**Differential Leucocyte Count and Total Colony Count Changes in Heat Stressed Broiler**

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**Abstract**

The present study aimed to explain the role of additives in alleviation of the negative effect of heat stress on differential leucocytes count, heterophil/lymphocytic ratio with especial studies on the microbial count of duodenum, jejunum, ileum and caecum. A total of 270 day-old (Ross308) broiler chickens randomly divided into 6 groups, which were kept under elevated temperature (34-36°C) and feed diet containing 1% Nigella Sativa (G2) or a mixture of sodium bicarbonate and potassium chloride (0.3% KCL + 0.5% NaHCO3) (G3) or 2% coriander seed (G4) or 0.03% Acetylsalicylic acid (aspirin) (G5) or 250 mg of Ascorbic acid (Vitamin C) (G6) for 6 weeks. The previous parameters were recorded after slaughtering to take microbial sample and collecting blood parameters. The results explained that, there was a significant increase lymphocyte percentage in case of G2, G3, G4, G5 and G6, while, there was significant decreases in Heterophil percentage, heterophil/lymphocyte ratio (H/L), eosinophils percentage, monocytes percentage, jejunum total colony count and caecum total colony in case of (G2, G3, G4, G5), (G6, G2, G3, G4, G5), (G6, G3), (G5; G2), (G3; G2) and (G2, G4) respectively in compared with control group (P<0.05). It could be concluded that black seed and coriander seed have a positive effect on heat stress broiler diet.

**Keywords:** Antioxidants, Acetylsalicylic acid, Vitamin C, Heat stressed broilers, Black seed

**Introduction**

The domestic fowl is a homoeothermic, which can live comfortably only in a relatively narrow zone of thermo-neutrality extending from 14.5°C to 25.5°C (Freeman, 1969). Heat stress may occur when ambient temperature exceeds 32°C (Ahmed and Maghbaby, 1995). So that, some physiological changes occur in broiler in Egypt during the summer season (Toyomizu *et al*., 2005)

Such as acute heat stress leads to decreased monocytes and lymphocytes proportions (Altan *et al*., 2000a,b). While, birds exposed to chronic heat stress (4 weeks) had a lower white blood cells count and higher heterophil/lymphocyte ratio (H/L) (Mashaly *et al*., 2004), a 70% decrease in the number of lymphocytes, increase about 2.5, 15, 20 and 44 % in eosinophils, monocytes, basophiles, and heterophiles respectively (Grey *et al*., 1989), reduced the number of heterophil, increased the numbers of basophiles and monocytes in birds (Maxwell, 1993) and increased heterophil percent and reduced basophiles percent (Osman, 1996; Vijayan and Rema, 1997).

Heterophil/lymphocyte ratio is a sensitive indicator of heat stress in fowls, which increased significantly at the first and last days of heat stress compared to control group (Altan *et al*., 2000a; 2008, Nadia 2003; Gharib *et al*., 2005). This in-
creasing measures a physiological change in organs such as a trophy of the bursa of Fabricius and thymus that is influenced by the effect of heterophils. (Aengwanich and Chinrasri, 2003).

On the other hand, the bad effect of the widely used antibiotics in broilers' diet that have a bad effect on broiler meat or production. So we try to use the previous feed additives to get rid of some bad effect of heat stress on broiler hematology with reference to their effect as an antimicrobial. One of the best methods used to control heat stress is the chemical management of acid-base balance by supplementing feed or water with different electrolyte salts as sodium bicarbonate (NaHCO₃), potassium chloride (KCl), which led to a higher carcass weight and dressing percentage than the control. (Ahmad et al., 2005). Dietary sodium bicarbonate at 0.25%, 0.50% and potassium chloride at 0.2%, 0.3% supplementation alone and combination between them give good and positive effects on body weight, feed conversion of golden motazah hens (Hassan et al., 2011) increased feed consumption values of laying hen and gave the best values of feed conversion (EL-Sheikh and Salama, 2010), improved significantly the average values of body weight, weight gain, feed intake and feed conversion (Ghazalah et al., 1998).

