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Intestinal development of early-weaned piglets receiving diets supplemented with selected amino acids or polyamines

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Ewtushik, A. L., Bertolo, R. F. P. and Ball, R. O. 2000. **Intestinal development of early-weaned piglets receiving diets supplemented with selected amino acids or polyamines**. Can. J. Anim. Sci. **80**: 653–662. Early-weaned piglets are subjected to various environmental and nutritional stresses that can result in overall poor performance. Several amino acids associated with the urea cycle have been shown to be critical to intestinal development and metabolism. The objective of this research was to examine performance and intestinal development in early-weaned piglets receiving diets supplemented with selected amino acids or polyamines. Forty-two Yorkshire piglets (3.94 ± 0.43 kg) weaned at ~12.5 d were randomly assigned to diets supplemented with either arginine, glutamate, citrulline, ornithine or polyamines, at levels of 0.93, 6.51, 0.94, 0.90 and 0.39%, respectively. Diets were fed for 12 d and various parameters to assess growth and intestinal development were measured. Glutamate supplementation enhanced both total and mucosal growth in several sections of the small intestine ($P < 0.05$), whereas polyamines were detrimental to intestinal growth. Arginine and glutamate supplementation prevented weaning-induced villus atrophy in the duodenum, compared with both the control and polyamine-fed pigs ($P = 0.004$). These results indicate that glutamate and arginine supplementation may enhance intestinal development of the early-weaned piglet, whereas polyamine supplementation at the ratios and concentrations used in this experiment is not recommended in typical early-weaned piglet diets.

Key words: Early-weaning, amino acids, piglet, polyamine, small intestine, development

Ewtushik, A. L., Bertolo, R. F. P. et Ball, R. O. 2000. **Développement intestinal chez des porcelets de sevrage précoce recevant des aliments complémentés de certains acides aminés ou de polyamines**. Can. J. Anim. Sci. **80**: 653–662. Nous avons exposé des porcelets de sevrage précoce à divers stress environnementaux et nutritionnels susceptibles de compromettre leurs performances zootechniques générales. On sait que plusieurs acides aminés associés au cycle de l'urée jouent un rôle critique dans le développement et le métabolisme intestinal. L'objet de nos travaux était d'examiner les performances de croissance et le développement intestinal chez des porcelets sevrés tôt recevant des aliments complémentés de certains acides aminés ou polyamines. Quarante-deux porcelets Yorkshire d'un poids moyen de 3,94 ± 0,43 kg, sevrés à l'âge approximatif de 12,5 j, étaient affectés à des aliments complémentés d'arginine, de glutamate, de citrulline, d'ornithine ou de polyamines aux doses respectives de 0,93, 6,51, 0,94, 0,90 et 0,39 %. Les aliments étaient servis pendant 12 j, durant lesquels divers paramètres d'appréciation du développement intestinal et de la croissance étaient mesurés. L'apport de glutamate stimulait à la fois la croissance générale et celle des muqueuses dans plusieurs segments de l'intestin grêle ($P < 0,05$), mais les polyamines avaient un effet négatif sur la croissance intestinale. L'apport d'arginine et de glutamate atténuait l'atrophie des villosités du duodénum résultant du sevrage, par rapport au traitement témoin et au régime complémenté de polyamines ($P = 0,004$). Ces observations montrent que la complémentation de glutamates et d'arginine peut stimuler le développement de l'intestin chez les porcelets conduits en sevrage précoce, tandis que l'apport de polyamines aux concentrations et dans les proportions utilisées dans l'expérience n'est pas à conseiller pour ce type de production.

Mots clés: Sevrage précoce, acides aminés, porcelet, polyamines, intestin grêle, développement

Early-weaned piglets are subjected to various environmental and nutritional stresses that can result in overall poor performance. The gastrointestinal tract of the early-weaned piglet is immature and is not well adapted to digest diets based primarily on grain and oilseed meal fed to the adult pig (Pettigrew et al. 1994). Therefore, highly-digestible and hence, high-cost diets must be fed to combat post-weaning problems, such as poor feed intake and diarrhea (Ball and

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Abbreviations: **ARG**, arginine-supplemented diet; **CIT**, citrulline-supplemented diet; **CON**, control diet; **GLU**, glutamate-supplemented diet; **ORN**, ornithine-supplemented diet; **POLY**, polyamine-supplemented diet

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Aherne 1987). Improving the early-weaning diet to reduce both nutritional stresses and at the same time lowering feed costs is highly desirable.

Arginine is important in protein synthesis and the excretion of nitrogen via the urea cycle, and stimulates various hormones involved in growth (Cynober 1994). Additionally, arginine is considered an essential amino acid in the young piglet due to inadequate biosynthesis (Ball et al. 1986). Arginine may also have additional roles in supporting gut function (Cynober 1994). In addition, Edmonds et al. (1987) found that citrulline could replace the arginine requirement in 4-wk-old pigs without any changes in growth performance.

Ornithine is a primary precursor for polyamine synthesis and an important component of protein and urea cycle metabolism. Ornithine supplementation may support gut function and structure through involvement in polyamine synthesis. Edmonds et al. (1987) observed significantly lower and unexplained feed intakes in pigs receiving diets supplemented with 0.912% ornithine. Polyamines (putrescine, spermidine, spermine) are essential for the growth and maturation of the gastrointestinal mucosa (McCormack and Johnson 1991), and are involved in both cell proliferation and differentiation (Cynober 1994). Several investigators have found that supplementation of polyamines in rat diets resulted in substantial decreases in lactase activity, as well as increases in maltase and sucrase activities, characteristic of intestinal maturation (Buts et al. 1993; Harada et al. 1994).

