2021

Evaluating Impacts of Tryptophan and Branched Chain Amino Acids in Swine Diets Containing Corn Based Dried Distillers Grains on the Growth Performance and Carcass Characteristics of Grow-Finish Pigs

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Recommended Citation

Clizer, David Alan, "Evaluating Impacts of Tryptophan and Branched Chain Amino Acids in Swine Diets Containing Corn Based Dried Distillers Grains on the Growth Performance and Carcass Characteristics of Grow-Finish Pigs" (2021). *Electronic Theses and Dissertations*. 5699.
https://openprairie.sdstate.edu/etd/5699

EVALUATING IMPACTS OF TRYPTOPHAN AND BRANCHED CHAIN AMINO ACIDS IN SWINE DIETS CONTAINING CORN BASED DRIED DISTILLERS GRAINS ON THE GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF GROW-FINISH PIGS

BY

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A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Animal Science

South Dakota State University

2021

DISSERTATION ACCEPTANCE PAGE
David Clizer

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

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ACKNOWLEDGEMENTS

I would like to give my greatest thanks to my family for all support they have given me through my years of education. Without their guidance, I would not have made it this far in my educational career. I would like to thank my significant other, Kaitlin Epperson, for her unconditional love and support in life. I must thank my dog, Maggie, for always being excited to see after getting home from school and providing me with daily companionship. I would like to thank Dr. Paul Cline for being a tremendous mentor throughout my Ph.D. journey. I cannot fathom where I would be in my career if Dr. Cline and Christensen Farms did not support my pursuit of a Ph.D. My appreciation goes out to Dr. Zachary Smith for his mentorship and for being a person that I can always talk to about research and much more. I cannot thank Dr. Julie Walker enough with being a mentor and resource I could always turn too. I would like to thank my advisor, Dr. Ryan Samuel, for giving me the opportunity to purse my Ph.D in swine nutrition. I would like to thank Dr. Crystal Levesque and Dr. Kenda Kattelmann for serving on my Ph.D committee. I would like to say thank you to Erin McCoy with Pipestone for all that she has taught me about the production of wean to finishing pigs. I cannot thank the front office ladies enough for all that they have done for me over the past years. Lastly, I would like to thank all the graduate students for being mentors and friends throughout my pursuit for a Ph.D.

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ABBREVIATIONS

AA	Amino Acids
ADFI	Average daily feed intake
ADG	Average daily gain
BCAA	Branched chain amino acids
BF	Back Fat
BIC	Bayesian information criteria
BW	Body weight
CI	Confidence interval
CP	Crude protein
DDGS	Dried distiller grains with solubles
DE	Digestible Energy
G:F	Gain to feed
HCW	Hot carcass weight
HPDDG	High protein dried distiller grains
Ile	Isoleucine
Leu	Leucine
LNAA	Large neutral amino acids
Lys	Lysine
ME	Metabolizable Energy
mTOR	mammalian target of rapamycin
NE	Net Energy
QBL	Quadratic broken line
QP	Quadratic polynomial

RFID	Radio-frequency identification
SBL	Straight broken line
SFFL	Standardized fat free lean
SID	Standardized ileal digestible
TID	True ileal digestible
Trp	Tryptophan
Val	Valine

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ABSTRACT

EVALUATING IMPACTS OF TRYPTOPHAN AND BRANCHED CHAIN AMINO ACIDS IN SWINE DIETS CONTAINING CORN BASED DRIED DISTILLERS GRAINS ON THE GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF GROW-FINISH PIGS

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A total of four studies were conducted in an effort to determine the impact of Trp and the BCAA in swine diets containing DDGS or HPDDG on the growth performance and carcass characteristics of the growing and finishing pig. The first study utilized 1,170 pigs (PIC 800 x PIC, initial BW 38.6 kg) in a 98-d grow-finish study to determine the performance response of pigs fed increasing levels of SID Trp:Lys in swine diets containing 40% DDGS. Treatments consisted of diets containing 40% DDGS with a SID Trp:Lys ratios of 15, 18, 21, or 24% or a corn-SBM diet for a total of five dietary treatments. Dietary treatments were replicated nine times and each pen contained 26 pigs balanced for sex. Data was analyzed as a randomized complete block design with the blocking factor of previous treatment considered as random. Pair-wise comparisons were used to evaluate dietary treatment impact on performance and carcass characteristics. Single degree of freedom orthogonal polynomials were used to evaluate the dose response of increasing the SID Trp:Lys in diets containing 40% DDGS. Increasing the SID Trp:Lys ratio in 40% DDGS diets increased (Linear, $P \leq 0.023$) ADG, ADFI, final BW, HCW, carcass gain, and standardized fat free lean weight. However, pigs fed the corn-SBM diet had greater ADG ($P<0.008$) and heavier ($P<0.002$) final BW compared to pigs fed 40% DDGS. Supplying a SID Trp:Lys ratio in 40% DDGS diets resulted in

similar (P=0.253) ADFI compared to pigs fed a corn-SBM diet. Corn-SBM fed pigs also had heavier HCW and standardized fat free lean weights, greater carcass yields and gains, and increased loin depths (*P*<0.001) compared to pigs fed diets containing 40% DDGS. The SID Trp:Lys ratio did not impact (*P*≥0.151) pigs with a lighter starting BW differently compared to average and heavy starting BW pigs. Data from this study would indicate that increasing the SID Trp:Lys ratio in diets containing 40% DDGS linearly improved ADG and ADFI until pigs reached approximately 115 kg, but feeding corn-SBM diets will still outperform pigs fed DDGS diets.

In the second experiment, a total of 2,430 (DNA 600 x PIC PN70, initial BW 39.4 kg) were used in a 28-d study to determine the SID Val:Lys requirement of pigs fed diets containing 30% DDGS. Dietary treatments consisted of five diets containing 30% DDGS with a SID Val:Lys ratios of 60, 65, 70, 75, or 80% and a corn-SBM diet. Pens of pigs were randomly assigned to one of six dietary treatments in a randomized complete block design with initial weight as the random blocking factor and each treatment was replicated 15 times. Pair-wise comparisons were used to evaluate dietary treatment impact on growth performance of pigs and single degree of freedom orthogonal polynomials were used to evaluate the dose response of increasing the SID Val:Lys ratio in 30% DDGS diets. Increasing the SID Val:Lys ratio in diets containing 30% DDGS increased (Quadratic, *P*<0.001) final BW, ADG, ADFI, and G:F of pigs. However, pigs fed the corn-SBM diet had heavier final BW and improved (*P*≤0.032) ADG, G:F, and ADFI compared to pigs fed the 30% DDGS diets with the exception of ADFI of pigs supplied a SID Val:Lys ratio of 75% (*P*=0.167). The SID Val:Lys requirement for the ADG response was estimated at 66.6% (95% CI: [65.9, 67.4]) from the SBL analysis and

69.9% (95% CI: [68.2, 71.5]) from the QBL analysis. Optimal SID Val:Lys ratio for the G:F response was estimated at 68.4% (95% CI: [66.0, 70.8]) and 72.8% (95% CI: [69.8, 75.8]) from the SBL and QBL methods. Data from this study would indicate that feeding a SID Val:Lys ratio of 68% in 30% DDGS diets will yield more than 99% and 98.5% of the maximum ADG and G:F response but a corn-SBM diet will still outperform DDGS diets for the 39 to 68 kg pig.

In the third experiment, 2,268 (DNA 600 x PIC PN70, initial BW 82.3 kg) pig were used in a 56-d study to quantify the SID Ile:Lys requirement in finishing swine diets containing 20% DDGS. A total of six dietary treatments were fed and consisted of five diets containing 20% DDGS with a SID Ile:Lys ratios of 55, 60, 65, 70, or 75% or a corn-SBM diet. Pens were assigned to dietary treatments within block, balancing for previous treatment, and each treatment was replicated 14 times. Pair-wise comparisons were used to evaluate dietary treatment impact on performance and carcass traits. Single degree of freedom orthogonal polynomials were used to evaluate the dose response of increasing the SID Ile:Lys ratio in 20% DDGS diets. Increasing the SID Ile:Lys ratio in 20% DDGS diets did not impact growth performance in a quadratic or linear fashion ($P \geq 0.153$). However, increasing the SID Ile:Lys ratio in 20% DDGS diets decreased back fat (Quadratic, $P=0.014$), increase loin depth (Quadratic, $P=0.029$), and tended to increase percent lean (Quadratic, $P=0.076$), with the optimal carcass parameters occurring when supplying a 65% SID Ile:Lys ratio in 20% DDGS diets. Pig fed the corn-SBM diet had similar final BW compared to pigs fed 20% DDGS diets containing 60 and 70% SID Ile:Lys ratios ($P>0.060$) and greater ADFI compared to pigs receiving diets with SID Ile:Lys ratios of 65 and 75% ($P<0.001$). This data would indicate that the optimal SID

Ile:Lys requirement to maximize carcass parameters would be 65%, while the optimal Ile requirement for growth performance is less clear.

In the fourth experiment, a total of 1,170 pigs (PIC 359 x PIC, initial BW 59.5 kg) were utilized in a 79-d grow-finish study to evaluate the impact of HPDDG (NexPro® protein ingredient, Flint Hills Resources, Wichita, KS) and SBM inclusion level on the performance and carcass traits of growing-finishing pigs when BCAA ratios were adjusted. Pen of pigs were allotted to one of five dietary treatments which included: 1) corn-SBM diet, 2) diet containing HPDDG with an SID Ile:Lys ratio of 56%, or diets containing HPDDG with a SID Val:Lys and Ile:Lys ratios of 75 and 65% met through the inclusion of 3) SBM (HPSBM), 4) 50% SBM and 50% crystalline AA blend (HP50/50), or 5) crystalline AA (HPAA). The inclusion of HPDDG in diets was 15% for phase one and 10% for phases two and three. Data was analyzed as a randomized complete block design with previous treatment considered as the random blocking factor. Pair-wise comparisons were used to evaluate dietary treatments impact on performance and carcass traits. Single degree of freedom orthogonal polynomials were used to evaluate dose response of SBM in HPDDG diets where SID Val:Lys and Ile:Lys ratios were held at 75 and 65%. Dietary treatment did not impact final BW, cumulative ADG, ADFI, G:F, or carcass traits ($P \geq 0.118$) with the exception of the pigs fed the corn-SBM and HP50/50 dietary treatments having a greater ($P \leq 0.043$) carcass yield compared to the HPSBM treatment. The reduction of SBM in HPDDG diets when SID Val and Ile were held constant relative to Lys resulted in a decrease (Linear, $P \leq 0.046$) in ADG and G:F and tended to reduce (Linear, $0.094 \geq P \geq 0.065$) final BW, carcass yield, and standardized fat free lean. Reduction of SBM inclusion in these diets also tended to reduce (Quadratic,

P=0.075) back fat, but did not impact HCW (*P*=0.142). Data from this study indicates HPDDG is a suitable feedstuff for grow-finish swine diets at low dietary inclusion levels and that, when adjusting BCAA ratios to mitigate negative impacts of excess dietary Leu, utilizing SBM provides a benefit compared to crystalline AA.

In overall conclusion, increasing the SID Trp:Lys ratio in 40% DDGS diets lead to an increase in ADG as a result of an increased ADFI and no difference in G:F. Providing diets with a SID Trp:Lys ratio of 24% lead to similar ADFI between pigs fed 40% DDGS diets and corn-SBM fed pigs. but ADG of pigs fed corn-SBM diets will be greater and lead to pigs with heavier final BW. When feeding 30% DDGS during the growing period, the SID Val:Lys requirement was determined to be 68%, but DDGS fed pigs still had worse performance compared to corn-SBM fed pigs. During the finishing period, supplying a SID Ile:Lys ratio of 65% would provide optimal carcass characteristics when feeding diet containing 20% DDGS. Finally, when adjusting the BCAA ratios in HPDDGS diets, it is better to utilize SBM compared to crystalline AA and low inclusion levels of HPDDG have minimal impact on growth performance and carcass characteristics of growing-finishing pigs.

CHAPTER 1

LITERATURE REVIEW

The Ideal Protein Concept

The quality of dietary protein is a function of the amino acids (AA) that comprise the protein, along with the digestibility and availability of these AA (Wang and Fuller, 1989). Wang and Fuller (1989) further defines “quality” as the degree to which the absorbed AA aligns with those required by the animal. Therefore, in order to evaluate the quality of protein sources, a reference of the animals optimal AA pattern or ideal protein is required (Wang and Fuller, 1989). The ideal protein concept was first introduced by Mitchell (1964) and is an attempt to quantify an ideal profile of AA that are required to optimize performance of pigs (Kendall, 2004). However, the AA that are required to maximize growth performance of pigs involves two components: 1) AA needed to meet requirements for maintenance and 2) AA needed to meet the requirements of tissue protein accretion (Fuller et al., 1989). The AA requirements for maintenance was described by Moughan (1999) as the combination of basal endogenous intestinal AA losses, AA utilized for skin and hair regeneration, and AA lost due to a minimum rate of body protein turnover. The NRC (2012) utilized the studies of Batterham et al. (1990), Kyriazakis et al. (1993), Bikker et al. (1994), and Mahan and Shields Jr (1998) to determine the AA requirement of whole body protein over various BW for the grow-finish pig. Therefore, when calculating the ideal AA pattern the proportion of AA required for maintenance and protein deposition need to be accurately described, which will be a function of the physiological state of the animal and production level (NRC, 2012). The ideal AA pattern to describe the ideal protein is usually expressed relative to a reference AA and Lys is typically used as the reference AA because it is usually the first-limiting AA in swine diets (van Milgen and Dourmad, 2015). Furthermore, factors affecting the amount of dietary AA that can be utilized to meet these AA requirements, such as digestibility, transport efficiency, and initial catabolism prior to utilization, need to be considered when targeting an optimal supply of dietary AA (van Milgen and Dourmad, 2015). Specifically, decreases in digestibility and transport efficiency or increases in initial catabolism prior to utilization will decrease the AA available to meet requirement. Recent advances by Stein et al. (2007) has allowed for both AA requirements and the dietary supply of AA to be expressed on the same SID basis, thus allowing for more accurate estimates of AA requirements by reducing inconsistencies due to differences in dietary AA digestibility. More research is required to accurately quantify other factors impacting discrepancies observed between dietary AA supply and AA requirements, along with the validation of current AA requirements through empirical means.

The Relationship Between Energy and Protein

It is understood that voluntary feed intake of pigs is largely impacted by dietary energy density and that pigs will adjust their feed intake in order to meet a certain daily energy intake, until limited by physical capacity or other environmental factors (Henry, 1985; Beaulieu et al., 2009). Due to feed intake being the primary factor determining total consumption of AA, it is generally assumed there should be a relationship between energy and AA concentrations in swine diets (Chiba et al., 1991). The expression of AA requirements as a ratio to energy would ensure that sufficient concentrations of AA are consumed in relation to the requirement for protein synthesis, regardless of dietary energy concentrations (Smith et al., 1999). However, this concept is only valid if there is a linear relationship between the rate of protein deposition and energy intake (SCA, 1987). The work by Rao and McCracken (1991) and Bikker (1994) indicated that were was a linear relationship between energy intake and protein deposition for pigs with a BW less than 90 kg. Therefore, a limit in energy supply will lead to a plateau in growth performance, given adequate amounts of AA are supplied to meet the requirement for protein deposition (Black and De Lange, 1995). Ultimately, there will be a point at which energy supply will not limit protein deposition but rather protein deposition will plateau in response to protein supply (Whittemore et al., 2001). This is the animal's maximum capacity for protein deposition or Pd_{max}, and this value is only derived when neither energy intake nor AA intake are limiting (Van der Peet-Schwering et al., 1999). Pigs during the growing period, less than 50 kg, generally lack the ability to reach Pd_{max} as a result of energy intake being limited due to physical capacity of the digestive system limiting feed intake, thus allowing for AA requirements to be defined in relation to energy (Möhn and De Lange, 1998). During the finishing period, the energy intake of pigs can exceed the requirements for protein deposition and, at this point, AA requirements for protein synthesis becomes independent from energy intake, leading to AA requirements having to be described on a daily intake basis to avoid over supplementation (Lewis and Southern, 2000).

The requirement for Lys in swine diets is commonly expressed as a Lys to calorie ratio (Main et al., 2008). It is not necessary to define other individual AA requirements on an energy basis because the utilization of the ideal protein concept allows for one to define other AA requirements in relation to Lys and, therefore, adequately suppling all dietary AA in relation to energy intake (Cline et al., 2016). Understanding these relationships becomes increasingly important when alternative feed ingredients are utilized due to their impact on dietary energy concentrations (Lewis and Southern, 2000). The correct application of these concepts would allow swine producers to be flexible in dietary ingredient composition while in turn maintaining adequate feed efficiency and carcass characteristics and, thus, profitability (Cline et al., 2016).

The Use of Corn Based DDGS in Swine Diets

A by-product of dry-grind ethanol plants is corn dried distillers grains with solubles (DDGS) and this alternative feedstuff has become popular in grow-finish swine diets (Xu et al., 2010). In the 1970s, the construction of ethanol plants resulted in the increased production of DDGS (Stein and Shurson, 2009). This led to the work by Wahlstrom et al. (1970), Smelski (1972), and Wahlstrom and Libal (1980) who attempted to quantify the feeding value of DDGS and suggested that Lys may need to be supplemented in diets containing DDGS to maintain performance. It is necessary to have accurate values for the nutrient composition of feed ingredients to ensure diets are formulated to meet the requirements of the animal (Spiehs et al., 2002).

Energy in DDGS

The energy density of feed is the most expensive aspect within total feed cost and, therefore, accurate quantification of the energy content of a feedstuff is crucial in developing a feeding value (Noblet and Van Milgen, 2013; Graham et al., 2014b). Pedersen et al. (2007) estimated the DE and ME value of DDGS at 4,410 and 3,897 kcal per kg of DM and these estimates are relatively close to the DE and ME of corn (Stein and Shurson, 2009). The chemical composition of DDGS can vary between ethanol plants which is likely the result of differences in processing methods and, therefore, the accuracy of a singular DE or ME value applied to different DDGS sources is limited (Spiehs et al., 2002; Rausch and Belyea, 2006). Anderson et al. (2012) set out to correct the limitation of a singular value and derived models that utilized GE, ether extract, total starch, and organic matter digestibility to estimate DE content of DDGS; only GE and total dietary fiber was required to estimate ME content, but to a less accurate degree than DE. However, these values of energy do not take into consideration how nutrients are metabolically utilized and the differences between the heat increment of nutrients (Noblet et al., 1994). Therefore, the NE is the most accurate energy system and should be used to describe the energy content of feed ingredients (Nitikanchana et al., 2015). Currently the NRC (2012) has categorized DDGS based on oil content into three main groups, including: low (<4% oil), medium (6 to 9% oil), and high oil (>10%). In order to increase the accuracy of DDGS energy content on a NE basis, Graham et al. (2014b) modeled the NE of DDGS with oil contents between 5.4 and 12.1% and concluded that for every 1% change in oil content, the NE of DDGS differs by 115 kcal/kg on an as-fed basis. Continued research on the energy content of DDGS will be required as the ethanol industry continues to improve their production methods and efficiency.

Amino Acids in DDGS

The precise formulation of AA content in a complete diet relies on the accurate estimation of the AA content of feed ingredients (Spiehs et al., 2002). The AA profile of corn DDGS is similar to that of corn itself (Liu, 2011). However, considerable variation in the digestibility of AA exists between different samples of DDGS (Stein et al., 2005; Fastinger and Mahan, 2006). The digestibility of Lys is more variable compared to other AA in DDGS (Pahm et al., 2008). The most likely explanation for the variation in Lys is the occurrence of the Maillard reaction; a reaction that happens in the presence of reducing sugars and heat, such as during the heating process of ethanol production (Mauron, 1981). Additionally, there is variability in the digestibility of the other AA in DDGS. However, the variation is not any more severe as compared to other feed ingredients (Stein et al., 2006). Relative to corn, the digestibility of most AA in DDGS are approximately ten percent lower and this has been suggested to be a result of higher dietary fiber concentrations in DDGS (Stein and Shurson, 2009). Urriola et al. (2009) attempted to develop prediction equations for digestible AA from the concentrations of total AA in corn DDGS. While Van Kempen et al. (2002) was successful in doing this for SBM, Urriola et al. (2009) was unable to derive accurate prediction equations and this was suggested to be a result in the variation in heat damage in DDGS samples. Due to the AA variation between DDGS sources and the inability to accurately derive prediction equations for digestible AA concentrations, nutritionists need to familiarize themselves with specific DDGS sources to ensure accurate feeding values are being used for DDGS in diet formulation (Spiehs et al., 2002).

Phosphorus in DDGS

Phosphorus is considered the third most expensive nutrient in swine diets (Spiehs et al., 2002). Phosphorus concentrations in DDGS range from 0.60 to 0.70% (Stein and Shurson, 2009). Pedersen et al. (2007) observed the apparent total tract digestibility of phosphorus in DDGS to be between 50 and 68% with an average of 59%, which is greater than phosphorus digestibility of corn. This is due to the phosphorus phytate bonds being hydrolyzed during the fermentation process in ethanol production and thus leading to an increase in available phosphorus (Pedersen et al., 2007). As a result, the inclusion of

DDGS in swine diets will lead to a reduction in the amount of supplemental inorganic phosphorus required and, therefore, a potential reduction in feed costs (Stein, 2007).

Growth Performance of Pigs Fed DDGS

The inclusion of DDGS at 20 or 30% in grow-finish diets have led to inconsistent responses in pig performance (Xu et al., 2010). Early reports of feeding DDGS in corn-SBM diets indicated that feeding up to 30% DDGS does not affect the performance of grow-finish pigs (Cook et al., 2005; DeDecker et al., 2005). However, Whitney et al. (2006) demonstrated that increasing the inclusion of DDGS up to 30% led to a linear reduction in ADG and G:F with no impact to ADFI. They concluded that DDGS should not be included in grow-finish swine diets at or above 20% if growth performance was not to be affected (Whitney et al., 2006). The work of Widmer et al. (2008) also indicated the inclusion of 10 or 20% DDGS does not impact growth performance. Linneen et al. (2008) demonstrated that increasing DDGS up to 30% in grow-finish diets linearly decreased ADFI and tended to decrease ADG with no impact on G:F. They were also able to detect a linear decrease in ADG and ADFI as DDGS levels increased up to 20% (Linneen et al., 2008). Ultimately, Linneen et al. (2008) came to the conclusion that DDGS starts to impact the growth rate of pigs after 10 or 15% inclusion level in the diet. The inconsistency in the performance results of these studies is hard to explain, but may have been a result of variation in nutrient composition of DDGS utilized (Stein and Shurson, 2009). During this period of research, the majority of DDGS sources contained approximately 10% oil due to lack of implementation of centrifuge processing technology in the ethanol industry (Graham et al., 2014a). Due to the inconsistencies of prior work and adaption of new technology in the ethanol industry, there is a renewed interest in evaluating the impact of DDGS on the performance of pigs.

Revaluation of DDGS containing a medium concentration of oil (7.6%) indicated that increasing dietary inclusion level of these DDGS linearly decreased ADG and G:F and tended to linearly decrease ADFI (Graham et al., 2014a). The inclusion of low oil DDGS (5.4%) resulted in a linear increase in ADFI and decrease in G:F as DDGS inclusion level increased from 0 to 20 to 40% of the diet, but there was no impact on performance when DDGS contained 9.6% oil (Graham et al., 2014b). In a second study completed by Graham et al. (2014b), results indicated that increasing dietary inclusion of DDGS containing 9.4% oil impacts ADG in a quadratic fashion while there was no impact of increasing 12.1% oil DDGS on ADG. However, regardless of oil concentration, increasing inclusion of DDGS in the diet decreased ADFI and tended to increase G:F in linear fashions in the second experiment (Graham et al., 2014b). This data suggests that when DDGS with lower oil content are fed, pigs respond by increasing ADFI and when DDGS with high oil are fed, pigs respond by decreasing ADFI which is in agreement with how pigs adjust feed intake in accordance with dietary energy level (Beaulieu et al., 2009; Graham et al., 2014b). It has also been demonstrated that the inclusion of fat will result in a linear increase in G:F (De la Llata et al., 2001). When pigs were fed increasing levels of high oil DDGS, G:F tended to linearly increase which is in agreement with the previous statement (Graham et al., 2014b). However, when pigs were fed increasing levels of low oil DDGS and, therefore, also an increase in dietary energy coming from a lipid source vs carbohydrate source, G:F linearly decreased which is not in agreement with pigs response to fat content (Graham et al., 2014b). The discrepancy in this response is likely a result of the low oil DDGS not being as calorically dense as the dietary ingredients it replaced therefore, reducing energy available for growth if similar caloric intakes were unable to be achieved. The increased fiber concentrations contributed by DDGS could have restricted overall caloric intake due to bulk volume of digesta in the intestinal tract (Nyachoti et al., 2004; Avelar et al., 2010).

The variation in the pig responses to increasing medium oil DDGS inclusion levels in diets is harder to explain, but may be a result of dietary CP concentrations (Stein and Shurson, 2009). All of the studies discussed above did not balance for CP when DDGS were included in diets. A response that was consistently observed across DDGS studies was the reduction in carcass yield due to the inclusion DDGS (Cook et al., 2005; Whitney et al., 2006; Linneen et al., 2008; Jacela et al., 2011; Graham et al., 2014a; Graham et al., 2014b). The increase in dietary CP as a result of increased inclusion of DDGS could be a contributing factor to the increase in visceral organ weight and, thus, reduced carcass yields (Linneen et al., 2008; Graham et al., 2014b). However, other studies have indicated that increasing dietary fiber can also led to an increase in organ and intestinal weights (Agyekum et al., 2012; Asmus et al., 2014). It is difficult to quantify the proportion that fiber or CP contribute to changes in organ weights in DDGS diets due to lack of balancing for fiber or CP across dietary treatments in the studies discussed above. Regardless, Pond et al. (1988) suggested that high dietary fiber content can increase basal metabolic rate and the results of Noblet et al. (1987) and Chen et al. (1996) have also indicated that increased dietary CP content can increase metabolic activity. The increase in metabolic rate due to these factors is explained by the large majority of energy required for maintenance being utilized by the visceral organs

(Johnson et al., 1990; Mahr-un-nisa and Feroz, 1999). Therefore, the increase in visceral organ weight due to concentrations of fiber and CP in DDGS diets could lead to an increase in the energy required for maintenance and reduce the energy available for gain (Jacela et al., 2011). It could therefore be suggested that, for pigs fed DDGS to perform similarly to that of a corn-SBM based diets, the differences in net energy required for maintenance must be considered and similar concentrations of net energy available for gain must be provided. However, the ability to correct the profile of AA in DDGS to agree with the ideal protein required by the pig and balance the concentration of AA in relation to the dietary energy would aid in reducing the amount of AA in excess of requirement. This would result in reducing the metabolic energy required to break down and excrete excess AA, leading to an increase in the energy available for gain of DDGS.

The AA concentrations of Lys and Trp are relatively low in corn while the concentrations of Met and Cys are relatively high when compared to the total corn CP concentration (NRC, 2012). Soybean-meal contains relatively high concentrations of Lys and Trp and lower concentrations of Met and Cys in relation to the total CP concentration (NRC, 2012). Therefore, the AA profiles of these two feedstuffs are complementary which aids in providing an AA profile that is balanced and of more biologically value to pigs.

In grow-finish swine diets it is common to utilize DDGS to partially replace corn, SBM, and inorganic phosphorus (Davis et al., 2015). This can result in an imbalance in the AA profile of these diets with respect to the daily AA requirements of the animals due to DDGS having a similar AA profile to that of corn (Liu, 2011). Due to the low concentration of Lys and Trp in DDGS, the increased inclusion of DDGS in diets will require the supplementation of crystalline Lys and Trp to ensure adequate levels are supplied (Stein, 2007). Crystalline AA can be used to correct for deficiencies in the supply of AA by feedstuffs containing similar AA profiles. However, greater concerns may be placed on the inability to reduce the excess amounts of AA supplied by feed ingredients with similar AA profiles due to their potential to reduce ADFI and negatively impact growth performance (Edmonds and Baker, 1987; Li and Patience, 2017). The inclusion of DDGS in swine diets can led to the excess supply of the BCAA, more specifically Leu, due to the high concentrations in corn and DDGS (Cemin et al., 2019). This may result in poor pig performance due to the antagonistic relationship that exists between the BCAA when provided in excess (Harper et al., 1984; Wiltafsky et al., 2010). In conjunction with the increase in the BCAA, dietary levels of Phe and Tyr also increase due to DDGS inclusion resulting in high concentrations of LNAA and low amounts of Trp which has been suggested to impact pig performance (Salyer et al., 2013; Kwon et al., 2019). Increasing the understanding of how to mitigate the negative impacts of excess AA supply on pig performance through the adjustment in other AA levels will provide a better understanding on how to efficiently utilize DDGS in swine diets.

Inclusion of HPDDG in Swine Diets

While conventional DDGS are commonly produced by ethanol plants, some ethanol plants have the capability of producing HPDDG. The major difference in between the production of conventional DDGS and HPDDG is that prior to the fermentation and distilling process, corn grain is dehulled and degermed (NRC, 2012). This is done to increase the efficiency of ethanol production by increasing the concentration of starch through the reducing in unfermentable components such as fiber and fat (Rausch and Belyea, 2006; Rosentrater et al., 2012).

Nutrient Composition of HPDDG

The nutrient composition of HPDDG presented in the NRC (2012) was based on the data presented in three studies conducted by Widmer et al. (2007), Kim et al. (2009), and Jacela et al. (2010). These studies indicated that the concentration of ME ranged from 3,426 to 3,821 kcal per kg which is greater than the ME value of corn and conventional DDGS (Widmer et al., 2007; Kim et al., 2009; Jacela et al., 2010; NRC, 2012). Jacela et al. (2010) was the only one to estimate the NE value of HPDDG using the equation by Noblet et al. (1994) for their two different HPDDG sources and estimated a NE value of 2,131 and 2,256 kcal per kg. These values are lower than corn and slightly lower than traditional DDGS (NRC, 2012). The lower NE value of HPDDG compared to corn and conventional DDGS can be attributed to the low oil concentrations of these HPDDG which ranged from 5.4 to 3.0%, but averaged 3.8% (Widmer et al., 2007; Widmer et al., 2008; Kim et al., 2009; Jacela et al., 2010). The concentration of CP in the HPDDGS used in this period was between 40.8 and 48.2% and the digestibility of AA was similar to traditional DDGS (Widmer et al., 2007; Widmer et al., 2008; Kim et al., 2009; Jacela et al., 2010). The amount of phosphorus in HPDDG is approximately half that of conventional DDGS, but the digestibility of phosphorus is similar between the two feedstuffs (Widmer et al., 2007; Almeida and Stein, 2012). The adoption of new processing technologies in the US ethanol industry had led to the production of HPDDG with different nutrient profiles compared to the one initial reported by the NRC (2012) (Yang et al., 2020). The energy concentration of the new generation HPDDG has been estimated between 4,157 and 3,544 kcal per kg for DE and between 3,271 and 3,698 kcal per kg for ME (Rho et al., 2017; Son et al., 2017; Espinosa and Stein, 2018). Considerable variation between exists between the energy concentrations and this may be a result of different oil concentrations which ranged from 5.2 to 9.3% (Rho et al., 2017; Son et al., 2017; Espinosa and Stein, 2018). Therefore, prediction equations may be required to aid in accurately predicting energy content of HPDDG as Graham et al. (2014b) demonstrated with conventional DDGS. Work by Cemin et al. (2021) estimated the NE value of HPDDGS at 2,600 kcal per kg or 97.3% the energy value of corn for the nursery pig while Rao et al. (2020) estimated the NE value of HPDDG at 103.4% the value of corn or 2,763 kcal per kg for the grow-finish pig through means of caloric efficiency calculations. The CP concentration of the newer generation of HPDDG is usually lower than what is published in the NRC (2012) but are approximately 38% and the digestibility of AA are typically greater than that of DDGS (Adeola and Ragland, 2016; Rho et al., 2017; Son et al., 2017; Espinosa and Stein, 2018). With the variation between the nutrient composition of HPPDG, nutritionists should develop nutrient loadings for specific sources of HPDDG which will ensure accurate diet formulation, similar to that suggest by Spiehs et al. (2002) for DDGS.

