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# Effects of Glutamine on Growth Performance and Intestinal Development of Immune Challenged Weanling Pigs Fed Chemically Defined Diets

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# Effects of Glutamine on Growth Performance and Intestinal Development of Immune Challenged Weanling Pigs Fed Chemically Defined Diets

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## Summary and Implications

Glutamine is currently considered a nonessential amino acid for pigs. In this study we investigated whether glutamine is essential during an acute immune challenge. Thirty-six individually housed 20-day old pigs were blocked by location and allotted to one of three purified dietary treatments: 1) contained no L-glutamine (CON), 2) contained 5% L-Glutamine (GLN), or 3) contained no L-glutamine but was equalized to GLN diet on a nitrogen basis with other nonessential amino acids (AA). Pigs were fed these diets for a 14-day growth assay. On day 7, one half of the pigs from each treatment were injected with 200 µg • kg BW<sup>-1</sup> lipopolysaccharide (LPS; the endotoxin synthesized from *E. coli*) and the remaining pigs were injected with an equal volume of physiologic saline (SAL). Average daily gain (ADG;  $P > 0.21$ ), average daily feed intake (ADFI;  $P > 0.79$ ), and feed efficiency (ADG/ADFI;  $P > 0.26$ ) were similar among treatments prior to LPS or SAL injection. During the period after LPS or SAL injection, LPS reduced ADG (0.46 vs. 0.24 lb/d;  $P < 0.0001$ ), ADFI (0.63 vs. 0.47 lb/d;  $P < 0.005$ ) and ADG/ADFI (0.74 vs. 0.50;  $P < 0.001$ ) by 48%, 25%, and 32%, respectively. However, there were no differences for ADG ( $P > 0.39$ ), ADFI ( $P > 0.95$ ), or ADG/ADFI ( $P > 0.24$ ) between pigs injected with LPS and SAL and fed GLN (Diet × LPS interaction,  $P < 0.06$ ). Intestine length ( $P < 0.0001$ ), full weight ( $P < 0.005$ ), and empty

Table 1. Ingredient and calculated nutrient composition of diets, as-fed basis.

Ingredients, %	Treatment <sup>d</sup>		
	CON	GLN	AA
Corn starch	32.92	27.92	27.00
Lactose	15.00	15.00	15.00
Sucrose	15.00	15.00	15.00
Corn oil	5.00	5.00	5.00
Solka floc	3.00	3.00	3.00
L-Arginine	0.64	0.64	0.64
L-Histidine•HCl•H <sub>2</sub> O	0.55	0.55	0.55
L-Lysine•HCl	1.95	1.95	1.95
L-Tyrosine	0.60	0.60	0.60
L-Tryptophan	0.21	0.21	0.21
L-Phenylalanine	0.66	0.66	0.66
DL-Methionine	0.47	0.47	0.47
L-Cystine	0.47	0.47	0.47
L-Threonine	0.86	0.86	0.86
L-Leucine	1.33	1.33	1.33
L-Isoleucine	0.86	0.86	0.86
L-Valine	0.92	0.92	0.92
Glycine	1.33	1.33	2.51
L-Proline	0.44	0.44	1.63
L-Glutamic Acid	10.82	10.82	10.82
L-Glutamine		5.00	
L-Alanine			1.18
L-Asparagine			1.18
L-Serine			1.18
Dicalcium phosphate	3.03	3.03	3.03
Limestone	0.46	0.46	0.46
Sodium bicarbonate	1.20	1.20	1.20
Magnesium potassium sulfate	0.22	0.22	0.22
Salt	0.91	0.91	0.91
Potassium chloride	0.51	0.51	0.51
Trace mineral premix <sup>a</sup>	0.15	0.15	0.15
Vitamin premix <sup>b</sup>	0.30	0.30	0.30
Choline chloride	0.20	0.20	0.20
Calculated nutrient composition			
Lysine, %	1.54	1.54	1.54
ME <sup>c</sup> , kcal/lb	1,269	1,269	1,269
Crude fat, %	5.00	5.00	5.00
Crude fiber, %	3.00	3.00	3.00
Ca, %	0.90	0.90	0.90
P, %	0.58	0.58	0.58
P, avail. %	0.55	0.55	0.55

<sup>a</sup>Supplied per lb of diet: Zn (as ZnO), 57.5 mg; Fe (as FeSO<sub>4</sub>•H<sub>2</sub>O), 57.5 mg; Mn (as MnO), 13.6 mg; Cu (as CuSO<sub>4</sub>•5 H<sub>2</sub>O), 4.75 mg; I (as Ca(IO<sub>3</sub>)•H<sub>2</sub>O), .13 mg; Se<sup>d</sup> (as Na<sub>2</sub>SeO<sub>3</sub>), .135 mg.

