Evaluation of soybean meal, distillers dried grains with solubles, and the interactions among branched-chain amino acids in swine diets

by

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M.V., Federal University of Rio Grande do Sul, 2013
M.Sc., Federal University of Rio Grande do Sul, 2016

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

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Abstract

This dissertation consists of 8 chapters involving studies with branched-chain amino acids (BCAA), His requirements for nursery pigs, soybean meal (SBM) inclusion in nursery diets, and Zn source and level for grow-finish pigs. The first chapter presents a review of the literature on the interactions among branched-chain amino acids for growing pigs. Chapter 2 describes a meta-regression analysis conducted to develop prediction equations for growth performance based on BCAA, large neutral amino acids (LNAA), and their interactions. The results suggest that increasing Leu negatively impacts growth performance due to insufficient levels of other BCAA and LNAA relative to Leu. The addition of Val, Ile, and Trp, alone or in combination, has the potential to counteract the negative effects of high Leu. Chapter 3 describes two experiments that determined the His requirements of 7- to 11-kg nursery pigs. The results suggest that the His requirement is no more than 31% of Lys. Chapter 4 describes four experiments that evaluated the effects of increasing SBM in diets with or without distillers dried grains with solubles (DDGS). In general, DDGS reduced growth performance, although the magnitude was different across experiments. Increasing inclusions of SBM consistently improved G:F and caloric efficiency. Chapter 5 presents two experiments that estimated the energy value of SBM relative to corn. The results suggest that the energy value of SBM ranges from 105 and 125% of corn energy, which indicates that the NRC (2012) underestimates SBM energy. Chapter 6 describes a study that estimated the energy of high protein DDG for nursery pigs and found that it contains 97.3% of corn energy. Chapter 7 presents a Zn titration from 50 to 200 mg/kg for grow-finish pigs. There were no improvements in ADG beyond 50 mg/kg added Zn; however, providing 125 mg/kg added Zn resulted in the greatest G:F. Finally, chapter 8 evaluated Zn sources (Zn sulfate and Zn hydroxychloride) and levels (50 to 150 mg/kg) for grow-finish pigs. There were small improvements in ADG of pigs fed added Zn beyond 50 mg/kg. Zinc source did not influence growth performance, but Zn hydroxychloride improved carcass characteristics compared with Zn sulfate.

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Preface

This dissertation is original work completed by the author, Henrique Scher Cemin. Chapters 1, 7, and 8 were published in Translational Animal Science, chapters 2 and 3 were published in Journal of Animal Science. All chapters were formatted according to the required standards of the corresponding journal.

Chapter 1 - Branched-chain amino acid interactions in growing pig diets[1]

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ABSTRACT: The branched-chain amino acids (**BCAA**) Leu, Ile, and Val share the first steps of their catabolism due to similarities in their structure. The BCAA are reversibly transaminated in skeletal muscle through the activity of branched-chain amino-transferase and transported to the liver. There they undergo an irreversible decarboxylation catalyzed by the branched-chain α-keto acid dehydrogenase complex. Both enzymes are common to Leu, Ile, and Val and increased enzymatic activity stimulated by one of them will increase the catabolism of all BCAA, which can result in antagonisms. Leucine and its keto-acid are the most potent stimulators of BCAA catabolic enzymes. Moreover, BCAA and large neutral amino acids (**LNAA**) share common brain transporters. Research has shown that high concentrations of BCAA, especially Leu, can decrease the absorption of LNAA, such as Trp, which is a precursor of serotonin and can have a significant impact in feed intake regulation. Finally, high Leu concentrations have the ability to over-stimulate the mTOR signaling pathway, resulting in an inhibitory effect on feed intake. Most of the research conducted to evaluate the impact of BCAA on growth performance of pigs seems to agree that high levels of Leu decrease weight gain, mostly due to a reduction in feed intake. However, some studies, mostly with finishing pigs, observed no evidence for an impact on growth performance even with extremely high levels of Leu. It could be hypothesized that these inconsistencies are driven by the dietary amino acid profile. Grow-finish diets normally contain high levels of Leu, but the other BCAA are also well above the requirement and could potentially mitigate the negative impact of Leu on BCAA catabolism. Indeed, some studies suggest that when diets contain high levels of Leu, more Ile and Val are needed to optimize growth performance. However, the precise relationship between BCAA and their balance in swine diets is not fully understood. More research is needed to understand and quantify the relationship between LNAA and BCAA.

Key words: antagonism, branched-chain amino acids, pig, performance

INTRODUCTION

Formulation of diets with low crude protein (**CP**) while maintaining adequate amino acid supply through the addition of crystalline amino acids is a well-established practice in swine diets. The use of crystalline amino acids reduces diet costs and nitrogen excretion while maintaining growth performance. Today, L-Lys, L-Thr, DL-Met, L-Trp, and L-Val are commonly used in the swine industry. After Val, Ile is likely the next limiting amino acid in corn-soybean meal diets (Figueroa et al., 2003). However, while Val and Ile are limiting in low CP corn-soybean meal diets, Leu is usually in excess due to its high concentration in corn and corn by-products (NRC, 2012). The branched-chain amino acids (**BCAA**) Leu, Ile, and Val are structurally similar and share the first steps of their catabolism. Therefore, excess of one BCAA, particularly Leu, may result in increased degradation of all three BCAA. The objective of this review is to summarize the current state of knowledge on BCAA metabolism and their interactions on growth performance of pigs.

BRANCHED-CHAIN AMINO ACIDS METABOLISM AND ANTAGONISM

The metabolism of BCAA amino acids is well described (Harper et al., 1984). While most amino acids are metabolized in the liver, BCAA are transported to the skeletal muscle to be degraded. Through the action of branched-chain amino-transferase (**BCAT**), BCAA are reversibly converted to α-keto acids. The α-keto acids of Ile, Leu, and Val are α-keto-β-methylvalerate, α-ketoisocaproate, and α-ketoisovalerate, respectively. The transamination also forms glutamate, which can be transformed to glutamine or alanine and used in protein synthesis.

In the next step, α-keto acids are transported to the liver, where they are irreversibly decarboxylated by branched-chain α-keto acid dehydrogenase complex (**BCKD**). The final products of this reaction are ketogenic (for α-ketoisocaproate), glucogenic (for α-ketoisovalerate), or both (for α-keto-β-methylvalerate) and can be used in the TCA cycle.

Both enzymes, BCAT and BCKD, are common to all three BCAA. Stimulation of enzymatic activity by one of the BCAA will increase the catabolism of all BCAA, possibly creating antagonisms among them. The most potent stimulator of BCAT and BCKD is Leu and its α-keto acid, α-ketoisocaproate (Harper et al., 1984). Excessive Val and Ile seem to have little effect on increasing the catabolism of the other BCAA (D'Mello and Lewis, 1970). The first study to demonstrate BCAA antagonism was conducted in 1955 with rats (Harper et al., 1955). Researchers observed that including 3% L-Leu to a low protein diet impaired growth and supplementation of L-Ile could partially alleviate the decrease in growth. Allen (1971) and Allen and Baker (1972) demonstrated in chicks that excess Val does not affect the utilization of Ile or Leu; however, excess Ile and especially Leu impair the utilization of the other BCAA and cause reduction in feed intake and growth. A similar observation was made by Harper et al. (1984) in rats, where excess Leu reduced growth and supplementation of Ile and Val was able to partially reverse the decrease in growth.

In a recent study (Wessels et al., 2016), nursery pigs with an initial body weight (**BW**) of 10.4 kg were fed diets with 100, 186, or 353% standardized ileal digestible (**SID**) Leu:Lys. In this experiment, 186% SID Leu:Lys was enough to produce a reduction ADFI and the effect was even more pronounced with 353% SID Leu:Lys (9 or 23% reduction compared to 100% SID Leu:Lys). Feeding high Leu diets increased Leu and reduced Ile and Val in plasma and several other tissues. The activity of BCKD was also increased in most tissues. Taken together, results indicate that high Leu increases the catabolism not only of itself but of the other BCAA. Interestingly, pigs fed 353% SID Leu:Lys diets had decreased plasma serotonin and numerically lower brain serotonin. A possible explanation for the decrease in serotonin is the lower concentration of Trp observed in the brain of piglets fed the 353% SID Leu:Lys diet. Branched-chain amino acids and other large neutral amino acids (**LNAA**), such as Trp, His, Phe, and Tyr, share the same brain transporters (Pardridge, 1977). Increasing levels of one of the LNAA raises its brain uptake and decreases the uptake of the other LNAA (Fernstrom, 2013). Therefore, excessive Leu may decrease the absorption and utilization of Trp, which ultimately results in reduced serotonin activity in the brain and decreased feed intake (Henry et al., 1992). It is well reported that low dietary Trp has profound effects on feed intake (Ettle and Roth, 2004, Gonçalves et al., 2015). However, Wessels et al. (2016) hypothesize that Trp and serotonin were not the main drivers of reduced feed intake because pigs fed the 186% SID Leu:Lys diet had similar brain Trp concentration to pigs fed 100% SID Leu:Lys but lower feed intake. Instead, the reduction in appetite was attributed to excessive mTOR activation, stimulating anorectic signals (Cota et al., 2006), and deficiency of Val and Ile caused by increased catabolism due to excess Leu.

A study was conducted by Millet et al. (2015) to evaluate the interaction between BCAA and Trp for nursery pigs (initially 9.9 kg). Treatments consisted of two ratios of SID Val:Lys (63 or 70%), SID Leu:Lys (97 or 192%), SID Ile:Lys (54 or 66%), and SID Trp:Lys (18 or 23%). High concentrations of dietary Leu negatively affected growth performance (Table 1.1), but some of the performance was recovered with the addition of high Val concentrations. Addition of Ile did not improve performance. Interestingly, there was an interaction between Leu and Trp due to a negative effect of adding Trp to a high Leu diet compared to a low Leu diet. This may indicate some degree of antagonism between the BCAA and Trp.

EFFECTS OF BCAA ON GROWTH PERFORMANCE

Kerr et al. (2004) fed nursery pigs (7 kg initial BW) diets with increasing levels of spray-dried blood cells (**SDBC**), an ingredient with very high concentration of Leu and Val and low concentration of Ile. Diets contained 2.5 or 5% SDBC and were supplemented with or without L-Ile. Pigs fed diets with 2.5% SDBC without or with L-Ile had similar average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F;** Table 1.2). However, pigs fed diets with 5% SDBC without L-Ile had reduced ADG, ADFI, and G:F. The inclusion of L-Ile to the 5% SDBC diets resulted in performance similar to the 2.5% SDBC diets. Comparable results were observed in other experiments (Kerr et al., 2004). These results suggest that diets with moderate levels of Leu can be fed to nursery pigs without compromising growth performance as long as Ile is above the requirement. Diets with 136% SID Leu:Lys (2.5% SDBC) did not reduced growth performance, but diets with 150% SID Leu:Lys (5% SDBC) impaired growth. It is important to observe that 136% SID Leu:Lys diets had Ile:Lys at or above the requirement (52 or 66% Ile:Lys), whereas the diet with 150% Leu:Lys without added L-Ile was below the Ile:Lys requirement (46% Ile:Lys).

In a summary of Ile requirement studies, Htoo (2012) observed that experiments where diets contained high levels of SDBC and consequently high SID Leu:Lys, i.e., greater than 150%, the estimated SID Ile:Lys requirements were considerably higher than in trials where SID Leu:Lys was held at more moderate levels (diets not containing blood products), regardless of BW and genetics. Data from 12 trials was combined to create a regression equation to estimate the SID Ile:Lys requirement based on the dietary SID Leu:Lys (Figure 1.1). The equation suggests that as Leu increases, Ile requirement increases proportionately. For example, in a diet with 110% SID Leu:Lys, the SID Ile:Lys requirement would be approximately 54% and if the SID Leu:Lys is 200%, the SID Ile requirement increases to 61% of Lys. However, it should be acknowledged that the regression equation was constructed with data from different classes of pigs, from approximately 6 to 113 kg BW, and there is not sufficient evidence to support the response of different classes of pigs to BCAA is comparable. Moreover, although the equation can be useful from a practical standpoint, the statistical procedures used to produce it were not described.

Gloaguen et al. (2013) fed nursery pigs (11.3 kg initial BW) diets with increasing SID Leu:Lys from 80 to 130%. The Ile:Lys ratio was 52%. Results show that 80% SID Leu:Lys reduced ADFI and ADG. However, no evidence for differences were observed between 90 and 130% SID Leu:Lys. This indicates that Leu levels 10% below the NRC (2012) requirement estimates do not seem to impact growth performance. Moreover, 130% SID Leu:Lys was not sufficient to produce a reduction in feed intake. Note the SID Ile:Lys in this study was not in excess but very close to the estimated requirement for nursery pigs. In another study (Gloaguen et al. 2011), nursery pigs (initially 11.7 kg BW) were fed diets with 2 levels of SID Val:Lys (60 or 70%) and 2 levels of SID Leu:Lys (111 or 165%). Treatments were created by replacing soybean meal with corn gluten meal. There were significant SID Val:Lys and Leu:Lys effects (Table 1.3). Increasing SID Val:Lys from 60 to 70% improved growth performance, indicating the requirement was greater than 60%. Pigs fed diets with high SID Leu:Lys had decreased growth performance, and the reduction was much more severe when SID Val:Lys was deficient.

However, it can be hypothesized that other differences between soybean meal and corn gluten meal were confounded and could play a role in the observed results.

Another experiment was conducted (Gloaguen et al., 2011) to determine the SID Val:Lys requirement of nursery pigs (initially 12.1 kg BW) when Leu is in excess (169% SID Leu:Lys). The estimated SID Val:Lys requirement was 72%. Although this observation is higher than the NRC (2012) requirement estimates, it is similar to the observations of Barea et al. (2009), conducted in the same laboratory using a diet with 118% SID Leu:Lys, and to other SID Val:Lys studies with moderate SID Leu:Lys levels (Soumeh et al., 2015, Clark et al., 2017). Therefore, it seems that 169% SID Leu:Lys was not enough to cause an increase in the Val requirement for nursery pigs. Wiltafsky et al. (2010) also conducted two experiments with nursery pigs with high SID Leu:Lys ratios ranging from 102 to 204% in Exp. 1 or 99 to 198% in Exp. 2. In Exp. 1 and 2, diets were marginal for Ile (44% SID Ile:Lys) or Val (55% SID Val:Lys), respectively. In both experiments, there was a linear reduction in ADFI and ADG in response to increasing SID Leu:Lys. Contrary to Gloaguen et al. (2011, 2013), the authors observed important reductions in feed intake even with intermediate SID Leu:Lys, i.e., around 150%. It can be hypothesized that the feed intake decrease was observed with lower SID Leu:Lys by Wiltafsky et al. (2010) because diets were limiting in Ile or Val, which exacerbated the effects of excess Leu.

Duan et al. (2016) studied the effects of BCAA ratios on growth performance of nursery pigs (Table 1.4). The authors compared diets with 20% CP and Leu:Ile:Val ratios of 1:0.51:0.63 and 17% CP diets with ratios of 1:1:1, 1:0.75:0.75, 1:0.51:0.63, or 1:0.25:0.25 Leu:Ile:Val. Results show that reducing CP from 20 to 17% while maintaining the 1:0.51:0.63 ratio did not affect growth performance. Pigs fed diets with 1:0.25:0.25 ratio had poorer performance than the other treatments. However, it is important to observe that the 1:0.25:0.25 treatment had 200%

SID Leu:Lys but only 50% SID Ile:Lys and 50% SID Val:Lys, much lower than the estimated requirement. Conversely, all other treatments also had moderately high SID Leu:Lys but Ile and Val levels well above the estimated requirement. Thus, this seems to support the fact that negative effects of high Leu are more evident when the other BCAA are below the estimated requirement. Moreover, although not statistically significant, ADFI decreased by 15% for the 1:0.25:0.25 treatment and an SID Leu:Lys of 141% (1:0.51:0.63 treatment) did not produce a reduction in ADFI.

Data for grow-finish pigs is less abundant. Morales et al. (2016) fed growing pigs (31.8 kg initial BW) a basal diet with 99% SID Leu:Lys, 58% SID Ile:Lys, and 73% SID Val:Lys, the basal diet supplemented with L-Leu (137% SID Leu:Lys, 58% SID Ile:Lys, and 73% SID Val:Lys), or the basal diet supplemented with L-Leu, L-Ile, and L-Val (133% SID Leu:Lys, 74% SID Ile:Lys, and 95% SID Val:Lys). Ratios were calculated from the analyzed diet composition. There was no evidence for differences in performance across dietary treatments, which indicates that 137% SID Leu:Lys was not sufficient to provoke a reduction in feed intake in growing pigs. However, it is important to acknowledge that both Ile and Val were above the estimated requirements.

Hyun et al. (2003) fed finishing pigs (initially 78.4 kg BW) corn-soybean meal diets (171% SID Leu:Lys) or corn-soybean meal diets supplemented with 2.1% L-Leu (424% SID Leu:Lys). Other ratios were 66% SID Ile:Lys, 20% SID Trp:Lys, 66% SID Met and Cys:Lys, and 62% SID Thr:Lys. Valine level was not reported. Pigs fed diets with added L-Leu had significantly lower ADG (829 vs 930 g), but there was no evidence for differences in ADFI (2.75 vs 2.89 kg). It is important to observe that the Leu levels in the L-Leu supplemented diet were extremely high and do not correspond to practical conditions. Also, although not significant, there was a 5% numerical reduction in ADFI.

In another finishing study, Hyun et al. (2007) fed finishing pigs (73 kg initial BW) in a 2 × 3 factorial treatment structure with two levels of SID Lys (0.5% or 0.7%) and three levels of SID Leu (1, 2, or 3%). Diets were corn-soybean meal based and treatments were achieved by manipulating the inclusion of feed-grade L-Lys and L-Leu. Calculated ratios were not provided, but analyzed values resulted in Leu:Lys of 218, 365, and 501% with 0.5% Lys and 166, 244, and 330% with 0.7% Lys. Other amino acid levels were above the estimated requirement. Interestingly, growth performance was not affected by Lys or Leu levels. In this study, even the highest SID Leu:Lys of 501% did not impact ADFI. A similar finding was observed by Cisneros et al. (1996), where no evidence for differences were observed in growth performance of finishing pigs fed diets with extremely high SID Leu:Lys, greater than 500%. However, it is important to note that in this study there was a numeric reduction in ADFI of 9%.

Rojo et al. (2011) conducted an experiment using growing pigs (42.4 kg initial BW) fed a corn-soybean meal diet (control), control supplemented with L-Leu, control supplemented with L-Leu and L-Ile, or control supplemented with L-Leu, L-Ile, and L-Val. Ratios of SID Leu:Lys, SID Ile:Lys, and SID Val:Lys were 128, 61, and 68% in control; 192, 61, and 68% in control supplemented with L-Leu; 192, 62, and 68% in control supplemented with L-Leu and L-Ile; and 192, 62, and 74% in control supplemented with L-Leu, L-Ile, and L-Val. The Trp:Lys was 18% for all treatments. There was a reduction in ADG in all treatments compared to the control. However, there was no evidence for differences in feed intake among treatments, which is in agreement with Hyun et al. (2007) and Cisneros et al. (1996).

Several studies were conducted to evaluate the effects of increasing distillers dried grains with solubles (**DDGS**) for grow-finish pigs. Although they were not designed as BCAA trials, data can be utilized to evaluate the effects of increasing SID Leu:Lys due to the amino acid profile of DDGS. Increasing DDGS will also result in high Val and Ile content, but not to the same extent as Leu. However, it should be recognized that there are a number of confounding factors when doing such evaluation. Whitney et al. (2006) fed grow-finish pigs (28.4 kg initial BW) diets with 0, 10, 20, or 30% DDGS. The inclusion of L-Lys HCl was held constant for all treatments. Ratios of SID Leu:Lys were not reported but they can be estimated to range from approximately 150% to greater than 250%, depending on the feeding phase. Overall, there was no evidence for differences in ADFI, although the inclusion of DDGS linearly reduced ADG and G:F. This suggests that the high SID Leu:Lys in these diets did not affect ADFI. The observed reduction in ADG and G:F could have been caused by overestimating amino acid content and digestibility or underestimating energy level of the DDGS source. In a study by Graham et al. (2014), grow-finish pigs (initially 46.1 kg BW) were fed diets with 0, 20, or 40% DDGS. The inclusion of L-Lys HCl was increased in diets with increasing DDGS. The SID Leu:Lys ranged from approximately 150 to 250%, depending on the feeding phase. Similar to Whitney et al. (2006), feeding high levels of DDGS did not have any negative impact on ADFI. It appears that high levels of DDGS can be used without negative impact on performance parameters (Stein and Shurson, 2009). It is unclear why high levels of Leu do not seem to impact feed intake as much in grow-finish compared to nursery pigs. It can be hypothesized that in the available research Val and Ile are also in a level high enough that the negative impact of exacerbated catabolism caused by excessive Leu is reduced. However, it is important to note that cost-effective practical diets are currently formulated with high inclusion of feed-grade AA to replace intact protein sources as well as alternative ingredients such as DDGS. Using this strategy, diets can be formulated with Val and Ile close to the estimated requirement, while Leu can fluctuate to levels well above the estimated requirement and may potentially impact growth performance.

Taken together, results suggest that there is strong evidence to support the negative effects of feeding high Leu levels when diets are marginal or below the Ile and/or Val estimated requirements. However, the effects on pigs fed diets with adequate or high Ile and Val, such as corn-soybean meal-DDGS grow-finish diets, are less clear. Furthermore, there is also no definition of exactly where the breakpoint is, i.e., at what SID Leu:Lys ratio growth performance is impaired. Similarly, other ratios, such as among BCAA and between BCAA and LNAA are not fully understood.

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Table 1.1. Effects of changing branched-chain amino acids and Trp for nursery pigs, adapted from Millet et al. (2015)[1]

	Treatment							
Item[2]	Control	Control +Val	Control +Val +Trp	Control +Leu	Control +Leu +Val	Control +Leu +Val +Ile	Control +Leu +Val +Trp	Control +Leu +Val +Ile +Trp
Diet composition, %								
SID Lys	1.11	1.11	1.11	1.11	1.11	1.11	1.11	1.11
SID Leu:Lys	97	97	97	192	192	192	192	192
SID Ile:Lys	54	54	54	54	54	66	54	66
SID Val:Lys	63	70	70	63	70	70	70	70
SID Trp:Lys	18	18	23	18	18	18	23	23
Results								
ADG[3], g	695	659	695	511	623	625	589	626
ADFI[4], g	1,142	1,110	1,142	974	1,055	1,058	1,055	1,034
G:F[5], g/kg	609	594	609	525	591	591	558	605

[1] A total of 32 pigs (initially 9.9 kg BW) were fed dietary treatments in a cross-over design during 8 weeks.

[2] SID = standardized ileal digestible. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio.

[3] ADG, $P < 0.05$ for Leu and Leu × Val; $P > 0.05$ for Val, Ile, Trp, Leu × Trp, and Ile × Trp.

[4] ADFI, $P < 0.05$ for Val, Leu, and Val × Leu; $P > 0.05$ for Ile, Leu × Trp, and Ile × Trp.

[5] G:F, $P < 0.05$ for Val, Leu, and Val × Leu; $P > 0.05$ for Ile, Trp, Leu × Trp, and Ile × Trp.

Table 1.2. Effects of spray-dried blood cells (SDBC) in diets with or without L-Ile for nursery pigs, adapted from Kerr et al. (2004)[1]

	2.5% SDBC		5% SDBC		Low-CP control	High-CP control	SEM
Item[2]	- L-Ile	+ L-Ile	- L-Ile	+ L-Ile			
Diet composition, %							
Crude protein	21.0	21.0	21.0	21.0	21.0	24.7	---
SID Lys	1.25	1.25	1.25	1.25	1.25	1.25	---
SID Leu:Lys	136	136	150	150	122	141	---
SID Ile:Lys	52	66	46	66	66	74	---
SID Val:Lys	74	74	83	83	71	78	---
SID Trp:Lys	18	18	18	18	18	21	---
Results[3]							
ADG, g	419^{a}	399^{ab}	336^{c}	393^{ab}	388^{b}	382^{b}	9.8
ADFI, g	505^{x}	481^{x}	440^{y}	479^{x}	481^{x}	473^{x}	13.9
G:F, g/kg	831^{a}	838^{a}	759^{b}	824^{a}	817^{a}	818^{a}	16.4

[1] A total of 936 pigs (initially 7.3 kg BW) were used in a 16-d trial with 12 replicates per treatment.

[2] SID = standardized ileal digestible. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio.

[3] Means with different superscripts differ (^{a,b,c} $P < 0.05$) or tend to differ ($^{x,\ y}$ $P < 0.10$).

Table 1.3. Effects of standardized ileal digestible (SID) Leu:Lys and SID Ile:Lys on growth performance of nursery pigs, adapted from Gloaguen et al. (2011)[1,2]

SID Leu:Lys, %	111		165		SEM	Probability, $P <$		
SID Val:Lys, %	60	70	60	70		SID Val:Lys	SID Leu:Lys	SID Val:Lys × Ile:Lys
Initial BW, kg	11.8	12.2	11.3	11.6	1.6	0.19	0.34	0.90
Final BW, kg	18.6	22.0	16.4	20.4	2.2	0.01	0.01	0.54
ADG[3], g	325^{b}	465^{a}	242^{c}	420^{a}	85	0.01	0.01	0.28
ADFI[4], g	634^{b}	736^{a}	563^{c}	715^{a}	69	0.01	0.04	0.24
G:F[5], g/kg	0.51^{b}	0.63^{a}	0.44^{c}	0.59^{a}	0.09	0.01	0.01	0.39

[1] A total of 64 pigs (initially 11.7 kg) were used in a 21-d trial with 16 replicates per treatment.

[2] Differences in SID Leu:Lys were obtained by replacing soybean meal with corn gluten meal.

[3] ADG = average daily gain.

[4] ADFI = average daily feed intake.

[5] G:F = gain-to-feed ratio.

Table 1.4. Effects of branched-chain amino acid ratio on growth performance of grow-finish pigs, adapted from Duan et al., (2016)[1]

Crude protein	20%	17%				SEM
SID[2] Leu:Ile:Val	1:0.51:0.63	1:1:1	1:0.75:0.75	1:0.51:0.63	1:0.25:0.25	
Diet composition, %						
SID Lys	1.23	1.23	1.23	1.23	1.23	---
SID Leu:Lys	141	100	120	141	200	---
SID Ile:Lys	72	100	90	72	50	---
SID Val:Lys	89	100	90	89	50	---
SID Trp:Lys	16	16	16	16	16	---
Results[3,4]						
Initial weight, kg	9.9	9.9	9.7	9.8	9.9	0.35
Final weight, kg	36.7^{a}	33.4^{ab}	35.6^{a}	36.6^{a}	31.2^{b}	0.66
ADG, kg	0.62^{a}	0.55^{ab}	0.60^{a}	0.63^{a}	0.50^{b}	0.03
ADFI, kg	1.15	1.12	1.06	1.16	0.99	0.14
G:F, g/kg	539^{xy}	491^{x}	566^{y}	543^{xy}	505^{xy}	0.18

[1] A total of 40 pigs (initially 9.9 kg) were used in a 45-d trial with 8 replicates per treatment.

[2] SID = standardized ileal digestible.

[3] ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio.

[4] Means with different superscripts differ (^{a,b,c} $P < 0.05$) or tend to differ ($^{x,\,y}$ $P < 0.10$).

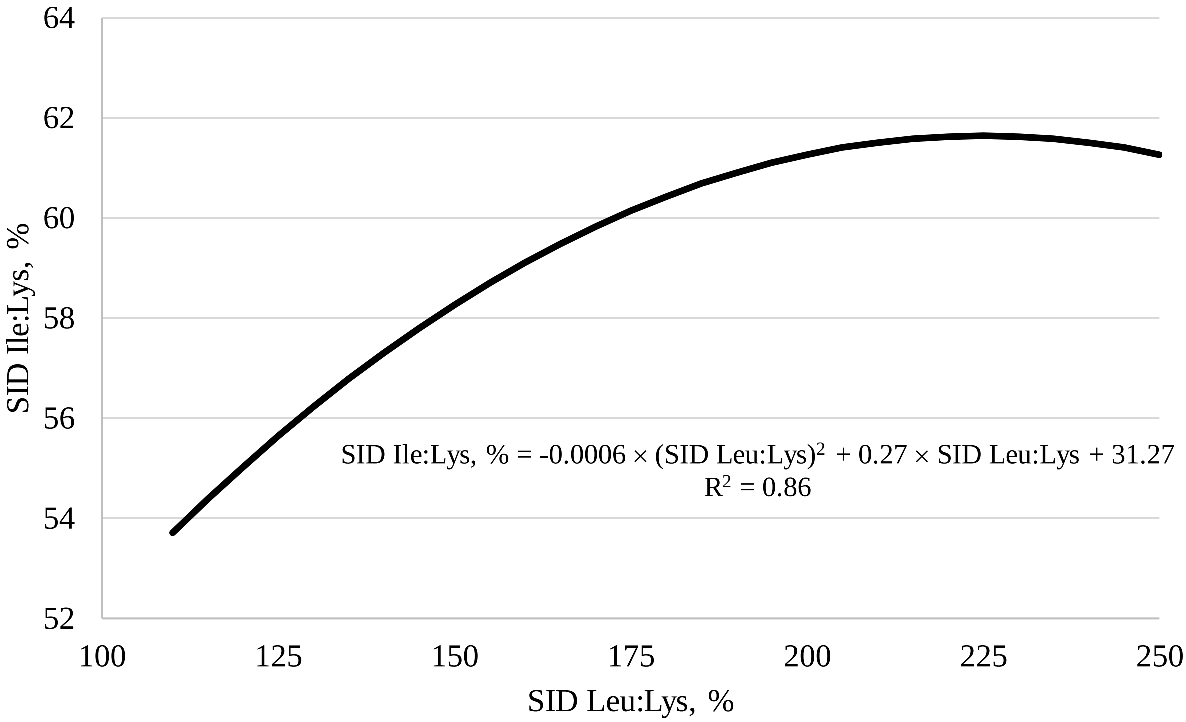

Figure 1.1. Regression equation of the estimated SID Ile:Lys requirement as a function of SID Leu:Lys, adapted from Htoo (2012).

Chapter 2 - Meta-regression analysis to predict the influence of branched-chain and large neutral amino acids on growth performance of pigs[1]

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ABSTRACT: A meta-analysis was conducted to evaluate the effects of branched-chain amino acids (**BCAA**), their interactions, and interactions with large neutral amino acids (**LNAA**) to develop prediction equations for growth performance of pigs. Data from 25 papers, published from 1995 to 2018, for a total of 44 trials and 210 observations were recorded in a database. Diets were reformulated using the NRC (2012) loading values to estimate nutrient concentrations. The response variables were average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). The predictor variables tested included average body weight (**BW**), crude protein, neutral detergent fiber, Ile:Lys, Leu:Lys, Val:Lys, BCAA:Lys, Ile:Leu, Val:Leu, Ile:Val, (Ile+Val):Leu, Trp:Lys, Leu:Trp, Ile:Trp, Val:Trp, BCAA:Trp, Met:Lys, Leu:Met, Ile:Met, Val:Met, BCAA:Met, His:Lys, Leu:His, Ile:His, Val:His, BCAA:His, Thr:Lys, Leu:Thr, Ile:Thr, Val:Thr, BCAA:Thr, (Phe+Tyr):Lys, Leu:(Phe+Tyr), Ile:(Phe+Tyr), Val:(Phe+Tyr), BCAA:(Phe+Tyr), LNAA:Lys, Leu:LNAA, Ile:LNAA, Val:LNAA, and BCAA:LNAA. Amino acids were expressed on standardized ileal digestible basis. The MIXED procedure of SAS was used to develop the equations. The inverse of squared SEM was used to account for heterogeneous errors using the WEIGHT statement. Models were selected with a step-wise manual forward selection. In order to be included in the final model, predictor variables had to be statistically significant ($P < 0.05$) and provide an improvement of at least 2 points in Bayesian information criterion. The optimum equations were: ADG, g = –985.94 + (15.2499 × average BW (kg)) – (0.08885 × average BW × average BW) + (1.063 × Leu:Lys) + (20.2659 × Ile:Lys) – (0.1479 × Ile:Lys × Ile:Lys) + (9.2243 × (Ile+Val):Leu) – (0.03321 × (Ile+Val):Leu × (Ile+Val):Leu) – (0.4413 × Ile:Trp); G:F, g/kg = 648.3 – (6.2974 × average BW (kg)) + (0.02051 × average BW × average BW) + (0.5396 × Ile:Lys) + (1.7284 × Val:Lys) – (0.00795 × Val:Lys × Val:Lys) – (1.7594 × Met:Lys); and ADFI, kg = predicted

ADG/predicted G:F. Overall, the prediction equations suggest that increasing Leu:Lys negatively impacts ADG due to a reduction in G:F and ADFI caused by insufficient levels of other BCAA and LNAA relative to Leu. According to the model, the addition of Val, Ile, and Trp, alone or in combination, has the potential to counteract the negative effects of high dietary Leu concentrations on growth performance.