Growth Performance of Pigs fed HPDDG

Early reports on the impact of feeding HPDDG to pigs indicated that replacing 100% of the SBM in grow-finish diets with HPDDG did not impact the overall performance of pigs (Widmer et al., 2008). However, Widmer et al. (2008) did observe a linear decrease in ADG, ADFI and a tendency for a linear decrease in G:F during the growing period but not the finishing period as HPDDG replaced SBM at 50 and 100%.

Kim et al. (2009) report the same response as Widmer et al. (2008) and indicated that HPDDG can replace 100% of SBM in grow-finish swine diets. The work by Gutierrez et al. (2014) indicated that 30% HPDDG can be fed in growing or finishing swine diets without impacting growth performance, body composition, or the retention of energy, protein, and lipids by pigs. More recent research on HPDDG has reported feeding up to 30% HPPDG in nursery pig diets will result in a linear decrease in BW, ADG, ADFI, and G:F (Yang et al., 2019). Cemin et al. (2021) observed the same linear decrease in ADG, ADFI, G:F, and final BW in nursery pigs fed HPDDG up to 40% of the diet. Both studies suggested that the decrease in performance was likely due to the increase in dietary fiber concentrations as HPDDG inclusion increased and an imbalance in the BCAA (Yang et al., 2019; Cemin et al., 2021). Yang et al. (2020) showed that feeding 30% HPDDGS during the grow-finish period resulted in a decrease in final BW, ADG, and tended to reduced G:F as compared to a corn-SBM diet, but this may have been a result of inaccurate diet formulations resulting in a less than adequate supply of AA to support growth performance. Most recently, Rao et al. (2020) demonstrated feeding 15 or 30% HPDDG to grow-finish pigs will result in similar performance to that of pigs fed a corn-SBM diet if AA concentrations were adjusted using the prediction equation by Cemin et al. (2019) to account for excess dietary Leu levels. However, a closer look at their performance results indicate that an increase of HPDDG up to 30% linearly decreased ADG and ADFI while linearly improving G:F during the growing period when pigs were between the BW of 27.1 and 75.2 kg, respectively (Rao et al., 2020). This is the same response that Widmer et al. (2008) observed during the growing period, with the exception of G:F, and from the observed performance responses during the nursery periods, it could be suggested that HPDDG inclusion negatively impacts the performance of lighter BW pigs. Continued research with HPDDG in both nursery and grow-finish pigs is required to determine an accurate feeding value for HPDDG in swine diets.

The Amino Acid Tryptophan

Tryptophan (Trp) is an essential amino acid and therefore, must be supplied through the diet because the animal cannot synthesis it (Koopmans et al., 2009). Tryptophan is involved in multiple different biological roles which include: protein synthesis, production of the neuromediator serotonin, and the immune response through the kynurenine pathway (Sainio et al., 1996; Le Floc'h and Seve, 2007). In corn-SBM based swine diets, Trp is typically considered the second or third limiting AA (Burgoon et al., 1992). However, the optimal Trp requirement is quite variable across studies and this may be a result of Trp role in the various biological pathways (Susenbeth, 2006).

Tryptophan and the Kynurenine Pathway

Following the utilization of Trp for protein synthesis, the kynurenine pathway is most important pathway for the metabolism of Trp and it is responsible for over 90% of Trp catabolism (Sainio et al., 1996). In basic description of the kynurenine pathway, Trp is broken down into kynurenine by either Trp dioxygenase located in the liver or indoleamine dioxygenase expressed by immune cells and tissues targeted by inflammation (Le Floc'h and Seve, 2007). Kynurenine can then be converted to kynurenic acid, an antagonist at glutamate receptors, quinolinic acid which is a glutamate agonist, further degraded to create ATP and carbon dioxide or be a precursor to NAD and NADP production (Moroni et al., 1990; Moffett and Namboodiri, 2003; Bryleva and Brundin, 2017). When under an inflammatory state, indoleamine dioxygenase is up regulated and

Trp dioxygenase is down regulated (Maes et al., 2007). This reduces Trp available for other functions such as protein synthesis and, therefore, potentially changes the Trp requirement when the immune system is stimulated in pigs (Le Floc'h and Seve, 2007). Le Floc'h et al. (2009) indicated that housing pigs in unsanitary conditions would result in poor pig performance because of inflammation reducing concentration of plasma Trp to be utilized for protein synthesis. More recently, De Ridder et al. (2012) found that the Trp requirement would increase seven percent in pigs under immune stimulation due to a lower efficiency of Trp utilization for protein deposition. Therefore, when targeting a certain supply of Trp in swine diets, the health status and sanitary conditions of the barn should be considered.

Tryptophan and Serotonin

The AA Trp also serves as the precursor to the production of serotonin, a neurotransmitter that is associated with the stress and feed intake response (Fernstrom, 1985; Adeola and Ball, 1992). The production of serotonin occurs mainly within the gut and, to a lesser extent, in the brain and platelets (Mohammad-Zadeh et al., 2008; Jenkins et al., 2016). The amount of Trp used for the production of serotonin is very low and estimated to be less than ten percent of the Trp catabolized (Le Floc'h and Seve, 2007). Wolf (1974) suggested the total dietary Trp consumed used to produce serotonin is approximately one percent. The synthesis of serotonin from Trp occurs in two enzymatic steps. First Trp is hydroxylated to 5-hydroxytryptophan by tryptophan hydroxylase and this enzyme is considered the rate limiting step (Mohammad-Zadeh et al., 2008). The second step is the decarboxylation of 5-hydroxytryptophan by L-aromatic amino acid decarboxylase to form 5-hydroxytryptamine (serotonin) and these two steps occur almost instantaneously in the presences of Trp (Clark et al., 1954). The synthesis and storage of serotonin in the brain occurs in the presynaptic neurons (Mohammad-Zadeh et al., 2008). Brain serotonin levels are dependent on the availability of tryptophan, due to the inability of serotonin to cross the blood brain barrier (Salyer et al., 2013). Therefore, the availability of Trp in the brain would be considered the rate limiting factor for hypothalamic serotonin synthesis prior to the enzyme Trp hydroxylase (Meunier-Salaün et al., 1991). Tryptophan competes with other LNAA to be transported across the blood brain barrier because they share the same competitive AA transporter (Pardridge, 1977; Fernstrom, 2005). More specifically the AA transporter at the blood brain barrier is the L-type amino acid transporter 1 (LAT1) which is primary expressed in the brain, placenta, and tumors (del Amo et al., 2008). The LAT1 is an AA transporter that is Na^+ independent that transports one AA out of the cell in exchange for another AA into the cell (Verrey, 2003; del Amo et al., 2008). Fernstrom and Wurtman (1972) correlated the concentration of brain Trp and the plasma ratio of Trp to the five competing LNAA Phe, Leu, Ile, Val, and Tyr with a high degree of accuracy. Therefore, the concentration of brain Trp levels will increase when either plasma levels of Trp increase or plasma levels of LNAA decrease (Fernstrom, 1985). Adeola and Ball (1992) indicated that concentrations of hypothermic serotonin increased when there was a large excess of dietary Trp and Henry et al. (1996) showed that serotonin production was impaired when there was a deficiency in Trp. Recently, Kwon et al. (2019) revealed that the excess of dietary Leu resulted in a linear decrease in hypothalamic serotonin and quadratic decrease in plasma serotonin. Due to the association between serotonin and animal behavior, proper dietary Trp supply is necessary to ensure adequate synthesis of serotonin.

Serotonin is associated with various behavioral and physiological processes such as the stress response, regulation of mood and feed intake along with behavioral changes (Cortamira et al., 1991; Lepage et al., 2005; Zhang et al., 2007; Poletto et al., 2010; Shen et al., 2012). Hypothalamic serotonin can aid in the stress response by reducing the secretion of stress hormones, such as cortisol and noradrenaline (Adeola et al., 1993; Lepage et al., 2003; Koopmans et al., 2005). Stress hormones in general are considered to be antagonistic to insulin and can stimulate catabolic pathways including glycogenolysis, lipolysis, and specific to cortisol, induce proteolysis (Bratusch-Marrain, 1983; Simmons et al., 1984; Strack et al., 1995; Ruzzin et al., 2005). Therefore, during periods of stress such as weaning or social mixing, the optimal Trp requirement may be increased (Le Floc'h et al., 2011). Koopmans et al. (2005) indicated that increasing dietary concentrations of Trp was helpful in lowering stress in pigs. More recent research has shown that the increased supplementation of Trp and/or the reduction of dietary concentrations of LNAA resulted in an improvement in ADG and feed efficiency of pigs under stress (Shen et al., 2012; Shen et al., 2015). A diet deficient in Trp has also been shown to negatively impact feed intake and therefore, growth performance (Henry et al., 1992; Henry et al., 1996; Eder et al., 2001). Henry et al. (1992) also showed that CP levels affected voluntary feed intake and concluded that a Trp to LNAA imbalance likely explained the lower concentrations of serotonin and, thus, resulting in a reduction in feed intake. Diets rich in carbohydrates have also been indicated to increase brain concentrations of serotonin because of the insulin response and clearing of plasma concentration of other LNAA competing with Trp transport into the brain (Fernstrom and Wurtman, 1971). However, the relationship between serotonin and appetite is still controversial due to serotonin usually being considered a mediator of satiety (Le Floc'h and Seve, 2007). The serotonin receptor 5-HT$_1$ is the primary receptor associate with feed intake, is negatively coupled with adenylyl cyclase, and, therefore, downregulates cyclic AMP when activated (Mohammad-Zadeh et al., 2008). It has been demonstrated that a central injection of a cyclic AMP analog results in the increase of neuropeptide Y protein levels in the arcuate nucleus (Akabayashi et al., 1994). The increase in the expression of neuropeptide Y promotes feeding, decreases energy expenditure, and silences neurons expressing proopiomelanocortin that promote satiety (Gao and Horvath, 2007). Therefore, the activation of 5-HT$_1$ by serotonin would downregulate cyclic AMP resulting in the down regulation of neuropeptide Y expression, increased expression of proopiomelanocortin leading to the promotion of satiety. This is not to say Trp is not involved in the feed intake response, rather Trp my promote feed intake through a different mechanism. Zhang et al. (2007) demonstrated that in pigs, oral ingestion of Trp lead to an increase in plasma ghrelin levels and an increase in ghrelin mRNA expression. Ghrelin, an orexigenic hormone produced in the stomach, up regulates the appetite stimulating neurons that express neuropeptide Y and, simultaneously, reduce the activity of neurons expressing proopiomelanocortin leading to the promotion of feed intake (Chen et al., 2004). While the mechanism by which Trp regulates feed intake is not clear, the consistent response of increased ADFI in pigs due to increased dietary Trp levels cannot be ignored. Overall, Trp is a unique AA due its functional diversity and the Trp requirement may vary in accordance with the targeted biological function being optimized (Le Floc'h and Seve, 2007).

Tryptophan Requirement in Pigs

Susenbeth (2006) conducted a meta-analysis on a total of 33 studies up till 2005 and concluded that an optimal Trp:Lys ratio for growing pigs was 17.4%. This is in agreement with the current NRC (2012) estimate of 17.6% SID Trp:Lys for the grow-finish pig. Nørgaard et al. (2015) studied the Trp requirement in the seven to 14 kg pig and indicated that increasing dietary Trp will result in a quadratic increase in ADG, ADFI, and G:F with optimal performance occurring at a SID Trp:Lys ratio of 18 and 20% from the broken-line and curvilinear-plateau models. Results from the first experiment of Gonçalves et al. (2015) showed ADG and G:F was maximized at 23.9 and 20.4% SID Trp:Lys for the six to ten kg pig. The second experiment of Gonçalves et al. (2015) for the 11 to 20 kg pig indicated that maximum ADG was achieved at 21.2% SID Trp:Lys while maximum G:F was achieved at 16.6 to 17.1% depending on which response model is utilized. In the late nursery pig from 15 to 30 kg, the optimal SID Trp:Lys was estimated at 17.5% for G:F by Pasquetti et al. (2015) but they were unable to define the break point for ADG due to the linear response of the parameter. In all of the studies above, the optimal SID Trp:Lys for G:F occurred before the optimal SID Trp:Lys ratio for ADG and this was due to the linear or quadratic ADFI response observed in response to increasing dietary Trp levels (Gonçalves et al., 2015; Nørgaard et al., 2015; Pasquetti et al., 2015). In growing pigs between 25 and 50 kg fed low CP diets, optimal SID Trp:Lys for growth performance was estimated at 20 and 23% from broken-line and curvilinear-plateau analyses (Zhang et al., 2012). Kendall et al. (2007) reported that the TID Trp:Lys requirement was at least 14.5% but less than 17% for the 90 to 125 kg pig. While Xie et al. (2014) indicated that for the finishing barrow and optimal SID Trp:Lys ratio for ADG and G:F was 20.3 and 19.7% from the broken-line model or 25.1 and 22.4% when using the quadratic polynomial model. Fewer studies have looked at the Trp requirement in diets containing DDGS. Early reports suggested that if DDGS are included at 30% of the diet, a minimum Trp:Lys ratio of 16% was necessary for growing-finishing pigs when using ADG as the response criteria (Hinson et al., 2010). Results from Salyer et al. (2013) indicated that 16.5% SID Trp:Lys was required in growing diets containing 30% DDGS but finishing pigs required at least 19.5% SID Trp:Lys. It should be pointed out that Hinson et al. (2010) supplied Trp in the form of crystalline L-Trp while Salyer et al. (2013) provided Trp through the inclusion of SBM. Salyer et al. (2013) did show in their second study that supplying a SID Trp:Lys ratio of 18% either through crystalline L-Trp or SBM resulted in similar performance. Gonçalves et al. (2018a) fed growing-finishing gilts 30% DDGS and determined the optimal SID Trp:Lys to be 16.9% for G:F and 23.5% for the ADG response. Most recently, Sespere Faria Oliveira et al. (2021) indicated that when feeding 35% DDGS in growing pig diets, the optimal SID Trp:Lys for ADG was 20.9 and 23.4% while optimal SID Trp:Lys for G:F was 18.7 and 20.2% from the broken-line and curvilinear-plateau models, respectively. This data indicates that Trp requirement may be greater then what the NRC (2012) currently recommends when DDGS are included in the diet. This could be explained due to the increase in the LNAA concentrations in these diets. However, due to the variation that exists in the nutrient composition of DDGS, modeling the response of Trp in DDGS in relation to the LNAA concentration may allow for greater applicability to swine producers.

The Branched Chain Amino Acids

The BCAA cannot be synthesized by animals and therefore, they are considered essential AA and must be supplied through dietary means to support growth and good health (Harris et al., 2004). The BCAA are comprised of Leu, Val, and Ile and this is because to the structurally similar side chains these AA share (Harper et al., 1984). As a result of this, all three BCAA share the first two enzymes of their catabolic pathway (Harris et al., 2005). In addition to their use for protein synthesis, the BCAA are important nutrient signals that are involved in the regulation of BW, protein synthesis, glucose homeostasis, and nutrient-sensitive signaling pathways such as mTOR (Jewell et al., 2013; Lynch and Adams, 2014).BCAA transport

Prior to AA being utilized for protein synthesis, they must be absorbed and transported to their desired location which required certain AA transporters. The large majority of dietary protein is absorbed as of di- and tripeptides by the peptide transporter PEPT1 located on the apical membrane of intestinal enterocytes (Daniel, 2004). However, free AA are also transported across the apical membrane of intestinal enterocytes by specific AA transporters. The transporter B^0AT1 is a major transporter of BCAA and other neutral AA in the intestine (Bröer et al., 2004). This AA transporter is a part of the B^0 system and depends on Na^+ to transport AA across the apical membrane of intestinal enterocytes via a symport manner (Bröer, 2008). Neutral AA and cationic AA also share a common AA transporter named $ATB^{0,+}$, for symport transport across the apical membrane through a Na^+ and Cl^- dependent mechanism (Bröer, 2008), However, this transporter has a greater affinity for neutral AA compared to cationic AA (Sloan and Mager, 1999). The rBAT/$b^{0,+}$AT transporter is another AA transporter on the apical membrane but this AA transporter transports cationic AA in exchange for neutral AA which always for redistribution of individual AA without affecting total pool size (Bröer, 2008). Fewer studies have analyzed the AA transporters across the basolateral membrane of intestinal enterocytes (Bröer, 2008). The L-system AA transporter LAT2 with the 4F2 heavy chain is found on the basolateral membrane and is responsible for antiport transport of neutral AA (Bröer, 2008). Both 4f2hc/y^+LAT1 and 4f2hc/y^+LAT2 are part of the y^+L AA transport system and are responsible for the transport of cationic AA across the basolateral membrane in exchange for Na^+ and neutral AA (Bröer, 2008). Understanding the transport of AA at the enterocyte level is important in understanding the efflux of AA in the plasma. It has been demonstrated that the L system transporters, LAT1 and LAT2, facilitate the transport of BCAA along with other neutral AA into skeletal muscle (Hamdi and Mutungi, 2011; Drummond et al., 2012). The difference between the two transporters is that LAT1 mainly transports LNAA while the LAT2 transports both LNAA and small neutral AA (del Amo et al., 2008). These transporters are Na^+ independent and transport one AA in exchange for another; however, the net direction of AA transport is suggested to depend on unidirectional transporters that are co-expressed in the cell (del Amo et al., 2008). Suryawan et al. (2013) gave an example of glutamine transporters, such as System A transporter (SNAT2) and the System N transporter (SNAT3), being used to maintain a glutamine gradient across the plasma membrane and thus allowing for intracellular transport of neutral AA via LAT1 and or LAT2 in exchange for glutamine. Therefore, it has been suggested that the function of LAT1 an LAT2 is to maintain an equilibrium between intracellular and extracellular AA concentrations (Meier et al., 2002). The transport of AA is complex and has yet to be fully described, but understanding these AA transporters could provide some clarification on the efficiency of AA utilization.

BCAA Metabolism

In general, the catabolism of AA can be classified into two groups based on the pathway at which its carbon skeleton is degraded (D’mello, 2003). The AA that are degraded incompletely or completely, directly or indirectly to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, or oxaloacetate net glucoses and are termed glucogenic or glycogenic (D'Andrea, 2000). The carbon skeleton from AA broken down completely or incompletely, directly or indirectly to acetyl-CoA, or acetoacetate give rise to ketone bodies and are termed ketogenic (D'Andrea, 2000). Valine is glucogenic because its catabolism yields succinyl-CoA while Leu catabolism yields acetoacetate and acetyl-CoA, therefore is ketogenic (Harper et al., 1984). Isoleucine is both ketogenic and glucogenic because the end products of its catabolism is propionyl-CoA and acetyl-CoA (Harper et al., 1984).

The metabolism of BCAA is unique because all three of the BCAA share the first two initial enzymes of their catabolic pathway (Harris et al., 2005). The first step in BCAA catabolism is catalyzed by branched chain aminotransferase (BCAT) (Harris et al., 2005). During this step the amino group of BCAA are transferred to α-ketoglutarate to form branched chain keto acids (BCKA) and glutamate (Harris et al., 2005). The α-keto acids for Leu, Val, and Ile are α-keto isocaproate (KIC), α-keto isovalerate (KIV), and α-keto-β-methylvalerate (KMV) respectively (Harper et al., 1984). The enzyme BCAT occurs in both the cytosol and the mitochondria (Harper et al., 1984). The concentration of mitochondrial based BCAT is high in the skeletal muscle and other organs except for the liver while the cytosol based BCAT is primarily found in the nervous system, ovary, placenta, and mammary tissue (Hutson et al., 2005; Li et al., 2009). It is important to understand the localization of BCAT because it determines the sources of α-ketoglutarate utilized as a nitrogen acceptor. In the mitochondria, BCAT utilizes the TCA cycle intermediate α-ketoglutarate while in the cytosol BCAT utilizes the α-ketoglutarate derived from the conversion of pyruvate to alanine. There is no mechanism for the regulation of BCAT, but rather it has been suggested that the concentration of substrates directly regulate the activity of BCAT (Harper et al., 1984; Wiltafsky et al., 2010). While there is basis for this, it could be further proposed that the active of BCAT is to increase intracellular concentrations of glutamate that will be converted to glutamine via glutamine synthetase. This would in turn allow for greater transport of LNAA into the cell through the transport systems previously described and, therefore, allow for redistribution of intracellular AA concentrations to better fit those needed to support cellular function. This theory would be partially supported by the previous research indicating that excess Leu does not impact cellular uptake of Ile and Val even though similar AA transporters are utilized (Langer et al., 2000).

The second catabolic step is the decarboxylation of the carboxyl groups of the BCAA α-ketoacid and, therefore, producing branched chain acyl-CoA esters specific to each BCAA (Harris et al., 2005). The enzyme response for this step is branched chain α-ketoacid dehydrogenase complex (BCKDC) and, unlike BCAT, the enzyme BCKDC is highly regulated and irreversible (Harper et al., 1984). The phosphorylation of the BCKDC E1 subunit by branched chain kinase (BCK) results in the inactivation of the complex while dephosphorylation of the E1 unit by branched chain phosphatase (BDP) results in its activation (Harris et al., 2005). The activity of BCK is inhibited by KIC, the α-ketoacid of Leu, and thus results in the activation of BCKDC due to BDP activity (Harris et al., 2005). However, the other BCAA ketoacids also allosterically inhibit BCK but are less effective than the inhibition by KIC (Brosnan and Brosnan, 2006). It has been demonstrated that excess Leu and/or KIC have shown to reduced plasma concentration of the other BCAA α-ketoacids (Crowell et al., 1990; Langer et al., 2000; Wiltafsky et al., 2010; Kwon et al., 2020). The BCDKC concentration and activity is highest in the liver and lowest in the skeletal muscle (Harper et al., 1984). This, in combination with low concentrations of BCAT in the liver, result in the majority of ingested BCAA to bypass initial metabolism at the liver and pass into the systemic circulation after a meal (Platell et al., 2000). The uniqueness of the metabolism of BCAA allows for these AA to act as nutrient signals and play a role in other important physiological functions in addition to their utilization for protein synthesis.

Valine Requirement in Pigs

Valine has generally been considered the fifth limiting AA in corn-SBM based swine diets (Figueroa et al., 2003). The majority of the research on the Val requirement has been conducted on nursery pigs. Currently, the NRC (2012) estimated the Val requirement of the 25 to 100 kg pig at 65% relative to Lys on a SID basis. While the requirement for the 100 to 130 kg is suggested to be 67% SID Val:Lys (NRC, 2012). Early estimates of the Val requirement by Chung and Baker (1992) suggested a 68% Val:Lys requirement for the nursery pig and this is in agreement with more recent research (Wiltafsky et al., 2009b; Xu et al., 2018). Several studies have suggested a slightly higher Val:Lys requirement of 70 to 71% for optimal ADG of nursery pigs

(Barea et al., 2009a; Soumeh et al., 2015a). Gaines et al. (2011) suggest the Val:Lys requirement of the 13 to 32 kg pig was 64 and 65% for ADG and G:F. Clark et al. (2017) estimated the optimal Val:Lys ratio at 63, 72 and 74% for ADG, G:F, and ADFI for the nursery pig. Gonçalves et al. (2018b) indicated that suppling a SID Val:Lys ratio of 68% would achieve more than 99% of the maximum ADG response and estimated maximum G:F at 69% SID Val:Lys. This would agree with the meta-analysis conducted by Van Milgen et al. (2013) suggesting an optimal SID Val:Lys ratio of 69% and of other studies looking at the Val requirement (Waguespack et al., 2012; Liu et al., 2015). Overall, the research on the Val requirement has been fairly consistent with an estimated requirement for pigs around 68% SID Val:Lys ratio and the data above indicated that the optimal Val to Lys ratio is not fluctuating with increases in BW. However, dietary levels of Leu might have an influence on the Val requirement due to the BCAA antagonism described above. It has been demonstrated that Val deficiency results in a dramatic decrease in ADFI and this response is exacerbated when dietary Leu is in excess (Gloaguen et al., 2010; Gloaguen et al., 2011; Gloaguen et al., 2012). More recently, Cemin et al. (2019) developed a predication equation that indicated the inclusion of Val, Ile, and Trp has the potential to mitigate the negative effects of excess dietary concentrations of Leu on pig performance. A study to validate this prediction equation was recently conducted and suggested that high inclusion levels of Val, around 76 to 78% SID Val:Lys, can reduce the negative impacts of excess dietary Leu during the growing period (Kerkaert et al., 2021). This could be a result of an increase in the efficiency of AA utilization as described by the work of Kwon et al. (2020).

Functions of Valine Beyond a Substrate for Protein Synthesis

The functions of Val beyond that of protein synthesis have not been clearly defined as compared to that of Leu. Some research has indicated that the catabolites of Val act as signaling molecules (Neinast et al., 2019). The Val catabolite 3-hydroxyisobutyrate (3-HIB) has been suggested to induce fatty acid transport into skeletal muscle while the Val catabolite beta-amino-isobutyric acid (BAIBA) promotes osteocyte survival, hepatic β oxidation, and adipocyte thermogenesis (Neinast et al., 2019). However, there is still much to learn about the functions Val beyond protein synthesis and the functions of Val catabolites.

Isoleucine Requirement in Pigs:

The inclusion of blood products, such as spray-dried blood cells, in swine diets led to the research on the Ile requirement. This was due to the AA imbalances caused by lower concentrations of Ile in these products compared to that of Leu, Val, and Lys (NRC, 1998). Current NRC (2012) recommendations on the Ile:Lys requirement are 51% for the nursery pigs, 53% for growing pigs, and 54% for finishing pigs. The published literature on the optimal Ile:Lys ratio is quite variable and could be potentially explained by studies being conducted with or without spray-dried blood cells. Kerr et al. (2004) showed the feeding spray-dried blood cells above 2.5% of the diet in nursery pigs negatively impacts growth performance unless diets were supplemented with crystalline Ile at a ratio of 66% relative to Lys. However, their experimental approach did not allow for an estimate of the optimal Ile:Lys ratio, but rather it was less than 66% in diets with spray-dried blood cells. Barea et al. (2009b) conducted three experiments on the Ile:Lys requirement in nursery pigs and suggested that the optimal Ile:Lys ratio was less than

50% in diets containing either corn gluten meal or spray-dried blood cells, but less than 48% when these feedstuffs were not included in the diet. Wiltafsky et al. (2009a) showed that the Ile:Lys requirement increased from 54 to 59% when dietary Leu concentrations were in excess (110 vs 160% Leu:Lys) as a result of the inclusion of spray-dried blood cells. These results are in strong agreement with the recent work of Htoo et al. (2017) that indicated increasing the Leu:Lys ratio from 110 to 160% resulted in the Ile:Lys ratio to increase from 54 to 58% for the nursery pig. Research on the late nursery, early growing pigs has indicated that the Ile:Lys requirement is between 52 and 54% when dietary Leu concentrations are not in excess (Waguespack et al., 2012; Htoo et al., 2014). Interestingly, Parr et al. (2003) suggested that when feeding 7.5% spray-dried blood cells to pigs between 25 and 47 kgs, the Ile:lys requirement was only 55% even when there was a Leu:Lys ratio of 187%. Parr et al. (2004) also indicated that the Ile requirement in late finishing pigs was 31% TID Ile while the work by Kendall et al. (2004) and Dean et al. (2005) suggested that 36% TID Ile was the optimal requirement in late finishing. Most recently, Zier-Rush et al. (2018) indicated that the SID Ile:Lys requirement for the late finishing pigs is approximately 60 to 61%. This would agree with the empirical estimates of 60 to 62% for the 90 kg barrow by Kendall (2004) when converted to a SID basis. The research discussed above suggests that the Ile requirement must be adjusted when dietary Leu concentrations are in excess (i.e. >130% Leu:Lys) and might need to be adjusted as the BW of pigs increase. A study by Kerkaert et al. (2021) was conducted to validate the BCAA / LNAA prediction equation derived by Cemin et al. (2019) and results from their study indicated that, in late finishing, the excess dietary Leu concentrations negative impacts on growth performance can be overcame by supplying a dietary SID Ile:Lys ratio of 66 to 68%. However, research is required to accurately describe the optimal Ile:Lys ratio in late finishing when dietary Leu is in excess due to the inclusion of DDGS rather than spray-dried blood cells.

Functions of Isoleucine Beyond a Substrate for Protein Synthesis

The BCAA have been demonstrated to be involved in glucose metabolism through enhancing glucose consumption and utilization (Doi et al., 2005). Work by Doi et al. (2003) demonstrated that in C_2C_{12} myotubes, Leu and Ile stimulate glucose uptake in an insulin-independent manner and that the impact of isoleucine was greater than that of leucine. In their study, Ile was suggested to increase cellular glucose uptake by increasing the activity of phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC), but not mTOR (Doi et al., 2003). Isoleucine also was shown to decrease the activity of 5’-AMP-activated protein kinase (AMPK) α2 which was suggested to be a result of an increase in cellular concentrations of ATP, therefore, decreasing the AMP:ATP ratio leading to a reduction in AMPK activity (Doi et al., 2005). This would suggest that the increase in glucose consumption was not due to an AMPK mediated mechanism (Doi et al., 2005). Zhang et al. (2016) indicated that a deficiency in Ile in the weaned pig down regulated the protein expression of GLUT1 in red muscle and GLUT4 in red, white, and intermediate muscles. They also indicated that a deficiency Ile resulted in the down regulation of intestinal glucose transporter SGLT-1 and GLUT2 protein expression (Zhang et al., 2016). This research indicates that Ile is involved in glucose consumption and utilization; however, the mechanisms through which Ile works is not yet clear.

