Glutamine and glutamic acid supplementation enhances performance of broiler chickens under the hot and humid tropical condition

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Abstract

Day-old (day 1) commercial broiler chickens were fed i) basal diet (control), ii) basal diet +0.5% AminoGut (AG), or iii) basal diet +1% AG from 1 to 42 d of age under the hot and humid tropical environment. AminoGut is a commercial dietary supplement containing a mixture of L-glutamine (Gln) and L-glutamic (Glu) acid. Weight gain and feed conversion ratio during the starter (1 to 21 d) and overall (1 to 42 d) periods improved linearly and quadratically with AG supplementation when compared to control. Supplementing birds with AG significantly reduced overall mortality rate. At 21 and 42 d of age, intestinal (duodenum and ileum) villi height and crypt depth showed both linear and quadratic positive responses to AG supplementation. Intestinal amylase activity increased linearly and quadratically on d 21, and linearly only on d 42. In conclusion, Gln and Glu supplementation was beneficial in improving the growth performance and survivability of broiler chickens under the hot and humid tropical environment.

Introduction

The prevailing hot and humid climate is one of the major constraints for optimum poultry production in the tropics. The fast growing commercial broiler chickens are particularly susceptible to heat stress related problems because metabolic heat production increases with growth rate, while heat dissipation does not. One of the most affected tissues during heat stress in chickens is the small intestine (Lambert, 2009). This is mainly due to its high proliferative activity (Murakami et al., 2007). Intestinal epithelium undergoes a continuous process of proliferation and self-renewal cycle of about 48 h (Morini et al., 2000; Skrzypek et al., 2005). Therefore, protecting the small intestine and its function is critical in heat-stressed poultry.

Glutamine (Gln) is an abundant amino acid in the body and is regarded as a conditionally essential nutrient in animals under stress conditions, such as infection, injury, high temperature and weaning (Murakami et al., 2007; Soubia et al., 1999; Wu et al., 1995; Soltan, 2009; Dai et al., 2009; Fischer da Silva et al., 2007; Bartell and Batal, 2007). Dietary supplementation of Gln has been reported to play an important role in preserving gut integrity in rodents (Larson et al., 2007) and pigs (Wu et al., 1996). Supplying of Gln enables a high metabolic activity of enterocytes as well as the proliferation, maturation, and migration of crypt cells, and it maintains the function of intestinal lymphocytes (Alverdy, 1990; Dai et al., 2009; Wu et al., 2011). Previous studies in poultry suggested that the effect of Gln supplementation on growth performance is consistent. Murukami et al. (2007), Bartell and Batal (2007), and Soltan (2009) showed that Gln supplementation improved the development of the gastrointestinal tract and growth performance in birds. On the contrary, Maiorka et al. (2000) and Sakamoto et al. (2006) failed to note any beneficial effect of Gln supplementation on the growth performance of broiler chickens. These discrepancies could be attributed to variations in the dosage and duration of dietary Gln supplementation practising. Most of these studies have been conducted under temperate condition and there is a dearth of information on Gln supplementation in broiler chickens reared under hot and humid climate. To date, the only work on Gln supplementation in heat-stressed chickens is Dai et al. (2009). The author subjected Gln supplemented broiler to heat stress from 35 to 42 days of age and studied growth performance and meat quality. In the small intestine surface Gln is readily converted to glutamic acid (Glu) by the action of glutaminase (Wu, 1998). Dietary Glu is a precursor for the intestinal synthesis of glutathione, arginine and proline (Reeds et al., 1997; Wu and Morris, 1998) and thus, it is of critical importance in intestinal metabolism and physiology. It has been reported that chronic heat stress may adversely affect intestinal morphology (Mitchell and Carlisle, 1992) and digestive enzymes activities (Osman and Tanios, 1983) in chickens. Devi-Priya et al. (2010) reported that digestive enzyme activity in broiler chickens, particularly amylase, was increased by Gln supplementation. Hence, the current study was designed to investigate the effect of Gln and Glu supplementation on the performance, intestinal morphology and amylase activity in broiler chickens under the hot and humid tropical conditions.

Materials and methods

Ethical note

This study was undertaken following the guidelines of the Research Policy of the Universiti Putra Malaysia on animal ethics.

Birds and husbandry

A total of 300 one-day old male broiler chicks (Cobb 500) were purchased from a local hatchery. Upon arrival, chicks were weighed and randomly assigned in groups of 10 to 30 battery cages (45x120x60 cm, height x length x width) with wire floors. Floor space allowed was 720 cm² per bird. The chicks were housed in a conventional open-sided house with cyclic temper-
atures. The profile of in-house temperature and relative humidity was recorded daily and means are shown in Table 1. The chicks were fed with starter mash from 1 to 21 days of age, and finisher mash from 22 to 42 days of age. Feed and water were available ad libitum. Birds were provided 12 h of natural lighting.