<sup>b</sup>Supplied per lb of diet: Vitamin A (as retinyl acetate), 2,993 IU; Vitamin D (as cholecalciferol), 299 IU; Vitamin E (as α-tocopherol acetate), 16.3 IU; Vitamin K (as menadione sodium bisulfite), 2.39 mg; riboflavin, 6; d-pantothenic acid, 12 mg; niacin, 18 mg; vitamin B<sub>12</sub>, 17.7 µg.

<sup>c</sup>ME = Metabolizable energy

<sup>d</sup>CON = Control; GLN = Control + 5% L-Glutamine; AA = Control + equalized with GLN on nitrogen from nonessential amino acids. <sup>h</sup>NS =  $P > 0.10$ .

weight ( $P < 0.0005$ ) were reduced by LPS injection compared to SAL injection. Pigs fed GLN and injected with LPS had similar empty small

intestine weight compared to pigs fed GLN and injected with SAL (diet × LPS interaction,  $P < 0.07$ ). These data suggest that glutamine is beneficial to



improve the growth and health of weanling pigs after an immune challenge.

### Background and Introduction

Glutamine is considered a nonessential (not required in the diet) amino acid for pigs. However, it has been documented that glutamine is an important energy source for the absorptive cells of the small intestine and cells of the immune system. Other researchers have shown that pigs fed diets with four concentrations of crystalline glutamine and abdominally inoculated with *E. coli* showed increased (in a dose-response fashion) serum IgG against *E. coli* antigens. Therefore, during an acute immune challenge, glutamine may be required in the weanling pig diet. Our aim was to investigate the effects of glutamine on growth performance of immune-challenged weanling pigs fed diets with or without glutamine. Because glutamine is ubiquitous in all protein sources and there is currently no assay to quantify glutamine, it was necessary to use purified diets.

### Procedures

Thirty-six individually-housed pigs were used in this 14-day experiment. Pigs were weaned at 20 days of age, blocked by location ( $n = 6$ ), and randomly assigned to one of three purified dietary treatments (Table 1) that: 1) contained no L-glutamine (CON), 2) contained 5% L-Glutamine (GLN), or 3) contained no L-glutamine but was equalized to GLN diet on a nitrogen basis with other nonessential amino acids (AA). On day 7, one-half of the pigs from each treatment were injected intramuscularly with 200  $\mu\text{g} \cdot \text{kg BW}^{-1}$  lipopolysaccharide (LPS) from *E. coli* or an equal volume of physiologic saline (SAL). LPS is the endotoxin that is produced by *E. coli*. Injection of LPS causes clinical symptoms of a septic state such as vomiting, diarrhea, and lethargy. Pigs and feeders were weighed on day 0, 7, and 14 to assess average daily gain (ADG), average daily feed intake

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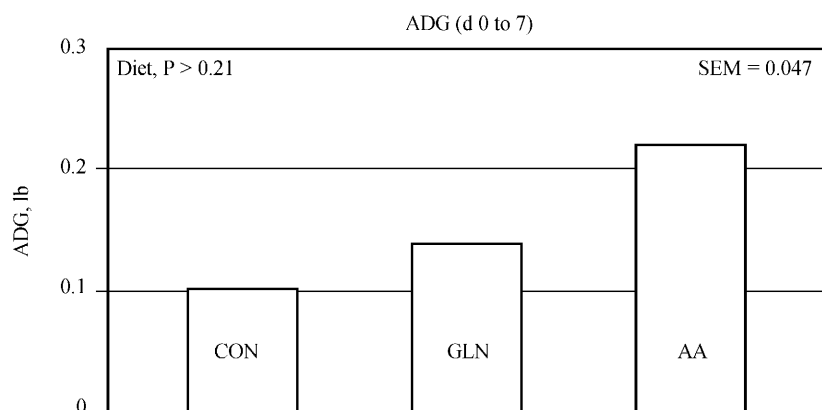


Figure 1. Effect of diet on average daily gain (d 0 to 7); Con = Control diet; GLN = 5 % Glutamine diet; AA = Nonessential amino acid diet (isonitrogenous to GLN).

