Influence of Supplemental Glutamine on Nutrient Digestibility and Utilization, Small Intestinal Morphology and Gastrointestinal Tract and Immune Organ Developments of Broiler Chickens

Sutisa Khempaka, Supattra Okrathok, Laddawan Hokking, Buntita Thukhanon and Wittawat Molee

Abstract—This study was conducted to investigate the optimum levels of glutamine (Gln) supplementation in broiler diets. A total of 32 one-day-old male chicks with initial body weight 41.5 g were segregated into 4 groups (8 chicks per group) and subsequently distributed to individual cages. Feed and water were provided ad libitum for 21 days. Four dietary treatments were as follows: control and supplemented Gln at 1, 2 and 3%, respectively. The results found that the addition of Gln had no negative effects on dry matter, organic matter, ash digestibility or nitrogen retention. Birds fed with 1% Gln had significantly higher villi width and villi height: crypt depth ratio in duodenum than the control chicks had significantly higher villi width and villi height: crypt depth ratio in duodenum than the control chicks. It is suggested that the addition of Gln at 1% indicated a beneficial effect on improving small intestinal morphology, in addition Gln may stimulate immune organ development of broiler chickens.

Keywords—broiler chicken, digestibility, gastrointestinal tract glutamine, glutamine

I. INTRODUCTION

Due to the ban on the use of antibiotics as growth promoters to improve growth performance and to control diseases in poultry feed, there have been numerous problems leading to depressed growth performance and an increased the incidence of disease. The supplementation of glutamine (Gln) is an alternative feed additive that should be studied in broiler diets. Glutamine is the most prevalent amino acid in the bloodstream, accounting for 30-35% of the amino acid N in the plasma and in the free amino acid pool in the body [1]. Numerous literatures reported that Gln is the principle metabolic fuel for small intestine enterocytes, lymphocytes, macrophages and fibroblasts and is considered an essential amino acid in some species under inflammatory conditions [2], [3]. Many benefits have been observed due to Gln supplementation in the diet of humans and rats, however, little research has been done with poultry. Therefore, this study was aimed to investigate the effect of Gln supplementation on nutrient digestibility and retention, small intestinal morphology and gastrointestinal tract and immune organ developments of broiler chickens.

II. MATERIALS AND METHODS

A total of 32 one-day-old male chicks with initial body weight 41.5 g were segregated into 4 groups (8 chicks per group) and subsequently distributed to individual cages. Feed and water were provided ad libitum for 21 days. Four dietary treatments were as follows: control and supplemented Gln at 1, 2 and 3%, respectively (Table 1). All nutrients were formulated to meet or exceed the minimum NRC [4] requirements for broiler chickens.

Excreta were collected on 18 to 21 days of age. The excreta were sprayed with 5% HCl and dried at 55°C. Dried excreta were stored at -20°C for later analyses. DM, organic matter and N in the diets and excreta were measured to assess their digestibilities and retention according to standard methods [5]. At the end of the experiment, the birds were weighed and killed by cervical dislocation and then the abdominal cavity was open. The thymus and spleen were removed and weighed. For intestinal weight measurements, the small intestine was removed and divided into 3 segments: duodenum, jejunum and ileum. The ileum was flushed with 10 to 20 ml of deionized water and the empty weight was recorded. While duodenum and jejunum were flushed with 20 ml saline solution and the empty weigh was recorded. Organ weights were expressed on a weight relative to live body weight (g/100g of BW). For morphologic analysis, approximately 5 cm of the middle portion of the duodenum and jejunum was excised and fixed with 10% formalin. The cross sections of 70% ethanol-preserved segments for each duodenal and jejuna sample were...
The effect of Glu on digestive and immune organ relative weights of broilers is summarized in Table 3. The data revealed that Glu supplementation had no effect on bursa, small intestine and cecum relative weights of broilers (P>0.05). While the spleen relative weight was significantly heavier with the addition of 3% Glu compared with the control and 1 to 2% Glu diets. The weights of spleen (g/100g BW) were 0.12, 0.09, 0.11 and 1.99 in broilers fed with control and supplemented with 1, 2 and 3% Glu, respectively.

**Table III**

**EFFECT OF GLUTAMINE SUPPLEMENTATION ON DIGESTIVE AND IMMUNE ORGAN WEIGHS OF BROILERS (G/100G BW)**

<table>
<thead>
<tr>
<th>Glutamine levels (%)</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>0.12</td>
<td>0.09</td>
<td>0.11</td>
<td>1.99</td>
<td>0.0533</td>
</tr>
<tr>
<td>Bursa</td>
<td>0.24</td>
<td>0.21</td>
<td>0.28</td>
<td>0.32</td>
<td>0.0541</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1.16</td>
<td>0.96</td>
<td>0.88</td>
<td>0.97</td>
<td>0.1131</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.69</td>
<td>1.50</td>
<td>1.48</td>
<td>1.58</td>
<td>0.1422</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.33</td>
<td>1.27</td>
<td>1.24</td>
<td>1.22</td>
<td>0.0722</td>
</tr>
<tr>
<td>Cecum</td>
<td>1.24</td>
<td>0.73</td>
<td>0.81</td>
<td>0.69</td>
<td>0.1855</td>
</tr>
</tbody>
</table>

