The Influence of Dietary Lysosome on the Growth Performance, Blood Constituents, Intestinal Morphology and Resistance Against Escherichia Coli in the Broiler Chicks

R.A. Hassan1*; Y.Z. Eid2; Zeinab, M. Farouk1, M. El-Gbaly1 and M. A. M. Mousa1

1Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. 2Department of Poultry Production, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.

*Corresponding author e-mail address (redaalihasan@yahoo.com)

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SUMMARY

One of the main Hazardous reasons the chicken sector suffers significant financial losses is Escherichia coli (E. coli). The benefits of lysozyme addition in the diet for preventing avian colibacillosis are not well understood. The purpose of the current study was to compare the effects of lysozyme dietary supplementation with antibiotic treatment in broiler chicks infected with E. coli. Eight groups of 600 one-day-old unsexed Ross chicks were created: The first group (control) was not infected and was given a balanced diet free of additives, the second and third groups were also not infected and were treated with 50 mg and 100 mg of lysozyme, respectively, the fourth group was non-infected and treated with 10 mg avilamycin / kg diet, the fifth group was infected and non-treated, the sixth and seventh group was infected and were treated with 50 mg and 100 mg of lysozyme, respectively, and the eighth group was infected and treated with 10 mg avilamycin / kg diet. The infection by E. coli was given orally to the chicks at days 5 and 16 of age. The experimental period was 35 days for all examined groups. The E. coli-infected group showed decreased phagocytic activity, dressing percentage, relative weight of thymus and bursa, and serum total antioxidant capacity. The liver enzymes, renal function tests, MDA, relative weights of the liver, heart, and spleen, as well as changes in gut morphology and intestinal microbial counts, were all significantly elevated in the same group. The lysozyme-pretreated infected chick showed improvements in body performance metrics in addition to a noteworthy reduction in the tests for renal and hepatic enzymes. Significant improvements were observed in gut morphology, intestinal microbial counts, antioxidant enzymes, and serum immunological parameters in the treated groups. It is possible to draw the conclusion that adding lysozyme (at 50 and 100 mg/kg) to the diet of broiler chickens improves their immune system, performance, and reduces the pathological lesions caused by E. coli infections.

Keywords: Escherichia coli, lysozyme, antibiotic, broilers, performance, immunity

INTRODUCTION

One of the most significant bacterial infections to affect the poultry production, avian colibacillosis causes significant financial losses in broilers. The disease is measured one of the major causes of mortality and morbidity in broilers that reaching up to 50%, either as a main or as a secondary pathogen. Escherichia coli causes avian colibacillosis, which is considered a native commensal occupant of the chicken’s intestinal tract and the trachea to a lesser degree. Generally, 10–15% of intestinal E. coli are avian pathogenic E. coli with different virulence factors, which might result in systemic diseases such as peripneumitis, pericarditis, peritonitis and airsacculites (Abo El-magd et al., 2019).

When a bird’s defence mechanism fails, infection is promoted and is exacerbated by a number of variables, including poor management, concomitant infections, and immunosuppression. Due to the wide range of avian pathogenic E. coli strains present in the field, there is currently no effective vaccine against colibacillosis. As a result, colibacillosis must be controlled with preventative measures and antibiotic therapy. The most typical method for treating colibacillosis is thought to involve the use of antibiotics.
And this approach may have significant negative consequences on the bird and consumers combined with the development of drug resistance (Hashem et al., 2022).

Lysozyme is one feed component that may eventually replace dietary antibiotics (Zhang et al., 2006). This enzyme separates the glycosidic bond between N-acetylg glucosamine and N-acetylmuramic acid in the bacterial peptidoglycan of the cell wall (Phillips, 1996). Commercially, lysozyme can be made from avian egg whites. It frequently appears in a variety of animal tissues and fluids (Grossowicz and Ariel, 1983). According to numerous studies, lysozyme in several organisms plays a protector against bacteria (Ibrahim et al., 1996). Lysozymes have an antibacterial effect that is achieved by their direct inducing the phagocytic activity of macrophages or by bacteriolytic action (Biggar and Sturgess, 1977). There are a few number of studies have examined the effects of exogenous lysozyme supplementation on broiler chicks performance and digestive health. The current study aims to understand how these feed additives, like lysozyme, can be used to control E. Coli infections and raise chicken productivity.

