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Effects of Glutamine on Growth Performance and Serum Biochemical Indices in Lipopolysaccharide-Challenged Piglets: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary glutamine (Gln) supplementation on growth performance of weaned piglets at different stages, and its effects on serum biochemical indices and growth performance of weaned piglets after lipopolysaccharide (LPS)-induced intestinal injury. Twenty-four healthy 28-day-old Duroc × Landrace × Yorkshire (DLY) crossbred weaned piglets were selected and randomly divided into 3 groups with 8 replicates per group and 1 piglet per replicate. The control group and LPS group were fed the basal diet, while the Gln+LPS group was fed the basal diet supplemented with 1% exogenous Gln; on days 22, 25, 28, and 30 of the experiment, the LPS group and Gln+LPS group received intraperitoneal injection of 100 g/kg BW LPS, while the control group received injection of the same dose of physiological saline. The experimental period was 30 days. The results showed that: 1) Before LPS treatment (days 1-21 of the experiment), compared with the control group, the Gln+LPS group significantly increased the average daily feed intake (ADFI) and average daily gain (ADG) of weaned piglets during days 1-7 ($P<0.05$), and significantly increased the ADFI of weaned piglets during days 8-14 and days 1-21 ($P<0.05$). 2) After LPS treatment (days 22-30 of the experiment), the ADFI, ADG, and body weight on day 30 of weaned piglets in the control group were significantly higher than those in the LPS group and Gln+LPS group ($P<0.05$), while the ADFI, ADG, and body weight on day 30 of weaned piglets in the Gln+LPS group were higher than those in the LPS group ($P>0.05$). 3) The small intestine length of weaned piglets in the LPS group was significantly lower than that in the control group and Gln+LPS group ($P<0.05$), while there was no significant difference between the control group and Gln+LPS group ($P>0.05$). 4) Compared with the control group, the Gln+LPS group showed significantly decreased serum high-density lipoprotein cholesterol (HDLC) content and alkaline phosphatase (ALP) activity ($P<0.05$), while there was no sig-

nificant difference between the Gln+LPS group and LPS group (P>0.05); the serum immunoglobulin M (IgM) content of weaned piglets in both the Gln+LPS group and LPS group was significantly increased (P<0.05). These results indicate that dietary supplementation with 1% Gln can significantly improve the growth performance of piglets during days 1-7 post-weaning, with no obvious effects thereafter. Dietary supplementation with 1% Gln can regulate serum biochemical indices, improve growth performance and small intestine length in stressed piglets, thereby alleviating weaning stress in piglets.

Full Text

Effects of Glutamine on Growth Performance and Serum Biochemical Parameters of Lipopolysaccharide Challenged Piglets

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Abstract

This study investigated the effects of dietary glutamine (Gln) supplementation on growth performance during different stages, and on serum biochemical parameters and growth performance following lipopolysaccharide (LPS)-induced intestinal injury in weaned piglets. Twenty-four healthy crossbred (Duroc × Landrace × Yorkshire) piglets weaned at 28 days of age were randomly allocated into three groups with eight replicates per group and one pig per replicate. Pigs in the control and LPS groups received a basal diet, while those in the Gln+LPS group received the basal diet supplemented with 1% exogenous Gln. On days 22, 25, 28, and 30 of the trial, piglets in the LPS and Gln+LPS groups were intraperitoneally injected with 100 g/kg body weight (BW) of LPS, while the control group received an equivalent volume of sterile saline. The experiment lasted for 30 days. The results showed: (1) Before LPS challenge (days 1-21), compared with the control group, the Gln+LPS group exhibited significantly increased average daily feed intake (ADFI) and average daily gain (ADG) during days 1-7 ($P < 0.05$), and significantly elevated ADFI during days 8-14 and 1-21 ($P < 0.05$). (2) After LPS challenge (days 22-30), the control group showed significantly higher ADFI, ADG, and body weight on day 30 compared with the LPS and Gln+LPS groups ($P < 0.05$), while the Gln+LPS group had higher ADFI, ADG, and day-30 body weight than the LPS group ($P > 0.05$). (3) The small intestine length in the LPS group was significantly shorter than in the control and Gln+LPS groups ($P < 0.05$), with no significant difference between the latter two groups ($P > 0.05$). (4) Compared with the control group, serum high-density lipoprotein cholesterol (HDLC) content and alkaline phosphatase (ALP) activity in the Gln+LPS group were significantly decreased ($P < 0.05$), though no significant difference existed between the Gln+LPS and LPS groups ($P > 0.05$). Serum immunoglobulin M (IgM) content in both the Gln+LPS and LPS groups was significantly elevated ($P < 0.05$). These findings indicate that dietary supplementation with 1% Gln significantly improves growth performance during the first week post-weaning, though the effect diminishes thereafter. Supplementation with 1% Gln can modulate serum biochemical parameters, improve growth performance and small intestine length, thereby alleviating weaning stress in piglets.

