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Non Ruminant Nutrition

Water- and feed-based arginine impacts on gut integrity in weanling pigs

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Abstract

Two hundred and forty newly weaned pigs (PIC, Hendersonville, TN) were used to determine if supplementing additional arginine (Arg) either in the water or in the feed, and the combinations thereof, improved intestinal integrity and growth performance in nursery pigs. Each of the 80 pens contained three pigs (21 ± 2 d of age) which were randomly allotted to treatments in 4 × 3 factorial arrangement consisting of four water treatments (0%, 4%, 8%, and 12% Arg stock delivered through a 1:128 medication delivery system) in combination with three dietary Arg treatments (1.35%, 1.55%, and 1.75% standardized ileal digestible Arg; SID). Pigs and feeders were weighed at the d0, d6 (water and diet change), d20 (diet change), and d41 for the calculation of average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed efficiency (G:F). Eighty pigs, 1 pig/pen, were euthanized at d6 for ileum evaluation of villus height and crypt depth. The remaining pigs were taken off the Arg-water treatment and fed phase-2 diets formulated to contain 1.35%, 1.55%, and 1.75% SID Arg. All pigs received a common diet from d20 to d41. Data were analyzed by pen as repeated measures (SAS 9.4). No interaction between water- and dietary-Arg was detected on nursery pig growth performance. There was a significant quadratic effect of SID Arg in the feed on pig final body weight (**BW**), ADG, ADFI, and G:F ($P \leq 0.037$), where feeding 1.55% dietary Arg tended to improve growth performance compared to the 1.35% level for the 41 d of the trial ($P \leq 0.088$). The use of the stock 8% Arg in the water resulted in a reduction in crypt depth (0:132.5, 4:140.7, 8:117.3, 12:132.0; $P \leq 0.01$) and an improvement in intestinal permeability. The 4% oral Arg significantly reduced villous height:crypt depth ratio (0:2.50, 4:2.09, 8:2.56, 12:2.43; $P \leq 0.02$). In conclusion, the feeding of 1.55% Arg resulted in an improvement in nursery pig ADG, ADFI, G:F, and final BW but did not alter intestinal villi morphology; however, the use of Arg in the water resulted in an improvement in intestinal villi, but no phenotypical change in piglet growth in the nursery.

Key words: arginine, nursery, pigs, water

Introduction

The weaning process for pigs is a stressful event due to changes in diet type and consistency, social distress, environmental challenges, or lack of exposure to dry feed prior to weaning. In addition, various studies have documented that the age of weaning can influence intestinal integrity (Spreeuwenberg et al., 2001; Moeser et al., 2007, 2017; Xun et al., 2018). The disruption of gastrointestinal function can influence piglet growth and livability. Furthermore, the greater the disruption typically is associated with subsequent growth performance. Many dietary strategies have been suggested to prevent or alleviate challenges associated with an impaired intestinal barrier. As the intestinal barrier repairs itself, some amino acids have been shown to have benefits.

Low levels of certain amino acids, such as arginine (Arg), from nursery pig diets have been shown to significantly restrict growth (Mertz et al., 1952; Roth et al., 1995). Arginine plays an important role in intestinal immunity and barrier function by stimulating protein synthesis and reducing transepithelial permeability, thus improving intestinal absorptive area (Corl et al., 2008; Zhu et al., 2013). Data are conflicted regarding the appropriate level of amino acids that benefit intestinal repair and growth relative to whole-body tissue deposition. Supplementing Arg in the feed has been shown to negatively impact the growth performance of weaned pigs (Southern and Baker, 1982; Hagemeier et al., 1983). However, little is known about the balance of Arg relative to amino acids besides lysine. More recent work did not indicate an adverse effect on feed intake of up to 2% dietary Arg (Hu et al., 2015).