MATERIALS AND METHODS

In accordance with an agreement between the Faculty of Agriculture in Kafr El-Sheikh and Animal Production Research Institute, this research was carried out on a private farm under the administration of the Poultry Department of the Faculty of Agriculture, Kafr El-Sheikh University. In order to conduct this study, 600 ROSS broiler chicks that were one-day old were divided into 8 treatments, each of which had five repetitions and each replicate contained 15 chicks that were housed in a 1.3 × 3.5-meter area that was covered in wood shavings to a depth of 5 cm. The treatments were as follows: The 1st group was (control) non-infected and fed balanced diet without additives, 2nd group was non-infected and treated with 50 mg lysozyme/kg diet, 3rd group was non-infected and treated with 100 mg lysozyme/kg diet, 4th group was non-infected and treated with 10 mg avilamycin/kg diet, 5th group was infected and non-treated, 6th group was infected and treated with 50 mg lysozyme/kg diet, 7th group was infected and treated with 100 mg lysozyme/kg diet and 8th group was infected and treated with 10 mg avilamycin/kg diet. The infection by E. coli was given orally to the chicks at 4x10^10 CFU/ml per chick at days 5 and 16 of age. By the third week of age, the birds' incubation temperature of 32 degrees Celsius had been gradually dropped to 26 degrees Celsius and they had been exposed to 23 hours of light. During the course of the experiment, feed was available ad libitum.

Diet:

According to the NRC (1994) standards, two basal diets were created for the starter stage (1–21 days) and the grower stage (22–35 days). Table 1 displays the components and nutrient makeup of the basal diets as determined by AOAC (2000).

Lysozyme:

Egg white lysozyme (activity 20000 U/mg); Beijing Solarbio Science & Technology Co., Ltd., Beijing, China.

Antibiotic:

Avilamycin 10%, an antibiotic growth promoter. Produced by Kavilamycin®, Kartal Kimya Sanayiye Ticaret AS Gebze-Kocaeli / Turkey.

At the 5th and 16th days of age, each chick received an oral infection with 0.5 ml of E. coli (O111:K58) containing (4x10^10 CFU/ml). Throughout the trial, unlimited access to feed and water was supplied (35 days).

E. coli (O111:K58):

The Animal Health Research Institute's Strains Bank, located in Dokky, Giza, is where the E. coli (O111:K58) strain was acquired. This used strain was isolated from omphalitis chicks.

Preparation of E. coli (O111:K58) inoculum:

According to Macfaddin (1980), E. coli colonies were cultivated in nutrient broth for 24 hours at 37 °C, and the viable number was adjusted to 4x10^10 colony-forming units CFU viable organism/ml. Following the procedure outlined by Peighambari et al. (2000), chicks were inoculated with 0.5 ml by intranasal route at days 5 and 16 of age.
Table (1): Composition and calculated analysis of the experimental starter and finisher diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter (%)</th>
<th>Grower (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grains</td>
<td>53.71</td>
<td>61.92</td>
</tr>
<tr>
<td>Soybean meal (44%)</td>
<td>33.42</td>
<td>28.05</td>
</tr>
<tr>
<td>Corn gluten meal (60%)</td>
<td>5.22</td>
<td>3.20</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>3.32</td>
<td>2.94</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.28</td>
<td>1.15</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.84</td>
<td>1.68</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.39</td>
<td>0.22</td>
</tr>
<tr>
<td>Vitamins and minerals premix*</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>L-lysine HCl</td>
<td>0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Analyzed and calculated composition (NRC, 1994)

<table>
<thead>
<tr>
<th></th>
<th>Starter (%)</th>
<th>Grower (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein %</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Metabolizable energy (Kcal/kg diet)</td>
<td>3094</td>
<td>3142</td>
</tr>
<tr>
<td>Methionine %</td>
<td>0.80</td>
<td>0.58</td>
</tr>
<tr>
<td>Calcium %</td>
<td>1.00</td>
<td>0.90</td>
</tr>
<tr>
<td>Available phosphorus %</td>
<td>0.49</td>
<td>0.45</td>
</tr>
<tr>
<td>Lysine %</td>
<td>1.25</td>
<td>1.11</td>
</tr>
</tbody>
</table>

* Composition (per 3 kg): vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 100000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 40000 mg, iodine 300 mg, selenium 100 mg, and cobalt 100 mg.

Measurements:

Average daily weight gain (ADWG), Average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated at the end of the experimental period. Throughout the study time, the death rate was noted, and the ratio for each treatment was computed.

Five chicks per treatment were randomly selecting (bird per replicate) during slaughter at 35 d of age. All the organs, comprising the liver, hearts, bursa, spleen, and thymus, were weighed, and then estimated the relative weight to live BW. By dividing the carcass weight plus the edible weight by the live body weight and multiplying by 100, the dressing percentage was calculated.

