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Effects of Different Dietary Levels of Glutamate and Aspartate on Organ Indices, Serum Biochemical Parameters, and Hormone Content in Piglets: Postprint

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Abstract

This experiment was conducted to investigate the effects of different dietary glutamic acid and aspartic acid levels on organ indices, serum biochemical indices, and hormone content in piglets. Forty-two healthy 35-day-old three-way crossbred (Duroc × Landrace × Yorkshire) weaned piglets were randomly allocated to 6 groups with 7 replicates per group and 1 pig per replicate. The control group (NC group) had dietary glutamic acid and aspartic acid levels of 2.9% and 1.5%, respectively. The other groups were modified from the NC group to have dietary glutamic acid and aspartic acid levels of 2.9% and 1.3% (LA group), 2.9% and 1.7% (HA group), 2.6% and 1.5% (LG group), 3.2% and 1.5% (HG group), and 3.5% and 1.5% (HHG group), respectively. The trial lasted 21 days. At the end of the trial, organ indices, serum biochemical indices, and hormone content in piglets were determined. The results showed: 1) Different dietary glutamic acid and aspartic acid levels had no significant effects on various organ indices in piglets ($P>0.05$). 2) Serum triglyceride content in the LA and NC groups was significantly lower than that in the HA group ($P<0.05$). Serum triglyceride content in the HG and HHG groups was significantly lower than that in the LG group ($P<0.05$). Serum albumin content in the LG group was significantly lower than that in the NC and HG groups ($P<0.05$). Serum glucose content in the LG and HG groups was significantly lower than that in the HHG group ($P<0.05$). 3) Serum gastrin content in the HA group was significantly lower than that in the LA group ($P<0.05$). Serum insulin content in the LG and HG groups was significantly higher than that in the HHG group ($P<0.05$). Serum glucagon content in the LG and HG groups was significantly lower than that in the HHG group ($P<0.05$). Serum growth hormone content in the LG and NC groups was significantly lower than that in the HHG group

(P<0.05). In conclusion, different dietary glutamic acid and aspartic acid levels affected serum biochemical indices and hormone content in piglets, but had no significant effect on organ development.

Full Text

Effects of Dietary Different Glutamate and Aspartate Levels on Organ Indexes, Serum Biochemical Parameters and Hormone Contents of Piglets

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Abstract

This study investigated the effects of dietary different glutamate and aspartate levels on organ indexes, serum biochemical parameters, and hormone contents in piglets. Forty-two healthy crossbred weaned piglets (Duroc × Landrace × Yorkshire) at 35 days of age were randomly allocated into 6 groups with 7 replicates per group and 1 pig per replicate. The control group (NC) received a diet containing 2.9% glutamate and 1.5% aspartate, while the other groups were fed diets with adjusted glutamate and aspartate levels: 2.9% and 1.3% (LA group), 2.9% and 1.7% (HA group), 2.6% and 1.5% (LG group), 3.2% and 1.5% (HG group), and 3.5% and 1.5% (HHG group). The experimental period lasted 21 days. At the end of the trial, organ indexes, serum biochemical parameters, and hormone contents were measured. The results showed that: (1) dietary different glutamate and aspartate levels had no significant effects on organ indexes of piglets ($P > 0.05$); (2) serum triglyceride content in the LA and NC groups was significantly lower than that in the HA group ($P < 0.05$), serum triglyceride content in the HG and HHG groups was significantly lower than that in the LG group ($P < 0.05$), serum albumin content in the LG group was significantly lower than that in the NC and HG groups ($P < 0.05$), and serum glucose content in the LG and HG groups was significantly lower than that in the HHG group ($P < 0.05$); (3) serum gastric inhibitory polypeptide content in the HA group was significantly lower than that in the LA group ($P < 0.05$), serum insulin content in the LG and HG groups was significantly higher than that in the HHG group ($P < 0.05$), serum glucagon content in the LG and HG groups was significantly lower than that in the HHG group ($P < 0.05$), and serum growth hormone content in the LG and NC groups was significantly lower than that in the HHG group ($P < 0.05$). In conclusion, dietary different glutamate and aspartate levels affected serum biochemical parameters and hormone contents but had no significant effects on organ development in piglets.