The transition from milk to solid food can be delayed due to the change in the animal's environment and the stress associated with new social hierarchy development, but newly weaned piglets will seek out water (Brooks et al., 1984). Therefore, many swine production facilities utilize water medication systems to deliver electrolytes and other potentially beneficial nutrients to aid in the transition period. Because Arg can improve intestinal immunity and barrier function, supplementing Arg in water may be a feasible option. However, there are no data published concerning Arg in the water. Therefore, the objective of this study was to determine if supplementing Arg, either in feed, water, or both, improves intestinal integrity and growth performance in nursery pigs.

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Materials and Methods

Animal Care Statement

All procedures in this experiment adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC #20-143).

Animals and Experimental Design

Two hundred and forty barrows and gilts with an average body weight (**BW**) of 5.17 kg (PIC 337 × 1050, PIC Genus, Hendersonville, TN) arrived immediately after weaning at approximately 21 d of age. Pigs were randomly allocated (mixed gender and gate cut with three pigs per pen) into 80 test pens at the Iowa State Swine Nutrition Research Facility (Ames, IA) upon arrival. Room configuration allowed for four Arg-water treatments with 20 pens per water line. Within each waterline, pens were randomly assigned into an incomplete randomized design to one of three Arg-dietary treatments across the 20 pens to allow for 20 replications per water treatment, 26–27 replications per feed treatment, and 6–7 replications of water × feed. Once allotted into pens, pigs were placed onto the treatment regimens (4 × 3 factorial). Each pen had one single-hole feeder and two nipple drinkers. The pigs were porcine reproductive and respiratory syndrome and porcine epidemic diarrhea virus negative and were vaccinated against *Mycoplasma hyopneumoniae* and porcine circovirus prior to weaning.

Dietary Treatments

The three dietary treatments consisted of 1.35%, 1.55%, and 1.75% standardized ileal digestible (SID) Arg (CJ America – Bio, Fort Dodge, IA) designed to provide 0, 0.33, and 0.65 additional g Arg/d above the NRC (2012) recommendations using feed-grade Arg. For the water Arg treatments, feed-grade Arg was placed into clean, plastic buckets filled with fresh water to constitute the four levels of water treatment (0%, 4%, 8%, and 12% Arg). After the solution was thoroughly mixed, a 1:128 proportioner (D25 Fixed Dosatron Injector, Dosatron International, Clearwater, FL) was utilized to distribute the solution into one of the four water lines. The solution was delivered into each row of trial pens at a final concentration of 0.03%, 0.06%, and 0.09% Arg designed to provide 0, 0.22, 0.44, and 0.66 additional g Arg/d, respectively. Diets were formulated to be isocaloric with a constant level of soybean meal. All nutrients were calculated to meet or exceed NRC (2012) requirements. Upon arrival, pigs were placed on both the water and dietary treatments. Water treatments were removed at the end of phase 1 (days 0–6). Pigs remained on the dietary Arg treatments during phase 2 (days 6–20). Following phase 2, pigs were placed on a common diet for the remaining 21 d of the nursery period (days 20–41).

Sample Collection and Analysis

Pigs and feeders were weighed at the beginning of the trial, day 6 (water and diet change), day 20 (diet change), and day 41 (end of trial) to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (G:F). On day 6 (prior to diet and water treatment change), one pig per pen was orally administered a solution of lactulose (500 mg/kg BW) and mannitol (50 mg/kg BW) via a gastric tube at a dose of 1 mL/kg of BW. Four hours post-gavage, blood was collected via a sterile jugular venipuncture using a 10-mL serum vacutainer tube (Becton Dickinson, Franklin Lakes, NJ). The blood was separated via centrifugation (2000 × *g* for 10 min at 4 °C), and the serum was collected and stored at −80 °C for later analysis of lactulose and mannitol concentration. Following bleeding, these same pigs were euthanized by captive bolt stunning, followed by exsanguination. Post-euthanasia, the abdomen was opened, and a 2-cm segment of the terminal ileum was dissected, washed with phosphate-buffered saline (pH 7.4), and stored in 10% neutral buffered formalin solution at room temperature for 24 h before being submitted to Iowa State University Diagnostic Laboratory (Ames, IA) for villus and crypt depth evaluation. In short, the samples were removed from formalin, and the tissue was processed following standard histological procedures. In short, the formalin-fixed ilea were processed and embedded in paraffin wax. A 5-µ transverse section was cut, stained with hematoxylin and eosin, and mounted on glass slides. Images of ileal sections were taken. Ten well-oriented villi and crypt pairs were selected. The height of each villus was measured from the top of the villus to the crypt transition. The crypt depth was defined as the invagination between two villi. The 10 individual measurements were taken from each pig, and the average of the 10 observations was used in the statistical analysis. A villous height:crypt depth ratio was calculated from the measurements of crypt depth and villous height for each sample.

