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# Effects of Glutamine on Growth Performance and Small Intestine Villus Height in Weanling Pigs

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## Summary and Implications

*The pig's small intestinal structure and function is altered during the days that follow weaning. As a consequence, the digestive and absorptive capacity of weanling pigs may decrease during this period, and this may be partially responsible for the postweaning growth lag. Additionally, health benefits may be associated with an improved small intestinal structure and function during the early postweaning period. This experiment was conducted to evaluate the effects of crystalline glutamine and (or) diet complexity on small intestine villus height and growth performance of 18-day-old pigs. During the 21-day trial, no differences in villus height were observed between pigs fed diets with or without supplemental glutamine or between pigs fed a complex diet or a simple diet. Pigs fed the complex diet had improved ( $P < 0.01$ ) average daily gain during days 0 to 4, 7 to 14, and 14 to 21. The majority of improvement in average daily gain of pigs fed the complex diet was likely due to the improvement in average daily feed intake; in as much as average daily feed intake was improved ( $P < 0.05$ ) during days 0 to 4, 4 to 7, 7 to 14, and*

*14 to 21. Pigs fed the simple diet had improved ( $P < 0.05$ ) feed efficiency during days 7 to 14 and 14 to 21. Although supplemental glutamine did not improve villus height, it did improve ( $P < 0.05$ ) feed efficiency during days 14 to 21, regardless of diet complexity. The glutamine-induced improvement in feed efficiency may have been related to other improvements in intestinal structure and function that were not measured in this experiment.*

## Background and Introduction

Villus atrophy (degeneration of the absorptive organelles) and crypt hyperplasia (increased cellular growth of the cells that replace villus epithelial cells) is observed in the small intestine of the weanling pig. During this time, it is thought that a temporary decrease in digestive and absorptive capacity ensues for several days. Additionally, it has been estimated that the majority of the immune response of a weanling pig is mediated in the small intestine.

Glutamine is an amino acid that is considered nonessential for swine. However, in humans glutamine promotes gastrointestinal growth during intravenous feeding and after traumatic events such as gastrointestinal surgery. Rapidly dividing cells, including the absorptive and immune cells of the small intestine, use glutamine (in preference to glucose) as an energy source. Additionally, free (unbound to protein) glutamine is the most abundant amino

acid in sow milk, particularly in late lactation. The addition of 1% crystalline glutamine to a corn-soybean meal diet has been reported to reduce villous atrophy in the jejunum (mid portion of the small intestine) on the seventh day after weaning. Other studies have demonstrated improvements in intestinal lamina propria depth (region associated with immune cell synthesis) and increased crypt depth. Cell culture experiments have shown that glutamine decreased intestinal permeability after an endotoxin (toxin synthesized by *E. coli*) challenge and *E. coli* challenge suggesting that glutamine decreases the ability of enteric pathogens and their toxins to enter the portal blood circulation. Animal experiments have confirmed an improved immune response, with elevated IgG (a indicator of immune system activation) after an *E. coli* challenge.

The goal of this study was to determine the effect of crystalline glutamine on villus height and growth performance in nursery pigs fed simple or complex Phase-I diets.

## Procedures

A total of 115, 18-day-old ( $\pm 2$ ) weanling pigs were used in this experiment. On day 0, four pigs were killed to determine initial villus height. The remaining 111 pigs were blocked ( $n = 4$ ) by weight and randomly assigned to pen ( $n = 16$ ; 7 pigs/pen). Treatments (Table 1) were arranged in a  $2 \times 2$

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**Table 1. Composition of diets, % (as fed).**

Ingredient	Simple		Complex	
	Control	Glutamine	Control	Glutamine
Corn	46.65	45.65	33.79	32.79
Dried whey	0	0	35.75	35.75
Soybean meal, 46.5% CP	45.78	45.78	13.45	13.45
Spray-dried plasma	0	0	6.00	6.00
Menhaden fish meal	0	0	6.00	6.00
Corn oil	3.00	3.00	3.00	3.00
Dicalcium phosphate	2.54	2.54	0.06	0.06
Limestone	0	0	0.20	0.20
Salt	0.74	0.74	0.35	0.35
L-Lysine•HCl	0.13	0.13	0.09	0.09
DL-Methionine	0.10	0.10	0.11	0.11
L-Threonine	0.08	0.08	0	0
Vitamin premix <sup>a</sup>	1.00	1.00	1.00	1.00
Mineral premix <sup>b</sup>	0.20	0.20	0.20	0.20
L-Glutamine	0	1.00	0	1.00

**Calculated composition:**

	Simple	Complex
ME <sup>c</sup> , kcal/lb	1,539	1,559
Lactose, %	0	25.0
CP, %	25.5	22.0
Lysine, %	1.60	1.60
Ca, %	0.90	0.90
P, %	0.92	0.73
P avail., %	0.55	0.55
Na, %	0.30	0.70

<sup>a</sup>Supplied per lb of diet: retinyl acetate, 2,000 IU/lb; cholecalciferol, 250 IU;  $\alpha$ -tocopherol acetate, 10 IU; menadione sodium bisulfite, 1.50 mg; riboflavin, 2.50 mg; d-pantothenic acid, 10.0 mg; niacin, 15.0 mg; choline, 50 mg; vitamin B<sub>12</sub>, 10.0  $\mu$ g.

