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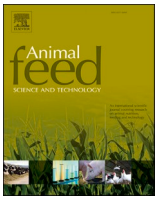

Including dietary valine and tryptophan, but not isoleucine, above the requirement for growing pigs may partly ameliorate negative effects of excess leucine from corn protein on nitrogen balance and growth performance

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ABSTRACT

Two experiments were conducted to test the hypothesis that increasing concentrations of dietary L-valine, L-isoleucine, or L-tryptophan in diets containing excess leucine (Leu) from corn protein will alleviate negative effects of excess dietary Leu on N balance and growth performance of growing pigs. In Exp. 1, 72 barrows (body weight: 33.9 ± 2.6 kg) were housed in metabolism crates and randomly allotted to 8 dietary treatments in a 12-day experiment. A basal diet based on corn and a high-protein corn product was formulated (16.9 mega joule/kg gross energy and 12.7 g/kg lysine). Two levels of crystalline L-isoleucine (0 or 1.0 g/kg), two levels of crystalline L-valine (0 or 1.0 g/kg), and two levels of crystalline L-tryptophan (0 or 0.5 g/kg) were added to the basal diet for a total of 8 diets that were used in a 2 × 2 × 2 factorial. Results indicated that fecal N output increased if isoleucine (Ile) was added to diets without adding valine (Val), but that was not the case if Val was added (interaction, $P < 0.05$). Isoleucine addition to diets reduced N retention, but N retention increased with tryptophan (Trp) addition to diets without valine addition, but not if Trp was added to diets with added Val (interaction, $P < 0.05$). The biological value of protein increased if Trp was added to diets without addition of Ile, but if Ile was added, Trp addition did not increase the biological value of protein (interaction, $P < 0.05$). In Exp. 2, a total of 288 growing pigs (body weight: 28.6 ± 2.5 kg) were randomly assigned to 9 dietary treatments in a 28-day growth performance experiment. There were 2 barrows and 2 gilts per pen and 8 replicate pens per treatment. A control diet based on corn and soybean meal (16.5 mega joule/kg gross energy and 11.1 g/kg lysine) was used in Exp. 2 in addition to the 8 diets used in Exp. 1. Results indicated that final body weight and average daily gain of pigs fed the control diet were greater ($P < 0.001$) than for pigs fed all other diets, except pigs fed the diet with addition of both L-valine and L-tryptophan. Pigs fed the control diet also had greater ($P < 0.001$) plasma

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; ATTD, apparent total tract digestibility; BCAA, branched-chain amino acids; BCKA, branched chain keto acids; EDTA, Ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunoassay; G:F, gain to feed ratio; HPCP, high-protein corn product; Ile, isoleucine; KIC, α-keto isocaproate; KIV, α-keto isovalerate; KMV, α-keto β-methylvalerate; Leu, leucine; PUN, Plasma urea nitrogen; SID, standardized ileal digestible; Trp, tryptophan; Val, valine.

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concentrations of Trp and Val than pigs fed the basal diet or diets not containing L-tryptophan or L-valine, respectively. In conclusion, adding Ile alone reduced N retention, but adding Trp alone or in combination with Ile or Val increased N retention. The combination of Val and Trp supplementation may be beneficial for preventing detrimental effects of excess Leu on growth performance of pigs.

1. Introduction

Leucine (Leu) is an indispensable amino acid (AA) that stimulates catabolism of branched-chain amino acids (BCAA) in the liver (Harper et al., 1984). Therefore, if pigs are fed diets with excess Leu, degradation of not only Leu, but also valine (Val) and isoleucine (Ile), may increase because of the stimulating effects of Leu or its metabolite on BCAA degrading enzymes (Wiltafsky et al., 2010). As a consequence, excess dietary Leu may result in reduced pig feed intake and growth performance (Gatnau et al., 1995; Wiltafsky et al., 2010) because of the imbalanced supply of BCAA that results from increased degradation of Val and Ile. Excess dietary Leu also reduces protein synthesis, which is likely a result of reduced availability of Val and Ile (Kwon et al., 2019).

Synthesis of serotonin in the brain may also be compromised by excess dietary Leu (Wessels et al., 2016) because excess Leu may prevent tryptophan (Trp), which is the precursor for serotonin, from being transported from the blood to the brain, and therefore reduce the availability of Trp for serotonin synthesis in the brain. Serotonin is a cerebral neurotransmitter that is important for feed intake regulation (Zhang et al., 2007). Therefore, if dietary Leu is in excess of the requirement, serotonin in the hypothalamus is linearly reduced (Kwon et al., 2019) and extra dietary Trp may be needed to overcome this reduction in serotonin synthesis and prevent reduced feed intake of pigs. Indeed, increasing dietary Trp had positive effects on average daily gain (ADG) and average daily feed intake (ADFI) of pigs fed diets with excess Leu (Kwon et al., 2022). Increasing concentrations of dietary Val and Ile alone or in combination also have the potential to alleviate negative effects of excess dietary Leu on growth performance of pigs (Cemin et al., 2019).