Leucine Requirement in Pigs

Research on the optimal Leu requirement in pigs is very limited and this is likely a result of Leu generally being the AA in greatest concentrations in feed ingredients. Early estimates of the Leu requirement suggested that a Leu:Lys ratio of 100% was ideal in the young pig (Chung and Baker, 1992). Augspurger and Baker (2004) also indicated that the ideal ratio of Leu:Lys was one for one in pigs from 10 to 20 kg. More recently, Gloaguen et al. (2013) estimated the optimal SID Leu:Lys ratio in 11 to 22 kg pig was 102% for growth performance. Soumeh et al. (2015b) indicated that growth was maximized at 93% SID Leu:Lys, maximal G:F was achieved at 80% SID Leu:Lys, but lowest plasma AA concentrations were achieved at 90 to 100% and lowest PUN tended to occur at 100% SID Leu:Lys in the 8 to 12 kg pig. Wessels et al. (2016) indicated that the optimal SID Leu:Lys for growth performance ranged from 95 to 108% depending on the statistical model utilized to estimate the requirement. However, the model that best describes the dose response was the quadratic polynomial and the optimal SID Leu:Lys was estimated at 108% for the 10 to 28 kg pig (Wessels et al., 2016). Currently, the NRC (2012) estimates the SID Leu:Lys requirement at 100 and 101% for the nursery and grow-finish pig.

Functions of Leucine Beyond a Substrate for Protein Synthesis

While research on the Leu requirement in pigs is limited, the research on Leu functions beyond protein synthesis is more extensive. Leucine is a potent activator of mTOR complex one activity and this complex is involved in numerous cellular processes, most notably protein synthesis and cellular growth (Neinast et al., 2019). The mTOR complex one promotes the activation of many anabolic processes including protein, lipid, and organelles synthesis and limits the activity of catabolic processes such as autophagy, therefore, increasing cell growth and proliferation (Laplante and Sabatini, 2009). Leucine but not Val, Ile, or the α-ketoacids of BCAA, stimulate the activation of mTOR complex one by directly binding to sestrin2, a negative regulator of mTOR complex one (Wolfson et al., 2016). Sestrin2 binds to GAP activity toward the Rag GTPases 2 (GATOR2), a positive regulator of mTOR complex one activity, when Leu is absent (Neinast et al., 2019). However, when Leu is at physiological concentrations, sestrin2 releases GATOR2 leading to the activation of mTOR complex one (Saxton et al., 2016). Upon activation of mTOR complex one, various downstream effectors of mTOR complex one are phosphorylated leading to their activation (Laplante and Sabatini, 2009). Leucine has been demonstrated to activate mTOR complex one in the hypothalamus leading to a decreased feed intake of rats in a similar manner as leptin (Cota et al., 2006). Leucine also plays a role in the glucose metabolism. The increase of Leu above that of physiological concentrations has been demonstrated to have a dose-related impact on insulin secretion (Platell et al., 2000). This effect has been suggested to be regulated through Leu acting in the islet cells as a substrate for energy and an allosteric activator of glutamate dehydrogenase (Sener and Malaisse, 1981). The increase in the deamination of glutamate to α-ketoglutarate and its entry into the TCA cycle, lead to the production of ATP, inhibition of KATP channels, depolarization of the plasma membrane, and vesicular release of insulin (Sener and Malaisse, 1980; Gao et al., 1999; Wilson et al., 2018). An additive effect on insulin secretion had been suggested due to the infusion of both Leu and glucose (Platell et al., 2000). Leucine has also demonstrated to increase glucose uptake into muscle cells of rats through the up regulating the translocation of

GLUT4 and GLUT1 to the plasma membrane (Nishitani et al., 2005). However, the mechanism through which Leu regulates translocation of glucose transporters is not clear. Leucine also has been shown to be involved with the postprandial rise in plasma leptin, however, this AA is only responsible for a part of the increase in leptin after a meal (Lynch et al., 2006). In the study by Lynch et al. (2006), a Leu deficient diet resulted in a 40% decrease in leptin secretion and this reduction was not further reduced when other AA were removed, therefore suggesting Leu regulated most of the dietary AA impact on leptin secretions. In the neonatal pig, the infusion of either insulin or AA increased protein synthesis in the skeletal muscle but the combination of both did not have an additive effect (Davis et al., 2002). Their lab later indicated that the increased protein synthesis in the skeletal muscle of the neonatal pig by AA infusion was due to the activation of mTOR complex one by Leu (Suryawan et al., 2008). More recently, they have demonstrated that supplemental Leu increased the activation of mTOR complex one in the skeletal muscle of neonatal pigs, but this did not lead to an increase in protein synthesis when protein or energy is restricted (Manjarín et al., 2016). Furthermore, they suggested that protein synthesis was limited due to an insufficient supply of some AA and the lack of energy may have resulted in an increase in catabolism of AA for energy utilization (Manjarín et al., 2016). The functional roles of Leu beyond a substrate for protein synthesis are extensive and have yet to be fully described, but have important impacts on the physiological development of pigs.

Summary

The inclusion of DDGS in swine diets causes two issues in swine production with regards to dietary AA concentrations. Firstly, the inclusion of DDGS in a corn-SBM based diet causes two similar AA profiles to make up a larger portion of the dietary CP. This leads to an imbalance in the dietary AA profile as a result of increased concentrations of LNAA. While the inclusion of crystalline AA can be used to rebalance for the AA not supplied by corn protein sources, of more notable concern is the excess of other AA. As dietary corn protein concentrations increase, the LNAA increase, most specifically Leu. Simultaneously, the concentrations of Leu increase at a faster rate compared to that of Val and Ile. This leads to a greater differentiation between Leu and Val along with Leu and Ile, but the differentiation between Leu and Ile occurs at a faster degree than that of Leu and Val. This becomes a concern due to the antagonistic relationship between the BCAA and an increase in the Val and or Ile dietary inclusion may be needed to maintain adequate growth performance. The increase in LNAA concentrations also may require that dietary Trp levels be adjusted to meet the demand for various biological functions due to a shared AA transporter between Trp and the other LNAA.

The second issue with the inclusion of DDGS is that, in general, the dietary concentration of energy decreases due to the lower caloric density of DDGS compared to the feedstuffs replaced. Therefore, to ensure that dietary AA are not supplied in excess of what the animal can utilize, the Lys concentration of the diet is decreased to account for an increase in feed intake and to maintain a constant Lys to calorie ratio. This in turn further exacerbates the increase in LNAA and differentiation between BCAA. While adjustments in dietary Trp, Val, and Ile levels relative to Lys may be required to maintain performance, other factors may play a role in the efficiency of utilization of these AA. The similarity amongst AA transporters and the functions of other non-essential AA, specifically glutamine and glutamate, in AA transport and BCAA metabolism may provide some explanation on this matter. Furthermore, the functions of these AA beyond a substrate for protein synthesis may provide some insight on why adjustments in these AA may be required in pigs at different physiological states. Continual research on DDGS and the AA Trp, Val, Ile, and Leu will be required to further understand the BCAA interaction and how to best utilize the feed ingredient DDGS.

CHAPTER 2

PERFORMANCE RESPONSE OF THE GROW-FINISH PIG FED DIFFERENT LEVELS OF TRYPTOPHAN:LYSINE IN DIETS CONTAINING 40% DRY DISTILLER GRAINS WITH SOLUBLES

ABSTRACT

A total of 1,170 pigs (PIC 800 x PIC, initial BW 38.6 kg) were used in a 98-d grow-finish study to determine the performance response of pigs fed increasing levels Trp:Lys in 40% DDGS diets. Pigs were fed one of four diets containing 40% DDGS with a Trp:Lys ratio of 15, 18, 21, or 24% or a diet being comprised of corn and SBM. Each dietary treatment was replicated nine times and pens contained 26 pigs with equal number of gilts and barrows. Data was analyzed as a randomized complete block design with previous nursery treatment as a random blocking factor. Pair-wise comparisons were used to evaluate dietary treatments impact on performance and carcass traits. Single degree of freedom orthogonal polynomials were used to evaluated dose response of SID Trp:Lys ratio in 40% DDGS diets. Increasing the SID Trp:Lys ratio in diets containing 40% DDGS increased (Linear, $P \leq 0.023$) ADG, ADFI, final BW, hot carcass weight, carcass gain, and standardized fat free lean weight. However, pigs fed the corn-SBM diet had greater ADG ($P<0.008$) and heavier ($P<0.002$) final BW compared to pig fed diets containing 40% DDGS. Diets that contained 40% DDGS with a SID Trp:Lys ratio of 24% had similar ($P=0.253$) ADFI compared to corn-SBM dietary treatment. Pigs receiving the corn-SBM diet also had heavier HCW, standardized fat free lean weights, greater carcass yields, carcass gain, and increased loin depths ($P<0.001$) compared to diets containing 40% DDGS. There was no interaction ($P \geq 0.151$) between dietary SID Trp:Lys ratio and starting BW classification on the growth performance of pigs fed 40%

DDGS. In conclusion, increasing the SID Trp:Lys ratio in 40% DDGS diets improved ADG and ADFI until pigs reached approximately 99 and 115 kgs; however, growth performance of pigs fed 40% DDGS was worse compared to pigs receiving a corn-SBM diet

INTRODUCTION

Byproducts from the ethanol industry such as DDGS are commonly used in commercial swine diets to replace portions of corn and SBM when economical. Previous research has shown that feeding up to 30% DDGS can result in linear reductions in ADG (Cromwell et al., 1993; Fu et al., 2004; Whitney et al., 2006). Work by Linneen et al. (2008) showed that feeding 15% DDGS resulted in no difference in ADG, ADFI, or G:F compared to a standard corn-SBM diet. Multiple studies have confirmed that DDGS can be included in swine diets up to 20% without negatively impacting ADG, ADFI, and G:F during the growing and finishing periods, provided that diets were adequately supplied with AA (Augspurger et al., 2008; Drescher et al., 2008; Widmer et al., 2008; Duttlinger et al., 2012). The negative impacts of feeding DDGS above 20% have yet to be fully understood, but factors such as the presence of mycotoxins, the fibrous components of DDGS, and potential AA imbalances are all reasonable to consider.

In order to determine how to economically use alternative feedstuffs, it is crucial to understand their nutritional value. The concentration of Trp in corn byproducts is low and, hence, the inclusion of DDGS in a corn-based diet can result in Trp becoming the second limiting AA (Stein, 2007). The high concentration of dietary corn protein can also lead to high concentrations of other LNAA. The LNAA are comprised of Val, Ile, Leu, Trp, Tyr, and Phe and all compete for transport across the BBB via the L-type AA carrier

(Pardridge, 1998b). Therefore, low concentrations of Trp and high concentrations of other LNAA results in a low Trp:LNAA ratio. This ratio has been highly correlated with brain Trp levels and its product hypothalamic serotonin but overall Trp intake has also been shown to influences serotonin concentrations (Fernstrom and Wurtman, 1972; Adeola and Ball, 1992; Henry et al., 1996). Hypothalamic serotonin plays a role in the stress response by reducing secretion of stress hormones and altering aggressive behavior (Mason, 1968; Cortamira et al., 1991; Adeola et al., 1993; Lepage et al., 2003; Koopmans et al., 2005; Koopmans et al., 2009; Poletto et al., 2010). Stress hormones are considered to be insulin antagonistic and can stimulate catabolic pathways such as glycogenolysis, lipolysis and specific to cortisol, stimulate proteolysis (Bratusch-Marrain, 1983; Simmons et al., 1984; Strack et al., 1995; Ruzzin et al., 2005). Therefore, inadequate production of serotonin has the potential to negatively impact growth performance.

Currently the NRC (2012) recommends a SID Trp:Lys ratio of 17.6% for the growing and finishing pig. However, results from experiments indicate that the optimal SID Trp:Lys ratio ranges between 17 and 23.6% for growing-finishing pigs (Susenbeth, 2006; Kendall et al., 2007; Simongiovanni et al., 2012; Zhang et al., 2012). Fewer studies have looked at the Trp requirement in swine diets containing DDGS but several studies have indicated that the optimal SID Trp:Lys is between 16 and 23.5% when DDGS are included in the diet at 30% (Hinson et al., 2010; Salyer et al., 2013; Gonçalves et al., 2018a). With increased utilization of DDGS in commercial swine diets, optimal SID Trp:Lys ratios need to be verified over a range of DDGS inclusion levels to ensure adequate amounts are being supplied for both protein synthesis and other physiological functions. Therefore, the objective of this study was to evaluate the dose response of increasing the SID Trp:Lys in swine diets containing 40% DDGS.

MATERIALS AND METHODS

The South Dakota State University Institutional Animal Care and Use committee approved the protocol (19-043E) used in this study.

An experiment was conducted at the South Dakota State University commercial wean to finish research facility to evaluate the impact of increasing the SID Trp:Lys ratio in grow-finish swine diets containing 40% DDGS on growth performance and carcass characteristics. Pen dimensions were 3.1m x 6.9m and contained a 5-slot stainless steel dry feeder (SDI, Inc., Alexandra, SD) and two cup waterers, providing ad libitum access to feed and water. Daily feed allowances were delivered to individual pens by a robotic feeding system (FeedPro, Feedlogic ComDel Innovation, Wilmar, MN). Prior to the start of the study, pigs were fed a corn-SBM based diet containing 30% DDGS that provided nutrients that met or exceeded NRC (2012) nutrient recommendations.

A total of 1,170 pigs (PIC 800 x PIC) were used in a 98-d grow-finish study. Pens were stocked with 26 pigs (38.6±0.37 kg initial BW) with equal number of barrows and gilts and blocked by previous nursery treatment. One of five dietary treatments were randomly allotted to pens within block and each treatment was replicated nine times. Dietary treatments included a corn-SBM diet (CS) or diets containing 40% DDGS with a SID Trp:Lys ratio of 15, 18, 21, or 24%. All diets were provided in meal form and dietary treatments were fed in six phases. Dietary phase changes occurred every 14 days in accordance with weigh periods. The addition of crystalline L-Trp was used to create the titrated levels of SID Trp:Lys in DDGS diets. Lysine was supplied at requirement (PIC, 2016) during each dietary phase and all diets were formulated to contain similar NE and SID Lys concentrations within phase.

Diet samples were collected from every batch delivered during all phases. Samples were stored in a freezer (-20°C) until subsamples were pooled together and sent for analysis. Complete AA, CP, fat, and fiber content of diets were determined at the University of Missouri Chemical Laboratories (University of Missouri, Columbia MO) for each phase. Dietary subsamples across phases were pooled together and sent to the North Dakota State University Veterinary Diagnostic Laboratory (North Dakota State University, Fargo ND) for analysis of mycotoxins (Table 2.7).

Individual pigs were tagged with a RFID ear tag (Allflex, Merck Animal Health Inc., Madison, NJ) and weighted individually 10 days prior to the start of the study. Pen weights and feed disappearance were measured every 14 days to calculate ADG, ADFI, and G:F. Feed intake was determined from feed delivery data reported by the automated feeding system and the amount of remaining feed in each feeder on the weigh day. Weight of feed remaining in feeders was calculated using a feed density equation that utilized feed height and density in calculation. Groups of pigs were marketed in two cuts, with the initial cut occurring on day 84 of the study with the remaining pigs marketed on day 98. Pen inventory was standardized within block during the initial cut and represented approximately 25% of the total barn inventory. Prior to being shipped to a commercial abattoir for processing, pigs selected for market were individually weighed allowing for calculation of individual ADG, carcass ADG, and carcass yield. At the commercial abattoir HCW, BF measured via Fat-O-Meater, and percent lean were recorded for every pig. The carcass parameters HCW and BF were used to calculate SFFL weight by utilizing the equation of Burson and Berg (2001) for carcasses measured with a Fat-O-Meater.

Data was analyzed as a randomized complete block design with pen as the experimental unit. Previous nursery treatment was included in the statistical model as a random blocking factor. Analysis of variance was performed using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Pair-wise comparisons were utilized to compared treatment response of the CS diet to that of DDGS diets with graded levels of SID Trp:Lys. Single degree of freedom orthogonal contrasts were used to evaluate the does response of increasing the SID Trp:Lys ratio in diets containing 40% DDGS. Contrast coefficients for single degree of freedom orthogonal polynomials were based on equally spaced treatments. The GLM procedure of SAS was utilized to perform regression analysis and derive predictive parameter estimates. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.10 \geq P \leq 0.05$.

RESULTS

Diet analysis verified that levels of fat, fiber, and free Trp, Leu, Iso, Met, and Val were within five to ten percent of expected dietary formulated values. The AA values used in diet formulation were derived from a historical profile of source specific feed ingredients obtained from Cargill. Analyzed mycotoxin level of dietary treatments are present in Table 2.7.

Dietary treatment had an impact on all growth performance responses measured during the growing period (39 to 83 kg) with the exception of the G:F response

($P=0.255$) from 14 to 28 d (Table 2.8). Feeding pigs the CS diet resulted in heavier BW for all time points during the growing period ($P\leq0.003$). Pigs fed the CS diet had a greater ADG ($P<0.001$) for the first 28d and overall growing period compared to pigs fed the 40% DDGS diets. Providing a SID Trp:Lys ratio of 24% resulted in similar ADFI ($P>0.064$) as pigs receiving the CS diet during the first 14 d and overall growing period. The feed efficiency of pigs was improved ($P\leq0.002$) by feeding the CS diet compared to diets containing DDGS for the first 14 d and overall growing period. Increasing the SID Trp:Lys ratio in diets containing 40% DDGS linearly increased ADFI ($P\leq0.008$) for all growing periods (Table 2.9). The ADG of pigs linearly increased ($P\leq0.021$) during the first and last 14 d along with the overall growing period in response to increasing the SID Trp:Lys ratio. As a result, increasing the SID Trp:Lys ratio tended to impact 14 d BW (Linear, $P=0.088$), while BW for 28d and 42d linearly increased ($P\leq0.004$). Increasing the SID Trp:Lys ratio tended to improve G:F (Linear, $P=0.052$) from 28 to 42 d. but had no impact during the other growing periods ($P\geq0.211$).

During the finishing period, pigs receiving the CS dietary treatment continued to have heavier BW ($P<0.001$) compared to diets containing 40% DDGS. From 42 to 56 d, the CS fed pigs had greater ADG and feed efficiency ($P\leq0.005$) and tended to have greater ADFI ($P=0.061$) then pigs receiving a 15% SID Trp:Lys ratio in 40% DDGS diets. Pigs receiving the CS diet had similar intakes ($P=0.763$) compared to the pigs fed 24% SID Trp:Lys DDGS diet but greater intakes ($P\leq0.043$) then all other dietary treatments for the 56 to 70 d period. From 42 to 56 d ADG (Quadratic, $P=0.030$) and G:F (Linear, $P=0.024$) increased in response to increasing the SID Trp:Lys ratio in diets containing 40% DDGS with ADG plateauing at 18%. Increasing the SID Trp:Lys ratio in

DDGS diets increased ADFI (Linear, $P\leq0.011$) during the finishing period until pigs reached approximately 115 kgs of BW. From approximately 99 to 115 kg of BW, increasing the SID Trp:Lys ratio decreased (Linear, $P=0.028$) G:F in pigs fed 40% DDGS. In the two weeks prior to marketing, differences were not detected due to dietary treatments or an increase in the SID Trp:Lys ratio ($P\geq0.163$). Over the course of marketing, CS fed pigs had greater ADG ($P\leq0.038$) compared to pigs fed 40% DDGS diets with a SID Trp:Lys ratio of equal to or greater than 18% and greater ADFI ($P=0.003$) than pigs receiving a SID Trp:Lys ratio of 15%. The feed efficiency of the CS group was intermediate ($P=0.017$) between the 15% SID Trp:Lys treatment and the other 40% DDGS treatments during this period. Increasing the SID Trp:Lys ratio in 40% DDGS diets did not impact ADG or ADFI ($P\geq0.145$), however, increasing the SID Trp:Lys ratio in DDGS diets decreased G:F (Quadratic, $P=0.013$) with the worst G:F occurring when 21% SID Trp:Lys was supplied.

For the overall finishing period (42 to 98 d), dietary treatment did not impact ADG or G:F ($P\geq0.216$) but pigs fed CS diets did have greater ADFI ($P=0.002$) compared to pigs fed the 40% DDGS diet with a SID Trp:Lys ratio of 15%. However, increasing the SID Trp:Lys ratio in 40% DDGS diets increased ADFI (Linear, $P=0.021$) and tended to increase ADG (Linear, $P=0.074$) with no impact ($P\geq0.203$) to G:F (Table 2.11). Overall (0 to 98 d), pigs fed the CS diet had heavier final BW ($P<0.002$) compared to pigs fed DDGS diets and increasing the SID Trp:Lys ratio in 40% DDGS diets also increased final BW (Linear, $P=0.001$). Increasing the SID Trp:Lys ratio in 40% DDGS diets increased ADG (Linear, $P=0.002$) but did not result in similar ADG ($P<0.008$) compared to the CS diet. In 40% DDGS diets, increasing the SID Trp:Lys ratio also increased ADFI (Linear, $P=0.004$) and providing a SID Trp:Lys ratio of 24% resulted in similar ADFI ($P=0.253$) compared to the pigs fed the CS dietary treatment. Feed efficiency of pigs was not impacted by increasing the SID Trp:Lys ratio in DDGS diets ($P\geq0.650$) and no difference was detected between dietary treatments ($P=0.315$).

Pigs fed the CS dietary treatment had heavier HCW, higher carcass yields, greater carcass gain both total and daily, increased loin depth, and heavier fat free lean weights ($P<0.001$) compared to pigs fed diets containing 40% DDGS (Table 2.12). Back fat of pigs fed the CS diet was greater ($P\leq0.042$) than pigs fed DDGS diets containing 15 or 21% SID Trp:Lys. Increasing the SID Trp:Lys ratio in 40% DDGS diets increased HCW, total and daily carcass gain, fat free lean weight (Linear, $P\leq0.023$) and tended to increase back fat (Linear, $P=0.061$). Loin depth was not impacted ($P\geq0.532$) by dietary SID Trp:Lys percent in diets containing 40% DDGS. Differences in percent lean was also not impacted ($P\geq0.162$) by dietary treatments.

Starting BW classes were determined by average starting BW and the number of standard deviations from the mean (Table 2.13). The average starting BW class was a function of all pigs starting plus or minus one standard deviation away from the mean. Light BW pigs were characterized by being more than one standard deviation below the average barn pig BW. Heavy BW pigs were characterized by pigs being more than one standard deviation above the average barn pig BW. There was no interaction between SID Trp:Lys ratio and the starting BW class for all performance and carcass responses observed ($P\geq0.151$). Performance and carcass responses followed their starting BW classification for final BW, HCW, fat free lean weight, daily carcass gain, and percent lean ($P\leq0.001$). Heavy and average starting BW pigs had similar carcass yield, total carcass gain, and loin depth ($P \geq 0.142$) while lighter starting BW pigs had lower response values for the previously stated variables ($P \leq 0.047$). Back fat was similar for light and average BW pigs ($P=0.096$). At time of harvest, HCW influenced the differences in back fat and loin depth because when HCW was used as a covariate, there were no differences in starting BW class on these carcass characteristics ($P \geq 0.217$).

DISCUSSION

The amino acid Trp is more than a substrate for protein synthesis and plays a crucial role in multiple biological pathways. Following the use of Trp for protein synthesis, the second most important role for Trp is the kynurenine pathway which is responsible for over 90% of Trp catabolism as well as regulation of immune responses (Sainio et al., 1996). Tryptophan also serves as the precursor for the production of the neuromediator serotonin, which is associate with the stress and feed intake response (Fernstrom, 1985; Adeola and Ball, 1992; Heisler et al., 2003). The quantity of Trp utilized for the production of serotonin is very low, less than 10% of metabolized Trp, and has even been estimated to be less than one percent of consumed Trp (Wolf, 1974). Dietary intake of Trp has been shown to influence brain concentrations of serotonin (Adeola and Ball, 1992; Henry et al., 1996). However, an increase in dietary Trp also increases the amount of Trp metabolized in the kynurenine pathway and currently there is no estimate of Trp partitioning between the various metabolic pathways (Le Floc'h and Seve, 2007).

The majority of serotonin synthesis occurs within the gut and to a lesser extent in the brain and platelets (Mohammad-Zadeh et al., 2008; Jenkins et al., 2016). The concentration of brain serotonin is dependent on the availability of its precursor, Trp, due to the inability of serotonin to cross the blood brain barrier (Salyer et al., 2013). Tryptophan is transported across the blood brain barrier using a L-type amino acid carrier and competes with other LNAA which encompass Val, Ile, Leu, Trp, Tyr, and Phe (Fernstrom and Wurtman, 1972; Pardridge, 1998a). Therefore, excess amounts of LNAA can reduce the amount of Trp transported into the brain, thus decreasing serotonin production and leading to potential negative impacts on animal growth performance. Shen et al. (2012; 2015) showed that the supplementation of Trp and/or the reduction of LNAA improved ADG and feed conversion during periods of stress. Diets deficient in Trp are also know to reduce appetite and feed intake resulting in reduced growth performance (Eder et al., 2001).

The inclusion of DDGS in swine diets usually result in the increase of dietary concentrations of LNAA due to the increase in dietary CP being comprised from corn protein (NRC, 2012). Concentrations of Trp in corn protein is low as well (Stein, 2007). This leads to a decrease in the Trp:LNAA ratio and, therefore, lowering the amount of Trp transported into the brain as a result of increased competition with LNAA at the BBB (Fernstrom, 2005). In the current study, DDGS were included in the diets at 40% and provided a large majority of the dietary CP. This lead to high dietary concentrations of LNAA and Leu which has been shown to decrease both plasma and hypothalamic serotonin levels and negatively impact ADFI and growth performance (Kwon et al., 2019). In this study, increasing the SID Trp:Lys ratio increased ADFI which might be explained through an increase in the production of serotonin.

Early research on the Trp:Lys ratio for growing pigs indicated that 19% was an optimal SID Trp:Lys ratio while finishing pigs required 17% SID Trp:Lys for maximal

ADG (Lorschy et al., 1999; Susenbeth, 2006). The research of Kendall et al. (2007) indicated that the optimal SID Trp:Lys ratio for G:F was greater than 14.5% but less than 17% for pigs between the BW of 90 to 125 kg. The current recommendation for the SID Trp:Lys requirement in growing and finishing swine diets is 17.6% (NRC, 2012). However, the inclusion of DDGS in swine may require an increase in the optimal Trp:Lys ratio in order to maintain performance. Initial studies that utilized 30% DDGS reported that 16 or 16.5% SID Trp:Lys is sufficient for the maximal ADG of growing pig regardless of if supplied through protein bound sources or crystalline L-Trp (Hinson et al., 2010; Salyer et al., 2013). Results from the growing period in this study indicated that increasing the SID Trp:Lys ratio in diets containing 40% DDGS will result in a linear increase in ADFI and ADG with no impact to G:F (Table 2.9). Due to the linear responses within this study, an optimal SID Trp:Lys could not be defined. Salyer et al. (2013) suggested that a Trp:LNAA ratio at or below 3.1% may negatively affect growth performance and diets fed in this study were at or slightly above this level. This may have contributed to the inability to define the optimal SID Trp:Lys. The results in this study are more in agreement with the results observed in Salyer et al. (2013) for the finishing period, where a linear response was observed due to increasing the SID Trp:Lys ratio and therefore, not allowing for one to define the apex of the response curve. While there was a linear increase in ADFI and a tendency for an increase in ADG during the finishing period, after pigs reached approximately 115 kg, the increase in SID Trp:Lys ratio no longer impacted growth performance of finishing pigs (Table 2.11). The decrease in floor space due to the increase in pig BW could have resulted in a decrease in voluntary feed intake or restricted the impact that SID Trp:Lys has on the feed intake response (Li and

Patience, 2017). It could be suggested that an optimal SID Trp:Lys requirement in late finishing should be defined on a gram per kilogram of weight gain basis, a value accounting for environmental factors impacting ADFI. This would allow the determination at which point increasing the SID Trp:Lys will no longer positively impact during late finishing. Gonçalves et al. (2018a) also fed diets containing 30% DDGS when determining the optimal SID Trp:Lys ration in gilts between 30 to 125 kg of BW when raised under commercial conditions. Results from their study indicated that providing SID Trp:Lys at 23.5% resulted in maximum ADG and a minimum of 16.9% was needed to maximize G:F (Gonçalves et al., 2018a). In agreement with Gonçalves et al. (2018a), the maximum ADFI in our study was greater than 24% as a linear increase was observed but a breakpoint was not able to be defined for the maximum ADG or G:F in the current study.

Unlike other studies on the optimal SID Trp:Lys ratio, a corn-SBM diet was used as the control group in the current study when evaluating the dose response of SID Trp:Lys in 40% DDGS diets. This allowed for investigation if the addition of crystalline L-Trp in diets containing 40% DDGS could restore performance relative to a corn-SBM diet. The dietary inclusion of fibrous feedstuffs, such as DDGS, may reduce feed intake of pigs due to the increase in bulk volume of digesta in the gastrointestinal tract (Nyachoti et al., 2004; Avelar et al., 2010). During the growing period, providing a SID Trp:Lys ratio of 24% resulted in similar ADFI compared to corn-SBM diet which brings to question the true impact of dietary fiber concentration’s impact on voluntary feed intake. However, it was observed that during the growing period, pigs fed the corn-SBM diet had improved ADG and G:F.

One potential explanation for the difference in growth performance between pigs fed diets containing 40% DDGS and corn-SBM fed pigs could be dietary concentrations of mycotoxins. Mycotoxins are the carcinogenic or toxic secondary metabolites produced by fungi that colonize crops (Liu, 2011). In the current study, concentrations of the mycotoxin deoxynivalenol (DON) averaged 0.538 ppm in diets that contained 40% DDGS while concentrations of DON in the corn-SBM diet were 0.378 ppm (Table 2.7). While there were marginal differences between DON concentrations of the dietary treatments, these concentrations were below the one ppm advisory levels of DON in complete diets for swine (Food and Administration, 2011). All other mycotoxin levels were at or below the detectable concentrations of the mycotoxin assays (Table 2.7). Therefore, it can be suggested that the dietary concentrations of mycotoxins did not impact growth performance of pigs and mycotoxins are not an explanation for different pig growth performance of the DDGS fed pigs compared to the corn-SBM fed pigs.

The decrease in ADG and G:F of the DDGS fed pigs could be explain by an imbalance in the BCAA. The excess dietary concentrations of Leu fed in this study would have resulted in increased catabolism of Val and Ile resulting in limited Val and Ile supply for protein synthesis due to the antagonistic relationship that exists between the BCAA (Harper et al., 1984; Cemin et al., 2019; Kwon et al., 2020). However, for the finishing period pigs fed 40% DDGS with an SID Trp:Lys ratio of 18% or greater resulted in similar ADFI as the corn-SBM diet. This may have been the result of the corn-SBM fed pigs containing greater amounts of adipose tissue due to their greater BW which would be supported by greater back fat at time of harvest (Table 2.12). The increase in adipose tissue would have resulted in greater amounts of circulating leptin and, therefore, lead to a decrease in ADFI due to the impact of leptin on feed intake (Houseknecht et al., 1998; Gao and Horvath, 2007). The pigs fed corn-SBM diets also had greater carcass yields which was expected due to the fiber concentration in DDGS diets. Fiber is known to increase gastrointestinal tract mass the weight and volume of intestinal contents at time of harvest (Agyekum et al., 2012; Asmus et al., 2014; Coble et al., 2018).

