Amino Acids (2013) 44:911–923
DOI 10.1007/s00726-012-1420-x

ORIGINAL ARTICLE

Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs

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Received: 5 October 2012 / Accepted: 9 October 2012 / Published online: 2 November 2012

Abstract Dietary intake of glutamate by postweaning pigs is markedly reduced due to low feed consumption. This study was conducted to determine the safety and efficacy of dietary supplementation with monosodium glutamate (MSG) in postweaning pigs. Piglets were weaned at 21 days of age to a corn and soybean meal-based diet supplemented with 0, 0.5, 1, 2, and 4 % MSG ($n = 25$/group). MSG was added to the basal diet at the expense of cornstarch. At 42 days of age (21 days after weaning), blood samples (10 mL) were obtained from the jugular vein of 25 pigs/group at 1 and 4 h after feeding for hematological and clinical chemistry tests; thereafter, pigs ($n = 6$/group) were euthanized to obtain tissues for histopathological examinations. Feed intake was not affected by dietary supplementation with 0–2 % MSG and was 15 % lower in pigs supplemented with 4 % MSG compared with the 0 % MSG group. Compared with the control, dietary supplementation with 1, 2 and 4 % MSG dose-dependently increased plasma concentrations of glutamate, glutamine, and other amino acids (including lysine, methionine, phenylalanine and leucine), daily weight gain, and feed efficiency in postweaning pigs. At day 7 postweaning, dietary supplementation with 1–4 % MSG also increased jejunal villus height, DNA content, and antioxidative capacity. The MSG supplementation dose-dependently reduced the incidence of diarrhea during the first week after weaning. All variables in standard hematology and clinical chemistry tests, as well as gross and microscopic structures, did not differ among the five groups of pigs. These results indicate that dietary supplementation with up to 4 % MSG is safe and improves growth performance in postweaning pigs.

Keywords Monosodium glutamate · Piglet · Growth · Safety · Efficacy

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Introduction

Glutamate is an acidic amino acid with multiple roles in cell metabolism and physiology. This nutrient participates in both synthetic and oxidative pathways in tissues (Blachier et al. 2009; Wu 1998), serves as a major energy substrate for the small intestine (Burrin and Stoll 2009) and an excitatory neurotransmitter (Kirchgessner 2001), activates taste receptors in the digestive tract (San Gabriel et al. 2009), enhances diet-induced thermogenesis in brown adipose tissue of young adult rats (Smriga et al. 2000), regulates the release of certain hormones [e.g., norepinephrine (Smriga and Torii 2000) and glucagon-like peptide-1 (Iwatsuki and Torii 2012)], and reduces white-fat deposition in adult rats (Kondoh and Torii 2008). Thus, glutamate has been recognized as a functional amino acid for humans and other animals (Brosnan and Brosnan 2012; San Gabriel and Uneyama 2012; Wu 2010).

Glutamate is particularly abundant in sow's milk to support neonatal growth and development (Wu and Knabe 1995). Because there is no uptake of arterial blood glutamate

by the gut, the enteral diet is the primary source of glutamate for enterocytes (Wu 1998). In young pigs, the supply of dietary glutamate to the gut is limited after weaning due to a marked reduction of food intake (Wu 2010), which is associated with severe intestinal atrophy, inflammation, malabsorption, and death (Lalles et al. 2007). Given a crucial role for glutamate in intestinal physiology, its deficiency may contribute to impaired function and reduced growth of the small intestine as well as high rates of morbidity and mortality after weaning. However, there is limited information regarding effects of dietary glutamate supplementation on weanling pigs. Therefore, the objective of the present study was to fill in this important gap of knowledge about glutamate nutrition and metabolism in animals.

Materials and methods

Three series of experiments were conducted with post-weaning pigs. All experimental procedures were approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University.

Experiment 1: effects of dietary MSG supplementation for 21 days on growth performance in post-weaning pigs

Animals and diets

Piglets were offspring of Yorkshire × Landrace dams and Duroc × Hampshire sires, nursed by sows fed an 18.4 % crude protein diet (Mateo et al. 2008), and weaned at 21 days of age to a conventional corn and soybean meal-based diet (Table 1). This basal diet contained 21.0 % crude protein and 1.91 % glutamate, which were analyzed as we described (Li et al. 2011). The basal diet was supplemented with 0, 0.5, 1, 2 or 4 % MSG at the expense of cornstarch. Isonitrogenous amounts of L-alanine were not added to the diets containing 0–2 % MSG because one of the goals of this study was to determine whether dietary MSG supplementation might affect concentrations of alanine in the plasma of postweaning pigs. Five piglets with similar body weights were selected from each of 25 litters and assigned randomly to one of the five treatment groups. There were 25 piglets per treatment group. During the entire experimental period, post-weaning pigs had free access to drinking water and their respective diets. Their body weights were recorded weekly and feed intake was determined daily. The incidence of diarrhea (indicated by the occurrence of watery feces) was recorded daily.

Collection of tissues

At 42 days of age (i.e., 21 days after weaning), blood samples (10 mL) were obtained from the jugular vein of 25 pigs/group at 1 and 4 h after feeding for hematological and clinical chemistry tests (including urea, sodium, potassium, iron, albumin, and transaminases in serum), which were conducted at Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, Texas). At the end of the second blood collection, six pigs were selected randomly from each group to be humanely euthanized by intracardiac administration of 10 mL saturated KCl after anesthesia induced by intramuscular administration of Telazol (5 mg/kg body weight). Criteria used to indicate that animals were deceased were the absence of all ocular reflexes as well as audible heart and breath sounds for longer than 5 min using a stethoscope. Immediately after euthanasia, the abdomen of individual pigs was opened to obtain tissues (brain, spinal cord, heart, lungs, spleen, liver, kidneys, adrenal gland, stomach, cecum, small intestine (duodenum, jejunum and ileum), large intestine, gonads, and bone marrow) for histopathological examinations by Texas A&M Veterinary Medical Diagnostic Laboratory.

Table 1 Composition of the basal experimental diet

Ingredient	Percentage (g/100 g)
Corn	52.37
Soybean meal	37.0
Soybean oil	2
Cornstarch	4
Lysine-HCl, 98.8 %	0.15
Dicalcium phosphate	1.56
Limestone	1.02
Medication[a]	1
Vitamin premix[b]	0.5
Trace mineral premix[c]	0.15
NaCl	0.25
Calculated content (%)	
Crude protein[d]	21.0
Digestible energy (Kcal/kg)	3630
Calcium	0.85
Phosphorus	0.70

Values are expressed on an as-fed basis. Dry matter content was 89.8 %

[a] Containing the following (mg/kg diet): carbadox, 55

[b] Containing the following (mg/kg diet): retinyl palmitate 4.24, cholecalciferol 0.019, all-rac-α-tocopheryl acetate 44, menadione sodium bisulfate complex 9, riboflavin 7.7, D-calcium pantothenate 33, niacin 33, choline 287, vitamin B-12, 0.044, D-Biotin 0.22

[c] Containing the following (mg/kg diet): Cu 15, Fe 100, I 0.6, Mn 20, Zn 100, Se 0.1

[d] The analyzed contents (% of diet; on an as-fed basis) of amino acids were as follows: arginine 1.36, alanine 1.29, aspartate 1.36, asparagine 0.95, cysteine 0.38, glutamate 1.91, glutamine 1.60, glycine 0.87, histidine 0.57, isoleucine 0.88, leucine 1.75, lysine 1.40, methionine 0.36, phenylalanine 1.00, proline 1.64, serine 0.77, threonine 0.84, tryptophan 0.24, tyrosine 0.75, and valine 0.98

Examination of small-intestinal morphology

Segments of duodenum, jejunum, and ileum (~10 cm in length for each segment) were obtained as we previously described (Wang et al. 2008). After their luminal contents were removed, the mucosa was rinsed gently with saline. A portion of the intestinal segment (~3 cm) was placed in 4 % paraformaldehyde for subsequent analysis of morphology, as we previously described (Wu et al. 1996), whereas the remaining portions of the small intestine were placed in liquid nitrogen and stored at −80 °C until analysis for DNA, RNA, and protein.

