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Efficacy of Neem Leaf (*Azadirachta indica*) Meal as an Alternative to Antibiotic in Broiler Ration

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MAHB and MZUR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MAHB, MZUR, TAN, MAZ and MB managed the analyses of the study. Author MZUR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present work aimed at studying growth performance, carcass traits and health status in broiler chicken fed on dietary Neem (*Azadirachta indica*) leaf Meal (NLM) over a period of 4 weeks. Day old broiler chicks (180) were randomly assigned to six treatment groups, each with 3 replicates (10). The last treatment was designated as control (T6) in which no supplement was added to the feed, on the other hand T5 named as antibiotic group which contain antibiotic supplement with feed, while in treatments T1, T2 and T3, T4 NLP was provided as 1.0%,1.5%, 2.0% and 2.5% of feed, respectively. All the data were analyzed statistically at 5% level of significance. The results revealed a significant ($P<0.05$) decrease in feed intake (2101 g and 2104 g) at 4 weeks in T3 and T4 group bird, but produced live weight (1708 g and 1712 g) which had no significant ($P>0.05$) difference with birds consumed highest amount of feed. The significantly ($P<0.05$) highest hemoglobin (16.33 gm/dl) was in 2.0% NLM broiler chicken than other *Neem*, control and antibiotic groups. No

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significant (P>0.05) difference was found in glucose and cholesterol for any treatment groups of broiler chicken, but significantly lowest (P<0.05) uric acid was observed in 1.50% NLM treated group than antibiotic group. The NLM treated groups broiler chicken showed no significant (P>0.05) difference in Neutrophils, Lymphocytes, Monocytes and Eosinophils counts comparing with antibiotic and control groups. *Neem* treated groups showed significantly (P<0.05) higher liver weight (43.67 g to 46.67 g) than antibiotic group (31.0 g). Spleen weights were not affected (P>0.05) by any treatments. The highest (P<0.05) viable bacteria was found in control group (163 x 10^4) than antibiotic (33 x 10^4) and *Neem* treated groups. But, *Neem* and antibiotic treated groups showed no significant (P>0.05) difference among them. The results of the study demonstrate the beneficial effects of supplementing NLM on body weight gain and dressed yield in the treated groups in broiler chicken. NLM is, therefore, suggested to be used as an alternative of antibiotics on broiler chicken ration for higher profitability.

Keywords: Antibiotic alternative; broiler; growth performance; Neem (Azadirachta indica) leaf meal; hematological parameter.

1. INTRODUCTION

The use of antibiotic in the animal feed has been controversial for a long time due to its link to the development of pathogenic resistance to human health [1]. Evidence shows that antibiotic resistance genes can be transmitted from animal to human microbiota [2]. As a result, every year a huge economic loss for the hospital cost due to less effective of antibiotic for saving the human lives. This problem has also been increasing day by day due to the misuse of antibiotics in animal and poultry feeds. On the other hand, about 90% of antibiotics given to livestock are excreted into the environment and may be a source of pollution [3]. It is documented that antibiotic resistance bacteria can transmit directly, and indirectly through the food chains, air, water, and soil. As a consequence, several countries have been banned or restricted the use of human health related antibiotics in food animal production for the public health safety concern. However, the poultry industry is now facing a great challenge to maintain production performance of birds due to increased feed costs, and find alternative supplements replacing antibiotic uses in feeds. In poultry industry, antibiotic growth promoters (AGP) have been used as a feed additive to enhance gut health and control sub-clinical diseases. Synthetic growth enhancers and supplements in poultry nutrition are expensive, usually unavailable and possess adverse effects in bird and human. Sub-therapeutic levels of antibiotics given to poultry as growth enhancer may result to the development of antibiotic-resistant bacteria, which are hazardous to animal and human health [4]. Currently, anumber of possible alternatives to AGPs is used. Some of the alternatives may include significant changes in husbandry practices or the strategic use of enteric microflora conditions, including acidifiers, probiotics, enzymes, algae and herbal products, microflora enhancers, and immuno-modulators [5-7]. Stimulation of the immune system and increased vitality, regulation of the intestinal micro-flora, etc. Moreover, herbs contain active substances that can improve digestion and metabolism and possess bacterial and immunostimulant activities [8].