In conclusion, this data suggests that increasing the SID Trp:Lys ratio in diets containing 40% DDGS will result in a linear increase in ADFI and, subsequently, ADG with no impact to feed efficiency for the overall grow-finish period. Increasing the SID Trp:Lys ratio had a greater impact on ADFI during the growing period compared to that of the finishing period. This was a result of SID Trp:Lys having no impact on ADFI or growth performance after pigs reached approximately 115 and 99 kgs of BW. Prior to pigs reaching this BW, optimal SID Trp:Lys is greater than 24% in diets containing 40% DDGS for the ADFI and ADG responses. The difference in magnitude of response to SID Trp:Lys during the growing and finishing period may suggest different approaches to maximize performance and economic return over the course of the grow-finish period. However, pigs fed diets containing 40% DDGS had lighter final BWs and worse ADG compared to pigs receiving the corn-SBM diet but providing a 24% SID Trp:Lys ratio in 40% DDGS diets did result in similar ADFI relative to the corn-SBM diet.

Table 2.1. Dietary Ingredient and Calculated Nutrient Composition of the Common diet and Phase One (0 to 14 d)

Item:	Common[1]	CS	SID Trp:Lys, %			
			15	18	21	24
Ingredients %						
Corn	47.57	69.21	43.80	43.76	43.72	43.68
Soybean meal	19.26	27.99	11.48	11.49	11.49	11.50
DDGS	30.00	-	40.00	40.00	40.00	40.00
Choice white grease	0.50	0.47	2.21	2.21	2.21	2.21
Salt	0.50	0.50	0.38	0.38	0.38	0.38
VTM premix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	1.10	0.75	1.13	1.13	1.13	1.13
Dicalcium phosphate	-	0.38	-	-	-	-
Lysine HCL	0.64	0.30	0.64	0.64	0.64	0.64
L-Threonine	0.16	0.13	0.18	0.18	0.18	0.18
L-Methionine	0.07	0.12	0.03	0.03	0.03	0.03
L-Tryptophan	0.05	-	0.02	0.05	0.09	0.12
Calculated analysis						
NE, Kcal/kg	2,316	2,406	2,411	2,411	2,411	2,411
CP, %	21.10	18.15	20.38	20.41	20.44	20.47
Ca, %	0.57	0.50	0.50	0.50	0.50	0.50
P, %	0.47	0.38	0.45	0.45	0.45	0.45
ATTD P, %	0.23	0.38	0.45	0.45	0.45	0.45
SID Amino Acid, %						
Lys	1.28	1.13	1.13	1.13	1.13	1.13
Met:Lys	32	34	31	31	31	31
Met + Cys:Lys	56	57	57	57	57	57
Thr:Lys	61	62	65	65	65	65
Trp:Lys	18	18	15	18	21	24
Val:Lys	67	67	70	70	70	70
Ile:Lys	58	61	59	59	59	59
Leu:Lys	143	116	149	149	149	149
Phe + Tyr:Lys	122	115	125	125	125	125
Trp:LNAA	4.4	4.8	3.6	4.3	5.0	5.6

[1] Common diet was fed from -14 d to 0 d

[2] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 2.2. Dietary Ingredient and Calculated Nutrient Composition of Phase Two (14 to 28 d)

Item:	CS	SID Trp:Lys, %			
		15	18	21	24
Ingredients %					
Corn	74.59	48.27	48.23	48.20	48.17
Soybean meal	22.52	7.06	7.07	7.07	7.07
DDGS	-	40.00	40.00	40.00	40.00
Choice white grease	0.65	2.35	2.35	2.35	2.35
Salt	0.50	0.38	0.38	0.38	0.38
VTM premix[1]	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.63	1.04	1.04	1.04	1.04
Dicalcium phosphate	0.45	-	-	-	-
Lysine HCL	0.30	0.60	0.60	0.60	0.60
L-Threonine	0.11	0.14	0.14	0.14	0.14
L-Methionine	0.09	-	-	-	-
L-Tryptophan	0.01	0.02	0.05	0.08	0.11
Calculated analysis					
NE, Kcal/kg	2,466	2,465	2,465	2,465	2,465
CP, %	15.84	18.56	18.58	18.61	18.63
Ca, %	0.45	0.45	0.45	0.45	0.45
P, %	0.38	0.45	0.45	0.45	0.45
ATTD P, %	0.37	0.44	0.44	0.44	0.44
SID Amino Acid, %					
Lys	0.98	0.98	0.98	0.98	0.98
Met:Lys	33	31	31	31	31
Met + Cys:Lys	57	59	59	59	59
Thr:Lys	62	65	65	65	65
Trp:Lys	18	15	18	21	24
Val:Lys	67	72	72	72	72
Ile:Lys	60	60	60	60	60
Leu:Lys	120	163	163	163	163
Phe + Tyr:Lys	118	133	133	133	133
Trp:LNAA	4.7	3.4	4.0	4.7	5.3

[1] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 2.3. Dietary Ingredient and Calculated Nutrient Composition of Phase Three (28 to 42 d)

Item:	CS	SID Trp:Lys, %			
		15	18	21	24
Ingredients %					
Corn	80.01	52.75	52.75	52.69	52.66
Soybean meal	17.34	2.56	2.56	2.57	2.57
DDGS	-	40.00	40.00	40.00	40.00
Choice white grease	0.55	2.40	2.40	2.40	2.40
Salt	0.50	0.38	0.38	0.38	0.38
VTM premix[1]	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.59	1.04	1.04	1.04	1.04
Dicalcium phosphate	0.38	-	-	-	-
Lysine HCL	0.30	0.58	0.58	0.58	0.58
L-Threonine	0.11	0.13	0.13	0.13	0.13
L-Methionine	0.06	-	-	-	-
L-Tryptophan	0.01	0.03	0.05	0.08	0.11
Calculated analysis					
NE, Kcal/kg	2,490	2,491	2,491	2,491	2,491
CP, %	13.74	16.71	16.73	16.75	16.77
Ca, %	0.40	0.44	0.44	0.44	0.44
P, %	0.35	0.44	0.44	0.44	0.44
ATTD P, %	0.35	0.43	0.43	0.43	0.43
SID Amino Acid, %					
Lys	0.85	0.85	0.85	0.85	0.85
Met:Lys	32	33	33	33	33
Met + Cys:Lys	57	64	64	64	64
Thr:Lys	63	66	66	66	66
Trp:Lys	18	15	18	21	24
Val:Lys	67	75	75	75	75
Ile:Lys	58	60	60	60	60
Leu:Lys	123	175	175	175	175
Phe + Tyr:Lys	117	137	137	137	137
Trp:LNAA	4.7	3.3	3.9	4.5	5.1

[1] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 2.4. Dietary Ingredient and Calculated Nutrient Composition of Phase Four (42 to 56 d)

Item:	CS	SID Trp:Lys, %			
		15	18	21	24
Ingredients %					
Corn	82.65	54.19	54.16	54.13	54.11
Soybean meal	14.81	0.95	0.95	0.96	0.96
DDGS	-	40.00	40.00	40.00	40.00
Choice white grease	0.60	2.60	2.60	2.60	2.60
Salt	0.50	0.38	0.38	0.38	0.38
VTM premix[1]	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.56	1.04	1.04	1.04	1.04
Dicalcium phosphate	0.26	-	-	-	-
Lysine HCL	0.30	0.56	0.56	0.56	0.56
L-Threonine	0.11	0.12	0.12	0.12	0.12
L-Methionine	0.05	-	-	-	-
L-Tryptophan	0.01	0.03	0.05	0.08	0.10
Calculated analysis					
NE, Kcal/kg	2,509	2,509	2,509	2,509	2,509
CP, %	12.77	16.02	16.04	16.06	16.08
Ca, %	0.35	0.43	0.43	0.43	0.43
P, %	0.33	0.43	0.43	0.43	0.43
ATTD P, %	0.33	0.43	0.43	0.43	0.43
SID Amino Acid, %					
Lys	0.79	0.79	0.79	0.79	0.79
Met:Lys	32	35	35	35	35
Met + Cys:Lys	58	67	67	67	67
Thr:Lys	64	67	67	67	67
Trp:Lys	18	15	18	21	24
Val:Lys	67	77	77	77	77
Ile:Lys	58	61	61	61	61
Leu:Lys	126	183	183	183	183
Phe + Tyr:Lys	116	141	141	141	141
Trp:LNAA	4.7	3.2	3.8	4.4	4.9

[1] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 2.5. Dietary Ingredient and Calculated Nutrient Composition of Phase Five (56 to 70 d)

Item:	CS	SID Trp:Lys, %			
		15	18	21	24
Ingredients %					
Corn	83.82	55.06	55.03	55.01	54.99
Soybean meal	13.53	-	-	-	-
DDGS	-	40.00	40.00	40.00	40.00
Choice white grease	0.70	2.70	2.70	2.70	2.70
Salt	0.50	0.38	0.38	0.38	0.38
VTM premix[1]	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.56	1.04	1.04	1.04	1.04
Dicalcium phosphate	0.28	-	-	-	-
Lysine HCL	0.30	0.54	0.54	0.54	0.54
L-Threonine	0.11	0.11	0.11	0.11	0.11
L-Methionine	0.04	-	-	-	-
L-Tryptophan	0.02	0.03	0.05	0.07	0.10
Calculated analysis					
NE, Kcal/kg	2,520	2,519	2,519	2,519	2,519
CP, %	12.23	15.61	15.63	15.65	15.66
Ca, %	0.35	0.43	0.43	0.43	0.43
P, %	0.33	0.43	0.43	0.43	0.43
ATTD P, %	0.33	0.43	0.43	0.43	0.43
SID Amino Acid, %					
Lys	0.76	0.76	0.76	0.76	0.76
Met:Lys	31	36	36	36	36
Met + Cys:Lys	58	69	69	69	69
Thr:Lys	64	67	67	67	67
Trp:Lys	18	15	18	21	24
Val:Lys	67	78	78	78	78
Ile:Lys	57	61	61	61	61
Leu:Lys	127	189	189	189	189
Phe + Tyr:Lys	116	144	144	144	144
Trp:LNAA	4.7	3.1	3.7	4.3	4.8

[1] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 2.6. Dietary Ingredient and Calculated Nutrient Composition of Phase Six (70 to 98 d)

Item:	CS	SID Trp:Lys, %			
		15	18	21	24
Ingredients %					
Corn	84.88	54.98	54.96	54.94	54.92
Soybean meal	12.68	-	-	-	-
DDGS	-	40.00	40.00	40.00	40.00
Choice white grease	0.65	2.80	2.80	2.80	2.80
Salt	0.50	0.38	0.38	0.38	0.38
VTM premix[1]	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.51	1.04	1.04	1.04	1.04
Dicalcium phosphate	0.16	-	-	-	-
Lysine HCL	0.30	0.52	0.52	0.52	0.52
L-Threonine	0.12	0.11	0.11	0.11	0.11
L-Methionine	0.04	-	-	-	-
L-Tryptophan	0.02	0.02	0.05	0.07	0.09
Calculated analysis					
NE, Kcal/kg	2,526	2,524	2,524	2,524	2,524
CP, %	11.90	15.58	15.60	15.61	15.63
Ca, %	0.30	0.43	0.43	0.43	0.43
P, %	0.30	0.43	0.43	0.43	0.43
ATTD P, %	0.30	0.42	0.42	0.42	0.42
SID Amino Acid, %					
Lys	0.74	0.74	0.74	0.74	0.74
Met:Lys	31	37	37	37	37
Met + Cys:Lys	58	71	71	71	71
Thr:Lys	66	69	69	69	69
Trp:Lys	18	15	18	21	24
Val:Lys	67	80	80	80	80
Ile:Lys	57	63	63	63	63
Leu:Lys	128	193	193	193	193
Phe + Tyr:Lys	115	147	147	147	147
Trp:LNAA	4.7	3.0	3.6	4.2	4.7

[1] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 2.7. Mycotoxin concentrations of dietary treatment diets (as-fed basis, ug/kg)[1]

Item:	SID Trp:Lys, %				CS
	15	18	21	24	
Alfatoxin B1	< 20	< 20	< 20	< 20	< 20
Alfatoxin B2	< 20	< 20	< 20	< 20	< 20
Alfatoxin G1	< 20	< 20	< 20	< 20	< 20
Alfatoxin G2	< 20	< 20	< 20	< 20	< 20
Fumonisin B1	< 200	< 200	200	< 200	< 200
Fumonisin B2	< 200	< 200	< 200	< 200	< 200
HT-2 Toxin	< 200	< 200	< 200	< 200	< 200
T-2 Toxin	< 20	< 20	< 20	< 20	< 20
Ochratoxin A	< 20	< 20	< 20	< 20	< 20
Sterigmatocystin	< 20	< 20	< 20	< 20	< 20
Zearalenone	< 100	< 100	< 100	< 100	< 100
Vomitoxin (DON)	548	583	544	477	378

[1] Representative samples of each dietary treatment for both phases were pooled and analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay.

Table 2.8. Performance response of growing pigs fed differing levels of SID Trp:Lys in diets containing 40% DDGS compared to pigs fed a corn-SBM diet.

Item:	SID Trp:Lys, %				CS	SEM	Diet
	15	18	21	24			
BW, kg							
d 0	38.6	38.6	38.6	38.6	38.5	0.37	0.999
d 14	52.3^{b}	53.0^{b}	52.7^{b}	53.2^{b}	54.3^{a}	0.47	0.003
d 28	66.4^{c}	67.6^{b}	67.5^{bc}	68.0^{b}	69.8^{a}	0.54	< 0.001
d 42	81.2^{c}	83.0^{b}	82.9^{b}	83.6^{b}	85.8^{a}	0.62	< 0.001
d 0 to 14							
ADG, kg	0.98^{c}	1.03^{bc}	1.01^{bc}	1.04^{b}	1.13^{a}	0.026	< 0.001
ADFI, kg	2.01^{c}	2.04^{bc}	2.07^{bc}	2.10^{ab}	2.16^{a}	0.032	0.001
G:F	0.488^{c}	0.505^{ab}	0.489^{bc}	0.497^{bc}	0.522^{a}	0.007	0.002
d 14 to 28							
ADG, kg	1.01^{c}	1.04^{bc}	1.06^{b}	1.04^{bc}	1.11^{a}	0.019	< 0.001
ADFI, kg	2.55^{c}	2.64^{b}	2.66^{b}	2.66^{b}	2.75^{a}	0.042	0.001
G:F	0.396	0.394	0.398	0.389	0.404	0.006	0.255
d 28 to 42							
ADG, kg	1.02^{c}	1.10^{ab}	1.08^{b}	1.11^{ab}	1.14^{a}	0.024	< 0.001
ADFI, kg	2.96^{c}	3.00^{bc}	3.03^{abc}	3.06^{ab}	3.09^{a}	0.034	0.007
G:F	0.343^{b}	0.368^{a}	0.355^{ab}	0.364^{a}	0.369^{a}	0.008	0.007
d 0 to 42							
ADG, kg	1.00^{c}	1.06^{b}	1.05^{b}	1.06^{b}	1.13^{a}	0.017	< 0.001
ADFI, kg	2.50^{c}	2.56^{bc}	2.58^{b}	2.61^{ab}	2.66^{a}	0.031	< 0.001
G:F	0.400^{c}	0.413^{b}	0.406^{bc}	0.409^{b}	0.422^{a}	0.004	< 0.001

$^{a\text{-}c}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

Table 2.9. Dose response of increasing the SID Trp:Lys ratio in diets containing 40% DDGS during the growing period.

Item:	SID Trp:Lys, %				SEM	Contrast	
	15	18	21	24		Linear	Quadratic
BW, kg							
d 0	38.6	38.6	38.6	38.6	0.36	0.926	0.967
d 14	52.3	53.0	52.7	53.2	0.45	0.088	0.641
d 28	66.4	67.6	67.5	68.0	0.56	0.004	0.325
d 42	81.2	83.0	82.9	83.6	0.70	0.001	0.189
d 0 to 14							
ADG, kg	0.98	1.03	1.01	1.04	0.017	0.021	0.564
ADFI, kg	2.01	2.04	2.07	2.10	0.029	0.008	0.971
G:F	0.488	0.505	0.489	0.497	0.003	0.601	0.408
d 14 to 28							
ADG, kg	1.01	1.04	1.06	1.04	0.015	0.108	0.073
ADFI, kg	2.55	2.64	2.66	2.66	0.037	0.006	0.113
G:F	0.396	0.394	0.398	0.389	0.002	0.461	0.551
d 28 to 42							
ADG, kg	1.02	1.10	1.08	1.11	0.025	0.005	0.226
ADFI, kg	2.96	3.00	3.03	3.06	0.032	0.006	0.978
G:F	0.343	0.368	0.355	0.364	0.003	0.052	0.165
d 0 to 42							
ADG, kg	1.00	1.06	1.05	1.06	0.015	0.002	0.109
ADFI, kg	2.50	2.56	2.58	2.61	0.028	0.002	0.444
G:F	0.400	0.413	0.406	0.409	0.002	0.211	0.130

Table 2.10. Performance response of finishing pigs fed differing levels of SID Trp:Lys in diets containing 40% DDGS compared to pigs fed a corn-SBM diet.

Item:	SID Trp:Lys, %				CS	SEM	Diet
	15	18	21	24			
BW, kg							
d 42	81.2^{c}	83.0^{b}	82.9^{b}	83.6^{b}	85.8^{a}	0.62	< 0.001
d 56	96.7^{c}	99.2^{b}	99.1^{b}	100.1^{b}	102.4^{a}	0.67	< 0.001
d 70	112.7^{c}	114.9^{b}	115.1^{b}	116.1^{b}	118.9^{a}	0.74	< 0.001
d 84	125.9^{c}	128.4^{b}	128.4^{b}	129.7^{b}	131.9^{a}	0.80	< 0.001
Final BW	139.0^{c}	140.4^{c}	140.4^{c}	142.4^{b}	145.4^{a}	0.87	< 0.001
d 42 to 56							
ADG, kg	1.08^{b}	1.15^{a}	1.16^{a}	1.17^{a}	1.19^{a}	0.020	< 0.001
ADFI, kg	3.24^{y}	3.30^{xy}	3.33^{x}	3.36^{x}	3.33^{x}	0.040	0.061
G:F	0.333^{b}	0.349^{a}	0.349^{a}	0.349^{a}	0.356^{a}	0.006	0.005
d 56 to 70							
ADG, kg	1.14	1.12	1.14	1.11	1.11	0.029	0.731
ADFI, kg	3.62^{c}	3.65^{c}	3.66^{bc}	3.74^{ba}	3.76^{a}	0.044	0.017
G:F	0.315^{x}	0.308^{xy}	0.311^{xy}	0.297^{y}	0.296^{y}	0.008	0.095
d 70 to 84							
ADG, kg	1.07	1.10	1.07	1.11	1.06	0.026	0.248
ADFI, kg	3.55	3.63	3.52	3.63	3.59	0.055	0.163
G:F	0.301	0.302	0.304	0.305	0.295	0.007	0.623
d 84 to 98							
ADG, kg	1.10^{ab}	1.05^{b}	1.06^{b}	1.08^{b}	1.14^{a}	0.029	0.028
ADFI, kg	3.68^{b}	3.88^{ab}	3.94^{a}	3.84^{ab}	4.02^{a}	0.105	0.034
G:F	0.304^{a}	0.270^{b}	0.269^{b}	0.280^{b}	0.283^{ab}	0.011	0.017
d 42 to 98							
ADG, kg	1.09	1.10	1.10	1.11	1.11	0.012	0.216
ADFI, kg	3.50^{b}	3.60^{a}	3.60^{a}	3.63^{a}	3.66^{a}	0.047	0.023
G:F	0.311	0.305	0.306	0.305	0.305	0.004	0.472
d 0 to 98							
ADG, kg	1.05^{c}	1.08^{b}	1.08^{b}	1.09^{b}	1.12^{a}	0.011	< 0.001
ADFI, kg	3.06^{c}	3.14^{b}	3.14^{b}	3.17^{ab}	3.21^{a}	0.034	0.001
G:F	0.345	0.346	0.344	0.344	0.350	0.003	0.315

$^{a-c}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

$^{x-y}$ Means within a row lacking common superscript tend to differ significantly, $P \leq 0.05$

Table 2.11. Dose response of increasing the SID Trp:Lys ratio in diets containing 40% DDGS during the finishing period.

Item:	SID Trp:Lys, %				SEM	Contrast	
	15	18	21	24		Linear	Quadratic
BW, kg							
d 42	81.2	83.0	82.9	83.6	0.70	0.001	0.189
d 56	96.7	99.2	99.1	100.1	0.78	< 0.001	0.068
d 70	112.7	114.9	115.1	116.1	0.85	< 0.001	0.231
d 84	125.9	128.4	128.4	129.7	0.92	< 0.001	0.249
Final BW	139.0	140.4	140.4	142.4	0.62	0.001	0.658
d 42 to 56							
ADG, kg	1.08	1.15	1.16	1.17	0.015	< 0.001	0.030
ADFI, kg	3.24	3.30	3.33	3.36	0.032	0.007	0.586
G:F	0.333	0.349	0.349	0.349	0.004	0.024	0.064
d 56 to 70							
ADG, kg	1.14	1.12	1.14	1.11	0.019	0.332	0.810
ADFI, kg	3.62	3.65	3.66	3.74	0.030	0.011	0.364
G:F	0.315	0.308	0.311	0.297	0.005	0.028	0.504
d 70 to 84							
ADG, kg	1.07	1.10	1.07	1.11	0.019	0.323	0.719
ADFI, kg	3.55	3.63	3.52	3.63	0.043	0.460	0.746
G:F	0.301	0.302	0.304	0.305	0.005	0.586	0.945
d 84 to 98							
ADG, kg	1.100	1.05	1.06	1.08	0.031	0.554	0.150
ADFI, kg	3.68	3.88	3.94	3.84	0.113	0.145	0.082
G:F	0.304	0.270	0.269	0.280	0.008	0.057	0.013
d 42 to 98							
ADG, kg	1.09	1.10	1.10	1.11	0.009	0.074	0.890
ADFI, kg	3.50	3.60	3.60	3.63	0.037	0.021	0.402
G:F	0.311	0.305	0.306	0.305	0.003	0.203	0.414
d 0 to 98							
ADG, kg	1.05	1.08	1.08	1.09	0.010	0.002	0.210
ADFI, kg	3.06	3.14	3.14	3.17	0.027	0.004	0.335
G:F	0.345	0.346	0.344	0.344	0.002	0.650	0.975

Table 2.12. Carcass characteristics of pigs fed differing levels of SID Trp:Lys in diets containing 40% DDGS compared to pigs fed a corn-SBM diet.

Item:	SID Trp:Lys, %				CS	SEM	Diet
	15	18	21	24			
Initial BW, kg[1]	33.2	33.6	33.3	33.3	33.2	0.45	0.897
Final BW, kg	139.5^{c}	140.8^{bc}	140.8^{bc}	142.6^{b}	145.8^{a}	0.91	< 0.001
ADG, kg	1.06^{c}	1.07^{c}	1.08^{bc}	1.09^{b}	1.12^{a}	0.008	< 0.001
HCW, kg	102.0^{c}	103.3^{bc}	103.2^{bc}	104.0^{b}	108.7^{a}	0.75	< 0.001
Carcass Yield, %[2]	73.2^{b}	73.4^{b}	73.1^{b}	73.1^{b}	74.8^{a}	0.18	< 0.001
Carcass Gain, kg	80.5^{c}	81.5^{bc}	81.5^{bc}	82.4^{b}	87.2^{a}	0.70	< 0.001
Daily Carcass Gain, kg	0.80^{c}	0.82^{bc}	0.82^{b}	0.83^{b}	0.87^{a}	0.007	< 0.001
Back Fat, mm	18.4^{c}	19.1^{ab}	18.7^{bc}	19.1^{ab}	19.4^{a}	0.29	0.024
Adj. Back Fat, mm[3]	18.7	19.2	18.9	19.2	18.8	0.34	0.313
Loin Depth, mm	60.4^{b}	60.1^{b}	60.7^{b}	60.5^{b}	64.2^{a}	0.51	< 0.001
Adj. Loin Depth, mm[3]	60.7^{b}	60.2^{b}	60.8^{b}	60.6^{b}	63.6^{a}	0.62	0.001
Percent Lean, %	52.6	52.3	52.5	52.3	52.6	0.17	0.162
Fat Free Lean, kg	50.0^{c}	50.6^{bc}	50.6^{bc}	50.9^{b}	53.2^{a}	0.35	< 0.001

$^{a-c}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

[1] Individual initial BW was taken on -6d.

[2] Utilized BW collected at barn in calculation.

[3] Adj. means values were adjusted by using HCW as a covariate.

Table 2.13. Dose response of increasing the SID Trp:Lys ratio in diets containing 40% DDGS on carcass characteristics.

Item:	SID Trp:Lys, %				SEM	Contrast	
	15	18	21	24		Linear	Quadratic
Initial BW, kg[1]	33.2	33.6	33.3	33.3	0.38	0.942	0.591
Final BW, kg	139.5	140.8	140.8	142.6	0.66	0.003	0.651
ADG, kg	1.06	1.07	1.08	1.09	0.010	0.001	0.795
HCW, kg	102.0	103.3	103.2	104.0	0.56	0.017	0.682
Carcass Yield, %[2]	73.2	73.4	73.1	73.1	0.20	0.514	0.452
Carcass Gain, kg	80.5	81.5	81.5	82.4	0.60	0.020	0.875
Daily Carcass Gain, kg	0.81	0.82	0.82	0.83	0.007	0.006	0.598
Back Fat, mm	18.4	19.1	18.7	19.1	0.23	0.061	0.537
Adj. Back Fat, mm[3]	18.7	19.2	18.9	19.2	0.23	0.299	0.608
Loin Depth, mm	60.4	60.1	60.7	60.5	0.37	0.532	0.861
Adj. Loin Depth, mm[3]	60.7	60.2	60.8	60.6	0.38	0.782	0.822
Percent Lean, %	52.6	52.3	52.5	52.3	0.13	0.249	0.461
Fat Free Lean, kg	50.0	50.6	50.6	50.9	0.27	0.023	0.602

[1] Individual initial BW was taken on -6d.

[2] Utilized BW collected at barn in calculation.

[3] Adj. means values were adjusted by using HCW as a covariate.

Table 2.14. Impact of increasing the SID Trp:Lys ratio in diets containing 40% DDGS on initial BW classification

Item:	Body Weight Classification[1]			SEM	Wt. Class	Wt. Class by Trp:Lys
	Light	Average	Heavy			
Initial BW, kg[2]	26.4^{c}	33.4^{b}	39.7^{a}	0.23	< 0.001	0.820
Final BW, kg	132.6^{c}	141.7^{b}	146.1^{a}	0.91	< 0.001	0.624
ADG, kg	1.03^{c}	1.08^{b}	1.12^{a}	0.008	< 0.001	0.843
HCW, kg	96.8^{c}	103.8^{b}	107.0^{a}	0.70	< 0.001	0.708
Carcass Yield, %[3]	72.9^{b}	73.3^{a}	73.4^{a}	0.18	0.048	0.161
Carcass Gain, kg	79.6^{b}	82.1^{a}	81.2^{a}	0.68	< 0.001	0.642
Daily Carcass Gain, kg	0.77^{c}	0.82^{b}	0.86^{a}	0.007	< 0.001	0.721
Back Fat, mm	18.3^{b}	18.8^{b}	19.5^{a}	0.33	0.005	0.180
Adj. Back Fat, mm[4]	19.2	18.7	19.1	0.34	0.217	0.151
Loin Depth, mm	59.3^{b}	60.7^{a}	60.8^{a}	0.50	0.007	0.710
Adj. Loin Depth, mm[4]	60.6	60.6	59.9	0.51	0.284	0.642
Percent Lean, %	52.8^{c}	52.5^{b}	52.0^{a}	0.17	0.001	0.152
Fat Free Lean, kg	47.5^{c}	50.9^{b}	52.2^{a}	0.34	< 0.001	0.912

$^{a\text{-}c}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

[1] Light: 20.2-28.9 kg (n=178), Average: 29.0-37.7 kg (n=774), Heavy: 37.8-46.2 (n=198).

[2] Individual initial BW was taken on -6d.

[3] Utilized BW collected at barn in calculation.

[4] Adj. means values were adjusted by using HCW as a covariate.

CHAPTER 3

IMPACT OF INCREASING STANDARDIZED ILEAL DIGESTIBLE VALINE:LYSINE IN DIETS CONTAINING 30% DRIED DISTILLER GRAINS WITH SOLUBLES ON EARLY GROW-FINISH PIG PERFORMANCE

ABSTRACT

A total of 2,430 pigs (DNA 600 x TopigsNorsvin TN70, initial BW 39.4 kg) were used in a 28-d trial to determine the SID Val:Lys requirement for pigs fed diets containing 30% DDGS. Treatments included five diets containing 30% DDGS with a SID Val:Lys ratio ranging from 60 to 80% in five percent increments plus a corn-SBM based diet, for a total of 6 dietary treatments. Pens were assigned to dietary treatment in a randomized complete block design with initial weight as the blocking factor and each treatment was replicated 15 times. Pair-wise comparisons were used to evaluate dietary treatment impact on performance while single degree of freedom orthogonal polynomials were used to evaluate dose response of SID Val:Lys in 30% DDGS diets. Increasing SID Val:Lys in diets containing 30% DDGS increased (Quadratic; $P<0.001$) final BW, ADG, ADFI and G:F with maximum growth performance occurring when 75% SID Val:Lys was supplied in 30% DDGS diets. Pigs fed CS had heavier final BW and greater ADG, G:F, and ADFI ($P\leq0.032$) compared to pigs fed diets containing 30% DDGS except for cumulative ADFI of pigs receiving 75% SID Val:Lys ($P=0.167$). The SID Val:Lys requirement for the ADG response was estimated at 66.6% (95% CI: [65.9, 67.4]) by the SBL analysis and 69.9% (95% CI: [68.2, 71.5]) by the QBL analysis. Optimal SID Val:Lys ratio for the G:F response was estimated at 68.4 (95% CI: [66.0, 70.8]) and 72.8% (95% CI: [69.8, 75.8]) for the SBL and QBL methods, respectively. This data

suggests that when feeding 30% DDGS during the swine growing period, a SID Val:Lys ratio of 68% would yield more than 99% and 98.5% of the maximum mean ADG and G:F response for the 39 to 68 kg pig. However, growth performance of pigs fed diets containing 30% DDGS did not equate to pigs consuming the corn-SBM diet regardless of SID Val:lys ratio.

INTRODUCTION

Valine has been reported to be the fifth limiting AA in corn-SBM diets with Ile being the next limiting AA (Figueroa et al., 2003). However, the inclusion of alternative ingredients in a corn-SBM based diet may change the order of limitation (Lordelo et al., 2008). The inclusion of corn based DDGS in swine diets results in Ile becoming the fifth limiting AA before that of Val, but this depends on what Ile:Lys ratio is targeted in the diet. The AA Leu is usually found in higher concentration than the other BCAA due to its higher concentration in corn and corn byproducts (Cemin et al., 2019). Due to the antagonistic relationship between the BCAA, emphasis has been put on adjusting the Ile requirement relative to dietary Leu concentrations. However, little is known on whether the Val requirement should also be adjusted relative to dietary Leu concentrations.

