

DETERMINATION OF THE FOURTH-LIMITING AMINO ACID IN SWINE DIETS CONTAINING NUTRIDENSE® CORN¹

A.W. Duttlinger, J.R. Bergstrom, M.D. Tokach, J.L. Nelssen, S.S. Dritz²
R.D. Goodband, J.M. DeRouchey, and J. Snow³

Summary

Two studies were conducted to determine the fourth-limiting amino acid in swine diets containing NutriDense® corn. Both experiments were conducted at a commercial swine research facility in southwest Minnesota. In Exp. 1, 1,259 pigs (initially 82.1 lb, PIC) were used in a 28-d study. Pigs were blocked by weight, and were randomly allotted to one of six dietary treatments. Pigs were fed NutriDense® corn-soybean meal-based diets. The treatments were 1) a positive control diet containing 0.15% L-lysine HCl and 0.015% added L-threonine; 2) a negative control diet with 0.45% L-lysine HCl, 0.085% added DL-methionine, and 0.15% added L-threonine; 3) treatment 2 with 0.05% L-isoleucine; 4) treatment 2 with 0.05% L-valine; 5) treatment 2 with 0.05% L-tryptophan; and 6) treatment 2 with a combination of 0.05% L-isoleucine, 0.05% L-tryptophan, and 0.05% L-valine. Pigs fed the positive control and the diet with the combination of added isoleucine, tryptophan, and valine had greater ADG ($P<0.05$) than all other treatments. Also, pigs fed added isoleucine or tryptophan had greater ADG ($P<0.05$) than pigs fed the negative control

with those fed added valine being intermediate. Pigs fed the diet with the combination of added isoleucine, tryptophan, and valine had greater ADFI ($P<0.05$) than pigs fed the negative control. There were no significant differences in F/G.

In Exp. 2, 1,038 pigs (initially 170.4 lb, PIC) were used in the 28-d study with six dietary treatments similar to Exp. 1 to determine the fourth-limiting amino acid in late finishing pigs. Overall, pigs fed the positive control diet had greater ($P<0.05$) ADG and lower ($P<0.05$) F/G than pigs fed the negative control diet and those fed either L-isoleucine, L-tryptophan, or L-valine. Pigs fed the diet containing added tryptophan or the combination of isoleucine, tryptophan, and valine had improved ($P<0.05$) ADG and F/G compared with those fed the negative control, or added isoleucine or valine. Pigs fed added isoleucine and valine had greater ($P<0.05$) ADG than pigs fed the negative control diet. There was no difference amongst the treatments for ADFI. These results suggest that in the 80 to 130 lb growing pig, tryptophan and isoleucine are the co-limiting fourth amino acid in diets containing NutriDense® corn. In 170 to 220 lb

¹Appreciation is expressed to New Horizon Farms for use of pigs and facilities and Richard Brobjorg, Cal Hulstein, and Marty Heintz for technical assistance. Also to BASF Plant Science, Research Triangle Park, NC for providing the corn used in these studies and Ajinomoto-Heartland LLC, Chicago, IL for providing the amino acids.

²Food Animal Health and Management Center, College of Veterinary Medicine.

³BASF Plant Science, Research Triangle Park, NC.

pigs, tryptophan appears to be fourth-limiting followed by isoleucine and valine.

(Key words: amino acids, feed ingredients, NutriDense[®] corn.)

Introduction

NutriDense[®] (ND) corn is a nutritionally enhanced product containing a stacked set of traits to provide greater nutrient density than conventional yellow dent (YD) corn. Specifically, it contains approximately 23% more lysine, 19% more sulfur amino acids, 18% more threonine, almost 34% more tryptophan, and 5% more energy than normal corn. Because ND corn contains greater levels of amino acids, inclusion of ND corn in the diet lowers soybean meal use and alters the amino acid balance, which should decrease the need for secondary amino acids when high levels of synthetic L-lysine are used. NutriDense[®] corn also contains higher levels of other amino acids relative to lysine, which may allow for higher levels of synthetic amino acids to be used in diets containing ND corn.