Keywords: acid amino acids; glutamate; aspartate; piglets; organ indexes; serum biochemical parameters; hormone

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Glutamate (Glu) and aspartate (Asp) are acidic amino acids with isoelectric points below 7. Traditionally, they have been considered non-essential amino acids in nutrition [**?**]. In the nervous system, Glu and Asp function as excitatory neurotransmitters and are recognized as excitatory amino acids [**?**]. However, recent studies have demonstrated that Glu and Asp play important roles as functional amino acids in the body [**?**]. As primary energy sources for tissues and organs, or through decarboxylation or transamination to form other nutrients such as glutamine, glutathione, citrulline, -ketoglutarate, and carbon dioxide, Glu and Asp contribute significantly to improving tissue structure and function [**?**] and alleviating stress [**?**, **?**]. Shi et al. [**?**] reported that supplementing 0.5% or 1.0% Asp to basal diets significantly alleviated the decrease in average daily gain induced by lipopolysaccharide (LPS) challenge in piglets. Wu et al. [**?**] found that dietary supplementation with 2% Glu mitigated the reduction in average daily feed intake caused by deoxynivalenol (DON) and significantly improved average daily gain and feed efficiency in piglets fed DON-contaminated diets. Duan [**?**] also observed that dietary supplementation with 1% Asp and 2% Glu significantly relieved growth performance inhibition caused by stress. Our previous research indicated that dietary Asp levels of 1.3%–1.5% and Glu levels of 2.6%–2.9% promoted growth and improved amino acid utilization in weaned piglets, whereas dietary Glu levels of 3.2%–3.5% or Asp levels of 1.7% inhibited growth. Using Ussing chamber techniques, we further demonstrated that high concentrations of Glu or Asp inhibited the absorption of Asp or Glu during transport [**?**]. These findings suggest that Glu and Asp serve as functional amino acids with important physiological roles. However, few studies have investigated the effects of different dietary Glu and Asp levels on organ indexes, serum biochemical parameters, and hormone contents in healthy weaned piglets. Therefore, this experiment was conducted to explore these effects and provide a scientific basis for the rational use of Glu and Asp in piglet diets.

1.1 Experimental Design

Forty-two healthy crossbred weaned piglets (Duroc × Landrace × Yorkshire) at 35 days of age (half male and half female, body weight 13.24 ± 0.25 kg) were randomly divided into 6 groups with 7 replicates per group and 1 pig per replicate, housed individually. The dietary standardized ileal digestible Glu and Asp levels were designed as follows: control group (NC) with 2.9% Glu and 1.5% Asp; low Asp group (LA) with 2.9% Glu and 1.3% Asp; high Asp group (HA) with 2.9% Glu and 1.7% Asp; low Glu group (LG) with 2.6% Glu and 1.5% Asp; high Glu group (HG) with 3.2% Glu and 1.5% Asp; and highly high Glu group (HHG) with 3.5% Glu and 1.5% Asp. The composition and nutrient levels of experimental diets are shown in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (air-dry basis)

Items	Groups
Ingredients	
Extruded soybean	
Soybean meal	
Fish meal	
Extruded corn	
Corn	
Soybean oil	
Glucose	
CaHPO	
Limestone	
NaCl	
Whey powder	
Sucrose	
Citric acid	
ZnO	
Choline chloride	
Lysine	
Methionine	
Threonine	
Tryptophan	
Multi-vitamin	
Microelement	
Aspartate	
Glutamate	
Rice mill by-product	
Total	
Nutrient levels	

Items	Groups
Digestible energy (MJ/kg)	14.61
Crude protein	
Total phosphorus (TP)	
Available phosphorus (AP)	
Lysine	
Methionine	
Threonine	
Tryptophan	
Glutamate (Glu)	
Aspartate (Asp)	

Multi-vitamin and microelement provided the following per kg of diets: nicotinic acid 50 mg, pantothenic acid 5 mg, folic acid 2 mg, biotin 0.2 mg, VA 10,800 IU, VD 4,000 IU, VE 40 IU, VK 4 mg, VB 6 mg, VB 12 mg, VB 6 mg, VB 0.05 mg, Cu 5 mg, Fe 80 mg, Mn 3 mg, Zn 85 mg, Mn 0.1 mg, Se 0.3 mg.