Significant increase in broiler body weight was obtained in groups that fed diet contain 0.2% of acetylsalicylic acid (Al-Obaidia and AL-Shadeedi, 2010), which improved the performance and physiological traits. (Abdel-Fattah, 2006) and improved feed conversion ratio (Hassan et al., 2003).

White Leghorn layers fed diets supplemented with vitamin C/kg diet during the hot summer season recorded a significantly higher body weight, increased feed intake and live weight gain than the control (Konka et al., 2009; Ajakaiye et al., 2010), beneficial effects on the performance and economics of production of broiler chicks exposed to heat stress. (Onu, 2009), improved the weight gain of birds (Sobayo et al., 2008), a slight increase in feed intake of the birds (Oruseibo and Alu, 2006) and a positive significant effect on final body weight and feed conversion (Metwally, 2005).

Finally, our result of previous work of adding 1% black seed or 2% coriander seed in diet of heat stress broiler for 6 weeks led to significant increases in feeding behavior, feed intake, average final body weights, average body weight gain and dressing percentage, and significant decreases in panting behavior, water to feed ratio, T₃ and corticosterone. Also, no significant difference was indicated in drinking behavior and T₄. In addition, there was numerically, but not significant decrease in feed conversion ratio and numerically, but a non-significant increase in slaughter and carcass weight. (Ramadan et al., 2014). The present study was undertaken to explain the role of these additives in alleviation the negative effect of heat stress on te differential leucocytes count, heterophil / lymphocytic ratio with especial reference to the microbial count in the duodenum, jejunum, ileum and caecum.

**Materials and methods**

**Groups classification**

A total number of 270, one unsexed day old "Ross 308" broiler chicks reared in a laboratory animal house in the Veterinary Teaching Hospital at Faculty of Veterinary Medicine, Assiut University, Egypt. The chicks were divided into four groups each one consisted of 15 birds/ replicate (Table 1).

<table>
<thead>
<tr>
<th>Group name</th>
<th>Numbers</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (G₁)</td>
<td>45 birds (3 replicate each one 15 birds)</td>
<td>Basal diet only</td>
</tr>
<tr>
<td>Nigella sativa (black seed) (G₂)</td>
<td>45 birds</td>
<td>Basal diet + 1% Nigella sativa</td>
</tr>
<tr>
<td>Sodium bicarbonate and Potassium chloride (KCl-NaHCO₃) (G₃)</td>
<td>45 birds</td>
<td>Basal diet - 0.3% potassium chloride - 0.5% Sodium bicarbonates</td>
</tr>
<tr>
<td>Coriander seed (G₄)</td>
<td>45 birds</td>
<td>Basal diet - 2% coriander seed</td>
</tr>
<tr>
<td>Acetylsalicylic acid (aspirin) (G₅)</td>
<td>45 birds</td>
<td>Basal diet - 0.03% Acetylsalicylic acid</td>
</tr>
<tr>
<td>Ascorbic acid (Vit. C) (G₆)</td>
<td>45 birds</td>
<td>Basal diet - 250mg of Vit. C</td>
</tr>
</tbody>
</table>

Table 1. Show the classification of different groups included in the experiment
Management and cleaning

Day to day management carried out in keeping the facility clean. After daily cleaning, cleaned feeders and drinkers were dried and filled with food and water. Also, the contaminated wastes and dead birds were hygienically disposed by incineration.

Lighting

Continuous lighting program (23 hours lighting: 1 hour darkness) used. 60 watt bulb suspended 2.20 meters at head height of the birds.

Temperature and relative humidity

In the first 14 days of age, temperature and relative humidity of the room maintained according to the recommendations of broiler breeder company (Aviagen, 2002). Then the heat stress experiment (34°C) started from 14: 42 days old. The heat provided by using an electrical heater. Ventilation was done by electrical ventilator to remove any excess of ammonia gases or heat.

Ambient temperature determined by maximum and minimum thermometer and indoor relative humidity measured by using wall mount wet and dry bulb hygrometer. The readings were taken every day and the average of weekly readings was calculated (Fig. 1).