Recent reductions in the price of L-threonine have made it feasible to add L-threonine and DL-methionine with L-lysine to further reduce the soybean meal level in corn-soybean meal based diets. In order to formulate ND corn-based diets with the maximum amount of added L-lysine, DL-methionine and L-threonine the fourth-limiting amino acid must be determined. Therefore, the objective of these studies was to determine the fourth-limiting amino acid in diets containing ND corn and to evaluate whether pig performance could be maintained with the inclusion of high levels of synthetic amino acids in diets containing ND corn.

Procedures

Procedures used in these experiments were approved by the Kansas State University

Animal Care and Use Committee. The two experiments were conducted at a commercial research facility in southwest Minnesota. The facility had a totally slatted floor, with approximately 7.2 ft² provided per pig. Each pen was equipped with a four-hole dry self feeder and one cup waterer. The facility was a double curtain-sided, deep-pit barn that operated on mechanical ventilation during the summer and on automatic ventilation during the winter. Experiments 1 and 2 were conducted in summer and spring, respectively. Pigs were randomly allotted and blocked to one of six dietary treatments with seven pens per treatment. Pigs and feeders were weighed on d 0, 14, and 28 to determine the response criteria of ADG, ADFI, and F/G. All pigs were fed diets containing ND corn. A single lot of ND corn was stored and used in both trials. The ND corn was sampled for amino acid analysis (Table 1) and the values used in diet formulation.

Table 1. Composition of NutriDense[®] Corn^a

Item	NutriDense [®] Corn
Dry matter, %	86.36
Crude Fat, %	6.16
CP, %	7.98
ME, kcal/lb	1,628
Fiber, %	3.06
Calcium, %	0.01
Total P, %	0.32
Available P, %	0.33
Amino acids,%	
Lysine	0.30
Cystine	0.20
Isoleucine	0.29
Leucine	0.97
Methionine	0.20
Tryptophan	0.07
Threonine	0.30
Valine	0.42

^aValues represent the mean of 2 samples analyzed in duplicate.

Experiment 1. A total of 1,259 pigs (initially 82.1 lb, PIC L 337 × 1050) were used in the 28-d study. Each pen contained 26 to 28 pigs with an equal distribution of barrows and gilts in each pen with 7 replicate pens per experimental diet. Experimental diets (Table 2) were fed in meal form. Pigs were fed ND corn-soybean meal-based diets. The treatments were: 1) a positive control diet containing 0.15% added L-lysine and 0.015% added L-threonine; 2) a negative control diet with 0.45% added L-lysine, 0.085% added DL-methionine, and 0.15% added L-threonine; 3) treatment 2 with 0.05% added L-isoleucine; 4) treatment 2 with 0.05% added L-valine; 5) treatment 2 with 0.05% added L-tryptophan; and 6) treatment 2 with a combination of 0.05% added L-isoleucine, 0.05% L-tryptophan, and L-valine, 0.05%. All experimental diets were balanced to maintain a constant true ileal digestible (TID) lysine:ME ratio and available P level.

Experiment 2. A total of 1,038 pigs (initially 170.4 lb, L 327 × C22) were used in the 28-d study. Each pen contained 23 to 25 pigs with an equal distribution of barrows and gilts in each pen with 7 replicate pens per experimental diet. Experimental diets (Table 3) were fed in meal form and based on the same formulation concept as Exp. 1. The treatments were: 1) a positive control diet containing 0.15% added L-lysine and 0.02% added L-threonine; 2) a negative control diet containing 0.40% added L-lysine, 0.03% added DL-methionine, and 0.13% added L-threonine; 3) treatment 2 with 0.05% added L-isoleucine; 4) treatment 2 with 0.05% added L-valine; 5) treatment 2 with 0.05% added L-tryptophan; 6) treatment 2 with a combination of 0.05% added L-isoleucine, 0.05% L-tryptophan and 0.05% L-valine. All other procedures were identical to Exp. 1.

Statistical Analysis. In both experiments, data were analyzed as a randomized complete-block design by using the PROC MIXED pro-

cedure of SAS, with pen as the experimental unit.