Microbial enumerations:

Sections from the ileum were taken at the conclusion of the experiment from 1 chick per replication (5 chicks from each group) that was acquired during slaughter. The Collins and Lyne (1970) method was used to conduct the E. coli, Clostridium, and Lactobacillus counts.

Biochemical assays:

One chick per replicate, or five chicks per group, was used to collect the blood. The blood samples were centrifuged for 15 minutes at 3500 rpm to extract blood plasma, which was then stored at -20 °C for examination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cholesterol, creatinine, and uric acid levels, using commercial kits (Diamond Diagnostics, Egypt) and a spectrophotometer. Oxidative capacity parameters, involving activity of total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS) were analyzed in blood plasma using commercially available kits. The phagocytic activity was detected according to Goddeeris et al. (1986).

Morphological analysis:

Following the birds' slaughter at the end of experimental period, 5 intestinal sections from each group were obtained in order to calculate the Crypt depth (µm), Villus height (µm), and Villus to crypt ratio. for 48 hours, jejunum samples were treated with 4 percent paraformaldehyde and fixed in paraffin. Hematoxylin and eosin was used to stain tissue samples (5 µm) for morphological investigation. Each segment evaluated nine full villi. The crypt depth was calculated from the bottom of the villus to the lamina propria, and the villus height was calculated from the tip of the villus to the villus-crypt junction.
The villus to crypt ratio was estimated after that. ProgRes CapturePro software and an Olympus optical microscope were utilized for all measurements and evaluations (version 2.7; Jenoptik, Jena, Germany).

**Immune response against Newcastle disease (ND):**

At 35 days of age, antibody titers for the immune response for Newcastle disease virus (NDV) were evaluated in each group (bird per replicate) using the HI test, as recommended by King and Seal (1998).

**Statistical analysis:**

The trial was operated using a completely random design. Duncan's multiple range tests (Duncan, 1955) were employed to evaluate the differences in means (P < 0.05) and were used in conjunction with The General Linear Model (GLM) approach of SAS (2003).

**RESULTS**

**Growth performance:**

Body weight gain and feed intake significantly decreased while FCR significantly damaged in the E. coli-infected group when compared to the control group. Treatments with lysozyme and antibiotics reduce mortality, while improving body weight, weight increase, and FCR throughout the trial period (Table 2). The infected non-treated groups demonstrated mortality (10%), while groups that received treatment did not record any mortality.

**Table (2): Effect of experimental treatments on growth performance in broiler chickens during whole the experimental period.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Daily weight gain, g</th>
<th>Daily feed intake (g/bird/day)</th>
<th>FCR (g feed/g gain)</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NLyz50</td>
<td>55.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>104.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NLyz100</td>
<td>56.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.87&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NANTI</td>
<td>56.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>INT</td>
<td>48.85&lt;sup&gt;d&lt;/sup&gt;</td>
<td>98.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ilyz50</td>
<td>52.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>101.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ilyz100</td>
<td>54.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>103.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IANTI</td>
<td>54.78&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>103.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.428</td>
<td>0.321</td>
<td>0.018</td>
<td>0.257</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<sup>*Mean with different superscripts in a column differ significantly (P < 0.05). *SEM = Standard Error of the mean; NLyz50: Non infected treated with lysozyme (50mg). NLyz100: Non infected treated with lysozyme (100mg). NANTI: Non infected treated with antibiotic. INT: Infected with E. coli non treated group. Ilyz50: infected treated with lysozyme (50mg). Ilyz100: infected treated with lysozyme (100mg). IANTI: Infected with E. coli and treated with antibiotic.</sup>

**Microbial enumeration:**

As demonstrated in Table 3, the substantial increases in the ileal Clostridium spp. and E. coli, in addition to the declines in Lactobacillus bacteria counts at 35 days of age, were caused by E. coli infection. The outcomes demonstrated that regardless of the lysozyme dose effect of lysozyme was considerable on the E. coli bacteria, Clostridium spp. and total Lactobacillus bacteria.

**Jejunal morphological indices:**

The effects of lysozyme inclusion in the feed on the jejunal morphological parameters in broilers with E. coli challenges are shown in Table 3. At 35 days of age, it is evident that E. coli infection significantly decreased the VH:CD ratio and the height of the jejunal villi, although it significantly increased the depth of the jejunal crypt. At the age of 35 days, lysozyme and antibiotic supplements were introduced to the diet, which reduced the adverse effects of the E. coli challenge on the jejunal villi width, crypt depth, villi height, and VH:CD ratio.
considerably outperformed the control group in characterizes of phagocytic percent and phagocytic antibiotic.

