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Effect and Mechanism of Glutamine on Productive Performance and Egg Quality of Laying Hens

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ABSTRACT : This study was conducted to investigate the effects of dietary glutamine (Gln) on the productive performance and egg quality of laying hens. A total of four hundred Lingnan Yellow laying hens aged 34 weeks were randomly assigned into four groups (100 laying hens/group), and fed, respectively, with diets supplemented with 0% (control group), 0.2%, 0.4%, and 0.8% Gln during the 6-week feeding period. The results were as follows. First, the productivity of laying hens fed with 0.8% Gln in diet was significantly increased ($p<0.05$); however, the egg quality (egg weight, yolk weight, shell weight, egg shape index, shell thickness, shell density, shell breaking strength, yolk color, yolk index, and Haugh unit) was not affected compared with that of the control group ($p>0.05$). Second, luteinizing hormone (LH) ($p<0.01$), follicle stimulating hormone (FSH) ($p<0.01$), triiodothyronine (T_3), and tetraiodothyronine (T_4) contents ($p<0.05$) in blood of laying hens fed with 0.8% Gln in diets were also significantly improved, and greater improvement in the duodenum and oviduct structure was observed in that treatment group. This study indicated for the first time that diets with 0.8% Gln were able to increase the productive performance of laying hens through stimulating hormone secretion and better development of both the duodenum and oviduct structure in laying hens. (**Key Words :** Glutamine, Laying Hen, Productive Performance, Egg Quality, Hormone Secretion, Duodenum, Oviduct)

INTRODUCTION

Glutamine, a semi-essential or conditionally essential amino acid, has two mobilizable N groups in its structure (Smith, 1990); it is also the amino acid maximally utilized in the intestine of healthy animals (Windmueller, 1980). Recently, Gln has come to be regarded as an essential amino acid for the gut in some species of animals when their body is in some specific physiological or developmental conditions, such as infections and injuries (Van der Hulst et al., 1996; Newsholme, 2001). Numerous observations indicated that Gln administration could suppress intestinal inflammation, protect intestinal mucosal structure and reduce mucosal apoptosis after injury in rodents (Sukhotnik et al., 2007; Kessel et al., 2008). Exogenous Gln had the effect of antioxidant protection for rats with the implanted tumor (Kaufmann et al., 2007) and mice with dystrophic muscles (Mok et al., 2008). As a precursor of Glutathione (GSH), Gln also showed its anti-inflammatory and anticancer effects by up-regulating the gut GSH metabolism in the post-sepsis or murine models of asthma (Kaufmann et al., 2008; Singleton et al., 2008), containing the spread of Escherichia coli from the intestine to such organs as the liver, lung, and spleen (de Oliveira et al., 2006), and lowering the mortality of i.p. Escherichia coli-challenged rats (Inoue et al., 1993). In addition, Gln was a necessary precursor for molecular synthesis, such as DNA and protein (Ardawi, 1983). Glutamine could synthesize a variety of amino acids to accelerate protein synthesis through deamination and transamination (Boza et al., 2001), which thus partially prevented animal nutrition deficiency. Glutamine stimulated protein synthesis could improve nitrogen balance, which was effective against the hurt from feed restriction and illness (Yeh et al., 2001; Johnson et al., 2003). Glutamine availability could modulate glucose homeostasis during and after exercise, implying that Gln was good for post-exercise intake (Iwashita et al., 2005). Therefore, it is quite important to

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ensure adequate intakes of this amino acid in order to meet the increased physiological demands of laying hens.

Many studies investigated the effects of Gln supplementation in the diet of livestock and poultry (Lackeyram et al., 2001; Doepel et al., 2006; Jafari et al., 2006; Fischer da Silva et al., 2007a; Murakami et al., 2007; Wang et al., 2008). However, there has been no research reported on Gln supplementation in the diet of laying hens. In this study, the effects and the mechanism of Gln on productive performance, egg parameters, hormone secretion, and the development of duodenum and oviduct in laying hens were investigated for the first time.

MATERIALS AND METHODS

Experimental design and treatments

A total of 400 Lingnan Yellow laying hens with almost the same BW were collected from a commercial breeding company (Donglian, Shaoguan, China), and randomly assigned into four groups, with 100 laying hens in each group. The hens were fed daily with a commercial layer diet and water *ad libitum* before the 6-week study. Both the ingredients and the nutrient composition of the basal diet formulated in accordance with the NRC requirement (1994) were shown in Table 1. All data were measured except for metabolic energy (ME).