Glutamate is a precursor for glutamine, which is a primary fuel for growth and renewal of the intestinal mucosal (Souba et al. 1990). As well, glutamate is a carbon precursor for proline and arginine, which are essential in the young piglet. Reeds et al. (1996) found that the intestine of the young piglet is an active user of enteral glutamate, since almost no [U^{13}-C]glutamate reached the portal circulation and the majority had been transaminated. Horvath et al. (1996) found that feeding a glutamate-free diet to rats had detrimental effects on the intestine. Thus, the potential benefits of glutamate supplementation on intestinal growth need to be explored.

The aim of this experiment was to examine intestinal development in the early-weaned piglet following dietary supplementation of arginine, glutamate, citrulline, ornithine and polyamines (putrescine, spermidine and spermine). The effect of supplementation on portal plasma amino acid concentrations was also assessed.

MATERIALS AND METHODS

Animals and Diets

All procedures in this study were approved by the University of Guelph Animal Care Committee. Forty-two male Yorkshire piglets from a minimal-disease herd were removed from the sow at 12 d of age (± 1.1 d), weighing 3.9 kg (± 0.42 kg); creep feed was provided from ~11 d of age.

Formulation of the diets is given in Table 1 (basal diet manufactured by ADM, Animal Health and Nutrition Division, Woodstock, ON); the basal diet was typical for an early-weaning diet and by industry standards would be considered a high-quality diet. Following a 2-d adaptation period, piglets were placed in separate pens and randomly assigned to one of six treatments: 1) control (CON); 2) control + 0.93% arginine (ARG); 3) control + 6.51% glutamate (GLU); 4) control + 0.94% citrulline (CIT); 5) control + 0.90% ornithine hydrochloride (ORN); 6) control + 0.39% polyamines (0.08% putrescine, 0.13% spermidine, 0.18% spermine) (POLY). All diets were formulated to be

Table 1. Composition of diet[z]

Diet Ingredient	g kg^{-1}	Nutreient (analyzed)[y]	% DM
Corn, ground	213	Dry matter (DM)	91.1
Whey, dried	187	Protein	24.9
Oats, ground	140	Fat (ether extract)	10.1
Wheat middlings	93	Crude fiber	1.71
Spray dried plasma[x]	70	Calcium	0.84
Fat, pork	65	Phosphorus	0.75
Soybean meal (48%)	47	Arginine[w]	1.36
Soy protein concentrate[v]	47	Glycine[w]	0.63
Herring meal (70%)	37	Histidine	0.29
Soybean oil	9	Isoleucine	0.89
Limestone	7	Leucine	1.75
Dicalcium phosphate	7	Lysine	1.47
Pellet binder[u]	2	Methionine	0.40
Butter	2	Phenylalanine	0.45
Mineral premix[t]	1.7	Threonine	0.80
Vitamin premix[s]	1.2	Total sulfur amino acids	0.83
Vitamin/mineral premix[r]	1.1	Tryptophan	0.31
Lysine HCl (98%)	0.8	Tyrosine	0.50
Sugar[q]	0.5	Valine	1.24
Choline chloride	0.5		
Mold inhibitor[p]	0.5	Calculated ME (kcal kg^{-1})	3540
Copper sulfate	0.4		
DL-methionine	0.4		
Selenium	0.3		
Anti-oxidant[o]	0.2		
Vitamin E	0.03		
Chromic oxide[n]	4.7		
Supplement[n,m]	65.1		

[z] Manufactured by Archer-Daniels-Midland, Woodstock, ON.
[y] Amino acid concentrations provided by manufacturer; other parameters analyzed by authors.
[x] AP-920, American Protein Corp., Ames, IA.
[w] Concentrations in basal diet.
[v] Profine E, Central Soya, Fort Wayne, IN.
[u] Ligno-pel, Tembec Inc., Temiscaming, QC.
[t] As mg kg^{-1} of diet: $ZnSO_4@7H_2O$, 187; $FeSO_4@7H_2O$, 140; $CuSO_4@5H_2O$, 117; Mn oxide, 65; calcium iodate, 1.35; $NaSeO_3$, 0.28.
[s] As mg kg^{-1} of diet: choline Cl, 243; niacin, 47; cyanocobalamin, 37; Ca pantothenate, 28; riboflavin, 11; menadione Na bisulfate, 5; folic acid, 4; pyridoxine, 4; thiamine mononitrate, 1.4.
[r] Starter/breeder pac (0.1%): Vitamin/mineral booster (confidential), Archer-Daniels-Midland, Woodstock, ON.
[q] Hy-sugar-ade (flavor), Feed Flavors, Inc. (Division of Hays Ingredients), Wheeling, IL.
[p] Myco-curb dry, Kemin Industries, Inc., Des Moines, IA.
[o] Endox, Kemin Industries, Inc., Des Moines, IA.
[n] Added by researchers.
[m] CON diet: 33.2 g glycine, 31.9 g corn starch; ARG diet: 9.3 g arginine, 17.6 g glycine, 38.2 g corn starch; GLU diet: 65.1 g glutamate; CIT diet: 9.4 g citrulline, 21.6 g glycine, 34.1 g corn starch; ORN diet: 9.0 g ornithine HCl, 25.6 g glycine, 30.5 g corn starch; POLY diet: 0.8 g putrescine, 1.3 g spermidine, 1.8 g spermine, 27.4 g glycine, 33.8 g corn starch.

