Journal of Animal Science, 2021, Vol. 99, No. 1, 1–9

doi:10.1093/jas/skab006
Advance Access publication January 11, 2021
Received: 18 September 2020 and **Accepted:** 8 January 2021
Non Ruminant Nutrition

NON RUMINANT NUTRITION

Improving performance of finishing pigs with added valine, isoleucine, and tryptophan: validating a meta-analysis model

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Abstract

Based on results of a recent meta-analysis, we hypothesized that increased dietary Val, Ile, or Trp could correct possible amino acid interactions because of excess Leu in diets containing high levels of corn protein, namely dried distiller's grains with solubles (DDGS). A total of 1,200 pigs (PIC TR4 × (Fast LW × PIC L02); initially 33.6 ± 0.6 kg) were used in a 103-d study. The 6 dietary treatments were corn–soybean meal (SBM)-DDGS-based as follows: (1) high SBM and low level of L-Lys HCl (HSBM), (2) high L-Lys HCl and moderate Ile, Val, Trp (AA above NRC 2012 estimates; NC), (3) moderate L-Lys HCl and high Ile, Val, and Trp (PC), and PC with either increased (4) L-Val (PC+Val), (5) L-Ile (PC+Ile), or (6) L-Trp (PC+Trp). Pigs fed the NC diet were predicted to have the poorest average daily gain (ADG), the PC diet to be intermediate, and pigs fed the HSBM, PC+Val, PC+Ile, and PC+Trp have the same and highest predicted ADG. In the grower period (34 to 90 kg), ADG was greater ($P < 0.05$) for the pigs fed HSBM and PC+Val diets than the NC with pigs fed other diets intermediate. Pigs fed HSBM were more ($P < 0.05$) efficient (G:F) than the NC and PC with pigs fed other diets intermediate. In the finisher period (90 to 136 kg), ADG was greater ($P < 0.05$) for pigs fed PC+Ile than that of the NC with pigs fed other diets intermediate. Pigs fed PC+Val had greater ($P < 0.05$) average daily feed intake (ADFI) than the NC with pigs fed other diets intermediate. However, PC+Ile pigs were more ($P < 0.05$) efficient than PC+Val with pigs fed other diets intermediate. Overall, ADG was greater ($P < 0.05$) for pigs fed HSBM, PC+Val, and PC+Ile diets than the NC with pigs fed other diets intermediate. Pigs fed the PC+Val diet had greater ($P < 0.05$) ADFI than the NC with pigs fed other diets intermediate. No differences were detected between treatments for overall G:F or other carcass characteristics. In conclusion, increasing Val or Ile in high L-Lys-HCl-DDGS-based diets improved growth performance compared with pigs fed diets containing high levels of L-Lys HCl without added Val and Ile. These results present evidence that the recently developed meta-analysis can predict the relative differences in overall ADG for pigs fed the NC, PC, PC+Val, and PC+Ile diets; however, the predicted G:F was less accurate. The data demonstrate that the negative effects of high Leu concentrations in corn-DDGS-based diets can be reversed by increasing the ratios of Val and Ile relative to Lys.

Key words: branch chain amino acids, finishing pig, isoleucine, leucine, tryptophan, valine

Abbreviations

AA	amino acids
ADFI	average daily feed intake
ADG	average daily gain
BCAA	branched-chain amino acids
BCAT	branch-chain amino acid transferase
BCKD	branched-chain α-keto acid dehydrogenase complex
BW	body weight
DDGS	dried distillers grains with solubles
G:F	feed efficiency
HCW	hot carcass weight
LNAA	large neutral amino acids
mTOR	mammalian target rapamycin receptors
SBM	soybean meal
SID	standardized ileal digestible

Introduction

Branched-chain amino acids (BCAA) are a collective group of structurally similar amino acids and are comprised of isoleucine (Ile), leucine (Leu), and valine (Val); all of which also share the same first steps in catabolism (Harris et al., 2005). Excess of any one of the BCAA leads to an increase in catabolism of all the BCAA, with Leu being the most potent stimulator of the muscle-containing enzyme, branched-chain amino acid transferase (BCAT), which is responsible for the first step of BCAA catabolism (Harper et al., 1984). This becomes increasingly important in diets containing high amounts of corn protein, as Leu is disproportionally higher than Val and Ile in corn by-products (NRC, 2012). Large neutral amino acids (LNAA), such as tryptophan (Trp), also share the same brain transporters as BCAA (Pardridge, 1977; Fernstrom, 2013). Tryptophan is a precursor for the neurotransmitter serotonin, which is involved in feed intake regulation (Henry et al., 1992; Fernstrom, 2013). In turn, an excess of BCAA may affect the transport of Trp into the brain and lead to a decrease in serotonin activity and, thus, feed intake.

Although not consistent, diets containing high levels of Leu have been shown to have negative effects on pig growth performance (Wiltafsky et al., 2010; Millet et al., 2015; Kwon et al., 2019a). The decrease in growth performance has been hypothesized as a result of an imbalance in BCAA. Based on an extensive literature review, Cemin et al. (2019) developed a growth prediction model, suggesting that high Leu:Lys negatively impacts growth performance due to insufficient levels of other BCAA and LNAA relative to Leu. However, the addition of different combinations of Ile, Val, and/or Trp can reverse the decrease in growth performance. If this model is accurate, it will create a platform for further advancements in diet formulation, which will allow nutritionists to create more nutritionally balanced diets. Therefore, our hypothesis was that dietary additions of Val, Ile, or Trp can ameliorate the poor performance of pigs fed diets containing high concentrations of Leu, which in turn would also validate the model developed by Cemin et al. (2019).