Results

Experiment 1. Overall (d 0 to 28), pigs fed the positive control and the diet with the combination of added isoleucine, tryptophan, and valine had greater ADG ($P<0.05$) than pigs fed all other treatments (Table 4). Pigs fed added isoleucine or tryptophan had greater ADG ($P<0.05$) than pigs fed the negative control with those fed valine being intermediate. Average daily feed intake was greatest for pigs fed the combination of isoleucine, tryptophan, and valine compared with pigs fed the negative control diet, with those fed the other dietary treatments intermediate. There were no significant differences in F/G with numerical differences following the ADG response. Final weight reflects the differences in ADG with pigs fed the positive control diet or the diet with the combination of added isoleucine, tryptophan, and valine being heavier ($P<0.05$) than those fed the negative control diet, with those fed the other treatments being intermediate.

Experiment 2. Overall (d 0 to 28), pigs fed the positive control diet had greater ($P<0.05$) ADG than pigs fed the negative control diet and those fed added isoleucine, tryptophan, or valine. Pigs fed the combination of isoleucine, tryptophan, and valine had greater ADG than those fed the negative control, or added isoleucine or valine. Also, pigs fed added tryptophan had greater ($P<0.05$) ADG than pigs fed the negative control diet and those fed additional isoleucine or valine. Furthermore, pigs fed added isoleucine or valine had greater ($P<0.05$) ADG than pigs fed the negative control diet. There was no difference amongst the treatments for ADFI. Pigs fed the positive control diet had better ($P<0.05$) F/G than pigs fed all other treatments, with those fed the combination of added isoleucine, tryptophan and valine, or tryptophan being intermediate followed by those fed the negative

control, added isoleucine or valine. Pigs fed the positive control diet had greater ($P < 0.05$) final BW than pigs fed the negative control diet and pigs fed diets with added isoleucine or valine.

Discussion

The true ileal digestible (TID) amino acid ratios for isoleucine, valine, and tryptophan recommended for grow-finish pigs by Kansas State University are 55, 16.5 and 65% relative to lysine, respectively. During the grower trial, the negative control diet was calculated to be deficient in isoleucine (51% of lysine) and tryptophan (14% of lysine), and marginally deficient in valine (63% of lysine). The growth data indicates that these amino acid ratios and recommendations appear to be accurate. Adding tryptophan or isoleucine to the diet increased ADG to a similar extent with only a minor numerical increase in ADG with the addition of valine. Adding all three amino acids together allowed performance to return to the level achieved by the positive control diet. Thus, very high levels of synthetic amino acids can be added to diets formulated with ND corn for grower pigs without sacrificing performance.

As TID lysine levels were lowered in the finishing phase to match the amino acid requirements, more ND corn and less soybean meal was used in the diets. Thus, the ratios of amino acids also changed. The negative control diet for the finishing experiment were calculated to be most deficient in tryptophan (12.5% of lysine), deficient in isoleucine (51% of lysine), and adequate in valine (66% of lysine). Performance data again indicates that the amino acid ratios and recommendations appear to be accurate. Adding tryptophan to the negative control diet resulted in the greatest improvement in ADG and F/G with the addition of isoleucine providing a small bene-

fit over the negative control. Adding valine to the diet did not influence performance. Adding all three amino acids provided only a small benefit over the addition of tryptophan alone and was not successful in returning F/G back to the level achieved by the positive control diet. The lack of completely returning performance of the tryptophan supplemented treatments diets does not appear to be because of a tryptophan, isoleucine or valine deficiency. These results are similar to other trials with late finishing pigs where additions of high levels (0.45% in this experiment) of L-lysine HCl with other amino acids were not able to equal the performance of finishing pigs fed diets with lower levels of synthetic amino acids. However, the results in this trial indicate that the use of ND corn allows performance to be returned closer to the performance of the control pigs than in much of the previous research with normal yellow dent corn.

Interestingly, a response was obtained in both the grower and finisher experiments with the addition of either tryptophan or isoleucine alone to the negative control diet. In the classical interpretation of an amino acid deficiency, a response to a secondary limiting amino acid should not be present until the most limiting amino acid is added to the diet. In both of these trials, a response was found to either amino acid added alone. This suggests that the amino acids may be acting through different mechanisms. For example, tryptophan appears to have a bigger impact on ADFI. Thus, the classical interpretation of an amino acid deficiency may be wrong.

In conclusion, these results suggest that in the 80 to 130 lb growing pig, tryptophan and isoleucine are the co-limiting fourth amino acid in diets containing ND corn. In 170 to 220 lb pigs, tryptophan appears to be the fourth-limiting amino acid followed by isoleucine.

