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Effects of Supplemental Glutamine on Growth Performance, Plasma Parameters and LPS-induced Immune Response of Weaned Barrows after Castration

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ABSTRACT: Two experiments were conducted to investigate the effects of supplemental glutamine on growth performance, plasma parameters and LPS-induced immune response of weaned barrows after castration. In experiment 1, forty-eight weaned male piglets were used and fed maize and soybean meal diets supplemented with 0 (Control) or 2% L-Gln (Gln+) for 25 days. The results indicated that the Gln+ group tended to increase average daily gain compared to control in stages of days 7 to 14 and 0 to 25. The Gln+ had significantly better feed efficiency than the control group did during days 14 to 25 and 0 to 25. The plasma blood urea nitrogen and alkaline phosphatase contents of Gln+ group were higher than those of the control group on day 14 post-weaning. In experiment 2, sixteen weaned male piglets were injected with *E. coli* K88+ lipopolysaccharide (LPS) on day 14 post-weaning. The results showed that the Gln+ group had lower concentrations of plasma adrenocorticotrophic hormone and cortisol than the control group on day 14 pre-LPS challenge. In addition, Gln+ group had higher plasma IgG concentration than the control group for pre- or post-LPS challenged on day 14 post-weaning. In summary, dietary supplementation of Gln was able to alleviate the stressful condition and inflammation associated with castration in weaned barrows, and to improve their immunity and growth performance in the early starter stage. (**Key Words:** Glutamine, Weaned Piglets, Growth Performance, Castration)

INTRODUCTION

Inadequate nutrient intake after weaning often damages the intestinal villi and contributes to the poor growth of weanling pigs (Pluske et al., 1997). Amino acids (glutamine (Gln) and arginine) and polysaccharide from Chinese herbs/Chitosan are an energy source and act immunologically; the intestinal mucosa are quantitatively more important in the abrupt change of weaning (Kong et al., 2007; Guo et al., 2008; Liu et al., 2008; Yin et al., 2008; Han et al., 2009; Yao et al., 2011).

Dietary Gln is metabolized by the small intestine and essentially all Gln within the body is synthesized de novo through the action of Gln synthetase. The major sites of net Gln synthesis are lungs, adipose tissue, skeletal muscle and liver (Hall et al., 1996; Holecek, 2002). It is also the most abundant free AA found in the blood of animals and in sow milk (Wu and Knabe, 1994).

Glutamine provides energy to rapidly dividing cells, such as intestinal epithelial cells, activates lymphocytes and is regarded as a conditionally essential AA (Ardawi and Newsholme, 1983; Wu et al., 1995; Komatsu et al., 2007; Li et al., 2007). Glutamine is known to stimulate protein synthesis in intestinal epithelial cells via activating the mammalian target of the rapamycin signaling pathway (Wu et al., 2011). In addition, a key intermediate in L-glutamine catabolism, alpha-ketoglutarate, could inhibit glutamine degradation and enhance protein synthesis in intestinal porcine epithelial cells (Yao et al., 2011). The requirement of Gln increased especially under certain physiological stresses such as weaning, castration and infection, which cannot be countered by endogenous synthesis (Lacey and Wilmore, 1990; Hall et al., 1996; Newsholme, 2001). Previous reports revealed that Gln reduces the susceptibility to infections and enhances the recovery of wounds for patients (Newsholme, 2001; Wilmore, 2001). In the swine industry, castration is usually carried out at 2 to 5 weeks of

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age without any anesthesia or analgesia, which results in painful and great stress to the barrows. Therefore, castrated barrows normally have an impaired growth performance than the gilts during the period immediately after the castration (Bruininx et al., 2001). Alleviation of the stress associated with castration can definitely minimize the economic losses. The objective of the present study was to investigate the effects of dietary supplementation of Gln on growth performance, plasma parameters and LPS-induced immune response of weaned barrows after castration.