The BCAA are a group of structurally similar amino acids which include Val, Leu, and Ile. The structural similarity in the BCAA side chains makes them unique compared to the other indispensable AA in that they share the first two initial enzymatic steps of catabolism (Hutson et al., 2005). Therefore, the excess of a BCAA, particularly Leu, can result in the increased catabolism of the others (Brosnan and Brosnan, 2006). The two common enzymes involved in BCAA catabolism are BCAA aminotransferase (BCAT) and branched chain α-ketoacid dehydrogenase complex (BCKDC). There are two forms of BCAT which include mitochondria based BCAT mainly found in skeletal muscle and cytosolic based BCAT mainly found in the brain but also in the kidney and mammary gland tissue (Harper et al., 1984). The first step of BCAA catabolism is fully reversible and does not commit the BCAA to degradation (Harris et al., 2005). The second enzyme involved in the BCAA catabolism is BCAKD, which is a multienzyme complex found on the inner membrane surface of the mitochondria (Harper et al., 1984). This decarboxylation step is irreversible and, therefore, commits the BCAA to degradation (Harris et al., 2005). The activity of this enzyme is found highest in the liver followed by the heart and kidneys, with relatively low activity in brain, muscle, and adipose tissue (Harper et al., 1984). The pathway for BCAA catabolism is unique when compared to other AA in that initial catabolism starts in the skeletal muscle and, therefore, suggests that the BCAA might play a role as nutrient signals.

The dietary intake of Leu above requirement has been shown to reduce pig performance in a dose dependent manor as a result of an AA imbalance due to increased BCAA catabolism (Wiltafsky et al., 2010). Recently, Cemin et al. (2019) conducted a meta-analysis and developed a performance prediction model for BCAA levels in swine diets. Their model suggested that in order to counteract the negative effects of high dietary Leu concentrations, the supplementation of additional Val, Ile, and/or Trp separately or in combination would be needed to correct growth performance (Cemin et al., 2019). A study to validate their model was conducted by Kerkaert et al. (2021) and results from their study indicated that high levels of Val during the grower period aided in mitigating the negative effects of excess dietary Leu. Therefore, the objective of this study was to determine the optimal SID Val:Lys ratio in diets containing 30% DDGS during the growing period of swine production.

MATERIALS AND METHODS

The South Dakota State University Institutional Animal Care and Use committee approved the protocol (2001-002E) used in this study.

An experiment was conducted to evaluate the effects of increasing the SID Val:Lys ratio in swine diets containing 30% DDGS on the growth performance of pigs during the growing period. The study was conducted in a double long, curtain sided commercial research facility located in southwestern Minnesota. Each pen (3.2m x 5.6m) was equipped with a 4-slot stainless steel dry feeder (Hog Slat Inc., Newton Grove, NC) and two cup waterers, providing ad libitum access to feed and water. Daily feed rations were delivered to individual pens by an automated feeding system (DryExact Pro; Big Dutchman Inc., Holland, MI) capable of measuring and mixing feed. Prior to the start of the trial, pigs were fed a corn-SBM based diet containing 30% DDGS and met or exceeded NRC (2012) nutrient requirements.

A total of 2,425 pigs (DNA 600 x TopigsNorsvin TN70) were used in a 28-day growing study. Pens were stocked with 27 pigs with approximately equal number of barrows and gilts. Pens were blocked by average pig weight per pen and 15 pens were used per treatment. One of six dietary treatments were randomly allotted to pens within block. Dietary treatments included a corn-SBM diet (CS) or diets containing 30% DDGS with a SID Val:Lys ratio of 60 to 80% in five percent increments. All diets were provided in meal form and were fed in two phases. The dietary phase change occurred on day 14 in accordance with a weigh period. The addition of crystalline L-Val was utilized to achieve the desired 80% SID Val:Lys ratio. Crystalline Val and Ile were measured out by hand and delivered to the mill to ensure accurate inclusion rates. A total of three dietary treatments were milled, which included the CS diet, a diet containing 30% DDGS with a SID Val:Lys ratio of 60%, and a diet containing 30% DDGS with a SID Val:Lys ratio of 80%. The two diets containing DDGS were blended on site with the automated feeding system at ratios of 100/0, 75/25, 50/50, 25/75, and 0/100 percent (60/80 SID Val:Lys) to achieve SID Val:Lys ratios of 60, 65, 70, 75, and 80%. Lysine was formulated to 95% of requirement (PIC, 2016) to ensure the valine requirement was not underestimated. All diets were formulated to contain similar dietary net energy and SID Lys concentrations within phase.

Diet samples were collected from every batch delivered during both phases. Samples were stored in a freezer (-20°C) until subsamples were pooled together and sent for analysis. Complete AA analyses, CP, fat, and fiber analyses were performed by the University of Missouri Chemical Laboratories (University of Missouri, Columbia MO). Dietary samples for the three milled diets were pooled together and sent to the North Dakota State University Veterinary Diagnostic Laboratory (North Dakota State University, Fargo ND) for analysis of mycotoxins.

Pigs were weighed by pen and feed disappearance was measured on day 0, 14, and 28 to calculate ADG, ADFI, and G:F. Feed intake and G:F were determined from feed delivery data reported by the automated feeding system and the feed amount remaining in the feeder during the weigh period. Weight of feed remaining in feeders were determined by regression curve which utilized feed height in calculation. Two regression curves were developed to account for differences in feed density between diets that included DDGS and the diet with zero percent inclusion of DDGS (CS).

Data was analyzed as a randomized complete block design with pen as the experimental unit and initial BW as a random blocking factor. Analysis of variance was performed using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC). Single degree of freedom orthogonal polynomials were used to evaluate treatment response of SID Val:Lys levels on diets containing DDGS. Contrast coefficients for single degree of freedom orthogonal polynomials were based on equally spaced treatments. Pair-wise comparisons were used to evaluate treatment response of the CS diet relative to diets containing DDGS with differing SID Val:Lys ratios. The GLIMMIX procedure of SAS was utilized to fit the dose response to a QP model. The SBL and QBL analysis was conducted with the NLMIXED procedure of SAS to estimate valine requirement. Statistical models were compared using maximum-likelihood-based fit criteria and the BIC (Milliken and Johnson, 2009). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.10 \geq P < 0.05$.

RESULTS

Diet analysis verified that levels of CP, fat, fiber, and free levels of Lys, Trp, Met, Val, Ile, and Leu were within five to ten percent of expected dietary formulated values. The AA values used in diet formulation were supplied by Cargill and represented a historical profile of feed ingredients from sources specific manufacturers of the major ingredients utilized in this study. Mycotoxin levels were analyzed and are present in Table 3.3.

During the first 14 days, increasing the SID Val:Lys in diets containing DDGS resulted in an increase (quadratic; *P*<0.001) in BW, ADG, ADFI, and G:F (Table 3.5). Greatest numerical BW, ADG, and ADFI was achieved at a SID Val:Lys ratio of 75% while G:F plateaued at a SID Val:Lys ratio of 70% in DDGS diets. The estimated break point for the ADG response was 66.4% (95% CI: [65.5, 67.3]) and 69.4% (95% CI: [67.3, 71.5]) SID Val:Lys for the SBL and QBL methods (Table 3.6). The G:F response plateaued at a value of 0.456 and break point was determined to be at a SID Val:Lys ratio of 67.4% (95% CI: [65.0, 69.8]) and 71.2% (95% CI: [67.4, 75.0]) for the SBL and QBL methods (Table 3.6). Estimated break point for the ADFI response was estimated at a value of 65.4% (95% CI: [64.3, 66.6]) SID Val:Lys for the SBL analysis and 67.0% (95% CI: [63.6, 70.3]) SID Val:Lys for the QBL analysis (Table 3.6). The Val intake on a daily basis estimated that a SID Val intake of 14.6 g/d (SBL, 95% CI: [14.2, 15.0] and 16.1 g/d (QBL, 95% CI: [15.3, 16.9]) would yield an ADG of 1.01 kg (Figure 3.1). Pigs fed the CS diet had greater (*P*<0.003) BW, ADG, and G:F compared to pigs fed diets containing DDGS (Table 3.4). Feed intake of pigs receiving the CS diet was similar (P=0.288) compared to pigs fed DDGS diets containing 75% SID Val:Lys, but greater ($P\leq$0.02) than other DDGS dietary treatments (Table 3.4).

From 14 to 28 days, ADG, ADFI, and G:F increased (quadratic; $P\leq$0.007) as the SID Val:Lys ratio increased in diets containing DDGS (Table 3.5). The performance responses for ADG and ADFI plateaued at a SID Val:Lys ratio of 75% while the G:F response plateaued at 70% SID Val:Lys. Break point for ADG was estimated at 66.8% (95% CI: [65.6, 68.0]) for the SBL and 70.5% (95% CI: [67.3, 73.7]) for the QBL analyses (Table 3.7). The G:F response plateaued at 0.388 and break point analysis estimated a SID Val:Lys ratio of 70.5% (95% CI: [66.7, 74.23]) and 75.4% (95% CI: [69.9, >80]) for the SBL and QBL methods (Table 3.7). Estimated break point for the ADFI response was 65.7% (95% CI: [65.0, 66.5]) and 67.7% (95% CI: [65.8, 69.6]) for the SBL and QBL methods (Table 3.7). The Val intake was modeled and estimated that a SID Val intake of 15.7 g/d (SBL, 95% CI: [15.1, 16.3]) and 17.6 g/d (QBL, 95% CI: [14.1, 21.0]) would result in ADG of 1.02 kg (Figure 3.2). Pigs receiving the CS dietary treatment had improved (P<0.038) ADG and G:F compared to pig fed diets containing DDGS (Table 3.4). Providing pigs SID Val:Lys ratios of 75% and 80% in DDGS diets resulted in a similar ($P \geq$0.132) ADFI to that of pigs fed the CS diet.

Overall (days 0 to 28), increasing the SID Val:Lys ratio in diets containing 30% DDGS increased (quadratic P<0.001) final BW, ADG, ADFI and G:F (Table 3.5). Providing a SID Val:Lys ratio of 75% in DDGS diets resulted in the greatest numerical final BW, cumulative ADG, and cumulative ADFI. Cumulative G:F response plateaued when a SID Val:Lys ratio of 70% was supplied in DDGS diets. Break point analysis for the cumulative ADG response estimated the plateau to occur at a SID Val:Lys ratio of 66.6% (95% CI: [65.9, 67.4]) and 69.9% (95% CI: [68.2, 71.5]) for the SBL and QBL methods (Table 3.8). The G:F response plateaued at a value of 0.419 and break point was estimated at 68.4% (95% CI: [66.0, 70.8]) and 72.8% (95% CI: [69.8, 75.8]) SID Val:Lys for the SBL and QBL methods (Table 3.8). Estimated break point for the cumulative ADFI response occurred at a SID Val:Lys ratio of 65.7% (95% CI: [64.8, 66.5]) for the SBL and 67.6% (95% CI: [65.4, 69.8]) for the QBL (Table 3.8). Pigs fed diets containing DDGS had a lower (P<0.001) final BW, cumulative ADG, and G:F compared diets containing no DDGS (Table 3.4). Pigs fed the CS diet had similar (P=0.167) cumulative feed intake compared to pig fed DDGS diets with a SID Val:Lys ratio of 75% but a greater ($P \leq 0.033$) cumulative intake then other DDGS dietary treatments (Table 3.4).

DISCUSSION

In the present research, pigs that received diets with a SID Val:lys ratio of 60% had the lowest performance and the addition of Val into the diet improved pig performance, therefore validating that pigs were deficient in Val when receiving a SID Val:Lys ratio of 60%. This is an important aspect to validate when conducting titration studies to ensure accurate conclusions are drawn (Gaines et al., 2011).

The majority of the previous research focusing on the Val requirement in swine has been conducted with nursery pigs. The current NRC (2012) summarizes only six studies of which pig BW did not exceed 33 kg. The AA requirements for the growing-finishing pig were generated based on models in the swine NRC (2012) and, therefore, require validation through empirical means. Results from this study would suggest that the current NRC (2012) recommendations of 65.3% SID Val:Lys for the 25 to 50 kg pig is not adequate for maximum ADG based on the 95% CI for models used. However, this value presented by the NRC would be adequate in achieving maximum ADFI regardless of model used and G:F when utilizing the SBL model. Results from the second period (14 to 28 d), would suggest that the current NRC (2012) recommendations of 64.7% would not be adequate for achieving maximum performance of the 50 to 75 kg pig when DDGS are included at 30% of the diet.

While results from this study are not in agreement with the swine NRC (2012), these results are in agreement with other published research on the Val requirement (Wiltafsky et al., 2009b; Waguespack et al., 2012; Van Milgen et al., 2013; Liu et al., 2015; Soumeh et al., 2015a; Gonçalves et al., 2018b). Liu et al., (2015) estimated the SID Val:Lys requirement of the 49 to 70 kg pig at 67% using the SBL or 72% from the quadratic method. This value of 67% would be in strong agreement with our finding of a SID Val:Lys ratio of 66.4% and 66.8% in periods 1 and 2, respectively, for the SBL methods. Fewer studies have reviewed the SID Val:Lys requirement of pigs when utilizing corn protein sources from by-products in diet formulation. Gonçalves et al. in 2018 included 15% DDGS in diets when evaluating the SID Val:Lys requirement of the 25 to 45 kg pig. Their results indicated that a SID Val:Lys ratio of 68% would be required to obtain 99% of the maximum ADG response. Results from this study would agree, as in this study a SID Val:Lys ratio of 68% would yield more than 99% and 98.5% of the maximum mean ADG and G:F response, respectively, for the 39 to 68 kg pig. Interestingly, in this study the order in which ADG, ADFI, and G:F were optimized by the increase in SID Val:Lys ratio did not agree with Gonçalves et al., (2018b) and with the work of Barea et al., (2009a). In both of these studies, the optimal SID Val:Lys ratio for the G:F response was less than the ADG response. This is contradictory to our results where a higher SID Val:Lys ratio was required to maximize G:F compared to the SID Val:Lys ratio required to maximize ADG. This was speculated to be a result of difference in the ADFI response across studies, as Barea et al., (2009a) ADFI response was maximized at a SID Val:Lys ratio of 73.7% and 80.5% for the SBL and QP models, while Gonçalves et al., (2018b) did not model the ADFI response but highest numerical ADFI occurred at the upper titrated levels. The inconsistency in the ADFI response across studies may be worthy of increased attention as deficiencies in Val are known to result in decreased feed intake, particularly when Leu is supplied in excess (Wiltafsky et al., 2010; Gloaguen et al., 2011; Gloaguen et al., 2012).

Recently, Kerkaert et al. (2021) conducted a study to validate the BCAA / LNAA prediction model derived through a meta-analysis by Cemin et al. (2019). In this study, Kerkaert et al. (2021) concluded that high inclusion of Val, 76% to 78% SID Val:Lys, can mitigate the negative effects of excess dietary leucine during the growing period. Results from this study would suggest that while supplying 75% SID Val:Lys in diets containing 30% DDGS did provide the numerically greatest ADG, it did not statistically differ compared to that of the 70% and 80% SID Val:Lys treatments. As described before, the break point for the ADG response for both periods was 66.4% and 66.8% SID Val:Lys, respectively, which is marginally below the Kerkaert et al. (2021) negative control diet of 68% SID Val:Lys. The composition of diets have the potential to explain these differences in optimal SID Val:Lys, as the average dietary SID levels of Ile (60% vs 59%), Leu (141% vs 151%), and Trp (21% vs 19%) relative to Lys were slightly different in the current study compared to Kerkaert et al. (2021) (Tables 3.1 and 3.2). Htoo et al. (2014) defines Leu to be in excess when dietary concentration of greater than 130% SID Leu:Lys. Therefore, marginal differences in the amount of excess Leu may have reduced the necessity of an increase in the SID Val:Lys ratio required for optimal protein deposition in this study. While the increase in Trp inclusion in the current study could have decreased Val role in the intake response by reducing Leu transport across the BBB and mitigating the negative effects on intake and/or ensuring adequate Trp utilization by the brain (Cota et al., 2006; Kwon et al., 2019). The current results, along with research from other labs, continue to suggest that the AA Val, Ile, and Trp all play an integral role in correcting the negative effect of excess dietary Leu from diets with high inclusion of corn co-products (Cemin et al., 2019; Kwon et al., 2019; Kwon et al., 2020; Kerkaert et al., 2021). However, further research is required to accurately describe the upper and lower bounds of the AA Val, Ile, Leu, and Trp in relation to BCAA/LNAA to obtain optimal performance of pigs.

In an effort to increase the consistency of determining AA requirements on a g intake per kg gain basis, SID Val intake in g per day was calculated on a pen basis for each period and modeled against ADG for diets containing 30% DDGS (Figures 3.1 & 3.2). The SBL and QBL models allowed for determination of break point and plateau values which were used to calculate the g SID Val required per kg of gain in periods 1 and 2. The results of this analysis for period 1 (0 to 14 d) would suggest 14.4 grams of SID Val are required per kg of gain for the SBL and 15.9 grams of SID Val are required per kg of gain when utilizing the QBL model. For period 2 (14 to 28 d), an estimate of 15.4 and 17.2 grams of SID Val are required per kg of gain for the SBL and QBL models. These estimated values are greater than that of Gaines et al. (2011) published values in the swine NRC (2012) and Gonçalves et al. (2018b).

The inclusion of Val in diets containing 30% DDGS did not result in achieving similar growth performance compared to the corn-SBM diets. Some of the factors that have the potential to explain this include mycotoxin levels, fibrous components of DDGS, another unknown nutrient involved with BCAA metabolism, and/or a potential unknown intrinsic component of SBM.

The mycotoxin deoxynivalenol (DON) is one of the most prevalent contaminants in cereal grains and can result in decreased animal performance, nutritional efficiency, and altered immune function with swine being the most sensitive to DON concentrations (Pestka, 2007; Ghareeb et al., 2015). Current Food and Drug Administration advisory levels of DON for swine are 5ppm for feed ingredients and 1ppm for complete diets (Food and Administration, 2011). Diets fed in this study had DON levels below 1ppm but diets containing 30% DDGS had approximately 0.30 ppm greater concentrations than the corn-SBM diet (0.57 and 0.64 vs 0.31 ppm). It can be suggested that due to the lower levels of DON in the diets fed in the study, mycotoxins are probably not a major factor impacting performance differences between DDGS and corn-SBM diets.

The inclusion of DDGS in diets result in an increase in dietary fiber concentrations. The increase in dietary fiber concentration is usually accompanied by a decrease in caloric density unless corrected for through the inclusion of a highly calorically dense ingredients, such as fat. Pigs attempt to maintain a constant daily caloric intake and, therefore, pigs will increase feed intake until feed intake is limited by physical feed intake capacity or other environmental factors (Beaulieu et al., 2009). Even though diets in this study were isocaloric, the high fiber content of diets containing 30% DDGS (12.6% vs 6.2% NDF) may have caused a reduction in feed intake due to the increased bulk volume of intestinal digesta and therefore, reducing growth (Nyachoti et al., 2004; Avelar et al., 2010).

In summary, this data suggests that when feeding diets that contain 30% DDGS during the growing period, a SID Val:Lys ratio of 68% should be targeted in formulation to achieve optimal pig growth performance. When requirement is calculated on a g intake per kg of gain basis, the optimal SID Val intake per kg of gain ranges between 14.4 and 17.2 g. However, pigs fed a standard corn-SBM diet will still outperform pigs receiving diets with 30% DDGS regardless of SID Val:Lys ratio. Continued research is this area is needed to accurately describe the factors contributing to differences in pig performance between DDGS and corn-SBM diets.

Table 3.1. Dietary Ingredient and Calculated Nutrient Composition of the Common Diet and Phase One (0 to 14 d)

Item:	Common[1]	SID Val:Lys, %		CS
		60	80	
Ingredients, %				
Corn	46.64	59.65	59.34	72.29
Soybean meal	19.90	5.32	5.32	23.89
DDGS	30.00	30.00	30.00	-
Choice white grease	0.70	1.75	1.85	0.95
Calcium carbonate	1.03	1.29	1.29	0.95
Salt	0.44	0.38	0.38	0.57
Monophosphate 21%	-	-	-	0.51
VTM premix[2]	0.10	0.10	0.10	0.10
Magnesium oxide 54%	0.30	0.30	0.30	0.30
Lysine HCL	0.56	0.69	0.69	0.27
L-Threonine	0.15	0.22	0.22	0.10
L-Methionine	0.11	0.09	0.09	0.09
L-Tryptophan	0.07	0.10	0.10	-
L-Valine	-	-	0.20	-
L-Isoleucine	-	0.11	0.12	-
Calculated Analysis				
NE, Kcal/kg	2,309	2,426	2,426	2,424
CP, %	21.62	15.33	15.46	16.00
NDF, %	12.59	12.63	12.61	6.25
Ca, %	0.50	0.55	0.55	0.55
P, %	0.46	0.41	0.41	0.41
Available P, %	0.34	0.32	0.32	0.33
SID Amino Acid, %				
Lys	1.25	0.98	0.98	0.98
Met:Lys	34	34	34	33
(Met+Cys):Lys	58	57	57	57
Thr:Lys	63	65	65	62
Trp:Lys	21	21	21	18
Val:Lys	69	60	80	69
Ile:Lys	60	60	60	62
Leu:Lys	143	137	137	124
Val:Leu	48	44	58	55
(Val+Ile):Leu	90	88	102	105

[1] Common diet was fed from -10d to 0 d

[2] Provided per kilogram of the diet: 600 FTU Axtra Phytase Gold 65G, 60.4 g Danisco® Xylanase 60000 G, 2,527 IU vitamin A, 1,134 IU vitamin D3, 32.4 IU vitamin E, 0.97 mg vitamin K3, 32.4 mg niacin, 13.2 mg pantothenic acid, 4.5 mg riboflavin, 21 ug vitamin B12, 40 mg Mn from manganous oxide, 50 mg Zn from zinc hydroxychloride, 100 mg Fe from ferrous sulfate, 10 mg Cu from copper sulfate, 1.0 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 3.2. Dietary Ingredient and Calculated Nutrient Composition of Phase Two (14 to 28 d)

Item:	SID Val:Lys, %		CS
	60	80	
Ingredients, %			
Corn	64.06	63.78	76.28
Soybean meal	1.05	1.05	19.97
DDGS	30.00	30.00	-
Choice white grease	1.55	1.65	0.90
Calcium carbonate	1.27	1.27	0.96
Salt	0.38	0.38	0.57
Monophosphate 21%	0.15	0.15	0.56
VTM premix[1]	0.10	0.10	0.10
Magnesium oxide 54%	0.30	0.30	0.30
Lysine HCL	0.67	0.67	0.24
L-Threonine	0.20	0.20	0.08
L-Methionine	0.05	0.05	0.05
L-Tryptophan	0.10	0.10	-
L-Valine	-	0.18	-
L-Isoleucine	0.12	0.12	-
Calculated Analysis			
NE, Kcal/kg	2,435	2,435	2,440
CP, %	13.59	13.70	14.38
NDF, %	12.63	12.61	6.24
Ca, %	0.55	0.55	0.55
P, %	0.43	0.43	0.41
Available P, %	0.34	0.34	0.33
SID Amino Acid, %			
Lys	0.86	0.86	0.86
Met:Lys	32	32	31
(Met+Cys):Lys	57	57	57
Thr:Lys	65	65	62
Trp:Lys	21	21	18
Val:Lys	60	80	70
Ile:Lys	60	60	62
Leu:Lys	144	144	130
Val:Leu	42	55	54
(Val+Ile):Leu	83	97	101

[1] Provided per kilogram of the diet: 600 FTU Axtra Phytase Gold 65G, 60.4 g Danisco® Xylanase 60000 G, 2,527 IU vitamin A, 1,134 IU vitamin D3, 32.4 IU vitamin E, 0.97 mg vitamin K3, 32.4 mg niacin, 13.2 mg pantothenic acid, 4.5 mg riboflavin, 21 ug vitamin B12, 40 mg Mn from manganous oxide, 50 mg Zn from zinc hydroxychloride, 100 mg Fe from ferrous sulfate, 10 mg Cu from copper sulfate, 1.0 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 3.3. Mycotoxin concentrations of dietary treatments (as-fed basis, ug/kg)[1]

Item	SID Val:Lys, %		CS
	60	80	
Alfatoxin B1	< 20	< 20	< 20
Alfatoxin B2	< 20	< 20	< 20
Alfatoxin G1	< 20	< 20	< 20
Alfatoxin G2	< 20	< 20	< 20
Fumonisin B1	< 200	< 200	200
Fumonisin B2	< 200	< 200	< 200
HT-2 Toxin	< 200	< 200	< 200
T-2 Toxin	< 20	< 20	< 20
Ochratoxin A	< 20	< 20	< 20
Sterigmatocystin	< 20	< 20	< 20
Zearalenone	< 100	< 100	< 100
Vomitoxin (DON)	568	635	313

[1] Representative samples of each dietary treatment for both phases were pooled and analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay.

Table 3.4. Performance response of growing pigs fed differing levels of SID Val:Lys in diets containing 30% DDGS compared to pigs fed a corn-SBM diet.

Item:	SID Val:Lys, %					CS	SEM	Diet
	60	65	70	75	80			
BW, kg								
Initial	39.3	39.5	39.4	39.5	39.4	39.4	0.21	0.94
d 14	51.0^{d}	53.1^{c}	53.5^{bc}	53.7^{b}	53.4^{bc}	54.6^{a}	0.26	< 0.001
Final	62.4^{d}	66.6^{c}	67.6^{b}	68.0^{b}	67.6^{b}	69.6^{a}	0.32	< 0.001
d 0 to 14								
ADG, kg	0.83^{d}	0.97^{c}	1.01^{b}	1.02^{b}	1.00^{b}	1.08^{a}	0.010	< 0.001
ADFI, kg	2.02^{c}	2.20^{b}	2.20^{b}	2.23^{ab}	2.20^{b}	2.26^{a}	0.018	< 0.001
G:F	0.417^{d}	0.444^{c}	0.458^{b}	0.455^{b}	0.456^{b}	0.480^{a}	0.005	< 0.001
d 14 to 28								
ADG, kg	0.82^{d}	0.96^{c}	1.00^{b}	1.02^{b}	1.01^{b}	1.07^{a}	0.013	< 0.001
ADFI, kg	2.22^{c}	2.56^{b}	2.59^{b}	2.62^{ab}	2.62^{ab}	2.66^{a}	0.022	< 0.001
G:F	0.365^{c}	0.375^{c}	0.387^{b}	0.390^{b}	0.387^{b}	0.404^{a}	0.005	< 0.001
d 0 to 28								
ADG, kg	0.82^{d}	0.96^{c}	1.01^{b}	1.02^{b}	1.01^{b}	1.08^{a}	0.008	< 0.001
ADFI, kg	2.12^{c}	2.38^{b}	2.40^{b}	2.42^{ab}	2.41^{b}	2.46^{a}	0.018	< 0.001
F:G	0.386^{d}	0.406^{c}	0.419^{b}	0.420^{b}	0.418^{b}	0.440^{a}	0.004	< 0.001

$^{a\text{-}d}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

Table 3.5. Dose response of increasing the SID Val:Lys ratio in diets containing 30% DDGS fed to pigs during the growing period.

