Evaluation of Branched-Chain Amino Acids Interactions in 10 to 20kg Nursery Pigs Using a Central Composite Design

Abstract

Two groups of 240 pigs (PIC 337 × 1050, PIC Genus, Hendersonville, TN) were used to investigate the interactions between leucine (Leu), isoleucine (Ile), and valine (Val) on the growth performance of approximately 10 to 20kg nursery pigs. Day 21 post-weaning, pens were randomly assigned to one of 15 dietary treatments in a central composite design.
Diets were formulated with varying levels of standardized ileal digestible (SID) branched-chain amino acids (BCAAs) by using L-Leu, L-Ile, and L-Val in place of corn starch. Levels BCAAs ratios to SID Lys ranged from 98 to 180% (Leu), 46 to 64% (Ile), and 51 to 78% (Val). Diets were formulated to be iso-Lysine, isonitrogenous, and isocaloric. Vitamin and mineral levels met or exceeded NRC (2012) recommendations. Pig weights and feed intake were measured for the 21-day experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Growth performance data were analyzed using the lm() function in R version 4.2.2 (R Core Team, 2022). The model included the linear and quadratic effects of Leu, Ile, and Val, their three two-way interactions, and initial body weight (BW). Pen was the experimental unit, and parameters were considered significant at $P \leq 0.10$. A linear and quadratic effect of Val (51 to 78% of SID Lys) was observed for ADG and G:F ($P < 0.001$).

There was an interaction between Leu and Ile for ADG ($P = 0.069$) and G:F ($P = 0.032$), where increasing Leu and decreasing Ile, and the inverse, improved ADG and G:F. However, growth and efficiency were negatively impacted as Leu and Ile increased in the diet.

There was no evidence of an effect of Ile increment on ADFI.
However, there was an interaction between Leu and Val (P = 0.060), where Leu negatively impacted feed intake at low levels of Val but had little impact as Val increased above NRC (2012) recommendations.
Overall Val positively impacts ADG and G:F, regardless of Leu and Ile levels in the diet. , However, ADG and G:F are reduced with high levels of Leu and Ile, which is ellivated as either Leu or Ile are reduced.
Furthermore, ADFI is negatively impacted by increased Leu when Val is below NRC (2012) recommendations but is not affected by higher Leu when feeding higher Val levels.

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Introduction

The branched-chain amino acids (BCAAs) are a group of essential amino acids that includes leucine, isoleucine, and valine. Aside from their requirement for protein synthesis, BCAAs serve regulatory roles by stimulating protein synthesis through activating the mammalian target for rapamycin (mTOR) pathway (Nie et al., 2018). The first two steps in the catabolic pathway of BCAAs degradation are the same for Leu, Ile, and Val, which is a reversible transamination by branched-chain aminotransferase (BCAT) and irreversible decarboxylation by the branched-chain α-keto acid dehydrogenase complex (BCKD) (Brosnan and Brosnan, 2006). Therefore, excess consumption of one BCAA will result in the catabolism of all three BCAAs, resulting in the BCAAs antagonism observed across various species (D'Mello, 2003). The α-keto acid of Leu degradation, α-ketoisocaproate, is the most potent allosteric inhibitor of the BCKD kinase, which regulates BCKD activity (Brosnan and Brosnan, 2006). Therefore, excess leucine consumption may elicit an antagonistic response more easily than high levels of Ile or Val.

