AMINO ACIDS

Impact of Arginine in the Water and/or Feed on Weanling Pig Performance and Gut Integrity

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Abstract

A proof of concept study was conducted to evaluate the impact of water and/or feed supplementation of L-arginine on weanling pig performance and gut integrity. A total of 240 newly weaned PIC pigs were used in a 4x3 factorial arranged experiment. Treatments included four levels of water supplementation (0, 2, 4, or 8%) and three different dietary levels of Arginine (basal, or increased by 0.20 or 0.40%) in a three-phase feeding program. Experimental diets were fed in Phases 1 and 2 (21 days total) with a common Phase 3 diet being fed for additional 20 days. Water treatments were offered only during the first 7 days. On days 7 and 21, lactulose mannitol was administered to one pig per pen to test for plasma level 4-hour post-gavage for indication of intestinal tight junction integrity. Increasing dietary SID arginine by 0.20% increased final body weight over the 41-day trial. Supplementing Arginine via the water decreased villus crypt depth (8%) and altered villus height to crypt depth ratio (4%) and decreased lactulose mannitol appearance in the blood. These results strongly suggest that the dietary arginine requirement for the weanling pig is higher than current estimates. Additionally, the use of water-supplemented arginine may benefit gut barrier integrity.

Background

Arginine plays many important roles beyond growth, including immune function and reproductive efficiency (Wu et al., 2018). Current NRC (2012) guidelines estimate the dietary SID arginine requirement at 0.68% for weaned piglets (5 - 7kg). This requirement was based on the work of Southern and Baker which is about 40 years old (1983) and little work has been conducted since to assess the arginine requirement for modern weanling pigs. However, recent work (Wu et al., 2018, Chapman et al., 2012, Konieczka et al., 2022) suggests arginine plays an important role in gut health, especially tight junctions (Chapman et al., 2012). The objective of this study was to determine the impact of supplemental arginine in the diet and/or oral administration via drinking water on weanling pig performance and gut integrity.

Methods

A proof of concept study was performed to evaluate supplemental arginine in weanling pigs using a 4 x 3 factorial arrangement of treatments; four levels of water administration (0, 4, 8, or 12% solution dosed through a 1:128 water mediator) and three levels of dietary SID arginine (1.35% (basal), 1.55%, or 1.75%). A total of 240 PIC-weaned pigs (21 ± 2 days) were allotted over 80 pens (3 pigs/pen). Pens were arranged in 20 pens for each water treatment with feed treatments randomly allotted within each water treatment. Each pen had one single-hole feeder and one nipple drinker.

The experimental timeline is shown in Figure 1. Pigs and feeders were weighed at the beginning of the trial on day 0 and again on day 7 with Phase 1 experiment diets fed and Arginine water treatments administered. On day 7 after pigs and feeders were weighed, pigs were changed to respective phase 2 diets and the water treatments were stopped. On day 21 pigs and feeders were weighed and all pens were switched to a common phase 3 diet until the end of the trial at day 41 and final body weight was recorded. Mortalities or removals were weighed with a date recorded to adjust growth and feed intake data.

Experimental diets are shown in Table 1. On day7 (before the phase 2 diet change), one pig per pen was gavaged with lactulose mannitol solution. Four hours post-gavage, blood was collected and plasma separated for determination of lactulose and mannitol concentration as an indication of gut permeability.

