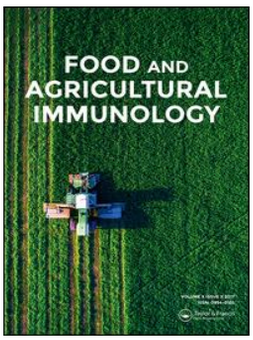

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Effects of glutamate and aspartate on growth performance, serum amino acids, and amino acid transporters in piglets

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ABSTRACT

This study mainly investigated the effects of different dietary levels of glutamate (Glu) and aspartate (Asp) on growth performance, blood amino acids, and amino acid transporters in piglets. Forty-two healthy piglets were randomly divided into six groups ($n = 7$): a control group in which piglets were fed 2.9% Glu and 1.5% Asp and other groups in which piglets received 1.3% or 1.7% Asp and 2.6%, 3.2%, or 3.5% Glu for 21 days. Growth performance, serum amino acid profiles from the mesenteric vein, portal vein, and anterior vena cava, and amino acid transporters in the liver were determined. The results showed that lower doses of Asp promoted growth and enhanced the amino acids, while high doses of Asp and Glu reduced growth and the amino acid pool in piglets ($P < 0.05$). Meanwhile, 3.2% Glu increased branched chain amino acids in the portal vein and anterior vena cava ($P < 0.05$). 3.5% Glu downregulated SLC7A1, SLC7A7, and SLC6A19 expression in the liver ($P < 0.05$). Collectively, these results indicated that different dietary doses of Glu and Asp influenced growth performance and serum amino acid.

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Abbreviations: Ala: alanine; Arg: arginine; Asp: aspartate; ADFI: average daily feed intake; ADG: average daily gain; Cys: cystine; F/G: feed intake/gain; Glu: glutamate; Gly: glycine; His: histidine; Ile: isoleucine; Leu: leucine; Lys: lysine; Met: methionine; Phe: phenylalanine; Pro: proline; Ser: serine; TAA: total amino acid; Thr: threonine; Try: tryptophan; Tyr: tyrosine; Val: valine; SLC1A1: solute carrier family 1 member 1; SLC7A1: solute carrier family 7 member 1; SLC7A7: solute carrier family7 member 7; SLC6A19: solute carrier family 6 member 19

Introduction

Glutamate (Glu) and aspartate (Asp), two acidic amino acids, are considered as non-essential amino acids in classical nutrition (Wu, 2010). Glu and Asp have been indicated to play important roles in physiological and biochemical processes (Duan et al., 2014; Wang et al., 2016). For example, Glu and Asp serve as major sources of energy and improve intestinal and liver function by alleviating oxidative stress (Duan et al., 2016; Leng et al., 2014; Lin et al., 2014). Meanwhile, dietary supplementations with Glu and Asp have been reported to enhance growth performance (Yin, Liu, et al., 2015), act as excitatory neurotransmitters in the brain (Wang, Wang, Xia, & Wood, 2014), and mediate feeding behaver (Khropycheva, Andreeva, Uneyama, Torii, & Zolotarev, 2011). Bin *et al* reported that 1% Asp lowered the ratio of *Firmicutes*: *Bacteroidetes* in mice and affected the innate immunity(Bin et al., 2017). Duan *et al* reported that 2% glutamate acted as a nutritional regulating factor to ameliorate the adverse effects of mycotoxins and improved growth performance in mycotoxins-challenged pigs (Duan et al., 2014) . Although various studies indicate that Glu and Asp protect piglets against different pathological conditions, such as oxidative stress and mycotoxin infection, little is known about the effects of different dietary contents of Glu and Asp on growth performance and amino acid metabolism in healthy piglets. Thus, the present study examined the amino acid metabolic responses to dietary Glu and Asp in piglets and growth performance was also focused.

Materials and methods

Animal and diets

All animal procedures were approved by the Committee on Animal Care of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (Changsha, Hunan Province, China). Forty-two piglets (Duroc × Landrace × Yorkshire, male, 13.24 ± 0.25 kg) (Hunan New Wellful Co., Ltd., Hunan Province, China) were randomly assigned into one of six treatment groups (n = 7/group). Piglets in the control group (CG) were fed a basic diet containing 2.9% Glu and 1.5% Asp according to our previous study (Wu et al., 2015). Other piglets received 1.3% (LA) or 1.7% (HA) Asp and 2.6% (LG), 3.2% (HG), or 3.5% (HHG) Glu for 21 days, respectively (Table 1). Each animal was housed in a single cage. Piglets had free access to drinking water and diets during the experimental period.