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at a commercial research facility owned and operated by New Fashion Pork (Jackson, MN). The 2 barns were tunnel-ventilated with completely slatted concrete flooring and deep pits for manure storage. Each pen (2.4 × 5.8 m) was equipped with adjustable gates and a 3-hole, dry feeder (Thorp Equipment, Inc., Thorp, WI), and a pan waterer. Feed and water were offered ad libitum and feed additions were delivered and recorded using a robotic feeding (FeedPro; Feedlogic Corp., Willmar, MN). The study was conducted from July 17, 2019 to November 28, 2019.

Approximately 1,200 growing pigs (PIC TR4 × (Fast LW × PIC L02); PIC, Hendersonville, TN; Fast Genetics, Saskatoon, SK, Canada; initial body weight [BW] 33.6 ± 0.63 kg) in 2 barns were used in a 103-d growth trial. Pigs were housed in mixed gender (10 barrows and 10 gilts) pens with 20 pigs per pen and 10 replicates per treatment. Pens were assigned to 1 of 6 dietary treatments in a complete randomized block design with initial BW and pen location within barn as blocking factors.

Prior to diet formulation, a composite sample of corn, soybean meal (SBM), and dried distiller's grains with solubles (DDGS) was collected and submitted to Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed for a complete AA profile (Table 1; Method 982.30, AOAC Int., 2006). These total AA values for corn, SBM, and DDGS were multiplied by NRC (2012) standardized ileal digestibility (SID) coefficients and these values were used in diet formulation. Experimental diets were fed in 4 phases (Tables 2 and 3) from days 0 to 16, 16 to 40, 40 to 64, and 64 to 103, which correspond to BW of ~34 to 51, 51 to 75, 75 to 99, and 99 kg to market, respectively. Experimental diets were corn–SBM–DDGS based with 30% DDGS fed in phases 1 to 3 and 20% DDGS fed in phase 4.

Experimental treatments consisted of: (1) high SBM and low feed grade AA (HSBM) with Val:Lys, Ile:Lys and Trp:Lys ranging from 85 to 90, 76 to 78, and 19.3% to 19.9%, respectively, across the 4 dietary phases; (2) negative control (NC) with low SBM and high levels of feed grade AA with Val:Lys, Ile:Lys, and Trp:Lys ranging from 64 to 68, 51 to 53, and 17.0% to 17.5%,

Table 1. Amino acid analysis of corn, SBM, and DDGS; as-fed basis[1]

Amino acid, %	Corn	SBM	DDGS
Alanine	0.43	1.95	1.54
Arginine	0.26	3.34	1.18
Aspartic acid	0.44	5.15	1.62
Cysteine	0.15	0.66	0.55
Glutamic acid	1.02	7.92	2.95
Histidine	0.18	1.18	0.75
Isoleucine	0.21	2.04	1.02
Leucine	0.66	3.41	2.68
Lysine	0.21	3.41	0.97
Methionine	0.12	0.61	0.46
Phenylalanine	0.29	2.33	1.06
Proline	0.53	2.42	1.85
Serine	0.30	2.24	1.07
Threonine	0.22	1.78	1.01
Tryptophan	0.05	0.62	0.19
Tyrosine	0.08	1.14	0.83
Valine	0.28	2.07	1.31

[1]A representative sample of each ingredient was collected, homogenized, and submitted to Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed in duplicate. These values, multiplied by SID coefficients derived from NRC (2012), were used in diet formulation.

Table 2. Phases 1 and 2 diet composition (as-fed basis)[1,2]

