Effects of amino acid biomass or feed-grade amino acids on growth performance of growing swine and poultry[1,2]

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ABSTRACT: Three experiments were conducted to determine the effect of three fermented amino acids (AA) with their respective biomass compared to crystalline AA on the growth performance of swine and poultry. In experiment 1, 315 barrows (DNA 200 × 400, initially 11.3 ± 0.69 kg) were allotted to 1 of 4 dietary treatments with 5 pigs per pen and 15 or 16 pens per treatment. Dietary treatments included a negative control (16% standardized ileal digestible [SID] Tryptophan:lysine [Trp:Lys] ratio), positive control (21% SID Trp:Lys ratio from crystalline Trp), or diets containing Trp with biomass to provide 21 or 23.5% SID Trp:Lys ratios, respectively. Pigs fed the positive control or low Trp with biomass diet had increased ($P < 0.05$) ADG compared to pigs fed the negative control diet, with pigs fed the high Trp with biomass diet intermediate. Pigs fed the low Trp with biomass diet had increased ($P < 0.05$) G:F compared to the negative control diet, with others intermediate. In experiment 2, 1,320 1-d-old male broilers (Cobb 500, initially 45.2 g) were allotted to one of four dietary treatments with 33 birds per pen and 10 pens per treatment. Dietary treatments included a negative control (58/58% Threonine:lysine [Thr:Lys] ratio), positive control (65/66% Thr:Lys ratio from crystalline Thr), or diets containing Thr with biomass to provide 65/66 or 69/70% Thr:Lys ratios in starter and grower diets, respectively. Broilers fed the positive control or Thr with biomass diets had increased ($P < 0.05$) ADG compared to broilers fed the negative control diet. Broilers fed the positive control or the low Thr with biomass diet had increased ($P < 0.05$) G:F compared to the negative control and high Thr with biomass treatments. In experiment 3, 2,100 one-day-old male broilers (Cobb 500, initially 39.4 g) were allotted to one of four dietary treatments with 35 birds per pen and 15 pens per treatment. Dietary treatments included a negative control (59/63% Valine:lysine [Val:Lys] ratio), positive control (75/76% Val:Lys ratio from crystalline Val), or diets containing Val with biomass to provide 75/76 or 84/83% Val:Lys ratios in starter and grower diets, respectively. Broilers fed the positive control or Val with biomass diets had increased ($P < 0.05$) ADG, ADFI, and G:F compared to those fed the negative control diet. In conclusion, Trp, Thr, or Val with their respective biomass appear to be equally bioavailable and a suitable alternative to crystalline AA in swine and poultry diets.

Key words: biomass, pigs, poultry, threonine, tryptophan, valine

[1]Contribution no. 20-033-J of the Kansas Agricultural Experiment Station, Manhattan, KS 66506-0201.

[2]The authors would like to thank CJ America – Bio for providing partial financial support for these studies.

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Received August 2, 2019.
Accepted October 6, 2019.

Transl. Anim. Sci. 2020.4:49–58
doi: 10.1093/tas/txz163

INTRODUCTION

Crystalline amino acids (AA) are often used as a replacement for intact protein sources, such as soybean meal, in swine and poultry diets. This allows for a reduction in crude protein (CP) concentration which has both environmental and economic benefits (Miranda et al., 2015).

The synthesis of crystalline, or feed-grade, AA occurs through the fermentation of bacteria in a culture medium containing carbon, nitrogen, sulfur and phosphorus sources (Leuchtenberger et al., 2005). Due to advances in genome sequencing, most AA can be produced using mutant strains of *Corynebacterium glutamicum* or *Escherichia coli* (Leuchtenberger et al., 2005). Following fermentation, the first step of purification is the separation of the AA from the fermented biomass (Herman, 2003). Through this process, residual amounts of CP and indispensable AA are retained in the biomass, which results in a nutrient-rich byproduct that often ends up as waste (Almeida et al., 2014). Using the AA rich biomass, before extraction of the specific AA, in place of crystalline sources is a viable option because of its opportunity to decrease manufacturing inputs while still providing an AA rich product (Herman, 2003). Lysine sulfate containing 54.6% L-lysine with fermented biomass is commonly used in swine and poultry diets (Schutte et al., 1994). The relative bioavailability of free lysine from lysine sulfate with fermented biomass has been shown to be equivalent to L-Lysine-HCl (Smiricky-Tjardes et al., 2004; Htoo et al., 2016).