Growth performance

During the experimental period, feed intake was recorded daily and all piglets were weighed individually at day 0 and 21. Average daily feed intake (ADFI), average daily

Table 1. Composition and nutrient levels of experimental diets for piglets (air-dried basis, %).

Feed ingredients (%)	LA	CG	HA	LG	HG	HHG
Extruded soybean	7	7	7	7	7	7
Soybean meal	13	12	11	12.5	11	10
Fish meal	3	3	3	3	3	3
Extruded Maize	33	33	33	33	33	33
Corn	32.6	33	33.9	33	33.9	34
Soybean oil	0.95	1	0.85	0.9	0.85	0.85
Glucose	0.5	0.5	0.5	0.5	0.5	0.5
$CaHPO_4$	1	1	1	1	1	1
Limestone	0.5	0.5	0.5	0.5	0.5	0.5
NaCl	0.40	0.40	0.40	0.40	0.40	0.40
Dried whey	3	3	3	3	3	3
Sucrose	2	2	2	2	2	2
Citric acid	0.8	0.8	0.8	0.8	0.8	0.8
ZnO	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1
Lysine	0.655	0.685	0.715	0.67	0.715	0.745
Methionine	0.2	0.21	0.22	0.205	0.22	0.23
Threonine	0.23	0.24	0.26	0.235	0.255	0.27
Tryptophan	0.06	0.065	0.07	0.06	0.065	0.07
Multi-vitamin[a]	0.04	0.04	0.04	0.04	0.04	0.04
Microelement[a]	0.15	0.15	0.15	0.15	0.15	0.15
Aspartate	0.07	0.32	0.57	0.2	0.26	0.31
Glutamate	0	0.07	0.13	0	0.5	1
Rice mill by-product	0.445	0.62	0.495	0.44	0.445	0.735
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition						
Digestible energy MJ/kg	14.61	14.61	14.61	14.61	14.61	14.61
Crude protein	17.16	17.16	17.16	17.16	17.16	17.16
Calcium	0.70	0.70	0.70	0.70	0.70	0.70
Total phosphorus	0.58	0.58	0.58	0.58	0.58	0.58
Available phosphorus	0.33	0.33	0.33	0.33	0.33	0.33
Lys	1.23	1.23	1.23	1.23	1.23	1.23
Met	0.68	0.68	0.68	0.68	0.68	0.68
Thr	0.73	0.73	0.73	0.73	0.73	0.73
Trp	0.20	0.20	0.20	0.20	0.20	0.20
Asp	1.3	1.5	1.7	1.5	1.5	1.5
Glu	2.9	2.9	2.9	2.6	3.2	3.5

[a]The premix provided the following per kg of diet:nicotinic acid 50 mg, pantothenic acid 5 mg, folic acid 2 mg, biotin 0.2 mg, VA 10800IU, VD_3 4000IU, VE 40IU, VK_3 4 mg, VB_1 6 mg, VB_2 12 mg, VB_6 6 mg,VB_{12} 0.05 mg, Cu 5 mg, Fe 80 mg, Mn 3 mg, Zn 85 mg,0.1 mg, Se 0.3 mg.

weight gain (ADG), and the ratio of feed intake to weight gain (F/G) were calculated according to the feed consumption and weight of each piglet.

Amino acid determination

Blood samples were obtained from the mesenteric vein, hepatic portal vein, and anterior vena cava after anesthetic treatment using Zoletil 50 (Virbac S.A., France). Serum was prepared via centrifugation at 3000 g and 4°C for 10 min and stored at −80°C until analysis (Fang et al., 2017). Eighteen amino acids (Lys, Met, Thr, Trp, Glu, Asp, Val, Ile, Leu, Phe, Arg, Ser, His, Gly, Ala, Pro, Cys, and Tyr) in the serum were detected via 1260 liquid chromatograph (Agilent 1260) according to our previous reports (Duan et al., 2016; Liao et al., 2017).