Common ingredients included used in swine diets have wide variation are variable in their BCAAs concentration and balance. For example, soybean meal contains a SID Leu:Ile:Val of 1.0:0.61:0.63, while dried distillers grain with solubles (< 4% oil) contains a SID Leu:Ile:Val of 1.0:0.25:0.33 (NRC; 2012). There has been extensive work evaluating the Val and Ile requirements of nursery pigs (Mavromichalis et al., 2001; Theil et al., 2004; Barea et al., 2009a; Barea et al., 2009b; Gloaguen et al., 2011; Gloaguen et al., 2013; Clark et al., 2017). However, there has been limited work evaluating interactions between BCAA in nursery pigs, which may have important implications when interpreting results of trials evaluating the requirements of BCAA separately. Gloaguen et al. (2011) investigated the SID Val:Lys requirement of 11 to 20 kg nursery pigs in combination with high Leu (SID Leu:Lys 165%) and found an average SID Val:Lys requirement of 72%, which was similar to the requirement determined by Barea et al. (2009a) in pigs fed diets with a moderate level of Leu (SID Leu:Lys 110%). Furthermore, BCAA levels are often manipulated through changing ingredient profiles of the diet, which confounds the response to BCAA with other amino acids and nutrients, such as fiber, and may contribute to the variability in observed responses. The current NRC (2012) SID BCAA recommendations for 11 to 25 kg pigs are 100%, 51%, and 63% for Leu:Lys, Ile:Lys, and Val:Lys, respectively; however, these relationships may not hold in diets containing unbalance levels of BCAA. Therefore, a paucity of data remains to fully elucidate the complex relationships between BCAA in nursery pigs. Thus, this trial aimed to investigate the interactions of leucine, isoleucine, and valine and their impacts on the growth performance of approximately 10 to 20 kg nursery pigs.

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Methods

All experimental protocols adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC 22-074). Pigs were housed in pens equipped with one two-space feeder and two nipple waterers, ensuring ad libitum access to feed and water for the duration of the study.

General design

The experiment was conducted at the Iowa State University Swine Nutrition Farm (Ames, IA). Two groups of 240 pigs (PIC 337 1050, PIC Genus, Hendersonville, TN) newly weaned pigs were placed into 40 pens with three barrows and three gilts per and fed a common diet for three weeks.

The pigs were porcine reproductive and respiratory virus and Mycoplasma hyopneumoniae stable and influenza A virus, porcine epidemic diarrhea virus, and deltacoronavirus negative.

On day 21-post weaning, pens were randomly assigned to one of 15 dietary treatments arranged in a rotatable central composite design (Table 1).

Pigs and feeders were weighed on days 0 and 21 of the trial to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Experimental design

Animal

Pigs

(PIC 337 × 1050, PIC Genus, Hendersonville, TN)

Periods

3 weeks

Virus management

No reproductive and respiratory virus and Mycoplasma hyopneumoniae stable and influenza A virus, porcine epidemic diarrhea virus, and deltacoronavirus negative.

Experimental diets and measurements

Experimental diets were formulated to various levels of SID Leu, Ile and Val by replacing corn starch and/or L-glycine . In the base diet (Table 2). BCAAs in the diets were expressed as ratios to SID lysine and ranged from 98 to 180% for Leu, 46 to 64% for Ile, and 51 to 78% for Val (Table 1). The experimental diets were also supplemented with L-lysine HCl, L-threonine, DL-methionine, L-tyrosine, L-histidine HCl, L-tryptophan, and L-phenylalanine. In order to evaluate BCAAs levels relative to lysine. Diets were formulated to be limiting in SID lysine (1.19% SID Lys) and exceed NRC (2012) recommendations for all other essential amino acids (Boisen, 2003). Additionally, diets were formulated to be isocaloric (3.40 Mcal/kg), isonitrogenous (17.38% crude protein; CP), and vitamin, and mineral levels to metmeet or exceeded NRC (2012) recommendations. Diets were manufactured at the Iowa State Swine Nutrition Farm feed mill. Feed samples for each experimental diet were collected after mixing and stored at -20°C.