MATERIALS AND METHODS

Animals and management

The animal feeding protocol of this research was approved by the Animal Care and Use Committee of Kaohsiung propagation station, Livestock Research Institute, Council of Agriculture, involving 64 crossbred (Landrace×Yorkshire×Duroc) weaned at 28±2 days of age and initial body weight was approximately 7.0 kg per pig from the commercial piglet farm. All male piglets were fed with a commercial mash diet during the 3 day adaptation period. The pigs were raised in a nursery house with concrete floor. Each pen contained a self feeder and a nipple water cooler. Mash feed and water were provided for ad libitum during the entire experimental period. The average temperature during the experiment was 26.3°C. All of the piglets were castrated on day 7 post-weaning by the same technician to avoid stress due to different sill between surgeons. The castration procedure was completed within 2 min per piglet.

Experimental design

In experiment 1, 48 weaned male piglets were randomly allocated into 12 pens for two dietary treatments with supplemented with 0 (Control) or 2% L-Gln supplemented diet (Gln+). The control starter diet was maize-soybean meal diet formulated according to the NRC (1998) standard (Table 1). Pigs were weighed individually at day 0 (initial of the trial), 7, 14 and 25 (end of the trial). Feed intake was recorded by period, days 0 to 14 and days 15 to 25. Blood samples of two pigs randomly selected from each pen were taken via the vena cava puncture at day 0, 7, 14 and 25 of the experimental period. Blood samples were centrifuged at 2,000×g for 20 min at 4°C, and the plasma was separated and stored at -20°C until the analysis of the biochemical parameters. On d 14, four piglets from each treatment were randomly selected after weighing and blood collection, sacrificed and the intestinal morphology was measured as described below. In experiment 2, 16 male piglets were randomly divided into 2 groups (4 piglets/pen, 2 pens/group) after weaning. The experimental dietary treatment and castration procedure was similar to

Table 1. Ingredient composition and calculated values of starter ${\rm diet}^{\rm a}$

| Ingredient (%) | Starter diet |
|----------------------------------|--------------|
| Maize, dent yellow | 49.05 |
| Soybean meal, solvent, 44% of CP | 23.70 |
| Dried skim milk | 16.00 |
| Whey | 5.00 |
| Soybean oil | 1.00 |
| Dicalcium phosphate | 1.60 |
| Limestone, pulverized | 0.80 |
| Salt | 0.50 |
| Vitamin premix ^b | 0.10 |
| Mineral premix ^c | 0.15 |
| Choline chloride, 50% | 0.10 |
| Maize starch | 2.00 |
| Calculated values (%) | |
| Crude protein | 20.30 |
| Digestible energy (kcal) | 3,450.00 |
| Calcium | 0.75 |
| Total phosphorus | 0.65 |
| Lysine | 1.17 |

^a Gln+treatment diet used 2% of glutamine to replace maize.

^b Supplied the following vitamins per kg of diet: Vitamin A, 6,000 IU; vitamin D₃, 800 IU; vitamin E, 20 mg; vitamin K₃, 4 mg; vitamin B₂, 4 mg; vitamin B₆, 1 mg; vitamin B₁₂, 0.02 mg; Niacin, 30 mg; calcium pantothenate, 16 mg; folic acid, 0.6 mg; biotin, 0.01 mg; choline chloride, 50 mg.

^c Supplied the following minerals per kg of diet: Fe, 140 mg; Cu, 7 mg; Mn, 20 mg; Zn, 120 mg; I, 0.45 mg.

experiment 1. On d 14, all of the piglets received an intramuscular injection of *E. coli* K88+ lipopolysaccharide (LPS, serotype 055:B5, Sigma Chemical, St Louis, MO) at 25 μ g/kg body weight. Rectal temperatures and blood samples were taken on d 7 (before castration) and d 14 (before and at 4 h after LPS challenge) post-weaning. Blood samples and plasma preparation were the same as mentioned above. The plasma was stored at -20°C for subsequent analysis of stress-related hormones (adrenocorticotrophic hormone (ACTH) and cortisol), IgG and cytokines (TNF- α , IL-1 β and IL-10).