Diets based on corn and corn co-products and sorghum and sorghum co-products are rich in Leu (Sotak et al., 2015; Stein et al., 2016) due to the high concentration of Leu in corn and sorghum protein. Therefore, if diets are formulated based on high-protein corn co-products, dietary Leu often is provided in an amount that is up to twice as high as the requirement for pigs (NRC, 2012). In many experiments conducted to evaluate the effect of excess dietary Leu, crystalline L-Leu was the source of dietary Leu (Gatnau et al., 1995; Wiltafsky et al., 2010; Wessels et al., 2016; Kwon et al., 2019). In these experiments Leu was included at levels up to three times of the

Table 1
Analyzed nutrient composition of ingredients, as-fed basis[a], Exp. 1 and 2.

Item	Corn	Soybean meal	HPCP[b]
Gross energy, mega joule/kg	16.0	17.6	20.9
Dry matter, g/kg	872	886	928
Crude protein, g/kg	65.0	459.4	496.0
Acid-hydrolyzed ether extract, g/kg	29.8	17.2	58.8
Insoluble dietary fiber, g/kg	110.0	165.0	349.0
Soluble dietary fiber, g/kg	1.0	13.0	27.0
Total dietary fiber, g/kg	111.0	178.0	376.0
Ash, g/kg	12.4	68.6	37.6
Indispensable amino acids, g/kg			
Arginine	3.0	33.2	20.7
Histidine	2.0	12.2	12.7
Isoleucine	2.6	22.6	21.2
Leucine	8.3	35.9	58.8
Lysine	2.5	30.5	17.7
Methionine	1.4	6.6	11.2
Phenylalanine	3.4	24.2	26.5
Threonine	2.5	18.2	19.1
Tryptophane	0.5	6.2	4.2
Valine	3.3	22.7	26.1
Dispensable amino acids, g/kg			
Alanine	5.1	20.2	34.8
Aspartate[c]	4.6	52.8	34.3
Cysteine	1.5	6.8	9.5
Glutamate[c]	12.5	83.7	79.2
Glycine	2.7	19.8	18.3
Proline	5.9	23.1	37.4
Serine	3.2	20.3	21.3
Tyrosine	1.9	16.5	21.2

[a] Ingredients were analyzed in duplicate.
[b] HPCP, high-protein corn product (NexPro, Flint Hills Resources, Wichita, KS, USA).
[c] Aspartate includes aspartic acid and asparagine; Glutamate includes glutamic acid and glutamine.

presumed requirement. However, to our knowledge, no information about interactive effects between dietary BCAA and Trp on N balance and growth performance have been reported for pigs fed diets containing excess Leu that was supplied by corn protein. Therefore, the objective of this research was to test the hypothesis that increasing concentrations of dietary Val, Ile, or Trp in diets containing excess Leu from corn protein will prevent negative effects of excess dietary Leu supplied by corn protein on N balance, plasma urea N (PUN), and growth performance of growing pigs.

2. Materials and methods

Protocols for two experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois (Urbana-Champaign, IL, USA). Growing pigs that were the offspring of Line 359 boars and Camborough sows (Pig Improvement Company, Henderson, TN, USA) were used in the experiments. A locally grown hybrid of yellow dent corn and bin-run soybean meal were obtained from the University of Illinois Feed Mill (Champaign, IL, USA). A high-protein corn product (HPCP), which contained 496 g/kg crude protein, 17.7 g/kg lysine, and 58.8 g/kg Leu, was also obtained (NexPro; Flint Hills Resources, Wichita, KS, USA; Table 1). The same batches of these ingredients were used in both experiments. The HPCP that was used was sourced from the same facility as the HPCP used by Acosta et al. (2021) and the digestibility values reported by Acosta et al. (2021) were used in diet formulation.

2.1. Exp. 1. Nitrogen balance

Seventy-two growing castrated male pigs (initial body weight: 33.9 ± 2.6 kg) were assigned to eight dietary treatments with nine replicate pigs per treatment in a randomized complete block design. There were three blocks of 24 pigs with three pigs per diet in each block and diets were fed for 12 days. Corn and HPCP were the sources of Leu in the diets. A basal diet based on corn and 260 g/kg HPCP was formulated to contain Leu in an amount that was 1.7-times greater than the requirement for standardized ileal digestible (SID) Leu (Table 2), which is 9.9 g/kg (NRC, 2012). Two levels of crystalline L-Ile (0 or 1.0 g/kg), two levels of crystalline L-Val (0 or 1.0 g/kg), and two levels of crystalline L-Trp (0 or 0.5 g/kg) were added to the basal diet for a total of 8 diets that were used in a 2 × 2 × 2 factorial arrangement of treatments. The added levels of AA resulted in diets containing 0.15 to 0.20 times more Val and Ile, and approximately 0.25 times more Trp than the presumed requirements (NRC, 2012). Crystalline L-Ile, L-Leu, and L-Trp were procured from Ajinomoto Health and Nutrition Inc., Itasca, IL, 60143, USA, and each AA had a purity of at least 980 g/kg.

The basal diet had SID Ile:lysine, SID Val:lysine, and SID Trp:lysine ratios of 0.53:1, 0.70:1, and 0.18:1. However, addition of Ile, Val, or Trp to the basal diet resulted in diets with SID Ile:lysine, SID Val:lysine, and SID Trp:lysine ratios of 0.63:1, 0.80:1, and 0.23:1, respectively. The increase in the concentration of these AA, therefore, was between 150 and 300 g/kg relative to the presumed requirement.