Scientists are again concentrating on the use of our ancient medicinal system to find beneficial herbs and plants, which can be safely used to increase the production. Many plants also produce secondary metabolites such as phenolic compounds, essential oils and sarasaponins [9]. Herbs normally used are picorhiza, garlic, cloves, *Neem* (*Azadirachta indica)* fruit and leaves, sophora flavescens, nutmeg, cinnamon, ginger, peppermint, sage, thyme, mustard and fenugreek. These plants are used as digestive stimulants, antidiarrhoic, antiseptic, anti-inflammatory, antiparasitic and appetite stimulants in human beings as well as animals. The Neem leaves are very bitter to taste, and possess a garlic-like smell. Its leaves also contain compounds with proven antimicrobial activity [10-11]. The antimicrobial activity of extracts of neem leaves against such micro-organisms as *Staphylococcus spp*, *Streptococcus spp, Pseudomonas spp and Escherichia coli*, and some fungal strains have been reported many researchers [11,12,13]. Studies on the effects of *Neem* on poultry production especially of broilers and laying hens also exist [14,15]. Antimicrobial studies on the effects of neem leaves and their extracts on cultured micro-organisms *in vitro* have also been carried out [13].

Neem (Azadirachta indica) is a tropical evergreen tree native to India, Bangladesh, Thailand, Nepal and Pakistan. It is an indigenous plant of Asian subcontinent and is also found in other countries of the world. Although it is a tropical tree plant, but widely distributed in Africa and available all year round [13,16-18]. It is a tree in the mahogany family Meliaceae. *Azadirachta indica* is locally known as *Neem*. The family includes about 50 genera and 550 species. Fruit, seeds, oil, leaves, roots, bark and almost every part of the tree is bitter and contain compounds with proven antiviral, antiretroviral, antiinflammatory, anti-ulcer and antifungal, antibacterial, anti plasmodial, antiseptic, antipyretic and anti-diabetic properties [19-21].

One of such plants is useful for medicinal properties like antibacterial, antiviral, antifungal, antiprotozoal, hepatoprotective, immunomo-dulator and various other properties without showing any adverse effects [22]. The feeding *Neem* leaves to immunosuppressed birds increase their humoral and cell mediate immune responses [23]. Low dose of *Neem* leaves powder have an inhibitory action on wide spectrum of microorganisms [24] and immuomo-dulator actions that induce cellular immune reaction [25].

1.1 Aims and Objectives

Considering the vast benefits of *Neem* on poultry health and management the present study was aimed to evaluate the growth performance of broilers supplemented with neem leaf meal.

Hence, the objective of the study was

- To investigate the efficacy of *Neem* supplemented diets on general performance index
- To determine some haematological and biochemical properties of broiler chicken by feeding *Neem* leaves as an alternative to antibiotics.

2. MATERIALS AND METHODS

2.1 Experiment Site

The experiment was conducted at Sher-e-Bangla Agricultural University Poultry Farm, Dhaka. The broiler rearing period was for 28 days and it was in the month of May- June 2017.

2.2 Experimental Deign

A total of 180 day-old Cobb broiler chicks were purchased from commercial hatchery. The chicks were carried to the university poultry early in the morning by university vehicles. They were kept in two electric brooders equally for one week by maintaining standard brooding protocol. In one brooder 1.0% (minimum dose of the treatments) *Neem* leaf meal (NLM) were supplied and another brooder with no *Neem* leaf meal (NLM). After one week 120 chicks were selected from NLM treated brooders and distributed randomly in four dietary treatments of NLM; another 60 chicks were selected from non NLM treated brooder and distributed randomly in one treatment for antibiotic and another treatment for control. Each treatment had three replications with 10 birds in each. So, the total numbers of treatments were six and replications were eighteen.

T1: 1.0% of *Neem* leaf meal (1.0 kg NLM/100 kg of the feed)
T2: 1.5.0% of *Neem* leaf meal (1.5 kg NLM/100 kg of the feed)
T3: 2.0 % of *Neem* leaf meal (2.0 kg NLM/ 100 kg of the feed)
T4: 2.5 % of *Neem* leaf meal (2.5 kg NLM/ 100 kg of the feed)
T5: Basal Diets + Oxytetracycline
T6: Basal Diets/ Control

Starter and grower commercial Kazi broiler feed were purchased from the market. Starter diet was enriched with minimum 21.0% protein, 6.0% fat, 5.0% fiber, 8.0% ash and the amino acids contents were lysine 1.20%, methionine 0.49%, cystine 0.40%, tryptophan 0.19%, threonine 0.79% and arginine 1.26%. Grower ration was enriched with minimum 19.0% protein, 6.0% fat, 5.0% fiber, 8.0% ash and the amino acids contents were lysine 1.10%, methionine 0.47%, cystine 0.39%, tryptophan 0.18%, threonine 0.75% and arginine 1.18%. Feed were supplied 4 times daily by following Cobb 500 Management Manual and *ad libitum* drinking water 2 times daily.