The diets were respectively supplemented with four levels of Gln: the diet with 0% of Gln was set as control group, and those with 0.2%, 0.4%, and 0.8% Gln were set as treatment groups. The diet of 120 g per bird was provided daily (considering 5% excess of requirement), and clean drinking water was supplied *ad libitum* during the 6-week feeding period. And the birds were exposed to a period of 16-hour (16L+8B) incandescent light each day. Temperature and ventilation were controlled. The temperature was set at 25-30°C throughout the experiment.

Table 1. Compositions and nutrient levels of the experimental basal diets %

Items	Ingredient compositions % DM
Corn	62.50
Soybean meal	24.10
Fish meal	1.00
Soybean oil	1.25
Calcium phosphate	1.45
Limestone meal	7.87
Lysine (78%)	0.28
DL-methionine (98%)	0.15
NaCl	0.32
Choline chloride (75%)	0.08
Mineral and vitamin premix[1]	1.00
Analyzed chemical composition	
Crude protein (%)	18.29
Calcium (%)	3.62
Available phosphorus (%)	0.46
Lysine (%)	0.81
Methionine (%)	0.42
Methionine+cystine (%)	0.67
ME[2] (MJ/kg)	11.68

[1] Provided per kilogram of diet: vitamin A, 11,000 IU; vitamin E, 20 IU; vitamin K_3, 2.0 mg; thiamin, 2.0 mg; riboflavin, 5.0 mg; niacin, 25.0 mg; pantothenic acid,10.0 mg; vitamin B_6, 3.5 mg; vitamin B_{12}, 0.015 mg; folic acid, 1.0 mg; biotin, 0.05 mg; choline, 1,000.0 mg; vitamin C, 100.0 mg; Mn, 80.0 mg; Zn, 60.0 mg; Fe, 50.0 mg; Cu, 7.5 mg; I, 1.2 mg; Co, 0.8 mg; and Se, 0.1 mg.

[2] The data is calculated.

Sample collection

Daily feed intake was recorded, and daily egg production and individual egg weight were recorded to determine the egg mass production (egg production×egg weight/100, g/h/d); moreover, feed conversion efficiency (feed intake/egg mass production) was calculated during the 6-week feeding period.

Eggs from each group of laying hens were collected at the end of the feeding period for egg quality measurement (egg weight, egg shape index, eggshell weight, eggshell thickness, yolk weight, yolk index, yolk color, and Haugh unit). Both the egg shape index and the yolk index were calculated with a micrometer. The Haugh unit reading was determined by the height of albumen measured with a micrometer. The Haugh unit was calculated with the following Haugh formula (Elsen et al., 1962): Haugh unit = 100 log (H-1.7 $W^{0.37}$+7.57), where H = height, and W = weight of the egg. When eggshell density was calculated (eggshell weight per surface area), eggshells were dried in a microwave oven and weighed in grams. The surface area of the egg was calculated from the weight of the egg with the formula $S = 4.67\ G^{2/3}$ (Paganelli et al., 1974), where S = surface area, and G = egg weight. The eggshell density (mg/cm) was calculated by dividing eggshell weight by the surface area. The egg color was measured with the Roche shade selection fan. Moreover, the eggshell breaking strength was measured with the eggshell strength meter (a product of FHK Company, Tokyo, Japan) which forced the egg until the shell broke.

Blood collection and analysis

Blood samples (8.0 ml) from each individual laying hen were collected from the heart with sterilized syringes and needles at the last day of the feeding period and then centrifuged (4000 rpm) for 6min. Then the obtained serum samples were stored at -70°C for further analysis. The levels of T_3, T_4, LH and FSH in serum were analyzed with the method of radio-immunity (RI). The assay kits of hormones were provided by Beijing North Institute of Biological Technology (20a Panjiamiao, Fengtai District, Beijing, China).

