Update on Amino Acids in High Fiber Diets: Threonine and Branch Chained Amino Acids

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Summary

Using co-products from the grain processing industries has become more common in swine diets to take advantage of less feed costs. However, most of these co-products contain high dietary fiber which can affect nutrient utilization by pigs. In particular, there are questions of how dietary fiber affects amino acid utilization in pigs. In addition to dietary fiber, co-products from corn and sorghum have high leucine concentrations. If large amounts of corn or sorghum co-products are used in swine diets, pigs will have excess dietary leucine which may result in reduced feed intake and growth performance. Effects of dietary fiber on threonine requirement has been determined, and the effects of elevated dietary concentrations of leucine on metabolism of isoleucine, valine, and tryptophan has also been reported. Results of these experiments have indicated that increased fiber levels in diets increase the requirement for threonine, and excess dietary leucine in diets reduce growth performance, protein retention, and serotonin synthesis for growing pigs. Increased dietary tryptophan levels in diets alleviate negative impact of excess dietary leucine on growth performance and serotonin synthesis for growing pigs.

Introduction

Co-products from corn- or wheat processing are widely used in diet formulation for swine to reduce feed costs. These co-products typically contain a larger proportion of dietary fiber, which can affect nutrient utilization by pigs (Urriola et al, 2013). An increase in dietary fiber will increase endogenous losses of nutrients including amino acids (**AA**; Cervantes-Pahm et al., 2014), particularly Thr, because endogenous protein that is lost from the small intestine is rich in mucin, which contains high levels of Thr (de Lange et al., 1989; Stein et al., 1999). Thus, high dietary fiber concentration that is introduced by using grain co-products may increase the endogenous losses of Thr, which may increase the requirement for Thr in the diet (Zhu et al., 2005; Mathai et al., 2016).

Leucine, Val, and Ile are categorized as the branched-chain AA (**BCAA**) because of the structural similarity of their side chains (Harper et al., 1984). All 3 BCAA share the enzymes that are involved in the first 2 steps of their catabolic pathway (Wiltafsky et al., 2010). Among the BCAA, Leu has been considered a key regulator that stimulates catabolism of all 3 BCAA in the liver (Harper et al., 1984). In general, co-products from corn and sorghum have high leucine concentrations compared with other ingredients (NRC, 2012). Thus, it is more likely that diets have excess leucine if large amounts of corn or sorghum co-products are used. When excess Leu in diets is offered to pigs, degradation of all 3 BCAA may increase by stimulating effects of Leu or its metabolite (α-keto isocaproate) on BCAA catabolizing enzymes (Wiltafsky et al., 2010). Excess dietary leucine may also reduce pig feed intake and growth performance (Gatnau et al., 1995; Wiltafsky et al., 2010) because of reduced synthesis of serotonin in the brain. Excess Leu may prevent Trp, which is the precursor for serotonin, from being transported from blood to brain, and therefore reduce the availability of Trp for serotonin synthesis (Henry et al., 1992). Serotonin is a cerebral neurotransmitter that plays an important role in feed intake regulation (Le Floc'h and Sève, 2007). As a consequence, feeding high-fiber diets may change requirements for a number of indispensable AA and it is the objective of the current contribution to summarize current knowledge about the requirement for Thr, Trp, and BCAA in high fiber diets fed to growing pigs.

Effects of Dietary Fiber on the Thr:Lys Ratio in Diets for Growing Pigs

Effects of dietary fiber on Thr:Lys ratio were determined (Mathai et al, 2016) by using a low-fiber basal diet with approximately 0.40% SID Thr and 0.90% SID Lys. Five additional diets were formulated by adding crystalline L-Thr to the basal diet in increments of 0.08% to create diets containing approximately from 0.49 to 0.81% SID Thr. A high-fiber basal diet was also formulated by adding 15% soybean hulls to the low-fiber basal diet at the expense of corn starch and 5 additional diets were formulated by adding crystalline Thr to this diet. The 12 diets were fed for 28 days to pigs that were 26.29 ± 4.64 kg at the start of the experiment with 2 pigs per pen and 8 replicate pens per treatment.