Table (4): Effect of experimental treatments on some blood constituents

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.77^a</td>
</tr>
<tr>
<td>NLyz50</td>
<td>2.55^c</td>
</tr>
<tr>
<td>NLyz100</td>
<td>3.67^a</td>
</tr>
<tr>
<td>NANTI</td>
<td>3.25^c</td>
</tr>
<tr>
<td>INT</td>
<td>1.52^c</td>
</tr>
<tr>
<td>Ilyz50</td>
<td>2.58^c</td>
</tr>
<tr>
<td>Ilyz100</td>
<td>3.08^abc</td>
</tr>
<tr>
<td>IANTI</td>
<td>2.85^bc</td>
</tr>
<tr>
<td>SEM</td>
<td>0.225</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Means with different superscripts in a column differ significantly (P < 0.05). *SEM = Standard Error of the mean;

Blood biochemical parameters:

Table 4 summarizes data indicating the impact of dietary treatments on serum uric acid, creatinine, ALT, and AST at 35 days of age. Serum levels of creatinine, uric acid, ALT, and AST in the E. coli-infected group that wasn't treated increased significantly at the end of the experimental stage as compared with the control group. In contrast, lysozyme-treated E. coli infection groups demonstrated a significant diminution in serum creatinine, uric acid, ALT, and AST when compared with the untreated E. coli infection treatment. Regarding antioxidant measures, comparing the E. coli-infected non-treated group to the control treatment, there was a substantial drop in TAC activity and a rise in TBARS concentration. At 35 days of age, the E. coli-infected chicks treated with lysozyme and antibiotics showed a significant drop in TBARS concentration and a rise in TAC activity (4).

Table (4): Effect of experimental treatments on some blood constituents

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>Uric acid mg/dl</th>
<th>Creat. mg/dl</th>
<th>MDA nmol/ml</th>
<th>TAC mmol/l</th>
<th>Phagocytic activates (%)</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>182^a</td>
<td>22.0^bc</td>
<td>3.60^a</td>
<td>0.260^b</td>
<td>1.05^c</td>
<td>1.47^b</td>
<td>69.8^a</td>
<td>6.08^ab</td>
</tr>
<tr>
<td>NLyz50</td>
<td>180^b</td>
<td>23.0^bc</td>
<td>3.62^a</td>
<td>0.280^b</td>
<td>1.00^c</td>
<td>1.55^ab</td>
<td>70.2^a</td>
<td>6.58^ab</td>
</tr>
<tr>
<td>NLyz100</td>
<td>181^c</td>
<td>21.0^c</td>
<td>3.45^c</td>
<td>0.260^b</td>
<td>0.80^c</td>
<td>1.78^a</td>
<td>71.05^a</td>
<td>7.0^c</td>
</tr>
<tr>
<td>NANTI</td>
<td>186^c</td>
<td>22.6^bc</td>
<td>3.72^c</td>
<td>0.270^b</td>
<td>1.00^c</td>
<td>1.50^b</td>
<td>70.31^a</td>
<td>6.09^ab</td>
</tr>
<tr>
<td>INT</td>
<td>230^a</td>
<td>31.0^c</td>
<td>6.50^b</td>
<td>0.380^a</td>
<td>1.98^c</td>
<td>1.07^a</td>
<td>38.65^d</td>
<td>3.45^c</td>
</tr>
<tr>
<td>Ilyz50</td>
<td>215^ab</td>
<td>26.1^bc</td>
<td>5.76^ab</td>
<td>0.280^b</td>
<td>1.40^b</td>
<td>1.36^b</td>
<td>50.66^a</td>
<td>4.98^bc</td>
</tr>
<tr>
<td>Ilyz100</td>
<td>190^c</td>
<td>24.0^bc</td>
<td>4.05^b</td>
<td>0.263^b</td>
<td>1.18^c</td>
<td>1.58^b</td>
<td>62.55^b</td>
<td>5.76^bc</td>
</tr>
<tr>
<td>IANTI</td>
<td>206^b</td>
<td>25.0^c</td>
<td>4.80^bc</td>
<td>0.290^b</td>
<td>1.30^c</td>
<td>1.42^b</td>
<td>50.75^a</td>
<td>5.00^bc</td>
</tr>
<tr>
<td>SEM</td>
<td>3.924</td>
<td>0.733</td>
<td>0.258</td>
<td>0.008</td>
<td>0.084</td>
<td>0.046</td>
<td>2.471</td>
<td>0.279</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Means with different superscripts in a column differ significantly (P < 0.05). *SEM = Standard Error of the mean;

In terms of phagocytic activity, non-infected groups treated with lysozyme and antibiotics considerably outperformed the control group in characterizes of phagocytic percent and phagocytic

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index. While they significantly decreased in the E. coli-infected treatment that was not treated compared with the control treatment. When compared with the infected E. coli non-treated group, the E. coli infection groups that received lysozyme and antibiotic treatment showed a significant rise in phagocytic index and phagocytic percent, as shown in Table (4).