Item:	SID Val:Lys, %					SEM	Contrast	
	60	65	70	75	80		Linear	Quadratic
BW, kg								
Initial	39.3	39.5	39.4	39.5	39.4	0.22	0.830	0.569
d 14	51.0	53.1	53.5	53.7	53.4	0.25	< 0.001	< 0.001
Final	62.4	66.6	67.6	68.0	67.6	0.33	< 0.001	< 0.001
d 0 to 14								
ADG, kg	0.83	0.97	1.01	1.02	1.00	0.008	< 0.001	< 0.001
ADFI, kg	2.02	2.20	2.20	2.23	2.20	0.017	< 0.001	< 0.001
G:F	0.417	0.444	0.458	0.455	0.456	0.004	< 0.001	< 0.001
d 14 to 28								
ADG, kg	0.82	0.96	1.00	1.02	1.01	0.012	< 0.001	< 0.001
ADFI, kg	2.22	2.56	2.59	2.62	2.62	0.020	< 0.001	< 0.001
G:F	0.365	0.375	0.387	0.390	0.387	0.004	< 0.001	0.007
d 0 to 28								
ADG, kg	0.82	0.96	1.01	1.02	1.01	0.007	< 0.001	< 0.001
ADFI, kg	2.12	2.38	2.40	2.42	2.41	0.016	< 0.001	< 0.001
G:F	0.386	0.406	0.419	0.420	0.418	0.003	< 0.001	< 0.001

Table 3.6. Period 1 (0 to 14 d) modeled swine growth performance response of increasing the SID Val:Lys ratio in diets containing 30% DDGS

Item:	Model Equation	Break Point Value	Apex / Plateau	95% Confidence Interval		BIC	R^2
				LCL	UCL		
ADG							
QP	$-4.3632+0.1456*(Val:Lys)-0.00098*(Val:Lys)^2$	74.29	1.045	-	-	-222.2	0.7726
SBL	$1.0088+0.02864*((Val:Lys<66.39)*(Val:Lys-66.39))$	66.39	1.009	65.46	67.32	-267.3	0.7927
QBL	$1.0088-0.00208*((Val:Lys<69.38)*(Val:Lys-69.38))^2$	69.38	1.009	67.29	71.46	-267.3	0.7927
ADFI							
QP	$-4.0298+0.1705*(Val:Lys)-0.00116*(Val:Lys)^2$	73.5	2.235	-	-	-138.7	0.5368
SBL	$2.2129+0.0366*((Val:Lys<65.43)*(Val:Lys-65.43))$	65.43	2.213	64.26	66.6	-173.4	0.5703
QBL	$2.2129-0.0041*((Val:Lys<66.96)*(Val:Lys-66.96))^2$	66.96	2.213	63.61	70.32	-173.4	0.5703
G:F							
QP	$-0.8162+0.03424*(Val:Lys)-0.00023*(Val:Lys)^2$	74.44	0.458	-	-	-332.5	0.4984
SBL	$0.4561+0.006208*((Val:Lys<67.39)*(Val:Lys-67.39))$	67.39	0.456	64.99	69.79	-377.1	0.5104
QBL	$0.4561-0.00037*((Val:Lys<71.15)*(Val:Lys-71.15))^2$	71.15	0.456	67.35	74.95	-377.0	0.5096

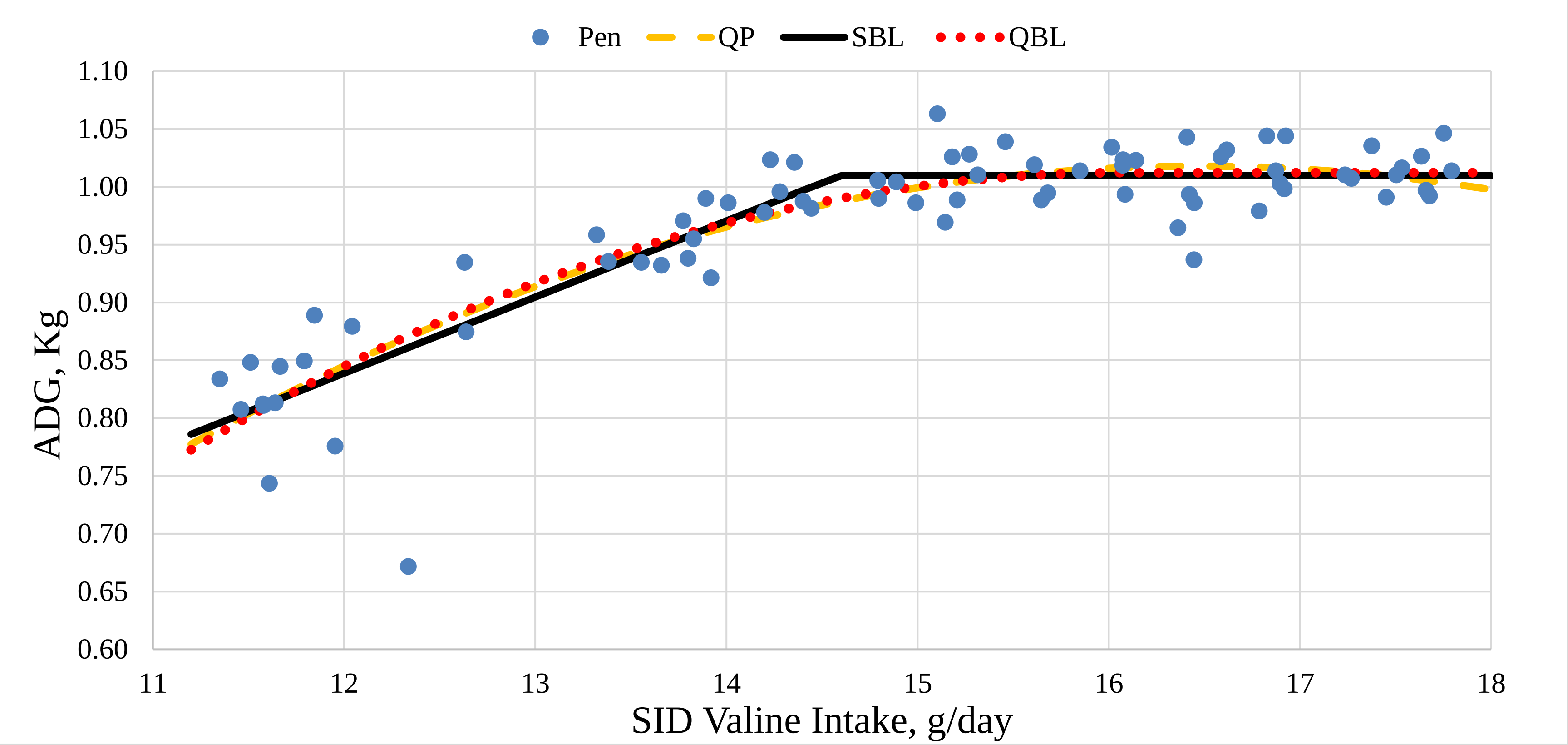
Table 3.7. Period 2 (14 to 28 d) modeled swine growth performance response of increasing the SID Val:Lys ratio in diets containing 30% DDGS

Item:	Model Equation	Break Point Value	Apex / Plateau	95% Confidence Interval		BIC	R^2
				LCL	UCL		
ADG							
QP	$-4.2809+0.1422*(Val:Lys)-0.00095*(Val:Lys)^2$	74.85	1.040	-	-	-194.8	0.7355
SBL	$1.011+0.02858*((Val:Lys<66.81)*(Val:Lys-66.81))$	66.81	1.011	65.61	68.01	-235.9	0.7494
QBL	$1.0118-0.00177*((Val:Lys<70.48)*(Val:Lys-70.48))^2$	70.48	1.012	67.30	73.66	-236.0	0.7495
ADFI							
QP	$-8.2931+0.2951*(Val:Lys)-0.00199*(Val:Lys)^2$	74.15	2.647	-	-	-99.58	0.7310
SBL	$2.6077+0.06783*((Val:Lys<65.72)*(Val:Lys-65.72))$	65.72	2.608	64.98	66.46	-152.7	0.7953
QBL	$2.6077-0.00647*((Val:Lys<67.74)*(Val:Lys-67.74))^2$	67.74	2.608	65.84	69.64	-152.7	0.7953
G:F							
QP	$-0.20365+0.015689*(Val:Lys)-0.00010381*(Val:Lys)^2$	75.61	0.389	-	-	-360.6	0.3169
SBL	$0.3881+0.002241*((Val:Lys<70.47)*(Val:Lys-70.47))$	70.47	0.388	66.70	74.23	-405.2	0.3198
QBL	$0.3883-0.0001*((Val:Lys<75.42)*(Val:Lys-75.42))^2$	75.42	0.388	69.92	80.91	-404.6	0.3133

Table 3.8. Cumulative (0 to 28 d) modeled swine growth performance response of increasing the SID Val:Lys ratio in diets containing 30% DDGS

Item:	Model Equation	Break Point Value	Apex / Plateau	95% Confidence Interval		BIC	R^2
				LCL	UCL		
ADG							
QP	$-4.332+0.1442*(Val:Lys)-0.00097*(Val:Lys)^2$	74.33	1.027	-	-	-250.8	0.8519
SBL	$1.0101+0.02862*((Val:Lys<66.60)*(Val:Lys-66.60))$	66.60	1.010	65.85	67.35	-300.8	0.8714
QBL	$1.0101-0.00195*((Val:Lys<69.85)*(Val:Lys-69.85))^2$	69.85	1.010	68.21	71.49	-300.8	0.8714
ADFI							
QP	$-5.9591+0.2274*(Val:Lys)-0.00154*(Val:Lys)^2$	73.84	2.436	-	-	-129.0	0.6940
SBL	$2.4106+0.05016*((Val:Lys<65.66)*(Val:Lys-65.66))$	65.66	2.411	64.80	66.51	-176.7	0.7445
QBL	$2.4106-0.00494*((Val:Lys<67.58)*(Val:Lys-67.58))^2$	67.58	2.411	65.35	69.81	-176.7	0.7445
G:F							
QP	$-0.4645+0.02365*(Val:Lys)-0.0001577*(Val:Lys)^2$	75.00	0.422	-	-	-414.5	0.6456
SBL	$0.4191+0.003923*((Val:Lys<68.39)*(Val:Lys-68.39))$	68.39	0.419	66.01	70.77	-462.0	0.6503
QBL	$0.4193-0.0002*((Val:Lys<72.78)*(Val:Lys-72.78))^2$	72.78	0.419	69.79	75.78	-461.3	0.6470

Figure 3.1. The impact of SID Val intake on the ADG response of individual pens during period one (0 to 14 d)



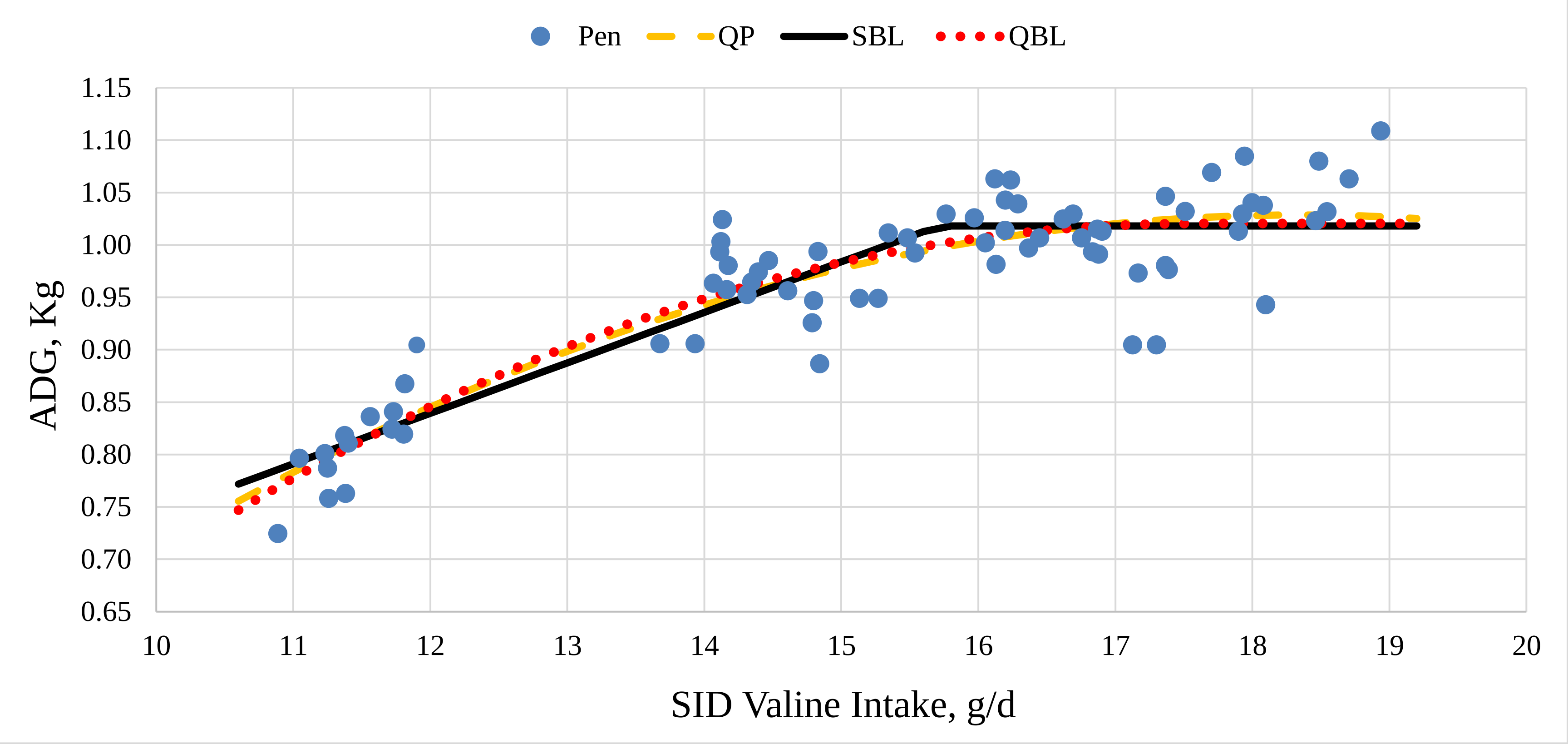
Pen represents the individual pen SID Val intake and ADG response.

QP model: ADG (kg) = -1.33265+0.28549*SID Val intake (g/d) -0.0086683*(SID Val intake (g/d))^2, BIC = -237.1, R^2 = 0.796

SBL model: ADG (kg) = 1.0096+0.0659*((SID Val intake (g/d)<14.59)*(SID Val intake (g/d)-14.59)) Plateau = 1.0096,
Break point = 14.59, 95% CI: [14.22, 14.97], BIC = -273.9, R^2 = 0.810

QBL model: ADG (kg) = 1.0122-0.01008*((SID Val intake (g/d)<16.08)*(SID Val intake (g/d)-16.08))^2, Plateau = 1.0122,
Break point = 16.08, 95% CI: [15.29, 16.86], BIC = -269.9, R^2 = 0.800

Figure 3.2. The impact of SID Val intake on the ADG response of individual pens during period two (14 to 28 d)



Pen represents the individual pen SID Val intake and ADG response.

QP model: ADG (kg) = -0.503995+0.167095*SID Val intake (g/d) -0.00455459*(SID Val intake (g/d))^2, BIC = -216.82, R^2 = 0.799

SBL model: ADG (kg) = 1.0181+0.04848*((SID Val intake (g/d)<15.71)*(SID Val intake (g/d)-15.71)) Plateau = 1.018, Break point = 15.71, 95% CI: [15.14, 16.28], BIC = -245.0, R^2 = 0.792

QBL model: ADG (kg) = 1.0205-0.00563*((SID Val intake (g/d)<17.57)*(SID Val intake (g/d)-17.57))^2, Plateau = 1.0205, Break point = 17.57, 95% CI: [14.13, 21.01], BIC = -248.8, R^2 = 0.802

CHAPTER 4

THE EFFECT OF STANDARDIZED ILEAL DIGESTIBLE ISOLEUCINE:LYSINE IN DIETS CONTAINING 20% DRIED DISTILLER GRAINS WITH SOLUBLES ON FINISHING PIG PERFORMACE AND CARCASS CHARACTERISTICS

ABSTRACT

In order to determine the SID Ile:Lys requirement in finishing diets containing 20% DDGS, a 56 d study was conducted utilizing 2,268 pigs (DNA 600 x TopigsNorsvin TN70, initial BW 82.3 kg). A total of six dietary treatments were fed, which included a corn-SBM based diet and five diets containing 20% DDGS with SID Ile:Lys ratios of 55, 60, 65, 70, and 75%. Dietary treatments were assigned to pens, balancing for previous treatment with each treatment being replicated 14 times. Pair-wise comparisons were used to evaluate the impact of dietary treatment on performance and carcass traits while single degree of freedom orthogonal polynomials were used to evaluate dose response of SID Ile:Lys in 20% DDGS diets fed to pigs. Increasing the SID Ile:Lys ratio in 20% DDGS diets did not impact pig growth performance criteria in a quadratic or linear fashion during this study ($P \geq 0.153$). However, increasing the SID Ile:Lys ratio in 20% DDGS diets fed to pigs did decrease back fat (Quadratic, $P=0.014$), increase loin depth (Quadratic, $P=0.029$), and tended to increase percent lean (Quadratic, $P=0.076$) with optimal carcass parameters occurring when 65% SID Ile:Lys was supplied in 20% DDGS diets. Pigs fed the corn-SBM diet had a similar final BW compared to pig fed 20% DDGS diets containing 60 and 70% SID Ile:Lys ratio ($P \geq 0.060$) and greater ADFI compared to pigs receiving diets with SID Ile:Lys ratios of 65 and 75% ($P<0.001$). In conclusion, it appears that optimal ADG of pigs fed 20% DDGS might be achieved with a SID Ile:Lys ratio of 60% during early finishing and 70% during late finishing.

However, responses in carcass parameters indicated that the optimal SID Ile:Lys ratio is 65% for the finishing pig fed diets containing 20% DDGS. Feeding 20% DDGS in late finishing still resulted in numerically lower growth performance compared to the corn-SBM fed pigs.

INTRODUCTION

The BCAA Val and Ile are typically considered the fifth or sixth limiting AA after Lys, Thr, Met, and Trp in low CP swine diets (Liu et al., 2000; Lordelo et al., 2008). Figueroa et al. (2003) has suggested that Val is limiting before Ile in common corn-SBM diets, however, the inclusion of by-product protein sources can affect order of limitation of AA (Lordelo et al., 2008). The inclusion of corn by-products, such as DDGS, ultimately leads to higher concentrations of dietary corn protein which can affect the order of limitation of AA. As the proportion of dietary CP contributed by corn protein increases, the concentration of dietary Ile decreases at a faster rate compared to Val and can result in Ile to become the fifth limiting AA prior to that of Val. The higher concentrations of corn protein can also result greater concentration of dietary Leu which has been shown to impact the Ile requirement (Htoo et al., 2017). An antagonistic relationship exists between the BCAA because they share the first two catabolic steps by the same degrading enzymes (Hutson et al., 2005). Due to this, the excess of any one of the BCAA can result in the increased catabolism of all the BCAA, leading to a potential AA deficiency (Harper et al., 1984). Therefore, accurate estimates of the optimal SID Ile to Lys requirement are necessary to ensure adequate performance when diets contain high amounts of corn protein.

The published literature on the Ile requirement reports a wide range of optimal levels ranging from less than 50% (Barea et al., 2009b) to 62% Ile:Lys (Fu et al., 2006) relative to Lys. This is most likely a result of studies conducted with and without spray-dried blood cells. Spray-dried blood cells contains high concentrations of Leu, Lys and Val, but a low concentration of Ile allowing for simple Ile deficient test diets (NRC, 1998). Wiltafsky et al. (2009a) showed that the inclusion of spray-dried blood cells at 7.5% resulted in an estimated SID Ile:Lys requirement of 59% while a SID Ile:Lys ratio of 54% was sufficient when Leu was not supplied in excess by spray-dried blood cells. Furthermore, research that utilized little to no spray-dried blood cells estimated the SID Ile:Lys requirement between 51 and 54% (Waguespack et al., 2012; Htoo et al., 2014; Soumeh et al., 2014). More recently, Htoo et al. (2017) confirmed that the optimal SID Ile:Lys ratio increases from 54% to 58% as the dietary Leu:Lys ratio increases from 110 to 160% in the eight to 21 kg pig. This research continues to suggest that the Ile:Lys requirement needs to be adjusted according to dietary Leu concentrations. However, dietary Leu concentration might not be the only factor affecting the Ile:Lys requirement. Research by Zier-Rush et al. (2018) indicated that the SID Ile:Lys requirement may be closer to 60 to 61% for the late finishing pig which is in agreement with the empirical estimate of 60 to 62% for the 90 kg barrow by Kendall et al. (2004). This would suggest that the optimal SID Ile:Lys ratio may need to be increased as BW of the pig increases. A study conducted by Kerkaert et al. (2021) indicated that in late finishing, the negative effects of high dietary Leu can be mitigated by high levels of Ile. Therefore, the objective of this study was to evaluate the impact of increasing the SID Ile:Lys ratio in swine diets containing 20% DDGS on late finishing pig performance and carcass characteristics.

MATERIALS AND METHODS

The South Dakota State University Institutional Animal Care and Use committee approved the protocol (2001-002E) used in this study.

A study was conducted to determine the SID Ile requirement of finishing pigs fed diets containing DDGS. The study was performed at a commercial research facility located in southwestern Minnesota. The barn was a 2,400-hd double long, curtain sided building with slatted flooring. Each pen (3.2 m x 5.6 m) contained a 4-slot stainless steel dry feeder (Hog Slat Inc., Newton Grove, NC) and two cup waterers allowing for ad libitum access to feed and water. Feed was delivered daily by an automated feeding system (DryExact Pro; Big Dutchman Inc., Holland, MI) which is capable of weighing feed, blending diets, and delivering feed to individual pens. Prior to the start of the study pigs were fed a common diet, comprised of a corn-SBM based diet containing 20% DDGS and provided nutrients at or above NRC (2012) recommendations.

A total of 2,268 pigs (DNA 600 x TopigsNorsvin TN70) were used in a 56 d finishing study. Pens were stocked with 26 or 27 pigs with approximately equal number of barrows and gilts. There were 14 replicate pens per treatment and pens were blocked based on pen location within the barn. Pens were assigned to one of six dietary treatments, partially balancing for previous treatment. Dietary treatments consisted of a corn-SBM diet (CS) or diets containing 20% DDGS with a SID Ile:Lys ratio of 55, 60, 65, 70, or 75%. All diets were provided in the meal form and were fed in two phases with the dietary phase changed occurring on day 28. Three main diets were milled, which included the CS diet and two diets containing 20% DDGS with a SID Ile:Lys ratios of 55 and 75%. The two DDGS diets were blended on site using the automated feeding system to achieve the other DDGS treatments with a SID Ile:Lys ratios of 60, 65, and 70%. Diets were formulated to supply Lys at 95% of requirement (PIC, 2016) to ensure that the Ile requirement was not underestimated. All diets were formulated to contain similar dietary NE and SID Lys concentrations within phase. The addition of crystalline L-Ile was utilized to achieve the 75% SID Ile:Lys ratio. Crystalline Ile and Val were measured out by hand and delivered to the mill to ensure accurate inclusion rates.

Diet samples were collected from every batch milled and delivered for both dietary phases. Samples were stored in a freezer (-20°C) until subsamples were pooled together and sent for analysis. The University of Missouri Chemical Laboratories (University of Missouri, Columbia MO) determined the complete AA, CP, fat, and fiber contents of the diets. Dietary samples for the three milled diets were pooled together and sent to the North Dakota State University Veterinary Diagnostic Laboratory (North Dakota State University, Fargo ND) for analysis of mycotoxins.

Weigh periods occurred on days 0, 14, 28, 42, and 56 to calculate ADG, ADFI, and G:F. Pigs were weighed by pen via pen scale. Feed disappearance was determined by feed delivery data reported by the automated feeding system minus the feed amount remaining int the feeder on the weigh day. The weight of feed remaining in feeders was determined using a regression equation which utilized feed height in calculation. Two regression equations were developed to account of differences in feed density between the CS diet and diets containing DDGS. Groups of pigs were marketed in two cuts, with the initial cut occurring on day 28 of the study and remaining pigs marketed on day 56. Pen inventory was standardized within block during initial cut which represented approximately 15% of pen inventory. Prior to shipment to the commercial abattoir, pigs selected for market were weighted by pen groups via pen scale. At the commercial abattoir, HCW, BF measured with a Fat-O-Meater, and percent lean were recorded for every pig.

Data was analyzed as a randomized complete block design with pen as the experimental unit and block as a random factor. Previous treatment was included in the model to test for any interaction between previous and current treatments. Analysis failed to detect any significant interactions between previous and current treatments. However, analysis did reveal that previous treatment was significant for initial BW. Therefore, initial BW was utilized as a covariate for remaining statistical analyzes. This model was determined to be the best based of statistical model comparison using the BIC fit statistic (Milliken and Johnson, 2009). Analysis of variance was performed using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Single degree of freedom orthogonal polynomials were used to evaluate SID Ile:Lys dose response in diets containing DDGS. Contrast coefficients for single degree of freedom orthogonal polynomials were based on equally spaced treatments. Pair-wise comparisons were used to evaluate treatment response of the CS diet relative to diets containing DDGS with differing SID Ile:Lys ratios. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.10 \geq P \leq 0.05$.

RESULTS

The analysis of dietary treatments verified that CP, fat, fiber, and free levels of Lys, Trp, Thr, Met, Val, Ile, and Leu were within five to ten percent of the expected dietary formulated values. The nutrient profile of major feed ingredients utilized in diet formulation were supplied by Cargill which represented a historical profile of feed ingredients from specific manufacturing sources that were used in this study.

Pigs fed the CS diet had greater ADFI ($P \leq 0.004$) then pigs fed diets containing 20% DDGS from 0 to 14 d and tended to have greater ADG ($P=0.053$) than pigs fed DDGS diets with different SID Ile:Lys ratios except for DDGS diets containing 60% SID Ile:Lys during the 14 to 28 d period. There was no impact of dietary treatment on other interim growth performance measurements ($P \geq 0.101$). However, pigs that received the CS diet had greater ADG and ADFI ($P \leq 0.033$) from 0 to 28 d when compared to pigs fed diets containing 20% DDGS. From 0 to 42 d, pigs that consumed the CS diet had similar ADG ($P=0.120$) to pigs that consumed DDGS diets containing 60% SID Ile:Lys and similar ADFI ($P \geq 0.060$) to pigs fed DDGS diets with a SID Ile:Lys ratio of 60 and 55%. The BW of CS fed pigs was heavier ($P \leq 0.05$) than pigs receiving DDGS diets at day 28 but similar ($P=0.167$) to 42 d BW of the 60% SID Ile:Lys DDGS treatment. A performance response to increasing the SID Ile:Lys ratio in diets containing 20% DDGS was not detected ($P \geq 0.153$) in any of the periods observed.

Overall (0 to 56 d), pigs fed the CS diet had greater ADFI ($P \leq 0.010$) than 20% DDGS diets that contained a SID Ile:Lys ratio of 65 and 75%. Pigs that received the CS diet tended to have greater ADG ($P=0.084$) compared to 55 and 75% SID Ile:Lys DDGS diets but dietary treatment did not impact ($P=0.427$) cumulative feed efficiency. Final BW of pigs were similar ($P \geq 0.060$) between the CS diet and DDGS diets containing a SID Ile:Lys ratio of 60 and 70%. Increasing the SID Ile:Lys ratio in diets containing 20% DDGS did not impact performance response ($P \geq 0.188$) for the cumulative period.

Pigs that consumed the CS diet had greater HCW ($P \leq 0.031$) than pigs that consumed DDGS diets containing a SID Ile:Lys ratios of 55, 65, 70, or 75% while the HCW of DDGS diets with a SID Ile:Lys ratio of 60% was intermediate (Table 4.2). Increasing the SID Ile:Lys ratio did not impact the HCW of pigs fed diets containing 20% DDGS (Table 4.3). The backfat of CS fed pigs was greater ($P=0.018$) then the backfat of pigs fed DDGS diets with a SID Ile:Lys ratio of 65%. Increasing the SID Ile:Lys ratio in 20% DDGS diet decreased (Quadratic, $P=0.014$) backfat and increased (Quadratic, $P=0.029$) loin depth with the 65% SID Ile:Lys ratio providing the lowest back fat and greatest loin depth. Pigs fed the CS diet had the greatest ($P \leq 0.019$) loin depth compared to all other dietary treatments. Increasing the SID Ile:Lys ratio in diets containing 20% DDGS tended to increase (Quadratic, $P=0.076$) percent lean and a ratio of 65% SID Ile:Lys provided the greatest percent lean.

DISCUSSION

The design of a nutrient requirement study general encompasses two treatment levels below and above the theoretical requirement allowing to quantify the response curve. The NRC (2012) estimates the SID Ile:Lys requirement at 53.4% for the 75 to 100 kg pig and 54.1% for the 100 to 135kg. The major ingredients in this study did not allow for a lower titration level than 55% SID Ile:Lys without reduction in dietary DDGS inclusion levels. That is why the large majority of the early research on the Ile requirement was conducted utilizing spray-dried blood cells due to the ease of formulating Ile deficient diets (NRC, 1998). While the inclusion of spray-dried blood cells in diets make excellent diets for studying the Ile requirement and BCAA interaction, concerns with extrapolation of these results to more practical industry diets are relevant

(Kendall et al., 2004). Therefore, understanding the Ile requirement in finishing diets that included 20% DDGS is necessary to maintaining adequate performance and profitability.

The research on the Ile requirement is somewhat variable and appears to be a multifaceted equation that includes both concentrations of the other BCAA and the BW of pigs. Previous research has demonstrated that the dietary Leu concentration can impact the optimal Ile requirement for growth performance (Wiltafsky et al., 2009a). More recent research by Htoo et al. (2017) showed that when the Leu:Lys ratio increased from 110 to 160%, the optimal Ile:Lys ratio changed from 54 to 58%. In the current study, diets provided 161 and 170% SID Leu:Lys (Tables 4.1 & 4.2). Based off the research of Htoo et al. (2017), the optimal SID Ile:Lys ratio in our study would be approximately 58%. While linear or quadratic trends were not detected in this study (Table 4.5), providing and SID Ile:Lys ratio of 60% in diets containing 20% DDGS resulted in the greatest numerical ADG until 42 d (Table 4.4). However, during the last 14 d (42 to 56 d) supplying a SID Ile:Lys ratio of 70% resulted in the greatest numerical ADG (Table 4.4). This could be potentially be explained due to pigs being at a heavier BW, as previous research has suggested that optimal SID Ile:Lys ratio is closer to 60 or 61% for the late finishing pig (Kendall et al., 2004; Zier-Rush et al., 2018). The combination of excess dietary SID Leu:Lys at 170% in the late finishing diet (Table 4.2) and pigs being a heavier BW could explain why 70% SID Ile:Lys ratio resulted in maximize numerical performance during the last 14 d. The inability to detect linear or quadratic trends in this study was most likely a result of unexpected performance of the SID 65% Ile:Lys treatment group (Table 4.5). Due to this, a more precise estimate of the optimal SID Ile:Lys requirement could not be detected. However, the SID Ile:Lys ratio of 60% early and 70% late provided the maximum numerical ADG. These ratios are both greater than the current NRC (2012) recommendations but the 60% SID Ile:Lys ratio is in reasonable agreement with the research of Kendall et al. (2004), Htoo et al. (2014), and Zier-Rush et al. (2018). Further research is warranted to understand if the optimal SID Ile:Lys ratio for growth performance in the last dietary phases prior to marketing is closer to 70% when dietary concentrations of Leu:Lys are in excess.

During the first 28d, pigs that received the CS diet had greater ADG and ADFI compared to diets containing 20% DDGS (Table 4.4). The difference in ADFI between the CS diet and diets containing 20% DDGS could be explained by feed intake reaching physical capacity due to the bulk volume of feed in the intestinal tract (Nyachoti et al., 2004; Li and Patience, 2017). This reduction in ADFI explains the reduction in ADG observed for this period. The decrease in ADFI could also be a result of feed contaminants such as mycotoxins. However, as seen in Table 4.3, the mycotoxin concentrations in these complete diets are lower than concentrations known to negatively affect performance (Accensi et al., 2006; Ensley and Radke, 2019). Overall (0 to 56 d), pigs receiving the CS diet had greater intakes compared to DDGS diets with a SID Ile:Lys of 65 and 75%. The lower ADFI of the 75% SID Ile:Lys treatment group could be due to Ile being supplied in excess but there is not a great explanation for the lower intake of the 65% SID Ile:Lys group (Li and Patience, 2017).

While not able to detect linear and quadratic trends in response to increasing the SID Ile:Lys ratio in 20% DDGS diets for performance criteria, they were detected in carcass characteristics (Table 4.7). Increasing the SID Ile:Lys ratio in DDGS diet led to a quadratic decrease in backfat and quadratic increase in loin depth with the optimal SID

Ile:Lys level being the 65% treatment group (Table 4.7). This improvement in carcass traits would suggest that the SID Ile:Lys requirement is closer to 65% when feeding 20% DDGS during the finishing period and is in reasonable agreement with the work of Kendall et al. (2004) and Zier-Rush et al. (2018) when using carcass parameters to define the SID Ile:Lys requirement. However, results from Dean et al. (2005) showed no impact of increasing the Ile level in late finishing diets with the exception a linear increase in kg of fat free lean. Therefore, utilizing carcass characteristics to define the optimal SID Ile:Lys ratio requires an increase in published literature to ensure repeatability of results. Compared to the CS treatment group, diets containing DDGS had lighter HCW and lower loin depths with the exception of the 60% SID Ile:Lys treatment group (Table 4.6). This was a result of pigs fed the CS diet having the heaviest final BW, as seen in Table 4.4.

In conclusion, this data suggests that when feeding 20% DDGS, the optimal SID Ile:Lys ratio may need to be increased from 60% to 70% over the course of the finishing period for maximum growth performance. However, when attempting to maximize the carcass characteristics of pigs at harvest, providing a SID Ile:Lys ratio of 65% would be optimal in 20% DDGS diet. Feeding a corn-SBM diet will still allow pigs to outperform pigs fed diets containing 20% DDGS regardless of SID Ile:Lys ratio during the initial finishing period. More research is required with DDGS and Ile to accurately define the optimal SID Ile:Lys ratio in finishing swine diets.

