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Valine, Isoleucine, and Histidine Supplementation of Low-Protein, Amino Acid-Supplemented Diets for Growing Pigs

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Summary and Implications

Previous experiments have shown that when the crude protein of a growing diet is reduced from 16 to 11% there is reduced growth performance of pigs, even though the diets are supplemented with lysine, tryptophan, threonine, and methionine. To determine which amino acid was next limiting in a corn-soybean meal, low-protein (11%), amino acid-supplemented diet, we conducted three experiments. In each of the three experiments, 36 growing gilts were individually penned and fed one of six diets. The three first diets were used in all three experiments: 1) 16% crude protein (CP) positive control, 2) 12% CP neutral control, and 3) 11% CP negative control. In Experiment 1, the 11% CP diet was supplemented with: 4) isoleucine, 5) valine, and 6) isoleucine + valine. In Experiment 2, the 11% CP diet was supplemented with: 4) histidine, 5) histidine + valine, and 6) histidine + valine + isoleucine. In Experiment 3, the 11% CP diet was supplemented with: 4) valine, 5) valine + histidine, and 6) valine + isoleucine. All low-protein diets were supplemented with lysine, tryptophan, threonine, and methionine. The supplementation of isoleucine or histidine alone reduced feed intake, daily gain, feed/gain, daily lean gain, longissi-

mus muscle area, and backfat thickness. Valine supplementation improved growth performance, but the combination of valine with isoleucine or histidine increased growth performance to levels similar to those of gilts fed the 16% CP diet. Plasma urea nitrogen decreased as the crude protein decreased from 16 to 11%. These results suggest that dietary crude protein can be reduced from 16 to 11% with little or no effect on pig performance if amino acids such as valine and isoleucine or valine and histidine are added. This reduction in dietary crude protein will reduce nitrogen excretion in feces and urine.

Introduction

Previous experiments reported in Nebraska Swine Reports have indicated that the crude protein (CP) content of diets for growing pigs can be reduced if they are supplemented with crystalline amino acids (lysine, tryptophan, threonine, and methionine). However, a reduction in protein content from 16 to 11% caused a reduction in other amino acids, and, because of that, growth performance was reduced. Amino acids that may be limiting in an 11% CP diet are valine, isoleucine, and histidine.

Other researchers have reported improvements in pig performance with supplementation of both valine and isoleucine to an 11% CP diet, but they did not obtain an improvement in growth performance with either valine or isoleucine supplementation alone. In fact,

isoleucine decreased pig performance, and valine improved average daily gain (ADG) but not feed efficiency.

Materials and Methods

Three experiments were conducted to determine the fifth and sixth limiting amino acids in a low-protein (11% CP), corn-soybean meal, amino acid-supplemented diet for growing pigs. In all experiments, 36 crossbred gilts weighing 46 lb were penned individually and fed one of six diets for 35 days. The diets (Tables 1, 2, and 3) were a standard corn-soybean meal diet with 16% CP and two low-CP amino acid-supplemented (lysine, tryptophan, threonine, and methionine) diets with 12 and 11% CP. In addition, in Experiment 1, the 11% CP diet was supplemented with isoleucine, valine, or isoleucine + valine; in Experiment 2, the 11% CP diet was supplemented with histidine, histidine + valine, or histidine + valine + isoleucine; and in Experiment 3, the 11% CP diet was supplemented with valine, valine + histidine, or valine + isoleucine to make up the other three diets in each experiment. All low-protein diets were supplemented with lysine, tryptophan, threonine, and methionine to have the same total concentration as in the standard (16% CP) diet. The supplements of isoleucine, valine, and histidine were also to approximately the same levels as in the 16% CP diet. Average daily gain (ADG) and average daily feed intake (ADFI) were measured weekly.

(Continued on next page)



Fat-free lean gain was calculated from backfat thickness and longissimus muscle area (obtained on the first and the last day of the experiment using real-time ultrasound) using the National Pork Producers Council equation, and plasma urea and plasma amino acid concentrations were determined in blood collected on the last day of each experiment.