Recently, fermented Tryptophan (Trp), Threonine (Thr), and Valine (Vale) biomass products (CJ America – Bio, Downers Grove, IL) have been developed for use in livestock and poultry diets. However, there is limited research available to determine their effectiveness as AA sources for growing pigs and broilers. Therefore, our objective was to compare the effect of each fermented AA with its respective biomass compared to the crystalline AA form on the growth performance of swine and poultry.

MATERIALS AND METHODS

Experiment 1: Tryptophan

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. Each pen contained a four-hole, dry self-feeder and cup waterer for ad libitum access to feed and water.

A total of 315 barrows (Line 200 × 400, DNA, Columbus, NE, initially 11.3 kg) were used in a 21 d growth trial. Pigs were weaned at approximately 21 d of age and following arrival to the research facility, were randomized to pens based on initial BW and fed common starter diets for 21 d. On day 21 after weaning, considered day 0 of the study, pigs were weighed and pens were allotted to 1 of 4 dietary treatments with five pigs per pen and 15 or 16 pens per treatment. Dietary treatments consisted of a negative control (16% standardized ileal digestible [SID] Trp:Lys ratio), positive control (21% SID Trp:Lys ratio from crystalline Trp), or diets containing Trp with biomass to provide 21 or 23.5% SID Trp:Lys ratios (0.104 or 0.156% added biomass, respectively). Diets were corn-soybean meal-based and formulated to contain 1.25% SID Lys. Other AA provided by the Trp biomass were not considered in diet formulation. Ingredient nutrient profiles and SID digestibility coefficients were used from NRC (2012). Diets were formulated to be slightly below or at requirement estimates based on previous data with pigs in this facility so as not to under-estimate the Trp:Lys ratio (Clark et al. 2017a, 2017b). The Trp with biomass (CJ America – Bio, Downers Grove, IL) had a granulated, cream-colored appearance and contained 60% Trp (assumed to have 100% SID coefficient) per the supplier's specifications.

All dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS and were formulated to meet or exceed NRC (2012) requirement estimates for nutrients other than Lys and Trp (Table 1). Samples of complete diets were collected during bagging of experimental diets with

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

	Tryptophan source			
	None	Crystalline	Biomass	
Item SID[2] tryptophan:lysine, %:	16	21	21	23.5
Ingredient, %				
Corn	69.51	69.44	69.40	69.34
Soybean meal	25.43	25.44	25.44	25.44
Choice white grease	1.00	1.00	1.00	1.00
Calcium carbonate	0.65	0.65	0.65	0.65
Monocalcium phosphate	1.20	1.20	1.20	1.20
Sodium chloride	0.60	0.60	0.60	0.60
L-Lysine-HCl	0.55	0.55	0.55	0.55
DL-Methionine	0.19	0.19	0.19	0.19
L-Threonine	0.28	0.28	0.28	0.28
L-Tryptophan	—	0.06	—	—
L-Valine	0.15	0.15	0.15	0.15
L-Isoleucine	0.04	0.04	0.04	0.04
Trace mineral premix[3]	0.15	0.15	0.15	0.15
Vitamin premix[4]	0.25	0.25	0.25	0.25
Phytase[5]	0.02	0.02	0.02	0.02
Tryptophan biomass[6]	—	—	0.104	0.156
Total	100	100	100	100
Calculated analysis				
SID Lys, %	1.25	1.25	1.25	1.25
Total Lys, %	1.38	1.38	1.38	1.38
Total Trp, %	0.22	0.29	0.29	0.32
SID amino acid ratios				
Ile:lys	57	57	57	57
Leu:lys	111	111	111	111
Met:lys	36	36	36	36
Met & cys:lys	57	57	57	57
Thr:lys	65	65	65	65
Trp:lys	16	21	21	23.5
Val:lys	70	70	70	70
His:lys	34	34	34	34
ME, kcal/kg	3,333	3,336	3,329	3,329
NE, kcal/kg	2,502	2,502	2,500	2,498
SID Lys:NE, g/Mcal	5.00	4.99	5.00	5.00
Crude protein, %	18.8	18.9	18.8	18.8
Calcium, %	0.72	0.72	0.72	0.72
Phosphorus, %	0.62	0.62	0.62	0.62
Available phosphorus, %	0.42	0.42	0.42	0.42
STTD P, %[7]	0.46	0.46	0.46	0.46

[1]Diets were fed for 21 d from approximately 11 to 23 kg BW.