Table 1. List of dietary treatments in central composite design

Treatment	n	Code[1]	Leucine[2]	Isoleucine	Valine
1	4	-1 -1 -1	115	50	56
2	4	1 -1 -1	163	50	56
3	4	-1 1 -1	115	60	56
4	4	1 1 -1	163	60	56
5	4	-1 -1 1	115	50	73
6	4	1 -1 1	163	50	73
7	4	-1 1 1	115	60	73
8	4	1 1 1	163	60	73
9	4	-α 0 0	98	55	65
10	4	α 0 0	180	55	65
11	4	0 -α 0	139	46	65
12	4	0 α 0	139	64	65
13	4	0 0 -α	139	55	51
14	4	0 0 α	139	55	78
15	24	0 0 0	139	55	65

[1]Coded levels represent standardized values of SID BCAA:Lys; α = 1.682 for rotatable design
[2]Dietary treatments expressed as ratios to standardized ileal digestible (SID) lysine, which was set at 1.19%

Statistical analysis

Growth performance data were analyzed according to the following linear model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \sum \beta_{ij} X_i X_j + \varepsilon$$

Where Y is the response variable (ADG, ADFI, G:F), X_i represents the input variables (SID Leu:Lys, SID Ile: Lys, SID Val:Lys, initial BW), β_0 is the intercept term, β_i is the estimated coefficients of the linear parameters, β_{ii} is the estimated coefficients of the quadratic parameters, β_{ij} is the estimated coefficients of the interaction parameters, and ε is the random error associated with Y, assuming $\varepsilon \sim N(0, I\sigma_e^2)$.

The model was fit using the lm() function in R version 4.2.2 (R Core Team, 2022). Pen was the experimental unit, and model parameters were considered significant at $P \leq 0.10$.

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Experimental diets and measurements

Table 2. Ingredient and nutrient composition of experimental diets

Ingredients (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Corn	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26	67.26
Soybean meal 47.5 CP	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43	23.43
Soybean oil	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34	3.34
Monocalcium phosphate	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Calcium carbonate	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
L-lysine	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-threonine	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
DL- methionine	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
L-tyrosine	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Trace mineral premix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-histidine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-tryptophan	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
L-phenylalanine	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Phytase[3]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Corn starch	0.41	0.16	0.34	0.09	0.34	0.08	0.27	0.02	0.43	—	0.27	0.16	0.27	0.15	0.21
L-glycine	0.55	0.21	0.48	0.14	0.43	0.09	0.35	0.02	0.57	—	0.35	0.22	0.39	0.18	0.28
L-leucine	0.20	0.79	0.20	0.79	0.20	0.79	0.20	0.79		0.99	0.50	0.50	0.50	0.50	0.50
L-isoleucine	0.05	0.05	0.19	0.19	0.05	0.05	0.19	0.19	0.12	0.12	—	0.24	0.12	0.12	0.12
L-valine	0.07	0.07	0.07	0.07	0.27	0.27	0.27	0.27	0.17	0.17	0.17	0.17	—	0.33	0.17

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Table 2. Ingredient and nutrient composition of experimental diets

Calculated composition	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ME, Mcal/kg	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40	3.40
Crude protein, %	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38	17.38
Total calcium, %	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Available phosphorus, %	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
SID Lys, %	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19	1.19
SID Ile, %	0.59	0.59	0.72	0.72	0.59	0.59	0.72	0.72	0.66	0.66	0.55	0.76	0.66	0.66	0.66
SID Leu, %	1.36	1.94	1.36	1.94	1.36	1.94	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
SID Val, %	0.67	0.67	0.67	0.67	0.86	0.86	0.86	0.86	0.77	0.77	0.77	0.77	0.61	0.93	0.77
SID Arg:Lys	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81	0.81
SID His:Lys	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
SID Ile:Lys	0.50	0.50	0.60	0.60	0.50	0.50	0.60	0.60	0.55	0.55	0.46	0.64	0.55	0.55	0.55
SID Leu:Lys	1.15	1.63	1.15	1.63	1.15	1.63	1.15	1.63	0.98	1.80	1.39	1.39	1.39	1.39	1.39
SID Met + Cys:Lys	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
SID Phe:Lys	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
SID Tyr:Lys	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
SID Thr:Lys	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
SID Trp:Lys	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
SID Val:Lys	0.56	0.56	0.56	0.56	0.73	0.73	0.73	0.73	0.65	0.65	0.65	0.65	0.51	0.78	0.65