**Internal organ weights:**

Thymus and bursa weight significantly decreased in infected broilers compared to non-infected broilers (Table 5). However, compared to non-infected broilers, the relative weight of the spleen, heart, and liver was greater in the infected broilers (Table 5). On the other hand, the lysozyme and antibiotic restored the negative effects of infection with E. coli on organ weights.

**Hemagglutination inhibition (HI) test:**

When compared with the control group, the E. coli-infected treatment that was not treated had a considerably lower antibody titer against NDV. When compared with the infected E. coli non-treated group, the E. coli infection groups that received lysozyme and antibiotic treatment showed a significant rise in antibody titer against NDV, as shown in Table (5).

**Table (5): Effect of experimental treatments on carcass traits and lymphoid organs**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carcass traits %</th>
<th>Lymphoid organs weights %</th>
<th>Immune response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dressing</td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70.00(a)</td>
<td>2.37(c)</td>
<td>0.43(b)</td>
</tr>
<tr>
<td>NLyz50</td>
<td>70.50(a)</td>
<td>2.36(c)</td>
<td>0.42(b)</td>
</tr>
<tr>
<td>NLyz100</td>
<td>71.60(a)</td>
<td>2.33(c)</td>
<td>0.42(b)</td>
</tr>
<tr>
<td>NANTI</td>
<td>70.80(a)</td>
<td>2.40(b)</td>
<td>0.43(b)</td>
</tr>
<tr>
<td>INT</td>
<td>68.55(b)</td>
<td>3.16(a)</td>
<td>0.50(a)</td>
</tr>
<tr>
<td>Ilyz50</td>
<td>69.00(ab)</td>
<td>2.65(b)</td>
<td>0.42(b)</td>
</tr>
<tr>
<td>Ilyz100</td>
<td>70.05(a)</td>
<td>2.51(bc)</td>
<td>0.43(b)</td>
</tr>
<tr>
<td>IANTI</td>
<td>69.25(a)</td>
<td>2.63(bc)</td>
<td>0.42(b)</td>
</tr>
<tr>
<td>SEM</td>
<td>3.65</td>
<td>0.11</td>
<td>0.02</td>
</tr>
<tr>
<td>p-value</td>
<td>0.015</td>
<td>0.002</td>
<td>0.045</td>
</tr>
</tbody>
</table>

\*Means with different superscripts in a column differ significantly (P < 0.05). \( SEM = \) Standard Error of the mean; NLyz50: Non infected treated with lysozyme (50mg). NLyz100: Non infected treated with lysozyme (100mg). NANTI: Non infected treated with antibiotic. INT: Infected with E. coli non treated group. Ilyz50: infected treated with lysozyme (50mg). Ilyz100: infected treated with lysozyme (100mg). IANTI: Infected with E. coli and treated with antibiotic.

**DISCUSSION**

**Growth performance:**

According to Remus et al. (2014), E. coli serotypes could have been the major and secondary reasons for feed intake rejection, which in turn caused a drop in body weight (Shah et al., 2004). Second, the synthesis of acute phase proteins, proteolysis, and gluconeogenesis might have been triggered by the release of interleukin 6 and C reactive protein in response to pathogen invasions. These subsequently caused the chicks’ feed consumption to drop, which in turn caused their growth to slow (Bodell et al., 2009). Third, slow feed transit may have been a result of the corticosterone produced by microbial stress (Hu and Guo, 2008), which may have also been a factor in the increased proteolysis and glucogenesis as well as the decreased feed intake in chicks (Lin et al., 2004). Fourth, malabsorption may have occurred as a result of the chicks’ decreased villi height and VH:CD ratio, which may be what caused the decline in ADWG (Table 3). Our findings support those of Huff et al. (2002), who exhibited that an E. coli infection dramatically reduced avian body weight. By comparison to the control group, the chicks exposed to E. coli had lower body weights, according to Shah et al. (2004). Lysozyme and antibiotic dietary inclusion during trial phases enhanced ADFI and ADWG, which improved FCR levels in their chicks. According to Miles et al. (2006), adding virginiamycin to a diet consisting of maize and soy meal encouraged an increase in the body weight of male and female broiler chicks as well as the proportion of absorptive cells per unit length in their intestines. Better nutrition absorption is made possible by this physical change, which supports performance stimulation.
Lysozyme's beneficial effects on growth performance in the present study may be attributable to better gut antioxidant and antibacterial activities as well as improved nutritional digestibility in chick’s gut, which may be related to enhanced nutrient absorption in the intestine (Remus et al., 2014). Additionally, the stronger immunological responses provided by the advantageous microbiota may be responsible for the higher growth performance.

