

# Antiviral efficacies of neem (*Azadirachta indica* J.) and scent (*Ocimum sanctum* L.) leaf meals against Newcastle and infectious bursal disease in broiler chickens

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**ABSTRACT:** The effects of neem (*Azadirachta indica*) and scent (*Ocimum santum*) leaf meals fed singly or in combination on the growth response, haemato-biochemical parameters and their antiviral potencies in broiler chickens were investigated. Two hundred 1-day old Ross 308 unsexed broiler chicks were randomly allotted to 5 treatments, consisting of 5 replicates with 8 birds each. Treatments 1 and 2 were basal diets with no leaf meals, treatments 3, 4 and 5 had the basal diets plus 3% neem leaf meal (NLM), 3% scent leaf meal (SLM) and the blend of 2% NLM + 2% SLM, respectively. Birds in treatments 2-5 were unvaccinated against Newcastle disease and Infectious bursal disease while birds in treatment 1 were vaccinated. On days 21 and 42, blood samples were randomly collected from 2 birds per replicate for haemato-biochemical assay, haemagglutination inhibition and agar gel precipitation tests. Data were subjected to descriptive statistics and ANOVA at  $p < 0.05$ . At the starter phase, an improved feed conversion ratio ( $p < 0.05$ ) was recorded in birds fed the blend at the starter (1.83) and finisher (1.92) phases and the overall performance (1.88) compared to other treatment groups. The least platelet counts ( $11.08 \times 10^4/\mu\text{L}$ ) were recorded in birds placed on the blend of 2% neem leaf meal + 2% scent leaf meal while the 3% scent leaf meal group recorded the lowest lymphocyte counts (53.60%). At the finisher phase, lymphocyte counts of birds that received the blend of 2% neem leaf meal + 2% scent leaf meal (63.00%) were higher than the other treatments. In conclusion, birds fed diets supplemented with 3% neem and 3% scent leaf meals singly had haemagglutination inhibition titre values and conferred immunity above the protective threshold against Newcastle disease and produced antibodies against Infectious bursal disease. Birds fed the blend of 2% neem + 2% scent leaf meal diets had improved overall performance.

**Keywords:** Broilers, herbal plants, infectious diseases, vaccination.

## INTRODUCTION

Poultry is regarded as the fastest means of bridging the gap of the prevalent animal protein deficiency in many developing countries as its production provides different types of animal proteins such as meat and eggs, and global production has tripled in the past 20 years (Atteh, 2002; Gabrielli *et al.*, 2020). It is a major source of protein in sub-Saharan Africa and many other lower-income regions and has been recognized as one of the possible solutions to malnutrition (Abedullah and Bukhsh, 2007). However, poultry birds are prone to different types of

diseases, most especially, infectious poultry illnesses, which are a serious concern to poultry farmers and the business at large, resulting in significant economic losses to the poultry industry (Nigest and Habin, 2020).

Vaccination has been in use for decades and it is one of the most essential techniques for preventing infections and lowering poultry producers' financial losses (Bosha and Nongo, 2012). This involves injecting attenuated, dead, or recombinant organisms into the body in order to stimulate an immune response that detects the injected organism as

a foreign antigen, which in turn, causes the antigen to be cleared and memory cells to grow (Ramirez *et al.*, 2013). Consequently, low-cost vaccines that can be combined to protect poultry against multiple infections are currently global initiatives (Mauri *et al.*, 2021).

According to Daniel *et al.* (2009), Newcastle Disease and Infectious Bursal Disease are two of Nigerian's most feared viral diseases of poultry, causing severe losses in their populations due to illness, reduced egg production, immunosuppression, and death after infection with pathogenic strains of their respective causative viruses. These two viruses never cease to wreak havoc on chicken flocks around the world and this led to the continual production of vaccines in a bid to combat them. However, there have been records of viral reversion and acute spread within the vaccinated flocks (Mortensen *et al.*, 2002). Some vaccines regarded as being stable also require strong adjuvants and several injections to induce the required level of immunity and are often effective in controlling only clinical signs rather than infections (Minke *et al.*, 2004), while some pose a greater risk of causing autoimmune diseases, allergic disorders and vaccine injection site sarcomas (Day, 2006), hence the need for alternatives.

To this effect, animal nutritionists are bewildered by the unintended consequences that accompany the use of synthetic drugs and vaccines, especially in poultry production, this has spurred researchers around the world to develop interest in natural (plant-based) vaccines. The use of plants as traditional medicine against viral diseases in animal production has been described and practised extensively (Yasmin *et al.*, 2020). The United States Department of Agriculture (USDA) already approved the production of plant-based vaccine against Newcastle Disease (Natsumi *et al.*, 2015).

Plants, such as *Azadirachta indica* (Neem), *Commiphora swynnertonii* (Burt), Aloe species, *Ocimum sanctum*, *Argemone mexicana*, *Dryopteridis crassirhizomatis* and *Fructus mume*, among others, are of immense medicinal importance in poultry production (Gill, 2022; Egbeyale *et al.*, 2022). However, information on the use of neem and scent leaves as viable alternatives to vaccines in poultry has not been fully elucidated. Therefore, this study evaluated the efficacy of *Azadirachta indica* (neem leaf) and *Ocimum sanctum* (scent leaf) as substitutes to vaccines, against Newcastle Disease Virus and Infectious Bursal Disease Virus in broiler chickens. Also, to determine the effects of neem and scent leaf meals on growth response, blood metabolites and specific antibody titre levels in broiler chickens.

## MATERIALS AND METHODS

### Experimental site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan,

Ibadan, Oyo State, Nigeria. The University of Ibadan (latitude 7° 23' 28.19" N and longitude 3° 54' 59.99"E) lies at an elevation of about 205-227 m above sea level in the sub-humid tropical rainforest zone. The study complies with the University of Ibadan's ethics requirements for animal handling and experimentation.

### Test materials and preparation method

Fresh neem (*Azadirachta indica*) and scent (*Ocimum sanctum*) leaves were harvested from the surroundings of the university teaching and research farm and were sorted, cleaned, and air-dried separately at room temperature until they became crispy but still retained the greenish tint. The leaves were turned on at regular intervals to prevent uneven drying and possible decay. Then, the dry leaves were later milled with a hammer mill and stored/labelled separately in different air-tight containers until they were incorporated into broiler starter and finisher diets (Tables 1 and 2), respectively.