The remaining pigs in the pen were then taken off the Arg-water treatment and continued on phase 2 dietary treatments, being the same dietary SID Arg level in phase 1 but a slightly different diet composition, Table 1. On day 20 (prior to diet change), one pig per pen was gavaged with lactulose and mannitol using previously described methods. Four hours post-gavage, blood was collected via sterile jugular venipuncture using a serum vacutainer tube. The blood was separated as described above to determine lactulose and mannitol concentration at day 20. After collecting blood, pigs were taken off phase 2 diets and fed a common diet for the next 21 d, with pigs and feeders weighed on day 41 to determine overall pig performance. Mortalities or removals were weighed with a date recorded to adjust growth and feed intake data.

Serum samples were submitted to the University of Illinois (Champaign, IL) for the measurement of lactulose and mannitol concentration via high-performance liquid chromatography (Dionex ICS-3000/ICS-5000; Dionex Corp., Sunnyvale, CA). Measurements below the lower limit of detection (**LOD**; 0.1 µg/mL) were imputed by randomly sampling from a lognormal distribution starting at zero and truncated at the LOD. The parameters of the lognormal distribution were calculated from the original data, with samples below the LOD set equal to the LOD (Cohen and Ryan, 1989).

Feed was manufactured at the Iowa State University Swine Nutrition farm feed mill. Feed samples were collected at the completion of mixing for each phase, and a core feed sample from each treatment was taken from the feed storage containers after each dietary treatment in each phase. Samples were then stored at −20 °C for subsequent analysis. Feed samples were ground to 1 mm particle size (Variable Speed Digital ED-5 Wiley Mill; Thomas Scientific, Swedesboro, NJ). Processed feed samples were homogenized and analyzed, in duplicate, for nitrogen (N; method 990.03; AOAC, 2007; Trumac; LECO Corp., St. Joseph, MI). For N analysis,

Table 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 (d 0–6)			Phase 2 (d 6-20)			Phase 3 (d 20-41)
	1.35% Arg[1]	1.55% Arg	1.75% Arg	1.35% Arg	1.55% Arg	1.75% Arg	Basal
Corn	32.555	32.351	32.150	47.766	47.556	47.356	58.580
Soybean meal 47.5% CP[2]	20.000	20.000	20.000	28.000	28.000	28.000	34.000
Oat groats	15.000	15.000	15.000	5.000	5.000	5.000	—
DairyLac 80[3]	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	—
Soybean 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[4]	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	—
Sodium chloride	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[5]	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[5]	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, Mcal/kg	3.32	3.32	3.32	3.39	3.39	3.39	3.45
Crude protein, %	22.48	22.66	22.84	21.67	21.85	22.03	21.00
SID Lys[6]	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
Available phosphorus, %	0.60	0.60	0.60	0.40	0.40	0.40	0.37
SID Arg, %	1.35	1.55	1.75	1.35	1.55	1.75	1.30