Chicks with E. coli O78:K80 infection were treated with antibiotics, which improved FCR levels while also raising DFI and DWG levels. In terms of BWG, FCR, and survival rate, antibiotic therapy has been shown to be beneficial for broiler hens exposed to Clostridium spp (Sen et al., 2012).

Mortality in infected group with E. coli was 10%. This result was in agreement with that published by Shen et al. (2002), who said that E. coli causes mortality rates of 8%. No mortality was noted in those that were infected or given Ca Fosfomycin treatment. This finding validates the use of antibiotics to manage E. coli infection.

**Intestinal microbial count:**

The findings exhibited that lysozyme had a significant impact on the overall amount of Clostridium spp, E. coli bacteria and Lactobacillus bacteria irrespective of the lysozyme dosage. Our findings concur with those of Ibrahim et al. (2011) who stated that lysozyme plays as protector against bacteria. They added that, the pathogenic bacterial cell wall's peptidoglycan is hydrolyzed by lysozyme as an antibiotic agent. When broilers were gavaged with Clostridium perfringens, Liu et al. (2010) discovered that 40 mg lysozyme efficiently decreased the amount of Clostridium pp in the ileum and inhibited intestinal injuries. Lysozyme can successfully aid broiler chickens in coping with Eimeria infection so that coccidiosis is avoided, as demonstrated by Sotirop and Koinarski (2003).

According to reports, E. coli levels in birds fed antibiotics to stimulate growth have significantly decreased (Rahimi et al., 2011). Salmonella and E. coli load in the digesta are reduced by the antimicrobial agent bacitracin methylene disalicylate, which also drives them toward the mucosal surface (Ghosh et al., 2012).

**Gut morphological:**

Concerning to intestinal morphology, E. coli infection significantly diminished the VH:CD ratio and the height of the jejunal villi, although it significantly raised the depth of the jejunal crypt. The findings of Wang et al. (2017), who obtained that broilers exposed to E. coli had smaller villi heights and lower VH:CD ratios than control chicks, are in keeping with the current results. Zhang et al. (2016) assert that an E. coli K88 challenge changed intestinal structure. Although, Miles et al. (2006) asserted that an enhancement in intestinal morphology encourages greater feed absorption, this leads to less energy being needed for tissue maintenance, which can then be employed for growth or better nutritional absorption.

Dietary lysozyme supplementation raised the VH:CD ratio and villi height in the jejunum. A larger VH:CD ratio indicates slower tissue turnover, which lowers feed required to make up for villi deteriorate or injury brought on by pathogen-induced irritation (Mahdavi et al., 2010). Lysozyme's positive impact on gut shape may be due to altered intestinal microbiota. Previous research supported the positive impact of lysozyme on gut microflora. Additionally, ingestion of lysozyme milk led to a rise in the number of Bifidobacteriaceae and Lactobacillaceae, both of which have been shown to be advantageous for the development of the digestive tract (Maga et al., 2012). According to findings from earlier studies, adding antibiotics to meals promotes the growth of cells that absorb nutrients in the intestine. In the ileum and jejunum after antibiotic therapy, we observed increased villus height and crypt depth (Xia et al., 2004).

**Blood biochemical parameters:**

The infected non-treated treatment exhibited a statistically significant rise in the serum activity of ALT and AST when compared to the control treatment. These findings support those of Abd-Allah et al. (2018). Higher hepatic enzyme activity may arise from the microorganism altering the hepatocytes’ membrane permeability, which causes the cell membrane to lose its functional integrity and spill these enzymes into circulation (Gahalain et al., 2011). Comparing the E. coli-treated group to the infected non-treated group, the lysozyme-treated group showed a substantial reduction in serum AST and ALT values. According to the research, lysozyme is an excellent source of antioxidants that can protect cells from free radicals, reduce toxicity, and possibly even safeguard liver health by preventing liver damage.

The fact that uric acid and creatinine levels in the lysozyme-treated groups did not significantly differ from those in the control, healthy group showed that this treatment was safe for the kidneys. Ibrahim and Banaee (2014) claim that using lysozyme on the kidney is safe. According to Abdelhady and El-Abasy (2015), the E. coli-infected non-treated group has higher serum renal function parameters.
than the other groups. This difference could be attributed to renal injury, an imbalance in protein metabolism, or an imbalance in amino acid concentration. These findings concur with those of Abd El-Ghany and Ismail (2014) and may be related to the kidney’s reaction to the bacteria and their toxin (Abd-Allah et al., 2018). When compared with the infected non-treated treatment, the lysozyme-treated infected group demonstrated a considerably lower level of serum uric acid and serum creatinine.