2.3 Analysis of Neem leaf Meal

Azadirachtin content in the neem leaves, seeds, kernels and hull was determined after its extraction, purification and analysis. Azadirachtin was extracted in a batch reactor equipped with a mechanical stirrer from 80 g of crushed seeds with a volume of 400 mL of methanol 4 hours (in

three successive extractions). The methanolic extract was defatted using hexane and the azadirachtin was extracted with dichloromethane. These operations were performed 3 times. The dichloromethane extract was dry concentrated in a rotary evaporator at 35°C. The solid was taken up in acetonitrile and filtered with a PTFE filter of 0.22 µm. The analysis was performed whit a Dionex type Ultimate 3000 HPLC equipped with a C18 column (100 × 3 mm 3 Omnispher C18), maintained at 30°C and a UV-visible detector (λ = 215 nm). The mobile phase consisted of acetonitrile/water at a flow rate of 0.8 mL/min. The injection volume was 20 µL. The mobile phase flow rate gradient programming was: 20% acetonitrile from 0 to 5 min, increased from 20 to 65% acetonirile from 5 to15 min and maintained at 65% for 5 min more.

2.4 The Measured Parameters

Parameters which were taken to determine the production performances were feed consumption, final live weight, feed conversion ratio, dressing percent and mortality. The hematological parameters were blood glucose, hemoglobin, cholesterol, uric acid and counts of leukocytes. Liver and spleen weight were taken for immunological study. The caecal contents were collected to analyze microbial load of gut health. During collection of all samples, standard methods were followed as described by Lamberg and Rothstein [26] to find out our desired data.

2.5 Statistical Analysis

MSTAT-C computer package program [27] was used for data analysis. Data were analyzed in Randomized Completely Block Design (RCBD 1 factor) for ANOVA table. Where analysis of variance indicated significant treatment effects, the means were separated using Duncan's New Multiple Range Test as described by Steel and Torrie [28]. The least significant difference (LSD) was used to separate treatment means at 5% significance level. Excel Program was practiced for preliminary data calculation.

3. RESULTS AND DISCUSSION

3.1 Production Performances of Broiler Chicken

The production performances like feed consumption (FC), live weight (LW), feed conversion ratio (FCR), dressing percent and mortality percent data of broiler chicken treated with *Neem* leaf meal and antibiotic (NLM) are presented in following Table 1.

3.1.1 Feed consumption (FC)

Different treatment groups (Table-1) showed significant (P<0.05) differences in FC of broiler chicken. Lower doses of NLM (1.00% and 1.50%) supplemented groups T1 (2207 g), T2 (2217 g) and control group T6 (2227 g) consumed significantly higher (P<0.05) amount of feed than higher doses (2.00% and 2.50%) of NLM supplemented groups T3 (2101 g) and T4 (2104 g). Antibiotic treated group T5 (2154 g) showed no significant (P>0.05) difference in FC with all other treatment groups.

Adeyemo, Alam, Bonsu [29,30,31] and Landy [32] found contrary findings and reported that feed consumption of the broiler chickens improved in the treatments fed diets supplemented with *Neem* leaf meal and were non-significant (P>0.05) compared to that of control group.

Table 1. Production performance of broiler chicken treated with *Neem* and antibiotic

Treatments	FC (g)	LW(g)	FCR	Mortality%	DP%
T1 (1.0% Neem Leaf)	2207^{a}	1697^{a}	1.29^{a}	0.00^{a}	72.41^{a}
T2 (1.5% Neem Leaf)	2217^{a}	1732^{a}	1.27^{a}	0.00^{a}	71.65^{a}
T3 (2.0% Neem Leaf)	2101^{b}	1708^{a}	1.23^{a}	3.33^{a}	70.42^{a}
T4 (2.5% Neem Leaf)	2104^{b}	1712^{a}	1.22^{a}	3.33^{a}	71.92^{a}
T5 (Antibiotic)	2154^{ab}	1666^{a}	1.29^{a}	3.33^{a}	71.80^{a}
T6 (Control)	2227^{a}	1718^{a}	1.29^{a}	3.33^{a}	72.29^{a}
Mean ±SE	2168.33 ±29.49	1705.33 ±19.27	1.26 ±0.02	2.22 ±2.50	71.74 ±0.78
CV%	2.36	1.96	3.38	195.58	1.91
LSD (0.05)	92.92	60.71^{NS}	0.08^{NS}	7.90^{NS}	2.48^{NS}

Mean with different superscripts are significantly different (P<0.05), Mean within same superscripts don't differ (P>0.05) significantly, SE= Standard Error, CV= Coefficient of Variation, LSD= Least Significant Difference NS= Non-significant

3.1.2 Live weight (LW)

All the treatment showed no significance (P>0.05) difference in final live weight of broiler chicken, but better LW were found in NLM supplemented and control group than that of antibiotic group. Alam et al. [30] and Ansari found significantly higher LW in Neem leaf treated groups compared to control group. Ansari et al. [33] found no significant (*p*>0.05) difference between BW when birds fed diets with Neem leaf meal and antibiotic like present research finding. Adeyeri [34] recommended that the neem leaf meal inclusion in the diets of the broiler chickens can be used as growth promoters during the chick phase or growth [31]. Reported that body weight gain was significantly depressed in birds fed by NLM when compared to control grour.

