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ORIGINAL ARTICLE

# Oral administration of MSG increases expression of glutamate receptors and transporters in the gastrointestinal tract of young piglets

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**Abstract** Glutamate receptors and transporters, including T1R1 and T1R3 (taste receptor 1, subtypes 1 and 3), mGluRs (metabotropic glutamate receptors), EAAC-1 (excitatory amino acid carrier-1), GLAST-1 (glutamate-aspartate transporter-1), and GLT-1 (glutamate transporter-1), are expressed in the gastrointestinal tract. This study determined effects of oral administration of monosodium glutamate [MSG; 0, 0.06, 0.5, or 1 g/kg body weight (BW)/day] for 21 days on expression of glutamate receptors and transporters in the stomach and jejunum of sow-reared piglets. Both mRNA and protein levels for gastric T1R1, T1R3, mGluR1, mGluR4, EAAT1, EAAT2, EAAT3, and EAAT4 and mRNA levels for jejunal T1R1, T1R3, EAAT1, EAAT2, EAAT3 and EAAT4 were increased ($P < 0.05$) by MSG supplementation. Among all groups, mRNA levels for gastric EAAT1, EAAT2, EAAT3, and EAAT4 were highest ($P < 0.05$) in piglets receiving 1 g MSG/kg BW/day. EAAT1 and EAAT2 mRNA levels in the stomach and jejunum of piglets receiving 0.5 g MSG/kg BW/day, as well as jejunal EAAT3 and EAAT4 mRNA levels in piglets receiving 1 g MSG/kg BW/day, were higher ($P < 0.05$) than those in the control and in piglets receiving 0.06 g MSG/kg BW/day. Furthermore, protein levels for jejunal T1R1 and EAAT3 were higher ($P < 0.05$) in piglets receiving 1 g MSG/kg BW/day than those in the control and in piglets receiving 0.06 g MSG/kg BW/day. Collectively, these findings indicate that dietary MSG may beneficially stimulate glutamate signaling and sensing in the stomach and jejunum of young pigs, as well as their gastrointestinal function.

**Keywords** Monosodium glutamate · Receptors · Transporters · T1R · mGluR · EAAT

**Abbreviations**

| | |
|---|---|
| AA | Amino acid |
| EAAC-1 | Excitatory amino acid carrier 1 |
| GLAST | Glutamate-aspartate transporter |
| GLT-1 | Glutamate transporter 1 |
| mGluRs | Metabotropic glutamate receptors |
| MSG | Monosodium glutamate |
| RT-PCR | Real-time polymerase chain reaction |
| SDS | Sodium dodecyl sulfate |
| T1R1 | Taste receptor 1, subtype 1 |
| T1R3 | Taste receptor 1, subtype 3 |
| TTBS | Tris-Tween buffered saline |

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## Introduction

Glutamate, a major amino acid in animal and plant proteins (Li et al. 2011) as well as in the milk (Haynes et al. 2009; Wu and Knabe 1994), is extensively degraded in the small intestine of mammals, including piglets (Wu 2009, 2010a; Wu et al. 2007, 2013a). Emerging evidence also shows that a small amount of dietary glutamate is metabolized in the stomach (Torii et al. 2013). T1R1 and T1R3 (taste receptor 1, subtypes 1 and 3) are expressed in epithelial cells of the gastrointestinal tract (Bezenc et al. 2007; Kawai et al. 2012). In addition, mGluR-1 (metabotropic glutamate receptor-1) is highly expressed in the rat gastric fundus (San Gabriel et al. 2007). Glutamate transporters, including EAAC-1 (excitatory amino acid carrier 1), GLAST-1 (glutamate-aspartate transporter-1), GLT-1 (glutamate transporter 1), VGLUT1 (vesicular transporter-1), and VGLUT2 (vesicular transporter-2) are present in the stomach (Aoyama and Nakaki 2013; Falalyeyeva and Beregova 2007). Glutamate transport by the enterocyte's apical membrane takes place mainly via the high-affinity $X_{AG}^{-}$ system and to a lesser extent by the low-affinity $B^{0}$ system, with the $X_{AG}^{-}$ system transporting both glutamate and aspartate (Had-Aissouni 2012; Gras et al. 2012; Lewerenz et al. 2012; Wu 2013b).

