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Effects of a Low Allergenic Soybean Variety on Gut Permeability, Digestibility and Growth Performance in Pigs

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EFFECTS OF A LOW ALLERGENIC SOYBEAN VARIETY ON GUT PERMEABILITY, DIGESTIBILITY AND GROWTH PERFORMANCE IN PIGS

BY

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THESIS ACCEPTANCE PAGE

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis 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.

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TABLE OF CONTENTS

ABBREVIATIONS

ΔIsc	Change in short circuit current
AA	Amino acid
ADG	Average daily gain
ADFI	Average daily feed intake
BBI	Bowman-Birk Trypsin inhibitor
^{o}C	Degrees Celsius
CON	Conventional
CONGR	Conventional full fat, ground soybeans
CONSBM	Conventional soybean meal
d	Day(s)
FD4	Fluorescein isothiocyanate-dextran
FM	Fish meal
g	Gram(s)
G:F	Gain to feed ratio
GI	Gastrointestinal
h	Hour(s)
IL	Interleukin
INFγ	Interferon gamma
Isc	Short circuit current
kg	Kilogram(s)
KTI	Kunitz Trypsin inhibitor
LA	Low allergenic
LAGR	Low allergenic full fat, ground soybeans

LASBM	Low allergenic soybean meal
L:M	Lactulose:mannitol ratio
m	Meter(s)
NCI	Northern Crops Institute
SAS	Statistical analysis system
SBM	Soybean meal
TER	Transepithelial resistance
TGF-β	Transforming growth factor beta
TI	Trypsin inhibitor
TNF-α	Tumour Necrosis Factor alpha

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

EFFECTS OF A LOW ALLERGENIC SOYBEAN VARIETY ON GUT PERMEABILITY, DIGESTIBILITY AND GROWTH PERFORMANCE IN PIGS

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Soybeans are the 'gold standard' protein source in pig diets, with lower inclusion levels in weaned pig diets due to transient inflammatory and hypersensitivity responses. This study evaluated a low allergenic (LA) soybean seed containing low Trypsin inhibitors, lectins, and P34 protein. The objective was to determine the impact of weaned pig diets containing LA soybean in both meal (LASBM) and full fat ground (LAGR) forms on the intestinal permeability and gut microbial composition, digestibility of protein and amino acids, and growth performance, in comparison to conventional (CON) soybeans and animal proteins (ANIM). In study 1, 60 weaned barrows (20.9 ± 1.0 d of age, 6.65 ± 0.3 kg, n=12/diet) were randomly assigned to one of five experimental diets containing one of 5 test proteins (CONSBM, CONGR, LASBM, LAGR and ANIM). Gut permeability measurements (Ussing Chambers and lactulose:mannitol ratio) were collected over 4d (1 pig/diet/d), beginning at d 11. No differences were detected in ileal or jejunal permeability among dietary treatments. Pigs fed ANIM had highest ($P < 0.05$) urine lactulose:mannitol ratio. Daily gain and feed disappearance were greatest ($P < 0.05$) for pigs fed ANIM-based diets. There were no differences in taxonomy or relative abundance of operational taxonomic unit's (OTU's) in digesta microbial content. In Exp. 1 of study 2, 10 ileal-cannulated barrows (17.63 ± 1.18 kg BW) were used in a cross-over design and randomly assigned to one of five experimental diets (FM, CONSBM1,

LASBM, LAGR and nitrogen-free), where the test ingredients were included as the sole protein source, to determine standardized ileal digestibility. Each pig received 3 of the 5 diets (1 diet/collection period; n = 6 per diet). In Exp. 2, the methods used in Exp. 1 were replicated, except 5 barrows and 5 gilts were used (19.40 ± 1.65 kg). In Exp. 1, SID of CP and AA was greater ($P < 0.05$) in FM than soy products. There were minor differences in digestibility between soy products where SID of LYS, MET, and HIS were greater ($P < 0.05$) in LASBM than CONSBM1. In Exp. 2, SID of CP and AA was similar between FM and CONSBM2 and lower ($P < 0.05$) in LASBM and LAGR than FM and CONSBM2. Overall SID tended ($P < 0.10$) to be lower in gilts than barrows. Growth performance was determined in 112 weaned pigs (7.30 ± 0.43 kg BW; 2 barrows and 2 gilts per pen; study 3) assigned to one of four dietary treatments in 2 phases (Ph1 = 5d, Ph2 = 13d). The control diet contained FM (7.25%, Ph1; 6%, Ph2); LASBM, LAGR and VOLGA-SBM replaced FM to supply equivalent dietary crude protein. Pigs received a common Ph3 diet (18d). Overall daily gain was greater ($P < 0.01$) in LAGR-fed pigs compared to LASBM, but not different from FM- or SBM-fed pigs; daily gain tended to be greater ($P < 0.10$) in SBM- than FM-fed pigs. Overall daily intake tended to be greater ($P < 0.10$) in SBM-fed pigs compared to FM and LASBM but was not different from LAGR. There was a tendency for greater ($P < 0.10$) overall G:F in LAGR- versus LASBM-fed pigs. Low allergenic soybean products may be considered a replacement for CON soybean products in weaned pig diets due to their similarity in gut permeability, digesta microbial content and similar growth performance. LAGR does not impact pig performance and could serve as a suitable replacement to FM in weaned pig diets.

Chapter 1

LITERATURE REVIEW

Weaning is a critical time for the health of the pigs as they are undergoing several different stressors and have immature immune and digestive systems. When pigs are weaned, they undergo stress of being removed from the sow, transportation to a new facility, new surroundings and mixing of pigs, as well as dietary stress transitioning from a complete milk diet to a dry diet (Lallès et al., 2004). A common weaning age in the U.S. is 21 days of age, a time point where passive immunity from the sow is low and their own adaptive immune system has not completely caught up yet. Their digestive systems are at a stage where they are adapted to milk constituents and less adapted to digesting plant-based ingredients (Lallès et al., 2004). All these factors need to be taken into consideration when feeding weaned pigs.

1.1 NEGATIVE IMPACTS OF WEANING

This part of the review will focus on the nutrient absorption capabilities, immune response, gut permeability, normal gut microbiome, and the growth performance of pigs following weaning. Most of the discussion of nutrient absorption will center around protein, even though absorption of other nutrients also plays an important role in pig health, particularly around weaning. The gastro-intestinal system of the pig is responsible for many different functions, including digestion and absorption of nutrients, serving as a barrier between the host animal and outside pathogens and antigens, and supplying necessary digestive enzymes, mucin and other components needed in the digestion and absorption process (Campbell et al., 2013).

The small intestine is responsible for breakdown of macromolecules (carbohydrates, proteins and lipids) into their specific monomers, which will later be absorbed by different pathways in the intestine to enter the blood stream (Feher, 2017). The intestines provide a large surface area for the nutrients to be absorbed. Inside of the intestinal wall there is a layer of columnar epithelial cells, or enterocytes that are primarily responsible for the transport of nutrients into the bloodstream. These enterocytes are part of the villi, which are finger-like projects within the lumen that allow for increased surface area in the intestine. On the luminal side of the enterocytes, are micro-villi which continue to add surface area, and are also known as the brush border. The brush border is responsible for the production and release of digestive enzymes. In between each villus, there are the crypts of Lieberkuhn. The crypt is responsible for creating new epithelial cells as the cells are constantly maturing as they move up the villus, and ultimately they will be sloughed off within a few days of being produced (Feher, 2017).

During weaning, the pigs are undergoing a period of physiological stress as they are switching from a milk-based diet to a plant-based diet. As part of this dietary change, only about 50% of the pigs eat within the first 24 hours after weaning and approximately 10% of pigs do not consume feed until 48 hours post wean (Brooks et al., 2001). Long periods without nutrients, or fasting periods, can play a large role in gut structure and function changes. Specifically, the villus height decreases (villus atrophy) and there is an increase in crypt depth (crypt elongation) (Cera et al., 1988; Li et al., 1990; Kelly et al., 1991; Li et al., 1991a; Li et al., 1991b; van Beers-Schreurs et al., 1998; McCracken et al., 1999; Tang et al., 1999; Hedemann et al., 2003; Boudry et al., 2004; Pié et al., 2004;

Verdonk et al., 2007). When villus height decreases, absorptive capacity of the intestinal epithelium also decreases. New epithelial cells are continuously being produced regardless of the size of villi. When the villi decrease in height, there is less replacement of sloughed off cells by new cells, causing a backup in the crypt resulting in elongation. Less cells present in the villi decreases the rate of absorption.

The brush border enzymes change in synthesis rate when a new diet is introduced, which can interrupt digestion and absorption (Lallès et al., 2004; Campbell et al., 2013). Specifically, lactase activity decreases after weaning, which may solely be related to the change in dietary composition (Pluske et al., 1997; Pié et al., 2004). On the other hand, enzymes such as aminopeptidase and dipetidylpeptidase, two of the proteolytic enzymes responsible for protein digestion, increase within 5 to 9 days post weaning (Hedemann et al., 2003). Other enzymes such as sucrase (Pié et al., 2004), maltase and glucoamylase have been shown to increase around 6 days post weaning (Kelly et al., 1991). As the villi are shortened post weaning, the number of mature enterocytes available in those villi is also decreased. With a smaller number of mature enterocytes, there are less cells to effectively produce digestive enzymes (Hedemann et al., 2003). The activity of brush-border enzymes has been an indicator of cell maturation and digestive capabilities for quite some time (Henning, 1985; Hampson and Kidder, 1986).

1.1.1 Immune response

In nature, pigs are typically weaned from the sow at about 10 to 12 weeks, whereas in commercial production, the common wean age is 14 to 30 days of age (Moeser et al., 2017). Weaning age has been shown to influence antibody synthesis

where serum antibody levels were lower in pigs weaned at 21 days compared to pigs weaned at older ages (Blecha et al., 1983). Early weaning imposes additional stressors beyond changes in diet to the animal, which occurs at a time point in the pigs life when the passive immunity pigs received from sow's milk is decreasing (Figure 1.1) (Moeser et al., 2017). Also at this time, the gastrointestinal barrier development is rapidly increasing, but is not fully developed until 12 to 14 weeks of age (Moeser et al., 2017). Due to gastrointestinal tract immaturity and low passive immunity, in conjunction with weaning, epithelial barrier function can be compromised.

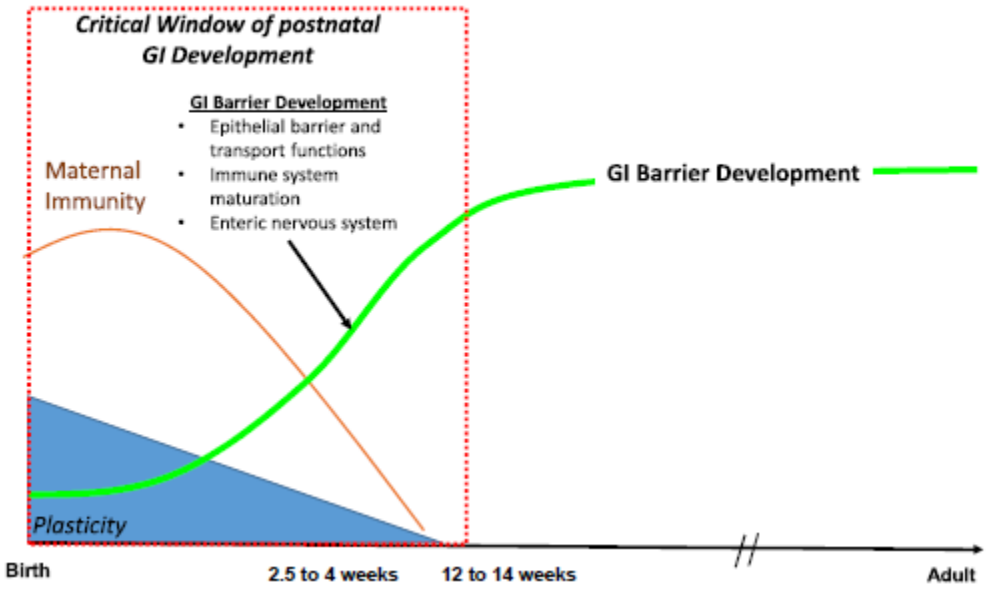

Figure 1.1: Critical window of postnatal GI development in correlation with maternal immunity over time. Adapted from Moeser et al. (2017).

In addition, pro-inflammatory cytokines increase after weaning and affect barrier function of the epithelium (McKay and Baird, 1999). In particular, Tumour Necrosis Factor alpha (TNF-α), Interferon gamma (INF-γ), and Interleukin 6 (IL-6) are all pro-inflammatory cytokines and have been demonstrated to increase intestinal permeability

(Youakim and Ahdieh, 1999; Al-Sadi et al., 2009). On the other hand, IL-10 and transforming growth factor beta (TGF-β) are anti-inflammatory cytokines that work to stabilize the barrier function (Madsen et al., 1997; Howe et al., 2005), and have not been found to increase after weaning (Hu et al., 2013). While serum cytokine levels increase, mast cell production also increases post weaning, as early as 24 hours post wean (Moeser et al., 2007a). Mast cells are found in the gut mucosa and play an active role in stimulating the immune response within the gut (Abraham and St. John, 2010). They act as a defense system for the gut, but when produced in large amounts, create additional inflammatory responses that can increase intestinal permeability (Moeser et al., 2017).

1.1.2 Gut permeability

The intestinal epithelium is responsible for digestion and absorption of nutrients into the blood, but they are also responsible for maintaining a strong barrier between the host animal and outside toxins, pathogens and antigens that are exposed to the small intestine (Lallès et al., 2004; Campbell et al., 2013; Moeser et al., 2017). There are two ways that particles can move through the epithelium: paracellular or transcellular. Paracellular transport is when nutrients travel between two epithelial cells and transcellular is when nutrients travel through the cells. Typically, epithelial cells contain a signal that only allow small nutrients with the correct polarity to flow between the cells paracellularly, including water and mannitol. However, large molecules, such as nutrient polymers, toxins, pathogens and antigens need protein transporters to move through the cell transcellularly. Tight junction proteins control the flow of large particles, toxins, or pathogens between cells. Tight junctions connect adjacent epithelial cells and limit passage of materials via paracellular transport across the epithelium (McKay and Baird,

1999). Tight junctions are made up of several proteins that create a complete, unregulated barrier between epithelial cells (Anderson and Van Itallie, 1995). Ultimately the tight junctions are responsible for maintaining the integrity and permeability of the intestine (Blikslager et al., 2007), only allowing the passage of predetermined particles (Moeser et al., 2017). Tight junctions are also important to regulate the epithelium to prevent "leaky gut" (Moeser et al., 2017), which can cause enteric related diseases. Following weaning, the already discussed physiological and dietary stresses on intestinal morphology and immune response can also influence intestinal permeability.

To determine these effects, intestinal permeability can be measured using both *in-vivo* and *ex-vivo* methods. The *ex-vivo* method is typically include the Ussing chambers which measure transepithelial resistance (TER), short circuit current (Isc) and paracellular flux of molecules across the epithelial membrane (McKay and Baird, 1999). TER measures how resistant the epithelial membrane is to the transport of ions through the cell. This ultimately measures how secure the tight junctions are and how well they are inhibiting movement of charged molecules (Li et al., 2004). Short circuit current is defined (Li et al., 2004) as 'the charge per unit time when the tissue is short-circuited'. Short circuit current measures the number of charged ions that are passing through the membrane over time, typically Na+ for active transport (Clarke, 2009). Specific molecules can be added to the chambers to determine flux across the membrane, such as mannitol, fluoresceine isothiocyanate dextran (FD4) and horseradish peroxidase (HRP) (Lallès et al., 2004). Mannitol is a small molecule that can pass through the epithelium via paracellular transport when tight junctions are fully functional, thus measuring paracellular permeability (Galipeau and Verdu, 2016). On the other hand, FD4 and HRP

are larger molecules that cannot pass through the cell paracellularly unless there is an interruption in the tight junction barriers (Wijtten et al., 2011). The impact of weaning on intestinal permeability in pigs has been widely researched and has been shown to be increased in comparison to levels pre-weaning (Kelly et al., 1991; McCracken et al., 1995; Spreeuwenberg et al., 2001; Boudry et al., 2004; Moeser et al., 2007b; Hu et al., 2013). This increase in intestinal permeability has still been observed at 14-15 days post-weaning (Boudry et al., 2004; Hu et al., 2013). An increase in permeability can allow passage of unwanted toxins, antigens and pathogens across the epithelial membrane, which can result in inflammatory responses and systemic diseases throughout the body (Deitch et al., 1996) which can lead to post weaning diarrhea symptoms.

The *in-vivo* method of assessing gut permeability is typically accomplished by determining lactulose:mannitol concentration in the urine. Lactulose is a sugar that does not undergo carbohydrate digestion like other sugars, and thus can pass through the epithelial membrane via paracellular transport when tight junctions are broken. Because mannitol can pass through the cells either paracellularly or transcellularly (Wijtten et al., 2011) it's ratio to lactulose can be used as a predictor of gut permeability. These two sugars are not metabolized by the pig and absorbed as whole molecules into the blood, transported to the kidney and excreted via urine. When the ratio of lactulose:mannitol increases, there is an increase in intestinal permeability (Wijtten et al., 2011). After weaning, an increase in the ratio of lactulose:mannitol was observed (Zhang and Guo, 2009; Li et al., 2018). Pigs exhibiting enterocolitis, or inflammation of the intestines, which can occur after weaning, have been found to have a higher level of

lactulose:mannitol ratios in their urine compared to pigs without inflammation (Nguyen et al., 2014)

1.1.3 Growth performance

It has been well established that feed intake decreases after weaning, primarily within the first two days post weaning, causing an impact on intestinal morphology, immune response, brush border enzyme activity, and absorption. All of these factors related to the lack of feed intake, induces post weaning diarrhea and negatively impacts gain (Vente-Spreeuwenberg et al., 2003). Recovery of feed intake has been observed by day 5 post wean (McCracken et al., 1995; McCracken et al., 1999). However, that loss in feed intake and gain within the first week post-wean may never be recovered and thus impacts the time it takes for the pigs to get to market compared to their counterparts that consume feed post-weaning (Tokach et al., 1992).