[1]Arginine.
[2]Crude protein.
[3]DairyLac 80 is a granular, high-lactose ingredient manufactured through the process of dry rolling liquid whey permeate that contains 3% crude protein and 80% lactose.
[4]Arg was provided as free base.
[5]Vitamin and trace mineral; provided 7,656 IU vitamin A, 875 IU vitamin D, 63 IU vitamin E, 3.8 mg vitamin K, 70 mg niacin, 33.8 mg pantothenic acid, 13.8 mg riboflavin, 0.06 mg vitamin B12, 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 16.5 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.
[6]SID, standardized ileal digestible; Lys, lysine.

ethylenediaminetetraacetic acid (9.56% N) was used as the standard for calibration and was determined to contain 9.59 ± 0.02% N. Crude protein was calculated as N × 6.25. Feed samples were analyzed at commercial laboratories for proximate analysis (Eurofins Scientific, Des Moines, IA) and amino acids (University of Missouri, Columbus, MO).

Statistical Analysis

Growth performance and intestinal integrity data were analyzed as repeated measures according to the following model:

$$y_{ijkl} = \mu + w_i + f_j + (w*f)_{ij} + d_k + (w*d)_{ik} + (f*d)_{jk} + (w*f*d)_{ijk} + e_{ijkl},$$

where y_{ijkl} is the observed value for lth experimental unit within ith level of Arg in the water and jth level of Arg in the feed on kth day; μ is the overall mean; w_i is the fixed effect of the ith level of Arg in the water (i = 1–4); f_j is the fixed effect of the jth level of Arg in the feed (j = 1–3); $(w*f)_{ij}$ is the interaction between Arg in the water and Arg in the feed; d_k is the fixed effect of fixed effect of the kth day (k = 1–3); $(w*d)_{ik}$ is the interaction between Arg in the water and day; $(f*d)_{jk}$ is the interaction between Arg in the feed and day; $(w*f*d)_{ijk}$ is the three-factor interaction between Arg in water, Arg in the feed, and day; e_{ijkl} is the random error associated withy_{ijkl}, assuming $e_{ijkl} \sim N[0, I_l \otimes \mathrm{ARH}(1)]$ for growth performance and $e_{ijkl} \sim N(0, I_l \otimes \mathrm{CS})$ for lactulose-to-mannitol ratio. $I_l$is the identity matrix, ARH(1) is the first-order autoregressive covariance matrix with heterogeneous variances, and CS is the compound symmetry

covariance matrix. Lactulose-to-mannitol ratios were natural log-transformed to achieve normality.

Histology data were analyzed according to the following statistical model:

$$y_{ijk} = \mu + w_i + f_j + (w * f)_{ij} + e_{ijk},$$

where y_{ijk} is the observed value for the kth experimental unit within ith level of Arg in the water and jth level of Arg in the feed; μ is the overall mean; w_i is the fixed effect of the ith level of Arg in the water (i = 1–4); f_j is the fixed effect of the jth level of Arg in the feed (j = 1–3); $(w * f)_{ij}$ is the interaction between Arg in the water and Arg in the feed; e_{ijk} is the random error associated with y_{ijk}, assuming $e_{ijk} \sim N\left(0, I\sigma_e^2\right)$.

All models were implemented in SAS 9.4 (SAS Inst., Cary, NC) using the GLIMMIX procedure. Covariance matrices were selected as the best fit for the repeated measures models according to Bayesian Information Criterion for each response variable. Normality of the 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 excluded from the analysis. Data were reported as least squares means, and the SLICE statement was used to perform F-tests between treatments at each day. Means separation was conducted using the PDIFF option with Tukey adjustment for multiplicity. When protected F-test $P \leq 0.10$, orthogonal polynomial contrasts were constructed to test linear and quadratic effects of arginine in the feed or the water. The pen was considered the experimental unit. Results were considered significant if $P < 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

Pigs arrived from the sow facility with an average weaning weight of 5.17 ± 0.48 kg. At the end of the 41 d trial, pigs weighed approximately 21 ± 3.31 kg. Overall, the pigs had a 0.35 ± 0.08 kg average daily gain and a 0.70 ± 0.04 G:F. Analyzed dietary analysis for the Lys and Arg were lower than that of the calculated diets; however, Lys was consistent across treatments, and the Arg increased across treatments as expected (Table 2). Furthermore, total and SID Arg intake (g/d) was similar using calculated or analyzed feed values across all phases (Table 3).

