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Effect of dietary supplementation of neem leaf powder (*Azadirachta indica*) on the haematological and biochemical parameters of commercial broiler chicken

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Abstract
A total of 144 day-old commercial broiler chicks (Cobb 400) from a single hatch were procured and chicks were randomly divided into four groups *viz*. T_0, T_1, T_2 and T_3 consisting of 36 number of birds in each group. The birds under T_0, T_1, T_2 and T_3 groups were fed Neem Leaf Powder at the rate of 0.0, 0.1, 0.2 and 0.3% in the feed (on dry matter basis), respectively. At the end of six weeks, for estimation of haematological and biochemical parameters were performed and found that the haematological parameters (Haemoglobin, PCV, Total RBC count, Total WBC count and WBC differential count) and biochemical parameters (Total Serum Urea, Serum Creatinine, Total Protein, Total Albumin and Total Globulin) did not differ significantly (P>0.05) among the different experimental groups of broiler chicken.

Keywords: Neem leaf powder, haematological, broiler chicken

Introduction
Researchers and scientist are now-a-days showing interest on the use of our traditional ancient medicinal system to find beneficial herbs and plants which can be safely used to increase the production. Different herbs used are garlic, ginger, cloves, turmeric, moringa, neem fruit and leaves, gooseberry, sage, thyme, mustard and fenugreek. These plants posses' innumerable properties like digestive stimulants, antidiarrheal, antiseptic, anti-inflammatory, antiparasitic and appetite stimulants in human beings as well as animals. It has been found that these herbal agents could serve as safer alternatives to antibiotic growth promoters due to their lower cost of production, reduced risks of toxicity and minimum health hazards. One of such plants, Neem (*Azadirachta indica*) is an indigenous plant of Asian subcontinent known for its useful medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective, immunomodulator and various other properties without showing any adverse affects (Kale *et al.*, 2003; Sadekar *et al*., 1998) [8, 17]. Neem promotes growth and feed efficiency of poultry because of its antibacterial and hepatoprotective properties (Padalwar, 1994) [16]. Neem leaf powder fed to poultry have been reported by Sadre *et al*., (1984) [18] and Gowda *et al*., (1998) [6] to significantly reduce the content of hemoglobin, erythrocyte count and packed cell volume. Recent biological trials of certain herbal formulations in India as growth have shown encouraging results and some of the reports have demonstrated improvement with respect to weight gain, feed efficiency, lowered mortality, increased immunity and increased livability in poultry birds (Kumar, 1991) [10]. Neem (*Azadirachia indica*) dry leaves powder as medical herbs could be beneficial in immunosuppressant diseases of poultry. The feeding neem leaves to immunosuppressed birds increase their humoral and cell mediate immune responses (Sadekar *et al*., 1998) [7]. Low dose of neem leaves powder have an inhibitory action on wide spectrum of microorganisms (Talwar *et al.*, 1997) [20] and immuomodulator actions that induce cellular immune reaction (Devakumar and Sukt, 1993) [5]. Considering the vast benefits of neem on poultry health and management the present study was aimed to evaluate the haematological and biochemical parameters of broilers supplemented with Neem leaf powder.

Materials and Method

The experiment was carried out on 144 day old commercial broiler chicks (Cobb 400) procured from the local hatchery of Guwahati city and having similar body weight from a single hatch. The chicks were weighed individually; wing banded and was distributed randomly into four groups *viz*. T_0, T_1, T_2 and T_3 containing 36 chicks in each group. Each group was further subdivided into 3 replicates of 12 chicks. Birds were maintained following standard feeding and uniform managemental practices under deep litter system of rearing. The experimental diets were T_0 (Control group with no Neem leaf powder supplementation) while diet T_1, T_2 and T_3 contained 0.1, 0.2 and 0.3% of Neem leaf powder respectively. The feeding trial was conducted for 6 weeks using broiler starter (0-4 weeks) and broiler finisher (5-6 weeks) ration as per BIS (1992). During the period of the experiment, daily feed intake, weekly feed consumption and weekly body weight changes at different stages were recorded.

The composition and nutrient levels of basal diets are shown in Table 01.

Table 1: Ingredients and Nutrient Composition of Basal Diet (Broiler Starter and Broiler Finisher) As Per Bis (1992)

Ingredients (Kg.)	Starter (0-28 days)	Finisher (29-42 days)
Maize	50.0	52.5
Rice polish	7.0	7.0
Ground nut cake	17.0	12.0
Soya bean meal	22.5	24.0
Vegetable oil	1.0	2.0
Mineral mixture	2.0	2.0
Common salt	0.5	0.5
Nutrient composition		
Dry matter (%)	88.4	86.6
Crude protein (%)	22.04	20.99
Ether extract (%)	3.5	5.0
Crude fibre (%)	4.0	3.5
Nitrogen free extract (%)	63.16	64.51
Total ash (%)	7.3	6.0
Metabolizable energy (Kcal/Kg.)*	2803.11	2901.53

*Calculated values.

(N.B. Vitamin premix Provita M was added @ 20g per 100 parts of the ration in both starter and finisher diet.)