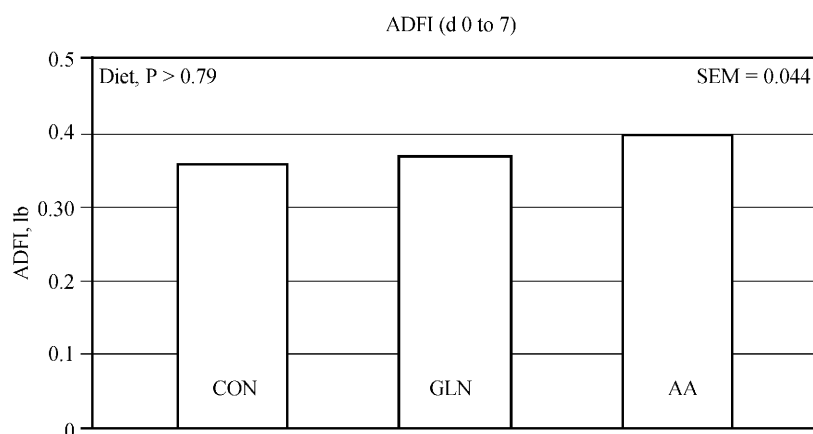


Figure 2. Effect of diet on average daily feed intake (d 0 to 7); Con = Control diet; GLN = 5 % Glutamine diet; AA = Nonessential amino acid diet (isonitrogenous to GLN).

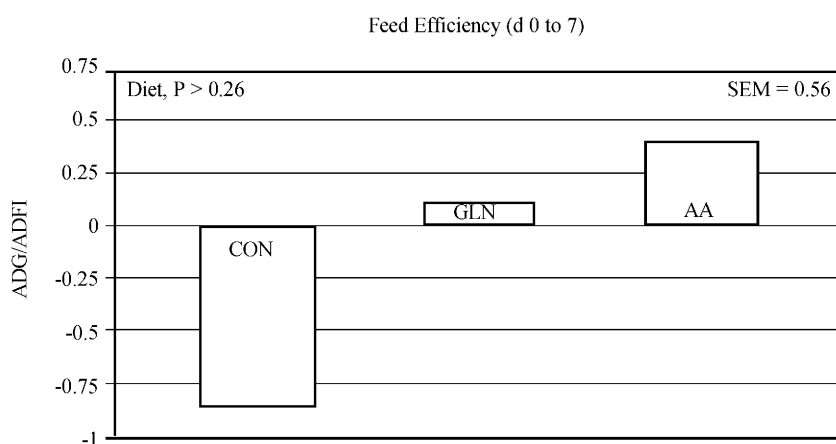


Figure 3. Effect of diet on feed efficiency (d 0 to 7); Con = Control diet; GLN = 5 % Glutamine diet; AA = Nonessential amino acid diet (isonitrogenous to GLN).

maintaining growth performance after an acute immune challenge. In the future, it will be important for researchers to quantify glutamine in

typical feedstuffs (spray-dried animal plasma, bloodmeal, fish meal, soybean meal, etc.). This may allow the use protein-bound glutamine as a way to



**Table 2. Day 0 to 14 combined growth performance and small intestine characteristics at day 14.**

Criteria, units	CON <sup>a</sup>		GLN <sup>b</sup>		AA <sup>c</sup>		SEM	P value <sup>h</sup>		
	SAL <sup>d</sup>	LPS <sup>e</sup>	SAL	LPS	SAL	LPS		Diet	LPS	Diet x LPS
ADG <sup>f</sup> , d 0 to 14, lb	0.26	0.15	0.25	0.27	0.34	0.20	0.06	NS	< 0.05	NS
ADFI <sup>g</sup> , d 0 to 14, lb	0.53	0.38	0.42	0.51	0.50	0.42	0.04	NS	NS	< 0.05
ADG/ADFI, d 0 to 14	-0.49	0.20	0.42	0.34	0.60	0.44	0.40	NS	NS	NS
Small intestine length, m	10.48	8.92	10.44	9.51	10.54	9.13	0.29	NS	< 0.0001	NS
Small intestine full wt., g	292.33	229.50	281.67	290.83	340.67	256.67	16.34	< 0.09	< 0.005	< 0.03
Small intestine empty wt., g	334.15	258.00	327.83	314.00	358.00	257.33	18.34	NS	< 0.0005	< 0.07

<sup>a</sup>CON = Control diet.

<sup>b</sup>GLN = 5% glutamine diet.

<sup>c</sup>AA = Nonessential amino acid diet (isonitrogenous to GLN).

<sup>d</sup>SAL = Saline injection.

<sup>e</sup>LPS = Lipopolysaccharide injection.