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isocaloric by adding corn starch (0 to 3.82%) and isonitrogenous to the GLU by adding glycine (0 to 3.32%). In formulating experimental diets, it was assumed that a 3.8% addition of corn starch to a diet already containing ~50% starch ingredients would not additionally induce disaccharidase expression. In addition, although there is evidence that glycine, alanine and serine induce ornithine decarboxylase activity in the intestine and liver of rats fed large doses of individual amino acids (i.e., 10% of diet as sole nitrogen source) (Jain et al. 1987; Minami et al. 1985), it has been demonstrated that ornithine decarboxylase activity per se is not the stimulus for mucosal growth (Jain et al. 1987). Thus, we assumed that glycine (added at up to 3.3%) would not lead to increased mucosal growth or crypt cell proliferation in the current study. Basal diet glutamate concentrations were approximately 3.5%; because glutamate is non-toxic and is almost completely metabolized by the pig gut (Reeds et al. 1996), we added glutamate to ~200% above basal concentrations. An approximately 1% addition of arginine was considered a moderate and non-toxic supplementation of ~75% above basal concentrations; the citrulline and ornithine additions were isomolar to arginine. Our preliminary studies supplemented spermine alone at a concentration isomolar to ornithine; however, this diet was unpalatable and feed intakes were very low. In a second preliminary study, two diets containing an isomolar combination of putrescine, spermine and spermidine were fed at total concentrations that were equal to either one or one-half of ornithine molarity; the combination was employed to overcome unpalatability and to accommodate possible stimulation of gut growth by more than one type of polyamine. The half isomolar concentration of polyamines was chosen because feed intakes were comparable to those of other treatments. Toxic concentrations of polyamines in swine are not known; however, based on an average body weight of 5 kg and daily feed intake of 0.28 kg, polyamine supplementation approximated 0.5 μmol kg^{-1} d^{-1} for each polyamine, similar to levels that gave positive responses in rat studies (Buts et al. 1993; Harada et al. 1994). Glutamate, arginine, ornithine, citrulline and glycine were analytical grade (United States Biochemical Corp., Cleveland, OH), as were putrescine, spermidine and spermine (Sigma Chemical Co., St. Louis, MO). Chromic oxide (Fisher Scientific, Nepean, ON) was added to each diet at 0.47%, to allow determination of apparent digestibility of the diets.

Animals were housed individually in approximately $1 \times 1 \times 1$ m wire mesh cages with plastic mesh flooring and a steel urine collection tray beneath the flooring. The room was maintained at 24°C and supplemental heat was provided with heat lamps. The room was lit from 0800 to 2000 h. Fresh feed and water was provided ad-libitum twice daily in stainless steel self-feeders and bowls. A 2-d adaptation period with the CON was used to teach the pigs to eat and experimental diets were fed for 10 d. Feed intake and body weights were determined daily.

Sample Collection

During the last 4 d of the treatment period, fresh uncontaminated fecal samples were collected for determining nutrient digestibility, and urine was collected quantitatively each 24 h for determining whole-body nitrogen retention. Urine flasks (with fine wire mesh filters) were acidified to prevent ammonia loss. Following the 12-d feeding period, piglets were sedated with intramuscular injections of ketamine hydrochloride (0.20 mg kg^{-1} BWT; Rogarsetic™: Rogar STB Inc., Montreal, QC), and acepromazine maleate (0.25 mg kg^{-1} BWT; Atravet™: Ayerst Laboratories, Montreal, QC) and then anesthetized with 5% halothane (Fluothane™: Ayerst Laboratories, Montreal, QC). Blood samples were obtained from the portal vein and then transferred into heparinized tubes (Hepalean™: Organon Teknika, Toronto, ON). After centrifugation, plasma samples were collected and frozen at –80°C until further analysis. Animals were then killed with 100 mg kg^{-1} BWT of sodium pentobarbital (Euthanyl™: MTC Pharmaceuticals, Cambridge, ON) via intra-cardiac injection.

The entire small intestine was excised, rinsed in ice-cold saline (0.9% NaCl), and blotted dry; weight and length were measured for the duodenum (defined as the section from the pylorus to the ligament of Treitz) and the remainder of the small intestine. Samples (1 cm) of the duodenum, ileum, and proximal and mid-jejunum were fixed in 10% buffered formalin solution (Sigma Chemical Co., St. Louis, MO) for histological measurements. The remaining duodenum and samples measuring 10% of the total small intestinal length were taken from each of the proximal jejunum (caudal from the ligament of Treitz) mid-jejunum (middle of total small intestine) and ileum (cranial from the ileo-cecal junction). All four intestinal sections were weighed, emptied, cut lengthwise and the remaining chyme was removed by gentle scraping with a glass slide; mucosa was subsequently scraped from the muscularis. Mucosal samples were then weighed and frozen at –80°C for later analysis. The liver, kidneys, spleen, stomach and large intestine were also removed, emptied and weighed. Organs were returned to the carcasses and were frozen at –20°C for later analyses.

Analytical Procedures

Feed, feces and urine samples were analyzed for nitrogen using the LECO method (LECO Instruments Ltd., Mississauga, ON) and for energy using bomb calorimetry [Association of Official Analytical Chemists (AOAC) 1990]. To determine apparent digestibilities of protein and energy, feed and fecal samples were also analyzed for chromic oxide using the method by Fenton and Fenton (1979). Apparent digestibility (%) was calculated as:

$$[1 - (N_F \times Cr_D)/(N_D \times Cr_F)] \times 100$$

where N_F = fecal nutrient concentration, Cr_D = dietary chromic oxide concentration, N_D = dietary nutrient concentration, and Cr_F = fecal chromic oxide concentration. Nitrogen retention (%) was calculated as:

$$[(N_{in} - N_{out})/N_{in}] \times 100$$

where N_{in} = nitrogen intake and N_{out} = nitrogen excretion via feces and urine.