The molecular identities of the four proteins possessing an $X_{AG}^{-}$ system activity have been described in various tissues, including GLAST-1, GLT-1, EAAC-1, EAAC-4, and EAAC-5 (Torii et al. 2013). Studies with pigs and rodent species show that EAAC-1 is the most abundant glutamate transporter in the small intestine and is expressed on the apical, brush border membrane throughout the gut (Fan et al. 2004). At present, little is known about effects of monosodium glutamate (MSG) on expression of glutamate receptors and transports in the gastrointestinal tract. The main goal of this study was to determine mRNA and protein levels for T1R1, T1R3, mGluR1, mGluR4, EAAT1, EAAT2, EAAT3, and EAAT4 in the stomach and jejunum of sow-reared piglets receiving oral administration of 0, 0.06, 0.5, or 1 g MSG/kg body weight/day.

## Materials and methods

MSG was purchased from Henan Lianhua Gourmet Powder Co., Ltd (Henan, China). Its purity was 99.9 %. Unless indicated, all chemicals were obtained from Sigma (St. Louis, MO, USA).

### Animals and diets

Twenty-four Duroc × Large White × Landrace newborn piglets with an average body weight of 1.55 ± 0.20 kg were assigned randomly to one of four treatments. There was one sow with her offpsring per pen in an environmentally controlled facility. Lactating sows had free access to a corn- and soybean meal-based diet (Wu et al. 2011a) and drinking water. The piglets were nursed by their mothers and received oral administration of 0 (control), 0.06 (low dose), 0.5 (intermediate dose), or 1 g (high dose) MSG/kg body weight/day twice daily after birth until 21 days of age. There were six piglets per treatment group. On each day, the first and second MSG administration were given between 08:00 and 09:00 and between 18:00 and 19:00, respectively, after piglets were nursed by their mothers. The MSG was dissolved in 2 ml water per kg of body weight before gavage. Piglets in the 0, 0.06, and 0.5 g MSG/kg body weight/day groups received the same amount of sodium in the form of NaCl as piglets in the 1 g MSG/kg body weight/day group, as previously described (Rezaei et al. 2013). The study was conducted in accordance with the Chinese guidelines for animal welfare and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, The Chinese Academy of Sciences (Yin et al. 2010b).

### Tissue collection

At 21 days of age, 24 piglets were sacrificed for tissue collection. Approximately 5 g each of the jejunum and the stomach was collected, their contents were removed, and their mucosal surface was cleaned with sapline. Thereafter, the tissues were immediately frozen in liquid nitrogen, and stored at −80 °C until mRNA analysis (Liu et al. 2012).

### RNA extraction and cDNA synthesis

Total RNA was extracted from the jejunal and stomach tissues using Trizol Reagents according to the instructions from Invitrogen Corporation (He et al. 2013). The quantity of the RNA obtained was checked by measuring optical density at 260 and 280 nm (Geng et al. 2011). Before reverse transcription (RT), all RNA samples were treated with Dnase I enzyme (amplification grade) to remove any residual DNA according to the manufacturer's instructions (Invitrogen). Each RNA (1 μg) sample was combined with 1 μl of 10× reaction buffer, 1 μL of DNase I (1 U/μL), and diethyl pyrocarbonate-treated $H_2O$ up to 10 μL, and incubated at 37 °C for 30 min. Next, 1 μl of 50 mM EDTA was added to stop the reaction by incubation at 65 °C for 10 min. Subsequently, the DNase-treated RNA samples were reverse-transcribed to cDNA in accordance with the manufacturer's instructions (TakaRa Biotechnology, Dalian Co., Ltd, China). The reaction mixture, which included 5× PrimeScript® Buffer (4 μl), PrimeScript® RT Enzyme Mix 1 (1 μl), RT Primer Mix (1 μl), and diethyl

pyrocarbonate-treated $H_2O$ up to 20 μL, was incubated at 37 °C for 15 min and at 85 °C for 5 s.

