

Evaluation of a novel threonine source for nursery pig diets

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ABSTRACT: Four hundred and eighty (PIC 337 X 1050, PIC Genus, Hendersonville, TN) pigs were used to evaluate a novel threonine source (ThrPro, CJ America Bio, Fort Dodge, IA) for nursery pigs from approximately 7 to 20 kg body weight (BW). After weaning, pigs were sorted by sex and fed a common diet for 1 wk. Upon completion of the first week, pigs were sorted into randomized complete blocks, equalized by weight, within 16 replications. Pigs were allocated to one of three dietary treatments: positive control (POS)—standard ileal digestible threonine-to-lysine ratio (SID; Thr:Lys) 0.60, negative control (NEG)—SID Thr:Lys ≤ 0.46, and alternative Thr source (TEST)—SID Thr:Lys 0.60. The alternative Thr source included fermentative biomass and was assumed to contain 75% Thr and a digestibility coefficient of 100% based on the manufacturer's specifications. All other nutrients met or exceeded the NRC recommendations. Growth and intake data were analyzed as repeated measures with a compound symmetry covariance structure using the MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC) with pen as the experimental unit. Treatment, phase, the interaction

between treatment and phase, and block were included as fixed effects in the model. Differences in total removals were tested using Fisher's Exact Test of PROC FREQ. Results were considered significant at $P \leq 0.05$ and considered a trend at $P > 0.05$ and $P \leq 0.10$. During the first 14 d, pigs fed TEST had decreased gain-to-feed ratio (G:F; 0.77 vs. 0.80, $P = 0.022$) compared to POS and increased G:F (0.77 vs. 0.73, $P < 0.001$) compared to NEG. Over days 14–28, pigs fed TEST had similar G:F (0.71 vs. 0.70, $P = 0.112$) compared to POS and increased G:F (0.71 vs. 0.63, $P < 0.001$) compared to NEG. Overall (days 0–28), pigs fed TEST had similar average daily gain (ADG; 0.47 vs. 0.47 kg/d, $P = 0.982$) and G:F (0.76 vs. 0.74, $P = 0.395$) compared to POS and increased ADG (0.47 vs. 0.43 kg/d, $P < 0.001$) and G:F (0.76 vs. 0.67, $P < 0.001$) compared to NEG. The average daily feed intake was not significantly different across treatments for the entirety of the study. In conclusion, the replacement of crystalline L-Thr with a novel Thr source resulted in similar growth performance in nursery pigs from approximately 7 to 20 kg.

Key words: nursery, pig, threonine

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INTRODUCTION

Supplementing swine diets with crystalline amino acids allows for the reduction of dietary crude protein, which has been shown to be advantageous for both the pig and the producer

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by increasing nitrogen (N) utilization, reducing feed costs and N excretion, and supporting gut health without impairing growth (Kerr et al., 2003; Nyachoti et al., 2006; Heo et al., 2009).

The manufacturing of crystalline amino acids can be accomplished through multiple processes, including extraction, synthesis, fermentation, and enzymatic catalysis (Leuchtenberger et al., 2005). Due to economic and ecological benefits, fermentation is the most prominently used of these processes. This method utilizes modified strains of bacteria in the presence of a carbohydrate source, N, phosphorus, sulfur, and trace elements to produce the desired amino acid (Leuchtenberger et al., 2005). Following fermentation, the amino acid must be further processed and refined. The first step in these downstream processes is usually the separation of the amino acid from the fermentative biomass (Hermann, 2003). However, this separation can leave behind a significant amount of the amino acid, thus decreasing the cost efficiency of production. Therefore, simplified downstream processes have been developed, which exclude biomass separation, resulting in a granular amino acid product containing the fermentative biomass (Hermann, 2003). This technique represents a cost-effective way to increase product yield and reduce input costs. Based on this, biomass-containing amino acid products represent an economical alternative to crystalline sources while potentially remaining equally bioavailable. However, limited investigation has been done with respect to utilizing these biomass-containing products as an amino acid source in livestock diets.