[2]Standardized ileal digestible.

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

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

[5]Ronozyme HiPhos 2700 (DSM Nutrition Products, Parsippany, NJ) provided 405 FTU per kg of feed.

[6]CJ America – Bio, Downers Grove, IL.

[7]Standardized total tract digestible phosphorus.

a subsample collected from every fourth bag and pooled into one homogenized sample per dietary treatment. Samples were stored at −20°C until they were subsampled and submitted for analysis (Eurofins Scientific Inc., Des Moines, IA) of complete AA profile (excluding Trp and methionine, method 994.12; AOAC International, 2012), Trp (method 988.15; AOAC International, 2012), and methionine (method 994.12; AOAC International, 2012). Diet samples were also analyzed for CP (method 990.03; AOAC International, 1990).

Data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Weight block was included in the model as a random effect. LS Means were applied to estimate the effects of Trp source and level. Results were considered significant at $P \leq 0.05$.

Experiment 2 and 3: Threonine and Valine

The Texas A&M University Institutional Animal Care and Use Committee approved the protocol used in these experiments. The studies were conducted at the Texas A&M University Poultry Research Facility in College Station, TX. Each pen contained a dry tube feeder and four nipple waterers for ad libitum access to feed and water. Minimum ventilation was run to supply necessary air exchange. Additionally, all birds were raised on used litter from two previous flocks.

In experiment 2, a total of 1,320 one-day-old male broilers (Cobb 500, initially 45.2 g) were used in a 28 d growth study. Upon arrival to the research facility, birds were randomized to pens based on weight and allotted to 1 of 4 dietary treatments with 33 birds per pen and 10 pens per treatment. Dietary treatments were fed in two phases. The starter phase was fed from days 0 to 14 and the grower phase was fed from days 14 to 28. Starter diets consisted of a negative control (58% Thr:Lys ratio), positive control (65% Thr:Lys ratio from crystalline Thr), or diets containing Thr with biomass to provide 65% or 69% Thr:Lys ratios (included at 0.117% or 0.175% of the diet, respectively). Grower diets consisted of a negative control (58% Thr:Lys ratio), positive control (66% Thr:Lys ratio from crystalline Thr), or diets containing Thr with biomass to provide 66% or 70% Thr:Lys ratios (0.113% or 0.170% added biomass, respectively). Diets were corn-soybean meal-based and formulated to contain 1.18 or 1.05% apparent digestible Lys in the starter and grower diets, respectively. Ingredient nutrient profiles and apparent digestibility coefficients were used from NRC (1994). The Thr with biomass (CJ America – Bio, Downers Grove, IL) had a granulated, brown cream-colored appearance and contained 75% Thr (assumed to have 100% digestibility coefficient) per the supplier's specifications.

In experiment 3, a total of 2,100 one-day-old male broilers (Cobb 500, initially 39.4 g) were used in a 28 d growth study. Upon arrival to the research facility, birds were randomized to pens based on weight and allotted to one of four dietary treatments with 35 birds per pen and 15 pens per treatment. Dietary treatments were fed in two phases. The starter phase was fed from days 0 to 14 and the grower phase was fed from days 14 to 28. Starter diets consisted of a negative control (59% Val:Lys ratio), positive control (75% Val:Lys ratio from crystalline Val), or diets containing Val with biomass to provide 75 or 84% Val:Lys ratios (0.28% or 0.41% added biomass, respectively). Grower diets consisted of a negative control (63% Val:Lys ratio), positive control (76% Val:Lys ratio from crystalline Val), or diets containing Val with biomass to provide 76% or 83% Val:Lys ratios (0.20% or 0.30% added biomass, respectively). Diets were corn-soybean meal-based and formulated to contain 1.18% or 1.05% apparent digestible Lys in the starter and grower diets, respectively. Ingredient nutrient profiles and apparent digestibility coefficients were used from NRC (1994). The Val biomass (CJ America – Bio, Downers Grove, IL) had a granulated, dark brown colored appearance and contained 70% Val (assumed to have 100% digestibility coefficient) per the supplier's specifications.