Diet and feeding

The basal diets formulated using NRC (1994) guidelines. It contained 21 proteins (starter type from 0 – 14 days) and 23% (grower type from 15 – 42 days) with 3200 kcal/kg.

The birds fed on antibiotic-free mash diet (containing thoroughly mixing treatment substance) ad libitum. Bird was given free access to fresh and clean water throughout the experimental period. The feed was offered daily and residual feed was measured weekly.

The composition and metabolizable energy value of the diet (physical and chemical) were mentioned in Tables 2 and 3.

Feed additives sources and the percentage used

The feed additives such as black seed and coriander seed were purchased from commercial market while the vitamin C, aspirin, potassium chloride and sodium bicarbonate were purchased from the medical company, the percentage of the feed additives were used according to previous studies. These additives were mixed thoroughly with the diet.

![Fig. 1. Average temperature and average relative humidity during the experiment period](image)
Bird identifications were carried by wing band.

Medication and vaccination

Medication was given to the experimined chicks according to the design in Table 4, while the vaccination program was done according to the design in Table 5.

Work items or measurements

Differential Leucocyte Count

At 42 days old, three blood smears from each treatment group, prepared immediately after slaughter and stained using Giemsa stain. A total of 100 white cells were counted by using an oil immersion lens of electrical microscope. Heterophil / Lymphocyte (H/L) ratio: H/L ratio was calculated using the following equation:

\[ \text{H/L ratio} = \frac{\text{number of heterophil cells}}{\text{number of lymphocyte cell}}. \]

Microbial counts

Intestinal contents from the duodenum, jejunum, ileum and caecum were evacuated and mixed immediately after slaughter, and then was stored separately in previously weighed screw-capped sterile plastic cups. The sealed containers were kept on ice until they transported to the laboratory for enumeration of microbial populations. One milliliter (1 ml) sample of each digesta was transferred into 9 ml of sterile normal saline and further dilutions were made. Aliquots (0.1 ml) of the appropriate dilutions of the serially diluted samples were then inoculated onto nutrient agar plates in duplicates.

The spread plate method using a sterile bent glass rod was used. Inoculated plates were incubated at 37°C for 24 hours, discrete colonies that developed on each plate were counted, and their

<table>
<thead>
<tr>
<th>Nutrient (%)</th>
<th>Starter diet</th>
<th>Grower diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>88.8</td>
<td>89.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>23.1</td>
<td>21.1</td>
</tr>
<tr>
<td>Crude fat</td>
<td>5.89</td>
<td>7.71</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>6.07</td>
<td>5.73</td>
</tr>
<tr>
<td>Crude ash</td>
<td>2.80</td>
<td>2.62</td>
</tr>
<tr>
<td>NFE</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.5</td>
<td>1.24</td>
</tr>
<tr>
<td>Lysine 2</td>
<td>1.39</td>
<td>.45</td>
</tr>
<tr>
<td>Ca</td>
<td>0.98</td>
<td>0.92</td>
</tr>
<tr>
<td>P</td>
<td>0.84</td>
<td>0.68</td>
</tr>
<tr>
<td>ME (MJ kg DM)</td>
<td>12.3</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Birds' identifications

Bird identifications were carried by wing band.

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Age</th>
<th>Dose of drug</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enroflox 10%</td>
<td>1 to 3 days of age</td>
<td>0.5 ml liter</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Roycl colistin</td>
<td>1 to 3 days of age</td>
<td>1 g liter</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Vitamins AD3E</td>
<td>3 days / week</td>
<td>1 ml liter</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Liquid minerals</td>
<td>3 days / week</td>
<td>1 ml liter</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Neo terramycin</td>
<td>4 to 10 days of age</td>
<td>3 g liter</td>
<td>Drinking water</td>
</tr>
</tbody>
</table>
averages from two plates were calculated. Numbers of colony-forming units (cfu) expressed as log colony-forming units per gram of digesta content and expressed as cfu/mL (APHA, 2005).

