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ORIGINAL ARTICLE

L-Glutamine regulates amino acid utilization by intestinal bacteria

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Abstract Catabolism of amino acids (AA) by intestinal bacteria greatly affects their bioavailability in the systemic circulation and the health of animals and humans. This study tests the novel hypothesis that L-glutamine regulates AA utilization by luminal bacteria of the small intestine. Pure bacterial strains (*Streptococcus* sp., *Escherichia coli* and *Klebsiella* sp.) and mixed bacterial cultures derived from the jejunum or ileum of pigs were cultured in the presence of 0–5 mM L-glutamine under anaerobic conditions. After 3 h of incubation, samples were taken for the determination of AA utilization. Results showed concentration-dependent increases in the utilization of glutamine in parallel with the increased conversion of glutamine into glutamate in all the bacteria. Complete utilization of asparagine, aspartate and serine was observed in pure bacterial strains after the 3-h incubation. The addition of glutamine reduced the net utilization of asparagine by both jejunal and ileal mixed bacteria. Net utilization of lysine, leucine, valine, ornithine and serine by jejunal or ileal mixed bacteria decreased with the addition of glutamine in a concentration-dependent manner. Collectively, glutamine dynamically modulates the bacterial metabolism of the arginine family of AA as well as the serine and aspartate families of AA and reduced the catabolism of most AA (including nutritionally essential and nonessential AA) in jejunal or ileal mixed bacteria. The beneficial effects of glutamine on gut nutrition and health may involve initiation of the signaling pathways related to AA metabolism in the luminal bacteria of the small intestine.

Keywords Amino acids · Gut bacteria · Small intestine · Nutrition · Swine

Abbreviations

AA	Amino acids
CFU	Colony forming unit
EAA	Nutritionally essential amino acid

Introduction

L-Glutamine is the most abundant free alpha-amino acid (AA) in the body and turnovers rapidly in plasma (Van Acker et al. 1998; Watford 1999), which reflects a crucial role of this AA in whole-body nutrient metabolism and health (Wu 2009). Studies over the last three decades indicated that glutamine is a major fuel for the small-intestinal mucosal cells and is crucial for maintaining the integrity and function of the small intestine by regulating gene expression, protein turnover, immune function, cell proliferation and apoptosis (Burrin and Davis 2004; Wang et al. 2008; Wu et al. 1996). Because catabolism of AAs by intestinal microorganisms greatly affects their bioavailability in the systemic circulation and the health of mammals (including pigs and humans) (Wu 1998), and other animal species (Dai et al. 2011c) it is of nutritional and physiological importance to determine effects of glutamine on AA utilization by luminal bacteria of the small intestine (Wu 2010).

Increasing evidence indicates that AA metabolism in the small intestine plays important roles in the regulation of

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whole-body AA homeostasis (Fuller and Reeds 1998; Hou et al. 2011; Stoll et al. 1998). However, the mechanism and the cell types responsible for the intestinal AA metabolism are not clear (Chen et al. 2007, 2009). Recent studies suggest that bacteria in the small intestine are active in the metabolism of AA, especially lysine, threonine, arginine, glutamate and glutamine (Dai et al. 2010, 2011a; Metges and Petzke 2005). The rapid utilization and metabolism of glutamine by small-intestinal bacteria supports the view that glutamine is a key regulator of the survival and growth of bacteria in the intestine through the regulation of the bacterial metabolism of nitrogenous compounds particularly AA (Forchhammer 2007). Moreover, glutamine may also affect AA utilization and metabolism in small-intestinal bacteria, thereby modulating the production and profile of nitrogenous compounds in the lumen of small intestine and whole-body AA homeostasis. However, the metabolic routes and the mode of action of glutamine in small-intestinal bacteria are not clear.

Therefore, this study employed the culture of small-intestinal bacteria to test the novel hypothesis that glutamine regulates the bacterial metabolism of AA and could reduce AA utilization in pig small-intestinal bacteria. Results from this study may help to explain the possible beneficial effects of glutamine on the nutrition and health of humans and animals (Wu et al. 2011a).

Materials and methods

Chemicals

HPLC-grade water and methanol were obtained from Fisher Scientific (Houston, TX, USA). Unless stated otherwise, all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Bacterial strains and culture conditions

Bacteria derived from the pig small intestine including *Streptococcus* sp., *Escherichia coli*, *Klebsiella* sp., jejunal mixed bacteria or ileal mixed bacteria were used in this study (Dai et al. 2010). All bacterial cultures were maintained in an anaerobic semi-defined medium (Dai et al. 2011a; Williams et al. 2005) with modifications of some chemicals as described previously (Dai et al. 2011b).

Subculture of bacteria

Stock bacterial cultures were subcultured in a semi-defined medium at 37 °C for 24 h. Aliquots of 1 mL of the 24-h cultures were further subcultured in 9 mL of anaerobic AA basal media twice (each for 24 h). The composition of the anaerobic AA basal media was similar as the semi-defined medium described above except the casitone and yeast extract was omitted and replaced by AA mixtures with glutamine concentrations at 0, 0.5, 1, 2 or 5 mmol/L in the media (Dai et al. 2011b). Concentrations of the carbon sources and ammonia in the media were also modified as described previously (Dai et al. 2011a).

In vitro incubation

Anaerobic AA basal media containing doses of glutamine (0, 0.5, 1, 2 or 5 mmol/L) were used for the experiment. After the second subculture, 0.1 mL of the 24-h cultures (ca. 10^8 bacteria cells) of *Streptococcus* sp., *E. coli*, *Klebsiella* sp., jejunal mixed bacteria or ileal mixed bacteria were inoculated into tubes containing 0.9 mL of corresponding sterile anaerobic AA basal media. Tubes containing AA mixtures plus doses of glutamine but no bacteria served as control. All tubes were incubated at 37 °C for 3 h. Tubes were taken before and after the 3-h incubation and put on ice immediately for the enumeration of bacteria and the determination of AA concentrations (Wu et al. 1997, 2007a).

Enumeration of bacteria

Numbers of bacteria were determined using the Hungate roll-tube method (Eller et al. 1971). The semi-defined media described above (liquid media or solidified media containing 1.5 % agar) were used for sample dilution and bacteria enumeration. Tubes were incubated at 37 °C for 24 h before counting the colony. In experiments involving pure bacterial cultures, bacteria numbers in the culture were calculated as described previously (Dai et al. 2011a, b).

Calculations and statistical analysis

Rates of net utilization/production of AA after a 3-h incubation were calculated on the basis of differences in AA concentrations between initial (0 h) and final (3 h) incubation media. Data were analyzed by one-way ANOVA and the general linear model procedure to evaluate the effects of doses of glutamine on the net utilization or production of AA in the media by pig small-intestinal bacteria. Statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA). P values ≤ 0.05 indicate statistical significance.