Table 2. Effects of dietary glutamine on productive performance and egg quality in laying hens (n = 10)

Items	0 (control)	0.2% Gln	0.4% Gln	0.8% Gln	SEM
Egg production (%)	83.81[c]	84.11[c]	84.97[b]	87.01[a]	0.21
Egg mass[1] (g/hen per d)	47.95[b]	48.17[b]	48.76[b]	51.31[a]	0.48
Feed intake (g/hen per d)	101.11[b]	103.25[b]	103.48[b]	106.85[a]	0.97
Feed conversion efficiency[2] (g/g)	2.15[b]	2.13[b]	2.14[b]	2.06[a]	0.02
Egg weight (g)	57.20	57.26	57.38	58.97	0.49
Yolk weight (g)	14.56	14.59	14.65	14.78	0.34
Shell weight (g)	5.50	5.46	5.53	5.66	0.12
Egg shape index[3]	1.32	1.32	1.34	1.33	0.01
Shell density (mg/cm^2)	96.77	97.05	96.87	96.92	1.56
Shell thickness (mm)	0.34	0.34	0.34	0.33	0.01
Shell breaking strength (kg/cm^2)	3.57	3.59	3.60	3.60	0.11
Yolk index[4]	0.43	0.43	0.42	0.43	0.14
Yolk color	8.37	8.36	8.50	8.49	0.08
Haugh unit	83.61	83.81	83.83	83.64	1.26

[a,b,c] Different letters for the same line denote significant differences ($p<0.05$).

[1] Egg mass = (egg production×egg weight)/100. [2] Feed conversion efficiency = Feed intake/egg mass (g/g).

[3] Egg shape index = Egg height/egg width. [4] Yolk index = Yolk height/yolk width.

Duodenum and oviduct morphometry

Three birds per replicate were killed with exsanguination. Segments of approximately 4 cm were obtained from the region of the pylorus to the distal portion of the duodenal loop, and from the tubal isthmus. According to the method described by Becak and Paulete (1976), collected segments were placed on polystyrene sheets, opened longitudinally, washed in saline solution, fixed in Bouin's solution for 24 h, and processed until paraffin embedding. Each fragment was submitted to semiseriate cuts (5 mm thick) and stained using the hematoxylin-eosin method.

In the morphometric study, images were captured using a light microscope and a system that analyzes computerized images. The height and width of 30 villi, the depth of 30 crypts, and the thickness of 30 muscular layers were measured from each replicate per segment.

Statistical analysis

The experiment was conducted under a completely randomized design. The SAS (Statistical Analysis System, SAS Institute Inc, Cary, NC, USA, Version 9.1.3) software package was used for all statistical analysis. Duncan's multiple range test ($p<0.05$) was used to test the significance of difference between means. All data were expressed as means±SD. Differences were considered significant at the level of $p<0.05$.

RESULTS

The results of egg production, egg mass, feed intake and feed conversion efficiency of the laying hens fed with the diets supplemented with 0%, 0.2%, 0.4%, and 0.8% Gln were shown in Table 2. After a feeding period of six weeks, the productivity of laying hens was not significantly ($p>0.05$) affected by adding 0.2% and 0.4% Gln in diet; however, egg production of laying hens ($p<0.01$), egg mass ($p<0.05$), feed intake ($p<0.05$), and feed conversion efficiency ($p<0.05$) were significantly improved by 0.8% dietary Gln treatments.

The effect of Gln on egg quality in laying hens was also shown in Table 2. Egg weight, yolk weight, shell weight, egg shape index, shell density, shell thickness, shell breaking strength, yolk index, yolk color, and Haugh unit did not differ significantly among laying hens fed with Gln supplemented diets comparing with the control group ($p>0.05$).

The hormone levels of laying hens in different treatments were shown in Table 3. Treatment of 0.2% dietary Gln had little effect on hormone secretion ($p>0.05$);

Table 3. Effect of dietary glutamine on hormone levels of serum in laying hens (n = 10)

Items	0 (control)	0.2% Gln	0.4% Gln	0.8% Gln	SEM
Triiodothyronine (ng/ml)	2.08[c]	2.11[c]	2.34[b]	2.76[a]	0.03
Tetraiodothyronine (ng/ml)	13.04[b]	13.12[b]	13.54[a]	13.67[a]	0.18
Luteinizing hormone (μg/ml)	2.40[c]	2.46[bc]	2.52[b]	2.66[a]	0.02
Follicle stimulating hormone (μg/ml)	2.17[c]	2.24[c]	2.46[b]	2.75[a]	0.01

[a,b,c] Different letters for the same line denote significant differences ($p<0.05$).