Results indicated that ADG and G:F increased (linear and quadratic, $P < 0.05$) as the Thr:Lys ratio increased in both low and high fiber diets (Table 1). There were no effects of Thr level on ADFI among low-fiber diets, but ADFI increased (linear, $P < 0.05$) as Thr concentration increased in high-fiber diets. Regression analysis estimated the ideal SID Thr:Lys ratio at 0.66 and 0.63 for ADG and G:F, respectively, for pigs fed low-fiber diets and at 0.71 and 0.63, respectively, for pigs fed high-fiber diets (Figure 1). The estimated requirement for the ideal Thr:Lys ratio for optimizing ADG was greater for pigs fed the high-fiber diets (0.71) than for the pigs fed the low-fiber diets (0.66). This increase in the estimated requirement indicates that dietary fiber may increase the requirement for Thr in growing pigs. The reason for this observation is that fiber may have negative effects on energy, lipid, and N digestibility (Urriola et al., 2013; Cervantes-Pahm et al., 2014). Dietary fiber may also result in a greater requirement of Thr in animals fed high-fiber diets because of increased endogenous losses and increased microbial activity in the hindgut (Zhu et al., 2005).

A follow-up experiment was conducted to determine the N balance in pigs fed low-fiber or high-fiber diets that were formulated to have SID Thr:Lys ratios of 45:100 or 60:100. Thirty-six growing pigs with initial body weight of 29.0 ± 0.74 kg were housed in metabolism crates that were equipped with a slatted floor, a feeder, and a nipple drinker. Pigs were allotted to 4 diets with 9 replicate pigs per diet using a randomized complete block design. All pigs were fed 810g of feed twice daily, which was believed to be approximately 90% of ad libitum feed intake for pigs. Urine and fecal samples were collected for 5 d following a 7-d adaptation period.

Results confirmed that retention of N was greater ($P < 0.05$) for pigs fed the low-fiber diets compared with pigs fed the high-fiber diets regardless of the Thr:Lys ratio (Table 2). This indicates that dietary fiber may af-