	Phase 1						Phase 2					
Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Ingredients, %												
Corn	41.92	55.53	50.94	50.87	50.89	50.92	47.00	60.35	56.03	55.91	55.92	56.01
SBM (46.5% CP)	24.27	10.31	15.07	15.08	15.08	15.07	19.34	5.71	10.14	10.15	10.15	10.14
DDGS, >6 and <9% oil[3]	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Choice white grease	1.50	1.00	1.15	1.15	1.15	1.15	1.55	1.05	1.20	1.25	1.25	1.20
Calcium carbonate	1.25	1.20	1.22	1.22	1.22	1.22	1.19	1.14	1.15	1.15	1.15	1.15
Monocalcium phosphate, 21% P	0.29	0.44	0.40	0.40	0.40	0.40	0.14	0.30	0.25	0.25	0.25	0.25
Salt	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-Lysine HCl	0.15	0.58	0.43	0.43	0.43	0.43	0.16	0.58	0.44	0.44	0.44	0.44
DL-Methionine	—	0.08	0.05	0.05	0.05	0.05	—	0.05	0.04	0.04	0.04	0.04
L-Threonine	—	0.14	0.08	0.08	0.08	0.08	—	0.14	0.08	0.08	0.08	0.08
L-Tryptophan	—	0.05	0.04	0.04	0.04	0.06	—	0.05	0.05	0.05	0.05	0.07
L-Valine	—	0.06	—	0.07	—	—	—	0.03	—	0.07	—	—
L-Isoleucine	—	—	—	—	0.05	—	—	—	—	—	0.06	—
Vitamin–mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase[5]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis												
SID amino acids, %												
Lysine	0.98	0.98	0.98	0.98	0.98	0.98	0.87	0.87	0.87	0.87	0.87	0.87
Isoleucine:lysine	76	53	61	61	66	61	77	51	59	59	66	59
Leucine:lysine	168	133	145	145	145	145	175	137	150	150	150	150
Methionine:lysine	29	30	30	30	30	30	30	29	30	30	30	30
Methionine + cysteine:lysine	60	55	57	57	57	57	63	55	58	58	58	58
Threonine:lysine	67	62	62	62	62	62	68	62	62	62	62	62
Tryptophan:lysine	19.9	17.5	19.0	19.0	19.0	21.1	19.5	17.0	19.0	19.0	19.0	21.8
Valine:lysine	85	68	70	76	70	70	86	64	70	77	70	70
Lysine:net energy, g/Mcal	3.92	3.92	3.92	3.92	3.92	3.92	3.45	3.45	3.45	3.45	3.45	3.45
Net energy kcal/kg	2,499	2,499	2,499	2,499	2,499	2,499	2,524	2,524	2,524	2,524	2,524	2,524
STTD P, %[6]	0.39	0.39	0.39	0.39	0.39	0.39	0.35	0.35	0.35	0.35	0.35	0.35
Chemical analysis[7]												
Crude protein, %	21.44	16.52	18.00	18.38	17.78	18.42	20.34	15.48	17.38	17.94	17.08	16.83
Total Ca, %	0.67	0.76	0.86	0.76	0.60	0.86	0.69	0.82	0.83	0.70	0.78	0.95
Total P, %	0.60	0.61	0.60	0.57	0.56	0.57	0.59	0.56	0.55	0.57	0.58	0.65

[1]Phase 1 diets were fed from days 0 to 16 (33.6 to 50.8 kg) and phase 2 diets were fed from days 16 to 40 (50.8 to 74.9 kg).
[2]HSBM, high soybean meal, NC, negative control, PC, positive control, PC+Val, positive control + valine, PC+Ile, positive control + isoleucine, PC+Trp, positive control + tryptophan.
[3]DDGS, dried distillers grains with solubles.
[4]Vitamin and mineral premix provided per kilogram of complete diet: 90 mg Zn, 37 mg Fe, 11 mg Mn, 15 mg Cu, 0.18 mg I, 0.30 mg of Se, 2,507 IU vitamin A, 318 IU vitamin D,12 IU vitamin E, 0.01 mg vitamin B12, 11.6 mg niacin, 7.4 mg pantothenic acid, and 2.0 mg riboflavin.
[5]Smizyme TS G5 2,500 (Origination Inc., St. Paul, MN) provided 626 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.
[6]Standardized total tract digestible P.
[7]A composite sample of each dietary treatment for each phase was collected, homogenized, and submitted to Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed.

respectively, across the 4 dietary phases; (3) positive control (PC) with a medium feed grade AA inclusion with Val:Lys and Trp:Lys held constant at 70 and 19.0%, respectively, and with Ile:Lys ranging from 58% to 61% across the 4 dietary phases; (4) PC with high Val:Lys (PC+Val) ranging from 76% to 80% across the 4 dietary phases; (5) PC with a high Ile:Lys (PC+Ile) ranging from 66% to 68% across the 4 dietary phases, and (6) PC with a high Trp:Lys (PC+Trp) ranging from 21.1% to 23.1% across the 4 dietary phases.

The formulated AA levels were entered in the ADG prediction equation developed by Cemin et al. (2019). Briefly, the equation was developed to determine the effects of BCAA and their interactions on relative differences in growth performance. Based on that, pigs fed the NC diet were predicted to have the poorest ADG, the PC diet to be intermediate, and pigs fed the HSBM, highest predicted ADG. The PC+Val and PC+Trp treatments were developed by increasing the Val:Lys and Trp:Lys, respectively, until the model of Cemin et al. (2019) predicted the same ADG of the HSBM treatment. Because the model predicts that the response to Ile is quadratic, the PC+Ile treatment was developed by increasing the Ile:Lys to come as close as possible to the predicted ADG of the HSBM diet.

Each pig was tagged with an RFID tag at the beginning of the trial in order to be individually identified. Pigs were individually weighed on days 0 and 76 in order to evaluate if the response to dietary treatment was influenced by pig sex. Pens of pigs were weighed approximately every 14 d to determine ADG, ADFI, and G:F. On day 83, 4 to 6 of the heaviest pigs in each pen were selected and marketed to achieve a consistent inventory of 14 pigs remaining in each pen. The pigs marketed on day 83 were included in the growth data, but not in the final pen carcass data. On the last day of the trial (day 103), final pen weights were obtained, and the remaining pigs were transported to a U.S. Department of Agriculture-inspected packing plant (Triumph Foods, St. Joseph, MO) for carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat, and percentage lean. Loin depth and back fat depth were determined ultrasonically using a Fat-O-Meater (SFK; Herlev, Denmark) inserted ~7 cm off the mid-line of the pig and between the 10th and 11th rib. 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 obtained at the farm.