Results

Bacterial utilization of the arginine family of amino acids

The utilization of glutamine by pig small-intestinal bacteria was dose-dependent (Table 1). The addition of glutamine

Table 1 Effects of L-glutamine on the utilization of the arginine family of AA by pig small-intestinal bacteria

L-Glutamine (mmol/L)	*Streptococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
nmol/(10^8 cells 3 h)					
L-Arginine					
0	-10.1 ± 1.0^{dC}	-77.2 ± 3.2^{aA}	-50.2 ± 4.7^{bB}	-21.8 ± 1.7^{c}	-15.4 ± 0.3^{cdB}
0.5	-18.5 ± 1.9^{cB}	-65.6 ± 3.0^{bAB}	-108.3 ± 1.0^{aA}	-19.1 ± 0.8^{c}	-21.3 ± 0.8^{cA}
1	-18.4 ± 1.3^{cB}	-61.3 ± 3.1^{bB}	-103.7 ± 2.7^{aA}	-19.2 ± 0.7^{c}	-20.4 ± 0.6^{cA}
2	-21.4 ± 1.9^{cB}	-33.8 ± 5.4^{bC}	-105.4 ± 4.9^{aA}	-19.9 ± 2.0^{c}	-19.4 ± 2.6^{cA}
5	-26.1 ± 0.7^{bA}	-31.3 ± 9.5^{bC}	-104.2 ± 2.5^{aA}	-19.8 ± 1.9^{b}	-19.7 ± 0.6^{bA}
L-Citrulline					
0	-16.6 ± 0.1^{bA}	-75.0 ± 6.2^{aA}	-9.4 ± 0.5^{bc}	-7.2 ± 0.2^{cC}	-13.6 ± 0.1^{bc}
0.5	-16.5 ± 0.2^{bA}	-33.8 ± 6.0^{aB}	-10.1 ± 1.9^{b}	-9.5 ± 0.1^{bB}	-14.0 ± 0.6^{b}
1	-16.3 ± 0.2^{aA}	-14.4 ± 3.4^{abC}	-9.5 ± 0.9^{b}	-13.9 ± 0.3^{abA}	-13.8 ± 0.3^{ab}
2	-14.2 ± 0.5^{aB}	$+1.9 \pm 2.4^{cD}$	-9.0 ± 1.3^{b}	-13.0 ± 0.3^{aA}	-14.2 ± 0.5^{a}
5	-12.7 ± 0.6^{aC}	$+20.0 \pm 5.0^{bE}$	-6.7 ± 0.9^{a}	-13.7 ± 0.2^{aA}	-13.6 ± 0.2^{a}
L-Glutamate					
0	-30.8 ± 4.4^{bA}	-95.8 ± 3.0^{a}	-36.4 ± 7.2^{bA}	-42.6 ± 0.8^{bA}	-11.8 ± 1.1^{dA}
0.5	$+23.6 \pm 1.4^{dB}$	-92.0 ± 10.8^{a}	-31.2 ± 2.7^{bA}	-8.5 ± 2.1^{cB}	$+20.6 \pm 3.0^{dB}$
1	$+24.9 \pm 4.8^{cB}$	-91.5 ± 14.4^{a}	$+59.3 \pm 1.7^{dB}$	-7.9 ± 1.3^{bB}	$+23.5 \pm 4.5^{cB}$
2	$+30.0 \pm 0.9^{bcB}$	-80.2 ± 14.4^{a}	$+92.4 \pm 9.1^{dC}$	$+13.7 \pm 2.1^{bC}$	$+39.9 \pm 5.3^{cC}$
5	$+30.2 \pm 1.0^{cB}$	-70.1 ± 9.4^{a}	$+138.4 \pm 1.4^{eD}$	$+14.4 \pm 1.0^{bC}$	$+46.9 \pm 2.2^{dC}$
L-Glutamine					
0	ND	ND	ND	ND	ND
0.5	-48.5 ± 5.1^{dC}	-229.6 ± 1.7^{aD}	-127.3 ± 2.2^{bD}	-37.2 ± 4.5^{eC}	-66.3 ± 0.0^{cB}
1	-85.3 ± 5.9^{cB}	-397.4 ± 2.9^{aC}	-206.2 ± 0.4^{bC}	-33.8 ± 2.4^{eC}	-66.9 ± 1.3^{dB}
2	-83.7 ± 2.9^{dB}	-650.7 ± 7.6^{aB}	-329.5 ± 3.6^{bB}	-46.8 ± 3.7^{eB}	-109.2 ± 5.6^{cA}
5	-107.7 ± 2.5^{cA}	-667.6 ± 8.9^{aA}	-447.9 ± 6.2^{bA}	-104.4 ± 2.6^{cA}	-108.8 ± 4.6^{cA}
L-Ornithine					
0	$+13.1 \pm 1.9^{bB}$	$+90.2 \pm 7.4^{dA}$	$+40.8 \pm 4.8^{cB}$	-44.0 ± 0.1^{aA}	-51.4 ± 1.0^{aA}
0.5	$+13.5 \pm 1.6^{cB}$	$+94.1 \pm 3.9^{eA}$	$+39.8 \pm 2.8^{dB}$	-23.5 ± 1.8^{bB}	-32.4 ± 1.2^{aB}
1	$+4.7 \pm 0.5^{bA}$	$+94.8 \pm 9.6^{dA}$	$+37.1 \pm 1.7^{cB}$	-22.6 ± 0.7^{aB}	-28.5 ± 0.3^{aB}
2	$+5.8 \pm 0.6^{bA}$	$+122.5 \pm 5.7^{dB}$	$+21.0 \pm 1.0^{cA}$	-22.3 ± 1.8^{aB}	-28.6 ± 1.4^{aB}
5	$+5.0 \pm 1.0^{bA}$	$+141.3 \pm 6.9^{dB}$	$+19.2 \pm 1.9^{cA}$	-23.4 ± 1.3^{aB}	-28.5 ± 2.2^{aB}
L-Proline					
0	-38.9 ± 3.0^{cB}	-198.2 ± 3.7^{aA}	-56.1 ± 5.3^{b}	-16.3 ± 1.7^{d}	-18.9 ± 1.1^{d}
0.5	-75.8 ± 2.4^{bA}	-191.6 ± 5.2^{aA}	-58.9 ± 6.2^{c}	-16.8 ± 0.8^{d}	-18.2 ± 1.8^{d}
1	-78.0 ± 1.2^{bA}	-184.5 ± 10.0^{aA}	-56.6 ± 0.7^{c}	-16.1 ± 1.1^{d}	-17.7 ± 1.0^{d}
2	-83.3 ± 2.8^{aA}	-86.7 ± 4.7^{aB}	-57.3 ± 4.1^{b}	-15.5 ± 0.5^{c}	-17.2 ± 0.9^{c}
5	-81.8 ± 3.0^{aA}	-74.5 ± 3.8^{aB}	-57.7 ± 4.9^{b}	-17.7 ± 1.7^{c}	-17.0 ± 1.1^{c}

Values are mean ± SEM, $n = 4$

− utilization, + production, *ND* not detectable

[a–e] Mean values in a row with superscripts without a common letter differ, $P < 0.05$; [A–D]mean values in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-glutamine for 3 h

to the media reduced the net utilization of glutamate by small-intestinal bacteria. Net production of glutamate was observed in *Streptococcus* sp. and ileal mixed bacteria with the addition of glutamine, while production of glutamate by *Klebsiella* sp. or jejunal mixed bacteria was observed only in the presence of high concentrations of glutamine.