### Experimental animals, diets, design and management

A total of 200 one-day-old Ross 308 unsexed broiler chicks were sourced from a reputable commercial hatchery. The birds were tagged, weighed, and randomly allotted to five dietary treatments, consisting of five replicates each with eight birds per replicate in a complete randomized design. The basal diet was a corn-soya bean meal diet formulated to meet the nutrient requirements (NRC, 1994) for broiler chickens. Treatment 1 was the positive control (Basal diet only + vaccinated against Newcastle Disease (NCD) Virus and Infectious Bursal Disease (IBD) Virus), treatment 2 was the negative control (Basal diet + unvaccinated against NCD and IBD), treatment 3 (Basal diet + 3% Neem Leaf Meal + unvaccinated against NCD and IBD), treatment 4 (Basal diet + 3% Scent Leaf Meal + unvaccinated against NCD and IBD) while treatment 5 (Basal diet + 2% Neem Leaf Meal + 2% Scent Leaf Meal + unvaccinated against NCD and IBD). The test ingredients were added over the top. A starter diet was offered to the birds from day 0-21 while a finisher diet was fed from day 22-42. The birds were raised on a deep litter system with *ad libitum* access to drinking water and experimental diets in controlled ventilation. Basal diets were formulated as shown in Tables 1 and 2.

### Vaccination procedure employed in the study

In this study, only the birds in the positive control were vaccinated. Gumboro vaccine (infectious bursal disease vaccine) was administered twice, on day 7 and day 18 of the experiment while Lasota vaccine (Newcastle disease vaccine) was administered on day 10 and day 20 of the experiment. The vaccines were administered orally through drinking water. The drinking water was withdrawn

**Table 1.** Gross composition (g/100gDM) of experimental broiler starter diet (day 0 to 21).

Ingredients	PC+0%LM	NC+0%LM	3%NLM	3%SLM	2% NLM + 2%SLM
Maize	54.50	54.50	54.50	54.50	54.50
Soyabean meal (46% CP)	38.00	38.00	38.00	38.00	38.00
Meat and Bone meal (50% CP)	3.00	3.00	3.00	3.00	3.00
Soya oil	1.00	1.00	1.00	1.00	1.00
Limestone	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	0.40	0.40	0.40	0.40	0.40
*Broiler starter premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.22	0.22	0.22	0.22	0.22
L-Lysine	0.18	0.18	0.18	0.18	0.18
Table salt	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.20	0.20	0.20	0.20	0.20
Scent Leaf Meal	0.00	0.00	0.00	3.00	2.00
Neem Leaf Meal	0.00	0.00	3.00	0.00	2.00
Total	100.00	100.00	103.00	103.00	104.00
Calculated Nutrient					
Crude protein (%)	23.74	23.74	23.74	23.74	23.74
Crude fat (%)	5.16	5.16	5.16	5.16	5.16
Crude fibr (%)	3.84	3.84	3.84	3.84	3.84
Calcium (%)	1.14	1.14	1.14	1.14	1.14
Avail. Phosphorus (%)	0.43	0.43	0.43	0.43	0.43
ME (Kcal/kg)	2914.00	2914.00	2914.00	2914.00	2914.00

\*Composition of premix per kg of the diet: Vitamin A (IU): 10,000,000, Vitamin D3 (IU): 2,500,000, Vitamin E (IU): 40,000, Vitamin K (mg): 2,250, Vitamin B1 (mg): 1,750, Vitamin B2 (mg): 5,000, Vitamin B6 (mg): 4,000, Vitamin B12 (mg):15, Niacin (mg): 40,000, Panth. Acid (mg): 10,000, Folic Acid (mg):3000, Biotin (mg):100, Choline Chloride (mg): 400,000, Manganese (mg):80,000, Zinc (mg):60,000, Iron (mg):40,000, Copper (mg):8000, Iodine (mg):1200, Selenium (mg):200, Cobalt (mg):300, Antioxidant (g):125. T<sub>1</sub>-Positive Control (0% Leaf Meal/Vaccinated); T<sub>2</sub> – Negative Control (0% Leaf Meal/Unvaccinated); T<sub>3</sub>-3% Neem Leaf Meal/Unvaccinated; T<sub>4</sub>-3% Scent Leaf Meal/Unvaccinated; T<sub>5</sub>-2% Neem Leaf Meal + 2% Scent Leaf Meal/Unvaccinated.

**Table 2.** Gross composition (g/100gDM) of experimental broiler finisher diet (Day 22 - 42).

Ingredients	PC+0%LM	NC+0%LM	3%NLM	3%SLM	2% NLM + 2%SLM
Maize	64.40	64.40	64.40	64.40	64.40
Soyabean meal (46% CP)	28.00	28.00	28.00	28.00	28.00
Meat and Bone meal (50% CP)	2.50	3.00	3.00	3.00	3.00
Soya oil	1.50	1.50	1.50	1.50	1.50
Limestone	2.00	2.00	2.00	2.00	2.00
Dicalcium Phosphate	0.40	0.40	0.40	0.40	0.40
*Broiler finisher premix	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.10	0.10	0.10	0.10	0.10
Table salt	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.20	0.20	0.20	0.20	0.20
Scent Leaf Meal	0.00	0.00	0.00	3.00	2.00
Neem Leaf Meal	0.00	0.00	3.00	0.00	2.00
Total	100.00	100.00	103.00	103.00	104.00
Calculated Nutrient					
Crude protein (%)	19.45	19.45	19.45	19.45	19.45
Crude fat (%)	4.37	4.37	4.37	4.37	4.37
Crude fibre (%)	3.42	3.42	3.42	3.42	3.42
Calcium (%)	1.07	1.07	1.07	1.07	1.07
Avail. Phosphorus (%)	0.41	0.41	0.41	0.41	0.41
ME (Kcal/kg)	3021.96	3021.96	3021.96	3021.96	3021.96

\*Composition of premix per kg of the diet: Vitamin A (IU): 10,000,000, Vitamin D3 (IU): 2,500,000, Vitamin E (IU): 40,000, Vitamin K (mg): 2,250, Vitamin B1 (mg): 1,750, Vitamin B2 (mg): 5,000, Vitamin B6 (mg): 4,000, Vitamin B12 (mg):15, Niacin (mg): 40,000, Panth. Acid (mg): 10,000, Folic Acid (mg):3000, Biotin (mg):100, Choline Chloride (mg): 400,000, Manganese (mg): 80,000, Zinc (mg): 60,000, Iron (mg): 40,000, Copper (mg):8000, Iodine (mg):1200, Selenium (mg):200, Cobalt (mg):300, Antioxidant (g):125. T<sub>1</sub>-Positive Control (0% Leaf Meal/Vaccinated); T<sub>2</sub>-Negative Control (0% Leaf Meal/Unvaccinated); T<sub>3</sub>-3% Neem Leaf Meal/Unvaccinated; T<sub>4</sub>-3% Scent Leaf Meal/Unvaccinated; T<sub>5</sub>-2% Neem Leaf Meal + 2% Scent Leaf Meal/Unvaccinated.

from the birds 6-8 hours prior to the vaccination, this was to ensure that the birds were thirsty and could finish the vaccine in their drinking water within 2 hours of administration.