Table 2
Ingredient composition of experimental diets, as-fed basis, Exp. 1 and 2.[a, b]

	Control diet[c]	High-protein corn product diets							
Item		Basal	+ Val	+ Ile	+ Trp	+ Val and Ile	+ Val and Trp	+ Ile and Trp	+ Val, Ile, and Trp
Ground corn	676.4	693.6	693.6	693.6	693.6	693.6	693.6	693.6	693.6
Soybean meal	270.0	-	-	-	-	-	-	-	-
High-protein corn product	-	260.0	260.0	260.0	260.0	260.0	260.0	260.0	260.0
Soybean oil	25.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
L-lysine•HCl	2.7	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
DL-methionine	0.6	-	-	-	-	-	-	-	-
L-threonine	0.8	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
L-tryptophan	-	0.7	0.7	0.7	1.2	0.7	1.2	1.2	1.2
L-isoleucine	-	-	-	1.0	-	1.0	-	1.0	1.0
L-valine	-	-	1.0	-	-	1.0	1.0	-	1.0
L-glycine	-	2.5	1.5	1.5	2.0	0.5	1.0	1.0	-
Limestone	10.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Monocalcium phosphate	9.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin-mineral premix[1]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

[1]Provided the following quantities of vitamins and micro minerals per kg of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

[a] Crystalline amino acids were procured from Ajinomoto Health and Nutrition Inc., Itasca, IL, 60143 USA. L-lysine-HCL contained 780 g/kg lysine and 220 g/kg chloride. The other crystalline amino acids had a purity of 980 g/kg. DL-methionine was procured from Evonik Animal Health, Hanau, Germany and had a purity of 980 g/kg.

[b] Val, valine; Ile, isoleucine; Trp, tryptophan.

[c] The control diet was only used in Exp. 2.

All diets were formulated to be isoenergetic (14.0 mega joule metabolizable energy/kg) and to contain 10.0 g/kg SID lysine, which was assumed to be slightly above the SID Lys requirement for 25 to 50 kg pigs (NRC, 2012). Other indispensable AA, except the three BCAA and Trp, were included in all diets in excess of requirements (NRC, 2012). Glycine was included in all diets to maintain a constant concentration of dietary crude protein at 185 g/kg.

Pigs were individually housed in metabolism crates (0.81 × 2.59 m) that were equipped with a feeder and a nipple drinker. Throughout the 13-day experiment, pigs were fed at 2.8-times the energy requirement for maintenance (i.e., 0.82 mega joule/kg × body weight$^{0.60}$; NRC, 2012), which was provided each day in two equal meals at 0800 and 1600 h. The daily consumption of feed was recorded, and water was available at all times.

The initial five days were considered an adaptation period to the experimental diets. Urine and fecal samples were collected quantitatively from the feed provided during the following five days according to standard procedures for the marker to marker method (Adeola, 2001). In short, the start marker was included in the morning meal of day 6 and fecal collection was initiated when the marker first appeared in the feces. The stop marker was included in the morning meal of day 11 and fecal collections ceased when that marker appeared in the feces. Urine was collected in buckets with 50 mL of 3 *N* HCl as a preservative. Fecal samples and 100 g/kg of the collected urine were stored at − 20 °C immediately after collection. At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet.

In the morning of day 13, pigs were fed 400 g of their experimental diet and 2.5 h later, a blood sample was collected from each pig. Blood samples were collected from the jugular vein of all pigs using heparinized vacutainers (BD, Franklin Lakes, NJ, USA). All samples were centrifuged at 1500*g* at 4 °C for 15 min and plasma was collected and stored at − 80 °C until analyzed for PUN.

The apparent total tract digestibility (ATTD) of N in each experimental diet was calculated and retention of N for each pig was calculated as the ratio of retained N relative to N-intake (Pedersen et al., 2007). The biological value of protein in the diets was calculated by expressing the retention of N relative to the difference between N intake and N output in feces (Mitchell, 1924).

2.2. Exp. 2. Growth performance

A total of 288 growing pigs with an initial body weight of 28.6 ± 2.5 kg were divided into two blocks of 72 pigs and one block of 144 pigs and randomly assigned to nine dietary treatments in a randomized complete block design. There were two barrows and two gilts per pen (1.84 × 2.59 m) and eight replicate pens per treatment. Treatments included a control diet based on corn and soybean meal and the eight diets that were used in Exp. 1. The requirement for SID Leu for 25 to 50 kg pigs is 9.9 g/kg (NRC, 2012), but by adding 260 g/kg HPCP to the diet, the basal diet contained 1.7-times more SID Leu than the requirement (Table 2).

Pigs were housed in pens with partly slatted concrete floors. Each pen was equipped with a feeder and a nipple drinker. Pigs had free access to feed and water throughout the experiment. Daily feed allotments were recorded, and the weight of feed left in the feeders was recorded on the last day of the experiment to calculate feed consumption. Individual pig weights were recorded at the beginning and at the end of the experiment.