Net utilization of arginine and proline increased ($P < 0.05$) in *Streptococcus* sp. with the addition of glutamine (Table 1). However, the utilization of citrulline and the production of ornithine by *Streptococcus* sp. decreased with high concentrations of glutamine. In *E. coli*, the net utilization of arginine and proline decreased ($P < 0.05$) in the presence of high concentration of glutamine. Net

utilization of citrulline decreased ($P < 0.05$) with increasing concentrations of glutamine. High concentrations of glutamine increased ($P < 0.05$) the production of citrulline and ornithine in *E. coli*. The net utilization of arginine increased ($P < 0.05$) in *Klebsiella* sp. with the addition of glutamine. Increased dose of glutamine reduced the net production of ornithine in *Klebsiella* sp., and significant rates were observed at 2 and 5 mmol/L glutamine. In jejunal mixed bacteria, net utilization of ornithine decreased ($P < 0.05$) with the addition of glutamine. However, net utilization of citrulline by jejunal mixed bacteria increased in the presence of glutamine. Net utilization of arginine increased in ileal mixed bacteria with the addition of glutamine. In the presence of glutamine, net utilization of ornithine by ileal mixed bacteria decreased.

Effects of glutamine on the bacterial utilization of the serine and aspartate family of amino acids

Complete utilization of asparagine in the culture of *E. coli* was observed after the 3-h incubation (Table 2). Serine in the culture media was completely utilized by *Streptococcus* sp. after 3 h of incubation. Complete utilization of aspartate and serine was observed in *E. coli* and *Klebsiella* sp.

The net utilization of asparagine and glycine decreased ($P < 0.05$) in *Streptococcus* sp. with increasing concentrations of glutamine (Table 2). Net utilization of alanine, aspartate and threonine by *Streptococcus* sp. increased ($P < 0.05$) with the addition of glutamine. However, dose-dependent effect was only observed for alanine. The addition of glutamine reduced ($P < 0.05$) the utilization of alanine and glycine by *E. coli* while stimulated ($P < 0.05$) the utilization of threonine in the bacteria. The net utilization of asparagine decreased ($P < 0.05$) with the addition of glutamine in culture of *Klebsiella* sp. after the 3-h incubation. Net utilization of alanine, glycine and threonine increased ($P < 0.05$) in *Klebsiella* sp. with the addition of glutamine.

The utilization of asparagine and serine by jejunal mixed bacteria was reduced with the addition of glutamine (Table 2). In the presence of glutamine, the net utilization of aspartate by jejunal mixed bacteria decreased ($P < 0.05$) and net production of aspartate was observed. Net production of aspartate by the jejunal mixed bacteria increased with increasing doses of glutamine. However, the net utilization of threonine by jejunal mixed bacteria increased ($P < 0.05$) with the addition of glutamine. The addition of glutamine reduced ($P < 0.05$) the net utilization of asparagine, aspartate, glycine, serine and threonine by ileal mixed bacteria.

Effects of glutamine on the bacterial utilization of branched-chain amino acids

The addition of glutamine reduced ($P < 0.05$) the net utilization of isoleucine by jejunal mixed bacteria after 3 h of incubation (Table 3). In contrast, net utilization of isoleucine increased ($P < 0.05$) in *Streptococcus* sp., *E. coli* and *Klebsiella* sp. with the addition of glutamine. Reduced utilization of leucine by *Streptococcus* sp. was observed with glutamine concentration at 5 mmol/L. Net utilization of leucine by jejunal or ileal mixed bacteria decreased ($P < 0.05$) with increased concentrations of glutamine. Utilization of leucine by *E. coli* increased ($P < 0.05$) with increased concentrations of glutamine, while dose-dependent effect was only observed with glutamine concentrations at 1 mmol/L and below. Increased concentrations of glutamine reduced ($P < 0.05$) the net utilization of valine by *Klebsiella* sp. or jejunal mixed bacteria. However, increased utilization of valine was observed in *Streptococcus* sp. with increased concentrations of glutamine.

Effects of glutamine on the bacterial utilization of sulfur amino acids

The utilization of cystine and methionine was greater in *E. coli* and *Klebsiella* sp. than other bacteria after 3 h of incubation (Table 4). The utilization of cystine by *Streptococcus* sp. and jejunal mixed bacteria decreased ($P < 0.05$) with the addition of glutamine. However, dose-dependent effect was only observed in *Streptococcus* sp. Net utilization of cystine increased ($P < 0.05$) in *E. coli* with increased dose of glutamine. Utilization of methionine by *E. coli*, *Klebsiella* sp. and jejunal mixed bacteria was reduced with increasing concentrations of glutamine. Net utilization of methionine by *Streptococcus* sp. increase ($P < 0.05$) with the addition of glutamine in a dose-dependent manner. There was no synthesis of taurine by all the bacteria studied.

Effects of glutamine on the bacterial utilization of aromatic amino acids

The addition of glutamine reduced ($P < 0.05$) the net utilization of phenylalanine by *Streptococcus* sp., *E. coli* or ileal mixed bacteria, while a dose-dependent effect was only observed in *Streptococcus* sp. or *E. coli* (Table 5). Net utilization of phenylalanine increased ($P < 0.05$) in *Klebsiella* sp. with increased dose of glutamine. The net utilization of tryptophan decreased ($P < 0.05$) in *E. coli* and *Klebsiella* sp. with increasing concentrations of glutamine. However, the addition of glutamine enhanced ($P < 0.05$) net utilization of tryptophan in *Streptococcus* sp. The net utilization of tyrosine increased ($P < 0.05$) in *Streptococcus* sp. and *E. coli* with the addition of glutamine.

Effects of glutamine on the bacterial utilization of lysine and histidine

Net utilization of lysine by *E. coli*, jejunal or ileal mixed bacteria decreased ($P < 0.05$) with increasing

Table 2 Effects of L-glutamine on the utilization of the serine and aspartate family of AA by pig small-intestinal bacteria

