Is Nitrogen the Limiting Factor to Maintain Feed Efficiency when Feeding Low Protein, Amino Acid Fortified Diets?

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Summary

A total of 981 mixed-sex pigs ([Fast LW × PIC L02] × PIC 800; initially 22.8 ± 0.42 lb) were used in a 21-d study to determine if nitrogen, derived from non-protein nitrogen or amino nitrogen, is the limiting factor to maintain feed efficiency when feeding low protein, amino acid fortified diets. Pens of pigs were randomly allotted to one of five dietary treatments in a randomized complete block design with BW as a blocking factor. There were 19 to 20 pigs per pen and 10 pens per treatment. The experimental diets were corn-soybean meal-based and were fed when pigs reached an approximate BW of 23 lb, considered d 0 of the study. The five treatments consisted of: 1) low level of feed-grade AAs with a SID Lys:CP ratio of 6.0:1; 2) moderate level of feed-grade AAs with a SID Lys:CP ratio of 6.5:1; 3) high level of feed-grade AAs with a SID Lys:CP ratio of 7.0:1; 4) diet 3 with di-ammonium phosphate (DAP) added to achieve a SID Lys:calculated CP ratio of 6.5:1; 5) diet 3 with glycine added to achieve a SID Lys:calculated CP ratio of 6.5:1. All diets contained 0.4% titanium dioxide for determination of apparent total tract digestibility (ATTD) of dry matter (DM) and crude protein (CP). Treatment diets were fed for 21 days. Feces were collected on d 21 from three pigs per pen to determine fecal DM. Overall (d 0 to 21), ADG was not influenced by treatment. Feed efficiency worsened (linear, $P = 0.002$; quadratic, $P = 0.054$) as the SID Lys:CP ratio was increased above 6.5% with additional feed-grade AAs. Adding DAP or glycine to the high feed-grade AA diet improved ($P \leq 0.003$) feed efficiency compared to pigs fed the high feed-grade AA diet because of a reduction ($P = 0.007$) or numerical reduction ($P = 0.109$), respectively, in ADFI. There was a decrease (linear, $P < 0.001$) in blood urea nitrogen (BUN) as feed-grade AAs increased. Adding additional nitrogen to the high feed-grade AA diet increased (DAP; $P = 0.038$) or tended to increase (glycine, $P = 0.091$) BUN. There was a tendency (quadratic, $P = 0.051$) in fecal DM with pigs fed the moderate feed-grade AA diet having the lowest fecal DM. Pigs fed the diet containing DAP had increased ($P = 0.005$) fecal DM compared with pigs fed the high feed-grade AA diet. There was a tendency for an increase ($P = 0.060$)

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in the ATTD of DM for pigs fed the DAP diet compared with the pigs fed the high feed-grade AA diet. The ATTD of CP decreased (linear, $P = 0.048$) as the SID Lys:CP ratio increased. Pigs fed the diets containing either DAP or glycine had increased ($P \leq 0.026$) CP digestibility compared with pigs fed the high feed-grade AA diet. These data suggest that diets for 25 to 50 lb pigs should be formulated to a SID Lys:CP ratio of 6.5 or lower and that adding a non-protein nitrogen source or non-essential AA to diets formulated above this ratio can improve feed efficiency and CP digestibility.