All dietary treatments for experiments 2 and 3 were manufactured at Texas A&M University in College Station, TX and were formulated to meet or exceed the Cobb (2017) recommendations (Tables 2 and 3). Other AA provided by the Thr or Val biomass were not considered in diet formulation. Diets were mixed in a horizontal mixer then conditioned for 15 s prior to pelleting at 75 °C. Starter diets were crumbled following pelleting. Complete diet samples were taken during bagging of experimental diets with five 1 kg grab samples collected and pooled into one homogenized sample per dietary treatment. Samples were stored at −20 °C until they were split into three equal samples per dietary treatment and submitted for analysis (ATC Scientific, North Little Rock, AR) of complete AA profile (excluding Trp and methionine, method 994.12; AOAC International, 2012), Trp (method 988.15; AOAC International, 2012), and methionine (method 994.12; AOAC International, 2012). Diet samples were also analyzed for CP (method 990.03; AOAC International, 1990).

Data were analyzed as a randomized complete block design using the GLM procedure of SPSS Statistics version 24.0 (IBM Corporation, Armonk, NY) with pen as the experimental unit. Weight block was included in the model as a random effect. Duncan's Multiple Range Test were applied to

Table 2. Male broiler starter and grower diet composition, experiment 2 (as-fed basis)[1]

	Experimental diet	
Item	Starter[2]	Grower[3]
Ingredient, %		
Corn	61.00	66.20
Soybean meal	33.15	27.95
Soybean oil	1.52	1.72
Limestone	1.33	1.27
Monocalcium phosphate	1.61	1.51
Sodium chloride	0.46	0.46
L-Lysine-HCl	0.23	0.23
DL-Methionine	0.30	0.27
L-Threonine	+/-	+/-
Trace mineral premix[4]	0.05	0.05
Vitamin premix[5]	0.13	0.13
Salinomycin – SaCox[6]	0.05	0.05
Cellulose, filler[7]	0.18	0.17
Threonine biomass[8]	+/-	+/-
Total	100	100
Calculated analysis		
Apparent digestible Lys, %	1.18	1.05
Apparent digestible Thr, %	0.68	0.61
Apparent digestible amino acid ratios		
Meth:lys	49	50
Meth & cys:lys	74	76
Arg:lys	108	107
Thr:lys	58	58
Val:lys	75	76
AME[9], kcal/kg	3,036	3,102
Crude protein, %	21.04	18.9
Calcium, %	0.90	0.84
Total phosphorus, %	0.69	0.65
Sodium, %	0.19	0.19

[1]Starter diets were fed from days 0 to 14 from approximately 45.2 to 458 g BW and grower diets were fed from days 14 to 28 from approximately 458 g to 1.55 kg BW.

[2]Starter diets consisted of a negative control (58% Thr:Lys ratio), positive control to provide 65% Thr:Lys ratio from crystalline Thr (0.088% added crystalline Thr), or diets containing Thr with biomass to provide 65% or 69% Thr:Lys ratios (0.117% or 0.175% added biomass, respectively).

[3]Grower diets consisted of a negative control (58% Thr:Lys ratio), positive control to provide 66% Thr:Lys ratio from crystalline Thr (0.085% added crystalline Thr), or diets containing Thr with biomass to provide 66% or 70% Thr:Lys ratios (0.113% or 0.170% added biomass, respectively).

[4]Trace mineral premix per kg of diet: 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[5]Vitamin premix per kg of diet: 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin.

[6]Active drug ingredient salinomycin sodium, 132 g/kg (66 mg/kg inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunette*, and *Eimeria mivati*.

[7]Cellulose, filler was used to equalize the addition of crystalline Thr and Thr biomass across experimental diets.

[8]CJ America – Bio, Downers Grove, IL.

[9]Apparent metabolizable energy.