L-Glutamine (mmol/L)	*Streptococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
nmol/(10^8 cells 3 h)					
L-Alanine					
0	-7.6 ± 1.4^{dD}	-144.6 ± 12.5^{aA}	-52.1 ± 5.2^{bB}	-15.6 ± 0.7^{cd}	-29.0 ± 1.4^{c}
0.5	-23.4 ± 0.8^{bC}	-87.6 ± 5.9^{aB}	-84.7 ± 2.7^{aA}	-14.0 ± 1.2^{c}	-28.8 ± 0.6^{b}
1	-33.4 ± 1.5^{bB}	-72.7 ± 11.2^{aB}	-85.1 ± 2.1^{aA}	-14.6 ± 1.2^{c}	-30.3 ± 1.2^{b}
2	-41.4 ± 4.8^{bA}	-73.3 ± 12.3^{aB}	-86.5 ± 2.9^{aA}	-11.6 ± 0.6^{c}	-31.0 ± 0.9^{b}
5	-39.4 ± 1.8^{bAB}	-74.4 ± 10.0^{aB}	-82.0 ± 5.1^{aA}	-12.7 ± 0.7^{c}	-30.1 ± 1.5^{b}
L-Asparagine					
0	-126.0 ± 0.0^{1cA}	-408.9 ± 0.0^{1a}	-183.7 ± 2.9^{bC}	-94.0 ± 0.0^{1dA}	-76.8 ± 0.5^{eA}
0.5	-96.0 ± 3.7^{cB}	-419.5 ± 0.0^{1a}	-169.2 ± 4.6^{bB}	-41.7 ± 0.6^{dB}	-36.6 ± 4.4^{dB}
1	-97.0 ± 3.1^{cB}	-415.1 ± 0.0^{1a}	-174.0 ± 1.1^{bB}	-42.6 ± 1.3^{dB}	-29.9 ± 0.8^{eBC}
2	-85.5 ± 4.3^{cC}	-430.6 ± 0.0^{1a}	-169.0 ± 4.0^{bB}	-42.6 ± 1.0^{dB}	-29.7 ± 2.4^{eBC}
5	-81.8 ± 2.9^{cC}	-465.2 ± 0.0^{1a}	-129.5 ± 1.6^{bA}	-43.1 ± 0.4^{dB}	-26.0 ± 1.3^{eC}
L-Aspartate					
0	-29.9 ± 2.9^{dB}	-909.1 ± 0.0^{1a}	-324.3 ± 0.0^{1b}	-4.4 ± 0.3^{eA}	-50.1 ± 1.3^{cA}
0.5	-33.4 ± 1.0^{cB}	-919.9 ± 0.0^{1a}	-354.6 ± 0.0^{1b}	$+12.3 \pm 0.9^{eB}$	-24.4 ± 1.4^{dB}
1	-44.3 ± 2.3^{cA}	-951.1 ± 0.0^{1a}	-325.7 ± 0.0^{1b}	$+13.4 \pm 1.6^{eB}$	-14.9 ± 0.4^{dC}
2	-44.0 ± 5.4^{cA}	-946.2 ± 0.0^{1a}	-326.4 ± 0.0^{1b}	$+16.5 \pm 3.1^{eB}$	-13.3 ± 0.8^{dC}
5	-44.4 ± 1.6^{cA}	-991.7 ± 0.0^{1a}	-319.2 ± 0.0^{1b}	$+30.0 \pm 3.0^{eC}$	-13.7 ± 0.4^{dC}
Glycine					
0	-36.3 ± 1.5^{bA}	-116.0 ± 2.7^{aA}	-42.4 ± 5.5^{bB}	-18.0 ± 0.6^{c}	-21.5 ± 1.2^{cA}
0.5	-36.4 ± 1.5^{bA}	-94.5 ± 13.9^{aA}	-90.4 ± 3.7^{aA}	-15.7 ± 2.0^{c}	-19.2 ± 0.3^{bcAB}
1	-23.1 ± 2.6^{bB}	-81.2 ± 9.0^{aAB}	-90.5 ± 5.6^{aA}	-14.7 ± 1.2^{b}	-16.2 ± 1.4^{bB}
2	-18.6 ± 1.9^{cB}	-52.7 ± 17.5^{bBC}	-92.8 ± 9.5^{aA}	-14.1 ± 0.7^{c}	-16.4 ± 1.1^{cB}
5	-18.8 ± 2.4^{cB}	-29.1 ± 6.9^{bC}	-93.3 ± 5.6^{aA}	-14.8 ± 1.0^{c}	-17.4 ± 0.6^{cB}
L−serine					
0	-196.1 ± 0.0^{1c}	-895.7 ± 0.0^{1a}	-410.0 ± 0.0^{1b}	-21.2 ± 0.4^{eA}	-25.1 ± 1.4^{dA}
0.5	-208.3 ± 0.0^{1c}	-922.3 ± 0.0^{1a}	-411.1 ± 0.0^{1b}	-14.9 ± 1.7^{dB}	-17.4 ± 1.4^{dB}
1	-204.4 ± 0.0^{1c}	-918.4 ± 0.0^{1a}	-407.8 ± 0.0^{1b}	-14.1 ± 1.6^{eB}	-17.7 ± 1.0^{dB}
2	-192.0 ± 0.0^{1c}	-926.4 ± 0.0^{1a}	-407.5 ± 0.0^{1b}	-14.2 ± 0.6^{eB}	-17.3 ± 1.5^{dB}
5	-187.0 ± 0.0^{1c}	-930.7 ± 0.0^{1a}	-397.6 ± 0.0^{1b}	-14.4 ± 1.4^{dB}	-14.1 ± 0.5^{dB}
L-Threonine					
0	-48.7 ± 3.1^{aB}	-52.6 ± 4.7^{aC}	-16.6 ± 4.0^{cB}	-6.2 ± 0.4^{dC}	-27.3 ± 0.6^{bA}
0.5	-72.0 ± 4.2^{aA}	-51.7 ± 6.6^{bC}	-33.1 ± 2.8^{cA}	-9.5 ± 0.9^{dC}	-19.4 ± 1.0^{dB}
1	-77.3 ± 2.6^{aA}	-62.1 ± 7.2^{bB}	-33.1 ± 1.3^{cA}	-16.4 ± 2.3^{dB}	-20.5 ± 0.9^{dB}
2	-76.0 ± 1.7^{aA}	-60.6 ± 3.2^{bB}	-33.0 ± 3.8^{cA}	-20.1 ± 2.5^{dB}	-19.9 ± 0.9^{dB}
5	-78.8 ± 0.8^{aA}	-81.7 ± 9.3^{aA}	-32.0 ± 1.0^{bA}	-27.6 ± 2.2^{bA}	-18.8 ± 0.6^{bB}

Values are mean ± SEM, $n = 4$

− utilization, + production

[a–e] Mean values in a row with superscripts without a common letter differ, $P < 0.05$; [A–D]mean values in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-glutamine for 3 h

[1] Complete utilization of the corresponding amino acid was observed after 3 h of incubation

concentrations of glutamine (Table 6). However, net utilization of lysine increased ($P < 0.05$) in *Klebsiella* sp. with the addition of glutamine. The utilization of histidine by *Streptococcus* sp., *E. coli* and *Klebsiella* sp. decreased ($P < 0.05$) with increased dose of glutamine.