1.2 Feeding Management and Slaughter

The experiment was conducted at the animal housing facility of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. The pigs were housed in a fully enclosed building with slatted metal flooring, stainless steel adjustable feeders, and nipple drinkers. Piglets were individually housed and managed according to standard pig farm procedures for deworming and vaccination. They had ad libitum access to water and feed, which was provided in powder form. The facility used natural ventilation, maintained cleanliness, and was disinfected periodically during the trial. The experimental period lasted 21 days. At the end of the feeding trial, 6 piglets were randomly selected from each group (total of 42 piglets) and slaughtered after a 24-hour fast.

1.3.1 Organ Index Measurement

On day 21, after a 24-hour fast, the live weight of each piglet was recorded before slaughter. The heart, liver, spleen, and kidneys were removed during necropsy, and surface moisture was blotted before weighing. Organ indexes were calculated using the following formula:

Organ index (%) = organ weight (g) / [live weight (kg) × 10]

1.3.2 Serum Biochemical Parameter Analysis

At the end of the feeding trial, 10 mL of blood was collected from the anterior vena cava of fasting piglets. The blood was centrifuged at 3,000 r/min for 10 minutes at 4°C, and serum was stored at -20°C. Serum total protein (TP), albumin (ALB), urea nitrogen (UN), glucose (GLU), and triglyceride (TG) contents were measured using a CX4 automatic biochemical analyzer (Beckman, USA) according to the kit instructions (Nanjing Jiancheng Bioengineering Institute).

1.3.3 Serum Hormone Content Analysis

Serum insulin (INS), glucagon (GC), gastric inhibitory polypeptide (GIP), glucagon-like peptide-I (GLP-I), growth hormone (GH), and insulin-like growth factor-I (IGF-I) contents were determined by enzyme-linked immunosorbent assay (ELISA) (Nanjing Huadong Electronic Group Medical Equipment Co., Ltd.) following the ELISA kit instructions (Nanjing Jiancheng Bioengineering Institute).

1.4 Statistical Analysis

All data were compiled using Excel 2010 software and analyzed by one-way ANOVA using SPSS 18.0 software. Duncan' s multiple comparison test was used to assess significant differences. Data are expressed as means ± standard error, with $P < 0.05$ considered statistically significant.

2 Results

2.1 Effects of Dietary Different Glu and Asp Levels on Organ Indexes of Piglets

As shown in Table 2 , dietary different Asp levels had no significant effects on heart index, liver index, spleen index, or kidney index of piglets ($P > 0.05$).

Table 2 Effects of dietary different Asp levels on organ indexes of piglets

Items	Groups	Heart index	Liver index	Spleen index	Kidney index
		0.52±0.02	2.73±0.11	0.22±0.01	0.47±0.02
		0.54±0.02	2.55±0.09	0.21±0.01	0.43±0.02
		0.55±0.01	2.56±0.04	0.20±0.01	0.44±0.03
P-value					

In the same row, values with different small letter superscripts mean significant difference (P < 0.05), while with the same or no letter superscripts mean no significant difference (P > 0.05). The same as below.

As shown in Table 3 , dietary different Glu levels had no significant effects on heart index, liver index, spleen index, or kidney index of piglets ($P > 0.05$).