Table 3. Male broiler starter and grower diet composition, experiment 3 (as-fed basis)[1]

	Experimental diet	
Item	Starter[2]	Grower[3]
Ingredient, %		
Corn	69.53	72.76
Soybean meal	23.46	21.00
Soybean oil	0.55	0.70
Limestone	1.40	1.36
Monocalcium phosphate	1.61	1.49
Sodium chloride	0.45	0.45
L-Lysine-HCl	0.49	0.40
L-Methionine	0.42	0.35
L-Threonine	0.26	0.20
L-Tryptophan	0.02	0.02
L-Isoleucine	0.21	0.16
L-Arginine	0.29	0.21
L-Valine	+/-	+/-
Trace mineral premix[4]	0.05	0.05
Vitamin premix[5]	0.13	0.13
Salinomycin – SaCox[6]	0.05	0.05
Cellulose, filler[7]	0.41	0.30
Choline chloride	0.05	0.05
Copper sulfate	0.05	0.05
Glycine	0.39	0.27
Valine biomass[8]	+/-	+/-
Total	100	100
Calculated analysis		
Apparent digestible Lys, %	1.18	1.05
Apparent digestible Val, %	0.70	0.66
Apparent digestible amino acid ratios		
Met:lys	58	58
Met & cys:lys	84	77
Arg:lys	107	107
Thr:lys	69	70
Val:lys	59	63
AME[9], kcal/kg	3,042	3,086
Crude protein, %	19.25	17.70
Calcium, %	0.88	0.84
Total phosphorus, %	0.44	0.42
Sodium, %	0.18	0.18

[1]Starter diets were fed from days 0 to 14 from approximately 39.4 g to 0.44 kg BW and grower diets were fed from days 14 to 28 from approximately 0.44 to 1.64 kg BW.

[2]Starter diets consisted of a negative control (59% Val:Lys ratio), positive control to provide 75% Val:Lys ratio from crystalline Val (0.20% added crystalline Val), or diets containing Val with biomass to provide 75% or 84% Val:Lys ratios (0.28 or 0.41% added biomass, respectively).

[3]Grower diets consisted of a negative control (63% Val:Lys ratio), positive control to provide 76% Val:Lys ratio from crystalline Val (0.15% added crystalline Val), or diets containing Val with biomass to provide 76% or 83% Val:Lys ratios (0.20 or 0.30% added biomass, respectively).

[4]Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[5]Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg

Table 3. Contined

riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, and 0.08 mg biotin per kg diet.

[6]Active drug ingredient salinomycin sodium, 132 g/kg (66 mg/kg inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by *Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti*, and *Eimeria mivati*.

[7]Cellulose, Filler (wt:wt) was used to equalize the addition of crystalline Thr and Thr biomass across experimental diets.

[8]CJ America – Bio, Downers Grove, IL.

[9]Apparent metabolizable energy.

estimate the effects of Thr or Val source and level. Mortality was transformed using arcsine prior to statistical analysis. Results were considered significant at $P \leq 0.05$.

RESULTS

Experiment 1: Tryptophan

Analysis of manufactured diets (Table 4) resulted in Trp values consistent with diet formulation, as the diet with no crystalline AA had the lowest level of analyzed Trp while the addition of either fermented Trp with biomass or crystalline Trp increased the total analyzed dietary Trp concentration.

Overall (days 0 to 21) pigs fed the 21% Trp:Lys ratio from crystalline Trp or Trp with biomass had increased ($P < 0.05$) ADG compared to those fed the negative control diet, with pigs fed the 23.5% SID Trp:Lys ratio with biomass intermediate (Table 5). There was no evidence for difference in overall ADFI, but pigs fed the 21% Trp:Lys ratio from Trp with biomass had improved ($P < 0.05$) G:F compared to those fed the negative control diet with the others intermediate.

Experiment 2: Threonine

Analysis of manufactured diets (Table 6) resulted in Thr values consistent with diet formulation, as the diet with no crystalline AA had the lowest level of analyzed Thr while the addition of either fermented Thr with biomass or crystalline Thr increased the total analyzed dietary Thr concentration.