The infected non-treated group exhibited a noticeably greater level of TBARS and a noticeably lower concentration of TAC as compared to the control group. Zheng et al. (2016) found similar outcomes in broiler chickens experimentally infected with lipopolysaccharide (LPS) of E. Coli O55: B5. They observed a drop in GPx activity and an increase in serum MDA. The reason for these outcomes can be attributed to the fact that LPS generates large amounts of reactive oxygen species (ROS), which can cause oxidative damage to cells and tissues by upsetting the delicate balance between the pro-oxidant and antioxidant systems. On the other hand, serum TAC activity significantly decreased in E. coli-infected non-treated chickens, which is exactly in line with the findings of Kumari et al. (2020), they found that an E. Coli infection lowered the antioxidant defense system. The lysozyme-treated E. coli-infected groups (groups 6 and 7) demonstrated a significant decline in TBARS and a significant rise in TAC when compared to the E. coli-infected non-treated group (group 5). These results are in harmony with those of Mezbani et al. (2019), they discovered that adding lysozyme (100 mg/kg) to broiler diet caused MDA levels to significantly decrease and CAT and GPx activities to significantly rise when compared to control birds.

Furthermore, this investigation showed that the birds treated with lysozyme (50 and 100 mg/kg) and the antibiotic showed a significant increase in both the phagocytic index and phagocytic percentage. These findings are in line with a previous study (Ali et al., 2018), which found that broiler chicks' phagocytic activity and phagocytic index could be increased by adding lysozyme to the feed in comparison to the control group. In contrast, the phagocytic activity and phagocytic percentage of the E. coli-challenged birds showed a significant decline after 35 days of age when compared to the control group. These outcomes were consistent with those of an earlier investigation (Abd El-Tawab et al., 2015), which linked these outcomes to pathophysiological effects of bacterial endotoxins and the suppression of antibacterial defense mechanisms. These outcomes could also be linked to the immune system's weariness from an E. coli infection (Lee et al., 2015).

Internal organ weights:

A vital part of the chicken immune system is the bursa. Furthermore, the morphological reaction to the immunological state in broilers is represented by the bursal weight, as stated by Manafi et al. (2016). The current study's findings were supported by Gottardo et al. (2017), who also found that the infected group's thymus and bursa had considerably smaller relative weights. Nevertheless, these results are at odds with those of Manafi et al. (2016). The relative weight of the spleens in the infected group increased dramatically on day 35. These results might be connected to the infection, as they were supported by Huff et al. (1998) findings in turkeys. However, in the Lysozyme groups, the spleen's relative weight was significantly lower and the thymus and bursa's relative weights were significantly higher. These results agree with those of Sohail et al. (2013). Dysfunction of lymphoid organs reduces immunity against bacterial, viral, parasitic, and fungal infections. Because of this, birds are more susceptible to a variety of diseases, which raises mortality and morbidity rates and results in large financial losses (Wan et al., 2017).

The weight of the bursa and thymus significantly increased in non-infected broilers compared to infected broilers (Table 5). The findings supported those made by Sadeghi et al. (2013), who asserted that a salmonella exposure diminished the growth of the lymphoid organs. Low bursa weight may be used as an indicator of low immunological activity because the bursa is a significant lymphoid organ in birds and immune tissue weight affects immune cell morphologies, immune cell proliferation, and antibody generation (Ghaderi-Joybari et al., 2014). Immunosuppression in poultry is typically indicated by clinically obvious E. coli infection (Mc Gruder and Moore, 1998). The chickens' immune systems were harmed by E. coli infection, which resulted in lymphocyte diminution in both the thymus and bursa (Nakamura et al., 1990).

The relative liver and heart weights of infected broilers were higher in the current study than those of non-infected broilers. Similar conclusions were reached by Abalaka et al. (2017), who found that broiler hens with E. coli infections had larger livers. Furthermore, Rocha et al. (2013) found that broiler chicks exposed to Salmonella typhimurium had an increased liver relative weight. The enlarged relative weight of the liver is thought to be due to the necrotic lesions and inflammation caused by E. coli infections (Abalaka et al., 2017). This supports research by Fernandez et al. (2002), who found that broiler chicks exposed to E. coli O78:K80 had increased relative weights of the liver, heart, and air sacs.
Similar to this, adding lysozyme to the diet seems to increase the relative carcass weights while decreasing the weight of the liver, pancreas, and heart. In line with our findings, Sarica et al. (2005) found no differences in the liver and pancreatic weight of broiler chicks fed antibiotic-added diets.