Introduction

The addition of dietary feed-grade amino acids has significantly benefited the swine industry by reducing feed costs, nitrogen excretion, and environmental impact. Extensive research has shown that pig performance, specifically feed efficiency, can either be maintained or enhanced by substituting a portion of intact protein sources, like soybean meal, with feed-grade amino acids. However, pig performance may be compromised when high amounts of feed-grade amino acids are used in the diets. This decrease in performance is not entirely understood, but it has been hypothesized that the amount of nitrogen needed to synthesize non-essential amino acids may become limiting. Using nitrogen requirements suggested by the NRC (2012),[4] the SID Lys:CP ratio should be lower in older pigs because of their increased maintenance requirement and lower needs for protein synthesis than in young pigs. The NRC suggests that a SID Lys:CP ratio of 6.5 is optimal for pigs weighing between 25 to 55 lbs. Research conducted by Mansilla et al. (2018)[5] demonstrated that ammonium phosphate can serve as a nitrogen source in diets deficient in non-essential amino acids. Mansilla[6] fed diets that were deficient in non-essential amino acids with or without a non-protein nitrogen (NPN) source. Feed efficiency was poorer for pigs fed the diet deficient in non-essential amino acids compared to when NPN was added to the diet. Therefore, the objective of this study was to determine the impact of a NPN source (DAP) or amino nitrogen source (glycine) on growth performance, feed efficiency, digestibility, and BUN of 25 to 50 lb pigs fed low protein, amino acid fortified diets.

Materials and Methods

General

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. This study was conducted at the New Fashion Pork research nursery facility in Round Lake, MN. The facility contained 52 pens and is completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 3-hole, dry self-feeder and a bowl waterer for ad libitum access to feed and water.

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[4] Nutrient Requirements of Swine: Eleventh Revised Edition. 2012. National Academies Press, Washington, D.C.

[5] Mansilla, W. D., K. E. Silva, C. Zhu, C. M. Nyachoti, J. K. Htoo, J. P. Cant, and C. F. De Lange. 2018. Ammonia-Nitrogen added to low-crude-protein diets deficient in dispensable amino acid–nitrogen increases the net release of alanine, citrulline, and glutamate post–splanchnic organ metabolism in growing pigs. J. Nutr. 148:1081–1087. doi:10.1093/jn/nxy076.

[6] Mansilla, W. D. 2013. Non-protein nitrogen is used efficiently for improving protein deposition and feed efficiency in growing pigs. M.S. Thesis. University of Guelph, Guelph, Ontario, Canada.

Animals and diets

A total of 981 pigs ([Fast LW × PIC L02] × PIC 800; initially 22.8 ± 0.42 lb) were used in a 21-d growth trial. Pigs were housed in mixed-gender pens with 19 to 20 pigs per pen and 10 replications per treatment in a randomized complete block design with BW as a blocking factor. Pens of pigs were randomly allotted to one of five dietary treatments (Table 1). Diets were corn-soybean meal-based and consisted of: 1) low level of feed-grade AAs with a SID Lys:CP ratio of 6.0:1; 2) moderate level of feed-grade AAs with a SID Lys:CP ratio of 6.5:1; 3) high level of feed-grade AAs with a SID Lys:CP ratio of 7.0:1; 4) diet 3 with di-ammonium phosphate (DAP) added to achieve a SID Lys:CP ratio of 6.5:1; 5) diet 3 with glycine added to achieve a SID Lys:CP ratio of 6.5:1. The NE of soybean meal was considered to be 100% of corn NE in diet formulation. Prior to diet formulation, samples of corn and soybean meal were submitted to Minnesota Valley Testing Laboratories, Inc. (New Ulm, MN) for a complete mineral panel analysis (AOAC 985.01) and to Market 1, Inc. (Ag State; Cherokee, IA) for proximate and AA analysis. Dietary additions of feed-grade AA were adjusted to meet or exceed AA requirements in relation to Lys for Met and Cys, Thr, Trp, Val, Ile, and His. Pigs were fed treatment diets in meal form. Treatment diets were manufactured at the New Fashion Pork feed mill in Round Lake, MN. Titanium dioxide was included at 0.4% in the treatment diets as an indigestible marker to determine ATTD of DM and CP. Feed samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory). Fecal samples were analyzed for DM, N, and TiO_2 at the Kansas State University Swine Laboratory.

