Effect of Supplementation of Garlic (*Allium sativum*) and Nilavembu (*Andrographis paniculata*) on Hematological and Serum Biochemical Parameters of Commercial Broiler Chicken

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Abstract

The effect of supplementation of garlic and nilavembu on haematological and biochemical parameters of commercial broiler chicken was studied in 300 commercial broiler for a period of six weeks. There were 5 treatment groups and each treatment had three replicates with 20 chicks. The treatment groups containing garlic and nilavembu at different levels, such as 0 g garlic + 0 g nilavembu (T₁ - control), 5 g garlic + 1 g nilavembu (T₂), 10 g garlic + 1 g nilavembu (T₃), 5 g garlic + 2 g nilavembu (T₄) and 10 g garlic + 2 g nilavembu (T₅) / kg of basal diet, respectively. WBC (x 10³) count revealed a highly significant (P<0.01) difference between treatment groups. Similarly, birds in T₅ group had significantly highest lymphocyte count and lowest cholesterol and triglyceride levels (P<0.01). Hence, it may be concluded that supplementation of garlic and nilavembu had positive effect on lowering cholesterol, triglyceride and enhance the immunity.

Keywords

*Allium sativum*, *Andrographis paniculata*, Haematological, Biochemical, Broiler

Introduction

Commercial broiler is one of the fastest growing birds; its growth and development are dynamic processes. Presently a need has arisen to completely avoid usage of antibiotics in poultry feeding due to increasing consumer concern for poultry drug residues in meat and egg. Hence, an alternative to antibiotics like probiotics, prebiotics, synbiotics and phytobiotics are being tried in poultry rations to improve growth, productivity and immunity against diseases. In this connection, phytobiotic such as, garlic (*Allium sativum*) and nilavembu (*Andrographis paniculata*) could be used as an alternative. It is expected to produce positive effects on production parameters and immune responses. Garlic is a spice proven as herbal medicine for the prevention and treatment of various diseases. It has anti-bacterial, anti-viral, anti-fungal, anti/protozoal, anti-cancer, anti-oxidant, immunomodulatory, anti-inflammatory, hypoglycemic and hypocholesteremic effect. Moreover, it improves body weight gain and digestibility. It decreases the bad cholesterol
and also augments meat quality parameters (Rehman and Munir, 2015). Similarly, nilavembu reported to possess anti-inflammatory, hepato-protective, astringent and anti-pyretic properties and helps in arresting dysentery, influenza and bronchitis (Salna et al., 2011).

The effect of garlic and nilavembu has been studied separately in a few researches. But the screening of garlic and nilavembu revealed the presence of some phytochemical compounds which are common to each other. Hence, the present experiment was conducted to study the combined effect of garlic and nilavembu on haematological and biochemical parameters of commercial broiler chicken.

Materials and Methods

The present study was performed in the Department of Poultry Science, Madras Veterinary College, TANUVAS (Chennai, India). Day old Cobb chicks (300) were randomly allotted to 3 replicate with 20 chicks each and these replicate were distributed randomly to 5 dietary treatments as shown in Table 1.

Experimental ration

The standard recommended commercial diet was prepared as per BIS 2007 broiler standard with similar nutrient composition for all the treatments. Fresh garlic were purchased from local market and pure nilavembu powder was obtained from the Government Siddha Medical College, Chennai. All the experimental feeds were prepared at the Central Feed Technology Unit, Kattupakkam, Tamil Nadu Veterinary and Animal Sciences University, as presented in Table 2. The experimental birds were fed ad libitum throughout the experimental period. The birds were fed with broiler pre-starter (0-7 days), starter (8-21 days) and finisher (22-42 days).