Discussion

Given the important role for glutamine in intestinal health (Haynes et al. 2009; Xi et al. 2011a), there is growing interest in glutamine nutrition in mammals, including

Table 3 Effects of L-glutamine on the utilization of branched-chain AA by pig small-intestinal bacteria

L-Glutamine (mmol/L)	*Streptococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
nmol/(10^8 cells 3 h)					
L-Isoleucine					
0	-21.3 ± 0.7^{cB}	-69.1 ± 7.7^{aB}	-44.6 ± 2.9^{bB}	-14.2 ± 0.8^{dA}	-13.7 ± 0.9^{d}
0.5	-23.4 ± 0.7^{cAB}	-79.7 ± 9.2^{aAB}	-46.6 ± 2.7^{bAB}	-10.8 ± 1.0^{dB}	-14.2 ± 0.9^{d}
1	-27.2 ± 0.4^{cA}	-92.5 ± 3.6^{aA}	-48.5 ± 0.9^{bAB}	-11.6 ± 0.4^{dB}	-13.3 ± 0.4^{d}
2	-27.3 ± 3.1^{cA}	-91.3 ± 3.9^{aA}	-52.1 ± 1.8^{bA}	-10.6 ± 0.6^{dB}	-14.6 ± 2.7^{d}
5	-26.1 ± 0.9^{cA}	-90.8 ± 9.4^{aA}	-53.0 ± 0.8^{bA}	-11.6 ± 1.2^{dB}	-14.0 ± 1.0^{d}
L-Leucine					
0	-26.5 ± 1.9^{cA}	-80.2 ± 9.2^{aB}	-64.5 ± 2.9^{b}	-20.1 ± 2.5^{cA}	-26.9 ± 1.7^{cA}
0.5	-27.8 ± 2.4^{cA}	-95.2 ± 6.5^{aB}	-63.6 ± 5.3^{b}	-18.2 ± 0.1^{cA}	-17.0 ± 0.9^{cB}
1	-26.7 ± 0.7^{cA}	-156.4 ± 5.7^{aA}	-61.2 ± 1.1^{b}	-19.4 ± 1.2^{cdAB}	-16.1 ± 1.1^{dB}
2	-26.0 ± 2.2^{cA}	-155.8 ± 10.3^{aA}	-61.5 ± 4.4^{b}	-17.1 ± 1.1^{dAB}	-14.8 ± 0.7^{dB}
5	-19.4 ± 0.7^{cB}	-154.0 ± 11.3^{aA}	-60.3 ± 2.6^{b}	-14.2 ± 1.1^{dB}	-14.8 ± 0.9^{dB}
L-Valine					
0	-23.1 ± 2.0^{cB}	-39.8 ± 7.7^{b}	-82.9 ± 2.9^{aA}	-16.1 ± 0.4^{cA}	-20.0 ± 0.8^{c}
0.5	-27.7 ± 3.6^{bB}	-35.2 ± 5.6^{b}	-80.7 ± 3.0^{aA}	-12.3 ± 0.8^{cB}	-15.4 ± 0.9^{c}
1	-39.9 ± 0.4^{bA}	-32.1 ± 5.7^{b}	-73.4 ± 1.1^{aAB}	-9.6 ± 1.7^{cB}	-16.3 ± 0.8^{c}
2	-41.3 ± 5.7^{bA}	-33.3 ± 2.2^{b}	-68.0 ± 4.9^{aB}	-8.8 ± 0.8^{cB}	-15.7 ± 3.0^{c}
5	-42.2 ± 1.8^{bA}	-32.2 ± 4.9^{c}	-55.5 ± 1.4^{aC}	-9.9 ± 1.4^{dB}	-16.4 ± 1.1^{d}

Values are mean ± SEM, $n = 4$

− utilization

[a–e] Mean values in a row with superscripts without a common letter differ, $P < 0.05$; [A–D]mean values in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-glutamine for 3 h

Table 4 Effects of L-glutamine on the utilization of sulfur AA by pig small-intestinal bacteria

L-Glutamine (mmol/L)	*Streptococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
nmol/(10^8 cells 3 h)					
L-Cystine					
0	-9.0 ± 1.8^{cA}	-100.8 ± 8.9^{aB}	-33.2 ± 1.5^{b}	-9.1 ± 0.6^{cA}	-2.1 ± 0.8^{d}
0.5	-7.2 ± 2.5^{cA}	-106.8 ± 4.7^{aB}	-30.3 ± 1.5^{b}	-8.2 ± 0.2^{cAB}	-2.5 ± 0.7^{d}
1	-5.0 ± 0.6^{cdAB}	-114.0 ± 2.1^{aB}	-28.9 ± 1.8^{b}	-7.4 ± 0.7^{cB}	-2.5 ± 0.3^{d}
2	-3.6 ± 0.3^{dB}	-146.9 ± 4.5^{aA}	-29.5 ± 1.1^{b}	-7.1 ± 0.3^{cB}	-2.6 ± 0.5^{d}
5	-2.5 ± 1.1^{dB}	-149.8 ± 5.2^{aA}	-29.0 ± 1.0^{b}	-7.9 ± 0.4^{cB}	-2.1 ± 0.2^{d}
L-Methionine					
0	-13.7 ± 0.9^{cB}	-67.6 ± 2.5^{aA}	-49.6 ± 1.9^{bA}	-8.1 ± 0.4^{dA}	-7.4 ± 0.7^{d}
0.5	-13.4 ± 0.5^{cB}	-58.1 ± 6.4^{aA}	-48.4 ± 2.7^{bAB}	-7.6 ± 1.1^{dAB}	-6.6 ± 0.5^{d}
1	-15.8 ± 0.7^{cA}	-24.6 ± 2.0^{bB}	-42.8 ± 1.1^{aAB}	-7.8 ± 0.3^{dAB}	-7.0 ± 0.5^{d}
2	-17.0 ± 0.6^{bA}	-22.7 ± 5.3^{bB}	-41.6 ± 2.9^{aB}	-5.8 ± 0.7^{cBC}	-6.7 ± 1.3^{c}
5	-17.2 ± 0.8^{bA}	-19.5 ± 4.2^{bB}	-33.0 ± 2.3^{aC}	-5.1 ± 0.5^{cC}	-6.2 ± 0.2^{c}

Values are mean ± SEM, $n = 4$

− utilization

[a–e] Mean values in a row with superscripts without a common letter differ, $P < 0.05$; [A–D]mean values in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-glutamine for 3 h

humans (Kalhan and Bier 2008; Kudsk 2006), pigs (Li et al. 2011; Wu et al. 2011a; Yin et al. 2010), and mice (Ren et al. 2011). Glutamine plays an important role in nitrogen balance and protein synthesis in bacteria (Forchhammer 2007) and small intestine (Wu et al. 2011a; Xi et al. 2011a, b; Yao et al. 2011). However, little is known about possible effects of glutamine on AA metabolism in gut bacteria particularly bacteria in the lumen of

Table 5 Effects of L-glutamine on the utilization of aromatic AA by pig small-intestinal bacteria