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For protein and disaccharidase activity analyses, 0.2 g of tissue was homogenized for 2 min at 50% output (Janke & Kunkel, Stafen, Germany) in 3 mL of 0.1 M phosphate buffer (pH 6.1) containing 0.05% sodium azide. After homogenization, samples were centrifuged (800 × g) at 4°C for 5 min, and supernatants were collected and stored at –80°C. Protein content was determined (Bradford 1976) using porcine albumin as a standard (Sigma Chemical Co., St. Louis, MO). Sucrase, maltase and lactase activities were measured according to Dahlqvist (1968), using 0.056 M substrate concentrations and 30 min incubation times.

After fixation of tissues in 10% buffered formalin, histological samples were processed by the standard paraffin method. Cross-sectional samples were cut and stained with hematoxylin and eosin. Villi and crypts were measured only when there was a complete longitudinal section of a villus and its associated crypt. The heights of at least 10 best-oriented villi were measured from the tip to the crypt mouth, and the depths of associated crypts were measured from the crypt mouth to the base.

Plasma amino acid concentrations were measured using reverse-phase high-performance liquid chromatography as described by Bidlingmeyer et al. (1984). Feed amino acid concentrations were provided by the manufacturer (ADM, Animal Health and Nutrition Division, Woodstock, ON). Concentrations of dry matter, protein, fat, crude fiber, calcium and phosphorus in the feed were analyzed using AOAC methods (1990). Piglet carcasses were ground individually, freeze-dried and then analyzed for nitrogen using the LECO method. Analysis of carcass fat, ash, energy and analytical dry matter were performed according to AOAC (1990) methods.

Statistical Analysis

The study was designed as a randomized complete block design with six treatments and seven replicates (blocked by treatment). Data were analyzed using the general linear models procedure of the SAS Institute, Inc. (Version 6.06, Statistical Analysis Systems). Differences were considered statistically significant at $P < 0.05$ and treatment means were compared by Student-Newman-Keuls multiple comparisons procedure. Intestinal mucosal content, disaccharidase activities and mucosal protein content could not be measured in animals of the first replicate, due to a failure during sample storage. Both initial weight and age were tested as covariates and were found to not significantly affect any of the results; therefore these covariates were not included in the model.

RESULTS

Piglet Performance

Regardless of treatment group, all piglets lost weight during day 1, and by day 2 an increase in body weight was achieved in all treatments. By day 4, all treatments achieved initial mean body weight again. Daily body weights were not different ($P > 0.05$) among groups from days 3 to 12 of the experimental period. There were no differences ($P > 0.05$) in growth rate during any time period tested (days 3–12, 5–12 or 8–12). Because all pigs were eating and growing well by day 5, the intake and growth data for the day 5–12 period were evaluated. For this period there were no differences ($P > 0.05$) in initial weight (pooled mean, 3.94 kg; pooled SD, 0.29), average daily gain (pooled mean, 253 g; pooled SD, 60), average daily feed intake (pooled mean, 257 g; pooled SD, 70) or gain:feed ratio (pooled mean, 1.02; pooled SD, 0.13) between treatments. Apparent nitrogen digestibility (pooled mean, 82.3%; pooled SD, 2.0), nitrogen retention (pooled mean, 59.6%; pooled SD, 5.2) and carcass protein content (pooled mean, 51.3 g 100 g^{-1} dry matter; pooled SD, 2.8) were not different between treatments ($P > 0.05$). In addition, apparent energy digestibility (pooled mean, 87.7%; pooled SD, 1.3) and carcass energy content (pooled mean, 2.74 MJ 100 g^{-1} dry matter; pooled SD, 0.10) were similar across treatments ($P > 0.05$). There were no differences in carcass fat content (pooled mean, 43.9 g 100 g^{-1} dry matter; pooled SD, 4.4) among treatments; however, carcass ash content of pigs on all treatments averaged 9.7 ± 1.0 % (g 100 g^{-1} dry matter) and was greater ($P = 0.03$) in the POLY pigs, compared with the GLU pigs (10.6 vs. 8.7%, respectively).

Organ and Tissue Measurements

Although total weights of the small ($P = 0.15$) and large intestines ($P = 0.19$) did not differ across treatments, total small intestinal mucosa weight was lower ($P < 0.05$) in the POLY group than all other treatment groups (Table 2). Small intestinal length did not vary among treatments ($P = 0.63$). The weights of the liver, kidneys, spleen or stomach were not different among treatments, either as absolute values, or when corrected for final body weight (Table 2).

Mucosa weights per section of small intestine are displayed in Fig. 1. The GLU pigs had heavier duodena than the POLY pigs and more duodenal mucosa than either POLY or ORN pigs (Fig. 1). Percent mucosa in the duodenum was significantly higher ($P < 0.05$) in the GLU pigs (70.2%), compared with CON (59.0%), CIT (60.2%) and POLY (62.8%) pigs (Fig. 1). In the proximal jejunum, total weight ($P = 0.06$) and mucosal weight ($P = 0.10$) were not different (Fig. 1). Heavier total weights were observed in the mid-jejunum of GLU pigs, compared with POLY pigs (Fig. 1); mucosal weights were not different ($P = 0.08$). Although mucosal weights were lower in the POLY than in the GLU group ($P < 0.05$), percent mucosa and total weights for the ileum did not differ among treatments ($P > 0.05$) (Fig. 1). Percent mucosa did not differ among treatments in either the proximal jejunum, mid-jejunum or ileum ($P > 0.05$) (Fig. 1).

Disaccharidase Assays

There were no differences ($P > 0.05$) in protein content (mg protein/g mucosa) among treatments (Table 3). Sucrase and lactase specific activities were not different ($P > 0.05$) among treatments, expressed as either μmol (of disaccharide hydrolyzed) min^{-1} g^{-1} (of mucosa) or g^{-1} (of protein). However, maltase activity was significantly higher ($P < 0.05$) in the ORN group, compared with the POLY group when expressed per gram mucosa (Table 3).