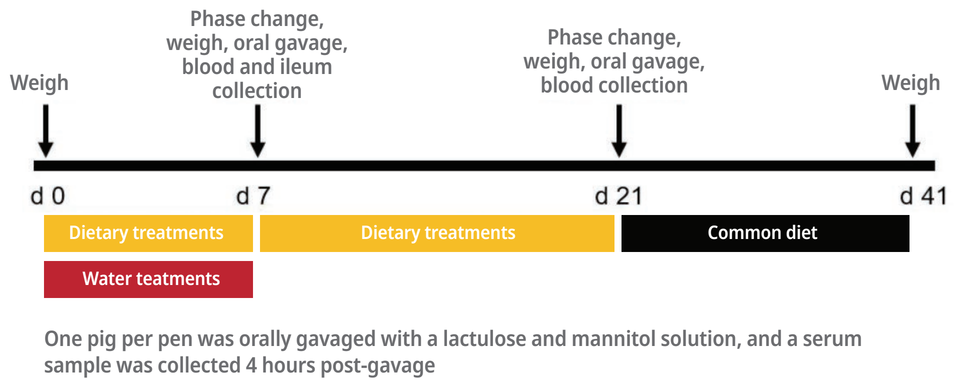


Figure 1. Experimental Timeline

Tabel 1. **Diet composition for the trial to assess arginine levels in nursery pig diets. Ingredients are listed as a percent inclusion in the diet and reported on an "as-fed" basis.**

Ingredient, %	Phase 1			Phase 2			Phase 3
	Control	1.55% Arg	1.75% Arg	Control	1.57% Arg	1.77% Arg	Control
Corn	32.555	32.351	32.150	47.766	47.556	47.356	58.580
SBM 47.5 CP	20.000	20.000	20.000	28.000	28.000	28.000	34.000
Oat grouts	15.000	15.000	15.000	5.000	5.000	5.000	-
DairyLac 80	12.906	12.906	12.906	5.406	5.406	5.406	-
Fish meal	5.000	5.000	5.000	2.500	2.500	2.500	-
Soy oil	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Plasma protein	3.985	3.985	3.985	1.290	1.296	1.296	-
Dried whey	2.500	2.500	2.500	2.500	2.500	2.500	-
Monocalcium phosphate	1.270	1.270	1.270	0.957	0.957	0.957	1.410
Calcium carbonate	0.800	0.800	0.800	0.903	0.903	0.903	1.100
Arginine	0.614	0.817	1.020	0.328	0.531	0.733	-
L-Lysine HCl	0.500	0.500	0.500	0.500	0.500	0.500	0.450
Zinc oxide	0.375	0.375	0.375	0.375	0.375	0.375	-
Salt	0.300	0.300	0.300	0.300	0.300	0.300	0.500
DL Methionine	0.294	0.294	0.294	0.282	0.282	0.282	0.252
Vitamin premix[1]	0.250	0.250	0.250	0.250	0.250	0.250	0.250
L-Threonine	0.209	0.209	0.209	0.205	0.205	0.205	0.179
Trace mineral premix[1]	0.150	0.150	0.150	0.150	0.150	0.150	0.150
L-Valine	0.146	0.146	0.146	0.152	0.152	0.152	0.121
L-Tryptophan	0.076	0.076	0.076	0.067	0.067	0.067	0.040
Copper sulfate	0.070	0.070	0.070	0.070	0.070	0.070	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Composition							
Metabolizable energy, kcal/kg	3,316	3,319	3,321	3,390	3,392	3,394	3,453
Crude protein, %	22.48	22.66	22.84	21.67	21.85	22.03	21.00
SID Lys[2]	1.50	1.50	1.50	1.42	1.42	1.42	1.33
Calcium, %	0.93	0.93	0.93	0.79	0.79	0.79	0.77
attdP[3], %	0.60	0.60	0.60	0.40	0.40	0.40	0.37
SID Arg, %	1.35	1.55	1.75	1.37	1.57	1.77	1.30

[1]Vitamin and trace mineral; Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, 0.023 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet

[2]SID, Standard ileal digestible; Lys, lysine

[3]attdP, apparent total tract digestibility

These same pigs (80) were euthanized, and an ileal sample was taken for histology evaluation of villus height and crypt depth by the Iowa State Veterinary Histology Department. Also, on day 21 (prior to a change to the common phase 3 diet), one pig per pen was gavaged with lactulose and mannitol, and the same procedure as on day 7 was repeated.

All statistical models were implemented in SAS 9.4 (SAS Inst., Cary, NC) using the GLIMMIX procedure. The ARH(1) covariance structure was selected as the best fit for the repeated measures model according to Bayesian Information Criterion for all response variables. The normality of Studentized residuals was verified using the Shapiro-Wilk test from the UNIVARIATE procedure. Studentized residuals greater than three standard deviations from the mean were considered outliers and removed from the analysis. Data were reported as least squares means and the SLICE statement was used to perform F-tests between treatments on each day with Tukey adjustment for multiplicity. The pen was considered the experimental unit. Results were considered significant if $P < 0.05$ and a tendency if $0.05 \geq P < 0.10$.