## Data collection

### *Growth parameters*

Weekly body weight and feed consumption of the birds in each replicate were recorded. These values were used to estimate the feed conversion ratio and body weight gain.

**Weight gain (g):** Final weight of the birds - Initial weight of the birds.

**Feed intake (g):** This is the measurement of the quantity of feed consumed weekly.

Feed intake = Quantity of feed given (g) – Quantity of feed left over (g).

**Feed Conversion Ratio (FCR):** This was estimated as feed intake per body weight (in grams).

$$FCR = \frac{\text{Feed intake (g)}}{\text{Body weight (g)}}$$

## Blood collection

### *Haematology and serum biochemistry*

At the end of both the starter (day 21) and finisher (day 42) phases of the experiment, 2 mL blood samples were collected from the jugular vein of two birds per replicate. The blood samples were collected into vials containing Ethylene diamine tetra-acetic acid (EDTA) for haematological assay and another 2 mL into plain vials without EDTA for serum separation. The following serum biochemical parameters were evaluated using an automatic analyzer (Shimadzu CL-7200, Shimadzu, Kyoto, Japan): alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin, globulin, and creatinine.

### *Haemagglutination inhibition and agar Gel precipitation tests*

Also, blood samples were collected from each replicate into plain vials for the assessment of Newcastle Disease Virus and Infectious Bursal Disease Virus – specific antibody levels which were made by conventional haemagglutination-inhibition test (4 HA unit of Ag) and Agar gel precipitation test as described by Thayer and Beard (1998).

## Proximate analysis

The proximate composition of the diets was carried out according to the methods of AOAC (2000).

## Statistical analysis

Data obtained were analyzed using ANOVA (SAS, 2012) at a significant level of  $p < 0.05$ . Treatment means were separated using Duncan's Multiple Range Test.

## RESULTS

### Proximate composition of experimental diets

Table 3 presents the proximate composition of the starter and finisher diets. The starter diet compositions are dry matter (92.52%), crude protein (24.68%), crude fat (4.45%), crude fibre (2.53%), ash (8.33%), nitrogen free extract (52.53%) and metabolizable energy (2923kcal/kg) while the broiler finisher diet compositions are dry matter (91.85%), crude protein (20.53%), crude fat (4.58%), crude fibre (2.53%), ash (8.23%), nitrogen free extract (55.98%) and metabolizable energy (3067kcal/kg).

### Growth performances of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination

The growth performances of broiler chickens are shown in Table 4. At the starter phase, birds fed blend of 2% neem + 2% scent leaf meal had the highest ( $p < 0.05$ ) final weight (859.73 g/b) and weight gain (819.49 g/b) while the least final weight (500.74 g/b) and weight gain (460.56 g/b) were recorded in birds that received neem leaf meal. Birds on the positive control consumed more feed (1897.56 g/b) while the lowest feed intake (1495.75g/b) and feed conversion ratio (1.83) were recorded in birds on the blend of 2% neem + 2% scent leaf meal. However, there was no significant difference in percentage livability across the treatment groups.

A similar trend was observed in the performance of birds at the finisher phase. The blend of 2% neem + 2% scent leaf meal had the highest final weight (2257.00 g/b) while the least was recorded in birds fed a 3% neem leaf meal supplemented diet (1905.60 g/b) though similar to the positive (2034.80 g/b) and negative control (2047.40 g/b). The diet did not influence the weight gain of birds across the treatment groups. The lowest feed consumption was recorded in birds that received the blend (2679.06 g/b) while the highest was in birds fed negative control (3342.18 g/b). The birds fed a 3% neem leaf meal supplemented diet (2.10) and the blend of 2% neem leaf meal + 2% scent leaf meal (1.92) had the lowest feed conversion ratio compared to those on the other treatment

**Table 3.** Proximate composition of experimental broiler diets.

Parameters	Starter	Finisher
Dry matter (%)	92.52	91.85
Crude Protein (%)	24.68	20.53
Crude Fat (%)	4.45	4.58
Crude Fibre (%)	2.53	2.53
Ash (%)	8.33	8.23
Nitrogen Free Extract (%)	52.53	55.98
Metabolizable Energy (kcal/kg)	2923.00	3067.00

**Table 4.** Growth performances of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination.

Growth period	Growth parameter	Treatment diet					SEM	p-value
		PC+0%LM	NC+0%LM	3%NLM	3%SLM	2% NLM + 2%SLM		
Day 0-21	Initial weight (g/b)	40.18	40.66	40.18	40.52	40.24	0.12	0.6323
	Final weight (g/b)	734.24 <sup>b</sup>	742.56 <sup>b</sup>	500.74 <sup>c</sup>	771.84 <sup>b</sup>	859.73 <sup>a</sup>	26.23	<0.0001
	Weight gain (g/b)	694.06 <sup>b</sup>	701.90 <sup>b</sup>	460.56 <sup>c</sup>	731.32 <sup>b</sup>	819.49 <sup>a</sup>	26.21	<0.0001
	Feed intake (g/b)	1897.56 <sup>a</sup>	1727.80 <sup>b</sup>	1601.70 <sup>c</sup>	1767.38 <sup>b</sup>	1495.75 <sup>d</sup>	28.92	<0.0001
	Feed conversion ratio	2.74 <sup>b</sup>	2.49 <sup>b</sup>	3.49 <sup>a</sup>	2.42 <sup>b</sup>	1.83 <sup>c</sup>	0.12	<0.0001
	Liveability (%)	98.32	99.16	100	100	100	0.6	0.213
Day 21-42	Initial weight (g/b)	734.24 <sup>b</sup>	742.56 <sup>b</sup>	500.74 <sup>c</sup>	771.84 <sup>b</sup>	859.73 <sup>a</sup>	26.23	<0.0001
	Final weight (g/b)	2034.80 <sup>bc</sup>	2047.40 <sup>bc</sup>	1905.60 <sup>c</sup>	2088.40 <sup>b</sup>	2257.00 <sup>a</sup>	43.3	0.0017
	Weight gain (g/b)	1300.56	1304.84	1404.86	1316.56	1397.27 <sup>a</sup>	22.33	0.3882
	Feed intake (g/b)	3165.79 <sup>b</sup>	3342.18 <sup>a</sup>	2950.75 <sup>c</sup>	3144.15 <sup>b</sup>	2679.06 <sup>d</sup>	49.58	<0.0001
	Feed conversion ratio	2.42 <sup>a</sup>	2.58 <sup>a</sup>	2.10 <sup>b</sup>	2.41 <sup>a</sup>	1.92 <sup>b</sup>	0.06	0.0001
	Liveability (%)	99.16	98.32	97.48	95.82	97.48	1.06	0.2802
Day 0-42	Initial weight (g/b)	40.18	40.66	40.18	40.52	40.24	0.1186	0.6323
	Final weight (g/b)	2034.80 <sup>bc</sup>	2047.40 <sup>bc</sup>	1905.60 <sup>c</sup>	2088.40 <sup>b</sup>	2257.00 <sup>a</sup>	30.7617	0.0017
	Weight gain (g/b)	1994.62 <sup>bc</sup>	2006.74 <sup>b</sup>	1865.42 <sup>c</sup>	2047.88 <sup>b</sup>	2216.76 <sup>a</sup>	30.7835	0.0017
	Feed intake (g/b)	5038.35 <sup>a</sup>	5069.97 <sup>a</sup>	4552.47 <sup>b</sup>	4911.52 <sup>a</sup>	4174.81 <sup>c</sup>	72.9276	<0.0001
	Feed conversion ratio	2.53 <sup>a</sup>	2.54 <sup>a</sup>	2.44 <sup>a</sup>	2.41 <sup>a</sup>	1.88 <sup>b</sup>	0.0552	<0.0001
	Liveability (%)	99.16	98.32	97.48	95.82	97.48	1.0593	0.2802