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Table 2. Ingredient and nutrient composition of experimental diets

Analyzed composition	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dry matter (%)	87.45	87.14	87.14	87.32	87.22	87.63	87.24	87.44	87.33	87.39	87.32	87.42	87.46	87.55	87.61
Gross energy (Mcal/kg)															
Crude protein (%)	17.61	16.27	16.60	17.25	17.08	18.13	17.75	17.58	17.40	17.27	17.57	17.52	17.14	16.74	16.89
Total Lys (%)	1.32	1.34	1.32	1.32	1.28	1.49	1.30	1.27	1.41	1.31	1.33	1.42	1.38	1.41	1.34
Total Arg (%)	0.96	0.93	0.98	1.00	0.95	1.00	0.95	0.97	1.01	0.97	1.01	0.97	1.01	0.99	0.97
Total His (%)	0.48	0.47	0.47	0.53	0.56	0.51	0.49	0.47	0.49	0.53	0.51	0.47	0.50	0.49	0.51
Total Ile (%)	0.73	0.71	0.87	0.91	0.73	0.77	0.83	0.85	0.84	0.78	0.72	0.89	0.83	0.81	0.80
Total Leu (%)	1.59	2.13	1.60	2.17	1.58	2.15	1.59	2.12	1.48	2.33	1.90	1.85	1.90	1.88	1.84
Total Met + Cys (%)	0.84	0.82	0.82	0.84	1.02	0.91	0.85	0.81	0.83	0.83	0.89	0.80	0.85	0.82	0.79
Total Phe (%)	0.86	0.84	0.85	0.88	0.84	0.90	0.84	0.86	0.89	0.84	0.87	0.85	0.89	0.85	0.84
Total Tyr (%)	0.74	0.72	0.75	0.76	0.73	0.76	0.72	0.74	0.76	0.75	0.75	0.74	0.77	0.75	0.73
Total Thr (%)	0.97	0.97	1.01	1.15	1.10	1.09	1.02	1.06	0.93	0.98	0.94	0.98	1.04	1.09	0.94
Total Trp (%)	0.27	0.27	0.27	0.26	0.27	0.26	0.27	0.26	0.26	0.27	0.27	0.26	0.26	0.27	0.26
Total Val (%)	0.82	0.79	0.79	0.83	0.98	1.01	0.99	0.97	0.94	0.89	0.91	0.89	0.77	1.06	0.88

[1]Provided 12 mg Cu (copper sulfate), 0.28 mg I (potassium iodate), 160 mg Fe (ferrous sulfate), 0.30 mg Se (sodium selenate), and 160 mg Zn (zinc sulfate) per kg of the diet
[2]Provided 4,594 IU vitamin A, 525 IU vitamin D, 37.5 IU vitamin E, 2.25 mg vitamin K, 8.25 mg riboflavin, 42 mg niacin, 20.25 mg pantothenic acid, 0.04 mg vitamin B12 per kg of the diet
[3]Phytase activity 450 FTU/kg

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Results

Initial average BW was 13.2 ± 3.05 kg and ending weight on day 21 was 23.4 ± 3.77 kg. The tests for lack of fit were not significant for any of the fitted models ($P \geq 0.167$; Table 4), indicating that the second-order polynomial equations adequately described the response surfaces. The corresponding regression equations for final BW, ADG, ADFI, and G:F are presented in Table 3. Increasing valine linearly and quadratically positively impacted ADG, G:F, and, consequently, ending with BW improvement, regardless of Leu and Ile levels ($P \leq 0.001$; Table 4). There was an interaction between Leu and Ile for ADG ($P = 0.069$), G:F ($P = 0.032$), and ending BW ($P = 0.047$) increasing levels of Leu and decreasing Ile improved ADG (Fig. 2), G:F (Fig. 3), and final BW (Fig. 1). Conversely, ADG, G:F, and final BW were decreased as both dietary SID Leu and Ile were increased. Isoleucine did not impact ADFI ($P \geq 0.391$; Table 3); however, there was an interaction between Leu and Val ($P = 0.060$), where Leu negatively impacted ADFI at low SID levels of Val but did not influence ADFI at higher levels of SID Val (Figure 4).