Real-time quantitative (RT–PCR)

Total RNA from liver samples was isolated with TRIZOL reagent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) (Meng et al., 2017; Reschke et al., 2017).

Table 2. Primers used in this study.

Gene	Gene bank no.	Sequence (5′–3′)	Product length (bp)
SLC1A1	NM_001164649.1	F:AGTGAGCCAGAGACGAATGG R:AAACAATCAAGCCCAGGACA	73
SLC7A1	NM_001012613.1	F:TCTGGTCCTGGGCTTCATAA R:ACCTTCGTGGCATTGTTCAG	123
SLC7A7	NM_001110421.1	F:GAGTGCCAGAACACAAACGA R:TCCTCCATCTTCCAAATCCA	116
SLC6A19	XM_003359855.3	F:TCATCTTCCTCTTCTTCTTCGTG R:CTTGACCTTCTGGGATTTGG	101
β-Actin	XM_003124280.4	F:CTGCGGCATCCACGAAACT R:AGGGCCGTGATCTCCTTCTG	147

All primer sequences were designed based on the accession numbers given above.

Reverse transcription was performed at 37°C for 15 min, 95°C 5 sec. Primers used in this study were designed using Primer 5.0 according to the pig gene sequence (Table 2). β-actin was chosen as the house-keeping gene to normalize target gene levels. The PCR cycling conditions were 36 cycles at 94°C for 40 sec, 60°C for 30 sec and 72°C for 35 sec. Relative expression was expressed as a the ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct=(Ct_{Target}\text{-}Ct_{\beta\text{-actin}})_{treatment}\text{-}(Ct_{Target}\text{-}Ct_{\beta\text{-actin}})_{control}$ (Feng et al., 2015; Gupta et al., 2017; Lin et al., 2016; Sohail, Doran, Riedemann, Macaulay, & Southern, 2003). Relative expression was normalized and expressed relative to the expression in the control group according to our previous report (Gupta et al., 2017; Reschke et al., 2017; Yin et al., 2013; Yin et al., 2017).

Statistical analysis

All statistical analyses were performed using IBM SPSS 20 software. The data were performed by using the one-way analysis of variance (ANOVA) to test homogeneity of variances via Levene's test and followed with student's t test Data are expressed as mean ± SEM. Values in the same row with * are significant ($P < 0.05$)

Results

Effect of Asp and Glu on growth performance in piglets

Effect of Asp on growth performance was summarized in Table 3. There was no significant difference in body weight of piglets compared with control group ($P > 0.05$). Interestingly, ADG in the LA group was higher in the control group and F/G was markedly lower in the LA group ($P < 0.05$). However, dietary high Asp (1.7%) markedly decreased ADFI and ADG but increased F/G ($P < 0.05$), suggesting that low Asp improve growth but high dosage of Asp exert negative effect.

Table 3. Effects of Asp on growth performance in piglets.

Item	LA	CG	HA
Initial body weight (kg)	13.31 ± 1.10	13.21 ± 0.58	13.31 ± 0.84
Final body weight (kg)	23.23 ± 1.50	21.44 ± 1.05	18.58 ± 1.00
Average daily feed intake (g/d)	911 ± 49	840 ± 44	688 ± 47*
Average daily gain (g/d)	551 ± 26*	457 ± 28	330 ± 28*
Feed conversion ratio	1.66 ± 0.05*	1.85 ± 0.06	2.12 ± 0.11*

Data are expressed as the mean ± SEM ($n = 7$). *means the difference was significant compared with the control group.

Dietary supplementation with different dosages of Glu failed to affect body weight in piglets ($P > 0.05$; Table 4). However, dietary high Glu significantly reduced ADG and increased F/G ($P < 0.05$). Moreover, ADFI in the HHG group was markedly lower than that in the control group ($P < 0.05$). These results suggested that excess Glu intake inhibit growth performance in piglets.

Effect of dietary Asp on the circulating amino acids in piglets

The levels of amino acids in the mesenteric vein, hepatic portal vein, and anterior vena cava were determined to evaluate amino acid metabolic response to dietary amino acids. In the mesenteric pool, different doses of Asp in the diet failed to affect the levels of Asp and other amino acids ($P > 0.05$; Table 5). In the hepatic portal, Trp and Tyr levels were significantly higher in the LA group than those in the control group ($P < 0.05$). In the anterior vena cava, Glu level in the HA group was markedly decreased compared with the control group ($P < 0.05$).

