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The Regulation of Glutamine Supplementation on Growth Performance, Liver Inflammatory Response and Protein Synthesis and Degradation in Muscle of Lipopolysaccharide-Challenged Broilers

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Simple summary: Our previous study suggested that Gln, defined as a conditionally essential amino acid, contributed to improving the growth performance, alleviating the inflammatory responses and ameliorating the intestinal permeability and rescuing the destroyed intestinal mucosal induced by LPS stimuli. In addition, it was demonstrated that LPS-induced immune stress led to a severe loss of muscle mass. Moreover, glutamine (Gln), is proven to be functionalized in regulating inflammatory responses, protein synthesis and degradation during sepsis. Our results showed that Gln administration attenuated liver inflammatory reactions, elevated protein synthesis and inhibited protein degradation of broilers subjected to LPS challenge.

Abstract: The aim of our present study was to investigate the effects of Gln supplementation on liver inflammatory responses and protein synthesis and degradation in muscle of LPS-challenged broilers. A total of 120 1d-old male broilers (Arbor Acres Plus) were randomly arranged in a 2 × 2 factorial design with five replicates per treatment and six broilers per replicate, containing two main factors: immune challenge (injected with LPS in a dose of 0 or 500 μg/kg of body weight) and dietary treatments (supplemented with 1.22% alanine or 1% Gln). After feeding with alanine or Gln diet for 15 days, broilers were respectively administrated with LPS or saline injection at 16 and 21 d. The results showed that Gln supplementation significantly reversed the adverse effects of LPS administration on growth performance, as evidenced as the increased ADFI and ADG, as well as the lowered F/G. Furthermore, Gln supplementation alleviated the increased mRNA expression of interleukin-6, interleukin-1β and tumor necrosis factor-α in liver induced by LPS. Moreover, the increased activities of aspartate aminotransferase, combined with the decreased expression of glutaminase in muscle, were observed by Gln addition. In addition, in comparison with saline treatment, LPS challenge altered signaling molecules mRNA expressions associated with protein synthesis and degradation. However, Gln supplementation reversed the negative effects on protein synthesis and degradation in muscle of LPS-challenged broilers. Taken together, Gln supplementation had benefit effects on improving growth performance, alleviating inflammatory responses, promoting protein synthesis and inhibiting protein degradation of LPS-challenged broilers.

Keywords: lipopolysaccharide challenge; glutamine; liver inflammatory; protein synthesis; protein degradation

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1. Introduction

Under intense breeding conditions, broilers commonly suffered from immune stress induced by diverse pathogenic and nonpathogenic microorganisms, which caused adverse changes in body metabolism and immune system and ultimately resulted in the impaired growth performance [1]. Lipopolysaccharide (LPS), an active component of gram-negative bacterial cell membrane and an effective immunostimulatory component, was preferentially used to establish immune-stress model via intraperitoneal injection in broilers [2,3]. Previous studies suggested that an acute systemic inflammatory response such as the production of inflammatory factors and the altered expression of genes involved in the immune system occurred in response to LPS stimulation [4]. Our previous study showed that LPS challenge resulted in the impaired growth performance, the elevated inflammatory cytokines in serum and the destructed intestinal mucosal barrier in broilers [5]. In a previous study, it was demonstrated that LPS-induced immune stress led to a severe loss of muscle mass [6]. Moreover, LPS challenge also altered the molecular pathways regulating the catabolism of muscle mass, which contained the signaling molecules responsible for the increased protein degradation and the decreased protein synthesis [7].

Glutamine (Gln), the most abundant amino acid in plasma and skeletal muscle, falls dramatically in circulation and tissue during stress conditions [8]. Therefore, Gln becomes a conditionally essential amino acid during inflammatory conditions [9]. It has been demonstrated that Gln has a wide range of biological functions and serves important roles in promoting growth performance, maintaining intestine health and improving immune response [5,10–12]. Previous studies suggested that 1% Gln supplementation improved growth performance of broilers [8,13]. Besides, Gln addition contributed to alleviating the inflammatory reactions evidenced as the decreased concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) in plasma [5]. Additionally, Gln is considered to be one of the important amino acids in regulation of mTOR and autophagy [14,15], which are respectively responsible for protein synthesis and degradation. However, the regulation of Gln on protein synthesis and degradation of muscle in LPS-challenged broilers was still not well understood.