Key words: branched-chain amino acids, growth, prediction, swine

INTRODUCTION

Low crude protein (**CP**), amino acid (**AA**) fortified diets are a well-established practice to reduce diet cost and nitrogen excretion. Currently, Lys, Thr, Met, Trp, and Val are commonly used in swine diets to replace a portion of intact protein sources, such as soybean meal. After Val, Ile is likely the next limiting AA in low CP, AA-fortified diets (Figueroa et al., 2003). Of the three branched-chain amino acids (**BCAA**), Val and Ile are frequently limiting and thus their amount is minimized. In contrast, Leu is usually in excess in corn-based diets due to its high concentration in corn and corn by-products, such as distillers dried grains with solubles (**DDGS**).

The BCAA are structurally similar and share the first steps of their catabolism. Therefore, excess of a BCAA, particularly Leu, may result in increased degradation of the others (Harper et al., 1984; Brosnan and Brosnan, 2006). Moreover, BCAA and the other large neutral amino acids (**LNAA**), such as Trp, Tyr, Phe, Met, Thr, and His, share the same brain transporters (Pardridge, 1977; Fernstrom, 2005). Therefore, excess BCAA may decrease the AA absorption and subsequent utilization as neurotransmitter precursors that play an important role in appetite regulation. For instance, Trp is a precursor of serotonin, Tyr forms catecholamines, and His is essential for the synthesis of histamine, all of which are involved in feed intake regulation (Henry et al., 1992; Kurose and Terashima, 1999; Fernstrom, 2013).

Although data on the effects of high Leu diets on pig performance is inconsistent (Cisneros et al., 1996; Hyun et al., 2007; Morales et al., 2016), we hypothesize that practical corn-soybean meal diets supplemented with high feed-grade AA, especially with inclusion of DDGS, can potentially create imbalances and negatively impact growth performance. Therefore, the objective of this study was to evaluate prediction equations to quantify the effects of dietary BCAA, their interactions, and the potential interactions between BCAA and LNAA for nursery and growing-finishing pigs.

MATERIAL AND METHODS

Database

A literature search similar to that described by Nitikanchana et al. (2015) and Flohr et al. (2018) was conducted to identify published literature that could be used to directly or indirectly evaluate the effects of BCAA on growth performance. The initial screening process included trials that directly evaluated BCAA as part of the treatment structure and also those that indirectly created diet formulations with variation in one or more of the BCAA, such as Ile, Val, and Leu requirement studies. In addition, trials that assessed the inclusion of corn by-products (corn germ meal, corn gluten meal, DDGS) were considered. All data was derived exclusively from peer-reviewed publications. In order to be included in the final database, trials had to provide treatment means and SEM or SD, pigs had to have ad libitum access to feed and water, and enough diet formulation detail to recreate the diet formulation. Trials that used blood products were eliminated based on preliminary model evaluation based on model fit and residual analysis. The reason for this is not clear but could be driven by the AA profile of blood products, high in Leu and low in Ile, that results in a substantial imbalance between BCAA.

Data from each trial was recorded in a spreadsheet template. Data included average daily gain (**ADG**), average daily feed intake (**ADFI**), gain-to-feed ratio (**G:F**), average body weight (**BW**), SEM, replicates per treatment, and number of pigs per pen. For papers that reported feed efficiency as feed-to-gain ratio (**F/G**), the inverse proportion was calculated based on the ADG and ADFI values provided. In some cases, growth performance was reported by period. In those situations, each period was identified in the database as a trial. For trials that reported only overall performance but diet formulation by period, the average nutrient content was pooled across periods to be used with overall growth performance.

The final database contained data from 25 papers, published from 1995 to 2018, that comprised 44 trials for a total of 210 observations. Dietary treatments from each trial were reformulated using a spreadsheet-based formulator with NRC (2012) nutrient loading values for ingredients to achieve a common basis for dietary nutrient concentrations. All AA were expressed on a standardized ileal digestible basis. Dietary factors evaluated included CP, neutral detergent fiber, Ile:Lys, Leu:Lys, Val:Lys, BCAA:Lys, Ile:Leu, Val:Leu, Ile:Val, (Ile+Val):Leu, Trp:Lys, Leu:Trp, Ile:Trp, Val:Trp, BCAA:Trp, Met:Lys, Leu:Met, Ile:Met, Val:Met, BCAA:Met, His:Lys, Leu:His, Ile:His, Val:His, BCAA:His, Thr:Lys, Leu:Thr, Ile:Thr, Val:Thr, BCAA:Thr, (Phe+Tyr):Lys, Leu:(Phe+Tyr), Ile:(Phe+Tyr), Val:(Phe+Tyr), BCAA:(Phe+Tyr), LNAA:Lys, Leu:LNAA, Ile:LNAA, Val:LNAA, and BCAA:LNAA.

Statistical analysis

The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used to develop the regression equations. The meta-regression was conducted as described by Flohr et al. (2018). Using the method of maximum likelihood, potential variables were selected by evaluating single variable equations. The statistical significance for inclusion of terms in the models was determined at $P < 0.05$. The WEIGHT statement of PROC MIXED was used to account for heterogeneous errors as the inverse of squared SEM (St-Pierre, 2001). Trial was included as a random effect. The single variable model with the lowest Bayesian information criterion (**BIC**) was selected and additional terms were added in a step-wise manual forward selection. In order to be included in the model, a reduction of 2 points or more in BIC was required (Kass and Raftery, 1995). Linear and quadratic terms as well as interactive effects were evaluated. If the quadratic or interactive terms were statistically significant and provided an improvement in BIC, the single order variables were also included in the model. Once the model with the lowest BIC was obtained, the method of residual maximum likelihood was used to obtain the parameter estimates. These methods were used to develop regression equations to predict ADG and G:F. Once ADG and G:F models were obtained, predicted values were used to estimate ADFI by dividing predicted ADG by predicted G:F. The adequacy of the models was examined by evaluating studentized residuals and residuals vs. predicted values plots.

RESULTS AND DISCUSSION

A summary of the publications used in the analysis is presented in Table 2.1. The values describe average initial and final BW and ranges of Leu:Lys, Ile:Lys, Val:Lys, and Trp:Lys. The studies in the final database comprised an average BW range of 6.5 to 126.7 kg, 82 to 715% Leu:Lys, 46 to 103% Ile:Lys, 38 to 124% Val:Lys, and 16 to 27% Trp:Lys. The inspection of residuals vs. predicted values relative to the line of equality (Figure 2.1) suggest the predictions were precise and not biased. The studentized residuals plots indicate that normality assumption was met and no evidence for outliers and heteroscedasticity was observed.

For ADG, the model with average BW as a single predictor had the lowest BIC value (2258.3) and it was a significant predictor of ADG ($P < 0.01$). Therefore, average BW was selected as the first predictor variable and other variables were subsequently added to the model. The step-wise inclusion of Leu:Lys (linear term; $P < 0.01$, BIC = 2244.7), Ile:Lys (linear and quadratic terms; $P < 0.01$, BIC = 2238.6), (Ile+Val):Leu (linear and quadratic terms; $P < 0.01$, BIC = 2214.6), and Ile:Trp (linear term; $P < 0.01$, BIC = 2208.6) resulted in improvements in BIC. Other variables did not further improve BIC. Therefore, the model with average BW, Leu:Lys, Ile:Lys, (Ile+Val):Leu, and Ile:Trp was selected as the final equation with a BIC of 2208.6 (Table 2.2).

For the G:F model, average BW was the single predictor with the lowest BIC (2023.5). The step-wise inclusion of Val:Lys (linear and quadratic terms; $P < 0.01$, BIC = 2000.2), Met:Lys (linear term; $P < 0.01$, BIC = 1990.7), and Ile:Lys (linear term; $P < 0.01$, BIC = 1985.8), resulted in improvements in BIC. The inclusion of other variables to the model did not further improve BIC. The final equation for G:F included average BW, Ile:Lys, Val:Lys, and Met:Lys (BIC = 1985.8; Table 2.2).

Surprisingly, we could not develop an equation to predict ADFI with the current database. Average BW was the single predictor with the lowest BIC, but the step-wise inclusion of other predictor variables did not improve BIC. Therefore, ADFI estimates were obtained by dividing predicted ADG by predicted G:F, in a procedure similar to Flohr et al. (2018).

The equations suggest that increasing Leu:Lys ratio has a negative effect on predicted performance. This agrees with the well-described metabolism of BCAA (Harper et al., 1984; Brosnan and Brosnan, 2006). While most AA are metabolized in the liver, BCAA are transported to the skeletal muscle to be degraded. Through the action of branched-chain amino-transferase (**BCAT**), BCAA are reversibly converted to α-keto acids. The α-keto acids of Ile, Leu, and Val are α-keto-β-methylvalerate, α-ketoisocaproate, and α-ketoisovalerate, respectively. In the next step, α-keto acids are transported to the liver, where they are irreversibly decarboxylated by branched-chain α-keto acid dehydrogenase complex (**BCKD**). Both enzymes BCAT and BCKD are common to all three BCAA. Stimulation of enzymatic activity by one of the BCAA will increase the catabolism of all BCAA, possibly creating antagonisms among them.

Antagonisms among BCAA are expected to be especially detrimental when the excess of a BCAA occurs while the others are marginally meeting or below the requirement. In practice, this situation commonly occurs in swine diets based on corn and/or corn-by products supplemented with feed-grade AA. Because of the AA profile of these ingredients and the increasing use of feed-grade AA in the swine industry, it is common to have diets with high concentrations of Leu while Ile and Val are close to the requirement estimate. This becomes an important issue if we consider that the most potent stimulator of BCAT and BCKD is Leu and its α-keto acid (Harper et al., 1984). In a recent study, Wessels et al. (2016a) fed nursery pigs with 1, 2, or 4% dietary Leu (approximately 100, 200, and 400% SID Leu:Lys) and observed that increasing Leu increased the activity of BCKD and reduced Ile and Val in plasma and several other tissues. This indicates that Leu stimulates not only its own catabolism but also of the other BCAA. As a consequence, Wessels et al. (2016a) observed that ADFI and ADG decreased by approximately 9 and 23% for pigs fed diets with 2 and 4% Leu, respectively, with no impact on G:F.

Excessive Val and Ile seems to have little effect on increasing the catabolism of the other BCAA (D'Mello and Lewis, 1970), which may be partially explained by their lower affinity to BCAT and BCKD compared to Leu (Harper et al., 1984). Moreover, early research (Benton et al., 1956) showed that Leu utilization for growth was decreased by high levels of Val or Ile only when Leu was growth-limiting, which is not a common circumstance in practical swine diets. Therefore, while Leu is undoubtedly the critical BCAA in swine diets regarding antagonisms, Val and Ile seem to require more careful manipulation of AA composition to cause antagonisms (Harper et al., 1984), which seems unrealistic in practical swine diets.

Contrary to Leu, the equations indicate that Ile and Val have positive effects on predicted performance and can potentially overcome the negative effects of high concentrations of Leu. Although both AA present diminishing returns due to the quadratic terms, Ile seems to have smaller capacity to counteract the negative effects of Leu compared to Val. This is mainly driven by the quadratic term for Ile:Lys and in the negative linear term for Ile:Trp, which indicates that higher Ile relative to Trp results in decreased ADG. The ability of Val and Ile to overcome the negative impact of high levels of Leu is not a new concept. Harper et al. (1954) demonstrated that Ile partially recovered decreased growth caused by high Leu diets in rats. Later, Benton et al. (1956) demonstrated that a combination of Ile and Val was needed to fully restore growth performance in rats fed high Leu diets. Although, our meta regression quantifies the magnitude of the effect under practical conditions of diet formulation with corn by-products and these predictions seem to agree well with the biochemical pathway data reported earlier. Recently, Millet et al. (2015) conducted a study with 10- to 45-kg pigs to evaluate the effects of SID Leu

(1.08 or 2.13%), Val (0.70 or 0.78%), Ile (0.60 or 0.73%), and Trp (0.20 or 0.25%), which resulted in ratios to Lys of approximately 97 to 192% SID Leu:Lys, 63 to 70% SID Val:Lys, 54 to 66% SID Ile:Lys, and 18 to 22.5% SID Trp:Lys. Increasing Leu greatly decreased ADG, ADFI, and G:F and Val effectively counteracted the ADG and G:F reductions and partially recovered ADFI. The addition of Ile or Trp did not provide further improvements in growth performance. Thus, it seems that in diets with high Leu concentrations, more Val is needed to ensure optimal growth performance, which may indicate a different Val requirement depending on Leu level (Millet et al., 2015). In a study with nursery pigs, Gloaguen et al. (2011) provided diets with 60 or 70% Val:Lys and 111 or 165% Leu:Lys and observed that pigs fed diets with high Leu:Lys ratios had decreased growth performance, but the reduction was lessened with higher Val:Lys.

Based on our model, a similar inference can be made for Ile. However, our equations indicate that Ile alone cannot overcome the negative impact of high levels of Leu on performance and needs to be added in combination with Val and/or Trp, which seems to agree with early research (Harper et al., 1954; Benton et al., 1956; Harper et al., 1984). The potential counteractive effects of Ile and Val in high Leu diets may be explained by transport competition into the brain. Hjelle et al. (1978), using an *in vitro* model, observed that the transport of Leu through the blood-brain barrier is inhibited by Val. This finding was confirmed by Hargreaves and Pardridge (1988), who observed that Val and to a lesser extent Ile, Tyr, Phe, and Trp significantly decreased the transport of Leu into the brain.

The ratio between Ile and Trp is the final component of the ADG prediction equation. As this ratio increases, due to a reduction in Trp and/or an increase in Ile to very high levels, predicted performance is negatively impacted. The LNAA enter the brain via a shared transporter

(Pardridge et al., 1977). Increasing levels of one of the LNAA raises its brain uptake and decreases the uptake of the other LNAA (Fernstrom, 2013). Carlsoon and Lindqvist (1978) observed lower Trp concentration in the brain of rats after intraperitoneal injection of Ile, Leu, or Val. Thus, it seems that Ile:Trp in the equations can be at least partially explained by the competition between Ile and Trp for brain uptake, which ultimately results in reduced serotonin activity in the brain and decreased feed intake (Henry et al., 1992). It is well described that low dietary Trp has profound effects on feed intake (Ettle and Roth, 2004, Gonçalves et al., 2015), and high Leu seems to be negatively correlated with brain Trp and serotonin (Wessels et al., 2016a,b). Rogers et al. (1967) observed that in addition to Val and Ile, the inclusion of the LNAA (Trp, Phe, and Thr) was required to recover growth depression caused by a high Leu diet in rats. However, the specific interactions between Ile and Trp is not fully understood. Interestingly, the ratios of Leu or Val to Trp were not found to be significant predictors of performance in either model. However, it is important to note this only means the magnitude was not sufficiently large to be captured, it does not exclude the possibility that Leu:Trp or Val:Trp affect growth performance.

The Met:Lys ratio was a significant predictor of G:F but not ADG. It is important to note that the trials selected for this meta-regression were not designed as Met requirement studies, thus the range of Met:Lys was fairly narrow (i.e., 27 to 56% SID Met:Lys). The NRC (2012) requirement estimate for SID Met:Lys is 29%. Based on our equation, a decrease in G:F is observed as SID Met:Lys increases. Edmonds and Baker (1987) observed that high levels of Met result in decreased ADFI and ADG but not G:F in young pigs and Edmonds et al. (1987) observed lower ADG, ADFI, and G:F in weanling pigs fed diets with 4% DL-Met. The relationship between Met and the BCAA is not fully understood. Surprisingly, Langer and Fuller

(2000) observed that an excess of Leu or all BCAA improved N utilization in pigs fed Met-limiting diets. In a subsequent study, (Langer et al. 2000) suggested that this may be driven by BCKD, which catabolizes BCAA keto-acids but also Met keto-acids. The addition of excess BCAA would affect Met catabolism by supplying alternative substrates to BCKD, resulting in more Met available for protein synthesis. However, it is important to note that in our model there was no evidence for interactive terms between Met and any BCAA.

A hypothetical scenario that simulates a practical situation is shown in Table 2.3, where grower pig diets based on corn, soybean meal, and DDGS were formulated with low or high addition of feed-grade AA to manipulate the BCAA levels. The diet with low feed-grade AA has a greater Leu:Lys ratio compared to the diet with high inclusion of feed-grade AA. However, the predicted ADG is greater for the low than the high feed-grade AA diet because Ile:Lys and Val:Lys are also increased in the low feed-grade AA diet. The predicted ADG of the high feed-grade AA diet can be restored to the same level of the low feed-grade AA diet by supplementing Val alone or a combination of Val, Ile, and Trp.

Our equations for ADG and G:F suggest that ADFI is reduced in high Leu diets. Other research also suggests that ADFI is decreased when pigs are fed diets with high Leu or imbalanced BCAA concentrations (Harper et al., 1984; Gloaguen et al., 2011; Millet et al., 2015; Wessels et al., 2016a; Meyer et al., 2017). A possible explanation for this response is the excessive stimulation of the mammalian target for rapamycin (**mTOR**), a signaling pathway that stimulates protein synthesis and cell growth in the presence of adequate nutrients (Schmelze and Hall, 2000). Leucine is a potent stimulator of mTOR and consequently plays an important role in protein synthesis (Suryawan et al., 2011). However, excessive mTOR signaling caused by high levels of Leu seems to impact growth performance through a strong inhibitory effect on feed intake (Cota et al., 2006). Interestingly, the effect on feed intake is not observed for Val (Cota et al. 2006). The available literature also supports the negative effects of increasing Leu on G:F (Gloaguen et al., 2011; Millet et al., 2015; Duan et al., 2016).

However, a few studies did not observe evidence for changes in any growth performance variable even at moderate to high Leu concentrations (Cisneros et al., 1996; Hyun et al., 2007; Morales et al., 2016). Although reasons are not well-defined, those diets were also well above the Val and Ile requirement estimates. According to our model, this could indicate that Val and Ile could be potentially counteracting the negative effect of high Leu on growth performance.

In our prediction equations, the combination of AA used to minimize the negative impact of Leu can be changed by the nutritionist to optimize feed cost depending on current AA costs. Other hypothetical scenarios with corn-soybean meal diets or different amounts of DDGS produce similar results. The implementation of prediction equations in a diet formulation is a straightforward process that would allow the nutritionist to visualize the predicted performance based on BCAA and LNAA concentrations. Field research trials are required to validate the predicted growth performance provided by the models. If proven correct, the prediction equations provide an important tool that allows nutritionists to formulate practical diets with high inclusion levels of feed grade AA and overcome the negative effects of excessive Leu:Lys.

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Table 2.1. Summary of publications used in the meta-regression to predict growth performance from branched-chained and large neutral amino acids[1,2]

Publication	Trials	Average initial BW, kg	Average final BW, kg	Range of SID Leu:Lys	Range of SID Ile:Lys	Range of SID Val:Lys	Range of SID Trp:Lys
Lewis and Nishimura, 1995	1	67.0	80.0	102	70	38-93	19
Cisneros et al., 1996	1	80.8	110.6	229-715	81	99	19-24
Mavromichalis et al., 2001	1	10.7	17.0	82-127	50-63	39-88	19-20
Whitney et al., 2004	4	8.2	16.0	130-176	66-78	71-87	19-22
Dean et al., 2005	3	82.3	114.3	160-251	48-69	64-124	19-24
Whitney et al., 2006	1	28.3	115.1	171-251	71-89	81-109	20-21
Hyun et al., 2007	1	73.3	126.7	204-594	77	91	16-24
Lineen et al., 2008	4	52.3	89.2	145-221	70-87	76-101	20-22
Weber et al., 2010	1	30.8	54.6	92-139	49-64	52-71	19-27
Xu et al., 2010	3	53.4	84.3	162-239	70-73	80-93	19-20
Cromwell et al., 2011	3	61.2	89.8	176-383	81-103	90-133	23-27
Gaines et al., 2011	3	16.1	28.1	110-145	58-74	54-79	18-22
Castilha et al., 2012	1	15.4	30.3	111	46-67	67	16
Lee et al., 2012	1	68.1	95.2	137-218	63-74	76-91	16-20
Waguespack et al., 2012	1	21.8	42.2	132	61	61-73	21
Bergstrom et al., 2014	1	35.1	125.0	186-247	71-81	83-100	17-19
Graham et al., 2014	1	68.9	125.6	167-244	70-82	80-101	18-19
Liu et al., 2015	4	60.0	81.8	113-138	52-56	54-79	18-23
Morales et al., 2016	1	31.8	46.8	94-140	57-78	69-96	24
Overholt et al., 2016	3	58.4	87.4	133-230	60-78	66-96	17-20
Clark et al., 2017	1	6.5	9.8	104	57	53-87	20
Coble et al., 2017	1	105.7	125.4	165-209	62-66	75-81	17-19
Huepa et al., 2017	1	11.1	24.8	91-142	51	64	17
Lazzeri et al., 2017	1	15.0	31.1	111	46-75	68	17
Xu et al., 2018	1	8.8	20.1	113	53	60-90	21

[1] For trials that reported growth performance by period, each period was identified in the database as a trial.

[2] Standardized ileal digestible (SID) amino acid ranges presented are based on reformulated diets using NRC (2012) nutrient loading values.

Table 2.2. Regression equations to predict growth performance of grow-finish pigs[1]

Variable[2]	Equation[3]	BIC[4]
ADG, g	$= -985.94 + (15.2499 \times \text{average BW (kg)}) - (0.08885 \times \text{average BW} \times \text{average BW}) + (1.063 \times \text{Leu:Lys}) + (20.2659 \times \text{Ile:Lys}) - (0.1479 \times \text{Ile:Lys} \times \text{Ile:Lys}) + (9.2243 \times \text{(Ile+Val):Leu}) - (0.03321 \times \text{(Ile+Val):Leu} \times \text{(Ile+Val):Leu}) - (0.4413 \times \text{Ile:Trp})$	2208.6
G:F, g/kg	$= 648.3 - (6.2974 \times \text{average BW (kg)}) + (0.02051 \times \text{average BW} \times \text{average BW}) + (0.5396 \times \text{Ile:Lys}) + (1.7284 \times \text{Val:Lys}) - (0.00795 \times \text{Val:Lys} \times \text{Val:Lys}) - (1.7594 \times \text{Met:Lys})$	1985.8
ADFI, kg	$= \text{Predicted ADG} \div \text{Predicted G:F}$	---

[1] Model adjusted for heterogeneous errors using the inverse of squared SEM.

[2] ADG = average daily gain. G:F = gain-to-feed-ratio. ADFI = average daily feed intake.

[3] Average BW = average body weight. Amino acids ratios to lysine are expressed on standardized ileal digestible basis.

[4] BIC = Bayesian information criterion.

Table 2.3. Hypothetical scenario for prediction of average daily gain (ADG), gain-to-feed ratio (G:F), and average daily feed intake (ADFI) of 75 kg pigs based on branched-chain and large neutral amino acid concentrations[1]

	Low AA	High AA	High AA+Val	High AA+Val, Ile	High AA +Val, Ile, Trp
Ingredient, %					
Corn	53.71	65.91	65.69	65.72	65.76
Soybean meal	14.42	1.55	1.56	1.56	1.56
Distillers dried grains with solubles	30.00	30.00	30.00	30.00	30.00
Calcium carbonate	0.98	0.90	0.90	0.90	0.90
Monocalcium phosphate	---	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	0.22	0.63	0.63	0.63	0.63
DL-Methionine	---	0.02	0.02	0.02	0.02
L-Threonine	---	0.16	0.16	0.16	0.16
L-Tryptophan	---	0.07	0.07	0.07	0.09
L-Valine	---	---	0.20	0.11	0.06
L-Isoleucine	---	---	---	0.07	0.06
Vitamin-mineral premix	0.43	0.43	0.43	0.43	0.43
Calculated analysis					
Crude protein, %	19.7	15.2	15.3	15.3	15.2
SID Lysine	0.82	0.82	0.82	0.82	0.82
SID Isoleucine:lysine	78	52	52	60	58
SID Leucine:lysine	211	173	173	173	173
SID Valine:lysine	93	67	91	79	74
SID Tryptophan:lysine	18.9	18.9	18.9	18.9	21.3
SID Isoleucine:tryptophan	413	275	275	319	274
SID Methionine:lysine	37	32	32	32	32
Predicted ADG[2], g	911	852	911	911	911
% relative to low AA diet	---	93.5%	100%	100%	100%
Predicted G:F[3], g/kg	360	343	355	354	351
% relative to low AA diet	---	95.3%	98.6%	98.3%	97.5%
Calculated ADFI[4], kg	2.53	2.48	2.57	2.57	2.60
% relative to low AA diet	---	98.0%	101.6%	101.6%	102.7%

[1] Diets were formulated with the NRC (2012) nutrient loading values to meet or exceed the nutrient requirements.

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[2] ADG, g = – 985.94 + (15.2499 × average BW (kg)) – (0.08885 × average BW × average BW) + (1.063 × Leu:Lys) + (20.2659 × Ile:Lys) – (0.1479 × Ile:Lys × Ile:Lys) + (9.2243 × (Ile+Val):Leu) – (0.03321 × (Ile+Val):Leu × (Ile+Val):Leu) – (0.4413 × Ile:Trp).

[3] G:F, g/kg = 648.3 – (6.2974 × average BW (kg)) + (0.02051 × average BW × average BW) + (0.5396 × Ile:Lys) + (1.7284 × Val:Lys) – (0.00795 × Val:Lys × Val:Lys) – (1.7594 × Met:Lys).

[4] Calculated ADFI, kg = predicted ADG ÷ predicted G:F.

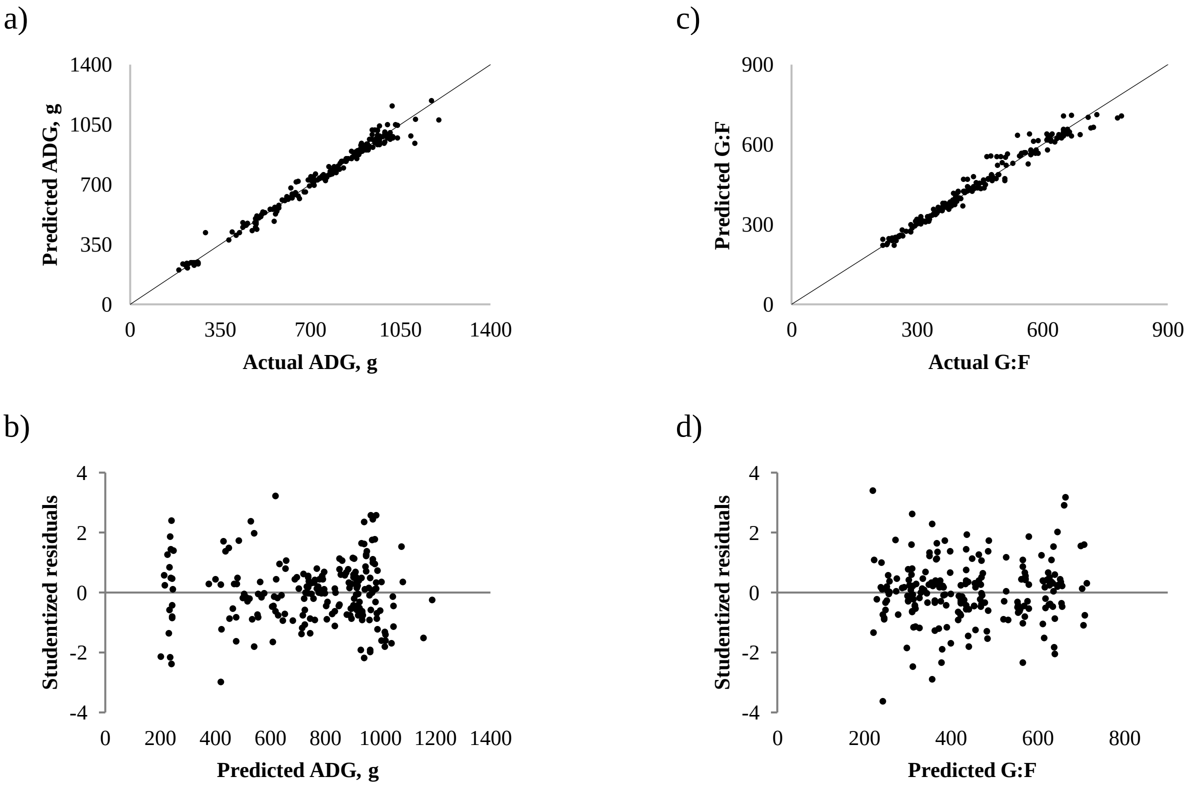

Figure 2.1. Plots of actual vs. predicted values relative to the line of equality and studentized residual of average daily gain (ADG) and gain-to-feed ratio (G:F). Plots a) and b) are for ADG and plots c) and d) for G:F.

Chapter 3 - Effects of standardized ileal digestible histidine to lysine ratio on growth performance of 7- to 11-kg nursery pigs[1]

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ABSTRACT: Histidine may be the sixth limiting amino acid in practical nursery diets supplemented with high amounts of feed-grade amino acids. Therefore, two experiments were conducted to determine the standardized ileal digestible (**SID**) His:Lys ratio requirement estimate for growth performance of 7- to 11-kg nursery pigs. A total of 360 and 350 pigs (DNA 241 × 600, Columbus, NE; initially 7.1 ± 0.31 and 6.6 ± 0.36 kg) were used in Exp. 1 and 2, respectively. There were 5 pigs per pen with 12 replicates per treatment in Exp. 1 and 10 replicates per treatment in Exp. 2. After weaning, pigs were fed a common pelleted diet for 10 d in Exp. 1 and 7 d in Exp. 2. Then, pens were assigned to treatments in a randomized complete block design with body weight (**BW**) as the blocking factor. Dietary treatments consisted of SID His:Lys ratios of 24, 28, 32, 36, 40, and 44% in Exp. 1 and 24, 28, 30, 32, 34, 36, and 42% in Exp. 2. Experimental diets were fed in pellet form for 10 or 14 d in Exp. 1 and 2, followed by a common mash diet for 15 or 14 d, respectively. Data were analyzed using the GLIMMIX and NLMIXED procedures of SAS, fitting data with heterogeneous variance when needed. The competing statistical models utilized were quadratic polynomial (**QP**), broken-line linear (**BLL**), and broken-line quadratic (**BLQ**). In Exp. 1, increasing SID His:Lys ratio increased (quadratic, $P = 0.001$) ADG, ADFI, G:F, and d 10 BW. In Exp. 2, ADG, G:F, and d 14 BW increased (quadratic, $P = 0.001$), and ADFI increased linearly ($P = 0.001$) with increasing SID His:Lys ratio. The best-fitting model for all response variables analyzed was the BLL. In Exp. 1, requirement estimates were 29.7, 29.1, and 29.8% SID His:Lys ratio for ADG, ADFI, and G:F, respectively. In Exp. 2, the SID His:Lys ratio requirement estimates were 31.0% for ADG and 28.6% for G:F. These results suggest that the SID His requirement estimate for growth performance is no more than 31% of Lys and that the NRC (2012) SID His requirement of 34% of Lys may be overestimated for 7- to 11-kg pigs.

