



## Evaluation of the Effects of Clove Essential Oil on Antibody, Haematological and Serum Biochemistry of Cockerels Challenged with Newcastle Disease Virus

Ifeoma Chinyere Ugwu<sup>1\*</sup>, Emmanuel Chukwudi Okwor<sup>1</sup>, Patience Chinasa Eze<sup>1</sup>, Amarachukwu Olejieme Igwe<sup>2</sup>, Ifeanyi Onyema<sup>1</sup>, Thaddeus Ofilibe Okeja<sup>1</sup>, Wilson Uchenna Anike<sup>3</sup>, Chidozie Clifford Ugwu<sup>4</sup> and Didacus Chukwuemeka Eze<sup>1</sup>

<sup>1</sup>Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

<sup>2</sup>Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

<sup>3</sup>Department of Veterinary Pathology and Microbiology, Federal University of Agriculture, Makurdi, Nigeria

<sup>4</sup>Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria

\*Corresponding author: Ifeoma Chinyere Ugwu, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria, Tel: +2348034480707; E-mail: ifeoma.cugwu@unn.edu.ng

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### Abstract

Newcastle Disease (ND) is an important disease of poultry worldwide. It is a devastating disease that may cause up to 100% mortality in susceptible chickens. The aim of this study was to investigate the effects of oral administration of clove essential oil on blood biochemical and hematological parameters and on antibody responses of chickens to ND challenge. A total of 120 white leghorn chicks were obtained at days old. They were randomly allocated into four groups of 30 chicks each. Groups I, II and III chicks were treated with 330 Pass Per Million (PPM) of clove essential oil through drinking water while group IV chicks were not treated. At 6 weeks of age, chicks in groups I, II, III and IV were inoculated intramuscularly in the breast muscle with 0.2 ml of NDV Kudu 113 containing  $10^{8.46}$  embryo infective dose 50% end point per ml. On day 24 post challenge, the results of the mean total white blood cell counts were  $1.50 \pm 0.27$ ,  $3.00 \pm 0.91$ ,  $1.88 \pm 0.23$  and  $1.00 \pm 0.00$  for groups A, B, C and D respectively. The mean haemagglutination inhibition titres of chicks in group B were significantly ( $P \leq 0.05$ ) higher than that of groups D but not significantly ( $P \geq 0.05$ ) different from those of groups A and C chicks. Clove essential oil had significantly high enhancement effects on antibody responses but no significant effects on serum creatinine and uric acid concentration, serum enzymes (Alkaline Phosphatase-ALP, Alanine Aminotransferase-ALT and Aspartate Transaminase-AST), serum proteins (total protein,

albumins and globulin levels), serum calcium and phosphorus concentrations, total red blood cell, packed cell volume and differential leucocytes counts.

**Keywords:** Antibody; Blood cells; Clove essential oil; Newcastle disease virus; Serum enzymes; Cockerels

### Introduction

Newcastle Disease (ND) as defined during the office des epizootics 67<sup>th</sup> general session in May 1999 is an infection of avian that has Avian Paramyxovirus (APMV) serotype-1 (APMV-1) as the aetiologic agent [1,2]. It is among the viral diseases of poultry that has an economic importance [3]. Newcastle disease affects domestic poultry and other avian species of all ages and gender [4-6]. It is among the diseases causing threat to poultry farming production due of large economic losses globally [7,8]. The disease affects almost all the systems and organs of the host [9]. There are focal annual outbreaks of ND primarily among chickens, pigeons and other domesticated birds [10]. The disease has become endemic in poultry population with annual outbreak thereby inflicting heavy losses [11]. The disease can be controlled by vaccination of which immune response of chickens to the vaccines and subsequent development of antibodies against the virus is essential.

Essential oils increase secretion and absorption of nutrients from the digestive systems decrease the microbial load in the gut, exert antioxidant properties and increase the immunity of the animal [12]. Essential oil from clove is known to have a great antimicrobial attributes [13]. Clove has also been reported to have antiseptic, anti-inflammatory and antioxidant properties. It can also serve as appetite and digestion stimulant [14,15]. To our knowledge, there is no report of antibody enhancement property of cloves essential oil in chickens. The aim of this study was to investigate the effects of oral administration of clove essential oil on blood biochemical and hematological parameters and on antibody responses of chickens challenged with newcastle disease virus.