Therefore, the present study was to investigate the effects of Gln supplementation on liver inflammatory responses and protein synthesis and degradation in muscle of LPS-challenged broilers.

2. Materials and methods

2.1. Diets, Experimental design and Animal management

The protocols of this experiment were performed according to the guidelines of the Institutional Animal Care and Use Committee of Qingdao agricultural university (QAU1121010) and approved by the Animal Care and Use Committee.

The dosage of Gln (1.0%) supplementation and Ala (1.22%) as isonitrogenous control were according to our previous study [5]. Both Ala and Gln were purchased from Shanghai Feiya Technology Development Co., Ltd. (Shanghai, China).

120 1d-old male broilers (Arbor Acres Plus) were randomly arranged in a 2 × 2 factorial design, containing five replicates per treatment and six broilers per replicate. Broilers were fed with either Ala or Gln diet for 15 days. However, broilers were intraperitoneally injected with LPS solution at a dosage of 500 μg LPS/kg of body weight or the equal volume of 0.9% sterile saline on 8.00 a.m. of d 16 and d 21, respectively. Therefore, the four experimental treatments were as follows: (1) Ala-saline group, in which birds were fed with diet containing 1.22% Ala and received intraperitoneal administration of sterile saline; (2) Gln-saline group, in which birds were fed with diet containing 1% Gln and received intraperitoneal administration of sterile saline; (3) Ala-LPS group, in which birds were fed with diet containing 1.22% Ala and received intraperitoneal administration of LPS; (4) Gln-LPS group, in which birds were fed with diet containing 1% Gln and received intraperitoneal administration of LPS. The ingredients of diets and nutrition contents were presented in Table 1. During the entire experiment, broilers were ad libitum access to feed in mash form and fresh water.

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Table 1. Ingredients and nutrient content of the basal diets.

Ingredients (g/kg diet)		Nutrient content (g/kg diet)	
Maize	563.0	Crude protein‡	210.8
Wheat bran	51.30	Metabolism energy（MJ/kg）	121.2
Soybean meal	285.0	Calcium（%）	10.00
Corn gluten meal	43.0	Phosphorus（%）	4.50
DL-Methionine	1.50	DL-Methionine（%）	8.60
Phytase	0.40	L-Lysine（%）	10.60
Choline	1.50	Threonine（%）	8.0
Dicalcium phosphate	18.70		
Limestone	12.60		
Salt	1.50		
Soybean oil	16.50		
Vitamin and mineral premix†	5.00		

†Premix per kg diet provided: Vitamin A 12 000IU; Vitamin D3 2 500IU; Vitamin E 30 mg; menadione 2.8 mg; thiamin 2.21 mg; riboflavin 7.8 mg; nicotinamide 40 mg; Calcium pantothenate 10 mg; pyridoxine·HCl 4 mg; biotin 0.04 mg; folic acid 1.2 mg; Vitamin B12 0.015 mg; Fe 80 mg; Cu 8 mg; Mn 110 mg; Zn 65 mg; I 0.35 mg; Se 0.3 mg. ‡Nutrient contenst of the diets were the value of measurement.

2.2. Sample Collection

At 21 d of age after LPS injection for 2 h, 10 broilers (2 broilers per replicate) were randomly selected to obtain blood samples from the wing vein. The collected blood samples were centrifuged at 4 °C 3000 × g for 10 min to collect plasma and stored at -20°C for further analysis. Immediately after blood sampling, the selected broilers were sacrificed by cervical dislocation followed by exsanguination. About 10 g. pectoralis muscle were collected into sterile tube and stored in liquid nitrogen for subsequent analysis. All samples were collected within 10 minutes after killing.

2.3. Growth performance

At 16 and 21 d of age, broilers were weighed after a 12-h feed withdraw. The total feed consumption and the total weight of birds for each replicate were respectively recorded to calculate average feed daily intake (ADFI), average daily gain (ADG) and the ratio of feed to gain (F/G).