Smiricky-Tjardes et al. (2004) reported no difference in growth performance between pigs fed L-lysine (L-Lys) HCl and pigs fed L-Lys sulfate with biomass, which resulted in a similar relative bioavailability between the two amino acid sources. Furthermore, work conducted by Wensley et al. (2020) reported no evidence for a difference in performance when pigs were fed a source of tryptophan with biomass compared to pigs fed the same amino acid requirement using crystalline tryptophan. However, there has been no work investigating the effectiveness of a threonine (Thr) biomass product on the growth performance of pigs as compared to pigs supplemented with crystalline Thr. Based on the literature from other amino acid biomass research, the hypothesis would be that this novel Thr biomass would be able to substitute crystalline Thr. Therefore, the objective of this study was to evaluate the performance of 7–20 kg nursery

pigs in response to replacing crystalline L-Thr with a novel Thr product that included biomass.

MATERIALS AND METHODS

General

All procedures in this experiment adhered to guidelines for the ethical and humane use of animals for research and were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC #19-267). Pigs were housed in pens (1.22 × 2.44 m) fitted with one four-space feeder and two nipple waterers, which ensured ad libitum access to feed and water.

Animals and Experimental Design

Four hundred and eighty barrows and gilts (PIC 337 X 1050, PIC Genus, Hendersonville, TN) arrived immediately after weaning at approximately 21 d of age. Upon arrival, pigs were sorted by sex and placed on a common diet for 1 wk. During acclimation, pigs were vaccinated for *Escherichia coli* (Entero Vac and Edema Vac, ARKO Laboratories, LTD, Jewell, IA). The pigs were porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus negative, which was confirmed through source herd diagnostics. Upon completion of the first week, pigs were organized into randomized complete blocks by sex, equalized by weight, with 10 pigs per pen within 16 replications. Within blocks, pens were randomly assigned to one of three dietary treatments.

Diets

Pigs were fed a common diet (Mid-State Milling, State Center, IA) from day –7 to day 0, which provided 1.65% Lys and 21.50% crude protein. Experimental diets were fed in two phases, with phase 1 diets being fed from day 0 to 14 (Table 1) and phase 2 diets being fed from day 14 to 28 (Table 2). The experimental diets consisted of a positive control supplemented with L-Thr (POS), a negative control (NEG), and an alternative Thr source (TEST; ThrPro, CJ America Bio, Fort Dodge, IA). The alternative Thr source was a granular product, which included the fermentative biomass from production. This product was assumed to contain 75% Thr and a digestibility coefficient of 100% based on the manufacturer's specifications. Diets within each phase were

Table 1. Diet composition for phase 1 (days 0–14)

Ingredients, %	Dietary treatment		
	Positive control	Novel Thr source	Negative control
Corn	47.13	47.06	47.33
Soybean meal	26.09	26.09	26.09
Zinc oxide ^a	0.38	0.38	0.38
Monocalcium phosphate	0.90	0.90	0.90
Calcium carbonate	0.91	0.91	0.91
Salt	0.35	0.35	0.35
Fishmeal	2.00	2.00	2.00
L-Lys	0.50	0.50	0.50
L-tryptophan	0.04	0.04	0.04
Soybean oil	2.50	2.50	2.50
Copper sulfate	0.08	0.08	0.08
Whey permeate	7.91	7.91	7.91
Dried whey	2.50	2.50	2.50
Oat groats	5.00	5.00	5.00
VTM premix ^b	0.50	0.50	0.50
Blood plasma	2.50	2.50	2.50
L-isoleucine	0.08	0.08	0.08
DL-methionine	0.28	0.28	0.28
Thr source ^c	0.20	0.27	–
L-valine	0.15	0.15	0.15
Total	100.00	100.00	100.00
Calculated composition			
Metabolizable energy, Mcal/kg	3.126	3.126	3.126
Crude protein, %	21.10	21.10	21.10
Fat, %	5.00	5.00	5.00
Calcium, %	0.76	0.76	0.76
Available phosphorus, %	0.40	0.40	0.40
Sodium, %	0.32	0.32	0.32
Lactose, %	8.00	8.00	8.00
SID Lys, %	1.42	1.42	1.42
SID Ile:Lys	0.55	0.55	0.55
SID Leu:Lys	0.87	0.87	0.87
SID M+C:Lys	0.58	0.58	0.58
SID Thr:Lys	0.60	0.60	0.46
SID Trp:Lys	0.18	0.18	0.18
SID Val:Lys	0.67	0.67	0.67

Ingredients are listed as percentage of inclusion in the diet and reported on an “as-fed” basis.

^aProvided 3,000 ppm zinc.