### Quantification of mRNA levels for AA and peptide transporters

Components of a 10 μL real-time PCR mixture included 5 μl SYBRR Premix Ex TaqTM (2×), 0.4 μL PCR Forward Primer (10 μM), 0.4 μL PCR Reverse Primer (10 μM), 0.2 μL ROX Reference Dye (50×), 1 μL template DNA, and nuclease-free water up to 10 μl. The PCR conditions used for amplification and quantification included an initial denaturing stage (95 °C for 30 s), followed by the PCR (95 °C for 5 s, 60 °C for 34 s), fusion (95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s), and continuous fluorescence measurements. The primers are shown in Table 1. The amplification of GAPDH was used for each sample to normalize the expression of selected genes. The relative expression ratios of mRNA were calculated as previously described (Bustin et al. 2009; Liu et al. 2012; Yin et al. 2010a).

### Quantification of protein amounts of AA and peptide transporters

The frozen samples were powdered under liquid nitrogen, and lysed in an assay buffer (150 mM NaCl, 1 % Triton X-100, 0.5 % sodium deoxycholate, 0.1 % SDS, 50 mM Tris-HCl at pH 7.4, and a protease inhibitor cocktail) purchased from Roche (Shanghai, China). After centrifugation at 10,000×*g* and 4 °C for 10 min, protein concentration in the supernatant fluid was determined using the Bicinchoninic Acid assay (Beyotime Biotechnology, China). All samples were adjusted to an equal protein concentration and then diluted with the 2× loading buffer [0.63 ml of 0.5 M Tris-HCl (pH 6.8), 0.42 ml 75 % glycerol, 0.125 g sodium dodecyl sulfate (SDS), 0.25 ml β-mercaptoethanol, 0.2 ml 0.05 % solution of bromphenol blue, and 1 ml water] to a final volume of 2.5 ml and heated in boiling water for 5 min. The solution was cooled on ice before use for Western blot analysis as previously described (Fu et al. 2013).

The denatured proteins were separated using SDS-PAGE (10 % gradient gel), transferred to PVDF membranes (Millipore, Billerica, MA, USA) overnight at 12 V using the Bio-Rad Transblot apparatus (Hercules, CA, USA). The membranes were blocked in 5 % fat-free milk in Tris-Tween buffered saline (TTBS: 20 mM Tris/150 mM NaCl, pH 7.5, and 0.1 % Tween-20) for 3 h and then incubated with an antibody for T1R1, mGluR1, EAAT3 or β-actin (Table 2) at 4 °C overnight with gentle rocking. After washing three times with TTBS, the membranes were incubated at room temperature for 2 h with horseradish peroxidase-linked secondary antibodies. Finally, the membranes were washed with TTBS, followed by development using Supersignal West Dura Extended Duration Substrate according to the manufacturer's instructions (Pierce, Rockford, IL, USA). The images were detected on chemiluminescence (Applygen Technologies Inc., Beijing, China). Multiple exposures of each Western blot were performed to ensure linearity of chemiluminescence signals. Western blots were quantified by measuring the intensity of correctly sized bands using AlphaImager 2200 (Alpha Innotech Corporation, CA, USA) software (Yang et al. 2013).

**Table 2** Antibodies used for Western blot analyses

| Anti-protein | Company | Catalog number | Dilution |
|---|---|---|---|
| Primary antibodies | | | |
| EAAT3 | Santa Cruz, CA, USA | sc-25658 | 1:200 |
| mGluR-1a/b | Santa Cruz, CA, USA | sc-47131 | 1:200 |
| T1R1 | Santa Cruz, CA, USA | sc-50307 | 1:200 |
| β-Actin | Santa Cruz, CA, USA | 600008-1 | 1:4000 |
| Secondary antibodies | | | |
| Goat IgG | Proteintech, USA | Sc-2020 | 1:5000 |
| Rabbit IgG | Proteintech, USA | Cw0103 | 1:3000 |

**Table 1** Primers used for real-time PCR analyzing

| Primer | Forward | Reverse |
|---|---|---|
| T1R1 | TCCCTGGGCTTCATACTGG | TTCTCTGGCAAGTCCTTACCC |
| T1R3 | AGCTGCAGCAGTCTAAAATGT ACTGGCCAGGCAACCA | GGTGCCAGTCTCCCAGTGTTCC CGCCAGTGC |
| mGluR1 | CATGCCCATTCTTTCCTACCC | TTTCTTTCTTCGGAAAATGTTG |
| mGluR4 | CCCAGAATGAGAAGAGTACC | TCTGCGAAGGTCGTCATGGT |
| POMC | GGAAAGTAACTTGCTGGCG | CCGTTGGGATACACCTTCAC |
| EAAT1 | GATGGGACCGCCCTCTAT | CGTGGCTGTGATGCTGATG |
| EAAT2 | GGCTGCTGGACAGGATGA | TAAATGGACTGGGTCTTGGT |
| EAAT3 | GGCACCGCACTCTACGAAGCA | GCCCACGGCACTTAGCACGA |
| EAAT4 | TAACCAGGACCATTGTGAGG | CATTGATGCCATTAGCCG |
| β-Actin | TCTGGCACCACACCTTCTACA | ATCTGGGTCATCTTCTCACGG |