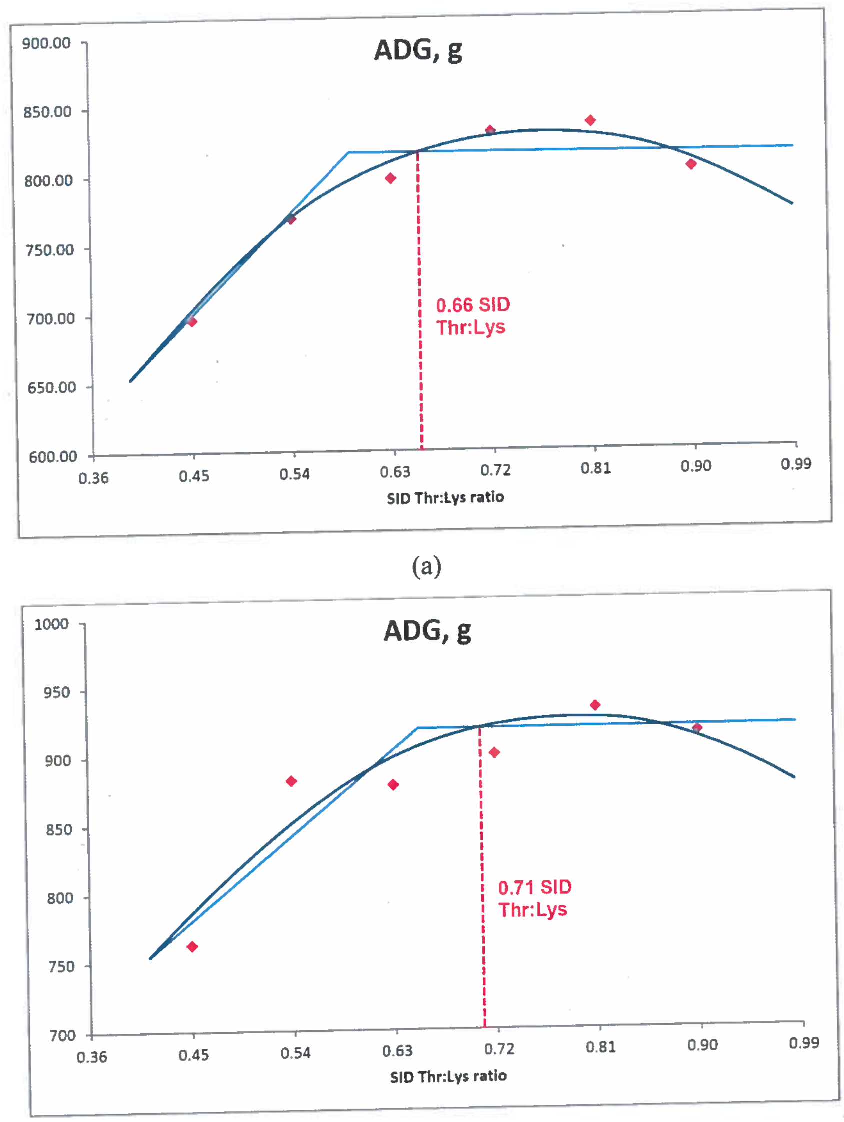

Figure 1. Fitted linear breakpoint and quadratic plots of average daily gain (ADG) as a function of standardized ileal digestible (SID) Thr to Lys ratio with observed treatment means in pigs fed low fiber diets (a) and high fiber diets (b; from Mathai et al., 2016).

fect the flow and retention of N in the pig (Urriola et al., 2013), because inclusion of fiber in the diet increases total N output and simultaneously decreases urinary N excretion by shifting N excretion towards fecal excretion. Results also confirmed that N retention was greater ($P < 0.05$) for pigs fed the high-Thr diets compared with pigs fed the low-Thr diets regardless of inclusion of dietary fiber. This indicates that the high-Thr diets was closer to the requirement for optimal protein accretion than the low-Thr diets in 25- to 50-kg growing pigs. However, the difference in N retention between the high-Thr diets indicates that pigs on the high-fiber, high-Thr diets were not receiving enough Thr to meet the requirement of the pigs.

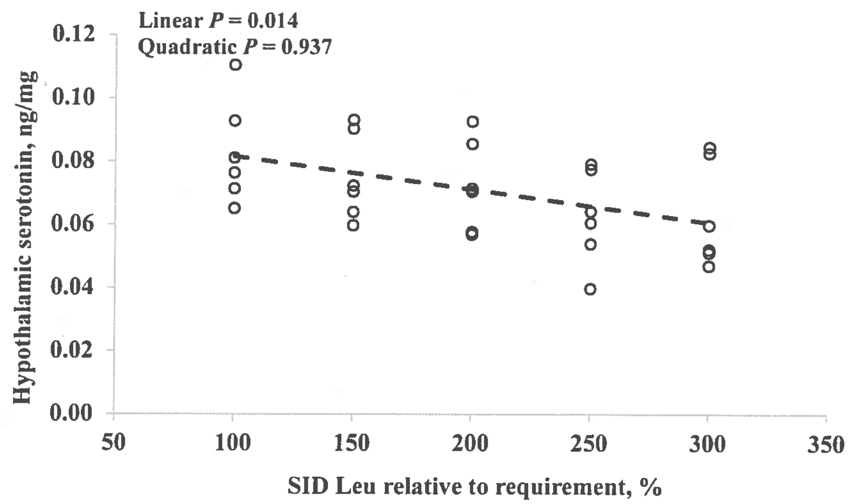

Figure 2. Hypothalamic serotonin of growing pigs (N = 30; n = 6) fed diets with increasing concentrations of standardized ileal digestible (SID) Leu relative to the requirement (NRC, 2012; from Kwon et al., 2019b).

Branched-chain Amino Acid Interactions in Diets Fed to Growing Pigs

Two experiments were conducted to test the hypothesis that elevated dietary concentrations of Leu impacts metabolism of Ile, Val, and Trp (Kwon et al., 2019a, b). Five experimental diets based on identical quantities of corn, soybean meal, wheat, and barley and formulated to contain 100, 150, 200, 250, or 300% of the requirement for SID Leu were used in Exp. 1. Forty pigs with initial body weight of 30.0 ± 2.7 kg were housed individually in metabolism crates and allotted to the 5 dietary treatments (8 replicates per treatment).