Key words: histidine, growth, lysine, modeling, swine

INTRODUCTION

Practical nursery diets are formulated with high inclusion of crystalline amino acids (**AA**). In many situations, it is economical to add L-Lys, L-Thr, L-Trp, DL-Met, and L-Val. The NRC (2012) AA requirement estimates suggest that His may become the sixth limiting AA in many diets fed to 7- to 11-kg body weight (**BW**) pigs when supplemented with high amounts of these feed-grade AA. Therefore, the SID His:Lys ratio requirement estimate could dictate the maximum inclusion of crystalline AA in nursery diets.

Amino acid requirements are often expressed as a standardized ileal digestible (**SID**) ratio to Lys. The NRC (2012) estimates the SID His:Lys ratio requirement at 34% for nursery pigs from 7 to 11 kg. Recent research suggests that the NRC (2012) recommendations may overestimate the His requirement. Gloaguen et al. (2013) determined that a 32% SID His:Lys ratio was ideal for 11- to 20-kg pigs and Wessels et al. (2016) estimated the SID His:Lys ratio at 28% for 8- to 21-kg pigs. However, there is limited data validating these ratios or investigating the SID His:Lys ratio requirement for lighter pigs. Therefore, the objective of our study was to determine the SID His:Lys ratio requirement for growth performance of 7- to 11-kg pigs.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Two experiments were conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS.

Animals and diets

All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Corn, soybean meal, spray-dried whey, and whey protein concentrate were submitted to Ajinomoto Heartland, Inc. (Chicago, IL) for total AA content analysis (excluding Trp, method 994.12; AOAC International, 2012) and Trp (method 13904:2005; ISO, 2005) prior to diet formulation (Table 3.1). These values were multiplied by NRC (2012) standardized ileal digestibility coefficients and used in diet formulation.

In Exp. 1, 360 pigs (DNA 241 × 600, Columbus, NE; initial average BW of 7.1 ± 0.31 kg) were used in a 25-d growth trial, where test diets were fed for 10 d and a common diet was then fed for 15 d. In Exp. 2, 350 pigs (DNA 241 × 600, Columbus, NE; initial average BW of 6.6 ± 0.36 kg) were used in a 28-d growth trial, where test diets were fed for 14 d and a common diet was fed for the next 14 d. Pigs in both trials were weaned at approximately 21 d of age and placed in pens of 5 pigs each based on initial BW and sex. A common phase 1 pelleted diet was fed for 10 d in Exp. 1 and 7 d in Exp. 2. At d 7 or 10 after weaning, which was considered d 0 of the trial, pens of pigs were allotted to treatment in a randomized complete block design with BW as the blocking factor. There were 12 replicates per treatment in Exp. 1 and 10 replicates per treatment in Exp. 2.

In Exp. 1 and 2, the same basal diet containing corn, spray-dried whey, and whey protein concentrate was formulated to 24% SID His:Lys ratio (Table 3.2). Then, a high SID His:Lys ratio diet (44 or 42% in Exp. 1 and 2, respectively) was formulated. Crystalline L-His replaced corn to form the high SID His:Lys ratio diet. Within each experiment, the low and high diets were blended at the feed mill to achieve the intermediate SID His:Lys ratio diets. In brief, large batches of the low and high SID His:Lys ratio diets were manufactured and bagged in 23 kg bags. Then, bags were randomly selected and blended in different proportions to achieve the desired treatment diet. In Exp. 1, six dietary treatments were created to contain SID His concentrations at 24, 28, 32, 36, 40, and 44% of Lys. To add more data points around the suggested requirement from Exp. 1, seven dietary treatments containing SID His at 24, 28, 30, 32, 34, 36, and 42% of Lys were used in Exp. 2. Based on Clark et al. (2017b), who determined the SID Lys requirement of 7- to 11-kg pigs at 1.45%, the experimental diets were formulated to contain 1.25% SID Lys to ensure Lys was the second limiting AA. All other AA met or exceeded the NRC (2012) requirement estimates. Experimental diets were pelleted and the average processing parameters were: 50.6°C conditioning temperature, 68.9°C hot pellet temperature, 330/1625 mm die size (length/diameter ratio = 5.0), 707 kg/h production rate, 29.8°C ambient temperature, and 82% relative humidity. The common diet was provided in mash form.

Each pen (1.5 × 1.5 m) was equipped with a 4-hole, dry self-feeder and a cup waterer to provide ad libitum access to feed and water. Pigs were weighed and feed disappearance was measured on d 0, 7, 10, 18, and 25 in Exp. 1 and on d 0, 7, 14, 21, and 28 in Exp. 2 to determine ADG, ADFI, and G:F.

Chemical analysis

Representative diet samples were obtained from all feeders of each treatment and stored at -20□C until analysis. Samples were analyzed (Ward Laboratories, Inc., Kearney, NE) for DM (method 935.29; AOAC International, 1990), CP (method 990.03; AOAC International, 1990), Ca (method 985.01; AOAC International, 1990), P (method 985.01; AOAC International, 1990), Na (Kovar, 2003), and Cl (method 969.10, AOAC International, 1990). Feed samples were also analyzed for total AA content (excluding Trp, method 994.12; AOAC International, 2012) and Trp (method 13904:2005; ISO, 2005) at Ajinomoto Heartland, Inc. (Chicago, IL).

Statistical analysis

Data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. Polynomial contrasts were constructed to evaluate the linear and quadratic effects of increasing SID His:Lys ratio on ADG, ADFI, G:F, and BW. Contrast coefficients were adjusted for unequally spaced treatments in Exp. 2. Data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$. Competing dose response models consisted of quadratic polynomial (**QP**), broken-line linear (**BLL**), and broken-line quadratic (**BLQ**). Broken-line regression models were fitted using the NLMIXED procedure of SAS according to the procedures of Gonçalves et al. (2016). Models were expanded to account for heterogeneous variance when needed. Competing models were compared using the Bayesian information criteria, with decreases by 2 or more units considered an improved fit. Results reported correspond to the best fitting model.

RESULTS

Chemical analysis

The analyzed DM, CP, Ca, P, Na, Cl, and total AA were consistent with formulated values (Tables 3.3 and 3.4). As expected, AA analysis showed a stepwise increase in total His concentrations.

Experiment 1

From d 0 to 10, when experimental diets were fed, ADG, ADFI, and G:F increased, then plateaued (quadratic, $P = 0.001$) with increasing SID His:Lys ratio (Table 3.5). For all response variables, the best fitting model was the BLL. For ADG, the estimated breakpoint was 29.7% (95% CI: [27.8, 31.6%]) SID His:Lys ratio and the regression equation (Fig. 1) was:

ADG, g = 463.23 – 23.96 × (29.69 – SID His:Lys) if SID His:Lys < 29.7%,

ADG, g = 463.23 if SID His:Lys ≥ 29.7%

For ADFI, the estimated breakpoint was 29.1% (95% CI: [27.6, 30.6%]) SID His:Lys ratio and the regression equation for the BLL model (Fig. 2) was:

ADFI, g = 562.24 – 19.45 × (29.1 – SID His:Lys) if SID His:Lys < 29.1%,

ADFI, g = 562.24 if SID His:Lys ≥ 29.1%

For G:F, the maximum mean value was estimated at 29.8% (95% CI: [27.6, 31.2%]) SID His:Lys ratio and the estimated regression equation for the BLL model (Fig. 3) was:

G:F, g/kg = 815.95 – 18.34 × (29.8 – SID His:Lys) if SID His:Lys < 29.8%,

G:F, g/kg = 815.95 if SID His:Lys ≥ 29.8%

During the post-test period (d 10 to 25), pigs previously fed low SID His:Lys ratios appeared to have a compensatory response in growth performance. Average daily gain and G:F increased (linear, $P < 0.05$) in pigs previously fed diets with the lower SID His:Lys ratios compared to pigs previously fed adequate SID His:Lys ratio. There was a quadratic response ($P < 0.01$) for BW on d 10 and 25. Overall (d 0 to 25), ADG and ADFI were greater (quadratic, $P < 0.05$) and there was a marginally significant improvement in G:F (quadratic, $P = 0.096$) with increasing SID His:Lys ratio.

Experiment 2

From d 0 to 14, when experimental diets were fed, ADG and G:F increased, then plateaued (quadratic, $P = 0.001$) and ADFI linearly increased ($P = 0.001$) with increasing SID His:Lys ratio (Table 3.6). The response for ADFI was not modeled due to its linear nature.

Similar to Exp. 1, the best fitting model was the BLL for ADG and G:F. The maximum mean ADG was estimated at 31.0% (95% CI: [29.7, 32.3%]) SID His:Lys ratio and the estimated regression equation (Fig. 4) was:

ADG, g = 355.01 – 17.22 × (31.0 – SID His:Lys) if SID His:Lys < 31.0%,

ADG, g = 355.01 if SID His:Lys ≥ 31.0%

For G:F, the estimated breakpoint was 28.6% (95% CI: [27.1, 30.0%]) SID His:Lys ratio and the regression equation for the BLL model (Fig. 5) was:

G:F, g/kg = 726.40 – 38.48 × (28.6 – SID His:Lys) if SID His:Lys < 28.6%,

G:F, g/kg = 726.40 for SID His:Lys ≥ 28.6%

During the post-test period (d 14 to 28), ADFI decreased (linear, $P = 0.003$) and G:F increased (quadratic, $P = 0.001$) in pigs previously fed diets with the lower SID His:Lys ratios compared to pigs previously fed adequate SID His:Lys ratio. There was a quadratic response ($P < 0.01$) for BW on d 14 and 28. Overall (d 0 to 28), ADG, ADFI, and G:F were greater (quadratic, $P < 0.05$) with increasing SID His:Lys ratio.

DISCUSSION

Most modern commercial nursery diets are formulated with high additions of crystalline AA. The replacement of intact protein sources by feed-grade AA increases as the feed-grade AA become available and economically justifiable. This strategy results not only in reduced diet cost but also lower CP diets and reduced N excretion to the environment (Kerr and Easter, 1995). Moreover, low CP diets may decrease fermentable protein in the hindgut and consequently decrease the incidence of post-weaning diarrhea (Heo et al., 2008). Considering the NRC (2012) SID His requirement estimate of 34% of Lys, His would be the sixth limiting AA after Val in many nursery diets. Therefore, in a practical diet formulated with L-Lys, L-Thr, L-Trp, DL-Met, and L-Val, the SID His:Lys ratio would dictate the maximum inclusion of crystalline AA.

In a requirement study, it is recommended that 25% of the treatments are placed below and above the anticipated requirement and 50% of the treatments around the anticipated requirement (Shearer, 2000). Based on the results of Exp. 1, Exp. 2 treatments were formulated with the intention to be more closely spaced around the expected breakpoint and ultimately provide a more precise requirement estimate. Moreover, it is critical that the basal diet is deficient in the test AA (Boisen, 2003). This may be especially true for His, because it has been shown that carnosine and hemoglobin degradation can provide His and partially alleviate the negative effects of a His deficient diet (Clemens et al., 1984). In this study, growth performance was dramatically reduced when pigs were fed the 24% SID His:Lys ratio diet, demonstrating it was deficient in the test AA. Finally, Lys should be the second limiting AA in requirement studies to avoid underestimation of the AA:Lys ratio requirement (Boisen, 2003). The SID Lys level of 1.25% was selected based on a study (Clark et al., 2017b) conducted in the same facilities with similar BW pigs.

A similar requirement estimate to our study was observed by Gloaguen et al. (2013), who determined the SID His:Lys requirement for 11- to 20-kg pigs at 31.6% for ADG and 28.8% for G:F using the BLQ model. Li et al. (2002) observed that the optimum His:Lys ratio for 10- to 20-kg pigs is 30%. In a N balance study, Heger et al. (2003) determined the ideal His:Lys ratio for 20-kg pigs at 33%. Wessels et al. (2016) determined the SID His:Lys requirement for 8- to 21-kg pigs at 26.5% using the BLL model and 27.9% using the BLQ model, which is moderately lower than our findings. The slightly greater breakpoint for ADG in Exp. 2 compared to Exp. 1 was driven by a marginal increase in growth rate of the pigs fed the SID His:Lys ratio of 32% and the addition of the diet with 30% SID His:Lys ratio. Furthermore, the requirement for maximum ADG was greater than that for maximum G:F, which is consistent with requirement studies for His (Gloaguen et al., 2013) and other AA, such as Ile (Gloaguen et al., 2013) and Trp (Gonçalves et al., 2015). Conversely, Wessels et al. (2016) found similar His requirements for ADG and G:F and some AA, such as Lys (Nemechek et al., 2012) and Val (Clark et al., 2017b) seem to have greater requirement for G:F than for ADG.

Taken together, these observations suggest that the NRC (2012) recommendation of 34% SID His:Lys ratio overestimates the His requirement of nursery pigs. Therefore, based on our results, practical nursery diets can be formulated with higher inclusions of crystalline AA before His becomes limiting. It is important to acknowledge that the NRC (2012) AA requirement estimates for nursery pigs, with the exception of Lys, are based on a factorial approach established by estimating the requirements for maintenance and growth rather than empirical studies. Moreover, in a summary of His requirement trials provided by the NRC (2012), only the early study of Izquierdo et al. (1988) is mentioned, clearly demonstrating the lack of research in this area.

The exact mode of action for the reduction in feed intake when pigs are fed diets deficient in histidine remains unknown. Histidine is a precursor of histamine, a neurotransmitter that plays an important role in appetite regulation (Kurose and Terashima, 1999). Dietary intake of neurotransmitter precursors is critical for normal growth. For instance, the amount of dietary Trp, a precursor of serotonin, has a profound effect on feed intake (Ettle and Roth, 2004; Gonçalves et al., 2015). Excessive neural histamine has been reported to suppress feed intake through H1 receptors in the brain satiety centers, namely ventromedial hypothalamic nucleus and paraventricular nucleus (Sakata et al., 1997). High levels of dietary L-His (Kasaoka et al., 2004) or injection of L-His (Sheiner et al., 1985; Yoshimatsu et al., 2002) have been shown to cause acute anorectic effects in rats. In the current study, we did not observe reduction in intake even when the highest levels of His were fed. It seems that a substantial amount of dietary L-His is required to provoke anorectic effects (Okusha et al., 2017). In our study, pigs fed diets with low SID His:Lys presented a dramatic decrease in intake, which is similar to that observed by Li (2002), Gloaguen et al. (2013) and Wessels et al. (2016), and seems the primary responsible for decreased growth performance. Radcliffe and Morrison (1981) observed a decrease in feed intake in rats fed His-free diets and Tobin and Boorman (1978) reported that the infusion of L-His in cockerels receiving a His deficient diet resulted in increased feed intake. Interestingly, knock-out mice, unable to synthesize histamine, presented no differences in caloric intake and body weight compared to normal mice (Provensi et al., 2016). At this point, it is unclear if histamine is involved in the anorectic response of pigs fed low His diets.

We observed compensatory growth, defined as an accelerated gain that occurs after a period of nutritional restriction (Heyer and Lebret, 2007), during the post-test period. Pigs previously fed the low SID His:Lys ratio diets had improved ADG (Exp. 1) and G:F (Exp. 1 and 2). The reasons for differences in post-treatment growth performance between experiments as well as the ADFI response in Exp. 2 are unclear. Nevertheless, the improvement in growth performance in the post-test period was not enough to change the overall results. Interestingly, pigs fed diets deficient in Ile (Clark et al., 2017a) and Val (Clark et al., 2017b) did not show evidence of compensatory growth during the post-test period.

In summary, our results suggest that the SID His required to optimize growth performance is no more that 31% of Lys for 7- to 11-kg pigs. The SID His requirement estimates observed in these studies are lower than the current NRC (2012) estimates of 34% of Lys.

Therefore, low CP, amino acid fortified nursery diets can be balanced to meet the pig's SID His:Lys ratio requirement for growth performance, allowing for greater use of the currently available feed-grade amino acids.

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Table 3.1. Total amino acid analysis of ingredients (as-fed basis)[1]

Item	Corn	Soybean meal	Whey protein concentrate	Spray-dried whey
Amino acids, %				
Ala	0.62	1.95	3.49	0.47
Arg	0.40	3.12	2.95	0.28
Asp	0.58	5.01	7.21	0.99
Cys	0.22	0.69	1.64	0.25
Glu	1.51	7.80	10.93	1.58
Gly	0.34	1.86	1.91	0.23
His	0.22	1.13	1.66	0.20
Ile	0.30	2.09	3.54	0.56
Leu	0.99	3.46	7.49	1.03
Lys	0.26	2.73	5.97	0.78
Met	0.20	0.63	1.41	0.15
Met and Cys	0.42	1.32	3.05	0.40
Phe	0.39	2.31	2.92	0.36
Pro	0.74	2.23	3.94	0.52
Ser	0.40	2.23	4.55	0.53
Thr	0.30	1.75	4.74	0.62
Tyr	0.17	1.37	1.91	0.15
Val	0.39	2.11	3.81	0.53
Trp	0.07	0.64	1.34	0.18

[1] A representative sample of each ingredient was obtained, homogenized, and submitted for amino acid analysis (Ajinomoto Heartland, Inc., Chicago, IL) prior to diet formulation.

Table 3.2. Diet composition, Exp. 1 and 2 (as-fed basis)[1]

Item	Exp. 1 and 2 24% SID[2] His:Lys	Exp. 1 44% SID His:Lys	Exp. 2 42% SID His:Lys
Ingredients, %			
Corn	60.20	59.94	59.97
Whey protein concentrate	7.75	7.75	7.75
Spray-dried whey	7.25	7.25	7.25
Soybean meal, 45% CP	5.63	5.63	5.63
Sucrose	10.00	10.00	10.00
Monocalcium phosphate, 21.5% P	1.43	1.43	1.43
Calcium carbonate	0.98	0.98	0.98
Sodium chloride	0.30	0.30	0.30
Sodium bicarbonate	0.75	0.75	0.75
Potassium chloride	0.11	0.11	0.11
L-Lys HCl	0.65	0.65	0.65
DL-Met	0.24	0.24	0.24
L-Thr	0.24	0.24	0.24
L-Trp	0.07	0.07	0.07
L-Val	0.26	0.26	0.26
L-Ile	0.14	0.14	0.14
L-Phe	0.34	0.34	0.34
L-His	---	0.25	0.23
Glutamic acid	1.50	1.50	1.50
Glycine	1.50	1.50	1.50
Zinc oxide	0.25	0.25	0.25
Vitamin premix[3]	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15
Phytase[5]	0.03	0.03	0.03
Total	100	100	100
Calculated analysis			
SID AA, %			
Lys	1.25	1.25	1.25
Ile:Lys	55	55	55
Leu:Lys	105	105	105
Met:Lys	39	39	39
Met and Cys:Lys	60	60	60
Thr:Lys	65	65	65
Trp:Lys	19.8	19.8	19.8
Val:Lys	70	70	70
His:Lys	24	44	42
NE, kcal/kg	2,511	2,504	2,504
CP, %	18.2	18.3	18.3
Ca, %	0.72	0.72	0.72
STTD P, %	0.52	0.52	0.52

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[1] In Exp. 1, diets were fed from 7.1 to 11.4 kg BW. Diets were blended to form the intermediate treatments: 28, 32, 36, and 40% SID His:Lys ratio. In Exp. 2, diets were fed from 6.6 to 11.2 kg BW. Diets were blended to form the intermediate treatments: 28, 30, 32, 34, and 36% SID His:Lys ratio.

[2] SID = standardized ileal digestible.

[2] Provided per kg of premix: 3,527,399 IU vitamin A; 881,850 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[3] Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[4] Ronozyme HiPhos 2700 (DSM Nutritional Products, Basel, Switzerland) provided 676 FTU per kg of feed.

Table 3.3. Chemical analysis of diets (as-fed basis; Exp 1)[1]

	SID[2] His:Lys ratio, %					
Item	24	28	32	36	40	44
Proximate analysis, %						
DM	90.5	90.9	91.0	91.6	91.4	91.5
CP	17.3	17.0	17.4	17.3	17.9	18.6
Ca	0.82	0.77	0.85	0.83	0.84	0.85
P	0.60	0.58	0.62	0.57	0.58	0.57
Na	0.35	0.37	0.37	0.39	0.35	0.42
Cl	0.48	0.51	0.48	0.48	0.51	0.60
Amino acids, %						
Lys	1.28	1.32	1.38	1.33	1.33	1.32
Ile	0.68	0.70	0.72	0.70	0.70	0.72
Leu	1.36	1.39	1.42	1.40	1.39	1.43
Met	0.45	0.46	0.46	0.45	0.47	0.45
Met and Cys	0.75	0.75	0.77	0.76	0.77	0.77
Thr	0.87	0.90	0.90	0.89	0.91	0.89
Trp	0.24	0.24	0.26	0.25	0.26	0.26
Val	0.88	0.91	0.92	0.90	0.91	0.92
His	0.33	0.37	0.43	0.45	0.50	0.55

[1] A representative sample of each diet was collected from all feeders for each treatment, homogenized, and submitted for proximate analysis (Ward Laboratories, Inc., Kearney, NE). Amino acid analysis was conducted on composite samples by Ajinomoto Heartland, Inc., Chicago, IL.

[2] SID = standardized ileal digestible.

Table 3.4. Chemical analysis of diets (as-fed basis; Exp 2)[1]

	SID[2] His:Lys ratio, %						
Item	24	28	30	32	34	36	42
Proximate analysis, %							
DM	91.8	91.4	91.2	92.1	91.4	91.2	91.7
CP	16.6	17.2	17.6	17.4	17.5	16.9	18.1
Ca	0.90	0.78	0.84	0.84	0.83	0.84	0.90
P	0.58	0.59	0.60	0.59	0.57	0.59	0.61
Na	0.43	0.40	0.39	0.41	0.46	0.41	0.42
Cl	0.53	0.54	0.53	0.53	0.57	0.54	0.54
Amino acids, %							
Lys	1.19	1.25	1.26	1.20	1.22	1.24	1.27
Ile	0.67	0.70	0.71	0.68	0.69	0.69	0.73
Leu	1.29	1.31	1.31	1.28	1.29	1.29	1.30
Met	0.41	0.43	0.42	0.41	0.42	0.42	0.44
Met and Cys	0.66	0.70	0.69	0.66	0.68	0.69	0.69
Thr	0.80	0.82	0.81	0.81	0.82	0.82	0.83
Trp	0.22	0.24	0.23	0.23	0.21	0.23	0.24
Val	0.84	0.86	0.87	0.84	0.85	0.86	0.87
His	0.33	0.36	0.38	0.39	0.39	0.44	0.50

[1] A representative sample of each diet was collected from all feeders for each treatment, homogenized, and submitted to Ward Laboratories, Inc., Kearney, NE for proximate analysis. Amino acid analysis was conducted on composite samples by Ajinomoto Heartland, Inc., Chicago, IL.

[2] SID = standardized ileal digestible.

Table 3.5. Least square means for growth performance of nursery pigs fed increasing standardized ileal digestible (SID) His:Lys ratio from 7 to 11 kg body weight (BW), Exp. 1[1,2]

	SID His:Lys ratio, %							Probability, *P* <	
Item	24	28	32	36	40	44	SEM	Linear	Quadratic
BW, kg									
d 0	7.1	7.1	7.1	7.1	7.1	7.1	0.31	0.910	0.679
d 10	10.3	11.3	11.8	11.8	11.5	11.7	0.42	0.001	0.001
d 25	18.2	18.9	19.3	19.1	19.1	19.0	0.60	0.010	0.003
Experimental period (d 0 to 10)									
ADG, g	327	423	469	474	448	462	15.16	0.001	0.001
ADFI, g	463	541	570	572	567	566	19.59	0.001	0.001
G:F, g/kg	709	782	826	829	791	818	11.34	0.001	0.001
Post-test period (d 10 to 25)									
ADG, g	524	505	506	488	505	488	15.11	0.025	0.440
ADFI, g	802	801	807	792	810	791	25.14	0.745	0.789
G:F, g/kg	653	631	627	617	624	618	7.62	0.002	0.071
Overall (d 0 to 25)									
ADG, g	445	472	491	482	482	477	13.02	0.007	0.002
ADFI, g	667	697	712	704	712	701	20.89	0.043	0.038
G:F, g/kg	668	678	690	685	678	683	6.80	0.224	0.096

[1] A total of 360 pigs (DNA 241 × 600, Columbus, NE; initially 7.1 kg) were used in a 25-d growth trial 5 pigs per pen and 12 replicates per treatment.

[2] Experimental diets were fed from d 0 to 10 and a common diet was fed from d 10 to 25.

Table 3.6. Least square means for growth performance of nursery pigs fed increasing standardized ileal digestible (SID) His:Lys ratio from 7 to 11 kg body weight (BW), Exp. 2[1,2]

	SID His:Lys ratio, %								Probability, $P <$	
Item	24	28	30	32	34	36	42	SEM	Linear	Quadratic
BW, kg										
d 0	6.6	6.6	6.6	6.6	6.6	6.6	6.6	0.36	0.826	0.857
d 14	9.8	10.9	11.0	11.5	11.8	11.5	11.6	0.58	0.001	0.001
d 28	17.2	18.3	18.5	18.7	19.1	18.7	18.9	0.81	0.001	0.003
Experimental period (d 0 to 14)										
ADG, g	232	309	321	350	372	351	357	16.80	0.001	0.001
ADFI, g	428	442	466	470	492	483	493	21.39	0.001	0.168
G:F, g/kg	550	704	689	740	754	729	723	20.17	0.001	0.001
Post-test period (d 14 to 28)										
ADG, g	524	526	533	519	522	517	528	20.61	0.960	0.831
ADFI, g	754	797	826	813	840	826	841	30.58	0.003	0.106
G:F, g/kg	694	660	645	640	623	626	627	8.62	0.001	0.001
Overall (d 0 to 28)										
ADG, g	378	417	427	435	447	434	442	17.17	0.001	0.003
ADFI, g	591	620	646	641	666	654	667	23.70	0.001	0.050
G:F, g/kg	639	674	660	677	672	664	662	9.31	0.209	0.016

[1] A total of 350 pigs (DNA 241 × 600, Columbus, NE; initially 6.6 kg) were used in a 28-d growth trial with 5 pigs per pen and 10 replicates per treatment.

[2] Experimental diets were fed from d 0 to 14 and a common diet was fed from d 14 to 28.

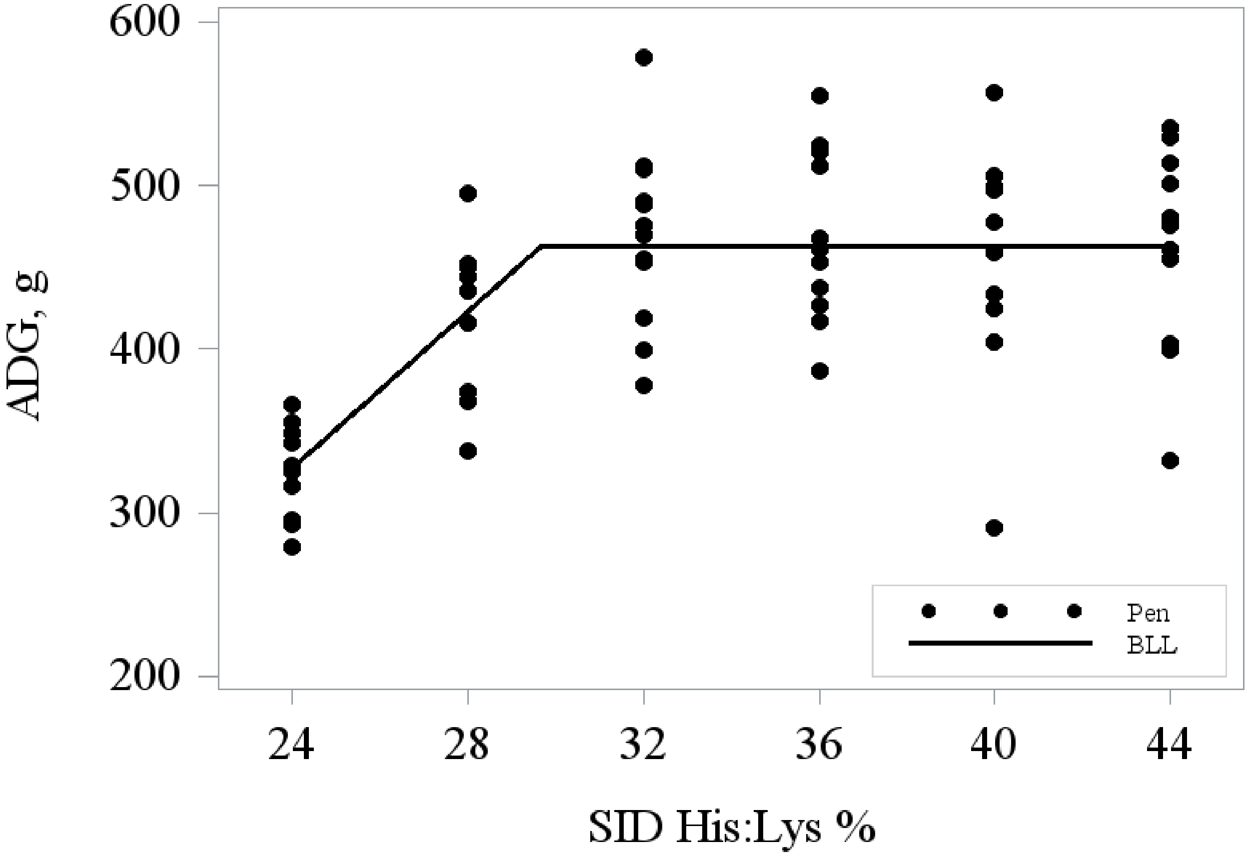

Figure 3.1. Fitted broken-line linear (BLL) regression model on ADG as a function of increasing SID His:Lys ratio for 7- to 11-kg pigs in Exp. 1. The maximum mean ADG was estimated at 29.7% (95% CI: [27.8, 31.6%]) SID His:Lys ratio. The estimated regression equation was ADG, g = 463.23 – 23.955 × (29.69 – SID His:Lys) if SID His:Lys < 29.7% and ADG, g = 463.23 if SID His:Lys ≥ 29.7%.

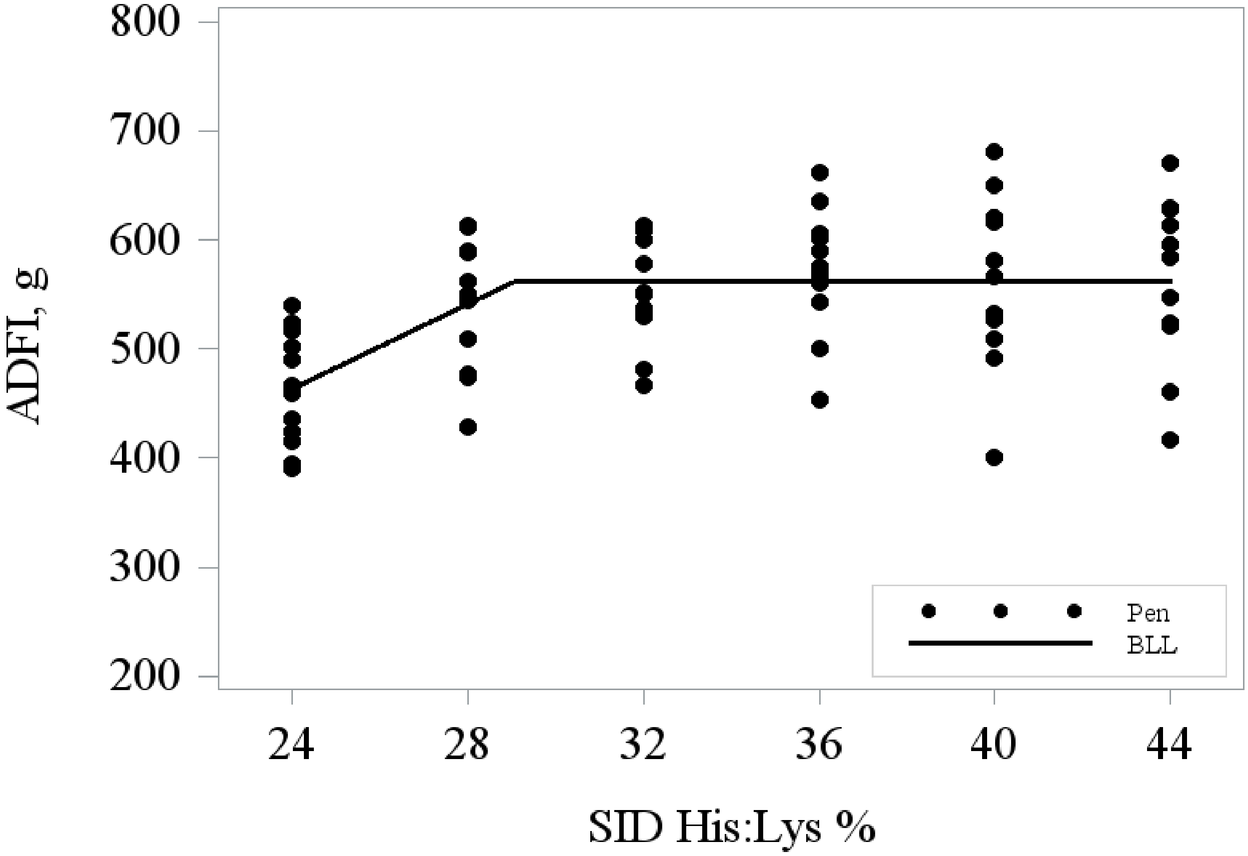

Figure 3.2. Fitted broken-line linear (BLL) regression model on ADFI as a function of increasing SID His:Lys ratio for 7- to 11-kg pigs in Exp. 1. The maximum mean ADFI was estimated at 29.1% (95% CI: [27.6, 30.6%]) SID His:Lys ratio. The estimated regression equation was ADFI, g = 562.24 – 19.448 × (29.1 – SID His:Lys) if SID His:Lys < 29.1% and ADFI, g = 562.24 if SID His:Lys ≥ 29.1%.