L-Glutamine (mmol/L)	*Streptococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
nmol/(10^8 cells 3 h)					
L-Phenylalanine					
0	-25.1 ± 1.9^{bA}	-101.4 ± 7.3^{aA}	-27.0 ± 3.5^{bC}	-5.4 ± 0.3^{d}	-17.0 ± 0.8^{cA}
0.5	-19.6 ± 0.1^{cB}	-108.9 ± 3.2^{aA}	-33.4 ± 2.0^{bC}	-7.6 ± 0.8^{e}	-14.1 ± 0.6^{dB}
1	-15.5 ± 0.3^{cC}	-96.8 ± 4.8^{aA}	-56.4 ± 6.1^{bB}	-5.0 ± 0.8^{d}	-15.8 ± 0.4^{cB}
2	-13.3 ± 1.7^{cCD}	-56.2 ± 7.9^{bB}	-72.7 ± 2.5^{aA}	-6.3 ± 1.4^{d}	-14.2 ± 0.8^{cB}
5	-10.8 ± 1.5^{cD}	-51.8 ± 4.6^{bB}	-70.1 ± 3.1^{aA}	-5.5 ± 0.8^{d}	-15.4 ± 0.4^{cB}
L-Tryptophan					
0	-15.8 ± 0.8^{cB}	-132.2 ± 3.8^{aA}	-43.1 ± 1.8^{bA}	-4.0 ± 0.3^{d}	-6.9 ± 0.4^{d}
0.5	-19.5 ± 0.4^{cA}	-102.6 ± 3.2^{aB}	-41.8 ± 1.5^{bA}	-4.8 ± 1.0^{d}	-6.6 ± 0.6^{d}
1	-20.5 ± 0.7^{cA}	-78.6 ± 1.6^{aC}	-37.5 ± 1.3^{bA}	-4.7 ± 0.2^{d}	-7.4 ± 0.4^{d}
2	-20.1 ± 0.4^{cA}	-78.1 ± 1.9^{aC}	-30.9 ± 1.0^{bB}	-4.3 ± 0.2^{d}	-6.9 ± 0.4^{d}
5	-20.0 ± 0.3^{bA}	-75.7 ± 4.7^{aC}	-25.9 ± 3.0^{bB}	-4.3 ± 0.2^{c}	-5.7 ± 0.3^{c}
L-Tyrosine					
0	-21.7 ± 2.7^{cB}	-67.2 ± 5.9^{bB}	-108.4 ± 2.1^{a}	-15.8 ± 0.7^{d}	-12.0 ± 0.4^{d}
0.5	-25.2 ± 2.5^{cB}	-137.2 ± 14.2^{aA}	-111.6 ± 1.6^{b}	-14.7 ± 0.1^{d}	-12.1 ± 0.6^{d}
1	-26.5 ± 2.9^{cB}	-134.2 ± 4.5^{aA}	-108.2 ± 0.6^{b}	-15.0 ± 0.5^{d}	-13.0 ± 1.5^{d}
2	-35.7 ± 1.0^{cA}	-141.7 ± 16.1^{aA}	-109.3 ± 1.4^{b}	-13.9 ± 1.3^{d}	-11.0 ± 1.2^{d}
5	-32.0 ± 3.4^{cA}	-138.4 ± 10.0^{aA}	-110.3 ± 3.3^{b}	-14.3 ± 0.7^{d}	-11.2 ± 0.8^{d}

Values are mean ± SEM, $n = 4$

− utilization

[a–e] Mean values in a row with superscripts without a common letter differ, $P < 0.05$; [A–D]mean values in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-glutamine for 3 h

Table 6 Effects of L-glutamine on the utilization of histidine and lysine by pig small-intestinal bacteria

L-Glutamine (mmol/L)	*Streptococcus* sp.	*Escherichia coli*	*Klebsiella* sp.	Jejunal bacteria	Ileal bacteria
nmol/(10^8 cells 3 h)					
L-Histidine					
0	-40.0 ± 1.6^{cA}	-205.3 ± 8.9^{aA}	-54.0 ± 4.6^{bA}	-13.1 ± 0.4^{d}	-16.2 ± 1.0^{d}
0.5	-37.0 ± 0.7^{cA}	-187.6 ± 2.9^{aB}	-58.4 ± 4.2^{bA}	-12.5 ± 1.4^{d}	-14.1 ± 0.6^{d}
1	-34.6 ± 0.9^{cB}	-155.7 ± 4.4^{aC}	-53.1 ± 1.5^{bAB}	-14.1 ± 0.6^{d}	-14.3 ± 0.8^{d}
2	-35.0 ± 1.9^{bB}	-157.3 ± 5.4^{aC}	-42.7 ± 4.6^{bBC}	-13.7 ± 0.9^{c}	-14.1 ± 1.0^{c}
5	-32.8 ± 0.4^{bB}	-151.4 ± 4.9^{aC}	-36.9 ± 0.9^{bC}	-14.5 ± 2.1^{c}	-14.2 ± 0.6^{c}
L-Lysine					
0	-35.7 ± 2.6^{c}	-70.3 ± 3.2^{bA}	-168.7 ± 2.6^{aB}	-38.5 ± 1.3^{cA}	-73.1 ± 3.9^{bA}
0.5	-40.1 ± 7.0^{d}	-71.3 ± 1.8^{cA}	-256.0 ± 2.9^{aA}	-23.2 ± 3.1^{eB}	-51.9 ± 2.6^{bB}
1	-40.1 ± 4.2^{d}	-69.9 ± 5.4^{bA}	-266.5 ± 1.5^{aA}	-20.3 ± 0.9^{eB}	-51.4 ± 0.4^{cB}
2	-39.3 ± 1.2^{b}	-34.5 ± 6.0^{bB}	-266.1 ± 5.1^{aA}	-21.4 ± 1.6^{cB}	-40.1 ± 2.5^{bC}
5	-40.3 ± 6.1^{b}	-27.8 ± 7.5^{bcB}	-264.9 ± 2.8^{aA}	-21.2 ± 0.9^{cB}	-39.4 ± 3.2^{bC}

Values are mean ± SEM, $n = 4$

− utilization

[a–e] Mean values in a row with superscripts without a common letter differ, $P < 0.05$; [A–D]mean values in a column with superscripts without a common letter differ, $P < 0.05$. Bacteria were incubated in the presence of 0–5 mmol/L L-glutamine for 3 h

the small intestine. Our previous work revealed that bacteria in the small intestine were active in the utilization of glutamine/glutamate and the utilization was species- and gut compartment-specific (Dai et al. 2010, 2011a, b). Results from this study showed dose-dependent utilization of glutamine by pig small-intestinal bacteria (Table 1). Moreover,

the activity of AA utilization was higher in *E. coli* and *Klebsiella* sp. than *Streptococcus* sp., jejunal mixed bacteria and ileal mixed bacteria. This may be partially because *E. coli* and *Klebsiella* sp. respond faster than other bacteria to the changes of the extracellular concentrations of substrates (e.g. carbon source or AA) (Lara et al. 2009) and grow faster than other bacteria (this study, data not shown). Therefore, the activity and the numbers of bacteria are two important factors influencing the bacterial utilization and metabolism of AA in the gut (Dai et al. 2011c; Wallace 1996).