Measurements and sampling

Pig weights and feed disappearance were measured on d 0, 13, and 21 to determine ADG, ADFI, and F/G. Fecal samples were collected on d 21 from three pigs per pen to determine percentage DM. Samples were dried at 131°F (55°C) in a forced-air oven for 48 h, and the ratio of dried-to-wet fecal weight determined the fecal dry matter. Fecal samples were analyzed separately for each pig and the average of the three samples from each pen was then used for statistical analysis. Blood samples were collected on d 21 from four pigs per pen (two barrows and two gilts of medium size) to measure serum BUN.[7]

Digestibility analysis

Following fecal DM determination, both ground feed and pen-level pooled fecal samples were dried in a 275°F (135°C) oven for 2 h to determine percentage DM of the samples used for titanium analysis. Titanium dioxide concentration in both dried feed and fecal samples was determined and ATTD of DM and N was calculated using the index method.

Statistical analysis

Growth and digestibility data were analyzed as a randomized complete block design as a one-way ANOVA with pen serving as the experimental unit, dietary treatment as fixed effect, and BW as a random intercept. Linear and quadratic contrasts were tested within increasing levels of feed-grade AAs without addition of added N. The effect of DAP and glycine were tested using pairwise comparisons to compare their responses to

[7] DetectX Urea Nitrogen Detection Kit; Arbor Assays, Ann Arbor, MI.

moderate and high levels of feed-grade AAs individually. Fecal DM data were analyzed with dietary treatment as fixed effect and block as a random intercept. BUN data were analyzed with pig serving as the observational unit, dietary treatment and gender as fixed effects, and block and microtiter plates as random intercepts. Pen was also included as a random effect to account for subsampling with multiple pigs measured from each pen. When treatment was a significant source of variation, differences were determined by pairwise comparison using the Tukey-Kramer multiplicity adjustment to control for Type I Error. All statistical models were fit using the lmer function from the lme4 package in R Studio (Version 3.5.2, R Core Team. Vienna, Austria). Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Growth performance

Overall (d 0 to 21), there was no evidence ($P > 0.05$) for ADG to be affected by treatment. Feed efficiency worsened (linear, $P = 0.002$; quadratic, $P = 0.054$) with the greatest change occurring as the SID Lys:CP ratio increased from 6.5:1 to 7.0:1 corresponding with the addition of increased feed-grade AAs. Incorporating either DAP or glycine to the high feed-grade AA diet led to an improvement ($P \leq 0.003$) in feed efficiency compared to the pigs fed the high feed-grade AA diet due to a reduction ($P = 0.007$) or numerical reduction ($P = 0.109$), respectively, in ADFI.

Blood urea nitrogen analysis

There was a decrease (linear, $P < 0.001$) in serum blood urea nitrogen (BUN) as the SID Lys:CP ratio increased, indicating that the pigs were more efficient at retaining dietary nitrogen. Adding nitrogen to the high feed-grade AA diet in the form of DAP or glycine increased ($P = 0.038$) or tended to increase ($P = 0.091$) BUN, respectively.

Fecal dry matter analysis

There was a tendency ($P = 0.051$) for a quadratic effect in fecal DM with pigs fed the moderate feed-grade AA diet having the lowest fecal DM. The pigs fed the diet containing DAP had increased ($P = 0.005$) fecal DM compared with the pigs fed the high feed-grade AA diet, which could be due to the differences in N in the two diets or feed intake.

DM and CP digestibility

There was a tendency for improvement ($P = 0.060$) in ATTD of DM for the pigs fed the diet containing DAP compared to the pigs fed the high feed-grade AA diet without DAP or glycine. Additionally, the ATTD of CP decreased (linear, $P = 0.048$) with increasing feed-grade AAs. Pigs fed the diets containing either DAP or glycine had increased ($P \leq 0.026$) CP digestibility compared to pigs fed the high feed-grade AA diet. Pigs fed the diet containing DAP had increased ($P = 0.005$) CP digestibility compared to the pigs fed the moderate feed-grade AA diet.

In summary, these data suggest that diets for 25 to 50 lb pigs should be formulated to a SID Lys:CP ratio of 6.5 or lower and that adding a non-protein nitrogen source or non-essential AA to diets formulated above this ratio can improve feed efficiency and CP digestibility.