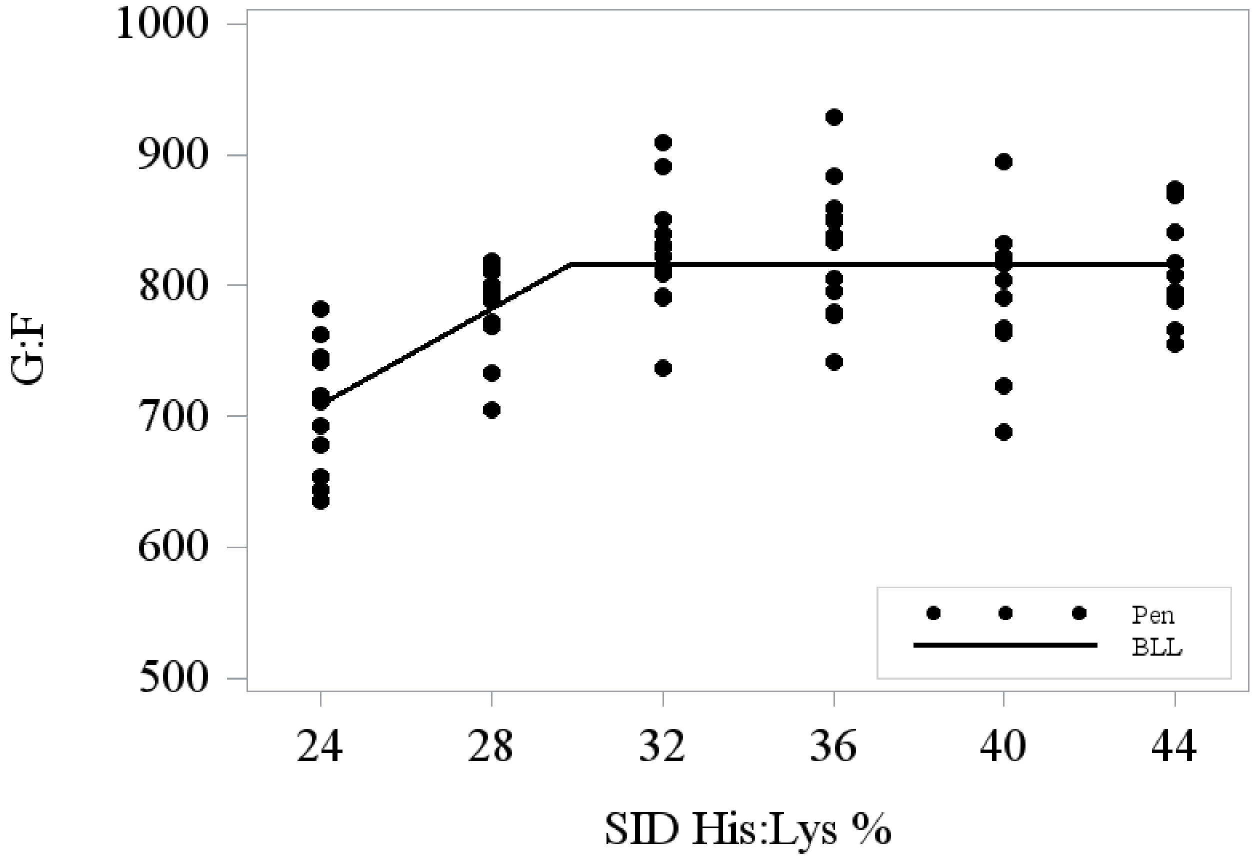

Figure 3.3. Fitted broken-line linear (BLL) regression model on G:F as a function of increasing SID His:Lys ratio for 7- to 11-kg pigs in Exp. 1. The maximum mean G:F was estimated at 29.8% (95% CI: [27.6, 31.2%]) SID His:Lys ratio. The estimated regression equation was G:F, g/kg = 815.95 – 18.344 × (29.8 – SID His:Lys) if SID His:Lys < 29.8% an G:F, g/kg = 815.95 if SID His:Lys ≥ 29.8%.

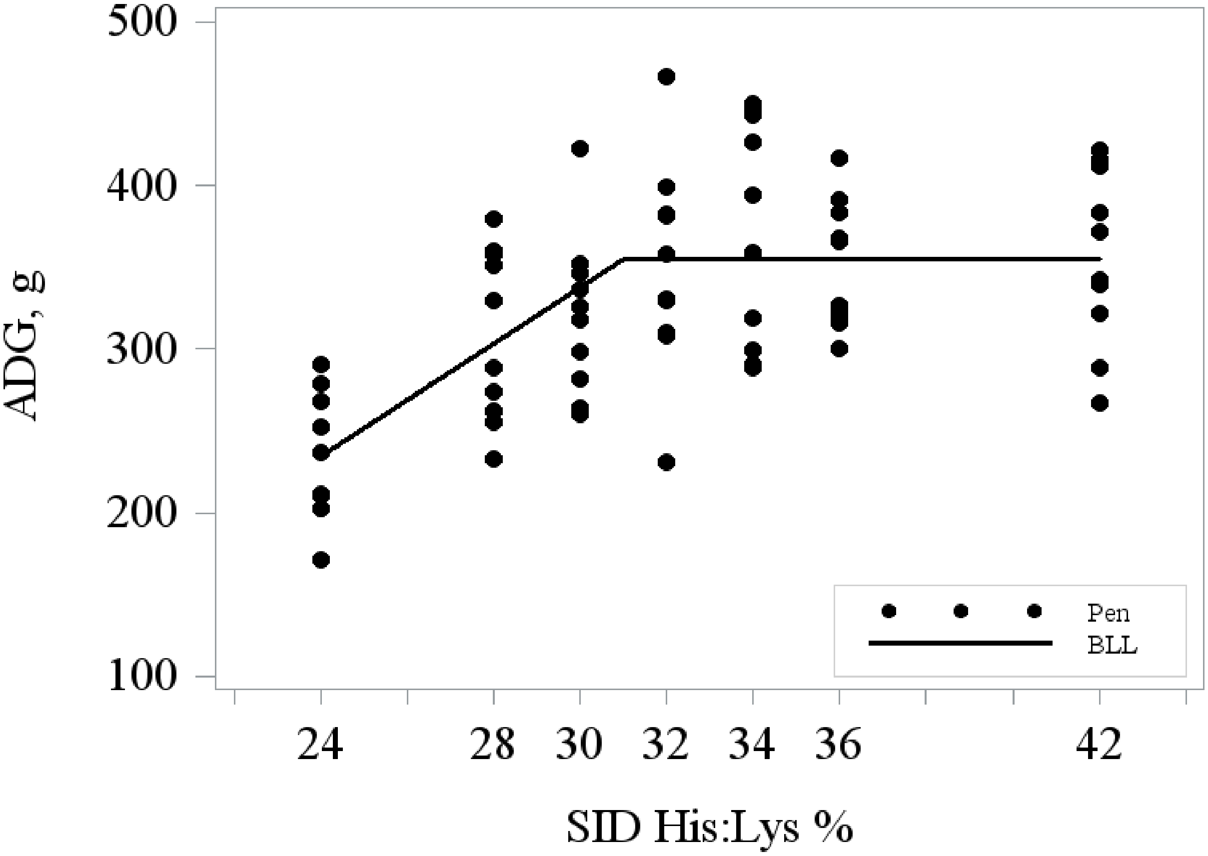

Figure 3.4. Fitted broken-line linear (BLL) regression model on ADG as a function of increasing SID His:Lys ratio for 7- to 11-kg pigs in Exp. 2. The maximum mean ADG was estimated at 31.0% (95% CI: [29.7, 32.3%]) SID His:Lys ratio. The estimated regression equation was ADG, g = 355.0 – 17.22 × (31.0 – SID His:Lys) if SID His:Lys < 31.0% and ADG, g = 355.0 if SID His:Lys ≥ 31.0%.

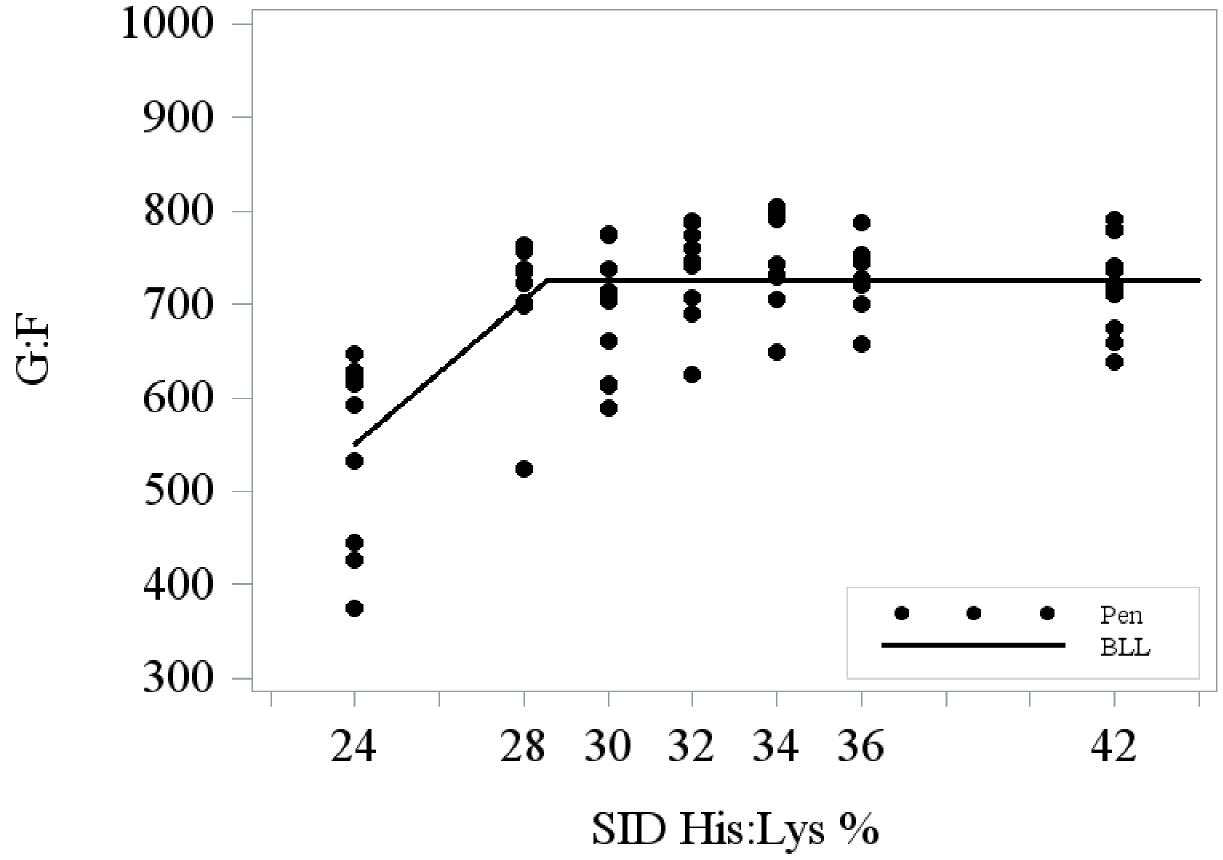

Figure 3.5. Fitted broken-line linear (BLL) regression model on G:F as a function of increasing SID His:Lys ratio for 7- to 11-kg pigs in Exp. 2. The maximum mean G:F was estimated at 28.6% (95% CI:[27.1, 30.0%]) SID His:Lys ratio. The estimated regression equation was G:F, g/kg = 726.4 – 38.48 × (28.6 – SID His:Lys) if SID His:Lys < 28.6% and G:F, g/kg = 726.4 if SID His:Lys ≥ 28.6%.

Chapter 4 - Effects of soybean meal level on growth performance of 11- to 25-kg nursery pigs[1,2]

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ABSTRACT: Four experiments were conducted to determine the effects of increasing soybean meal (**SBM**) level in diets with or without 25% distillers dried grains with solubles (**DDGS**) on growth performance of nursery pigs raised in university or commercial facilities. Treatments were arranged in a 2 × 3 factorial with main effects of SBM (27.5, 32.5, or 37.5% of the diet) and DDGS (0 or 25% of the diet). A total of 296, 2,502, 4,118, and 711 pigs initially 10.6, 11.7, 12.5, and 12.3 kg body weight (**BW**) were used in Exp. 1, 2, 3, and 4, respectively. There were 10, 16, 13, and 12 replicates per treatment in Exp. 1, 2, 3, and 4, respectively. After weaning, pigs were fed common diets for approximately 21 d. Then, pens of pigs were assigned to treatments in a randomized complete block design with BW as the blocking factor and experimental diets were fed for 21 d. Pigs were weighed and feed disappearance measured to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), gain-to-feed ratio (**G:F**), and caloric efficiency (**CE**). Data were analyzed using the GLIMMIX procedure of SAS with block as a random effect and treatment as a fixed effect. Single degree-of-freedom contrasts were constructed to test the linear and quadratic effects of increasing SBM and their interactions with DDGS. Pigs used in all experiments did not undergo major health challenges during the experimental period and due to the low number of mortality and cull events, statistical analysis was not performed on these variables. The average cull rate was 0.7, 0.5, 0.2, and 0% and the mortality rate was 0.7, 0.3, 0.4, and 0% in Exp. 1 to 4, respectively. There were interactions ($P \leq$ 0.039) between SBM and DDGS for G:F and CE in Exp. 2 and for ADG and ADFI in Exp. 3. These were mostly driven by increasing SBM negatively affecting performance in a greater magnitude when diets contained DDGS compared to diets without DDGS. The main effects of DDGS and SBM were more consistently observed across experiments. Pigs fed diets with 25% DDGS had decreased ($P \leq 0.001$) ADG and ADFI in all experiments as well as poorer ($P \leq$

0.028) G:F and CE except for Exp. 3. Feeding increasing amounts of SBM generally did not result in any major impact in ADG, but consistently improved (linear, $P \leq 0.078$) G:F and CE across experiments. The mechanism for this response is unclear but could be driven by intrinsic components of SBM, such as isoflavones, or by underestimation of SBM energy value.

Key words: caloric efficiency; growth; protein; soybean meal; swine

INTRODUCTION

Soybean meal (**SBM**) is the primary plant-protein source for swine diets in the United States (Stein et al., 2013; Pettigrew et al., 2017). The amino acid (**AA**) profile of SBM is highly digestible and complements major dietary cereal grain AA profiles, such as those of corn and wheat (NRC, 2012). Moreover, the processing techniques to remove SBM antinutritional factors are well-described and consistent. Additionally, research suggests health benefits when feeding high SBM levels. Trials with nursery (Rocha et al., 2013; Rochell et al., 2015) and finishing pigs (Johnston et al., 2010) infected with porcine reproductive and respiratory syndrome (**PRRS**) suggest health-challenged pig growth performance is improved by feeding high SBM levels. Although the mechanisms are not fully understood, it is suggested that SBM bioactive compounds, namely isoflavones and saponins, may be involved in this response (Smith and Dilger, 2018).

Distillers dried grains with solubles (**DDGS**) is a co-product of the ethanol industry widely used in swine diets. It is generally accepted 30% DDGS can be included in late nursery diets without significantly compromising growth performance (Stein and Shurson, 2009), although factors such as fat and fiber content and mycotoxin levels must be considered. Diets today are frequently formulated with higher amounts of DDGS amounts and increasing feed-grade AA replacing intact protein sources such as SBM, which typically reduces diet costs. However, given the potential benefits of SBM, a minimum amount may be desirable. We hypothesize that SBM may be especially beneficial for pigs raised under the rigors of commercial conditions. Therefore, the objective of the current study was to determine the effects of increasing SBM in diets with or without DDGS on growth performance of 11- to 25-kg nursery pigs across different environmental conditions.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments.

Ingredients and chemical analysis

Samples of corn, SBM, and DDGS were obtained from each location and submitted to the Agricultural Experimental Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for total AA content analysis (method 982.30; AOAC International, 2006) prior to diet formulation (Table 4.1). The total AA values for corn and SBM were multiplied by NRC (2012) SID coefficients and used in diet formulation. Corn, SBM, and DDGS were also analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter (method 935.29; AOAC International, 1990), crude protein (method 990.03; AOAC International, 1990), neutral detergent fiber (Ankom, 1998), and ether extract (Ankom, 2004). Samples of DDGS from each location were analyzed (North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND) for mycotoxin concentrations through extraction in acetonitrile and water followed by liquid chromatography with tandem mass spectrometry (LC/MS/MS) detection (Table 4.2).

Representative diet samples were obtained from each treatment within experiment and stored at -20°C until analysis. Samples were analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter, crude protein, calcium (method 985.01; AOAC International, 1990), phosphorus (method 985.01; AOAC International, 1990), neutral detergent fiber, and ether extract.

Animals and diets

A total of four experiments were conducted, one in a university facility and three in commercial research facilities. In all experiments, pigs were weaned at approximately 21 d of age, placed in pens based on initial body weight (**BW**), and fed common diets for approximately 21 d. On d 21, which was considered d 0 of the trials, pens of pigs were allotted to 1 of 6 dietary treatments in a randomized complete block design with BW as the blocking factor. Treatments were arranged in a 2 × 3 factorial with main effects of SBM (27.5, 32.5, or 37.5% of the diet) and DDGS (0 or 25% of the diet). The increasing levels of SBM were obtained by changing the amount of feed-grade amino acids and corn. Diets (Tables 4.3 to 4.6) were formulated to contain the same net energy (**NE**). The NE value for DDGS was estimated as a function of the oil content based on Graham et al. (2014) equation. The NE of SBM used in diet formulation was 88% of corn NE (as-fed basis) or 2,351 kcal/kg NE. Diets were provided ad libitum in mash form. There were 10, 16, 13, and 12 replicates per treatment in Exp. 1, 2, 3, and 4, respectively.

Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center (Manhattan, KS). A total of 296 pigs (DNA 241 × 600, Columbus, NE; initially 10.6 kg) were placed in pens of 4 or 5 mixed gender pigs each and used in a 24-d trial. Pens (1.52 × 1.52 m) had metal slatted floors and were equipped with a four-hole stainless steel dry feeder and a nipple waterer. Experiment 2 was conducted at New Horizon Farms Nursery Research (Pipestone, MN). In Exp. 2, 2,502 pigs (PIC 337 × 1050, Hendersonville, TN; initially 11.7 kg) were placed in pens with 24 to 27 mixed gender pigs each and used in a 21-d trial. Each pen (3.7 × 2.3 m) had plastic floors and was equipped with a six-hole stainless steel dry feeder and a pan waterer. Experiment 3 was conducted at Hord Family Farms nursery research facility (Bucyrus, OH). A total of 4,118 pigs (PIC 337 × 1050, Hendersonville, TN; initially 12.5 kg) were used in a 21-d trial. Two pens sharing a fence line feeder were considered the experimental unit and had 48 to 54 mixed gender pigs each. Pens (2.3 × 2.7 m) had plastic slatted floor and were equipped with a double-sided five-hole stainless steel feeder and a cup waterer. Experiment

4 was conducted at the Cooperative Research Farm's Swine Research Nursery (Kalmbach Feeds, Inc., Sycamore, OH). A total of 711 pigs (PIC 380 ×1050, Hendersonville, TN; initially 12.3 kg) were placed in pens with 9 or 10 mixed gender pigs and used in a 21-d trial. Each pen (1.52 × 1.83 m) had slated metal floors and was equipped with a four-hole stainless steel dry feeder and a nipple-cup waterer.

In all experiments pens of pigs were weighed and feed disappearance was measured weekly to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Mortality and culls were recorded daily. Caloric efficiency (**CE**) was calculated by multiplying ADFI by kcal of NE per kg of diet and dividing by ADG.

Statistical analysis

Data were analyzed as a randomized complete block design in a 2 × 3 factorial treatment arrangement. There was significant treatment × experiment interaction, thus each experiment was analyzed separately. Single degree-of-freedom contrasts were constructed to test the linear and quadratic effects of increasing SBM and their interactions with DDGS. Block was included as a random effect and treatment as a fixed effect. Pen was considered the experimental unit in all experiments except in Exp. 3 where two pens shared a feeder, the feeder was considered the experimental unit. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

RESULTS

Chemical analysis

The analyzed total SBM AA concentration was similar across locations and the values were comparable to those presented in the NRC (2012). The corn AA profile was also similar across locations and, in general, slightly lower than NRC (2012) values. In general, DDGS used in Exp. 1 had the highest AA content, and the DDGS used in Exp. 2, 3, and 4 had a similar AA profile. All DDGS sources had higher total AA content than the values reported in the NRC (2012), especially total Lys. The DDGS sources had variation in fiber and oil content, thus the NE estimates were different for each source. The differences in ingredient composition across locations were accounted for in diet formulation and are not expected to have influenced the outcome of the study. The analyzed dietary crude protein, Ca, P, and neutral detergent fiber were consistent with formulated values (Tables 4.3 to 4.6).

There was variation in mycotoxin content in DDGS across locations (Table 4.2). The DDGS used in Exp. 1 had significant concentration of deoxynivalenol (**DON**) and total fumonisin, 1,047 and 6,347 ppb, respectively. Similarly, the DDGS used in Exp. 3 and 4 had high levels of DON (4,093 and 4,231 ppb, respectively) and contained detectible levels of zearalenone (328 and 274 ppb, respectively). The DDGS used in Exp. 2 did not contain particularly high levels of any mycotoxin.

Experiment 1

There was a tendency ($P = 0.086$) for a SBM × DDGS interaction for G:F (Table 4.7). Gain-to-feed ratio increased then decreased as SBM increased in diets without DDGS. However, in diets with DDGS, G:F was similar in pigs fed 32.5 and 37.5% and both were better than those fed 27.5%. There was no evidence ($P > 0.10$) for interactions for ADG, ADFI, or CE. Pigs fed diets with DDGS had decreased ($P < 0.01$) ADG, ADFI, and final BW, as well as poorer CE

(Table 4.8). Pigs fed increasing SBM had a tendency (*P* = 0.078) for a linear improvement in CE.

Experiment 2

There was an SBM × DDGS interaction (*P* = 0.039) for G:F (Table 4.7). Pigs fed diets without DDGS had increasing improvements in G:F as SBM concentration increased. However, for pigs fed diets with DDGS, increasing SBM from 27.5 to 32.5% resulted in similar G:F but it was improved for pigs fed diets with 37.5% SBM. A similar interaction (*P* = 0.032) was observed for CE. There was a tendency (*P* = 0.063) for a SBM × DDGS interaction for ADG, where ADG increased in pigs fed increasing SBM in diets without DDGS, whereas ADG decreased as SBM increased in diet with DDGS. There was no evidence (*P* > 0.10) for interactions for ADFI and final BW. Pigs fed diets with DDGS had decreased (*P* = 0.001) ADFI and final BW (Table 4.8). Increasing SBM resulted in a decrease (linear, *P* = 0.015) in ADFI.

Experiment 3

There were SBM × DDGS interactions (*P* < 0.01) for ADG, ADFI, and final BW (Table 4.7). Pigs had decreased ADG, ADFI, and final BW as SBM increased; however, the magnitude of the decrease was greater for pigs fed diets with DDGS than those fed diets without DDGS. There was no evidence for interactions for G:F or CE. Pigs fed diets with DDGS had poorer (*P* ≤ 0.028) G:F and CE and those fed increasing SBM had improved (linear, *P* ≤ 0.014) G:F and CE (Table 4.8).

Experiment 4

There was a tendency (*P* = 0.076) for a SBM × DDGS interaction for ADG (Table 4.7). Pigs fed diets without DDGS had decreased ADG when fed 32.5% SBM compared to 27.5 or 37.5% SBM, whereas pigs fed diets with DDGS had higher ADG when diets contained 27.5 or

37.5% SBM. There was no evidence ($P > 0.10$) for interactions for ADFI, G:F, or CE. Pigs fed diets containing DDGS had decreased ($P \leq 0.002$) ADFI, G:F, and poorer CE (Table 4.8). Increasing SBM resulted in an improvement (linear, $P \leq 0.027$) in G:F and CE.

Culls and mortality

In general, pigs used in all experiments were healthy and did not undergo major health challenges during the experimental period. The average cull rate was 0.7, 0.5, 0.2, and 0% and the mortality rate was 0.7, 0.3, 0.4, and 0% in Exp. 1 to 4, respectively (Table 4.9). Due to the low number of events, the statistical analysis for cull rate was not performed and only descriptive statistics are presented.

DISCUSSION

The United States is the world's largest producer of soybeans, with an annual production of approximately 120 million tons in 2017, followed by Brazil and Argentina with 113 and 47 million tons, respectively (ASA, 2018). The majority of soybeans are destined to oil and SBM production, and almost 8 million tons of SBM were fed to pigs in the United States in 2017 (ASA, 2018). Typically, swine nutritionists formulate diets with dehulled solvent-extracted SBM, which contains approximately 47.5% crude protein and a balanced AA profile particularly rich in Lys, Thr, and Trp (NRC, 2012). These AA are limiting in typical swine diets and are relatively low in corn and wheat, thus SBM complements their AA profiles well. Also, SBM AA digestibility is high for swine, with essential AA standardized ileal digestible coefficients ranging from 85 to 94% (NRC, 2012). Finally, protein quality, expressed as AA concentration as a percentage of crude protein, is higher for SBM relative to other protein sources (Stein et al., 2013). Taken together, these characteristics contribute to the prevalent SBM use as a primary swine diet protein source in the United States and globally.

The addition of SBM is typically restricted to less than 20% of the diet in the period immediately postweaning due to a transient type II hypersensitivity reaction (Engle, 1994). This reaction is caused by antigenic proteins present in SBM, namely glycinin and conglycinin, and results in decreased growth performance (Li et al., 1990; Engle, 1994). Nevertheless, after initial exposure, there is little evidence for negative effect of feeding high SBM levels. Thus, it is not necessary to restrict its inclusion in late nursery diets or above approximately 11 kg BW.

Diets with high feed-grade AA inclusion are commonly used with the lower cost commercial availability of L-Lys, L-Thr, DL-Met or L-Met, L-Trp and L-Val (Clark et al., 2017a; Menegat et al., 2019) at the expense of intact protein sources such as SBM. Moreover, as our knowledge of the next limiting AA requirements such as Ile (Clark et al., 2017b) and His (Cemin et al., 2018) develops and AA prices become more competitive, formulation strategies with higher inclusion of feed-grade AA are expected. Although the use of feed-grade AA has potential benefits regarding diet costs, research suggests that there may be benefits of feeding high levels of SBM, especially for health-challenged pigs. Porcine respiratory and reproductive syndrome is one of the most prevalent diseases of swine globally (Lunney et al., 2010) and causes estimated annual losses of over $600 million in the United States (Holtkamp et al., 2012). Therefore, strategies to mitigate the economic impact of PRRS can greatly benefit the swine industry. Johnston et al. (2010) were the first to describe the advantages of feeding high SBM for naturally PRRS infected pigs. The authors observed a 10% improvement in ADG and 8% improvement in G:F for grow-finish pigs fed diets with 32% SBM compared to 21% SBM supplemented with feed-grade AA. Later, Rocha et al. (2013) observed that nursery pigs inoculated with PRRS virus had similar ADG but improved G:F when SBM was increased from 12.5 to 22.5% of the diet. This effect was observed during the first week post-inoculation, but no differences were observed in subsequent periods. Conversely, Rochell et al. (2015) observed that PRRS infected nursery pigs had improved growth when SBM increased from 17.5 to 29% of the diet, as well as lower serum PRRS virus load. In our study, only marginal improvements in ADG were observed with increasing SBM, and a reduction was observed in some cases. It is important to note that the pigs used in the current experiments had relatively high health status, as evidenced by the low number of culls and mortality, were not inoculated with PRRS virus, and were not exposed to significant health challenges throughout the experimental period. Therefore, our results may not be directly comparable to previous research. Interestingly, Rochell et al. (2015) observed that pigs not infected with PRRS did not benefit from the high inclusion of SBM and even presented reduced ADG in some periods, which is in agreement with our findings. Taken together, it seems that pigs raised under high health conditions do not seem to benefit from high inclusions of SBM to the same extent as PRRS infected pigs.

The reasons behind the benefits of feeding higher SBM diets to pigs are unclear. The improvement in growth performance of PRRS-infected pigs fed increasing SBM does not seem to be related to changes in nutrient or AA digestibility (Schweer et al., 2018). One of the modes of action could be explained by the presence of bioactive components in SBM, namely isoflavones and saponins. A review of these components was recently published (Smith and Dilger, 2018) and will not be described in detail. Briefly, isoflavones and saponins have been reported to have anti-inflammatory, antioxidant, and anti-viral properties as well as the ability to modulate intestinal permeability. However, the available research shows uncertainty regarding the effects of isoflavones. In a wean-to-finish trial, Kuhn et al. (2004) compared SBM and soy protein concentrate, an ingredient with markedly lower isoflavones relative to SBM. The authors observed no evidence for differences in growth performance in any stage of production, although plasma isoflavone concentration was higher in pigs fed SBM than those fed soy protein concentrate. On the other hand, in a grow-finish study, Payne et al. (2001) observed reduced growth in late finishing pigs fed soy protein concentrate diets supplemented with isoflavones compared to pigs fed soy protein concentrate- or SBM-based diets, but no significant differences overall. It appears that isoflavones could be more beneficial when fed to health challenged pigs, but results are also inconsistent. Greiner et al. (2001a,b) observed improvements in performance of PRRS positive pigs driven by increasing isoflavones, but mostly during periods of peak viremia. Conversely, Smith et al. (2017) evaluated diets with or without supplementation of isoflavones for PRRS-infected nursery pigs and found no improvements in growth performance, although some immunological changes were observed.

A consistent finding in our experiments was an improvement in G:F and CE as SBM increased. Yet again, the reasons for these responses are unclear as they could be driven by the intrinsic bioactive components, but also by an underestimation of the energy value assigned for SBM (Boyd et al., 2011; Li et al., 2017). Under- or overestimating NE can be detected if pigs fed diets with increasing amount of a test ingredient present differences in G:F or CE (De Jong et al., 2014; Gonçalves et al., 2016). Our findings suggest that the energy value assigned for SBM could have been underestimated. The NRC (2012) NE estimate for SBM is 2,087 kcal/kg or 78% of corn NE. Our diets were assuming SBM had 2,351 kcal/kg or 88% of corn NE and balanced for NE. Therefore, this suggests that the NRC (2012) considerably underestimates the NE value of SBM and this has important ramifications in diet formulation as it increases the value of SBM. A comparable result was reported by Cemin et al. (2019), who also formulated diets with SBM

NE at 88% of corn NE and observed approximately 4% improvement in G:F of nursery pigs when SBM inclusion increased from 27 to 35%, suggesting a SBM NE value greater than corn. Moran et al. (2017) conducted two trials evaluating increasing SBM for nursery pigs. In the first trial, pigs were PRRS negative and the authors observed a consistent improvement in G:F, in agreement with our findings. However, the results were not repeated in a subsequent study with pigs originated from a PRRS-positive sow farm, where increasing SBM in the diet did not improve growth performance but reduced the percentage of pigs removed for medical treatment from 11.1 to 8.4%.