Effect of dietary Glu on the circulating amino acids in piglets

In the mesenteric pool, Met and Tyr were decreased in the HHG group, while Glu was increased in the HG group compared with the control group ($P < 0.05$; Table 6). In the hepatic portal, Trp, Val, Ile, Leu, and Ser levels were higher in the HG group than those in the control group. Trp in HHG group and Ser in LG group were markedly increased ($P < 0.05$). In the anterior vena cava, Ile and Tyr were decreased in the LG group while His was increased compared with the control group ($P < 0.05$). Moreover, Glu and Tyr in the HG group were decreased ($P < 0.05$) and Ile, Leu, and Tyr in the HHG group were also decreased compared with control group ($P < 0.05$).

Liver expression of amino acid transporters

In this study, expressions of SLC1A1, SLC7A1, SLC7A7, and SLC6A19 were determined via RT–PCR (Figure 1). Low-dose Asp down-regulated SLC6A19 expression in the liver ($P < 0.05$). Moreover, expressions of SLC7A1, SLC7A7 and SLC6A19 were markedly down-regulated in the HHG group ($P < 0.05$) mRNA in the liver. Also, dietary high Glu significantly down-regulated SLC6A19 expression compared with the control group ($P < 0.05$).

Table 4. Effects of Glu on growth performance in piglets.

Item	LG	CG	HG	HHG
Initial body weight (kg)	13.24 ± 0.54	13.21 ± 0.58	13.24 ± 0.50	13.31 ± 0.54
Final body weight (kg)	20.38 ± 0.42	21.44 ± 1.05	19.90 ± 0.84	20.20 ± 0.44
Average daily feed intake (g/d)	771 ± 33	840 ± 44	790 ± 42	687 ± 38*
Average daily gain (g/d)	400 ± 19	457 ± 28	364 ± 26*	374 ± 27
Feed conversion ratio	1.99 ± 0.06	1.85 ± 0.06	2.16 ± 0.02*	1.88 ± 0.04

Data are expressed as the mean ± SEM ($n = 7$). *means the difference was significant compared with the control group.

Table 5. Effect of dietary Asp on the circulating amino acids in piglets (μg/mL).

