14.8	14.2	1.07	0.772	0.907	0.650
Stillborns	2.4	1.2	0.8	0.54	0.041	0.016	0.014
Mummified	0.3	0.4	0.2	0.13	0.739	0.670	0.963
Weaned	11.8	12.6	13.2	0.56	0.090	0.029	0.053
Farrowing duration, mins	315	353	377	53.0	0.641	0.350	0.394

Abbreviations: IgG = immunoglobulin G; IgA = immunoglobulin A.

[1] Contrasts between with and without YC supplementation.

[2] Nutrient and immune marker composition of colostrum and milk were conducted solely on samples from the 0.0% and 0.2% YC treatment groups.

[3] Determined as the ratio of the precipitate length divided by the total length of diluted colostrum, then doubled to account for prior colostrum dilution.

Table 5-3. Serum concentration of immunoglobulins (Ig) and colostrum and milk nutrient composition in sows fed diets supplemented with yeast carbohydrate (YC) from day 105 of gestation to weaning.

	YC inclusion, %				*P*-value		
Item	0.0	0.1	0.2	SEM	YC[1]	Linear	Quadratic
Serum Ig, mg/mL							
IgG, day 105 gestation	25.0	16.1	18.9	3.66	0.222	0.246	0.101
IgA, weaning	1.1	1.1	1.0	0.05	0.456	0.309	0.595
Colostrum composition[2]							
Immunocrit ratio[3]	0.5	-	0.7	0.071	0.085	-	-
Fat, %	2.6	-	2.5	0.16	0.661	-	-
Protein, %	8.6	-	8.5	0.38	0.964	-	-
Lactose, %	2.5	-	2.5	0.09	0.953	-	-
Total Solids, %	15.7	-	15.6	0.38	0.848	-	-
Solids not Fat, %	11.9	-	11.9	0.32	0.987	-	-
IgG, mg/ml	35.8	-	32.8	6.20	0.739	-	-
Milk composition[1]							
Fat, %	7.1	-	6.8	0.81	0.547	-	-
Protein, %	4.4	-	4.0	0.20	0.137	-	-
Lactose, %	5.1	-	4.9	0.15	0.455	-	-
Total Solids, %	17.6	-	16.8	0.72	0.306	-	-
Solids not Fat, %	9.9	-	9.4	0.27	0.238	-	-
IgA, mg/ml	4.5	-	4.9	0.76	0.598	-	-

Abbreviations: IgG = immunoglobulin G; IgA = immunoglobulin A.

[1] Contrasts between with and without YC supplementation.

[2] Nutrient and immune marker composition of colostrum and milk were conducted solely on samples from the 0.0% and 0.2% YC treatment groups.

[3] Determined as the ratio of the precipitate length divided by the total length of diluted colostrum, then doubled to account for prior colostrum dilution.

Table 5-4. Piglet performance and serum immunoglobulins (Ig) at weaning of pigs nursing sows supplemented with yeast carbohydrate (YC).

	YC inclusion, %				*P*-value		
Item	0.0	0.1	0.2	SEM	YC[1]	Linear	Quadratic
BW, kg							
Day 1 lactation[2]	1.40	1.48	1.49	0.041	0.037	0.414	0.398
Day 7 lactation	2.62	2.57	2.57	0.103	0.804	0.575	0.509
2 days pre-weaning	5.85	5.53	5.37	0.247	0.045	0.076	0.094
ADG, kg[3]							
Week 1	0.18	0.35	0.33	0.094	0.445	0.300	0.206
Overall	0.24	0.22	0.22	0.012	<0.001	0.311	0.569
Age, 2 days prior to weaning[4]	19.6	18.8	19.2	0.20	<0.001	0.841	0.722
Day 2 immunocrit ratio[5]	0.16	0.16	0.17	0.020	0.553	0.364	0.614
Serum Ig, µg/mL[6,7]							
IgG	171.0	117.4	135.6	15.24	0.068	0.372	0.271
IgA	24.4	23.8	31.1	2.12	0.489	0.300	0.054

Abbreviations: BW = body weight; ADG = average daily gain; IgG = immunoglobulin G; IgA = immunoglobulin A.

[1] Contrasts between with and without YC supplementation.

[2] Average weight of pigs born alive.

[3] Based on litter performance after cross-fostering.

[4] Used as a covariate for 2 days pre-weaning BW and Overall ADG.

[5] Immunocrit ratio was determined as the ratio of the precipitate length divided by the total length of diluted serum.

[6] Blood samples for serum analysis were collected 2 days before weaning.

[7] Presented in µg/mL due to smaller body mass.