**Experimental diets**

Diets were formulated to meet or exceed the nutritional recommendation of broiler chicken (National Research Council, 1994; Table 2). Diets were prepared on a weekly basis in the University feed mill. From 1 to 42 days of age, equal number of birds (10 replicate cages per diet) was fed i) basal diet (control), ii) basal diet +0.5% AminoGut (AG), or iii) basal diet +1% AG (Table 1). AminoGut is a commercial dietary supplement (Ajinomoto, Tokyo, Japan) containing a mixture of L-Gln (minimum 10%) and L-Glu (minimum 10%) (crude protein: 60%; metabolisable energy: 3434 kcal/kg).

**Gut morphology**

On days 21 and 42, 10 birds per dietary groups (one bird per cage) were randomly selected and immediately decapitated for collection of small intestines. Approximately 5 cm of the middle portion of the duodenum (apex section), jejunum (midway between the point of entry the bile ducts and Meckel's diverticulum) and ileum was cut gently and washed with phosphate buffer solution (PBS) and fixed in 10% formalin. Samples were then dehydrated for 16 h in an automatic tissue processor (Leica ASP 3000; Leica Biosystems GmbH, Nussloch, Germany) and embedded in paraffin wax using a paraffin embedding system (Leica EG 1160; Leica Biosystems GmbH). Each sample was cut into 4 m-thick sections using a rotary microtome machine (Leica RM 2155; Leica Biosystems GmbH). The sections were placed on glass slides, heated at 57°C until dried, and stained with haematoxylin and eosin (Uni, 1999). The stained sections were examined using light microscope (Dialux, Lüdenscheid, Germany) fitted with a digital camera (Leica Camera AG, Solms, Germany). A total of 4 intact, well-oriented villi sections per slide were evaluated in each of 6 replicate slides per intestinal sample (24 measurements for each sample). Villi height was measured from the tip of the villi to the villus crypt junction. Villus height (VH) and crypt depth (CD) were determined using Image-Pro Plus software as described in details by Touche et al. (2002).

**Table 1. Mean environmental temperatures and relative humidity inside the cages during the experimental period.**

| Parameter       | 08:00 | 14:00 | 20:00 |
|-----------------|-------|-------|-------|
| Temperature, °C | 24    | 35    | 29    |
| Humidity, %     | 90    | 65    | 78    |

**Table 2. Composition of experimental diets.**

| Ingredients, % | Starter diet | Finisher diet |
|----------------|--------------|---------------|
| Corn           | 48.50        | 48.50         |
| Soybean meal   | 40.00        | 40.00         |
| Palm oil       | 6.15         | 6.15          |
| Limestone      | 1.21         | 1.21          |
| Dicalcium phosphate | 1.95   | 1.95         |
| Sodium chloride | 0.44      | 0.44          |
| Mineral premix | 0.30         | 0.30          |
| Vitamin premix | 0.30         | 0.30          |
| DL-Methionine  | 0.15         | 0.15          |
| AG             | 1.00         | 1.00          |
| Inert (sand)   | 0.50         | 0.50          |

**Table 3. Effect of AminoGut supplementation on feed intake, body weight, weight gain and feed conversion ratios in broiler chickens.**

| Dietary treatment | SEM | Linear | Quadratic |
|-------------------|-----|--------|-----------|
| FL, g              |     |        |           |
| Control            | 1024| 1070   | 1081      |
| AG0.5              | 2907| 3013   | 2964      |
| AG1                | 3931| 4083   | 4045      |
| 1 to 21 d          | 8   | 0.034  | 0.039     |
| 21 to 42 d         | 31  | 0.221  | 0.953     |
| 1 to 42 d          | 34  | 0.110  | 0.596     |
| BW, g              |     |        |           |
| Control            | 670 | 822    | 835       |
| AG0.5              | 2077| 2266   | 2261      |
| AG1                |     |        |           |
| 1 to 21 d          | 0.1 | 0.680  | 0.250     |
| 21 to 42 d         | 13  | <0.001 | <0.001    |
| 42 d               | 22  | <0.001 | <0.001    |
| WG, g              |     |        |           |
| Control            | 631 | 784    | 797       |
| AG0.5              | 1407| 1443   | 1426      |
| AG1                | 2038| 2227   | 2222      |
| 1 to 21 d          | 13  | <0.001 | <0.001    |
| 21 to 42 d         | 17  | 0.451  | 0.975     |
| 1 to 42 d          | 22  | <0.001 | <0.001    |
| FCR                |     |        |           |
| Control            | 1.62| 1.36   | 1.36      |
| AG0.5              | 2.07| 2.09   | 2.08      |
| AG1                | 1.93| 1.83   | 1.82      |
| 1 to 21 d          | 0.02| <0.001 | <0.001    |
| 21 to 42 d         | 0.02| 0.720  | 0.991     |
| 1 to 42 d          | 0.01| 0.015  | 0.037     |