Table 2: Composition of Provita M

Vitamin A	6,000 IU
Vitamin D_3	2,500 IU
Vitamin E	1.5 IU
Thiamine	0.2 mg
Riboflavin	1 mg
Pantothenic Acid	3 mg
Niacin	10 mg
Pyridoxine	0.5 mg
Folic Acid	0.2 mg
Vitamin B_{12}	10 mcg
Choline	5 mg
Lactic Acid Bacteria*	100 million CFU**

**Lactobacillus acidophilus, Enterococcus faecium, Lactobacillus plantarum, Lactobacillus casei*

Table 3: Nutrient Composition of Neem Leaf Powder

Nutrient composition	Neem Leaf Powder
Dry matter (%)	92.08
Crude protein (%)	16.69
Ether extract (%)	1.59
Crude fibre (%)	14.61
Nitrogen free extract (%)	60.27
Total ash (%)	6.85

Haematological parameter

At the end of the experiment, for estimation of hematological parameter like hemoglobin (Hb), Packed Cell Volume (PCV), total Red Blood Corpuscles (RBC), total White Blood Corpuscles (WBC) and WBC differential count, about 2 ml of blood was collected aseptically with anticoagulant from 5 birds of each group. The blood was estimated for the haematological parameters using the instrument "Automatic Haematolyzer" in Teaching Veterinary Clinical Complex, College of Veterinary Science, AAU, Khanapara.

Biochemical parameter

Total serum Protein

Twenty µl of serum or BSA standard (6 g/dl) or distilled water was taken in a test tube, 1 ml of Biuret reagent was added and mixed properly. The absorbance was measured at 555 nm. The absorbance of BSA standard or serum was normalized against the absorbance of distilled water.

Serum Albumin

For estimation of total serum albumin, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. The blood samples were centrifuged for separation of serum. Then the total serum albumin was estimated using spectrophotometer at 630 nm with Albumin Kit (BCG method) supplied by Coral Clinical Systems, Gitanjali, Dr. Antonio Do RegoBagh.

Serum Creatinine

For estimation of serum creatinine, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. The blood samples were centrifuged for separation of serum. The serum creatinine was estimated using spectrophotometer at 505 nm with AUTOSPAN Liquid Gold Creatinine Kit, Modified Jaffes Reaction, Initial Rate Assay supplied by ARKRAY Healthcare Pvt. Ltd.

$$\text{Serum creatinine (mg/dl)} = \frac{\text{Final OD of test sample} - \text{Initial OD of test sample}}{\text{Final OD of standard sample} - \text{Initial OD of test sample}} \times 2$$

Serum Globulin

For estimation of total serum globulin, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. The blood samples were centrifuged for separation of serum. Globulin was calculated by subtracting total serum albumin from total serum protein.

Serum Urea

For estimation of serum urea, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. The blood samples were centrifuged for separation of serum. The urea was estimated using spectrophotometer at 340 nm with Urea GLDH DIATEK Kit (GLDH method) supplied by DIATEK Healthcare Pvt. Ltd.

Calculation

$$\text{Urea (mg/dl)} = \frac{\Delta \text{ Absorbance of test sample}}{\Delta \text{ Absorbance of standard}} \times 50$$

Statistical Analysis

Completely Randomized Design (CRD). The mean, SE (Standard Error) were calculated with the standard statistical procedure. One way Analysis of Variance was performed by the software SAS system (Local, X64_7PRO).

Results and Discussion

The haematological parameters comprising of haemoglobin (g/dl), Packed Cell Volume (%), total RBC count (million/mm^3), total WBC count (thousand/mm^3) and WBC differential count (Neutrophil, Eosinophil, Monocyte and Lymphocyte) were studied at the end of the experimental period of 42 days and mean (± SE) values have been presented in Table 04 & 05.

All the haematological parameters (Haemoglobin Hb, PCV, total RBC, total WBC, Neutrophil, Eosinophil, Monocyte and Lymphocyte) estimated in the present study did not differ significantly (P>0.05) among the different experimental groups (Table 04.). The present findings were in agreement with the observations of Nnenna and Okey, (2013) [18], Alam *et al.* (2015) [1] and Nodu *et al.* (2016) [14] who found no significant (P>0.05) differences in the haematological parameters due to supplementation of NLP or NLE in broiler chicken. On the other hand, Nayaka *et al.* (2013) [12] and Khulbey *et al.* (2015) [9] found significantly (P≤0.05) higher values of Hb and PCV in broiler chicken due to supplementation of NLP at 0.8 and 0.2% levels. The increase in Hb content in treated groups was reported due to blood purifying properties of neem (Bhowmik *et al.*, 2010) [3] or due to the mineral content specifically Fe, Mg, Zn, Cu, Ca and P in leaves (Atangwho *et al.*, 2009) [2] which were important for synthesis of Haemoglobin (Hb). Similarly, Zanu *et al.* (2011) [22] and Bonsu *et al.* (2012) [4] reported that total WBC count and lymphocyte values decreased significantly (P≤0.05) in neem treated group as compared to control. The lower value of WBC in treated groups was reported due to lower probable disease condition in the birds.