The real-time PCR primers are designed by the author with Primer Premier 5.0

### Statistics

Data are expressed as mean ± standard deviation (SD). Data were subjected to one-way analysis of variance using the General Linear Model procedures of SAS (SAS 9.1, SAS Institute, Cary, USA). Log transformation of variables was performed when variance of data was not homogenous among treatment groups, as assessed using the Levene's test (Wei et al. 2012). Differences among treatment means were determined by the Tukey multiple comparison test (Fu et al. 2010). Differences were considered significant at $P < 0.05$.

## Results

### Overall observations

The body weights of piglets at 0, 7, 14, and 21 days of age are shown in Table 3. Compared with the control group, dietary supplementation with 0.5 and 1 g MSG/kg body weight/day did not affect ($P > 0.05$) the weight gains of piglets during the 21-day period. Oral administration of 0.5 g MSG/kg body weight/day over a 3-week period increased ($P < 0.05$) the average day weight gain of piglets than that for the 1 g MSG/kg body weight/day group. No adverse effects of oral MSG administration on any piglet were observed during the entire experimental period.

### mRNA levels for AA transporters in the stomach

The mRNA levels for T1R1, T1R3, mGluR1, mGluR4, EAAT1, EAAT2, EAAT3 and EAAT4 in the stomach were increased ($P < 0.05$) by dietary MSG supplementation (Fig. 1). Gastric T1R1 mRNA levels in the High-MSG group and intermediate-MSG group were higher ($P < 0.05$) than those in the other groups. Compared with the control group, mRNA levels for gastric T1R3, mGluR1, mGluR4, EAAT1, EAAT2, EAAT3 and EAAT4 in the Low-MSG,

**Table 3** Effects of oral MSG administration on growth performance in sucking pigs

| Item | CN | LMG | MMG | HMG | *P* value |
|---|---|---|---|---|---|
| BW on Day 0, kg | 1.55 ± 0.20 | 1.56 ± 0.18 | 1.54 ± 0.16 | 1.57 ± 0.21 | 0.990 |
| BW on Day 7, kg | 2.62 ± 0.33 | 2.73 ± 0.40 | 2.51 ± 0.48 | 2.67 ± 0.43 | 0.627 |
| BW on Day 14, kg | 4.55 ± 0.47 | 4.78 ± 0.42 | 4.91 ± 0.44 | 4.62 ± 0.20 | 0.393 |
| BW on Day 21, kg | 6.57 ± 0.61 | 6.80 ± 0.64 | 7.05 ± 0.34 | 6.18 ± 0.53 | 0.067 |
| ADG between Days 21 and 0, g/day | 244 ± 26$^{ab}$ | 252 ± 27$^{ab}$ | 262 ± 17$^{a}$ | 221 ± 24$^{b}$ | 0.046 |

Values are mean ± SD, $n = 6$

Different letters within a line significantly differ from each other ($P < 0.05$)

*ADG* average daily gain of body weight, *BW* body weight, *CN* control, LMG = 0.06 g MSG/kg BW/day; MMG = 0.5 g MSG/kg BW/day; HMG = 1 g MSG/kg BW/day