Samples of complete diets were obtained from 5 feeders of each treatment in each barn ~4 d after the beginning and 4 d prior to the end of each phase. Feed samples were delivered to the Kansas State University Swine Laboratory (Manhattan, KS) and stored at –20 °C until analysis. Samples of diets were combined within dietary treatment, and a composite sample from each phase for each treatment was analyzed (University of Missouri-Columbia, MO; Tables 2 and 3). Samples were analyzed for crude protein (Method 990.03; AOAC Int., 2006), P (Method 966.01; AOAC Int., 2006), and Ca (Method 985.01; AOAC Int., 1990).

Data were analyzed as a randomized complete block design for 1-way ANOVA using the lmer function from the lme4 package in R (version 3.5.1; 2018-07-02, R Foundation for Statistical Computing, Vienna, Austria) with pen considered as the experimental unit and initial BW and pen location within barn as blocking factors. Treatment was considered a fixed effect, and blocking factors were considered random effects. Preplanned pairwise comparisons using the Tukey–Kramer adjustment were used to evaluate differences in treatment means. Results were considered significant at $P \leq 0.05$.

At the conclusion of the study, in order to validate and compare the relative differences in predicted ADG to the actual ADG observed, the equation's intercept term was adjusted until the predicted ADG matched the actual ADG for the pigs fed the HSBM diets. The equation with the adjusted intercept term was then used to predict the ADG of the remaining 5 treatments. The relationship between the actual and predicted ADG was calculated (actual ADG/predicted ADG) to illustrate the accuracy of the prediction model.

Results

Chemical analysis

Results of AA analysis of the corn, SBM, and DDGS used in diet formulation indicated a lower Lys content in corn but greater in SBM than listed by NRC (2012; Table 1). These values, multiplied by NRC (2012) SID digestibility coefficients, were used in diet formulation.

Growth performance and carcass characteristics

In the grower period from 34 to 90 kg (days 0 to 54), pigs fed the HSBM and PC+Val diets had greater ($P < 0.05$) ADG than those fed the NC, with pigs fed PC, PC+Ile, and PC+Trp intermediate (Table 4). Pigs fed HSBM were also more ($P < 0.05$) efficient than the NC and PC pigs with those fed PC+Val, PC+Ile, and PC+Trp intermediate. There was no difference ($P > 0.05$) in ADFI among pigs fed any of the treatments during the grower period.

During the finishing period from 90 to 136 kg (days 54 to 103), ADG was greater ($P < 0.05$) for pigs fed PC+Ile than that of pigs fed the NC with those fed HSBM, PC, PC+Val, and PC+Trp intermediate. Pigs fed PC+Val had greater ($P < 0.05$) ADFI than pigs fed NC with those fed HSBM, PC, PC+Ile, and PC+Trp intermediate. Pigs fed the PC+Ile treatment were more ($P < 0.05$) efficient than pigs fed PC+Val with those fed HSBM, NC, PC, and PC+Trp intermediate.

Overall, pigs fed the HSBM, PC+Val, and PC+Ile diets had greater ($P < 0.05$) ADG and final BW than the pigs fed the NC diet with those fed PC and PC+Trp intermediate. Pigs fed the PC+Val diets had greater ($P < 0.05$) ADFI than pigs fed NC with those fed HSBM, PC, PC+Ile, and PC+Trp intermediate. There were no differences ($P > 0.05$) between treatments for G:F. Similar to overall ADG and final BW, pigs fed the HSBM, PC, PC+Val, and PC+Ile diets had heavier ($P < 0.05$) HCW than the pigs fed the NC diet with those fed PC+Trp intermediate. There was no evidence for treatment differences ($P > 0.05$) observed for any other carcass characteristic or percentage carcass yield.

There was no ($P > 0.10$) treatment × sex interaction on growth performance (data not shown). Day 76 BW standard deviation was also not influenced by sex within treatment. The day 76 BW standard deviation for barrows for barrows averaged 25.0 and averaged 25.6 for gilts.

Model validation

When comparing the model predicted ADG proposed by Cemin et al. (2019) to actual ADG in the grower period (Table 5), the model accurately predicted the relative differences in ADG of pigs fed the NC and PC+Val diets, but overpredicted ADG for the pigs fed the PC (2.2%), PC+Ile (2.5%), and PC+Trp (3%) diets. In the finisher period, the model accurately predicted ADG of the PC+Val treatment and overpredicted the ADG for the PC+Trp treatment by 1.6%, but underpredicted the ADG for

Table 3. Phases 3 and 4 diet composition (as-fed basis)[1,2]