Acknowledgments

Appreciation is expressed to New Fashion Pork (Jackson, MN) for providing the nursery facility and CJ America for partial financial support of this trial.

Table 1. Diet composition of experimental diets (as-fed basis)[1]

Feed-Grade AA:	Low	Moderate	High	High	High
Added N:	None	None	None	DAP[2]	Glycine
Ingredient, % — SID Lys:CP:	6.0:1	6.5:1	7.0:1	6.5:1	6.5:1
Corn	62.10	66.80	70.10	69.50	69.00
Soybean meal (46% CP)	33.70	28.60	24.70	24.70	24.70
Calcium carbonate	0.68	0.70	0.70	1.20	0.70
Monocalcium P (21% P)	0.95	1.00	1.05	---	1.05
Salt	0.60	0.60	0.61	0.61	0.61
L-Lys-HCl	0.40	0.55	0.66	0.66	0.66
DL-Met	0.25	0.29	0.32	0.32	0.32
L-Trp	0.04	0.06	0.09	0.09	0.09
L-Val	0.13	0.21	0.28	0.28	0.28
L-Ile	---	0.06	0.13	0.13	0.13
Thr[3]	0.27	0.35	0.42	0.42	0.42
L-His-HCl	---	0.03	0.08	0.08	0.08
Vitamin premix with phytase	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Glycine	---	---	---	---	1.14
Di-ammonium phosphate (18% N)	---	---	---	1.14	---
Titanium dioxide	0.40	0.40	0.40	0.40	0.40
Copper chloride[4]	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100

continued

Table 1. Diet composition of experimental diets (as-fed basis)[1]

Feed-Grade AA:	Low	Moderate	High	High	High
Added N:	None	None	None	DAP[2]	Glycine
Ingredient, % — SID Lys:CP:	6.0:1	6.5:1	7.0:1	6.5:1	6.5:1
Calculated analysis					
SID AA, %					
Lys, %	1.25	1.25	1.25	1.25	1.25
Ile:Lys	57	55	55	55	55
Leu:Lys	117	108	101	100	100
Met:Lys	39	40	42	42	42
Met and Cys:Lys	58	58	58	58	58
Thr:Lys	67	67	67	67	67
Trp:Lys	20.3	20.0	20.3	20.3	20.3
Val:Lys	72	72	72	72	72
His:Lys	36	34	34	34	34
Arg:Lys	93	81	73	73	73
Phe and Tyr:Lys	111	99	90	90	90
Total Lys, %	1.39	1.38	1.37	1.37	1.37
Total EAA:NEAA	0.84	0.89	0.94	0.94	0.83
NE, kcal/lb	1,011	1,038	1,057	1,050	1,054
SID Lys:NE, g/Mcal	5.61	5.46	5.36	5.39	5.37
CP, %	20.9	19.2	17.9	19.2	19.2
Ca, %	0.63	0.63	0.63	0.63	0.63
STTD P, %	0.47	0.47	0.47	0.47	0.47
Ca:P	1.03	1.06	1.07	1.08	1.07
Na, %	0.28	0.28	0.28	0.28	0.28
Cl, %	0.48	0.51	0.53	0.53	0.53

[1] Diets were fed from d 0 (22 lb) to d 21 (48 lb).
[2] Di-ammonium phosphate.
[3] Thr Pro; CJ America-Bio, Downers Grove, IL.
[4] Tribasic copper chloride, 58% Cu; SAM Nutrition, Bloomington, MN.