Small intestinal morphology

Four 4-cm segments from the front section (one-tenth), middle section (a half) and last section (nine-tenth) of the small intestine representing duodenum, jejunum and ileum, respectively, were taken for histological measurement. These samples were first rinsed with 0.1 M phosphate buffered saline (PBS) at pH 7.2, and then fixed with 10% neutral formaldehyde. After 24 h, the samples were removed from the fixative, cut into 1 cm² sections (two per location) and stored in fresh fixative. They were then embedded in paraffin, sectioned at 6 μ m thickness and stained with hematoxylin and eosin for a light microscopy

examination. The villous height (VH), crypt depth (CD), and the muscular layer thickness were measured based on 15 apparently intact villi from each section according to the procedure described by Yu et al. (1999).

Plasma biochemical parameters

The concentrations of blood urea nitrogen (BUN), creatinine, glucose, and alkaline phosphatase (ALP) activity were measured by an automatic biochemical analyzer (Automatic Analyzer, HITACHI 7150. Tokyo, Japan). The concentrations of ACTH and cortisol were determined by chemiluminescent immunoassay (Immulite Chemiluminescent immunoassay; DPC, Los Angeles, CA) (Webel et al., 1997).

Plasma IgG and cytokines

The plasma concentrations of total IgG and cytokines (TNF- α , IL-1 β and IL-10) were measured by commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's instructions. Plasma samples were diluted (1:100,000 for IgG; 1:1 or 1:10 for cytokines) and analyzed in duplicate.

Statistical analysis

Data were analyzed by SAS programs (1999). Unpaired Student's t-test was used to determine differences between treatments, and p<0.05 was considered as statistically significant; p<0.1 was considered as a trend.

Table 2. Effect of glutamine supplementation on growthperformance of weaned piglets

| Item | Control | Gln+ | SEM ^c | p value | | |
|------------------------|---------|-------|------------------|---------|--|--|
| Mean body weight (kg) | | | | | | |
| $0 d^{a}$ | 8.18 | 8.13 | 0.21 | 0.878 | | |
| 7 d ^a | 9.16 | 9.11 | 0.25 | 0.893 | | |
| 14 d ^a | 10.96 | 11.34 | 0.34 | 0.412 | | |
| 25 d ^b | 15.02 | 16.27 | 0.51 | 0.100 | | |
| Mean daily gain (k | g) | | | | | |
| 0-7 d ^a | 0.14 | 0.14 | 0.02 | 0.993 | | |
| 7-14 d ^{a,} * | 0.27 | 0.32 | 0.02 | 0.061 | | |
| 0-14d ^a | 0.20 | 0.23 | 0.01 | 0.169 | | |
| 14-25 ^b | 0.41 | 0.44 | 0.03 | 0.287 | | |
| 0-25 ^{b,} * | 0.29 | 0.33 | 0.02 | 0.097 | | |
| Daily feed intake (l | kg) | | | | | |
| 0-14 | 0.37 | 0.38 | 0.02 | 0.604 | | |
| 14-25 | 0.78 | 0.77 | 0.05 | 0.955 | | |
| 0-25 | 0.54 | 0.54 | 0.03 | 0.867 | | |
| Feed efficiency (G/F) | | | | | | |
| 0-14* | 0.54 | 0.63 | 0.03 | 0.099 | | |
| 14-25** | 0.52 | 0.58 | 0.02 | 0.022 | | |
| 0-25** | 0.53 | 0.61 | 0.02 | 0.033 | | |

^{a,b} Mean value is obtained from 24 and 14 piglets, respectively. ^c Standard error of means.

* Means in the treatments are numerically different (p<0.1). ** Means in the treatments are different significantly (p<0.05).

RESULTS

Growth performance in experiment 1

Glutamine supplementation on growth performance of weaned piglets is shown in Table 2. There were no significant differences in initial body weight between treatments; however, the body weight of Gln+ group tended to be higher than the control at d 25 post-weaning. The ADG was compared based on different stages (0 to 7, 7 to 14, 0 to 14, 14 to 25 and 0 to 25 d) of growth. The Gln+ group had trended to increase ADG compared to the control on days 7 to 14 and days 0 to 25. In general, the feed intakes in all stages did not differ between treatments. However, the Gln+ group had significantly higher G/F than the control group during the 14 to 25 day and 0 to 25 day periods.