A novel observation of the present study is that glutamine regulated the utilization and metabolism of the arginine family of AA in pig small-intestinal bacteria (Fig. 1). With the addition of glutamine, the net utilization of glutamate decreased in *E. coli* and net glutamate production increased in other bacteria investigated (Table 1). These findings suggested that the conversion of glutamine to glutamate is a common event in small-intestinal bacteria. This finding is of nutritional importance because glutamate has versatile functions in metabolism and physiology (Brosnan and Brosnan 2012). The production of ammonia might increase the extracellular pH or served as a nitrogen source for the microbial synthesis of AA and protein (Forchhammer 2007) and the production of citrulline and arginine from proline and glutamate by intestinal mucosal epithelial cells (Wu 1997). Meanwhile, increased concentration of glutamine in the

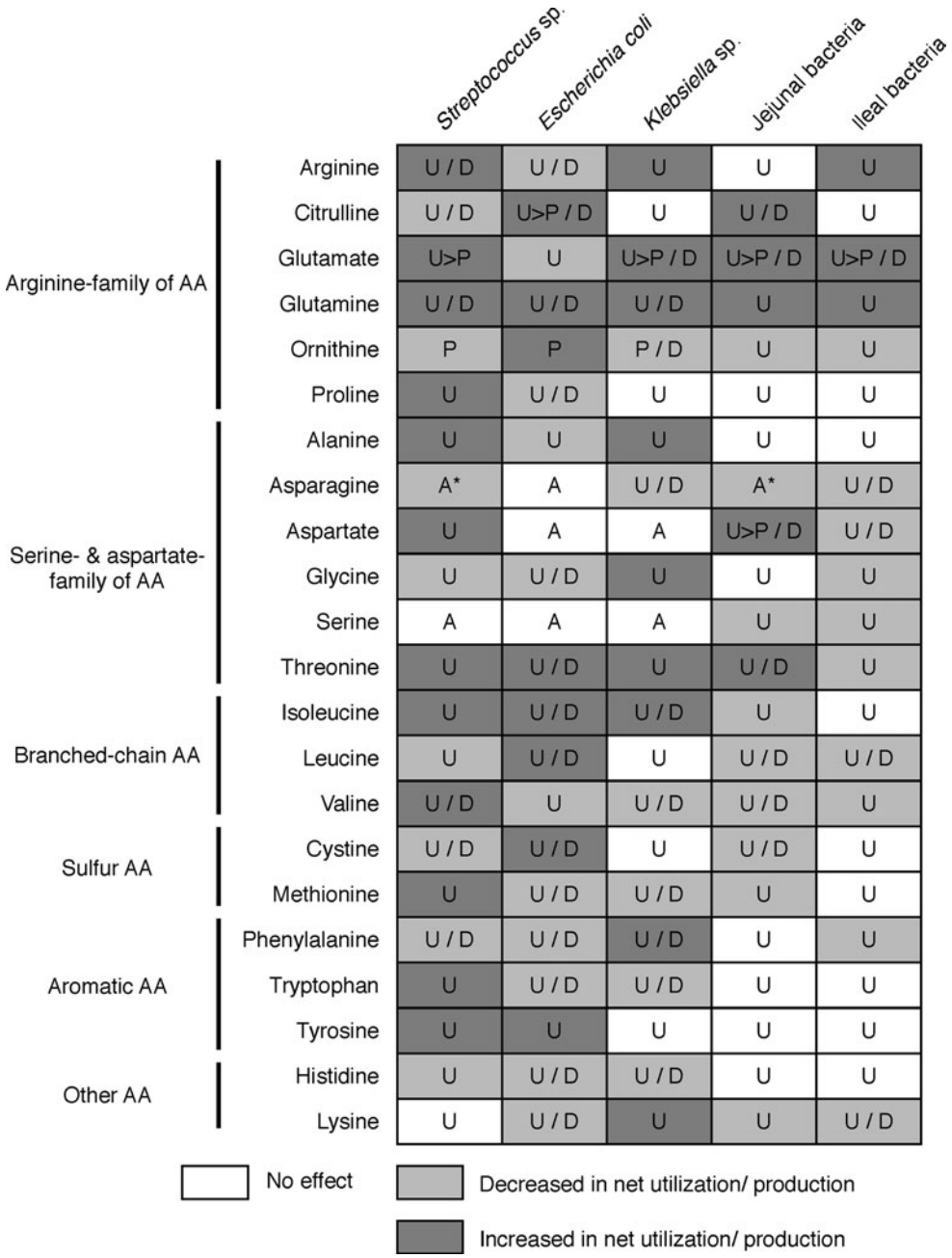

Fig. 1 Overall effects of L-glutamine on the utilization or production of amino acids in small-intestinal bacteria. A, complete utilization; D, dose dependent; P, net production; U, net utilization; U > P, shift from net utilization to net production over time; *, complete utilization of asparagine in cultures of *Streptococcus* sp. and jejunal mixed bacteria after 3 h of incubation was only observed in 0 mmol/L of glutamine

culture might increase the activity of small-intestinal bacteria. In support of this notion, the addition of glutamine to the culture increased the utilization of threonine and isoleucine in pure bacterial strains (Fig. 1). Moreover, the current results suggested that the addition of glutamine affected the conversion of arginine into citrulline and ornithine. There is coordination of glutamine and arginine metabolism in intestinal mucosal cells (Wu 1998, 2009), which suggests that the metabolism of the arginine family of AA in small-intestinal bacteria might occur via similar metabolic pathways to those in enterocytes and play important roles in the metabolism of nitrogenous compounds within the small intestine (Bergen and Wu 2009). These findings indicate that the effect of glutamine on the metabolism of the arginine family of AA in the small intestine might be partially mediated by the small-intestinal bacteria (Reeds and Burrin 2001; Wu et al. 1994, 1996; Wu 1998). However, the contribution of the bacterial metabolism of the arginine family of AA to the recycling of nitrogenous compounds in the small intestine is not clear. Further investigations are required to uncover the regulatory role of glutamine and the extent of utilization of the arginine family of AA, including ornithine and proline (Wu et al. 2011b), in the intestinal nitrogen cycling (Dai et al. 2011b).

The regulatory role of glutamine in the metabolism of the arginine family of AA by small-intestinal bacteria indicates that glutamine is not only nutritionally important but also crucial for maintaining gut health and function. First, although the high requirements of glutamine in the potential pathogenic bacteria *E. coli* and *Klebsiella* sp. are nutritionally disadvantage to the host, dietary supplementation of glutamine may reduce the bacterial utilization and degradation of host-derived glutamine or modulate the utilization of AA by the host and gut bacteria. This, in turn, helps maintain gut integrity and function and also reduce the occurrence of bacterial invasion and infection. Second, differences in the regulatory role of glutamine in the utilization and production of the arginine family of AA in pure bacterial strains (Table 1) suggest that different bacteria species play their different specific roles in the utilization and metabolism of AA and contribute to the homeostasis of the luminal pool of nitrogenous compounds especially AA. The altered luminal pools of AA, especially the arginine family of AA, and their related metabolites (e.g., agmatine and polyamines) brought about by dietary glutamine supplementation and small-intestinal bacteria may intervene the signaling pathways in small-intestinal cells, therefore affecting the function and health of the small intestine (Rhoads and Wu 2009; Wu 2010). However, further studies are warranted to uncover specific effects of glutamine (e.g., phosphorylation of protein kinases and transport of nutrients by the small-intestinal mucosa) and possible mechanisms.