Results and Discussion

Growth Performance

In general, ADG was higher in gilts fed 16 or 12% CP diets than in gilts fed 11% CP diets, even when supplemented with valine, isoleucine, histidine, or various combinations (Tables 4, 5, and 6). Supplementation of isoleucine or histidine alone decreased ADG, whereas supplementation of valine alone increased ADG compared with the 11% CP diet. However, the combination of valine and isoleucine; valine and histidine; or valine, histidine, and isoleucine in an 11% CP diet increased ADG to levels similar to those of gilts fed the 12% CP diet or even the 16% CP diet.

There was no effect of dietary CP concentration on ADFI. Supplementation of isoleucine or histidine alone reduced ADFI compared with the supplementation of valine alone or the control diets. However, gilts fed the 11% CP diet with the combination of valine and isoleucine had higher ADFI than gilts fed the 12 and 16% CP diets.

Feed efficiency (ADFI/ADG) was affected by dietary CP concentration, with better values in gilts fed 16 and 12% CP diets than in gilts fed the 11% CP diets. Supplementation of isoleucine or histidine alone had a negative effect on feed efficiency. Supplementation of both isoleucine and valine or histidine and valine resulted in better feed efficiency than the 11% CP diet.

Fat-free lean gain was affected by dietary CP concentration, with higher values in gilts fed the 16 or 12% CP diets than in gilts fed the 11% CP diets. Supplementation of either isoleucine or histidine alone decreased lean gain

Table 1. Composition of diets^a, Exp. 1.

Item	Dietary protein concentration, %					
	16	12	11	11+I	11+V	11+IV
Ingredient, %						
Corn	71.36	82.41	85.22	84.95	84.95	84.67
Soybean meal (46.1% CP)	23.70	11.50	8.50	8.50	8.50	8.50
Tallow	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.17	1.46	1.47	1.47	1.47	1.47
Limestone	0.68	0.60	0.60	0.60	0.60	0.60
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix ^b	0.70	0.70	0.70	0.70	0.70	0.70
Trace mineral premix ^c	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine•HCl	—	0.48	0.57	0.57	0.57	0.57
L-Tryptophan	—	0.08	0.095	0.095	0.095	0.095
L-Threonine	—	0.23	0.27	0.27	0.27	0.27
DL-Methionine	—	0.15	0.175	0.175	0.175	0.175
L-Isoleucine	—	—	—	0.275	—	0.275
L-Valine	—	—	—	—	0.275	0.275
Calculated nutrient composition, %						
Crude protein	16.00	12.00	11.00	11.20	11.20	11.50
Lysine	0.92	0.92	0.92	0.92	0.92	0.92
Tryptophan	0.20	0.20	0.20	0.20	0.20	0.20
Threonine	0.67	0.67	0.67	0.67	0.67	0.67
Methionine + cystine	0.60	0.60	0.60	0.60	0.60	0.60
Isoleucine	0.69	0.47	0.42	0.69	0.41	0.69
Valine	0.79	0.57	0.52	0.52	0.79	0.79
Calcium	0.61	0.61	0.61	0.61	0.61	0.61
Phosphorus	0.56	0.57	0.57	0.57	0.57	0.57
Available phosphorus	0.32	0.32	0.32	0.32	0.32	0.32
Metabolizable energy, Mcal/lb	1.52	1.52	1.52	1.51	1.51	1.51
Net energy, Mcal/lb	1.06	1.07	1.07	1.07	1.07	1.07

^aAs-fed basis. 11+I means 11% CP supplemented with isoleucine; 11+V, 11% CP diet and valine; 11+IV, 11% CP and isoleucine and valine.

^bSupplied per kilogram of diet: retinyl acetate, 3,858 IU; cholecalciferol, 386 IU; all-rac- α -tocopheryl acetate, 19.3 IU; menadione (as menadione sodium bisulfite complex), 2.3 mg; folic acid, 1.54 mg; niacin, 23 mg; riboflavin, 3.9 mg; cyanocobalamin, 15.4 μ g; d-pantothenic acid (as d-calcium pantothenate), 15.4 mg; D-biotin, 77 μ g; choline (as choline chloride), 386 mg.