**HI titer:**

The stress of infection and diarrhoea, which may alter the acid-base balance, may be to blame for the decrease in HI titer in the E. coli-infected groups (Sil et al., 2002). Clinically, clear Immunosuppression in birds is frequently indicated by E. coli infection (Mc Gruder and Moore, 1998). E. coli infection compromised the chickens' immune systems and reduced the number of lymphocytes in their thymus and bursa (Nakamura et al., 1990). Both innate and adaptive host immune responses are influenced by immune-regulating peptides known as cytokines (Lee et al., 2015). Sadeyen et al. (2014) reported that compared to control birds, birds exposed to the E. coli-O78:H9 strain exhibited increased levels of IL-1β, IFNγ, and IL-10. This could be as a result of phagocytic cells in the innate immune system being stimulated by an E. Coli infection, which results in the release of IL-10. Lysozyme-fed groups had higher antibody titers against NDV vaccination, which may indicate that the bird's immune system and antioxidant defences had improved. These results back up those by Fritz et al. (2009). Regarding the antibiotic, Perez (2014) corroborated this conclusion by pointing out that fosfomycin had an immunomodulatory effect and encouraged phagocytosis.

**CONCLUSIONS**

The results of this study showed that the experimental infection of chicks with E. coli caused notable alterations in the gut's shape, as well as in antioxidant, metabolic, immunological, and immune markers. It's interesting to note that giving non-infected bird group 100 mg/kg of lysozyme increased immune response, enhanced bodily function, and increased poultry production efficiency. Based on the information provided above, lysozyme (100 mg/kg) may be a promising supplement to prevent E. coli infection by improving growth performance and returning the aforementioned parameters to values that are near normal.

**REFERENCES**


Hassan et al.


تأثير الليزوزيم الغذائي على أداء النمو ومكونات الدم وشكل الأمعاء ومقاومة بكتيريا الإشريكية القولونية في كتاكيت اللحم

رضي حسن1، يحيى زكريا عيد2، زينب محمد فاروق1، محمود الجبالي1 و محمد عبد العظيم محمد موسى1

1معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، الدقي، الجيزة، مصر.
2قسم إنتاج الدواجن، كلية الزراعة، جامعة كفر الشيخ، مصر

عنوان البريد الإلكتروني (redaalihasan@yahoo.com)

أعراض الإشريكية القولونية أحد الأسباب الرئيسية لتفاقم نقص حساسية كبيرة، وانتشار الأشريكية القولونية في النظام الغذائي للوقاية من داء الإصابة القولونية لدى الطيور ليست مفهومة جيدا. لذلك كان الغرض من الدراسة الحالية هو تأثير الليزوزيم كأيكلة مكملات غذائية في كتاكيت اللحم المصاب بالإشريكية القولونية. تم إنشاء ثماني مجموعات مكونة من 600 قرش روس غير منجس عمر يوم واحد: المجموعة الأولى (المجموعة الأولى) لم تتعرض للإصابات، كما لم تفحص المجموعة الثانية والثالثة بـ 500 ملم و 100 ملم من الليزوزيم على التوالي، المجموعة الرابعة كانت غير مصابة وعولجت بـ 10 ملم من الأفيلامايسين، المجموعة الخامسة أصيبت بـ 100 ملم من الأفيلامايسين، بينما كانت المجموعة السادسة والسابعة أصيبت وعولجت بـ 50 ملم و 100 ملم من الليزوزيم على التوالي، أما المجموعة الثامنة فقد أصيبت وعولجت بجرعة 10 ملم من الأفيلامايسين/كجم علف.

تعرضت المجموعة المصابة بالإشريكية القولونية للإصابة والعلاج، بينما كانت المجموعات الأخرى (المجموعة المنفحة) لم تكن مصابة ولم تكن ملحة. حيث انتشرت الإصابة في جميع المجموعات، ولكن المجموعة المصابة والإحالة كانت أقل في الحال. استمرت المجموعة المصابة بالإشريكية القولونية في تعزيز الوزن النسبية للثدي والبدة، بالإضافة إلى مراقبة النتائج في حالات التحريك، ونتائج الكشف على ملازمة تكون معينة في مثل هذه الحالات. هذه النتائج تشير إلى أن استخدام الليزوزيم (بجرعة 50 و 100 ملم/كجم) يحسن الأداء وتقلل من الأعراض المرضية التي تسببها عدوى الإشريكية القولونية.