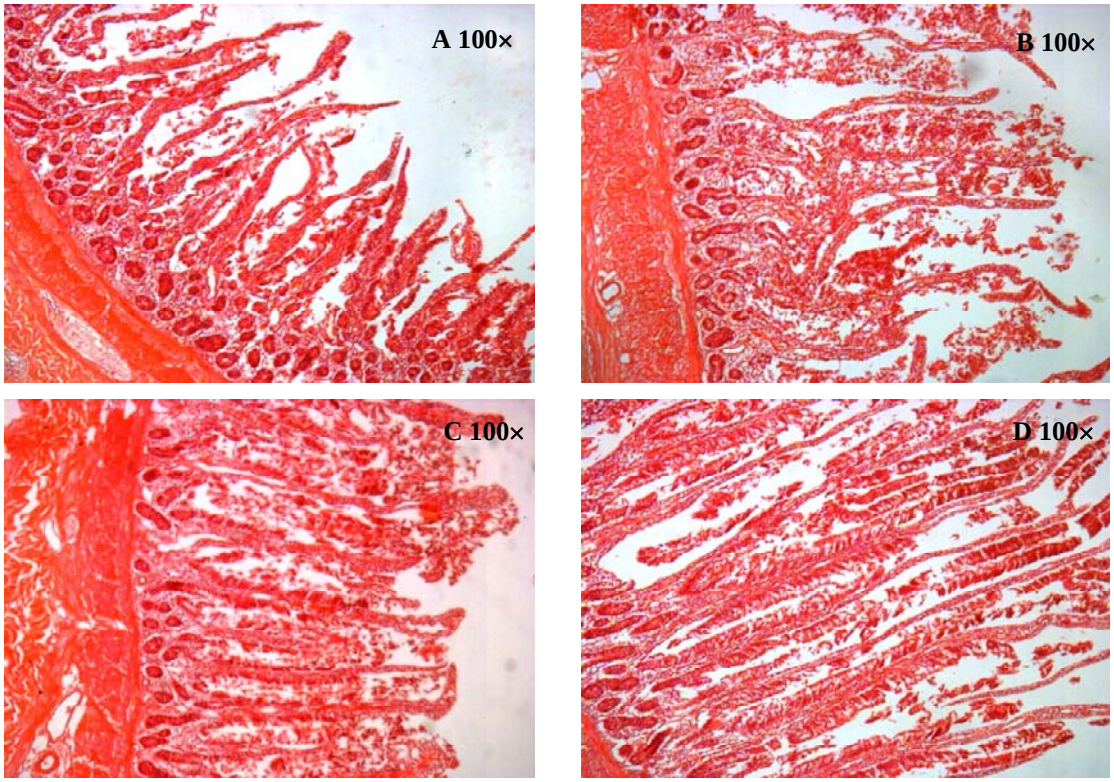

Figure 1. Light microscopy of duodenum in laying hens. A-D Birds fed with different levels of Gln for 6 weeks. A: Control diet, B: Control diet plus 0.2% Gln, C: Control diet plus 0.4% Gln, D: Control diet plus 0.8% Gln.

0.4% Gln in diet could increase the levels of hormone secretion but not significantly ($p>0.05$). LH ($p<0.01$), FSH ($p<0.01$), T_3 ($p<0.05$), and T_4 ($p<0.05$) contents in blood increased significantly when the hens were fed with 0.8% Gln in diet.

The intestinal morphology of the duodenum in different treatments was shown in micrographs A, B, C, and D of Figure 1. In Figure 1A, birds in the control group had a villus height lower than those fed with Gln. Figures 1B and C indicated that 0.2% and 0.4% Gln were able to promote the development of duodenum, but failed to match the performance of 0.8% Gln. Figure 1D demonstrated that 0.8% Gln clearly improved the growth of duodenum and led to the longest villus height and largest absorptive surface area. The results of villus width, villus height, crypt depth and muscular layer thickness were shown in Table 4. Compared with those in the control group, the birds in 0.8% Gln group had a higher villus height of duodenum ($p<0.05$), but there was no statistically significant difference in villus width, crypt depth, and muscular layer thickness ($p>0.05$).

The structure of oviduct was shown in Figure 2. Compared with the birds in the control group (Figure 2A), birds fed with different levels of Gln had a higher villus height and good oviductal integrity. 0.2% and 0.4% Gln in the diets were able to induce the recovery of oviduct (Figure 2B, C), but the effect of 0.8% Gln were more significant (Figure 2D).