Analyses of amino acids and glucose in plasma

Plasma (0.5 mL) were deproteinized with an equal volume of 1.5 M $HClO_4$, followed by addition of 0.25 ml 2 M K_2CO_3. Amino acids in the extract were determined by fluorometric HPLC methods involving precolumn derivatization with *o*-phthaldialdehyde as described previously (Wu et al. 1997). The integration of chromatographic peaks was performed using Millenium-32 Software (Waters, Milford, MA, USA). Glucose in plasma was determined enzymatically by a spectrophotometric method involving hexokinase and glucose-6-phosphate dehydrogenase (Satterfield et al. 2012).

Analyses of total lipids in tissues

Total lipids in tissues (~100 mg liver and ~500 mg skeletal muscle, heart, and small intestine) were analyzed using chloroform extraction as we previously described (Jobgen et al. 2009).

Analysis for DNA, RNA, protein, glutathione, and ATP in tissues

A tissue (~100 mg) was homogenized in 2 mL of Tris buffer (0.1 M Tris/10 mM EDTA/0.1 M NaCl). The homogenizer was rinsed with 2 mL of the same buffer. The combined solution was centrifuged at 600*g* for 5 min. An aliquot of the supernatant fluid was diluted 10 times with the Tris buffer for analysis of DNA and RNA using ethidium bromide and RNase, as described by Prasad et al. (1972). Another aliquot was used for analysis of protein using bovine serum albumin as standard (Yao et al. 2012). Glutathione and ATP were analyzed using high-performance liquid chromatography as we described (Haynes et al. 2009).

Experiment 2: effects of dietary MSG supplementation for 7 days on small-intestinal morphology in post-weaning pigs

We previously reported that dietary supplementation with 1 % L-glutamine to postweaning pigs prevented jejunal atrophy at day 7 postweaning and that such an effect of glutamine supplementation was not observed at day 14 postweaning (Wu et al. 1996). To evaluate possible effects of dietary MSG supplementation on the morphology of the small intestine during the first week of weaning, experiment 2 was conducted as experiment 1, except that all pigs were euthanized at 4 h post feeding after a 7-day period of dietary supplementation with MSG. There were 20 piglets per treatment group.

Experiment 3: effects of dietary NaCl supplementation for 21 days on growth performance in post-weaning pigs

This experiment was conducted as experiment 1, except that 0.000, 0.172, 0.343, 0.686, or 1.372 % NaCl (Sigma Chemicals, St. Louis, MO, USA) was added to the basal diet at the expense of cornstarch, providing 0.000, 0.068, 0.136, 0.272, or 0.544 % Na, respectively. These amounts of sodium were equivalent to those in 0.0, 0.5, 1, 2, or 4 % MSG. There were 20 piglets per treatment group.

Statistical analyses

Results are expressed as mean ± SEM. The pen is the experimental unit for analyzed data on growth performance (body weight, average daily gain, feed intake, and gain:feed ratio). The pig is the experimental unit for analyzed data on plasma metabolites and hormones, intestinal morphology, and nucleotides in tissues. Statistical analyses of data were performed by one-way analysis of variance using the General Linear Models procedures of the Statistical Analysis System (SAS Institute, Cary, NC, USA). Differences among treatment means were determined using the Student–Newman–Keuls multiple comparison method (Wei et al. 2012). Comparisons of means between 1- and 4-h time points were analyzed by the paired *t* test. Differences in the incidence of diarrhea among the treatment groups were analyzed by Chi-square analysis. A probability value ≤0.05 was taken to indicate statistical significance.

Results

Effects of MSG on growth performance of pigs

Supplementing 0–2 % MSG to the diet did not affect feed intake by postweaning pigs (Table 2). In weeks 2–3, feed intake of pigs supplemented with 4 % MSG was 13–17 % lower ($P < 0.05$) compared with pigs in the control and 0.5 % MSG groups. In weeks 1–3, body weight or average daily gain (ADG) of pigs was not affected by dietary supplementation with 0.5 % MSG. However, compared with the control group, dietary supplementation with 1, 2, and 4 % dose-dependently increased ($P < 0.05$) body

Table 2 Effects of dietary MSG supplementation on growth performance in postweaning pigs

Variable	Supplemental MSG in diet (%)				
	0 (0.00 % Na)	0.5 (0.068 % Na)	1 (0.136 % Na)	2 (0.272 % Na)	4 (0.544 % Na)
Body weight (kg) of pigs					
Day 0	5.49 ± 0.13	5.53 ± 0.08	5.52 ± 0.08	5.55 ± 0.09	5.60 ± 0.08
Day 7	6.82 ± 0.17^{d}	6.88 ± 0.15^{c,d}	7.06 ± 0.15^{b,c}	7.15 ± 0.14^{a,b}	7.35 ± 0.13^{a}
Day 14	9.01 ± 0.19^{d}	9.20 ± 0.20^{c,d}	9.31 ± 0.21^{c}	9.61 ± 0.18^{b}	9.91 ± 0.22^{a}
Day 21	12.2 ± 0.26^{c}	12.5 ± 0.28^{b,c}	12.7 ± 0.30^{b}	13.0 ± 0.33^{a,b}	13.4 ± 0.31^{a}
Average daily gain (g/day)					
Days 0–7	190 ± 7^{c}	193 ± 6^{c}	220 ± 7^{b}	228 ± 7^{b}	250 ± 8^{a}
Days 7–14	314 ± 6^{b}	332 ± 5^{b}	321 ± 6^{b}	351 ± 6^{a}	365 ± 7^{a}
Days 14–21	460 ± 8^{b}	463 ± 9^{b}	485 ± 8^{a}	485 ± 8^{a}	497 ± 9^{a}
Days 0–21	321 ± 6^{d}	329 ± 6^{c,d}	342 ± 7^{b,c}	355 ± 7^{a,b}	371 ± 8^{a}
Feed intake (g/kg body weight per day)					
Days 0–7	26.7 ± 2.3	27.2 ± 2.4	26.3 ± 2.8	25.8 ± 2.6	24.7 ± 1.9
Days 7–14	43.8 ± 2.5^{a}	42.3 ± 3.0^{a}	38.7 ± 2.6^{a,b}	38.3 ± 2.4^{a,b}	36.4 ± 2.3^{b}
Days 14–21	55.7 ± 3.2^{a}	54.1 ± 3.5^{a}	52.8 ± 3.4^{a,b}	50.4 ± 3.3^{a,b}	48.2 ± 3.1^{b}
Days 0–21	41.3 ± 2.4^{a}	40.6 ± 2.1^{a}	38.4 ± 2.5^{a,b}	38.1 ± 2.2^{a,b}	36.5 ± 2.0^{b}
Gain:Feed ratio (g/g)					
Days 0–7	0.931 ± 0.08^{c}	0.939 ± 0.08^{c}	1.08 ± 0.09^{b,c}	1.29 ± 0.12^{a,b}	1.38 ± 0.11^{a}
Days 7–14	0.842 ± 0.06^{c}	0.905 ± 0.07^{b}	0.913 ± 0.05^{b}	1.11 ± 0.07^{a}	1.16 ± 0.09^{a}
Days 14–21	0.766 ± 0.05^{c}	0.792 ± 0.05^{c}	0.860 ± 0.05^{b}	0.894 ± 0.04^{b}	0.957 ± 0.06^{a}
Days 0–21	0.812 ± 0.02^{e}	0.846 ± 0.02^{d}	0.887 ± 0.01^{c}	0.934 ± 0.02^{b}	0.990 ± 0.02^{a}