1.1.4 Normal gut microbiome

Pigs are born with little diversity in their microbiome as they are not exposed to many antigens *in utero*. When born, they are exposed to both bacterial and dietary antigens that influence the development of their microbiome (Kelly and King, 2001). The first colonizers in the pig gut are typically *Lactobacillus*, *Enterobacteria*,and *Proteobacteria* (Lallès et al., 2007; Frese et al., 2015). These colonizers remain stable throughout the nursing period (Frese et al., 2015), until abrupt events such as dietary and environmental changes, commonly associated with weaning (Hopwood and Hampson, 2003; Gresse et al., 2017). A study done by Inoue et al. (2005) examined the diversity of intestinal microbiota from birth to 49 days. In this study they discovered that the total

number of bacteria remained stable from birth to weaning and increased in total number by day 49.

Weaning negatively influences the microbial community within the intestine and can increase the amount of anaerobic bacteria which can impact the intestinal epithelial barrier (Winter et al., 2013). Beneficial bacteria such as *Lactobacillus*, which assist in fighting off pathogens, decrease after weaning (Gresse et al., 2017) which can increase the instance of diseases (Konstantinov et al., 2006). This can lead to an increase in harmful bacteria passing through the intestinal barrier, leading to systemic diseases for the pigs, particularly *E. coli*, which has been observed as a pathogen causing diarrhea (Konstantinov et al., 2006).

1.2 FEEDSTUFFS IN WEANED PIG DIETS

At weaning, the pigs quickly undergo a change from a liquid milk diet to a solid plant-based diet. In order to allow for a smooth transition to a plant-based diets, milk products are typically added to the first 3 phases of weaned pig diets. In addition, plasma and other similar animal by-products are added to help with immature immune system. FM is sometimes added to diets as a highly digestible protein source in the post-weaning period. Soybeans are the ‘gold-standard’ protein source found in pig diets. However, there are some negatives to feeding soybeans to weaned pigs due to the transient hypersensitivity response and immune response such as increased inflammation and intestinal permeability, decrease in villus height and increase in crypt depth, and growth performance (Li et al., 1990; Li et al., 1991a; Freisen et al., 1993; McCracken et al., 1995).

Growth performance was lower after weaning for pigs receiving soy protein compared to milk protein, but improved by week 5 after weaning due to a transient

hypersensitivity response to the soy protein (Li et al., 1990). As previously noted, immediately after weaning villus height decreases, and crypt depth increases which is associated with a decrease in nutrient absorption, morphological changes that also occur when feeding soy proteins to nursery pigs (Li et al., 1990; Li et al., 1991a). McCracken et al. (1995) determined that villus height: crypt depth ratio linearly decreased from day 1 to day 7 post wean in pigs fed cereal grains.

Friesen et al. (1993) determined that it is important to add soybean meal to weaned pig diets immediately post weaning to allow the pigs to adapt. While there was no difference in growth performance when pigs were fed soybean meal during the first 14 days after weaning, there was an increase in ADG, ADFI and G:F from days 14-35 post weaning, suggesting the adaptation to soybeans (Freisen et al., 1993). If pigs are sensitized to soy protein within the first week of weaning, they have been reported to exhibit a lower ADG compared to milk proteins, but compensate for that loss in gain by week 5 after weaning (Li et al., 1990). Li et al. (1991a) also determined that ADG, ADFI and G:F were lower in soybean fed pigs up to 14 days after weaning compared to those fed milk protein, but had no difference from days 14-35. Villus height, an indicator of absorption in the small intestine, has been positively correlated with ADG from 0-14 days post weaning (Li et al., 1991a).

Antibody mediated immune responses occur when feeding soybeans to pigs. A common measurement is anti-soy IgG titers in the blood sera. The immunoglobulins can only pass through the intestinal epithelium into the blood when the intestinal epithelium barrier is broken, as discussed in the gut permeability section of this review. When pigs were fed high levels of soybean meal after weaning, their anti-soy IgG titers were higher

in comparison to other soy proteins and milk protein (Li et al., 1991a). Anti-soy IgG titers have been negatively correlated with villus height (Li et al., 1991a), impacting nutrient absorption. It has been researched that after exposure to soybeans, pigs have an increase in the level of soy IgG titers, which reduce the measurable hypersensitivity response to the soy proteins (Freisen et al., 1993).

Soybeans also contain anti-nutritional factors and can have an impact on nutrient utilization, which will be discussed later in this review. Due to the negative impacts that soybeans have on the weaned pig, other feedstuffs have been widely researched. Other feedstuffs that are fed in weaned pig diets include fish meal, animal proteins (blood plasma, blood meal) and different versions of processed soy proteins (enzymatically treated, soy protein concentrate, hydrolyzed protein).

1.2.1 Anti-nutritional factors and allergens of soybeans

Soybeans, like other legumes, contain anti-nutritional factors (ANF) that are defined as "biological compounds in feeds that reduce nutrient utilization or feed intake, thereby contributing to impaired gastrointestinal and metabolic performance" (Dunlop and Malbert, 2004). ANF's found in soybeans include trypsin inhibitors, lectins, and others, all of which contribute to decreasing nutrient utilization (Mekbungwan, 2007). There are also different allergens within the soybean that are primarily responsible for hypersensitivity in pigs and humans.

1.2.2 Trypsin Inhibitors

The most commonly discussed group of ANF's found in the soybean seed is the protease inhibitors, specifically important in swine nutrition is the trypsin inhibitor (TI). The TI in the soybean plant has been found to be a defense mechanism for the plant to

fight off predators (Ryan, 1990). TI acts to inhibit proper functioning of proteolytic enzymes, which may relate more with the species consuming the soybean rather than the plant itself (Liener, 2012).

Proteins are broken down in two different phases, starting in the stomach with chemical digestion or the activity of acid denaturing the proteins and the activation of pepsinogens to pepsins (Feher, 2017). Pepsinogens is a class of enzymes known as endopeptidases which break down peptide bonds in the proteins. In the intestinal phase, the endopeptidases and exopeptidases are released in large quantities by the pancreas into the intestine where they become activated. Already in the intestine, sitting on the intestinal border is enterokinase, which activates trypsinogen to trypsin. This is an extremely important step as the increase in trypsin availability activates all other proteolytic enzymes (Feher, 2017). Another reason that this is important, especially in swine nutrition, is because trypsin breaks down two amino acids, lysine, the most limiting amino acid in swine diets (Lewis and Southern, 2000; NRC, 2012), and arginine. Chymotrypsin is also an important enzyme as it breaks the peptide bonds in aromatic amino acids (Feher, 2017).

The presence of TIs affect proteolytic digestive enzymes in mammals and their capability to break down proteins by blocking the active site (Huisman and Jansman, 1991), decreasing the activation of trypsin from trypsinogen. When there is a high level of TI present, the level of trypsin available to break down proteins is decreased. Due to this decrease in trypsin availability, the hormone cholecystokinin (CCK) sends a response to the pancreas which then releases more trypsinogen in a positive feedback mechanism (Liener, 1994; Morisset, 2008). In broilers, pancreatic weight increases when the diet

contains soybeans with high TI levels (Palacios et al., 2004). It is expected that the increase in weight is due to an increase in pancreatic activity as a result of the increase in CCK which increases the endogenous secretion of all pancreatic enzymes. The relative pancreas weight in pigs fed soybeans was higher than in pigs fed a casein based diet which is low in TI's (Salgado et al., 2002). Because of the high metabolic activity that is occurring when additional enzymes are synthesized in the pancreas, there is increased energy expenditure for maintenance rather than using that energy towards muscle tissue deposition (Ferrell, 1988).

There are two types of TI found in soy proteins; Kunitz trypsin inhibitor (KTI) and Bowman-Birk trypsin inhibitor (BBI) with each having its own properties and functions. The more common TI is the KTI, which is found at 3 to 4-fold more per gram of sample compared to BBI (Anderson and Wolf, 1995). KTI consists of 181 amino acids and has two disulfide bridges. KTI are primarily responsible for the inactivation of trypsin but also some chymotrypsin (Huisman and Jansman, 1991). The KTI can be inactivated using heat processing or inactivated in acidic gastric juices found in the stomach of monogastric animals (Kunitz, 1947; Kassell, 1970). The BBI is made up of 71 amino acids and has seven disulfide bridges. BBI has two different binding sites one for trypsin and one for chymotrypsin (Huisman and Jansman, 1991), where protein synthesis can be interrupted. Unlike the KTI, BBI cannot be inactivated by heat or the acidic pH found in the stomach (Kassell, 1970), due to the difference in disulfide bridges between the two inhibitors. Both KTI and BBI inhibit the digestion of proteins. KTI is the primary TI that is found in soybeans and primarily inhibits trypsin with little inactivation of

chymotrypsin. BBI on the other hand, has two different active sites, so it is more likely to be the inactivator of chymotrypsin compared to KTI (Huisman and Jansman, 1991).

Most soybeans that are fed to pigs are in the form of soybean meal which has been heat treated to remove the KTI. However, heat treatment of soybean meal, does not completely inactivate all TI; residual TI, measured as trypsin inhibitor units (TIU), is primarily in the form of BBI. A TI level of 1-8 mg TIU/g is considered a good standard for soybean meal (Peisker, 2001; Banaszkiewicz, 2011), where there are minimal negative effects on protein digestion. When TI levels are low, or void, the digestibility of amino acids increase (Wright, 1981; Herkelman et al., 1992; Li et al., 1998; Goebel and Stein, 2011). When removing the KTI, improvements in growth performance and feed efficiency have been observed in both chicks (Palacios et al., 2004) and pigs (Herkelman et al., 1992; Friesen et al., 1993; Palacios et al., 2004) which is attributed to the increase in amino acid digestibility. However, TI are not the only ANF that have been attributed to a decrease in growth performance or total nutrient availability, as they only account for about 40% of growth interference (Kakade et al., 1973) .

1.2.3 Lectins

Another ANF important in swine feeding are lectins, also commonly known as soybean agglutinins (Liener, 1994). Lectins found in soybeans are responsible for 25% of all ANF growth interference exhibited by pigs (Liener, 1994). Lectins are proteins found in the soybean that are not broken down by proteolytic enzymes and can stay in their functional form throughout the entire intestinal tract. These proteins have the capability to bind to carbohydrates, as well as agglutination of cells, which is why they are considered an ANF (Liener, 1994). Lectins have high affinity for carbohydrates and can

attach to carbohydrate receptors on the intestinal epithelium, typically on the upper lining of the small intestine. When lectins are attached to the intestinal lining, the digestive and nutrient absorption capabilities are impaired; specifically lectins interfere with transportation of macronutrients through the intestinal wall (Huisman and Jansman, 1991), most notably glucose and amino acids (Liener, 1986). When the lectins attach to the intestinal lining, they also impact intestinal permeability and the morphology of the brush border (Liener, 1986). In addition, the epithelial cells in the villi are shed into the lumen and replaced by new cells. When cells with lectins attached to them are shed into the lumen, the cell constituents are metabolized as they move down the lumen, except for the lectins as they are still resistant to proteolytic enzymes (Pusztai et al., 1990). Thus, the lectin can attach to more carbohydrate receptors further down the gut (Bardocz et al., 1995). Like TI's, lectins can be inactivated using heat treatment, with moist heat being more effective at inactivation than dry heat (Huisman and Jansman, 1991).

In a study done by Douglas et al. (1999) they found feeding a low lectin raw soybean to chicks increased weight gain, but did not affect feed intake compared to feeding a conventional soybean meal that was heat treated. An increase in the percentage of lectins fed to weaned pigs was found to increase intestinal permeability (Zhao et al., 2011). When lectins are still present in the soybean products, the digestibility of protein decreases (Lajolo and Genovese, 2002). If both lectins and TI are removed from a soybean source, the resultant soybean product can be expected to have a larger impact on improving gain, feed intake and feed efficiency in both poultry and pigs compared to removing them independently (Douglas et al., 1999; Palacios et al., 2004), due to the increase in protein digestibility (Lajolo and Genovese, 2002).

1.2.4 Glycinin and β-conglycinin

Glycinin (11S) and β-conglycinin (7S) are storage proteins that are found within the soybean seeds, also referred to as globulins, that have allergenic effects and cause hypersensitivity responses in both humans (Burks Jr et al., 1988) and pigs (Sun et al., 2008a; Sun et al., 2008b). Glycinin is the larger protein fraction of the soybean that has a molecular mass of 320kd with 6 subunits which accounts for 40% of the total 11S fraction of the soy protein (L'Hocine and Boye, 2007). The 11S fraction makes up 31-52% of the soluble proteins. β-conglycinin is a glycoprotein that has about half the molecular mass of glycinin at 180kd and consists of 3 subunits (Breiteneder and Ebner, 2000), making up 85% of the 7S fraction of the soy protein, which is about 35% of the total soluble proteins (L'Hocine and Boye, 2007). Together, these two storage proteins make up 42 to 51% of the total soy protein. Glycinin is soluble in acid whereas β-conglycinin is not soluble (Wolf, 1976).

When glycinin levels increase in the diets fed to pigs, a linear decrease in average daily gain and feed conversion was reported (Sun et al., 2008a). In addition, CD4+ lymphocytes (Sun et al., 2008a), IgE (Sun et al., 2008b) and IgA (Sun et al., 2008a) levels in jejunal mucosal tissues increased when diets contained higher glycinin levels. Pro-inflammatory cytokines IL-4, IL-6 (Sun et al., 2008a) and anti-inflammatory cytokine IL-10 (Sun et al., 2008b) increased with increasing glycinin levels.

A decreased gain and feed intake in rats, as well as, increased serum IgE concentrations with increasing levels of β-conglycinin and over time have been reported (Guo et al., 2007). Pro-inflammatory cytokines IL-4, IL-5 and TNF-α in the plasma and spleen of rats also increased with β-conglycinin as well as the CD4+ lymphocytes in plasma and blood (Guo et al., 2007). The increase in serum IgE concentration, pro-

inflammatory cytokines and lymphocytes can interrupt the intestinal function and inhibit gastrointestinal absorption, which can impact growth, as discussed earlier in this review. Growth performance measures of average daily gain, average daily feed intake and feed conversion decline when pigs are fed diets that contain β-conglycinin (Zhao et al., 2010).

In a soybean meal diet that was fed to pigs without glycinin and β-conglycinin compared to a diet that had those allergens, there was an increase in villus height and villus height:crypt depth ratio (Dréau et al., 1994). Li et al. (1990) determined that dietary antigens such as glycinin and β-conglycinin play a large role in the delayed hypersensitivity response to weaned pigs fed soy proteins. Hypersensitivity to these dietary antigens has also been linked to post weaning diarrhea (Miller et al., 1983).

1.2.5 P34 and other allergens

Another portion of the 7S storage protein of the soybean is the allergen called Gly m Bd 30k, commonly referred to as P34 protein. As previously noted, β-conglycinin represents 85% of the total 7S fraction, leaving the other 15% to other proteins such as P34, Cytochrome *c*, β-amylase, lipoxygenases and lectins (Nielson, 1985), therefore, the P34 protein makes up less than 5% of the total soybean storage proteins. This protein was first discovered as the major soy protein allergen in humans by Ogawa et al. (1991) when patients with atopic dermatitis had large amounts of P34 in their serum.

P34 protein is the main soy specific immunodominant antigen in humans with soy allergies. In a recent *in vitro* study by Sewekow et al. (2012) it was reported that some of the P34 proteins resisted proteolysis and can bind to the epithelium to be endocytosed into the cell. Since the P34 is attaching to the epithelium and resisting digestion, it is likely to suppress the immune system and elicit an inflammatory response.

1.2.6 Other anti-nutritional factors

Additionally, there are other ANF's that are associated with soybeans that play a role in reducing nutrient availability for the pig. These include phytic acid and saponins. Phytic acid is a compound found in soybeans that inhibits the availability of phosphorous to the pig. Despite phytic acid inhibiting available phosphorous, it also impacts availability of minerals as minerals can attach to the phosphate groups on the phytic acid and then become unavailable for absorption (Liener, 1994). Higher amounts of phytic acid have been shown *in vivo* to inhibit the amount trypsin activity (Singh and Krikorian, 1982; Caldwell, 1992). Phytase is commonly used in swine diets to break down the phytic acid and increase the availability of phosphorous.

Saponins are compounds that consist of a steroid linked to one or more oligosaccharides. Saponins have hemolytic activity and can make the soybeans taste bitter if found in large quantities (Liener, 1994), which can reduce feed intake. Saponins are heat stable and make up approximately 0.5% of the total soybean (Liener, 1994). If saponins are present at higher levels in pig diets, there are negative effects to the mucosal membranes due to depolarization. Depolarization causes a change in charge of the cell which can decrease nutrient absorption thus decreasing growth (Smith and Dilger, 2018).

1.3 ALTERNATIVE SOY PRODUCTS

Over the years there have been many new soybean products that have entered the swine feed market, typically using different processing methods. While FM and animal by-products are high quality sources of amino acids, these processed soy products have played a major role in the reduction of FM and animal by-products in nursery pig diets. There is concern with using FM in weaned pig diets including the increased cost, decrease in availability and variation in growth response between FM sources (Jones et

al., 2010). Animal by-products have also been reduced due to concern with biosecurity and increased cost. Soybeans can be processed to increase nutrient digestibility (Min et al., 2004) by inactivating ANF's, making processed soybeans a potential replacement for FM and animal proteins in weaned pig diets. Some of the products that have been produced and successfully used in nursery pig diets include: HP300, microbially enhanced soybeans, soy protein isolate, fermented soy protein, among others (Li et al., 1991a; Li et al., 1991b; Zhu et al., 1998; Sinn et al., 2017).

An alternative soybean, also known as triple null or low allergenic (LA), was developed by breeding out the KTI, soybean agglutinins (lectins) and P34/*Gly m* BD 30k protein, thus eliminating them from the soybean seed (Schmidt et al., 2015). This LA soybean was developed for both human consumption and livestock feeding purposes. As discussed earlier, P34 protein is the main cause of soy allergies in humans, thus removal of P34 from the soybean seed may suppress the allergic reaction. In livestock feeding practices, the LA soybean could be beneficial as a full fat, ground soybean null of TI and lectins, removing the need for heat treatment. The LA ground full fat soybeans could potentially reduce the cost of processing for producers. This LA soybean could possibly be beneficial as a value-added product like HP300 and microbially enhanced soybean products that are good sources of amino acids to replace FM and other animal proteins in weaned pig diets, without the hypersensitivity and anti-nutritional effects of conventional soybean meal.