Small intestinal morphology in experiment 1

Table 3 presents the small intestinal morphological criteria observed on d 14. There were no differences in villous height, crypt depth and VH/CD ratio between treatments at day 14 post-weaning. However, in comparison with the control, dietary supplementation of Gln significantly increased the muscular layer thickness in the jejunum and ileum.

Plasma biochemical parameters in experiment 1

For all the plasma biochemical parameters, there was no significant difference due to weaning (d 0 and d 7 post-weaning) (Table 4). The Gln+ group showed a trend toward

| Table | 3. | Effec | t of | glutamine | suppleme | ntation | on | the | intestinal |
|-------|-----|-------|------|-------------|-----------|---------|------|-----------------|------------|
| morph | olo | gy of | weat | ned piglets | on day 14 | post-w | eani | ng ^a | |

| Item | Control | Gln+ | SEM ^b | p value |
|--------------------------|---------|------|------------------|---------|
| Duodenum | | | | |
| Villous height (µm) | 377 | 450 | 40 | 0.248 |
| Crypt depth (µm) | 329 | 343 | 34 | 0.783 |
| VH/CD value | 1.22 | 1.42 | 0.27 | 0.621 |
| Muscular thickness (µm) | 444 | 460 | 57 | 0.854 |
| Jejunum | | | | |
| Villous height (µm) | 394 | 445 | 32 | 0.206 |
| Crypt depth (µm) | 242 | 312 | 29 | 0.119 |
| VH/CD value | 1.73 | 1.48 | 0.19 | 0.409 |
| Muscular thickness (µm)* | 245 | 336 | 25 | 0.046 |
| Ileum | | | | |
| Villous height (µm) | 319 | 358 | 19 | 0.155 |
| Crypt depth (µm) | 209 | 216 | 14 | 0.757 |
| VH/CD value | 1.59 | 1.75 | 0.17 | 0.536 |
| Muscular thickness (µm)* | 451 | 736 | 67 | 0.024 |

* Means in the treatments are different significantly (p<0.05).

^a Values of intestinal morphology assay are means of 12 pigs per treatment.

^b Standard error of means.

Table 4. Effect of glutamine supplementation on the plasma biochemical parameters of weaned piglets^a

| e 11 | • | • | | |
|-------------------------------------|---------|-------|------------------|---------|
| Item | Control | Gln+ | SEM ^b | p value |
| Day 0 post-weaning | | | | |
| Creatinine (mg/dl) | 1.00 | 1.07 | 0.04 | 0.220 |
| BUN (mg/dl) | 5.5 | 5.9 | 0.5 | 0.579 |
| Total protein (g/dl) | 4.9 | 5.1 | 0.1 | 0.155 |
| Glucose (mg/dl) | 132.0 | 131.0 | 4.0 | 0.963 |
| Alkinine phosphatase (IU/L) | 9.8 | 12.1 | 2.1 | 0.439 |
| Day 7 post-weaning (pre-castration) | | | | |
| Creatinine (mg/dl) | 0.93 | 0.89 | 0.03 | 0.413 |
| BUN (mg/dl)* | 12.5 | 14.9 | 0.9 | 0.064 |
| Total protein (g/dl)* | 4.8 | 5.1 | 0.1 | 0.094 |
| Glucose (mg/dl) | 112.0 | 115.0 | 3.6 | 0.539 |
| Alkinine phosphatase (IU/L) | 5.9 | 6.2 | 1.2 | 0.840 |
| Day 14 post-weaning | | | | |
| Creatinine (mg/dl) | 0.79 | 0.80 | 0.04 | 0.892 |
| BUN (mg/dl)** | 10.0 | 13.0 | 0.6 | 0.003 |
| Total protein (g/dl) | 4.8 | 4.9 | 0.1 | 0.431 |
| Glucose (mg/dl) | 117.0 | 110.0 | 3.3 | 0.206 |
| Alkinine phosphatase (IU/L)** | 4.7 | 14.6 | 1.4 | 0.001 |
| Day 25 post-weaning | | | | |
| Creatinine (mg/dl) | 0.77 | 0.69 | 0.03 | 0.126 |
| BUN (mg/dl) | 10.2 | 9.7 | 0.6 | 0.589 |
| Total protein (g/dl) | 5.2 | 5.2 | 0.2 | 0.926 |
| Glucose (mg/dl) | 115.0 | 114.0 | 3.1 | 0.928 |
| Alkinine phosphatase (IU/L) | 8.2 | 6.4 | 1.1 | 0.234 |

* Means in the treatments are numerically different (p<0.1). ** Means in the treatments are different significantly (p<0.05).

