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Effects of Sow Dietary Glutamine Supplementation on Sow and Litter Performance, Subsequent Weanling Pig Performance and Intestinal Development After an Immune Challenge

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Summary and Implications

Sixteen sows were randomly assigned to two treatments: CON: Control corn-soybean meal diet; GLN: Corn-soybean meal diet + 2.5% crystalline glutamine. No differences ($P > 0.10$) between treatments were observed for sow weight loss, sow feed intake, or litter weight gain. Sow plasma glutamine concentration tended to be increased on days 7 and 21 ($P < 0.13$) in sows fed GLN. Milk glutamine concentration was increased ($P < 0.08$) on days 7 and 21 of lactation. However, suckling pig plasma glutamine concentration was not altered ($P > 0.38$) on day 21 by glutamine enriched milk consumption. On day 21, pigs were weaned to a common starter diet, sow treatment structure was maintained, and two additional treatments were imposed on weanling pigs and arranged in a 2×2 factorial: SAL: Saline injection on days 1 and 3; Lipopolysaccharide (LPS) $91 \mu\text{g} \cdot \text{lb BW}^{-1}$ injection on days 1 and 3. Lipopolysaccharide injection on days 1 and 3 reduced ($P < 0.05$) ADG during days 0 to 3, 3 to 7, and 7 to 14. Daily feed intake was reduced ($P < 0.005$) during days 0 to 3, 3 to 7, 7 to 14, and 14 to 21 by LPS injection. However, LPS increased ADG/ADFI during days 3 to 7 ($P < 0.0001$) and days 7 to 14 ($P < 0.02$). Progeny of sows fed CON diet gained 0.14 lb/d ($P < 0.03$) more weight during days 3 to 7, and consumed 0.33 lb/d more feed ($P < 0.09$)

during days 7 to 14 versus progeny of GLN-fed sows. Small intestine length measured on day 3 was not affected ($P > 0.23$) by sow diet or injection type. Pigs injected with LPS had reduced ($P < 0.01$) small intestine empty weight. Progeny from sows that consumed CON had 10% greater empty weight on day 7 compared to progeny from sows fed GLN. Pigs injected with LPS had reduced ($P < 0.01$) small intestine weights on day 7 compared to pigs injected with SAL. Lipopolysaccharide challenge reduced ($P < 0.01$) duodenum villus height. However, progeny of sows that consumed GLN had 12% greater ($P < 0.05$) villus height on day 3 compared to progeny of sows fed CON. Duodenum villus height on day 7 was similar in progeny from sows fed GLN and injected with SAL; whereas, progeny from sows fed GLN injected with SAL had reduced villus height (Diet \times LPS, $P < 0.05$). Collectively, these data suggest that dietary glutamine increases sow milk glutamine concentration, but does not positively influence progeny growth performance during lactation or immediately following weaning during an immune challenge.

Introduction

Previous research conducted at this station (see Kitt et al., Nebraska Swine Report 2003) suggested that glutamine may be a dietary essential amino acid during an immune challenge immediately following weaning. However, currently no assay is available to quantify glutamine in feedstuffs; additionally, crystalline glutamine is not

economically practical to include in weanling pig diets. One method to heighten piglet glutamine intake is via increased piglet glutamine composition of sow milk. Therefore, we proposed to investigate whether increased sow glutamine intake alters milk glutamine composition and subsequently affects growth performance and intestinal characteristics of immune-challenged weanling pigs.

Procedures

Sow and Litters.

On approximately day 106 of gestation, 16 sows were transported from the University of Nebraska Agricultural Research Division Swine Farm to the Animal Science Complex. Upon arrival, sows were weighed and randomly allotted to treatments; whereby, treatments were initiated on the day of parturition. Treatments were: 1) Corn-soybean meal (CON), or 2) Corn-soybean meal + 2.5% L-glutamine (GLN), where L-glutamine replaced corn in the diet (Table 1). Until parturition, sows were fed 6.0 lb/d of a standard 14% CP corn-soybean meal gestation diet. All sows were induced to farrow via intramuscular injection of 10 mg prostaglandin $F_2\alpha$ (In-Synch, Pro Labs, St. Joseph, MO) on day 112 of gestation. All sows farrowed within 25 h of the first sow farrowed. Sows were weighed on days -3, 7, 14 and 21 relative to farrowing and litters were weighed on days 0, 7, 14 and 21. By day 1 post-farrowing, litters were standard-



Table 1. Ingredient and calculated nutrient composition of sow diets (as-fed basis).