<sup>f</sup>ADG = average daily gain.

<sup>g</sup>ADFI = average daily feed intake.

<sup>h</sup>NS = P > 0.10.

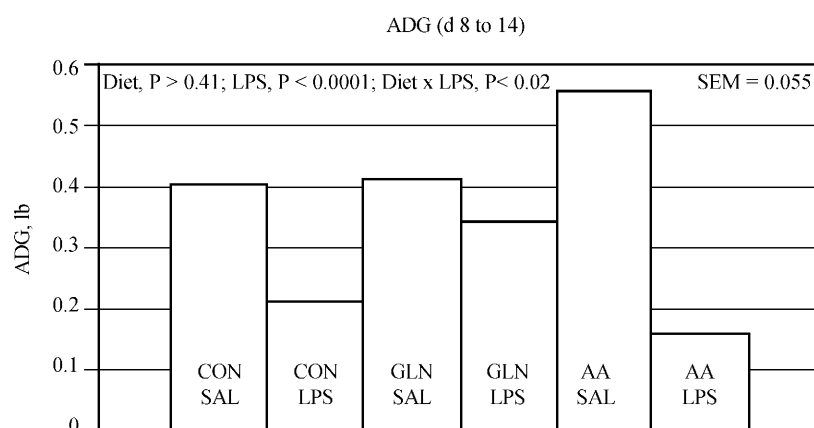
(ADFI), and feed efficiency (ADG/ADFI). On day 14, all pigs were anesthetized, the body cavity was opened, and the small intestine was removed. After samples were collected from the small intestine, pigs were euthanized. The small intestine length, full (including feed) intestine weight, and empty intestine weight were recorded.

## Results and Discussion

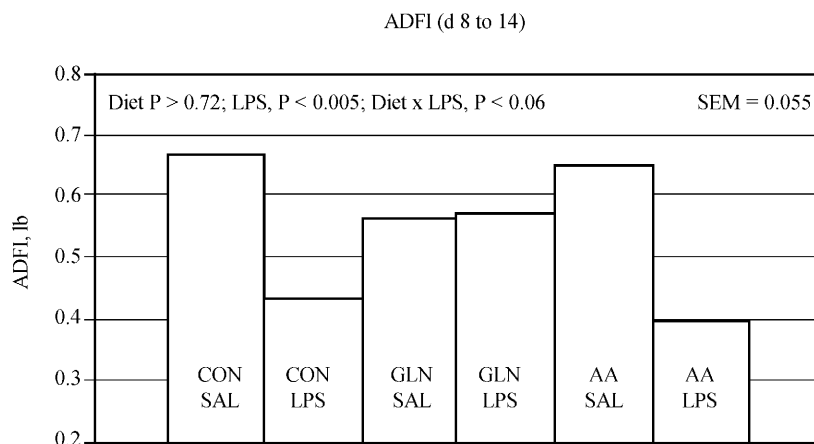
During days 0 to 7, diet did not affect ADG (Figure 1; P > 0.21), ADFI (Figure 2; P > 0.79), or ADG/ADFI (Figure 3; P > 0.26). This suggests that pigs do not possess a dietary requirement for glutamine in situations where the immune system is not vigorously activated.

After the LPS injection (day 8 to 14), pigs grew 48% slower (Figure 4; P < 0.0001) compared to pigs injected with SAL. Additionally, LPS reduced ADFI (Figure 5; P < 0.005) and ADG/ADFI (Figure 6; P < 0.001) by 25 and 32%, respectively. Pigs fed GLN and injected with LPS had similar ADG (P > 0.39), ADFI (P > 0.95), and ADG/ADFI (P > 0.24) compared to pigs fed GLN and injected with SAL (Diet × LPS; P < 0.06).

Average daily gain was decreased (P < 0.05) by LPS versus SAL during days 0 to 14 (Table 2). However, LPS did not decrease feed efficiency (P > 0.64) of pigs when days 0 to 7 and 8 to 14 were combined. Pigs fed GLN and injected with LPS had greater ADFI compared pigs fed GLN and injected