^a Values of plasma biochemical parameters are means of 12 pigs per treatment. ^b Standard error of means.

higher BUN and total protein concentrations on d 7, and BUN and ALP activity showed a significantly higher than control group on d 14. However, no differences in all plasma parameters between the groups were observed on d 25.

Plasma stress-related hormones and rectal temperature in experiment 2

The concentration of plasma stress-related hormones (ACTH and cortisol) and rectal temperature of pigs in experiment 2 are shown in Table 5. The rectal temperature

Table 5. Effect of glutamine supplementation on the plasma stress-related hormones and rectal temperature of barrows with LPS challenge^a

| Item | Control | Gln+ | SEM ^b | p value |
|-------------------------------------|---------|-------|------------------|---------|
| Day 7 post-weaning (Pre-castration) | | | | |
| Rectal temperature (°C) | 40.0 | 40.1 | 0.1 | 0.659 |
| ACTH (pg/ml) | 27.1 | 24.7 | 5.3 | 0.642 |
| Cortisol (µg/dl) | 2.9 | 2.6 | 0.4 | 0.727 |
| Day 14 post-weaning | | | | |
| Pre-LPS challenge | | | | |
| Rectal temperature (°C) | 40.0 | 39.9 | 0.1 | 0.489 |
| ACTH (pg/ml)* | 81.3 | 44.1 | 12.1 | 0.044 |
| Cortisol (µg/dl)* | 6.1 | 3.2 | 0.6 | 0.003 |
| Post-LPS challenge | | | | |
| Rectal temperature (°C)* | 41.4 | 41.0 | 0.1 | 0.025 |
| ACTH (pg/ml) | 142.9 | 134.2 | 33.1 | 0.854 |
| Cortisol (µg/dl) | 8.5 | 10.3 | 1.4 | 0.355 |

* Means in the treatments are different significantly (p<0.05).

^a Values of plasma stress-related hormones and rectal temperature are means of 8 pigs per treatment. ^b Standard error of means.

and the concentrations of ACTH and cortisol were no different between the groups before castration on d 7 postweaning. In contrast, the Gln+ barrows had significantly lower concentrations of ACTH and cortisol when compared to control barrows on d 14 post-weaning before LPS challenge. When the pigs were challenged with LPS, the plasma level of ACTH and cortisol dramatically increased, but no difference was found between treatments 4 h later. Nevertheless, the rectal temperature of the control group was significantly higher than the Gln+ group after LPS challenge.

Responses of plasma IgG and cytokines after the LPS challenge in experiment 2

In experiment 2, the plasma concentrations of IgG and cytokines (TNF-a, IL-1ß and IL-10) on d 14 post-weaning are shown in Table 6. There were no significant differences in plasma concentrations of IgG and cytokines between groups on d 7 (before castration, data not shown). On d 14, before the LPS challenge, the plasma IgG concentration in Gln+ group was significantly higher than that of the control; however, the plasma TNF- α in Gln+ group exhibited no significant effects compared to the control (p = 0.113). After the LPS challenge, the concentrations of IgG appeared to decline in all treatments, but the Gln+ group was significantly higher than the control. For the cytokines, although no differences were found between groups after the LPS challenge, the concentrations of TNF- α , IL-1 β and IL-10 increased in the treatments compared to the prechallenged status.