2.4. Real-Time PCR analysis

Total RNA extraction from pectoralis muscle samples isolated using RNAiso Plus reagent (catalogue no. 9108, TaKaRa Biotechnology (Dalian) CO., LTD. Dalian China). were reverse transcribed into cDNA using PrimeScript TM RT Master Mix (catalogue no. RR037A, TaKaRa), followed by a Real time RT-PCR analysis with TB Green Premix Ex Taq (catalogue no. RR420A) according to the instructions of manufacture. PCR program consisted of one cycle at 95°C for 30s, 40 cycles of denaturation at 95°C for 5s, followed by a 60°C annealing step for 30s. The expression of selected genes relative to the housekeeping gene (β-actin) were analyzed according to the method by Livak and Schmittgen [16]. The primer sequences for the target genes toll-like receptor 4 (TLR4), muscle atrophy F-box (MAFbx), muscle ring finger 1 (MuRF1), forkhead Box O1 (FOXO1), forkhead Box O4 (FOXO4), protein kinase B (Akt), mammalian target of rapamycin (mTOR), eIF-4E binding protein-1 (4E-BP1), ribosomal protein S6 kinase (S6K1) and β-actin are shown in Table 2.

Table 2. Primer sequences for RT-PCR analysis.

Genes	ID	Primers sequences（5′-3′）	Product size (bp)
MAFbx	NM_001030956.1	F: GCCAGTACCACTTCACAGACAGAC R: GCGTGTCACCATACTGCTCCTTC	132
MuRF1	XM_424369.4	F: GAACGACCGCATCCAGACCATC R: TCCGTCTTCTTCTCCTCCAGCAG	138

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FOXO1	NM_204328.1	F: GACCTCATCACCAAGGCCATCG R: GCACGCTCTTGACCATCCACTA	85
Akt	NM_205055.1	F: GGCTACAAGGAACGACCGCAAG R: TACTGTGGTCCACTGGAGGCATC	141
TLR4	NM_001030693.1	F: TTCGGTTGGTGGACCTGAATCTTG R: ACAGCTTCTCAGCAGGCAATTCC	114
GA	NM_001031248.1	F: TCCTCGCAGAGAAGGTGGTGATC R: TACGTGCAATGCTGTTCGTGAGTC	154
S6K1	NM_001030721.1	F: GTTCAGGCTCACCCGTTCTTCAG R: TGGCTCACATCCTCTTCAGATTGC	107
FOXO4	XM_015278657.2	F: CAACGTTCCACCACCCGTGA R: TGGAGGCAGATTGCTGGGTA	101
TNF-α	NM_204267.1	F: TGTGTATGTGCAGCAACCCG R: AACAACCAGCTATGCACCCC	178
mTOR	XM_417614.6	F: AACCACTGCTCGCCACAATGC R: CATAGGATCGCCACACGGATTAGC	120
4E-BP1	XM_424384.6	F: GACCGTAAGTTCCTGATGGAGTGC R: ATTGGGCTGGTAACACCTGGAATG	92
IL-1β	NM_204524.1	F: AAGCCTCGCCTGGATTCTAG R: TCAGGTCGCTGTCAGCAAAG	90
IL-6	NM_204628.1	F: TCCCTCCTCGCCAATCTGAA R: AAATAGCGAACGGCCCTCAC	80
β-actin	NM_205518.1	F: ATTGTCCACCGCAAATGCTTC R: AAATAAAGCCATGCCAATCTCGTC	113

MAFbx, muscle atrophy F-box; MuRF1, muscle Ring finger 1; FOXO1, Forkhead Box O1; Akt, protein kinase B, also named PKB; TLR4, Toll-like receptor 4; GA, glutaminase; S6K1, ribosomal protein S6 kinase; FOXO4, Forkhead Box O4; TNF-α: Tumor necrosis factor-α; mTOR, mammalian target of rapamycin; 4E-BP1, eIF-4E binding protein-1; IL-1β: Interleukin-1β; IL-6: Interleukin-6.

2.5. Statistical analysis

Data analysis was conducted by a 2×2 factorial arrangement (SPSS version 20.0). The statistical model contained diet (1.2% Ala or 1.0% Gln) and intraperitoneal injection challenge (LPS or saline) and their interactions. $P<0.05$ was considered significant. All values are shown as means and standard error of the mean.

3. Results

3.1. The growth performance of broilers challenged with LPS

As reported in our previous study [5], there were no differences in the control group and Gln addition group before LPS-challenge treatment ($P<0.05$). However, lower body weight, ADFI and ADG, but higher F/G were observed in broilers received LPS challenge in comparison with those receiving saline-injection treatment ($P<0.05$). Moreover, compared with the control group, Gln addition significantly increased body weight of 21 d, ADFI and ADG, as well as the lowered F/G ($P<0.05$). There were no interactions for body weight of 16 d and 21d, ADG, ADFI and F/G of broilers between Gln addition and LPS challenge ($P>0.05$).