Item		LA	CG	HA
mesenteric vein.	Lys	52.48 ± 3.60	47.59 ± 4.32	50.96 ± 1.04
	Met	12.12 ± 0.94	13.62 ± 1.17	11.94 ± 1.67
	Thr	23.72 ± 2.41	20.06 ± 2.43	25.28 ± 2.55
	Trp	7.24 ± 0.41	6.40 ± 0.71	6.47 ± 0.87
	Asp	8.33 ± 0.98	6.43 ± 0.62	7.00 ± 0.57
	Glu	99.28 ± 9.78	83.64 ± 7.85	82.48 ± 3.89
	Val	25.68 ± 2.52	24.88 ± 3.64	26.02 ± 2.91
	Ile	19.36 ± 2.05	16.77 ± 2.27	17.17 ± 1.85
	Leu	33.07 ± 2.63	28.18 ± 3.38	30.09 ± 2.69
	Phe	16.14 ± 0.94	14.91 ± 1.36	15.30 ± 0.82
	Arg	24.58 ± 4.70	22.02 ± 4.98	19.20 ± 3.57
	Ser	32.02 ± 2.98	32.30 ± 1.92	38.34 ± 4.29
	His	8.09 ± 0.71	6.56 ± 0.98	7.07 ± 0.65
	Gly	127.99 ± 9.16	131.60 ± 9.99	129.70 ± 18.67
	Ala	91.90 ± 7.15	84.29 ± 7.57	79.49 ± 6.25
	Pro	35.89 ± 1.24	35.33 ± 2.50	32.86 ± 2.76
	Cys	1.67 ± 0.39	1.47 ± 0.34	1.89 ± 0.57
	Tyr	22.89 ± 1.45	21.71 ± 1.67	19.79 ± 0.6
portal vein	Lys	53.52 ± 4.57	46.69 ± 5.04	49.58 ± 2.37
	Met	12.36 ± 0.96	12.97 ± 1.28	11.70 ± 1.50
	Thr	23.72 ± 2.70	19.27 ± 2.67	24.38 ± 3.43
	Trp	7.75 ± 0.62**	5.67 ± 0.67	6.09 ± 0.62
	Asp	8.56 ± 1.54	7.26 ± 1.18	7.85 ± 1.54
	Glu	107.07 ± 12.41	81.94 ± 7.13	88.92 ± 10.24
	Val	26.58 ± 3.22	23.37 ± 3.61	24.98 ± 2.51
	Ile	19.81 ± 2.51	16.53 ± 2.16	16.17 ± 1.41
	Leu	33.76 ± 3.52	27.71 ± 3.28	28.37 ± 2.21
	Phe	16.65 ± 1.41	14.17 ± 1.20	14.32 ± 1.06
	Arg	21.80 ± 4.69	16.60 ± 6.02	14.58 ± 2.60
	Ser	32.17 ± 2.79	31.39 ± 2.28	40.37 ± 6.39
	His	7.96 ± 1.06	6.76 ± 0.86	7.28 ± 0.62
	Gly	124.97 ± 8.57	128.29 ± 10.56	134.82 ± 22.14
	Ala	91.52 ± 7.72	79.12 ± 6.94	78.80 ± 7.96
	Pro	35.96 ± 2.19	32.99 ± 2.23	31.98 ± 2.48
	Cys	2.18 ± 0.56	1.65 ± 0.41	2.05 ± 0.84
	Tyr	23.25 ± 1.65*	19.29 ± 1.67	19.61 ± 1.21
anterior vena cava	Lys	43.50 ± 2.49	41.42 ± 3.00	43.99 ± 2.18
	Met	10.93 ± 0.98	13.02 ± 1.10	10.98 ± 1.52
	Thr	19.76 ± 2.26	18.02 ± 1.65	22.50 ± 3.04
	Trp	6.46 ± 0.38	5.70 ± 0.50	5.01 ± 0.56
	Asp	6.67 ± 0.72	6.20 ± 1.12	4.82 ± 0.36
	Glu	85.11 ± 9.36	73.96 ± 4.40	61.49 ± 5.33*
	Val	21.89 ± 2.41	22.97 ± 2.79	21.98 ± 2.56
	Ile	16.17 ± 1.73	15.12 ± 1.48	13.56 ± 1.44
	Leu	26.46 ± 2.13	24.85 ± 2.01	24.08 ± 1.50
	Phe	13.37 ± 0.82	13.35 ± 0.72	12.71 ± 0.87
	Arg	16.94 ± 3.13	18.52 ± 3.27	19.19 ± 2.00
	Ser	28.25 ± 2.78	29.86 ± 1.67	33.66 ± 4.33
	His	5.33 ± 0.71	4.47 ± 0.45	5.00 ± 0.26
	Gly	116.17 ± 8.89	126.23 ± 9.51	129.18 ± 18.94
	Ala	77.04 ± 6.24	71.78 ± 6.44	64.00 ± 4.35
	Pro	29.43 ± 1.09	30.45 ± 1.29	27.10 ± 1.30
	Cys	1.47 ± 0.29	1.28 ± 0.37	0.88 ± 0.30
	Tyr	19.54 ± 0.85	18.64 ± 1.53	18.65 ± 0.89

Data are expressed as the mean ± SEM ($n = 6$). *means the difference was significant compared with the control group.

Table 6. Effect of dietary Glu on the circulating amino acids in piglets(μg/mL).