Parameters studied

Haematological and biochemical parameters

On the 42nd day of study, blood samples were randomly collected from one male and one female of each replicate, total of six birds per treatment. The blood samples were collected via the wing veins using sterile needles and syringes. The blood samples for haematological parameters were collected into well-labeled vacutainer containing ethylene diamine tetra acetic acid (EDTA), as anti-coagulant. The samples were investigated for the following haematological parameters – packed cell volume (PCV), red blood cell count (RBC), white blood cell (WBC), haemoglobin, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Differential leucocyte Count (Bancroft and Marilyn, 2008). Serum samples were analyzed for total protein, serum cholesterol and triglyceride. The total protein level in the serum samples were estimated in A15 Biosystem auto analyser by using commercial available AGAPPE kit based on Direct Biuret method (Gornall et al., 1949) and BCG method (Doumas et al., 1970). Cholesterol and Triglyceride in the serum were estimated using A15 Biosystem auto analyser using commercially available Kits from AGAPPE Diagnostic Ltd based on CHOD- PAP methodology (Allain et al., 1974) and GPO – PAP method (Buccolo and David, 1973) respectively.

Statistical analysis

All the statistical analysis were performed by using SPSS software (version 20.0) as per Snedecor and Cochran (1994). The mean were compared by one way ANOVA for significant difference among treatment. All the per cent
values in the experiment were transformed to their arcsine roots before subjecting to statistical analysis.

Results and Discussion

Haematological parameters

The effect of supplementation of garlic and nilavembu on the haematological parameters (Mean ± S.E) such as PCV (%), Hb (g/dl), RBC (x 10^6) and WBC (x 10^3) in commercial broiler chicken were presented in Table 3. The result on WBC (x 10^3) count revealed a highly significant (P<0.01) difference between the treatment groups with highest count in (T_5) 10 g of garlic and 2 g of nilavembu fed group (11.60 ± 0.49) when compared with control (7.21 ± 0.28) group. Whereas treatment groups T_1, T_2 and T_3 had comparable WBC count; however, it was not significant. It was also found that, there was a non-significant difference exist among the various treatment groups with respect to PCV (%), Hb (g/dl) and RBC (x 10^6) count. Mean (±S.E) value of MCV, MCH and MCHC of commercial broiler chicken as influenced by supplementation of garlic and nilavembu was presented in Table 4. It was observed that, there is a non-significant different exist among the different treatment groups with respect to MCV, MCH and MCHC, respectively.

The effect of supplementation of garlic and nilavembu on differential count mean (±S.E) of commercial broiler chicken was presented in Table 5. Statistical analysis of data on differential count showed that only lymphocyte had a significant (P<0.05) influence in the group fed with (T_5) 10 g of garlic and 2 g of nilavembu (58.80 ± 0.73) and it was comparable with T_4 (57.83 ± 1.19) and control (56.00 ± 1.06) group. However, heterophil, eosinophil, basophil and monocyte count showed a non-significant difference among the various treatment groups.

The above results were in accordance with the earlier reports of Fadlalla et al., (2010) and Eid and Iraqi (2014) who also observed that total WBC count had a significant (P<0.05) differences among the dietary treated groups. On contrary to the above finding, Jimoh et al., (2012) and Elagib et al., (2013) who observed that supplementation of garlic and nilavembu had a non-significant difference on blood haematological parameters.

The mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) expressed a non-significant difference among various treatment groups. These finding were in agreement with Toghyani et al., (2011) who observed that mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly influenced by the supplementation of garlic.

Serum bio-chemical parameters

The effect of supplementation of garlic and nilavembu on serum biochemical parameters (Mean ±S.E) such as total serum protein (mg/dl), total serum cholesterol (mg/dl) and triglyceride (mg/dl) of commercial broiler chicken at sixth week of age was presented in Table 6. Statistical analysis of data on total serum cholesterol and triglyceride revealed a highly significant (P<0.01) difference upon the supplementation of garlic and nilavembu among the different treatment group, where as a non-significant difference was noticed among the various treatment groups with the respect to total serum protein. The lowest total serum cholesterol and triglyceride values were found in 10 g of garlic and 2 g of nilavembu (T_5) supplemented group (132.81 ± 0.86) and (66.42 ± 1.09) respectively, and it was significantly (P<0.01) lower than all the other groups.
Table 1: Experimental design