Table 5-5. Maternal yeast carbohydrate (YC) supplementation on the distribution of suckling piglets in weight categories, removed (death) and treated.

	YC inclusion, %			
Item	0.0	0.1	0.2	*Chisq*
Birth weight category, %				
Light (< 1.2 kg)	31.3	15.6	17.2	0.001
Average (1.2-1.65 kg)	45.8	59.7	56.7	
Heavy (> 1.65 kg)	22.9	24.7	26.1	
Wean weight category, %				
Light (< 5.0 kg)	19.1	21.8	24.1	0.007
Average (5.0 – 6.4 kg)	46.6	45.4	56.4	
Heavy (> 6.4 kg)	34.3	32.9	19.6	
Piglet mortality, %				
Overall	9.5	9.1	6.7	0.514
Light born (< 1.2 kg)	20.1	39.5	14.6	0.027
Average born (1.2-1.65 kg)	6.5	7.6	8.2	0.891
Heavy born (> 1.65 kg)	2.2	1.7	1.6	0.973
Treated piglets, %	9.0	9.9	7.1	0.548

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

The overall objective for these sets of trial was to determine the value of fibrous technologies to improve the feeding of commercial breeding sows. This included evaluating fiber degrading enzymes in gestation diets and commonly fed feedstuffs to increase dietary energy and nutrient availability (such as standardized ileal digestibility of amino acids [**AA**]) for gestating sows, comparison of a novel post-protein separate corn distiller grain with solubles (**post-MSC DDGS**) as a fiber and energy source for gestating sows to soybean hulls (**SH**) and sugar beet pulp (**SBP**), and determining the effects of a yeast carbohydrate (**YC**) comprised of hydrolyzed mannan-oligosaccharide and β-glucan on sow and litter performance when supplemented into sows diets.

When gestating sows were fed complete corn-soybean meal diets that included soybean hulls and corn DDGS as fibrous ingredients, the supplementation of a multienzyme blend (**ME$_{\mathbf{blend}}$**) (a combination of xylanase, glucanase, cellulase, amylase, protease, invertase, and pectinase) increased the metabolizable energy (**ME**) content by an average of 195 kcal/kg and net energy (**NE**) content by 142 kcal/kg (as-fed basis). However, the improvement was dependent on dietary neutral detergent fiber (**NDF**) content where the ME$_{blend}$ inclusion of 0.1% improved the diet metabolizable and net energy content by 10% in a low-fiber diet (10% dietary NDF) and 3% in a high-fiber diet (18% dietary NDF). The total tract digestibility of fiber fraction and non-starch polysaccharide sugar components was increased with ME$_{blend}$ on average by 10%. A ME-$_{blend}$ (xylanase, glucanase, cellulase, amylase, protease, and invertase) supplemented at 0.1% of the diet increased ME and NE of corn, wheat, soybean meal, field peas by approximately 100 kcal/kg (as-fed). The uplift in ME and NE of canola meal was 400 kcal/kg as-fed greater) with ME_{blend}. The energy content of sorghum and sugar beet pulp was not impacted by multienzyme supplementation.

The utilization of exogenous fiber-degrading enzymes in commercial swine diets is a strategy to reduce ingredient usage and lower diet costs by increasing the nutrient and energy density of poorly digested ingredients. Based on the results of the energy digestibility trial conducted in gestating sows, supplementation of a ME_{blend} can be applied to in commercial production to increase the dietary energy in gestating sow diets. The NE content (as-fed) of corn, irrespective of enzyme supplementation, when fed to gestating sows was on average 2713 kcal/kg (Chapters 3 and 4). The expense of corn being removed by 0.1% in the diet results in a lower NE by 2.7 kcal/kg, which is minimal when considering an increase in diet NE by 100 kcal/kg (as-fed) with ME_{blend} inclusion at 0.1% in the diet. Thus, the supplementation of fiber-degrading enzymes can be a potential strategy to increase dietary energy when feeding poorly digested feedstuffs in gestation diets.

Dietary multienzyme supplementation had limited impact on the SID of crude protein and AA regardless of dietary fiber level. However, the SID of 7 out of 10 indispensable amino acids (His, Ile, Lys, Phe, Thr, Trp, and Val) was 3-6% lower in the high-fiber than the low-fiber diet independent of enzyme supplementation level. This should be considered when formulating high fiber diets for gestating sows that contain similar ingredients.

Based on the results in Chapter 2, the response to the ME_{blend} observed in growing pigs does not necessarily reflect the same response in gestating sows. Therefore, extrapolating the effects of enzyme supplementation from growing pigs to gestating sows should be taken with caution. The efficacy of enzymes is also in part related to a balance between substrate availability and enzyme concentration. It is possible that substrate availability in the high-fiber diet overwhelmed potential enzyme activity at the 0.08% inclusion. This concept might also apply to feedstuffs with greater fiber and non-starch polysaccharide content, such as what was seen with the sugar beet pulp in Chapter 3.