3.1.3 Feed conversion ratio (FCR)

No significant (P>0.05) difference were found in FCR data (Table-1) of broiler chicken among different treatment groups of T1, T2 ,T3 ,T4, T5 and T6 but better FCR were found in most of the NLM supplemented groups than antibiotic and control groups. Alam et al. [30] found identical non-significant FCR in all Neem treated groups compared to that of control group of broilers. Zanu et al. [35] also got no significant effect of Neem decoctions on feed conversion efficiency. But Ansari et al. [33] found contrary result and reported that at 28 days birds fed diets supplemented with 2.5 g/kg of leaf meal had significantly greater better FCR than those fed diets with 1.25, 5.0 g/kg of *Neem* leaf meal and controls.

3.1.4 Mortality (%)

The mortality data (Table-1) of broiler chicken presented in the table showed no significant difference among the treatment groups. The mortality percent of different treatment groups were T1 (.00%), T2 (0.00%), T3 (3.33%), T4 (3.333%), T5 (3.33%) and T6 (3.33%) which were not affected significantly by treated with NLM and antibiotic. Similar finding was reported by Ansari et al. [33] and they found no significant (*p*>0.05) effect on mortality at any time during the study. Zanu et al. [35] also got no significant effect of *Neem* decoctions on mortality.

3.1.5 Dressing percent (DP)

The dressing percent data (Table-1) of broiler chicken were not significantly affected by NLM and antibiotic. The treatment groups of T1 (72.41%), T2 (71.65%), T3 (70.42%), T4 (71.92%), T5 (71.81%) and T6 (72.29%) showed no significance (P>0.05) difference in dressing percent of broiler chicken. Many researchers [31,32,33] also got alike findings by using *Neem* products that had no significant influence on the dressing percentage of broiler chicken. Alam et al. [36] also found that polyherbal (including neem) extrat did not exhibit any effect on the dressing percentage values of broiler chicken.

3.2 Biochemical analysis of broiler chickens

Some biochemical properties of broiler chicken such as glucose, hemoglobin, cholesterol and uric acid data from blood of broiler chicken treated with *Neem* leaf meal and antibiotic (NLM) are presented in Table 2.

3.2.1 Glucose

Different treatment groups (Table 2) of broiler chicken treated with NLM and antibiotic showed no significance (P>0.05) difference in blood glucose in broiler chicken. The blood glucose data in different treatment groups are T1 (10.55 mmol/L), T2 (10.71 mmol/L), T3 (10.33 mmol/L), T4 (10.40 mmol/L), T5 (10.83 mmol/L) and T6 (10.50 mmol/L). Here the broiler chicken of antibiotic treated group T5 showed the tendency of increasing the blood glucose level. Obikaonu et al. [37] found different findings and reported that blood sugar was significantly (P<0.05) increased by supplementing NLM to broiler diets.

3.2.2 Hemoglobin

The hemoglobin data (Table-2) of broiler chicken were affected significantly (P<0.05) treated by NLM and antibiotic. The NLM treated groups T3 showed the highest hemoglobin level than other treatment groups of T1, T2, T4,T5 and T6 respectively, but no significant (P>0.05) difference was found among most of the *Neem* treatment with control and antibiotic groups except 2% NLM treatment. The current results supported by Bonsu et al. [31] who found that hemoglobin were not significantly influenced by NLM. Similarly Odo and Bratte [38] also found that NLM had no significant (P>0.05) effect on Haemoglobin of layer chicken.

3.2.3 Cholesterol

The blood cholesterol data (Table-2) of broiler chicken treated with NLM and antibiotic showed no significant (P>0.05) difference among different treatment groups of T1 (225 mg/dl), T2 (197 mg/dl), T3 (207 mg/dl), T4 (205 mg/dl), T5 (219 mg/dl) and T1 (219 mg/dl). Alike (P>0.05)

Table 2. Biochemical properties of broiler chicken treated with *Neem* and antibiotic

Treatments	Glucose (mmol/L)	Hemoglobin (gm/dl)	Cholesterol (mg/dl)	Uric Acid (mg/dl)
T1 (1.0% Neem Leaf)	10.55^{a}	13.60^{b}	225^{a}	5.10^{ab}
T2 (1.5% Neem Leaf)	10.71^{a}	13.17^{b}	197^{a}	4.80^{b}
T3 (2.0% Neem Leaf)	10.33^{a}	16.33^{a}	207^{a}	5.03^{ab}
T4 (2.5% Neem Leaf)	10.40^{a}	14.50^{b}	205^{a}	5.06^{ab}
T5 (Antibiotic)	10.83^{a}	14.23^{b}	219^{a}	5.70^{a}
T6 (Control)	11.50^{a}	13.17^{b}	219^{a}	5.20^{ab}
Mean ±SE	10.72±0.95	14.16±0.50	212.22±15.59	5.15±0.23
CV%	15.46	6.20	12.72	7.87
LSD (0.05)	3.01^{NS}	1.59	49.12^{NS}	0.73