Table 3 Effects of dietary different Glu levels on organ indexes of piglets

Items	Groups	Heart index	Liver index	Spleen index	Kidney index
		0.54±0.02	2.55±0.09	0.21±0.01	0.43±0.02
		0.52±0.02	2.54±0.09	0.26±0.03	0.47±0.02
		0.54±0.02	2.76±0.18	0.23±0.02	0.49±0.04
		0.53±0.02	2.75±0.18	0.22±0.02	0.45±0.00
P-value					

2.2 Effects of Dietary Different Glu and Asp Levels on Serum Biochemical Parameters of Piglets

As shown in Table 4 , dietary different Asp levels had no significant effects on serum TP, ALB, UN, or GLU contents ($P > 0.05$), but serum TG content in the LA and NC groups was significantly lower than that in the HA group ($P < 0.05$).

Table 4 Effects of dietary different Asp levels on serum biochemical parameters of piglets

Items	Groups	TP (g/L)	ALB (g/L)	UN (mmol/L)	GLU (mmol/L)	TG (mmol/L)
		57.98±1.70	41.42±1.97	3.48±0.41	5.00±0.22	0.47±0.05
		58.83±1.77	41.28±2.25	3.99±0.52	5.72±0.33	0.47±0.04
		59.33±1.37	37.67±0.45	4.03±0.69	6.60±0.98	0.72±0.07
P-value						

As shown in Table 5 , dietary different Glu levels had no significant effects on serum TP or UN contents ($P > 0.05$). However, serum ALB content in the LG group was significantly lower than that in the NC and HG groups ($P < 0.05$), serum GLU content in the LG and HG groups was significantly lower than that in the HHG group ($P < 0.05$), and serum TG content in the HG and HHG groups was significantly lower than that in the LG group ($P < 0.05$).

Table 5 Effects of dietary different Glu levels on serum biochemical parameters of piglets

Items	Groups	TP (g/L)	ALB (g/L)	UN (mmol/L)	GLU (mmol/L)	TG (mmol/L)
		58.28±2.09	38.84±0.77	3.79±0.38	4.88±0.18	0.63±0.07
		58.83±1.77	41.28±2.25	3.99±0.52	5.72±0.33	0.47±0.04
		54.03±1.29	41.00±0.79	3.54±0.25	4.70±0.17	0.54±0.04
		56.24±0.16	35.95±2.63	3.06±0.27	6.61±0.90	0.42±0.03

Items	Groups	TP (g/L)	ALB (g/L)	UN (mmol/L)	GLU (mmol/L)	TG (mmol/L)
P-value						

2.3 Effects of Dietary Different Glu and Asp Levels on Serum Hormone Contents of Weaned Piglets

As shown in Table 6 , dietary different Asp levels had no significant effects on serum INS, GC, GLP-I, GH, or IGF-I contents ($P > 0.05$), but serum GIP content in the HA group was significantly lower than that in the LA group ($P < 0.05$).

Table 6 Effects of dietary different Asp levels on serum hormone contents of piglets

Items	Groups	INS (mIU/L)	GC (pg/mL)	GIP (pg/mL)	GLP-I (pg/mL)	GH (ng/mL)	IGF-I (U/mL)
		7.86±0.17	115.42±1.53	87.09±4.34	217.06±16.71	1.03±0.11	8.95±0.59
		7.72±0.22	145.03±9.87	84.10±3.18	211.13±13.61	1.04±0.12	8.24±0.58
		7.14±0.46	161.14±22.69	69.18±7.81	202.07±17.53	1.13±0.11	7.63±0.65
P-value							

As shown in Table 7 , dietary different Glu levels had no significant effects on serum GIP, GLP-I, or IGF-I contents ($P > 0.05$). However, serum INS content in the LG and HG groups was significantly higher than that in the HHG group ($P < 0.05$), while serum GC content showed the opposite trend, with the LG and HG groups having significantly lower GC content than the HHG group ($P < 0.05$). Additionally, serum GH content in the LG and NC groups was significantly lower than that in the HHG group ($P < 0.05$).