Pigs were weaned at 21 days of age (the day of weaning = Day 0). Values are mean ± SEM, $n = 5$ pens/group with 5 piglets per pen

[a–e] Within a row, means sharing different superscript letters differ ($P < 0.05$)

weight and ADG of pigs in weeks 2–3. During the 3-week experimental period, increasing the supplemental dosage of MSG from 0 to 4 % progressively enhanced ($P < 0.05$) the gain:feed ratio in postweaning pigs.

Effects of MSG on concentrations of amino acids in pig plasma

Table 3 summarizes plasma concentrations of amino acids in 42-day-old control and MSG-supplemented pigs (i.e., 21 days after weaning) at 1 and 4 h after feeding. Dietary MSG supplementation increased ($P < 0.05$) concentrations of aspartate, glutamate, glutamine, histidine, citrulline, arginine, taurine, alanine, methionine, valine, phenylalanine, isoleucine, leucine, proline, cysteine, ornithine, and lysine in plasma to various extents at both 1 and 4 h after feeding. Supplementing MSG to the basal diet also increased ($P < 0.05$) concentrations of asparagine, serine, threonine, tryptophan, and tyrosine in plasma of weaned pigs at 1 h after feeding (Table 3). Compared with values obtained at 4 h after feeding, concentrations of alanine, citrulline, glutamate, methionine, ornithine, phenylalanine, proline, and tryptophan in plasma were higher ($P < 0.05$) at 1 h after feeding.

Effects of MSG on hematology of pigs

Compared with the control group, dietary supplementation with 1, 2, and 4 % MSG increased ($P < 0.05$) plasma concentration of urea at 1 h and 4 h after feeding (Table 4), while decreasing ($P < 0.05$) concentrations of total lipids in liver and skeletal muscle in a dose-dependent manner (Table 5). Concentrations of total protein, creatinine, glucose, electrolytes, and select enzymes (Table 4), as well as the numbers of white blood cells, red blood cells, and platelets (Table 6), in blood samples obtained at both 1 and 4 h after feeding were not affected by dietary MSG supplementation. Interestingly, concentrations of blood hemoglobin were higher ($P < 0.05$) in pigs supplemented with 2 and 4 % MSG compared with the control and 0.5 % MSG groups.

Effects of MSG on jejunal morphology and small-intestine weight at day 21 after weaning

Concentrations of DNA, RNA, and protein in the small intestine of 42-day-old pigs (21 days after weaning) did not differ ($P > 0.05$) among the five treatment groups (Table 7). Likewise, dietary supplementation with up to

Table 3 Concentrations of free amino acids in the plasma of control and MSG-supplemented pigs obtained at 1 and 4 h after feeding

Amino acid	Supplemental MSG in diet (%)					Supplemental MSG in diet (%)				
	0	0.5	1	2	4	0	0.5	1	2	4
	Blood samples obtained at 1 h after feeding					Blood samples obtained at 4 h after feeding				
Ala	498 ± 15^{c}	516 ± 16^{c}	575 ± 13^{b,*}	604 ± 9^{a,*}	620 ± 10^{a,*}	481 ± 13^{d}	506 ± 15^{c,d}	524 ± 15^{c}	558 ± 16^{b}	589 ± 17^{a}
Arg	123 ± 5^{e}	144 ± 6^{d}	165 ± 5^{c}	196 ± 5^{b}	221 ± 7^{a}	120 ± 6^{e}	143 ± 7^{d}	157 ± 5^{c}	188 ± 8^{b}	205 ± 10^{a}
Asn	113 ± 4^{d}	117 ± 5^{c,d}	126 ± 3^{c}	140 ± 2^{b}	162 ± 4^{a}	116 ± 7	125 ± 10	129 ± 8	132 ± 5	127 ± 8
Asp	30 ± 2^{c}	33 ± 2^{c}	40 ± 2^{b}	42 ± 2^{b}	49 ± 2^{a}	29 ± 2^{d}	30 ± 3^{c,d}	33 ± 1^{c}	39 ± 2^{b}	48 ± 3^{a}
β-Ala	39 ± 1	40 ± 2	40 ± 1	40 ± ±1	42 ± 1	40 ± 3	42 ± 3	44 ± 5	45 ± 4	41 ± 5
Cit	76 ± 2^{e}	89 ± 3^{d}	108 ± 5^{c,*}	130 ± 3^{b,*}	148 ± 4^{a,*}	75 ± 3^{e}	86 ± 4^{d}	98 ± 5^{c}	115 ± 5^{b}	137 ± 7^{a}
Cys	69 ± 2^{c}	72 ± 2^{c}	83 ± 3^{b}	89 ± 4^{a,b}	94 ± 3^{a}	70 ± 2^{c}	75 ± 3^{b,c}	80 ± 3^{b}	92 ± 4^{a}	98 ± 5^{a}
Gln	543 ± 7^{e}	596 ± 17^{d}	644 ± 10^{c}	695 ± 12^{b}	742 ± 14^{a}	537 ± 13^{e}	586 ± 19^{d}	637 ± 21^{c}	683 ± 24^{b}	723 ± 26^{a}
Glu	227 ± 9^{b,*}	239 ± 10^{b,*}	243 ± 8^{b,*}	250 ± 12^{b,*}	302 ± 14^{a,*}	102 ± 5^{c}	106 ± 6^{c}	113 ± 6^{c}	130 ± 7^{b}	158 ± 8^{a}
Gly	1050 ± 22	1087 ± 19	1106 ± 17	1213 ± 20	1268 ± 9	1094 ± 27	1107 ± 42	1276 ± 25	1241 ± 43	1138 ± 31
His	72 ± 2^{c}	72 ± 2^{c}	75 ± 2^{c}	84 ± 3^{b}	95 ± 2^{a}	74 ± 4^{c}	75 ± 3^{c}	77 ± 4^{c}	86 ± 5^{b}	98 ± 6^{a}
Ile	152 ± 4^{d}	155 ± 5^{d}	170 ± 7^{c}	201 ± 9^{b}	221 ± 8^{a}	154 ± 7^{d}	163 ± 5 ^{cd}	176 ± 7^{c}	198 ± 6^{b}	216 ± 9^{a}
Leu	166 ± 5^{d}	173 ± 4^{d}	195 ± 5^{c}	214 ± 6^{b}	232 ± 5^{a}	165 ± 5^{d}	177 ± 6^{c,d}	183 ± 8^{c}	207 ± 7^{b}	235 ± 8^{a}
Lys	110 ± 4^{d}	118 ± 4^{c,d}	129 ± 4^{c}	149 ± 6^{b}	177 ± 7^{a}	103 ± 5^{d}	114 ± 6^{c,d}	121 ± 8^{c}	142 ± 9^{b}	165 ± 10^{a}
Met	105 ± 5^{e,*}	117 ± 6^{d,*}	133 ± 4^{c,*}	149 ± 3^{b,*}	170 ± 5^{a,*}	94 ± 5^{d}	102 ± 6^{c,d}	113 ± 6^{c}	134 ± 7^{b}	156 ± 8^{a}
Orn	80 ± 2^{e,*}	93 ± 2^{d,*}	110 ± 4^{c,*}	130 ± 4^{b,*}	149 ± 5^{a,*}	71 ± 3^{e}	80 ± 4^{d}	91 ± 5^{c}	103 ± 4^{b}	120 ± 6^{a}
Phe	112 ± 4^{d,*}	115 ± 3^{d,*}	126 ± 5^{c,*}	148 ± 4^{b,*}	172 ± 5^{a,*}	98 ± 5^{c}	103 ± 7^{c}	101 ± 6^{c}	126 ± 7^{b}	144 ± 8^{a}
Pro	287 ± 7^{d,*}	302 ± 8^{c,d*}	324 ± 10^{c,*}	368 ± 9^{b,*}	402 ± 11^{a,*}	236 ± 6^{d}	253 ± 10^{c,d}	270 ± 12^{c}	305 ± 9^{b}	346 ± 10^{a}
Ser	161 ± 3^{c}	174 ± 4^{c}	193 ± 6^{b}	208 ± 4^{b}	236 ± 7^{a}	171 ± 14	176 ± 12	182 ± 15	185 ± 11	180 ± 12
Taurine	53 ± 2^{e}	72 ± 2^{d}	83 ± 2^{c}	87 ± 3^{b,c}	91 ± 2^{a}	49 ± 3^{d}	64 ± 4^{c}	71 ± 6^{c}	86 ± 6^{b}	103 ± 8^{a}
Thr	188 ± 4^{d}	208 ± 4^{c}	231 ± 6^{b,*}	257 ± 7^{a,*}	262 ± 4^{a,*}	188 ± 9	202 ± 12	208 ± 10	196 ± 8	193 ± 9
Trp	81 ± 2^{e,*}	95 ± 3^{d,*}	107 ± 4^{c,*}	124 ± 5^{b,*}	137 ± 4^{a,*}	74 ± 4	77 ± 5	73 ± 4	68 ± 6	72 ± 5
Tyr	109 ± 6^{b}	115 ± 4^{b}	119 ± 6^{b}	126 ± 5^{b}	149 ± 3^{a,*}	102 ± 9	108 ± 8	113 ± 7	124 ± 10	116 ± 12
Val	149 ± 7^{d}	155 ± 3^{d}	176 ± 4^{c}	198 ± 7^{b}	229 ± 5^{a}	152 ± 8^{d}	162 ± 6^{c,d}	171 ± 7^{c}	195 ± 6^{b}	218 ± 7^{a}