Item		LG	CG	HG	HHG
mesenteric vein	Lys	54.84 ± 7.67	47.59 ± 4.32	64.28 ± 5.55*	50.15 ± 1.82
	Met	12.64 ± 1.79	13.62 ± 1.17	13.90 ± 1.83	10.29 ± 1.10*
	Thr	26.09 ± 3.44	20.06 ± 2.43	24.82 ± 3.35	21.35 ± 1.99
	Trp	6.75 ± 0.95	6.40 ± 0.71	7.70 ± 1.09	5.92 ± 0.38
	Asp	7.57 ± 1.13	6.43 ± 0.62	7.86 ± 1.20	6.78 ± 0.91
	Glu	89.79 ± 6.97	83.64 ± 7.85	86.18 ± 8.08	84.06 ± 10.40
	Val	28.72 ± 3.35	24.88 ± 3.64	31.94 ± 3.57	21.82 ± 1.04
	Ile	19.19 ± 2.09	16.77 ± 2.27	22.35 ± 2.82	14.89 ± 1.09
	Leu	33.12 ± 3.95	28.18 ± 3.38	37.57 ± 3.75	27.15 ± 1.65
	Phe	16.73 ± 2.11	14.91 ± 1.36	18.34 ± 2.09	14.91 ± 0.31
	Arg	25.00 ± 3.76	22.02 ± 4.98	33.35 ± 7.16	18.06 ± 4.33
	Ser	35.92 ± 3.48	32.30 ± 1.92	39.41 ± 3.17	33.10 ± 2.41
	His	8.37 ± 1.36	6.56 ± 0.98	9.31 ± 1.22	7.00 ± 0.31
	Gly	127.56 ± 10.84	131.60 ± 9.99	132.63 ± 2.64	132.59 ± 15.79
	Ala	77.99 ± 8.89	84.29 ± 7.57	82.42 ± 7.49	80.88 ± 3.67
	Pro	33.44 ± 3.59	35.33 ± 2.50	37.13 ± 3.43	31.94 ± 1.81
	Cys	2.56 ± 0.51	1.47 ± 0.34	1.72 ± 0.28	1.97 ± 0.57
	Tyr	20.08 ± 2.24	21.71 ± 1.67	21.94 ± 2.06	16.65 ± 0.74*
portal vein	Lys	46.51 ± 3.46	46.69 ±5.04	53.70 ± 2.07	50.05 ± 2.36
	Met	10.78 ± 0.82	12.97 ± 1.28	12.05 ± 1.15	10.84 ± 0.97
	Thr	23.03 ± 3.89	19.27 ± 2.67	19.84 ± 1.82	21.35 ± 1.69
	Trp	5.75 ± 0.88	5.67 ± 0.67	6.77 ± 0.47*	6.09 ± 0.24*
	Asp	7.24 ± 1.03	7.26 ± 1.18	6.57 ± 0.59	7.55 ± 0.99
	Glu	94.62 ± 12.15	81.94 ± 7.13	85.60 ± 7.84	94.59 ± 12.74
	Val	25.17 ± 2.04	23.37 ± 3.61	28.15 ± 1.71**	20.76 ± 1.43
	Ile	16.08 ± 3.03*	16.53 ± 2.16	18.49 ± 1.33*	13.46 ± 1.57
	Leu	28.55 ± 1.93	27.71 ± 3.28	31.13 ± 1.33*	25.90 ± 2.25
	Phe	14.16 ± 1.00	14.17 ± 1.20	15.08 ± 0.55	14.61 ± 0.62
	Arg	17.41 ± 1.26	16.60 ± 6.02	22.14 ± 3.86	22.03 ± 4.28
	Ser	32.76 ± 3.21	31.39 ± 2.28	37.11 ± 2.45*	39.48 ± 3.37*
	His	6.72 ± 0.98	6.76 ± 0.86	6.77 ± 0.49	7.35 ± 0.46
	Gly	116.37 ± 12.60	128.29 ± 10.56	116.74 ± 5.17	139.78 ± 15.58
	Ala	70.11 ± 6.68	79.12 ± 6.94	71.72 ± 3.43	84.15 ± 4.30
	Pro	29.05 ± 2.46	32.99 ± 2.23	31.34 ± 1.16	32.42 ± 1.83
	Cys	2.78 ± 0.32*	1.65 ± 0.41	1.53 ± 0.25	1.67 ± 0.45
	Tyr	17.27 ± 1.03	19.29 ± 1.67	14.61 ± 3.40	16.54 ± 0.65
anterior vena cava	Lys	43.12 ± 2.84	41.42 ± 3.00	48.63 ± 3.23	44.62 ± 1.00
	Met	10.75 ± 1.23	13.02 ± 1.10	11.55 ± 1.67	9.80 ± 0.78
	Thr	21.49 ± 3.15	18.02 ± 1.65	19.65 ± 2.27	19.53 ± 1.21
	Trp	5.00 ± 0.95	5.70 ± 0.50	5.57 ± 0.69	5.06 ± 0.28
	Asp	5.00 ± 0.52	6.20 ± 1.12	5.09 ± 0.39	6.05 ± 0.58
	Glu	66.62 ± 5.42	73.96 ± 4.40	60.99 ± 6.17*	70.50 ± 6.37
	Val	23.31 ± 2.78	22.97 ± 2.79	24.02 ± 2.33	18.31 ± 1.89
	Ile	14.86 ± 2.62*	15.12 ± 1.48	15.57 ± 1.78	11.26 ± 1.51*
	Leu	25.08 ± 1.8	26.64 ± 2.01	26.61 ± 1.70	21.29 ± 1.89*
	Phe	13.18 ± 0.91	13.35 ± 0.72	13.58 ± 0.97	12.62 ± 0.56
	Arg	20.66 ± 1.52	18.52 ± 3.27	23.57 ± 2.68	14.89 ± 2.48
	Ser	28.54 ± 2.20	29.86 ± 1.67	34.12 ± 1.86	33.50 ± 2.91
	His	4.95 ± 0.60	4.47 ± 0.45	5.48 ± 0.49*	5.50 ± 0.28
	Gly	117.91 ± 10.92	126.23 ± 9.51	125.82 ± 2.54	135.23 ± 13.86
	Ala	63.66 ± 3.27	71.78 ± 6.44	65.86 ± 4.45	72.59 ± 3.45
	Pro	26.82 ± 1.40*	30.45 ± 1.29	28.69 ± 1.42	28.54 ± 1.18
	Cys	1.61 ± 0.29	1.28 ± 0.37	0.94 ± 0.21	0.73 ± 0.18
	Tyr	15.84 ± 1.44	18.64 ± 1.53	14.13 ± 2.41*	14.43 ± 0.52*

