Immune status of broiler chickens experimentally infected with avian *E.coli* supplemented with amino acid mixture rich in Glycine

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1. Abstract

Meat producing broiler chickens may needs dietary amino acids of animal origin to obtain high growth, good health, muscles yield and healthy immune response. To investigate the effects of the amino acids mixture from animal origins rich in glycine on performance and immunity of broiler chickens an in vivo experiment was conducted at which T1 and T2 groups supplemented with amino acid mixture in every 10 days for successive 3 days, 8 hr. daily interval at dose 1 ml/L in drinking water and the second (T2 group) was experimentally challenged with avian pathogenic *E.coli* (APEC) and the third group (T3) had no supplementation. The obtained results showed that amino acids mixture treated groups (T1 and T2) had a remarkable immune-modulation as noticed by significant elevation of phagocytic count and activities (lysozymes and nitric oxide production), expression of IL-4, IL-10 and interferon- gamma-genes using RT-PCR. Performance studies proved that there was an improvement of body weight gain and lowered mortality rate in amino acids treated group.

Key words: Glycine amino acid, Avian *E. coli*, Immunomodulation, growth enhancing, Broiler chickens.

2. Introduction

Broiler chickens average daily gain during the last decades, increased to more than 50 g/day, and the market age was reduced to six weeks due to improvement the genetic potential [1,2]. Availability of amino acids in feedstuffs is an important feature of dietary protein quality, as all dietary sources of protein are heterogeneous mixtures of different proteins, therefore, these proteins would be digested and different amino acids were taken up from the gut [3]. Digestible amino acids used in diet formulation as it makes it possible to increase the inclusion levels of different ingredients in poultry diets [4]. There are ten amino acids cannot be synthesized by poultry and must be supplied in the diet to
cover maintenance, growth, and production requirements. The ten essential amino acids are lysine, methionine, tryptophan, arginine, threonine, isoleucine, histidine, leucine, phenylalanine, and valine, while glycine is considered to be essential for the modern broiler chickens, since the rate of its synthesis is not adequate to support growth and maximum muscles production [5]. Undoubtedly that immune system requires proper nutrition [6], as studies have shown that random level of protein or of essential amino acids in birds even deficiencies or excesses can lead to shift of immune responses [7]. Among amino acids, both arginine and tryptophan have been proven to positively influence the avian systemic immune response [8]. Among amino acids, both arginine and tryptophan has been proven to positively influence the avian systemic immune response [8]. Although most E. coli strains belong to the normal flora of the intestines and are non-pathogenic, there are some strains that are able to establish themselves outside of the intestines and cause diseases, these falls into the extra-intestinal pathogenic E. coli (ExPEC) [9]. Colibacillosis is the extra-intestinal infections caused by APEC which lead to reduced yield, quality and reduce immune response with high mortality and morbidity rate [10]. Glycine act as an important compound in the synthesis of many physiologically molecules as nucleotides as purines and haem [11], also, glycine itself is a potent antioxidant as has a role in, glutathione synthesis [12] and also improve leucocytes anti-oxidative capability. The pharmacological importance of glycine represented in glycine-gated chloride channel in leucocytes cells helping in cytokines production regulation and improves immune response [13,14], so the current work studied the effect of supplementing amino acids mixture rich in glycine on performance, and immune status of broiler chickens challenged with avian pathogenic E. coli compared with non-challenged group.

3. Materials and Methods

Ethical approval

This investigation was performed in accordance with the recommendations in the updated Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. All procedures were approved by the Beni-Sueif University Ethical Committee in compliance with the United Kingdom (UK) Animals.

Broiler chickens husbandry

According to [15] with little modification, a total of 3 hundred (-day-old) Cobb broiler chickens were obtained from a commercial hatchery. Birds had free access to pelleted feed and water. Random distribution of chicks into 3 groups of 100 birds each, the experimental groups were as follows: T1; Amino acid mixture rich in Glycine treated T2 Amino acid mixture rich in Glycine treated and avian Pathogenic E. coli challenged and T3; Amino acid mixture rich in Glycine untreated.