Table 4.1. Dietary Ingredient and Calculated Nutrient Composition of the Common Diet and Phase One (0 to 28 d)

Item:	Common[1]	SID Val:Lys, %		CS
		60	80	
Ingredients, %				
Corn	62.45	72.52	72.38	85.15
Soybean meal	14.18	3.66	3.66	11.85
DDGS	20.00	20.00	20.00	-
Choice white grease	0.75	1.20	1.20	0.35
Calcium carbonate	1.09	1.05	1.05	0.87
Salt	0.48	0.41	0.41	0.57
Monophosphate 21%	-	-	-	0.31
VTM premix[2]	0.10	0.10	0.10	0.10
Magnesium oxide 54%	0.30	0.30	0.30	0.30
Lysine HCL	0.42	0.46	0.46	0.31
L-Threonine	0.13	0.13	0.13	0.11
L-Methionine	0.05	0.03	0.03	0.07
L-Tryptophan	0.05	0.07	0.07	0.02
L-Valine	-	0.07	0.07	-
L-Isoleucine	-	-	0.15	-
Calculated Analysis				
NE, Kcal/kg	2,384	2,464	2,464	2466
CP, %	16.87	12.83	12.96	11.7
NDF, %	10.47	10.90	10.89	6.37
Ca, %	0.50	0.45	0.45	0.45
P, %	0.38	0.33	0.33	0.33
Available P, %	0.30	0.28	0.28	0.27
SID Amino Acid, %				
Lys	0.95	0.73	0.73	0.73
Met:Lys	33	32	32	34
(Met+Cys):Lys	59	58	58	58
Thr:Lys	66	67	67	64
Trp:Lys	21	21	21	18
Val:Lys	72	78	78	67
Ile:Lys	61	55	75	56.5
Leu:Lys	150	161	161	133
Ile:Leu	41	34	47	42
(Val+Ile):Leu	89	83	95	93

[1] Common diet was fed from -14d to 0d

[2] Provided per kilogram of the diet: 600 FTU Axtra Phytase Gold 65G, 60.4 g Danisco® Xylanase 60000 G, 2,527 IU vitamin A, 1,134 IU vitamin D3, 32.4 IU vitamin E, 0.97 mg vitamin K3, 32.4 mg niacin, 13.2 mg pantothenic acid, 4.5 mg riboflavin, 21 ug vitamin B12, 40 mg Mn from manganous oxide, 50 mg Zn from zinc hydroxychloride, 100 mg Fe from ferrous sulfate, 10 mg Cu from copper sulfate, 1.0 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 4.2. Dietary Ingredient and Calculated Nutrient Composition of Phase Two (28 to 56 d)

Item:	SID Val:Lys, %		CS
	60	80	
Ingredients, %			
Corn	74.89	74.76	86.42
Soybean meal	1.42	1.42	10.86
DDGS	20.00	20.00	-
Choice white grease	1.25	1.25	0.40
Calcium carbonate	0.94	0.94	0.81
Salt	0.41	0.41	0.57
Monophosphate 21%	-	-	0.15
VTM premix[1]	0.10	0.10	0.10
Magnesium oxide 54%	0.30	0.30	0.30
Lysine HCL	0.44	0.44	0.25
L-Threonine	0.13	0.13	0.09
L-Methionine	0.01	0.01	0.04
L-Tryptophan	0.06	0.06	0.01
L-Valine	0.05	0.05	-
L-Isoleucine	-	0.13	-
Calculated Analysis			
NE, Kcal/kg	2,481	2,481	2,479
CP, %	11.9	12.02	11.23
NDF, %	10.91	10.90	6.39
Ca, %	0.40	0.40	0.40
P, %	0.33	0.33	0.30
Available P, %	0.27	0.27	0.24
SID Amino Acid, %			
Lys	0.66	0.66	0.66
Met:Lys	30	30	32
(Met+Cys):Lys	58	58	58
Thr:Lys	69	69	66
Trp:Lys	21	21	18
Val:Lys	78	78	72
Ile:Lys	55	75	60
Leu:Lys	170	170	144
Ile:Leu	32	44	42
(Val+Ile):Leu	78	90	91

[1] Provided per kilogram of the diet: 600 FTU Axtra Phytase Gold 65G, 60.4 g Danisco® Xylanase 60000 G, 2,527 IU vitamin A, 1,134 IU vitamin D3, 32.4 IU vitamin E, 0.97 mg vitamin K3, 32.4 mg niacin, 13.2 mg pantothenic acid, 4.5 mg riboflavin, 21 ug vitamin B12, 40 mg Mn from manganous oxide, 50 mg Zn from zinc hydroxychloride, 100 mg Fe from ferrous sulfate, 10 mg Cu from copper sulfate, 1.0 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 4.3. Mycotoxin concentrations of dietary treatments (as-fed basis, ug/kg)[1]

Item	SID Ile:Lys, %		CS
	55	75	
Alfatoxin B1	< 20	< 20	< 20
Alfatoxin B2	< 20	< 20	< 20
Alfatoxin G1	< 20	< 20	< 20
Alfatoxin G2	< 20	< 20	< 20
Fumonisin B1	< 200	< 200	< 200
Fumonisin B2	< 200	< 200	< 200
HT-2 Toxin	< 200	< 200	< 200
T-2 Toxin	20	< 20	< 20
Ochratoxin A	< 20	< 20	< 20
Sterigmatocystin	< 20	< 20	< 20
Zearalenone	< 100	< 100	< 100
Vomitoxin (DON)	540	571	308

[1] Representative samples of each dietary treatment for both phases were pooled and analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) by LC/MS/MS assay.

Table 4.4. Performance response of finishing pigs fed differing levels of SID Ile:Lys in diets containing 20% DDGS compared to pigs fed a corn-SBM diet.

Item:	SID Ile:Lys, %					CS	SEM	Diet
	55	60	65	70	75			
BW, kg								
Initial	82.4	82.6	82.7	82.3	81.7	82.3	0.64	-
d 14	95.7	95.8	95.7	95.7	95.6	96.3	0.28	0.172
d 28	107.8^{b}	108.0^{b}	107.6^{b}	107.8^{b}	107.5^{b}	108.8^{a}	0.41	0.030
d 42	119.5^{bc}	120.1^{ab}	118.9^{c}	119.6^{bc}	119.0^{bc}	121.0^{a}	0.60	0.012
Final	131.0^{bc}	131.3^{abc}	129.4^{d}	131.6^{ab}	130.1^{cd}	132.6^{a}	0.69	< 0.001
d 0 to 14								
ADG, kg	0.95	0.96	0.96	0.95	0.94	0.99	0.019	0.173
ADFI, kg	2.88^{b}	2.90^{b}	2.89^{b}	2.87^{b}	2.84^{b}	3.00^{a}	0.034	< 0.001
G:F	0.331	0.330	0.332	0.331	0.333	0.331	0.0044	0.986
d 14 to 28								
ADG, kg	0.89^{y}	0.90^{xy}	0.88^{y}	0.89^{y}	0.89^{y}	0.93^{x}	0.017	0.053
ADFI, kg	2.90	2.91	2.86	2.87	2.88	2.97	0.047	0.268
G:F	0.308	0.310	0.307	0.312	0.307	0.314	0.0043	0.485
d 0 to 28								
ADG, kg	0.92^{b}	0.93^{b}	0.92^{b}	0.92^{b}	0.92^{b}	0.96^{a}	0.015	0.028
ADFI, kg	2.89^{b}	2.91^{b}	2.88^{b}	2.87^{b}	2.86^{b}	2.98^{a}	0.034	0.006
G:F	0.319	0.320	0.320	0.322	0.320	0.323	0.0030	0.910
d 28 to 42								
ADG, kg	0.98	1.02	1.00	1.00	0.98	1.01	0.031	0.798
ADFI, kg	3.11	3.11	3.00	3.15	3.05	3.10	0.055	0.101
G:F	0.315	0.327	0.334	0.318	0.323	0.327	0.0088	0.332
d 0 to 42								
ADG, kg	0.94^{b}	0.96^{ab}	0.94^{b}	0.94^{b}	0.94^{b}	0.98^{a}	0.014	0.032
ADFI, kg	2.96^{ab}	2.97^{ab}	2.91^{b}	2.95^{b}	2.91^{b}	3.02^{a}	0.033	0.025
G:F	0.318	0.322	0.324	0.320	0.321	0.324	0.0033	0.456
d 42 to 56								
ADG, kg	0.82	0.79	0.75	0.86	0.79	0.83	0.042	0.163
ADFI, kg	2.99	3.02	2.94	3.02	2.99	3.01	0.064	0.808
G:F	0.274	0.262	0.254	0.284	0.265	0.275	0.0115	0.132
d 0 to 56								
ADG, kg	0.86^{y}	0.90^{xy}	0.88^{xy}	0.89^{xy}	0.86^{y}	0.92^{x}	0.024	0.084
ADFI, kg	2.96^{ab}	2.98^{ab}	2.92^{b}	2.97^{ab}	2.93^{b}	3.01^{a}	0.032	0.048
G:F	0.290	0.302	0.300	0.299	0.294	0.306	0.0078	0.427

$^{a\text{-}c}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

$^{x\text{-}y}$ Means within a row lacking common superscript tend to differ significantly, $P \leq 0.05$

Table 4.5. Dose response of swine fed increasing the SID Ile:Lys ratio in diets containing 20% DDGS during the finishing period.

Item:	SID Ile:Lys, %							
	55	60	65	70	75	SEM	Linear	Quadratic
BW, kg								
Initial	82.4	82.6	82.7	82.3	81.7	0.66	-	-
d 14	95.7	95.8	95.7	95.7	95.6	0.22	0.569	0.695
d 28	107.8	108.0	107.6	107.8	107.5	0.34	0.513	0.719
d 42	119.5	120.1	118.9	119.6	119.0	0.55	0.353	0.817
Final	131.0	131.3	129.4	131.6	130.1	0.61	0.389	0.683
d 0 to 14								
ADG, kg	0.95	0.96	0.96	0.95	0.94	0.015	0.701	0.549
ADFI, kg	2.88	2.90	2.89	2.87	2.84	0.027	0.229	0.222
G:F	0.331	0.330	0.332	0.331	0.333	0.003	0.610	0.761
d 14 to 28								
ADG, kg	0.89	0.90	0.88	0.89	0.89	0.023	0.583	0.981
ADFI, kg	2.90	2.91	2.86	2.87	2.88	0.042	0.477	0.640
G:F	0.308	0.310	0.307	0.312	0.307	0.005	0.997	0.551
d 0 to 28								
ADG, kg	0.92	0.93	0.92	0.92	0.92	0.014	0.544	0.678
ADFI, kg	2.89	2.91	2.88	2.87	2.86	0.028	0.272	0.772
G:F	0.319	0.320	0.320	0.322	0.320	0.003	0.720	0.719
d 28 to 42								
ADG, kg	0.98	1.02	1.00	1.00	0.98	0.024	0.864	0.368
ADFI, kg	3.11	3.11	3.00	3.15	3.05	0.042	0.444	0.625
G:F	0.315	0.327	0.334	0.318	0.323	0.007	0.774	0.154
d 0 to 42								
ADG, kg	0.94	0.96	0.94	0.94	0.94	0.012	0.528	0.316
ADFI, kg	2.96	2.97	2.91	2.95	2.91	0.027	0.232	0.987
G:F	0.318	0.322	0.324	0.320	0.321	0.003	0.749	0.153
d 42 to 56								
ADG, kg	0.82	0.79	0.75	0.86	0.79	0.034	0.967	0.521
ADFI, kg	2.99	3.02	2.94	3.02	2.99	0.044	0.977	0.907
G:F	0.274	0.262	0.254	0.284	0.265	0.010	0.876	0.457
d 0 to 56								
ADG, kg	0.86	0.90	0.88	0.89	0.86	0.018	0.979	0.188
ADFI, kg	2.96	2.98	2.92	2.97	2.93	0.025	0.317	0.971
G:F	0.290	0.302	0.300	0.299	0.294	0.006	0.709	0.175

Table 4.6. Carcass characteristics of pigs fed differing levels of SID Ile:Lys in diets containing 20% DDGS compared to pigs fed a corn-SBM diet.

Item:	SID Ile:Lys, %					CS	SEM	Diet
	55	60	65	70	75			
HCW, kg	94.2^{b}	94.6^{ab}	94.0^{b}	94.4^{b}	93.9^{b}	95.5^{a}	0.53	0.040
Backfat, mm	16.5^{a}	16.4^{a}	15.8^{b}	16.0^{ab}	16.3^{a}	16.3^{a}	0.22	0.031
Loin Depth, mm	58.0^{cd}	57.9^{d}	58.7^{b}	58.5^{bc}	58.0^{cd}	59.4^{a}	0.30	< 0.001
Percent Lean, %	53.4^{y}	53.4^{y}	53.7^{x}	53.6^{xy}	53.5^{xy}	53.6^{xy}	0.14	0.057

$^{a\text{-}d}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

$^{x\text{-}y}$ Means within a row lacking common superscript tend to differ significantly, $P \leq 0.05$

Table 4.7. Dose response of pigs fed increasing SID Ile:Lys ratios in diets containing 20% DDGS on carcass characteristics.

Item:	SID Ile:Lys, %							
	55	60	65	70	75	SEM	Linear	Quadratic
HCW, kg	94.2	94.6	94.3	94.3	93.9	0.19	0.428	0.525
Backfat, mm	16.5	16.4	15.8	16.0	16.3	0.20	0.162	0.014
Loin Depth, mm	58.0	57.9	58.7	58.5	58.0	0.21	0.404	0.029
Percent Lean, %	53.4	53.4	53.7	53.6	53.5	0.12	0.131	0.076

CHAPTER 5

IMPACT OF HIGH PROTEIN DRIED DISTILLER GRANS AND SOYBEAN MEAL INCLUSION LEVEL ON GROW-FINISH PIG PERFORMACNE AND CARCASS TRAITS

ABSTRACT

A total of 1,170 pigs (PIC 359 x PIC, initial BW 59.5 kg) were used in a 79 d grow-finish study to determine how high protein dried distillers grains (HPDDG; NexPro® protein ingredient, Flint Hills Resources, Wichita, KS) and SBM inclusion level when adjusting BCAA ratios effect grow-finish pig performance and carcass characteristics. Pen of pigs were allotted to one of five dietary treatments which consisted of 1) corn-SBM diet, 2) diet containing HPDDG with an SID Ile:Lys ratio of 56%, or diets containing HPDDG with a SID Val:Lys and Ile:Lys ratios of 75 and 65% met through the inclusion of 3) SBM, 4) 50% SBM and 50% crystalline AA blend, or 5) crystalline AA. The HPDDG were included in diets at 15% in phase one and 10% in phases two and three. Data was analyzed as a randomized complete block design. Pair-wise comparisons were used to evaluate the impact of dietary treatment on pig growth performance and carcass traits. Single degree of freedom orthogonal polynomials were used to evaluate dose response of SBM in HPDDG diets where SID Val:Lys and Ile:Lys ratios were held at 75 and 65%. A difference in cumulative ADG, ADFI, G:F, final BW, or carcass traits was not detected ($P \geq 0.118$) due to diet except for dressing percentage (P=0.040). The reduction in SBM in HPDDG diets where SID Val and Ile were held constant relative to Lys decreased (Linear, $P \leq 0.046$) cumulative ADG and G:F and tended to reduce (Linear, $0.094 \geq P \geq 0.065$) final BW, dressing percent, standardized fat free lean, and back fat (Quadratic, P=0.075) while not impacting hot carcass weight

(*P*=0.142). This data indicates HPDDG is a suitable feedstuff for grow-finish swine diets at low dietary inclusion levels due to minimal impact on performance and carcass characteristics. When adjusting BCAA ratios to alleviate the negative impacts of excess dietary Leu, utilizing SBM provided a greater benefit compared to crystalline AA.

INTRODUCTION

Conventional dried distillers grains with solubles (DDGS) is a commonly utilized co-product of the ethanol industry in swine diets. Recent advances in processing methods have been developed to increase the efficiency ethanol production and result in the production of high protein dried distillers grains with solubles (HPDDGS) (NRC, 2012; Sekhon et al., 2015). The variation of processing methods, type of yeast utilized in fermentation, complexity of the dry-grind process, and quantity of solubles added back have led to differences in the nutrient composition of HPDDG (Liu, 2011). The NRC (2012) nutrient composition of HPDDG was derived from the early studies of Widmer et al. (2008), Kim et al. (2009), and Jacela et al. (2010) which utilized HPDDG produced from the old front-end fractionation method. Recent research has indicated that the new generation of HPDDG have higher CP, digestible and metabolizable energy, and digestibility of various nutrients compared to conventional DDGS (Rho et al., 2017; Espinosa and Stein, 2018). These aspects may prove to be beneficial to pig performance, however, more research is required to validate the feeding value of HPDDG during the growing and finishing periods.

Early work by Widmer et al. (2008) indicated that the inclusion of HPDDGS up to 40% of the diet resulted in a linear reduction of ADG, ADFI, and final BW during the growing period. However, the inclusion of HPDDGS did not impact growth performance of pigs during the finishing period (>58 kg BW) or the overall cumulative period (Widmer et al., 2008). Kim et al. (2009) showed that HPDDGS can replace 100% of the dietary SBM during the growing and finishing periods with no impact on performance or carcass traits provided that the AA Lys, Thr, and Trp were balanced. Similarly, results from Gutierrez et al. (2014) demonstrated that HPDDGS could be fed up to 30% with no impact on body composition or the retention of energy, protein, and lipids. However, more recent research using the new generation of HPDDGS showed that feeding 30% HPDDGS negatively affected final BW and cumulative ADG compared to a corn-SBM diet (Yang et al., 2020). Differences in growth performance could be attributed to an imbalance in dietary AA levels. More specifically, an imbalance in the BCAA as a result of high Leu levels due to the greater dietary protein concentrations being comprised of corn protein. An antagonistic relationship exists between the BCAA due to their structural similarity and shared catabolic pathway (Hutson et al., 2005). Therefore, dietary concentrations and ratio the BCAA must be considered when including higher levels of corn protein in swine diets. A unique study conducted by Rao et al. (2020) utilized the predication equation derived by Cemin et al. (2019) to balance for dietary BCAA ratios when evaluating the impact of HPDDGS on pig growth performance. Results from their study showed that increased inclusion of HPDDGS reduced ADFI but improved G:F and caloric efficiency, which was suspected to be a result of differences in energy content of the HPDDGS compared to the expected (103.4 vs 97.3% NE) value of corn. Therefore, indicating that growth performance of pigs can be maintained when high levels of corn protein sources are used by adjusting BCAA ratios based on the predication equation of Cemin et al. (2019). However, little is known on whether the method in which the BCAA ratio are adjusted, intact protein source verses crystalline AA, have an impact on growth performance of pigs. Therefore, a study was conducted to evaluate the impact of HPDDG and SBM inclusion level on growth performance and carcass characteristics of the grow-finish pig.

MATERIALS AND METHODS

The South Dakota State University Institutional Animal Care and Use Committee reviewed and approved the protocol (2008-034E) used in this study.

This study was conducted at the South Dakota State University commercial wean to finish swine research facility. Each pen (3.1 m x 6.9 m) was equipped with a 5-slot stainless steel dry feeder (SDI, Inc., Alexandra, SD) and 2 cup waterers, providing ad libitum access to feed and water. Daily feed rations were delivered to individual pens through a robotic feeding system (FeedPro, Feedlogic ComDel Innovation, Wilmar, MN). A total of 1,170 pigs (PIC 359 X PIC) were used in a 79 d grow-finish study. Pens were stocked with 26 pigs (59.5 ± 0.5 kg initial BW) and sex was balanced within block (13 barrows and 13 gilts). Pens were blocked by previous nursery treatment and dietary treatments were randomly assigned to pens within block. Prior to the start of the study, pigs were fed a common corn-SBM diet that contained 10% HPDDG (NexPro® protein ingredient, Flint Hills Resources, Wichita, KS) and supplied AA above NRC (2012) recommendations in an effort to acclimate pigs to diets containing HPDDG and reduced any prior nutrient deficiencies.

Diets were provided in meal form and were fed in three phases: Phase one was fed from 59.5 to 72.6 kg BW, Phase two was fed from 72.6 to 95.3 kg BW, and Phase three was fed from 95.3 kg BW to market. Dietary treatments consisted: 1) a corn-SBM based diet (CS); 2) a diet containing HPDDG (HP); diets containing HPDDG with a standard ileal digestible (SID) valine (Val) to lysine (Lys) ratio of 75% and isoleucine (Ile) to Lys ratio of 65% met through 3) SBM (HPSBM); 4) 50% SBM and 50% crystalline amino acids (HP50/50); 5) crystalline amino acids (HPAA). The inclusion level of HPDDG was 15% for Phase one and 10% for Phases two and three. Dietary treatments were formulated to contain similar NE, through the inclusion of corn oil, and SID Lys concentrations within phase. The PIC nutrient specifications manual (2016) was used to determine the Lys requirement in this study. Lysine was formulated to 95% of the requirement for maximum protein deposition of the given weight bracket to ensure responses in growth performance were due to changes in AA concentrations. Crystalline Val, Ile, and Trp was weighed out by hand and mixed to create AA premix bags, which were delivered to the feed mill to ensure accurate dietary inclusion rates.

Diet samples were collected from each batch of feed delivered to the unit for all dietary phases. Samples were stored in a freezer (-20°C) until subsamples of each batch were pooled and sent for nutrient analysis. Complete AA, CP, fat, and fiber analyses were performed by the University of Missouri Chemical Laboratories (University of Missouri, Columbia MO).

Pen weights and feed disappearance were measured on d 0, 16, 30, 44, 58, 65, 72, and 79 to calculate ADG, ADFI, and G:F. Feed intake was determined from feed delivery data reported by the automated feeding system and the amount of remaining in each feeder on weigh days. Weight of feed remaining in feeders was calculated using a custom feed density equation which included feed height and feed density in the calculation. Groups of pigs were sent to a commercial abattoir for processing starting on day 58 and every seven days after for three weeks. Prior to shipment to the processing facility, groups of pigs selected for market within pens were weighed via pen scale. Pen inventory was standardized within block over the course of marketing through number of pigs selected for processing. Hot carcass weight and back fat at the 10th rib measured by ruler was collected at the processing facility. These two carcass parameters were used to calculate standardized fat free lean weight by utilizing the equation of Burson and Berg (2001) for unribbed carcasses measured by ruler.

Data was analyzed as a randomized complete block design with pen as the experimental unit. Previous nursery treatment was incorporated into the model as a random blocking factor. Analysis of variance was performed using the GLIMMIX procedure (SAS Inst. Inc., Cary, NC) to conduct pair-wise comparisons. Single degree of freedom orthogonal contrasts were used to evaluate the effects of decreasing SBM in HPDDG diets when SID Val and Ile levels were held constant. Results were considered significant at $P \leq 0.05$ and a tendency at $0.10 \geq P > 0.05$.

RESULTS

Diet analysis verified that levels of fat, fiber, and free Lys, Trp, Val, and Ile levels were within five to ten percent of expected dietary formulated values. The AA values and SID coefficients used for HPDDG were obtained from the manufacturer. Analysis of HPDDG verified nutrient composition of fat, CP, free AA levels of Lys, Iso, Met, Val, Thr, Phe, and Arg were within five to ten percent of nutrition content supplied by the manufacturer (Table 5.1). The nutrient loadings for corn and SBM were supplied by Cargill and represented a historical profile of these feed ingredients from sources specific manufacturers.

From 0 to 30 d, reducing the SBM inclusion in diets with SID Val:Lys and Ile:Lys inclusions held constant at 75% and 65%, respectively, tended to decrease ADG (Linear; P=0.065) with no impact on ADFI (P≥0.609) and decreased G:F (Linear; P=0.001). Pigs fed the HP diet with no adjustments to the SID Val and Ile levels had lower ADG (P≤0.006) compared to pigs consuming the HPSBM and HP50/50 diets. Feed efficiency of the HP fed pigs was reduced (P=0.022) compared to the pigs on the HPSBM treatment, but similar to all other dietary treatments (P≥0.109). Pigs receiving the CS diet had an intermediate ADG between pigs within the HPSBM and HP50/50 treatment groups at the high end and the HP and HPAA treatment groups at the low end. The G:F of the CS fed pigs was greater (P=0.020) than pigs consuming HPAA diets but similar to (P≥0.155) other dietary treatments.

From 30 to 58 d, reducing the dietary concentration of SBM in diets where SID Val and Ile were held constant decreased ADG (Linear; P=0.014), ADFI (Linear; P=0.013) and feed efficiency (Quadratic; P=0.037). Pigs receiving the HP diet with no adjustments in the SID Val and Ile levels had greater ADG (P≤0.016) compared to pigs fed the HP50/50 and HPAA diets but similar ADG (P≥0.169) to the pigs fed the CS and HPSBM dietary treatments. There was a tendency for pigs receiving the HPSBM diet to have greater ADFI (P=0.096) compared to pigs in the HPAA treatment group, while other dietary treatments had intermediate ADFI. Pigs fed the HP50/50 dietary treatment had a lower feed efficiency (P≤0.033) compared pigs receiving the CS, HP, and HPSBM diets, while the HPAA fed pigs had an intermediate feed conversion.

For the cumulative period prior to marketing (0 to 58 d), reducing the SBM inclusion in diets where the SID Val and Ile levels were held constant resulted in a decrease in ADG (Linear; P=0.028) and feed efficiency (Linear; P=0.037) with no impact on ADFI (P≥0.218). There was a tendency for reductions of dietary inclusion of SBM to decrease BW (Linear; P=0.099) at day 58. Pigs receiving the HPSBM dietary treatment tended to have greater ADG (P=0.092) compared to pigs fed the HPAA diet while other dietary treatments had intermediate ADG. Dietary treatment had no impact on ADFI of pigs (P=0.434). The CS and HPSBM treatment groups tended to have greater feed efficiency (P=0.081) compared to pigs fed the HPAA diet, while pigs receiving the HP and HP50/50 diets had intermediate rates of feed conversion.

Over the course of marketing (58 to 79 d), dietary treatment did not impact pig performance (P≥0.403). However, pigs fed the CS diet had numerically greater ADG, ADFI, and rate of feed conversion. Dietary inclusion level of SBM also did not impact performance of pigs (P≥0.554) when SID Val and Ile levels in the diet were held constant.

Overall (0 to 79 d), reductions in the dietary SBM inclusions in diets where SID Val and Ile were held constant resulted in decreased ADG (Linear; P=0.035), no impact on ADFI (P≥0.244), and decreased feed efficiency (Linear; P=0.046). Lower inclusion levels of SBM in diets where SID Val and Ile levels were held constant also tended to decrease final BW (P=0.065). Pigs fed the CS and HP diets had similar final BW (P=0.179), ADG (P=0.130), ADFI (P=0.584), and feed conversation (P=0.160) compared to diets containing HPPDG where the SID Val:Lys and Ile:Lys ratios were held at 75 and 65%.

Dietary treatment did not have an impact on hot carcass weight ($P \geq 0.142$). The reduction in SBM concentration in diets where SID Val and Ile levels were held constant tended to increase carcass yield (Linear; P=0.071) and back fat thickness (Quadratic; P=0.075). Lowering the inclusion level of SBM in diets that maintained a constant level of SID Val and Ile also tended to decrease standardized fat free lean weight (Linear; P=0.094) and percent fat free lean (Quadratic; P=0.081). Pigs fed the CS and HP50/50 diets had greater carcass yield ($P \leq 0.043$) compared to pigs that received the HPSBM diet, while the HP and HPAA fed pigs had intermediate carcass yields. Pigs that received the CS and HP diets had similar back fat thickness (P=0.199), standardized fat free lean weight (P=0.118) and percent fat free lean (P=0.226) compared to pigs that were fed diets containing HPDDG with a SID Val:Lys and Ile:Lys ratio of 75 and 65%.

DISCUSSION

Early research on the use of HPDDG in swine diets have shown HPPDG can replace 50 to 100% of SBM with no impacts to pig performance (Widmer et al., 2008; Kim et al., 2009). More recently, Gutierrez et al. (2014) suggested that HPDDG can be fed at 30% in swine diets and pigs will perform equivalent to that of pigs fed a corn-SBM diet. However, these studies utilized limited number of pigs ($n \leq 84$). For the studies of Kim et al. (2009) and Widmer et al. (2008), pigs were housed individually or in groups of two which is known to impact feed intake and growth performance and potentially result in less repeatable results when applied to an industry setting (Bustamante et al., 1996). While the work of Gutierrez et al. (2014) suggested that 30% HPDDG and corn-SBM diet will results in similar pig performance even though large numerical differences existed between the two treatments which speaks to the precision of their study. In the current study, feeding 15% HPDDG during the first period tended to result in lower ADG compared to the corn-SBM diet when not adjusting the BCAA ratios (Table 5.8). However, supplying Val and Ile at 75% and 65% relative to Lys on a SID basis in HPDDG diets restored performance to that of the corn-SBM diet (Table 5.8).These results demonstrate that growth performance was being restricted by SID Val and Ile levels in the HPDDG diets. Interestingly, the method of supplying these AA in either a protein bound form, or a crystalline AA form influenced growth performance. The reduction in SBM and increased inclusion of crystalline AA resulted in a reduction in ADG and feed efficiency (Table 5.9). Suggesting the reduction in performance was due to the inadequate supply of a certain nutrient and resulted in a reduction in rate of lean tissue deposition and an increase in adipose tissue deposition. In HPDDG diets where SID Val and Ile were held at 75 and 65%, the supply of AA down to the sixth limiting AA were formulated to be held constant in relation to Lys (Tables 5.2, 5.4, & 5.6) . Therefore, the reduction in SBM inclusion would have resulted in a decrease in the supply of Leu, His, Phe, Tyr, and the non-essential AA (Tables 5.3, 5.5, & 5.7).

Histidine has been suggested to be the next limiting AA after the first six (Figueroa et al., 2003). The current NRC (2012) suggests that the His requirement for the growing finishing pig is 34% relative to Lys. In the current study, diets provided a His:Lys ratio at or above 35% and, therefore, His supply should not have affected lean tissue deposition. However, there is potential that current recommendations are inaccurate and that His levels in this study did impact growth performance in this study. More research is required on the His requirement in grow-finish pigs to validate or dispute current recommendations.

The AA Leu has shown to have negative impacts to pig performance in a dose dependent manner when supplied in excess (Wiltafsky et al., 2010). Htoo et al. (2014) has defined Leu to be in excess when supplied above 130% relative to Lys on as SID basis. In all HPDDG diets, Leu would be considered to be in excess for this study. Due to the antagonistic relationship that exists between the BCAA, excess dietary Leu concentrations would have required an increase in Val and Ile supplementation in order to maintain pig growth performance (Harper et al., 1984). Results from the first 30 d of this study confirmed that increased levels of SID Ile and Val were needed to maximize lean tissue deposition (Table 5.8). Results of Kerkaert et al. (2021) showed this in diets containing DDGS and Rao et al. (2020) showed this in diets containing HPDDG. However, this study indicated that utilizing SBM to meet the increased SID Val and Ile ratio provided a benefit over that of crystalline AA (Table 5.9). It could be suggested that the levels of Val and Ile were oversupplied in the HPAA diet relative to Leu and therefore, reducing growth performance. However, and excess of an AA is usually accompanied by a decrease in voluntary feed intake (Li and Patience, 2017). Due to the lack of difference in ADFI, it could be suggested that dietary levels of Val and Ile relative to Leu was not in excess and thus not an influential factor on growth performance of the HPAA dietary treatment.