Table 3. Regression equations for nursery pig ADG, ADFI, and G:F

Response variable	Regression equation[1]
Final BW, kg	$= -49.200 + (1.433\times10^{-2} \times \text{Leu}) + (5.584\times10^{-1} \times \text{Ile}) + (1.213 \times \text{Val}) + (2.082\times10^{-4} \times \text{Leu}^2) - (3.860\times10\text{-}3 \times \text{Ile}^2) - (1.086\times10^{-2} \times \text{Val}^2) - (2.951\times10^{-3} \times \text{Leu} \times \text{Ile}) + (1.126\times10^{-3} \times \text{Leu} \times \text{Val}) + (3.638\times10^{-3} \times \text{Ile} \times \text{Val}) + (1.102 \times \text{initial BW (kg)})$
ADG, kg	$= -2.441 - (7.901\times10^{-4} \times \text{Leu}) + (3.008\times10^{-2} \times \text{Ile}) + (6.058\times10^{-2} \times \text{Val}) + (9.632\times10^{-6} \times \text{Leu}^2) - (2.043\times10^{-4} \times \text{Ile}^2) - (5.285\times10^{-4} \times \text{Val}^2) - (1.248\times10^{-4} \times \text{Leu} \times \text{Ile}) + (6.322\times10^{-5} \times \text{Leu} \times \text{Val}) + (1.275\times10^{-4} \times \text{Ile} \times \text{Val}) + (4.880\times10^{-3} \times \text{initial BW (kg)})$
ADFI, kg	$= -2.124 - (5.136\times10^{-3} \times \text{Leu}) + (2.811\times10^{-2} \times \text{Ile}) + (6.277\times10^{-2} \times \text{Val}) + (6.017\times10^{-6} \times \text{Leu}^2) - (2.578\times10^{-4} \times \text{Ile}^2) - (6.102\times10^{-4} \times \text{Val}^2) - (9.660\times10^{-5} \times \text{Leu} \times \text{Ile}) + (1.155\times10^{-4} \times \text{Leu} \times \text{Val}) + (1.895\times10^{-4} \times \text{Ile} \times \text{Val}) + (1.998\times10^{-2} \times \text{initial BW(kg)})$
G:F	$= -0.6675 + (2.714\times10^{-3} \times \text{Leu}) + (8.069\times10^{-3} \times \text{Ile}) + (3.123\times10^{-2} \times \text{Val}) + (7.975\times10^{-6} \times \text{Leu}^2) + (6.795\times10^{-6} \times \text{Ile}^2) - (2.266\times10^{-4} \times \text{Val}^2) - (8.274\times10^{-5} \times \text{Leu} \times \text{Ile}) - (7.040\times10^{-6} \times \text{Leu} \times \text{Val}) + (2.268\times10^{-5} \times \text{Ile} \times \text{Val}) - (1.038\times10^{-2} \times \text{initial BW(kg)})$

[1]Leucine, isoleucine, and valine levels expressed as SID ratios to lysine .