Blood samples were obtained from 42-day-old pigs (21 days after weaning) at 1 and 4 h after feeding. Values, expressed as nmol/ml, are mean ± SEM, $n = 10$/group

* $P < 0.05$ versus the 4 h value, as analyzed by the paired t test

[a–e] Within a row, means sharing different superscript letters differ ($P < 0.05$)

Table 4 Concentrations of total protein, glucose, urea, creatinine, electrolytes, and enzymes in the serum of control and MSG-supplemented pigs at 1 and 4 h after feeding

Variable	Time after feeding (h)	Supplemental MSG in diet (%)				
		0	0.5	1	2	4
Total protein (g/dL)	1	3.96 ± 0.15	3.90 ± 0.10	3.82 ± 0.25	4.04 ± 0.10	4.05 ± 0.13
	4	4.06 ± 0.16	3.98 ± 0.08	3.85 ± 0.26	3.96 ± 0.14	3.91 ± 0.15
Albumin (g/dL)	1	2.50 ± 0.13	2.56 ± 0.10	2.38 ± 0.21	2.54 ± 0.12	2.44 ± 0.15
	4	2.42 ± 0.14	2.50 ± 0.13	2.42 ± 0.23	2.48 ± 0.16	2.36 ± 0.16
Globulins (g/dL)	1	1.45 ± 0.16	1.41 ± 0.07	1.54 ± 0.07	1.42 ± 0.05	1.43 ± 0.10
	4	1.57 ± 0.18	1.48 ± 0.09	1.42 ± 0.05	1.48 ± 0.10	1.54 ± 0.17
Total bilirubin (mg/dL)	1	<0.1	<0.1	<0.1	<0.1	<0.1
	4	<0.1	<0.1	<0.1	<0.1	<0.1
Calcium (mg/dL)	1	11.8 ± 0.16	11.6 ± 0.19	12.3 ± 0.46	12.5 ± 0.37	12.6 ± 0.14
	4	12.1 ± 0.22	12.0 ± 0.36	11.2 ± 0.23	11.4 ± 0.20	11.6 ± 0.18
Phosphorus (mg/dL)	1	7.13 ± 0.24	7.15 ± 0.41	7.21 ± 0.55	7.23 ± 0.26	7.04 ± 0.42
	4	6.97 ± 0.33	6.95 ± 0.40	7.04 ± 0.76	6.99 ± 0.34	7.10 ± 0.49
Glucose (mg/dL)	1	141 ± 8	137 ± 10	135 ± 9	144 ± 5	140 ± 6
	4	89.5 ± 2.6*	87.7 ± 2.1*	87.4 ± 1.6*	88.5 ± 3.2*	89.1 ± 2.3*
BUN (mg/dL)	1	6.74 ± 0.48^{e}	8.68 ± 0.52^{d}	9.94 ± 0.62^{c}	10.9 ± 0.51^{b}	12.8 ± 0.54^{a}
	4	5.63 ± 0.41^{d,*}	5.70 ± 0.46^{d,*}	8.83 ± 0.57^{c,*}	10.2 ± 0.59^{b}	12.6 ± 0.43^{a}
Creatinine (mg/dL)	1	0.56 ± 0.02	0.59 ± 0.03	0.54 ± 0.04	0.61 ± 0.04	0.59 ± 0.05
	4	0.58 ± 0.02	0.62 ± 0.02	0.56 ± 0.05	0.60 ± 0.04	0.61 ± 0.06
Sodium (mM)	1	138 ± 0.6	138 ± 0.9	137 ± 1	139 ± 0.8	139 ± 0.2
	4	139 ± 1	138 ± 1	138 ± 1	138 ± 1	140 ± 1
Potassium (mM)	1	5.26 ± 0.14	5.35 ± 0.23	5.23 ± 0.08	5.27 ± 0.21	5.20 ± 0.29
	4	5.13 ± 0.14	5.20 ± 0.25	5.12 ± 0.10	5.16 ± 0.19	5.24 ± 0.33
Chloride (mM)	1	100 ± 0.6	101 ± 0.7	98.2 ± 0.7	98.4 ± 0.5	99.5 ± 0.7
	4	99.2 ± 0.4	100 ± 1	97.6 ± 0.9	97.8 ± 0.9	99.4 ± 0.8
Magnesium (mM)	1	2.13 ± 0.08	2.19 ± 0.08	2.05 ± 0.11	2.02 ± 0.08	2.07 ± 0.05
	4	2.10 ± 0.07	2.06 ± 0.09	2.04 ± 0.07	2.08 ± 0.18	2.16 ± 0.10
ALP (U/L)	1	404 ± 53	416 ± 56	407 ± 29	427 ± 33	439 ± 18
	4	371 ± 41	402 ± 46	398 ± 31	412 ± 32	420 ± 29
ALT (U/L)	1	40.6 ± 3.2	44.4 ± 2.5	43.2 ± 3.3	43.2 ± 2.8	40.8 ± 4.1
	4	42.8 ± 4.0	42.6 ± 1.9	42.2 ± 4.5	43.4 ± 3.4	41.5 ± 3.8
AST (U/L)	1	39.2 ± 3.1	38.8 ± 2.3	37.3 ± 2.4	39.0 ± 4.0	41.0 ± 3.2
	4	37.6 ± 2.8	40.4 ± 2.0	36.4 ± 4.7	38.6 ± 2.2	38.2 ± 2.5
CK (U/L)	1	520 ± 83	549 ± 60	527 ± 71	562 ± 85	594 ± 77
	4	485 ± 51	513 ± 47	502 ± 58	508 ± 62	521 ± 63
LDH (IU/L)	1	729 ± 37	784 ± 22	713 ± 42	752 ± 18	753 ± 19
	4	717 ± 21	750 ± 44	722 ± 48	748 ± 23	760 ± 32

