EFFECT OF GARLIC (ALLIUM SATIVUM) SUPPLEMENTATION ON HAEMATOLOGICAL PARAMETERS IN CHICKEN (GALLUS DOMESTICUS)

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ABSTRACT
Garlic has been shown to have several effects in the body including its role in diabetes, blood coagulation, metabolism and immune functions. But there are scanty reports regarding its effect on haematological parameters in poultry. The study was conducted on 24 broiler chicks divided randomly into 3 groups, each group consisting of 8 birds. Group I birds were used as control kept on conventional diet. Group II and III birds were supplemented with garlic at the rate of 1.5% and 3.0% respectively (on dry matter basis) of total feed for a period of 8 weeks. Haematological parameters like hemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DLC) were studied. Values of Hb and TEC were found to be insignificantly lower in garlic treated groups till the end of the experiment. These effects may probably be due to the presence of some hemolytic bioactive constituents and/or their metabolites in garlic. Slight rise in lymphocyte and heterophil counts were observed in garlic supplemented groups which may be due to immuno-stimulatory effects of garlic. In conclusion, haematological parameters are not significantly affected by garlic supplementation in feed and slight variation in DLC may be due to the interaction of garlic with immune system and Hb and TEC variations may be due to hemolytic constituents of the garlic.

Key words: Broiler, Chicken, Garlic, Haematology.

INTRODUCTION
Garlic is one of the oldest cultivated plant (Moyers, 1996). The virtues of garlic (Allium sativum), as a medicinal plant, are known to most cultures of the world. Garlic has been shown to have several effects in the body. These include inhibition of platelet aggregation (Apitz-Castro et al., 1983), reduction in arterial blood pressure (Mc Mohan and Vargas, 1993) and prevention of fat infiltration of liver (Sand et al., 1995). Also invivo and invitro studies showed that aged garlic extracts stimulate immune functions (Sumiyoshi, 1997). Broiler industry is a source of nutrition, provides rural employment and fulfils all nutritional standards. The success of poultry as industry in Indian scenario is evident from the fact that with 10 Kg of similar feed, broiler type chicken gives 450 gm of protein while swine gives 160 gm, beef type cow gives 96 gm and sheep and goat around 225 gm of protein (Qureshi et al., 1983). Therefore, chicks and eggs are inexpensive sources of protein among all animal products.

Due to scanty information on the effect of garlic on haematological parameters in chicken, this study was undertaken to investigate the influence of garlic on certain haematological parameters in chicken.

MATERIAL AND METHODS
Investigations were carried out on the 24, day old, unsexed and healthy commercial broiler chicks, procured from a commercial breeding farm of Hisar. All the chicks belonged to the same batch and the same breeding stock. The chicks were reared under strict hygienic conditions. Before housing the chicks; rooms, brooder battery and cages were thoroughly cleaned with 2.5% phenol and subsequently fumigated with formaldehyde gas (35 ml of commercial formalin and 17.5 g potassium permanganate per hundred cubic feet area). Electric bulbs were used as source of heat and light.

The experimental chicks were reared for eight weeks on the prepared feed procured from Department of Animal Nutrition, CCS Haryana Agricultural University, Hisar. Garlic purchased from...
Table 1: Differential leucocyte count (%). Mean ± SEM) of broiler chickens of experimental groups at different time intervals following garlic powder supplementation in feed.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Leucocyte Count</th>
</tr>
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<tbody>
<tr>
<td>04</td>
<td>L</td>
<td>69.80±0.66a</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>21.60±1.03a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.80±0.80x</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.16±0.40x</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.20±0.20</td>
</tr>
<tr>
<td>06</td>
<td>L</td>
<td>70.60±0.92a</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>21.60±0.98a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.80±0.37x</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.60±0.40x</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>08</td>
<td>L</td>
<td>71.20±0.60a</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>21.56±1.01a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.64±0.06a</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.16±0.40a</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>OVERALL AVERAGE</td>
<td>L</td>
<td>70.00±0.12a</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>21.56±1.01a</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.64±0.06a</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.16±0.40a</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

Values bearing common superscripts within a row (a) and within a column (x) except overall average do not differ significantly at 5% level of significance.

Collection of Blood Sample for Haematological Parameters

Blood samples from all the three groups were collected on 4th, 6th and 8th week of age by cardiac puncture for haematological studies. Blood was collected in clean glass vials using dried heparin @ 15 IU/ml of blood as an anticoagulant. Haematological parameters were determined on the fresh blood.

Haemoglobin (Hb) concentration

Haemoglobin concentration was estimated by cyanomet–haemoglobin method as described by Schalm et al., (2000) using Digital Haemoglobinometer-185, Systronics, India Limited. The results are expressed as gm/dl.

Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC)

Total erythrocyte count and total leucocyte count were estimated by the method described by Natt and Herrick, (1952). The results of TEC are expressed in millions per μl (10⁶/μl), and those of TLC as thousands per μl (10³/μl).
Total erythrocyte and leucocyte counts were directly made from the same blood sample placed in haemocytometer by Natt and Herrick diluent solution using erythrocyte diluting pipette. The diluent used in the study consisted of the following: [NaCl-3.88g; Na₂SO₄-2.50g; Na₂HPO₄·12H₂O-2.91g; Formalin (37%)-7.50ml; Methyl violet (2B)-0.10g; Distilled water-1000ml].