DISCUSSION

In this study, lower levels of Gln in the diets of laying hens showed no difference on productive performance compared with the control group. Supplementation of 0.8% Gln in the diets of laying hens could improve egg

Table 4. Effects of glutamine on duodenum morphology of laying hens (n = 3) [1]

Items	0 (Control)	0.2% Gln	0.4% Gln	0.8% Gln	SEM
Villus width (μm)	131.15±0.12	131.32±0.24	131.56±0.36	132.03±0.15	0.47
Villus height (μm)	876.35±1.34[c]	884.56±0.25[c]	927.34±0.53[b]	945.42±2.14a	2.65
Crypt depth (μm)	126.51±0.24	126.41±2.89	125.12±1.45	125.25±2.68	1.76
Muscular layer thickness (μm)	145.25±3.62	147.25±2.38	146.25±0.56	148.25±2.32	1.68

[a,b,c] Different letters for the same line denote significant differences ($p<0.05$).

[1] The height and width of 30 villi, the depth of 30 crypts and the thickness of 30 muscular layers were measured from each replicate.

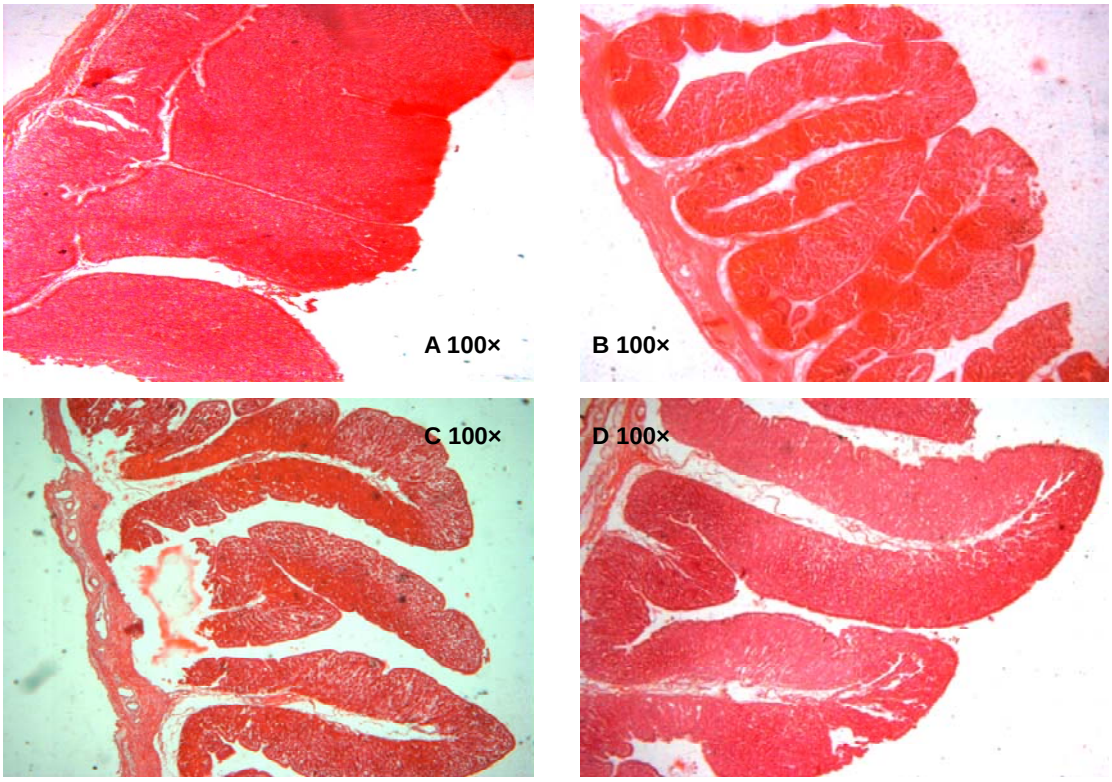

Figure 2. Light microscopy of oviduct in laying hens. A-D Birds fed with different levels of Gln for 6 weeks. A: Control diet, B: Control diet plus 0.2% Gln, C: Control diet plus 0.4% Gln, D: Control diet plus 0.8% Gln.

production, egg mass, feed intake, and feed conversion efficiency, but it had little effect on egg quality. It was also observed that the dietary supplementation of 0.8% Gln could stimulate hormone secretion and induce better development of duodenum and oviduct in laying hens.