It is unclear why growth performance was negatively impacted with high amounts of SBM in some of the current experiments, especially when diets contained DDGS. The available research generally does not agree with this finding, as most of the studies found no change or improvements in ADG with increasing SBM, it is important to note that the current study evaluated higher SBM additions than the majority of previous research. Therefore, a possible explanation for our finding is the dietary crude protein level. The diets with the highest inclusion of SBM contained on average 27% crude protein. It is well known that pigs do not have a crude protein requirement but rather a need for AA. Protein or AA provided in excess will be deaminated and excreted, thus representing an inefficient use of nutrients and an energy cost to the animal (Van Milgen and Dourmad, 2015). Moreover, undigested protein can contribute to the proliferation of nitrogen-utilizing pathogenic bacteria in the gastrointestinal tract (Ball and Aherne, 1987) and high crude protein diets have been shown to increase the incidence of diarrhea in nursery pigs (Heo et al., 2009). Finally, dietary crude protein has the ability to impact gut morphology and gut microbiota (Opapeju et al., 2009). Therefore, it may be important to limit the inclusion of SBM, especially in diets formulated with DDGS, to avoid excess dietary crude protein. Taken together, it is challenging to identify the reason for the decreased growth of pigs fed high protein diets, and it is likely driven by multiple factors.

Our experiments showed that pigs fed diets with 25% DDGS had decreased growth performance compared to those fed corn-SBM diets. In contrast, the literature suggests that feeding DDGS to late nursery pigs is typically not detrimental to growth performance (Stein and Shurson, 2009). Whitney and Shurson (2004) observed no evidence for differences in late nursery performance for pigs fed up to 25% DDGS. A similar observation was made by Jones et al. (2010) when feeding up to 30% DDGS and Cemin et al. (2019) when diets contained 23% DDGS. The negative response to DDGS found in the current study could have been driven by the higher fiber content of the ingredient, although the DDGS sources used in our experiments were comparable in fiber content to previous research (Whitney and Shurson, 2004; Jones et al., 2010; Cemin et al., 2019). It could also be hypothesized that the energy value of DDGS was underestimated, which would help explain the G:F and CE responses observed in 3 of the 4 experiments. The presence of mycotoxins could also explain the reduced growth performance observed in pigs fed diets with 25% DDGS. The U.S. Food and Drug Administration (**FDA**) recommends that feed ingredients contain less than 5,000 ppb DON and that these ingredients do not exceed 20% of the diet, for a maximum of 1,000 ppb DON in complete feed (FDA, 2010). The DDGS used in the current experiments contained 1,047, 825, 4,093, and 4,231 ppb DON in Exp. 1, 2, 3, and 4, respectively. These levels are below the FDA recommendation but DDGS was included at 25% of the diet, thus resulting in dietary concentrations slightly greater than 1,000 ppb in Exp. 3 and 4. Furthermore, the recommended total fumonisin levels in feed ingredients is 20,000 ppb and that these ingredients do not exceed 50% of the diet (FDA, 2001). Therefore, all DDGS sources were under the recommended levels, with the highest concentration of total fumonisin observed in the DDGS used in Exp. 1 (6,347 ppb). Although the individual mycotoxin levels were generally below the recommended by the FDA, some mycotoxins can interact and potentially present additive or synergistic toxicity (Huff et al., 1988; Pierron et al., 2016), thus their impact on growth performance cannot be predicted upon individual concentrations. Other factors for the negative DDGS response include variability among sources (Spiehs et al., 2002), changes in palatability (Hastad et al., 2005), or feed intake limitation to the lower bulk density (Ndou et al., 2012).

In conclusion, a common observation from these studies is that DDGS generally reduced growth performance, possibly influenced by mycotoxin levels. On the other hand, increasing addition of SBM from 27.5 to 37.5% of the diet did not result in major changes in ADG, but consistently improved G:F and CE. The underlying mechanism for this response is unclear but could be driven by intrinsic SBM components such as isoflavones or by underestimating SBM energy value.

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Table 4.1. Proximate and total amino acid analysis of soybean meal, distillers dried grains with solubles (DDGS), and corn (as-fed basis)[1]

	Soybean meal				DDGS				Corn			
Item, %	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 1[2]	Exp. 2	Exp. 3	Exp. 4
Dry matter	89.7	89.5	88.84	88.4	90.0	90.8	89.1	89.5	88.3	87.8	89.2	85.9
Crude protein	47.5	47.5	48.5	47.6	31.2	28.7	27.5	27.2	8.2	6.3	7.3	7.0
Neutral detergent fiber	8.1	8.0	6.7	9.7	25.5	27.9	30.6	30.5	9.1	7.0	5.2	6.8
Ether extract	1.4	1.5	1.7	1.6	6.6	8.8	6.9	7.1	3.5	3.6	3.7	2.8
Calcium	0.61	0.54	0.54	0.62	0.10	0.08	0.06	0.06	0.02	0.07	0.07	0.04
Phosphorus	0.71	0.69	0.63	0.60	1.01	0.88	0.91	0.84	0.26	0.23	0.23	0.20
Amino acids												
Alanine	2.02	2.07	2.08	2.01	2.75	1.86	1.85	1.85	0.60	0.45	0.52	0.48
Arginine	3.40	3.46	3.39	3.34	1.58	1.27	1.25	1.22	0.37	0.30	0.34	0.28
Aspartic acid	5.24	5.43	5.39	5.25	2.46	1.79	1.79	1.81	0.54	0.44	0.48	0.43
Cysteine	0.73	0.73	0.69	0.69	0.80	0.60	0.60	0.65	0.19	0.16	0.18	0.16
Glutamic acid	8.34	8.69	8.64	8.29	6.05	3.64	4.18	4.20	1.48	1.11	1.27	1.15
Glycine	1.97	2.00	2.04	1.96	1.49	1.11	1.13	1.16	0.31	0.26	0.29	0.28
Histidine	1.23	1.26	1.22	1.22	1.06	0.78	0.79	0.79	0.24	0.19	0.21	0.19
Isoleucine	2.28	2.30	2.31	2.26	1.60	1.09	1.04	1.07	0.28	0.24	0.26	0.24
Leucine	3.59	3.70	3.66	3.58	4.90	3.19	3.02	3.10	0.96	0.71	0.83	0.75
Lysine	3.05	3.14	3.03	3.01	1.22	1.08	1.04	1.04	0.25	0.25	0.26	0.24
Methionine	0.67	0.67	0.65	0.63	0.80	0.50	0.53	0.53	0.18	0.13	0.14	0.14
Phenylalanine	2.44	2.52	2.46	2.39	2.21	1.69	1.33	1.27	0.39	0.31	0.35	0.31
Proline	2.36	2.48	2.42	2.26	3.04	2.07	2.20	2.25	0.71	0.56	0.59	0.56
Serine	2.03	2.19	2.12	1.94	1.66	1.26	1.14	1.11	0.38	0.29	0.33	0.28
Threonine	1.80	1.88	1.83	1.79	1.46	1.10	1.02	1.05	0.28	0.23	0.27	0.24
Tryptophan	0.72	0.69	0.62	0.67	0.38	0.22	0.18	0.21	0.06	0.06	0.05	0.06
Tyrosine	1.74	1.77	1.51	1.61	1.51	1.03	1.06	0.90	0.26	0.18	0.18	0.13
Valine	2.32	2.38	2.40	2.34	2.08	1.45	1.38	1.37	0.38	0.31	0.34	0.32

[1] A representative sample of each ingredient was obtained, homogenized, and submitted to the Agricultural Experimental Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for amino acid analysis and Ward Laboratories (Kearney, NE) for proximate analysis prior to diet formulation.

[2] NRC (2012) amino acid values were used for corn in Exp. 1.

Table 4.2. Mycotoxin analysis of distillers dried grains with solubles[1]

Mycotoxins	Practical quantitation limit, ppb	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Aflatoxin B1	20	< 20	< 20	< 20	< 20
Aflatoxin B2	20	< 20	< 20	< 20	< 20
Aflatoxin G1	20	< 20	< 20	< 20	< 20
Aflatoxin G2	20	< 20	< 20	< 20	< 20
Deoxynivalenol	200	1,047	825	4,093	4,231
Fumonisin B1	200	5,031	214	961	895
Fumonisin B2	200	1,316	< 200	244	244
HT-2 toxin	200	< 200	< 200	< 200	< 200
Ochratoxin A	20	< 20	< 20	< 20	< 20
T-2 toxin	20	< 20	< 20	< 20	< 20
Sterigmatocystin	20	< 20	< 20	< 20	< 20
Zearalenone	100	111	< 100	328	274

[1] A representative sample of each source was collected, homogenized, and submitted to North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND).

Table 4.3. Diet composition of Exp. 1 (as-fed basis)

DDGS[1] :	0%			25%		
Soybean meal:	27.5%	32.5%	37.5%	27.5%	32.5%	37.5%
Ingredient, %						
Corn	66.67	61.76	56.86	40.66	35.69	30.71
Soybean meal	27.52	32.51	37.48	27.50	32.52	37.50
DDGS	---	---	---	25.00	25.00	25.00
Choice white grease	1.60	2.00	2.40	3.80	4.15	4.50
Calcium carbonate	0.80	0.78	0.75	1.18	1.15	1.13
Monocalcium phosphate, 21.5% P	1.03	0.95	0.90	0.30	0.23	0.15
Sodium chloride	0.68	0.68	0.68	0.50	0.50	0.50
L-Lysine HCl	0.545	0.385	0.225	0.400	0.240	0.080
DL-Methionine	0.225	0.180	0.130	0.070	0.025	---
L-Threonine	0.280	0.215	0.150	0.140	0.075	0.010
L-Tryptophan	0.065	0.035	0.005	0.030	---	---
L-Valine	0.175	0.090	---	---	---	---
Vitamin premix[2]	0.250	0.250	0.250	0.250	0.250	0.250
Trace-mineral premix[3]	0.150	0.150	0.150	0.150	0.150	0.150
Phytase[4]	0.025	0.025	0.025	0.025	0.025	0.025
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis						
SID[5] amino acids, %						
Lysine	1.30	1.30	1.30	1.30	1.30	1.30
Isoleucine:lysine	55	62	69	67	74	81
Leucine:lysine	110	119	128	149	158	167
Methionine:lysine	38	36	34	32	30	30
Methionine & cystine:lysine	58	58	58	58	58	60
Threonine:lysine	65	65	65	65	65	65
Tryptophan:lysine	21.3	21.3	21.4	21.2	21.2	23.6
Valine:lysine	72	72	72	75	81	88
Histidine:lysine	34	37	41	42	45	49

Net energy, kcal/kg	2,590	2,590	2,590	2,590	2,590	2,590
Crude protein, %	19.5	21.2	22.8	24.7	26.4	28.2
Neutral detergent fiber, %	8.3	8.3	8.2	12.3	12.3	12.2
Calcium, %	0.74	0.74	0.75	0.78	0.78	0.79
STTD P[6], %	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed values, %						
Dry matter	90.2	90.0	90.3	90.8	90.9	90.5
Crude protein	19.9	21.7	21.9	23.9	25.9	28.4
Neutral detergent fiber	5.2	5.5	5.5	13.4	12.8	13.5
Ether extract	4.3	4.7	5.0	7.8	8.0	7.9
Calcium	0.79	0.73	0.87	1.02	0.90	1.01
Phosphorus	0.53	0.56	0.60	0.61	0.60	0.62

[1] DDGS = distillers dried grains with solubles.

[2] Provided per kg of premix: 1,654,468 IU vitamin A; 661,387 IU vitamin D; 17,637 IU vitamin E; 1,323 mg vitamin K; 13.2 mg vitamin B12; 19,842 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[3] Provided per kg of premix: 73 g Zn from zinc sulfate; 73 g Fe from ferrous sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[4] Ronozyme HiPhos 2700 (DSM Nutritional Products, Inc., Parsipanny, NJ).

[5] SID = standardized ileal digestible.

[6] STTD P = standardized total tract digestible phosphorus.

Table 4.4. Diet composition of Exp. 2 (as-fed basis)

DDGS[1] :	0%			25%		
Soybean meal:	27.5%	32.5%	37.5%	27.5%	32.5%	37.5%
Ingredient, %						
Corn	66.81	61.91	56.91	42.07	37.08	32.05
Soybean meal	27.49	32.48	37.49	27.50	32.50	37.50
DDGS	---	---	---	25.00	25.00	25.00
Beef tallow	1.60	2.00	2.45	2.45	2.85	3.25
Calcium carbonate	0.80	0.78	0.75	1.18	1.15	1.13
Monocalcium phosphate, 21.5% P	1.03	0.95	0.90	0.30	0.23	0.15
Sodium chloride	0.68	0.68	0.68	0.50	0.50	0.50
L-Lysine HCl	0.513	0.352	0.190	0.365	0.204	0.043
DL-Methionine	0.260	0.210	0.165	0.115	0.065	0.020
L-Threonine	0.285	0.215	0.145	0.135	0.065	---
L-Tryptophan	0.073	0.045	0.015	0.045	0.018	---
L-Valine	0.205	0.125	0.025	---	---	---
Vitamin trace-mineral premix[2]	0.150	0.150	0.150	0.150	0.150	0.150
Phytase[3]	0.015	0.015	0.015	0.015	0.015	0.015
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis						
SID[4] amino acids, %						
Lysine	1.30	1.30	1.30	1.30	1.30	1.30
Isoleucine:lysine	54	61	68	66	73	79
Leucine:lysine	101	111	121	141	150	160
Methionine:lysine	38	36	35	33	31	29
Methionine & cystine:lysine	58	58	58	58	58	58
Threonine:lysine	65	65	65	65	65	65
Tryptophan:lysine	21.3	21.4	21.3	21.3	21.4	22.2
Valine:lysine	73	73	73	73	80	87
Histidine:lysine	32	36	40	41	44	48
Net energy, kcal/kg	2,590	2,590	2,590	2,590	2,590	2,590

Crude protein, %	18.2	20.0	21.7	23.3	25.1	26.9
Neutral detergent fiber, %	8.8	8.6	8.4	13.2	12.9	12.7
Calcium, %	0.71	0.72	0.74	0.74	0.75	0.76
STTD P[5], %	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed values, %						
Dry matter	88.6	89.0	89.0	90.6	90.4	91.1
Crude protein	19.0	19.7	21.8	22.0	25.2	28.2
Neutral detergent fiber	7.4	6.4	5.7	10.9	12.3	13.2
Ether extract	4.2	3.9	4.3	5.7	6.5	6.6
Calcium	0.62	0.61	0.67	0.72	0.67	0.56
Phosphorus	0.55	0.52	0.56	0.53	0.59	0.59

[1] DDGS = distillers dried grains with solubles.

[2] Provided per kg of premix: 5,344,543 IU vitamin A; 1,336,137 IU vitamin D; 100,211 IU vitamin E; 1,671 mg vitamin K; 21.4 mg vitamin B12; 29,061 mg niacin; 15,366 mg pantothenic acid; 4,008 mg riboflavin; 66.8 mg biotin; 668 mg folic acid; 1202 mg vitamin B6; 73 g Zn from zinc sulfate; 67 g Fe from ferrous sulfate; 27 g Mn from manganese oxide; 10 g Cu from copper sulfate; 0.5 g I from calcium iodate; 0.2 g Se from sodium selenite.

[3] Optiphos 2000 (Huvepharma, Inc., Peachtree City, GA).

[4] SID = standardized ileal digestible.

[5] STTD P = standardized total tract digestible phosphorus.

Table 4.5. Diet composition of Exp. 3 (as-fed basis)

DDGS[1]:	0%			25%		
Soybean meal:	27.5%	32.5%	37.5%	27.5%	32.5%	37.5%
Ingredient, %						
Corn	66.34	61.51	56.56	40.64	35.66	30.68
Soybean meal	27.50	32.50	37.50	27.50	32.50	37.50
DDGS	---	---	---	25.00	25.00	25.00
Corn oil	1.60	1.95	2.35	3.45	3.80	4.15
Calcium carbonate	0.85	0.83	0.80	1.23	1.20	1.20
Monocalcium phosphate, 21.5% P	1.15	1.05	1.00	0.45	0.40	0.33
Sodium chloride	0.50	0.50	0.50	0.33	0.33	0.33
L-Lysine HCl	0.547	0.387	0.228	0.408	0.249	0.090
DL-Methionine	0.255	0.210	0.165	0.090	0.045	0.000
L-Threonine	0.280	0.215	0.150	0.150	0.080	0.015
L-Tryptophan	0.095	0.065	0.040	0.065	0.040	0.010
L-Valine	0.185	0.090	0.000	0.000	0.000	0.000
Vitamin trace-mineral premix[2]	0.175	0.175	0.175	0.175	0.175	0.175
Phytase[3]	0.025	0.025	0.025	0.025	0.025	0.025
Sodium metabisulfite	0.500	0.500	0.500	0.500	0.500	0.500
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis						
SID[4] amino acids, %						
Lysine	1.30	1.30	1.30	1.30	1.30	1.30
Isoleucine:lysine	54	61	69	65	72	80
Leucine:lysine	105	115	124	139	149	159
Methionine:lysine	38	36	34	31	30	28
Methionine & cystine:lysine	57	57	57	56	56	57
Threonine:lysine	65	65	65	65	65	65
Tryptophan:lysine	21.2	20.9	21.1	20.6	20.7	20.4
Valine:lysine	72	72	72	73	80	87
Histidine:lysine	32	36	39	41	44	48

Net energy, kcal/kg	2,590	2,590	2,590	2,590	2,590	2,590
Crude protein, %	19.2	21.0	22.7	23.8	25.6	27.4
Neutral detergent fiber, %	5.3	5.4	5.4	11.6	11.7	11.7
Calcium, %	0.72	0.72	0.72	0.74	0.75	0.76
STTD P[5], %	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed values, %						
Dry matter	87.8	88.0	88.3	88.4	88.2	88.4
Crude protein	19.2	19.2	21.3	20.8	24.2	26.7
Neutral detergent fiber	7.1	6.8	6.8	11.3	13.1	13.2
Ether extract	4.4	4.7	4.8	7.3	7.4	7.3
Calcium	0.74	0.78	0.79	0.97	0.83	0.97
Phosphorus	0.52	0.56	0.53	0.57	0.58	0.56

[1] DDGS = distillers dried grains with solubles.

[2] Provided per kg of premix: 1,653,468 IU vitamin A; 551,156 IU vitamin D; 17,637 IU vitamin E; 1,323 mg vitamin K; 13.2 mg vitamin B12; 22,046 mg niacin; 11,023 mg pantothenic acid; 3,086 mg riboflavin; 88 g Zn from zinc sulfate; 77 g Fe from ferrous sulfate; 6.6 g Mn from manganese oxide; 9.9 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[3] Quantum Blue 2500 (AB Vista, Marlborough, UK).

[4] SID = standardized ileal digestible.

[5] STTD P = standardized total tract digestible phosphorus.

Table 4.6. Diet composition of Exp. 4 (as-fed basis)

DDGS[1]:	0%			25%		
Soybean meal:	27.5%	32.5%	37.5%	27.5%	32.5%	37.5%
Ingredient, %						
Corn	66.15	61.17	56.33	40.26	35.23	30.21
Soybean meal	27.51	32.52	37.51	27.52	32.52	37.52
DDGS	---	---	---	25.00	25.00	25.00
Corn oil	1.80	2.25	2.60	3.80	4.20	4.60
Calcium carbonate	0.75	0.73	0.70	1.10	1.08	1.05
Monocalcium phosphate, 21.5% P	1.20	1.15	1.05	0.55	0.50	0.45
Sodium chloride	0.50	0.50	0.50	0.35	0.35	0.35
L-Lysine HCl	0.565	0.406	0.247	0.422	0.264	0.105
DL-Methionine	0.280	0.235	0.190	0.110	0.070	0.025
L-Threonine	0.305	0.235	0.165	0.165	0.100	0.030
L-Tryptophan	0.085	0.055	0.025	0.060	0.030	0.000
L-Valine	0.185	0.095	0.015	0.000	0.000	0.000
Vitamin premix[2]	0.050	0.050	0.050	0.050	0.050	0.050
Trace-mineral premix[3]	0.090	0.090	0.090	0.090	0.090	0.090
Phytase[4]	0.025	0.025	0.025	0.025	0.025	0.025
Sodium metabisulfite	0.500	0.500	0.500	0.500	0.500	0.500
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis						
SID[5] amino acids, %						
Lysine	1.30	1.30	1.30	1.30	1.30	1.30
Isoleucine:lysine	53	60	67	64	71	78
Leucine:lysine	100	110	119	137	147	156
Methionine:lysine	39	38	36	32	31	29
Methionine & cystine:lysine	58	58	58	58	58	58
Threonine:lysine	65	65	64	65	65	65
Tryptophan:lysine	21.8	21.7	21.6	21.8	21.7	21.6
Valine:lysine	71	71	71	71	78	85

Histidine:lysine	31	35	39	40	44	47
Net energy, kcal/kg	2,590	2,590	2,590	2,590	2,590	2,590
Crude protein, %	18.8	20.5	22.3	23.3	25.1	26.9
Neutral detergent fiber, %	7.9	8.0	8.1	13.5	13.6	13.7
Calcium, %	0.69	0.70	0.70	0.72	0.73	0.74
STTD P[6], %	0.45	0.45	0.45	0.45	0.45	0.45
Analyzed values, %						
Dry matter	87.8	88.0	88.1	88.5	88.7	88.8
Crude protein	17.2	18.8	22.2	23.4	25.3	25.8
Neutral detergent fiber	6.5	6.1	5.9	12.6	12.9	12.4
Ether extract	4.2	4.1	4.7	6.8	7.3	7.6
Calcium	0.53	0.61	0.72	0.67	0.89	0.79
Phosphorus	0.49	0.52	0.61	0.62	0.61	0.62

[1] DDGS = distillers dried grains with solubles.

[2] Provided per kg of premix: 28,660,117 IU vitamin A; 4,409,249 IU vitamin D; 105,822 IU vitamin E; 8,009 mg vitamin K; 79.4 mg vitamin B12; 308,647 mg niacin; 66,139 mg pantothenic acid; 15,432 mg riboflavin;

[3] Provided per kg of premix: 112 g Zn from zinc sulfate; 104 g Fe from ferrous sulfate; 30 g Mn from manganese sulfate; 16 g Cu from copper sulfate; 0.16 g I from ethylenediamine dihydriodide; 0.2 g Se from sodium selenite.

[4] Quantum Blue 2500 (AB Vista, Marlborough, UK).

[5] SID = standardized ileal digestible.

[6] STTD P = standardized total tract digestible phosphorus.

Table 4.7. Interactive effects of distillers dried grains with solubles (DDGS) and soybean meal (SBM) on growth performance of nursery pigs

	0% DDGS			25% DDGS				Probability, $P <$	
Item[1]	27.5% SBM	32.5% SBM	37.5% SBM	27.5% SBM	32.5% SBM	37.5% SBM	SEM	DDGS × SBM linear	DDGS × SBM quadratic
Initial BW, kg									
Exp. 1[2]	10.5	10.6	10.5	10.6	10.6	10.5	0.20	0.758	0.926
Exp. 2[3]	11.7	11.7	11.7	11.7	11.6	11.7	0.18	0.999	0.736
Exp. 3[4]	12.5	12.5	12.5	12.5	12.5	12.5	0.25	0.845	0.875
Exp. 4[5]	12.3	12.3	12.3	12.3	12.3	12.3	0.57	0.992	0.984
Final BW, kg									
Exp. 1	25.4	25.8	25.0	23.3	24.3	23.6	0.54	0.251	0.595
Exp. 2	22.7	23.0	22.9	22.4	22.3	22.2	0.25	0.220	0.459
Exp. 3	26.2	26.1	25.9	25.5	24.8	24.5	0.38	0.013	0.271
Exp. 4	24.7	24.3	24.9	23.0	23.4	23.4	0.99	0.668	0.205
ADG, g									
Exp. 1	621	620	603	519	558	535	20.4	0.263	0.389
Exp. 2	524	539	533	510	508	497	6.9	0.063	0.568
Exp. 3	650	648	637	618	586	571	7.7	0.003	0.198
Exp. 4	592	570	598	510	531	529	21.5	0.553	0.076
ADFI, g									
Exp. 1	919	904	888	839	852	814	26.3	0.895	0.421
Exp. 2	794	798	783	767	771	732	11.3	0.190	0.476
Exp. 3	967	955	930	927	870	847	11.5	0.001	0.016
Exp. 4	869	835	858	786	802	782	32.9	0.813	0.111
G:F, g/kg									
Exp. 1	677	686	679	616	655	656	12.3	0.086	0.563
Exp. 2	660	676	681	665	661	679	5.1	0.460	0.039
Exp. 3	673	678	686	666	674	674	5.0	0.500	0.519
Exp. 4	682	685	698	651	664	675	11.1	0.675	0.690
CE, kcal/kg gain									
Exp. 1	3,829	3,780	3,819	4,231	3,967	3,980	80.9	0.102	0.454

Exp. 2	3,925	3,836	3,809	3,897	3,928	3,819	76.2	0.470	0.031
Exp. 3	3,852	3,821	3,778	3,895	3,846	3,846	28.8	0.615	0.477
Exp. 4	3,815	3,794	3,714	3,999	3,908	3,843	63.5	0.601	0.642

[1] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. CE = caloric efficiency.

[2] A total of 296 pigs (initially 10.6 kg) were used in a 24-d study with 4 or 5 pigs per pen and 10 replicates per treatment.

[3] A total of 2,502 pigs (initially 11.7 kg) were used in a 21-d trial with 24 to 27 pigs per pen and 16 replicates per treatment.

[4] A total of 4,118 pigs (initially 12.5 kg) were used in a 21-d trial with 48 to 54 pigs per feeder (experimental unit) and 13 replicates per treatment.

[5] A total of 711 pigs (initially 12.3 kg) were used in a 21-d trial with 9 or 10 pigs per pen and 12 replicates per treatment.

Table 4.8. Main effects of distillers dried grains with solubles (DDGS) and soybean meal (SBM) on growth performance of nursery pigs

	DDGS		SEM	Probability, *P* <	SBM			SEM	Probability, *P* <	
Item[1]	0%	25%			27.5%	32.5%	37.5%		Linear	Quadratic
Initial BW, kg										
Exp. 1[2]	10.5	10.6	0.184	0.602	10.5	10.6	10.5	0.19	0.727	0.514
Exp. 2[3]	11.7	11.7	0.162	0.980	11.7	11.7	11.7	0.17	0.951	0.763
Exp. 3[4]	12.5	12.5	0.244	0.779	12.5	12.5	12.5	0.25	0.462	0.559
Exp. 4[5]	12.3	12.3	0.561	0.947	12.3	12.3	12.3	0.57	0.988	0.991
Final BW, kg										
Exp. 1	25.4	23.7	0.461	0.001	24.3	25.0	24.3	0.48	0.838	0.014
Exp. 2	22.9	22.3	0.214	0.001	22.6	22.7	22.5	0.23	0.927	0.356
Exp. 3	26.1	25.0	0.359	0.001	25.9	25.5	25.2	0.36	0.001	0.459
Exp. 4	24.6	23.3	0.955	0.001	23.9	23.9	24.1	0.97	0.426	0.626
ADG, g										
Exp. 1	615	537	16.21	0.001	570	589	569	17.4	0.915	0.137
Exp. 2	532	505	5.02	0.001	517	524	515	5.6	0.726	0.127
Exp. 3	645	591	6.40	0.001	634	617	604	6.8	0.001	0.612
Exp. 4	587	523	19.32	0.001	551	550	564	19.9	0.271	0.500
ADFI, g										
Exp. 1	904	835	21.80	0.001	879	878	851	23.0	0.123	0.397
Exp. 2	792	757	8.41	0.001	780	784	757	9.2	0.015	0.057
Exp. 3	951	881	10.67	0.001	947	913	889	11.0	0.001	0.289
Exp. 4	854	790	30.03	0.001	827	819	820	30.8	0.666	0.727
G:F, g/kg										
Exp. 1	681	642	8.44	0.001	647	670	667	9.6	0.067	0.166
Exp. 2	672	668	3.51	0.290	663	668	680	4.0	0.001	0.456
Exp. 3	679	671	3.63	0.028	669	676	680	4.0	0.014	0.733
Exp. 4	688	663	8.24	0.001	666	675	687	9.0	0.027	0.841
CE, kcal/kg gain										
Exp. 1	3,809	4,059	55.33	0.001	4,030	3,873	3,899	63.0	0.078	0.152
Exp. 2	3,857	3,881	20.15	0.258	3,911	3,882	3,814	23.0	0.001	0.403
Exp. 3	3,817	3,863	20.96	0.025	3,874	3,834	3,812	23.1	0.013	0.657

Exp. 4	3,774	3,917	46.84	0.002	3,908	3,851	3,778	51.4	0.017	0.858

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[1] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. CE = caloric efficiency.

[2] A total of 296 pigs (initially 10.6 kg) were used in a 24-d study with 4 or 5 pigs per pen and 10 replicates per treatment.

[3] A total of 2,502 pigs (initially 11.7 kg) were used in a 21-d trial with 24 to 27 pigs per pen and 16 replicates per treatment.

[4] A total of 4,118 pigs (initially 12.5 kg) were used in a 21-d trial with 48 to 54 pigs per feeder (experimental unit) and 13 replicates per treatment.

[5] A total of 711 pigs (initially 12.3 kg) were used in a 21-d trial with 9 or 10 pigs per pen and 12 replicates per treatment.

Table 4.9. Effects of distillers dried grains with solubles (DDGS) and soybean meal (SBM) on cull and mortality rate of nursery pigs[1,2]

	0% DDGS			25% DDGS		
Item	27.5% SBM	32.5% SBM	37.5% SBM	27.5% SBM	32.5% SBM	37.5% SBM
Culls, %						
Exp. 1	0.0	2.0	2.0	0.0	0.0	0.0
Exp. 2	0.5	0.7	0.7	0.0	0.5	0.5
Exp. 3	0.1	0.4	0.1	0.3	0.4	0.1
Exp. 4	0.0	0.0	0.0	0.0	0.0	0.0
Mortality, %						
Exp. 1	2.0	0.0	0.0	0.0	2.0	0.0
Exp. 2	0.2	0.0	0.2	0.0	0.2	1.0
Exp. 3	0.4	0.3	0.3	0.3	0.3	0.6
Exp. 4	0.0	0.0	0.0	0.0	0.0	0.0
Total, %						
Exp. 1	2.0	2.0	2.0	0.0	2.0	0.0
Exp. 2	0.7	0.7	1.0	0.0	0.7	1.5
Exp. 3	0.5	0.7	0.4	0.6	0.7	0.7
Exp. 4	0.0	0.0	0.0	0.0	0.0	0.0

[1] A total of 296, 2,502, 4,118, and 711 pigs were used in Exp. 1, 2, 3, and 4, respectively, in 21-d duration nursery trials.

[2] Descriptive data is presented. Due to the low number of events, statistical analysis was not performed.