Mean with *differensuperscripts are significantly different (P<0.05), Mean within same superscripts don't differ (P>0.05) significantly, SE= Standard Error, CV= Coefficient of Variation, LSD= Least Significant Difference NS= Non-significant*

findings noted by Odo and Bratte [38]. Ansari et al. [33] found contrary results by investigating the serum cholesterol. They reported that serum cholesterol progressively decreased if dietary levels of *Azadirachta indica* leaf meal are increased. Alam et al. [30] also reported that Cholesterol was significantly (P<0.05) decreased by *Neem* leaf meal.

3.2.4 Uric acid

The blood uric acid data (Table-2) of broiler chicken treated with NLM and antibiotic showed significant (P<0.05) differences among the treatment groups of T1, T2, T3, T4, T5 and T6. The antibiotic treated group T5 (5.70 mg/dl) showed the significantly (P<0.05) highest uric acid level and T2 (4.80 mg/dl) showed the lowest uric acid level in comparison with other treatment groups. It can be concluded from the table that blood uric acid level showed a decreasing trend in *Neem* treated groups than antibiotic and control group. Here the NLM acted as hepatoprotecter in broiler physiology. Lower uric acid level in blood is a sign of good renal function and good health, so *Neem* leaf meal can be used in broiler ration instead of antibiotic. Jawad et al. [39] published similar opinion, "Serum uric acid values showed a decreasing trend with increased level of *Neem* Leaf Meal''.

3.3 Number of Leukocytes of Broiler Chicken

The neutrophils percent of broiler chicken presented in Table-3 ranges from 28.67 to 33.67 showed no significant (P>0.05) difference among the different treatment groups. Bonsu et al. [31] stated dissimilar result that NLM produced significant (P<0.05) differences between treatment means in the neutrophils of layer chicken.The Lymphocytes percent of broiler chicken presented in Table-3 ranges from 56.33 to 62.00 were not affected significantly (P>0.05) by NLM and antibiotic. Contrary findings published by [38] and they found NLM produced significant (P<0.05) differences between treatment means in the lymphocytes of layer chicken. Similarly, Zanu et al. [35] reported that Lymphocytes which were significantly (P<0.05) influenced by Neem decoction in broiler chickens. The Monocytes cell percent of broiler chicken presented in Table-3 ranges from 6.66 to 8.33 did not show any significantly (P>0.05) difference among the different treatment groups. This finding which is also in agreement with the findings of Alam et al. [30] who observed no significant difference in monocytes cell of broiler chicken. But, [38] found significant (P<0.05) differences between treatment means in the monocytes counts by treated NLM in layer chicken.

The Eosinophils cell percent of broiler chicken presented in Table 3 ranges from 2.00 to 4.33 were not affected significantly (P>0.05) by NLM and antibiotic. This result is in line with the findings of Obikaonu et al. [37] who observed no significant effect on mean values of eosinophils of broiler chicken. Similarly, Odo and Bratte [38] noted that NLM produced no significant (P>0.05) differences between treatment means in the eosinophils of layer chicken.

Table 3. Differential counts of broiler chicken treated with *Neem* and antibiotic

Treatments	Neutrophils%	Lymphocytes%	Monocytes%	Eosinophils%
T1 (1.0% Neem Leaf)	29.00^{A}	58.33^{A}	8.33^{A}	4.33^{A}
T2 (1.5% Neem Leaf)	31.67^{A}	56.33^{A}	8.33^{A}	3.66^{A}
T3 (2.0% Neem Leaf)	33.67^{A}	56.67^{A}	7.33^{A}	2.33^{A}
T4 (2.5% Neem Leaf)	28.67^{A}	59.67^{A}	8.00^{A}	3.66^{A}
T5 (Antibiotic)	29.33^{A}	62.00^{A}	6.66^{A}	2.00^{A}
T6 (Control)	32.00^{A}	58.67^{A}	7.33^{A}	2.00^{A}
Mean ±SE	30.72±2.24	58.61±1.81	7.66±1.32	3.00±0.73
CV%	12.63	5.37	30.03	43.60
LSD (0.05)	7.05^{NS}	5.73^{NS}	4.18^{NS}	2.32^{NS}

Mean with different superscripts are significantly different (P<0.05), Mean within same superscripts don't differ (P>0.05) significantly, SE= Standard Error, CV= Coefficient of Variation, LSD= Least Significant Difference NS= Non-significant

3.4 Weight of Liver and Spleen

The liver and spleen weight data of broiler chicken treated with NLM and antibiotic are presented in Table 4.