^cSupplied (mg/kg of diet): Cu (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 11; I (as $\text{Ca}[\text{IO}_3]_2 \cdot \text{H}_2\text{O}$), 22; Fe (as $\text{FeSO}_4 \cdot \text{H}_2\text{O}$), 110; Mn (as MnO), 22; Se (as Na_2SeO_3), 0.3; Zn (as ZnO), 110.

Table 2. Composition of diets^a, Exp. 2.

Item	Dietary protein concentration, %					
	16	12	11	11+H	11+HV	11+HVI
Ingredient, %						
Corn	71.56	83.15	85.94	85.73	85.45	85.19
Soybean meal (44.8% CP)	23.50	10.75	7.75	7.75	7.75	7.75
Tallow	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.16	1.45	1.47	1.47	1.47	1.47
Limestone	0.68	0.60	0.60	0.60	0.60	0.60
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix ^b	0.70	0.70	0.70	0.70	0.70	0.70
Trace mineral premix ^c	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine•HCl	—	0.47	0.57	0.57	0.57	0.57
L-Tryptophan	—	0.08	0.10	0.10	0.10	0.10
L-Threonine	—	0.23	0.29	0.29	0.29	0.29
DL-Methionine	—	0.15	0.19	0.19	0.19	0.19
L-Histidine•HCl•H ₂ O	—	—	—	0.21	0.21	0.21
L-Valine	—	—	—	—	0.28	0.28
L-Isoleucine	—	—	—	—	—	0.28
Calculated nutrient composition, %						
Crude protein	16.00	12.00	11.00	11.20	11.30	11.50
Lysine	0.91	0.91	0.91	0.91	0.91	0.91
Tryptophan	0.20	0.20	0.20	0.20	0.20	0.20
Threonine	0.67	0.67	0.67	0.67	0.67	0.67
Methionine + cystine	0.60	0.60	0.60	0.60	0.60	0.60
Histidine	0.45	0.32	0.29	0.45	0.45	0.45
Isoleucine	0.69	0.46	0.40	0.40	0.40	0.69
Valine	0.79	0.56	0.50	0.50	0.79	0.79
Calcium	0.61	0.61	0.61	0.61	0.61	0.61
Phosphorus	0.57	0.57	0.57	0.57	0.57	0.57
Available phosphorus	0.29	0.32	0.32	0.32	0.32	0.32
Metabolizable energy, Mcal/lb	1.52	1.52	1.52	1.52	1.51	1.51
Net energy, Mcal/lb	1.06	1.07	1.07	1.07	1.07	1.07

^aAs-fed basis. 11+H means 11% CP diet supplemented with histidine; 11+HV, 11% CP and histidine and valine; 11+HVI, 11% CP and histidine, valine, and isoleucine.

^bSupplied per kilogram of diet: retinyl acetate, 3,858 IU; cholecalciferol, 386 IU; all-rac- α -tocopheryl acetate, 19.3 IU; menadione (as menadione sodium bisulfite complex), 2.3 mg; folic acid, 1.54 mg; niacin, 23 mg; riboflavin, 3.9 mg; cyanocobalamin, 15.4 μ g; d-pantothenic acid (as d-calcium pantothenate), 15.4 mg; D-biotin, 77 μ g; choline (as choline chloride), 386 mg.

^cSupplied (mg/kg of diet): Cu (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 11; I (as $\text{Ca}[\text{IO}_3]_2 \cdot \text{H}_2\text{O}$), 22; Fe (as $\text{FeSO}_4 \cdot \text{H}_2\text{O}$), 110; Mn (as MnO), 22; Se (as Na_2SeO_3), 0.3; Zn (as ZnO), 110.



Table 3. Composition of diets^a, Exp. 3.