At the beginning of the experiment and on day 14, blood samples were collected from the jugular vein from one castrated male pig in each pen that had a body weight that was closest to the pen average at the start of the experiment using heparinized vacutainers (BD, Franklin Lakes, NJ, USA). At the end of the experiment, two blood samples were collected in heparinized vacutainers and vacutainers containing Ethylenediaminetetraacetic acid (EDTA; BD, Franklin Lakes, NJ, USA), respectively, from the same castrated male pig that was used for bleeding at the beginning of the experiment and on day 14. Blood samples were collected in the morning of the last day of the experiment and centrifuged at 1500*g* at 4 °C for 15 min and plasma was collected and stored at − 80 °C until analysis. After blood collection, all pigs from which blood samples had been collected were euthanized by electrocution. Brain tissue was removed, and the hypothalamus was isolated and collected into 2-mL cryogenic tubes and snap-frozen in liquid N. Samples were collected from the same spot in the hypothalamus and the same person collected all samples. All hypothalamus samples were stored at − 80 °C until analysis.

2.3. Sample analyses

Samples of corn, soybean meal, HPCP, and all experimental diets were analyzed for nitrogen by the Kjeldahl procedure (method 984.13; AOAC Int, 2007) using a Kjeltec 8400 apparatus (FOSS Inc., Eden Prairie, MN, USA), and crude protein was calculated as nitrogen × 6.25. Amino acids were analyzed in ingredients and diets [method 982.30 E (a, b, c); AOAC Int, 2007] using an Amino Acid Analyzer (model L-8800; Hitachi High Technologies America Inc., Pleasanton, CA, USA). Gross energy was determined using a bomb calorimeter (model 6400; Parr Instruments, Moline, IL, USA). Ingredient samples were also analyzed for dry matter (Method 930.15; AOAC Int, 2007), ash (Method 942.05; AOAC Int, 2007), acid hydrolyzed ether extract (Method AM 5–04; AOAC Int, 2007), and total dietary fiber (Method 991.43; AOAC Int, 2007) using standard procedures as described by Navarro et al. (2018).

The frozen fecal samples were dried in a forced-air drying oven at 55 °C until constant weight and ground for analysis. Fecal samples and thawed urine samples were analyzed for crude protein as explained for diets and feed ingredients. Plasma from blood in heparinized tubes was analyzed for PUN using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter Inc., Brea, CA, USA). Concentrations of BCAA and branched-chain α-keto acids (BCKA) in plasma from blood collected in EDTA tubes were measured by liquid chromatography-mass spectrometry analysis using a Sciex 5500 QTrap with an Agilent 1200 LC apparatus (AB Sciex, Framingham, MA, USA) according to the protocol described by Beals et al. (2016). Concentration of serotonin in the hypothalamus was analyzed using enzyme-linked immunoassay (ELISA) kits developed for porcine tissues according to the manufacturer's protocol (GenWay Biotech, Inc., San Diego, CA, USA). To obtain homogenates from the hypothalamus, frozen samples were weighed (0.5 g) and homogenized with buffer solution on ice using a handheld Tissue Tearor (Biospec Products, Inc., Bartlesville, OK, USA). The

homogenate was centrifuged at 15,000g at 4 °C for 30 min and the supernatant was used to determine the concentration of tissue-free serotonin in the hypothalamus.

2.4. Statistical analysis

Normality of data from Exp. 1 was verified and outliers were identified using the UNIVARIATE procedure (SAS Institute Inc, 2016). Data were analyzed using the PROC MIXED of SAS as a 2 × 2 × 2 factorial arrangement of treatments. The experimental unit was the pig and the model included SID Ile:lysine ratio, SID Val:lysine ratio, SID Trp:lysine ratio, and all possible interactions as fixed variables and block and replicate pen within block as random variables. Treatment means were calculated using the LSMEANS statement and if significant, means were separated using the PDIFF statement with the Tukey adjustment. Statistical significance was considered as $P < 0.05$.

In Exp. 2, data were summarized to calculate ADG, ADFI, and gain to feed ratio (G:F) for each pen of pigs at the conclusion of the experiment. Normality of data was verified, and outliers were identified using the UNIVARIATE procedure of SAS (SAS Institute Inc, 2016). Data were analyzed using the PROC MIXED of SAS with the pen as the experimental unit. The model included diet as fixed effect and block and replicate within block as random effects. Treatment means were calculated and separated as for Exp. 1. Statistical significance was considered at $P < 0.05$.

Table 3
Analyzed nutrient composition of experimental diets, as-fed basis, Exp. 1 and 2.