<sup>a,b,c,d</sup>: Means with different superscripts on the same row are significantly ( $P < 0.05$ ) different. SEM- Standard Error of Mean ASTT<sub>1</sub>-Positive Control (0% Leaf Meal/Vaccinated); T<sub>2</sub>-Negative Control (0% Leaf Meal/unvaccinated); T<sub>3</sub>-3% Neem Leaf Meal/unvaccinated; T<sub>4</sub>-3% Scent Leaf Meal/unvaccinated; T<sub>5</sub>-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated

groups. However, there was no significant difference in the livability across the treatments.

The overall growth performance result shows that final weight, body weight gain, and feed intake and feed conversion ratio were significantly ( $p < 0.05$ ) influenced by the dietary treatments. Birds fed with the combination of 2% neem leaf meal + 2% scent leaf meal supplemented diet had the highest final weight (2257.00 g/b) and weight gain (2216.76 g/b) compared to other treatment groups.

Feed intake was significantly ( $p < 0.05$ ) higher in birds fed positive control (5038.35 g/b), negative control (5069.97 g/b) and 2% scent leaf meal (4911.52 g/b) diets respectively while the least was recorded for birds fed the combination of neem and scent leaf meals (4174.81 g/b).

Similarly, the lowest feed conversion ratio (1.88) was recorded in birds on the blend. There were no significant differences in the livability across the treatments.

#### Haematological parameters of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination (Day 0 to 21)

Table 5 represents the results of the haematological indices of birds at the starter phase. Diets had to influence on the red blood cell, monocyte, eosinophil and basophil counts of birds across the treatment groups. The treatment groups fed a 3% scent leaf meal diet (22.00%) had the

**Table 5.** Haematological parameters of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination (Day 0 to 21).

Parameters	Treatments					SEM	p-value
	PC+0%LM	NC+0%LM	3%NLM	3%SLM	2% NLM+2%SLM		
Packed cell volume (%)	25.20 <sup>ab</sup>	24.00 <sup>bc</sup>	24.40 <sup>ab</sup>	22.00 <sup>c</sup>	26.60 <sup>a</sup>	0.72	0.004
Haemoglobin (g/dL)	8.20 <sup>ab</sup>	8.20 <sup>ab</sup>	8.00 <sup>b</sup>	7.00 <sup>c</sup>	8.80 <sup>a</sup>	0.21	<0.001
Red blood cell ( $\times 10^6/\mu\text{L}$ )	2.00	4.40	2.60	1.80	3.00	0.98	0.374
White blood cell ( $\times 10^6/\mu\text{L}$ )	12.29 <sup>c</sup>	13.35 <sup>a</sup>	13.20 <sup>ab</sup>	12.77 <sup>bc</sup>	12.97 <sup>ab</sup>	0.17	0.002
Platelet ( $\times 10^4/\mu\text{L}$ )	12.30 <sup>a</sup>	12.82 <sup>a</sup>	13.02 <sup>a</sup>	12.68 <sup>a</sup>	11.08 <sup>b</sup>	3759.26	0.012
Lymphocytes (%)	57.60 <sup>a</sup>	57.20 <sup>a</sup>	56.80 <sup>a</sup>	53.60 <sup>b</sup>	59.20 <sup>a</sup>	0.84	0.003
Neutrophil (%)	34.20 <sup>bc</sup>	34.00 <sup>bc</sup>	36.20 <sup>b</sup>	38.40 <sup>a</sup>	32.20 <sup>c</sup>	0.90	0.001
Monocyte (%)	3.60	4.00	2.80	3.60	4.20	0.45	0.229
Eosinophils (%)	4.40	4.40	4.20	4.20	4.20	0.45	0.958
Basophils (%)	0.20	0.40	0.00	0.20	0.20	0.19	0.697

<sup>abc</sup> Means on the same row with different superscripts are significantly ( $p < 0.05$ ) different, SEM- Standard Error of Mean, PC: Positive Control, NC: Negative Control, LM: Leaf Meal, NLM: Neem Leaf Meal, SLM: Scent Leaf Meal.