Table 7 Effects of dietary different Glu levels on serum hormone contents of piglets

Items	Groups	INS (mIU/L)	GC (pg/mL)	GIP (pg/mL)	GLP-I (pg/mL)	GH (ng/mL)	IGF-I (U/mL)
		8.12±0.22	133.26±4.17	75.19±2.24	232.22±10.26	1.13±0.09	8.80±0.46
		7.72±0.22	145.03±9.87	84.10±3.18	211.13±13.61	1.04±0.12	8.24±0.58
		8.46±0.13	129.66±6.51	78.09±5.48	231.16±12.93	1.16±0.13	9.31±0.62
		7.23±0.50	171.24±23.78	69.28±2.55	221.36±22.17	1.52±0.15	9.19±0.36
P-value							

3 Discussion

3.1 Effects of Dietary Different Glu and Asp Levels on Organ Indexes of Piglets

Organ index, the relative weight of visceral organs, is a direct indicator of animal health status [?]. Chen [?] reported that dietary supplementation with 2% Glu provided significant protective effects on organs in moldy diets. Wu et al. [?] also demonstrated that Glu had protective and reparative effects on the liver, kidneys, pancreas, and spleen. In the current study, dietary different Glu and Asp levels had no significant effects on organ indexes of piglets, possibly because the piglets were 35 days old and had passed the peak period of visceral organ development, resulting in normally developed organs that were less responsive to dietary modifications [?].

3.2 Effects of Dietary Different Glu and Asp Levels on Serum Biochemical Parameters of Piglets

Serum biochemical parameters are influenced by dietary nutrient levels and reflect nutritional metabolism and physiological function. Serum albumin content directly indicates protein synthesis and metabolism in the body [?]. Serum glucose content remains relatively stable under normal conditions but can become abnormally high or low under pathological or stress conditions [?]. Triglycerides directly participate in cholesterol synthesis and are a major component of blood lipids [?]. Wu et al. [?] reported that dietary supplementation with 306.64 mg/kg glutamine increased serum albumin content in rats. Wu et al. [?] found that dietary supplementation with 2% Glu significantly alleviated the elevation in serum glucose content caused by vomitoxin. In the present study, dietary Asp level of 1.7% significantly decreased serum triglyceride content, while dietary Glu level of 3.2% significantly increased serum albumin content and decreased serum glucose and triglyceride contents. However, our previous research indicated that dietary Glu levels of 3.2% or Asp levels of 1.7% inhibited growth performance, possibly because Glu and Asp, as important energy-yielding substances, were extensively oxidized to maintain intestinal health [?] without promoting body growth.

3.3 Effects of Dietary Different Glu and Asp Levels on Serum Hormone Contents of Piglets

Gastric inhibitory polypeptide (GIP) is an important satiety hormone released after feeding that enhances glucose-dependent insulin synthesis and secretion while inhibiting glucagon secretion, delaying gastric emptying, and reducing gastrointestinal motility and gastric acid secretion [?]. In this study, serum GIP content decreased with increasing dietary Asp levels, which aligns with our previous findings showing that growth performance trends were consistent with serum GIP content trends. Glu can act as a messenger to induce insulin secretion within certain ranges [?]. Peng [?] reported that dietary supplementation with

1% Glu significantly increased serum GH content [?]. In the current study, dietary Glu level of 3.5% decreased serum insulin content but increased serum glucagon and growth hormone contents, consistent with Peng's [?] results. These findings also parallel those of Matsunaga et al. [?], suggesting that elevated dietary Glu levels promote growth hormone secretion while decreasing serum glucose and insulin contents.

4 Conclusion

1. Dietary different Glu and Asp levels had no significant effects on organ indexes of piglets.
2. Dietary Asp level of 1.7% significantly decreased serum triglyceride content. Dietary Glu level of 3.2% significantly increased serum albumin content and decreased serum glucose and triglyceride contents.
3. Increasing dietary Asp levels reduced serum GIP content. Dietary Glu level of 3.5% decreased serum insulin content while increasing serum glucagon and growth hormone contents.

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