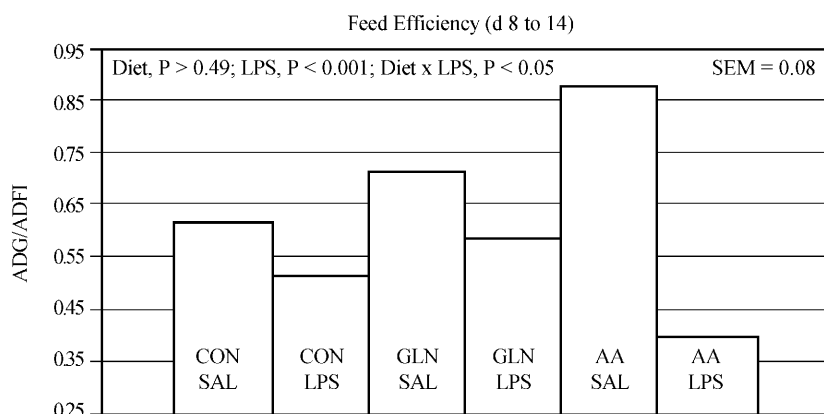
**Figure 4. Effect of diet and immune challenge on average daily gain (d 8 to 14); Con = Control diet; GLN = 5 % Glutamine diet; AA = Nonessential amino acid diet (isonitrogenous to GLN); SAL = Saline injection; LPS = Lipopolysaccharide injection.**



**Figure 5. Effect of diet and immune challenge on average daily feed intake (d 8 to 14); Con = Control diet; GLN = 5 % Glutamine diet; AA = Nonessential amino acid diet (isonitrogenous to GLN); SAL = Saline injection; LPS = Lipopolysaccharide injection.**

with SAL during days 0 to 14; whereas, pigs fed AA and CON and injected with LPS had decreased ADFI (LPS × Diet, P < 0.05).

Injecting pigs with LPS caused a 12 % reduction (P < 0.0001) in small intestine length compared to pigs injected with SAL (Table 2). Pigs fed



**Figure 6.** Effect of diet and immune challenge on feed efficiency (d 8 to 14); Con = Control diet; GLN = 5 % Glutamine diet; AA = Nonessential amino acid diet (isonitrogenous to GLN); SAL = Saline injection; LPS = Lipopolysaccharide injection.

GLN had similar small intestine weights (full and empty) compared to pigs fed GLN and injected with SAL; however, pigs fed either CON or AA and injected with SAL had reduced small intestine weight compared to their GLN counterparts (Diet x LPS,  $P < 0.07$ ). The response of small intestine

weight to treatments was similar to the response observed for ADG. It is possible that the effects observed on intestine weight may be related to body weight and (or) feed intake (indirect effects of glutamine) and not a direct effect of glutamine; however, glutamine is known to be an

important source of energy for the small intestine.

### Conclusion

From these data, it is apparent that dietary glutamine is an essential nutrient during an acute immune challenge. Whether all acute or chronic immune challenges would respond to dietary glutamine is unknown. However, dietary glutamine may play a role in modulating the immune response of *E. coli* infection and possibly other infections. It will be important to quantify glutamine concentrations in feedstuffs in order to better understand the function of glutamine and specific ingredients in improving growth and health of weaning pigs.

<sup>1</sup>Steven J. Kitt is a graduate student, Phillip S. Miller is an associate professor, and Robert L. Fischer is a graduate student and research technologist in the Department of Animal Science.

## Influence of Crystalline or Protein-Bound Lysine on Growth Performance, Body Protein Deposition and Lysine Utilization in Nursery Pigs

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### Summary and Implications

Experiments have shown that the efficiency of utilization of crystalline amino acids may be lower than that of amino acids bound in protein. A four-week experiment was conducted to determine whether the efficiency of utilization of crystalline lysine was lower than that of lysine in soybean meal for growth and body protein deposition in nursery pigs. A total of 30 pigs

(15 barrows and 15 gilts) with initial body weight of 13 lb were blocked by sex and randomly allotted, one per pen, to 30 pens in two nursery facilities. There were six replications per treatment. Six pigs (three barrows and three gilts) were killed at the beginning of the experiment to determine initial body composition. Pigs were fed five dietary treatments that consisted of a basal diet (1.05% lysine) and diets containing 1.15 and 1.25% lysine which were achieved by adding lysine to the basal diet from either soybean meal (SBM) or L-Lysine.HCl (crystalline). Blood samples were collected on the last day of the experiment and plasma was analyzed for

urea concentration. Average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG/ADFI) were similar ( $P > 0.10$ ) among treatments. The total lysine intake increased as the lysine concentration in the diet increased ( $P < 0.01$ ). Body protein content was affected by diet ( $P < 0.01$ ). For pigs fed diets containing 1.15% lysine, body protein percentage was greater ( $P < 0.01$ ) for pigs consuming crystalline lysine, versus SBM-supplemented diets. However, body deposition rates of protein were not different among treatments. Body fat concentration and body fat deposition were affected by

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