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Table 2. Intestinal parameters in piglets fed early-weaning diets: control (CON), supplemented with arginine (ARG), glutamate (GLU), citrulline (CIT), ornithine (ORN) or polyamines (POLY)[z]

	CON	ARG	GLU	CIT	ORN	POLY	SD
Large intestine							
(g)	101	91	97	96	77	91	17
(g kg^{-1} BWT)	15.4	14.5	14.4	15.6	13.6	15.3	2.1
Small intestine (SI)							
(g)	235	254	259	242	213	212	40
(gkg^{-1} BWT)	36.1	39.6	38.8	38.7	37.0	35.1	0.4
SI length							
(cm)	986	997	1018	1036	994	954	90
(cm kg^{-1} BWT)	154	158	153	167	176	161	18
Total SI mucosa							
(g)[y]	187*a*	195*a*	193*a*	191*a*	180*a*	143*b*	28
(g kg^{-1} BWT)[x]	26.9	29.8	28.0	30.4	30.4	24.6	3.6

[z]Values are means of $n = 7$ piglets. Data not sharing the same letter within a row differ ($P<0.05$).
[y]Total mucosa was calculated as described in **Materials and Methods**.
[x]Total small intestinal mucosa corrected for body weight tended to differ across treatments ($P = 0.06$); CIT and ORN treatments differed from POLY treatments.

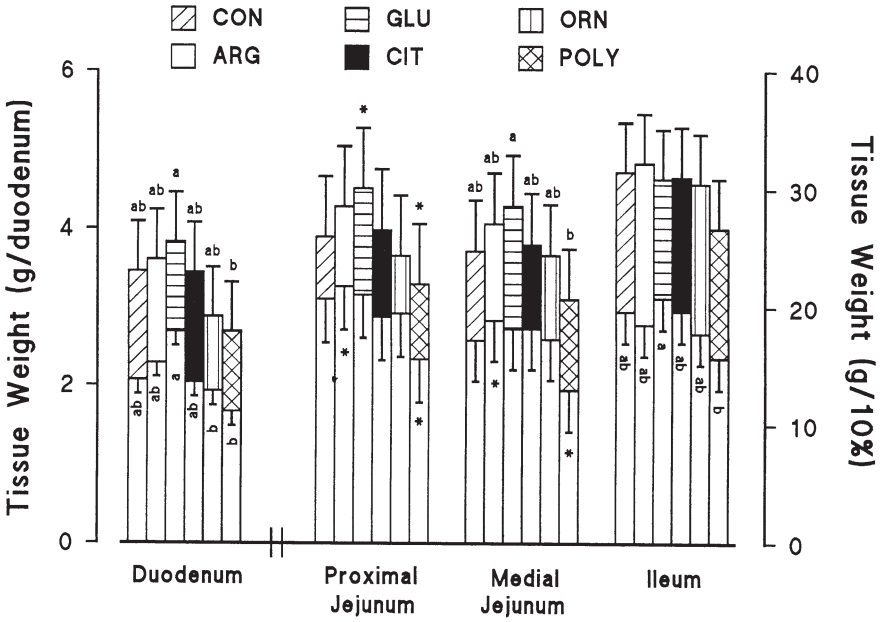

Fig. 1. Total and mucosal weights of the small intestine at four sites in piglets fed early-weaning diets: control (CON), supplemented with arginine (ARG), glutamate (GLU), citrulline (CIT), ornithine (ORN) or polyamines (POLY). Each bar represents the mean ± pooled SD for $n = 7$ piglets; bars not sharing the same letter are different ($P < 0.05$); * indicates $P < 0.10$. For the duodenum, the entire duodenal length was collected and these data are expressed as grams per duodenum (left axis). For the proximal jejunum, medial jejunum and ileum, a tissue sample equal to 10% of the entire small intestinal length was collected from each section and these data are expressed as gram per 10% (right axis). Open bars – pooled SD represent total mucosal weight per whole duodenum (left axis) or per 10% of total length for respective sections (right axis); whole bars + pooled SD represent total tissue weight per whole duodenum (left axis) or per 10% of total length from respective sections (right axis).

Histological Analyses

In the current experiment, distal gut segments had lower crypt depths and villus heights, consistent with previous studies in weanling pigs (Kelly et al. 1991; Pluske et al. 1996; Tang et al. 1999). As shown in Fig. 2, there were treatment effects ($P < 0.05$) when examining the histology of the duodenum and ileum. Greater villus height was measured in the duodenum of the ARG and GLU groups, compared with the POLY and CON groups. There were no differences ($P > 0.05$) in villus height in the proximal jejunal, mid-jejunal and ileal sections among treatments. In the ileum, CON pigs had deeper crypts ($P < 0.05$) than the pigs fed CIT and POLY (236 μm vs. 194 and 193 μm, respectively) (Fig. 2). However, crypt depth was not different ($P > 0.05$) among treatments in the other sections. The ratio of villus height to crypt depth was greater ($P < 0.05$) in the duodena of ARG pigs compared with CON pigs (1.25 vs. 0.89, respectively); there were no differences in villus height:crypt depth ratio in the other sections ($P > 0.05$).