Keywords: glutamine; piglets; lipopolysaccharide; growth performance; serum biochemical parameters

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Studies have shown that suckling piglets cannot synthesize sufficient glutamine (Gln) to meet their physiological demands, and their underdeveloped digestive tracts, combined with early weaning stress, result in weak capacity to obtain exogenous Gln. Therefore, timely supplementation of exogenous Gln in weaned piglet diets is crucial. As a conditionally essential amino acid, Gln participates in multiple metabolic pathways and serves as a precursor for ornithine, citrulline, proline, and arginine. It not only stimulates cell growth and antibody production but also represents a primary energy source for intestinal cells. Research by Liu et al. demonstrated that dietary Gln supplementation at various levels improved growth performance in nursery pigs, with 1.0% being optimal. Chen et al. found that Gln could mitigate the effects of immune stress on piglet growth performance seven days post-weaning. Although numerous studies have examined Gln' s role in alleviating weaning stress, few have investigated its effects during the late weaning period (after 21 days), particularly regarding immune stress induced by direct external stimuli in early-weaned piglets. Therefore, this study established a stress model by feeding piglets a diet supplemented with 1% Gln and administering multiple intraperitoneal injections of Escherichia coli LPS during the late weaning period to investigate Gln' s effects on growth performance at different stages and on post-challenge growth performance and serum biochemical parameters, thereby providing theoretical guidance for practical production.

Materials and Methods

Experimental Design

Twenty-four healthy crossbred (Duroc × Landrace × Yorkshire) piglets weaned at 28 days of age [initial body weight (6.24 ± 0.25) kg] were randomly divided into three groups with eight replicates per group (equal numbers of barrows and gilts) and one pig per replicate. Throughout the trial, the control and LPS groups received a basal diet, while the Gln+LPS group received the basal diet supplemented with 1% exogenous Gln. On days 22, 25, 28, and 30, piglets in the LPS and Gln+LPS groups were intraperitoneally injected with 100 g/kg BW LPS, while the control group received equivalent volumes of physiological saline. On day 30, blood samples were collected via anterior vena cava puncture before slaughter. The trial was conducted in the animal facility of the Institute of Subtropical Agriculture, Chinese Academy of Sciences. Piglets were housed individually, fed powdered feed ad libitum, and provided free access to water. Disinfection and immunization followed standard farm procedures. Feed intake and body weight were recorded throughout the trial. Piglets were weaned at 28 days of age, transferred to the laboratory animal facility, and allowed a 3-day adaptation period before the 30-day experimental period commenced.