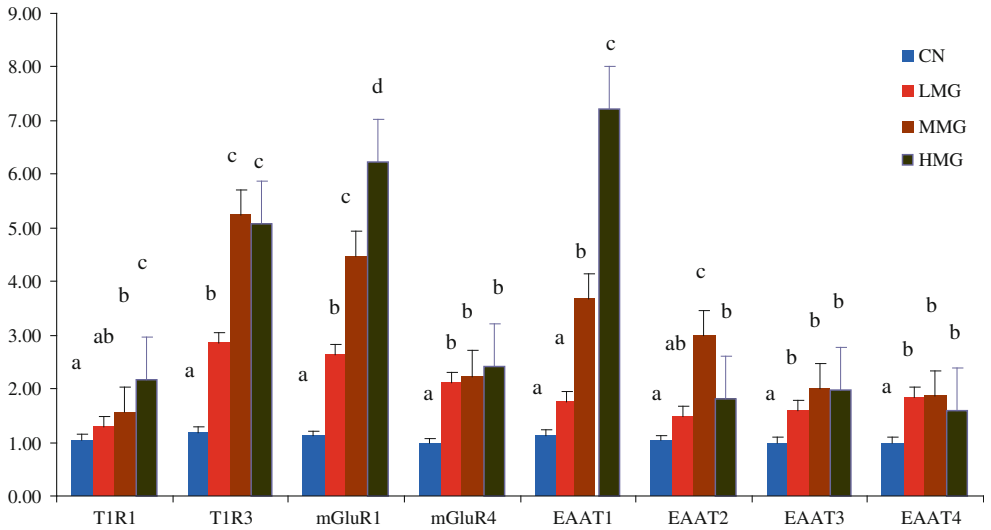


**Fig. 1** Effects of dietary supplementation with MSG on mRNA levels for AA and peptide transporters in the stomach of sow-reared piglets. mRNA abundances of *T1R1*, *T1R3*, *mGluR1*, *mGluR4*, *EAAT1*, *EAAT2*, *EAAT3*, *EAAT4* were normalized using *GAPDH* as an internal control. Data are expressed as mean ± SEM, $n = 6$. *a–c* Within a variable, values with different superscripts differ ($P < 0.05$). *CN* control; LMG = 0.06 g MSG/kg BW/day; MMG = 0.5 g MSG/kg BW/day; HMG = 1 g MSG/kg BW/day

intermediate-MSG, and High-MSG groups were elevated ($P < 0.05$). The mRNA levels for EAAT1, EAAT2, EAAT3, and EAAT4 in the stomach of piglets in the High-MSG group were higher ($P < 0.05$) than those in the other groups. Gastric EAAT1 and EAAT2 mRNA levels in the intermediate-MSG group were higher ($P < 0.05$) than those in the control and the low-MSG group.

mRNA levels for AA transporters in the jejunum

The T1R1 and T1R3 mRNA levels in the jejunum were increased ($P < 0.05$) by dietary MSG supplementation (Fig. 2). Supplementation with MSG had no effect on intestinal mGluR1 mRNA abundance. Jejunal EAAT1, EAAT2, EAAT3 and EAAT4 mRNA levels in the low-MSG, intermediate-MSG and high-MSG groups were higher ($P < 0.05$) than those in the control group. Abundances of EAAT1 and EAAT2 mRNA in the jejunum of piglets in the intermediate-MSG and high-MSG groups were higher ($P < 0.05$) than those in the control and low-MSG group. Similar results were obtained for jejunal EAAT3 and EAAT4 abundance.

Protein levels for AA transporters in the stomach

Abundances of proteins for T1R1, mGluR1 and EAAT3 in the stomach were higher ($P < 0.05$) in the MSG-supplemented piglets compared with the control group (Fig. 3). The values were the highest ($P < 0.05$) in the high-MSG group among all piglets.