Table 4. Predicted performance of nursery pigs fed various levels of leucine isoleucine, and valine

	Dietary treatment[1]			Predicted mean response			
	Leu	Ile	Val	Final BW, kg	ADG, kg	ADFI, kg	G:F
1	115	50	56	22.49	0.44	0.72	
2	163	50	56	21.97	0.41	0.63	0.64
3	115	60	56	22.50	0.45	0.71	0.62
4	163	60	56	20.44	0.35	0.58	0.60
5	115	50	73	20.45	0.54	0.83	0.65
6	163	50	73	24.81	0.55	0.83	0.67
7	115	60	73	25.07	0.56	0.85	0.67
8	163	60	73	23.90	0.51	0.81	0.64
9	98	55	65	24.96	0.56	0.85	0.66
10	180	55	65	23.53	0.49	0.74	0.66
11	139	46	65	23.96	0.51	0.78	0.66
12	139	64	65	23.20	0.48	0.75	0.64
13	139	55	51	19.34	0.30	0.53	0.58
14	139	55	78	24.19	0.52	0.82	0.64
15	139	55	65	23.89	0.51	0.79	0.65
SE center point[2]				0.219	0.010	0.014	0.006
SE factorial points				0.440	0.020	0.027	0.011
SE axial points				0.219	0.020	0.026	0.011
Adjusted R^2				0.92	0.64	0.70	0.64
P-values							
	Linear						
	Leu			0.005	0.002	0.001	0.637
	Ile			0.129	0.233	0.391	0.008
	Val			<0.001	<0.001	<0.001	<0.001
	Quadratic						
	Leu × Leu			0.386	0.385	0.685	0.201
	Ile × Ile			0.438	0.375	0.404	<0.001
	Val × Val			<0.001	<0.001	<0.001	0.958
	Cross product			0.11	0.11	0.11	0.11
	Leu × Ile			0.047	0.069	0.289	0.032
	Leu × Val			0.251	0.164	0.060	0.781
	Ile × Val			0.414	0.536	0.493	0.844
	Initial BW			<0.001	<0.001	<0.001	<0.001
	Lack of fit			0.436	0.317	0.363	0.167

[1]Dietary treatments expressed as ratios to standardized ileal digestible (SID) lysine, which was set at 1.19%
[2]SE: standard errors of center point means (treatment 15), standard errors of factorial point means (treatments 1 – 8), and standard errors of axial point means (treatments 9 – 14)

Evaluation of Branched-Chain Amino Acids Interactions in 10 to 20kg Nursery Pigs Using a Central Composite Design

Results

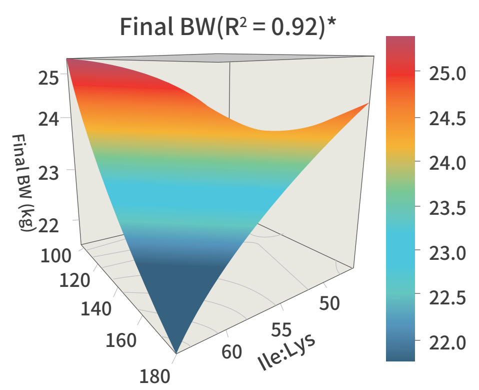

Figure 1. Response surface plot of SID Leu:Lys and Ile:Lys for final BW

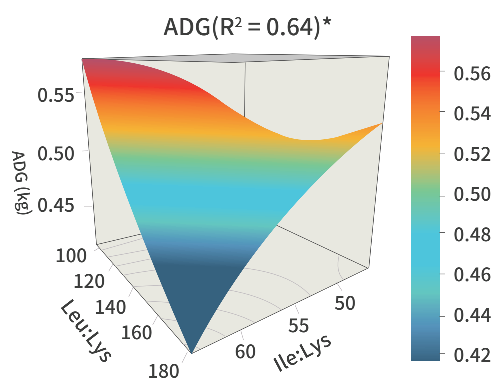

Figure 2. Response surface plot of SID Leu:Lys and Ile:Lys for ADG

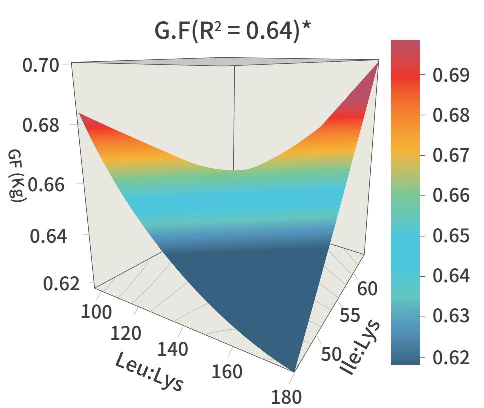

Figure 3. Response surface plot of SID Leu:Lys and Ile:Lys for G:F.