Two of the five pillars for sustainable swine production include utilizing non-renewable resources and mitigating negative impacts on the environment. Post-protein separation corn dried distiller grains with solubles (post-MSC DDGS) fed to gestating sows was approximately 45 and 12% greater in digestible, metabolizable, and net energy than soybean hulls and sugar beet pulp, respectively. The energy values of these fibrous feedstuffs are also 5% greater in gestating sows compared to published values in grower pigs. The post-MSC DDGS can be used as an alternative fiber and energy source in commercial gestation diets and promote sustainable swine production by utilizing a co-product of biofuel production while reducing the necessity for more arable land to raise grains for both biofuel and livestock production.

Cumulative gas production following *in-vitro* fermentation was also lower with the post-MSC DDGS compared to the other fibrous ingredients. A limitation to using *in-vitro* model is the potential of the model not being representative of the *in-vivo* actions occurring the animal. This study (Chapter 4) is also the first study conducted that used fecal samples from animals to be as inoculum for their respective dietary treatment. The gas kinetics and parameters collected based on sow fecal inoculum also differed from values reported in grower pigs.

Supplementation of a yeast carbohydrate (**YC**) at 0.2% dietary inclusion in sow diets benefited litter performance based on weaning more light born pigs as well as a higher colostral immunocrit ratio and offspring IgA levels at weaning. Yeast supplementation in sow diets may provide a means to enhance performance of lightweight, higher risk piglets. Other studies evaluating the effects yeast derived products in sow diets began supplementation period earlier than the current trial or for the entirety of gestation. Supplementation of the YC used in this current trial starting at an earlier period might produce additional and beneficial effects for both the dam and nursing offspring.

A lower pre-weaning mortality of light born pigs in litters nursing sows supplemented with YC at 0.2% was not an expected outcome. Blood serum at weaning were collected on the average size pig per litter believing the average size pigs would have benefitted more so. Thus, it cannot be presumed that light born pigs in the 0.2%YC supplementation had a better developed immunity based on the average pig having elevated serum IgA at weaning. Potential follow ups to validate beneficial effects of YC supplementation in sow diets for light born are focusing more on immune parameters at weaning of light born pigs and performance post-weaning.

In conclusion, the extent of enzyme efficacy was dependent on the feedstuff, where the enzyme effect was greater on protein feedstuffs than on cereal grains. A multienzyme complex at 0.1% inclusion in complete gestation diets increased total tract digestibility of nutrients and energy for gestating sows by 3 to 10%, depending on the dietary NDF level. Although multienzyme supplementation did not impact the ileal digestibility of amino acids, higher dietary fiber reduced standardized ileal digestibility of amino acids in gestating sows by approximately 3%. This information is crucial when formulating high-fiber diets for gestating sows that contain similar ingredients.

Overall, the findings from this dissertation can be incorporated in commercial production to benefit and improve the feeding of breeding herds.

BIBLIOGRAPHY

Garrin Lee Shipman was born June 1, 1995 to Scott and Teresa Shipman in Charlotte, NC. While visiting his grandparents' farm in Lake Toxaway, NC he began to love animals. Garrin's uttermost passion in life has been his Paso Fino horses. Starting at an early age, Garrin has been successfully showing Paso Fino horses as well as winning multiple regional and national titles within the Paso Fino Horse Association.

It was not until his junior year of college at North Carolina State University (NCSU) that Garrin was introduced to pigs.He immediately became intrigued with hogs while assisting pulling pigs from a sow. This led him to eventually work as an undergraduate research assistant under Dr. Eric van Heugten. In the summer of 2018, Garrin participated in a swine production internship with Prestage Farms, Inc. to learn more about the swine industry. During this internship, Garrin discovered his interest in sow nutrition. Garrin graduated with his Bachelor of Science degree in Animal Science in the Fall of 2018. Garrin completed his master's degree in Animal Science at NCSU in the fall of 2020 with Dr. van Heugten as his advisor. Under Dr. van Heugten's tutelage, Garrin was able to pursue his interest in sow nutrition. His M.S. thesis focused on the influence of dietary essential fatty acids on the subsequent reproduction of sows.

Garrin's passion and interests in sow nutrition led him pursue a Ph.D. program at South Dakota State University under the advisement of Dr. Crystal Levesque. He hopes to begin his career as a professional swine nutritionist in commercial swine production where he can have a direct impact on swine production and "help feed the world."