1.4 RESEARCH OBJECTIVES

The objectives of this research were to 1) determine the effect of feeding LA soybean meal, LA ground soybeans, conventional soybean meal, conventional ground soybeans and animal based protein meal on gut permeability and digesta microbial

population in weaned pigs; 2) determine standardized ileal digestibility of amino acids and digestible amino acid content of LA soybean meal and LA ground beans in comparison to conventional soybean meal and FM and 3) determine the effect of feeding LA soybean meal and ground beans as a substitute for conventional soybeans on growth performance of pigs in the first six weeks after weaning.

Chapter 2

Effect of low allergenic soybean products on gut permeability and digesta microbial composition in weaned pigs compared to conventional soybean meal and animal protein products

2.1 ABSTRACT

This study evaluated the effects of LA soybean seed in both the meal (LASBM) and full fat ground (LAGR) forms in comparison to conventional soybean meal (CONSBM), full fat soybeans (CONGR) and animal proteins (ANIM). The goal was to determine the impact of weaned pig diets containing LASBM and LAGR on the intestinal permeability, growth performance and microbial composition of digesta in comparison to CON soybean products or high-quality animal protein sources. Sixty weaned barrows (20.9 ± 1.0 d of age, 6.65 ± 0.3 kg, n=12/diet) were randomly assigned to one of five experimental diets (CONSBM, CONGR, LASBM, LAGR and ANIM) containing one of 5 test proteins where soybean meals made up 21.2% of the diet, ground soybeans made up 27% and animal proteins made up 19% such that test proteins contributed equivalent dietary crude protein. Pigs were individually housed in metabolism crates, with ad libitum access to feed and water for the 11d study. Gut permeability measurements (Ussing chambers and lactulose:mannitol ratio) were collected over 4d (1 pig/diet/d), beginning at d11. Data was analyzed using one-way ANOVA in SAS v9.4. The model included main effect of diet with pig as experimental unit. No differences were detected in ileal or jejunal permeability among dietary treatments. Pigs fed ANIM had higher ($P < 0.05$) urine lactulose:mannitol ratio compared to all other treatments. Daily gain and feed disappearance were greater ($P < 0.05$) for pigs fed ANIM vs all other treatments. There

were no differences in taxonomy or relative abundance of operational taxonomic unit's (OTU's) in digesta. Low allergenic soybean products may be considered a replacement for CON soybean products in weaned pig diets due to their similarity in gut permeability, digesta microbial content and similar growth performance.

Key words: intestinal permeability, soybean, trypsin inhibitors, weaned pig

2.2 INTRODUCTION

Soybeans are commonly fed to pigs as they are a high-quality source of amino acids. However, soybeans are typically included at a lower level in nursery diets with an increase in diet inclusion over time due to their abrasiveness (Cera et al., 1988), impact on intestinal morphology (Moeser et al., 2007a), suppression of immune response (Li et al., 1991b), and transient hypersensitivity response (Li et al., 1990; Friesen et al., 1993). Pigs undergo many stressors when they are weaned including abrupt removal from their mother, moving to a new location with new surroundings and pen mates, transportation stress and the change from a milk to a solid diet (Lallès et al., 2004). This weaning stress also impacts feed intake and growth performance of pigs in the early post-weaning period (McCracken et al., 1995; McCracken et al., 1999). These stressors can exacerbate the effects associated with feeding soybeans in weaned pig diets.

Soybeans contain ANFs that impact the availability of nutrients to the pigs, the most common ANF associated with soybeans is the TI. When TI's are found in large quantities in diets fed to pigs, digestibility of amino acids is inhibited (Wright, 1981; Herkelman et al., 1992; Li et al., 1998; Goebel and Stein, 2011). Soybean agglutinins, also known as lectins, are another common ANF found in soybeans. Lectins cannot be broken down via normal protein digestion and attach to carbohydrate receptors on

intestinal epithelium which can impair digestive and nutrient capabilities of pigs (Huisman and Jansman, 1991). Both TI and lectins can be inactivated by heat treatment (Huisman and Jansman, 1991). In addition, there are allergenic proteins (P34, glycinin and β-conglycinin) that are commonly associated with the transient inflammatory response to soybeans observed in humans and pigs (Li et al., 1990; Ogawa et al., 1991). The P34 protein is an important allergen in human nutrition as it is the main protein associated with soy allergies in patients with atopic dermatitis (Ogawa et al., 1991), and causes allergenic responses in humans, especially neonates (L'Hocine and Boye, 2007; Schmidt et al., 2015). Soy proteins are known as one of the eight major food allergens that account for 90% of all human food allergies (Hefle et al., 1996; Zarkadas et al., 1999).

To alleviate the effects of these ANF's on performance of pigs, an alternative soybean was developed through breeding and removal of alleles that express KTI, lectins and *Gly m* BD 28k (P34) protein (Schmidt et al., 2015). Removal of TI and lectins could potentially increase AA digestibility when fed to pigs, without having to heat treat the product. With the removal of KTI's, lectins and P34 protein, there is expected to be a lesser immune response in comparison to CON soybeans. Therefore, the objective of this study was to determine the effects of feeding LA soybean, in both meal and full fat ground form, on gut permeability, growth performance and gut microbiome of weaned pigs.

2.3 MATERIALS AND METHODS

South Dakota State University's Institutional Animal Care and Use Committee approved all protocols used in this experiment (IACUC #17-116A).

Animal housing, diets, and experimental design

Pigs were weaned from the South Dakota State University Swine Education and Research facility in three batches at 20.9 ± 1.0 d of age (n = 60 pigs, 20 pigs per batch; 6.65 ± 0.3 kg) and transferred to the pig metabolism room in the Animal Science Complex on South Dakota State University campus. Pigs were progeny of PIC 1050 (Landrace x Large White) sows and composite Duroc boars. Pigs in batch 1 and 2 were from sows on an unrelated nutrition trial. No more than 2 piglets/litter and only 5 pigs/sow treatment were used such that previous maternal treatment was balanced across experimental diets in this study. Pigs were assigned to one of five experimental diets (n = 12/dietary treatment) (Table 2.1). Conventional SBM (CONSBM), conventional full fat ground soybeans (CONGR), low allergenic SBM (LASBM), low allergenic full fat ground soybeans (LAGR), and animal protein sources (ANIM) were added to a base diet containing whey, HP300, casein and soybean oil, such that each test ingredient contributed equivalent dietary protein. The ANIM diet was included as a control with limited allergens and ANF's as the only soy product in the diet was HP300, which is null of allergens and ANF's (Zhu et al., 1998). All soy proteins for this trial were processed at the Northern Crops Institute in Fargo, ND; details of soy processing are included in Appendix A. Test ingredients and experimental diets were analyzed for AA, crude protein (CP), moisture, ash, ether extract, and crude fiber at a commercial laboratory (Experiment Station Chemical Laboratories, University of Missouri – Columbia). All diets were tested for total TI levels at Eurofins Nutrition Analysis Center (Des Moines, IA).

Pigs were individually housed with 0.45m^2/pig. Ten larger metabolism pens were separated by a mesh barrier to allow nose-to-nose contact; each half contained one nipple drinker and a stainless-steel feeder for ad libitum access to feed and water. Feed was provided twice daily (0800 and 1600h) simultaneous with daily care checks; feed orts were removed and weighed every other day. Room temperature was maintained at 26ºC and heat lamps were provided for the duration of the treatment period. Pigs were weighed upon arrival (d0) and just prior to tissue collection. Daily feed and intake were calculated over this same period.

Tissue Collection

Starting on day 11 of the trial, five pigs were sacrificed for tissue collection, which included one pig from each dietary treatment. This process continued over the course of four days (12, 13 and 14d after start of the trial). Five hours prior to euthanasia pigs were fed a bolus that contained 5% of both lactulose and mannitol provided at 15mL/kg (Nguyen et al. 2014). A collection pan placed under the pen was used to capture all urine prior to anesthesia. Tissues were collected under anesthesia and pigs were euthanized by captive bolt gun. Pigs were anesthetized by intramuscular injection of TKX (telazol:ketamine:xylazine – 50 mg/mL; 1 mL/pig). Urine was removed directly from the bladder using an 18-ga needle and 20 mL syringe. If urine could not be collected directly from the bladder, urine in the pan placed below the pig was retained.

The entire small intestine was removed from the abdominal cavity and laid out in equal loops to facilitate identification of the middle of the jejunum. 10 cm sections of mid-jejunum and ileum (beginning 5 cm proximal from the ileo-cecal junction) were removed for Ussing chamber analysis. These two sections of tissue were cut open along

the mesenteric border, rinsed, and placed in ice-cold Ringer (KBR) solution (115 mM NaCl, 2.4 mM K_2HPO_4, 0.4 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 25 mM $NaHCO_3^-$) with 10 µM of indomethacin and were transferred to the Ussing chamber room. Details on the Ussing chamber methods are provided in the *ex-vivo* section.

For the purpose of gene expression, 10 cm of duodenum (beginning 70 cm from the stomach), 10 cm of mid jejunum and 10 cm of ileum (beginning 15 cm proximal to ileo-cecal junction) were removed. Each section was rinsed with saline and patted dry before being placed in foil packets that were immediately frozen in liquid nitrogen. Digesta was obtained from the ileum, put into microcentrifuge tubes and frozen in liquid nitrogen and stored in the -80^{o}C freezer for later analysis.

In – vivo

Collected urine was analyzed using EnzyChrom™ intestinal permeability assay kits (BioAssay Systems, Hayward, CA) following manufacturer's instructions. Concentrations were calculated to provide a ratio of µM lactulose to µM mannitol (L:M) in the urine.

Ex – vivo

Tissue samples of jejunum and ileum were prepared for the Ussing chamber (Physiological Instruments Inc., San Diego, CA). The mucosal side of the tissue was placed onto a piece of parafilm on a Styrofoam sheet. Tissue was stretched and secured in placed using thumbtacks touching only the edges of the tissue to avoid tissue damage. The serosal layer was gently removed using microforceps. Each tissue was cut into three sections, mounted cassettes with an aperture of 1.00 cm^2, and immediately placed the chamber blocks with the mucosal side towards the left of the chamber and the serosal

side towards the right of the chamber. Cassettes were secured in chambers and 3 mL of Ringer's solution without indomethacin was added to each side. Chambers were gassed with carbogen (95% O_2 and 5% CO_2) to replicate respiration and keep the tissue alive during the experimental run.

Once all tissues were mounted in the Ussing chambers, 3 µL of D-mannitol was added to the mucosal side and 3 µL of glucose was added to the serosal side. The Ussing chamber was then referenced, and data collection began using the Acquire and Analyze program. Any issues with the chambers were noted at the start and throughout the procedure. After 15 minutes, the chambers reached equilibrium and 6 µL of glucose was added to the mucosal side and 6 µL of D-mannitol was added to the serosal side to determine the glucose induced short circuit change which was calculated over a 15-minute time period. At the 30-minute mark, 200 µL was removed from the serosal side of each chamber and placed into a 96-well plate to determine initial concentration of the buffer solution prior to the addition of dextran; 200 µL of KBR was added to the serosal side to replace the 200 µL removed. The lights in the room were shut off and 10 µL of FD4 was added to the mucosal side of each chamber, while 10 µL of KBR was added to the serosal side to balance chamber volume. 200 µL was removed from the serosal side of each chamber at time 15, 30, 45 and 60 and placed into the 96-well plate to measure the FD4 flux over the 60 minutes. Every time 200 µL was removed, it was replaced with 200 µL of KBR without indomethacin. Within 24 hours of plating, the 96-well plates with the standard curve were analyzed on a Synergy 2 Multi-detection Microplate Reader (BioTek, Winooski, VT) using the Gen 5 1.11 program in the fluorescence detection mode, with excitation of 485nm and emission of 528nm at the BioStress common

laboratory at South Dakota State University. The standard curve provided a range of 0 to 1 mg/mL of FD4.

Through the Acquire and Analyze data acquisition program (Physiological Instruments Inc., San Diego, CA), data for short circuit current (Isc), voltage (V) and total epithelial resistance (TER) was collected every 10 seconds over the course of the 90-minute sample collection described above. The average basal Isc was computed using the data points from 30-90 minutes. TER was calculated using the equation TER = V/Isc and averaged over the 30-90 minutes. From the three replicates of ileum and jejunum from each pig, the average of two samples with the best coefficient of variation was used for analysis.

Microbial analysis

Microbial DNA isolation and amplicon-based sequencing of the 16S rRNA gene

Microbial genomic DNA was isolated from ileal digesta samples from pigs fed CONSBM, LAGR and ANIM using DNeasy© PowerSoil© Kit (MO BIO laboratories, Qiagen, Venlo, Netherlands). In relation to potential impact on small intestinal microbial populations, processing technique was expected to have a greater impact than differences in soybean variety; therefore, digesta from pigs in one SBM group (CONSBM) and one full fat ground group (LAGR) were evaluated along with pigs from ANIM group. The V1-V3 region of the bacterial 16S rRNA gene was targeted for Next-Generation Sequencing using the 27F forward (Edwards et al., 1989) and 519R reverse (Lane et al., 1985) primer pair. Library preparations and corresponding sequencing runs using an Illumina MiSeq (2X300) platform to generate overlapping paired end reads were performed by the Genomics Sequencing Facility at South Dakota State University.

Computational analysis of PCR generated 16S rRNA amplicon sequences

Unless specified, sequence data analysis was performed using custom written Perl scripts (available upon request). Using the make.contigs command from the MOTHUR open source software package (Opdahl et al., 2018), raw bacterial 16S rRNA gene V1-V3 amplicon sequences were assembled into contigs from overlapping MiSeq (2X300) paired-end reads from the same flow cell clusters. Reads were then selected to meet the following criteria: presence of both intact 27F (forward) and 519R (reverse) primer nucleotide sequences, length between 400 and 600 nt, and a minimal average Phred quality of 33 or greater per read.

Following quality screens, sequence reads were aligned, then clustered into Operational Taxonomic Units (OTUs) at a genetic distance cutoff of 5% sequence dissimilarity (Opdahl et al., 2018). While 3% is the most commonly used clustering cutoff for 16S rRNA, it was originally recommended for full length sequences, and may not be suitable for the analysis of specific subregions since nucleotide sequence variability is not constant across the entire length of the 16S rRNA gene. In this context, if 3% is a commonly accepted clustering cutoff for V4 or V4–V5 regions, which are the least variable of the hypervariable regions, then a higher cutoff should be used for the V1-V3 region, since V1 is the most variable region of the 16S rRNA gene. OTUs were screened for DNA sequence artifacts using the following methods. Chimeric sequences were first identified with the chimera.uchime and chimera.slayer commands from the MOTHUR open source software package (Schloss et al., 2009). Secondly, the integrity of the 5’ and 3’ ends of OTUs was evaluated using a database alignment search-based approach; when compared to their closest match of equal or longer sequence length from

the NCBI nt database, as determined by BLAST (Altschul et al., 1997), OTUs with more than five nucleotides missing from the 5' or 3' end of their respective alignments were discarded as artifacts. Single read OTUs were subjected to an additional screen, where only sequences that had a perfect or near perfect match to a sequence in the NCBI nt database were kept for analysis, i.e. that the alignment had to span the entire sequence of the OTU, and a maximum of 1% of dissimilar nucleotides was tolerated.

After removal of sequence chimeras and artifacts, taxonomic assignment of valid OTUs was determined using a combination of RDP Classifier (Wang et al., 2007) and BLAST (Altschul et al., 1997). The List of Prokaryotic Names with Standing in Nomenclature (LPSN - http://www.bacterio.net) was also consulted for information on valid species belonging to taxa of interest (Parte, 2013).

Statistical analysis

Statistical analysis was performed using the PROC MIXED procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC). A randomized complete block design was used with individual pig as the experimental unit for all tested parameters. The LAGR treatment had 11 final observations as one pig was lost due to illness unrelated to experimental treatment. All other treatments had 12 observations. Main effect was treatment and random effect was block. Where there was a significant main effect of treatment, Tukey's adjustment was used as means separation test. Contrast statements were used to compare effect of ground soybeans versus soybean meals, effect of CON soy products versus LA soy products, and effect of ANIM dietary treatment compared to all soybean treatments. For microbial analysis, a one-way nonparametric ANOVA was used, with a Kruskal-Wallis rank sum test for unrepeated measures (Version 9.4, SAS

Inst. Inc., Cary, NC). Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

2.4 RESULTS AND DISCUSSION

Diets

Analyzed content of experimental diets can be found in Table 2.1. On the basis of total trypsin inhibitor levels (TIU/g) diets were ranked from highest to lowest as CONSBM, CONGR, LAGR, LASBM, and ANIM. Diets containing full fat soybeans would be expected to have more TIU than their meal counterparts due to the inactivation of TI by heat during meal processing (Witte, 1995). The LA soybean products followed this expectation, but the CON soybean products did not. The raw ingredients were also analyzed for nutrient content and TI levels (Table 2.2). Based on these analyses, there was a small difference between CONSBM and CONGR which may suggest heat application at the processing plant was not as expected. A sample of SBM processed at a commercial mill (VOLGA-SBM; South Dakota Soybean Processors, Volga, SD) was also analyzed and TIU value for this meal was very low in comparison to the test meals, supporting the lesser heat application during processing of test soybeans. Undercooked SBM's have TI levels exceeding 12 TIA mg/g (Witte, 1995) which equates to 22,800 TIU/g (Jiang et al., 2013). In the current study, all the SBM's had TIU indicative of undercooking. Regardless, the LA soybean products had lower levels of TIU compared to the CON soybean products.