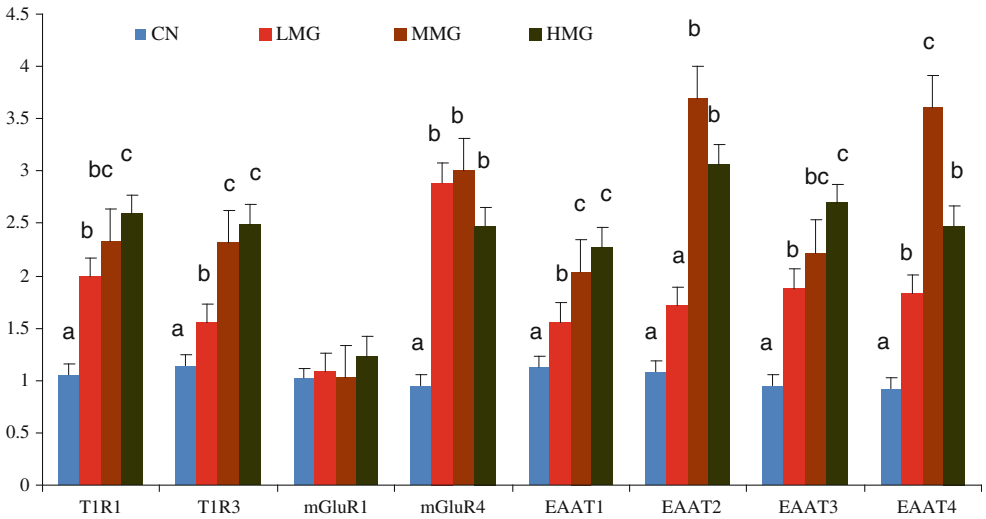


**Fig. 2** Effects of dietary supplementation with MSG on mRNA levels for AA and peptide transporters in the jejunum of sow-reared piglets. The mRNA expression abundances of *T1R1*, *T1R3*, *mGluR1*, *mGluR4*, *EAAT1*, *EAAT2*, *EAAT3*, *EAAT4* were normalized using *GAPDH* as an internal control. Data are expressed as mean ± SEM, $n = 6$. *a–c* Within a variable, values with different superscripts differ ($P < 0.05$). *CN* control; LMG = 0.06 g MSG/kg BW/day; MMG = 0.5 g MSG/kg BW/day; HMG = 1 g MSG/kg BW/day

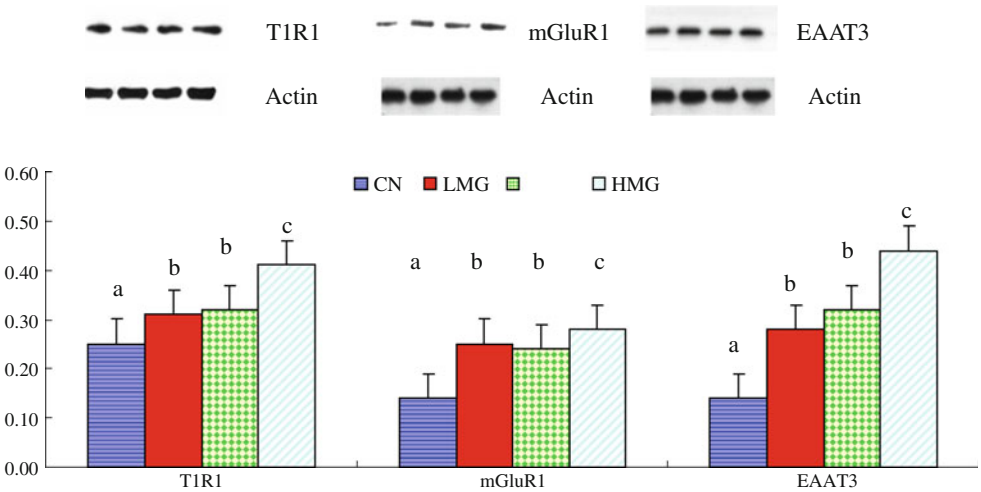


**Fig. 3** Effects of dietary supplementation with MSG on the protein abundance of AA and peptide transporters in the stomach of sow-reared piglets. The protein abundances of *T1R1*, *mGluR1 and EAAT3* were normalized using β-actin as an internal control. Data are expressed as mean ± SEM, $n = 6$. *a–c* Within a variable, values with different superscripts differ ($P < 0.05$). *CN* control; LMG = 0.06 g MSG/kg BW/day; MMG = 0.5 g MSG/kg BW/day; HMG = 1 g MSG/kg BW/day