Chapter 5 - Estimate of the energy value of soybean meal relative to corn based on growth performance of nursery pigs[1]

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ABSTRACT: Two experiments were conducted to estimate the energy value of soybean meal (**SBM**) and determine the effects of increasing amounts of SBM in swine diets. A total of 2,233 pigs (PIC 337 × 1050, Hendersonville, TN) and 3,796 pigs (PIC 359 × C40), initially 11.0 kg and 17.6 kg body weight (**BW**), were used in Exp. 1 and 2, respectively. In Exp. 1, pigs were placed in 92 pens each containing 20 to 27 pigs. In Exp. 2, pigs were placed in 84 pens each containing 37 to 43 pigs. Treatments were assigned in a randomized complete block design with BW as the blocking factor. Dietary treatments consisted of 21, 27, 33, or 39% SBM in Exp. 1 and 17.5, 22, 26.5, 31, 35.5, or 40% SBM in Exp. 2, obtained by changing the amount of feed-grade amino acids and corn. Treatment diets were fed for 21 and 22 d (Exp. 1 and 2) and there were 23 replicates in Exp. 1 and 14 replicates in Exp. 2. Pigs were weighed and feed disappearance measured to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), gain-to-feed ratio (**G:F**), and caloric efficiency (**CE**). Data were analyzed with block as a random effect and treatment as a fixed effect, and contrasts were constructed to test the linear and quadratic effects of increasing SBM. In Exp. 1, there was a tendency ($P = 0.090$) for a quadratic response for ADG, with a decrease in ADG observed with 39% SBM inclusion. There was a tendency (linear, $P = 0.092$) for a decrease in ADFI as SBM increased. Pigs fed diets with increasing SBM had a tendency (quadratic, $P = 0.069$) for an increase in G:F up to 33% SBM and an improvement (linear, $P = 0.001$; quadratic, $P = 0.063$) in CE with increasing SBM. Using CE to estimate the energy of SBM relative to corn, a value of 105.4% of corn energy or 2,816 kcal/kg NE was determined using all data points. When removing the CE value of the 39% SBM treatment due to the quadratic tendency, SBM was estimated to have 121.1% of corn energy or 3,236 kcal/kg NE. In Exp. 2, pigs fed increasing SBM had a tendency (linear, $P = 0.065$) for reduced ADG. There was a decrease (linear, $P = 0.001$) in ADFI but an improvement (linear, $P = 0.001$) in G:F and

CE as SBM increased. The energy value of SBM was estimated as 124.7% of corn energy or 3,332 kcal/kg NE. In conclusion, these results suggest that feeding increasing levels of SBM improves G:F and CE. The energy value of SBM is estimated to be between 105 and 125% of corn, which is much greater than the NRC (2012) would indicate.

Key words: caloric efficiency, energy, soybean meal, swine

INTRODUCTION

Soybean meal (**SBM**) is the primary plant-protein source for swine diets in the United States. The amino acid (**AA**) profile of SBM is well-balanced and complements the AA profile of grains such as corn and wheat, and these AA are highly digestible for pigs (NRC, 2012). The energy content of SBM has been reported (NRC, 2012) as 3,619 kcal/kg digestible energy (**DE**) and 3,294 kcal/kg metabolizable energy (**ME**), which suggests that SBM has 105% and 97% of corn DE and ME values, respectively. More recently, swine nutritionists have adopted the net energy (**NE**) system due to the higher correlation to performance relative to DE or ME systems (Nitikanchana et al., 2015). Using the NE system, SBM contains 2,087 kcal/kg, which is only 78% of the corn energy value (NRC, 2012). However, recent research shows improvements in gain-to-feed ratio (**G:F**) of pigs fed increasing levels of SBM (Moran et al., 2017; Cemin et al., 2019), which could indicate that the NRC (2012) underestimates the NE of SBM.

Calorimetry trials to measure NE involve labor-intensive procedures that require highly specialized equipment. A practical approach conducted under field conditions to estimate energy values has gained acceptance among swine nutritionists. Feeding increasing amounts of an ingredient and using the differences in caloric efficiency (**CE**) to estimate the energy content of a test ingredient relative to a known ingredient, usually corn, has been reported by others and sometimes termed productive energy (Boyd et al., 2010; 2011; Graham et al., 2014; Gonçalves et al., 2016; Estrada et al., 2017). As the inclusion of the test ingredient increases, CE should not change if its energy estimate is accurate. Increases or decreases in CE indicate over- or underestimation of the energy content. Therefore, the objective of this study was to determine differences in growth performance of pigs fed increasing amounts of SBM and, by using changes in CE, estimate SBM energy value relative to corn.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments.

Diets and experimental design

Representative samples of corn, SBM, and distillers dried grains with solubles were submitted to the Agricultural Experimental Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for determination of total AA content (method 982.30; AOAC International, 2006) prior to diet formulation (Table 5.1) in Exp. 1. The total AA values were multiplied by NRC (2012) standardized ileal digestible coefficients and used in diet formulation. Corn, SBM, and distillers dried grains were also analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter (method 935.29; AOAC International, 1990), crude protein (method 990.03; AOAC International, 1990), neutral detergent fiber (Ankom, 1998), and ether extract (Ankom, 2004). For Exp. 2, diets were formulated using NRC (2012) nutrient loadings.

There were 4 dietary treatments in Exp. 1 consisting of increasing amounts of SBM (21, 27, 33, or 39% of the diet) with 23 replicates per treatment. In Exp. 2, there were 6 dietary treatments (17.5, 22.0, 26.5, 31.0, 35.5, or 40.0% SBM) with 14 replicates per treatment. The increasing amounts of SBM were obtained by changing the inclusion of feed-grade AA and corn (Tables 5.2 and 5.3). Diets were formulated to meet or exceed the NRC (2012) requirement estimates and were not balanced for NE. The NRC (2012) NE value for SBM (2,087 kcal/kg) and corn (2,672 kcal/kg) were used in diet formulation. The NE value for DDGS was estimated as a function of the oil content based on Graham et al. (2014) equation. Diets were provided in mash form. The energy value of SBM relative to corn was estimated based on CE, which was obtained by multiplying ADFI by kcal of NE per kg of diet and dividing by ADG. In order to obtain an energy estimate, the energy value of SBM was adjusted for the slope of CE to be zero.

Animals and housing

Experiment 1 was conducted at New Horizon Farms Nursery Research (Pipestone, MN). A total of 2,233 pigs (PIC 337 × 1050, Hendersonville, TN) were placed in 92 pens containing 20 to 27 mixed gender pigs and used in a 21-d trial. Each pen (3.7 × 2.3 m) had plastic floors and was equipped with a six-hole stainless steel dry feeder and a pan waterer. Experiment 2 was conducted at the JBS Research Facility (Tipton, MO). A total of 3,796 pigs (PIC 359 × C40, Hendersonville, TN) were placed in 84 pens with 37 to 43 pigs per pen. Each pen (6.9 × 3.6 m) had fully slated floors and was equipped with a 4-hole stainless steel wet-dry feeder and a nipple waterer.

Pigs were weaned at approximately 21 d of age, placed in pens based on initial body weight (**BW**), and fed common diets until the start of the experiments. Pens of pigs were blocked by BW (initial BW = 11.0 kg in Exp. 1 and 17.6 kg in Exp. 2) and allotted to 1 of 4 or 6 treatments in Exp. 1 and 2, respectively, in a randomized complete block design. Pens of pigs were weighed and feed disappearance was measured weekly to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), G:F, and CE. Culls and mortality were recorded daily.

Chemical analysis

Representative diet samples were obtained from each treatment and stored at -20 °C until analysis. Samples were analyzed for dry matter (method 935.29; AOAC International, 1990), crude protein (method 990.03; AOAC International, 1990), calcium (method 985.01; AOAC International, 1990), phosphorus (method 985.01; AOAC International, 1990), neutral detergent fiber (Ankom, 1998), and ether extract (Ankom, 2004).

Statistical analysis

Data were analyzed as a randomized complete block design with initial BW as the blocking factor. Single degree-of-freedom contrasts were constructed to test the linear and quadratic effects of increasing SBM. Block was included as a random effect and treatment as a fixed effect. Pen was considered the experimental unit. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Chemical analysis

The analyzed amino acid profiles of corn, DDGS, and SBM used in Exp. 1 were, in general, within the expected values (Table 5.1). Soybean meal and DDGS had a similar amino acid composition to NRC (2012) values, whereas AA in corn were slightly lower than NRC (2012) estimates, especially for Met and Leu. The chemical analysis of diets was consistent with formulated values (Tables 5.2 and 5.3).

Growth performance and energy estimate

In Exp. 1, pigs fed diets with increasing SBM had a tendency (quadratic, $P = 0.090$) for an improvement in ADG up to 33% SBM, followed by a decrease in ADG when 39% SBM was fed (Table 5.4). There was a tendency (linear, $P = 0.092$) for a decrease in ADFI as dietary SBM increased. The changes in ADG and ADFI resulted in a tendency (quadratic, $P = 0.069$) for an improvement in G:F up to 33% SBM. There was an improvement (linear, $P = 0.001$; quadratic, P

= 0.063) in CE with increasing SBM. There was no evidence (*P* > 0.10) for difference in cull and mortality rate.

In Exp. 2, pigs fed increasing SBM had a tendency (linear, *P* = 0.065) for reduced ADG (Table 5.5). However, the differences were relatively small and did not result in evidence for differences (*P* ≥ 0.27) in final BW. There was a reduction (linear, *P* = 0.001) in ADFI and an improvement (linear, *P* = 0.001) in G:F and CE as SBM increased. There was a reduction (linear, *P* = 0.050) in cull and mortality rate as SBM increased.

A considerable amount of research has been conducted to evaluate the effects of SBM on growth performance of pigs. It is well known that the addition of SBM should be restricted in the diet immediately after weaning due to a hypersensitivity reaction (Li et al., 1990; Engle, 1994), but the restriction is not necessary after initial exposure. Nevertheless, the use of SBM is usually limited due to the high cost compared to diets formulated with high amounts of feed-grade AA as a replacement of intact protein sources. However, some research suggests feeding diets with higher amounts of SBM could prove beneficial, especially for health challenged pigs. Johnston et al. (2010) fed 21 or 32% SBM to grow-finish pigs that were infected with porcine reproductive and respiratory syndrome (**PRRS**) and observed that pigs fed 32% SBM had improved ADG and G:F compared with those fed 21% SBM. Similarly, Rocha et al. (2013) observed improvements in G:F of nursery pigs inoculated with PRRS virus and fed 22.5% SBM compared to 12.5% SBM, although the responses were only found in the first week after inoculation and no differences in ADG were observed. Rochell et al. (2015) found that PRRS positive nursery pigs had improved ADG when fed 29% SBM compared to 17.5% SBM. However, there was no evidence for benefits of SBM on growth performance of pigs that were not infected with PRRS. Conversely, Cemin et al. (2019) fed 27 or 35% SBM to PRRS negative nursery pigs and observed improvements in ADG and G:F as SBM increased. Moran et al. (2017) conducted two trials evaluating increasing amounts of SBM for nursery pigs. In the first trial, pigs were PRRS negative and the authors observed a consistent improvement in G:F. However, the results were not repeated in the subsequent study when pigs originated from a PRRS positive sow farm performance.

Interestingly, Moran et al. (2017) found a reduction in the percentage of pigs removed for medical treatment from 11.1 to 8.4% as SBM increased. This observation is in agreement to our finding in Exp. 2, where increasing SBM linearly reduced cull rate. The benefits of SBM on growth performance, especially for health challenged pigs, have also been hypothesized to be driven by bioactive components such as isoflavones and saponins, which have anti-inflammatory, antioxidant, and anti-viral properties (Smith and Dilger, 2018; Smith et al., 2019). However, the available research is inconsistent regarding the effects of isoflavones on growth performance of pigs. Kuhn et al. (2004) compared SBM and soy protein concentrate, an ingredient with markedly lower isoflavones relative to SBM, in a wean-to-finish study and observed higher plasma isoflavones in pigs fed SBM but no evidence for differences in growth performance. Greiner et al. (2001a,b) evaluated increasing dietary isoflavones and observed improvements in performance of PRRS positive pigs, mostly during periods of peak viremia. Smith et al. (2019) fed diets based on soy protein concentrate or enzyme-treated SBM with or without isoflavones and observed changes in activation of the adaptive immune system, although no impact on growth performance was observed.

There was a quadratic response in ADG with increasing SBM in Exp. 1, with a decrease observed in ADG of pigs fed the highest SBM inclusion. Similarly, in Exp. 2 there was a slight reduction in ADG with increasing SBM . However, the differences between treatments were relatively small and did not result in statistical differences in final BW. The reason for the negative impact of high SBM inclusion on ADG is unclear. Although the available literature generally does not agree with this finding, as the majority of studies (Johnston et al., 2010; Rocha et al., 2013; Rochell et al., 2015; Moran et al., 2017; Cemin et al., 2019) found no change or improvements in ADG with increasing SBM, the current experiment evaluated higher SBM levels than most of the previous research. It could be hypothesized that the high level of crude protein in the diet with 39% SBM provided excess nitrogen which needs to be metabolized and excreted by the animal (Van Milgen and Dourmad, 2015). The excess nitrogen represents an energy cost that may ultimately translate to decreased growth performance.

Improvements in feed efficiency with increasing amounts of SBM seem to be more consistently reported in the literature and agree with our findings. Energy is the most expensive component of any swine diet, thus it is critical to accurately determine the energy value of feedstuffs. Direct measurement of NE is a procedure that requires highly specialized equipment. Therefore, the estimation of the energy value of a test ingredient based on CE relative to a known ingredient such as corn is suggested as a practical approach, and is sometimes termed productive energy (Boyd et al., 2010; 2011; Graham et al., 2014; Gonçalves et al., 2016; Estrada et al., 2017). Besides the practical advantage, the estimates using CE conducted under field conditions may be more predictive of growth performance than other energy values. The diets used in our experiments were formulated using the NRC (2012) NE value for SBM and were not balanced for energy; thus, as SBM increased, dietary NE decreased. The resulting dietary NE values ranged from 2,475 to 2,362 kcal/kg in Exp. 1 and 2,455 to 2,344 kcal/kg in Exp. 2. Therefore, if the NE of SBM provided by the NRC (2012) is accurate, G:F should become worse as SBM level increased in the diet. However, the improvement in CE observed in the current experiments suggest that the NE value of SBM is underestimated. The NRC (2012) NE value for SBM is 2,087 kcal/kg or 78% of corn NE. Our findings from Exp. 1 based on CE suggest that the energy value of SBM is 105.4% of corn energy or 2,816 kcal/kg NE. It is important to note that, while CE response was significantly linear ($P = 0.001$), there was also a tendency ($P = 0.065$) for a quadratic response. Therefore, it could be hypothesized that the CE value of 39% SBM treatment should not be considered for estimating energy because slope-ratio assays should only include the linear portion of the response (Littell et al., 1997). By removing the 39% SBM diet and using the linear portion of the dataset results in an energy estimate of 121.1% of corn energy or 3,236 kcal/kg NE. A similar response was observed in Exp. 2, where energy value of SBM was estimated as 124.7% of corn energy or 3,332 kcal/kg. The energy estimates of both experiments are significantly greater than the NRC (2012) NE value, which may be driven by Noblet et al. (1994) equations too severely penalizing the NE content of high crude protein ingredients. However, it is important to note the using CE to estimate the energy value of an ingredient as a ratio to corn has limitations. This approach assumes that the NE values of corn are accurate and does not account for changes in body composition, which can influence the CE response as leaner pigs are more efficient (Campbell and Taverner, 1988). Using indirect calorimetry, Li et al. (2017) found that a NE of 2,709 kcal/kg for SBM, which is 101.4% of NRC (2012) corn NE and significantly greater than the NRC (2012) SBM NE value.

Another important consideration is that the responses in performance could have been driven by underestimation of the AA requirements relative to Lys. Our diets were formulated to meet or exceed the NRC (2012) requirement estimates; nevertheless, if any of these estimates is not accurate, by increasing the inclusion of SBM we could have potentially corrected an AA deficiency. However, most of the AA ratios were well above that recommended by the NRC (2012), thus the responses to SBM are unlikely to be driven by changes in AA ratios.

In conclusion, nursery pigs fed diets with increasing amounts of SBM presented inconsistent responses in ADG, but G:F and CE were improved in both experiments. The results of the current study suggest that the energy value of SBM may be estimated to range between 105 and 125% of corn energy, or 2,816 and 3,332 kcal/kg NE, which indicates that the NRC (2012) potentially underestimates the SBM NE value. This has important ramifications as it increases the value of SBM in diet formulation. However, it is unclear if the benefit of higher inclusion of SBM is entirely driven by energy or if another underlying mechanism, potentially involving intrinsic SBM components such as isoflavones, could be partially responsible for the response observed in this study.

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Table 5.1. Proximate and total amino acid analysis of corn, distillers dried grains with solubles (DDGS), and soybean meal used in Exp. 1 (as-fed basis)[1,2]

Item, %	Corn	DDGS	Soybean meal
Dry matter	87.8	90.8	88.8
Crude protein	6.3	28.7	48.0
Neutral detergent fiber	7.0	27.9	5.4
Ether extract	3.6	8.8	1.1
Calcium	0.07	0.08	0.42
Phosphorus	0.23	0.88	0.64
Amino acids			
Alanine	0.45	1.86	2.06
Arginine	0.30	1.27	3.42
Aspartic acid	0.44	1.79	5.39
Cysteine	0.16	0.60	0.73
Glutamic acid	1.11	3.64	8.44
Glycine	0.26	1.11	2.00
Histidine	0.19	0.78	1.27
Isoleucine	0.24	1.09	2.33
Leucine	0.71	3.19	3.71
Lysine	0.25	1.08	3.09
Methionine	0.13	0.50	0.66
Phenylalanine	0.31	1.69	2.52
Proline	0.56	2.07	2.41
Serine	0.29	1.26	1.91
Threonine	0.23	1.10	1.81
Tryptophan	0.06	0.22	0.73
Tyrosine	0.18	1.03	1.66
Valine	0.31	1.45	2.44

[1] A representative sample of each ingredient was obtained, homogenized, and submitted to the Agricultural Experimental Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for amino acid analysis and Ward Laboratories (Kearney, NE) for proximate analysis prior to diet formulation.

[2] For Exp. 2, the NRC (2012) amino acid values were used in diet formulation.

Table 5.2. Composition of experimental diets, Exp. 1 (as-fed basis)

	Soybean meal, %			
	21	27	33	39
Ingredient, %				
Corn	60.07	54.68	49.21	43.70
Soybean meal	21.00	27.00	33.00	39.00
DDGS[1]	15.00	15.00	15.00	15.00
Calcium carbonate	1.08	1.08	1.08	1.08
Monocalcium phosphate, 21.5% P	0.65	0.55	0.50	0.40
Sodium chloride	0.50	0.50	0.50	0.50
L-Lysine HCl	0.643	0.456	0.255	0.053
DL-Methionine	0.225	0.170	0.110	0.045
L-Threonine	0.295	0.215	0.135	0.040
L-Tryptophan	0.095	0.060	0.020	---
L-Valine	0.225	0.115	---	---
L-Isoleucine	0.040	---	---	---
Vitamin trace-mineral premix[2]	0.150	0.150	0.150	0.150
Phytase[3]	0.050	0.050	0.050	0.050
Total	100	100	100	100
Calculated analysis				
SID[4] amino acids, %				
Lysine	1.30	1.30	1.30	1.30
Isoleucine:lysine	55	61	69	78
Leucine:lysine	112	124	137	149
Methionine:lysine	37	34	32	30
Methionine & cystine:lysine	57	57	57	57
Threonine:lysine	65	65	65	65
Tryptophan:lysine	22.1	22.1	22.0	23.4
Valine:lysine	76	76	76	85
Histidine:lysine	33	37	42	47
Net energy[5], kcal/kg	2,475	2,437	2,398	2,362
Crude protein, %	19.2	21.3	23.4	25.6
Neutral detergent fiber, %	11.9	11.7	11.5	11.3
Calcium, %	0.69	0.70	0.72	0.74
STTD P[6], %	0.45	0.45	0.45	0.45
Analyzed values, %				
Dry matter	87.7	88.1	88.2	88.5
Crude protein	20.0	21.4	24.2	25.9
Neutral detergent fiber	9.2	9.8	9.8	9.3
Ether extract	2.9	3.0	2.8	2.6

[1] DDGS = distillers dried grains with solubles.

[2] Provided per kg of premix: 5,344,543 IU vitamin A; 1,336,137 IU vitamin D; 100,211 IU vitamin E; 1,671 mg vitamin K; 21.4 mg vitamin B12; 29,061 mg niacin; 15,366 mg pantothenic acid; 4,008 mg riboflavin; 66.8 mg biotin; 668 mg folic acid; 1202 mg vitamin B6; 73 g Zn from zinc sulfate; 67 g Fe from ferrous sulfate; 27 g

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Mn from manganese oxide; 10 g Cu from copper sulfate; 0.5 g I from calcium iodate; 0.2 g Se from sodium selenite.

[3] Optiphos 2000 (Huvepharma, Inc., Peachtree City, GA).

[4] SID = standardized ileal digestible.

[5] Net energy values were obtained from the NRC (2012).

[6] STTD P = standardized total tract digestible phosphorus.

Table 5.3. Composition of experimental diets, Exp. 2 (as-fed basis)

	Soybean meal, %					
	17.5	22.0	26.5	31.0	35.5	40.0
Ingredient, %						
Corn	62.69	58.78	54.86	50.90	46.98	43.07
Soybean meal	17.50	21.99	26.48	31.01	35.5	40.00
DDGS[1]	15.00	15.00	15.00	15.00	15.00	15.00
Calcium carbonate	1.35	1.36	1.36	1.37	1.37	1.37
Monocalcium phosphate, 21.5% P	0.46	0.37	0.28	0.19	0.09	---
Sodium chloride	0.43	0.42	0.42	0.41	0.40	0.40
L-Lysine sulfate	1.694	1.355	1.016	0.678	0.339	---
Methionine hydroxy analong	0.195	0.159	0.123	0.088	0.052	0.016
L-Threonine	0.247	0.198	0.148	0.099	0.050	---
L-Tryptophan	0.071	0.057	0.043	0.028	0.014	---
L-Valine	0.147	0.117	0.088	0.059	0.030	---
L-Isoleucine	0.061	0.049	0.037	0.024	0.012	---
Vitamin trace-mineral premix[2]	0.150	0.150	0.150	0.150	0.150	0.150
Total	100	100	100	100	100	100
Calculated analysis						
SID[3] amino acids, %						
Lysine	1.20	1.20	1.20	1.20	1.20	1.20
Isoleucine:lysine	55	60	66	71	76	81
Leucine:lysine	124	133	142	151	160	169
Methionine:lysine	37	36	35	34	33	32
Methionine & cystine:lysine	58	59	59	60	61	62
Threonine:lysine	64	65	66	67	68	69
Tryptophan:lysine	19.2	20.1	21.0	21.8	22.7	23.5
Valine:lysine	70	74	77	81	85	88
Histidine:lysine	34	38	42	45	49	52
Net energy, kcal/kg	2,455	2,433	2,411	2,388	2,366	2,344
Crude protein, %	18.9	20.5	22.1	23.7	25.3	26.9

Neutral detergent fiber, %	12.17	12.19	12.20	12.21	12.22	12.23
Calcium, %	0.69	0.69	0.69	0.69	0.69	0.69
STTD P[4], %	0.38	0.38	0.37	0.36	0.36	0.35
Analyzed values, %						
Dry matter	85.7	86.0	85.9	86.2	86.9	86.8
Crude protein	17.2	19.2	20.2	22.7	23.7	25.6
Crude fiber	2.2	2.4	2.7	3.1	3.0	3.2
Ether extract	3.1	3.1	3.1	3.1	3.3	3.3

[1] DDGS = distillers dried grains with solubles.

[2] Provided per kg of premix: 1,653,468 IU vitamin A; 661,387 IU vitamin D; 17,637 IU vitamin E; 1,323 mg vitamin K; 13.2 mg vitamin B12; 19,842 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 499,899 FTU phytase; 73 g Zn from zinc sulfate; 67 g Fe from ferrous sulfate; 27 g Mn from manganese oxide; 10 g Cu from copper sulfate; 0.5 g I from calcium iodate; 0.2 g Se from sodium selenite.

[3] SID = standardized ileal digestible.

[4] STTD P = standardized total tract digestible phosphorus.

Table 5.4. Effects of increasing soybean meal on growth performance and caloric efficiency of pigs, Exp. 1[1]

	Soybean meal, %				SEM	Probability, *P* <	
Item[2]	21	27	33	39		Linear	Quadratic
BW, kg							
d 0	11.0	11.0	11.0	11.0	0.15	0.894	0.993
d 21	22.3	22.3	22.4	22.0	0.28	0.263	0.180
d 0 to 21							
ADG, g	537	537	543	524	7.3	0.207	0.090
ADFI, g	824	822	815	804	11.7	0.092	0.579
G:F, g/kg	652	653	667	653	5.1	0.390	0.069
CE, kcal/kg gain	3,801	3,738	3,600	3,623	28.8	0.001	0.063
Culls and mortality, %	0.72	0.36	0.36	0.36	0.356	0.457	0.596

[1] A total of 2,233 pigs (initially 11.0 kg) were used in a 21-d study with 20 to 27 pigs per pen and 23 replicates per treatment.

[2] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. CE = caloric efficiency.

Table 5.5. Effects of increasing soybean meal on growth performance and caloric efficiency of pigs, Exp. 2[1]

	Soybean meal, %						SEM	Probability, $P <$	
Item[2]	17.5	22.0	26.5	31.0	35.5	40.0		Linear	Quadratic
BW, kg									
d 0	17.5	17.6	17.5	17.6	17.6	17.6	0.20	0.801	0.997
d 22	35.6	35.8	35.5	35.4	35.5	35.4	0.28	0.272	0.987
d 0 to 22									
ADG, g	820	825	818	809	812	809	7.4	0.065	0.922
ADFI, g	1,500	1,509	1,473	1,424	1,415	1,401	18.1	0.001	0.957
G:F, g/kg	547	548	556	568	574	578	5.4	0.001	0.893
CE, kcal/kg gain	4,491	4,450	4,342	4,203	4,126	4,055	43.6	0.001	0.955
Culls and mortality, %	0.58	0.89	0.71	0.43	0.14	0.14	0.447	0.050	0.377

[1] A total of 3,796 pigs (initially 17.6 kg) were used in a 22-d study with 37 to 43 pigs per pen and 14 replicates per treatment.

[2] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. CE = caloric efficiency.

Chapter 6 - Effects of high-protein distillers dried grains on growth performance of nursery pigs and initial estimates of its energy content relative to corn[1,2]

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ABSTRACT: A total of 300 pigs (DNA 400 × 200, Columbus, NE), initially 11.1 kg, were used in a study to evaluate the effects of increasing amounts of high-protein distillers dried grains (**HP DDG**) on growth performance and to estimate its energy value relative to corn based only on caloric efficiency. Pigs were weaned, placed in pens with 5 pigs each, and fed a common diet for 21 d after weaning. Then, pens were assigned to treatments in a randomized complete block design. There were 5 treatments with 12 replicates per treatment. Treatments consisted of 0, 10, 20, 30, or 40% HP DDG inclusion, formulated by changing amounts of corn and feed-grade amino acids. Pigs were weighed weekly for 21 d to evaluate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Caloric efficiency was obtained by multiplying ADFI by kcal of net energy (**NE**) per kg of diet and dividing by ADG. The NE values for corn and soybean meal were obtained from NRC (2012), and initial estimates for HP DDG NE were derived from Noblet et al. (1994) equations. The energy of HP DDG was estimated based on caloric efficiency relative to the diet without HP DDG. Changes in body composition were not measured. Pigs fed diets with increasing HP DDG had a linear decrease ($P < 0.01$) in ADG, ADFI, and final body weight. There was a tendency for a quadratic response ($P = 0.051$) in G:F, with the greatest G:F observed for pigs fed diets with 40% HP DDG. There was a linear reduction ($P < 0.05$) in caloric efficiency with increasing amounts of HP DDG, indicating an underestimation of the initial estimate of HP DDG NE. Based on the changes in caloric efficiency alone, the energy value of HP DDG in this study was estimated to be 97.3% of the energy value of corn. If a standard reference value for corn of 2,672 kcal/kg NE is used, the HP DDG would have an approximate NE value of 2,600 kcal/kg.

Key words: caloric efficiency, growth, high-protein distillers dried grains, performance

INTRODUCTION

Distillers dried grains with solubles (**DDGS**) is a co-product of the ethanol industry widely used in swine diets. Recently, new processing techniques have been developed to remove nonfermentable components before fermentation (Sekhon et al., 2015) resulting in a high-protein DDG (**HP DDG**) with approximately 40% crude protein. The HP DDG generated has a different chemical composition and nutritive value than DDGS for swine diets (NRC, 2012). Therefore, it is critical to characterize the nutrient profile of HP DDG and its effects on growth performance. Recently, Rho et al. (2017) determined the standardized ileal digestibility (**SID**) of amino acids (**AA**) and digestible energy (**DE**) content of HP DDG and observed similar AA digestibility coefficients, but approximately 25% greater DE than conventional DDGS. However, growth performance was not evaluated in that study and it is not clear if the higher DE content translates to improved growth performance.

Feeding increasing amounts of an ingredient and using the differences in caloric efficiency to estimate the energy content of the test ingredient relative to a known ingredient, usually corn, has been reported by others and sometimes termed productive energy (Boyd et al., 2010; 2011; Graham et al., 2014; Gonçalves et al., 2016; Estrada et al., 2017). This method is a more practical approach than determining net energy (**NE**) using direct or indirect calorimetry. Energy estimation from caloric efficiency calculations are done using growth assays under field conditions. Therefore, this method may be more predictive of growth performance than systems based on digestible or metabolizable energy. The objective of this study was to determine differences in growth performance of pigs fed increasing amounts of HP DDG in order to estimate its energy content relative to corn.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State University Swine Segregated Early Weaning Facility in Manhattan, KS.

Animals and diets

Samples of corn, soybean meal, and HP DDG (manufactured in St. Joseph MO, by ICM, Inc., Colwich, KS) were submitted to the Agricultural Experimental Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for total AA content analysis (method 982.30; AOAC International, 2006) prior to diet formulation (Table 6.1). The total AA values for corn and soybean meal were multiplied by NRC (2012) SID coefficients and the values for HP DDG were multiplied by Rho et al. (2017) SID coefficients and used in diet formulation. Corn, soybean meal, and HP DDG were also analyzed (Ward Laboratories, Inc., Kearney, NE) for dry matter (**DM**) (method 935.29; AOAC International, 1990), crude protein (**CP**) (method 990.03; AOAC International, 1990), acid detergent fiber (**ADF**) (Ankom, 1998), neutral detergent fiber (**NDF**) (Ankom, 1998), ether extract (**EE**) (Ankom, 2004), starch (Xiong et al., 1990), and ash (method 942.05; AOAC International, 1990). A sample of HP DDG was analyzed (North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND) for mycotoxin concentrations through extraction in acetonitrile and water followed by chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) detection (Table 6.2).

A total of 300 barrows (DNA 400 × 200, Columbus, NE; initially 11.1 kg) were used in a 21-d growth trial. Pigs were weaned at approximately 21 d of age, placed in pens of 5 pigs each based on initial body weight (**BW**), and fed common diets for 21 d. On d 21, which was considered d 0 of the trial, pens of pigs were allotted to 1 of 5 treatments in a randomized complete block design with BW as the blocking factor. There were 12 replicates per treatment. Treatments consisted of corn-soybean meal diets with increasing amounts of HP DDG at 0, 10, 20, 30, or 40% of the diet. The addition of HP DDG was made at the expense of corn and feed-grade amino acids while the amount of soybean meal in the diet was held constant (Table 6.3). Diets were not balanced for NE content. Each pen (1.2 × 1.2 m) was equipped with a 4-hole, dry self-feeder and a cup waterer to provide ad libitum access to feed and water. Pigs were weighed and feed disappearance was measured to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**).