Results

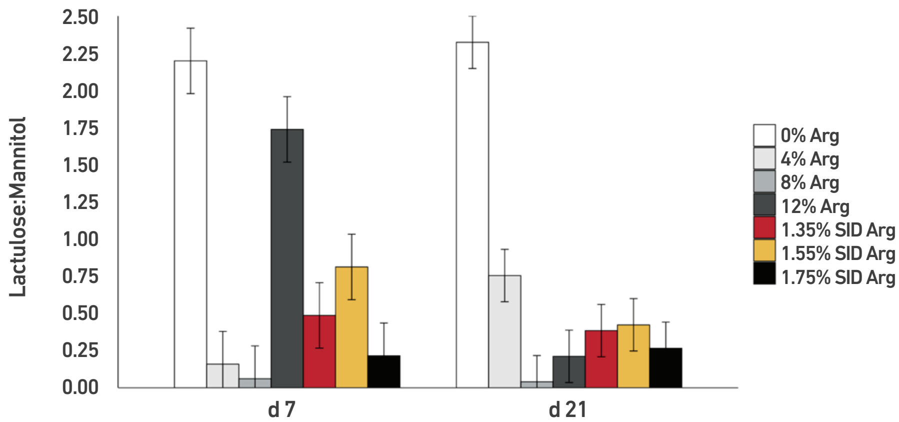
Performance results are shown in Table 2. Supplementing dietary arginine over the basal diet (1.35% SID) by 0.20% to 1.55% SID arginine resulted in a significant improvement ($P < 0.05$) in pig body weight at the end of the trial (day 41) compared to the basal diet. Further supplementation to 1.75% SID arginine resulted in numeric improvement compared to the basal diet ($P > 0.05$) There were no interactions when using arginine in the water and the feed simultaneously on pig performance. The use of 8% arginine in the water resulted in a reduction in crypt depth ($P < 0.01$). The 4% arginine water solution reduced the total villous height: crypt depth ratio ($P < 0.02$, Table 3). However, the supplementation of arginine in the water resulted in a significant reduction in the lactulose: mannitol ratio ($P < 0.01$) in the plasma when arginine was added at the level of 4% in the water (Figure 2) on day 7. Interestingly, the 8% solution decreased ($P < .05$) plasma lactulose mannitol ratio at day 21 as compared to the basal diet despite the fact that the water supplementation had ceased 14 days prior to the measurement.

Table 2. Main effects of either Arginine in the feed or the water on overall piglet performance

		Water				Feed			SEM	Feed	Water	FXW
		0	4	8	12	1.35	1.55	1.76		P Value	P Value	P Value
Start												
	Wt , kg	5.21	5.18	5.21	5.10	5.16	5.19	5.16	0.11	0.55	0.70	0.55
Day 6												
	Wt, kg	5.33	5.31	5.24	5.17	5.21	5.38	5.21	0.14			
	ADG, kg	0.02	0.03	0.01	0.01	0.00	0.03	0.01	0.01			
	ADFI, kg	0.08	0.08	0.07	0.08	0.07	0.08	0.07	0.01			
	G : F	0.20	0.19	-0.03	-0.04	-0.08	0.30	0.16	0.13			
Day 20												
	Wt, kg	9.02	9.39	9.39	8.84	8.65	9.57	9.26	0.38			
	ADG, kg	0.26	0.27	0.28	0.23	0.25	0.27	0.26	0.02			
	ADFI, kg	0.33	0.32	0.33	0.29	0.30	0.34	0.31	0.02			
	G : F	0.81	0.82	0.84	0.81	0.82	0.80	0.85	0.02			
Day 41												
	Wt, kg	20.95	21.20	21.68	20.84	20.35[b]	22.22[a]	20.93[ab]	0.71	0.04	0.84	0.21
	ADG, kg	0.56	0.56	0.59	0.56	0.55	0.60	0.55	0.02			
	ADFI, kg	0.82	0.81	0.85	0.80	0.79	0.87	0.80	0.03			
	G : F	0.69	0.69	0.69	0.70	0.69	0.69	0.69	0.01			
Overall												
	ADG, kg	0.35	0.35	0.37	0.33	0.33	0.37	0.34	0.02	0.13	0.41	0.61
	ADFI, kg	0.50	0.49	0.52	0.47	0.48	0.53	0.48	0.02	0.11	0.46	0.60
	G : F	0.69	0.70	0.71	0.70	0.70	0.70	0.71	0.01	0.73	0.65	0.37