| Treatments | Experimental design                                           | No of birds |
|------------|----------------------------------------------------------------|-------------|
| T<sub>1</sub> | Control group – Basal feed                                     | 20 20 20 60 |
| T<sub>2</sub> | Basal diet + 5 g of Garlic + 1 g of Nilavembu / kg of feed     | 20 20 20 60 |
| T<sub>3</sub> | Basal diet + 10 g of Garlic + 1 g of Nilavembu / kg of feed    | 20 20 20 60 |
| T<sub>4</sub> | Basal diet + 5 g of Garlic + 2 g of Nilavembu / kg of feed     | 20 20 20 60 |
| T<sub>5</sub> | Basal diet + 10 g Garlic + 2 g of Nilavembu / kg of feed       | 20 20 20 60 |
| **Total**   |                                                                | **100 100 100 300** |

Table 2: Ingredient and nutrient composition of the experimental broiler diet

| Ingredients (kg)             | Broiler                                                                 |
|------------------------------|-------------------------------------------------------------------------|
|                              | Pre-starter | Starter | Finisher |
| Maize                        | 50.50       | 53.50   | 54.00    |
| Cumbu/Bajra                  | 7.00        | 6.25    | 8.00     |
| Soya bean meal               | 29.75       | 26.00   | 22.00    |
| Dry fish                     | 9.00        | 9.00    | 9.00     |
| Mineral Mixture              | 1.50        | 1.50    | 1.50     |
| Oil                          | 1.75        | 3.25    | 5.00     |
| Salt                         | 0.50        | 0.50    | 0.50     |
| **TOTAL**                    | **100.00**  | **100.00** | **100.00** |
| Supplements (g)              |             |         |          |
| Vitamin AB<sub>2</sub>D<sub>3</sub>K<sub>1</sub> | 10.00      | 10.00   | 10.00    |
| B-complex vitamins<sup>2</sup> | 25.00      | 25.00   | 25.00    |
| Trace minerals<sup>3</sup>   | 50.00       | 50.00   | 50.00    |
| **NUTRIENT COMPOSITION**     |             |         |          |
| Crude Protein %              | 22.51       | 21.04   | 19.53    |
| ME (Kcal / Kg)               | 3000        | 3124    | 3252     |
| Crude Fiber %                | 3.75        | 3.52    | 3.40     |
| Ether extract %              | 5.27        | 6.75    | 8.47     |
| Calcium %                    | 0.99        | 0.96    | 0.90     |
| Available Phosphorus %       | 0.45        | 0.44    | 0.43     |
| Lysine %                     | 1.33        | 1.22    | 1.12     |
| Methionine %                 | 0.63        | 0.61    | 0.59     |
| Linoleic acid %              | 1.50        | 1.91    | 2.36     |

1. One gram of Vitamin AB<sub>2</sub>D<sub>3</sub>K<sub>1</sub> supplement contained 82500 IU of Vitamin-A, 50 mg of Vitamin-B2, 12000 IU of Vitamin-D3 and 10 mg of Vitamin-K.
2. One gram of B-Complex supplement contained 8 mg of Vitamin-B<sub>1</sub>, 16 mg of Vitamin-B<sub>6</sub>, 80 mg of Vitamin B<sub>12</sub>, 80 mg of Vitamin-E, 120 mg of Niacin, 5 mg of Folic acid, 80 mg of Calcium pantothenate, 120 mg of Calcium and 300 mg of Phosphate.
3. One gram of Trace Minerals contained 54 mg of manganese, 52 mg of zinc, 20 mg of iron, 2 mg of iodine and 1 mg of cobalt.
### Table 3: Effect of supplementation of garlic and nilavembu on PCV (%), Hb (g/dl), RBC (x 10^6) and WBC (x 10^3) (Mean ± S.E) of commercial broiler chicken at 6 weeks of age