Plasma Amino Acid Concentrations

Diet had a significant effect on portal plasma concentrations of many amino acids (Table 4). Compared with all other dietary treatments, pigs receiving the GLU had approximately twofold higher ($P < 0.05$) plasma glutamate and glutamine concentrations. In addition, plasma glutamine concentrations were lower ($P < 0.05$) in the CIT group, compared with either the GLU or ORN groups. CON and

Table 3. Specific activities of lactase, sucrase and maltase in the proximal jejunum of piglets fed early-weaning diets: control (CON), supplemented with arginine (ARG), glutamate (GLU), citrulline (CIT), ornithine (ORN) or polyamines (POLY)[z]

	CON	ARG	GLU	CIT	ORN	POLY	SD
Protein (mg g^{-1} mucosa)	50.3	50.9	49.9	50.9	51.2	49.3	5.3
Lactase (per g protein)[y]	106	111	109	143	112	117	53
Lactase (per g mucosa)	5.26	5.73	5.35	7.34	5.77	5.79	2.78
Sucrase (per g protein)	85	73	56	76	71	80	46
Sucrase (per g mucosa)	4.16	3.65	2.83	3.92	3.64	4.01	2.30
Maltase (per g protein)	108	104	110	106	108	99	19
Maltase (per g mucosa)	5.41*ab*	5.19*ab*	5.40*ab*	5.34*ab*	5.49*a*	4.87*b*	0.73

[z]Values are means of $n = 7$ piglets. Data not sharing the same letter within a row differ ($P<0.05$).
[y]Specific activity is calculated as μmol (of substrate hydrolyzed) min^{-1} g^{-1} (of protein or mucosa).

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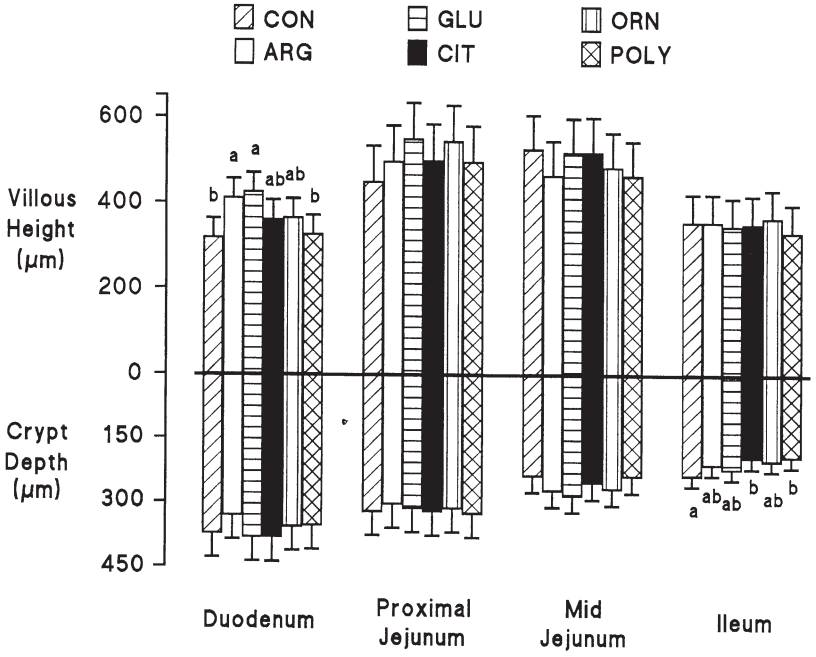

Fig. 2. Villus heights (bars above abscissa) and crypt depths (bars below abscissa) of the small intestine at four sites in piglets fed early-weaning diets: control (CON), supplemented with arginine (ARG), glutamate (GLU), citrulline (CIT), ornithine (ORN) or polyamines (POLY). Each bar represents the mean + pooled SD for $n = 7$ piglets; bars not sharing the same letter are different ($P < 0.05$).

POLY-fed pigs had lower ($P < 0.05$) plasma ornithine concentrations than other treatments. Greater ($P < 0.05$) plasma citrulline concentrations were observed in pigs receiving the CIT compared with all other treatments. Portal plasma arginine concentrations were highest ($P < 0.05$) in the ARG and CIT treatments. Portal plasma concentrations of aspartate ($P = 0.06$), hydroxyproline ($P = 0.11$), methionine ($P = 0.08$) and proline ($P = 0.12$) were not different. Reflective of glycine addition to the diets, plasma glycine concentrations were highest ($P < 0.05$) in the CON-fed pigs, followed by pigs fed ORN, POLY, ARG and CIT; pigs fed GLU had lower ($P < 0.05$) plasma glycine concentrations than all other treatments. Plasma alanine concentrations were higher ($P < 0.05$) in GLU pigs, compared with all other groups, and lower ($P < 0.05$) in CIT pigs (vs. GLU and ORN). Plasma serine concentrations were higher ($P < 0.05$) in CON pigs and lower ($P < 0.05$) in GLU pigs than in the other treatments. Of the indispensable amino acids, GLU pigs had higher ($P < 0.05$) plasma concentrations of histidine, isoleucine, leucine and valine concentrations compared with most other groups (Table 4).

DISCUSSION

There are few studies that have altered the level of arginine, glutamate, citrulline, ornithine, or polyamines in early-weaned piglet diets, because these amino acids are generally considered non-essential or semi-essential. However, recently there has been interest in supplementing these amino acids (and polyamines) to support gut morphology and functions during periods of stress (Cynober 1994; Gardiner et al. 1995).