Results indicated that excess dietary Leu reduced (linear, $P < 0.05$) ADG, ADFI, and G:F (Table 3). Reduced growth performance is most likely due to reduced feed intake caused by excess dietary Leu because excess dietary Leu may generate imbalanced supply of BCAA for protein synthesis that resulted from degradation of BCAA (Wiltafsky et al., 2010). Increased (linear, $P < 0.05$) plasma urea N as dietary Leu increased may be a result of the reduced availability of Val and Ile and further indicates that excess Leu creates AA imbalance (Table 4). In addition, decreased (linear, $P < 0.05$) N retention and biological value of protein in diets that were observed as dietary Leu increased is indicative of the reduced utilization of dietary N for protein deposition (Gatnau et al., 1995). Pigs can detect BCAA imbalances in a diet and they will avoid eating that diet, which indicates that there is an innate mechanism against imbalanced supply of indispensable AA in the diet (Gloaguen et al., 2012). Results also indicates that excess dietary Leu reduced (linear, $P < 0.05$) serotonin concentration in the hypothalamus (Figure 2). Serotonin is important for appetite regulation and is synthesized from Trp in the brain, and excess dietary Leu may hinder uptake of Trp in the brain (Henry et al., 1992). Thus, the decreased serotonin concentration in hypothalamus that was observed as dietary Leu increased, indicates that excess dietary Leu may reduce Trp uptake into the brain, resulting in decreased serotonin synthesis in the hypothalamus. This may have contributed to the reduced feed intake observed for pigs fed diets with excess Leu.

A follow-up experiment was conducted to test the hypothesis that increased dietary Trp is needed in diets containing excess dietary Leu to prevent a drop in hypothalamic serotonin concentrations and to maintain growth performance of animals (Kwon et al., 2019a). A basal diet based on corn, soybean meal, wheat, and barley was formulated to contain 100% of the requirement for SID Leu (NRC, 2012). Two additional diets were formulated by adding crystalline L-Leu to the basal diet to increase the concentration of SID Leu to 200 or 300% of the requirement. These 3 diets were formulated to have a SID Trp:Lys ratio of 18%. Six additional diets were formulated by adding either 0.05% or 0.10% crystalline L-Trp to each of the 3 original diets. Thus, there was a total of 9 diets that were arranged in a 3 × 3 factorial with 3 levels of Leu (100, 200, or 300% of the SID requirement) and 3 levels of SID Trp (18, 23, or 28% SID Trp:Lys). The 9 diets were fed for 21 days to pigs that were 28.2 ± 1.9 kg at the start of experiment with 2 pigs per pen and 8 replicate pens per treatment. Individual pig weights were recorded at the conclusion of the experiment and on the last day of the experiment, one pigs per pen was sacrificed and blood and hypothalamus samples were collected to measure plasma-free AA and serotonin concentration, respectively.

Results confirmed the negative effects of excess Leu in the diets and indicated that both ADG and ADFI is reduced as dietary Leu increases, whereas dietary Trp had no impact on ADG and ADFI (Table 5). However, there were no consistent impacts of dietary Trp or Leu on G:F in this experiment. The reduced model for prediction of ADG and ADFI indicated that the ADG and ADFI were positively affected by increased dietary Trp, but negatively affected by increased dietary Leu. However, the ADG and ADFI were positively affected by the interaction between dietary Trp and Leu. (Figure 3). This indicates that the negative effect of excess Leu may partially be ameliorated by increasing dietary Trp (Cemin et al., 2019). However, the observation that both ADG and ADFI were maximized at the lowest Leu concentration indicates that excess Trp cannot completely overcome the negative effects of excess Leu. Results also confirmed that excess dietary Leu reduces Ile and Val concentration and increase Leu concentration in plasma, whereas dietary Trp had no impact on concentration of the 3 BCAA in plasma. However, excess dietary Trp increases Trp concentration in plasma, whereas excess dietary Leu had no effect on Trp concentration in plasma. Reduced serotonin concentration in the hypothalamus that was observed as dietary Leu increased confirms the importance of Trp as a precursor for serotonin, but there was no significant effect of excess dietary Trp on hypothalamic serotonin. The reduced model for prediction of serotonin in the hypothalamus indicated that the hypothalamic serotonin was positively affected by increased dietary Trp, but negatively affected by increased dietary Leu. However, hypothalamic serotonin was negatively affected by the interaction between dietary Trp and Leu. (Figure 4). This is likely because excess Leu reduces Trp uptake in the brain due to competition for the shared L-type AA transporter from blood to brain (Le Floc'h and Sève, 2007).