Item	Control diet[a]	High-protein corn product diets[b]							
		Basal	+ Val	+ Ile	+ Trp	+ Val and Ile	+ Val and Trp	+ Ile and Trp	+ Val, Ile, and Trp
Gross energy, mega joule/kg	16.5	16.9	16.9	17.0	17.0	17.1	17.1	17.0	17.1
Metabolizable energy[c], mega joule/kg	3418	3393	3399	3399	3396	3405	3402	3402	3408
Crude protein, g/kg	173.0	183.2	183.6	182.8	185.8	185.5	184.2	183.5	183.7
Indispensable amino acids, g/kg									
Arginine	10.8	8.3	8.0	7.9	7.7	7.7	7.7	7.4	7.8
Histidine	4.6	5.1	5.0	4.9	4.8	4.9	4.8	4.7	4.9
Isoleucine	7.6	7.8	7.8	8.3	7.4	8.6	7.5	8.3	8.4
Leucine	15.4	22.6	22.6	22.0	21.6	21.7	21.4	21.1	21.5
Lysine	11.1	12.7	11.6	11.8	12.2	12.3	12.7	11.5	12.3
Methionine	3.0	4.1	4.1	4.0	4.0	3.9	4.0	3.9	4.0
Phenylalanine	8.7	9.9	9.8	9.5	9.4	9.4	9.3	9.1	9.3
Threonine	7.0	8.3	8.0	8.0	7.8	8.0	7.8	7.7	7.9
Tryptophane	2.1	2.1	2.1	2.1	2.5	2.1	2.5	2.6	2.4
Valine	8.2	9.3	10.4	9.4	9.2	10.5	10.1	9.1	10.3
Dispensable amino acids, g/kg									
Alanine	9.1	13.8	13.7	13.4	13.2	13.2	13.1	12.8	13.2
Aspartate[d]	17.2	13.6	13.0	12.8	12.5	12.6	12.5	12.3	12.5
Cysteine	2.8	3.8	3.7	3.6	3.5	3.7	3.6	3.5	3.5
Glutamate[d]	31.0	32.5	32.4	31.6	31.0	31.0	30.9	30.1	30.5
Glycine	7.1	10.8	8.3	8.7	9.6	7.7	7.8	7.5	7.0
Proline	10.4	15.0	15.2	14.8	14.8	14.6	14.4	14.0	14.3
Serine	7.5	8.4	8.3	8.1	8.1	7.7	7.8	7.6	7.8
Tyrosine	5.9	7.5	7.3	7.2	7.0	6.8	6.9	6.7	6.9
Calculated values[e]									
Arginine	10.1	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Histidine	4.1	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Isoleucine	6.2	5.5	5.5	6.5	5.5	6.5	5.5	6.5	6.5
Leucine	12.7	17.1	17.1	17.1	17.1	17.1	17.1	17.1	17.1
Lysine	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methionine	2.9	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Phenylalanine	7.3	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Threonine	6.0	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1
Tryptophane	1.8	1.8	1.8	1.8	2.3	1.8	2.3	2.3	2.3
Valine	6.7	7.1	8.1	7.1	7.1	8.1	8.1	7.1	8.1

[a] The control diet was only used in Exp. 2.
[b] Val, valine; Ile, isoleucine; Trp, tryptophan.
[c] Values for metabolizable energy were calculated (NRC, 2012; Acosta et al., 2021).
[d] Aspartate includes aspartic acid and asparagine; Glutamate includes glutamic acid and glutamine.
[e] Values indicated as standardized ileal digestible amino acids (NRC, 2012; Acosta et al., 2021).

Table 4
Effects of dietary isoleucine, valine, and tryptophan on N balance and plasma urea N of growing pigs[a, b, c], as-fed basis, Exp. 1.

Val supplementation, g/kg:	0				1.0				SEM						
Ile supplementation, g/kg:	0		1.0		0		1.0			P-values[c]					
Trp supplementation, g/kg:	0	0.5	0	0.5	0	0.5	0	0.5		Val	Ile	Trp	Val × Ile	Val × Trp	Ile × Trp
Initial body weight, kg	34.1	34.0	34.1	34.1	33.5	34.0	33.8	34.0	-	-	-	-	-	-	-
Feed intake, g/5 days	6378	6342	6519	6519	6423	6515	6358	6301	132.8	0.654	0.910	0.999	0.198	0.843	0.751
N intake, g/5 days	187	189	191	191	189	192	189	185	3.9	0.779	0.989	0.839	0.206	0.815	0.465
Fecal N output, g/5 days	40.1	40.4	47.7	43.8	38.6	43.2	39.7	40.2	2.06	0.054	0.092	0.792	0.018	0.111	0.120
Urinary N output, g/5 days	41.8	37.6	41.6	40.5	43.3	36.9	38.9	43.3	2.75	0.920	0.537	0.344	0.919	0.675	0.080
ATTD[d] of N	0.79	0.78	0.75	0.77	0.80	0.78	0.79	0.78	0.114	0.092	0.117	0.804	0.117	0.125	0.271
N retention, g/5 days	105	111	101	107	107	112	110	102	3.3	0.436	0.121	0.344	0.989	0.105	0.133
N retention[e]	0.56	0.59	0.53	0.56	0.57	0.58	0.58	0.55	0.129	0.233	0.029	0.258	0.303	0.045	0.159
Biological value[e]	0.72	0.75	0.71	0.73	0.72	0.75	0.74	0.70	0.148	0.940	0.195	0.173	0.990	0.261	0.032
Plasma urea N, mg/dL	8.1	8.8	7.0	8.3	8.6	7.9	7.8	8.6	0.63	0.731	0.342	0.253	0.364	0.342	0.225

[a] The experimental unit was the metabolism crate and data are least square mean values of 9 replicate metabolism crates per treatment (1 castrated male pig per replicate metabolism crate).
[b] No interactions among Val, Ile, and Trp (V × I × T) were observed.
[c] Val, valine; Ile, isoleucine; Trp, tryptophan.
[d] ATTD, apparent total tract digestibility.
[e] N-retension was calculated as the ratio of retained N relative to consumed N. Biological value was the ratio of retained N relative to absorbed N (Mitchell, 1924).

3. Results

3.1. Exp. 1. Nitrogen balance

During the adaptation period, one pig died and was excluded from analysis. All other animals remained healthy and easily consumed their diets without apparent problems. Crude protein and AA concentrations in corn and the HPCP were in agreement with expected values. Analyzed values for lysine and BCAA in all diets were also in agreement with formulated values (Table 3).