In order for the young piglet to maintain normal growth, it is essential that the organs grow proportionately to support the growth potential. For example, stomach size will limit feed intake, and intestinal size may limit nutrient absorption. In the present experiment, organ weights were similar when

Table 4. Free amino acid concentrations (μmol L^{-1}) in portal venous blood from piglets fed early-weaning diets: control (CON), supplemented with arginine (ARG), glutamate (GLU), citrulline (CIT), ornithine (ORN) or polyamines (POLY)[z]

Amino acid	CON	ARG	GLU	CIT	ORN	POLY	SD
Indispensable							
Histidine	67*b*	64*b*	87*a*	61*b*	68*b*	72*b*	13
Isoleucine	136*b*	130*b*	174*a*	122*b*	152*ab*	125*b*	29
Leucine	238*ab*	210*b*	293*a*	213*b*	261*ab*	225*ab*	50
Lysine	134	120	137	115	149	139	38
Methionine[y]	21	19	29	23	27	19	8
Phenylalanine	114	110	125	108	117	112	19
Threonine	273	301	290	225	248	278	61
Tryptophan	30	30	34	27	33	30	6
Valine	266*ab*	262*b*	342*a*	261*b*	316*ab*	274*ab*	50
Totals	1279*ab*	1246*ab*	1511*a*	1154*ab*	1371*ab*	1035*b*	242
Semi-indispensable							
Arginine	146*b*	274*a*	193*b*	249*a*	180*b*	147*b*	48
Cystine	70	64	65	52	67	58	22
Proline[y]	503	485	534	427	520	425	92
Tyrosine	107	109	126	102	110	110	20
Other							
Alanine	907*bc*	904*bc*	1659*a*	826*c*	1165*b*	906*bc*	201
Aspartate[y]	111	76	73	76	96	93	26
Citrulline	94*b*	82*b*	92*b*	181*a*	95*b*	93*b*	44
Glutamate	249*b*	212*b*	433*a*	209*b*	291*b*	233*b*	77
Glutamine	356*bc*	305*bc*	728*a*	252*c*	384*b*	357*bc*	79
Glycine	7857*a*	4830*b*	1294*c*	4651*b*	6079*b*	5687b	997
Hydroxyproline[y]	136	113	131	127	140	132	18
Ornithine	89*b*	141*a*	188*a*	157*a*	181*a*	85*b*	36
Serine	591*a*	413*b*	259*c*	402*b*	505*b*	453*b*	75
Taurine	117	113	125	102	104	107	23

[z]Values are means of n=7 piglets. Data not sharing the same letter within a row differ ($P < 0.05$).
[y]$P < 0.12$.

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compared to Kelly et al. (1991), who weaned piglets at 14 d and fed them for 7 d. As in the present experiment, other studies have found no significant changes in small intestinal weight following glutamate supplementation in pigs and rats (Burrin et al. 1991; Horvath et al. 1996). However, Horvath et al. (1996) demonstrated that glutamine or glutamate-free diets resulted in significantly lower small intestinal weights compared with controls. Buts et al. (1993) found significantly heavier intestinal weight (12% increase) and length (26% increase) in spermine-treated rats versus control rats. Those data suggested that rats respond differently to polyamine supplementation, compared with pigs. It appears that although the selected amino acids are essential for organ growth, their supplementation at the ratios and concentrations used in this experiment does not result in enhanced organ growth.

Because different sections of the small intestine perform different roles, it is important to divide the sections when examining the effect of amino acid supplementation on mucosal growth. Theoretically, an increase in mucosal weight will result in an increase in surface area available for nutrient absorption. Obvious and consistent trends were found when measuring total and mucosal weights of the four intestinal sections. Although the weight of the entire small intestine was not significantly different between treatments, several significant effects were found in specific regions of the intestine. The POLY-treated pigs had the lowest total and mucosal weights, both absolute and mg cm^{-1}, regardless of location. Unlike the current experiment, Buts et al. (1993) reported a 39% increase in mucosal weight (mg cm^{-1}) in spermine-treated rats, compared with controls. In the duodenum, proximal and mid-jejunum, GLU and ARG-fed pigs tended to have the heaviest intestinal sections. These results agree with the measurements of villus height and crypt depth, where improvements were observed for the pigs receiving GLU and ARG. Increased mucosal weight implies that an increased amount of tissue is available for digestive and absorptive processes. The results in this study suggest that glutamate and arginine supplementation to typical early-weaning diets are potentially beneficial for intestinal growth, whereas polyamines are detrimental at the ratios and concentrations fed in the current experiment.

Disaccharidase activities have often been used as indicators of gut maturity. In the present experiment, glutamate supplementation had no effect on disaccharidase activities, similar to data from adult rats (Horvath et al. 1996). Polyamines have been shown to evoke a significant rise in sucrase and maltase activities and a decline in lactase activity (Buts et al. 1993; Harada et al. 1994). However, Grant et al. (1990) found that the lowest total and specific sucrase activity occurred in pigs fed a putrescine-supplemented milk-soy diet, compared with a control group. The present

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experiment found less maltase activity (per g mucosa) in the POLY-treated pigs. Therefore, it may be speculated that intestinal disaccharidases in rats respond differently to polyamine supplementation than in swine.

At the time of weaning, gross changes often occur in intestinal morphology, characterized by decreases in villus height and increases in crypt depth (Miller et al. 1986; Wu et al. 1996). This indicates that there is less surface area present on the villi for nutrient absorption and an increase in immature villi being formed in the crypts, potentially resulting in a decrease in overall growth performance of the animal. In the current experiment, it was proposed that amino acid supplementation would prevent or lessen the effects of weaning on structural changes in the small intestine. GLU and ARG-fed pigs had significantly longer villi in the duodenum, compared with pigs fed CON and POLY. This suggests that both glutamate and arginine prevented the villus atrophy that normally occurs post-weaning (Bertolo et al. 1999). Miller et al. (1986) found that alanine uptake, as well as lactase activity, occurs mainly at the villus tip. Therefore, taller villi suggest that an increased surface area is available for nutrient absorption, as well as the greater presence of enzymes and transport mechanisms required for digestion and absorption. Wu et al. (1996) did not find any increase in duodenal villus height in a glutamine-supplemented group of pigs, at 7 or 14 d post-weaning (21-d weaning age). However, they found that glutamine supplementation prevented jejunal atrophy at 7, but not 14 d post-weaning. Following weaning, a decrease in villus height:crypt depth ratio is common, as an indication of villus atrophy. The higher villus height:crypt depth ratio measured in the duodenum of pigs receiving the ARG appears to be the result of a relative increase in villus height, since there were no significant differences in duodenal crypt depth between treatments. Again, this suggests that the duodenal morphology of pigs fed ARG may be superior to that of CON pigs, and could possess the ability for increased absorptive capacity.