The present values of haematological parameters fall within the normal range for healthy broiler chicken as reported by Mitruka and Rawnsely (1997) [11]. Hackbarth *et al.* (1983) [7] reported that there was a strong influence of diet on haematological traits with Hb and PCV being very strong indicators of the nutritional status of the animals. The results of the present study showed that NLP numerically increased the Hb and PCV of the birds. This indicated that these birds were not stressed by the Neem leaf powder. The values of these blood parameters obtained from broiler chicken fed NLP may indicate better nutrient availability and utilization by the birds. This suggests that the birds were properly nourished and were able to obtain essential amino acids and minerals necessary for the normal function of the haematopoietic tissues.

Table 4: Mean ± Se Values Of Haematological Parameters Of Broiler Chicken Under Different Treatment Groups

Parameters \ Groups	T_0 (Control)	T_1 (NLP-0.1%)	T_2 (NLP-0.2%)	T_3 (NLP-0.3%)
Haemoglobin (g/dl)	9.38^a ±0.37	9.70^a ±0.17	9.72^a ±0.23	9.62^a ±0.36
PCV (%)	33.06^a ±0.56	34.34^a ±1.35	33.92^a ±0.82	34.62^a ±1.26
Total RBC (10^6/mm^3)	2.47^a ±0.03	2.54^a ±0.02	2.57^a ±0.03	2.58^a ±0.05
Total WBC (10^3/mm^3)	14.63^a ±0.46	14.86^a ±1.26	13.98^a ±1.23	14.81^a ±0.77
Neutrophil (10^3 /mm^3)	2.24^a ±0.25	2.22^a ±0.28	2.32^a ±0.09	2.24^a ±0.32
Eosinophil(10^3 /mm^3)	0.25^a ±0.07	0.27^a ±0.04	0.25^a ±0.04	0.25^a ±0.04
Monocyte (10^3/mm^3)	1.41^a ±0.22	1.40^a ±0.16	1.39^a ±0.08	1.40^a ±0.20
Lymphocyte(10^3/mm^3)	9.71^a ±0.17	10.07^a ±0.69	11.20^a ±1.02	10.15^a ±1.20

Means bearing same superscripts in a row did not differ significantly (P>0.05).

The mean (±SE) values of all the biochemical parameters (urea, creatinine, total protein, total albumin and total globulin) estimated in the present study did not differ significantly (P>0.05) among the different experimental group (Table 05.). The present findings were in agreement with the reports of Nnenna and Okey (2013) [18] who indicated that supplementation of NLE did not significantly (P>0.05) influenced the serum biochemical parameters like urea, creatinine, total protein, total albumin and total globulin among the control and NLE treated group of broiler chicken. Similarly, Nodu *et al.* (2016) [14] also did not find any significant differences for most of the biochemical parameters except total protein when broiler chicken was supplemented with NLE for a period of 8 weeks. Contrary to the present findings Shihab *et al.* (2017) [19] reported that supplementation of NLP at the rate of 0.1 and 0.2% in the basal diet increased the levels of total protein, albumin and globulin significantly (P≤0.05) as compared to control group. However, they did not find any significant differences in values of these parameters between the control and 0.3% supplemented group. The findings of Obikaonu *et al.* (2012) [15] could not be compared with the present findings because they supplemented NLP at a very high level (up to 10.0%) and the results did not show much consistency in the measured biochemical parameters.

The values of all the biochemical parameters found in the present study (Table 05.) were within the normal ranges for healthy broiler chicken (Trinica *et al.*, 2012) [21] and this implied that use of neem leaves in broiler production with the

present levels is safe and healthy. Moreover, the non-significant (P>0.05) values for total protein, albumin and globulin obtained in the present study suggest nutritional adequacy of the dietary proteins for broiler chicken.

Table 5: Mean (±Se) Values of Biochemical Parameters of Broiler Chicken under Different Treatment Groups

Groups / Parameters	T_0 (Control)	T_1 (NLP-0.1%)	T_2 (NLP-0.2%)	T_3 (NLP-0.3%)
Serum urea (mg/dl)	5.60^a±1.60	5.20^a ±1.50	5.60^a ±1.47	5.20^a ±3.07
Serum creatinine (mg/dl)	0.50^a ±0.03	0.50^a ±0.07	0.49^a ±0.01	0.48^a ±0.10
Total serum protein(g/dl)	2.99^a ±0.34	2.99^a ±0.11	3.07^a ±0.42	3.03^a ±0.33
Total serum albumin(g/dl)	1.33^a ±0.12	1.31^a ±0.14	1.37^a ±0.11	1.32^a ±0.12
Total serum globulin(g/dl)	1.66^a ±0.37	1.69^a ±0.20	1.69^a ±0.51	1.71^a ±0.37

Means bearing same superscripts in a row did not differ significantly (P>0.05).

Conclusion

The study suggests the potential use of Neem leaf powder to improve the performance of broiler. However, further studies are needed to evaluate the effect of neem supplementation on the blood parameters of broilers taking into more number of treatment groups under the study.

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