Ingredients, %	Treatment ^a	
	CON	GLN
Corn	63.35	60.85
Soybean meal, 46.5% CP	30.00	30.00
Dicalcium phosphate	2.65	2.65
Bleachable tallow	2.50	2.50
Limestone	0.60	0.60
Salt	0.50	0.50
Breeding vitamin premix ^b	0.25	0.25
Trace mineral premix ^c	0.15	0.15
L-Glutamine	2.50	—
Calculated nutrient composition		
Lysine, %	1.00	1.00
ME, kcal/lb	1,534	1,495
Crude fat, %	5.86	5.76
Crude fiber, %	2.26	2.21
Ca, %	0.96	0.96
P, %	0.88	0.87
P, avail. %	0.55	0.55
Analyzed nutrient composition		
CP, %	19.86	22.43
Lysine, %	0.97	0.96

^aCON = Control; GLN = Control + 2.5% L-Glutamine.

^bSupplied per kg of diet: Vitamin A (as retinyl acetate), 6,600 IU; Vitamin D (as cholecalciferol), 659 IU; Vitamin E (as α -tocopherol acetate), 66.15 IU; Vitamin K (as menadione sodium bisulfite), 4.35 mg; riboflavin, 11.0; d-pantothenic acid, 22.0 mg; niacin, 33.0 mg; vitamin B₁₂, 22 ug; folate, 1.65 mg; biotin, 0.22 mg.

^cSupplied per kg of diet: Zn (as ZnO), 126.5 mg; Fe (as FeSO₄•H₂O), 126.5 mg; Mn (as MnO), 30.0 mg; Cu (as CuSO₄•5 H₂O), 10.45 mg; I (as Ca(IO₃)•H₂O), 0.29 mg; Se (as Na₂SeO₃), 0.30 mg.

Table 2. Ingredient and calculated nutrient composition of common nursery diet (as-fed basis).

Ingredients, %	
Corn	43.97
Dried whey, 12% CP	20.75
Soybean meal, 46.5% CP	20.00
Fish meal, 60% CP	6.00
Spray-dried animal plasma	4.00
Corn oil	2.50
Dicalcium phosphate	0.69
Salt	0.40
Limestone	0.35
Vitamin premix ^a	0.25
Trace mineral premix ^b	0.15
L-lysine•HCl, 78.8%	0.11
DL-Methionine, 99%	0.10
Calculated nutrient composition	
Lysine, %	1.60
ME, kcal/lb	1,554
Crude fat, %	5.59
NDF, %	6.44
Ca, %	0.90
P, %	0.79
P, avail. %	0.55
Analyzed nutrient composition	
CP, %	23.59
Lysine, %	1.42

^aCON = Control; GLN = Control + 2.5% L-Glutamine.

^bSupplied per kg of diet: Vitamin A (as retinyl acetate), 6,600 IU; Vitamin D (as cholecalciferol), 659 IU; Vitamin E (as α -tocopherol acetate), 66.15 IU; Vitamin K (as menadione sodium bisulfite), 4.35 mg; riboflavin, 11.0; d-pantothenic acid, 22.0 mg; niacin, 33.0 mg; vitamin B₁₂, 22 ug; folate, 1.65 mg; biotin, 0.22 mg.

^cSupplied per kg of diet: Zn (as ZnO), 126.5 mg; Fe (as FeSO₄•H₂O), 126.5 mg; Mn (as MnO), 30.0 mg; Cu (as CuSO₄•5 H₂O), 10.45 mg; I (as Ca(IO₃)•H₂O), 0.29 mg; Se (as Na₂SeO₃), 0.30 mg.

ized to 11 pigs. On day 2 post-farrowing, an injection of iron dextran was administered, needle teeth were clipped, and tails were docked. Boars were castrated on day 7 of parturition. Milk samples were collected on days 7, 14 and 21 after injection of 20 units of oxytocin (Pro Labs Ltd., St. Joseph, MO; 20 USP units/mL) from multiple teats. Blood samples were collected from sows on days 7, 14 and 21 and from weanling pigs (from 4 of 8 pigs per pen) on day 21 post-farrowing.