There were no significant water-by-day or feed-by-day interactions for ADG or ADFI ($P \geq 0.277$; Table 4). There was a significant three-way interaction for G:F; however, the lack of significance for ADG and ADFI suggests this interaction was driven by high variability in G:F during the first phase of the study; therefore, only main effect results are presented. Averaged over day, supplementing Arg in the feed tended to improve ADG, ADFI, and G:F ($P \leq 0.087$). Means separation indicated that feeding 1.55% SID Arg tended to improve ADG, ADFI, and G:F compared to 1.35% SID Arg ($P \leq 0.088$), which was further supported by the significant quadratic effect of feed ($P \leq 0.032$). Subsequently, there was a quadratic effect of Arg in the feed on final BW (P = 0.037), where pigs fed 1.55% SID Arg tended to be heavier on day 41 compared to pigs fed 1.35% SID Arg (P = 0.077). Supplementing Arg in the water did not impact nursery pig BW, ADG, ADFI, or G:F ($P \geq 0.444$). Additionally, there were no interactions between Arg in the feed and water ($P \geq 0.155$).

The use of 8% Arg in the water resulted in a reduction in crypt depth ($P < 0.01$; Table 5), and 4% Arg reduced total

Table 2. Diet calculated total and standardized ileal digestible lysine and arginine compared to analyzed values by phase

Item, %	Phase 1			Phase 2			Phase 3
	1.35% SID[1] Arg[2]	1.55% SID Arg	1.75% SID Arg	1.35% SID Arg	1.55% SID Arg	1.75% SID Arg	Basal
Calculated							
Total							
Lysine	1.66	1.66	1.66	1.57	1.57	1.57	1.47
Arginine	1.56	1.76	1.96	1.48	1.68	1.89	1.34
Arg:Lys	0.94	1.06	1.18	0.94	1.07	1.20	0.91
SID							
Lysine	1.50	1.50	1.50	1.42	1.42	1.42	1.33
Arginine	1.35	1.55	1.75	1.35	1.55	1.75	1.30
Arg:Lys	0.90	1.03	1.17	0.95	1.09	1.23	0.98
Analyzed							
Total							
Lysine	1.52	1.52	1.55	1.55	1.53	1.50	1.50
Arginine	1.68	1.93	2.14	1.49	1.69	1.90	1.34
Arg:Lys	1.11	1.27	1.38	0.96	1.10	1.27	0.89
SID[3]							
Lysine	1.37	1.37	1.40	1.40	1.38	1.35	1.34
Arginine	1.45	1.70	1.91	1.36	1.56	1.76	1.30
Arg:Lys	1.06	1.24	1.36	0.97	1.13	1.30	0.97

[1]Standardized ileal digestible.
[2]Arginine.
[3]Estimated from analyzed total values.

Table 3. Dietary treatment mean arginine intake by phase based on calculated and analyzed feed values

	Phase 1			Phase 2			Phase 3		
	1.35% SID[1] Arg[2]	1.55% SID Arg	1.75% SID Arg	1.35% SID Arg	1.55% SID Arg	1.75% SID Arg	1.35% SID Arg	1.55% SID Arg	1.75% SID Arg
Total arginine intake, g/d									
Calculated	1.02	1.47	1.40	4.48	5.67	5.95	10.54	11.69	10.75
Analyzed	1.10	1.61	1.53	4.51	5.71	5.98	10.54	11.69	10.75
SID arginine intake, g/d									
Calculated	0.89	1.29	1.25	4.09	5.24	5.51	10.22	11.34	10.43
Analyzed[3]	0.95	1.42	1.37	4.12	5.27	5.53	10.22	11.34	10.43

[1]Standardized ileal digestible.
[2]Arginine.
[3]Analyzed SID Arg in feed estimated from analyzed total values.