Dietary treatments

Amino acid mixture rich in Glycine is a plenty mixture of amino acids from animal origin with high concentration of glycine amino acids distributed by ATCO, veterinary pharmaceutical integration. It added to treated groups every 10 days for successive 3 days, 8 hr. daily interval 1 ml/L in drinking water.

Challenge strains

Avian pathogenic E. coli serogroup O78 used in the challenged study was local isolate full identified and classified phenotypically and genotypically and accession number on Genbank MW699361 (SEC-EGY/ORABI/Raheel/2020). The challenged dose was 1.5x10⁸ cfu/ml through drinking water according to [16].

Immune matrix of broiler chickens supplemented with amino acid mixture and experimentally infected with APEC
HI titer; Blood samples were collected and tested using HI test by using inactivated H5N1 antigen (A/chicken/Egypt/18-H/2009) was used for detection of AIV-H5 antibodies and Lasota strains (8HA units) for detection of NDV antibodies [17].

Phagocytic cell assay; 96-Well Phagocytosis Assay, CytoSelect™ used according to [18] and Nitric oxide, Lysozyme level recorded according to [19]. The results of all immune parameters were presented as mean±SE. All given parameters were compared between studied groups using the one-way ANOVA with fixed effects of the factors using (Start Soft Inc).

Cytokines assay using qRT-PCR; RNA extraction from tissue samples was applied according to (Yuan et al., 2006) and the reaction prepared by using specific primers for IL-4[20],IL-10[21] and IFNG [22].

Re-isolation of avian pathogenic E.coli
According to [23]; 10 g of solid sample in 90 ml of normal saline followed by enrichment for 24 h incubation at 37°C in non-differential broth such as nutrient broth. This procedure will allow multiplication of E. coli then streaked on MacConkey’s and EMB agar and biochemically and serologically identified.

Performance measurements
Feed intake (FI), BWG, FCR and mortality percent were weekly determined [24].

4. Results
Results of innate immunity parameters
the phagocytic cell count determined in T1, T2 and at which there were propagation in the count cells from $10^2$ to $10^6$ in these groups at 10 and 30 days old respectively in comparison with T3 that show slow cell count from $10^2$ to 103 and also the secretory mediators of activated macrophage increased as nitric oxide and lysozyme and were determined as follow, T1:lysozyme from 1.5±0.356 to 5.5±0.556/nitric oxide from 2.5±0.215 to 18.4±0.235 and T3: lysozyme from 1.5±0.365 to 78.3±0.256/nitric oxide from 2.4±0.117 to 28.5±0.525 at 10 and 30 day old respectively, while T2 showed low level of these mediator (table 2).

Results of humoral immunity parameters
HI test used for traceability of the vaccination program reaction success, the results in table (1) for AI antibodies revealed that there were increasement in T1 from 1.94 ± 1.34 at 10 day old to 3.25±1.65 then for 4.92±1.73 and at 20 and 30 day old, while in T2 from 1.82 ± 1.75 to 2.66±1.36 and 3.95±1.43 at 10, 20 and 30 day old respectively, in comparison with the ND antibodies titers there were also increasement in T1( from 4.82±1.22 to 5.43±1.54), T2 (from 3.92±1.46 to 4.34±1.52) at 20 and 30 day old. However the results of untreated control negative T3 showed little changes.

Results of Avian cytokines gene expression analysis using qRT-PCR
The fold change in some avian cytokines showed in table (2), which revealed that there were fold change increasement in T1: IL4; from 5.1337 to 11.1579 / IL10; from 3.9449 to 7.5685 / IFNG ; from 7.6741 to 13.5479, T2: IL4; from 4.3772 to 8.5742/ IL10; from 2.5847 to 5.6178/ IFNG; from 6.1903 to 10.4831) at 15 and 25 day old respectively with little change in T3.