There were no 3-way interactions among main effects, and feed intake and N intake were not different among treatments (Table 4). Fecal N output increased if Ile was added to diets without added Val, but that was not the case if Val was added to the diet (interaction, $P < 0.05$). Urine N excretion (g/5 days) tended to be reduced if Trp was added to diets with no added Ile, but if Ile was added, Trp did not tend to reduce N output in urine (interaction, $P < 0.10$). The ATTD of N tended ($P < 0.10$) to increase with added Val in diets. Addition of Ile to diets reduced (interaction, $P < 0.05$) N retention, and N retention increased with Trp addition to diets without Val addition, but not in diets with added Val (interaction, $P < 0.05$). The biological value of protein increased if Trp was added to diets without addition of Ile, but if Ile was added, Trp addition did not increase the biological value of protein (interaction, $P < 0.05$).

3.2. Exp. 2. Growth performance

All animals were healthy and readily consumed their assigned diets throughout the experiment. Final body weight and ADG of pigs fed the control diet were greater ($P < 0.05$) than for pigs fed all other diets except the pigs fed the diet with added Val and Trp (Table 5).

There was no difference among dietary treatments for PUN on day one or day 14, but on day 28, PUN of pigs fed the control diet was greater ($P < 0.05$) than that of pigs fed all other diets except the diet supplemented with Trp (Table 6). Pigs fed the basal diet had lower ($P < 0.05$) plasma free Trp compared with pigs fed all other diets except for pigs fed the diets with added Val, Ile, or Val and Ile.

Concentration of plasma α-keto isovalerate (KIV) was greater ($P < 0.05$) in pigs fed the control diet than in pigs fed the basal diet or diets with added Ile, Trp, or Ile and Trp. Concentration in plasma of α-keto β-methylvalerate (KMV) was greater ($P < 0.05$) in pigs fed the control diet than in pigs fed all other diets. Plasma free Val in pigs fed the control diets was not different from that of pigs fed diets with added Val, Val and Ile, Val and Trp, or Val, Ile, and Trp, but greater ($P < 0.05$) than that of pigs fed the other diets.

4. Discussion

The objective of this research was to test the hypothesis that increasing concentrations of dietary Val, Ile, or Trp, independently or in combination, in diets containing excess Leu from HPCP may mitigate negative effects on efficiency of AA utilization and growth performance of growing pigs. Therefore, the basal diet was formulated to contain dietary Leu well above the SID Leu requirement (NRC, 2012). A SID Val:lysine ratio of 0.70:1, a SID Ile: lysine ratio of 0.53:1, and a SID Trp:lysine ratio of 0.18:1 were used in the formulation of the basal diet, which were believed to be the optimal ratios of SID Val:lysine, SID Ile:lysine, and SID Trp:lysine to maximize growth performance of pigs (NRC, 2012; van Milgen et al., 2012, 2013). The fact that SID lysine was calculated to be 10 g/kg ensured that inclusions of Ile, Val, and Trp in the control diet were at least at the requirement. The HPCP used in the current research has greater concentrations of crude protein, lysine, and methionine, but contains less fat and fiber, compared with distillers dried grains with solubles that is usually produced by the ethanol industry (NRC, 2012; Kim et al., 2013; Espinosa and Stein, 2018).

Results from Exp. 1 indicating that there was no effect on N-balance of adding dietary Val, Ile, or Trp to excess Leu diets is likely a result of the fact that all pigs were fed similar amount of isonitrogenous diets. However, this is in contrast with results of previous research indicating that addition of Trp to a high-Leu diet increased feed intake, and therefore, N balance (Kwon et al., 2022). It is

Table 5
Effects of dietary isoleucine, valine, and tryptophan on growth performance of growing pigs[1, 2, 3], as-fed basis, Exp. 2.

Item	Control	High-protein corn product diets								SEM	P-value
		Basal	+ Val	+ Ile	+ Trp	+ Val and Ile	+ Val and Trp	+ Ile and Trp	+ Val, Ile, and Trp		
Body weight, kg											
Day 1	28.7	28.5	28.7	28.7	28.4	28.5	28.5	28.7	28.5	0.83	0.969
Day 28	55.3[a]	50.7[b]	51.0[b]	50.7[b]	50.7[b]	50.2[b]	52.6[ab]	50.0[b]	51.3[b]	1.35	< 0.001
ADG, g/day[4]	950[a]	793[b]	797[b]	785[b]	797[b]	776[b]	862[ab]	760[b]	813[b]	28.8	< 0.001
ADFI, g/day[4]	1816[a]	1621[b]	1650[ab]	1623[b]	1704[ab]	1617[b]	1758[ab]	1683[ab]	1695[ab]	57.7	0.034
G:F[2]	0.53	0.49	0.48	0.48	0.47	0.48	0.49	0.45	0.48	0.015	0.079

[1] The experimental unit was the pen and data are least square mean values of 8 replicate pens per treatment (2 barrows and 2 gilts per replicate pen).
[2] Means within a row without a common superscript letter differ ($P < 0.05$).
[3] Val, valine; Ile, isoleucine; Trp, tryptophan.
[4] ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 6
Effects of dietary isoleucine, valine, and tryptophan on plasma urea N, hypothalamic serotonin, and plasma free Trp of growing pigs,[1, 2, 3] as-fed basis, Exp. 2.