Table 4. Effect of arginine in the water or feed on piglet initial and final bodyweight (**BW**) and overall (days 0–41) average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (G:F)

Item	Arginine in water, %[1]				SEM	SID Arginine in feed, %[2]			SEM
	0	4	8	12		1.35	1.55	1.75	
BW									
Initial BW, kg	5.21	5.18	5.21	5.10	0.111	5.16	5.19	5.16	0.096
Final BW, kg	20.95	21.20	21.68	20.84	0.706	20.35	22.22	20.93	0.619
ADG, kg	0.28	0.28	0.29	0.26	0.014	0.26	0.30	0.27	0.012
ADFI, kg	0.41	0.40	0.42	0.39	0.416	0.39	0.43	0.40	0.015
G:F	0.56	0.57	0.50	0.49	0.044	0.48	0.60	0.52	0.038

P-values

			Feed[3]						
	Water	Feed	Lin.	Quad.	Water × feed	Day	Water × day	Feed × day	Water × feed × day
BW	0.822	0.139	—	—	0.285	<0.001	0.798	0.122	0.718
Initial BW, kg[4]	0.886	0.970	—	—	—	—	—	—	—
Final BW, kg[5]	0.838	0.089	0.502	0.037	—	—	—	—	—
ADG, kg	0.574	0.083	0.583	0.031	0.279	<0.001	0.222	0.473	0.805
ADFI, kg	0.685	0.087	0.624	0.032	0.322	<0.001	0.267	0.277	0.737
G:F	0.444	0.069	0.433	0.031	0.155	<0.001	0.558	0.066	0.013

[1]0%, 4%, 8%, and 12% arginine as the free base stock solution prior to 1:128 dilution into the water system.
[2]SID, standardized ileal digestible.
[3]Orthogonal polynomial contrasts tested when protected F-test $P \leq 0.10$.
[4]Main effects and polynomial contrasts sliced at d0 from repeated measures model.
[5]Main effects and polynomial contrasts sliced from d41 from repeated measures model.

villous height:crypt depth ratio ($P < 0.02$). The feeding of Arg did not change the villous height:crypt depth ratio. The delivery of Arg in the water resulted in a significant reduction of lactulose:mannitol ($P < 0.01$) in serum when Arg was added at the level of 4% (Figure 1), but not when provided in the feed.

Discussion

While the analyzed values of Lys and Arg differed from the calculated values, the ratios of Arg:Lys increased as expected across the treatments and maintained values higher than NRC requirements. Feeding 1.55% SID Arg improved ADG, ADFI, and G:F, which resulted in a 1.87 kg (9%) increase in final BW compared to the pigs fed the 1.35% Arg diet. Due to the quadratic effect of Arg on ADFI, average SID Arg intake based on analyzed feed values was the highest in pigs fed the 1.55% Arg diet (5.10 vs. 6.01 vs. 5.78 g/d for 1.35%, 1.55%, and 1.75% diets, respectively). Together, this may indicate that the improved growth performance resulting from Arg supplementation was largely driven by improvements in ADFI and, consequently, increased Arg intake. Furthermore, there were no feed-by-water interactions, suggesting the added Arg intake allowed through water was not enough to alter the growth response elicited by altering feed Arg levels.