Item	Dietary protein concentration, %					
	16	12	11	11+V	11+VH	11+VI
Ingredient, %						
Corn	70.99	82.35	85.19	84.91	84.71	84.64
Soybean meal (44.8% CP)	24.10	11.55	8.50	8.50	8.50	8.50
Tallow	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.16	1.45	1.47	1.47	1.47	1.47
Limestone	0.65	0.60	0.60	0.60	0.60	0.60
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix ^b	0.70	0.70	0.70	0.70	0.70	0.70
Trace mineral premix ^c	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine•HCl	—	0.47	0.57	0.57	0.57	0.57
L-Tryptophan	—	0.08	0.10	0.10	0.10	0.10
L-Threonine	—	0.23	0.29	0.29	0.29	0.29
DL-Methionine	—	0.15	0.19	0.19	0.19	0.19
L-Isoleucine	—	—	—	—	—	0.28
L-Valine	—	—	—	0.28	0.28	0.28
L-Histidine•HCl•H ₂ O	—	—	—	—	0.21	—
Calculated nutrient composition, %						
Crude protein	16.00	12.00	11.00	11.10	11.30	11.30
Lysine	0.93	0.92	0.92	0.91	0.91	0.91
Tryptophan	0.20	0.20	0.20	0.20	0.20	0.20
Threonine	0.68	0.68	0.68	0.68	0.68	0.67
Methionine + cystine	0.60	0.61	0.61	0.60	0.60	0.60
Isoleucine	0.70	0.47	0.42	0.41	0.41	0.69
Valine	0.80	0.57	0.52	0.79	0.79	0.79
Histidine	0.46	0.33	0.30	0.30	0.51	0.30
Calcium	0.60	0.61	0.61	0.61	0.61	0.61
Phosphorus	0.57	0.57	0.57	0.57	0.56	0.56
Available phosphorus	0.29	0.32	0.32	0.32	0.32	0.32
Metabolizable energy, Mcal/lb	1.52	1.52	1.52	1.51	1.51	1.51
Net energy, Mcal/lb	1.06	1.07	1.07	1.07	1.07	1.07

^aAs-fed basis. 11+V means 11% CP diet supplemented with valine; 11+VH, 11% CP and valine and histidine; 11+VI, 11% CP and valine and isoleucine.

^bSupplied per kilogram of diet: retinyl acetate, 3,858 IU; cholecalciferol, 386 IU; all-rac- α -tocopheryl acetate, 19.3 IU; menadione (as menadione sodium bisulfite complex), 2.3 mg; folic acid, 1.54 mg; niacin, 23 mg; riboflavin, 3.9 mg; cyanocobalamin, 15.4 μ g; d-pantothenic acid (as d-calcium pantothenate), 15.4 mg; D-biotin, 77 μ g; choline (as choline chloride), 386 mg.

^cSupplied (mg/kg of diet): Cu (as CuSO₄•5H₂O), 11; I (as Ca[IO₃]₂•H₂O), 22; Fe (as FeSO₄•H₂O), 110; Mn (as MnO), 22; Se (as Na₂SeO₃), 0.3; Zn (as ZnO), 110.

Table 4. Effect of protein concentration and amino acid supplementation on growth performance of growing gilts^{ab} (Exp. 1).

Item	Dietary protein concentration, % ^c						SEM ^d
	16	12	11	11+I	11+V	11+IV	
Growth performance							
ADG ^e , lb	1.72	1.59	1.47	1.12	1.50	1.58	0.0297
ADFI, lb	3.55	3.50	3.44	2.89	3.55	3.66	0.0574
ADFI/ADG	2.064	2.201	2.340	2.580	2.367	2.317	0.0109
FFLG ^g , lb/d	0.69	0.64	0.58	0.48	0.56	0.60	0.0111
Carcass traits							
Backfat, in	0.40	0.40	0.40	0.33	0.44	0.45	0.0551
LMA ^h , in ²	3.32	3.34	3.06	2.95	2.91	3.18	0.6857
Blood metabolites, mg/dL							
Urea	20.49	4.97	4.89	6.46	5.84	4.31	0.8544
Lysine	1.29	4.15	5.15	4.71	4.34	5.28	0.7434
Tryptophan	1.05	1.20	1.43	1.24	1.26	1.32	0.1358
Threonine	2.41	4.91	8.35	7.33	5.16	6.83	0.7525
Methionine	0.50	1.09	1.33	1.20	1.06	1.44	0.1830
Valine	3.10	0.93	0.83	0.62	6.99	6.85	0.2840
Isoleucine	1.68	0.69	0.54	2.45	0.64	3.20	0.1979
Histidine	1.34	0.63	0.61	0.54	0.61	0.57	0.0606