1 Values for each parameter represent mean values of 2 observations

Glutamine supplementation in diets did not affect the villi height and crypt dept both in duodenum and jejunum of broilers (P>0.05) (Table 4). The values of villi height in the duodenum and jejunum were 331, 449, 248, 180 µm and 257, 552, 333 and 320 µm in broilers fed control and supplemented with 1, 2 and 3% Glu, respectively. The values of crypt dept in duodenum and jejunum were 125, 132, 124 and 102 µm, and 93, 107, 97 and 98 µm in broilers fed control and supplemented with 1, 2 and 3% Glu, respectively. While the birds fed diets supplemented with 1% Glu had significantly higher villi width in the duodenum than control and 2 to 3% Glu birds. The values of villi width in the duodenum were 86, 92, 68 and 51 µm in broilers fed control and supplemented with 1, 2 and 3% Glu respectively, in which the addition of 2 and 3% Glu resulted in decreased villi width (P<0.05). However, the addition of Glu had no negative effect on decreased villi width in jejunum. The ratio of villi height : crypt dept in duodenum were increased according with the villi width. These ratios increased in 1% Glu but decreased in 2 and 3% Glu when compared to control birds (P<0.05). However, the ratios of villi height : crypt dept were not significantly in jejunum. The values of villi height : crypt dept ratio in the duodenum and jejunum were 2.7, 3.4, 2.0, 1.8 and 2.7, 5.0, 3.4, 3.0 in broilers fed control and supplemented with 1, 2 and 3% Glu, respectively.

**IV. DISCUSSION**

This study found that the addition of Glu at 1% showed the highest efficacy without any negative effects on dry matter, organic matter, ash digestibility or nitrogen retention. Birds fed with 1% Glu had significantly higher villi width and villi height : crypt dept ratio in duodenum than the control chicks. This finding according to many previous studies indicated the same effect of Glu supplementation in broiler chick diets on increased villi height in small intestine [8]-[10]. In addition,
Duodenum
Villi wide (µm)
Duodenum 86 92 68 51 1.5116
Jejunum 99 102 88 81 11.3400
Villi height : Crypt depth
Duodenum 2.7 3.4 2.0 1.8 0.0075
Jejunum 2.7 5.0 3.4 3.0 0.5408

**Means with different superscripts in a row are significantly different (P<0.05)**

1 Values for each parameter represent mean values of 2 observations
2 Small intestine sample of each treatment was taken from chickens aged 21 days

| Table IV
| EFFECT OF GLUTAMINE SUPPLEMENTATION ON SMALL INTESTINAL MORPHOLOGY**
<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Glutamine levels (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villi height (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>331</td>
<td>449</td>
<td>248</td>
</tr>
<tr>
<td>Jejunum</td>
<td>257</td>
<td>552</td>
<td>333</td>
</tr>
<tr>
<td>Crypt depth (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>125</td>
<td>132</td>
<td>124</td>
</tr>
<tr>
<td>Jejunum</td>
<td>93</td>
<td>107</td>
<td>97</td>
</tr>
<tr>
<td>Villi width (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>86</td>
<td>92</td>
<td>68</td>
</tr>
<tr>
<td>Jejunum</td>
<td>99</td>
<td>102</td>
<td>88</td>
</tr>
</tbody>
</table>

Bartell and Batal [11] also reported a beneficial effect of Gln on increased intestinal villi height when Gln was added to diets at 1% and 4%. Because Gln is an amino acid important for utilization as energy source for the development of mucosa and stimulate intestinal cell proliferation, thus it increasing the absorptive surface of gastrointestinal mucosa and the utilization of nutrients [11]. However, broilers fed diets supplemented with 2 and 3% Gln had a significantly lower intestinal villi width than the broilers fed control and 1% Gln diets. This phenomenon is still not clear. Soltan [10] investigated the effect of Gln supplementation at 0.5, 1.0, 1.5 and 2% in broiler diets and concluded that the addition of 1% Gln can be improved growth performance and may stimulate the development of gastrointestinal tract and immune response, while higher level had negative effects. Normally, if the intestinal villi height can be increased early in the chick’s life, then the chick may be able to utilize nutrients more efficiently earlier in life and thus have improved growth performance [11]. In addition, Nitsan et al. [12] also stated that birds with a faster growth rate have a high capability to secrete high levels of enzymes, implying that initial growth is only limited by the early development of the digestive organs, therefore, reducing the time for development of digestive organs, growth improvements could be achieved. Even though the birds fed with 1% Gln has increased villi width in comparison with the control or 2 and 3% Gln, nutrient digestibility and retention were not significantly different among treatments. This may be due to the fact that all dietary nutrients are balance and are sufficient for broiler requirements, or it could also suggest that increased villi width does not necessarily lead to increased nutrient utilization.

Immune tissue development is the basis of immune functionality. The supplementation of Gln at levels of 3% significantly promoted the growth of the spleen but had no effect on the bursa weight. These findings are in agreement with the previous studies which reported the improvement of spleen and thymus weights in broiler chicks fed on diets supplemented with Gln [10], [11], [13]. Glutamine is also the precursor for the net synthesis of arginine, which has been shown to increase thymus and spleen size in mice [14] increase cytokine production and enhance lymphocyte proliferation [15].

Based on the above studies it is suggested that the addition of Gln 1% indicated the most advantageous on improving small intestine morphology. In addition Gln may stimulate immune organ development of broiler chickens. This finding may be useful for newborn chicks, since their system, especially digestive and immune system is not fully developed and the chicks are more susceptible to disease or likely to be negatively impacted by their environment.

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REFERENCES