Experimental Materials and Basal Diet

The Gln used in this study contained 99.5% active ingredient and was purchased from Wuhan Yuancheng Co-Creation Technology Co., Ltd. LPS was purchased from Sigma-Aldrich (USA), serotype E. coli O55:B5. The basal diet was formulated according to NRC (2012) standards. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Item	Content
Ingredients	
Expanded corn	
Extruded soybean	
Maize starch	
Dried whey	
Plasma protein powder	
Emulsified oil powder	
Fish meal	
L-Lys	
Met	
L-Thr	
Trp	
White sugar	
Glucose	
Limestone	

Item	Content
CaHPO	
Premix[1])	
Acidifier	
Antioxidant	
Fungicide	
Total	
Nutrient levels[2])	
DE/(kJ/kg)	
CP	
Lys	
Met	
Thr	
Trp	
AP	

[1]) The premix provided the following per kg of diet: 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, biotin 0.15 mg, folic acid 2 mg, niacin 50 mg, D-calcium pantothenate 25 mg, Fe (as ferrous sulfate) 100 mg, Cu (as copper sulfate) 150 mg, Mn (as manganese sulfate) 40 mg, Zn (as zinc sulfate) 100 mg, I (as potassium iodide) 0.50 mg, Zn (as zinc sulfate) 75 mg.
[2]) Digestible energy (DE) was a calculated value, while other nutrients were measured values.

Measured Parameters

Feed intake was recorded throughout the trial. Piglets were weighed on days 1, 7, 14, 21, and 30 after overnight fasting to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G). On day 30, 10 mL of blood was collected via anterior vena cava puncture before slaughter. Serum was prepared after standing at 4 °C for 30 minutes and centrifugation at 3,000 rpm for 15 minutes. Serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), and -amylase (-AMY) activities, as well as urea (UREA), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), immunoglobulin M (IgM), and glucose (GLU) concentrations were determined using an automatic biochemical analyzer. After slaughter, the heart, liver, spleen, and kidneys were excised and weighed, and the small intestine was removed and measured for length. Organ index was calculated as organ weight/body weight.

Statistical Analysis

All data were analyzed using one-way ANOVA in SPSS 17.0 statistical software. Differences were considered significant at $P < 0.05$.

Results

Effects of Dietary Gln Supplementation on Growth Performance Before and After LPS Challenge

As shown in Table 2 , during days 1-7, the Gln+LPS group exhibited significantly higher ADFI and ADG compared with the control group (P < 0.05), representing increases of 20% and 34%, respectively, while F/G decreased by 16% (P > 0.05). During days 8-14, the Gln+LPS group showed significantly higher ADFI than the control group (P < 0.05), but ADG decreased by 10% (P > 0.05) and F/G increased by 20% (P > 0.05). During days 15-21, no significant differences were observed among the three groups in ADFI, ADG, or F/G (P > 0.05).

Overall, during days 1-21 (pre-LPS challenge), the Gln+LPS group demonstrated significantly increased ADFI compared with the control group (P < 0.05), though no significant differences were observed in day-21 body weight, ADG, or F/G (P > 0.05), with F/G actually increasing by 9% (P > 0.05). During the post-challenge period (days 22-30), the LPS group showed significantly lower ADFI, ADG, and day-30 body weight compared with the control group (P < 0.05). However, the Gln+LPS group exhibited 12%, 17%, and 7% improvements in ADFI, ADG, and day-30 body weight, respectively, compared with the LPS group (P > 0.05), with F/G decreasing by 9% (P > 0.05). Throughout the entire trial (days 1-30), the LPS group displayed significantly lower day-30 body weight than the control group (P < 0.05), with ADFI and ADG also lower (P > 0.05). Compared with the LPS group, the Gln+LPS group showed 7%, 12%, and 9% improvements in ADFI, ADG, and day-30 body weight (P > 0.05), respectively, with F/G decreasing by 5% (P > 0.05).