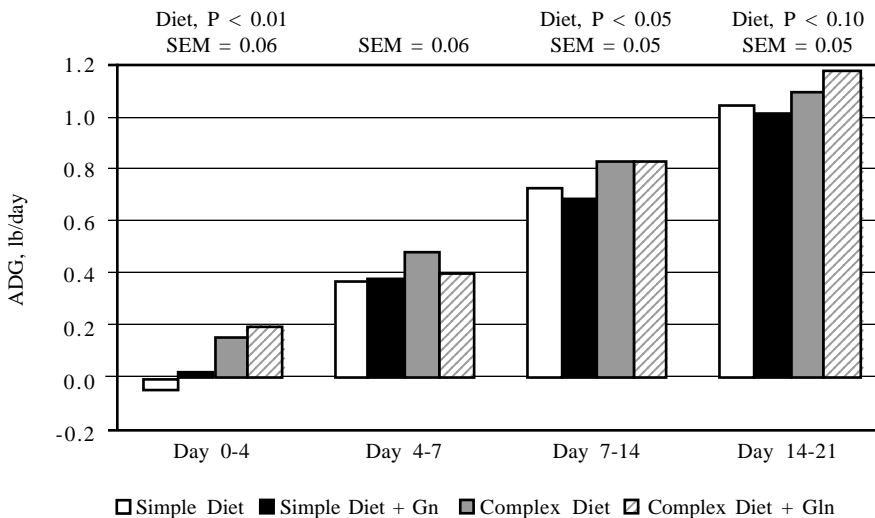
<sup>b</sup>Supplied per lb of diet: Zn (as ZnO), 100 mg; Fe (as FeSO<sub>4</sub>•H<sub>2</sub>O), 100 mg; Mn (as MnO), 20 mg; Cu (as CuSO<sub>4</sub>•5 H<sub>2</sub>O), 10 mg; I (as Ca(IO<sub>3</sub>)•H<sub>2</sub>O, 0.14 mg; Se (as Na<sub>2</sub>SeO<sub>3</sub>), 0.27 mg.

<sup>c</sup>Metabolizable energy.

factorial with the main effects being glutamine concentration (0% or 1%) and diet (simple or complex). Pigs and feeders were weighed and four pigs per treatment were killed on days 4, 7, 14, and 21. Pigs that were used for villus height determination were fully anesthetized, and an incision was made near the naval. The intact small intestine was removed from the body cavity and placed in physiologic saline solution, and the pig was then killed before it regained consciousness. Duodenum samples were taken at 5 cm from the beginning of the small intestine and jejunum samples were taken from the central point between the beginning and end of the small intestine. Samples were immediately fixed in a formalin solution and transferred to an ethanol solution. The duodenum and jejunum samples were imbedded in paraffin, sliced, mounted on a slide, and stained. A total of fifteen representative villi were measured from three sites of each sample using a microscope with a power of 25 times that of normal vision. Data were analyzed as a randomized complete block design with initial weight as the block, pen as the experimental unit, and individual pigs as the sampling unit for villus height determination. Data are reported as least squares means.

**Results and Discussion**

Pigs fed the complex diet had increased average daily gain (ADG) during days 0 to 4 ( $P < 0.01$ ), 7 to 14 ( $P < 0.05$ ), and 14 to 21 ( $P < 0.10$ ) compared to pigs fed the simple diet (Figure 1). Average daily feed intake (ADFI) of pigs fed the complex diet was increased during days 0 to 4 ( $P < 0.05$ ), 4 to 7 ( $P < 0.001$ ), 7 to 14 ( $P < 0.001$ ), and 14 to 21 ( $P < 0.05$ ) (Figure 2). Pigs fed the simple diet had greater ( $P < 0.05$ ) feed efficiency (ADG/ADFI) during days 7 to 14 and 14 to 21 (Figure 3). Greater ( $P < 0.05$ ) ADG/ADFI was observed during days 14 to 21 in pigs fed supplemental glutamine compared to pigs fed the diets without supplemental glutamine.