^bVitamin and trace mineral; provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, 0.023 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

^cL-Thr in positive control; ThrPro (CJ America Bio, Fort Dodge, IA) in novel Thr source.

formulated to a constant standard ileal digestible (SID) Lys level, and all SID ratios to Lys, with the exception of Thr, were held constant. The POS and TEST diets were formulated to an equal SID Thr:Lys of 0.60, while the NEG diet was formulated to be deficient, with a SID Thr:Lys of less than 0.50. All diets within a phase were formulated to be isocaloric and met or exceeded [NRC \(2012\)](#) recommendations for vitamins and minerals.

Sample Collection and Analysis

Pigs and feeders were weighed on days 0, 14, and 28 of the trial to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F). During the course of the trial, pigs showing signs of illness, such as lethargy, fever, or loss of body condition, were treated with ceftiofur (Exceede, Zoetis, Florham Park, NJ) or enrofloxacin (Baytril, Bayer HealthCare, Animal

Table 2. Diet composition for phase 2 (days 14–28)

Ingredients, %	Dietary treatment		
	Positive control	Novel Thr source	Negative control
Corn	62.14	62.07	62.36
Soybean meal	30.76	30.76	30.76
Monocalcium phosphate	1.43	1.43	1.43
Calcium carbonate	0.90	0.90	0.90
Salt	0.45	0.45	0.45
L-Lys	0.55	0.55	0.55
L-tryptophan	0.04	0.04	0.04
Soybean oil	2.50	2.50	2.50
VTM premix ^a	0.50	0.50	0.50
L-isoleucine	0.06	0.06	0.06
DL-methionine	0.28	0.28	0.28
Thr source ^b	0.22	0.29	–
L-valine	0.17	0.17	0.17
Total	100.00	100.00	100.00
Calculated composition			
Metabolizable energy, Mcal/kg	3.109	3.109	3.109
Crude protein, %	19.90	19.90	19.90
Fat, %	5.00	5.00	5.00
Calcium, %	0.70	0.70	0.70
Available phosphorus, %	0.37	0.37	0.37
Sodium, %	0.20	0.20	0.20
SID Lys, %	1.33	1.33	1.33
SID Ile:Lys	0.55	0.55	0.55
SID Leu:Lys	1.02	1.02	1.02
SID M+C:Lys	0.58	0.58	0.58
SID Thr:Lys	0.60	0.60	0.44
SID Trp:Lys	0.18	0.18	0.18
SID Val:Lys	0.67	0.67	0.67

Ingredients are listed as percentage of inclusion in the diet and reported on an “as-fed” basis.

^aVitamin and trace mineral; provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, 0.023 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

^bL-Thr in positive control; ThrPro (CJ America Bio, Fort Dodge, IA) in novel Thr source.

Health Division, Shawnee Mission, KS). Pigs that did not show signs of improvement 48 h posttreatment were deemed morbid and removed from the trial. The weight of any mortalities and morbidities were recorded along with the date the pigs were removed from the trial. The removal weights and days on trial were calculated back into growth data by adding the removal weight to the total pen weight and adding the number of days the morbidity or mortality was on trial to the total pig days.

Diets were manufactured at the Iowa State University Swine Nutrition Farm feed mill. Feed samples were collected at the completion of mixing for each dietary treatment in each phase. Samples were then stored at -20°C for subsequent analysis.

Feed samples were ground to 1 mm particle size using a Wiley Mill (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ). Processed samples were homogenized and analyzed,

in duplicate, for N (method 990.03; AOAC, 2007; Trumac; LECO Corp., St. Joseph, MI). For N analysis, ethylenediaminetetraacetic acid (9.56% N) was used as the standard for calibration and was determined to contain $9.59 \pm 0.02\%$ N. Crude protein was calculated as $\text{N} \times 6.25$. Diet samples were also submitted to Eurofins Scientific Inc. (Des Moines, IA) to be subject to ion exchange chromatography for amino acid profiling (AOAC 982.30 with modifications).

Statistical Analysis

Growth performance data by phase were analyzed as repeated measures using the MIXED procedure of SAS 9.4 (SAS Inst., Cary, NC). Pen was considered the experimental unit, with treatment, phase, the interaction between treatment and phase, and block included as fixed effects in the

statistical model. The compound symmetry covariance structure was selected for the repeated measures model according to Bayesian information criterion. Overall (days 0–28) growth performance data were analyzed by analysis of variance using the MIXED procedure. Pen was considered the experimental unit, with treatment and block included as fixed effects in the model. Data were reported as least squares means and means separation was done using the PDIFF option.