Table 2 Effects of glutamine on growth performance of weaned piglets

Item	Control group	LPS group	Gln+LPS group
Days 1-7			
Body weight on day 1/kg	6.24±0.19	6.24±0.24	6.24±0.16
Body weight on day 7/kg	6.79±0.24	6.83±0.2	6.98±0.13
ADFI/g	181.63±7.82	207.5±3.28	217.86±6.81
ADG/g	92.86±5.13	100.18±7.85	124.49±7.5
F/G	2.14±0.21	2.12±0.17	1.79±0.09
Days 8-14			
Body weight on day 14/kg	8.4±0.31	8.21±0.18	8.43±0.17
ADFI/g	319.64±8.83	321.43±8.1	350.39±3.84
ADG/g	230.36±18.2	218.37±17.28	207.14±9.35
F/G	1.43±0.08	1.52±0.12	1.72±0.09
Days 15-21			
Body weight on day 21/kg	11.09±0.34	11.11±0.25	11.16±0.33
ADFI/g	532.77±22.13	566.37±6.37	587.78±13.73
ADG/g	383.93±29.26	414.29±20.2	389.8±27.83

Item	Control group	LPS group	Gln+LPS group
F/G	1.42±0.07	1.38±0.06	1.54±0.09
Days 1-21			
Body weight on day 21/kg	11.09±0.34	11.11±0.25	11.16±0.33
ADFI/g	343.06±12.22	365.39±4.34	386.14±6.63
ADG/g	230.95±13.32	240.82±12.64	238.78±13.49
F/G	1.5±0.06	1.54±0.08	1.64±0.07
Days 22-30			
Body weight on day 30/kg	15.38±0.33	13.43±0.59	14.4±0.57
ADFI/g	758.04±33.9	574.83±41.84	643.35±40.2
ADG/g	444.45±12.51	285.04±38.45	333.33±24.6
F/G	1.71±0.09	2.16±0.25	1.96±0.13
Days 1-30			
Body weight on day 30/kg	15.38±0.33	13.43±0.59	14.4±0.57
ADFI/g	475.13±16	427.16±12.35	466.04±13.53
ADG/g	302.78±9.56	243.33±22.59	271.33±18.72
F/G	1.57±0.04	1.84±0.18	1.74±0.1

In the same row, values with no letter or the same letter superscripts indicate no significant difference ($P > 0.05$), while different lowercase letters indicate significant difference ($P < 0.05$). The same applies below.

Effects of Dietary Gln Supplementation on Organ Indices After LPS Challenge

As shown in Table 3 , compared with the control group, the LPS group exhibited increases of 9%, 33%, 37%, and 8% in cardiac, liver, spleen, and renal indices, respectively, though none were significant ($P > 0.05$). The Gln+LPS group showed reductions of 10%, 19%, 12%, and 4% in these indices compared with the LPS group, also without significant differences ($P > 0.05$).

Table 3 Effects of glutamine on organ index of weaned piglets

Item	Control group	LPS group	Gln+LPS group
Cardiac index	0.0044±0.0003	0.0048±0.0002	0.0043±0.0003
Liver index	0.0240±0.0010	0.0320±0.0021	0.0260±0.0020
Spleen index	0.0019±0.0001	0.0026±0.0003	0.0023±0.0002
Renal index	0.0051±0.0003	0.0055±0.0003	0.0053±0.0002

Effects of Dietary Gln Supplementation on Small Intestinal Length After LPS Challenge

As illustrated in Figure 1 [Figure 1: see original paper], small intestinal length in the LPS group was significantly shorter than in the control group, while no significant difference was observed between the Gln+LPS and control groups ($P > 0.05$). Both the control and Gln+LPS groups exhibited significantly greater small intestinal length than the LPS group ($P < 0.05$).

Figure 1 Effects of glutamine on small intestinal length of weaned piglets. Value columns with different lowercase letters indicate significant difference ($P < 0.05$).

Effects of Dietary Gln Supplementation on Serum Biochemical Parameters After LPS Challenge

As shown in Table 4 , serum ALP activity in the LPS group decreased by 16% compared with the control group ($P > 0.05$), while the Gln+LPS group exhibited a significant 32% reduction ($P < 0.05$). Serum HDLC content in the Gln+LPS group was significantly lower than in the control group (29% decrease, $P < 0.05$), though no significant difference existed between the LPS and control groups ($P > 0.05$). Serum IgM content was significantly elevated in both the Gln+LPS and LPS groups compared with the control group ($P < 0.05$), with the Gln+LPS group showing a 16% increase over the LPS group ($P > 0.05$). No significant differences were detected among the three groups in serum UREA, LDLC, or GLU content, or in ALT, AST, CK, or -AMY activities ($P > 0.05$).

