Effect of different infective doses of *Ascaridia galli* eggs on the total serum protein and weight gain in white lohman laying hens

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Abstract

The present study was conducted to investigate the effect of different infective doses of *Ascaridia galli* eggs on the total serum protein, weight gain and shedding of eggs per gram (EPG) in laying hens by using 75 layer birds, eight weeks old which divided randomly into 3 groups, each group included 25 chickens. 1st and 2nd groups were infected orally with 1500 and 250 viable *A. galli* eggs/ bird respectively. The 3rd group was given 0.5 ml physiological saline orally (control negative).

The results of the total serum protein concentration decreased significantly in both infected (1st and 2nd groups) as compared with the control non infected group and was decreased significantly in the first infected group than the second infected group.

Control group showed a higher (p<0.05) of weights than the first and second infected groups, while the first group recorded a significant decrease (p<0.05) than the second group. The number of eggs count (EPG) in feces of infected birds was higher in the first than in the second group.


تأثير الإصابة بجرع مختلفه بببوض طفيلي معدل *Ascaridia galli* على تركيز البروتين الكليو معدل

الوزان في الدجاج اللوهمان الإبيض

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Introduction

Ascaridia galli is an intestinal helminth parasite belonging to the group of ascarid worms (Phylum Nematoda; Class Secernentia; Order Ascaridida; Family Ascaridiidae). Members of the genus Ascaridia are essentially parasitic in birds, and most prevalent in fowl, particularly in chicken and turkey, geese, guinea fowl and a number of wild birds; the principal host manifestly being the chicken. (1) Studies have suggested that A. galli is the most common nematode in all types of production systems and has worldwide distribution. (2). Infections with Ascaridia spp. may cause, in addition to direct losses, reductions in growth rate, weight loss, reduced feed conversion rates and damage to the intestinal mucosa, leading to blood loss and secondary infections (3). partial or complete obstruction of the intestine, and increased mortality due to secondary bacterial infections.(4). The primary damage reduced efficiency of feed utilization, reduced egg production and weight loss are common symptoms in broiler chickens; Young birds are most susceptible, and heavier breeds seem more resistant than the lighter breeds such as leghorns and white minorecas (5).
third stage larvae. After ingestion, eggs reach the duodenum and hatch within 24 hours (6), and larvae are released into the lumen of the intestine. The larvae then enter the mucosa of the small intestine and most larvae initiate a histotrophic phase between day 8 to 17 post infection (p.i.) (7). *A. galli* may also play a role in transmission of *Salmonella* infections (8) and Avian Reo viruses (9) resulting in disease and economic loses. This study aimed to study effect of *A. galli* infection on in layer hens using different doses on the total serum protein and weight gain.

**Materials and Methods**

**1- Preparation of *Ascaridia galli* eggs:**

Adult *Ascaridia galli* female worms were collected from naturally infected chickens according to standard parasitological techniques accordig to (10,3). The uteri of graved female worms were dissected and the eggs were recovered. The eggs embryonated by incubation in 0.1% (w/v) potassium-dichromate solution at room temperature for 14 days(11). Larvae viability was assessed by observing the larvae spontaneous movement inside the egg after increasing the surrounding temperature. Infective *A. galli* L3 eggs were stored at +4 °C until use. On the day of infection another viability test was performed. Only larvae that were well developed, motile and not hatched were accounted as viable and infective. Eggs in the culture were suspended with tap water to get a final volume of 0.5 ml containing the infection dose to be given to each bird. Number of eggs/ml suspension was determined using a McMaster egg counting chamber. The chickens inoculated via oral gavage at 8 weeks old with an infection doses of 1500 and 250 embryonated eggs per bird.

**2- Experimental Design:-**

Seventy five chickens (8 weeks old ) white lohman laying hens were purchased from local layer field and kept in a room 25 m² of an experimental animal house - Veterinary Medicine College - Baghdad University ,which had free access to water, and the diet was good grounded seeds with supplements vitamins and amino acids with anticoccidial drugs . (12). Chickens were divided randomly into three groups (25 chicks each) ; The hens were orally infected using a plastic Pasteur pipette as described by (10); The 1st and 2nd groups
were inoculated with 1500 and 250 infected egg / bird respectively ,3rd group were inoculated with 0.1 ml of phosphate buffer saline( PBS) orally as negative control group .