Previous studies showed that Gln supplementation had limited effects on metabolism, immune status, milk yield, DM intake (Doepel et al., 2006; Jafari et al., 2006), and glucose metabolism (Doepel et al., 2007) during the experimental period in dairy cows. Treatment of 1% dietary Gln did not improve the average daily gain (ADG) and intestinal morphology of calves (Drackley et al., 2006). In contrast to the results in cows and calves, the findings in pigs indicated that feeding 0.8-4% Gln in diet had beneficial effects in alleviating growth depression of E. coli $K88^+$ -challenged pigs (Yi et al., 2002; Yi et al., 2005b), enhancing body weight gain and feed efficiency of weaned piglets (Lackeyram et al., 2001; Wang et al., 2008). In poultry, supplementing the diet with 1% Gln improved ($p<0.05$) the weight gain and feed efficiency of broilers (Yi et al., 2001), and increased the ornithine decarboxylase (ODC) expression (Fischer da Silva et al., 2007b). Bartell et al. (2007) further indicated that the addition of 1% Gln to the diet of broiler chicks improved the weight gain, 4% Gln in diet or water depressed the growth performance. It was also observed in our study that supplementation of 0.8% Gln in diets of laying hens could promote the productive performance but had no effects on the egg quality. In our study, 1% Gln used for broilers and livestock was lowered to the level of 0.8% for laying hens, but a better performance was still found. The mechanism was further investigated to support our findings above.

Glutamine had significant effects on maintaining intestinal integrity and function (Liu et al., 2002; Wang et al., 2008), helping nutrient digestion and absorption. As an energy source for the maturation of the mucosa cells of the chicken (Maiorka et al., 2000), Gln could improve the intestinal villus height, intestinal relative weights, villus density, microvilli width, and surface area of the tip of the enterocytes in poults (Yi et al., 2001; Bartell et al., 2007; Fischer da Silva et al., 2007a; Murakami et al., 2007). In addition to promoting the intestinal integrity, Gln acted as an intestinal barrier against bacteria attacks to help the immune system to kill bacteria, and ensured host survival during critical situations. Glutamine was responsible for gut mass and maintenance of a bacteria barrier (Belmonte et al., 2007). During illness such as colitis, biliary obstruction, trauma and endotoxemia, Gln supplementation significantly modulated intestinal permeability and reduced bacterial translocation barrier functions (White et al., 2005; Vicario

et al., 2007). It was found in our study that 0.8% Gln in the diets of laying hens could promote the development of duodenum and led to the longest villus height and largest absorptive surface area, thus increasing the absorptive surface of gastrointestinal mucosa and the utilization of dietary nutrients. Therefore, Gln could improve the productive performance of laying hens.

Glutamine could improve gonadal hormone levels in animal body for a better genital system growth. Glutamate (Glu) biosynthesis from Gln through binding to N-methyl-D-aspartate receptors (NMDAR) was an event contributing to the pubertal activation of luteinizing hormone-releasing hormone (LHRH) (Ottem et al., 2002; Roth et al., 2006) and pulsatile gonadotropin-releasing hormone (GnRH) secretion (Bourguignon et al., 1995). These data indicated that Gln was a prerequisite to the physiological mechanism of gonadal hormones. The oviduct of birds was the place where egg white and eggshell formed, and its development and functions could directly affect productive performance of layers. At the same time, the oviduct was the target organ of LH and FSH that could maintain higher secretion of the oviduct, thus increasing the quantity of egg laying, decreasing the feed conversion efficiency, and lengthening the crest-time of egg laying (Zuelke et al., 1993). LH in peripheral blood was directly correlated with the ovulation of layers. FSH could affect the growth and maturation of ovarian follicle, which had synergistic effects on ovulating with LH (Ooi et al., 2004). In this study, the morphology of oviduct, FSH and LH secretion was investigated, and it was found that 0.8% Gln in diets of laying hens kept a better morphology of oviduct, and enhanced FSH and LH secretion in blood. It was postulated that 0.8% Gln in diets could promote the productive performance through releasing higher levels of LH, FSH, keeping better development and secretion of oviduct.

T_3 and T_4 in peripheral blood of laying hens played their physiological functions in many ways such as facilitating the differentiation, growth and development of tissue, stimulating DNA transcription and mRNA formation, promoting the formation of protein and enzymes, increasing the utilization of carbohydrate, and enhancing the disintegration of amylon and fat (Ooi et al., 2004). After feeding with Gln for six weeks, the levels of T_3 and T_4 in the blood of hens were measured, and the results indicated that 0.8% Gln in diets could increase the concentration of T_3 and T_4, thus resulting in greater metabolisms and absorption of nutrients and enhanced performance of laying hens.

In conclusion, the results of this study showed that incorporating 0.8% Gln into a balanced layer diet could produce better development of the duodenum and recovery of the oviduct, maintain their integrity, promote hormone secretion, and lead to a better performance in laying hens.

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