Normality and homoscedasticity of the studentized residuals were tested using the UNIVARIATE procedure. Statistical outliers were identified as occurring greater than 3 SDs from the mean and were excluded from the analysis. Differences in total removals were tested using Fisher's Exact Test of the FREQ procedure. Results were considered significant at $P \leq 0.05$ and a trend at $P > 0.05$ and $P \leq 0.10$.

Due to mechanical error and the mixing of pens early in the first phase, growth and intake data of four pens were unable to be captured; therefore, these pens were excluded from the phase 1 data and statistical analysis.

RESULTS

Diet analysis indicated that calculated total Lys values of phase 1 diets were slightly lower than analyzed values across all diets; however, calculated and analyzed Thr:Lys ratios remained similar (Table 3). Analyzed total Lys and total Thr for phase 2 diets were similar to the calculated values (Table 4).

Pigs began the trial at an average BW of 6.9 ± 0.3 kg and ended the trial on day 28 at an average BW of 19.9 ± 1.2 kg. From days 0 to 14, pigs fed TEST had decreased ($P \leq 0.05$; Table 5) G:F compared to pigs fed POS and increased ($P \leq 0.05$) G:F compared to pigs fed NEG. From days 14 to 28, pigs fed TEST has similar ($P > 0.10$) G:F compared to pigs fed POS and increased ($P \leq 0.05$) G:F compared to pigs fed NEG. Overall (days 0–28), pigs fed

TEST had similar ($P > 0.10$; Table 6) ADG and G:F compared to pigs fed POS and increased ($P \leq 0.05$) ADG and G:F compared to pigs fed NEG. There was no evidence ($P > 0.10$) for an effect of dietary treatment on ADFI for the entirety of the study. The percentage of total removals did not differ between dietary treatments ($P > 0.10$; Table 6).

DISCUSSION

Threonine is an essential amino acid in swine nutrition and is considered the third or equally second limiting amino acid in pigs fed corn-soybean meal-based diets (Guzik et al., 2005). Aside from its role in protein accretion, Thr is a major constituent of immunoglobulin and intestinal mucins; therefore, it is of vital importance to supply pigs with the correct level of Thr to support optimal growth performance, immune function, and intestinal integrity (Mcgilvray et al., 2019).

The estimated total Thr requirement for pigs weighing between 7 and 11 kg is 0.95%, and the requirement for pigs weighing between 11 and 25 kg is 0.87% of the diet (NRC, 2012). The diet analysis (Tables 3 and 4) indicated that the POS and TEST diets were above these requirements, while the NEG diet was lower. Average daily feed intake during phase 2 (Table 5) was lower than estimated, which resulted in lower Lys consumption than expected across all treatments; however, the differences in Thr:Lys between dietary treatments remained as expected. Therefore, despite a reduction in Lys consumption, meaningful comparisons between dietary treatments were still able to be made.

The current NRC (2012) estimate of the Thr requirement for nursery pigs between 7 and 25 kg is 59% of Lys. A study conducted by Jayaraman et al. (2015), utilizing quadratic broken-line analysis, reported gain efficiency being optimized in nursery pigs at a Thr:Lys of 65–66.5% depending on environmental conditions. This study also saw a linear reduction in gain efficiency when Thr:Lys was

Table 3. Calculated versus analyzed nutrient composition (as fed) for phase 1 (days 0–14) diets

	Item	Dietary treatment		
		Positive control	Novel Thr source	Negative control
Calculated	Crude protein, %	21.10	21.10	21.10
	Total Lys, %	1.57	1.57	1.57
	Total Thr, %	0.99	0.99	0.79
	Total Thr:Lys	0.63	0.63	0.50
Analyzed	Crude protein, %	19.44	19.31	19.54
	Total Lys, %	1.73	1.60	1.71
	Total Thr, %	0.99	1.00	0.81
	Total Thr:Lys	0.57	0.63	0.47

Table 4. Calculated versus analyzed nutrient composition (as fed) for phase 2 (days 14–28) diets