Overall (days 0 to 28) broilers fed diets containing crystalline Thr and the two diets containing increasing Thr with biomass had increased ($P < 0.05$) ADG compared to broilers fed the negative control diet (Table 7). There was no evidence for difference in overall ADFI but broilers fed diets containing Thr from crystalline Thr or the low Thr with biomass diet had improved ($P < 0.05$) G:F compared to the negative control and high Thr with biomass treatments.

Experiment 3: Valine

Analysis of manufactured diets (Table 8) resulted in Val values consistent with diet formulation, as the diet with no crystalline AA had the lowest level of analyzed Val while the addition of either fermented Val with biomass or crystalline Val increased the total analyzed dietary Val concentration.

Overall (days 0 to 28) broilers fed diets containing crystalline Val and the two diets containing increasing Val with biomass had increased ($P < 0.05$) ADG, ADFI, and G:F compared to broilers fed the negative control diet (Table 9).

DISCUSSION

AA biomass is formed during the fermentation process. At the conclusion of fermentation, the liquid ferment is concentrated by heating with steam, which results in a wet product that is formed into granules, and then dried in a fluid bed dryer prior to packaging. The final AA with fermented biomass contains an accumulation of both dispensable and indispensable AA, in addition to CP and carbohydrates. Specifically, the SID coefficients for lysine and methionine, the first limiting AA in swine and poultry diets, in dried fermentation biomass is greater when compared to other protein sources such as fish meal (Sulabo et al., 2013). Similarly, Almeida et al. (2014), showed that when adding Thr biomass or fish meal as the sole source of AA in nursery pig diets, the SID of CP and indispensable AA, except Trp, was greater ($P < 0.05$) in the Thr with biomass than in fish meal. Additionally, the metabolizable energy values were greater ($P < 0.05$). Further evidence demonstrates that the standard total tract digestibility of phosphorus (P) in fermentation biomass is increased (Sulabo et al., 2013). This indicates that AA with biomass has high nutritional value, therefore, may be used as an alternative ingredient in nursery pig diets.

Tryptophan is often the second most limiting AA in corn-soybean meal diets for growing pigs (Lewis, 2000). Tryptophan is a precursor for the neurotransmitter serotonin, which is believed to play a role in mood, intestinal activity, and appetite. Therefore, supplying sufficient Trp in the diet is important for meeting the animal's protein

Table 4. Chemical analysis of diets, experiment 1 (as-fed basis)[1]

		Experimental diet				
	Tryptophan source:	None	Crystalline	Biomass		
Item, %[4]	SID[2] tryptophan:lysine, %	16	21	21	23.5	Trp Biomass[3]
Crude protein		18.19	17.31	18.56	19.13	77.69
Lysine		1.29	1.31	1.23	1.49	0.79
Isoleucine		0.80	0.72	0.71	0.79	0.84
Leucine		1.62	1.49	1.48	1.59	1.41
Methionine		0.48	0.46	0.42	0.45	0.18
Threonine		0.91	0.88	0.89	0.99	0.86
Tryptophan		0.22	0.27	0.29	0.33	52.56
Valine		1.03	0.96	0.92	0.99	1.05
Histidine		0.48	0.45	0.45	0.50	0.38
Phenylalanine		0.90	0.80	0.81	0.89	0.73
Arginine		1.13	1.04	1.05	1.13	—

[1]Diets were fed for 21 d from approximately 11 to 23 kg BW.

[2]Standardized ileal digestible.

[3]CJ America – Bio, Downers Grove, IL.

[4]A sample of all experimental diets and the Trp with biomass were submitted for AA analysis and crude protein (Eurofins Scientific Inc., Des Moines, IA).

Table 5. Effects of using tryptophan biomass as a source of tryptophan on nursery pig performance, experiment 1[1]

		Experimental diet					
	Tryptophan source:	None	Crystalline	Biomass[3]			
Item	SID[2] tryptophan:lysine, %:	16	21	21	23.5	SEM	Probability, $P <$
BW, kg							
Day 0		11.3	11.3	11.2	11.3	0.25	0.723
Day 21		22.8[b]	23.5[a]	23.5[ab]	23.0[ab]	0.37	0.019
Days 0 to 21							
ADG, g		549[b]	576[a]	580[a]	556[ab]	8.9	0.004
ADFI, g		836	865	854	833	18.0	0.132
G:F		0.658[b]	0.667[ab]	0.680[a]	0.668[ab]	0.0077	0.045

[1]A total of 315 barrows (DNA 200 × 400, initially 11.3 kg) were used in a 21 d nursery study with five pigs per pen and 15 or 16 pens per treatment.