### Materials and Methods

#### Newcastle disease virus inoculum

Velogenic NDV (KUDU strain) obtained from national veterinary research institute, Vom, Plateau state, Nigeria was used. It was reconstituted and administered as recommended by the research institute. Each chicken in the selected group was inoculated with 0.2 ml of NDV kudu 113 strain containing  $10^{8.46}$  EID<sub>50</sub>/ml intramuscularly in the breast muscle.

#### Chickens and management

One hundred and twenty (120) one day old white leghorn chicks were at random allocated into four groups of 30 chicks each; and were housed separately on deep litter system. The birds were not vaccinated against newcastle disease. Each chicken in groups I, II and III were treated with 330 Parts Per Million (PPM) of clove essential oil through drinking water while group IV was not treated. Group I chicks were treated for two weeks before NDV inoculation; group II chicks were treated from two weeks before inoculation to two weeks after

inoculation; group III chicks were treated for two weeks immediately after inoculation while group IV were not treated. At 6 weeks of age, chickens in groups I, II, III and IV were inoculated intramuscularly with NDV kudu 113 viruses. Feed and water were provided ad libitum.

### Haemagglutination inhibition test

On days 0, 5, 10, 17, 24 and 31 Post Inoculation (PI), anti-NDV antibody titers were determined using haemagglutination inhibition test. The HI titers were conducted according to standard procedures [16]. Controls were included as a positive serum, a negative serum, erythrocytes and antigens. The endpoint was regarded as the highest dilution of serum causing complete inhibition. The geometric mean titer as documented by villages and purchase was recorded as reciprocal log<sub>2</sub> values of the highest dilution that showed haemagglutination inhibition [17].

### Determination of serum biochemical parameters

On days 0, 5, 10, 17, 24 and 31 pi, five chicks from each group were randomly selected for sampling for the evaluation of blood biochemical parameters. Blood samples were collected from wing vein and stored at the room temperature for 30 minutes and then centrifuged for 15 min at 3000 rpm to separate the serum. The serum samples were then poured into eppendorf tube and stored at -20°C until needed for assays. Serum AST, ALT, ALP, calcium and phosphorus concentrations, serum proteins, uric acid and creatinine clearance test were determined calorimetrically using an auto-analyzer system (bio systems SA, Costa Brava 30, 08030 Barcelona, Spain) with aid of commercially available kits (bio systems co. Spain).

### Determination of hematological parameters

On days 0, 5, 10, 17, 24 and 31 pi, 2 ml of blood were collected from each of the four birds from each group into a sample bottle containing Ethylene Diamine Tetra Acetic acid (EDTA) for the haematological analysis. The Packed Cell Volume (PCV) was determined by the microhaematocrit method [18]. The Hemoglobin Concentration (HBC) was determined by the cyanomethaemoglobin method [19]. The Total Leukocyte Count (TLC) were carried out by the haemocytometer method, while the differential leukocyte count smears were stained by the leishman technique and counted with a differential cell counter [20].

### Data analysis

Data obtained were analyzed using one way Analysis of Variance (ANOVA) and means separated with Least Significant Difference (LSD) method on SPSS for windows version 16.0. Significance was accepted at P<0.05.

## Results

### Serology

The results of the mean haemagglutination inhibition titres were presented on Table 1. The mean haemagglutination inhibition titres of chickens in group B was significantly ( $P \leq 0.05$ ) higher than that of groups D but not significantly ( $P \geq 0.05$ ) different from those of groups A and C chickens.

	A	B	C	D
0	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>	0 ± 0.00 <sup>a</sup>
5	2.00 ± 0.60 <sup>a</sup>	2.13 ± 0.30 <sup>a</sup>	1.50 ± 0.65 <sup>a</sup>	0.75 ± 0.37 <sup>a</sup>
10	1.25 ± 0.25 <sup>a</sup>	1.75 ± 0.49 <sup>a</sup>	1.63 ± 0.32 <sup>a</sup>	1.13 ± 0.13 <sup>a</sup>
17	1.25 ± 0.16 <sup>a</sup>	1.50 ± 0.19 <sup>a</sup>	1.50 ± 0.19 <sup>a</sup>	1.25 ± 0.25 <sup>a</sup>
24	1.50 ± 0.27 <sup>ab</sup>	3.00 ± 0.91 <sup>b</sup>	1.88 ± 0.23 <sup>b</sup>	1.00 ± 0.00 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05; Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 1:** The mean haemagglutination inhibition titre of chickens treated with clove essential oil and experimental challenge with vNDV.