Blood samples were obtained from 42-day-old pigs (21 days after weaning) at 1 and 4 h after feeding. Values are mean ± SEM, $n = 6$/group

ALP alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *BUN* blood urea nitrogen, *CK* creatine kinase, *LDH* lactate dehydrogenase

* $P < 0.05$ versus the corresponding 1-h value

[a–e] Within a row, means sharing different superscript letters differ ($P < 0.05$)

4 % MSG did not affect intestinal morphology in these pigs (data not shown). At 21 days postweaning, the proportion of the small-intestine weight in the body did not differ ($P > 0.05$) among the five groups of pigs (38.2 ± 0.6 %). The absolute weight of the small intestine in 42-day-old pigs that had been supplemented with 1, 2, and 4 % MSG for 3 weeks was enhanced ($P < 0.05$) by 6.3, 10.5, and 15.7 %, respectively, when compared with the control group (468 ± 9 g).

Table 5 Concentration of total lipids in tissues of control and MSG-supplemented pigs obtained at 4 h after feeding

Plasma or tissue	Supplemental MSG in diet (%)				
	0	0.5	1	2	4
Liver (%)	12.8 ± 0.30^{a}	$12.4 \pm 0.25^{a,b}$	12.2 ± 0.19^{b}	11.5 ± 0.14^{c}	10.8 ± 0.12^{d}
Skeletal muscle (%)	3.31 ± 0.07^{a}	3.16 ± 0.06^{b}	$3.11 \pm 0.04^{b,c}$	3.05 ± 0.03^{c}	2.95 ± 0.05^{d}

Blood samples were obtained from 42-day-old pigs (21 days after weaning) at 4 h after feeding. Values are mean ± SEM, $n = 6$/group

[a–d] Within a row, means sharing different superscript letters differ ($P < 0.05$)

Table 6 Hematology variables in control and MSG-supplemented pigs at 1 and 4 h after feeding

Variables	Time after feeding (h)	Supplemental MSG in diet (%)				
		0	0.5	1	2	4
Total WBC, $\times 10^3/\mu L$	1	13.4 ± 0.86	12.7 ± 0.74	12.5 ± 1.2	13.5 ± 0.71	12.2 ± 0.68
	4	11.0 ± 0.32	11.2 ± 0.50	11.9 ± 0.82	11.3 ± 0.28	11.8 ± 0.64
Total RBC, $\times 10^6/\mu L$	1	5.62 ± 0.12	5.80 ± 0.10	5.61 ± 0.18	5.84 ± 0.11	6.06 ± 0.16
	4	5.84 ± 0.14	6.38 ± 0.09	6.07 ± 0.16	5.97 ± 0.10	6.08 ± 0.12
Platelets, $\times 10^3/\mu L$	1	729 ± 59	665 ± 74	752 ± 49	702 ± 70	696 ± 65
	4	369 ± 45*	324 ± 34*	318 ± 37*	327 ± 13*	353 ± 26*
Blood HG (g/dL)	1	9.42 ± 0.17^{b}	9.44 ± 0.26^{b}	9.31 ± 0.25^{b}	9.84 ± 0.25^{a}	9.93 ± 0.20^{a}
	4	9.54 ± 0.23^{b}	$9.85 \pm 0.28^{a,b}$	$9.83 \pm 0.22^{a,b}$	10.3 ± 0.26^{a}	10.4 ± 0.33^{a}
Plasma protein (g/dL)	1	4.83 ± 0.16	4.82 ± 0.09	4.75 ± 0.11	4.81 ± 0.10	4.54 ± 0.11
	4	4.50 ± 0.15*	4.58 ± 0.07*	4.38 ± 0.13*	4.31 ± 0.12*	4.22 ± 0.12*
Fibrinogen (mg/dL)	1	224 ± 13	203 ± 32	248 ± 36	201 ± 10	202 ± 22
	4	102 ± 5*	108 ± 12*	135 ± 19*	101 ± 4*	105 ± 6*
Hematocrit (%)	1	30.4 ± 0.5	30.3 ± 0.6	30.1 ± 0.6	31.3 ± 0.5	31.3 ± 0.5
	4	30.2 ± 0.6	30.7 ± 0.9	30.1 ± 0.5	30.6 ± 1	30.3 ± 1
MCV (fL)	1	52.1 ± 0.5	50.9 ± 1.1	51.7 ± 1.8	53.9 ± 0.3	51.9 ± 1
	4	51.3 ± 0.7	50.1 ± 0.9	50.2 ± 1.7	51.5 ± 0.8	48.7 ± 0.4
MCH (pg)	1	16.6 ± 0.3	16.0 ± 0.5	16.8 ± 0.5	16.9 ± 0.3	16.4 ± 0.4
	4	16.3 ± 0.3	16.2 ± 0.5	16.4 ± 0.5	16.8 ± 0.4	16.3 ± 0.2
MCHC (g/dL)	1	32.0 ± 0.4	31.4 ± 0.5	32.5 ± 0.6	31.4 ± 0.4	31.7 ± 0.3
	4	31.9 ± 0.3	31.5 ± 0.6	32.7 ± 0.4	32.6 ± 0.2	33.4 ± 0.3
Percent of WBC in blood						
Neutrophils	1	44.8 ± 2.5	48.0 ± 3.7	47.3 ± 2.8	49.5 ± 2.4	42.4 ± 3.2
	4	49.3 ± 5.7	47.5 ± 7.1	45.6 ± 6.2	47.2 ± 6.9	41.5 ± 5.0
Lymphocytes	1	48.8 ± 2.0	47.6 ± 3.7	42.8 ± 1.7	43.4 ± 1.3	53.4 ± 3.3
	4	46.3 ± 4.6	46.5 ± 6.6	44.6 ± 7.5	50.1 ± 4.2	54.2 ± 6.5
Monocytes	1	1.54 ± 0.28	1.72 ± 0.35	1.70 ± 0.48	1.58 ± 0.40	1.63 ± 0.37
	4	1.67 ± 0.26	1.50 ± 0.32	1.55 ± 0.22	1.50 ± 0.24	1.54 ± 0.30
Eosinophils	1	3.06 ± 0.52	2.59 ± 0.66	2.53 ± 0.40	3.01 ± 0.64	2.62 ± 0.58
	4	2.73 ± 0.45	3.03 ± 0.29	2.84 ± 0.37	3.15 ± 0.41	2.87 ± 0.43