The soybean-based diets had urease activity ranging from 1.54 to 1.76 (Table 2.1) with ANIM meal having a negligible amount of urease activity. Urease activity measures the pH unit rise and is used to evaluate processing techniques. The recommended urease

activity of SBM is 0-0.10 pH unit rise (Van Eys et al., 2004). Raw soybeans have a urease activity of 2.0, severely undercooked SBM is reported to have a urease activity of 0.51-2.0 (Witte, 1995). A higher pH rise has been correlated to higher TIU in SBM (Van Eys et al., 2004), but is less predictive of TIU in full fat soybeans (Ruiz et al., 2018). In this study, urease activity of the soybean products was >2, while VOLGA-SBM was <0.02, which may also explain the high TIU in the experimental diets. While there are small differences between diet and ingredient urease activity, the LA soybean products were slightly lower than the CON soybean products.

There were little differences in protein solubility of experimental diets, but there were some differences with the soy ingredients (Table 2.2). Protein solubility is known to decrease as extrusion temperature increases, with a commercial SBM protein solubility generally between 78-85% KOH (Van Eys et al., 2004). The CONSBM, LASBM and LAGR had values greater than 94%, suggesting insufficient heat was applied, with 100% meaning no heat was applied. VOLGA-SBM falls in the acceptable range at 84%. However, the CONGR ingredient had protein solubility of 57% which would imply more temperature was applied compared to all other soy ingredients. However, the ground soybeans were simply ground and should not have had any heat treatment. This may also explain why the TIU of CONGR is greater than that of CONSBM. Lectin levels were not measured in either soy protein products or diets used. The LA soy products had lower TIU compared to the CON soy products, suggesting that removal of the KTI through breeding of the LA seed was successful. Thus, we can assume the level of lectins followed the same pattern as the TIU; where the lectins may have been successfully removed from the LA seed through breeding.

Growth Performance

The ANIM fed pigs had the highest ADG ($P < 0.001$; Table 2.2) and ADFI ($P < 0.001$; Table 2.3) compared to pigs fed soy protein diets. There were no differences in ADG and ADFI between soy protein diets. Based on caretaker observations, it was difficult to get pigs to consume feed after weaning, but ANIM-fed pigs appeared to adapt to environmental, social, and dietary stressors faster than the soybean-fed pigs, which may be partly attributed to the soybean products, given relatively high dietary TI levels. High TI levels inhibit the ability of pigs to digest proteins and decrease AA digestibility in pigs (Wright, 1981; Herkelman et al., 1992; Li et al., 1998; Goebel and Stein, 2011). This may in part, explain the lesser gain in pigs fed soy-based diets because the small difference in feed intake between soy-fed and ANIM-fed pigs is insufficient to account for the large difference in gain. Digestibility values of these soybeans are provided in Chapter 3 of this thesis and will be discussed there. Overall gain and feed efficiency were similar between LA and CON soybean products.

Intestinal Permeability

Due to errors with measurements of the FD4, the flux was analyzed from 0 to 30 minutes rather than 0 to 60 minutes. The FD4 flux observed in the ileum over a 30-minute period was not different between individual treatments ($P > 0.05$); however, there was a greater FD4 flux in the ileum of pigs fed CON soy products compared to pigs fed LA soy products ($P = 0.046$;Figure 2.1A). In addition, after addition of glucose, there was no difference in ΔIsc between treatments in the ileum (Figure 2.1B). The basal TER in ileal tissue from ANIM-fed pigs tended to be greater than pigs fed soy protein diets ($P = 0.096$; Figure 2.1C). There were no other differences between treatments in basal TER

or basal Isc from ileal sections (Figure 2.1C-D). In the jejunal section, there were no differences between individual treatments in FD4 flux over the 30-minute time period ($P > 0.05$); however, there was a greater FD4 flux in the jejunum of pigs fed LA soy products compared to CON soy products ($P = 0.036$;Figure 2.2E). After adding glucose, there were no differences in ΔIsc of the jejunum between treatments (Figure 2.2F). There were no differences in basal jejunal TER or basal jejunal Isc between treatments (Figure 2.2G-H). While hypersensitivity wasn't explicitly determined in this study, differences in gut immune markers in a previous trial evaluating the same soy-based products were also not detected (Tauer, 2018). We expected there to be increased intestinal permeability when pigs were fed soybean diets compared to pigs fed ANIM diet, specifically a more permeable intestinal tract in pigs fed CON soybean products compared to pigs fed LA soybean products. The increase in FD4 flux in ileal tissue of pigs fed CON soybean products would support this hypothesis of increased permeability compared to LA soybean products while the response of greater FD4 in jejunal tissue of pigs fed LA soybean product seems contradictory. It is possible that the lack of differences in other measured variables between pigs fed soybean products is due to the similarity in diet allergen levels (glycinin and β-conglycinin content). On the other hand, the explanation behind why gut permeability in ANIM-fed pigs was the same as soybean-fed pigs may be due to low feed intake by all pigs relative to industry average 12-14 days post-weaning. A lower feed intake later in the post-weaning period can increase intestinal permeability (Pearce et al., 2013), which may be a reason for similarity between treatments.

Considering the *in-vivo* assessment of intestinal permeability, urine from pigs fed ANIM had a higher L:M ratio compared to the soybean treatments ($P < 0.05$; Figure 2.3).

This was not expected as an increase in the ratio means more lactulose was found in the urine because lactulose only travels paracellularly through opened tight junctions between the intestinal epithelial cells. As discussed previously, we expected an increase in intestinal permeability with soybean diets compared to ANIM diet. The L:M ratio suggests a more permeable intestine in pigs fed ANIM diet in comparison to soybean products, while a greater TER suggests a less permeable intestine in pigs fed ANIM. The ANIM diet contained a small amount of HP300, an enzymatically treated soybean meal, thus limited anti-nutritional factors and allergens were expected (Zhu et al., 1998). Further, the level of TIU present in the ANIM diet was the lowest among all dietary treatments. In pigs, glycinin and β-conglycinin proteins have been most commonly associated with intestinal hypersensitivity (Sun et al., 2008a; Sun et al., 2008b). The total percentage of both proteins was generally the same for the soybean products, which would explain the similarities in *in-vivo* gut permeability between the soy protein diets. The increased permeability based on the *in-vivo* technique compared in ANIM-fed pigs compared to the other treatments is unclear as all parameters expected to reduce gut permeability were lowest in ANIM diets.

Microbial analysis

Just over 2200 high quality reads were analyzed. There was no difference in the level of *Firmicutes*, *Actinobacteria* or *Proteobacteria* phyla among dietary treatments. The primary phylum observed in all samples was *Firmicutes*. Similarly, there was no difference at the family level including those that are part of the phylum *Firmicutes*: *Lactobacillaceae, Peptostreptococcaceae, Veillonellaceae, Streptococcacceae and*

Erysipelotrichaceae, or the single family associated with the phylum *Actinobacteria*: *Coriobacteriales* (Table 2.4).

Twenty of the twenty-four most prominent OTU's were part of the *Firmicutes* phylum. The three most prominent *Firmicute*s OTU's in all treatments were from the *Lactobacillus* family and had a 99% or greater validity with *Lactobacillus mucosae* (KZ1-03387), *Lactobacillus delbrueckii subsp. indicus* (KZ1-00045), and *Lactobacillus reuteri* (KZ1-00094; Table 2.5). *Lactobacillus mucosae* has been closely associated with *Lactobacillus reuteri* and is known as a microbe with mucus-binding capabilities (Roos et al., 2000). *Lactobacillus delbrueckii subsp. indicus* was isolated from an Indian dairy milk protein, but has similar activity to other *Lactobacillus delbrueckii* which are associated with lactose breakdown (Dellaglio et al., 2005). *Lactobacillus reuteri* is a microbe that has many probiotic effects in pigs, especially during the post-weaning period (Liu et al., 2014). Probiotics such as *Lactobacillus reuteri* help create stable gut microflora for the pig (Hou et al., 2015). *Lactobacillus* is typically found in the microbiome of healthy pigs (Huang et al., 2004) and associated with nutrient breakdown, probiotic effects and decreasing the presence of *E. coli* in the intestinal tract.

One of the other five most abundant OTU's found in the digesta was part of the phylum *Proteobacteria* which had validity with *Escherichia fergusonii* (KZ3-16886; Table 2.5). *Escherichia fergusonii* is closely related to *Escherichia coli* (Lawrence et al., 1991) and is considered a harmful pathogen to pigs (Gaastra et al., 2014). While there were no significant differences, the level of *Escherichia fergusonii* constituted <0.5% relative abundance in the LAGR treatment and >4% in CONSBM and ANIM pigs. An increase in *in-vivo* intestinal permeability of ANIM-fed pigs might be attributed to the

presence of this harmful pathogen; however, *E. fergusonii* was also present in CONSBM-fed pigs. There were no significant differences in the relative abundance of each OTU between dietary treatment. Overall, there was little difference in the microbial population of the digesta between treatments.

2.5 CONCLUSION

Pigs fed ANIM outperformed pigs fed soybean products due to a higher intake and lower dietary TI levels which can increase digestibility and thus impact growth. However, the greater urine L:M ratio from ANIM-fed pigs suggest that intestinal integrity may have been lower compared to pigs fed the soybean products. A clear explanation for this response cannot be elucidated at this time. Low allergenic soybean products may be considered as a replacement for CON soybean products due to their similarity in gut permeability, gut microbiome and supporting similar growth performance in weaned pigs.

Table 2.1: Ingredient composition and nutrient analysis of experimental diets fed to weaned pigs, as fed basis[1]

Item	CONSBM	CONGR	LASBM	LAGR	ANIM
Ingredient (%)					
Corn	50.93	44.92	50.93	44.92	59.41
LA soybean meal	-	-	21.20	-	-
LA soybeans, full fat	-	-	-	27.00	-
Soybean meal, dehulled	21.20	-	-	-	-
Soybeans, full fat	-	27.00	-	-	-
HP300	3.00	3.00	3.00	3.00	3.00
Whey, dried	15.00	15.00	15.00	15.00	15.00
Fishmeal	-	-	-	-	5.00
Blood plasma	-	-	-	-	5.00
Soya oil	2.00	2.00	2.00	2.00	2.00
Casein	5.00	5.00	5.00	5.00	5.00
Whey protein concentrate	-	-	-	-	4.00
L-Lys-HCl	0.30	0.40	0.30	0.40	-
DL-Met	0.12	0.15	0.12	0.15	0.04
L-Thr	0.07	0.13	0.07	0.13	-
Calcium carbonate	1.10	1.00	1.10	1.00	0.85
Salt	0.08	0.10	0.08	0.10	-
Monocalcium phosphate	1.00	1.10	1.00	1.10	0.05
Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Formulated Content					
Total Lys %	1.56	1.60	1.56	1.60	1.70
SID[4] Lys %	1.35	1.35	1.35	1.35	1.46
Met + Cys:Lys ratio, %	0.55	0.55	0.55	0.55	0.57
Thr:Lys ratio, %	0.58	0.59	0.58	0.59	0.73
Ile:Lys ratio, %	0.63	0.57	0.63	0.57	0.74
Val:Lys ratio, %	0.70	0.65	0.70	0.65	0.81
Trp:Lys ratio, %	0.18	0.17	0.18	0.17	0.22
Digestible energy (kcal/kg)	1490	1430	1490	1430	1543
Lysine:digestible energy (g/Mcal)	3.88	4.03	3.88	4.03	4.03
Analyzed Composition (%)					
Dry matter	92.03	91.94	92.07	91.90	90.69
CP	22.73	21.98	21.23	21.15	22.79
Ash	5.24	5.15	5.14	4.96	4.54
Crude fat	3.44	7.43	4.45	8.70	4.27
Crude fiber	2.13	2.36	2.42	2.31	1.33
Lys	1.61	1.66	1.55	1.61	1.84
Trypsin inhibitor (TIU/g)[5]	11600	8900	4900	6400	2100

Urease activity (pH rise)[5]	1.76	1.74	1.63	1.54	<0.02
Protein solubility (KOH %)[5]	88.40	86.52	93.18	86.09	84.17

[1]Test feedstuffs were added to the base diet to provide equivalent dietary crude protein from conventional soybean meal (CONSBM), conventional full fat ground soybeans (CONGR), low allergenic soybean meal (LASBM), low allergenic full fat ground soybeans (LAGR), and animal protein sources (ANIM). All soybean feedstuffs were processed at Northern Crops Institute, Fargo, ND.

[2]Provided per kg of complete diet: 10,991 IU vitamin A supplement, 1649 IU vitamin D_3 supplement, 55 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidional bisulfite, 9.9 mg riboflavin as riboflavin supplement, 60.5 mg $_D$-pantothenic acid as $_D$-calcium pantothenate, 55 mg niacin as niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin. (Nutra Blend, LLC, Neosho, MO)

[3]Provided per kg of the complete diet: 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 44.1 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg Se as sodium selenite. (J & R Distributing, Inc., Lake Norden, SD)

[4]SID = standardized ileal digestibility

[5]Trypsin inhibitor, urease activity and protein solubility were measured at Eurofins Nutrition Analysis Center (Des Moines, IA).

Table 2.2: Test ingredient analysis including amino acid composition and anti-nutritional factor content of soybean products used in the dietary treatments[1]

Ingredient composition, %	CONSBM	CONGR	LASBM	LAGR	VOLGA-SBM
Lysine	3.25	2.44	2.92	2.28	2.11
Methionine	0.70	0.52	0.63	0.48	0.48
Threonine	1.93	1.44	1.74	1.34	1.32
Tryptophan	0.67	0.42	0.54	0.30	0.48
Isoleucine	2.44	1.78	2.12	1.59	1.62
Leucine	3.85	2.83	3.49	2.62	2.67
Valine	2.48	1.84	2.20	1.66	1.64
Phenylalanine	2.61	1.91	2.33	1.79	1.68
Histidine	1.32	0.98	1.19	0.90	0.88
Arginine	3.66	2.74	3.26	2.46	2.34
Glutamate	8.91	6.37	7.93	5.82	5.97
Proline	2.47	1.84	2.22	1.66	1.84
Serine	2.06	1.56	1.82	1.45	1.48
Glycine	2.11	1.57	1.86	1.48	1.42
Cystine	0.81	0.60	0.67	0.51	0.52
Aspartic acid	5.71	4.20	4.97	3.69	3.67
Alanine	2.12	1.56	1.92	1.45	1.50
Tyrosine	1.85	1.43	1.69	1.28	1.21
CP	52.07	37.84	45.88	34.13	42.79
Dry Matter	97.60	92.25	97.78	95.20	90.90
Ash	6.09	5.47	5.91	4.52	5.82
Crude fat	2.13	18.34	3.44	19.96	1.59
Crude fiber	3.74	4.43	4.62	5.15	3.84
Total trypsin inhibitor (TIU/g)[2]	62600	56700	21110	31200	5000
Bowman-Birk trypsin inhibitor (mg/g)[3]	NDS	NDS	NDS	NDS	NDS
Kunitz trypsin inhibitor (mg/g)[3]	3514.3	13.5	NDS	NDS	NDS
Urease activity (pH rise)[2]	2.17	2.14	2.15	2.10	<0.02
Protein solubility (KOH %)[2]	97.70	57.32	99.07	94.45	83.84
β – conglycinin[4]					
α – subunit, %	0.63	0.69	0.97	1.06	1.59
α' – subunit, %	2.91	2.38	4.06	3.80	4.63
β – subunit, %	9.40	9.06	8.63	11.59	21.11
Total, %	12.94	12.13	13.66	16.45	27.33
Glycinin[4]					
A3 chain, %	6.84	7.89	5.86	6.66	2.02

A1, 2, 4 chains, %	13.40	17.88	14.52	21.57	31.76
Basic chain, %	19.62	20.70	21.39	27.4	32.68
Total, %	39.86	46.47	41.77	55.63	66.46

[1]Test soybeans used in the experiments were conventional soybean meal, conventional full fat ground soybeans, low allergenic soybean meal, low allergenic full fat ground soybeans. All soybean feedstuffs were processed at Northern Crops Institute, Fargo, ND, except for conventional soybean meal (VOLGA-SBM) which was processed at South Dakota Soybean Processors, Volga, SD.

[2]Total trypsin inhibitor, urease activity and protein solubility were measured by Eurofins Nutrition Analysis Center, Des Moines, IA

[3]Bowman-birk trypsin inhibitor and Kunitz trypsin inhibitor analysis completed by Dr. Vermont Dia's lab at the University of Tennessee

[4]β – Conglycinin and glycinin analyses completed by Dr. Vermont Dia's lab at the University of Tennessee. Values are reported as percentages relative to total band intensity per each lane [(intensity of band of interest/total band intensity) *100]

NDS: No detectable signal (< 2.0 µg/g)

Table 2.3: Growth performance of weaned pigs fed diets containing conventional soybean meal (CONSBM), conventional ground soybeans (CONGR), low allergenic soybean meal (LASBM), low allergenic ground soybeans (LAGR) and animal proteins (ANIM)[1]

Item	CONSBM	CONGR	LASBM	LAGR	ANIM	SEM	Diet	ANIM vs others	SBM vs GR	CON vs LA
N	12	12	12	11	12					
Gain, g/d	48.82	54.46	45.09	41.10	160.95	13.83	<0.001	<0.001	0.953	0.544
Feed disappearance, g/d	130.46	139.03	120.02	114.36	182.11	12.83	<0.001	<0.001	0.894	0.110

[1]One pig/treatment was euthanized for tissue collection at d 11, 12, 13, and 14 in each of 3 blocks of 20 pigs. Daily gain and feed disappearance were determined from weaning to tissue collection.

Table 2.4: Relative abundance (%) of phyla and family taxonomic classifications of ileal digesta samples from pigs fed diets containing conventional soybean meal (CONSBM), low allergenic full fat ground soybeans (LAGR), and animal proteins (ANIM)[1]

Item	CONSBM	LAGR	ANIM	*P*-value
Number of observations	12	11	12	
Firmicutes	88.60 ± 5.65	93.77 ± 2.55	89.82 ± 4.39	0.40
Lactobacillaceae	73.56 ± 7.47	70.92 ± 8.71	71.94 ± 7.24	0.92
Peptostreptococcaceae	1.95 ± 1.38	4.83 ± 3.16	1.10 ± 0.50	0.92
Veillonellaceae	4.67 ± 1.30	4.92 ± 1.21	7.77 ± 2.63	0.80
Streptococcaceae	1.60 ± 0.78	5.26 ± 3.96	1.23 ± 0.25	0.71
Erysipelotrichaceae	3.20 ± 0.69	4.70 ± 2.88	5.65 ± 2.22	0.76
Actinobacteria	3.71 ± 1.82	3.73 ± 1.75	1.57 ± 0.63	0.76
Coriobacteriales	3.28 ± 1.69	3.07 ± 1.73	1.07 ± 0.57	0.67
Proteobacteria	6.88 ± 5.25	1.95 ± 1.47	7.58 ± 3.81	0.13

[1]Data represent lsmeans and SEM of 12, 11, and 12 pigs for CONSBM, LAGR, and ANIM, respectively.