Control, basal diet; AG0.5, basal diet +0.5% AminoGut; AG1, basal diet +1% AminoGut; FL, feed intake; BW, body weight; WG, weight gain; FCR, feed conversion ratio. Ten cages were used per dietary treatment group. Significance was established at P<0.05.
**Enzyme activity**

Digesta samples were collected from distal end of the duodenum to the ileo-cecal junction by gentle massaging. The samples were homogenised, snap frozen in liquid nitrogen and stored at -70°C for further analysis. Amylase (α-1,4-glucanohydrolase, EC 3.2.1.1) activity was determined using modified method of Bernfeld (1955). Assay system containing 1.0 mL of small intestinal digesta homogenates (enzyme solution) with ice cold PBS (pH 7.0) was centrifuged at 18,000 g for 20 min at 4°C. Of soluble starch solution (pH 7.0 in PBS), 1.0 mL was incubated at 30°C for 30 min. The reaction was terminated by the addition of 2.0 mL 3,5-Dinitrosalicylic acid (DNS) reagent. The solution was heated at 100°C for 10 min and then allowed to cool to room temperature. Absorbance was measured using spectrophotometer (SPECORD® PLUS; Analytik Jena AG, Jena, Germany) at 540 nm. Blank was prepared in the same manner (1.0 mL soluble starch was incubated at 30°C for 30 min) and enzyme solution was added after the addition of DNS. A unit of enzyme activity was defined as the amount of enzyme required releasing 1 μmol of reducing sugar.

**Statistical analysis**

All data were presented as mean±SEM and analysed using GLM procedure of SAS (SAS, 2004). A model with the fixed effect of diet was used, and orthogonal contrasts were applied to test the effect of Gln and Glu across doses. The model included the linear and quadratic effects of diet. Cages were served as experimental units. Mortality data were analysed by Chi square test. Values of P<0.05 were considered statistically significant.

**Results and discussion**

The effect of diet on FI, BW, weight gain (WG) and FCR are presented in Table 3. AminoGut supplementation tended to improve FI, WG and FCR during the starter phase (d 1 to 21). However, during the finisher period (d 21 to 42), dietary treatment had no significant effect on FI, WG, and FCR. Supplementing diets with AG improved day 21 and 42 BW, and overall WG (d 1 to 42) linearly and quadratically. Jejunal VH and CD remained unaffected by dietary treatment throughout the experimental period (Table 5). On d 21, intestinal amylase activity showed both linear and quadratic increment with AG supplementation. Dietary treatment increased amylase activity linearly but not quadratically on day 42 (Table 6).

Generally, irrespective of diet, the WG and FCR of birds in the present study were poorer than the Cobb 500 standard performance. The retarded performance could be attributed to the hot, humid tropical climate, and the form of feed (mash) provided (Zulkifli and Ramlah, 1998). The present findings suggested that AG supplementation to chickens raised under the tropical climate was critical only during the starter period. Yi et al. (2001) reported that Gln supplementation at 1% improved WG only during the first week of life. Studies in pigs (Wu et al., 1995; Wang et al., 2008) suggested that Gln supplementation is important during the weaning period. The phenomenon could be associated with early development of the digestive system. Because of the rapid growth rate, broiler chicks require greater nutrient absorption and utilisation. Reducing the time for development of the digestive organs and functions would have a profound impact on growth performance (Nitsan et al., 1991; Sklan, 2001).

Regarding the effective dosage of Gln, Soltan (2009) supplemented broilers with 0.5, 1.0, 1.5 and 2.0% Gln and concluded that the inclusion rate of 1.0% resulted in the best growth performance in 6-week-old chickens.

### Table 4. Effect of AminoGut supplementation on mortality rate (%) in broiler chickens.

| Days | Dietary treatment | SEM | P       |
|------|------------------|-----|---------|
| 1 to 21 d | Control | 3   | 2       | 2       |
| 21 to 42 d | AG0.5 | 10   | 3       | 4       |
| 1 to 42 d | AG1 | 13   | 5       | 6       |

Control, basal diet; AG0.5, basal diet +0.5% AminoGut; AG1, basal diet +1% AminoGut. *Means within row with no common superscript differ at P<0.05.