**Figure 1. Average daily gain of pigs fed diets differing in complexity and crystalline glutamine concentration. Gln = glutamine.**



**Table 2. Effects of diet complexity and crystalline glutamine supplementation on weaning pig villus height and small intestine length.**

	Simple		Complex		Diet	Glutamine	Diet × Glutamine	SEM
	Control	Glutamine	Control	Glutamine	P <sup>d</sup> <			
Day 4 <sup>a</sup>								
Duodenum VH <sup>b</sup> , mm	0.438	0.493	0.488	0.512	NS	NS	NS	0.032
Jejunum VH, mm	0.338	0.327	0.324	0.334	NS	NS	NS	0.015
Day 7								
Duodenum VH, mm	0.162	0.160	0.221	0.162	0.05	0.05	0.05	0.012
Jejunum VH, mm	0.131	0.121	0.141	0.126	NS	0.20	NS	0.009
Day 14								
Duodenum VH, mm	0.232	0.204	0.232	0.226	NS	0.18	NS	0.012
Jejunum VH, mm	0.161	0.176	0.159	0.157	NS	NS	NS	0.010
Day 21								
Duodenum VH, mm	0.257	0.252	0.220	0.253	NS	NS	NS	0.022
Jejunum VH, mm	0.191	0.195	0.184	0.182	NS	NS	NS	0.014
SI <sup>c</sup> length, m	10.533	10.970	10.980	11.138	NS	NS	NS	0.459

<sup>a</sup>Day 0 duodenum = 0.475 mm; Day 0 jejunum = 0.420 mm.

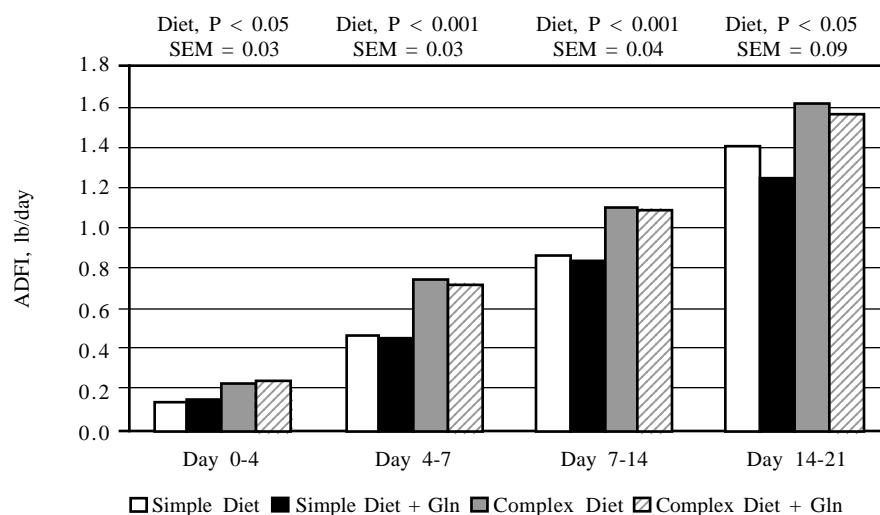
<sup>b</sup>VH = villus height.

<sup>c</sup>SI = small intestine.

<sup>d</sup>NS = P > 0.20.

Villus height (Table 2) on day 7 was reduced by 37% and 31% of day 0 villus height in the duodenum and jejunum, respectively. Villus height increased progressively after day 7, but by day 21 was still only 52% and 45% of day 0 duodenum and jejunum villus height, respectively. Surprisingly, diet complexity had little effect on villus height with the exception that the control-complex diet had greater duodenum villus height on day 7, resulting in an interaction of diet complexity and glutamine concentration ( $P < 0.05$ ). Duodenum villus height seemed to be greater on day 4 in pigs offered the complex diet and glutamine supplementation, but was not different ( $P > 0.27$ ) from pigs fed the simple or non-glutamine supplemented diet. No difference ( $P > 0.52$ ) in total small intestine length was observed among treatments on day 21.

The results from this trial confirmed that ADFI was increased when pigs were offered a complex diet compared to a simple diet. This increase in ADFI supported an improved ADG. However, pigs fed the simple diet had a greater ADG/ADFI. No improvement in villus height was attributed to diet complexity or glutamine supplementation; however, the variability in