All diets were above NRC (2012) requirement for Arg intake throughout the nursery period (3.72 g SID

Table 5. Main effects of arginine in the feed or the water on piglet villus height, crypt depth, and villus height:crypt depth ratio

Item	Arginine in water, %[1]				SEM	SID Arginine in Feed, %[2]			SEM
	0	4	8	12		1.35	1.55	1.75	
Villus height	342.4	294.2	299.7	316.7	15.89	300.2	323.1	316.4	13.94
Crypt depth	132.5[a]	140.7[a]	117.3[b]	132.0[a]	4.5	127.2	129.0	133.2	3.9
Villus:Crypt	2.5[a]	2.1[b]	2.6[a]	2.4[a]	0.11	2.4	2.5	2.4	0.10

P-values

		Water[3]			
	Water	Lin.	Quad.	Feed	Water × feed
Villus height	0.145	—	—	0.481	0.291
Crypt depth	0.0051	0.219	0.472	0.545	
Villus:Crypt	0.018	0.621	0.206	0.975	0.677

[1]0%, 4%, 8%, and 12% arginine as the free base stock solution prior to 1:128 dilution into the water system.
[2]SID, standardized ileal digestible.
[3]Orthogonal polynomial contrasts tested when protected F-test $P \leq 0.10$.
[a,b]Without a common superscript differ ($P \leq 0.05$).

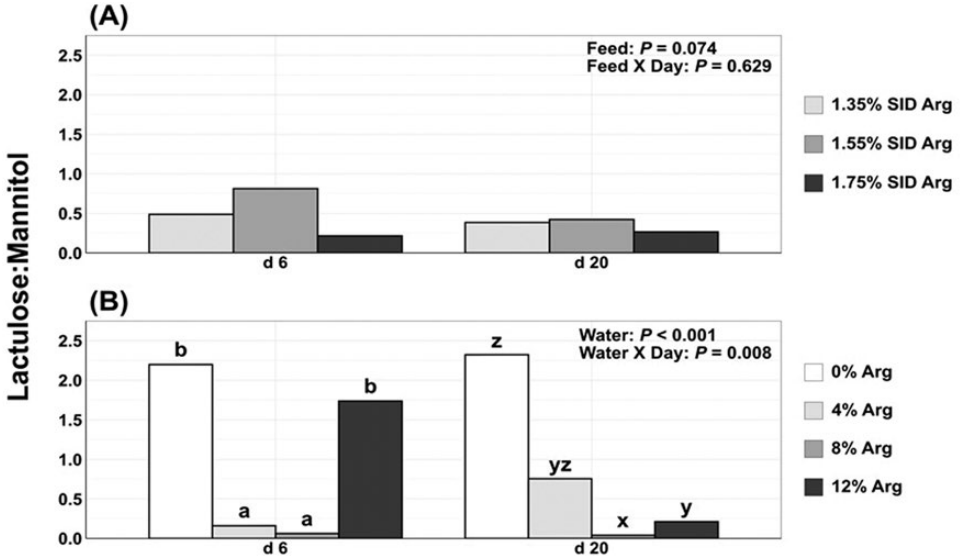

Figure 1. The impact of oral arginine (A) and dietary arginine (B) on serum lactulose:mannitol. Pigs were orally gavaged with a lactulose-mannitol solution, and blood samples were collected approximately four hours later to determine the lactulose:mannitol in the blood.

Arg/d), suggesting current recommendations may be underestimated. In this study, increasing Arg in the feed resulted in improved pig performance, with the optimal response occurring at approximately 2.74 g Arg/d higher than current NRC (2012) recommendations. The literature is conflicting regarding the response of nursery pig growth performance to increased Arg. Some studies have shown reduced performance when feeding excess levels of Arg relative to NRC recommendations, while others have observed no changes or improved performance with increased Arg, indicating the balance of other amino acids relative to lysine may determine the response to Arg supplementation (Southern and Baker, 1982; Hagemeier et al., 1983; Anderson et al., 1984; Zhan et al., 2008; Perez-Palencia et al., 2022).