	Phase 3						Phase 4					
Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Ingredients, %												
Corn	51.34	62.55	60.58	60.46	60.45	60.55	62.44	71.87	70.00	69.87	69.88	69.92
SBM (46.5% CP)	15.07	3.58	5.55	5.56	5.56	5.55	13.96	4.27	6.24	6.25	6.25	6.25
DDGS, >6 and <9% oil[3]	30.00	30.00	30.00	30.00	30.00	30.00	20.00	20.00	20.00	20.00	20.00	20.00
Choice white grease	1.50	1.10	1.20	1.25	1.25	1.20	1.45	1.10	1.15	1.20	1.20	1.20
Calcium carbonate	1.16	1.13	1.13	1.13	1.13	1.13	1.10	1.07	1.07	1.07	1.07	1.07
Monocalcium phosphate, 21% P	0.15	0.33	0.30	0.30	0.30	0.30	0.30	0.44	0.40	0.40	0.40	0.40
Salt	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-Lysine HCl	0.15	0.50	0.44	0.44	0.44	0.44	0.13	0.42	0.36	0.36	0.36	0.36
DL-Methionine	—	0.03	0.03	0.03	0.03	0.03	—	0.05	0.04	0.04	0.04	0.04
L-Threonine	—	0.11	0.10	0.10	0.10	0.10	—	0.11	0.09	0.09	0.09	0.09
L-Tryptophan	—	0.04	0.05	0.05	0.05	0.07	—	0.04	0.04	0.04	0.04	0.06
L-Valine	—	0.02	—	0.06	—	—	—	0.02	—	0.07	—	—
L-Isoleucine	—	—	—	—	0.08	—	—	—	—	—	0.07	—
Vitamin–mineral premix[4]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase[4]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Calculated analysis												
SID amino acids, %												
Lysine	0.76	0.76	0.76	0.76	0.76	0.76	0.67	0.67	0.67	0.67	0.67	0.67
Isoleucine:lysine	78	53	58	58	67	58	77	53	58	58	68	58
Leucine:lysine	187	150	157	157	157	157	183	148	155	155	155	155
Methionine:lysine	32	29	30	30	30	30	32	32	32	32	32	32
Methionine + cysteine:lysine	67	58	59	59	59	59	67	61	62	61	61	62
Threonine:lysine	70	64	66	66	66	66	69	65	66	66	66	66
Tryptophan:lysine	19.3	17.0	19.0	19.0	19.0	22.2	19.7	17.5	19.0	19.0	19.0	23.1
Valine:lysine	90	68	70	78	70	70	88	68	70	80	70	70
Lysine:net energy, g/Mcal	3.00	3.00	3.00	3.00	3.00	3.00	2.61	2.61	2.61	2.61	2.61	2.61
Net energy, kcal/kg	2,537	2,537	2,537	2,537	2,537	2,537	2,568	2,568	2,568	2,568	2,568	2,568
STTD P, %[6]	0.34	0.35	0.35	0.35	0.35	0.35	0.34	0.34	0.34	0.34	0.34	0.34
Chemical analysis[7]												
Crude protein, %	18.64	14.34	14.62	14.06	14.80	14.97	14.98	13.55	13.23	12.91	13.61	13.10
Total Ca, %	0.63	0.66	0.63	0.71	0.75	0.53	0.79	0.64	0.54	0.58	0.77	0.73
Total P, %	0.54	0.52	0.58	0.55	0.54	0.51	0.46	0.50	0.42	0.43	0.50	0.47

[1]Phase 3 diets were fed from d 40 to 64 (74.9 to 98.5 kg) and phase 4 diets were fed from d 64 to 103 (98.5 to market, respectively)
[2]HSBM, high soybean meal, NC, negative control, PC, positive control, PC+Val, positive control + valine, PC+Ile, positive control + isoleucine, PC+Trp, positive control + tryptophan.
[3]DDGS, dried distillers grains with solubles.
[4]Vitamin and mineral premix provided per kg of complete diet: 90 mg Zn, 37 mg Fe, 11 mg Mn, 15 mg Cu, 0.18 mg I, 0.30 mg of Se, 2507 IU vitamin A, 318 IU vitamin D,12 IU vitamin E, 0.01 mg vitamin B12, 11.6 mg niacin, 7.4 mg pantothenic acid, and 2.0 mg riboflavin
[5]Smizyme TS G5 2,500 (Origination Inc., St. Paul, MN) provided 626 units of phytase FTU/kg of diet with an assumed release of 0.12 available P.
[6]Standardized total tract digestible P.
[7]A composite sample of each dietary treatment for each phase was collected, homogenized, and submitted to Agriculture Experiment Station Chemical Laboratories (University of Missouri-Columbia, MO) and analyzed.

Table 4. Effects of supplemental Val, Ile, Trp on growth performance of growing-finishing pigs[1,2]

Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp	SEM	P-value
Initial BW, kg	33.5	33.5	33.6	33.6	33.6	33.5	0.63	0.994
Day 54 BW, kg	91.5[a]	88.1[c]	89.2[bc]	91.2[ab]	89.8[abc]	89.9[abc]	0.61	<0.001
Final BW, kg	136.3[a]	130.6[b]	134.3[ab]	136.0[a]	135.4[a]	133.9[ab]	0.96	<0.001
Grower (days 0 to 54)								
ADG, kg	1.078[a]	1.019[b]	1.040[ab]	1.074[a]	1.049[ab]	1.046[ab]	0.0107	<0.001
ADFI, kg	2.313	2.265	2.291	2.343	2.301	2.303	0.0202	0.175
G:F	0.466[a]	0.450[b]	0.454[b]	0.459[ab]	0.456[ab]	0.454[ab]	0.0038	0.007
Finisher (days 54 to 103)								
ADG, kg	0.983[ab]	0.956[b]	0.985[ab]	0.978[ab]	1.006[a]	0.967[ab]	0.0121	0.080
ADFI, kg	3.022[ab]	2.936[b]	3.011[ab]	3.047[a]	3.036[ab]	2.976[ab]	0.0296	0.042
G:F	0.325[ab]	0.326[ab]	0.327[ab]	0.321[b]	0.331[a]	0.325[ab]	0.0023	0.049
Overall (days 0 to 103)								
ADG, kg	1.035[a]	0.990[b]	1.015[ab]	1.031[a]	1.029[a]	1.010[ab]	0.0084	<0.001
ADFI, kg	2.629[ab]	2.563[b]	2.611[ab]	2.656[a]	2.629[ab]	2.602[ab]	0.0207	0.027
G:F	0.394	0.387	0.389	0.388	0.392	0.388	0.0021	0.060
Carcass characteristics								
HCW, kg	99.8[a]	95.9[b]	98.7[a]	100.0[a]	99.3[a]	98.7[ab]	0.78	0.005
Carcass yield, %	73.2	73.4	73.4	73.4	73.3	73.7	0.298	0.931
Backfat depth, mm[3]	15.1	15.6	15.2	15.8	15.3	15.4	0.28	0.335
Loin depth, mm[3]	65.5	64.2	65.0	64.5	65.2	65.1	0.41	0.136
Lean, %[3]	54.9	54.5	54.8	54.5	54.7	54.7	0.14	0.190