Data are expressed as the mean ± SEM ($n = 6$). *means the difference was significant compared with the control group.

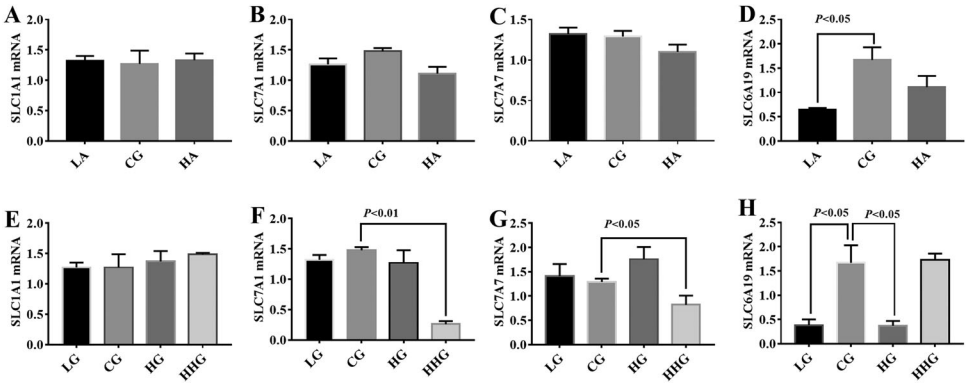

Figure 1. Effects of dietary Asp (A, B, C, D) and Glu (E, F, G, H) on amino acid transporters in the liver. Data are presented as mean ± SEM, $n = 6$. SLC1A1 solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system XAG, member 1), SLC7A1 solute carrier family 7 (cationic amino acid transporter, y + system; member 1), SLC7A7 solute carrier family7 (amino acid transporter light chain, y + L system, member 7), and SLC6A19 solute carrier family 6 (neurotransmitter/neutral amino acid transporter, member 19).

Discussion

Glu and Asp play important roles in the nervous system, immunity, and nutrition in animals and humans (Khropycheva et al., 2011; Leng, 2014; Lin et al., 2014; Wang et al., 2016; Yanni et al., 2010). Our previous studies showed that dietary supplementation with Asp and Glu reduced oxidative stress-induced growth suppression (Duan et al., 2016; Yin, Liu, et al., 2015). Shi *et al.* also reported that dietary 0.5% and 1% Asp improved ADG in piglets after exposure to lipopolysaccharide (Shi, 2013). Meanwhile, dietary 2% Glu attenuated mycotoxin-induced growth suppression by improving ADFI, ADWG, and the feed/weight ratio in piglets (Wu, 2014). The animals may change in diet and bring about poor growth performance under weaning stress (Li et al., 2017; Ren et al., 2018). Thus, we investigated dietary Asp and Glu in healthy piglets with initial weight 10 kg and pre-feeding for a week. In the current study indicated that dietary supplementation with high dosages of Asp and Glu reduced growth performance and diets with low levels of Asp or Glu improved growth performance in piglets. Together, we speculated that low doses of Asp and Glu may satisfy the nutritional requirements in healthy piglets, while supplementations with excess Asp and Glu exhibit a negative effect on growth performance under healthy conditions.