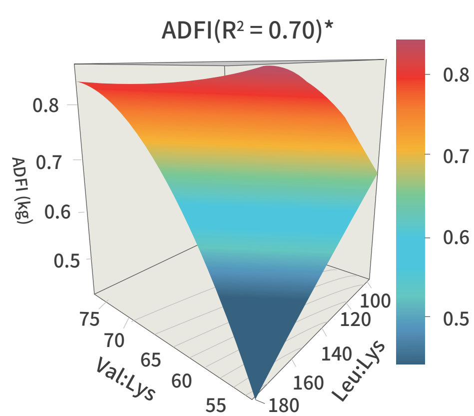

Figure 4. Response surface plot of SID Leu:Lys and Val:Lys for ADFI

*Valine was held at the midpoint (SID Val:Lys 64%) and starting BW at 13.2 kg
ADG : Average Daily Gain, G.F : Feed Effeciency, ADFI : Average Daily Feedintake

Discussion

This experiment utilized a central composite design to evaluate the relationships between Leu, Ile, and Val and their impact on the growth performance of 13 to 20 kg nursery pigs. This design allows for the estimation of linear, quadratic, and interaction terms without a full-factorial design. In this case, a three-factor central composite design reduced the number of dietary treatments required by a 5 × 5 × 5 full factorial design from 125 to 15. Previous work in poultry has successfully utilized the central composite design and similar response surface designs to evaluate the interactive effects of amino acids; however, to the authors' knowledge, this design had not been evaluated for use in swine nutrition research (Farran and Thomas, 1989; Ospina-Rojas et al., 2020; Kidd et al., 2021; Maynard et al., 2021; Chrystal et al., 2022). Based on the results of the tests for lack of fit, the second-order polynomial models fit with this design adequately described the growth response of nursery pigs fed varying levels of BCAA.

In the present study, ADFI was negatively impacted by increasing Leu in the diet and showed a linear and quadratic response to dietary Val increment. however, there was an interaction between Val and Leu, which indicated the negative impact of Leu was only present at low levels of Val. The contour plot of ADFI indicated that maximum ADFI could be achieved at a SID Val:Lys between approximately 65 and 75%, while ADFI was reduced at levels below 65%.
Results of the present study correspond to the observations of Gloaguen et al. (2011), where increasing SID Leu:Lys from 111 to 165% in diets with a SID Val:Lys of 60% resulted in an 11.2% reduction in feed intake, with no differences reported with increased Leu in diets with a SID Val:Lys of 70%. Similarly, the model of ADFI generated in this study would predict an 11.8% reduction in ADFI with the same SID Leu and Val ratios to Lys. In a subsequent study, Gloaguen et al. (2013) fed nursery pigs increasing levels of SID Leu:Lys from 80 to 130% with Ile and Val levels above NRC (2012) requirements and saw an improvement in growth when SID Leu:Lys was increased from 80 to 90%, but no differences as Leu was increased further.