### Table 5. Effect of AminoGut supplementation on villi height and crypt depth in broiler chickens.

| Days | Parameter, μm | Dietary treatments | SEM | P       |
|------|---------------|--------------------|-----|---------|
| 21   | VH            | Control | AG0.5 | AG1 | Linear | Quadratic |
|      | Duodenum      | 472      | 514    | 534 | 9      | 0.035 | 0.024 |
|      | Jejunum       | 649      | 715    | 715 | 15     | 0.076 | 0.286 |
|      | Ileum         | 461      | 596    | 597 | 18     | <0.001 | 0.034 |
|      | CD            | Duodenum | 73    | 129   | 150   | 8     | <0.001 | <0.001 |
|      | Jejunum       | 75       | 87     | 87   | 3      | 0.109 | 0.330 |
|      | Ileum         | 86       | 115    | 116  | 4      | 0.001 | 0.034 |
| 42   | VH            | Control | AG0.5 | AG1 | Linear | Quadratic |
|      | Duodenum      | 484      | 973    | 950 | 43     | <0.001 | <0.001 |
|      | Jejunum       | 491      | 512    | 543 | 11     | 0.451 | 0.086 |
|      | Ileum         | 65       | 140    | 145 | 7      | <0.001 | <0.001 |
|      | CD            | Duodenum | 108   | 178   | 183   | 9     | <0.001 | <0.001 |
|      | Jejunum       | 92       | 111    | 111  | 6      | 0.241 | 0.487 |
|      | Ileum         | 69       | 131    | 132  | 6      | <0.001 | <0.001 |

Control, basal diet; AG0.5, basal diet +0.5% AminoGut; AG1, basal diet +1% AminoGut; VH, villi height; CD, crypt depth. Ten cages were used per dietary treatment group. Significance was established at P<0.05.

### Table 6. Effect of AminoGut supplementation on intestinal amylase activity (U/g fresh digesta) in broiler chickens.

| Days | Dietary treatment | SEM | P       |
|------|------------------|-----|---------|
| 21   | Control | 0.307 | 0.437 | 0.453 | 0.019 | <0.001 | 0.009 |
|      | AG0.5 | 0.315 | 0.385 | 0.393 | 0.014 | 0.033 | 0.114 |

Control, basal diet; AG0.5, basal diet +0.5% AminoGut; AG1, basal diet +1% AminoGut. Ten cages were used per dietary treatment group. Significance was established at P<0.05.
On the contrary, Devi-Priya et al. (2010) observed an increase in WG of broiler chickens supplemented with 0.5% Gln when compared with those fed 1% Gln and control diets. Bartell and Batal (2007) supplemented broilers with 1.0 and 4.0% Gln and noted a depression in the WG of the latter. The authors suggested that Gln supplementation at 4.0% could be toxic to chickens. The present findings indicate that AG supplementation improves overall WG and FCR both linearly and quadratically.

It is interesting to note that AG supplementation significantly reduces the mortality rate of chickens under the hot and humid environment. Work in rats showed that oral Gln may enhance heat shock protein (hsp) 70 expression, decrease intestinal permeability, and improve survival from hyperthermia injury (Singleton and Wischmeyer, 2006). When living organisms are exposed to thermal stress, the synthesis of most proteins is retarded but a group of highly conserved proteins known as hsp are rapidly synthesised (Etches et al., 1995). It is well documented that one of the most important functions of hsp is to protect organisms from the toxic effects of heating (Barbe et al., 1988). Zulkifli et al. (2002, 2003) reported that enhanced hsp 70 expression may improve the ability of broiler chickens to withstand high temperatures.

The health and development of the gastrointestinal tract is crucial for optimum productivity in poultry. An increase in VH may enhance nutrient absorption by increasing the intestinal surface area (Bartell and Batal, 2007; Soltan, 2009; Ebadiasl, 2011). The benefit of Gln supplementation has been associated with a better development of the gastrointestinal tract. Chickens supplemented with 1% Gln have longer VH and deeper CH (Fischer da Silva et al., 2009; Ebadiasl, 2011). The benefit of AG supplementation improves structure and function of the gastrointestinal tract and increases the BWG of the latter. The authors suggested that AG supplementation improves overall WG and FCR both linearly and quadratically.

Similarly, works on Jian carp (Lin and Zhou, 1990) showed that activity of intestinal enzyme positively correlated with level of Gln supplementation.

Conclusions

Results from this study suggest that supplementing diets with AG, which contains Gln and Glu, may improve growth performance, survivability, intestinal morphology and amylase activity of broiler chickens under heat stress condition. Therefore, dietary supplementation of Gln and Glu is beneficial for broiler chickens reared under the hot and humid tropical environment.

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