| Treatments               | PCV (%)      | Hb (g/dl)    | RBC (x 10^6) | WBC (x 10^3) |
|--------------------------|--------------|--------------|--------------|--------------|
| T1 (control)             | 39.18 ± 0.49 | 10.39 ± 0.37 | 2.31 ± 0.27  | 7.21c ± 0.28 |
| T2 (G-5 g + N-1 g)       | 40.06 ± 0.26 | 10.77 ± 0.27 | 2.62 ± 0.10  | 8.23c ± 0.45 |
| T3 (G-10 g + N-1 g)      | 39.12 ± 0.35 | 10.54 ± 0.09 | 2.57 ± 0.19  | 8.23c ± 0.30 |
| T4 (G-5 g + N-2 g)       | 39.85 ± 0.29 | 10.77 ± 0.27 | 2.68 ± 0.17  | 10.53b ± 0.25|
| T5 (G-10 g + N-2 g)      | 39.18 ± 0.35 | 10.48 ± 0.26 | 2.54 ± 0.19  | 11.60a ± 0.49|
| F value                  | 1.050        | 0.417        | 0.534        | 21.456       |
| Significant              | NS           | NS           | NS           | **           |

Means bearing different superscripts within the same column differ significantly
NS - No Significant ** - Highly significant (P < 0.01) G- Garlic N- Nilavembu

### Table 4: Effect of supplementation of garlic and nilavembu on MCV (fl), MCH (pg) and MCHC (pg) (Mean ± S.E) of commercial broiler chicken at 6 weeks of age

| Treatments               | MCV (fl)      | MCH (pg)     | MCHC (pg)    |
|--------------------------|---------------|--------------|--------------|
| T1 (control)             | 143.06 ± 0.94 | 41.92 ± 0.36 | 26.53 ± 0.87 |
| T2 (G-5 g + N-1 g)       | 142.94 ± 1.83 | 41.12 ± 1.10 | 26.89 ± 0.77 |
| T3 (G-10 g + N-1 g)      | 142.23 ± 1.07 | 41.16 ± 1.42 | 26.96 ± 0.22 |
| T4 (G-5 g + N-2 g)       | 140.92 ± 1.47 | 41.28 ± 1.41 | 26.04 ± 0.14 |
| T5 (G-10 g + N-2 g)      | 143.07 ± 1.02 | 41.34 ± 1.20 | 26.77 ± 0.52 |
| F value                  | 0.489         | 0.022        | 0.406        |
| Significant              | NS            | NS           | NS           |

NS - No Significant G- Garlic N- Nilavembu FL- Femto litre; pg- Pico gram
Table.5 Effect of supplementation of garlic and nilavembu on differential count (Mean ± S.E) of commercial broiler chicken at 6 weeks of age

| Treatments          | Heterophils  | Eosinophils | Basophils | Lymphocytes  | Monocytes  |
|---------------------|--------------|-------------|-----------|--------------|------------|
| T<sub>1</sub> (control) | 26.16 ± 0.47 | 2.83 ± 0.30 | 1.16 ± 0.17 | 56.00<sup>ab</sup> ± 1.06 | 4.33 ± 0.33 |
| T<sub>2</sub> (G-5 g + N-1 g) | 26.00 ± 0.44 | 2.83 ± 0.40 | 1.33 ± 0.21 | 55.00<sup>b</sup> ± 1.09 | 4.16 ± 0.40 |
| T<sub>3</sub> (G-10 g + N-1 g) | 26.83 ± 0.40 | 2.50 ± 0.34 | 1.00 ± 0.25 | 55.00<sup>b</sup> ± 1.29 | 4.16 ± 0.30 |
| T<sub>4</sub> (G-5 g + N-2 g) | 26.66 ± 0.80 | 2.66 ± 0.21 | 1.16 ± 0.16 | 57.83<sup>ab</sup> ± 1.19 | 4.17 ± 0.31 |
| T<sub>5</sub> (G-10 g + N-2 g) | 25.60 ± 0.74 | 2.40 ± 0.24 | 1.40 ± 0.24 | 58.80<sup>a</sup> ± 0.73 | 4.20 ± 0.37 |
| F value             | 0.696        | 0.373       | 0.541     | 2.265        | 0.045      |
| Significant         | NS           | NS          | NS        | *            | NS         |