**Results**

**Differential leucocyte count**

Data illustrated in Table (6) showed the effect of Nigella Sativa (G2) or mix of sodium bicarbonate and potassium chloride (G3) or coriander seed (G4) or acetylsalicylic acid (G5) or ascorbic acid (G6) on differential leucocyte count and H/L ratio, the analysis of variance revealed that there were significant increases in lymphocyte percentages in cases of G2, G3, G4, G5 and G6 compared with the control group. While, there were significant decreases in Heterophil percentage, H/L ratio, eosinophiles and monocytes percentages in cases of (G2, G3, G4, G5 and G6), (G2, G3, G4, G5 and G6), (G3 and G5) and (G2 and G3) respectively. Moreover, there were numerical, but not significance decreases in eosinophiles, monocytes and basophils percentages in (G2 and G6), (G6) and (G2, G3, G4, G5 and G6) respectively as well as, a numerical but not significant increases in cases of the eosinophiles and monocytes percentages in G4 and (G4 and G5) respectively compared with the control group.

**Microbial total colony count**

Results shown in Table (7) revealed that the effect of black seed, sodium bicarbonate plus potassium chloride, coriander seed, acetylsalicylic acid and vitamin C on the total colony count. Where, the mean values of duodenum total colony count of treatment substances were 8.68, 9.31, 9.06, 9.93 and 8.58 respectively, while that of control group was 9.12 CFU x 10^3 / ml. Moreover, the mean values of ileum total colony count of treatment substances were 8.37, 9.12, 8.90, 9.00 and 10.21 CFU x 10^3 / ml, respectively, while it was 10.40 CFU x 10^3 / ml for the control group. At the same time, the mean values of the jejunum total colony count of treatment substances were 6.57, 9.27, 9.11, 9.27 and 9.51 CFU x 10^3 / ml, respectively, while that for the control group was 10.7 CFU x 10^3 / ml. Finally the mean values of caecal total colony count of treatment substances were 6.66, 9.86, 7.83, 9.41 and 10.85 CFU x 10^3 / ml, respectively, while that for the control group was 10.52 CFU x 10^3 / ml.

Biostatistics of the previously mentioned data showed that, there was no significant difference in the duodenum and ileum total colony count of treatment groups and control one, however, there was a significant decrease in jejunum total colony count among black seed treatment and control group. While there was a significant decrease in

<table>
<thead>
<tr>
<th>Age of bird</th>
<th>Name of vaccine</th>
<th>Route of vaccination</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>6th days old</td>
<td>Hitterer B1</td>
<td>Drinking water</td>
<td>Newcastle disease</td>
</tr>
<tr>
<td>17th days old</td>
<td>Lasota strain</td>
<td>Drinking water</td>
<td>Newcastle disease</td>
</tr>
<tr>
<td>29th days old</td>
<td>Lasota strain</td>
<td>Drinking water</td>
<td>Newcastle disease</td>
</tr>
<tr>
<td>12th days old</td>
<td>Gumboro</td>
<td>Drinking water</td>
<td>Gumboro disease</td>
</tr>
<tr>
<td>23rd days old</td>
<td>Gumboro</td>
<td>Drinking water</td>
<td>Gumboro disease</td>
</tr>
</tbody>
</table>

Table 6. Effect of black seed, sodium bicarbonate plus potassium chloride, Coriander seed, acetylsalicylic acid and vitamin C on Differential Leucocytes Count (%) and H/L ratio.