**Table 6.** Haematological parameters of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination (Day 22 to 42).

Parameters	Treatments					SEM	p-value
	PC+0%LM	NC+0%LM	3%NLM	3%SLM	2%NLM+2%SLM		
Packed cell volume (%)	20.80 <sup>d</sup>	28.80 <sup>b</sup>	24.40 <sup>c</sup>	36.00 <sup>a</sup>	31.00 <sup>b</sup>	1.07	<0.001
Haemoglobin (g/dL)	6.40 <sup>e</sup>	9.60 <sup>c</sup>	8.20 <sup>d</sup>	13.00 <sup>a</sup>	10.40 <sup>b</sup>	0.21	<0.001
Red blood cell ( $\times 10^6/\mu\text{L}$ )	2.00 <sup>c</sup>	3.00 <sup>b</sup>	2.80 <sup>b</sup>	3.60 <sup>a</sup>	3.00 <sup>b</sup>	0.14	<0.001
White blood cell ( $\times 10^6/\mu\text{L}$ )	12.99 <sup>d</sup>	14.69 <sup>c</sup>	13.32 <sup>d</sup>	18.98 <sup>a</sup>	16.30 <sup>b</sup>	248.09	<0.001
Platelet ( $\times 10^4/\mu\text{L}$ )	13.54 <sup>c</sup>	13.50 <sup>c</sup>	14.10 <sup>c</sup>	20.18 <sup>a</sup>	16.70 <sup>b</sup>	0.52	<0.001
Lymphocytes (%)	53.00 <sup>c</sup>	61.60 <sup>b</sup>	54.50 <sup>c</sup>	60.20 <sup>a</sup>	63.00 <sup>a</sup>	1.60	<0.001
Neutrophil (%)	40.00 <sup>a</sup>	31.40 <sup>c</sup>	38.20 <sup>ab</sup>	33.60 <sup>b</sup>	30.20 <sup>c</sup>	1.80	0.005
Monocyte (%)	3.20 <sup>ab</sup>	2.80 <sup>b</sup>	2.60 <sup>b</sup>	3.80 <sup>a</sup>	3.40 <sup>ab</sup>	0.30	0.045
Eosinophils (%)	3.60	3.80	4.50	2.00	3.40	0.56	0.012
Basophils (%)	0.20	0.40	0.20	0.40	0.00	0.20	0.601

<sup>abdec</sup> Means on the same row with different superscript are significantly ( $p < 0.05$ ) different, SEM- Standard Error of Mean, PC: Positive Control, NC: Negative Control, LM: Leaf Meal, NLM: Neem Leaf Meal, SLM: Scent Leaf Meal.

lowest packed cell volume values though similar to those on the negative control diet (24.00%). Similarly, those that received a 3% scent leaf meal supplemented diet (7.00 g/dL) had the least haemoglobin compared with other treatment groups. The WBC count was highest in the negative control (13.35  $\times 10^6/\mu\text{L}$ ) and lowest in the positive control group (12.29  $\times 10^6/\mu\text{L}$ ) while birds fed a 3% neem leaf meal diet and those fed a combination of 2% neem leaf meal + 2% scent leaf meal were similar but significantly higher than the negative control group. The least platelet counts (11.08  $\times 10^4/\mu\text{L}$ ) were recorded in birds placed on the blend of 2% neem leaf meal + 2% scent leaf meal while the 3% scent leaf meal group recorded the lowest lymphocyte counts (53.60%). The neutrophil count was highest in birds fed 3% scent leaf meal (38.40%) and lowest in those fed a combination of 2% neem leaf meal + 2% scent leaf meal (32.20%) though similar to the positive (34.20%) and negative (34.00%) control groups.

#### Haematological parameters of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination (Day 22 to 42)

The haematological parameters of birds at the finisher phase are presented in Table 6. The birds fed 3% scent leaf meal had the highest packed cell volume (36.00%), haemoglobin (13.00g/dL), red blood cell (3.60  $\times 10^6/\mu\text{L}$ ), white blood cell (18.98  $\times 10^6/\mu\text{L}$ ) and platelet (20.18  $\times 10^4/\mu\text{L}$ ) values compared with other treatment groups. Lymphocyte counts of birds that received the blend of 2% neem leaf meal + 2% scent leaf meal (63.00%) were higher than the other treatments. The neutrophil count of birds fed the positive control (40.00%) was significantly higher ( $p < 0.05$ ) than those fed the negative control (31.40%). The monocyte counts of birds fed the positive control (3.20%) and the blend of 2% neem leaf meal + 2% scent leaf meal (3.40%) supplemented diet were similar to

**Table 7.** Serum biochemical parameters of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination.

Growth period	Serum biochemical parameter	Treatment diet					SEM	p-value
		PC+0%LM	NC+0%LM	3%NLM	3%SLM	2%NLM + 2%SLM		
Day 0-21	Total protein (g/dL)	2.97 <sup>d</sup>	3.09 <sup>cd</sup>	3.61 <sup>a</sup>	3.35 <sup>b</sup>	3.30 <sup>bc</sup>	0.07	<0.001
	Albumin (g/dL)	1.64 <sup>b</sup>	1.63 <sup>b</sup>	1.68 <sup>ab</sup>	1.84 <sup>a</sup>	1.74 <sup>ab</sup>	0.05	0.010
	Globulin (g/dL)	1.33 <sup>b</sup>	1.46 <sup>b</sup>	1.94 <sup>a</sup>	1.52 <sup>b</sup>	1.56 <sup>b</sup>	0.11	0.013
	Creatinine (mg/dL)	0.68 <sup>a</sup>	0.65 <sup>a</sup>	0.69 <sup>a</sup>	0.57 <sup>b</sup>	0.71 <sup>a</sup>	0.02	0.001
	Cholesterol (mg/dL)	112.13 <sup>b</sup>	140.09 <sup>a</sup>	116.98 <sup>b</sup>	118.43 <sup>b</sup>	127.06 <sup>ab</sup>	5.62	0.012
	High density lipoprotein (mg/dL)	97.50 <sup>b</sup>	96.58 <sup>b</sup>	59.76 <sup>c</sup>	95.77 <sup>b</sup>	112.59 <sup>a</sup>	3.18	<0.001
	Low density lipoprotein (mg/dL)	33.71 <sup>b</sup>	47.24 <sup>a</sup>	43.49 <sup>a</sup>	37.48 <sup>a</sup>	27.52 <sup>c</sup>	1.13	<0.001
	AST (IU/L)	108.18 <sup>c</sup>	96.22 <sup>d</sup>	139.01 <sup>a</sup>	125.34 <sup>b</sup>	149.05 <sup>a</sup>	3.629	<0.001
	ALT (IU/L)	24.77 <sup>b</sup>	27.23 <sup>b</sup>	24.85 <sup>b</sup>	22.12 <sup>c</sup>	35.30 <sup>a</sup>	0.861	<0.001
ALP (IU/L)	20.70 <sup>bc</sup>	24.03 <sup>a</sup>	24.42 <sup>a</sup>	21.66 <sup>b</sup>	19.81 <sup>c</sup>	0.456	<0.001	
Day 21 to 42	Total protein (g/dL)	3.35 <sup>c</sup>	3.73 <sup>b</sup>	3.71 <sup>b</sup>	4.20 <sup>a</sup>	3.89 <sup>b</sup>	0.1	<0.001
	Albumin (g/dL)	1.68 <sup>b</sup>	1.63 <sup>b</sup>	1.83 <sup>a</sup>	1.53 <sup>c</sup>	1.50 <sup>c</sup>	0.03	<0.001
	Globulin (g/dL)	1.68 <sup>d</sup>	2.10 <sup>c</sup>	1.88 <sup>cd</sup>	2.67 <sup>a</sup>	2.39 <sup>b</sup>	0.09	<0.001
	Creatinine (mg/dL)	0.73 <sup>b</sup>	0.79 <sup>a</sup>	0.68 <sup>c</sup>	0.81 <sup>a</sup>	0.54 <sup>d</sup>	0.02	<0.001
	Cholesterol (mg/dL)	144.42 <sup>a</sup>	150.14 <sup>a</sup>	126.73 <sup>b</sup>	128.21 <sup>b</sup>	137.09 <sup>ab</sup>	6.71	0.018
	High density lipoprotein (mg/dL)	107.20 <sup>a</sup>	106.32 <sup>a</sup>	79.26 <sup>c</sup>	95.62 <sup>b</sup>	94.35 <sup>b</sup>	4.48	<0.001
	Low density lipoprotein (mg/dL)	49.41 <sup>b</sup>	57.24 <sup>a</sup>	49.44 <sup>b</sup>	57.68 <sup>a</sup>	43.42 <sup>c</sup>	1.63	<0.001
	AST (IU/L)	200.44	202.63	215.54	182.9	201.57	18.453	0.808
	ALT (IU/L)	26.05 <sup>b</sup>	30.42 <sup>a</sup>	22.30 <sup>c</sup>	20.67 <sup>c</sup>	15.99 <sup>d</sup>	0.937	<0.001
ALP (IU/L)	21.66 <sup>a</sup>	22.86 <sup>a</sup>	20.84 <sup>ab</sup>	13.51 <sup>c</sup>	19.13 <sup>b</sup>	0.687	<0.001	