3.2. mRNA expression of TNF-α、IL-6、IL-1β in liver

As illustrated in Figure 1, in comparison with those received saline treatment, LPS challenge significantly increased mRNA expression of TNF-α、IL-6 and IL-1β in liver ($P<0.05$). However, Gln supplementation reversed the increased expression of TNF-α、IL-6 and IL-1β induced by LPS

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stimulation (P<0.05). Significant interactions for IL-6 and IL-1β (P<0.05) except for TNF-α (P>0.05) were observed between Gln addition and LPS challenge.

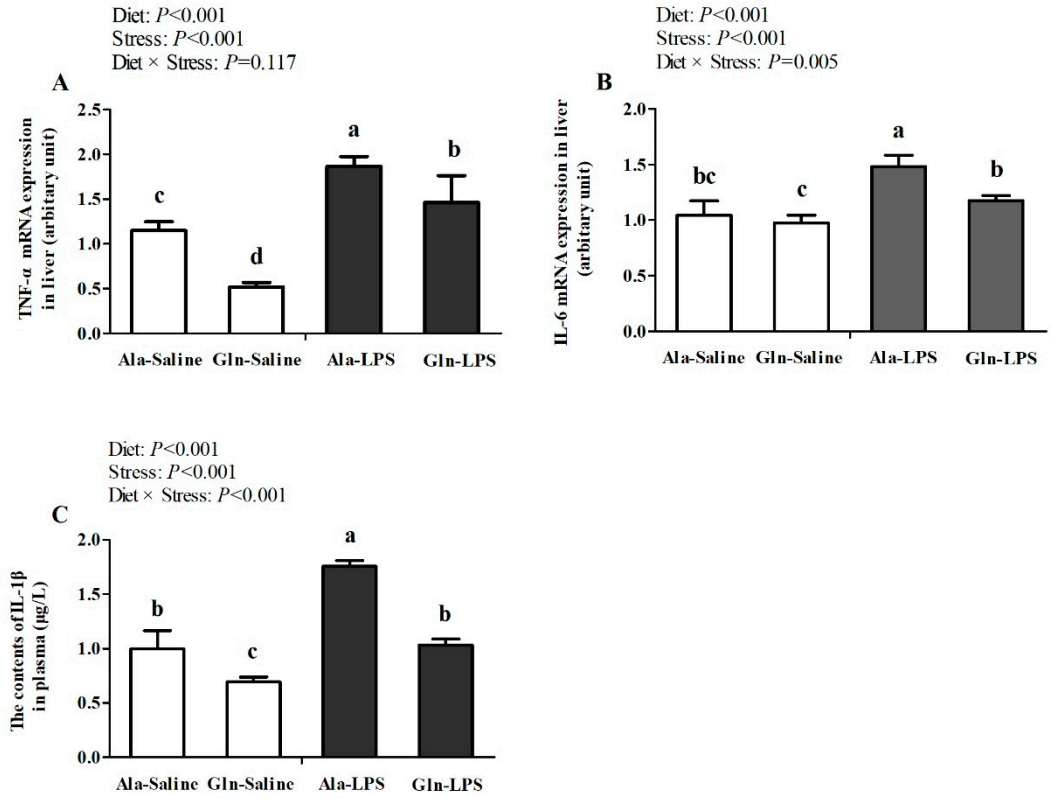

Figure 1. Effects of glutamine supplementation on the mRNA expression of TNF-α (A), IL-6 (B) and IL-1β (C) in liver of LPS-challenged broilers.Ala, alanine; Gln, glutamine; LPS, lipopolysaccharide. Values are means ± SEM, n=10. Different superscripts above the column means differences, *P*<0.05.

3.3. The activities of ALT and AST in muscle of broilers

The activity of AST in *Pecloralis Muscle* of broilers was decreased by LPS administration in comparison with those treated with saline injection (*P*<0.05, Table 3). However, no difference in the activity of ALT was observed in LPS treatment group (*P*>0.05). In contrast, dietary Gln supplementation significantly elevated the activity of AST of LPS-challenged broilers (*P*<0.05). But Gln supplementation did not affect the activity of ALT(*P*>0.05). In addition, no interaction for the activities of AST or ALT was observed between LPS treatment and Gln supplementation (*P*>0.05).