The BCAA are a subgroup of the LNAA. The LNAA share common transport systems and most notably compete for transport across the BBB (Fernstrom, 2005). It has been demonstrated that excess Leu can reduce other LNAA, such as Trp, transport in to the brain and thus affecting the production of amine neurotransmitters such as serotonin (Fernstrom, 2005; Kwon et al., 2019). The removal of SBM from HPDDG diets resulted in a decrease in the concentrations of LNAA and therefore, the increase in the Trp:LNAA ratio. This would have result in an increase in the production of serotonin leading to an increase feed intake due to the suggested role of serotonin in the feed intake response (Fernstrom, 1985). However, this was not observed in this study. It could be suggested that the increase in Trp:LNAA ratio could have elevated the negative effects of an AA imbalance on voluntary feed intake, resulting in similar intakes across dietary treatments. While this has the potential to explain the ADFI results, it does not explain why reducing SBM resulted a reduction in feed efficiency. The transport system B^0AT1 is a major transporter of the BCAA and other neutral AA in the intestine (Bröer et al., 2004). Therefore, as SBM was removed from HPDDG diets and the BCAA:LNAA ratio increased, the absorption of other LNAA by intestinal enterocytes could have been reduced. This could have leaded to a potential AA deficiency and negatively impact lean tissue deposition.

In summary, the inclusion of HPDDG in grow-finish swine diets at low inclusion had minimal impact on the performance and carcass characteristics of pigs. When adjusting the SID Val and Ile levels to reduce the negative impacts of excess dietary Leu, utilizing SBM provides a benefit over that of crystalline AA. Adjusting the dietary SID Val and Ile levels in the diet to correct for excess Leu concentrations required approximately one percent more SBM for the HPSBM diet. However, due to the experimental design of the study, marginal amounts of L-Ile were required to meet the targeted levels in HPSBM diets. It is hard to propose how pig performance may have been impacted if Val concentrations were allowed to float with ingredient inclusion when targeting a desired Ile level relative to Leu. More research is required to fully understand how methods in meeting optimal BCAA ratios impact pig performance and explain the mechanisms involved.

Table 5.1. Analyzed Nutrient composition of the NexPro® protein ingredient

Sample Date	1 5/25/2020	2 6/29/2020	3 7/2/2020	4 7/6/2020	5 7/13/2020	Avg.
CP, %	51.22	50.34	50.36	50.09	50.15	50.43
Fat, %	2.83	2.81	2.81	2.80	2.95	2.84
NDF, %	29.33	28.1	28.87	27.35	29.34	28.60
ADF, %	10.01	10.04	9.67	9.90	9.97	9.92
Starch, %	ND[1]	ND	ND	0.13	0.35	0.10
NE, Kcal/kg[2]	2,181	2,186	2,189	2,189	2,191	2,187
Free Amino Acids, %						
Taurine	0.09	0.09	0.09	0.08	0.10	0.09
Hydroxyproline	0.13	0.02	0.02	0.03	0.00	0.04
Aspartic Acid	3.62	3.50	3.46	3.48	3.52	3.52
Threonine	2.00	1.89	1.89	1.91	1.92	1.92
Serine	2.28	2.10	2.06	2.13	2.15	2.14
Glutamic Acid	7.88	7.86	7.89	8.04	7.95	7.92
Proline	3.61	3.50	3.54	3.60	3.60	3.57
Lanthionine	0.12	0.11	0.12	0.14	0.11	0.12
Glycine	2.09	2.03	1.97	1.98	1.98	2.01
Alanine	3.58	3.49	3.50	3.56	3.52	3.53
Cysteine	0.97	0.94	0.95	0.94	0.94	0.95
Valine	2.82	2.81	2.77	2.78	2.75	2.79
Methionine	1.14	1.10	1.11	1.12	1.13	1.12
Isoleucine	2.20	2.19	2.19	2.21	2.19	2.20
Leucine	5.78	5.64	5.75	5.86	5.77	5.76
Tyrosine	2.13	1.96	1.99	2.06	2.09	2.05
Phenylalanine	2.67	2.58	2.58	2.63	2.62	2.62
Hydroxylysine	0.04	0.03	0.04	0.03	0.04	0.04
Ornithine	0.04	0.04	0.04	0.04	0.04	0.04
Lysine	1.96	1.66	1.77	1.80	1.79	1.80
Histidine	1.43	1.39	1.37	1.38	1.37	1.39
Arginine	2.51	2.38	2.30	2.30	2.33	2.36
Tryptophan	0.52	0.49	0.48	0.49	0.48	0.49

[1] ND = Not Detected

[2] NE calculated using Noblet et al. 1994, assumed a ME value of 3,504 kcal/kg

[3] Near Infrared Spectroscopy used for analysis of CP, Fat, NDF, and ADF

[4] Free amino acid and starch concentration analyzed by the University of Missouri Chemical Laboratories (University of Missouri, Columbia MO).

Table 5.2. Ingredient composition of the common diet and phase one (59.5 to 72.6 kg BW) dietary treatments

Ingredients, %	Common[1]	Treatment Diets				
		CS	HP	HPSBM	HP50/50	HPAA
Corn	73.47	80.03	78.07	76.91	78.93	80.94
Soybean Meal	13.27	16.35	3.11	4.27	2.14	-
HPDDG[2]	10.00	-	15.00	15.00	15.00	15.00
Corn oil	0.50	0.99	0.81	0.87	0.69	0.50
Monophosphate 21%	0.24	0.64	0.18	0.17	0.19	0.22
Calcium carbonate	1.16	0.83	1.14	1.14	1.14	1.15
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Potassium Carbonate	-	-	0.29	0.25	0.32	0.39
VTM Premix[3]	0.15	0.15	0.15	0.15	0.15	0.15
Lysine HCL	0.46	0.32	0.55	0.51	0.58	0.65
L-Threonine	0.14	0.11	0.14	0.12	0.15	0.18
DL-Methionine	0.05	0.07	-	-	0.01	0.03
L-Tryptophan	0.06	0.02	0.07	0.06	0.08	0.09
L-Valine	-	-	-	-	0.04	0.07
L-Isoleucine	-	-	-	0.06	0.09	0.13

[1] Common diet was fed from -10d to 0d

[2] NexPro® protein ingredient, Flint Hills Resources, Wichita, KS

[3] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 5.3. Calculated nutrient composition (as-fed basis) of the common diet and phase one (59.5 to 72.6 kg BW) dietary treatments

Calculated Analysis	Common[1]	Treatment Diets				
		CS	HP	HPSBM	HP50/50	HPAA
NE, Kcal/kg	2,388	2,458	2,458	2,458	2,458	2,458
CP, %	16.36	13.26	14.37	14.84	13.96	13.09
Ca, %	0.55	0.50	0.50	0.50	0.50	0.50
P, %	0.42	0.42	0.42	0.42	0.42	0.42
Available P, %	0.28	0.28	0.28	0.28	0.28	0.28
Potassium, %	0.566	0.617	0.533	0.532	0.533	0.531
Chlorine, %	0.358	0.342	0.367	0.366	0.367	0.367
Sodium, %	0.231	0.229	0.234	0.234	0.234	0.234
DEB, meq/kg[2]	144.6	161.0	134.6	134.5	134.8	134.3
Lys:CP, %	5.99	6.34	5.85	5.66	6.02	6.42
SID Amino Acids, %						
Lys	0.98	0.84	0.84	0.84	0.84	0.84
Thr:Lys	65.0	63.0	66.0	66.0	66.0	66.0
Met:Lys	32.6	32.8	30.5	31.1	30.9	31.9
(Met+Cys):Lys	58.0	58.0	58.1	59.2	58.0	58.0
His:Lys	39.5	38.9	39.8	41.1	38.7	36.3
Trp:Lys	21.0	18.0	21.0	21.0	21.0	21.0
Val:Lys	70.8	67.0	72.6	75.0	75.0	75.0
Ile:Lys	59.0	58.1	56.0	65.0	65.0	65.0
Leu:Lys	145.6	131.0	160.7	164.2	157.7	151.2
(Val+Ile):Leu	89.2	95.6	80.0	85.3	88.8	92.6
Val:Leu	48.6	51.2	45.2	45.7	47.6	49.6
Ile:Leu	40.5	44.4	34.8	39.6	41.2	43.0
Trp:LNAA[3]	5.03	4.63	4.86	4.65	4.81	4.97
BCAA:LNAA[4]	65.96	65.90	66.93	67.37	68.13	68.94

[1] Common diet was fed from -10d to 0d

[2] Dietary Electrolyte Balance = [(Na*10,000/23) +(K*10,000/39)] / (Cl *10,000/35.4)

[3,4] LNAA = (Val+Ile+Leu+Trp+Phe+Tyr), BCAA = (Val+Ile+Leu)

Table 5.4. Ingredient composition of phase two (72.6 to 95.3 kg BW) dietary treatments

Ingredients, %	CS	HP	HPSBM	HP50/50	HPAA
Corn	83.53	81.71	80.31	82.86	85.40
Soybean Meal	13.00	4.48	5.89	3.19	0.50
HPDDG[1]	-	10.00	10.00	10.00	10.00
Corn oil	0.83	0.88	0.97	0.74	0.50
Monophosphate 21%	0.66	0.35	0.34	0.37	0.41
Calcium carbonate	0.84	1.05	1.05	1.05	1.06
Salt	0.50	0.50	0.50	0.50	0.50
Potassium Carbonate	-	0.23	0.18	0.28	0.37
VTM Premix[2]	0.15	0.15	0.15	0.15	0.15
Lysine HCL	0.32	0.46	0.42	0.50	0.59
L-Threonine	0.10	0.12	0.11	0.14	0.18
DL-Methionine	0.04	-	-	0.01	0.03
L-Tryptophan	0.02	0.06	0.05	0.07	0.08
L-Valine	-	-	-	0.05	0.09
L-Isoleucine	-	-	0.04	0.09	0.14

[1] NexPro® protein ingredient, Flint Hills Resources, Wichita, KS

[2] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 5.5. Calculated nutrient composition (as-fed basis) of phase two (72.6 to 95.3 kg BW) dietary treatments

Calculated Analysis	CS	HP	HPSBM	HP50/50	HPAA
NE, Kcal/kg	2,464	2,464	2,464	2,464	2,464
CP, %	11.91	12.76	13.33	12.23	11.13
Ca, %	0.50	0.50	0.50	0.50	0.50
P, %	0.41	0.42	0.42	0.42	0.42
Available P, %	0.28	0.28	0.28	0.28	0.28
Potassium, %	0.557	0.526	0.526	0.527	0.525
Chlorine, %	0.343	0.359	0.359	0.359	0.359
Sodium, %	0.229	0.232	0.232	0.233	0.233
DEB, meq/kg[1]	145.6	134.7	134.5	134.8	134.4
Lys:CP, %	6.34	5.92	5.66	6.17	6.78
SID Amino Acids, %					
Lys	0.755	0.755	0.755	0.755	0.755
Thr:Lys	63.0	66.0	66.0	66.0	66.0
Met:Lys	31.6	30.1	30.9	30.4	31.9
(Met+Cys):Lys	58.0	58.3	59.8	58.0	58.0
His:Lys	39.1	40.1	41.9	38.5	35.1
Trp:Lys	18.0	21.0	21.0	21.0	12.0
Val:Lys	67.0	71.8	75.0	75.0	75.0
Ile:Lys	56.9	56.0	65.0	65.0	65.0
Leu:Lys	134.7	157.6	162.3	153.2	144.1
(Val+Ile):Leu	92.0	81.1	86.3	91.4	97.2
Val:Leu	49.7	45.6	46.2	49	52.1
Ile:Leu	42.3	35.5	40.1	42.4	45.1
Trp:LNAA[2]	4.61	4.92	4.67	4.90	5.14
BCAA:LNAA[3]	66.28	66.89	67.26	68.34	69.52

[1] Dietary Electrolyte Balance = [(Na*10,000/23) +(K*10,000/39)] / (Cl *10,000/35.4)

[2,3] LNAA = (Val+Ile+Leu+Trp+Phe+Tyr), BCAA = (Val+Ile+Leu)

Table 5.6. Ingredient composition of phase three (95.3 kg BW to market) dietary treatments

Ingredients, %	CS	HP	HPSBM	HP50/50	HPAA
Corn	86.25	84.40	83.51	84.91	86.30
Soybean Meal	10.68	2.05	2.93	1.47	-
HPDDG[1]	-	10.00	10.00	10.00	10.00
Corn oil	0.63	0.70	0.75	0.63	0.50
Monophosphate 21%	0.58	0.27	0.26	0.28	0.30
Calcium carbonate	0.77	0.98	0.97	0.98	0.98
Salt	0.50	0.50	0.50	0.50	0.50
Potassium Carbonate	-	0.31	0.28	0.33	0.38
VTM Premix[2]	0.15	0.15	0.15	0.15	0.15
Lysine HCL	0.30	0.46	0.43	0.48	0.53
L-Threonine	0.09	0.12	0.11	0.13	0.15
DL-Methionine	0.03	-	-	-	-
L-Tryptophan	0.02	0.05	0.05	0.06	0.06
L-Valine	-	-	-	0.03	0.05
L-Isoleucine	-	-	0.05	0.07	0.10

[1] NexPro® protein ingredient, Flint Hills Resources, Wichita, KS

[2] Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Table 5.7. Calculated nutrient composition (as-fed basis) of phase three (95.3 kg BW to market) dietary treatments

Calculated Analysis	CS	HP	HPSBM	HP50/50	HPAA
NE, Kcal/kg	2,468	2,468	2,468	2,468	2,468
CP, %	11.00	11.80	12.15	11.55	10.96
Ca, %	0.45	0.45	0.45	0.45	0.45
P, %	0.39	0.39	0.39	0.39	0.39
Available P, %	0.26	0.26	0.26	0.26	0.26
Potassium, %	0.516	0.525	0.526	0.525	0.525
Chlorine, %	0.343	0.359	0.359	0.359	0.359
Sodium, %	0.229	0.233	0.232	0.233	0.233
DEB, meq/kg[1]	135.0	134.4	134.4	134.4	134.3
Lys:CP, %	6.18	5.76	5.60	5.89	6.21
SID Amino Acids, %					
Lys	0.68	0.68	0.68	0.68	0.68
Thr:Lys	64.0	67.0	67.0	67.0	67.0
Met:Lys	31.0	33.2	33.8	32.8	31.9
(Met+Cys):Lys	59.0	65.7	66.8	65.0	63.1
His:Lys	40.3	41.9	43.2	41.1	39.1
Trp:Lys	18.0	21.0	21.0	21.0	21.0
Val:Lys	68.7	72.8	75.0	75.0	75.0
Ile:Lys	57.4	56.0	65.0	65.0	65.0
Leu:Lys	141.4	168.8	172.0	166.5	161.0
(Val+Ile):Leu	89.2	76.3	81.4	84.1	87.0
Val:Leu	48.6	43.1	43.6	45.0	46.6
Ile:Leu	40.6	33.2	37.8	39.0	40.4
Trp:LNAA[2]	4.49	4.75	4.56	4.69	4.82
BCAA:LNAA[3]	66.67	67.37	67.80	68.44	69.11

[1] Dietary Electrolyte Balance = [(Na*10,000/23) +(K*10,000/39)] / (Cl *10,000/35.4)

[2,3] LNAA = (Val+Ile+Leu+Trp+Phe+Tyr), BCAA = (Val+Ile+Leu)

Table 5.8. Impact of HPDDG and SBM inclusion on pig growth performance

Item:	Dietary Treatment					SEM	Diet
	CS	HP	HPSBM	HP50/50	HPAA		
BW, kg							
d 0	59.7	59.4	59.4	59.4	59.4	0.48	0.984
d 16	73.3	71.6	73.2	73.2	72.8	0.81	0.223
d 30	87.7	86.8	88.1	88.0	87.0	0.77	0.327
d 44	103.5	102.5	103.4	103.5	102.4	1.14	0.633
d 58	118.4	118.4	119.6	118.4	117.6	1.04	0.454
Final	133.6	132.0	133.3	132.1	130.9	1.18	0.179
d 0 to 16							
ADG, kg	0.85^{x}	0.78^{y}	0.86^{x}	0.86^{x}	0.84^{xy}	0.035	0.099
ADFI, kg	2.09^{xy}	2.00^{y}	2.17^{x}	2.20^{x}	2.12^{xy}	0.070	0.070
G:F	0.410	0.388	0.397	0.392	0.396	0.010	0.261
d 16 to 30							
ADG, kg	1.03	1.05	1.07	1.06	1.01	0.025	0.202
ADFI, kg	2.70	2.72	2.71	2.68	2.71	0.046	0.910
G:F	0.380^{bc}	0.386^{ab}	0.393^{a}	0.395^{a}	0.374^{c}	0.005	0.004
d 0 to 30							
ADG, kg	0.94^{ab}	0.90^{b}	0.95^{a}	0.95^{a}	0.92^{b}	0.017	0.020
ADFI, kg	2.40	2.35	2.43	2.44	2.41	0.040	0.185
G:F	0.390^{ab}	0.385^{bc}	0.393^{a}	0.390^{ab}	0.381^{c}	0.003	0.012
d 30 to 44							
ADG, kg	1.13	1.13	1.09	1.10	1.10	0.025	0.525
ADFI, kg	3.04^{ab}	3.10^{a}	3.08^{a}	3.04^{ab}	2.96^{b}	0.041	0.034
G:F	0.371^{a}	0.364^{ab}	0.355^{b}	0.363^{ab}	0.370^{a}	0.006	0.049
d 44 to 58							
ADG, kg	1.09^{bc}	1.14^{ab}	1.16^{a}	1.06^{c}	1.09^{c}	0.020	< 0.001
ADFI, kg	3.16	3.16	3.23	3.14	3.16	0.043	0.306
G:F	0.346^{b}	0.359^{a}	0.359^{a}	0.339^{b}	0.344^{b}	0.005	0.001
d 30 to 58							
ADG, kg	1.11^{ab}	1.13^{a}	1.13^{a}	1.09^{b}	1.09^{b}	0.017	0.024
ADFI, kg	3.09^{xy}	3.13^{xy}	3.15^{x}	3.09^{xy}	3.05^{y}	0.038	0.096
G:F	0.359^{a}	0.361^{a}	0.359^{a}	0.352^{b}	0.357^{ab}	0.003	0.016
d 0 to 58							
ADG, kg	1.02^{xy}	1.01^{xy}	1.04^{x}	1.02^{xy}	1.00^{y}	0.012	0.092
ADFI, kg	2.74	2.72	2.78	2.76	2.72	0.033	0.434
G:F	0.373^{x}	0.372^{xy}	0.373^{x}	0.369^{xy}	0.368^{y}	0.002	0.081
Marketing[1]							
ADG, kg	1.23	1.18	1.18	1.18	1.16	0.039	0.403
ADFI, kg	3.65	3.58	3.56	3.57	3.54	0.063	0.535
G:F	0.339	0.329	0.331	0.332	0.328	0.008	0.642
d 0 to 79							
ADG, kg	1.06	1.04	1.06	1.04	1.03	0.013	0.130
ADFI, kg	2.90	2.87	2.91	2.90	2.86	0.033	0.584
G:F	0.365	0.362	0.364	0.361	0.359	0.003	0.160

[1] Marketing represents cumulative data from 58 to 79 d

$^{a\text{-}c}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

$^{x\text{-}y}$ Means within a row lacking common superscript tend to differ significantly, $P \leq 0.05$

Table 5.9. Dose response of pigs fed SBM in HPDDG diets when SID Val and Ile were constant.

Item:	Dietary Treatment			SEM	Contrast	
	HPSBM	HP50/50	HPAA		Linear	Quadratic
BW, kg						
d 0	59.4	59.4	59.4	0.79	0.959	0.971
d 16	73.2	73.2	72.8	0.95	0.646	0.731
d 30	88.1	88.0	87.0	1.08	0.250	0.532
d 44	103.4	103.5	102.4	1.24	0.418	0.560
d 58	119.6	118.4	117.6	1.41	0.099	0.792
Final	133.3	132.1	130.9	1.17	0.065	0.989
d 0 to 16						
ADG, kg	0.86	0.86	0.84	0.025	0.430	0.555
ADFI, kg	2.17	2.20	2.12	0.056	0.522	0.328
G:F	0.397	0.392	0.396	0.007	0.901	0.535
d 16 to 30						
ADG, kg	1.07	1.06	1.01	0.020	0.045	0.385
ADFI, kg	2.71	2.68	2.71	0.043	0.931	0.437
G:F	0.393	0.395	0.374	0.004	0.001	0.015
d 0 to 30						
ADG, kg	0.95	0.95	0.92	0.017	0.065	0.310
ADFI, kg	2.43	2.44	2.41	0.042	0.674	0.609
G:F	0.393	0.390	0.381	0.002	0.001	0.223
d 30 to 44						
ADG, kg	1.09	1.10	1.10	0.019	0.805	0.661
ADFI, kg	3.08	3.04	2.96	0.040	0.025	0.625
G:F	0.355	0.363	0.370	0.005	0.012	0.855
d 44 to 58						
ADG, kg	1.16	1.06	1.09	0.027	0.001	0.002
ADFI, kg	3.23	3.14	3.16	0.037	0.093	0.094
G:F	0.359	0.339	0.344	0.007	0.022	0.029
d 30 to 58						
ADG, kg	1.13	1.09	1.09	0.014	0.014	0.072
ADFI, kg	3.15	3.09	3.05	0.036	0.013	0.699
G:F	0.359	0.352	0.357	0.003	0.845	0.037
d 0 to 58						
ADG, kg	1.04	1.02	1.00	0.014	0.028	0.768
ADFI, kg	2.78	2.76	2.72	0.037	0.218	0.936
G:F	0.373	0.369	0.368	0.002	0.037	0.394
Marketing[1]						
ADG, kg	1.18	1.18	1.16	0.027	0.633	0.600
ADFI, kg	3.56	3.57	3.54	0.052	0.694	0.778
G:F	0.331	0.332	0.328	0.004	0.662	0.554
d 0 to 79						
ADG, kg	1.06	1.04	1.03	0.013	0.035	0.962
ADFI, kg	2.91	2.90	2.86	0.034	0.244	0.865
G:F	0.364	0.361	0.359	0.002	0.046	0.775

[1] Marketing represents cumulative data from 58 to 79 d

Table 5.10. Impact of HPDDG and SBM inclusion on carcass characteristics.

Item:	Dietary Treatments					SEM	Diet
	CS	HP	HPSBM	HP50/50	HPAA		
HCW, kg	100.2	98.6	99.3	98.8	97.9	0.89	0.150
DP, %[1]	75.0^{a}	74.7^{ab}	74.4^{b}	74.8^{a}	74.7^{ab}	0.18	0.040
Backfat , mm	25.4	25.2	25.2	25.7	25.3	0.19	0.199
SFFL, kg[2]	51.3	50.7	51.1	50.6	50.3	0.41	0.118

$^{a-d}$ Means within a row lacking common superscript differ significantly, $P \leq 0.05$

[1] Utilized data collected at barn in calculation.

[2] Standard Fat Free Lean; calculation: SFFL, lbs = 23.568 + (HCW,lbs x 0.503) – (backfat, in x 21.348)

Table 5.11. Dose response of pigs fed SBM in HPDDG diets when SID Val and Ile were constant on carcass characteristics.

Item:	Dietary Treatment			SEM	Contrast	
	HPSBM	HP50/50	HPAA		Linear	Quadratic
HCW, kg	99.3	98.8	97.9	0.90	0.142	0.772
DP, %[1]	74.4	74.8	74.7	0.12	0.071	0.197
Backfat , mm	25.2	25.7	25.3	0.21	0.550	0.075
SFFL, kg[2]	51.1	50.6	50.3	0.42	0.094	0.901

[1] Utilized data collected at barn in calculation.

[2] Standard Fat Free Lean; calculation: SFFL, lbs = 23.568 + (HCW,lbs x 0.503) – (backfat, in x 21.348)

CHAPTER 6

CONCLUSION

The increase in the SID Trp:Lys ratio in 40% DDGS diets promoted the linear increase in ADG and ADFI of pigs up until pigs reached approximately 99 and 115 kgs, respectively. The increase in the SID Trp:Lys ratio did not impact G:F for the overall growing period and finishing period or the cumulative period. This suggests that the increase in ADG was mainly due to the increase in ADFI caused by the increase in dietary SID Trp:Lys. Providing a diet with SID Trp:Lys ratio of 24% in the 40% DDGS diets resulted in a similar ADFI as the corn-SBM fed pigs during the growing period. However, the pigs receiving the corn-SBM diet had greater ADG due to a greater efficiency of utilization of nutrients as indicated by a greater G:F compared to the 24% SID Trp:Lys supplied pigs along with other SID Trp:Lys ratios in 40% DDGS diets. This suggested that a certain nutrient in dietary supply or a miscalculation in the nutrient value of a feed ingredient is negatively affecting performance. An imbalance in BCAA ratios in DDGS diets, leading to negative impacts on other BCAA in the diet, or inaccurate calculation of energy availability of DDGS are potential explanations for this. During the finishing period, increasing the SID Trp:Lys ratio linearly increase ADFI and tended to linearly increase ADG but did not impact G:F. However, supplying a SID Trp:Lys ratio of at least 18% resulted in similar ADFI compared to the corn-SBM diet. The transition of pigs from a period where caloric intake limits lean tissue deposition to a period where lean tissue deposition is not limited due to caloric intake might provide some clarification on how to efficiently utilize Trp. After pigs reach approximately 99 kg, increasing the SID Trp:Lys ratio did not improve ADG but rather it increased ADFI and decreased G:F of pigs between 99 and 115 kgs. Therefore, to efficiently utilize Trp in 40% DDGS diets, it might not be beneficial to feed a SID Trp:Lys above 18% after pigs reach approximately 99 kgs; until then, increasing the SID Trp:Lys can increase performance and potentially economical return depending on cost of feedstuffs.

When feeding diets containing 30% DDGS during the growing period, providing SID Val:Lys ratio of 68% will provide 99% of the maximal mean ADG and 98.5% of the maximal mean G:F. The value of 68% SID Val:Lys is similar to other current recommendations of published literature on the Val requirement. Even though DDGS were provided at 30% of the diet, the dietary Leu:Lys concentrations were at or below 144% which is considered to be only marginal excess. This was a result of lower dietary inclusion levels of SBM to supply the lowest titration level of SID Val:Lys. Therefore, the failure to conclude that the SID Val:Lys ratio needs to be increased due to the dietary inclusion of DDGS was likely a result of Leu concentration not being at levels to negatively impact Val metabolism. Interestingly, the inclusion of Val in DDGS diets did not restore performance to that of pigs fed a corn-SBM diet with the exceptions ADFI of pigs supplied a SID Val:Lys ratio of 75%. The difference in growth performance is likely a result of a miscalculation in the nutrient composition of a feedstuff or the lack of dietary supply of a certain nutrient. The energy value of DDGS could have been underestimated and this would move the Lys:NE value further away from requirement, leading to reduced performance compared to the corn-SBM diet, as seen in this study. The dietary concentrations of Leu were not considered to be in excess during this study and, therefore, it is less likely that one of the BCAA were supplied below the requirement for protein synthesis. Rather another nutrient, such as one of the non-essential AA, might have become conditionally essential, thus reducing protein synthesis. Overall, this study continues to provide an agreed upon SID Val:Lys estimate of 68% to the current published literature, but further research is required to understand the difference in performance between DDGS and corn-SBM fed pigs.

The inclusion of Ile in late finishing did not present a clear statistical response which was likely a result of unexpected performance of the 65% SID Ile:Lys group. However, it could be suggested that the optimal SID Ile:Lys ratio may need to be increased from 60 to 70% over the course of the finishing period to maximize the ADG response of pigs. When using carcass characteristics to determine the SID Ile:Lys requirement, supplying a SID Ile:Lys ratio of 65% in 20% DDGS diets would lead to optimized carcass traits. However, during this initial finishing period the corn-SBM diets still outperformed pigs fed diets containing 20% DDGS regardless of SID Ile:Lys ratio. Repeating this study along with other studies would aid in the clarification of the optimal Ile:Lys ratio during the finishing period.

Lastly, the inclusion of HPDDG in grow-finish swine diets at low dietary inclusion levels had minimal impact on the performance and carcass characteristics of pigs. It could be suggested that HPDDG inclusion was not high enough to illicit a response in performance based on the previous HPDDG literature. However, during the first 30 days, feeding HPDDG without adjusting BCAA levels did result in reduced ADG of pigs compared to that of the HPSBM and HP50/50 dietary treatments. Therefore, indicating that even at low dietary inclusion levels, HPDDG inclusion in partial replacement of SBM could negatively impact pig growth performance if BCAA are not adjusted for. Interestingly though, the method by which BCAA were adjusted had an impact on pig growth performance and this data suggests that adjusting diet formulation for BCAA levels though the inclusion of SBM provided a benefit over that of crystalline AA. This would suggest that protein synthesis might have been limited due to the inadequate supply of another nutrient, such as a non-essential AA. An imbalance in the optimal LNAA profile could also be a potential explanatory factor but further research is required to fully understand the mechanisms at play between the BCAA and LNAA. However, this research and the other studies above put emphasis on having the correct nutrient values for DDGS and speculates to another unknown nutrient playing a role in the efficiency of utilization of the BCAA and LNAA.

In conclusion, the results of this work indicate that the inclusion of DDGS into swine diets requires further study to determine what particular AA and/or nutrient(s) should be specifically included into the diet to remove the negative effects of DDGS as an important feedstuff.

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VITA

David Alan Clizer was born in Kansas City, Missouri on May 19, 1994. He graduated from Parkhill South High school in May 2012. He then pursued a Bachelor of Science degree in Animal Science with minors in Agriculture Economics and Agriculture Systems Management at the University of Missouri, Columbia Missouri, and graduate in May of 2016, becoming the third-generation college graduate from the University of Missouri. Following the completion of his Bachelor of Science, he continued his education at the University of Missouri in pursuit of master's degree in Poultry Production and Monogastric Nutrition. During his master's he served as the manager of the University of Missouri Rocheford Poultry Teaching and Research Facility where he conducted over ten research trials and taught undergraduate students of poultry production. His Masters research was focused on the effect of reduced nocturnal temperatures on the performance of broiler chicks and hen poults from zero to 21 days of age under guidance of Dr. Jeffre D. Firman. He completed his Masters in August of 2018 and continued his graduated training under Dr. Ryan S. Samuel at South Dakota State University, where he also served as the manager of the South Dakota State University 1,200-hd commercial wean to finish research facility. David's research at South Dakota State University has focused on the evaluating the impacts of tryptophan and branched chain amino acids in swine diets containing corn based dried distillers grains on the growth performance and carcass characteristics of growing and finishing pigs.