[1]A total of 1,200 pigs in 2 groups were used in a 103-d study with 20 pigs per pen and 10 replicates per treatment.
[2]HSBM, high soybean meal, NC, negative control, PC, positive control, PC+Val, positive control + valine, PC+Ile, positive control + isoleucine, PC+Trp, positive control + tryptophan.
[3]Adjusted using HCW as covariate.
[a,b,c]Means with different superscripts are significantly different ($P \leq 0.05$).

Table 5. Comparison of predicted ADG based on the model versus the actual ADG[1,2]

Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Grower (days 0 to 54)						
Actual ADG, kg	1.078	1.019	1.040	1.074	1.049	1.046
Predicted ADG, kg	1.078	1.025	1.063	1.078	1.076	1.078
Actual vs. predicted, %[3]	100	99.4	97.8	99.6	97.5	97.0
Finisher (days 54 to 103)						
Actual ADG, kg	0.983	0.956	0.985	0.978	1.006	0.967
Predicted ADG, kg	0.983	0.930	0.960	0.983	0.978	0.983
Actual vs. predicted, %[3]	100	102.8	102.6	99.5	102.9	98.4
Overall						
Actual ADG, kg	1.035	0.990	1.015	1.031	1.029	1.010
Predicted ADG, kg	1.035	0.990	1.015	1.035	1.033	1.035
Actual vs. predicted, %[3]	100.0	100.0	100.0	99.6	99.6	97.8

[1]Prediction equation used was derived by Cemin et al. (2019), the intercept term was adjusted until the predicted ADG matched the actual ADG of HSBM treatment. The adjusted intercept term equation was then used to predict the ADG of the remaining treatments.
[2]HSBM, high soybean meal, NC, negative control, PC, positive control, PC+Val, positive control + valine, PC+Ile, positive control + isoleucine, PC+Trp, positive control + tryptophan.
[3]Actual vs. predicted = actual ADG/predicted ADG.

pigs fed the NC, PC, and PC+Ile diets by 2.8%, 2.6%, and 2.9%, respectively. For the overall experimental period, the model was quite accurate for most treatments with ADG of the pigs fed the NC, PC, PC+Val, and PC+Ile predicted within 0.4% of actual. However, the model overpredicted ADG of pigs fed the PC+Trp diet by 2.2%.

Comparing the model predicted G:F to actual G:F the model accurately predicted G:F of pigs fed the different dietary treatments in the grower period (Table 6). However, in the finisher phase then model was much less accurate than predictions for ADG and off as much as 7.7%. For the overall period, the model underpredicted G:F by as much as 1.8 (PC+Val) to 4.5% (NC).

Discussion

An imbalance in BCAA can occur in swine diets comprised of corn by-products that are also supplemented with high amounts of feed-grade AA. The imbalance is a result of the high Leu content relative to Val and Ile in corn protein (NRC, 2012). Unlike most AA, BCAA are first transported to skeletal muscle to be degraded and through the action of BCAT are reversibly converted to α-keto acids. The α-keto acids are then transported to the liver where they are decarboxylated by branched-chain α-keto acid dehydrogenase complex (BCKD). Leucine is the most potent stimulator of BCAT and BCKD. Thus, high dietary

Table 6. Comparison of predicted G:F based on the model versus the actual G:F[1,2]

Item	HSBM	NC	PC	PC+Val	PC+Ile	PC+Trp
Grower (days 0 to 54)						
Actual G:F	0.466	0.450	0.454	0.459	0.456	0.454
Predicted G:F	0.466	0.445	0.451	0.454	0.452	0.450
Actual vs. predicted, %[3]	100	101.2	100.7	101.1	100.9	101.0
Finisher (days 54 to 103)						
Actual G:F	0.325	0.326	0.327	0.321	0.331	0.325
Predicted G:F	0.325	0.303	0.309	0.313	0.312	0.307
Actual vs. predicted, %[3]	100	107.7	105.9	102.5	106.0	105.9
Overall						
Actual G:F	0.394	0.387	0.389	0.388	0.392	0.388
Predicted G:F	0.394	0.370	0.376	0.381	0.379	0.375
Actual vs. predicted, %[3]	100	104.5	103.4	101.8	103.5	103.5

[1]Prediction equation used was derived by Cemin et al. (2019), the intercept term was adjusted until the predicted G:F matched the actual G:F of HSBM treatment. The adjusted intercept term equation was then used to predict the G:F of the remaining treatments.
[2]HSBM, high soybean meal, NC, negative control, PC, positive control, PC+Val, positive control + valine, PC+Ile, positive control + isoleucine, PC+Trp, positive control + tryptophan.
[3]Actual vs. predicted = actual G:F/predicted G:F.

concentrations of Leu would lead to catabolism not only of itself, but also Ile and Val (Harper et al., 1984). If ratios of Ile and Val are close to requirement estimates, as in the case in diets supplemented with L-Lys, DL-Met, L-Thr, and L-Trp, increased degradation may potentially cause deficiency in Ile and Val and reduce pig growth performance.