### Total white blood cell counts

The results of the mean Total White Blood Cell Counts (TWBC) were presented on Table 2. TWBC count of chicks in group A was significantly ( $P \leq 0.05$ ) higher than those of groups B and C but not significantly ( $P \geq 0.05$ ) different from that of group D chicks. The TWBC count of chicks in group A was significantly ( $P \leq 0.05$ ) higher than those of groups B, C and D. The TWBC count of chicks in group B was significantly ( $P \leq 0.05$ ) higher than that of group C. There were

no significant ( $P \geq 0.05$ ) differences in the TWBC counts of chicks among groups A, B and D. On day 17 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the TWBC counts of chicks among groups A, B, C and D. On day 24 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the TWBC counts of chicks among groups A, B, C and D.

	A	B	C	D
0	68.00 ± 6.31 <sup>b</sup>	30.63 ± 6.88 <sup>b</sup>	40.63 ± 4.83 <sup>b</sup>	60.88 ± 4.44 <sup>a</sup>
5	99.30 ± 4.68 <sup>a</sup>	72.00 ± 4.26 <sup>b</sup>	62.20 ± 3.10 <sup>bc</sup>	57.50 ± 5.86 <sup>c</sup>
10	109.80 ± 11.03 <sup>a</sup>	122.80 ± 10.72 <sup>a</sup>	60.10 ± 7.67 <sup>b</sup>	110.90 ± 5.67 <sup>a</sup>

17	102.00 ± 12.14 <sup>a</sup>	87.00 ± 5.23 <sup>a</sup>	96.40 ± 5.97 <sup>a</sup>	104.40 ± 2.23 <sup>a</sup>
24	95.90 ± 12.41 <sup>a</sup>	94.00 ± 7.02 <sup>a</sup>	88.00 ± 4.93 <sup>a</sup>	85.70 ± 7.21 <sup>a</sup>

<sup>a, b, c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05. Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 2:** Total white blood cell count (10<sup>3</sup>/μl) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Absolute eosinophil counts

The results of the Mean Absolute Eosinophil (MAE) counts were presented on Table 3. There were no significant (P ≥ 0.05) differences in the MAE counts among the groups. The MAE count of chicks in group D was significantly (P ≤ 0.05) higher than those of groups A, B and C. On day 10 after challenge, the MAE count of chicks in group B

was significantly (P ≤ 0.05) higher than those of groups A, C and D. On day 17 after challenge, there were no significant (P ≥ 0.05) differences in the MAE counts among groups A, B, C and D. On day 24 after challenge, there were no significant (P ≥ 0.05) differences in the MAE counts among groups A, B, C and D.

	A	B	C	D
0	0.00 ± 0.00 <sup>a</sup>	0.1 ± 0.10 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
5	0.22 ± 0.22 <sup>a</sup>	0.13 ± 0.13 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
10	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
17	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
24	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>

<sup>a, b, c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05. Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 3:** Absolute basophils counts (10<sup>3</sup>/μl) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Absolute heterophil counts

The results of the Mean Absolute Heterophil (MAH) counts were presented on Table 4. The MAH count of chicks in group A was significantly (P ≤ 0.05) higher than those of groups B and C but not significantly (P ≥ 0.05) different from that group D chicks. There was no significant (P ≥ 0.05) difference in the MAH counts between groups B and D. On day 5 post challenge, there were no significant (P ≥ 0.05) differences in the MAH counts among groups A, B, C and D. On day 10 post challenge, the MAH count of chicks in group D was significantly (P ≤ 0.05) higher than that of group C but not significant

(P ≥ 0.05) different from those of groups A and B chicks. There was no significant (P ≥ 0.05) difference in the MAH counts between groups A and B. On day 17 post challenge, there were no significant (P ≥ 0.05) differences in the MAH counts among groups A, B, C and D. On day 24 post challenge, the MAH count of chicks in group D was significantly (P ≤ 0.05) higher than those of groups A and B but not significant (P ≥ 0.05) different from that of group C chicks. There were no significant (P ≥ 0.05) differences in the MAH counts among groups A, B and C.