Blood samples were obtained from 42-day-old pigs (21 days after weaning) at 1 and 4 h after feeding. Values are mean ± SEM, $n = 6$/group

HG hemoglobin, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *MCV* mean corpuscular volume, *RBC* red blood cells, *WBC* white blood cells

[a–b] Within a row, means sharing different superscript letters differ ($P < 0.05$)

Table 7 Concentration of DNA and RNA in tissues of control and MSG-supplemented pigs obtained at 4 h after feeding

Variables	Supplemental MSG in diet (%)				
	0	0.5	1	2	4
DNA (μg/mg tissue)					
Liver	5.34 ± 0.75	5.14 ± 0.55	5.13 ± 0.83	5.86 ± 0.19	6.25 ± 0.31
Jejunum	4.77 ± 1.0	4.96 ± 0.70	4.76 ± 0.66	4.88 ± 0.07	4.89 ± 0.39
Muscle	1.93 ± 0.12	1.91 ± 0.16	2.02 ± 0.24	1.91 ± 0.10	1.95 ± 0.17
RNA (μg/mg tissue)					
Liver	6.14 ± 0.23	6.24 ± 0.40	5.68 ± 0.40	5.88 ± 0.29	6.14 ± 0.16
Jejunum	6.83 ± 0.24	6.87 ± 0.30	6.42 ± 0.35	7.38 ± 0.26	7.39 ± 0.32
Muscle	4.07 ± 0.11	3.94 ± 0.07	3.85 ± 0.08	4.15 ± 0.14	4.32 ± 0.23

Blood samples were obtained from 42-day-old pigs (21 days after weaning) at 4 h after feeding. Values are mean ± SEM, $n = 6$/group

Effects of MSG on jejunal morphology, small-intestine weight and the incidence of diarrhea in pigs after weaning

Dietary supplementation with 0.5, 1, 2 and 4 % MSG to 21-day-old pigs for 7 days increased ($P < 0.05$) jejunal villus height, as well as jejunal concentrations of DNA, RNA, ATP and glutathione (the reduced form), while decreasing oxidative stress (as indicated by the ratio of oxidized glutathione to reduced glutathione) in a dose-dependent manner (Table 8). Crypt depth and lamina propria depth were higher ($P < 0.05$) in the jejunum of pigs supplemented with 4 % MSG compared with the control group. Concentrations of protein in the jejunal tissue did not differ among the five groups of pigs. At day 7 postweaning, the proportion of the small-intestine weight in the body of pigs supplemented with 0.5, 1, 2 and 4 % MSG was higher ($P < 0.05$) than that in the control group (Table 9). Dietary supplementation with MSG enhanced ($P < 0.05$) the absolute weight of the small intestine in a dose-dependent manner (Table 9). Combining the data from experiments 1 and 2 (a total of 40 pigs per treatment group), the incidence of diarrhea in piglets during the first week after weaning was 35, 20, 15, 7.5, and 7.5 % when the diets were supplemented with 0.0, 0.5, 1, 2, and 4 % MSG, respectively. Dietary supplementation with 0.5 to 2 % MSG dose-dependently reduced ($P < 0.05$) the incidence of diarrhea pigs during the first week after weaning. During the second and third weeks after weaning, the incidence of diarrhea was 6.5 and 4.5 %, respectively, for all pigs and the rates did not differ among the treatment groups.

Effects of MSG on histopathology of pig tissues

All pigs appeared active and healthy at day 21 after weaning. No abnormalities were observed in sections of heart, skin, spleen, kidney, testis, pancreas, bone marrow, brain, spinal cord, or lymph node (Table 10). All sections of liver among the five groups of pigs were similar with no variation. There was mild vacuolar change in all hepatocytes that was interpreted as diffuse hepatic lipidosis. All levels of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum, and large intestine) had infiltrations of lymphocytes, plasma cells, macrophages, and/or eosinophils within the lamina propria which would be expected in pigs on traditional diets. In all groups of pigs, there was variation in relative numbers of these cell types by gastrointestinal site, with most of the eosinophils observed in the jejunum and ileum. Many sections of cecum and large intestine had small numbers of crypt abscesses and large filamentous bacteria. These changes are not to be expected to have clinical significance.

Effects of NaCl on growth performance of pigs

Adding up to 0.686 % NaCl (0.272 % Na) to the basal diet had no effect ($P > 0.05$) on feed intake, weigh gains, or feed efficiency in postweaning pigs (Table 11). However, dietary supplementation with 1.372 % NaCl (0.544 % Na) modestly reduced ($P < 0.05$) feed intake, weigh gains, and feed efficiency of the animals. Dietary supplementation with 0.172–1.372 % NaCl did not affect the rates of incidence of diarrhea in pigs during the first, second or third week after weaning, which were 33, 6, and 4 %, respectively.

Discussion

Glutamate was traditionally classified as a nutritionally nonessential amino acid for pigs, because it was assumed, without much experimental evidence, that this nutrient could be adequately synthesized by the body relative to its needs (Bergen 1974). Thus, glutamate had received little attention from animal nutritionists, until Windmueller and Spaeth (1975 and 1980) discovered that large amounts of

Table 8 Jejunal morphology and jejunal concentrations of DNA, RNA, protein, ATP, and glutathione in 28-day-old pigs weaned at 21 days of age

MSG in diet (%)	Villus height (μm)	Crypt depth (μm)	Lamina propria (μm)	DNA (μg/mg tissue)	RNA (μg/mg tissue)	Protein (%)	ATP (μmol/g tissue)	GSH (μmol/g tissue)	GSSG (μmol/g tissue)	GSSG to GSH ratio
0	273 ± 4.8^{e}	236 ± 5.4^{b}	217 ± 4.2^{b}	4.80 ± 0.10^{e}	6.84 ± 0.16^{e}	12.8 ± 0.4	1.86 ± 0.03^{e}	1.94 ± 0.05^{e}	0.17 ± 0.01^{a}	0.089 ± 0.005^{a}
0.5	295 ± 4.2^{d}	239 ± 4.7^{a,b}	224 ± 5.0^{a,b}	5.18 ± 0.12^{d}	7.21 ± 0.20^{d}	12.8 ± 0.5	2.13 ± 0.05^{d}	2.06 ± 0.06^{d}	0.16 ± 0.01^{a,b}	0.076 ± 0.004^{b}
1	317 ± 4.4^{c}	240 ± 7.0^{a,b}	229 ± 6.5^{a,b}	5.43 ± 0.13^{c}	7.65 ± 0.22^{c}	13.0 ± 0.6	2.39 ± 0.06^{c}	2.34 ± 0.05^{c}	0.16 ± 0.01^{a,b}	0.065 ± 0.003^{c}
2	344 ± 5.2^{b}	251 ± 6.3^{a,b}	233 ± 5.8^{a,b}	5.92 ± 0.16^{b}	8.28 ± 0.28^{b}	13.2 ± 0.7	2.57 ± 0.07^{b}	2.69 ± 0.08^{b}	0.14 ± 0.01^{b,c}	0.053 ± 0.002^{d}
4	360 ± 6.3^{a}	255 ± 5.9^{a}	237 ± 5.6^{a}	6.48 ± 0.24^{a}	8.94 ± 0.26^{a}	13.1 ± 0.6	2.88 ± 0.09^{a}	3.02 ± 0.11^{a}	0.13 ± 0.01^{c}	0.041 ± 0.001^{e}

Jejunum was obtained from 28-day-old pigs which were weaned at 21 days of age and fed a corn- and soybean meal-based diet supplemented with 0, 0.5, 1, 2, or 4 % MSG. Values are mean ± SEM, $n = 20$/group

GSH glutathione (reduced form), *GSSG* glutathione (oxidized form)

$^{a–e}$ Within a column, means sharing different superscript letters differ ($P < 0.05$)

glutamate in the jejunal lumen were utilized by the mucosal cells of rats as a major respiratory fuel and precursor of some amino acids. The past 35 years have witnessed increasing interest in glutamate nutrition and metabolism in animals and the experimental results not only support the seminal observations with rats but also quantify the utilization of dietary glutamate by the pig small intestine (Bertolo and Burrin 2008; Wu et al. 1995; Wu 2010). Data on growth performance, intestinal morphology, as well as intestinal concentrations of glutathione, ATP, DNA, and RNA (Tables 1, 8, 9) support the view that glutamate is a conditionally essential amino acid for weanling pigs.