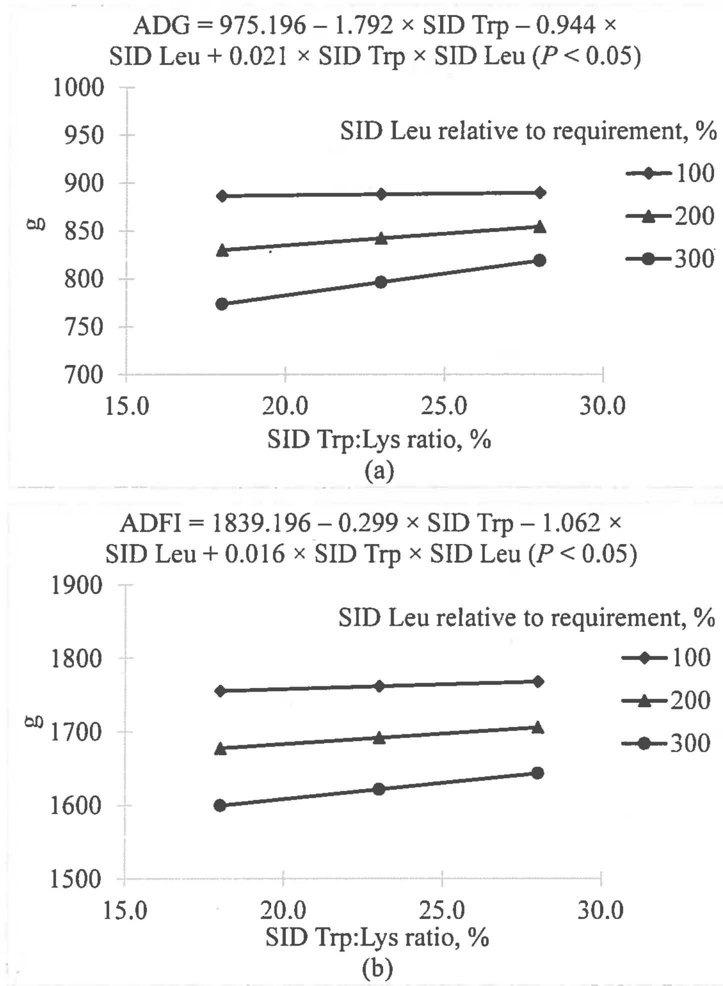

Figure 3. Predicted values, based on the interaction between SID Trp and SID Leu ($P < 0.05$), for (a) average daily gain (ADG) and (b) average daily feed intake (ADFI) in growing pigs fed diets containing from 18 to 28% standardized ieal digestible (SID) Trp:Lys and from 100 to 300% SID Leu relative to the requirement (NRC, 2012; from Kwon et al., 2019a).