Weanling Pigs

One hundred twenty-eight pigs (64 barrows and 64 gilts) were weaned on day 21 of lactation and within sow treatment, randomly assigned to one of two nursery treatments: 1) Saline (0.90%) injection on days 1 and 3 postweaning, or 2) 91 μ g/lb body weight *E. coli* Lipopolysaccharide (LPS) injection on days 1 and 3 postweaning. Eight pigs were placed into one of the 16 pens and fed a common starter nursery diet (Table 2). Pigs and feeders were weighed on days 3, 7, 14 and 21 to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (ADG/ADFI). On days 3 and 7 four pigs per treatment (one pig per pen) were used to measure small intestine characteristics. Small intestine sampling and measurements were performed as previously described in the 2003 Nebraska Swine Report.

Sample Analysis

Plasma and milk samples were deproteinized within 12 hours of collection and immediately frozen and stored at -80°C. Samples remained frozen until glutamine analysis.

Data Analysis

Sow and litter criteria data were analyzed as a completely randomized design with sow and litter as the experimental units, respectively. Nursery growth performance and

(Continued on next page)



intestine characteristics were analyzed as a randomized complete block design with treatments arranged in a 2 × 2 factorial. In the nursery experiment, the model included main effects of sow treatment and nursery treatment and interactions of main effects. Nursery pen was the experimental unit. Data are reported as least squares means.

Results

No differences for sow weight loss, sow feed intake, or litter weight gain (Table 3; $P > 0.15$) were observed between sows fed the CON diets versus sows fed the GLN diet. Sows fed 2.5% supplemental dietary glutamine tended to have increased plasma glutamine concentration on day 7 ($P < 0.11$) and 21 ($P < 0.13$). Additionally, sows fed increased glutamine had 46% and 265% (on days 7 and 21, respectively) greater ($P < 0.08$ and $P < 0.01$, respectively) milk glutamine concentration compared to sows fed the control diet. Weanling pig plasma glutamine concentration (Table 3) did not differ ($P > 0.38$) between pigs that suckled from dams consuming CON versus GLN diets. These data suggest that increased glutamine intake and increased milk glutamine has little effect on sow and litter performance.

Postweaning performance and small intestine characteristics are provided in Table 4. Endotoxin challenge on days 1 and 3 reduced ($P < 0.05$) ADG during days 0 to 3, 3 to 7, and 7 to 14. Daily feed intake was reduced ($P < 0.005$) during days 0 to 3, 3 to 7, 7 to 14, and 14 to 21 by LPS injection. Due to a greater reduction in ADFI relative to ADG, pigs injected with LPS had 40% and 12% greater ADG/ADFI than pigs injected with SAL during days 3 to 7 ($P < 0.0001$) and days 7 to 14 ($P < 0.02$), respectively.

Progeny of sows fed CON diet gained 0.14 lb/d ($P < 0.03$) more

Table 3. Sow and litter growth performance and plasma metabolite concentrations.

Item	CON ^a	GLN ^b	SEM	P-value ^c , <
d - 3 to 7 sow weight loss, lb	39.12	43.17	6.42	NS
d 7 to 14 sow weight loss, lb	7.56	15.94	6.20	NS
d 14 to 21 sow weight loss, lb	0	0.74	3.42	NS
d -3 to 21 sow weight loss, lb	46.66	59.89	9.02	NS
d 0 to 7 weekly sow feed intake, lb	78.45	74.71	5.31	NS
d 7 to 14 weekly sow feed intake, lb	103.37	100.44	6.72	NS
d 14 to 21 weekly sow feed intake, lb	109.57	104.12	4.85	NS
d 0 to 7 litter weight gain, lb	28.38	27.69	1.30	NS
d 7 to 14 litter weight gain, lb	44.67	45.97	1.94	NS
d 14 to 21 litter weight gain, lb	41.16	48.60	1.79	NS
d 0 to 21 litter weight gain, lb	121.19	122.27	4.41	NS
d 7 sow plasma glutamine, mg/dL	6.20	7.27	0.43	0.11
d 21 sow plasma glutamine, mg/dL	4.61	6.31	0.73	0.13
d 7 milk glutamine, mg/dL	1.90	2.77	0.32	0.08
d 21 milk glutamine, mg/dL	2.38	6.31	0.87	0.01
d 21 pig plasma glutamine, mg/dL	5.96	6.35	0.31	NS