Caloric efficiency was obtained by multiplying ADFI by kcal of NE per kg of diet and dividing by ADG. The NE value for HP DDG was estimated using proximate analysis values by three different methods:

a) Equation 1: DE was estimated from Noblet and Perez (1993) equation as DE, kcal/kg = 4,168 – (9.1 × ash) + (1.9 × CP) + (3.9 × EE) – (3.6 × NDF) and the DE value was used in the Noblet et al. (1994) equation to estimate NE as NE, kcal/kg = (0.700 × DE) + (1.61 × EE) + (0.48 × starch) – (0.91 × CP) – (0.87 × ADF), where all components are expressed as g/kg of dry matter (**DM**);

b) Equation 2: DE was estimated from Anderson et al. (2012) equation as DE, kcal/kg = –2,161 + (1.39 × gross energy) – (20.7 × NDF) – (49.3 × EE), where gross energy is expressed as kcal/kg DM and other components are expressed as percentages, and the DE value obtained was used in the Noblet et al. (1994) NE equation;

c) Equation 3: DE value was obtained from Rho et al. (2017; 4,555 kcal/kg DM) and used in the Noblet et al. (1994) NE equation.

The equations resulted in energy values on a DM basis, which were then multiplied by the analyzed DM content of HP DDG and presented on an as-fed basis. Corn and soybean meal were assigned NE values obtained from the NRC (2012), at 2,672 and 2,087 kcal/kg, respectively.

Chemical analysis

Representative diet samples were obtained from all feeders of each treatment and stored at -20°C until analysis. Samples were analyzed (Ward Laboratories, Inc., Kearney, NE) for DM (method 935.29; AOAC International, 1990), CP (method 990.03; AOAC International, 1990), calcium (method 985.01; AOAC International, 1990), phosphorus (method 985.01; AOAC International, 1990), and NDF (Ankom, 1998).

Statistical analysis

Data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. Polynomial contrasts were constructed to evaluate the linear and quadratic effects of increasing HP DDG on ADG, ADFI, G:F, BW, and caloric efficiency. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Chemical analysis

The total AA content of HP DDG was similar to that observed by Rho et al. (2017; Table 6.1). Mycotoxins were under the detectable values except for deoxynivalenol, which was present at 560 ppb (Table 6.2). The U.S. Food and Drug Administration recommends that feed ingredients contain less than 5,000 ppb deoxynivalenol and that these ingredients do not exceed 20% of the diet, for a maximum of 1,000 ppb deoxynivalenol in complete feed (FDA, 2010).

Therefore, the mycotoxin levels in HP DDG used in this study were not deemed to impact pig performance. The analyzed CP, calcium, phosphorus, and NDF of the complete diets were consistent with formulated values (Table 6.3).

Growth performance

Pigs fed diets with increasing HP DDG had a linear decrease ($P < 0.01$) in ADG, ADFI, and final BW (Table 6.4). There was a tendency ($P = 0.051$) for a quadratic response in G:F, with the greatest value observed for pigs fed the diet with 40% HP DDG. The negative impact of HP DDG on ADG resulted in a decrease (linear, $P < 0.01$) in d 21 BW with increasing HP DDG.

In a recent study, Yang et al. (2018) observed that the inclusion of up to 30% HP DDGS to diets for 7- to 22-kg pigs linearly decreased growth performance, which is in agreement with our findings. For finishing pigs, Kim et al. (2009) were able to completely replace soybean meal with HP DDG in diets for 58- to 130-kg pigs without compromising growth performance. However, it should be noted that the HP DDG source as well as age and BW range of pigs used in that experiment were not the same as in our study.

A potential cause for the decreased growth performance observed in our study is dietary fiber level. The calculated NDF in our diets ranged from 8.4 to 19.3%. Similarly, Yang et al. (2018) reported an NDF range of 6.3 to 13.5% with increasing HP DDGS from 0 to 30%. There are no specific requirements for fiber in swine diets, and although there may be potential benefits from functional properties of insoluble fiber, especially in weaned pigs (Molist et al., 2014), dietary fiber may have a negative effect on nutrient digestibility (Schulze et al., 1994) and it is possible that the ability to utilize fiber is lower in younger pigs (Le Goff et al., 2003).

Dietary Thr content also could have influenced the results. The NRC (2012) requirement estimate for 11- to 25-kg pigs is 59% SID Thr:Lys. However, Mathai et al. (2016) observed that the SID Thr:Lys requirement for ADG is approximately 8% greater (71 vs 66% SID Thr:Lys) in growing pigs fed high fiber diets, which was most likely driven by higher mucin production and endogenous Thr losses. Therefore, although our diets were well above the NRC (2012) recommendations with 65 to 67% SID Thr:Lys, Thr could have been limiting in diets with high levels of HP DDG according to Mathai et al. (2016).

Diets formulated with high levels of corn by-products, such as HP DDG, can result in a branched-chain AA (**BCAA**) imbalance due to the elevated Leu content relative to Val and Ile (NRC, 2012). The BCAA are structurally similar and share the first steps of their catabolism. Therefore, excess of one BCAA, particularly Leu, may result in increased degradation of all three BCAA (Harper et al., 1984). The BCAA requirements for nursery pigs have been recently reported as 102% to 108% SID Leu:Lys (Gloaguen et al., 2013; Wessels et al., 2016), 52% SID Ile:Lys (Soumeh et al., 2014; Clark et al., 2017a), and 63 to 74% SID Val:Lys (Clark et al., 2017b). However, Val and Ile requirements seem to depend on the dietary Leu level. Htoo et al. (2017) observed that 8- to 25-kg pigs fed diets with 110 or 160% SID Leu:Lys have a different SID Ile:Lys requirement of 54 or 58%, respectively. Our diets ranged from 105 to 210% SID Leu:Lys, 61 to 85% SID Ile:Lys, and 70 to 96% SID Val:Lys. It is important to note that while Leu levels were excessive in diets with high amounts of HP DDG, the other BCAA were also well above the estimated requirement. Cemin et al. (2019) suggested that the negative effects of high levels of Leu can be counteracted by concomitant increases in Ile, Val, or Trp. Millet et al. (2015) observed that 10- to 45-kg pigs fed diets with high Leu concentrations had decreased growth performance, but the addition of Val effectively counteracted the negative effects of excessive Leu. Thus, it could be hypothesized that the high Leu levels in our diets were at least partially compensated by the concomitant increase in Ile and Val.

Energy estimation

Using the DE value (3,298 kcal/kg) estimated by Equation 1 resulted in a NE estimate of 1,914 kcal/kg for HP DDG. The DE value (3,663 kcal/kg) from Equation 2 resulted in a slightly greater estimate of 2,170 kcal/kg NE. Using measured DE energy value (4,162 kcal/kg) from Rho et al. (2017) in Equation 3 resulted in the greatest NE estimate of 2,519 kcal/kg. The NE for soybean meal and corn used in diet formulation were obtained from the NRC (2012), thus these values were used to estimate the relative energy value of HP DDG.

All equations resulted in similar responses in caloric efficiency, with a decrease (linear, $P \leq 0.014$; quadratic, $P \leq 0.062$) in caloric efficiency as HP DDG increased. This result suggests that in order to obtain a caloric efficiency similar to the corn-soybean meal diet, the energy value of HP DDG would need to be 97.3% of the energy value of corn. If corn NE is assumed to be 2,672 kcal/kg (NRC, 2012), HP DDG would have a NE estimate of 2,600 kcal/kg. In comparison, the initial energy value of HP DDG was estimated as 71.6% of corn NE using Equation 1, 81.2% of corn NE using Equation 2, and 94.3% of corn NE using Equation 3.

Energy is the most expensive component of swine diets. Therefore, it is critical to accurately determine the energy content of feed ingredients and its availability to the animal. This is especially true for novel alternative feedstuffs that are available to the swine industry and can be important cost-effective alternatives to traditional corn-soybean meal-based diets. The NE system has the highest correlation to performance compared to digestible or metabolizable energy systems (Nitikanchana et al., 2015). However, direct measurement of NE is highly specialized and labor intensive. The utilization of caloric efficiency to estimate the energy value of a test ingredient relative to a known ingredient, typically corn, is sometimes termed productive energy (Boyd et al., 2010, 2011; Estrada et al., 2017). This method was developed as a more practical approach to energy estimations of any ingredient (Gonçalves et al., 2016). Under or overestimation of an energy value can be detected if pigs fed diets with increasing amounts of the test ingredient present differences in caloric efficiency (De Jong et al., 2014). In our study, caloric efficiency linearly decreased with increasing HP DDG, indicating that the initial energy value of HP DDG was underestimated. The closest energy estimate was obtained using actual DE values determined by Rho et al. (2017) in combination with Noblet et al. (1994) equation, whereas using the DE value estimated by Noblet and Perez (1993) equation in the Noblet et al. (1994) equation or the DE equation of Anderson et al. (2012) coupled with the Noblet et al. (1994) equation resulted in underestimated HP DDG energy, which may be driven by the equations too severely penalizing HP DDG due to its high CP and fiber concentrations.

It is important to note that the method presented in the current study using only caloric efficiency to estimate the energy value of HP DDG presents some limitations. This approach assumes that the assigned NE values of corn and soybean meal are accurate. In our study, reference values for corn and soybean meal were derived from the NRC (2012) which are estimated from their chemical composition using equations derived from Noblet and Perez (1993) and Noblet et al. (1994). A second limitation of using caloric efficiency to estimate NE is that it does not measure changes in body composition. Changes in G:F could also be the result of changes in body composition as leaner pigs are more efficient than fatter pigs (Campbell and Taverner, 1988).

In conclusion, feeding diets with increasing HP DDG resulted in decreased ADG. The improvement in caloric efficiency that was observed indicates that the initial HP DDG energy value was underestimated. Using caloric efficiency as a means to estimate NE suggests that the HP DDG used in this study contained approximately 97.3% of the energy value of corn. If a standard reference value for corn of 2,762 kcal/kg NE is used, the HP DDG would have an approximate NE value of 2,600 kcal/kg.

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Table 6.1. Chemical analysis of corn, soybean meal, and high-protein distillers dried grains (HP DDG; as-fed basis)[1]

Item, %	Corn	Soybean meal	HP DDG
Dry matter	87.1	89.8	91.4
Crude protein	7.4	47.3	39.0
Ether extract	2.7	1.3	8.4
Ash	1.2	5.9	3.1
Neutral detergent fiber	5.8	11.1	36.0
Acid detergent fiber	1.8	9.5	21.3
Starch	59.0	1.2	2.3
Amino acids			
Alanine	0.56	2.01	2.79
Arginine	0.37	3.38	1.64
Aspartic acid	0.53	5.27	2.52
Cysteine	0.19	0.70	0.83
Glutamic acid	1.39	8.48	6.26
Glycine	0.31	2.00	1.54
Histidine	0.22	1.20	1.06
Isoleucine	0.28	2.27	1.67
Leucine	0.88	3.58	4.93
Lysine	0.27	3.01	1.22
Methionine	0.18	0.66	0.83
Phenylalanine	0.38	2.38	2.08
Proline	0.67	2.37	3.21
Serine	0.34	2.01	1.63
Threonine	0.28	1.81	1.47
Tryptophan	0.06	0.63	0.32
Tyrosine	0.23	1.72	1.56
Valine	0.38	2.32	2.05

[1] A representative sample of each ingredient was obtained, homogenized, and submitted to Ward Laboratories, Inc. (Kearney, NE) for proximate analysis and to the Agricultural Experimental Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for amino acid analysis prior to diet formulation.

Table 6.2. Mycotoxins analysis of high-protein distillers dried grains (HP DDG)[1]

Mycotoxins	Practical quantitation limit, ppb	HP DDG, ppb
Aflatoxin B1	20	< 20
Aflatoxin B2	20	< 20
Aflatoxin G1	20	< 20
Aflatoxin G2	20	< 20
Deoxynivalenol	200	560
Fumonisin B1	200	< 200
Fumonisin B2	200	< 200
HT-2 toxin	200	< 200
Ochratoxin A	20	< 20
T-2 toxin	20	< 20
Sterigmatocystin	20	< 20
Zearalenone	100	< 100

[1] A representative sample of HP DDG was collected, homogenized, and submitted to North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND).

Table 6.3. Diet composition (as-fed basis)[1]

	HP DDG[2], %				
Item	0	10	20	30	40
Ingredient, %					
Corn	68.6	59.3	49.7	40.0	30.3
Soybean meal, 47% crude protein	26.5	26.5	26.5	26.5	26.5
HP DDG	---	10.0	20.0	30.0	40.0
Calcium carbonate	0.98	1.05	1.13	1.18	1.25
Monocalcium phosphate, 21.5% P	1.60	1.35	1.15	0.95	0.75
Sodium chloride	0.50	0.50	0.50	0.50	0.50
L-Lysine HCl	0.58	0.51	0.45	0.39	0.33
DL-Methionine	0.22	0.12	0.01	---	---
L-Threonine	0.30	0.21	0.13	0.06	---
L-Tryptophan	0.06	0.05	0.03	0.01	---
L-Valine	0.17	0.04	---	---	---
L-Isoleucine	0.10	---		---	---
L-Histidine	0.06	---	---	---	---
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
SID[5] amino acids, %					
Lysine	1.30	1.30	1.30	1.30	1.30
Isoleucine:lysine	61	61	69	77	85
Leucine:lysine	105	131	157	184	210
Methionine:lysine	37	33	29	33	37
Methionine & cystine:lysine	57	57	57	64	72
Threonine:lysine	65	65	65	65	67
Tryptophan:lysine	19.0	19.0	19.0	19.0	19.8
Valine:lysine	70	70	77	86	96
Histidine:lysine	36	36	41	45	50
Net energy[6], kcal/kg	2,437	2,360	2,285	2,212	2,138
Crude protein, %	19.5	22.2	25.1	28.1	31.0
Neutral detergent fiber, %	8.4	11.2	13.9	16.6	19.3
Calcium, %	0.82	0.82	0.82	0.82	0.82
STTD P[7], %	0.45	0.45	0.45	0.45	0.45
Analyzed values, %					
Dry matter	89.0	89.4	89.8	90.0	90.3
Crude protein	19.0	21.7	24.4	27.2	30.1
Neutral detergent fiber	5.2	7.2	10.5	15.4	17.4
Calcium	1.07	0.85	0.93	0.96	0.94
Phosphorus	0.65	0.56	0.57	0.57	0.57

[1] HP DDG = high-protein distillers dried grains.

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[2] Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin.

[3] Provided per kg of diet: 110 mg Zn from Zn sulfate; 110 mg Fe from iron sulfate; 33 mg Mn from manganese oxide; 17 mg Cu from copper sulfate; 0.30 mg I from calcium iodate; 0.30 mg Se from sodium selenite.

[4] SID = standardized ileal digestible.

[5] Initial net energy estimates were obtained using NRC (2012) values for corn and soybean meal. For HP DDG, digestible energy value was first derived using Noblet and Perez et al. (1993) equation: DE, kcal/kg = 4,168 – (9.1 × ash) + (1.9 × crude protein) + (3.9 × ether extract) – (3.6 × NDF) and net energy value was then derived from Noblet et al. (1994) equation: NE, kcal/kg = (0.700 × digestible energy) + (1.61 × ether extract) + (0.48 × starch) – (0.91 × crude protein) – (0.87 × acid detergent fiber), where all components are expressed as g/kg of dry matter, using analyzed values for ether extract, starch, crude protein, acid detergent fiber, and neutral detergent fiber.

[6] STTD P = standardized total tract digestible phosphorus.

Table 6.4. Effects of high-protein distillers dried grains (HP DDG) on nursery pig performance[1,2]

	HP DDG, %						Probability, $P <$	
Item[3]	0	10	20	30	40	SEM	Linear	Quadratic
BW, kg								
d 0	11.1	11.1	11.1	11.1	11.1	0.18	0.985	0.814
d 21	22.3	22.6	21.4	21.2	21.4	0.39	0.001	0.366
d 0 to 21								
ADG, g	536	550	493	483	490	12.2	0.001	0.385
ADFI, g	830	855	778	755	746	18.2	0.001	0.715
G:F, g/kg	645	644	634	640	657	7.1	0.365	0.051
CE, kcal/kg gain (Equation 1)[4]	3,782	3,669	3,607	3,463	3,258	38.3	0.001	0.068
CE, kcal/kg gain (Equation 2)[5]	3,782	3,709	3,687	3,584	3,415	39.2	0.001	0.067
CE, kcal/kg gain (Equation 3)[6]	3,782	3,764	3,798	3,747	3,626	40.6	0.014	0.062

[1] A total of 300 pigs were used in a 21-d study with 5 pigs per pen and 12 replicates per treatment.

[2] Diets contained 2,498, 2,414, 2,330, 2,249, and 2,165 kcal/kg calculated net energy, respectively. Net energy values for corn and soybean meal were obtained from NRC (2012) and for HP DDG, digestible energy value was first derived using Noblet and Perez et al. (1993) equation: DE = 4,168 – (9.1 × ash) + (1.9 × crude protein) + (3.9 × ether extract) – (3.6 × NDF) and net energy value was then derived from Noblet et al. (1994) equation: NE = (0.700 × digestible energy) + (1.61 × ether extract) + (0.48 × starch) – (0.91 × crude protein) – (0.87 × acid detergent fiber), using analyzed values for ether extract, starch, crude protein, acid detergent fiber, and neutral detergent fiber.

[3] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. CE = caloric efficiency.

[4] For CE (Equation 1), digestible energy value for HP DDG was first derived using Noblet and Perez et al. (1993) equation: DE, kcal/kg = 4,168 – (9.1 × ash) + (1.9 × crude protein) + (3.9 × ether extract) – (3.6 × NDF) and net energy value was then derived from Noblet et al. (1994) equation: NE, kcal kg = (0.700 × digestible energy) + (1.61 × ether extract) + (0.48 × starch) – (0.91 × crude protein) – (0.87 × acid detergent fiber), where all components are expressed as g/kg of dry matter, using analyzed values for ether extract, starch, crude protein, acid detergent fiber, and neutral detergent fiber.

[5] For CE (Equation 2), digestible energy value for HP DDG was first derived using values were obtained using Anderson et al. (2012) equation: DE, kcal/kg = –2,161 + (1.39 × gross energy) – (20.7 × neutral detergent fiber) – (49.3 × ether extract), where gross energy is expressed as kcal/kg and others are expressed as percentages, and net energy value was then derived from Noblet et al. (1994) equation: NE, kcal/kg = (0.700 × digestible energy) + (1.61 × ether extract) + (0.48 × starch) – (0.91 × crude protein) – (0.87 × acid detergent fiber), where all components are expresses as g/kg of dry matter, using analyzed values for gross energy, ether extract, starch, crude protein, acid detergent fiber, and neutral detergent fiber.

[6] For CE (Equation 3), digestible energy value for HP DDG was first obtained from Rho et al. (2017; 4,555 kcal/kg of dry matter) and net energy value was then derived from Noblet et al. (1994) equation: NE, kcal/kg = (0.700 × digestible energy) + (1.61 × ether extract) + (0.48 × starch) – (0.91 × crude protein) – (0.87 × acid detergent fiber), where all components are expressed as g/kg of dry matter, using analyzed values for ether extract, starch, crude protein, and acid detergent fiber.

Chapter 7 - Effects of increasing dietary zinc on growth performance and carcass characteristics of pigs raised under commercial conditions[1,2]

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ABSTRACT: A total of 2,430 pigs (PIC 337 × 1050, Hendersonville, TN; initially 30.1 kg) were used in a 113-d growth trial to determine the effects of increasing dietary Zn on growth performance and carcass characteristics of finishing pigs raised under commercial conditions. Pens of pigs were assigned to be fed 1 of 5 dietary treatments in a randomized complete block design. Treatments consisted of 50, 87.5, 125, 162.5, or 200 mg/kg added Zn from Zn hydroxychloride (IntelliBond Z, Micronutrients, Indianapolis, IN). Two identical barns were used for a total of 18 pens per treatment with 27 pigs per pen. Experimental diets were fed in 5 phases and contained a vitamin-trace mineral premix without added Zn. Pens of pigs were weighed approximately every 2 weeks to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). At the end of the experimental period, pigs were tattooed with a pen identification number and transported to a packing plant to measure hot carcass weight (**HCW**), backfat, loin depth, and calculate lean percentage. Data were analyzed block nested within barn as a random effect and pen as the experimental unit. From d 0 to 42, pigs fed diets with increasing added Zn had lower (linear, $P = 0.043$) ADFI and a tendency ($P = 0.092$) for lower ADG. From d 42 to 113, increasing added Zn resulted in a quadratic response ($P = 0.042$) for ADFI and a tendency (linear, $P = 0.056$) for improved G:F. Overall (d 0 to 113), there were tendencies for quadratic responses for ADFI ($P = 0.073$) and G:F ($P = 0.059$), with the greatest G:F observed when 125 mg/kg of Zn was fed. Increasing added Zn resulted in a linear increase ($P < 0.001$) in daily Zn intake. There were no differences ($P > 0.10$) in overall ADG, final BW, HCW, backfat, loin depth, lean percentage, mortality, and removal rate. In conclusion, there were no improvements in ADG when feeding beyond 50 mg/kg added Zn; however, providing 125 mg/kg added Zn resulted in the greatest G:F.

Key words: finishing pig, growth performance, zinc hydroxychloride

INTRODUCTION

Zinc is an essential mineral for protein, carbohydrate, and lipid metabolism due to its essentiality as a component of metalloenzymes, such as DNA and RNA synthetases, digestive enzymes, and insulin (NRC, 2012). The dietary Zn requirement for 25 to 135 kg grow-finish pigs ranges from 50 to 60 mg/kg depending on body weight (NRC, 2012). This requirement is usually met through the trace mineral premix, which is commonly considered the sole source of added Zn (Miller, 1991). According to a U.S. swine industry survey, added Zn levels in grow-finish diets range from 72 to 86 mg/kg (Flohr et al., 2016) thus it is relatively common practice to supplement more Zn in growing pig diets than the requirement.

Traditionally, inorganic Zn sources such as Zn oxide and Zn sulfate are most commonly used in swine diets. Zinc hydroxychloride is a novel inorganic source of Zn manufactured through the reaction of high purity forms of the metal with water and hydrochloric acid (Leisure et al., 2014). This process forms hydroxychloride crystals that contain Zn covalently bonded to hydroxyl groups and chloride. The covalent bonds are expected to reduce reactiveness with other components of the diet and to improve bioavailability (Cao et al., 2000).

Currently, there are few studies with grow-finish pigs, especially conducted under large-scale commercial conditions, evaluating dietary Zn concentrations. Therefore, the objective of this study was to determine the effects of increasing dietary Zn from Zn hydroxychloride on growth performance and carcass characteristics of grow-finish pigs housed under commercial conditions.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in this experiment.

Animals and diets

The study was conducted at a commercial research facility in southwestern Minnesota. Two identical barns, naturally ventilated and double-curtain sided, were used for a total of 90 pens with 27 pigs per pen. Pens (5.5 × 3.0 m) had completely slatted floors and were equipped with a 4-hole stainless steel dry self-feeder and a cup waterer. Feed additions were accomplished and recorded by a computerized feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

A total of 2,430 pigs (PIC 337 × 1050, Hendersonville, TN; initially 30.1 ± 0.70 kg BW) were used in a 113-d growth trial. Pens of pigs were blocked by BW within barn and randomly assigned to 1 of 5 treatments in a randomized complete block design. Treatments consisted of 50, 87.5, 125, 162.5, or 200 mg/kg added Zn from Zn hydroxychloride (IntelliBond Z; Micronutrients, Indianapolis, IN). A vitamin-trace mineral premix was formulated without Zn to ensure Zn hydroxychloride was the only source of added Zn. There were 18 replicates per treatment. Diets were manufactured at the New Horizon Farms feed mill (Pipestone, MN) and offered in 5 phases in meal form (Table 7.1).

Pens of pigs were weighed and feed disappearance measured approximately every 2 weeks to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). Daily zinc intake was calculated by multiplying overall ADFI by the inclusion of added Zn. Mortality and removals were recorded daily. On d 90, according to standard farm protocol, the 3 heaviest pigs in each pen were identified and transported to a packing plant (JBS Swift and Company, Worthington, MN) to be processed. At the end of the experimental period, final pen weights were recorded and remaining pigs were tattooed with a pen identification number and transported to a packing plant (JBS Swift and Company, Worthington, MN) for carcass data collection. Carcass measurements included hot carcass weight (**HCW**), loin depth, backfat, and percentage lean. Loin depth and backfat were measured using an optical probe (Fat-O-Meter; SFK, Herlev, Denmark). Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight.

Chemical analysis

Representative samples were collected from 12 feeders per dietary treatment during each of the 5 phases. Samples were stored at -20°C until analysis. Diet samples were analyzed for dry matter (method 935.29; AOAC International, 1990), crude protein (990.03, AOAC International, 1990), calcium and phosphorus (method 985.01; AOAC International, 1990), Zn, Fe, and Cu (985.01; AOAC International, 1990) in duplicate at both Midwest Laboratories Inc. (Omaha, NE) and Cumberland Valley Analytical Services (Hagerstown, MD).

Statistical analysis

Data were analyzed as a randomized complete block design with block nested within barn as a random effect and pen as the experimental unit. Polynomial contrasts were constructed to evaluate the linear and quadratic effects of increasing Zn on ADG, ADFI, G:F, BW, daily Zn intake, and carcass characteristics. All data were modeled as normally distributed with the exception pig removals and mortality were modeled using a binomial distribution. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Chemical analysis

Results of proximate analysis and total Zn analysis followed formulated values (Tables 7.2 and 7.3). Crude protein, total Ca, and total P levels were similar across treatments within dietary phase (Tables 7.2 and 7.3). The average analyzed total Zn across all phases was 110, 115, 142, 185, and 204 mg/kg for diets formulated to 50, 87.5, 125, 162.5, and 200 mg/kg added Zn, respectively. The analyzed total Fe and Cu were similar across treatments (Tables 7.2 and 7.3) and were, on average, 150 and 151 ppm, respectively.

Growth performance and carcass characteristics

From d 0 to 42, pigs fed diets with increasing added Zn had decreased (linear, $P = 0.043$; Table 7.4) ADFI. There was a tendency for reduced ADG (linear, $P = 0.092$) and d 42 BW (linear, $P = 0.078$) as added dietary Zn increased. From d 42 to 113, increasing added Zn resulted in a quadratic response ($P = 0.042$) for ADFI, with ADFI decreasing for pigs fed 87.5 mg/kg of Zn and then returning to control intake values as Zn increased. As a result, there was a tendency (linear, $P = 0.056$) for improved G:F with increasing added Zn. For overall growth performance (d 0 to 113), there were tendencies for quadratic responses for ADFI ($P = 0.073$) and G:F ($P = 0.059$). The lowest ADFI was observed at 87.5 mg/kg added Zn and the greatest G:F observed at 125 mg/kg added Zn. Pigs fed diets with increasing added Zn presented a linear increase in daily Zn intake ($P < 0.001$). There were no differences ($P > 0.10$) in overall ADG, final BW, HCW, backfat, loin depth, lean percentage. Pig mortality and removal were low (0.8 and 2.5%, respectively) and not influenced by dietary Zn inclusion.

Based on our results, there was no benefit to adding more than 50 mg/kg Zn for ADG and carcass characteristics, but 125 mg/kg of added Zn improved G:F. Cemin et al. (2019) evaluated 50, 100, and 150 mg/kg of added Zn from two sources (Zn sulfate and Zn hydroxychloride) for grow-finish pigs. In contrast to findings of the current study, it was observed that pigs fed diets with 100 mg/kg added Zn had a tendency for greater ADG and significantly greater HCW regardless of Zn source. Carcass yield increased linearly with increasing added Zn, and pigs fed Zn hydroxychloride had greater carcass yield and a tendency for heavier HCW than those fed Zn sulfate.

Although our experimental diets did not contain ractopamine hydrochloride, our results are in agreement with Paulk et al. (2015), who observed in one trial a tendency for a linear improvement in G:F of finishing pigs with increasing added Zn from Zn oxide from 50 to 150 mg/kg in diets with ractopamine. However, the results were inconsistent, and in a second trial Paulk et al. (2015) did not observe evidence for effects of increasing added Zn. A similar result was reported by Fry et al. (2013), where added Zn improved G:F for finishing pigs fed diets with ractopamine but results were not repeatable in subsequent trials. It is important to note that our diets did not contain ractopamine, thus these results may not be directly comparable

However, other research suggests there is little evidence for Zn effects on growth performance of grow-finish pigs. Feldpausch et al. (2016) observed no evidence for effects of 150 mg/kg added Zn from Zn oxide on growth performance and carcass characteristics of 48- to 136-kg pigs, as well as no additive effects of Zn and Cu. Holen et al. (2018) evaluated organic and inorganic Zn sources ranging from 60 to 140 mg/kg added Zn for grow-finish pigs raised under restricted floor space allowance and observed no effects on growth performance and carcass characteristics. Interestingly, Ma et al. (2012) observed no effects of removing supplemental trace minerals (Zn, Cu, Fe, and Mn) up to 6 weeks pre-slaughter on growth performance, although some carcass characteristics were negatively affected. Moreover, Gowanlock et al. (2013) found no evidence for differences between pigs fed a basal corn-soybean meal diet without supplemental Zn, Cu, Fe, and Mn or the basal diet with 50 or 100% of the NRC (2012) mineral requirement estimates.

The NRC (2012) presents Zn requirement estimates as mg/kg of diet as well as mg per day. These values range from 50 to 60 mg/kg and 90 to 139 mg per day for grow-finish pigs from 25 to 135 kg BW. In our study, pigs fed diets with 50 ppm added Zn had a daily Zn intake of 129 mg, which should meet the requirement estimated by the NRC (2012). Overall, available research suggests that there are no benefits of feeding higher levels of Zn to grow-finish pigs although in the current study G:F was improved with 125 mg/kg added Zn or 310 mg per day. It is important to note that most existing research evaluated other inorganic sources, such as Zn oxide and Zn sulfate, or organic Zn sources. It could be hypothesized that the contrasts observed between the available literature and our study were at least partially driven by differences in Zn availability in the Zn hydroxychloride used in our study. An important consideration is that excessive Zn excreted in the manure can pollute ground water by leaching or soil erosion (Hsu and Lo, 2001). As restrictions to nutrient excretion increase in the swine industry, the use of lower levels of added Zn close to the requirements may be beneficial.

In conclusion, our results suggest that supplementing grow-finish diets with greater than 50 mg/kg added Zn did not lead to improvements in ADG and carcass characteristics. However, there may be G:F benefits of supplementing up to 125 mg/kg added Zn. These results match some of the data found in the literature, but inconsistencies in response are apparent and differences in Zn source could at least partially explain the results observed in the current study. Further research is required to compare Zn hydroxychloride with other inorganic and organic Zn sources to determine if source of Zn has an impact on the response observed.

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Table 7.1. Composition of the basal diets (as-fed basis)[1]

	Feeding phase				
Ingredients, %	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Corn	50.39	55.56	58.37	61.77	78.35
Soybean meal, 47% crude protein	17.08	11.95	9.34	5.92	9.64
DDGS[2]	30.00	30.00	30.00	30.00	10.00
Calcium carbonate	1.35	1.35	1.25	1.25	1.10
Sodium chloride	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.50	0.50	0.45	0.45	0.30
DL-Methionine	0.05	0.02	---	---	0.01
L-Threonine	0.06	0.05	0.02	0.04	0.05
L-Tryptophan	0.04	0.04	0.03	0.03	0.02
Vitamin-trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Zn hydroxychloride[4]	+/-	+/-	+/-	+/-	+/-
Total	100.0	100.0	100.0	100.0	100.0
SID[5] amino acids, %					
Lysine	1.03	0.91	0.81	0.73	0.65
Isoleucine:lysine	63	62	64	63	64
Leucine:lysine	162	172	186	196	186
Methionine:lysine	30	29	29	31	30
Methionine & cystine:lysine	56	56	58	60	60
Threonine:lysine	61	61	61	63	66
Tryptophan:lysine	19	19	19	19	19
Valine:lysine	71	71	75	76	75
Net energy[6], kcal/kg	2,465	2,493	2,511	2,531	2,551
Crude protein, %	20.0	17.9	16.8	15.4	13.0
Calcium, %	0.59	0.56	0.52	0.50	0.46
STTD P[7], %	0.37	0.36	0.36	0.35	0.27

[1] Phases 1, 2, 3, 4, and 5 were fed from approximately 30 to 45 kg, 45 to 64 kg, 64 to 82 kg, 82 to 104 kg, and 104 kg to marketing, respectively.

[2] DDGS = distillers dried grains with solubles.