Table 4 Effects of glutamine on serum biochemical parameters of weaned piglets

Item	Control group	LPS group	Gln+LPS group
ALP/(U/L)	349.41±32.11	295.06±29.32	236.03±13.57
UREA/(mmol/L)	3.39±0.25	3.5±0.24	2.91±0.15
ALT/(U/L)	105.19±6.68	84.67±9.66	116.06±12.93
AST/(U/L)	91.78±20.94	83.71±4.71	70.51±5.47
HDLC/(mmol/L)	0.76±0.05	0.69±0.02	0.54±0.06
LDLC/(mmol/L)	1.26±0.09	1.12±0.09	0.99±0.09
CK/(U/L)	2,911.03±575.76	2,036.09±621.09	1,094.7±136.08
IgM/(g/L)	0.35±0.03	0.49±0.03	0.57±0.06
-AMY/(U/L)	2,850.85±287.33	2,578.85±140.63	2,791.82±159.95
GLU/(mmol/L)	5.72±0.43	4.63±1.06	4.8±0.38

Discussion

Effects of Dietary Gln Supplementation on Growth Performance Before and After LPS Challenge

As a conditionally essential amino acid and the most abundant amino acid in sow milk, Gln plays a vital role in piglet growth and development. Due to their immature gastrointestinal tracts, early-weaned piglets have insufficient capacity to digest and absorb dietary Gln, leading to Gln deficiency and compromised growth performance. This study found that dietary supplementation with 1% Gln significantly improved piglet growth performance only during the first week post-weaning, with no obvious effects thereafter. This phenomenon may occur because during the first week post-weaning, piglets fed the Gln-supplemented diet could obtain adequate exogenous Gln, thereby alleviating endogenous Gln deficiency and significantly improving growth performance. Additionally, piglets in the non-supplemented group gradually adapted to the post-weaning environment and exhibited compensatory growth after one week, which improved their ADG and F/G during the later experimental period and eliminated significant differences between the control and Gln-supplemented groups after the first week. These results are consistent with findings by Dai et al. and Liu et al., who reported that dietary Gln supplementation increased small intestinal villus length and reduced crypt depth at seven days post-weaning, though growth performance and intestinal development differences became non-significant by 14 days post-weaning. However, Zhang et al., in reviewing previous studies, noted inconsistent reports regarding which post-weaning stage shows more pronounced growth-promoting effects with 1% Gln supplementation. Wu et al. found that 1% dietary Gln had no significant effect during the early post-weaning period (days 21–35) but became effective during the late period (days 21–49), consistent with reports by Zhang et al. and Qian et al. Conversely, Yang et al. observed that 1% Gln significantly improved ADG during both the early post-weaning period and the entire experimental period. These discrepancies may relate to the physiological status of piglets at weaning, whether creep feed was provided pre-weaning, and weaning age. It is well established that incomplete physiological development makes piglets susceptible to stress from weaning, nutrition, immunity, and management, thereby affecting growth performance. Intraperitoneal LPS injection is a classic model for inducing stress in piglets. In this study, LPS injections on days 22, 25, 28, and 30 significantly reduced growth performance in the LPS and Gln+LPS groups, though the Gln-supplemented group showed improvements compared with the non-supplemented group. These findings align with Chen et al., who reported that immune stress reduced weaned piglet growth performance while Gln supplementation mitigated these effects. This demonstrates that 1% Gln supplementation can alleviate the impact of LPS-induced immune stress on growth performance.

The present results indicate that 1% dietary Gln supplementation significantly improves growth performance only during the first week post-weaning in 28-day-old piglets but can mitigate stress effects on growth performance throughout the physiological period. Therefore, in practical production, Gln supplementation levels could be increased during the first week post-weaning but potentially reduced during the late weaning period.