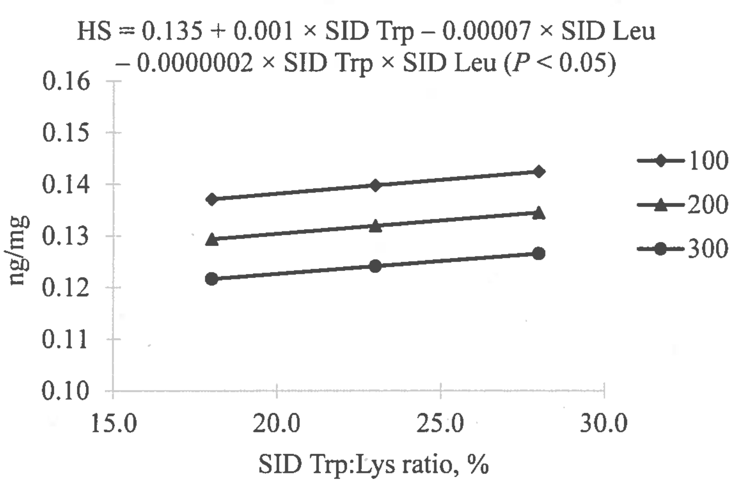

Figure 4. Predicted values, based on the interaction between SID Trp and SID Leu ($P < 0.05$), for hypothalamic serotonin (HS) concentrations in growing pigs fed diets containing from 18 to 28% standardized ieal digestible (SID) Trp:Lys and from 100 to 300% SID Leu relative to the requirement (NRC, 2012; from Kwon et al., 2019a).

Table 1. Growth performance of AA supplemented diets with low or high concentration of fiber[1,2,3]

Item	Standardized ileal digestible Thr:Lys ratio						SEM	*P*-value	
	0.45	0.54	0.63	0.72	0.81	0.90		Linear	Quadratic
Low fiber									
ADG, g	696	769	797	830	836	803	29	< 0.01	< 0.05
ADFI, g	1,785	1,799	1,777	1,812	1,862	1,830	102	0.376	0.917
G:F, g/g	0.38	0.42	0.45	0.46	0.45	0.44	0.02	0.001	< 0.001
High fiber									
ADG, g	763	882	878	900	933	763	35	< 0.001	< 0.05
ADFI, g	1,828	1,872	1,835	1,864	1,945	1,828	65	< 0.05	0.409
G:F, g/g	0.42	0.46	0.47	0.47	0.47	0.46	0.01	< 0.01	< 0.01

[1] Data from Mathai et al., 2016.
[2] Data are means of 8 observations per treatment.
[3] Values for ADG and G:F were greater ($P < 0.05$) for the high-fiber diets than for the low-fiber diets, but for ADFI, no differences between low- and high-fiber diets were observed.

Table 2. Nitrogen balance of pigs fed diets with major deficiency or marginal deficiency of Thr and with low or high concentrations of fiber[1,2]

Fiber level:	Low fiber		High fiber		Pooled	*P*-value		
SID[3] Thr:Lys ratio:	0.45	0.60	0.45	0.60	SEM	Fiber	Thr	Fiber × Thr
N intake, g/5 d	182	185	162	171	4.8	< 0.05	0.17	0.48
N output in feces, g/5 d	38[bc]	35.2[c]	41[b]	47[a]	2.6	< 0.05	0.3	< 0.05
N output in urine, g/5 d	29	23	26	15	2.1	< 0.05	< 0.05	0.22
ATTD[4] of N, %	80.1	81.6	75.6	73.2	1.5	< 0.05	0.69	0.06
N retention, g/5 d	119	131	99	113	3.6	< 0.05	< 0.05	0.85
N retention, %	64.5	69.8	59.5	64.9	2.1	< 0.05	< 0.05	0.99

[a–c] Means within a row lacking a common superscript letter differ ($P < 0.05$).
[1] Data are means of 9 observations per treatment, except for the treatment with high fiber and the 0.60 SID Thr:Lys ratio, which had only 7 observations.
[2] Data from Mathai et al., 2016.
[3] SID = standardized ileal digestible.
[4] ATTD = apparent total tract digestibility.