Different treatment groups of T1 (46.67 g), T2 (44.05 g), T3 (43.67 g), T4 (45.00 g), T5 (31.00 g) and T6 (37.67 g) showed (Table-4) significant (P<0.05) difference in liver weight among them. Significantly (P<0.05) higher liver weight was found in NLM treated groups than antibiotic group. NLM treated group T1 (46.67 g) showed significantly (P<0.05) highest liver weight; whereas antibiotic treated group T5 (31.00 g) was the lowest. Increased liver weight at NLM treated birds indicates better detoxification of blood, better health and meat quality. Similar findings noted by Steel and Torrie [28] and stated that liver weights significantly increased with the inclusion of *Neem* decoction in broiler diets. But, Talwar et al. [24] found no significant (P>0.05) difference in liver weight fed by the NLM. No significant (P>0.05) difference was found (Table-4) in spleen weight of broiler chicken at any treatment group of T1 (2.33 g), T2 (2.33 g), T3 (1.83 g), T4 (1.83 g), T5 (1.50 g) and T6 (1.66 g). Supplementation of NLM in broiler diets did not exert any effect on the mean relative values of spleen weights of the broilers used in this study. But relatively higher spleen weight was found in NLM treated groups than antibiotic and control groups. Larger size spleen produces more antibodies which results stronger immune system. The results of the study are consistent with those observed by Alam et al. [36], Ahmad [40] and Landy [32] also stated that internal organs weight was not influenced by the dietary treatments of *Neem*.

3.5 Total Bacterial Count (TBC) of the Caecal Bacterial Culture

The total bacterial count in caecal faeces of broiler chicken treated with *Neem* and antibiotic are presented in Table 5.

Table 4. Weight of liver and spleen of broiler chicken treated with *Neem* and antibiotic

Treatments	Liver (g)	Spleen(g)
T1 (1.0% Neem Leaf)	46.67^{A}	2.33^{A}
T2 (1.5% Neem Leaf)	44.00^{A}	2.33^{A}
T3 (2.0% Neem Leaf)	43.67^{A}	1.83^{A}
T4 (2.5% Neem Leaf)	45.00^{A}	1.83^{A}
T5 (Antibiotic)	31.00^{B}	1.50^{A}
T6 (Control)	37.67^{AB}	1.66^{A}
Mean ±SE	41.33±2.79	1.91±0.25
CV%	11.69	23.33
LSD (0.05)	8.79	0.81^{NS}

Mean with different superscripts are significantly different (P<0.05), Mean within same superscripts don't differ (P>0.05) significantly, SE= Standard Error, CV= Coefficient of Variation, LSD= Least Significant Difference NS= Non-significant

Table 5. Total viable count of bacteria from caecal faeces of broiler chicken treated with *Neem* and antibiotic (Using dilution factor 10^{-4})

Treatments	Colony forming unit of bacteria (cfu)/gram
T1 (1.0% Neem Leaf)	40×10^{4B}
T2 (1.5% Neem Leaf)	20×10^{4B}
T3 (2.0% Neem Leaf)	23×10^{4B}
T4 (2.5% Neem Leaf)	44×10^{4B}
T5 (Antibiotic)	33×10^{4B}
T6 (Control)	163×10^{4A}
Mean ±SE	$53.83 \times 10^4 \pm 11.68 \times 10^4$
CV%	37.58×10^4
LSD (0.05)	36.81×10^4

Mean with different superscripts are significantly different (P<0.05), Mean within same superscripts don't differ (P>0.05) significantly, SE= Standard Error, CV= Coefficient of Variation, LSD= Least Significant Difference NS= Non-significant

Total viable count of bacteria from caecal faeces of broiler chicken treated with *Neem* and antibiotic presented in Table 5. Different treatment groups showed significant (P<0.05) difference among treatments. The cfu/gram in Neem treated groups ranges from 20×10^4 to $44 \times 10^{4.}$. The highest (P<0.05) viable bacteria was found in control group (163×10^{4a}) than antibiotic (33×10^{4b}) and *Neem* treated groups. But, *Neem* and antibiotic treated groups showed no significant (P>0.05) difference among them. This findings confirmed by many researchers [41-42].

5. CONCLUSION

Analyzing the above research findings the production performance, carcass traits hematological parameter, weight of lymphatic organ and microbial load in feaces sample 1.5% *Neem leaf meal* was very effective. So *Neem leaf meal* could be used as an alternative of antibiotics on broiler ration. The study therefore recommends conducting field trial on commercial poultry farm to fix up inclusion level of *Neem leaf meal*.