Table 6

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heterophil</th>
<th>Lymphocyte</th>
<th>Monocytes</th>
<th>Eosinophiles</th>
<th>Basophils</th>
<th>H/L Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.33±0.88^a</td>
<td>34.33±1.52^a</td>
<td>10.00±1.15^a</td>
<td>4.33±0.88^a</td>
<td>0.00±0.00</td>
<td>1.37±0.11^a</td>
</tr>
<tr>
<td>Black seed</td>
<td>20.33±0.67^c</td>
<td>70.67±9.02^c</td>
<td>5.33±0.88^c</td>
<td>3.33±1.20^c</td>
<td>0.33±0.33</td>
<td>0.37±0.05^c</td>
</tr>
<tr>
<td>Sodium bicarbonate &amp; potassium chloride</td>
<td>12.67±1.45^d</td>
<td>45.00±5.0^a</td>
<td>2.00±1.15^c</td>
<td>1.00±0.58</td>
<td>0.67±0.33</td>
<td>0.17±0.03^d</td>
</tr>
<tr>
<td>Coriander seed</td>
<td>22.00±2.89^e</td>
<td>62.33±2.52^e</td>
<td>10.33±1.14^e</td>
<td>4.67±0.88^e</td>
<td>0.67±0.33</td>
<td>0.37±0.05^e</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>40.00±2.08^f</td>
<td>49.33±5.51</td>
<td>10.33±0.88^f</td>
<td>0.67±0.33</td>
<td>0.33±0.33</td>
<td>0.68±0.07^f</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>35.00±2.88^g</td>
<td>34.00±5.00^h</td>
<td>7.67±1.52^g</td>
<td>2.67±1.45^h</td>
<td>0.67±0.33</td>
<td>0.64±0.04^g</td>
</tr>
</tbody>
</table>
The effect of heat stress on increasing heterophil percentage, H/L ratio, eosinophiles percentage, monocytes percentage and basophils percentage was previously studied by Grey et al. (1989); Maxwell (1993); Vijayan and Rema (1997); Altan et al. (2000a); Nadia (2003); Gharib et al. (2005); Gharib et al. (2008). Moreover, the effect of heat stress on decreasing lymphocyte percentage was studied by Altan et al. (2000 a,b). This result was disagreed with the present data which explained the importance to use the different treatments in alleviation of negative effect of heat stress on differential leukocyte count.

The decreasing heterophils percentages may be related to the ability of the treatment substances to relieve the effect of heat stress, which decreases the corticosterone secretion by sequence increase the immunity through increasing the lymphocyte numbers (Satterlee et al., 1989). While, increasing of lymphocyte percentage may be due to the immune stimulant of that substance and increasing the effect on lymphoid organs weight, especially with black seed and coriander seed. Moreover, it may be due to the role of vitamin C in the synthesis of leukocytes (Null, 2001). Meanwhile, decreasing the effect of acetylsalicylic acid on of H/L was agreed with previous data of Abou-El-Soud et al. (2006). This result may be due to the depressing effect on corticosterone concentration, which cause increased H/L ratio (Aengwanich and Chirnrasri 2003). Moreover, the effect of Vitamin C to decrease H/L was agreed with the study of Satterlee et al. (1989); Zulkifli et al. (2000). This result may be related to the ability of the treatment substances in decreasing heterophils percentage and increasing lymphocytes percentage as mention before.

The caecum is one of the areas of greatest microbial activities in the gastrointestinal tract of chickens, and thus, can be described as the location for a very complex microbial ecosystem. Relative to other parts of the gastrointestinal tract, the caecum provides a stable environment for microorganisms, resulting in a large microbial population due to the slower transit time. In addition to that, intestinal microflora plays an important role in the health status of host animals. In general, intestinal bacteria may be divided into species that exert either harmful (pathogenic) or beneficial effects on host health. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria in order to inhibit colonization of invading pathogens (Guo et al., 2004).

The applity of black seed to decrease the microbial count may be due to the anti microbial activity of the black seed, which were studied by Lee et al. (2003); Guo et al. (2004); Gilani et al. (2004); Abu-Dieyeh and Abu-Darwish (2008). As known Nigella sativa contains different components such as thymoquinone and thymohydroquinone, which shown to posses antimicrobial activities. Also, the antimicrobial activity of coriander seed was studied by Delaquis et al. (2002); Singh et al. (2002).

**Conclusion**

It could be concluded that black seed or coriander seed can be used to alleviate the negative effect of heat stress in broiler hematology during the summer season in Egypt. Also, act as an antimicrobial agent.
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ters in broilers during natural summer temperature. Asian Journal of Animal and Veterinary Advances 4, 139-147.