Blood was drawn exactly to the 0.5 mark in RBC diluting pipette. Natt and Herrick diluting fluid was drawn into the pipette with the steady suction to 101 mark. This made 1:200 dilution. Then, the pipette was shaken well and discarded 3-4 drops from the stem of the pipette. Thereafter charged the improved neubar’s haemocytometer and counted RBC in high power (40X) in 5 of 25 small squares in the central area. For leucocytes, the cells were counted which appeared round light blue coloured, uniform objects in 4 large corner squares.

Differential Leucocyte Count (DLC)
Differential Leucocyte Count was estimated by counting one hundred cells (leucocytes) under oil immersion on a blood film prepared from fresh blood and fixed with methanol for 3 minutes. The smears were stained with modified Wright’s stain for poultry (Lucas and Jamroz, 1974).

Statistical Analysis
The data obtained was statistically analyzed using ANOVA followed by t-test for comparison of them among different group using Sigma stat software.

RESULTS AND DISCUSSION
Total erythrocyte count (TEC) and Haemoglobin (Hb)
The mean TEC in birds of different groups are presented graphically in Fig. 1. The mean haemoglobin (Hb) concentration in birds of different groups are depicted in Fig. 2. In the present study,
Fig. 2: Haemoglobin concentration (gm/dl, Mean ± S.E.) in broiler chickens of experimental groups at different time intervals following garlic supplementation in feed.

Fig. 3: Total leucocyte count (10^3/μl, Mean ± S.E.) in broiler chickens of experimental groups at different time intervals following garlic supplementation in feed.
dietary supplementation of garlic powder in groups i.e. II and III was found to cause an insignificant decrease in the mean values of total erythrocyte count (TEC) as compared to group II. There are reports in literature regarding the effect of garlic products administration on total erythrocyte count in many species as well as poultry. Ademola (2004) reported slight decrease in total erythrocyte count in garlic powder treated birds. Ohaeri (2001) reported an insignificant decrease in total erythrocyte count in rats after garlic supplementation. This might probably be due to the presence of hemolytic bioactive constituents in garlic or their respective metabolites (Ademola, 2004). There are reports which indicate significant increase in TEC due to garlic supplementation in feed in rats (Iranloye, 2002; Oluwole, 2001) which might be due to species variation.

However, there was insignificant increase in TEC with the advancement of age in control as well as garlic supplemented groups. It is evident from the present findings that TEC increases with the advancement of age. Dukes (1955) reported that total erythrocyte count was lowest at younger age and increases with the advancement of age in chicken. Kai and Pranklin (1984) detected that erythrocyte number is lower in early age and gradually increases with ages. Kundu et al. (1993) also reported a lowest RBC in day-old chicks and higher in 3 months old. Thus, it is suggested that slight decrease in TEC in the present study in garlic supplemented groups may be due to hemolytic action of garlic or its bioactive metabolites. And insignificant increase in TEC is in accordance with the previously reported work may be a function of age related factors.

In the present study, dietary supplementation of garlic powder in feed was found to cause insignificant decrease in the mean values of haemoglobin (Hb) as compared to control group. This may probably be due to the presence of hemolytic bioactive constituents in garlic or their respective metabolites (Ademola, 2004). There are reports in literature regarding the effect of garlic products administration on Hb value in many species as well as poultry. Ademola (2004) reported slight decrease in haemoglobin concentration in garlic powder treated birds. But there has also been seen significant rise in Hb concentration due to garlic supplementation in feed in rats (Iranloye, 2002) which might be due to species variation. It is evident from the present findings that hemoglobin concentration increases with the advancement of age. It is in accordance with the previous findings of Dukes and Schwarte (1931). Thus, as previously discussed (Ademola, 2004) hemolytic action of garlic may be responsible for insignificant decrease in Hb concentration in the garlic supplemented groups.

**Total leucocyte count (TLC) and differential leucocyte count (DLC)**

In the present study, dietary supplementation of garlic powder in broilers i.e. groups II and III showed slight increase in the mean values of total leucocyte count (TLC) as compared to control birds. And also slight rise in lymphocyte and heterophil count was observed in garlic supplemented groups. There are reports in literature regarding the effect of garlic products administration on total leucocyte count in many species as well as poultry. Ademola (2004) reported increase in total white blood cells and heterophils by about 18.7 per cent and 20.4 per cent respectively in garlic powder treated birds as compared to control birds. There was no significant difference in lymphocytes, monocytes and eosinophils in garlic treated birds as compared to control birds. While garlic supplemented rats showed a significant increase in total leucocyte count (Iranloye, 2002; Oluwole, 2001). Accordingly, the present study also revealed slightly increased lymphocytes and heterophils in the garlic fed groups in the broiler chickens. These data, therefore, support the earlier reports of Sumiyoshi (1997) that garlic extract stimulates immune functions. This observation may explain the role of garlic in activating the natural killer cells, the function of T-lymphocytes and the level of interleukin-2 (Tang et al., 1997). Conversely, garlic supplementation does not affect total and differential leucocyte counts in broiler chicks (Jafari et al., 2008).

**CONCLUSION**

The study was conducted on 24 broiler chicks divided randomly into 3 groups, each group consisting of 8 birds. Group I birds were used as
control kept on conventional diet. Group II and III birds were supplemented with garlic at the rate of 1.5% and 3.0% respectively (on dry matter basis) of total feed for a period of 8 weeks. Values of Hb and TEC were found to be insignificantly lower in garlic treated groups till the end of the experiment. These effects may probably be due to the presence of some hemolytic bioactive constituents and/or their metabolites in garlic. Slight rise in lymphocyte and heterophil counts were observed in garlic supplemented groups which may be due to immunostimulatory effects of garlic.

ACKNOWLEDGEMENTS
Authors are thankful to Mr. S. P. Sangwan for technical help rendered during research work.

REFERENCES