In contrast, Wiltafsky et al. (2010) evaluated the impact of excess Leu in two trials, with marginal levels of either Ile or Val and reported a linear decrease in nursery pig ADG and ADFI as Leu increased. In both trials, growth was improved in the high Leu diets by adding Ile or Val in the diets that were marginal in Ile or Val. Together, these results indicate that, within practical diets, high Leu does not negatively impact feed intake when Val and Ile levels are adequate .
The branched-chain amino acids are transported across the blood-brain barrier through the L-system amino acid transporters, which are shared by other large neutral amino acids (LNAA), such as Met, His, Trp, Phe, and Tyr (Pardridge, 1977).
Of the LNAA, the aromatic amino acids, tryptophan, tyrosine, and phenylalanine, are the precursors of serotonin, dopamine, and norepinephrine, respectively (Fernstrom, 1994). Therefore, competitive inhibition of LNAA transport in the brain and subsequent alterations in neurotransmitter biosynthesis play a role in the feed intake response observed with BCAA imbalance. However we do not suspect this is the case in the present study, as all essential amino acids except

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Discussion

Lys and BCAA were provided at 5 to 10% above requirements. Consequently, it is unlikely that the ADFI response to Leu and Val was due to competition with the LNAA.

In rats, injection of L-Leu into the brain resulted in mTOR activation and decreased feed intake, indicating that excessive mTOR activation may result in signals to reduce feed intake (Cota et al., 2006). Additionally, excess Leu supplementation has been shown to reduce Ile and Val plasma levels (Wessels et al., 2016; Morales et al., 2016; Duan et al., 2016; Kwon et al., 2019). Therefore, the reductions in ADFI observed in the present study with increased Leu at low levels of Val may be due to a combination of anorexigenic signals from mTOR activation and aggravated Val deficiency through increased BCAA catabolism with high Leu.

In the current experiment, there was an interaction between Leu and Ile for ADG and G:F. Where performance was reduced as levels of both amino acids (Leu and Ile) were increased in the diet, but were ADG and G:F were improved when either dietary Leu or Ile was decreased. A similar relationship was observed in poultry with Ile and Val, where BW gain and feed conversion was the poorest when Ile and Val were both at the highest or lowest levels evaluated (Maynard et al., 2021). The differences between swine and poultry are not clear but may be due to species-specific requirements for each BCAA. Little work has been conducted in swine evaluating the growth response to varying levels of Ile and Leu. Barea et al. (2009b) utilized a 2 × 2 factorial design to evaluate the effect of SID Ile:Lys level and protein source on the growth performance of nursery pigs (initial BW 11 kg). The two protein sources tested in the experiment were spray-dried blood cells and corn gluten meal, which resulted in slight differences in SID Leu:Lys and large differences in Val:Lys between diets. In this trial, Ile, protein source, or their interaction had no impact on growth performance. From these results, the authors concluded that the SID Ile:Lys requirement is not greater than 50% in diets with moderate levels of Val and Leu. However, the range in SID Leu levels evaluated by Barea et al. (2009b) may not have been large enough to elicit the negative response in ADG and G:F with increased Ile observed in the current trial.
Other researchers have supplemented Val and Ile in high Leu diets and observed positive responses in growth performance (Morales et al., 2016; Stas et al., 2022). In contrast, the results of the current experiment would indicate there is a negative relationship between Leu and Ile levels in the diet, where increasing Ile inclusion in high Leu diets would reduce performance. Our current findings suggest the positive performance responses are response to Val, which may outweigh the negative response of increasing Ile in those diets. Therefore, further research is warranted to validate the Leu and Ile interaction observed in the present study.

Conclusion

In conclusion, the response of nursery pig ADG and G:F to Val is linear and quadratic, regardless of Leu and Ile levels, while ADG and G:F are reduced at high levels of Leu and Ile, which is restored as either Leu or Ile levels are reduced. Furthermore, at low levels of Val, ADFI is negatively impacted by increased Leu.
however, the negative impacts of increasing Leu on ADFI are negated when Val is at or above the NRC (2012) requirement.
Additionally, similar growth performance can be achieved at various Leu, Ile, and Val levels.
Therefore, optimal levels of BCAA will ultimately depend on ingredient cost and which growth metric is being optimized. This experiment emphasizes the complexity of amino acid metabolism in nursery pigs and the importance of understanding potential interactions among amino acids when conducting studies to determine amino acid requirements.