Serum amino acid levels are highly associated with animal health and growth performance (Li et al., 2016; Liao et al., 2017; Wu et al., 2014; Yin, Ren, et al., 2015). Previous studies have shown that dietary supplementation with 2.0% Glu, 0.5% −2.0% glutamine, 1.0% Asp or their combinations reduced oxidized stress or a pathological amino acid profile in weaned piglets (Duan et al., 2016; Ren et al., 2014). Moreover, Glu and Asp can transform alanine, citrulline, glutathione, ornithine and arginine and other precursor substances (Wu et al., 2007; Zhang et al., 2012). A portion of the free amino acids in the intestine are selectively absorbed into the mesenteric vein, and some amino acids, such as Glu and Asp, are used by the intestine to maintain intestinal health (Deutz, Bruins, & Soeters, 1998; Pi et al., 2014; Stoll & Burrin, 2006; Xi et al., 2012). Portal vein blood is

collected by the stomach, intestine, spleen, and other organs (extracorporeal tissue, Portal-Drained Viscera, PDV) (Stoll et al., 1998). Studies have shown that the liver contains high levels of Glu and Asp under low crude protein diets (17% crude protein) (Zheng, 2015). Meanwhile, Glu and Asp are absorbed and oxidized in the intestine and liver to maintain the intestinal and liver structure and function (Jinap & Hajeb, 2010; Leng et al., 2014; Wang et al., 2015; Wang et al., 2017). In the current work, only Glu levels in the anterior chamber of the HA and HG group were markedly decreased. The reason may be explained by that low doses of Glu and Asp meet the needs of the body, thus extra Glu and Asp may be oxidized by intestinal epithelial cells and liver cells to provide energy.

Zhenyukh *et al.* found that pathological conditions increased plasma levels of branched chain amino acids (Zhenyukh et al., 2017). In this study, the hepatic portal vein in the HG group (3.2% Glu group) contained the highest levels of branched chain amino acids, which may explain why 3.2% Glu appeared to have a negative effect on growth performance. Try is a precursor of the neurotransmitter serotonin, which promotes feed intake (Burgoon, Knabe, & Gregg, 1992; Eder, Nonn, Kluge, & Peganova, 2003; Kendall, Gaines, Kerr, & Allee, 2007), reduces energy waste, and enhances feed utilization (Zhang et al., 2013). Meanwhile, tryptophan inhibits xanthine oxidase activity and reactive oxygen, as well as oxidative stress (Lv et al., 2012). The current study showed that the portal vein of the LA, HG, and HHG group showed a high tryptophan level, which might affect growth performance and amino acid metabolism and detailed mechanisms should be further investigated.

Amino acids are mainly sensed and transported by specific amino acid transporters (Burckhardt Birgitta & Burckhardt, 2017; Yin et al., 2017; Zuo et al., 2015). For example, SLC1A1 transports Asp and Glu and SLC7A1 contributes to Lys, Arg, and His uptake (Verrey, Meier, Rossier, & Kuhn, 2000). In this study, high dosage of Glu downregulated SLC7A1, SLC7A1, and SLC6A19 expressions, which may be associated with the feedback regulation as excess of amino acids are toxic to cells. SLC6A19 transports all neutral amino acids and is the only transporter of Trp in the small intestine (Bröer et al., 2004). Thus, we speculated that SLC6A19 may mediate Trp metabolism in this study.

Conclusions

Low levels of Glu and Asp in the diet increased growth performance of piglets and the serum tryptophan level. High doses of Glu and Asp, especially 3.2% Glu, inhibited the growth of piglets, increased BCAA levels in the hepatic portal vein and may have toxicological effects.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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