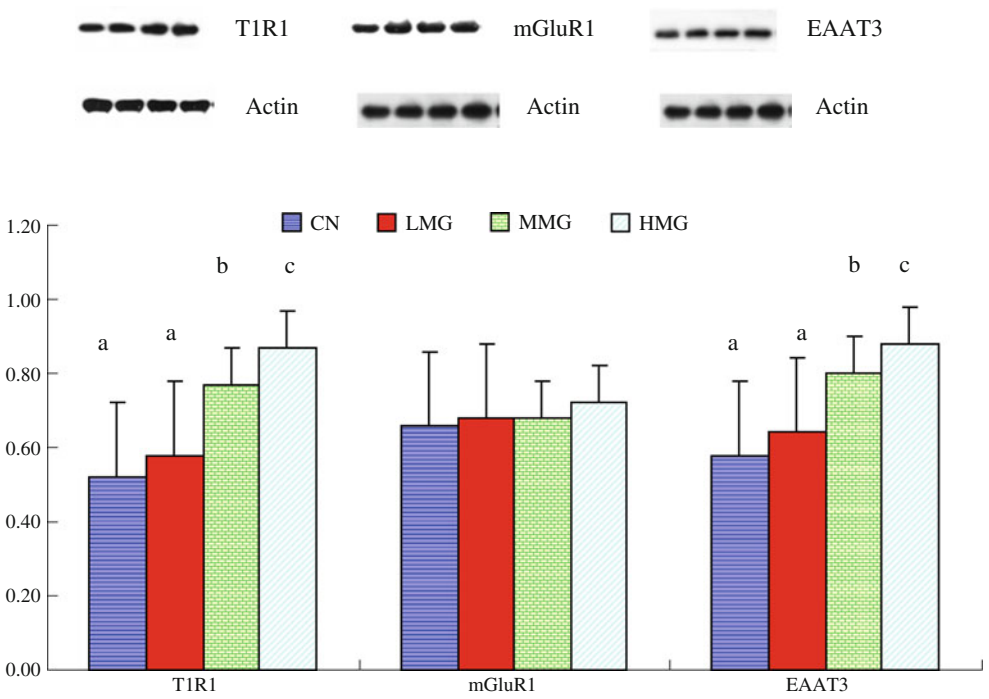


**Fig. 4** Effects of dietary supplementation with MSG on the protein abundance of AA and peptide transporters in the jejunum of sow-reared piglets. The protein abundances of *T1R1*, *mGluR1 and EAAT3* were normalized using β-actin as an internal control. Data are expressed as mean ± SEM, $n = 6$. *a–c* Within a variable, values with different superscripts differ ($P < 0.05$). *CN* control; LMG = 0.06 g MSG/kg BW/day; MMG = 0.5 g MSG/kg BW/day; HMG = 1 g MSG/kg BW/day

Protein levels for AA transporters in the jejunum

The amounts of jejunal T1R1 and EAAT3 proteins were higher ($P < 0.05$) in the High-MSG group compared with the control and the low-MSG group (Fig. 4). Dietary supplementation with MSG had no effect ($P < 0.05$) on the abundance of mGluR1 protein in the jejunum.

## Discussion

While glutamate was traditionally considered as a nutritionally nonessential amino acid for humans and other animals (see Wu 2010b for review), this amino acid plays an important role in physiology (Brosnan and Brosnan 2012; Parpura and Verkhratsky 2013; Yao et al. 2012), nutrient metabolism (Wu et al. 2011a, b), anti-oxidative responses (Aoyama et al. 2012; Had-Aissouni 2012), and immunity (Gupta et al. 2013). In addition, glutamate is a major energy substrate for the small intestine and an excitatory neurotransmitter (Burrin and Stoll 2009; Wu 1998). This AA activates taste receptors in the digestive tract (Kawai et al. 2012; San Gabriel and Uneyama 2012), enhances diet-induced thermogenesis in brown adipose tissue of young adult rats (Smriga et al. 2000), and regulates the release of certain hormones [e.g., norepinephrine (Smriga and Torii 2000) and glucagon-like peptide-1 (Iwatsuki and Torii 2012)] in animals. Interestingly, glutamate reduces white-fat deposition in adult rats (Kondoh and Torii 2008) and growing pigs (Rezaei et al. 2013). Thus, there is growing interest in glutamate nutrition in swine (Tan et al. 2012; Wu 2010a), humans (Shi et al. 2012), and other mammals (Boutry et al. 2012; Brosnan and Brosnan 2012; Wu 2013a, b).

The stomach has the capacity for the transport of glutamate into the blood circulation and also is a site for glutamate sensing and glutamate-mediated regulation of digestive function (Burrin and Stoll 2009). Humans have an ability to detect at least five basic taste qualities: sweet, umami, bitter, salty, and sour (Iwatsuki and Torii 2012). Receptors for umami taste and sweet taste are closely related to each other. The three subunits of the T1R family form two heteromeric receptors: umami (T1R1/T1R3) (Li et al. 2002; Nelson et al. 2002) and sweet (T1R2/T1R3) (Li et al. 2002; Nelson et al. 2001). Currently, two L-glutamate receptors have been identified in the cells lining the gut: metabotropic L-glutamate receptor type 1 is located in the chief cells (pepsinogen-secreting cells) of the stomach (San Gabriel et al. 2007), and a heterodimer L-glutamate taste receptor, T1R1 + T1R3 (taste receptor 1, subtypes 1 and 3), is found in epithelial cells of the stomach, small intestine, and colon (Bezenc et al. 2007).