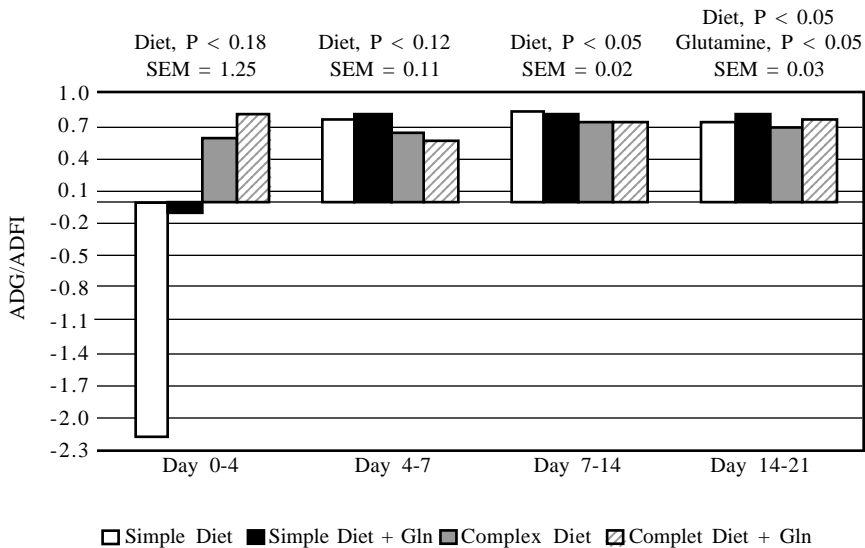
**Figure 2. Average daily feed intake of pigs fed diets differing in complexity and crystalline glutamine concentration. Gln = glutamine.**

villus height measurements appeared to be high. The improvement in ADG/ADFI in pigs offered the diet with supplemental glutamine coupled with known properties of glutamine on intestine metabolism suggests an improvement in intestine function or morphology.

Future research will attempt to examine the effects of glutamine on small intestine growth using more direct measurements. For example,

indices of protein and DNA concentrations may provide a more definitive answer to how glutamine is influencing intestinal growth. Also, it may be important to study the effects of glutamine during a pathogen challenge. Presently, we are working on an assay to quantify glutamine in feedstuffs to help objectively assess dietary glutamine concentration in a practical manner.

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**Figure 3. Feed efficiency of pigs fed diets differing in complexity and crystalline glutamine concentration. Gln = glutamine.**

## Conclusions

Data from this trial suggest that diet complexity had a significant effect on growth, but little to no effect on intestine villus height. Supplemental glutamine did not improve villus height but did improve feed efficiency in the third week of this 21-day growth study. Additional research is needed to examine the effects of glutamine on intestine metabolism and function to ascertain whether glutamine may be beneficial in practical situations.

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

# Influence of Linoleic Acid Isomers on Body Fat

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## Summary and Implications

*In two studies, mice were fed diets containing either individual conjugated linoleic acid (CLA) isomers or a mixture of isomers in the presence or absence of dietary essential fatty acids. Mice fed the C18:2 Δ10,12 CLA isomer lost as much body fat as mice fed a mixture of isomers. This effect was not observed when the mice were fed the C18:2 Δ9,11 isomer or when feed intake was restricted. The loss of body fat was much greater in mice consuming an essential fatty acid deficient diet versus a control diet. This supports our hypothesis that for CLA to deplete body fat, it must first be metabolized in a manner similar to linoleic acid. Furthermore, we sug-*

*gest that the loss of body fat may be mediated by metabolism of CLA to an isomer of arachidonic acid. Understanding the mechanism by which CLA causes body fat loss, in pigs as well as mice, will allow for greater regulation of body fat content.*

## Introduction

Conjugated linoleic acid (CLA) is a group of isomers of linoleic acid (C18:2Δ9,12), which, when consumed, produce health benefits such as reducing the incidence of cancer and cardiovascular disease and reducing body fat content. Furthermore, in swine, dietary CLA has resulted in firmer bellies, reduced backfat, and improved feed efficiency. Our group previously reported (Nebraska Swine Report 2001, pg 27 – 28) that not only did dietary CLA supplementation cause a loss of body fat in mice, but that it also resulted in programmed cell death, or apoptosis, of fat cells. The basis for the following

two studies was to further determine the mechanism by which CLA is causing both the body fat loss as well as the apoptosis. The predominant naturally occurring isomer is C18:2Δ9,11 (CLA 9/11), whereas commercially synthesized CLA products usually contain approximately equal amounts of C18:2Δ10,12 (CLA 10/12) and CLA 9/11 as well as smaller quantities of other isomers. The diverse benefits of CLA may depend on different isomers. Therefore our first objective was to determine which isomer(s) are responsible for the loss of body fat in mice.

Arachidonic acid (C20:4Δ5,8,11,14) is synthesized in animals from dietary linoleic acid. Similarly, CLA 10/12 can be metabolized to C20:4Δ5,8,12,14. This product of CLA metabolism could antagonize the normal production of prostaglandins from arachidonic acid. Therefore, mice fed a diet deficient in linoleic acid, and thus arachidonic acid, may be especially sensitive to the anti-