Table 3. Effects of Gln supplementation on the activities of ALT and AST in muscle of LPS-challenged broilers.

Treatment	ALT（u/g of protein）	AST（u/g of protein）
Ala-Saline	2.93	22.97
Ala-LPS	2.43	16.49
Gln-Saline	3.05	27.79
Gln-LPS	2.68	24.63
SEM	0.542	4.587
Main Effect		
Diet		
Ala	2.68	19.73[b]

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Gln	2.87	26.21[a]
Stress		
Saline	2.99	25.38[a]
LPS	2.56	20.56[b]
P Value		
Gln	0.603	<0.001
LPS	0.236	0.002
Gln × LPS	0.848	0.160

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ala, alanine; Gln, glutamine; LPS, lipopolysaccharide. Values are means and SEM, n=10. Different superscripts in an array means differences, $P<0.05$.

3.4. GS activity and mRNA expression of GA in Pecloralis Muscles of broilers

As presented by Figure 2, in compared with broilers received saline injection, LPS challenge significantly increased the activity of GS ($P<0.05$). Meanwhile, mRNA expressions of GA were also upregulated by LPS treatment ($P<0.05$). However, Gln supplementation downregulated the increased mRNA expression of GA induced by LPS stimulation ($P<0.05$). There were no interactions for GS activity and GA mRNA expression between LPS challenge and Gln supplementation ($P>0.05$).

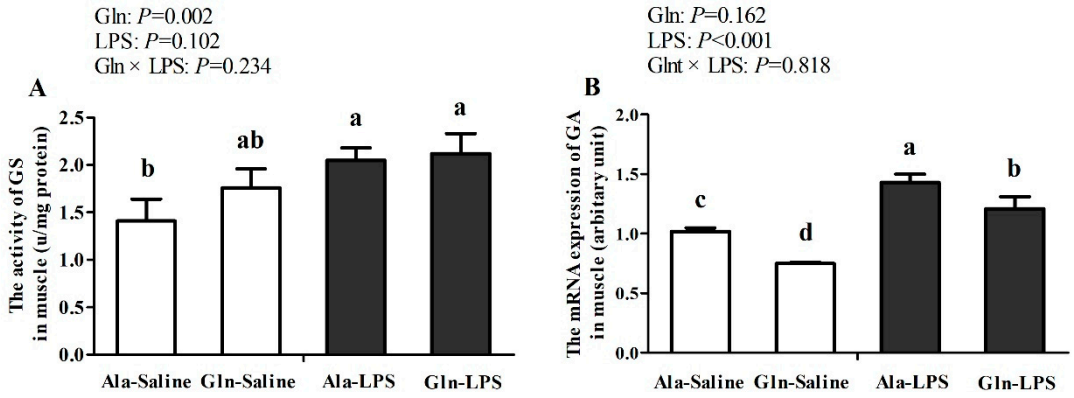

Figure 2. Effects of Gln supplementation on the GS activity (A) and GA mRNA expression (B) in muscle of LPS-challenged broilers.

GS, glutamine synthetase; GA, glutaminase; Ala, alanine; Gln, glutamine; LPS, lipopolysaccharide. Values are means ± SEM, n=10. Different superscripts above the column means differences, $P<0.05$.

3.5. mTOR signaling molecules in muscle

In comparison with saline treatment, LPS treatment significantly lowered mRNA expressions of mTOR, 4E-BP1 and S6K1 in *Pecloralis Muscles* of broilers ($P<0.05$, Figure 3). However, mRNA expressions of mTOR, 4E-BP1 and S6K1 were all elevated by Gln supplementation ($P<0.05$). A significant interaction for 4E-BP1 was observed between LPS challenge and Gln supplementation ($P<0.05$). But there were no interactions for mTOR and S6K1 between LPS challenge and Gln supplementation ($P>0.05$).