	A	B	C	D
0	5.54 ± 0.95 <sup>a</sup>	3.49 ± 0.55 <sup>a</sup>	2.73 ± 0.28 <sup>a</sup>	2.86 ± 1.55 <sup>a</sup>
5	4.02 ± 1.45 <sup>ab</sup>	3.63 ± 0.63 <sup>ab</sup>	2.83 ± 0.82 <sup>a</sup>	6.19 ± 1.00 <sup>b</sup>
10	3.22 ± 0.42 <sup>a</sup>	5.89 ± 1.23 <sup>b</sup>	2.36 ± 0.46 <sup>a</sup>	2.20 ± 0.32 <sup>a</sup>
17	7.14 ± 2.60 <sup>a</sup>	5.95 ± 2.10 <sup>a</sup>	5.50 ± 1.74 <sup>a</sup>	4.80 ± 0.91 <sup>a</sup>
24	6.36 ± 2.49 <sup>a</sup>	4.27 ± 0.18 <sup>a</sup>	7.53 ± 2.21 <sup>a</sup>	7.24 ± 1.82 <sup>a</sup>

<sup>a, b, c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05. Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 4:** Absolute eosinophils counts (10<sup>3</sup>/μl) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Absolute lymphocyte counts

The results of the Absolute Lymphocyte (ALC) counts were presented on Table 5. The ALC of chicks in group A was significantly (P ≤ 0.05) higher than that of group C but not significantly (P ≥ 0.05) different from those of groups B and D chicks. On day 5 post challenge, the ALC of chicks in group A was significantly (P ≤ 0.05) higher than those of groups B, C and D. There were no significant (P ≥ 0.05) differences in the ALC among groups B, C and D. On day 10

post challenge, the ALC of chicks in group B was significantly (P ≤ 0.05) higher than those of group A, C and D. However, there was no significant (P ≥ 0.05) difference in the ALC between groups A and D but their ALC were significantly (P ≤ 0.05) higher than that of group C chicks (Table 6). On day 17 post challenge, the ALC of chicks in group D was significantly (P ≤ 0.05) higher than those of group A, B and C. There were no significant (P ≥ 0.05) differences in the ALC

counts among groups A, B and C. On day 24 post challenge, the ALC of chicks in group B was significantly ( $P \leq 0.05$ ) higher than those of groups A, C and D. Also, ALC of chicks in group A was significantly ( $P \leq 0.05$ ) higher than that of group D. There was no significant ( $P \geq 0.05$ ) difference in the ALC between groups C and D.

	A	B	C	D
0	15.74 ± 2.22 <sup>a</sup>	10.07 ± 0.58 <sup>b</sup>	10.24 ± 1.28 <sup>b</sup>	12.21 ± 1.20 <sup>ab</sup>
5	23.77 ± 6.97 <sup>a</sup>	23.39 ± 6.80 <sup>a</sup>	22.45 ± 4.93 <sup>a</sup>	26.2 ± 4.56 <sup>a</sup>
10	34.05 ± 8.42 <sup>ab</sup>	31.19 ± 4.10 <sup>ab</sup>	23.68 ± 5.45 <sup>a</sup>	44.99 ± 4.81 <sup>b</sup>
17	49.48 ± 3.02 <sup>a</sup>	46.10 ± 1.35 <sup>a</sup>	41.89 ± 5.59 <sup>a</sup>	37.08 ± 4.34 <sup>a</sup>
24	18.75 ± 5.01 <sup>a</sup>	15.21 ± 4.60 <sup>a</sup>	25.61 ± 4.19 <sup>ab</sup>	38.38 ± 5.38 <sup>b</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 5:** Differential Heterophil counts ( $10^3$ )  $\mu$ l of chickens treated with clove essential oil and experimental challenge with vNDV.

	A	B	C	D
0	36.88 ± 3.67 <sup>a</sup>	26.63 ± 5.90 <sup>ab</sup>	21.32 ± 2.40 <sup>b</sup>	32.20 ± 2.22 <sup>ab</sup>
5	49.77 ± 7.71 <sup>a</sup>	33.85 ± 1.79 <sup>b</sup>	28.46 ± 1.30 <sup>b</sup>	23.65 ± 1.10 <sup>b</sup>
10	42.67 ± 4.55 <sup>a</sup>	64.01 ± 3.67 <sup>b</sup>	22.76 ± 3.10 <sup>c</sup>	46.78 ± 3.92 <sup>a</sup>
17	40.71 ± 6.82 <sup>ab</sup>	29.50 ± 2.22 <sup>a</sup>	39.65 ± 5.27 <sup>ab</sup>	52.96 ± 2.67 <sup>b</sup>
24	59.75 ± 5.64 <sup>ab</sup>	66.14 ± 4.89 <sup>a</sup>	48.49 ± 4.23 <sup>bc</sup>	34.78 ± 4.08 <sup>c</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 6:** Differential lymphocytes counts ( $10^3$ )  $\mu$ l of chickens treated with clove essential oil and experimental challenge with vNDV.