[2]Standardized ileal digestible.

[3]CJ America – Bio, Downers Grove, IL.

[ab]Values with different superscripts differ, $P < 0.05$.

requirements, as well as stimulating feed intake and growth performance. The current NRC (2012) Trp requirement estimate for 11 to 20 kg nursery pigs is 16% of Lys. Goncalves et al. (2015) concluded that increasing the SID Trp:Lys ratio up to 21% improved ADG, ADFI, and G:F in 11 to 20 kg nursery pigs, while formulating diets below 18% SID Trp:Lys had negative impacts on performance, when using commercially available crystalline Trp (98.5% Trp). In the current study, using L-Trp with dried fermented biomass demonstrated that the L-Trp is equally bioavailable as commercial crystalline L-Trp, indicating the dried biomass had no negative impacts on performance.

Threonine has long been recognized as the third limiting AA for broilers. Warnick et al (1968) demonstrated in a 12% CP semi-purified soybean meal-based diet that lysine and threonine were next limiting essential AA after methionine, with valine now recognized as the fourth most limiting (Corzo et al., 2007). Baker et al. (1994) proposed the first "ideal protein" concept for broilers with essential AA levels being expressed as a ratio to dietary lysine level. Their initial requirement estimate was 67% and 77% for threonine and valine. Two years after, Kidd et al. (1996) demonstrated that increasing dietary threonine improved breast yield, which led to widespread adoption of threonine supplementation in the broiler industry as

Table 6. Chemical analysis of starter and grower diets, experiment 2 (as-fed basis)

	Starter diet[1]				Grower diet[2]			
Threonine source:	None	Crystalline	Biomass		None	Crystalline	Biomass	
Item, %[3] Apparent digestible threonine:lysine, %:	58	65	65	69	58	66	66	70
Crude protein	20.23	21.69	21.64	21.41	18.52	19.06	18.89	18.57
Lysine	1.35	1.29	1.31	1.34	1.22	1.23	1.22	1.18
Isoleucine	0.90	0.87	0.85	0.93	0.81	0.81	0.81	0.75
Leucine	1.77	1.78	1.73	1.85	1.65	1.67	1.68	1.55
Methionine	0.53	0.61	0.54	0.57	0.54	0.49	0.50	0.53
Threonine	0.79	0.84	0.84	0.92	0.72	0.79	0.79	0.80
Tryptophan	0.31	0.28	0.33	0.31	0.28	0.26	0.27	0.29
Valine	1.00	1.02	0.97	1.06	0.95	0.96	0.94	0.88
Arginine	1.46	1.43	1.43	1.57	1.32	1.36	1.28	1.20

[1]Starter diets were fed from days 0 to 14 from approximately 45.2 to 458 g BW.

[2]Grower diets were fed from days 14 to 28 from approximately 458 g to 1.55 kg BW.

[3]A sample of all experimental diets were submitted for AA analysis and crude protein (Eurofins Scientific Inc., Des Moines, IA).

Table 7. Effects of using threonine biomass as a source of threonine on male broiler performance, experiment 2[1]

	Experimental diet					
Threonine source:	None	Crystalline	Biomass[2]			
Item Apparent digestible threonine:lysine, %[3]:	58/58	65/66	65/66	69/70	PSEM	Probability, $P <$
BW						
Day 0, g	45.2	45.1	45.2	45.2	0.04	0.683
Day 28, kg	1.524[b]	1.562[a]	1.563[a]	1.546[ab]	0.0066	0.038
Days 0 to 28						
ADG, g	52.8[b]	54.2[a]	54.2[a]	53.6[a]	0.38	0.041
ADFI, g	80.9	81.3	81.6	82.0	0.35	0.486
G:F	0.671[b]	0.685[a]	0.685[a]	0.677[ab]	0.0029	0.006

[1]A total of 1,320 male broilers (Cobb 500, initially 45.2 g) were used in a 28 d growth study with 33 birds per pen and 10 pens per treatment.