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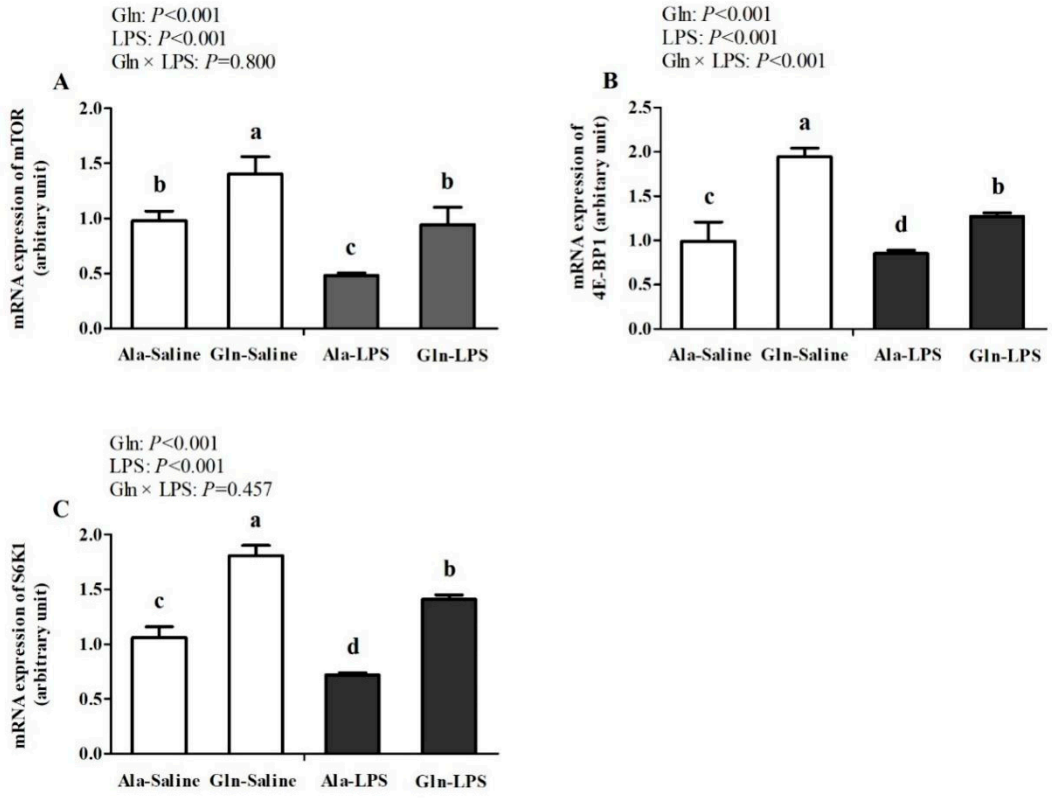

Figure 3. Effects of Gln supplementation on mRNA expressions of mTOR (A), 4E-BP1 (B) and S6K1 (C) in muscle of LPS-challenged broilers.

mTOR, mammalian target of rapamycin; S6K1, ribosomal protein S6 kinase; 4E-BP1, eIF-4E binding protein-1; Ala, alanine; Gln, glutamine; LPS, lipopolysaccharide. Values are means ± SEM, n=10. Different superscripts above the column means differences, $P<0.05$.

3.6. mRNA expression of Akt/FOXO signals mediated by TLR4

As shown in Table 4, when compared with broilers in saline treatment, LPS challenge significantly decreased Akt mRNA expression, but increased mRNA expressions of TLR4, FOXO4, FOXO1, MAFbx and MuRF1 ($P<0.05$). In contrast, Gln supplementation upregulated Akt mRNA expression and downregulated mRNA expressions of TLR4, FOXO4, FOXO1, MAFbx and MuRF1 ($P<0.05$). There were significant interactions for TLR4, FOXO1 and MuRF1 between LPS challenge and Gln supplementation ($P<0.05$), however, no interactions for Akt, FOXO4 or MAFbx was observed between LPS challenge and Gln supplementation ($P>0.05$).

Table 4. Effects of Gln supplementation on mRNA expressions of protein degradation related genes in muscle of LPS-challenged broilers.