3-Estimation of total serum protein concentration:

After collection of blood from the hans through a wing vein, blood was transferred into gel tube (without anticoagulant) and allowed to stand in refrigerator for 15 minutes and centrifuged at speed of up to 3500 RPM for 5 minutes. Serum was aspirated by a pipette and transferred into small clean tubes kept frozen until use. Colorimetrically total serum protein mg/dl was estimated by the Biuret method as discribed by (13) using SPAN diagnostic kit (Code N0.25931).

4- Body weight:-

Five chicks were randomly weighed of each group weekly for 7 intervals time (14).

5- Egg counts - Eggs per Gram of feces (EPG):

The eggs were counted using a modified McMaster method (15).

6-Statistical analysis:-

Statistical analysis of means were performed by using statistical package for social science (SPSS,2008) , Version 16, and for determination of a significant differences by using two way analysis ANOVA (16).

Results and discussion

1-Total protein mg /ml:

Table(1) shows a significant decrease (P<0.05) in total protien between the 1st and 2nd groups that infected with 1500 and 250 viable eggs at the 3rd and 4th weeks respectively also there is a significant decrease (P<0.05) within group in the 1st and 2nd groups at the 2nd , 3rd and 4th week. In the second month between groups; A significant decrease (P<0.05) was found between the 1st and control groups at 6th ,7th and 8th weeks , but only a significant decrease (P<0.05) was recorded between the second and control groups at 7th week . (first and second groups) showed no significant differences (P>0.05)in compare with control birds . (Table 2).
Table 1: Effect of *A.galli* infection on the Total proteins mg/ml in laying chickens in the First month:

<table>
<thead>
<tr>
<th>Weeks</th>
<th>First month</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>5.30 ± 0.31</td>
<td>3.80 ± 0.18</td>
<td>2.64 ± 0.18</td>
<td>2.38 ± 0.34</td>
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<tr>
<td></td>
<td>A a</td>
<td>B b</td>
<td>C b</td>
<td>B b</td>
<td></td>
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<tr>
<td>Group 2</td>
<td>5.5 ± 0.39</td>
<td>4.11 ± 0.18</td>
<td>3.63 ± 0.37</td>
<td>3.62 ± 0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A a</td>
<td>AB b</td>
<td>B b</td>
<td>B b</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>5.36 ± 0.42</td>
<td>4.66 ± 0.27</td>
<td>5.16 ± 0.41</td>
<td>4.77 ± 0.37</td>
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<tr>
<td></td>
<td>A a</td>
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</tbody>
</table>

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 viable egg / bird) G2= infected with (250 viable egg /bird) G3= Control negative.

Table 2: Effect of *A.galli* infection in Total proteins mg/ml in laying hens in the second month:

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Second month</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>2.88 ± 0.4</td>
<td>2.09 ± 0.13</td>
<td>2.3 ± 0.26</td>
<td>1.99 ± 0.27</td>
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<tr>
<td></td>
<td>A a</td>
<td>B a</td>
<td>C a</td>
<td>B a</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>2.68 ± 0.54</td>
<td>2.47 ± 0.33</td>
<td>3.77 ± 0.32</td>
<td>3.05 ± 0.36</td>
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<tr>
<td></td>
<td>A a</td>
<td>AB a</td>
<td>B a</td>
<td>AB a</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>4.94 ± 0.22</td>
<td>4.96 ± 0.51</td>
<td>5.51 ± 0.33</td>
<td>4.45 ± 0.42</td>
<td></td>
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<tr>
<td></td>
<td>A a</td>
<td>A a</td>
<td>A a</td>
<td>A a</td>
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</tbody>
</table>

Different capital letters show a significant difference (P<0.05) between groups. Different small letters show a significant difference (P<0.05) within group. SE= Standard error. N= 5 animals each group. G1= infected with (1500 viable egg / bird) G2= infected with (250 viable egg /bird) G3= Control negative.
Biochemical study showed that significantly decreased in total serum protein in both infected groups with *Ascardia galli* as compared with control group. Our finding was in agreed with that of [17]. Coles [18] reported that a considerable loss of digestive secretion and mucous due to intestinal parasitism in anaemic birds, which also cause inefficient protein absorption and utilization in the system to extent of leading to marked decrease in serum protein. The lowered of total protein values may result from a great loss of tissue protein occurring through leakage into gut with loss of digestive secretion and mucous due to intestinal parasitism in anaemic birds, which also caused inefficient protein absorption and utilization process [19]. Similar phenomena may occur in chicken following *A. galli* infection in this study.