Item	Control	High-protein corn product diets							SEM	P-value
		Basal	+ Val	+ Ile	+ Trp	+ Val and Ile	+ Val and Trp	+ Ile and Trp	+ Val, Ile, and Trp		
PUN[4], mg/dL											
Day 1	9.0	7.6	8.3	8.3	8.0	7.4	8.3	8.0	8.1	0.45	0.486
Day 14	7.9	8.0	8.9	7.6	7.5	8.5	8.8	7.8	7.4	0.71	0.773
Day 28	12.0^{a}	8.1^{b}	8.1^{b}	8.6^{b}	8.8^{ab}	7.6^{b}	8.3^{b}	8.3^{b}	7.8^{b}	0.76	0.004
HS[4], μg/mL	0.193	0.149	0.178	0.183	0.191	0.180	0.201	0.183	0.188	0.0126	0.070
Trp, μmol/L	60.5^{ab}	28.4^{c}	40.2^{bc}	43.9^{bc}	61.6^{ab}	45.9^{bc}	79.9^{a}	62.3^{ab}	59.4^{ab}	7.04	< 0.001

[1] The experimental unit was the pen and data are least square mean values of 8 replicate pens per treatment (1 barrow per replicate pen).
[2] Means within a row without a common superscript letter differ ($P < 0.05$).
[3] Val, valine; Ile, isoleucine; Trp, tryptophan.
[4] PUN, plasma urea N; HS, hypothalamic serotonin.

Table 7
Effects of dietary isoleucine, valine, and tryptophan on branched-chain α-keto acids (BCKA) and branched-chain amino acids (BCAA) in plasma of growing pigs[1,2,3], as-fed basis, Exp. 2.

Item	Control	High-protein corn product diets							SEM	P-value
		Basal	+ Val	+ Ile	+ Trp	+ Val and Ile	+ Val and Trp	+ Ile and Trp	+ Val, Ile, and Trp		
BCKA, μg/mL											
KIV[4]	0.76^{a}	0.38^{c}	0.63^{abc}	0.40^{bc}	0.43^{bc}	0.57^{abc}	0.65^{ab}	0.46^{bc}	0.60^{abc}	0.078	< 0.001
KMV[4]	2.04^{a}	0.71^{b}	0.63^{b}	1.21^{b}	0.78^{b}	1.14^{b}	0.72^{b}	1.26^{b}	1.11^{b}	0.164	< 0.001
KIC[4]	10.1	12.6	12.2	13.3	11.9	11.3	12.2	11.9	12.6	0.98	0.949
BCAA, μmol/L											
Val	398^{a}	173^{d}	332^{abc}	218 ^{cd}	246^{bcd}	353^{ab}	368^{ab}	229 ^{cd}	324^{abc}	27.1	< 0.001
Ile	282	202	213	226	185	236	242	183	240	40.1	0.244
Leu	402	463	489	496	416	495	540	434	507	77.4	0.534

[1]The experimental unit was the pen and data are least square mean values of 8 replicate pens per treatment (1 barrow per replicate pen).
[2]Means within a row without a common superscript letter differ ($P < 0.05$).
[3]Val, valine; Ile, isoleucine; Trp, tryptophan.
[4]KIV = α-keto isovalerate; KMV = α-keto-β-methylvalerate; KIC = α-keto isocaproate.

possible that the reason for the difference between the two experiments is that the excess Leu in the previous experiment was provided by crystalline L-Leu, which likely was rapidly absorbed. In contrast, the excess Leu in the current experiment was provided by intact corn protein, which may have been more slowly hydrolyzed and AA may, therefore, have been more slowly absorbed. In addition, dietary Leu was three times greater than the requirement for SID Leu in the previous experiment, but only 1.7-times greater than the requirement for SID Leu in the current experiment.

All three BCAA share not only the enzymes that are involved in their catabolism in skeletal muscle and liver (Harris et al., 2005), but also the AA transport system for absorption from the small intestine (Bröer, 2008). The AA transporter B^0 AT1 is Na^+-dependent transport system, which is the major transporter of BCAA, is located in the apical membrane of the enterocyte (Bröer et al., 2004). The large neutral AA are transported via the B^0 AT1 transporter with different affinities, but all three BCAA are transported with similar affinities (Bröer, 2008). As a consequence, if one of the BCAA is provided in excess, absorption of the other two BCAA may be reduced. However, excess dietary BCAA affects expression of the B^0 AT1 transporter in jejunum and ileum of pigs (Cervantes et al., 2015). Excess Leu by itself does not affect expression of the B^0 AT1 transporter, but combined excesses of all three BCAA appear to stimulate its expression in the jejunum and ileum (Cervantes et al., 2015). Therefore, the changes in fecal N output that were observed as an interactive effect between adding Val and Ile to the diet is most likely a result of increased competition for absorption in the small intestine. This may also partly explain the tendency for increased ATTD of N that was observed as dietary Val increased, and the decreased retention of N that occurred as dietary Ile increased.