Treatment	TLR4	Akt	FOXO4	FOXO1	MAFbx	MuRF1
Ala-Saline	1.00^{c}	1.00	0.99	1.04^{b}	1.0	1.07^{c}
Ala-LPS	1.43^{a}	0.59	1.42	2.93^{a}	1.4	2.81^{a}
Gln-Saline	0.86^{d}	1.39	0.92	0.94^{b}	0.69	1.03^{c}
Gln-LPS	1.01^{b}	0.96	1.11	1.28^{b}	1.14	1.47^{b}
SEM	0.234	0.301	0.249	0.868	0.292	0.762
Main Effect						
Diet						
Ala	1.22^{a}	0.80^{b}	1.21^{a}	1.99^{a}	1.29^{a}	1.94^{a}

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Gln	0.94[b]	1.18[a]	1.02[b]	1.11[b]	0.92[b]	1.25[b]
Stress						
Saline	0.93[b]	1.20[a]	0.96[b]	0.99[b]	0.89[b]	1.05[b]
LPS	1.22[a]	0.78[b]	1.27[a]	2.11[a]	1.32[a]	2.14[a]
P Value						
Gln	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LPS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Gln × LPS	0.002	0.932	0.091	<0.001	0.266	<0.001

TLR4: Toll-like receptors 4; Akt: protein kinase B; FOXO4: Forkhead Box O 4; FOXO1: Forkhead Box O 1; MAFbx: Muscle atrophy F-box; MuRF1: Muscle Ring finger 1; Ala, alanine; Gln, glutamine; LPS, lipopolysaccharide. Values are means ± SEM, n=10. Different superscripts in an array means differences, $P<0.05$.

4. Discussion

Under the condition of LPS exposure, animals generally were subjected to the reduced appetite [17], the destroyed integrity of intestinal mucosal barrier [18] and nutrients were redistributed from anabolic and body protein deposition towards utilization for immune system functioning [19], followed by the reduced ADFI and ADG combined with the increased F/G. Accordingly, compared with those received saline administration, LPS challenge reduced the retardation of the growth performance, evidenced as the decreased in ADFI and ADG as well as the increased F/G in our present study. Similar with this, it was reported that LPS challenge with intra-peritoneal injection significantly decreased the growth performance of broilers [20,21]. However, Gln supplementation reversed the negative effects on the growth performance of broilers indued by LPS challenge. Moreover, Gln, a neutral and multifunctional essential amino acid, is proven to be particularly prominent in the anti-stress response [9,22]. In addition, in previous studies, it was demonstrated that 1% Gln supplementation improved the growth performance of broilers under stress, accompanied by the increased in ADFI and ADG and the decreased F/G [8,13], which indicated that Gln administration contributed to ameliorating the adverse effects of LPS stimulation on the growth performance of broilers.

It had been demonstrated that LPS is a potential stimulation triggering the release of proinflammatory cytokines [23,24]. The secretion of proinflammatory cytokines are crucial for activating the innate host defense system and subsequently regulating the adaptive immune response, such as IL-6, TNF-α and IL-1β [21]. TNF-α, IL-6 and IL-1β, originated from macrophages, are the major regulators in diverse inflammatory responses [5]. The results of our present study showed that LPS challenge increased mRNA expressions of TNF-α, IL-6 and IL-1β in liver, indicating that LPS challenge induced an acute inflammatory response. It was demonstrated that LPS challenge resulted in higher mRNA expression TNF-α, IL-6 and IL-1β in liver of broilers [25]. In similar with this, the increased mRNA expressions of IL-6 and IL-1β were observed in LPS-challenged broilers [21]. The results of our previous study suggested that Gln supplementation decreased the contents of TNF-α, IL-6 and IL-1β in plasma of LPS-challenged broilers [5]. Moreover, it was proven that Gln deprivation exacerbates the production of proinflammatory cytokines, whereas Gln supplementation limits the inflammatory response *in vitro* [26]. The results aforementioned indicated that Gln supplementation might help to alleviate the inflammatory responses induced by LPS challenge.

Because of the physiological demand of Gln exceeded the synthesis capacity under catabolic stresses, it became the conditionally essential amino acid [27]. Skeletal muscle plays important roles in Gln metabolism and is quantitatively the most relevant site of Gln stock, synthesis and release site [28]. The two intracellular enzymes are glutamine synthetase (GS) and phosphate-dependent glutaminase (GA), which respectively are responsible for Gln synthesis and Gln hydrolysis [29]. It has been proven that there are a concomitant increase in GA expression under catabolic conditions, such as sepsis and infections [30,31]. Similarly, in our present study, LPS administration significantly increased mRNA expression of GA in muscle of broilers suggesting that Gln consumption accelerated induced by LPS challenge. In addition, the increased GS enzyme activity in skeletal muscle was

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observed during severe catabolic states [29]. In according with this, our results also showed that LPS challenge elevated the activity of GS in muscle. Moreover, Gln metabolism is also influenced by glutamine aminotransferase. In this study, Gln supplementation increased AST activity in muscle. Consistent with the result of ours, it was also reported that 1% Gln supplementation increased AST activity in muscle associated with Gln metabolism [32]. However, Gln addition significantly decreased GA mRNA expression in muscle, indicating that Gln supplementation might compensated for the decline of Gln in muscle under stress and partly contributed to inhibiting the catabolism of Gln.