Table 2. Analyzed composition of experimental diets (as-fed basis)[1]

Feed-Grade AA:	Low	Moderate	High	High	High
Added N:	None	None	None	DAP[2]	Glycine
SID Lys:CP:	6.0:1	6.5:1	7.0:1	6.5:1	6.5:1
Nutrient, %					
CP[3] (MU)	19.24	19.04	17.21	19.16	18.53
CP (K-State)	20.42	17.86	16.86	19.24	18.12
Moisture	12.73	13.20	13.04	13.02	12.89
Crude fat	2.28	1.97	2.22	2.15	2.38
Crude fiber	2.03	1.90	2.02	2.05	2.03
Ash	5.55	5.39	5.13	5.06	4.88
Essential AAs					
Arg	1.20	1.17	1.02	0.95	0.97
His	0.49	0.50	0.49	0.45	0.48
Ile	0.82	0.86	0.82	0.78	0.77
Leu	1.59	1.57	1.45	1.39	1.42
Lys	1.37	1.46	1.39	1.33	1.33
Met	0.47	0.56	0.55	0.49	0.53
Phe	0.92	0.91	0.82	0.77	0.78
Thr	0.89	0.93	0.93	0.84	0.87
Trp	0.27	0.27	0.27	0.28	0.27
Val	1.02	1.07	1.02	0.98	0.99
Non-essential AAs					
Ala	0.93	0.92	0.85	0.82	0.85
Asp	1.92	1.88	1.66	1.56	1.56
Cys	0.29	0.30	0.27	0.24	0.26
Glu	3.39	3.34	3.02	2.83	2.86
Gly	0.77	0.75	0.68	0.63	1.64
Pro	1.09	1.08	1.01	0.96	1.00
Ser	0.81	0.78	0.70	0.67	0.70
Tyr	0.64	0.63	0.55	0.54	0.55

[1] Samples were analyzed for proximate analysis and complete AA profile (University of Missouri Agricultural Experiment Station Chemical Laboratory). Samples were analyzed for CP in the Kansas State University Swine Laboratory.
[2] Di-ammonium phosphate.
[3] Crude protein = % N × 6.25.

Table 3. Effect of feed-grade AA inclusion with and without an added nitrogen source on growth performance, fecal DM, and serum BUN[1]

Feed-Grade AA:	Low	Moderate	High	High	High		*P* =			
Added N:	None	None	None	DAP[2]	Glycine		SID Lys:CP[3]			
SID Lys:CP:	6.0:1	6.5:1	7.0:1	6.5:1	6.5:1	SEM	Linear	Quadratic	DAP[4]	Glycine[5]
BW, lb										
d 0	22.7	22.8	22.7	22.8	22.8	0.42	0.715	0.649	0.354	0.297
d 21	48.9	48.7	48.5	48.1	48.8	0.71	0.344	0.965	0.377	0.518
Overall (d 0 to 21)										
ADG, lb	1.24	1.23	1.23	1.20	1.24	0.019	0.616	0.989	0.280	0.736
ADFI, lb	1.85	1.85	1.90	1.80	1.85	0.032	0.163	0.291	0.007	0.109
G:F	0.67	0.67	0.65	0.67	0.67	0.005	0.002	0.054	0.003	0.001
F/G[6]	1.50	1.50	1.55	1.50	1.49	0.011	---	---	---	---
Serum BUN	8.17	7.12	6.27	6.88	6.76	0.224	< 0.001	0.680	0.038	0.091
Fecal DM, %	20.49	19.61	21.99	24.81	22.87	0.660	0.118	0.051	0.005	0.351
DM digestibility	83.88	84.08	83.82	85.55	84.70	0.630	0.951	0.766	0.060	0.328
CP digestibility[7]	78.69	77.00	75.43	81.78	79.12	1.127	0.048	0.963	< 0.001	0.026

[1] A total of 981 pigs (initially 22.8 ± 1.26 lb) were used in a 21-d growth study with 19 to 20 pigs per pen and 10 replicates per treatment.
[2] DAP = di-ammonium phosphate.
[3] Comparing the main effects of low, moderate, and high feed-grade AA inclusion without added N.
[4] Pairwise comparison between high feed-grade AA + DAP and high feed-grade AA.
[5] Pairwise comparison between high feed-grade AA + glycine and high feed-grade AA.
[6] F/G was calculated taking the inverse of G:F. *P*-values are the same as reported for G:F.
[7] DAP to moderate feed-grade AA ($P = 0.005$).