Means bearing different superscripts within the same column differ significantly
* - Significant (P<0.05) NS- No Significant
G - Garlic N - Nilavembu

Table.6 Effect of supplementation of garlic and nilavembu on total serum protein (mg/dl), total serum cholesterol (mg/dl) and triglyceride (mg/dl) (Mean ± S.E) of commercial broiler chicken at 6 weeks of age

| Treatments          | Total serum protein (mg/dl) | Total serum cholesterol (mg/dl) | Triglyceride (mg/dl) |
|---------------------|-----------------------------|--------------------------------|---------------------|
| T<sub>1</sub> (control) | 3.45 ± 0.02                  | 154.19± 1.27                   | 105.17<sup>a</sup> ± 1.48 |
| T<sub>2</sub> (G-5 g + N-1 g) | 3.55 ± 0.12                  | 152.43± 0.58                   | 95.14<sup>b</sup> ± 1.14 |
| T<sub>3</sub> (G-10 g + N-1 g) | 3.50 ± 0.12                  | 144.49<sup>b</sup> ± 1.26     | 83.61<sup>c</sup> ± 1.04 |
| T<sub>4</sub> (G-5 g + N-2 g) | 3.55 ± 0.04                  | 142.00<sup>b</sup> ± 0.66     | 75.18<sup>d</sup> ± 1.66 |
| T<sub>5</sub> (G-10 g + N-2 g) | 3.55 ± 0.08                  | 132.81<sup>c</sup> ± 0.86     | 66.42<sup>e</sup> ± 1.09 |
| F value             | 0.275                       | 78.630                         | 138.840             |
| Significant         | NS                          | **                             | **                  |

Means bearing different superscripts within the same column differ significantly
** - Highly significant (P< 0.01) NS- No Significant
G - Garlic N - Nilavembu

From this experiment, it was found that with increase in garlic and nilavembu levels in the feed, there was decrease in cholesterol and triglyceride level in serum. However, the highest level of total cholesterol was obtained from control group.

Despite the fact that the nutrient levels of garlic and nilavembu, they had strong impact on the growth and serum lipid levels of broilers. The supplementation of garlic and nilavembu significantly improved the growth of the chicks. However, the mixtures of garlic
and nilavembu had reduced serum lipids and abdominal fat pad without any adverse effect on the growth of the broiler chickens. This reveals that addition of the mixture of garlic and nilavembu in broiler diet could be more beneficial in lowering lipids in broiler chickens than sole addition of garlic and nilavembu. Furthermore, the results of hypolipidemic action of the supplements showed that they could be used to lower some of the risk factors associated with the development of cardiovascular diseases and cancer whether in animals or human beings. It has been reported that lowering blood levels of cholesterol and fats may help to prevent heart disease, angina, stokes and heart attacks (Anonymous, 2008).

Various research’s reported that (Jimoh et al., 2012 and Puvaca et al., 2014) the supplementation of garlic significantly (P<0.05) decreased the level of total cholesterol, triglyceride which was in agreement with the finding of the present study. This might be due to garlic contains high levels of bioactive saponin which form insoluble complexes with cholesterol and inhibit intestinal absorption of endogenous and exogenous cholesterol. These key saponins in garlic also possess the ability to inhibit key enzymes in the cholesterol and lipid biosynthetic pathways. Allicin also inhibit the action of hydroxymethyl gutaryl-CoA reductase, which is the most important enzyme that participates in the synthesis of cholesterol and lipids. On contrary Anvar et al., (2012) who observed that there was a non-significant difference in total cholesterol and triglyceride among the garlic treated groups. Combination of garlic and nilavembu also gave lower total serum cholesterol and triglyceride level in commercial broilers.

It was concluded that the supplementation of garlic and nilavembu showed a hypolipidemic effects on the serum cholesterol and triglyceride levels without affecting the growth performance of the broilers and also enhance the immunity through increased leucocyte count. The combined effect of garlic and nilavembu showed a positive response in the broiler production.

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