^aThe average initial weight was 43 lb.

^bNumber of observations per treatment = 6.

^c11+I means 11% CP supplemented with isoleucine; 11+V, 11% CP diet and valine; 11+IV, 11% CP and isoleucine and valine.

^dSEM = Standard error of mean.

^eADG = Average daily gain.

^fADFI = Average daily feed intake.

^gFFLG = Fat-free lean gain.

^hLMA = Longissimus muscle area.

compared with supplementation of valine. In addition, the combination of isoleucine and valine or histidine and valine added to the 11% CP diet was more effective in restoring fat-free lean gain than was the single supplementation of either amino acid. Amino acid supplements restored lean gain to values that were between those of the 11 and 12% CP diets.

Carcass Traits

Backfat thickness increased with the supplementation of isoleucine plus valine to the 11% CP diet. This effect seemed to be due to the single supplementation of valine compared with isoleucine supplementation, because the single supplementation of isoleucine reduced backfat thickness. Supplementation of either histidine or valine with isoleucine tended to increase backfat thickness of gilts to levels similar to those of the control diet (Table 5), but in Experiment 3 (Table 6) there was no difference among gilts fed any diet.

Longissimus muscle area was affected by dietary CP concentration. It was similar in gilts fed 16 and 12% CP diets but lower in gilts fed 11% CP. There were only minor differences in longissimus muscle area among gilts fed 11% CP diets. Supplementation of valine alone was not enough to improve longissimus muscle area. Supplementation of isoleucine or histidine alone decreased longissimus muscle area, but the combination of valine with isoleucine or histidine tended to increase longissimus muscle area. However, this increase was not large enough to reach levels similar to those of gilts fed 16% CP diets. Supplementation of isoleucine to the diet with histidine and valine did not increase longissimus muscle area further.

Blood Metabolites

There was a large reduction in plasma urea concentration from the 16% CP diet to all other low-protein diets. Generally, plasma urea concentrations of gilts fed 11% CP diets were

(Continued on next page)



Table 5. Effect of protein concentration and amino acid supplementation on growth performance of gilts^{ab} (Exp. 2).

Item	Dietary protein concentration, % ^c						SEM ^d
	16	12	11	11+H	11+HV	11+HVI	
Growth performance							
ADG ^e , lb	1.94	1.67	1.50	1.40	1.80	1.68	0.2256
ADFI ^f , lb	3.94	3.88	3.59	3.42	4.00	3.76	0.0695
ADFI/ADG	2.031	2.323	2.393	2.443	2.222	2.238	0.0131
FFLG ^g , lb/d	0.752	0.644	0.567	0.540	0.666	0.615	0.0121
Carcass traits							
Backfat depth, in	0.45	0.43	0.41	0.46	0.51	0.45	0.0888
LMA ^h , in ²	3.92	3.64	3.41	3.43	3.69	3.48	0.8824
Blood metabolites, mg/dL							
Urea	27.42	8.94	8.29	8.50	6.97	6.61	1.3360
Lysine	1.92	4.26	5.26	5.73	4.56	5.38	0.8811
Tryptophan	1.26	1.32	1.51	1.50	1.24	1.25	0.1241
Threonine	2.60	3.90	5.56	6.06	5.29	5.88	0.6364
Methionine	0.56	1.0	1.28	1.34	1.10	1.08	0.0360
Valine	3.26	0.94	0.69	0.92	6.39	6.25	0.0616
Isoleucine	1.67	0.52	0.42	0.51	0.49	2.66	0.0156
Histidine	1.44	0.48	0.59	1.83	1.69	1.67	0.0214

^aThe average initial weight was 48.3 lb.