ETHICAL APPROVAL

Approved by the animal ethical committee of the Department of Poultry Science, Sher-e-Bangla Agricultural University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris Jr JG. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. National Academy of Science. USA. 2003; 99:6434–6439.
2. Greko C. Safety aspects on non-use of antimicrobials as growth promoters. In Gut Environment of Pigs. A. Piva, K. E. Bach Knudsen. Journal of E. Lindberg, ed. Nottingham University Press, Nottingham, UK. 2001;219–230.
3. Marshall BM, Levy SB. Clinical microbiology reviews. Food Animals and Antimicrobials: Impacts on Human Health. 2011;24:718–733.
4. Sarica S, Ciftci A, Demir E, Kilinc K, Yildirim Y. Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. South African Journal of Animal Science. 2005;35:61–72.
5. Salim HM, Kang HK, Akter N, Kim DW, Kim JH, Kim MJ, Na JC, Jong HB, Choi HC, Suh OS, Kim WK. Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. Poultry Science. 2013;92:2084–2090.
6. Kang HK, Salim HM, Akter N, Na JC, Kim DW, Bang HT, Kim MJ, Chae HS, Choi HC, Suh OS. Effect of various forms of dietary Chlorella supplementation on growth performance, immune response, and intestinal micro flora concentration of

broiler chickens. The Journal of Applied Poultry Research. 2013;22(1):100-108.
7. Kim DW, Kim JH, Kang HK, Akter N, Kim MJ, Na JC, Jong HB, Choi HC, Suh OS, Salim HM. Dietary supplementation of phenyllactic acid on growth performance, immune response, cecal microbial population, and meat quality attributes of broiler chicken. The Journal of Applied Poultry Research. 2014;23(4):661-670.
8. Ghazalah AA, Ali AM. Rosemary leaves as a dietary supplement for growth in broiler chickens. International Journal of Poultry Science. 2008;7(3):234-239.
9. Kamel CT. Modes of action and roles of plant extracts in non-ruminants. Recent advances in Animal nutrition; PC Garnssworthy and Journal. Wiseman, Nottingham University. Press: Nottingham, Uk; 2001.
10. Makeri HK, Maikai VA, Nok JA. Effect of topical application of neem seed (Azadirachta indica) extract on sheep infested with *Amblyomma variegatum.* African Journal of Biotechnology. 2007; 6(20):2324–2327.
11. Valarmathy K, Gokulakrishnan, Salma KM, Kusum DP. A study of antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species. International Journal of Pharm Sciences and Research. 2010;1(8):293–295.
12. Tuhin J, Zinnat AB, Sayeeda S. Effect of neem oil on some pathogenic bacteria. Bangdalesh Journal of Pharmacology. 2007;2(2):71–72.
13. Koona S, Budida S. Antibacterial potential of the extracts of the leaves of *Azadirachta indica* Linn. Notulae Scientia Biologicae. 2011;3(1):65–69.
14. Esonu BO, Opara MN, Odoli NC, Obikaonu HO, Udedibe C, Iheahiulor. Physiological response of laying birds to Neem (*Azadirachta indica*) leaf meal based diet, Body weight, Organ characteristics and Haematology. Life Science Journal. 2007;4(2):37-41.
15. Obun CO, Olatunji EA, Ukim CI. Utilization of sun-cured neem leaf meal (*Azadirachta indica, A. Juss*) based diets by finisher broiler chickens. Poultry industry Technical Article, Abuja, Nigeria; 2013.
16. Ganguli S. Neem: A therapeutic for all seasons. Current Science. 2002;82(11): 1304.
17. Kabeh JD, Jalingo MGDSS. Exploiting neem (*Azadirachta indica*) Resources for Improving the quality of Life in Taraba State, Nigeria. International Journal of Agriculture and Biology. 2007;9(3):530–532.
18. Ogbuewu IP, Odoemenam VU, Obikaonu HO, Opara MN, Emenalom O, Uchegbu MC, Okoli IC, Esonu BO, Iloje MU. The growing importance of neem (*Azadirachta indica, A. Juss*) in agriculture, industry, medicine and environment. A Review Research Journal of Medicinal Plants. 2011;5(3):230–245.
19. Subapriya R, Nagini S. Medicinal properties of Neem leaves. A Review Source, Current Medicinal Chemistry - Anti-Cancer Agents. 2005;5:149-156.
20. El-Hawary SS, El-Tantawy ME, Rabeh MA, Badr WK. Chemical composition and biological activities of essential oils of *Azadirachta indica A. juss.* International Journal of Applied Research in Natural Products. 2013;6:33-42.
21. Pandey IP, Ahmed SF, Chhimwal S, Pandey S. Chemical composition and wound healing activity of volatile oil of leaves of *Azadirachta indica*; *A. juss.* Advances in Pure and Applied Chemistry. 2012;62:2167-0854.
22. Kale BP, Kothekar MA, Tayade HP, Jaju JB, Mateeddin M. Indian Journal of Pharmacology. 2003;35:177.
23. Sadekar RD, Kolte AY, Barmase BS, Desai VF. Immunopotentiating effects of *A. indica* (Neem) dry leaves powder in broilers naturally infected with infectious bursal disease virus. Indian Journal of Experimental Biology. 1998;36(11):1151-3.
24. Talwar GP, Raguvanshi P, Misra R, Mukherjee S, Shah S. Plant immunomodulators for termination of unwanted pregnancy and contraception and reproductive health. Immunology & Cell Biology. 1997;75(2):190-192.
25. Devakumar C, Sukтt DV. Chemistry. In: Randhawa NS, Parmar BS (eds). Neem research and development. (India, Society of Pesticide Science). 1993;3:63-96.
26. Lamberg SL, Rothstein R. Laboratory manual of hematology and urine analysis 1st edition, AG publishing Company Inc, Westport, Conencticut, USA; 1977.
27. Russel DF. MSTAT-C statistical software program. Director Plant and Soil Sciences Department; Michigan State University, USA; 2004.