Results of re-isolation rate of avian E. coli
The re-isolation rate of the challenged serogroup was 12% in experimentally challenged group (T2)

Results of Performance parameters
Performance parameters recorded as feed intake(FI g/bird),BWG(kg/bird) and FCR, the results revealed that the FI in all experimented groups was 3300 g/bird, while the BWG was 2.4, 2.2 and 2.1 kg/bird in T1,T2 and T3 respectively and the FCR was 1.45, 1.5, 1.571 in T1,T2 and T3 respectively
5. Discussion

Consumer pressure for broilers raised has contributed to increased diets free of animal by-product meals being fed to broilers. Increase the use of the amino acid as feed ingredients has will continue in the coming periods, due to limited resources accompanied by excess requirements of amino acids levels in new broilers strains performance and efficiency assessments [25,26]. However diets for broiler chickens based mainly on cereals, oilseeds, and this raise least cost formulation on the total glycine or glycine plus serine amino acids [27]. In the present study Amino acid mixture rich in Glycine, which is a commercial amino acid combination rich in glycine explored as immune modulatory and performance enhancer agent in broilers chickens at which the immune status of the chicks under experiments evaluated by different parameters measurement. Humoral immune response depend on production of huge amount of antibodies against foreign bodies and vaccines which act as antigens, so in the current study the antibodies titer of Avian influenza and Newcastle used vaccine were measured using HI test at which the amino acid treated groups T1 showed best titer at different age old , the results in table (1) for AI antibodies revealed that there were increasement in T1: IL4 ; from 5.1337 to 11.1579 / IL10; from 3.9449 to 7.5685/ IFNG ; form 7.6741 to 13.5479 and in T2: IL4; from 4.3772 to 8.5742/ IL10; from 2.5847 to 5.6178/ IFNG; form 6.1903 to 10.4831) at 15 and 25 day old respectively with disappointed results in T3 as there were confinement of these mediators which reflect on immune response against infectious agents as IL4 act as a potent mediators of many immune cells as mast cell, eosinophil’s and Th2 cells [32], while IL10 has potent anti-inflammatory actions by preventing tissue damage and maintain normal cell homeostasis [33], in the other hand interferon gamma has a critical roles in modulation of innate and cellular immune response toward viral pathogens [34]. Glycine amino acid reduces inflammation in infected animals as low level of glycine in diets decrease immune responses in chickens treated with lipopolysaccharides[35], which was followed by glycine dietary supplementation [36], as it decrease TNFα levels in plasma improved survival rate and decrease morbidity. Interestingly, glycine protected animals against arthritis induced by peptidoglycan polysaccharide, help in control of stress-induced gastrointestinal mucosal injury, protect from the ischemia shock caused by endotoxin sepsis and hemorrhage [14]. Experimental infection
with avian pathogenic E.coli have been crucial in the study of immune modulation of amino acid mixture rich in Glycine by allowing investigation of the macrophage clearance ability and shedding of the challenged strains, in the current investigation the re-isolation rate of the challenged pathogenic serogroup was 12% in T2 group. Macrophages are one of the mononuclear phagocytic systems, that it is a crucial player in both the innate and adaptive immune responses, for this reasons the present investigation studied the phagocytic count and its mediators there were augmentation in the count cells from $10^2$ to $10^6$ in T1 and T2 groups at 10 and 30 days old respectively in comparison with T3 that showed slow cell count from $10^2$ to $10^3$ and exacerbate nitric oxide and lysozyme level in T1 and T2 more than T3 (table 2) which indicated that activation of macrophages cells by action of dietary supplementation of amino acid mixture rich in glycine [37]. The good immune response, the best performance criteria as broilers are among the most efficient feed converting livestock in the world, so During the present experimental study the performance parameter were determined as feed intake, body weight gain(kg/bird) and feed conversion at which the results showed that the weight gain were 2.4, 2.2 and 2.1 kg/bird in T1,T2 and T3 respectively and the FCR was1.45,1.5 and 1.571 in T1,T2 and T3 respectively which confirm that the treated group with amino acid mixture has potent muscle yield and health digestive system for feed conversion.