DISCUSSION

The growth performance of weaned castrated piglets is

Table 6. Effect of glutamine supplementation on the plasma IgG and cytokine profiles of barrows with LPS challenge on d 14 post-weaning^a

| Item | Control | Gln+ | SEM ^b | p value | |
|--------------------------------|----------|-------|------------------|---------|--|
| Day 14 post-weaning (| Pre-LPS) | | | | |
| IgG (mg/ml)* | 13.1 | 20.5 | 1.3 | 0.002 | |
| TNF-α (pg/ml) | 983 | 668 | 108 | 0.113 | |
| IL-1β (pg/ml) | 527 | 415 | 85 | 0.524 | |
| IL-10 (pg/ml) | 1,059 | 950 | 82 | 0.453 | |
| Day 14 post-weaning (Post-LPS) | | | | | |
| IgG (mg/ml)* | 11.7 | 15.6 | 1.0 | 0.011 | |
| TNF-α (pg/ml) | 1,674 | 1,317 | 177 | 0.332 | |
| IL-1 β (pg/ml) | 751 | 607 | 121 | 0.536 | |
| IL-10 (pg/ml) | 1,704 | 1,431 | 172 | 0.415 | |

* Means in the treatments are different significantly (p<0.05).

^a Values of plasma IgG and cytokine profiles are means of 8 pigs per treatment.

^b Standard error of means.

usually inferior to that of the gilts during the early stage after castration, even though the castration is done as early as 3 days of age (Bruininx et al., 2001). Castration of male piglets without anesthesia or analgesia induces a strong activation of the pituitary-adrenocortical axis, so that the piglets are under acute pain and stress (Prunier et al., 2005). Under stress or inflammation, animals elevate the proinflammatory cytokine concentrations in plasma, leading to depressed feed intake and growth performance (Le Floc'h et al., 2004). In the current study, the Gln+ group (2% Gln supplementation) had trended to increase ADG compared to the control at days 7 to 14 and days 0 to 25. These results may suggest that dietary Gln supplementation alleviates the piglets suffering from pain and stress due to castration and improves their growth performance.

Lower than 2% Gln supplementation may not enhance the performance of piglets. Zou et al. (2006) discovered that pigs supplemented with 1% Gln had a 12% lower feed/gain ratio during the first ten days after weaning. Wu et al. (1996) showed that 0.2 to 1.0% Gln supplementation did not have any significant effects on the daily feed intake, daily gain and gain/feed during the first week post-weaning. Moreover, Lee et al. (2003) also found that the 1.5% Gln supplementation did not affect the feed intake, ADG, and gain/feed of pigs weaned on 21 days of age. However, Alverdy (1990) showed that dietary provision of 2% Gln was essential for the maintenance of gut-associated lymphoid tissues and for the secretary IgA synthesis by small intestine, thereby preventing TNFa-induced bacterial translocation from lumen of gut into the circulatory system. Dietary supplementation with 2% Gln increased the survival of mice to bacterial challenges (Adjei et al., 1994) and improved tumor-directed NK cell cytotoxic activity in rats (Shewchuk et al., 1997). Therefore, 2% Gln supplementation was chosen in the current study. The improvement growth performance of weaned piglets by Gln supplementation may be attributed to the maintenance of intestinal villous function and morphology and the increasing ability in intestinal absorption (Liu et al., 2002; Yi et al., 2005). In the present study, the villous morphology in various intestinal segments of villous height and crypt depth were not affected by Gln supplementation, but did increase the muscular layer thickness in the jejunum and ileum (Table 3). We speculated that Gln may facilitate mucosal protein synthesis in the gut and promote the growth of intestinal muscular layer (Coëffier et al., 2003; Le Bacquer et al., 2003).

In the plasma BUN level, the Gln supplemented piglets had higher plasma BUN levels than the control piglets did after 7 days and 14 days post-weaning, respectively. Zou et al. (2006) suggested that higher level of blood BUN in weaned piglets may be a consequence of deficient nutrient supply with increasing protein catabolism, causing N losses to occur. One percentage of Gln supplement diet corrected these negative effects and decreased serum BUN concentration to some extent because Gln provides precursors of several kinds of amino acids and promotes protein synthesis (Yu et al., 2002). Currently, a higher supplementary level could suggest that excess provision might lead to an imbalance of amino acids, elevated catabolism and blood BUN concentration (Holecek, 2002). The Gln requirement for weaned male piglets might be in dynamic rapidly transient status; therefore, 2% of Gln supplementation may be adequate for requirement initially and over provision in the late stages (d 25 post-weaning). The results demonstrated that increasing the Gln supplement up to 2% obtained a positive growth perform effect, but elevated the BUN and increased the risk of metabolic imbalance of amino acids.