<sup>a,b,c,d</sup>: Means with different superscripts on the same row are significantly ( $P < 0.05$ ) different. SEM- Standard Error of Mean AST-Aspartate Aminotransferase; ALT- Alanine Aminotransferase; ALP-Alkaline Phosphatase; T<sub>1</sub>-Positive Control (0% Leaf Meal/Vaccinated); T<sub>2</sub>-Negative Control (0% Leaf Meal/unvaccinated); T<sub>3</sub>-3% Neem Leaf Meal/unvaccinated; T<sub>4</sub>-3% Scent Leaf Meal/unvaccinated; T<sub>5</sub>-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated.

other treatment groups. Birds fed 3% neem leaf meal had the highest ( $p < 0.05$ ) eosinophil counts (4.50%). The basophil count was not significantly different among the treatment groups.

#### Serum biochemical indices of broiler chickens fed diets supplemented with neem and scent leaf meals with or without vaccination

Table 7 shows the serum biochemical parameters of experimental birds at the starter phase (day 0 to 21). The result shows that the broiler chickens fed with 3% neem leaf meal (3.61g/dL) supplemented diet had the highest total protein compared to other treatment groups. The albumin values of birds fed 3% neem leaf meal (1.68 g/dL) and the blend of 2% neem leaf meal + 2% scent leaf meal (1.74 g/dL) were identical with other treatment groups. The highest ( $p < 0.05$ ) globulin (1.94 g/dL) was found in the 3% neem leaf meal group while the least creatinine (0.57 mg/dL) was recorded in the 3% scent leaf meal group. The negative control group had the least aspartate aminotransferase (96.22 iu/L). The alanine aminotrans-

ferase (22.12 iu/L) was lowest in the scent leaf meal group. Also, the highest ( $p < 0.05$ ) alkaline phosphatase was recorded in the negative control group (24.03 iu/L) and 3% neem leaf meal group (24.42 iu/L).

There were significant ( $p < 0.05$ ) differences in the serum total cholesterol, low density lipoprotein and high density lipoprotein across the treatments. The cholesterol value of the birds fed the blend of 2% neem leaf meal + 2% scent leaf meal (127.06 mg/dL) was similar to other treatment groups. The highest high-density lipoprotein (112.59 mg/dL) was observed in the blend while the least was found in the 3% neem leaf meal group (59.76 mg/dL). The lowest (27.57mg/dL) low-density lipoprotein was observed among birds fed with the combined 2% neem + 2% scent leaf meals while the highest low-density lipoprotein was recorded among birds fed the negative control diet (47.24 mg/dL) though similar to those that received 3% neem leaf meal (43.49 mg/dL).

The result of serum metabolites of birds fed supplemented diets of neem and scent leaf meals diets at the finisher phase (day 22-42) shows significant ( $p < 0.05$ ) differences in the serum biochemical parameters measured except for aspartate aminotransferase which

**Table 8.** Haemagglutination Inhibition antibody titres against Newcastle disease virus.

Age group (Days)	Treatment	Number of samples	Antibody titres obtained by HI test								Mean $\pm$ SE
			(2 <sup>1</sup> )	(2 <sup>2</sup> )	(2 <sup>3</sup> )	(2 <sup>4</sup> )*	(2 <sup>5</sup> )	(2 <sup>6</sup> )	(2 <sup>7</sup> )	(2 <sup>8</sup> )	
1-21	T1	5	-	-	2	-	-	3	-	-	41.60 $\pm$ 13.7171
	T2	5	-	-	2	-	-	3	-	-	41.60 $\pm$ 13.7171
	T3	5	-	-	2	-	3	-	-	-	22.40 $\pm$ 5.8787
	T4	5	-	-	-	-	-	5	-	-	64.00 $\pm$ 0.0000
	T5	5	-	2	3	-	-	-	-	-	6.40 $\pm$ 0.9797
22-42	T1	5	-	-	-	3	2	-	-	-	22.40 $\pm$ 3.9191
	T2	5	-	-	-	5	-	-	-	-	16.00 $\pm$ 0.0000
	T3	5	-	-	-	-	5	-	-	-	32.00 $\pm$ 0.0000
	T4	5	-	-	-	-	5	-	-	-	32.00 $\pm$ 0.0000
	T5	5	-	3	2	-	-	-	-	-	5.60 $\pm$ 0.9797

HI- Haemagglutination inhibition; \* - Protective threshold, T<sub>1</sub>-Positive Control (0% Leaf Meal/Vaccinated); T<sub>2</sub>-Negative Control (0% Leaf Meal/unvaccinated); T<sub>3</sub>-3% Neem Leaf Meal/unvaccinated; T<sub>4</sub>-3% Scent Leaf Meal/unvaccinated; T<sub>5</sub>-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated; SE-Stardard Error.