[2]CJ America – Bio, Downers Grove, IL.

[3]Apparent digestible threonine:lysine ratio in the starter/grower phase.

[ab]Values with different superscripts differ, $P < 0.05$.

Table 8. Chemical analysis of starter and grower diets, experiment 3 (as-fed basis)

	Starter diet[1]				Grower diet[2]			
Valine source:	None	Crystalline	Biomass		None	Crystalline	Biomass	
Item, % [3] Apparent digestible valine:lysine, %:	59	75	75	84	63	76	76	83
Crude protein	18.25	18.69	19.00	18.50	16.25	17.06	16.56	17.69
Lysine	1.25	1.35	1.35	1.39	1.09	1.27	1.16	1.22
Isoleucine	0.83	0.84	0.86	0.86	0.76	0.79	0.73	0.78
Leucine	1.46	1.40	1.46	1.39	1.36	1.39	1.25	1.40
Methionine	0.60	0.60	0.62	0.62	0.55	0.57	0.58	0.55
Threonine	0.86	0.87	0.91	0.87	0.79	0.80	0.76	0.78
Tryptophan	0.25	0.25	0.27	0.26	0.22	0.22	0.22	0.23
Valine	0.80	0.95	0.98	1.06	0.74	0.88	0.84	0.96
Arginine	1.28	1.29	1.33	1.31	1.16	1.20	1.12	1.6

[1]Starter diets were fed from days 0 to 14 from approximately 39.4 g to 0.44 kg BW.

[2]Grower diets were fed from days 14 to 28 from approximately 0.44 to 1.64 kg BW.

[3]A sample of all experimental diets were submitted for AA analysis and crude protein (Eurofins Scientific Inc., Des Moines, IA).

Table 9. Effects of using valine biomass as a source of valine on male broiler performance, experiment 3[1]

		Experimental diet					
	Valine source:	None	Crystalline	Biomass[2]			
Item	Apparent digestible valine:lysine, %[3]:	59/63	75/76	75/76	84/83	PSEM	Probability, P <
BW							
Day 0, g		39.4	39.4	39.5	39.3	0.03	0.764
Day 28, kg		1.551[b]	1.665[a]	1.684[a]	1.662[a]	0.0088	<0.001
Days 0 to 28							
ADG, g		54.0[b]	58.1[a]	58.7[a]	58.0[a]	0.34	<0.001
ADFI, g		78.0[b]	81.4[a]	82.4[a]	81.1[a]	0.39	<0.001
G:F		0.711[b]	0.729[a]	0.730[a]	0.728[a]	0.0031	<0.001

[ab]Values with different superscripts differ, $P < 0.05$.

[1]A total of 2,100 male broilers (Cobb 500, initially 39.4 g) were used in a 28 d growth study with 35 birds per pen and 15 pens per treatment.

[2]CJ America – Bio, Downers Grove, IL.

[3]Apparent digestible valine:lysine ratio in the starter/grower phase.

breast meat became the primary economic driver. The use of crystalline valine, however, did not gain attention until more recently. Corozo et al. (2011) and Miranda et al. (2015) demonstrated that broiler diets supplemented with crystalline valine resulted in growth performance and meat yields similar to diets derived from non-crystalline AA sources. The Cobb (2017) recommendations for digestible Thr:lys ratio in broilers for starter, grower, and finisher diets are 65%, 66%, and 67%. While the Cobb (2017) recommendations for digestible Val:lys in broilers for starter, grower, and finisher diets are 75%, 76%, and 77%, respectively.

In the present experiments, when growing pigs or poultry were fed diets containing high Trp or Val with biomass, there was a numeric decrease in feed intake when compared to the low added biomass diets. While the reason is unknown, this could be a result of too high of a digestible Trp or Val percentage, creating an imbalance to other AA. In contrast, there was no evidence for difference in performance when pigs or poultry were fed to the AA requirement at the same digestible Trp, Thr, or Val percent from either purified crystalline sources of the AA or from the AA with biomass source.

In conclusion, these data suggest that Trp, Thr, or Val with biomass appear to be equally bioavailable and are as suitable for use as crystalline AA in swine and poultry diets.

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