Table 2.5: Relative abundance (%) of the twenty-four most abundant OTU's of ileal digesta samples from pigs fed conventional soybean meal (CONSBM), low allergenic full fat ground soybeans (LAGR) and animal proteins (ANIM)[1]

OTU's, %	CONSBM	LAGR	ANIM	*P* – value	Closest valid taxon (id%)
Number of observations	12	11	12		
Firmicutes					
KZ1-03387	37.76 ± 5.17	39.37 ± 8.16	31.01 ± 7.49	0.56	*L. mucosae* (99%)
KZ1-00045	20.55 ± 6.42	14.96 ± 4.24	21.87 ± 4.39	0.49	*L. delbrueckii subsp indicus* (100%)
KZ1-00094	9.46 ± 3.79	8.20 ± 3.65	12.45 ± 4.45	0.52	*L. reuteri* (99%)
KZ2-16662	3.68 ± 2.38	4.77 ± 3.39	4.67 ± 2.12	0.72	*L. salivarius* (99%)
KZ2-13591	3.31 ± 1.01	2.58 ± 0.85	3.79 ± 1.48	0.91	*M. elsdenii* (99%)
KZ1-03885	2.24 ± 0.54	2.56 ± 1.73	3.76 ± 1.51	0.53	*Sh. azabuensis* (97%)
KZ2-02955	0.99 ± 0.50	3.61 ± 3.10	0.98 ± 0.48	0.88	*T. mayombei* (97%)
KZ1-10680	0.47 ± 0.14	1.11 ± 0.96	1.29 ± 0.62	0.66	*Sh. azabuensis* (97%)
KZ1-33674	0.75 ± 0.56	0.37 ± 0.31	2.20 ± 1.51	0.15	*V. caviae* (99%)
KZ1-01664	0.56 ± 0.33	0.40 ± 0.39	0.49 ± 0.37	0.12	*An. senegalensis* (92%)
KZ2-31575	0.95 ± 0.93	1.21 ± 1.11	0.10 ± 0.04	0.63	*R. timonensis* (98%)
KZ4-11606	1.01 ± 0.67	0.24 ± 0.23	0.01 ± 0.01	0.72	*E. hirae* (100%)
KZ2-05452	0.11 ± 0.06	1.96 ± 1.44	0.09 ± 0.09	0.25	*L. agilis* (99%)
KZ2-07936	0.26 ± 0.16	0.81 ± 0.51	0.46 ± 0.19	0.42	*Tu. sanguinis* (98%)
KZ2-10632	0.11 ± 0.09	0.89 ± 0.63	0.12 ± 0.05	0.60	*C. disporcium* (98%)
KZ1-02880	0.30 ± 0.15	1.61 ± 1.52	0.18 ± 0.04	0.29	*S. porcorum* (100%)
KZ1-01302	0.35 ± 0.13	1.06 ± 0.92	0.36 ± 0.12	0.57	*S. orisratti* (98%)
KZ2-05411	0.95 ± 0.61	0.23 ± 0.18	0.21 ± 0.10	0.29	*Mo. neglectum* (92%)
KZ4-01945	0.17 ± 0.12	0.33 ± 0.21	0.65 ± 0.41	0.39	*Se. bovis* (95%)
KZ21-02231	0.00 ± 0.00	0.01 ± 0.01	0.76 ± 0.52	0.34	*C. amylolyticum* (87%)
Actinobacteria					
KZ1-01800	1.50 ± 0.77	1.87 ± 0.90	0.77 ± 0.52	0.28	*O. profuse* (95%)
KZ2-10755	1.03 ± 0.69	0.98 ± 0.91	0.10 ± 0.06	0.17	*O. umbonate* (100%)
Proteobacteria					
KZ1-09927	0.92 ± 0.90	0.13 ± 0.09	0.53 ± 0.48	0.92	*H. equorum* (98%)
KZ3-16886	5.59 ± 5.10	0.22 ± 0.18	4.81 ± 3.10	0.55	*Es. fergusonii* (99%)

[1]Data represent lsmeans and SEM of 12, 11, and 12 pigs for CONSBM, LAGR, and ANIM, respectively.

Abbreviations: *L: Lactobacillus; M: Megasphaera; Sh: Sharpea; T: Terrisporobacter; V: Veillonella; An: Anaeromassilibacillus; R: Romboutsia; E: Enterococcus; Tu: Turicibacter; C: Clostridium; S: Streptococcus; Mo: Mogibacterium; Se: Selenomonas; O: Olsenella; Es: Escherichia; H: Heliobacter*

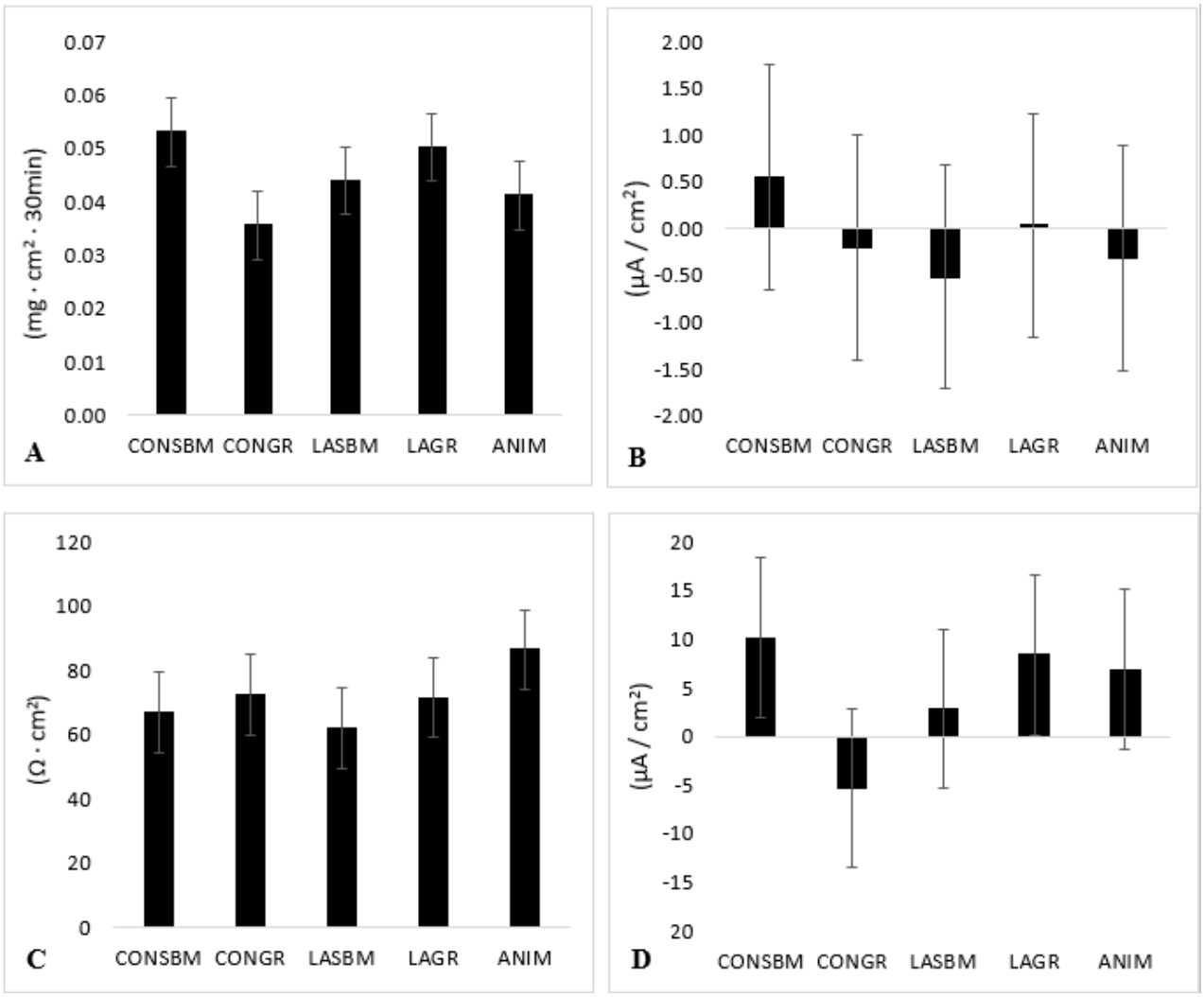

Figure 2.1: *Ex-vivo* intestinal permeability measures [**A**: fluorescein isothiocyanate-dextran (FD4) flux (CON vs. LA $P = 0.046$). **B**: glucose-induced ΔIsc. **C**: Basal transepithelial resistance (TER). **D**: Basal short circuit current (Isc) (ANIM vs. others $P = 0.096$)] of ileal tissues samples from pigs fed five dietary treatments.

Test feedstuffs were added to the base diet to provide equivalent dietary crude protein from conventional soybean meal (CONSBM), conventional full fat ground soybeans (CONGR), low allergenic soybean meal (LASBM), low allergenic full fat ground soybeans (LAGR), and animal protein sources (ANIM). All soybean feedstuffs were processed at Northern Crops Institute, Fargo, ND.

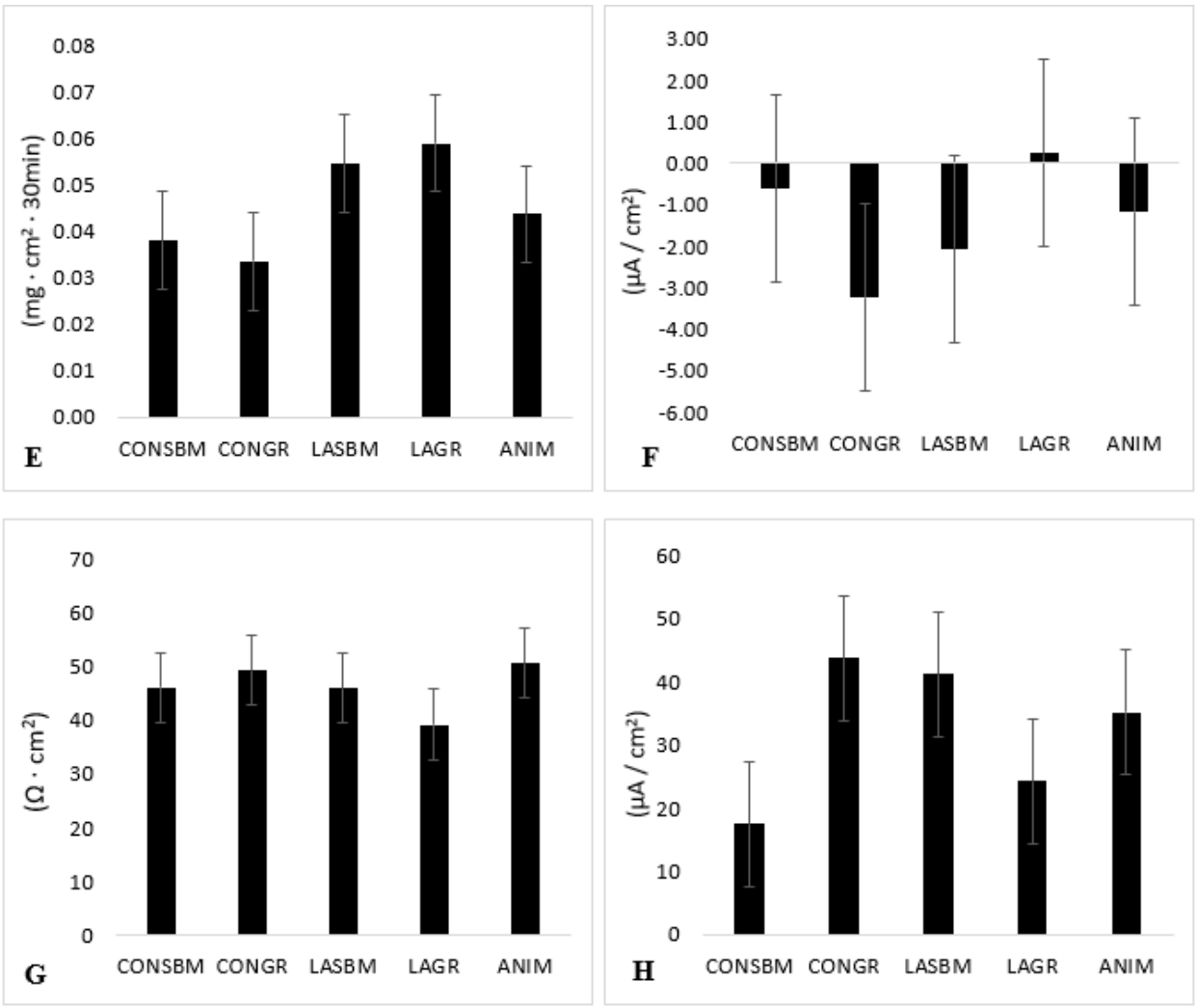

Figure 2.2: *Ex-vivo* intestinal permeability measures [**E:** fluorescein isothiocyanate-dextran (FD4) flux (CON vs LA $P = 0.031$). **F:** glucose-induced ΔIsc. **G:** Basal transepithelial resistance (TER). **H:** Basal short circuit current (Isc)] of jejunal tissue samples from pigs fed five dietary treatments.

Test feedstuffs were added to the base diet to provide equivalent dietary crude protein from conventional soybean meal (CONSBM), conventional full fat ground soybeans (CONGR), low allergenic soybean meal (LASBM), low allergenic full fat ground soybeans (LAGR), and animal protein sources (ANIM). All soybean feedstuffs were processed at Northern Crops Institute, Fargo, ND.

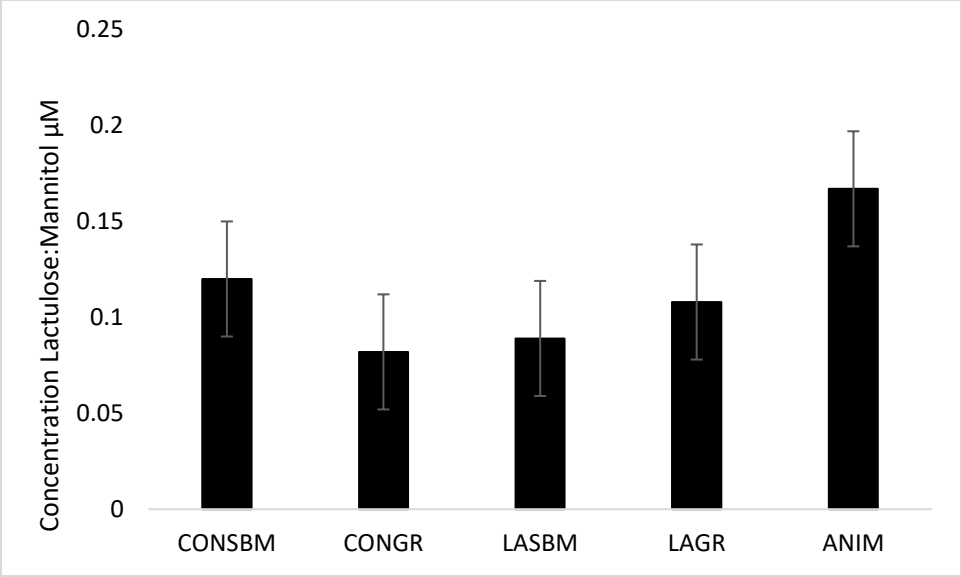

Figure 2.3: *In-vivo* intestinal permeability measure of lactulose:mannitol ratio in urine obtained from pigs fed five dietary treatments (ANIM vs. others $P < 0.05$).
Test feedstuffs were added to the base diet to provide equivalent dietary crude protein from conventional soybean meal (CONSBM), conventional full fat ground soybeans (CONGR), low allergenic soybean meal (LASBM), low allergenic full fat ground soybeans (LAGR), and animal protein sources (ANIM). All soybean feedstuffs were processed at Northern Crops Institute, Fargo, ND.

Chapter 3

Evaluation of crude protein and amino acid digestibility of a low allergenic soybean variety and impact of the low allergenic soybean variety on the growth performance of weaned pigs

3.1 ABSTRACT

This study determined digestibility and digestible amino acid content in LASBM and LAGR in comparison to a conventional solvent extracted SBM (CONSBM) and fish meal (FM). The LASBM and LAGR were also tested as substitutes for FM in commercially relevant weaned pig diets. In Exp. 1, ten ileal-cannulated barrows (17.63 ± 1.18 kg BW) were used in a cross over design and randomly assigned to one of five experimental diets (FM, CONSBM, LASBM, LAGR and nitrogen-free), where FM, CONSBM1, LASBM and LAGR were included as the sole protein source. The nitrogen-free diet was included to estimate endogenous AA losses and calculate standard ileal digestibility (SID). Each pig received 3 of the 5 diets (1 diet/collection period), totaling 6 replications per diet. Each collection period consisted of 5d diet adaptation and 2d ileal digesta collection (12h/d). Daily feed allowance/period was provided at 3x maintenance energy requirement (110 kcal/kg $BW^{0.75}$) based on BW at the beginning of each period. In Exp. 2, the methods used in Exp. 1 were replicated, except 5 barrows and 5 gilts were used (19.40 ± 1.65 kg) and a CONSBM from a commercial soybean processor (VOLGA-SBM) was used. Growth performance was determined in 112 weaned pigs (7.30 ± 0.43 kg BW; 2 barrows and 2 gilts per pen) assigned to one of 4 dietary treatments in 2 phases (Ph1 = 5d, Ph2 = 13d). The control diet contained FM (7.25%, Ph1; 6%, Ph2); LASBM, LAGR and VOLGA-SBM replaced FM to supply equivalent dietary crude protein. Pigs

received a common Ph3 diet (18d). Data from each trial was analyzed using PROC MIXED in SAS v9.4. In Exp. 1, SID of CP and AA was greater ($P < 0.05$) in FM than soy products. There were minor differences in digestibility between soy products where SID of LYS, MET, and HIS were greater ($P < 0.05$) in LASBM than CONSBM. In Exp. 2, SID of CP and AA was similar between FM and CONSBM and lower ($P < 0.05$) in LASBM and LAGR than FM and CONSBM. Overall SID tended ($P < 0.10$) to be lower in gilts than barrows. Overall daily gain was greater ($P < 0.01$) in LAGR-fed pigs compared to LASBM, but not different ($P > 0.05$) from FM- or SBM-fed pigs; daily gain tended to be greater ($P < 0.10$) in SBM- than FM-fed pigs. Overall daily intake tended to be greater ($P < 0.10$) in SBM-fed pigs compared to FM and LASBM but was not different ($P > 0.05$) from LAGR. There was a tendency for greater ($P < 0.10$) overall G:F in LAGR- versus LASBM-fed pigs. While digestibility of LA soy products varied greatly between trials, growth performance data suggest LAGR could serve as a suitable replacement to FM in weaned pig diets.