Table 2. Composition of Diets (Exp. 1; as-fed basis)

Item	Positive Control	Negative Control	Added Isoleucine	Added Valine	Added Tryptophan	Added Ile, Try and Val
NutriDense® corn	75.12	83.88	83.88	83.88	83.88	83.8
Soybean meal (46.5% CP)	22.70	13.25	13.25	13.25	13.25	13.25
Monocalcium P (21% P)	0.65	0.70	0.70	0.70	0.70	0.70
Limestone	0.90	0.90	0.90	0.90	0.90	0.90
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase	0.08	0.08	0.08	0.08	0.08	0.08
Trace mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine HCl	0.15	0.45	0.45	0.45	0.45	0.45
DL-methionine	---	0.09	0.09	0.09	0.09	0.09
L-threonine	0.015	0.15	0.15	0.15	0.15	0.15
L-tryptophan	---	---	---	---	0.05	0.05
L-isoleucine	---	---	0.05	---	---	0.05
L-valine	---	---	---	0.05	---	0.05
Total	100	100	100	100	100	100
Calculated analysis						
Total lysine, %	1.03	1.01	1.01	1.01	1.01	1.01
Lysine:ME ratio, g/Mcal	2.62	2.61	2.61	2.61	2.61	2.61
True ileal amino acids, %						
Lysine	0.91	0.91	0.91	0.91	0.91	0.91
Isoleucine:lysine ratio	69	51	57	51	51	57
Leucine:lysine ratio	154	129	129	129	129	129
Methionine:lysine ratio	30	34	34	34	34	34
Met & Cys:lysine ratio	60	60	60	60	60	59
Threonine:lysine ratio	62	62	62	62	62	62
Tryptophan:lysine ratio	19	14	14	14	19	19
Valine:lysine ratio	80	63	63	68	63	68
ME, kcal/lb	1,574	1,584	1,583	1,583	1,584	1,583
CP, %	16.8	13.5	13.5	13.5	13.6	13.6
Ca, %	0.55	0.52	0.52	0.52	0.52	0.52
P, %	0.53	0.51	0.51	0.51	0.51	0.51
Available P, %	0.25	0.26	0.26	0.26	0.26	0.26
Available P equivalent, %	0.31	0.32	0.32	0.32	0.32	0.32
Avail P:calorie ratio g/mcal	0.9	0.91	0.91	0.91	0.91	0.91

Table 3. Composition of Diets (Exp 2; as-fed basis)

Ingredient	Positive Control	Negative Control	Added Isoleucine	Added Valine	Added Tryptophan	Added Ile, Try and Val
NutriDense [®] corn	82.98	90.34	90.34	90.34	90.34	90.2
Soybean meal (46.5% CP)	15.00	7.15	7.15	7.15	7.15	7.15
Monocalcium P (21% P)	0.45	0.50	0.50	0.50	0.50	0.50
Limestone	0.95	1.00	1.00	1.00	1.00	1.00
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix with phytase	0.05	0.05	0.05	0.05	0.05	0.05
Trace mineral premix	0.05	0.05	0.05	0.05	0.05	0.05
L-lysine HCl	0.15	0.40	0.40	0.40	0.40	0.40
DL-methionine	---	0.03	0.03	0.03	0.03	0.03
L-threonine	0.02	0.13	0.13	0.13	0.13	0.13
L-tryptophan	---	---	---	---	0.05	0.05
L-isoleucine	---	---	0.05	---	---	0.05
Valine	---	---	---	0.05	---	0.05
Total	100	100	100	100	100	100
Calculated analysis						
Total lysine, %	0.82	0.80	0.80	0.80	0.80	0.80
TID Lysine:ME ratio, g/Mcal	2.06	2.05	2.05	2.05	2.05	2.05
True Ileal amino acids, %						
Lysine	0.72	0.72	0.72	0.72	0.72	0.72
Isoleucine:lysine ratio	69	51	58	51	51	58
Leucine:lysine ratio	170	144	144	144	144	144
Methionine:lysine ratio	33	32	32	32	32	32
Met & Cys:lysine ratio	66	60	60	60	60	60
Threonine:lysine ratio	65	65	65	65	65	65
Tryptophan:lysine ratio	18	12.7	12.7	12.7	19	19
Valine:lysine ratio	84	66	66	73	66	72
ME, kcal/lb	1,585	1,592	1,591	1,591	1,593	1,591
CP, %	13.8	11.1	11.1	11.1	11.2	11.2
Ca, %	0.5	0.51	0.51	0.51	0.51	0.51
P, %	0.46	0.44	0.44	0.44	0.44	0.44
Available P, %	0.21	0.21	0.21	0.21	0.21	0.21
Available P equivalent, %	0.24	0.25	0.25	0.25	0.25	0.25
Avail P:calorie ratio g/mcal	0.7	0.71	0.71	0.71	0.71	0.71