Effects of Dietary Gln Supplementation on Organ Indices and Small Intestinal Length After LPS Challenge

Organ indices represent biological characteristics that partially determine organ function. Chen et al. reported that immune stress significantly reduced spleen and thymus indices in piglets, while Gln supplementation significantly increased these indices and alleviated LPS-induced effects on immune organs. This study found no significant effects of Gln supplementation on cardiac, hepatic, splenic, or renal indices following LPS challenge, though the LPS group showed higher indices than the control group, while the Gln+LPS group had lower indices than the LPS group. This may be attributed to LPS-induced organ edema or congestion increasing organ weight, with Gln supplementation partially alleviating LPS-induced damage to these organs. Small intestinal length and weight are key indicators of intestinal development and absorptive capacity; longer intestines and greater absorptive surface area indicate stronger digestive function and more complete immune development. Dai et al. found that 1% dietary Gln significantly increased small intestinal weight and villus length while reducing crypt depth at one week post-weaning. Current research also indicates that Gln supplementation can improve intestinal morphology by increasing villus height and decreasing crypt depth, thereby promoting intestinal development. In this study, small intestinal length in both the control and Gln+LPS groups was significantly greater than in the LPS group, with no significant difference between the former two groups. This demonstrates that Gln supplementation effectively mitigates LPS-induced impairment of intestinal development, thereby maintaining intestinal health in weaned piglets.

Effects of Dietary Gln Supplementation on Serum Biochemical Parameters After LPS Challenge

Serum biochemical parameters are important indicators of metabolic status and can reflect animal health and growth performance, holding significant value in animal production research. Immunoglobulin M (IgM) in serum possesses both immune and nutritional functions; when piglets encounter various external antigens, immune responses are triggered, rapidly producing high antibody levels (globulins) to combat antigens. Ye et al. reported that dietary alanine combined with Gln increased IgA plasma cell numbers and secretory IgA secretion in the small intestinal lamina propria of weaned piglets. In this study, serum IgM content was significantly elevated in both the LPS and Gln+LPS groups compared with the control group, with the Gln+LPS group showing a 16% increase over the LPS group. These results suggest that dietary Gln supplementation can enhance serum antibody protein synthesis, thereby strengthening piglet stress resistance. Previous reports indicate that serum ALP activity in livestock typically decreases only when intestinal absorption is impaired, serving as an important indicator of malabsorption. Qian et al. found that weaning stress significantly reduced serum ALP activity, while Gln supplementation had no significant effect. The present results show that LPS challenge significantly decreased serum ALP activity, and Gln supplementation did not increase it. This suggests that LPS stress may cause intestinal absorptive dysfunction, with Gln supplementation exerting no effect on serum ALP activity. Research has shown that HDLC can bind LPS, thereby antagonizing its toxic effects. HDLC-LPS binding effectively blocks the active center of LPS, competitively inhibits LPS-receptor binding, reduces activation of target cells, decreases inflammatory mediator release, and alleviates LPS-induced inflammatory responses. In this study, LPS injection resulted in significantly lower serum HDLC content in both the LPS and Gln+LPS groups compared with the control group, with the Gln+LPS group showing a 22% reduction compared with the LPS group. This may be explained by either HDLC binding to LPS, thereby reducing serum HDLC levels, or by 1% Gln supplementation enhancing HDLC-LPS binding capacity, consequently lowering serum HDLC content and improving piglet capacity to resist LPS stimulation. Therefore, 1% Gln supplementation can modulate relevant serum biochemical parameters and further alleviate stress responses in challenged piglets.

Conclusion

Dietary supplementation with 1% Gln significantly improves growth performance only during the first week post-weaning, with no obvious effects thereafter. Supplementation with 1% Gln can increase serum IgM content, reduce HDLC levels, improve growth performance, and increase small intestinal length, thereby alleviating stress in weaned piglets.

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