Conclusions

Results of Thr experiments indicate that increased fiber levels in diets to growing pigs increase the requirement for Thr. For 25- to 50-kg growing pigs, the ideal Thr:Lys ratio is 0.71 to optimize ADG, but if low-fiber diets are fed, the ideal Thr:Lys ratio is 0.66 to optimize ADG. Results also indicate that dietary fiber affects the flow and retention of N resulting in increased total N output and simultaneously decreased urinary N excretion by shifting N- excretion towards fecal excretion in pigs. The increase in N retention in pigs fed the high-Thr diets indicates that those diets provided for the pigs that were closer to the requirement of the animals than the low-Thr diets. However, the difference in N retention between the high-Thr diets indicates that a higher fiber diet may require a greater inclusion level of Thr relative to Lys. Results of Leu experiments indicate that excess dietary Leu reduces growth performance of pigs, which is most likely due to reduced ADFI, lack of free Val and Ile as substrates for protein synthesis, and consequently reduced protein synthesis as dietary SID Leu increased. Excess dietary Leu also reduces serotonin synthesis in the hypothalamus, which may have contributed to the reduced ADFI observed for pigs fed diets with excess Leu. Results also indicate that increased dietary Trp levels alleviate negative impact of excess dietary Leu on growth performance and serotonin synthesis in growing pigs.

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Table 3. Growth performance of pigs fed diets with graded levels of standardized ileal digestible (SID) Leu relative to requirement[1,2,3]

	SID Leu relative to requirement, %						*P*-value	
Item	100	150	200	250	300	SEM	Linear	Quadratic
ADG, g	698	645	673	593	559	47	< 0.001	0.522
ADFI, g	1,416	1,409	1,411	1,360	1,278	31	< 0.001	0.050
G:F, g/g	0.50	0.46	0.48	0.44	0.44	0.03	0.023	0.835

1 Data from Kwon et al., 2019b.
2 The requirement for Leu was from NRC (2012).
3 Each least squares mean represents 8 observations.

Table 4. Plasma urea N and N balance of growing pigs fed diets with graded levels of standardized ileal digestible (SID) Leu relative to requirement[1] during a 5-d collection period[1,2,3]

	SID Leu relative to requirement, %						*P*-value	
Item	100	150	200	250	300	SEM	Linear	Quadratic
Plasma urea N, µg/mL	5.63	6.25	6.88	6.63	7.38	0.60	0.047	0.779
N balance								
N intake, g/5 d	165	165	163	163	159	5.9	0.187	0.729
N output in feces, g/5 d	29	29	27	29	26	1.7	0.151	0.732
N output in urine, g/5 d	28	30	30	30	31	2.5	0.235	0.528
ATTD[4] of N, %	82.4	82.7	83.3	82.1	83.7	0.7	0.315	0.776
N retention, g/5 d	108	106	106	103	102	3	0.082	0.994
N retention, %	65.4	64.3	64.9	63.6	64.3	1.3	0.136	0.447
Biological value[5], %	79.4	77.7	77.8	77.5	76.8	1.4	0.021	0.579

1 Data from Kwon et al., 2019b.
2 The requirement for Leu was from NRC (2012).
3 Each least squares mean represents 8 observations.
4 ATTD = apparent total tract digestibility.
5 Biological value was calculated as [N retained/(N intake – N output in feces)] × 100 (Rojas and Stein, 2013).

Table 5. Least squares means for growth performance of growing pigs fed diets with varying ratios between dietary standardized ieal digestible (SID) Leu and SID Trp[1,2]

SID Leu relative to requirement[1], %:	100			200			300			
SID Trp:Lys , %:	18	23	28	18	23	28	18	23	28	SEM
ADG, g[3]	867	898	852	845	869	905	750	815	777	61
ADFI, g[4]	1,675	1,724	1,630	1,657	1,656	1,720	1,519	1,584	1,506	95
G:F[5]	0.52	0.52	0.52	0.51	0.52	0.53	0.49	0.51	0.51	0.02

1 Data from Kwon et al., 2019a.
2 The requirement for Leu was from NRC (2012).
3 Results indicated that ADG from d 0 to d 21 at different combinations of SID Trp and SID Leu could be described by the following model: 975.196 – 1.792 × SID Trp – 0.944 × SID Leu + 0.021 × SID Trp × SID Leu ($P < 0.05$).
4 Results indicated that ADFI from d 0 to d 21 at different combinations of SID Trp and SID Leu could be described by the following model: 1839.196 – 0.299 × SID Trp – 1.062 × SID Leu + 0.016 × SID Trp × SID Leu ($P < 0.05$).
5 Results indicated that G:F could not be predicted from dietary SID Trp or SID Leu.

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