Tryptophan is an LNAA that is a precursor for serotonin, which plays a role in appetite regulation (Henry et al., 1992). Large neutral AA share the same brain transporters as BCAA and an excess in BCAA, specifically Leu, has been negatively correlated with Trp uptake and serotonin levels in the brain, ultimately leading to a decrease in ADFI and growth performance (Wessels et al., 2016a, 2016b).

A prediction model for ADG based on a meta-analysis by Cemin et al. (2019) suggests that increased concentrations of Val, Ile, or Trp might reverse the decreased performance of pigs fed diets containing high Leu. The current study focused on validating this model predicting the relative differences among treatments by adding Val, Ile, or Trp to diets containing high concentrations of Leu. The HSBM dietary treatment with addition of low feed grade AA contains a high Leu level, but also has high Val, Ile, and Trp ratios relative to Lys and therefore should have had the best ADG. In order to validate the model's prediction for lower ADG from high Leu along with an imbalance in BCAA, the NC diet was formulated to contain the most L-Lys HCl and the lowest amount of SBM and by doing so it resulted in a predicted decrease in ADG because of an imbalance in BCAA. The PC diet was the base for the remaining treatments and contained high levels of L-Lys HCl; however, less than that of the NC, and had slightly greater L-Val, L-Ile, and L-Trp than the NC. The PC was formulated to have intermediate predicted ADG compared with the HSBM and the NC, while the PC+ Val, PC+Ile, and PC+Trp where formulated to match the ADG of the HSBM treatment.

The HSBM diets in our experiment had the highest dietary Leu concentration; however, the increased levels of SBM and reduced L-Lys HCl also resulted in elevated dietary levels of Val, Ile, and Trp. These greater levels of other BCAA and Trp negate or lessen the negative effects of high Leu. Meanwhile, despite the reduction of Leu in the NC diet compared with the HSBM diets, the NC diets also had the lowest dietary levels of Val, Ile, and Trp with levels being above, but near NRC (2012) requirement estimates. Thus, the NC diet contained Val, Ile, and Trp that meet the pig's requirements relative to Lys in diets without excess Leu, but may not meet the needs when diet contains excess Leu. The model by Cemin et al. (2019) accurately predicted the actual 4.4% reduction in ADG for pigs fed the NC treatment when compared with those fed the HSBM diets. The reduction in ADG observed in our study is in agreement with observations by Kwon et al. (2019a), who also observed decreased ADG when an imbalance in BCAA arise, namely an increase in Leu concentrations with no change in Val or Ile.

The equation for ADG by Cemin et al. (2019) predicts that adding Val or Ile to the diet can reverse the negative effects of high Leu concentrations. However, the model predicts that Ile has a lesser ability to reverse the negative effects of Leu than Val. In our study, pigs fed the diets with increased Val were able achieve ADG that was almost identical to performance of pigs fed the HSBM diet as predicted by the model. The model accurately predicted the ADG in both the grower and finisher phases for the pigs fed PC+Val. The increased ADG of pigs fed increased Val in high Leu diets is in agreement with the results from Gloaguen et al. (2011) and Millet et al. (2015), where increased Val was observed to ameliorate the decrease in ADG of pigs fed excess Leu.

The improvement in overall ADG and lower G:F for pigs fed the PC+Val diets in our study was primarily driven by increased feed intake. High levels of Leu have also been shown to decrease ADFI (Gloaguen et al., 2011; Millet et al., 2015; Kwon et al., 2019a) possibly a result of overstimulating mammalian target rapamycin (mTOR) receptors, although the impact is variable (Hyun et al., 2003, 2007). Mammalian target rapamycin receptors are a signaling pathway that stimulates protein synthesis for cell growth (Schmelze and Hall, 2000) but overstimulation can lead to inhibition of feed intake (Cota et al., 2006). Valine, however, has been shown to decrease or inhibit the transport of Leu through the blood brain barrier (Hjelle et al., 1978; Hargreaves and Pardridge, 1988), which could lead to a reduction in mTOR stimulation. Although mTOR stimulation was not measured, the resulting increase in feed intake in the finishing period that occurred for the PC+Val treatment may have been a result to the reduction of Leu crossing the blood-brain barrier and preventing mTOR over stimulation.

According to the model of Cemin et al. (2019), Ile alone cannot reverse the negative effects of high Leu concentrations and may need to be used in combination with Val or Trp. This would be in agreement with Harper et al. (1954) where increased Ile was only able to partially recover growth in rats fed high dietary Leu. In the present study, the model of Cemin et al. (2019) accurately predicted ADG for the PC+Ile treatment, but when broken down into 2 different time periods, the PC+Ile dietary treatment underperformed in ADG in the grower period and then overperformed in the finisher period when compared with the predicted model. These results may indicate that Ile deficiency relative to Leu in the NC diet may have been more detrimental in the finisher period than during the growing period allowing for a greater response to dietary addition of Leu. Although the Ile:Lys ratio was similar in the grower and finisher phases, the Leu:Ile ratio was greater in the finisher phase as the Leu content of the diets increased with greater inclusion of corn.