	Item	Dietary treatment		
		Positive control	Novel Thr source	Negative control
Calculated	Crude protein, %	19.90	19.90	19.90
	Total Lys, %	1.47	1.47	1.47
	Total Thr, %	0.92	0.92	0.70
	Total Thr:Lys	0.63	0.63	0.48
Analyzed	Crude protein, %	17.31	19.17	18.78
	Total Lys, %	1.45	1.48	1.49
	Total Thr, %	0.88	0.90	0.74
	Total Thr:Lys	0.61	0.61	0.51

Table 5. Effect of dietary treatment by phase on nursery pig BW, ADG, ADFI, and G:F

Item	Dietary Treatment			SEM	P-value		
	Positive control	Novel Thr source	Negative control		Trt	Phase	Trt ×Phase
BW, kg				0.100	<0.001	<0.001	<0.001
Day 0	6.91	6.90	6.91				
Day 14	12.75 ^a	12.89 ^a	12.38 ^b				
Day 28	20.49 ^a	20.48 ^a	19.07 ^b				
ADG, kg				0.008	<0.001	<0.001	0.279
Day 0–14	0.41	0.41	0.38				
Days 14–28	0.53	0.52	0.48				
ADFI, kg				0.011	0.376	<0.001	0.656
Days 0–14	0.52	0.51	0.52				
Days 14–28	0.76	0.74	0.76				
G:F				0.008	<0.001	<0.001	0.015
Days 0–14	0.80 ^a	0.77 ^b	0.73 ^c				
Days 14–28	0.70 ^a	0.71 ^a	0.63 ^b				

Trt, treatment. Differing superscripts letters (^{a,b,c}) indicate statistical significance ($P < 0.05$) between the treatments.

Table 6. Overall (days 0–28) effect of dietary treatment on nursery pig ADG, ADFI, G:F, and removals

Item	Dietary treatment ^a			SEM	P-value
	Positive control	Novel Thr source	Negative control		
ADG, kg	0.47 ^a	0.47 ^a	0.43 ^b	0.007	<0.001
ADFI, kg	0.63	0.64	0.64	0.010	0.749
G:F	0.74 ^a	0.76 ^a	0.67 ^b	0.010	<0.001
Removals, % ^b	2.50	4.35	2.50	–	0.675

^aLeast squares means not connected by the same letter are significantly different ($P \leq 0.05$).

^bRemovals included the total number of mortalities and morbidities.

decreased to 55%. Results of the current study agree with these findings as the negative control diet, formulated to a Thr:Lys of less than 50%, resulted in suboptimal growth and efficiency compared to pigs fed the positive control diet, which was formulated slightly higher than [NRC \(2012\)](#) requirements.

In the current trial, it was observed that pigs fed TEST had a lower G:F compared to pigs fed POS from days 0 to 14; however, this difference did not carry over to the following phase or overall. During this phase, pigs were noted as exhibiting symptoms that indicated a health challenge in the barn. This also resulted in greater variation in feed intake, and

a greater number of outliers being removed for G:F from the TEST diet, which may have artificially produced this significant difference during phase 1.

Several studies have been conducted that demonstrate an equal bioavailability of L-Lys HCL and L-Lys sulfate with biomass in nursery, growing, and finishing pigs ([Smiricky-Tjardes et al., 2004](#); [Liu et al., 2007](#); [Htoo et al., 2016](#); [Li et al., 2019](#); [Palencia et al., 2019](#)). Similar results have been reported in pigs fed tryptophan and valine sources with biomass compared to pigs fed the same amino acid level using crystalline sources ([Oliveira et al., 2019](#); [Wensley et al., 2020](#)).

Furthermore, work conducted by Wensley et al. (2020), saw no evidence for a difference in performance in broilers fed a source of Thr or valine with biomass compared to broilers fed the same amino acid requirement using crystalline sources. These results are supported by the current trial in swine and indicate that amino acid sources with biomass do not appear to have any negative implications on the health and performance of monogastric animals.

In the current trial, pigs fed a diet containing a novel Thr source with residual fermentative biomass performed similarly to pigs fed a diet supplemented with crystalline L-Thr. This supports the concept that the Thr source with biomass did not have any negative consequences to performance and is equally bioavailable compared to the crystalline source of Thr. In conclusion, the replacement of crystalline L-Thr with a novel Thr source resulted in similar performance in nursery pigs from approximately 7 to 20 kg.

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