Tabel 3. Main effects of either Arginine in the feed or the water on piglet villus height, crypt depth, and villus height: crypt depth ratio

Item	Water				Feed			SEM	Feed	Water	FXW
	0	4	8	12	1.35	1.55	1.76		P Value	P Value	P Value
Villus height	342.42	294.23	299.73	316.68	300.24	323.14	316.41	28.932	0.145	0.481	0.291
Crypt depth	132.54[a]	140.70[a]	117.28[b]	131.99[a]	127.23	128.96	133.18	8.234	0.005	0.545	0.018
Villus:Crypt	2.50[a]	2.09[b]	2.56[a]	2.43[a]	2.38	2.49	2.39	0.204	0.018	0.975	0.677



Water P<.01, Feed P=.07, Day P=.54, Water x Feed P=.89, Feed x Day P=.63, and Water x Day P =01.

Figure 2. **The impact of oral Arg and dietary Arg on intestinal permeability. Pigs were orally gavaged with a lactulose mannitol solution and blood samples were collected approximately four hours later to determine the lactulose: mannitol solution in the blood.**

Discussion

The objective of this trial was to determine the impact of supplemental arginine provided both in the feed and water to young weanling pigs. The trial design was not specifically set to assess arginine requirement per sue. However, we did observe an increase ($P < 0.05$) in final body weight over the entire trial period when diets were supplemented with an additional 0.20% L-arginine fed in phase 1 and phase 2 compared to the current NRC (2012) SID arginine requirement of 0.68%. This data suggest that the SID arginine requirement is much higher (1.55%) to maximize body weight, which is similar to the findings of Perez-Palencia et al., (2022), who reported maximum performance when feeding 1.66% dietary SID Arginine for the modern weanling pig.

Interestingly, we noted histological changes in intestinal crypt depth ($P < .01$) and villus height to crypt depth ratio ($P < .02$) when supplementing arginine through the water. In addition, we observed lower blood lactulose mannitol levels when supplementing arginine (water $P < 0.02$) and feed ($P < 0.07$). These findings suggest an improvement in gut integrity with arginine supplementation. Wu et al. (2010) also noted increased intestinal villus height when supplementing arginine to weaning pigs fed a corn- soybean meal-based diet. Getty et al. (2015) reported that supplemental arginine (145 mg/kg of body weight) daily for 17 days via oral gavage, partially restored weight gains of pigs classified as low birth weight (> 0.90 kg at birth) to that of those classified as average birth weight pigs (1.30 – 1.50 kg). Arginine has been reported to stimulate neonatal porcine intestinal epithelial IPEC-J2 cells, arginine can also stimulate protein synthesis through activation of the mTOR pathway, but independently of NO production (Bauchart-Thevret et al., 2010). The imm- unological effects of arginine supply have been extensively described, primarily through the nitric oxide (NO) pathway. However, arginine may also support other vital functions, such as neonatal development and healing (Wu et al., 2009).

Conclusions

The results of this trial clearly suggest that current NRC (2012) dietary SID arginine recommendations for weanling pigs (5 - 7 kg) are too low. The current trial suggests they could be twice the current 0.68% SID recommendation when it comes to post-weaning performance. In addition, lactulose mannitol ratio measured in the plasma and intestinal histology data suggest the use of an arginine solution of 8% may improve gut barrier function. Additional work is needed to confirm these findings.

Declarations

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