^bNumber of observations per treatment = 6.

^c11+H means 11% CP diet supplemented with histidine; 11+HV, 11% CP and histidine and valine; 11+HVI, 11% CP and histidine, valine, and isoleucine.

^dSEM = Standard error of mean.

^eADG = Average daily gain.

^fADFI = Average daily feed intake.

^gFFLG = Fat-free lean gain.

^hLMA = Longissimus muscle area.

Table 6. Effect of protein concentration and amino acid supplementation on growth performance of growing gilts^{ab} (Exp. 3).

Item	Dietary protein concentration, % ^c						SEM ^d
	16	12	11	11+V	11+VH	11+VI	
Growth performance							
ADG ^e , lb	1.86	1.79	1.62	1.76	1.82	1.90	0.0355
ADFI ^f , lb	3.89	3.67	3.53	3.85	3.83	3.91	0.0681
ADFI/ADG	2.091	2.050	2.179	2.188	2.104	2.058	0.0077
FFLG ^g , lb/d	0.717	0.706	0.613	0.631	0.672	0.688	0.0105
Carcass traits							
Backfat depth, in	0.44	0.50	0.58	0.52	0.48	0.50	0.0812
LMA ^h , in ²	3.65	3.69	3.43	3.48	3.49	3.59	0.2014
Blood metabolites, mg/dL							
Urea	22.92	17.85	10.31	8.51	6.66	9.41	2.9701
Lysine	2.36	3.72	3.80	4.66	4.44	4.25	0.4245
Tryptophan	1.26	1.49	1.18	2.08	1.50	2.03	0.3328
Threonine	2.99	3.71	4.94	4.74	4.37	5.30	0.4758
Methionine	0.54	1.00	1.19	1.00	1.08	1.05	0.0955
Valine	3.46	1.35	0.87	4.52	5.12	5.30	0.2930
Isoleucine	1.72	0.77	0.56	0.57	0.55	2.40	0.1017
Histidine	1.30	0.74	0.58	0.49	1.75	0.44	0.0918

^aThe average initial weight was 43 lb.

^bNumber of observations per treatment = 6.

^c11+V means 11% CP diet supplemented with valine; 11+VH, 11% CP and valine and histidine; 11+VI, 11% CP and valine and isoleucine.

^dSEM = Standard error of the mean.

^eADG = Average daily gain.

^fADFI = Average daily feed intake.

^gFFLG = Fat-free lean gain.

^hLMA = Longissimus muscle area.

lower than those of gilts fed 12% CP. The supplementation of valine, histidine, isoleucine, or various combination did not change plasma urea concentrations.

The plasma concentrations of amino acids that were supplemented to all diets (lysine, tryptophan, threonine, and methionine) all responded in a similar manner, increasing as dietary CP was reduced.

Supplementation of valine with or without isoleucine, histidine, or the combination of these two amino acids dramatically increased plasma valine concentration. Values were higher than in pigs fed the control 16% CP diet, suggesting that valine-supplemented diets contained more available valine than the control diet. Similar effects were observed when either isoleucine or histidine were supplemented. The addition of these two amino acids either alone or in combinations resulted in higher plasma concentrations than in pigs fed the control diet. These effects were anticipated because diets were formulated to the same total amino acid concentrations and it is known that crystalline amino acids are more readily bioavailable than those in intact protein sources, such as soybean meal and corn.

These results suggest that valine and isoleucine may be the fifth and sixth limiting amino acids, respectively, in a low-protein, amino acid-supplemented, corn-soybean meal diet for growing pigs. Further research may provide additional insight about the optimal amounts of crystalline amino acids that should be supplemented to improve the performance and carcass traits of growing pigs.

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