The use of lactulose and mannitol to assess intestinal permeability has been widely documented in human trials (Travis and Menzies, 1992; Mishra and Makharia, 2012; Sequeira et al., 2014). Lactulose is a relatively large sugar molecule that cannot be easily transported across the intestinal lumen, while mannitol is a smaller molecule that can readily move across the intestinal epithelium. However, when the tight cell junctions are impaired, lactulose molecules can paracellularly migrate from the intestinal lumen into the bloodstream. Therefore, the ratio of lactulose to mannitol in circulation provides insight on intestinal permeability. A low lactulose:mannitol ratio indicates a tight intestinal barrier, while a high ratio suggests a leaky gut. Therefore, measuring these sugar molecules within the blood can provide insight into the degree of intestinal permeability.

Aside from its role as a constituent of protein synthesis, Arg may play regulatory roles in maintaining intestinal barrier and immune function. As described by Das et al. (2010), Arg is a

substrate for inducible nitric oxide synthase, which catalyzes the conversion of L-Arg to nitric oxide and L-citrulline. Nitric oxide is an important component of cytotoxic immune cell killing of pathogens. Therefore, Arg may aid in reducing inflammation in the gut by modulating the immune system through nitric oxide synthesis. Furthermore, nitric oxide stimulates angiogenesis and increased blood flow, which may aid tissue repair (Dai et al., 2013). Consequently, supplemental L-Arg has been shown to improve intestinal development in newly-weaned pigs (Zhan et al., 2008). Additionally, in vivo work has shown that L-Arg and L-Cit improve intestinal tight junction barrier function during hypoxia through nitric oxide production (Chapman et al., 2012).

The present study showed no differences in intestinal integrity measures when Arg was added to the feed. In contrast, other work has demonstrated that feeding Arg can maintain barrier function in mice (Viana et al., 2010). Further research has demonstrated that feeding mice a diet supplemented with 2% L-Arg decreased intestinal permeability during hyperthermia (Costa et al., 2014). Liu et al. (2008) demonstrated that feeding 1% supplemental Arg reduced IL-6 production within the intestine during an *Escherichia coli* challenge in nursery pigs.

The use of Arg in the water during the first week after weaning resulted in an improvement in intestinal morphology. These findings support other studies showing that 2% oral Arg can reduce intestinal injury (Sukhotnik et al., 2004; Koppelmann et al., 2012). Similarly, Yang et al. (2016) supplemented L-Arg in milk replacer fed to pre-weaned pigs and observed that 8 g/kg L-Arg increased villus height and relative intestine weight in the subsequent nursery period. The current study included Arg at 0%, 4%, 8%, and 12% in the water stock solution, allowing for approximately 0, 0.22, 0.44, and 0.66 g Arg/d intake through the water. Intestinal permeability was improved at both the 4% and 8% inclusion levels compared to pigs receiving the 0% Arg water. In addition, the crypt:villi ratio was improved at the 4% inclusion level in the water compared to pigs receiving the 0% Arg water, suggesting that pigs may benefit from supplementing Arg in the water at the time of weaning.

While the delivery of oral Arg improved intestinal permeability and villus height, this response did not depend on the level of Arg in the feed, further supporting the lack of interactive effects of feed and water Arg. The lack of response of oral Arg on phenotypical changes in growth rate could be because the piglets were not significantly challenged with enteric pathogens. Together, this may indicate Arg supplementation in the water or feed may work through different modes of action. The differences in response to Arg in the feed or the water observed in the present study are not clear; therefore, further research investigating these routes of supplementation is warranted.

In conclusion, nursery pig growth performance was improved with no changes in intestinal morphology when Arg was increased in the diet. However, the administration of Arg through the water resulted in improved intestinal integrity and morphology but no phenotypical changes in growth.

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Conflict of Interest Statement

The authors disclose that this project was funded by CJ Bio America which is the manufacturer of the arginine tested within the study. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by Iowa State University or the USDA and does not imply approval to the exclusion of other products that may be suitable. The USDA is an equal opportunity provider and employer.

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