[3] The premix did not contain Zn and provided per kg of premix: 4,116,034 IU vitamin A; 661,387 IU vitamin D; 26,455 IU vitamin E; 1,764 mg vitamin K; 16.2 mg vitamin B12; 17,637 mg niacin; 11,759 mg pantothenic acid; 5,880 mg riboflavin; 50.7 g Fe from iron sulfate; 19 g Mn from manganese oxide; 10.8 g Cu from copper sulfate; 0.25 g I from calcium iodate; 0.2 g Se from sodium selenite; 500,000 FTU phytase.

[4] IntelliBond Z (Micronutrients, Indianapolis, IN) was added at 0.009, 0.016, 0.022, 0.029, or 0.036% to achieve 50, 87.5, 125, 167.5, or 200 mg/kg of added Zn, respectively.

[5] SID = standardized ileal digestible.

[6] Net energy values for ingredients used in diet formulation were derived from the NRC (2012).

[7] STTD P = standardized total tract digestible phosphorus.

Table 7.2. Chemical analysis of Phase 1, 2, and 3 diets (as-fed basis)[1]

	Phase 1 added Zn, mg/kg					Phase 2 added Zn, mg/kg					Phase 3 added Zn, mg/kg				
Item	50	87.5	125	162.5	200	50	87.5	125	162.5	200	50	87.5	125	162.5	200
Dry matter[2], %	87.1	87.2	87.2	87.4	87.2	86.9	86.8	86.4	86.6	86.3	86.8	86.7	86.8	86.5	86.7
Crude protein[2], %	21.5	21.2	21.3	21.5	22.3	19.6	18.9	19.3	19.7	18.9	19.4	18.6	18.2	18.5	18.7
Ca[2], %	0.62	0.69	0.64	0.66	0.65	0.56	0.46	0.61	0.59	0.57	0.47	0.56	0.54	0.48	0.59
P[2], %	0.51	0.50	0.51	0.53	0.52	0.47	0.47	0.47	0.47	0.46	0.49	0.47	0.49	0.48	0.48
Zn[3], mg/kg	99	108	146	172	198	93	114	138	177	183	173	117	134	184	198
Fe[4], mg/kg	138	145	147	184	170	133	133	185	217	160	132	120	172	135	162
Cu[4], mg/kg	146	167	163	137	139	149	166	157	155	160	143	136	143	137	135

[1] For each treatment, samples were collected from multiple feeders, blended, subsampled, ground, and analyzed.

[2] Values represent means of 2 samples from Cumberland Valley Analytical Services (Hagerstown, MD) and 2 samples from Midwest Laboratories Inc. (Omaha, NE).

[3] Values represent means of 8 samples from Cumberland Valley Analytical Services (Hagerstown, MD) and 6 samples from Midwest Laboratories Inc. (Omaha, NE).

[4] Values represent means of 2 samples from Cumberland Valley Analytical Services (Hagerstown, MD).

Table 7.3. Chemical analysis of Phase 4 and 5 diets (as-fed basis)[1]

	Phase 4 added Zn, mg/kg					Phase 5 added Zn, mg/kg				
Item	50	87.5	125	162.5	200	50	87.5	125	162.5	200
Dry matter[2], %	86.3	86.4	86.6	86.4	86.6	85.5	86.4	85.3	85.7	85.8
Crude protein[2], %	16.9	17.1	16.8	16.0	17.2	12.9	13.4	13.3	12.9	13.0
Ca[2], %	0.33	0.46	0.43	0.57	0.48	0.53	0.65	0.50	0.62	0.61
P[2], %	0.45	0.45	0.45	0.44	0.45	0.32	0.35	0.33	0.34	0.32
Zn[3], mg/kg	89	116	147	201	208	94	121	147	190	231
Fe[4], mg/kg	132	184	169	177	136	123	127	123	130	128
Cu[4], mg/kg	125	146	150	154	145	159	170	154	178	172

[1] For each treatment, samples were collected from multiple feeders, blended, subsampled, ground, and analyzed.

[2] Values represent means of 2 samples from Cumberland Valley Analytical Services (Hagerstown, MD) and 2 samples from Midwest Laboratories Inc. (Omaha, NE).

[3] Values represent means of 8 samples from Cumberland Valley Analytical Services (Hagerstown, MD) and 6 samples from Midwest Laboratories Inc. (Omaha, NE).

[4] Values represent means of 2 samples from Cumberland Valley Analytical Services (Hagerstown, MD).

Table 7.4. Effects of increasing added Zn on grow-finish pig performance and carcass characteristics[1]

	Added Zn, mg/kg					SEM	Probability, *P* <	
Item[2]	50	87.5	125	162.5	200		Linear	Quadratic
BW, kg								
d 0	30.1	30.1	30.1	30.1	30.1	0.70	0.947	0.895
d 42	68.6	67.6	67.7	68.0	67.1	1.28	0.078	0.752
d 113	132.2	129.7	130.7	131.7	130.9	2.50	0.830	0.314
d 0 to 42								
ADG, kg	0.95	0.92	0.93	0.94	0.91	0.019	0.092	0.674
ADFI, kg	2.01	1.94	1.94	1.96	1.93	0.037	0.043	0.227
G:F	0.473	0.475	0.479	0.479	0.474	0.007	0.600	0.182
d 42 to 113								
ADG, kg	0.93	0.91	0.94	0.93	0.94	0.023	0.208	0.407
ADFI, kg	2.93	2.85	2.88	2.89	2.91	0.038	0.868	0.042
G:F	0.318	0.320	0.325	0.323	0.323	0.004	0.056	0.155
d 0 to 113								
ADG, kg	0.94	0.92	0.93	0.93	0.93	0.021	0.887	0.494
ADFI, kg	2.58	2.50	2.52	2.53	2.53	0.032	0.317	0.073
G:F	0.364	0.366	0.371	0.369	0.367	0.005	0.154	0.059
Zn intake, mg/d	129	215	310	412	507	4.58	0.001	0.142
Carcass characteristics								
HCW, kg	96.7	94.8	95.3	96.4	95.8	1.80	0.852	0.140
Yield, %	73.3	73.1	72.9	73.2	73.2	0.18	0.702	0.151
Backfat[3], mm	17.5	17.5	17.2	17.6	17.2	0.62	0.536	0.947
Loin depth[3], mm	68.6	69.0	68.0	69.1	68.7	1.18	0.817	0.767
Lean[3], %	56.4	56.5	56.5	56.4	56.6	0.51	0.470	0.851

[1] A total of 2,430 pigs were used in a 113-d study with 27 pigs per pen and 18 replicates per treatment.

[2] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.

[3] Adjusted using HCW as a covariate.

Chapter 8 - Effects of zinc source and level on growth performance and carcass characteristics of finishing pigs[1,2]

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ABSTRACT: An experiment was conducted to determine the effects of added Zn source and level on growth performance and carcass characteristics of finishing pigs. A total of 1,980 pigs divided into 2 groups, (group 1: 1,008 pigs, TR4 × (Fast Large White × PIC L02) and group 2: 972 pigs, PIC 337 × 1050), initially 33.3 kg, were used in a 103 or 114-d growth trial in groups 1 and 2, respectively. Treatments were arranged in a 2 × 3 factorial with two sources of added Zn, Zn hydroxychloride (ZnHyd; IntelliBond Z, Micronutrients, Indianapolis, IN) or Zn sulfate ($ZnSO_4$), and three levels of added Zn (50, 100, or 150 mg/kg). Diets contained a vitamin-trace mineral premix without added Zn and provided 76 and 162 mg/kg Fe and Cu, respectively. All diets contained 750 FTU/kg phytase. There was a total of 14 replicates per treatment. Pens of pigs were weighed approximately every 2 weeks to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**). At the end of the experiment, pigs were transported to a packing plant to determine hot carcass weight (**HCW**), backfat depth, loin depth, and lean percentage. Overall, there was no evidence ($P > 0.10$) for interactive effects of added Zn source and level for growth performance and carcass characteristics. Pigs fed diets with increasing added Zn had a tendency ($P = 0.093$) for a quadratic response in ADG, with the greatest ADG observed at 100 mg/kg added Zn. There was a linear improvement ($P = 0.010$) in carcass yield and a quadratic response ($P = 0.045$) in HCW, with pigs fed 100 mg/kg added Zn having the highest HCW. Pigs fed diets with ZnHyd had improved ($P = 0.017$) carcass yield and a tendency ($P = 0.058$) for greater HCW compared to pigs fed $ZnSO_4$. In summary, under the commercial conditions of the study and with diets containing 750 FTU/kg phytase, there were relatively small improvements in ADG of growing-finishing pigs fed added Zn beyond 50 mg/kg. Providing higher levels of added Zn improved carcass characteristics. Zinc source did not influence growth performance, but ZnHyd improved carcass characteristics compared with $ZnSO_4$.

Key words: grow-finish, mineral, performance, zinc

INTRODUCTION

The swine industry traditionally supplements Zn in diets through inorganic sources, namely Zn oxide and Zn sulfate (**$ZnSO_4$**). The supplemental source is commonly considered the only source of added Zn due to the low availability of the mineral from feed ingredients (Miller, 1991). Recently, novel Zn sources have become available, such as Zn hydroxychloride (**ZnHyd**), an inorganic source produced through the reaction of high purity forms of the metal with water and hydrochloric acid (Leisure et al., 2014). The process results in the formation of hydroxychloride crystals that contain Zn covalently bonded to hydroxyl groups and chloride. The covalent bonds are expected to reduce reactiveness with other components of the diet and to improve bioavailability (Cao et al., 2000).

According to the NRC (2012), the dietary Zn requirement for grow-finish pigs is 50 to 60 mg/kg from 25 to 135 kg of body weight (**BW**). However, supplementing Zn above the NRC (2012) recommendations is a common practice in the United States (Flohr et al., 2016) and there may be benefits of feeding higher levels of added Zn for grow-finish pigs. In a large commercial study, Cemin et al. (2019) observed that ADG was maximized at 50 mg/kg added Zn, but there was an improvement in gain-to-feed ratio (**G:F**) of pigs fed 125 mg/kg added Zn. Similarly, Fry et al. (2013) and Paulk et al. (2015) observed improvements in G:F of finishing pigs fed increasing added Zn. However, results are inconsistent and there is a lack of grow-finish studies comparing $ZnSO_4$, a traditional Zn source, with ZnHyd. Therefore, the objective of this study was to determine the effects of Zn source and level on growth performance and carcass characteristics of grow-finish pigs housed in a commercial environment.

MATERIAL AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments.

Animals and diets

An experiment was conducted at commercial research facilities in Minnesota. The research barns were double-curtain sided, naturally ventilated, had completely slatted floors, and each pen was equipped with a stainless steel dry self-feeder and a cup waterer. Feed additions were accomplished and recorded by a computerized feeding system (FeedPro; Feedlogic Corp., Wilmar, MN).

A total of 1,980 pigs divided into 2 groups (group 1: 1,008 pigs, TR4 × (Fast Large White × PIC L02) and group 2: 972 pigs, PIC 337 × 1050), with an average initial BW of 33.3 ± 0.55 kg, were used in a 103 or 114-d growth trial in groups 1 and 2, respectively. Pens of pigs were blocked by BW and randomly assigned to 1 of 6 treatments in a randomized complete block design. Treatments were arranged in a 2 × 3 factorial with two sources of added Zn, ZnHyd (IntelliBond Z, Micronutrients, Indianapolis, IN) or $ZnSO_4$ (Agrium Advance Technology, Loveland, CO for group 1 and Prince Agri Products Inc., Quincy, IL for group 2), and three levels of added Zn (50, 100, or 150 mg/kg). A vitamin-trace mineral premix was formulated without added Zn and utilized in all diets to provide other minerals and vitamins above the NRC (2012) requirement estimates. Diets (Table 8.1) were offered in 5 phases in meal form. A single source of corn and soybean meal were used in diets, but different between groups

1 and 2. The final phase contained ractopamine hydrochloride and was fed from approximately 104 kg BW to marketing. There were 14 replicates per treatment.

Pens of pigs were weighed and feed disappearance measured approximately every 2 weeks to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and G:F. Data is presented as grower period (d 0 to 66 in group 1 and d 0 to 72 in group 2), finisher period (d 66 to 103 in group 1 and d 72 to 114 in group 2), and overall period (d 0 to 103 in group 1 and d 0 to 114 in group 2). At the end of the experimental period, final pen weights were recorded and pigs were tattooed with a pen identification number and transported to a commercial packing plant for carcass data collection. Carcass measurements included hot carcass weight (**HCW**), loin depth, backfat, and percentage lean. Percentage lean was calculated from a plant proprietary equation. Carcass yield was calculated by dividing the pen average HCW by the pen average final live weight.

Chemical analysis

Representative samples were collected from each of the 5 dietary phases. Samples were stored at -20°C until analysis. Diet samples were analyzed for dry matter (method 935.29; AOAC International, 1990), crude protein (990.03, AOAC International, 1990), calcium and phosphorus (method 985.01; AOAC International, 1990), and Zn (985.01; AOAC International, 1990) at Ward Laboratories Inc. (Kearney, NE) and Cumberland Valley Analytical Services (Hagerstown, MD).

Statistical analysis

Data were analyzed as a randomized complete block design with block as a random effect and pen as the experimental unit. Polynomial contrasts were constructed to evaluate the interactive and main effects of added Zn source and level on ADG, ADFI, G:F, BW, and carcass characteristics. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Chemical analysis

Results of proximate analysis and total Zn analysis generally matched formulated values (Tables 8.2 and 8.3). The average total analyzed Zn across phases for diets formulated with 50, 100, and 150 mg/kg added Zn from ZnHyd were 120, 174, and 218 mg/kg, respectively. For diets formulated with $ZnSO_4$, averages were 112, 149, and 198 mg/kg, respectively.

Growth performance and carcass characteristics

In the grower period, there was an interaction (quadratic, $P < 0.05$) between added Zn source and level for ADFI and BW, and a tendency (quadratic, $P = 0.099$) for an interaction for ADG (Table 8.4). Pigs fed diets with ZnHyd had greater ADFI, BW, and ADG at 100 mg/kg added Zn, whereas pigs fed diets with $ZnSO_4$ presented greater ADFI, BW, and ADG at 150 mg/kg added Zn.

In the finisher period, there was an interaction (linear, $P = 0.020$) for G:F. Pigs fed diets with ZnHyd had improved G:F when fed increasing levels of added Zn, while pigs fed $ZnSO_4$ had similar G:F at all levels. In the finisher period and overall, there was a tendency ($P < 0.10$) for a quadratic response for ADG, with the greatest ADG observed at 100 mg/kg added Zn (Table 8.5).

Regarding carcass characteristics, pigs fed diets with ZnHyd had higher ($P = 0.017$) carcass yield and a tendency ($P = 0.058$) for heavier HCW than pigs fed $ZnSO_4$. Increasing added Zn resulted in a quadratic response (*P* = 0.045) in HCW, with the highest value observed at 100 mg/kg added Zn. Moreover, there was a linear response (*P* = 0.010) for carcass yield with increasing added Zn.

Our results suggest that there is no evidence for differences in growth performance between the tested Zn sources, although HCW and carcass yield improved when pigs were fed ZnHyd. Similarly, Fry et al. (2013) observed a tendency for improved carcass yield for pigs fed diets with an organic Zn source compared to $ZnSO_4$, although results were not consistent in subsequent trials. Ma et al. (2012) found no evidence for differences in growth performance or carcass traits of pigs fed organic or inorganic trace-mineral premixes. Holen et al. (2018) tested organic and inorganic Zn sources with Zn level ranging from 60 to 140 mg/kg for grow-finish pigs raised under restricted floor space allowance and observed no evidence for effects on growth performance and carcass characteristics. Feldpausch et al. (2018) evaluated inorganic and organic Zn sources at 50 and 130 mg/kg for grow-finish pigs under heat stress conditions and observed no evidence for source or level effect on growth performance and carcass characteristics. Similarly, Patience et al. (2013) found no evidence for differences in growth and carcass characteristics between organic and inorganic Zn added at 50 mg/kg for grow-finish pigs fed different lysine to calorie ratios. Overall, it seems there is little evidence in the literature to support differences in growth performance and carcass characteristics between Zn sources; however, the vast majority of available research compared inorganic and organic Zn rather than ZnHyd, which could at least partially explain our findings.

Research results are inconsistent regarding Zn level effects on growth performance of grow-finish pigs. We observed a marginal improvement in ADG when added Zn was increased from 50 to 100 mg/kg. Conversely, Cemin et al. (2019) observed no evidence for differences in

ADG for grow-finish pigs fed 50 to 200 mg/kg added Zn from ZnHyd but an improvement in G:F as added Zn increased up to 125 mg/kg. Paulk et al. (2015) added 50 to 150 mg/kg Zn from Zn oxide to finishing diets that contained 83 mg/kg of Zn from the premix, for a total of 133 to 233 mg/kg added Zn. The authors observed a tendency for a linear improvement in G:F in one trial, but the results were not reproduced in a second experiment. Interestingly, Paulk et al. (2015) also observed a tendency for quadratic improvement in loin weight but, contrary to our findings, no evidence for differences in HCW or carcass yield. Fry et al. (2013) had a similar observation regarding the lack of repeatability of Zn effects on G:F. Feldpausch et al. (2016) evaluated the addition of 0 or 150 mg/kg Zn to diets that contained 73 mg/kg Zn from the premix, for a total of 73 to 223 mg/kg added Zn. Similar to others, the authors also found no evidence for differences on growth performance and carcass characteristics. In contrast to our findings, the majority of research available found no evidence for an improvement in ADG with increasing added Zn. In fact, some researchers showed that even completely removing (Ma et al., 2012) or decreasing (Gowanlock et al., 2013) the supplementation of the trace-mineral premix containing Zn, Cu, Fe, and Mn would not result in significant differences in growth performance of finishing pigs.

There are several factors that can influence the Zn requirements (NRC, 2012), such as added phytase and dietary Cu and Fe level. Adeola et al. (1995) evaluated the supplementation of 1,500 FTU/kg phytase in diets with 0 or 100 mg/kg added Zn and observed that Zn balance is increased when diets contain phytase. In a study with growing pigs, Bikker et al. (2012) observed that the use of 500 FTU/kg phytase increased Zn digestibility, serum Zn level, and liver Zn content. However, the improvement in Zn digestibility observed by Bikker et al. (2012) did not result in changes in growth performance. In the current study, all diets contained 750 FTU/kg phytase, thus the potential impact of phytase on Zn digestibility needs to be considered. The complex interactions between Zn, Cu, and Fe and potential competitive inhibition of transport have also been recognized (Brewer et al., 1985). Arredondo et al. (2006) showed that Cu and Zn may inhibit Fe uptake, but Zn does not seem to inhibit Cu uptake in human cells. Abdel-Mageed and Oehme (1991) found that the dietary proportions of Zn, Cu, and Fe influence the intestinal and cellular transport levels of Zn, Cu, and Fe in rats. However, the ideal proportion of these minerals in swine diets is unclear.

In summary, our results suggest that supplementing grow-finish diets with greater than 50 mg/kg added Zn may result in a modest increase in ADG for mixed-gender pigs raised in commercial conditions and fed diets containing 750 FTU/kg phytase. However, HCW and carcass yield were improved by providing higher levels of added Zn. The use of ZnHyd did not impact growth performance, but improved HCW and carcass yield compared to ZnSO4.

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Table 8.1. Composition of the basal diets (as-fed basis)

	Feeding phase[1]				
Ingredients, %	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Corn	48.07	52.12	55.69	58.30	68.99
Soybean meal, 47% crude protein	19.56	15.69	12.24	9.66	18.67
DDGS[2]	30.00	30.00	30.00	30.00	10.00
Calcium carbonate	1.35	1.35	1.25	1.25	0.95
Monocalcium phosphate, 21.5% P	0.15	---	---	---	0.30
Sodium chloride	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.35	0.33	0.30	0.28	0.35
Methionine hydroxy-analog	---	---	---	---	0.10
L-Threonine	---	---	---	---	0.09
L-Tryptophan	0.01	0.01	0.01	0.01	0.02
Vitamin-trace mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Ractopamine HCl	---	---	---	---	0.03
Zn source[4]	+/-	+/-	+/-	+/-	+/-
Total	100.0	100.0	100.0	100.0	100.0
SID[5] amino acids, %					
Lysine	1.03	0.91	0.81	0.72	0.94
Isoleucine:lysine	70	72	74	77	63
Leucine:lysine	179	192	207	222	153
Methionine:lysine	32	34	37	39	37
Methionine & cystine:lysine	62	66	70	75	63
Threonine:lysine	61	63	66	68	64
Tryptophan:lysine	18.9	19.0	18.7	18.6	18.9
Valine:lysine	82	86	90	94	72
Net energy, kcal/kg	2,421	2,447	2,469	2,485	2,491
Crude protein, %	22.1	20.5	19.2	18.1	17.9
Calcium, %	0.66	0.62	0.57	0.56	0.52
STTD P[6], %	0.39	0.35	0.34	0.33	0.34

[1] Phases 1, 2, 3, 4, and 5 were fed from approximately 33 to 45 kg, 45 to 64 kg, 64 to 82 kg, 82 to 104 kg, and 104 kg to marketing, respectively.

[2] DDGS = distillers dried grains with solubles.

[3] The premix did not contain Zn and provided per kg of premix: 4,116,034 IU vitamin A; 661,387 IU vitamin D; 26,455 IU vitamin E; 1,764 mg vitamin K; 16.2 mg vitamin B12; 17,637 mg niacin; 11,759 mg pantothenic acid; 5,880 mg riboflavin; 50.7 g Fe from iron sulfate; 19 g Mn from manganese oxide; 10.8 g Cu from copper sulfate; 0.25 g I from calcium iodate; 0.2 g Se from sodium selenite; 500,000 FTU phytase.

[4] Zn hydroxychloride (IntelliBond Z, Micronutrients, Indianapolis, IN) or Zn sulfate (Agrium Advance Technology, Loveland, CO for group 1 and Prince Agri Products Inc., Quincy, IL for group 2) were included in the diets at 50, 100, or 150 mg/kg added Zn to form the dietary treatments.

[5] SID = standardized ileal digestible.

[6] STTD P = standardized total tract digestible phosphorus.

Table 8.2. Chemical analysis of phase 1, 2, and 3 diets (as-fed basis)[1]

	Phase 1						Phase 2						Phase 3					
Zn source[2]	ZnHyd			$ZnSO_4$			ZnHyd			$ZnSO_4$			ZnHyd			$ZnSO_4$		
Zn level, mg/kg	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
Dry matter, %[3]	88.5	88.5	88.7	88.2	88.1	88.5	87.9	87.9	88.0	88.0	87.9	87.7	88.1	88.1	88.2	87.9	88.0	88.8
Crude protein, %[3]	22.2	21.8	20.7	20.5	20.7	22.0	20.0	20.9	21.0	20.7	21.7	21.8	19.3	19.6	19.7	19.6	19.3	20.5
Calcium, %[3]	0.80	0.92	0.95	0.84	0.89	0.97	0.84	0.89	0.83	0.87	0.83	0.86	0.87	0.67	0.76	0.92	0.73	0.89
Phosphorus, %[3]	0.59	0.63	0.62	0.61	0.58	0.62	0.56	0.57	0.60	0.57	0.60	0.58	0.55	0.57	0.54	0.54	0.53	0.55
Zinc, mg/kg[4]	113	176	196	132	137	197	115	157	193	111	171	229	169	165	266	107	156	193
Iron[4], mg/kg	225	139	158	139	150	148	153	146	154	147	148	154	155	120	148	140	118	151
Copper[4], mg/kg	306	187	209	176	202	234	232	229	190	238	219	222	213	187	207	201	138	194

[1] For each treatment, samples were collected from multiple feeders, blended, subsampled, ground, and analyzed (Ward Laboratories Inc., Kearny, NE and Cumberland Valley Analytical Services, Hagerstown, MD).

[2] Zinc sources were Zn hydroxychloride (ZnHyd; IntelliBond Z, Micronutrients, Indianapolis, IN) or Zn sulfate ($ZnSO_4$; Agrium Advance Technology, Loveland, CO for group 1 and Prince Agri Products Inc., Quincy, IL for group 2).

[3] Values represent means of one analysis from group 1 and one analysis from group 2.

[4] Values represent means from three analysis from group 1 and five analysis from group 2.

Table 8.3. Chemical analysis of phase 4 and 5 diets (as-fed basis)[1]

	Phase 4						Phase 5					
Zn source[2]	ZnHyd			$ZnSO_4$			ZnHyd			$ZnSO_4$		
Zn level, mg/kg	50	100	150	50	100	150	50	100	150	50	100	150
Dry matter, %[3]	88.1	87.6	88.2	87.9	88.4	88.3	87.4	87.3	87.0	87.0	87.2	87.4
Crude protein, %[3]	19.3	19.0	19.8	18.2	18.9	18.2	19.1	17.6	17.1	17.7	19.1	18.7
Calcium, %[3]	0.56	0.78	0.59	0.63	0.75	0.68	0.67	0.71	0.69	0.66	0.70	0.68
Phosphorus, %[3]	0.54	0.55	0.53	0.55	0.56	0.55	0.50	0.49	0.50	0.50	0.50	0.51
Zinc, mg/kg[4]	122	187	206	117	141	174	84	187	228	95	139	198
Iron[4], mg/kg	127	140	129	122	133	112	137	153	139	147	144	194
Copper[4], mg/kg	205	231	195	221	224	174	208	221	210	179	211	243

[1] For each treatment, samples were collected from multiple feeders, blended, subsampled, ground, and analyzed (Ward Laboratories Inc., Kearny, NE and Cumberland Valley Analytical Services, Hagerstown, MD).

[2] Zinc sources were Zn hydroxychloride (ZnHyd; IntelliBond Z, Micronutrients, Indianapolis, IN) or Zn sulfate ($ZnSO_4$; Agrium Advance Technology, Loveland, CO for group 1 and Prince Agri Products Inc., Quincy, IL for group 2).

[3] Values represent means of one analysis from group 1 and one analysis from group 2.

[4] Values represent means from three analysis from group 1 and five analysis from group 2.

Table 8.4. Interactive effects of added Zn source and level on growth performance and carcass characteristics of grow-finish pigs[1,2]

	ZnHyd, mg/kg			ZnSO$_4$, mg/kg				Probability, *P* <			
								Source × level			
Item[3]	50	100	150	50	100	150	SEM	Linear	Quadratic	Source	Level
BW, kg											
d 0	33.3	33.3	33.3	33.3	33.3	33.3	0.55	0.934	0.931	0.967	0.998
Grower[4]	94.5	95.4	94.0	94.4	94.5	95.3	0.58	0.106	0.044	0.748	0.477
Finisher[5]	130.5	131.4	129.6	130.2	130.5	130.3	1.34	0.559	0.471	0.788	0.527
Grower											
ADG, kg	0.89	0.90	0.88	0.89	0.89	0.90	0.017	0.130	0.099	0.677	0.490
ADFI, kg	2.13	2.17	2.11	2.11	2.12	2.16	0.056	0.079	0.038	0.688	0.359
G:F, g/kg	418	415	420	421	421	418	3.979	0.310	0.246	0.348	0.852
Finisher											
ADG, kg	0.97	0.98	0.95	0.95	0.98	0.96	0.015	0.422	0.963	0.505	0.229
ADFI, kg	2.83	2.88	2.90	2.80	2.87	2.81	0.044	0.385	0.540	0.181	0.304
G:F, g/kg	344	341	330	340	340	341	4.477	0.020	0.408	0.420	0.115
Overall											
ADG, kg	0.92	0.93	0.91	0.91	0.92	0.92	0.012	0.158	0.351	0.859	0.241
ADFI, kg	2.36	2.41	2.38	2.35	2.37	2.38	0.047	0.673	0.425	0.348	0.254
G:F, g/kg	389	385	383	388	388	387	3.487	0.362	0.899	0.245	0.398
Carcass characteristics											
HCW, kg	95.5	97.3	95.7	94.0	95.6	95.6	0.759	0.322	0.486	0.058	0.061
Carcass yield, %	73.2	74.0	73.9	72.2	73.3	73.4	0.414	0.487	0.983	0.017	0.013
Backfat depth[6], mm	16.3	16.1	15.9	16.0	16.1	16.3	0.560	0.253	0.936	0.904	1.000
Lean[6], %	55.8	55.9	56.0	55.5	55.9	55.9	0.678	0.761	0.591	0.510	0.389
Loin depth[6], mm	66.1	66.1	66.4	64.8	66.5	66.4	0.986	0.273	0.296	0.554	0.222

[1] A total of 1,980 pigs (initial BW = 33.3 kg) were used in two groups with 21 to 27 pigs per pen and 14 replicates per treatment.

[2] Zn sources were Zn hydroxychloride (ZnHyd; IntelliBond Z, Micronutrients, Indianapolis, IN) or Zn sulfate (ZnSO$_4$; Agrium Advance Technology, Loveland, CO for group 1 and Prince Agri Products Inc., Quincy, IL for group 2)

[3] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.

[4] Grower period was from d 0 to 66 in group 1 and from d 0 to 72 in group 2.

[5] Finisher period was from d 66 to 103 in group 1 and from d 72 to 114 in group 2.

[6] Adjusted using HCW as covariate.

Table 8.5. Main effects of added Zn source and level on growth performance and carcass characteristics of grow-finish pigs[1,2]

Item[3]	Source		SEM	Probability, $P <$	Level, mg/kg			SEM	Probability, $P <$	
	ZnHyd	$ZnSO_4$			50	100	150		Linear	Quadratic
BW, kg										
d 0	33.3	33.3	0.55	0.967	33.3	33.3	33.3	0.55	0.987	0.946
Grower[4]	94.6	94.7	0.58	0.748	94.4	94.9	94.6	0.58	0.655	0.259
Finisher[5]	130.5	130.3	1.34	0.788	130.4	130.9	129.9	1.34	0.626	0.309
Grower										
ADG, kg	0.89	0.89	0.016	0.677	0.89	0.90	0.89	0.016	0.516	0.317
ADFI, kg	2.14	2.13	0.054	0.688	2.12	2.15	2.14	0.054	0.404	0.246
G:F, g/kg	418	420	3.355	0.348	420	418	419	3.521	0.829	0.602
Finisher										
ADG, kg	0.97	0.96	0.015	0.505	0.96	0.98	0.96	0.015	0.643	0.099
ADFI, kg	2.87	2.82	0.044	0.181	2.81	2.87	2.85	0.044	0.342	0.223
G:F, g/kg	338	341	4.477	0.420	342	341	336	4.477	0.050	0.501
Overall										
ADG, kg	0.92	0.92	0.012	0.859	0.91	0.92	0.91	0.012	0.940	0.093
ADFI, kg	2.38	2.37	0.047	0.348	2.35	2.39	2.38	0.047	0.321	0.185
G:F, g/kg	386	388	3.487	0.245	388	387	385	3.487	0.178	0.891
Carcass characteristics										
HCW, kg	96.2	95.1	0.759	0.058	94.8	96.4	95.7	0.762	0.198	0.045
Carcass yield, %	73.7	73.0	0.414	0.017	72.7	73.7	73.7	0.415	0.010	0.135
Backfat depth[6], mm	16.1	16.2	0.560	0.904	16.1	16.1	16.1	0.560	0.993	0.998
Lean[6], %	55.9	55.8	0.678	0.510	55.6	55.9	56.0	0.678	0.186	0.682
Loin depth[6], mm	66.2	65.9	0.986	0.554	65.4	66.3	66.4	0.986	0.109	0.473

[1] A total of 1,980 pigs (initial BW = 33.3 kg) were used in two groups with 21 to 27 pigs per pen and 14 replicates per treatment.

[2] Zn sources were Zn hydroxychloride (ZnHyd; IntelliBond Z, Micronutrients, Indianapolis, IN) or Zn sulfate ($ZnSO_4$; Agrium Advance Technology, Loveland, CO for group 1 and Prince Agri Products Inc., Quincy, IL for group 2)

[3] BW = body weight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.

[4] Grower period was from d 0 to 66 in group 1 and from d 0 to 72 in group 2.

[5] Finisher period was from d 66 to 103 in group 1 and from d 72 to 114 in group 2.

[6] Adjusted using HCW as covariate.