Keywords: anti-nutritional factors, digestibility, growth performance, weaned pig

3.2 INTRODUCTION

In Chapter 2.0 and in a study conducted by Tauer (2018), it was observed that the level of TI, lectins and P34 proteins did not influence the intestinal permeability and morphology, and gut microbiome of nursery pigs. With minimal impact observed on the pig immune system, the studies in this chapter were completed for nutritional evaluation and practical application of LA soybeans. Therefore, the first objective of this study was to determine AA digestibility of both LA soybean meal and LA full fat, ground soybeans in comparison to conventional SBM and fish meal (FM). The second objective of this

study was to determine the suitability of the LA soybean products as replacement for FM in commercially relevant weaned pig diets.

3.3 MATERIALS AND METHODS

South Dakota State University's Institutional Animal Care and Use Committee approved all protocols used in this experiment (IACUC #18-013A).

Exp.1 - Digestibility

Ten barrows (17.5 ± 1.76 kg) with t-cannula in distal ileum (Wubben et al., 2001) were individually housed in metabolism pens where each pen (1.22 m × 1.83 m) contained an individual feeder and nipple drinker. Room temperature was set at 23ºC for the duration of the study. Pigs were given one week to recover from surgery and adjust to treatment diets and housing before starting the collection periods. Daily care followed established South Dakota State University protocols for cannulated pigs.

Barrows were randomly allotted to one of five diets: fishmeal (FM), conventional soybean meal (CONSBM), low allergenic soybean meal (LASBM), low allergenic ground soybeans (LAGR), or a nitrogen-free diet (NF**)** (Table 3.1) in an incomplete block design where all pigs received three of the five diets (1 diet/collection period). There was a total of three 7-d collection periods (5 d diet adaptation and 2 d of 12 h digesta collection from 0800 to 2000 h), resulting in six replications per diet. All soy products were processed at the Northern Crops Institute (Fargo, ND) following the same protocol as discussed in Chapter 2. The FM, CONSBM, LASBM and LAGR were included as the sole protein source in their respective diets and the NF diet was used to determine basal endogenous AA losses (Stein et al. 2007). All diets contained 0.3% titanium (IV) oxide, as an indigestible marker (Sigma-Aldrich Co. LLC, St. Louis, MO).

Daily feed was provided at 3x maintenance energy requirement (110 kcal/kg $BW^{0.75}$; NRC, 1998) based on their body weight at the start of each collection period. Daily feed allowance was split into two equal allotments provided at 0800 h and 1600 h, and water was available ad libitum. Feed orts were weighed back at the end of each collection period. Digesta was collected using methods previously reported by Cervantes-Pahm and Stein (2010); 3 mL of 10% formic acid was added to each bag to minimize the amount of enzymatic activity (Fan et al., 1994). Bags were removed every 30 to 60 minutes or when the bag was full. Digesta was pooled within pig and period, and immediately stored at -20°C. At the end of each collection period, digesta was thawed, mixed and subsampled for further chemical analysis. Drop fecal samples were collected on days 5, 6 and 7 of each collection periods, pooled within pig and period and frozen at -20°C until further analysis.

Prior to analysis, digesta and feces were freeze dried (Dura-Dry, Fits Systems, Kinetics Thermal Systems) and finely ground (Ultra Centrifugal Mill ZM 200, Retsch, Haan, Germany). Samples of experimental diets, test ingredients and digesta were analyzed for AA at a commercial laboratory (Experiment Station Chemical Laboratories, University of Missouri – Columbia). Experimental diets and test ingredients were analyzed for CP, moisture, ash, ether extract, and crude fiber (Experiment Station Chemical Laboratories, University of Missouri – Columbia). Nitrogen content of experimental diets, test ingredients and digesta was determined (Rapid N III, Elementar, Hanau, Germany) and CP was calculated as N x 6.25%. Titanium dioxide was analyzed using standard AOAC procedures (AOAC, 1995). Apparent ileal digestibility (AID) was determined by using the following equation: AID = [(AA intake – ileal AA outflow) / AA

intake] × 100 (Stein, H.H. et al. 2007). Using AID and endogenous losses determined in pigs fed the NF diet, SID was calculated using the following equation: SID = [(AA intake – (ileal AA outflow – basal IAA_{end})) / AA intake] × 100 (Stein et al., 2007). Digestible content was calculated as (SID × AA in experimental diet) / 100.

Exp. 2 – Digestibility

SID digestibility values for CONSBM in Exp. 1 were considerably low relative to NRC (2012) values for solvent extracted soybeans. A second trial (Exp. 2) was conducted. However, there was insufficient CONSBM processed at the NCI facility thus conventional SBM from a commercial soy processor (VOLGA-SBM; South Dakota Soybean Processors, Volga, SD). Experimental methods used in Exp. 1 were replicated in Exp. 2 with five barrows and five gilts (19.40 ± 1.65 kg). The diets were fishmeal (FM), conventional soybean meal (VOLGA-SBM), (LASBM), (LAGR) with as the sole protein source; and a nitrogen-free diet (NF) (Table 3.1). The same LASBM and LAGR soy products from Exp. 1 were used in Exp. 2.

Exp. 3 - Growth Performance

Pigs were weaned from South Dakota State University Swine Education and Research Facility at 23.6 ± 1.3 d of age and transferred to two nursery rooms within the same facility. Each nursery room consisted of 14 raised pens (1.2 m × 1.2 m). A total of 112 pigs (7.30 ± 0.43 kg), progeny of PIC 1050 (Landrace x Large White) sows and composite Duroc boars, were allotted to pens by weight and treatments were randomly allotted to pens. Each pen contained two barrows and two gilts (n = 7 pens / dietary treatment).

The trial was conducted over three diet phases: Phase 1 (d0-5), Phase 2 (d6-18), and Phase 3 (d19-36). Four dietary treatments were utilized in the first 2 phases and pigs were fed a common diet in Phase 3. The base of both Ph1 and Ph2 diets included corn, VOLGA-SBM (13% Ph1; 20% Ph2), whey (23% Ph1; 10.5% Ph2), blood meal (≤2.25% Ph1; ≤1.10% Ph2) and FM (7.25% Ph1; 6% Ph2). LASBM, LAGR, and VOLGA-SBM replaced FM in their respective diets to provide an equivalent level of dietary crude protein. NRC (2012) SID values for conventional SBM conventional full fat soybeans were used for LASBM and LAGR, respectively, rather than the SID values obtained in Exp. 1 and 2. All diets were formulated to meet or exceed nutrient requirements and meet similar AA ratios and digestible energy within phase (NRC, 2012). Experimental diets and test ingredients were analyzed for AA, CP, moisture, ash, ether extract and crude fiber at a commercial laboratory (Experiment Station Chemical Laboratories, University of Missouri – Columbia). Phase 2 experimental diets were also analyzed for TIU (Eurofins Nutrition Analysis Center, Des Moines, IA) (Table 3.2). Test ingredients were analyzed for TIU, urease activity, protein solubility (Eurofins Nutrition Analysis Center, Des Moines, IA), as well as BBI, KTI, β – conglycinin and glycinin using gel electrophoresis and western blot (Dr. Vermont Dia's lab, University of Tennessee, Knoxville, TN) (Table 2.2).

Pigs were individually weighed on day 0, 5, 14, 18, 25, 32 and 36 to determine ADG by period. Daily gain per pig was calculated by determining the total pen gain divided by the number of pig days per pen. Feeders were weighed on the same days to determine ADFI on a pig basis similar to ADG. G:F ratio was calculated as total pen gain divided by the total feed consumed.

Statistical Analysis

Statistical analysis was performed using the PROC MIXED procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC). For Exp. 1 and 2, a cross-over design was used with individual pig as the experimental unit for all tested parameters. Main effect was treatment and random effect was pig nested within period. In Exp. 2, main effects were both treatment, gender and their interaction with pig nested within period as random effect. Where a significant main effect was observed, Tukey's adjustment was used as means separation test.

Statistical analysis for growth performance in Exp. 3 was performed using a one-way ANOVA in SAS (Version 9.4, SAS Inst. Inc., Cary, NC). An incomplete block design was used with pen as the experimental unit. Main effect was treatment and random effect was pen nested within room. Where a significant main effect of treatment was observed, Tukey's adjustment was used as means separation test. Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

3.4 RESULTS

Diets and test ingredients

Phase 2 diet TIU and urease activity levels were similar between LASBM and LAGR diets and almost 2-fold higher than the FM and SBM diets (Table 3.2). Protein solubility was similar among all diets.

Exp. 1 - Digestibility

Digestibility results are summarized by experiment and reported as SID and standard digestible content (g/kg). CP digestibility of FM was greater ($P < 0.05$; Table

3.3) than CONSBM1 and tended to be greater ($P < 0.10$; Table 3.3) than LAGR. LYS was greatest in FM ($P < 0.05$; Table 3.3) compared to all other ingredients, with CONSBM being lower ($P < 0.05$; Table 3.3) than LASBM but not different from LAGR. MET was greatest ($P < 0.05$; Table 3.3) in FM, CONSBM was lower ($P < 0.05$; Table 3.3) than LASBM and not different from LAGR. SID of MET in LASBM had a tendency ($P < 0.10$; Table 3.3) to be greater than LAGR. THR digestibility was greater ($P < 0.05$; Table 3.3) in FM compared to CONSBM and LAGR and tended to be greater ($P < 0.10$; Table 3.3) than LASBM. THR was greater in LASBM ($P < 0.05$; Table 3.3) than in LAGR. FM had the greatest ($P < 0.05$; Table 3.3) TRP digestibility compared to all other ingredients. SID of TRP was greater in the LASBM ($P < 0.05$; Table 3.3) compared to CONSBM and LAGR. ILE, LEU, PHE, and ARG digestibility were greater ($P < 0.05$; Table 3.3) in FM compared to all other ingredients. VAL digestibility was greater ($P < 0.05$; Table 3.3) in FM than all other ingredients and LASBM tended to be greater ($P < 0.10$; Table 3.3) than LAGR. SID of HIS was greatest ($P < 0.05$; Table 3.3) in FM, with LASBM being greater ($P < 0.05$; Table 3.3) than CONSBM but not different from LAGR. FM had a greater ($P < 0.05$; Table 3.3) GLU digestibility than CONSBM and LAGR but was not different from LASBM. SID of SER was greater ($P < 0.05$; Table 3.3) in FM than CONSBM and LAGR and tended to be greater ($P < 0.10$; Table 3.3) than LASBM. FM had greater ($P < 0.05$; Table 3.3) GLY digestibility than LAGR but was not different from the other ingredients. SID of ASP was greater ($P < 0.05$; Table 3.3) in FM than CONSBM and LAGR with LASBM intermediate. ALA digestibility was greater ($P < 0.05$; Table 3.3) in FM than all other ingredients and LASBM tended to be greater ($P < 0.10$; Table 3.3) than LAGR. FM had greater ($P < 0.05$; Table 3.3) TYR digestibility than

all other ingredients while LASBM tended to be greater ($P < 0.10$; Table 3.3) than CONSBM. There were no differences between ingredients for both PRO and CYS.

Digestible content of CP was greater ($P < 0.05$; Table 3.3) in FM compared to all other ingredients. LYS, THR and SER digestible content were greatest ($P < 0.05$; Table 3.3) in FM with LASBM having greater ($P < 0.05$; Table 3.3) digestible content than LAGR but not different from CONSBM. The digestible content of MET, ILE, LEU, VAL, PHE, HIS, ARG, GLY, ASP and TYR in FM was greater ($P < 0.05$; Table 3.3) than all other ingredients, with CONSBM and LASBM not different from each other but greater ($P < 0.05$; Table 3.3) than LAGR. FM had greater ($P < 0.05$; Table 3.3) digestible content than LASBM and LAGR and tended to be greater ($P < 0.10$; Table 3.3) than CONSBM. Digestible content of GLU was lowest ($P < 0.05$; Table 3.3) in LAGR and there was a tendency ($P < 0.10$; Table 3.3) for FM to have greater GLU than CONSBM. There was a tendency ($P < 0.10$; Table 3.3) for FM to have a greater digestible content of PRO than LAGR. LASBM had a greater ($P < 0.05$; Table 3.3) digestible content for CYS compared to LAGR but was not different from FM or CONSBM. Digestible content of CYS had a tendency to be greater ($P < 0.10$; Table 3.3) in CONSBM than LAGR. ALA digestible content was greatest ($P < 0.05$; Table 3.3) in FM compared to all other ingredients, with LASBM having greater ($P < 0.05$; Table 3.3) digestible content than LAGR and a tendency ($P < 0.10$; Table 3.3) for greater digestible content in CONSBM than LAGR.

Exp. 2 – Digestibility

The SID of CP, LYS, MET, THR, TRP, ILE, LEU, VAL, PHE, HIS, ARG, GLU, SER, GLY, ALA and TYR in FM and VOLGA-SBM were not different from each other,

but were greater ($P < 0.05$; Table 3.4) than LASBM and LAGR, which were also not different from each other. SID of PRO was greater ($P < 0.05$; Table 3.4) in FM than LASBM and LAGR but not different from VOLGA-SBM. FM had greater digestibility ($P < 0.05$; Table 3.4) of CYS and ASP than LASBM and tended to be greater ($P < 0.10$; Table 3.4) than LAGR but was not different from VOLGA-SBM. There was an effect of gender for CP, ARG, PRO, GLY, and ALA where SID was greater ($P < 0.05$; Table 3.4) in barrows than gilts. The SID of THR, TRP, HIS, and ASP tended to be greater ($P < 0.10$; Table 3.4) in barrows. There was a tendency for an interaction ($P = 0.0893$; Table 3.4) between treatment and gender for SID of PRO where LAGR was greater in barrows than in gilts but not different across gender for all other feedstuffs.

Digestible content for CP, LYS, THR, LEU, and VAL were not different in FM and VOLGA-SBM but were greater ($P < 0.05$; Table 3.4) than LASBM and LAGR, which were also not different from each other. FM had greater ($P < 0.05$; Table 3.5) digestible content for MET, ILE, PHE, HIS, ARG, GLU, SER, GLY, ASP, ALA and TYR than all other ingredients with VOLGA-SBM greater ($P < 0.05$; Table 3.4) than LASBM and LAGR which were not different from each other. Digestible content of PRO in FM was greater ($P < 0.05$; Table 3.4) compared to LASBM and LAGR but was not different from VOLGA-SBM. VOLGA-SBM tended to have a greater ($P < 0.10$; Table 3.4) digestible content of PRO than LASBM. Digestible content of CYS was the same between FM, LASBM and LAGR and they were all lower ($P < 0.05$; Table 3.4) than VOLGA-SBM. There was an effect of gender for CP, PRO and GLY where the digestible content was greater ($P < 0.05$; Table 3.4) in barrows than gilts. There was a tendency ($P < 0.10$; Table 3.4) for digestible content of ALA and ARG to be greater in barrows. All

other AA digestible contents were not different between barrows and gilts and there were no interaction effects between treatment and gender for the digestible AA content.

Exp. 3 - Growth Performance

There were no differences ($P > 0.05$; Table 3.5) in BW of pigs fed different dietary treatments in Phase 1. At the end of Phase 2, pigs fed SBM were heavier than all other treatments ($P < 0.05$; Table 3.5). At the end of the study, the BW of pigs fed SBM and LAGR did not differ, but SBM-fed pigs had higher BW than LASBM and FM ($P < 0.05$; Table 3.5). Pigs fed FM had lower ($P < 0.05$; Table 3.5) ADG during Phase 1 than LAGR- and SBM-fed pigs but not different from LASBM-fed pigs. During Phase 2, pigs fed SBM had greater ADG than all other treatments ($P < 0.05$; Table 3.5). There was a tendency for pigs fed LAGR to have greater ADG than LASBM-fed pigs during Phase 3 ($P < 0.10$; Table 3.5). Over the entire trial period, pigs fed LAGR had the same ADG as SBM- and FM-fed pigs and had greater ADG than LASBM-fed pigs ($P < 0.05$; Table 3.5). There was a tendency ($P < 0.10$; Table 3.5) for SBM-fed pigs to have greater overall ADG compared to FM-fed pigs. Pigs fed FM had lowest ADFI ($P < 0.05$; Table 3.5) compared to all other treatments during Phase 1. In Phase 2, pigs fed SBM had the greatest ADFI ($P < 0.05$; Table 3.5) compared to all other groups. There were no significant differences in ADFI during Phase 3. Considering overall ADFI, pigs fed SBM tended to have greater ($P < 0.10$; Table 3.5) intake than FM-fed pigs and LASBM-fed pigs but was not different from pigs fed LAGR. Lastly, there were no differences in G:F between groups in Phase 1, 2 or 3. However, when considering the overall G:F, there was a tendency for LAGR-fed pigs to have greater G:F than LASBM-fed pigs ($P < 0.10$; Table 3.5).