**Table 9.** Agar Gel Precipitation Test (AGPT) for IBDV at Day 21 and 42.

Treatment	T1	T2	T3	T4	T5
Day 21	-	-	-	-	-
Number of samples	5	5	5	5	5
Day 42	+	+	+	+	+
Number of samples	5	5	5	5	5

Negative to AGPT (-), Positive to AGPT (+), T<sub>1</sub>-Positive Control (0% Leaf Meal/Vaccinated); T<sub>2</sub>-Negative Control (0% Leaf Meal/unvaccinated); T<sub>3</sub>-3% Neem Leaf Meal/unvaccinated; T<sub>4</sub>-3% Scent Leaf Meal/unvaccinated; T<sub>5</sub>-2% Neem Leaf Meal + 2% Scent Leaf Meal/unvaccinated.

did not differ significantly across the treatments. The highest ( $p < 0.05$ ) total protein (4.20 g/dL) and globulin (2.67 g/dL) were observed among birds fed 3% scent leaf meal and the least was observed among birds fed positive control (3.35 g/dL and 1.68 g/dL), respectively. The highest ( $p < 0.05$ ) albumin (1.83g/dL) was recorded in birds fed neem leaf diet. The lowest creatinine level (0.54 mg/dL) was recorded in the blend treatment group. Birds ( $p < 0.05$ ) fed the blend of 2% neem + 2% scent leaf meals showed the lowest (22.12 iu/L) level of alanine aminotransferase. The least alkaline phosphatase level (13.51 iu/L) was recorded in the scent leaf meal group. The cholesterol level (1.83 mg/dL) in the blend group was similar to the other treatment groups. The lowest high density lipoprotein was recorded in birds fed a 3% neem leaf meal supplemented diet. Birds fed with combined 2% neem + 2% scent leaf meals had the least (43.42 mg/dL) low density lipoprotein.

#### Haemagglutination Inhibition antibody titres against Newcastle disease virus

The specific antibody level of the experimental broiler

chickens against the Newcastle Disease Virus is shown in Table 8. At day 21, about 3 out of 5 samples representing 60% of the samples in the positive and negative control groups and the group fed 3% neem leaf meal fell under protective antibody level against Newcastle Disease (ND). About 100% of the samples in the treatment fed 3% scent leaf meal indicated protective antibody levels against the ND. At day 42, all the samples (100%) in the positive control group, the negative control, treatment group fed 3% neem leaf meal and those fed 3% scent leaf meal had specific antibody titres that can prevent Newcastle Disease Virus.

#### Agar Gel Precipitation Test (AGPT) for IBDV at Day 21 and 42

The results of the agar gel precipitation test (AGPT) on day 21 and day 42 are presented in Table 9. All the samples tested negative for the presence of Infectious Bursal Disease (IBD) antibody on day 21. However, at day 42, all the samples across the 5 treatments tested positive for the presence of IBD antibody.

## DISCUSSION

The results obtained from this study revealed that the supplementation of neem and scent leaf meals in the diets of the experimental birds at the starter and finisher phase had a positive effect on the performance of the birds than the control groups. The birds fed with the combined 2% neem + 2% scent leaf meals had the highest weight gain and the best feed conversion ratio in the overall growth performance results. This result is in agreement with the observation of Nweze (2020) who fed Marshal white broiler chicks with scent and neem leaf extracts and reported that the weight gain of the chicks on both extracts was higher than the control group and the mortality rate of the chicks on scent leaf extract was less than chicks without the scent leaf extract. The results of the present study are consistent with the findings of Wankar *et al.* (2012) who reported a significant improvement in the average body weight when adding neem leaves powder of 1, 2, and 3 g/kg to the broiler diet. Alabi and Chime (2021) equally reported that scent leaf has high mineral and vitamin contents and can increase the digestibility of diets and consequently increase the utilization of the feed as indicated by the feed conversion ratio. Al-Harathi (2015) also reported improved growth performance of broiler chickens fed scent leaf supplemented diets. Biswas *et al.* (2020) averred that extracts from the leaves mixture of neem, tulsi, and ginger could serve as an alternative to antibiotic growth promoters in broiler chickens.

The observed improved weight gains of the birds fed with the combination of 2% neem + 2% scent leaf meals from this study could be attributed to the antimicrobial, digestion-stimulating and anti-bacterial properties of neem and scent leaves which help to improve the feed consumption and feed conversion efficiency of the birds (Prasannabalaji *et al.*, 2012). The feed intake of the birds fed with 3% neem leaf meal and a combination of neem and scent leaf meals at the end of the starter and finisher phases, was significantly low compared to birds in other treatments which can be related to the bitter taste of the neem leaf. Meanwhile, the birds in this treatment group had the improved weight gain and lowest feed conversion ratio. This agrees with the observations of Onyimanyi *et al.* (2009) that supplementation of neem leaf meal at 0.5% inclusion level gave better growth performance even with low feed intake because the active components of neem leaf which enhanced proper assimilation of digestive nutrients for improved growth of broiler chickens. Similar views were expressed by Iheukwumere *et al.* (2008). The feed conversion ratio was significantly lower among birds fed combined 2% neem + 2% scent leaf meals in the overall performance, this could be attributed to the synergistic effect of the active ingredient (azadirachtin) in neem and active biological ingredient (Eugenol {1-hydroxy-2-methoxy-4-allylbenzene}) in scent leaf.

Haematological variables give insight into the physiological state of animals in terms of blood cell