Another salient finding of this study is the high requirements of serine and aspartate by pig small-intestinal bacteria (Fig. 1). These results support the previous work that serine was the most rapidly metabolized AA in *E. coli* and stimulated the growth of *Streptococcus* sp. and *Klebsiella* sp. (Chaussee et al. 2003; Dai et al. 2011b; Prüß et al. 1994; Vining and Magasanik 1981). Specifically, *E. coli*, *Klebsiella* sp., and lactic acid bacteria including *Streptococcus* sp. harbor the activity of L-serine dehydratase/L-serine deaminase (Chaussee et al. 2003; Fernández and Zúñiga 2006; Sawers 1998; Vining and Magasanik 1981). The major products from the catabolism of serine via serine dehydratase (a pyridoxal phosphate-dependent enzyme) are pyruvate and ammonia. The production of pyruvate and ammonia functions to not only regulate nitrogen balance but also maintain the extracellular pH of the bacteria, and as a consequence, plays an important role in the bacterial adaption to the changes of the surrounding environments (Chaussee et al. 2003; Vining and Magasanik 1981). Serine can also be converted to glycine by serine hydroxymethyltransferase (Flynn et al.2010). Meanwhile, recent findings indicated the enrichment of one of the virulence phosphopeptides, the serine-aspartate-rich fibrinogen/bone sialoprotein binding protein (SdrE), in the pathogenic bacteria *Staphylococcus aureus* (Burnside et al. 2010). Interestingly, we found the complete utilization of aspartate in cultures of *E. coli* and *Klebsiella* sp., while the rate of aspartate utilization was quite low in *Streptococcus* sp. These results suggest variations in patterns of AA assimilation and the production of nitrogenous compounds by bacteria, especially pathogenic bacteria. To date, it is not clear how much serine and aspartate metabolism in small-intestinal bacteria contributes to the bacterial colonization and production of virulence factors in the intestine.

The high metabolic requirements of glutamine and asparagine in *E. coli* and glutamine affect the bacterial utilization of asparagine especially in *Streptococcus* sp. and *Klebsiella* sp. (Table 1; Fig. 1). These results suggest that the metabolism of glutamine and asparagine in small-intestinal bacteria might be closely related to each other. Recent studies indicated that the asparaginase from *E. coli* not only hydrolyzes asparagine but also glutamine at low rates (Fernández and Zúñiga 2006). In clinical practice, asparaginase has been used for the treatment of acute childhood lymphoblastic leukemia (Dhavala et al. 2008). However, due to the glutaminase activity of *E. coli* asparaginase, the depletion of glutamine in the circulation affected body protein synthesis and have deleterious influence on liver and immune functions. Thus, screening for asparaginase that has little glutaminase activity would be required in this regard (Dhavala et al. 2008; Ollenschläger et al. 1988). This might also help to explain the detrimental effect of the overgrowth of pathogenic

bacteria in the small intestine especially bacteria with high glutaminase activity. Moreover, it was also found that both *Streptococcus* sp. and *Klebsiella* sp. possessed asparaginase and glutaminase/glutamine cyclotransferase (Chen and Russell 1989; Fernández and Zúñiga 2006). However, the interaction of the asparaginase and glutaminase/glutamine cyclotransferase in both bacteria is not clear. It might be important to uncover the interplay of the two enzymes and the role in the bacterial metabolism of AA and adaptation to gut environment and their links to intestinal health.

Results from this study also indicate that glutamine might regulate the compartmental AA metabolism in the small intestine through different mechanisms. First, glutamine affected the flux distribution of arginine metabolism in both jejunal microbiota and ileal microbiota. The reduction in the net utilization of citrulline in the small-intestinal bacteria might result from the increased flux of ornithine into citrulline in jejunal microbiota and increased fluxes of arginine into ornithine and citrulline in the ileal microbiota. The result suggests that the effect of glutamine on the nitrogen utilization of the small intestine might partially result from changes in intestinal arginine degradation. This further supports the view that the compartmental AA metabolism in small-intestinal bacteria might play an important role in nitrogen cycling in the small intestine (Bergen and Wu 2009; Fuller and Reeds 1998). Second, the flux from threonine to glycine, serine, and isoleucine (Sawers 1998) in small-intestinal bacteria might be affected by glutamine in a compartmentation-dependent manner. In the jejunal microbiota, increased utilization of threonine and decreased utilization of serine and isoleucine indicate that the increased flux of threonine to glycine, serine and isoleucine. However, the overall flux of threonine to glycine and serine might be inhibited by glutamine in the ileal microbiota. The central domain of the gut epithelium mucin protein backbone is rich in threonine, serine and glycine (Montagne et al. 2004). On one hand, the modulation of the luminal threonine pool in the small intestine by glutamine might affect mucin structure and synthesis in the small intestine, thereby regulating the morphology of the gut epithelium and nutrient absorption (Wang et al. 2007). On the other hand, effects of bacterial glutamine metabolism on mucin formation along the small intestine might affect the bacterial colonization and community structure. Although it is not clear whether AA modulate microbial community composition in the intestine, the compartmental AA metabolism in the gut might partially be due to differences in the biochemical characteristics of the digesta and the microbial community composition and activity. Therefore, it is important to identify changes in the compartmental metabolism of dietary compounds in the intestine with an emphasis on the structure and activity of the microbiota (Claus et al. 2008).

Collectively, variations in AA utilization and metabolism occur in pig small-intestinal bacteria. Glutamine displays a divergent regulatory role in the bacterial metabolism of AA in a bacterial species- and gut compartment-dependent manner. The utilization and metabolism of glutamine by small-intestinal bacteria may function as a nitrogen donor in various biosynthetic reactions and may also control cellular nitrogen balance (Forchhammer 2007). Therefore, increased extracellular concentrations of glutamine might initiate the signaling pathways related to the metabolism of nitrogenous compounds in bacteria as previously reported for mammalian cells (Wu et al. 2011a; Xi et al. 2011a, b). As a consequence, the flux distributions of the metabolic networks of either individual bacterial species or complex microbiota in the small intestine could be altered to influence the microbial community structure and function (Almaas et al. 2004; Almaas 2007; Samal 2008). Additionally, studying the metabolic variations of different bacterial species and their response to the extracellular concentrations of substrates (e.g., glutamine and arginine) provide us with opportunities unraveling the metabolic interactions within the microbial community and the metabolic outcome (Dai et al. 2011b; Shiloach et al. 2010). New strategies could be developed for the modulation of the AA metabolic networks in small-intestinal bacteria with the goal of maintaining robust gut ecology and function.

In conclusion, this study showed the dose-dependent utilization of glutamine in porcine small-intestinal bacteria. Glutamine regulated the bacterial metabolism of the arginine family of AA, and serine and aspartate family of AA in a species- and gut compartment-dependent manner. These findings help to explain the functional role of glutamine in the regulation of the AA metabolism in the small intestine and promotion of gut health (Wang et al. 2008; Wu et al. 2007b, 2009) and may have important implications for protein nutrition in ruminants which have large amounts of bacteria in the rumen (Satterfield et al. 2011, 2012). Further studies are warranted to unravel the interplay between AA and small-intestinal bacteria and to better understand the functional aspects of dietary AA and the gut microbiota in nutrition and health.

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

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