MSG contains 13.6 % sodium. Supplemental 0.5, 1, 2, and 4 % MSG provide 0.068, 0.136, 0.272, and 0.544 % sodium in diets, respectively. Results of this study indicate that dietary supplementation with up to 4 % MSG did not affect concentrations of sodium in plasma (Table 4), indicating a high capacity of pigs to excrete dietary Na. Feed intake by pigs that were fed a diet supplemented with 4 % MSG was modestly reduced compared with other groups (Table 2), likely due to an effect of increased Na intake (Table 11). Notably, supplementing 0.068, 0.136, and 0.272 % Na in the form of NaCl to the basal diet for 3 weeks did not affect growth performance of postweaning pigs (Table 11). Similarly, Mahan et al. (1999) reported that feed intake, weigh gain, or feed efficiency did not differ between two groups of postweaning pigs that were fed for 3 weeks a corn soybean meal and lactose-based diet (containing 0.20 % Na) supplemented with 0.4 or 0.6 % NaCl (i.e., 0.159 or 0.238 % Na). Thus, the observed effects of MSG supplementation on postweaning pigs were attributed to by the provision of glutamate rather than sodium in the diets.

Nearly all of the dietary glutamate (e.g., 97 % in young pigs) in a typical ration is utilized by the portal-drained viscera (primarily the small intestine) and only 3 % of dietary glutamate enters the portal vein (Burrin and Stoll 2009). Thus, as reported by Janeczko et al. (2007), the small intestine has such a high capacity for catabolizing glutamate that even oral administration of glutamate at four times its normal dietary intake results in only a transient, modest increase in circulating levels of glutamate (Table 3). Because there is little uptake of arterial glutamate by the small intestine of young pigs (Wu et al. 1994), enteral glutamate is the primary source of glutamate for the small intestine in the fed state. When piglets are nursed by sows, the milk provides large amounts of glutamate to meet the needs of the neonatal small intestine (Haynes et al. 2009; Wu et al. 2011). However, the transition from sow's milk to solid feed during weaning (in addition to other stressors) results in reduced food intake and the subsequent occurrence of intestinal atrophy (Li et al. 2009;

Table 9 The length and weight of the small intestine in 28-day-old pigs weaned at 21 days of age

Variable	Supplemental MSG in diet (%)				
	0	0.5	1	2	4
Length of SI (cm)	716 ± 14	722 ± 18	720 ± 15	718 ± 12	725 ± 16
Weight of SI (g)	226 ± 3^{e}	240 ± 4^{d}	253 ± 4^{c}	269 ± 5^{b}	287 ± 5^{a}
SI weight as BW (%)	31.8 ± 0.2^{e}	32.6 ± 0.3^{d}	33.8 ± 0.2^{c}	34.6 ± 0.3^{b}	36.1 ± 0.4^{a}

The small intestine (duodenum + jejunum + ileum) was obtained from 28-day-old pigs which were weaned at 21 days of age to a corn- and soybean meal-based diet supplemented with 0, 0.5, 1, 2, or 4 % MSG. Values are mean ± SEM, $n = 20$/group (4 pigs/pen)

SI small intestine

[a–e] Within a column, means sharing different superscript letters differ ($P < 0.05$)

Table 10 Histopathology examination of tissues from postweaning pigs receiving dietary supplementation with 0, 0.5, 1, 2, or 4 MSG for 21 days

Tissue	Supplemental MSG in diet (%)				
	0	0.5	1	2	4
Brain	NSL	NSL	NSL	NSL	NSL
Spinal cord	NSL	NSL	NSL	NSL	NSL
Bone marrow	NSL	NSL	NSL	NSL	NSL
Skin	NSL	NSL	NSL	NSL	NSL
Spleen	NSL	NSL	NSL	NSL	NSL
Liver	DMVC in all pigs	DMVC in all pigs	DMVC in all pigs	DMVC in all pigs	DMVC in all pigs
Kidneys	NSL	NSL	NSL	NSL	NSL
Lung	NSL	NSL	NSL	NSL	NSL
Heart	NSL	NSL	NSL	NSL	NSL
Pancreas	NSL	NSL	NSL	NSL	NSL
Stomach	DMLG in all pigs	DMLG in all pigs	DMLG in all pigs	DMLG in all pigs	DMLG in all pigs
	EG in 4 pigs	EG in 3 pigs	EG in 3 pigs	EG in 4 pigs	EG in 4 pigs
Duodenum	DMLED in all pigs	DMLED in all pigs	DMLED in all pigs	DMLED in all pigs	DMLED in all pigs
Jejunum	MJ in all pigs	MJ in all pigs	MJ in all pigs	MJ in all pigs	MJ in all pigs
Ileum	DMLEI in all pigs	DMLEI in all pigs	DMLEI in all pigs	DMLEI in all pigs	DMLEI in all pigs
Large intestine and cecum	DMLEC in all pigs	DMLEC in all pigs	DMLEC in all pigs	DMLEC in all pigs	DMLEC in all pigs
	Typhlitis in all pigs	Typhlitis in all pigs	Typhlitis in all pigs	Typhlitis in all pigs	Typhlitis in all pigs
	MSCA in 5 pigs	MSCA in 4 pigs	MSCA in 5 pigs	MSCA in 5 pigs	MSCA in 4 pigs
	LFB in 5 pigs	LFB in 5 pigs	LFB in 4 pigs	LFB in 4 pigs	LFB in 5 pigs
Testis or ovary	NSL	NSL	NSL	NSL	NSL
Lymph node	NSL	NSL	NSL	NSL	NSL

Six pigs were selected randomly from each treatment group for histopathology examinations

DMLED diffuse mild lymphoplasmacytic and eosinophilic duodenitis, *DMLG* diffuse mild lymphoplasmacytic and eosinophilic gastritis, *DMLEI* diffuse mild lymphoplasmacytic and eosinophilic ileitis, *DMLEC* diffuse mild lymphoplasmacytic and eosinphilic colitis, *DMVC* diffuse mild vacuolar change (lipidosis), *EG* eosinophilic gastritis, *LFB* large filamentous bacteria, *MJ* mild jejunitis, *MSCA* multiple small crypt abscesses, *NSL* no significant lesions observed