^aCON = Control sow diet.

^bGLN = 2.5% sow glutamine diet.

^cNS = $P > 0.15$.

weight during days 3 to 7, and consumed 0.33 lb/d more feed ($P < 0.09$) during days 7 to 14 than progeny of GLN fed sows. During days 0 to 3, progeny of sows fed GLN had the greatest ADG/ADFI when injected with SAL and the lowest ADG/ADFI when injected with LPS (Diet × LPS, $P < 0.05$).

Small intestine length was not influenced ($P > 0.23$) by sow diet or injection type at day 3. Pigs injected with LPS had 34% lower ($P < 0.01$) small intestine empty weight on day 3 compared to pigs injected with SAL. Progeny from sows that consumed CON had 10% greater ($P < 0.10$) small intestine length and 12% greater empty weight on day 7 compared to progeny from sows fed GLN. Pigs injected with LPS had reduced ($P < 0.01$) small intestine weights on day 7 compared to pigs injected with SAL.

Endotoxin challenge reduced ($P < 0.01$) duodenum villus height on day 3 by 22%. Progeny of sows that consumed GLN had 12% greater ($P < 0.05$) villus height on day 3 compared to progeny of sows fed CON. Duodenum villus height on day 7 was similar in progeny from sows fed GLN and injected with LPS compared to progeny from sows injected with SAL (Diet × LPS, $P < 0.05$).

Discussion

Sow weight loss and feed intake were similar during days -5 to 14 compared to other research conducted at University of Nebraska and elsewhere. Litter weight gain of 121 lb during the 21-day lactation was above expected performance.

Our previous research showed improved growth performance and intestinal growth/maturation in weanling pigs when glutamine was present in the diet during an immune challenge. Therefore, improved growth performance (due to enhanced immune response) of immune challenged progeny from sows with greater glutamine intake was expected. However, in the present study, pigs that had previously suckled sows that had increased milk glutamine concentration had generally reduced ADG, ADFI and ADG/ADFI during the 21-day feeding trial. Additionally, progeny from sows fed supplemental glutamine had reduced small intestine length and empty weight on day 7. However, progeny of sows fed supplemental glutamine and injected with endotoxin appeared to maintain duodenum villus height on day 7 compared to progeny of sows fed the control diet.

Apparently, increased dietary glutamine was not metabolized by the sow's intestine because we



Table 4. Day 0 to 21 weanling pig growth performance and small intestine (SI) characteristics.