Plasma urea N is often used as a response criteria in AA requirement studies, because PUN responds rapidly to changes in dietary AA concentration and to changes in the efficiency of AA utilization in pigs (Coma et al., 1995). The increased PUN that was observed as dietary Leu increased is likely a result of increased catabolism of Ile and Val, which in turn reduces availability of these AA and causes an imbalance among other indispensable AA (Gatnau et al., 1995). A deficiency in Ile and Val also may have reduced protein synthesis as indicated by the reduced N retention, which may have resulted in increased deamination of other AA and a subsequent increase in

PUN (Kwon et al., 2019). Adding dietary Val to diets with excess Leu reduced PUN, but no effect of Ile supplementation on PUN was observed regardless of dietary Leu concentration (Kwon et al., 2019). This observation indicates that Val addition is more beneficial than Ile if diets with excess Leu are used, but current data indicated that there were no effects on PUN of adding dietary Val, Ile, or Trp to a diet containing 1.7-times more SID Leu than required.

Results of studies with pigs fed diets containing corn protein from the ethanol industry indicated that growth performance was negatively affected by the use of corn protein if a poor quality of the ingredient was used or if excess quantities of corn protein was fed to young pigs (Stein and Shurson, 2009; Woyengo et al., 2014). In general, the use of corn protein is restricted in hte diets for pigs because the relatively high fiber concentration in most corn-co products contributes to reduced digestibility of amino acids and energy by pigs (Woyengo et al., 2014). Recently, Yang et al. (2019) indicated that excess dietary Leu that was supplied by high inclusion of corn protein may contribute to a reduced ADG and ADFI of weanling pigs. Leucine stimulates catabolism of BCAA in skeletal muscle and liver (Harper et al., 1984). If diets fed to pigs contain excess Leu, catabolism of all three BCAA may increase because of the stimulating effect of the Leu metabolite, α-keto isocaproate (KIC), on the branched-chain α-keto acid dehydrogenase enzyme complex (Wiltafsky et al., 2010). The greater final body weight and ADG of pigs fed the control diet than of pigs fed the basal diet is likely a result of the imbalanced supply of BCAA that may have resulted from increased catabolism of Val and Ile by excess Leu (Wiltafsky et al., 2010; Kwon et al., 2019). The increased final body weight and ADG for pigs fed diets with Val and Trp addition to the basal diet that was observed indicates that Val and Trp supplementation partially overcame the negative impact on growth that was caused by excess dietary Leu. It is, however, also possible that reduced palatability contributed to the reduced growth performance of pigs fed the diets containing HPCP because pigs prefer to eat corn-soybean meal diets rather than diets containing distillers dried grains with solubles (Kim et al., 2012). However, we are not aware of preference tests determining the impact of feed intake of pigs fed a diet containing HPCP relative to a corn-soybean meal diet.

The reason for the increased PUN on day 28 in pigs fed the control diet compared with pigs fed the HPCP diets is likely a result of greater ADFI for pigs fed the control diet than for pigs fed some of the HPCP diets. The concentration of PUN is mostly dependent on the quantities and balance of AA that are absorbed (Nyachoti et al., 2006). The observation that adding Trp, either alone or in combination with Ile or Val, or Ile and Val, to the diets increased plasma free Trp indicates that there is a positive correlation between dietary Trp concentration and plasma free Trp, which has also been observed previously (Kwon et al., 2022).

The first step in the catabolism of BCAA, which is catalyzed by BCAA aminotransferase, produces α-keto isovalerate (KIV), α-keto-β-methylvalerate (KMV), and KIC, from Val, Ile, and Leu, respectively (Harris et al., 2005). If excess Leu is included in diets for pigs, the catabolism of all three BCAA may increase because of increased activities of BCAA aminotransferase. However, the greater activity of the transamination enzyme may produce more KIC, which activates the branched-chain α-keto acid dehydrogenase complex and changes concentrations of BCKA in plasma. Therefore, the reason for the low concentrations of plasma free Val and KIV that were observed in pigs fed the basal diet may be an increased catabolism of Val that is caused by excess dietary Leu. Likewise, the reason pigs fed the control diet had greater plasma KMV than pigs fed all diets containing HPCP may have been increased catabolism of Ile caused by excess dietary Leu in the basal diet. The reduced concentrations of KIV and KMV that were observed in pigs fed HPCP diets are also in agreement with previous data (Langer et al., 2000; Wiltafsky et al., 2010). It is likely that the increased stimulation of the branched-chain α-keto acid dehydrogenase enzyme complex, which was the result of the elevated concentration of KIC, increased decarboxylation of KIV and KMV, which resulted in the reduced KIV and KMV concentrations.

5. Conclusion

Adding approximately 0.20 times more isoleucine than the requirement to a diet with excess leucine reduced N retention, but if approximately 0.25 times more tryptophan than the requirement was added alone or in combination with isoleucine and valine, N retention increased. Adding both tryptophan and approximately 0.15 times more valine than the requirement to a high-leucine diet also prevented detrimental effects of excess leucine on growth performance of pigs. Thus, it appears that the successful use of corn protein in diets for growing pigs requires additional valine and possibly tryptophan in the diets, whereas there does not appear to be an advantage of including isoleucine above the requirement.

Declaration of Competing Interest

At the time the experiments were conducted, JAS was an employer at Ajinomoto Health and Nutrition, Inc., Itasca, IL, which is a global supplier of crystalline AA to livestock diets. WBK and HHS have no conflicts of interest.

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Author contributions

HHS and WBK conceptualized the experiment. WBK conducted the experiment and summarized data. HHS and JS contributed with data interpretation. WBK wrote the first draft of the manuscript. HHS and JS edited the final version of the manuscript. HHS supervised the project.

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