Wu et al. 1996). The latter is primarily a consequence of high rates of mucosal cell turnover and apoptosis in the young piglet that did not receive sufficient nutrients to meet the requirement for mucosal protein synthesis and growth, thereby impairing the digestion, absorption and utilization of nutrients by the small intestine (Plusk et al. 1997; Lalles et al. 2007; Ou et al. 2007). Based on the work of Klein and McKenzie (1983), the time required for the complete replacement of the intestinal mucosa in young pigs is 7–10 days in 1- to 7-day-old piglets, and approximately 4 days in 21- to 28-day-old piglets. In early-weaned pigs, the general maintenance, structure, and function of the small intestine are impaired immediately within the first days after weaning. Under these conditions, the intestinal

Table 11 Effects of dietary NaCl supplementation on growth performance in postweaning pigs

Variable	Amount of NaCl supplemented to the basal diet (%)				
	0.0 (0.00 % Na)	0.172 (0.068 % Na)	0.343 (0.136 % Na)	0.686 (0.272 % Na)	1.372 (0.544 % Na)
Body weight (kg) of pigs					
Day 0	5.62 ± 0.11	5.70 ± 0.10	5.66 ± 0.13	5.73 ± 0.12	5.68 ± 0.10
Day 21	12.5 ± 0.39[a]	12.8 ± 0.33[a]	12.5 ± 0.35[a]	12.6 ± 0.36[a]	11.8 ± 0.34[b]
Average daily gain (g/day)					
Days 0–21	329 ± 9[a]	337 ± 8[a]	326 ± 10[a]	327 ± 11[a]	292 ± 10[b]
Feed intake (g/kg body weight per day)					
Days 0–21	40.8 ± 2.0[a]	41.0 ± 1.8[a]	40.6 ± 1.6[a]	40.5 ± 1.9[a]	37.3 ± 1.5[b]
Gain:feed ratio (g/g)					
Days 0–21	0.824 ± 0.01[a]	0.832 ± 0.02[a]	0.823 ± 0.02[a]	0.828 ± 0.01[a]	0.801 ± 0.01[b]

Pigs were weaned at 21 days of age (the day of weaning = Day 0). Values are mean ± SEM, $n = 5$ pens/group with 4 piglets per pen

[a–b] Within a row, means sharing different superscript letters differ ($P < 0.05$)

mucosa cells undergo a rapid, progressive autolysis and shedding within a matter of 1 day, followed by a high rate of diarrhea within 3–10 days after weaning (Plusk et al. 1997). Therefore, reduced consumption of feed during the post-weaning period limits the dietary intake of glutamate and the precursors for glutamate synthesis in the neonates (Zimmerman 1975). This warrants supplementation of glutamate to the diet for weanling piglets to maintain the normal physiological activity of the small intestine (Liu et al. 2002). Likewise, glutamate can improve absorptive function of the gut, thereby preventing diarrhea in weanling pigs (this study) and in rats receiving intragastric feeding (Somekawa et al. 2012).

Unlike glutamine, glutamate is not a substrate for the synthesis of aminosugars, purines, or pyrimidines (Wu et al. 2011). However, glutamate can reduce glutaminase activity, therefore sparing glutamine for use through multiple metabolic pathways (Curthoys and Watford 1995). Thus, dietary supplementation with glutamate may enhance the availability of dietary glutamine in plasma (Table 3). As a versatile amino acid, glutamate participates in both synthetic and oxidative pathways in the small intestine (Blachier et al. 2009; Burrin and Stoll 2009), resulting in the production of proteins, ornithine, citrulline, proline, arginine, alanine, aspartate, glutathione, CO_2, and ATP (Wu 1998). Therefore, dietary supplementation with glutamate increased the plasma concentrations of these amino acids (Table 3) and jejunal concentrations of glutathione (Table 8) in weaned pigs. Compelling evidence shows that dietary glutamate is a major energy substrate for the small intestine, which is an organ with a particularly high metabolic rate (Bertolo and Burrin 2008). In support of this notion, we found that dietary MSG supplementation increased jejunal concentrations of ATP in weaned pigs (Table 8). Additionally, glutamate is an excitatory neurotransmitter, thereby regulating the motility of the gastrointestinal tract (Kirchgessner 2001). Thus, when a weaning diet is deficient in glutamate, gut atrophy occurs (Liu et al. 2002) and the efficiency of utilization of dietary protein for growth and other physiological functions is greatly decreased (Horvath et al. 1994).

An interesting observation from this study is that dietary supplementation with MSG beneficially increased the concentrations of several amino acids that cannot be synthesized in the body. These amino acids included histidine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine, which are extensively catabolized by bacteria in the lumen of the pig small intestine (Dai et al. 2010, 2011, 2012a, b, c). Additionally, branched-chain amino acids are actively transaminated by enterocytes of the pig small intestine (Chen et al. 2007, 2009). Thus, 30–50 % of nutritionally essential amino acids in the diet are degraded by the pig small intestine in the first pass (Burrin and Stoll 2009), leading to a reduced efficiency of utilization of dietary protein. It is possible that MSG reduces the catabolism of these amino acids in the small intestine, thereby enhancing their entry into the portal circulation. This may explain the recent finding that oral administration of MSG to adult humans increased the concentrations of leucine, isoleucine, valine, lysine, cysteine, alanine, tyrosine, and tryptophan in plasma (Boutry et al. 2011). Increases in the circulating levels of methionine and cysteine may promote hepatic synthesis of taurine (an antioxidant) in MSG-supplemented pigs, leading to elevated concentrations of taurine in plasma. An overall improvement in amino acid nutrition contributes to an increase in the synthesis and concentrations of blood hemoglobin in weaned pigs (Table 6).

Although administration of MSG directly into the brain of animals may result in local lesions, growing evidence

shows that dietary supplementation with appropriate dosages of MSG is generally safe in humans and other animals (Brosnan and Brosnan 2012; Boutry et al. 2011). This is because almost all glutamate in the diet is catabolized by the small intestine and, therefore, does not enter the portal circulation (Burrin and Stoll 2009). Results of the present study unequivocally indicate normal hematology in MSG-supplemented pigs, including (1) the numbers of red blood cells, white blood cells, and platelets; (2) concentrations of electrolytes, metabolites, proteins, and enzymes; and (3) hematocrit and hemoglobin (Table 6). Likewise, dietary supplementation with up to 4 % MSG did not affect the structure or appearance of internal organs in postweaning pigs (Table 10). Interestingly, pigs in the control and MSG groups exhibited diffuse mild lymphoplasmacytic and eosinophilic gastritis; diffuse mild lymphoplasmacytic eosinophilic duodenitis; mild jejunitis, ileitis, colitis; diffuse mild vacuolar change in liver (lipidosis); large filamentous bacteria in the large intestine; and multiple small crypt abscesses. These changes occurred in all groups of pigs to the similar extent and, thus, should not necessarily be considered pathologic alterations. Rather, the corn and soybean meal-based diet may play a major role in affecting the development of the liver and gastrointestinal tract in postweaning pigs.

In conclusion, supplementing up to 4 % MSG to the diet for 3 weeks did not have any adverse effect on postweaning piglets. Our results also indicate a promising effect of MSG on reducing the incidence of diarrhea during the first week after weaning, increasing intestinal absorptive capacity, and enhancing weight gain and feed efficiency in these animals. Glutamate is a nutritionally indispensable amino acid for weanling pigs to maintain the small-intestinal structure and function as well as maximize their growth performance.

Acknowledgments This research was supported by a grant from the International Glutamate Technical Committee (Brussels, Belgium). We thank our graduate students and technicians for assistance in this work.

Conflict of interest The authors declare that they have no conflict of interest.

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