formation and function. Normal haematological parameters of an animal are a direct indication of the absence of disease (Olafedehan, 2010). Birds fed a combination of neem and scent leaf meals at the starter phase had significantly higher PCV and Hb than birds in other treatments. Nayaka *et al.* (2013) and Khulbey *et al.* (2015) found significantly higher values of Hb and PCV in broiler chickens due to supplementation of neem leaf powder at 0.8 and 0.2% levels respectively. The increase in Hb content in treated groups was reported to be due to the blood purifying properties of neem (Bhowmik *et al.*, 2010) or due to the mineral content specifically Fe, Mg, Zn, Cu, Ca and P in neem leaves (Atangwho *et al.*, 2009) which were important for synthesis of haemoglobin. Hossain *et al.* (2021) also reported a significantly higher PCV in birds fed diets containing neem and scent leaves. Haematological parameters of birds at the finisher phase showed that birds fed scent leaf meal had a significantly higher PCV, Hb, RBC and WBC than birds in other treatment groups. The results of this present study are in tandem with the findings of Muthusamy *et al.* (2017) who reported a significant increase in the haemoglobin, packed cell volume, erythrocyte and leucocyte counts of layers in the treatment group supplemented with a blended herbal preparation (*Ocimum sanctum*, *Zingiber officinale*, *Allium sativum*, *Trigonella foenum graecum* and *Curcuma longa*) at 0.25 and 0.5% in basal feed as compared to the control group. Jeba *et al.* (2011) reported that *Ocimum sanctum* enhances the production of RBC and Hb. A remarkable increase in the WBC and platelets counts of birds fed scent leaf supplemented diets at the finisher phase agrees with the findings of Gole (2001) who reported an increase in total leukocyte count in broilers supplemented with 1% *Ocimum sanctum* and liquid preparation of vitamin E and Se. Naeem *et al.* (2021) had a similar report. This suggests that scent leaf enhanced birds' cellular immune organs and boosted their immunity level against infectious diseases. Singh and Doley (2014) reported that scent leaf improved immune responses which might be due to the immunostimulatory effects of eugenol and other essential oils present in *Ocimum sanctum*. However, Hossain *et al.* (2021) reported no significant difference in the RBC counts of broilers fed scent leaf diet. Lymphocytes are known to be the main effector cells of the immune system while eosinophils are commonly believed to play a role in destroying invading pathogens with immune regulatory properties (Jacobsen *et al.*, 2012). In this study, birds fed a combination of neem and scent leaves had significantly higher lymphocytes than birds in other treatments. However, birds fed neem leaf meal diet singly had a significantly higher eosinophil at the finisher phase compared to other treatment groups. Thange (2009) observed a non-significant effect of *Ocimum sanctum* and other herbal preparations on monocytes and eosinophils of broilers. Variations in results might be due to differences in strains of birds and differences in the percentage inclusion of test ingredients in the diet. No significant

difference in the monocytes, eosinophils and basophils of birds at the starter phase agrees with the work of Egbeyale *et al.* (2018) who recorded no significant difference in the monocytes, eosinophils and basophils of broilers fed neem leaf extracts.

Serum biochemical investigations have been explored extensively to distinguish normal states from stress and disease conditions in animals. In the current study, a significant increase in the total protein and albumin of birds fed scent leaves is in accordance with the report of Muthusamy *et al.* (2017) who averred an increase in albumin and total protein of layers in the treatment group supplemented with a blended herbal preparation (*Ocimum sanctum*, *Zingiber officinale*, *Allium sativum*, *Trigonella foenum* and *Curcuma longa*) at 0.25 and 0.5% in basal feed as compared to control group. Similarly, Obikaonu *et al.* (2011) reported an elevated serum protein level in birds fed neem leaf meal and this finding agrees with the result of this study. These results indicate that supplemented herbal ingredients may improve the immune response as globulin level has been used as an indicator of immune responses and a source of antibody production. A significant increase in the total protein and globulin levels of birds fed neem leaf at the starter phase and albumin level at the finisher phase agrees with the findings of Shihab *et al.* (2017) who reported that supplementation of neem leaf powder at the rate of 0.1 and 0.2% in the basal diet increased the levels of total protein, albumin, and globulin significantly as compared to the control group.

Birds fed scent leaf-based diet had a significantly lower ALT both at the starter and finisher phases with birds at the starter phase also having a lower AST. This report was in concurrence with the work of Arivuchelvan and Murugesan (2020). These results indicate that scent leaf supplementations decreased serum liver leakage enzymes, and liver toxicity and may enhance liver functions by improving cell membrane permeability (Mathivanan and Edwin, 2012). The positive effects of scent leaf powder on liver functions could be due to its antimicrobial and antioxidant activities (Grashorn, 2010). Eugenol and methyl eugenol present in *Ocimum sanctum* could have been responsible for its hepatoprotective properties (Govind and Madhuri, 2010) and the restoration of ALT and AST levels. Pingale *et al.* (2010) also reported that *Ocimum sanctum* has a high potential in healing liver parenchyma as well as regeneration of liver cells.

Birds fed neem and scent leaf meal singly had higher HI titre values which was above the protective threshold. The result of the present study is similar to the findings of Sadekar *et al.* (1998) who reported that feeding powdered neem leaves to broilers significantly enhanced antibody titers against the Newcastle Disease Virus (NDV) antigen. Similarly, Pande and Vijay (1994) and Pande (2000) also reported significantly improved antibody titres against Newcastle disease in broilers treated with Zeetress (a polyherbal combination of *Withania somnifera* (aswaghandha), *Ocimum sanctum* (scent) and *Embllica*

*officinalis* (amla). Neem is an herbal plant which is known to exhibit various beneficial pharmacological properties including immunomodulatory effects in broilers (Upadhyay *et al.*, 1992). The inclusion of scent improved HI titer value hence increasing the immunity status. Herbs can influence selectively the microorganism by an antimicrobial activity thus favouring better nutrient utilization and absorption or the stimulation of the immune system (Wenk, 2003). In the present study, supplementation diets with scent leaf improved immune responses which might be due to the immunostimulatory effect of eugenol and other essential oils present in scent leaf (Sen, 1993).

Birds fed neem and scent leaf meal either singly or in combination had antibodies against Infectious Bursal Disease (IBD). This result is in tandem with the work of Vishwas *et al.* (2021) who reported a numerical increase in the antibody titre of birds fed neem, ginger and garlic powder. Jawad *et al.* (2013) who supplemented neem leaves at the rate of 2, 4 and 6 % observed significant improvement in the antibody titre against the IBD in the groups fed with neem leaf-based diet compared to the control group at days 14, 21 and 42. This result validates the immunomodulatory and immunopotential properties of both neem and scent leaves (Upadhyay *et al.*, 1992; Jeba *et al.*, 2011).

## Conclusion

The results of this study showed that supplementation of neem (*Azadirachta indica*) and scent (*Ocimum sanctum*) leaf meals as feed additives improved the performance and haemato-biochemical indices of broiler chickens. The birds were found to be in good health with no signs of anaemia which revealed the valuable and significant effects of these herbs. It also proves that the test ingredients are not toxic. Neem and scent leaf meals also conferred immunity against Newcastle Disease and Infectious Bursal Disease when included singly.

## Recommendation

Further research should be carried out to evaluate the interactions between the active ingredients in *A. indica* (Azadirachtin) and *O. sanctum* (Eugenol) and other phytonutrients on the physicochemical and organoleptic properties of broiler chicken meat fed the leaf meals.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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