It is well documented that mTOR signaling pathway is an evolutionally conserved protein kinase and is important in regulating protein synthesis. The active of mTOR and its downstream regulators 4E-BP1 and S6K1 synergistically leads to the initiation of polypeptide formation [33]. But mTOR signaling is inhibited by sepsis and endotoxin-related inflammation [34]. It was found that LPS challenge downregulated mRNA expressions of mTOR, 4E-BP1 and S6K1 in muscle in our present study. It was also reported that the expressions of mTOR, 4E-BP1 and S6K1 were inhibited in septic rats or animals treated with LPS [34], indicating that protein synthesis was inhibited by LPS challenge. Independent of LPS administration, the increased in mRNA expressions of mTOR, combined with 4E-BP1 and S6K1 in muscle, were observed by Gln addition. It has been demonstrated that Gln is required for the activation of mTOR signaling [35]. In similar with this, in a previous study, Gln supplementation significantly elevated the protein expressions of mTOR, 4E-BP1 and S6K1 in skeletal muscle [27]. In addition, Gln addition to the medium stimulated protein synthesis through mTOR signaling pathway [36]. The results mentioned above suggested that Gln contributed to promoting protein synthesis associated with the activation of mTOR signaling.

The major of intra-cellular proteins are degraded by the ubiquitin-proteasome pathway in all tissues [37], which contributes to 75% protein degradation during skeletal muscle atrophy [38]. The activation of Akt and inactivation of FOXO transcriptionally upregulated of FOXO gene targets MAFbx and MuRF1, subsequently induced muscle protein degradation [39]. TLR4 was also demonstrated to be a master regulator of muscle wasting induced by endotoxemia [40]. The present study found that LPS challenge significantly decreased mRNA expression of Akt and increased mRNA expression of TLR4, FOXO1, FOXO4, MAFbx and MuRF1 in muscle. It has been demonstrated that the decreased in Akt expression and the increased expressions of MAFbx and MuRF1 occurred in response to LPS-induced endotoxaemia [41]. Besides, in a previous study, it was demonstrated that increased mRNA expression of FOXO1 and FOXO4, combined with the increased MAFbx and MuRF1 mRNA expression were induced by LPS challenge [7]. In similar, in a previous study about LPS-administrated rat, it was demonstrated that LPS challenge downregulated Akt expression and upregulated MAFbx and MuRF1 in skeletal muscle [39]. Currently, Gln has been shown to be an amino acid involved in the regulation of autophagy [42]. It was proven that Gln administration inhibited protein degradation of intestinal epithelial cells [36]. Our results also showed that Gln addition increased Akt mRNA expression and lowered mRNA expression of MAFbx and MuRF1 associated with protein degradation. Moreover, in our previous study in piglets, alanyl-glutamine (a dipeptide of Gln) supplementation decreased mRNA expressions of both MAFbx and MuRF1 in skeletal muscle under normal and LPS condition [7,27]. In addition, Gln administration induced the reduced mRNA expression of MAFbx and MuRF1, and the loss in the skeletal muscle mass was alleviated partially by Gln supplementation [43]. Therefore, based on the mentioned above, we could speculate that Gln supplementation could contribute to inhibiting protein degradation of skeletal muscle via TLR4/Akt/UPP signaling pathway.

5. Conclusion

In conclusion, Gln supplementation improved growth performance of broilers administrated with LPS and alleviated inflammatory response in liver. Moreover, Gln supplementation increased the expressions of signaling molecules in mTOR and Akt/FOXO/UPP pathway, indicating that Gln addition might contributed to promoting protein synthesis and inhibiting protein degradation of LPS-challenged broilers.

Author Contributions B. L. Zhang mainly wrote this manuscript and experimental administration. N. Liu assisted in data analysis and writing the manuscript. Q. Yang conducted in animal trial. Q.Z. Zhong and Z.W. Sun assisted in revising the manuscript. All authors reviewed the final manuscript and approved the submitted version. All authors have no conflict on the interest of this paper.

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