28. Steel RGD, Torrie JH. Principles and procedures of statistics. New York, McGrae Hill. 1980;137-269.
29. Adeyemo GO, Akanmu AM. Effects of Neem (*Azadirachta indica*) and pawpaw (*Carica papaya*) leaves supplementation on performance and carcass characteristics of broilers. Poultry Industry-A Technical Article. Department of Animal Science, Faculty of Agriculture, University of Ibadan, Nigeria; 2013.
30. Alam M, ATM, Rakib FK, Al-Hasan MA, Hasan MS, Ali MA. Effects of neem leave powder as a growth promoter in broilers. International Journal of Natural and Social Sciences. 2015;2:22-26.
31. Bonsu FRK, Kagya-Agyemang JK, Kwenin WKJ, Zanu HK. Medicinal response of broiler chickens to diets containing neem (*Azadirachta indica*) leaf meal, haematology and meat sensory analysis. World Applied Sciences Journal. 2012;19(6):800-805.
32. Landy N, Ghalamkari GH, Toghyani M. Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (*Azadirachta indica*) as alternative for an antibiotic growth promoter. Livestock Science. 2011;142(1):305-309.
33. Ansari J, Khan SH, Haq A, Yousuf M. Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on the growth performance and haemato-biochemical parameters in broiler chicks. J. of Applied Animal Research. 2012;40(4).
34. Adeyeri OP. The effect of neem (*Azadirachta indica*) leaf meal on the growth performance of broiler chickens. Undergraduate and Postgraduate Thesis. Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria; 2012.
35. Zanu HK, Kagya-Agyemang JK, Kwenin WKJ, Bonsu FRK, Antwi E, Ateni S. Physiological response of broiler chickens to neem (*Azadirachta indica*) and akakapenpen (*Rauvolfia vomitoria*) decoctions: Performance and carcass characteristics. International Journal of Poult. Sci. 2011;10(9):730-733.
36. Alam MN, Uddin J, Akter MTD, Moni MIZ, Alom F, Rahman A, Al Noman MA. Broiler production by using polyherbal medication (Neem, Nishyinda, Tulsi and Turmeric Extract). International Journal of Innovation and Applied Studies. 2014;9(3):1161-1175. [ISSN 2028-9324]
37. Obikaonu HO, Opara MN, Okoli IC, Okoro VM, Ogbuewu IP, Etuk EB, Udedibie. Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica*). Journal of Agriculture Technology. 2012;8(1):71-79.
38. Odo LI, Bratte L. Effects of varying levels of neem (*Azadirachta indica*) leaf meal in layer diets on haematological and serological indices, and faecal bacterial counts of layers. Journal of Natural Sciences Research. 2015;ISSN 2224-3186.
Available: www.iiste.org
39. Jawad Z, Younus M, Rehman MU, Munir R, Maqbool A, Shahzad W, Masood S, Muhammad K. Effect of *Azadirachta indica* on the hepato-renal functions in broilers chickens. The Journal of Animal & Plant Sciences. 2014;24(4):1012-1018.
40. Ahmad S. Comparative efficiency of turmeric, garlic, turmeric and kalongi as growth promoter in broiler. M.Sc. (Hons.) Thesis, Department of Poultry Sciences, University of Agriculture, Faisalabad, Pakistan; 2005.
41. Divya J, Lakshmi J, Venkatraya PM, Gopalkrishna K. Antibacterial effect of neem (*Azadirachta indica*) oil on multidrug resistant bacteria isolated from human infections. International Journal of Biological and Medical Research. 2013;4(4):3544-3546.
42. Abalaka M, Oyewole O, Kolawole AR. Antibacterial activities of *Azadirachta indica* against some bacterial pathogens. Advances in Life Sciences. 2012;2(2):5-8.

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