3.5 DISCUSSION

The overall goal of these studies was to determine the practical use of LA soybeans. The first objective was to determine the amino acid digestibility of LAGR and LASBM in comparison to FM and CONSBM. We hypothesized that the LAGR and the LASBM would have digestibility values like CONSBM. Due to the removal of KTI, lectins and P34 protein from the seed itself, the TI level of the LAGR and LASBM regardless of heat treatment, was expected to be lower than CONSBM. Lower levels of TI and lectins have been shown to increase digestibility (Wright, 1981; Herkelman et al., 1992; Li et al., 1998; Goebel and Stein, 2011). However, almost all SID values for CP and AA were the same between CONSBM, LASBM, and LAGR. In this experiment, the SID values for the LASBM were numerically greater than both CONSBM and LAGR, but were lower than FM. The CONSBM digestibility values determined in Exp. 1, were 35-40% lower than those presented in NRC (2012). The digestibility values of the VOLGA-SBM more closely align with those in the NRC (2012), leading us to conclude the CONSBM used in Exp.1 was likely undercooked (Witte, 1995) and supports the conclusion in Chapter 2 that processing conditions for the CON and LASBM's was less than optimal.

Similar to Exp.1, low AA digestibility was also observed in Exp. 2 with even greater reduction in digestibility than expected based on NRC (2012). Digestibility may have been impacted by the fact that in Exp. 2 both barrows and gilts were utilized. There were some significance and strong tendencies for AA digestibility's to be higher in barrows compared to gilts. The effect of gender may be more related to issues with initial cannulation surgeries, where greater difficulties locating the cecum was experienced with

the gilts and may have increased intestinal fusion after surgery. In at least 1 gilt, extensive intestinal fusion was noted at necropsy which could explain lower digestibility values in the gilts compared to the barrows. In contrast to this trial, a study by Kim et al. (2000) found that AA digestibility was higher in gilts in the grower phase, but not different from barrows in the finishing phase. In addition, the LASBM, LAGR and CONSBM were finely ground to the consistency of flour compared to a larger particle size exhibited by VOLGA-SBM, which may also attribute to a low AA digestibility observed in both experiments. In a study completed by Tauer (2018) using the same LA soybean, there was no difference in ATTD of AA's between the LA diet and CON diet, suggesting that LA soybean would be a suitable replacement for CON soybeans. Low digestibility values may be linked to the fine particle size of the feed stuffs, high TI levels and poor cannulation technique in Exp. 2. Despite the low digestibility values, there is potential for LA soybeans to be an alternative ingredient in niche markets.

The second goal of this study was to determine the growth performance of nursery pigs when fed the LA soybean products. We hypothesized that feeding LA soybeans in place of FM during the first few phases of the nursery would decrease the hypersensitivity response of newly weaned piglets, thus positively impacting their growth performance. It is important to note that when formulating the performance diets, the SID values determined in this study were not used. Instead, NRC (2012) values for conventional SBM conventional full fat soybeans were used for LASBM and LAGR, respectively. During Phase 1, there was no difference in BW, but gain was lower in pigs fed FM in comparison to pigs fed LAGR and SBM, which was related to the lower ADFI by pigs fed FM than the other treatments. In Phase 2, the SBM-fed pigs had significantly

higher BW which would be attributed to the better gain and feed intake compared to the other treatments. The LAGR-fed pigs had similar final performance compared to the SBM-fed pigs, which suggests that LAGR may have value as an alternative in weaned pig diets. The slower initial gain in pigs fed FM may be attributed to a possible aversion to the feed as their intake was almost 2-fold lower than pigs fed the other treatments in Phase 1. Their response overall reinforces the importance of gains in the first week or so after weaning on the long-term performance of pigs (Tokach et al., 1992).

3.6 CONCLUSION

As the level of TIU increases in the soy products, the AA digestibility values decrease. The overall response of the growth performance trial suggests that LAGR soybeans have value in nursery pig diets and does not appear to limit pig performance even in comparison to a high-quality protein source like FM.

Table 3.1: Ingredient composition and nutrient analysis of digestibility experimental diets, used in both Exp. 1 and Exp. 2, as fed basis[1]

Item	Exp. 1					Exp. 2				
	FM	CONSBM	LASBM	LAGR	NF	FM	VOLGA-SBM	LASBM	LAGR	NF
Cornstarch	36.50	32.80	32.80	32.80	51.62	36.50	33.80	32.80	32.80	49.50
LA-MEAL	-	-	30.00	-	-	-	-	30.00	-	-
LA-FF	-	-	-	30.00	-	-	-	-	30.00	-
CON-MEAL	-	30.00	-	-	-	-	-	-	-	-
VOLGA-SBM	-	-	-	-	-	-	30.00	-	-	-
Fishmeal	25.00	-	-	-	-	25.00	-	-	-	-
Sugar	36.00	32.00	33.00	33.00	40.00	36.00	32.00	33.00	33.00	40.00
Solka floc	-	-	-	-	2.00	-	-	-	-	4.00
Soya oil	1.00	2.00	1.00	1.00	2.00	1.00	1.00	1.00	1.00	2.00
Potassium carbonate	-	-	-	-	0.60	-	-	-	-	0.60
Calcium carbonate	0.30	1.00	1.00	0.90	0.70	0.30	1.00	1.00	0.90	0.70
Dicalcium phosphate	-	-	-	-	0.80	-	-	-	-	0.80
Salt	0.20	0.30	0.30	0.30	0.30	0.20	0.30	0.30	0.30	0.30
Monocalcium phosphate	0.50	1.40	1.40	1.50	1.60	0.50	1.40	1.40	1.50	1.60
Vitamin premix[2]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix[3]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Titanium (IV) oxide[4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Digestible energy (kcal/kg)	3842	3752	3701	3853	3713	3842	3705	3701	3853	3652
Analyzed composition (%)										
Dry matter	95.59	96.40	96.49	95.58	95.62	94.75	94.42	95.37	94.37	95.05
CP	16.45	16.08	13.53	10.64	0.17	15.21	13.11	14.04	10.06	0.19
Ash	5.64	5.60	4.66	4.62	3.68	6.47	4.51	3.85	3.37	3.72
Crude fat	4.62	3.52	2.33	8.20	1.98	3.53	1.62	2.07	7.42	2.42
Crude fiber	0.12	1.13	1.27	1.51	0.89	0.03	1.09	1.04	1.42	1.57
Lys	1.31	1.06	0.93	0.67	0.00	1.09	0.99	0.91	0.69	0.00

[1]Test ingredients were added to the diets as the sole dietary protein source from fishmeal (FM), conventional soybean meal (CONSBM1), low allergenic soybean meal (LASBM), low allergenic ground full fat soybean (LAGR) and nitrogen-free to account for basal endogenous losses (NF). CONSBM1, LASBM and LAGR were all processed at the Northern Crops Institute, Fargo, ND. CONSBM2 was processed at South Dakota Soybean Processors, Volga, SD. Refer to table 2.2 for test ingredient analysis.

[2]Provided per kg of complete diet: 32,973 IU vitamin A supplement, 4,947 IU vitamin D_3 supplement, 165 IU vitamin E supplement, 0.132 mg vitamin B_{12} supplement, 13.2 mg menadione as menadione dimethylpyrimidional bisulfite, 29.7 mg riboflavin as riboflavin supplement, 181.5 mg $_D$-pantothenic acid as $_D$-calcium pantothenate, 165 mg niacin as niacin supplement, 3.3 mg folic acid, 9.9 mg pyridoxine as pyridoxine hydrochloride, 9.9 mg thiamine as thiamine mononitrate and 0.513 mg biotin. (Nutra Blend, LLC, Neosho, MO)

[3]Provided per kg of the complete diet: 55 mg Zn as zinc sulfate, 55 mg Fe as ferrous sulfate, 14.7 mg Mn as manganese sulfate, 5.5 mg Cu as basic copper chloride, 0.12 mg I as ethylenediamine dihydriodide and 0.1 mg Se as sodium selenite. (J & R Distributing, Inc., Lake Norden, SD)

[4]Titanium (IV) oxide, anatase (Sigma-Aldrich Co. LLC, St. Louis, MO)

Table 3.2: Ingredient composition and nutrient analysis of growth performance experimental diets, as fed basis[1]

	Phase I				Phase II				
Item	FM	LASBM	LAGR	SBM	FM	LASBM	LAGR	SBM	Phase III
Corn	50.59	44.21	42.27	45.52	59.32	53.89	52.05	53.64	63.69
LA-MEAL	-	10.10	-	-	-	8.40	-	-	-
LA-FF	-	-	13.60	-	-	-	11.30	-	-
VOLGA-SBM	13.00	13.00	13.00	23.25	20.00	20.00	20.00	30.00	32.50
Whey, dried	20.00	20.00	20.00	20.00	10.00	10.00	10.00	10.00	-
Fishmeal	7.25	-	-	-	6.00	-	-	-	-
Blood meal	1.60	1.95	2.25	2.25	0.50	0.85	1.10	0.86	-
Soya oil	2.50	4.00	2.15	2.25	1.00	2.30	1.00	1.00	-
Whey protein concentrate (76.3% CP)	3.00	3.00	3.00	3.00	0.50	0.50	0.50	0.50	-
$_{L}$-Lys-HCl	0.15	0.36	0.34	0.35	0.24	0.41	0.40	0.39	0.37
$_{DL}$-Met	0.15	0.22	0.23	0.22	0.13	0.18	0.19	0.17	0.12
$_{L}$-Thr	0.02	0.12	0.13	0.13	0.06	0.14	0.14	0.13	0.12
$_{L}$-Trp	-	-	0.01	-	-	-	-	-	-
$_{L}$-Iso	0.01	-	-	-	-	-	-	-	-
Calcium carbonate	0.50	0.95	0.93	0.98	0.75	1.05	1.05	1.05	1.05
Dicalcium phosphate	0.63	0.40	0.40	0.40	-	-	-	-	-
Salt	-	0.10	0.10	0.10	0.40	0.48	0.47	0.46	0.85
Monocalcium phosphate	-	0.99	0.99	0.95	0.70	1.40	1.40	1.40	1.10
Vitamin premix[4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[5]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Zinc oxide	0.40	0.40	0.40	0.40	0.20	0.20	0.20	0.20	-
Formulated content									
Total Lys %	1.67	1.61	1.63	1.62	1.51	1.46	1.48	1.48	1.36
SID[4] Lys %	1.50	1.50	1.50	1.50	1.35	1.35	1.35	1.35	1.23
Met + Cys:Lys ratio, %	0.57	0.55	0.55	0.55	0.57	0.55	0.55	0.55	0.55
Thr:Lys ratio, %	0.58	0.59	0.59	0.59	0.59	0.59	0.58	0.59	0.59
Ile:Lys ratio, %	0.51	0.53	0.52	0.51	0.51	0.53	0.52	0.53	0.57
Val:Lys ratio, %	0.72	0.66	0.67	0.67	0.68	0.63	0.63	0.63	0.64

Trp:Lys ratio, %	0.20	0.17	0.17	0.18	0.19	0.17	0.16	0.18	0.19
Digestible energy (kcal/kg)	3662	3658	3668	3650	3619	3546	3639	3631	3598
Lysine:digestible energy (g/Mcal)	4.18	4.18	4.18	4.18	3.80	3.80	3.80	3.79	3.48
Analyzed Composition (%)									
Dry matter	90.88	91.62	91.48	90.72	89.46	90.14	89.88	89.45	87.93
CP	20.20	18.89	19.82	20.71	19.60	19.43	19.70	19.59	21.94
Ash	5.61	5.41	5.57	5.64	5.47	5.44	5.30	5.08	5.34
Crude fat	5.09	6.43	6.45	4.14	3.69	4.33	5.26	2.95	1.90
Crude fiber	1.57	1.61	1.92	1.88	1.85	2.06	2.52	2.19	2.14
Lys	1.60	1.58	1.58	1.57	1.32	1.55	1.50	1.52	1.53
Trypsin inhibitor (TIU/g)[5]	-	-	-	-	2400	4900	4600	2500	-
Urease activity (pH rise)[5]	-	-	-	-	<0.02	0.97	0.74	<0.02	-
Protein solubility (KOH %)[5]	-	-	-	-	74.93	83.83	79.70	82.92	-

Phase 1 (D0-5); Phase 2 (D6-18); Phase 3 (D19-36);

[1]Test ingredients were added to the base diet to provide equivalent dietary crude protein from fish meal (FM), low allergenic soybean meal (LASBM), low allergenic ground full-fat soybeans (LAGR) and a conventional soybean meal (SBM). LA-MEAL and LA-FF were processed at the Northern Crops Institute, Fargo, ND. VOLGA-SBM was processed at South Dakota Soybean Processors, Volga, SD. Refer to table 2.2 for test ingredient analysis.

[2]Provided per kg of complete diet: 10,991 IU vitamin A supplement, 1649 IU vitamin D_3 supplement, 55 IU vitamin E supplement, 0.044 mg vitamin B_{12} supplement, 4.4 mg menadione as menadione dimethylpyrimidional bisulfite, 9.9 mg riboflavin as riboflavin supplement, 60.5 mg $_D$-pantothenic acid as $_D$-calcium pantothenate, 55 mg niacin as niacin supplement, 1.1 mg folic acid, 3.3 mg pyridoxine as pyridoxine hydrochloride, 3.3 mg thiamine as thiamine mononitrate and 0.171 mg biotin. (Nutra Blend, LLC, Neosho, MO)

[3]Provided per kg of the complete diet: 165 mg Zn as zinc sulfate, 165 mg Fe as ferrous sulfate, 44.1 mg Mn as manganese sulfate, 16.5 mg Cu as basic copper chloride, 0.36 mg I as ethylenediamine dihydriodide and 0.3 mg Se as sodium selenite. (J & R Distributing, Inc., Lake Norden, SD)

[4]SID = Standardized ileal digestibility

[5]Trypsin inhibitor, urease activity and protein solubility were all measured at Eurofins Nutrition Analysis Center (Des Moines, IA).

Table 3.3: Exp. 1 Standard ileal digestibility (SID) and digestible content of CP and amino acids in fishmeal (FM), conventional soybean meal (CONSBM), low allergenic soybean meal (LASBM), and low allergenic ground soybeans (LAGR) on an as fed basis.

Items	FM	CONSBM	LASBM	LAGR	SEM	*P*-Value
SID%						
Crude protein	70.44^{ax}	44.63^{b}	60.36^{ab}	51.75^{aby}	5.20	0.009
Lysine	81.85^{a}	48.87^{c}	64.16^{b}	54.76^{bc}	3.51	<0.001
Methionine	87.57^{a}	53.77^{c}	66.45^{bx}	55.98^{cy}	2.88	<0.001
Threonine	75.34^{ax}	44.21^{bc}	58.59^{by}	40.93^{cd}	4.36	<0.001
Tryptophan	85.72^{a}	54.88^{b}	69.22^{c}	54.08^{b}	4.13	<0.001
Isoleucine	82.64^{a}	45.82^{b}	54.40^{b}	44.65^{b}	3.07	<0.001
Leucine	84.28^{a}	43.54^{b}	53.74^{b}	44.30^{b}	3.12	<0.001
Valine	79.54^{a}	45.24^{b}	56.64^{bx}	42.29^{by}	3.70	<0.001
Phenylalanine	82.25^{a}	45.34^{b}	53.76^{b}	46.89^{b}	3.14	<0.001
Histidine	83.26^{a}	56.60^{c}	68.89^{b}	59.51^{bc}	3.01	<0.001
Arginine	88.03^{a}	51.57^{b}	64.03^{b}	53.21^{b}	4.39	<0.001
Glutamate	78.97^{a}	55.09^{b}	63.94^{ab}	58.07^{b}	4.78	0.012
Proline	83.77	43.90	71.86	-6.30	38.89	0.370
Serine	74.14^{ax}	43.54^{b}	56.31^{by}	43.38^{b}	4.81	<0.001
Glycine	76.70^{a}	38.33^{ab}	58.07^{ab}	21.75^{b}	11.60	0.021
Cystine	45.28	36.26	50.85	27.20	8.12	0.219
Aspartic acid	73.29^{a}	50.99^{b}	59.52^{ab}	50.89^{bc}	4.13	0.004
Alanine	82.07^{a}	48.26^{b}	60.13^{bx}	42.01^{by}	4.57	<0.001
Tyrosine	79.17^{a}	42.35^{bx}	55.36^{by}	45.46^{b}	3.53	<0.001
Standard digestible content (g/kg)						
Crude protein	45.31^{a}	23.24^{b}	27.69^{b}	17.66^{b}	1.32	<0.001
Lysine	3.99^{a}	1.59^{bc}	1.87^{b}	1.25^{c}	0.13	<0.001
Methionine	1.45^{a}	0.38^{b}	0.42^{b}	0.27^{c}	0.02	<0.001
Threonine	1.85^{a}	0.85^{bc}	1.02^{b}	0.55^{c}	0.09	<0.001
Tryptophan	0.14^{ax}	0.11^{aby}	0.11^{b}	0.05^{c}	0.01	<0.001
Isoleucine	2.12^{a}	1.12^{b}	1.15^{b}	0.71^{c}	0.07	<0.001
Leucine	3.58^{a}	1.68^{b}	1.88^{b}	1.16^{c}	0.11	<0.001
Valine	2.29^{a}	1.12^{b}	1.25^{b}	0.70^{c}	0.09	<0.001
Phenylalanine	2.03^{a}	1.18^{b}	1.25^{b}	0.84^{c}	0.07	<0.001
Histidine	1.21^{a}	0.75^{b}	0.82^{b}	0.54^{c}	0.04	<0.001
Arginine	3.32^{a}	1.91^{b}	2.11^{b}	1.34^{c}	0.12	<0.001
Glutamate	6.24^{ax}	4.91^{ay}	5.07^{a}	3.38^{b}	0.36	<0.001
Proline	2.79^{x}	1.56	2.13	0.43^{y}	0.75	0.075
Serine	1.51^{a}	0.90^{bc}	1.03^{b}	0.63^{c}	0.09	<0.001
Glycine	3.64^{a}	0.81^{b}	1.08^{b}	0.32^{b}	0.23	<0.001
Cystine	0.24^{ab}	0.30^{abx}	0.34^{a}	0.14^{by}	0.05	0.026
Aspartic acid	4.01^{a}	2.91^{b}	2.96^{b}	1.88^{c}	0.21	<0.001

Alanine	3.19[a]	1.02[bx]	1.16[b]	0.61[cy]	0.10	<0.001
Tyrosine	0.52[a]	0.28[b]	0.30[b]	0.14[c]	0.02	<0.001

[a-d] Within a row, means lacking a common superscript differ ($P < 0.05$)

[xy] Within a row, means lacking a common superscript are tendencies ($0.05 \leq P \geq 0.10$)

Table 3.4: Exp. 2 Standard ileal digestibility (SID) and digestible content of CP and amino acids in fishmeal (FM), conventional soybean meal (VOLGA-SBM), low allergenic soybean meal (LASBM), and low allergenic ground soybeans (LAGR) on an as fed basis.