6. Conclusion

In conclusion for best growth, high muscle yield, alert immune response and less inflammatory reaction in broiler chickens amino acid mixture rich in glycine dietary supplement is required.

7. References


Table 1: Immune matrix of Amino acid rich in Glycine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND-HI</td>
<td>10 days</td>
<td>2.12±1.35</td>
<td>1.84±1.43</td>
<td>1.83±1.54</td>
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<tr>
<td></td>
<td>20 days</td>
<td>4.82±1.22</td>
<td>3.92±1.46</td>
<td>2.83±1.43</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>5.43±1.54</td>
<td>4.34±1.52</td>
<td>3.24±1.42</td>
</tr>
<tr>
<td>AI-HI</td>
<td>10 days</td>
<td>1.94±1.34</td>
<td>1.82±1.75</td>
<td>1.78±1.46</td>
</tr>
<tr>
<td></td>
<td>20 days</td>
<td>3.25±1.65</td>
<td>2.66±1.36</td>
<td>1.93±1.86</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>4.92±1.73</td>
<td>3.95±1.43</td>
<td>2.8±1.37</td>
</tr>
<tr>
<td>Phagocytic cell count</td>
<td>10 days</td>
<td>10³</td>
<td>10³</td>
<td>10²</td>
</tr>
<tr>
<td></td>
<td>20 days</td>
<td>10⁴</td>
<td>10⁵</td>
<td>10³</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>10⁶</td>
<td>10⁶</td>
<td>10³</td>
</tr>
<tr>
<td>Nitric oxide µmol/ml</td>
<td>10 days</td>
<td>2.5±0.215</td>
<td>2.4±0.117</td>
<td>2.4±0.235</td>
</tr>
<tr>
<td></td>
<td>20 days</td>
<td>15.2±0.145</td>
<td>25.6±0.415</td>
<td>6.8±0.315</td>
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<tr>
<td></td>
<td>30 days</td>
<td>18.4±0.235</td>
<td>28.5±0.525</td>
<td>9.5±0.415</td>
</tr>
<tr>
<td>Lysozyme µmol/ml</td>
<td>10 days</td>
<td>1.5±0.356</td>
<td>1.5±0.365</td>
<td>1.4±0.156</td>
</tr>
<tr>
<td></td>
<td>20 days</td>
<td>3.5±0.422</td>
<td>6.5±0.345</td>
<td>2.2±0.132</td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>5.5±0.556</td>
<td>8.3±0.256</td>
<td>3.2±0.254</td>
</tr>
</tbody>
</table>

T1: Amino acid rich in Glycine treated, T2: Amino acid rich in Glycine treated and avian Pathogenic E. coli challenged, T3: Amino acid rich in Glycine untreated and unchallenged (control negative)
Table 2: Results of cytokines indices

<table>
<thead>
<tr>
<th>Time point</th>
<th>Gp.</th>
<th>IL4</th>
<th>IL10</th>
<th>IFNG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>Fold change</td>
<td>CT</td>
</tr>
<tr>
<td>15 day</td>
<td>T1</td>
<td>19.21</td>
<td>5.1337</td>
<td>20.42</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>18.17</td>
<td>4.3772</td>
<td>19.76</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>21.33</td>
<td>-</td>
<td>22.16</td>
</tr>
<tr>
<td>25 day</td>
<td>T1</td>
<td>17.59</td>
<td>11.1579</td>
<td>18.96</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>19.08</td>
<td>8.5742</td>
<td>20.50</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>21.07</td>
<td>-</td>
<td>21.88</td>
</tr>
</tbody>
</table>

T1: Amino acid rich in Glycine treated, T2: Amino acid rich in Glycine treated and avian Pathogenic E. coli challenged, T3: Amino acid rich in Glycine untreated and unchallenged (control negative)