Results of our study indicate that expression of T1R1 and T1R3 as well as mGluR1 and mGluR4 in the stomach

and the jejunum was increased by dietary MSG supplementation. Thus, it is possible that glutamate receptors in the gastrointestinal tract may detect ingested glutamate, transmitting this information to adjacent cells and neurons. Some receptors, such as *N*-methyl-D-aspartate (NMDA) and mGluR1 to mGluR8, are present in the stomach and regulate gastrointestinal function (San Gabriel et al. 2007; Torii et al. 2013). In support of this view, there is evidence that dietary supplementation with 1 % L-glutamate increased pepsinogen C expression in the stomach mucosa and gastric secretions (Khropycheva et al. 2011; Zolotarev et al. 2009). Furthermore, Nakamura et al. (2010) reported that mGluR1 expression in gastric chief cells was much lower than that in parietal cells and the entire gastric mucosa. In contrast, the endocrine cells, possibly D cells, specifically expressed multiple metabotropic glutamate receptors such as mGluR2, 3, 4, and 7, although the relative level of expression was low (Nakamura et al. 2010). Taken together, these data suggest that mGluR1 is involved in the gastric phase control of protein digestion.

Glutamate transporters (EAAC-1, GLAST and GLT-1) and vesicular transporters (VGLUT1 and VGLUT2) are expressed in the stomach (Falalyeyeva and Beregova 2007). Burrin and Stoll (2009) compared the metabolic fate of dietary [$^{13}$C]-glutamate in young pigs when administered the same control diet and supplemental glutamate intakes by the intragastric and intraduodenal feeding route. They reported that the fractional rate of gastrointestinal glutamate absorption in pigs when given intragastrically was higher than that for intraduodenal feeding. This suggests glutamate transport by the stomach mucosal cells. Consistent with these data, we found that dietary supplementation with MSG increased the expression of EAAT1, EAAT2, EAAT3 and EAAT4 in the piglet stomach. At present, the metabolic fate of glutamate in the gastric mucosa is unknown. However, we surmise that dietary glutamate upregulates expression of key genes for glutamate transport and sensing in the stomach.

The first step for the utilization of glutamate by the small intestine is its transport from the lumen into enterocytes, which involves the $Na^+$-dependent high-affinity $X^-_{AG}$ system and/or the low-affinity $B^0$ system (Conrad and Sato 2012; Wu et al. 2013b). The $X^-_{AG}$ system transports both glutamate and aspartate. The molecular identities of the four proteins in the $X^-_{AG}$ system have been described in various tissues, including GLAST-1, GLT-1, EAAC-1, and excitatory amino acid transporters 4 and 5 (Beart and O'Shea 2007; Fan et al. 2004; Iwanaga et al. 2005; Kanai and Hediger 2003). Studies with pigs, rats and mice have demonstrated that EAAC-1 is the most abundant glutamate transporter in the intestine and is expressed on the apical, brush border membrane throughout the small intestine (Fan et al. 2004). In our work, we found that the expression of EAAT1, EAAT2, EAAT3 and EAAT4 in the jejunum was enhanced by dietary MSG supplementation. Whether glutamate directly or indirectly regulates gene expression in intestinal cells is unknown. Nonetheless, increased abundance of glutamate transporters in enterocytes aid in maximizing the absorption of dietary glutamate for utilization, thereby reducing its entry into the large intestine.

In conclusion, dietary supplementation with MSG increases expression of glutamate signaling receptors and glutamate transporters, including T1R1, T1R3, mGluR1, EAAT1, EAAT2, EAAT3, and EAAT4, in the stomach and small intestine of neonatal pigs. Taken together from previous studies, glutamate is a functional amino acid to beneficially enhance nutrient sensing and transport in the gastrointestinal tract. MSG is safe and may serve as a low-cost ingredient to improve the intestinal absorptive capacity in sucking piglets.

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

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