Portal amino acid concentrations reflected the amino acid composition for the respective dietary treatment groups, compared with the CON group. In the current experiment, portal arginine concentrations were significantly higher only in the ARG and CIT groups. These results are supported by previous work (Wu 1998), which demonstrated that the enzymes required for the conversion of citrulline to arginine, argininosuccinate synthase and argininosuccinate lyase are at high levels in the intestine of the young piglet. In the adult, the kidney is a net producer of arginine (via citrulline from the gut) because argininosuccinate synthase and argininosuccinate lyase activities are low in the intestine, but high in the kidneys (Jones 1985). In contrast, the activities of these enzymes are very low in the kidneys of neonates (Wu 1998). The present results are also in agreement with Edmonds et al. (1987) who found that dietary citrulline, but not ornithine, can totally replace the arginine requirement in young pigs. In the present study, supplemental ornithine or glutamate did not result in greater portal plasma arginine or citrulline concentrations. Conversion of glutamine to citrulline, which occurs via glutamate in the adult intestine, is not an active pathway in the young animal because of low pyrroline-5-carboxylate synthase activity (Wu 1998). These data also indicate that the conversion of ornithine to citrulline is low in the intestine of the young piglet. This observation is supported by the work of Blachier et al. (1993), who suggested that the low conversion of ornithine to citrulline is due to low activity of ornithine transcarbamylase, or limited accessibility of ornithine to ornithine transcarbamylase. Together, these factors suggest that arginine is indispensable in the neonatal, but not the adult pig, due to insufficient intestinal synthesis.

Reeds et al. (1996) reported that almost no orally infused [U–^{13}C]glutamate reached the portal circulation in weaned pigs. In the current experiment, the GLU group had significantly higher portal glutamate and glutamine concentrations than all other treatments, indicating that the amount of supplemented glutamate in the present study exceeded the metabolic capacity of the gut to metabolize glutamate. The high concentrations of portal glutamine also indicated that substantial conversion of glutamate to glutamine occurred in the gut of the young piglet. It is interesting to note that the CIT group had significantly lower glutamine concentrations than the remaining four treatment groups. This could occur by an increase in urea cycle metabolism due to citrulline supplementation requiring ammonia production from glutamine deamination, resulting in lower plasma glutamine concentrations. Interestingly, the response of portal plasma alanine concentrations paralleled that of plasma glutamine concentrations. Because alanine and glutamine are major ammonia carriers, these data suggest that these amino acids were responsible for the removal of excess nitrogen from the high gut catabolism of glutamate in the GLU pigs.

The GLU pigs also had higher portal plasma concentrations of total indispensable amino acids (as well as histidine, isoleucine, leucine and valine, individually) (Table 4). Because feed intake was not different among treatments, these data suggest that the absorption of these amino acids was greater, perhaps due to an improvement in gut absorptive capacity. These data may represent an improved functional outcome that corresponds to the improved anatomical outcomes observed in the GLU-fed pigs. Alternatively, it is possible that the supplemental glutamate was catabolized for energy by the small intestine (Reeds et al. 1996), thereby sparing the catabolism of indispensable amino acids for energy (Stoll et al. 1998); this sparing effect would allow greater transport of indispensable amino acids to the portal circulation. In either case, glutamate supplementation appears to have benefited the early-weaned pig by increasing the availability of dietary indispensable amino acids to the whole body for protein deposition.

We have been intentionally cautious in translating our results to net benefits for pig performance. We did not expect to observe any “functional” changes (i.e., growth, nitrogen retention) as a result of our dietary treatments because these methods are not very sensitive in small pigs over a short period of time. Our primary interest was in gut integrity because this is believed to be a significant factor in “growth lag” following weaning. This interest directed our choice of supplements and our choice of analyses. How these results translate to absorptive capacity or other

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functional outcomes is not yet known and will require additional research.

In conclusion, because of the consistently poorer gut development in POLY-fed pigs, polyamine supplementation at the ratios and concentrations used in this experiment is not recommended in typical early-weaning diets. This finding does not controvert the importance of polyamines in gut development. Other polyamines, or their ratios and concentrations, may have had different effects. In addition, although we could not measure the polyamine content of the basal diet, the high basal concentrations of whey protein, spray-dried plasma and fish meal would make this diet rich in amines before the supplementation of polyamines. It is possible that the polyamine supplementation to such an amine-rich diet was slightly toxic; however, there were no differences in feed intake among treatment groups, which suggests otherwise. Our choice of early-weaning diet was based on its commercial availability and widespread use in the swine industry. Although polyamine supplementation may have benefited a "lower quality" diet, such a scenario is not relevant to producers who generally employ "high quality" early-weaning diets.

In a similar manner, the benefits of ornithine and citrulline supplementation may have been masked by the high quality of our basal diet. Supplementation of these amino acids to inferior diets may have to be explored should the industry move towards employing such diets for early-weaned piglets. Indeed, the fact that arginine and glutamate supplementation were able to effect improvements in gut development when supplemented to a "high quality" diet emphasizes the potential importance and benefit of these amino acids to piglet performance. As a result, arginine and glutamate supplementation to typical early-weaned piglet diets should be further investigated.

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