Criteria, units	CON ^a		GLN ^b		SEM	P < ^e		
	SAL ^c	LPS ^d	SAL	LPS		Diet	LPS	Diet × LPS
ADG, d 0 to 3, lb	0.16	0.051	0.17	-0.27	0.10	NS	0.03	NS
ADFI, d 0 to 3, lb	0.332	0.17	0.32	0.10	0.03	NS	0.0001	NS
ADG/ADFI, d 0 to 3	0.46	0.27	0.52	-4.17	0.85	0.06	0.05	0.05
ADG, d 3 to 7, lb	0.82	0.68	0.73	0.49	0.05	0.03	0.005	NS
ADFI, d 3 to 7, lb	0.93	0.58	0.94	0.42	0.05	NS	0.0001	NS
ADG/ADFI, d 3 to 7	0.89	1.17	0.78	1.18	0.06	NS	0.0001	NS
ADG, d 7 to 14, lb	0.98	0.85	0.96	0.81	0.03	NS	0.02	NS
ADFI, d 7 to 14, lb	1.37	1.06	1.30	0.97	0.04	0.09	0.0001	NS
ADG/ADFI, d 7 to 14	0.71	0.79	0.74	0.83	0.03	NS	0.02	NS
ADG, d 14 to 21, lb	1.08	1.14	1.20	1.16	0.05	NS	NS	NS
ADFI, d 14 to 21, lb	1.74	1.57	1.65	1.48	0.04	0.09	0.005	NS
ADG/ADFI, d 14 to 21	0.63	0.73	0.73	0.79	0.05	NS	NS	NS
SI length, d 3, mm	8.37	8.10	8.94	8.04	0.47	NS	NS	NS
SI empty wt., d 3, lb	0.39	0.27	0.43	0.26	0.04	NS	0.01	NS
SI length, d 7, m	9.11	8.60	7.95	8.21	0.34	0.05	NS	NS
SI empty wt., d 7, lb	0.55	0.43	0.49	0.38	0.03	0.10	0.01	NS
Duodenum VH ^f , d 3, μm	429.67	391.09	515.58	408.57	22.94	0.05	0.01	NS
Jejunum VH, d 3, μm	314.27	325.67	341.65	279.70	28.70	NS	NS	NS
Duodenum VH, d 7, μm	621.64	429.78	545.16	521.43	28.22	NS	0.005	0.05
Jejunum VH, d 7, μm	437.39	347.23	334.02	311.74	40.49	NS	NS	NS

^aCON = Control sow diet.

^bGLN = 2.5% glutamine sow diet.

^cSAL = Saline injection.

^dLPS = Lipopolysaccharide injection.

^eNS = P > 0.10.

^fVH = Villus height; d 0 duodenum VH = 420.77; d 0 jejunum VH = 367.84.

observed a slight numerical increase in plasma glutamine concentration. Moreover, increased milk glutamine concentration was observed on day 7 and day 21 of lactation.

Greater glutamine intake (during suckling) may have altered the absorption or utilization of systemic (enteral and arterial) glutamine. Glutaminase is required for the catabolism of glutamine to glutamate and ammonium and is most likely the first step in use of glutamine as an energy source for cellular proliferation. Therefore, increased plasma glutamine may signal to the intestine to decrease glutamine catabolism and subsequently decrease intestinal growth.

It has been shown that glutamine synthetase (required for synthesis of glutamine from glutamate and ammonium) is important for intestinal differentiation. Therefore, absorption or arterial recruitment of glutamate and ammonia would be important for intestinal growth during an endotoxin challenge provided adequate glutamine synthetase protein is present. It is possible that the expression of glutamine synthetase or glutamate

transporters may decrease with the presence of high concentrations of enteral glutamine and this may correspond with changes in feed intake. The results observed in this experiment may be a result of decreased luminal glutamine absorption and/or glutamine synthesis capacity due to increased glutamine consumption while suckling (i.e., down regulation of amino acid transporter and/or glutamine synthetase expression). However, if this were the underlying mechanism, the maintenance of duodenum morphology cannot be explained. It may be possible that the arterial, in contrast to luminal glutamine (in pigs suckling glutamine supplemented sows) is required prevent a possible endotoxin block on intestinal glutaminase. However, the exchange of arterial versus lumenally derived glutamine for intestinal maintenance is unclear and in this experiment we did not observe an increase in plasma glutamine in newly weaned pigs. Additionally, the reduction in growth performance may be explained by a shift of glutamine (and possibly other nutrients)

towards the small intestine enterocytes and away from other tissues. However, without knowledge of first-pass (versus second pass) plasma glutamine and intestinal enzyme expression of glutamine synthetase and glutaminase, this theory can not be substantiated.

Conclusion

The data from this experiment suggest that increased sow consumption of glutamine does not improve the immune response of endotoxin-treated progeny following weaning. However, it appears that duodenum villus height may be maintained in pigs challenged with endotoxin if they previously consumed milk with greater concentrations of free glutamine (due to increased sow glutamine intake).

¹Steve J. Kitt was a graduate student and is currently employed by ADM, Des Moines, IA., Phillip S. Miller is a professor, and Robert L. Fischer is a research technologist and graduate student in the Department of Animal Science.