Van Milgen et al. (2012) demonstrated using a meta-analysis that the requirement for Ile increased when pigs were fed diets containing blood meal or blood cells and believed that this to be the result of the high concentration of Leu in these products creating an imbalance in BCAA. In support of this, Hargreaves and Pardridge (1988) observed that in the brain, the competition for Leu uptake is higher for Ile than Val. In that study, the Km value was highest for Val followed by Leu and Ile. This results in a lower clearance from the plasma pool for Val but a higher (faster) clearance for Ile and suggests that Ile might be more sensitive to excess Leu than Val (Hargreaves and Pardridge, 1988). Parr et al. (2004) observed a linear reduction in plasma urea nitrogen (PUN) as Ile increased in the diet for finishing pigs. Although Leu levels were not stated in the publication, the resulting decrease in PUN may have been due to a decrease in AA catabolism stimulated from a BCAA imbalance caused by excess Leu. A potential decrease in catabolism might be the reason for the improvement in G:F that was observed for the pigs fed PC+Ile in the finishing period. Over the course of multiple experiments, Dean et al. (2005) observed mixed results when evaluating the Ile requirement in finishing pigs and this may have been a result of different Leu levels across the 6 experiments. Retrospectively, the experiments where increasing dietary Ile improved growth performance may have been a result of correcting an imbalance in BCAA caused by true digestible Leu:Lys being at or greater than 1.32 in the diets and when no response observed; true digestible Leu:Lys levels were at or below 0.99 which may not have been high enough to create a BCAA imbalance, thus making incremental inclusion of Ile unnecessary.

Our results show that the model of Cemin et al. (2019) overpredicted the relative response to dietary addition of Trp during the grower, finisher, and overall periods. This result was unexpected and may have been due to not having a high enough added Trp in the PC+Trp diet. Kwon et al. (2019b) were partially able to overcome the negative effects on ADG and ADFI from excess dietary Leu with 23% and 28% Trp:Lys ratios, which were greater than the Trp:Lys ratios used in our experiment (~21% to 23% of Lys). Another possible reason for the model's overprediction may be because Val or Ile might be more deficient relative to Leu in the PC diets in this experiment and that correcting the BCAA imbalance was more important than correcting an LNAA imbalance. If we used greater levels of Val or Ile in the PC+Trp diet, we might have observed increased ADG for those fed the PC+Trp diets. Early research conducted by Rogers et al. (1967) observed that Trp needed to be supplemented in combination with Val and Ile in order to fully alleviate the decreased growth from excess dietary Leu in rats. However, additional research is needed to verify this response in pigs.

Tryptophan also plays a key role in feed intake as it is a precursor for serotonin. Because LNAA and BCAA compete for the same brain receptors, an imbalance in BCAA can lead to a decrease in Trp uptake in the brain and in turn reduce serotonin synthesis. Henry et al. (1992) observed ADFI and serotonin concentrations in the hypothalamus were more reduced in gilts than barrows when Trp was fed at deficient levels. More importantly, Wessels et al. (2016a, 2016b) observed a negative correlation between increasing amounts of Leu on Trp and serotonin levels in the brain. Our results show that the PC+Trp treatment did not improve ADFI in the presence of high Leu levels used in the current study, again possibly suggesting that Trp alone may not be able to overcome an imbalance in BCAA caused by excess Leu. Additionally, these data show that increased inclusion of Val, Ile, or Trp did not influence ADG of barrows and gilts differently.

Dietary treatments had no influence on any measured carcass characteristics except for HCW. The HCW response was directly correlated to the improvements in overall ADG. High Leu diets have not been shown to effect carcass yield, backfat, or loin depth (Hyun et al., 2003, 2007). Our results would suggest that the additions of Val, Ile, or Trp in diets with high Leu also do not affect carcass characteristics.

In conclusion, the ADG model proposed by Cemin et al. (2019) accurately predicted the relative differences in overall growth for pigs fed the NC, PC, PC+Val, and PC+Ile diets; however, the model overpredicted ADG for pigs fed the PC+Trp diet. While relative difference in G:F during the grower period was accurately predicted by the model, G:F in the finisher and overall periods was not. This suggests that the differences in ADG are driven by feed intake, not any changes in efficiency of gain. Pigs fed the high Val diets were able to reverse the negative effects of excess Leu on ADG starting in the grower period, whereas pigs fed PC+Ile diets overcame the negative effects of high dietary Leu in the finishing period. The overprediction for ADG of the PC+Trp treatment may have been result of also needing additional Val and/or Ile above the levels used herein to reduce the negative effects of Leu or the model may underestimate the amount of added Trp needed in the diet. Further research is needed to validate the ADG prediction model when combinations of Val, Ile, and Trp are used in high Leu diets.

Acknowledgements

The authors would like to thank CJ Bio America and New Fashion Pork for providing financial and technical support, respectively, for these studies. Contribution no. 20-183-J of the Kansas Agricultural Experiment Station, Manhattan, 66506-0201.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest. However, Keith D. Haydon is employed by CJ Bio-America, the company that provided partial financial support.

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