Table 4. Determination of the Fourth-limiting Amino Acid in Swine Diets Containing NutriDense® Corn (Exp.1)^a

Item	Positive Control	Negative Control	Added Isoleucine	Added Valine	Added Tryptophan	Added Ile, Val and Try	SE
D 0 to 28							
ADG, lb	1.93 ^b	1.69 ^d	1.81 ^c	1.76 ^{cd}	1.80 ^c	1.92 ^b	0.04
ADFI, lb	4.99 ^{bc}	4.62 ^c	4.87 ^{bc}	4.68 ^{bc}	4.81 ^{bc}	5.05 ^b	0.13
F/G	2.59	2.73	2.69	2.66	2.68	2.63	0.07
Final wt, lb	136.5 ^b	130.4 ^c	133.1 ^{bc}	131.4 ^{bc}	132.4 ^{bc}	136.0 ^b	1.83

^aA total of 1,259 pigs, initially 82.1 lb, were used in a 28 d experiment to evaluate growth performance and determine the 4th limiting amino acid for pigs fed diets containing NutriDense® corn. Experimental diets were formulated to contain 0.91% TID Lysine using either 0.15% added L-lysine HCl (Control), 0.45% added L-lysine HCl (Negative Control), 0.45% added L-lysine HCl + 0.05% added L-isoleucine (NC + Ile), 0.45% added L-lysine HCl + 0.05% added L-valine (NC + Val), 0.45% added L-lysine HCl + 0.05% L-tryptophan (NC + Try), or 0.45% added L-lysine HCl + 0.05% added L-isoleucine + 0.05% added L-valine + 0.05% added L-tryptophan (NC + Ile + Val + Try).

^{bcd}Means within a row containing different superscripts are different $P < 0.05$.

Table 5. Determination of the Fourth-limiting Amino Acid in Swine Diets Containing NutriDense® Corn (Exp. 2)^a

Item	Positive Control	Negative Control	Added Isoleucine	Added Valine	Added Tryptophan	Added Ile, Val and Try	SE Mean
D 0 to 28							
ADG, lb	1.94 ^b	1.62 ^e	1.72 ^d	1.65 ^{de}	1.85 ^c	1.88 ^{bc}	0.04
ADFI, lb	5.83	5.81	5.89	5.76	6.00	6.05	0.11
F/G	3.01 ^b	3.60 ^d	3.44 ^d	3.48 ^d	3.25 ^c	3.23 ^c	0.07
Final wt, lb	226.0 ^b	218.8 ^{cd}	218.7 ^{cd}	216.6 ^d	222.0 ^{bcd}	224.2 ^{bc}	2.74

^aA total of 1,038 pigs, initially 170.4 lb, were used in a 28 d experiment to evaluate growth performance and determine the 4th limiting amino acid for pigs fed diets containing NutriDense® corn. Experimental diets were formulated to contain 0.72% TID Lysine using either 0.15% added L-lysine HCl (Control), 0.40% added L-lysine HCl (Negative Control), 0.40% added L-lysine HCl + 0.05% added L-isoleucine (NC + Ile), 0.40% added L-lysine HCl + 0.05% added L-valine (NC + Val), 0.40% added L-lysine HCl + 0.05% L-tryptophan (NC + Try), or 0.40% added L-lysine HCl + 0.05% added L-isoleucine + 0.05% added L-valine + 0.05% added L-tryptophan (NC + Ile + Val + Try).

^{bcd}Means within a row containing different superscripts are different $P < 0.05$.