Both Gln and glucose are major sources of energy for enterocytes of piglets (Posho et al., 1994). It suggests that Gln can exert a "sparing effect" by reducing glucose contribution to the total ATP turnover (Posho et al., 1994). However, no difference was found in plasma glucose between treatments at each stage of post-weaning. The pain and stress associated with castration peaked between 30 and 60 min after the operation, and were reflected in the plasma cortisol concentration, which returned to the baseline level within 3 h (Prunier et al., 2005). The plasma ALP activity in Gln+ pigs was significantly higher than that of control pigs after two weeks of weaning. It is possible that higher dietary Gln can increase the ALP activity of barrows and facilitate the recovery of injured tissues.

The subsequent development of cortisol or ACTH concentration in barrows remained unclear. In this study, the ACTH and cortisol were higher than those of the barrows on d 7 after castration; moreover, Gln+ group was significantly lower than the control group without LPS, challenged on the d 14 post-weaning. We speculate that more pain or stress might exist in barrows after castration, and Gln could alleviate the stressful condition. Recently, research on molecular mechanisms has revealed that dietary Gln supplementation would increase the intestinal expression (120 to 124%) of genes that are necessary for cell growth and removal of oxidants, while reducing (34 to 75%) the expression of genes that promote oxidative stress (Wang et al., 2008). The results suggested that Gln supplementation may improve the intestinal oxidativedefense capacity and result in lower plasma stress-related hormones.

The significantly higher concentration of plasma IgG in the Gln+ group pre- and post- LPS challenged at d 14 postweaning indicated that male piglets could possess stronger anti-stress capacity to cope with the invasive organisms. Although the increase of IgG contents in weaned piglets after treatment cannot directly indicate biological significance, Yu et al. (2002) reported that dietary supplementation of 1% Gln was able to increase serum IgG level and to neutralize antibody titers of foot and mouth disease (FMD) in LPS-challenged weaned piglets. Thus, they speculated that Gln might play a role in enhancing the immune system especially in the humoral immunity of piglets. Similar results also showed that dietary 1% Gln supplementation could improve growth performance, facilitate the health of the GI tract, and increase the concentrations of sera IgG and IgA in chickens (Bartell and Batal, 2007).

On d 14 post-weaning (d 7 post-castrated), dietary Gln supplementation had somewhat modulated the expression of plasma TNF- α ; the Gln+ piglets seemed to have lower levels of inflammation after weaning and castration. In the response of the LPS challenge, the significant elevation of rectal temperature indicated that the inflammatory response of pigs had been induced, and the extent of fever was milder in Gln+ group (Table 5). The production of plasma proinflammatory cytokines peaked 2 to 4 h after LPS challenge in piglets and returned to normal levels within 12 h (Webel et al., 1997). In the present study, the concentrations of plasma cytokines in all groups obviously increased 4 h postchallenged, but they did not statistically differ between treatments (Table 6). Similar results were reported, that dietary supplementation of Gln and nucleotides did not affect the concentration of serum TNF- α in piglets challenged with LPS (Yu et al., 2002). It was speculated that under such a high dose of LPS challenge, the cytokines were acutely produced by macrophages and monocytes of piglets to modulate the inflammation. In short, dietary supplementation of Gln might not powerfully affect the plasma cytokine profiles during the short period of LPS challenge in our current study even if Gln could inhibit over-expression of pro-inflammatory genes in rats (Fillmann et al., 2007). Furthermore, the effect of Gln on the regulation of systemic inflammation suggested that it could be applied potentially to the inflammatory diseases (Singleton and Wischmeyer, 2008).

CONCLUSIONS

Dietary supplementation of 2% Gln was able to alleviate the stressful condition and inflammation associated with castration in weaned barrows, and to improve their immunity and growth performance in the early starter stage.

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