2. Body weight:

In the first month; there was a significant decrease (P<0.05) between the 1\(^{st}\) and the control groups at 3\(^{rd}\) and 4\(^{th}\) week, while no significant difference (p>0.05) was found between the 1\(^{st}\) and 2\(^{nd}\) group and between the 2\(^{nd}\) and control group.

In the second month there was a significant decrease (P<0.05) between the 1\(^{st}\) group and 2\(^{nd}\) group compared with the 3\(^{rd}\) group.

**Table 3: Effect of *A. galli* infection on body gains (gm/bird) in laying hens the first month**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Groups</th>
<th>First month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Group 1</td>
<td>710±36.7</td>
<td>A</td>
</tr>
<tr>
<td>Group 2</td>
<td>720±29.1</td>
<td>A</td>
</tr>
<tr>
<td>Group 3</td>
<td>690±25.4</td>
<td>A</td>
</tr>
</tbody>
</table>

Different capital letters show a significant difference (P<0.05) between groups. SE = Standard error. N = 5 animals each.
Table 4: Effect of *A. galli* infection on body gains (gm/bird) in laying hens in the second month:

<table>
<thead>
<tr>
<th>Weeks</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td>secondmonth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>1040±18.7 C</td>
<td>1130±19.7 C</td>
<td>1244 ± 16.91 C</td>
<td>1272±21.3 C</td>
</tr>
<tr>
<td>Group 2</td>
<td>1120±37.4 B</td>
<td>1270±29.8 B</td>
<td>1352±177.2 B</td>
<td>1412±14.2 B</td>
</tr>
<tr>
<td>Group 3</td>
<td>1340±50.9 A</td>
<td>1408±46.3 A</td>
<td>1709±24.41 A</td>
<td>1922±52.2 A</td>
</tr>
</tbody>
</table>

Different capital letters show a significant difference (P<0.05) between groups. SE= Standard error. N= 5 animals each group. G1= infected with (1500 viable egg / bird) G2= infected with (250 viable egg /bird) G3= Control negative.

The development of immunity to parasitic infections is a costly process and requires high metabolic inputs (20). Marcos-Atxutegi (21) have shown that infected chickens produce increasing amounts of specific IgG antibodies against both embryonated *A. galli* egg antigens and adult somatic antigens from the first week of infection to day 42. In this period, the activity of histotropic stages of the parasite and host immunity are developing, the histotropic phase is a normal part of the life cycle of *A. galli* and it lasts approximately 7–50 days, depending on a number of parasite and host-oriented factors (7). Also, the pathogenicity of *A. galli* is considered to be stronger during histotropic, larval development, resulting in inflammation and injury to the intestinal wall and to the host’s absorption of metabolic waste (22). Correspondingly, the allocation of nutrients may primarily be changed from growth to acquisition of immunity when growing animal first encounters parasitic (that may be assumed that the lower body weight development of infected compared to uninfected birds (23). *A. galli* infection may also influence digestion and absorption of nutrients by reducing proteolytic enzyme activity in the jejunum (24) being associated with an increased recycling of nitrogen (25). Reduced metabolizability of energy
and lowered nitrogen retention have been reported for *A. galli* infected chickens (26). Recent data suggest that *A. galli* infection impairs electrogenic transport of alanin and glucose in the intestinal epithelial tissues of growing chicks (27).

3. Shedding of eggs:

The means of egg per gram in the first group (received 1500 viable eggs) was higher than the second group (infected with 250 viable eggs) with a significant increase (P<0.05) at the 5th week till the end of the experiment. (Table 5)

Table 5: Number of egg per gram (EPG) in litter of infected groups with *A. galli* infection in laying hens

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>Weeks 5</td>
<td>Weeks 6</td>
</tr>
<tr>
<td>Group 1</td>
<td>6460 ± 441.13</td>
<td>6800 ± 187.08</td>
</tr>
<tr>
<td>Group 2</td>
<td>3640 ± 380.26</td>
<td>4150 ± 374.16</td>
</tr>
</tbody>
</table>

N= 5 animals each group. G1= infected with (1500 viable egg / bird) G2= infected with (250 viable egg /bird).

The number of EPG may be influenced by the number of adult worms in the gastrointestinal tract, worm age, host immunity, host age, host sex, stages of infection, fecundity, feed composition and consistency of the faeces and time of day of collecting the faeces, even though the EPG cannot be used directly to estimate the worm burden in either experimental infections or in natural infections, the described method proved adequate for our experiments, as the sensitivity was high making it possible for us to determine when the worms became mature (28).
References:


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