	Ingredient						Gender			
Items	FM	VOLGA-SBM	LASBM	LAGR	SEM	*P*-Value	Barrow	Gilt	SEM	*P*-Value
SID%										
Crude protein	78.17^{a}	84.04^{a}	38.03^{b}	50.23^{b}	5.97	<0.001	69.69	55.55	4.04	<0.001
Lysine	84.11^{a}	88.74^{a}	42.42^{b}	57.94^{b}	5.97	<0.001	72.46	64.14	4.49	0.153
Methionine	85.59^{a}	90.53^{a}	46.55^{b}	58.20^{b}	5.15	<0.001	73.65	66.78	3.86	0.168
Threonine	80.92^{a}	83.09^{a}	38.57^{b}	48.88^{b}	6.49	<0.001	68.17	57.57	5.03	0.096
Tryptophan	91.64^{a}	89.84^{a}	55.48^{b}	57.35^{b}	5.92	<0.001	78.71	68.45	4.94	0.077
Isoleucine	84.42^{a}	88.69^{a}	34.16^{b}	47.52^{b}	5.89	<0.001	68.34	59.05	4.41	0.108
Leucine	85.44^{a}	87.37^{a}	32.10^{b}	46.02^{b}	5.91	<0.001	67.32	58.13	4.47	0.112
Valine	82.11^{a}	86.58^{a}	35.03^{b}	47.35^{b}	5.96	<0.001	67.34	58.20	4.46	0.117
Phenylalanine	83.50^{a}	88.03^{a}	33.46^{b}	46.52^{b}	5.68	<0.001	67.37	58.38	4.23	0.107
Histidine	81.11^{a}	88.90^{a}	45.90^{b}	57.74^{b}	5.38	<0.001	72.84	63.99	3.84	0.097
Arginine	92.71^{a}	93.02^{a}	45.83^{b}	57.77^{b}	5.10	<0.001	77.64	67.03	3.44	0.041
Glutamate	83.84^{a}	89.13^{a}	42.51^{b}	56.88^{b}	5.80	<0.001	71.87	64.32	4.25	0.179
Proline*	148.83^{a}	107.75^{ab}	60.32^{b}	63.24^{b}	19.83	0.011	123.32	66.76	14.16	0.009
Serine	81.07^{a}	87.69^{a}	32.67^{b}	45.03^{b}	7.05	<0.001	66.36	56.87	5.83	0.162
Glycine	86.87^{a}	90.55^{a}	35.95^{b}	47.51^{b}	10.34	0.003	79.98	50.45	6.93	0.009
Cystine	64.17^{ax}	79.08^{a}	15.99^{b}	32.72^{by}	10.09	<0.001	55.89	40.09	8.16	0.108
Aspartic acid	71.57^{ax}	86.29^{a}	38.29^{b}	51.58^{by}	6.15	<0.001	66.89	56.98	4.89	0.099
Alanine	85.31^{a}	59.17^{a}	39.89^{b}	51.40^{b}	5.68	<0.001	71.41	59.97	3.65	0.048
Tyrosine	82.46^{a}	88.10^{a}	38.26^{b}	46.86^{b}	6.35	<0.001	68.86	58.98	5.14	0.110
Standard digestible content (g/kg)										
Crude protein	11.89^{a}	11.03^{a}	5.34^{b}	5.05^{b}	0.80	<0.001	9.18	4.74	0.54	0.037

Lysine	0.92[a]	0.88[a]	0.39[b]	0.40[b]	0.05	<0.001	0.68	0.61	0.04	0.193
Methionine	0.34[a]	0.18[b]	0.09[c]	0.08[c]	0.01	<0.001	0.18	0.17	0.01	0.235
Threonine	0.48[a]	0.49[a]	0.21[b]	0.20[b]	0.03	<0.001	0.37	0.32	0.02	0.121
Tryptophan	0.14[a]	0.20[b]	0.09[c]	0.05[d]	0.01	<0.001	0.13	0.11	0.01	0.110
Isoleucine	0.52[a]	0.68[b]	0.23[c]	0.23[c]	0.04	<0.001	0.44	0.39	0.02	0.134
Leucine	0.88[a]	1.05[a]	0.36[b]	0.37[b]	0.06	<0.001	0.71	0.62	0.05	0.138
Valine	0.56[a]	0.67[a]	0.24[b]	0.24[b]	0.04	<0.001	0.46	0.40	0.03	0.148
Phenylalanine	0.49[a]	0.71[b]	0.25[c]	0.24[c]	0.04	<0.001	0.45	0.39	0.03	0.130
Histidine	0.25[a]	0.36[b]	0.17[c]	0.16[c]	0.02	<0.001	0.25	0.22	0.01	0.124
Arginine	0.79[a]	0.98[b]	0.45[c]	0.41[c]	0.05	<0.001	0.70	0.61	0.03	0.060
Glutamate	1.64[a]	2.43[b]	1.08[c]	1.02[c]	0.14	<0.001	1.63	1.46	0.10	0.208
Proline	1.23[a]	0.98[ax]	0.45[by]	0.38[b]	0.16	0.001	0.96	0.57	0.12	0.016
Serine	0.40[a]	0.60[b]	0.20[c]	0.20[c]	0.04	<0.001	0.38	0.33	0.03	0.190
Glycine	1.03[a]	0.57[b]	0.21[c]	0.20[c]	0.06	<0.001	0.58	0.42	0.04	0.011
Cystine	0.08[a]	0.17[b]	0.03[a]	0.05[a]	0.02	<0.001	0.10	0.07	0.02	0.119
Aspartic acid	0.96[a]	1.50[b]	0.60[c]	0.59[c]	0.09	<0.001	0.96	1.50	0.07	0.125
Alanine	0.83[a]	0.57[b]	0.24[c]	0.23[c]	0.03	<0.001	0.50	0.44	0.02	0.078
Tyrosine	0.31[a]	0.42[b]	0.17[c]	0.14[c]	0.03	<0.001	0.28	0.24	0.02	0.135

[a-d] Within a row, means lacking a common superscript differ ($P < 0.05$)

[xy] Within a row, means lacking a common superscript are tendencies ($0.05 \leq P \geq 0.10$)

*Tendency found between ingredient x gender interaction ($P = 0.0893$)

Table 3.5: Growth performance of nursery pigs fed diets containing one of four different protein sources; fishmeal (FM), low allergenic soybean meal (LASBM), low allergenic ground beans (LAGR), or conventional soybean meal (SBM).

Item	FM	LASBM	LAGR	SBM	SEM	*P*-Value
BW, kg						
Phase 1 (d 5)	7.861	8.052	8.109	8.184	0.077	0.017
Phase 2 (d 17)	11.806^{b}	11.507^{b}	11.945^{b}	13.172^{a}	0.220	<0.001
Phase 3 (d 36)	22.967^{b}	22.870^{b}	24.326^{ab}	24.936^{a}	0.490	0.015
Daily gain, kg						
Phase 1	0.038^{b}	0.077^{ab}	0.099^{a}	0.118^{a}	0.017	0.006
Phase 2	0.305^{b}	0.267^{b}	0.297^{b}	0.386^{a}	0.015	<0.001
Phase 3	0.619^{xy}	0.592^{y}	0.685^{x}	0.650^{xy}	0.026	0.079
Overall	0.425^{aby}	0.402^{b}	0.464^{a}	0.482^{ax}	0.015	0.005
Daily feed disappearance, kg						
Phase 1	0.101^{b}	0.176^{a}	0.179^{a}	0.186^{a}	0.016	0.001
Phase 2	0.364^{b}	0.347^{b}	0.366^{b}	0.450^{a}	0.017	0.001
Phase 3	1.009	1.005	1.053	1.071	0.047	0.630
Overall	0.651^{y}	0.649^{y}	0.685^{xy}	0.725^{x}	0.023	0.051
Gain:feed						
Phase 1	-0.090	0.378	0.492	0.580	0.227	0.191
Phase 2	0.837	0.770	0.812	0.865	0.031	0.184
Phase 3	0.609	0.584	0.644	0.608	0.020	0.213
Overall	0.648^{xy}	0.614^{y}	0.672^{x}	0.661^{xy}	0.017	0.105

Phase 1 (d0-5); Phase 2 (d6-17); Phase 3 (d18-36)

^{ab} Within a row, means lacking a common superscript differ ($P < 0.05$)

^{xy} Within a row, means lacking a common superscript are tendencies ($0.05 \leq P \geq 0.10$)

Chapter 4

FINAL DISCUSSION

The primary focus of this project was to assess the potential feeding value of an alternative soybean variety (LA) that was bred to remove KTI, lectins and P34 protein from the seed. Assessment of feeding value was accomplished in 2 stages: gut health based on intestinal permeability in the post-weaning period, then nutritional evaluation including AA digestibility and growth performance of weaned pigs. The intestinal permeability section was completed as supplement to work conducted by Tauer (2018) to determine the overall effect of the LA soybeans on gut health. We hypothesized that the inflammatory response in pigs fed LA soybean would be lower than pigs fed conventional and subsequently improve intestinal permeability in comparison to CON diets with conventional soybeans yet similar to diets based on ANIM proteins. In addition, we hypothesized the LA soybean products would have similar digestibility values to that of conventional SBM and would be a good alternative to FM in weaned pig diets.

Work done by Tauer (2018) showed no difference between the LA soy protein and CON soy protein in intestinal morphology based on villus height or crypt depth, or inflammatory responses as indicated by gene expression and interleukin levels. With lower levels of TIU, lectins and P34 proteins in the LA soybean, we expected there to be less of an inflammatory response which would thus positively impact the morphology and permeability of the intestines. The lack of difference in villus height, crypt depth, gene expression, interleukin levels, Ussing chamber measurements of TER, Isc, ΔIsc and FD4, and L:M ratio suggest that the inflammatory response of soybeans is linked more

closely with allergens such as glycinin and β-conglycinin rather than ANF's. The allergens were analyzed in all soy ingredients and there was little difference between both LA and CON soybean products, which may explain the similar intestinal permeability. The LA soybeans used in Tauer (2018) versus the LA soybeans used in this study were from different growing seasons and processed at different times. Due to these differences, it may have been beneficial to measure intestinal morphology and inflammatory markers in this study as well to have a more defined understanding of gut health utilizing LA soybeans. Low allergenic and conventional SBM's used in Tauer (2018) had TIU levels of 6,500 and 16,700, respectively. In the current study, LASBM and CONSBM had TIU levels of 21,110 and 62,600, respectively, 3-4 fold higher compared to Tauer (2018). Despite differences in growing season and processing, results from both studies suggest that LA soybeans do not have a negative impact on intestinal morphology or permeability. One limitation in the intestinal permeability study may have been that the pigs were individually housed in metabolism crates with only nose to nose contact. This is not considered a 'normal' environment for the pigs as they are used to socializing with other pigs. When the pigs are in an environment by themselves, the stress could have been higher for those pigs which could have impacted the study. In addition, they are only exposed to their own feeder, waterer and manure which limits outside environmental factors that may be present in a commercial setting.

Based on the nutritional evaluation in combination with measures of intestinal permeability, between LA soy products, LAGR may be a suitable replacement in weaned pig diets. There was similar performance between pigs fed LAGR and pigs fed a conventional corn-soybean meal diet with no impact compared to FM. It may be

beneficial to test the LA soybean in a commercial nursery barn, with presumably greater overall impact, to assess practical feeding value.

Low allergenic full fat, ground soybeans may be beneficial as an alternative protein source for niche market pork producers, specifically those who produce antibiotic free, organic or all-natural pork. In all-natural pork production systems, the pigs are to be fed feed that has been minimally processed and does not include antibiotics, growth promotants, or animal by-products (Pork Checkoff, 2019). With these stipulations, there can be limited feed options available for niche producers, specifically all-natural. Due to the low levels of ANF's, LA soybeans can be fed to nursery pigs with minimal processing (i.e. just ground). This minimal processing also retains the oil which is valuable from a dietary energy supply perspective.

For LA soybeans to be used in pork production, a consistent supply of beans is necessary. Low allergenic soybeans are not genetically modified and thus could provide a premium to crop farmers in today's market. However, in order to make that premium a viable option, 75 cents to $1 per bushel over conventional soybeans would be optimal for them to make the switch, because of a slight increase in cost to produce (Youngerberg, 2019). For example, with a soybean that is not genetically modified, weed and pest control systems may need to be altered and separate storage bins would be required in comparison to conventional soybeans. If there is a demand for LA soybean and they have good yields, there may be potential for crop farmers to switch from conventional soybean production to LA production.

Although AA digestibility of LAGR may be lower compared to FM, crystalline AA's could be supplemented to meet the pig's nutrient requirements. For example,

considering the first four limiting AA, FM inclusion of 5% provides SID Lys, Met, Thr and Trp at levels of 0.21, 0.07, 0.10 and 0.03%, respectively. LAGR added to the diet at 16% with supplementation of 0.03% Met supplies equivalent AA. In 1-ton of feed, with FM priced at ~$0.66/lb. (MUNDI Index, October 18, 2019) and LAGR at same price as CONSBM (~$0.15/lb.; MUNDI Index, October 18, 2019), the cost to supply equivalent AA would decrease (FM: 5% of 2000 lbs. = 100 lbs. x $0.66/lb. = $66; LAGR: 16% of 2000 lbs. = 320 lbs. x $0.15/lb. = $48; Met: 0.03% of 2000lbs. = 0.6lbs x $1.23/lb. = $0.74). Adjustments in other ingredients are not considered in this example but does demonstrate potential cost savings replacing FM with LAGR. If LAGR was reduced to 10% of the diet and supplemented with Lys, Met, Thr, and Trp (0.10, 0.04, 0.04, 0.01%), the same SID AA content would be met. Despite changes in other ingredients added in the diet, the cost to supply equivalent AA would further decrease because AA's are relatively inexpensive (FM: $66; LAGR 10% of 2000 lbs. = 200 lbs. x $0.15/lb. = $30; AA: ~$4.25).

Further evaluation of LA soybeans should be done to look at inflammatory cytokines and blood IgE antibody and anti-soy antigen levels at two different time points, one prior to feeding the soybean to pigs and the other several days after soybeans have been fed to further analyze the effect of allergens (glycinin, β-conglycinin, and P34) on the overall immune response. Specifically, the P34 protein should have been researched more as the LA soybeans were null of this protein. In order to determine if this soybean has potential to be used in human nutrition, additional research in the inflammatory markers and blood antibody levels would assist in determining if the hypoallergenic effects have been eliminated for humans with soy sensitivities.

Based on the stated objective and the evidence supported in this thesis, I conclude that LA ground, full fat soybeans may be a suitable alternative protein source in weaned pig diets due to similar impact on intestinal permeability and growth performance compared to FM. The use of the LA ground beans would primarily be a suitable option for those who have limited feed resources in niche pork production.

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APPENDIX A

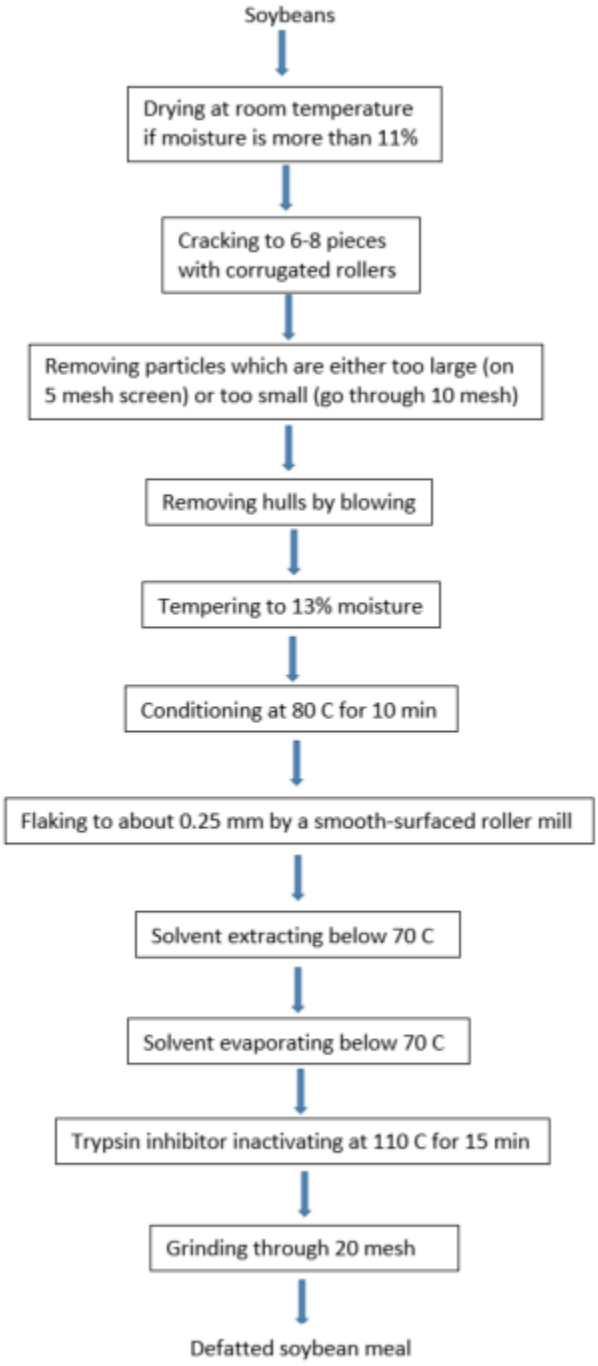

Figure A: Soybean meal processing methods as provided by Dr. Zhisheng (Zach) Liu, food scientist at the Northern Crops Institute, Fargo, ND.

Full fat, ground soybeans were simply ground using a dry extrusion process.