### Mean absolute monocyte counts

The results of the mean Absolute Monocyte Counts (AMC) were presented on Table 7. The AMC count of chicks in group A was significantly ( $P \leq 0.05$ ) higher than those of groups B and C but not significant ( $P \geq 0.05$ ) different from that of group D chicks. Also, there was no significant ( $P \geq 0.05$ ) difference in the AMC between group B and C chicks. On day 5 post challenge, the AMC of chicks in group A was significantly ( $P \leq 0.05$ ) higher than those of groups B, C and D. There were no significant ( $P \geq 0.05$ ) differences in the AMC among groups B, C and D chicks. On day 10 post challenge, the AMC of chicks in group A was significantly ( $P \leq 0.05$ ) higher than those of groups C and D but not significant ( $P \geq 0.05$ ) different from that of

group B chicks. The AMC of chicks in group B was significantly ( $P \leq 0.05$ ) higher than that of groups C but not significantly ( $P \geq 0.05$ ) different from that of group D chicks. On day 17 post challenge, the AMC of chicks in group D was significantly ( $P \leq 0.05$ ) higher than those of groups A and B but not significant ( $P \geq 0.05$ ) different from that of group C chicks. There were no significant ( $P \geq 0.05$ ) differences in the AMC among groups A, B and C chicks. On day 24 post challenge, the AMC of chicks in group A was significantly ( $P \leq 0.05$ ) higher than those of groups C and D but not significant ( $P \geq 0.05$ ) different from that of group B chicks. There were no significant ( $P \geq 0.05$ ) differences in the AMC among groups B, C and D chicks.

	A	B	C	D
0	11.19 ± 1.60 <sup>a</sup>	5.22 ± 0.60 <sup>b</sup>	4.83 ± 0.35 <sup>b</sup>	10.28 ± 1.65 <sup>a</sup>
5	19.66 ± 4.27 <sup>a</sup>	11.13 ± 2.13 <sup>b</sup>	8.45 ± 2.27 <sup>b</sup>	4.54 ± 0.45 <sup>b</sup>
10	29.63 ± 4.58 <sup>a</sup>	23.98 ± 5.30 <sup>ab</sup>	11.07 ± 2.98 <sup>c</sup>	15.60 ± 2.16 <sup>bc</sup>
17	4.69 ± 1.28 <sup>a</sup>	5.46 ± 0.71 <sup>a</sup>	7.93 ± 1.75 <sup>ab</sup>	10.24 ± 1.19 <sup>b</sup>
24	10.72 ± 1.63 <sup>a</sup>	8.39 ± 1.08 <sup>ab</sup>	6.38 ± 1.37 <sup>b</sup>	5.30 ± 0.65 <sup>b</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 7:** Absolute monocytes counts ( $10^3/\mu$ l) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Red blood cells ( $10^6/\mu$ l)

The results of the mean Red Blood Cell counts (RBC) were presented on Table 8. There were no significant ( $P \geq 0.05$ ) differences

in the RBC counts among groups A, B, C and D. On day 5 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the RBC



counts among groups A, B, C and D. On day 10 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the RBC among groups A, B, C and D. On day 17 post challenge, the RBC of chicks in group C was significantly ( $P \leq 0.05$ ) higher than those of groups A, B and D.

However, there were no significant ( $P \geq 0.05$ ) differences in the RBC among groups A, B and D. On day 24 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the RBC among groups A, B, C and D.

	A	B	C	D
0	3.33 ± 0.15	3.24 ± 0.17	3.12 ± 0.09	3.30 ± 0.29
5	2.98 ± 0.16	3.15 ± 0.14	3.13 ± 0.18	3.21 ± 0.23
10	2.98 ± 0.42	3.36 ± 0.34	3.56 ± 0.31	3.63 ± 0.27
17	3.24 ± 0.29	3.33 ± 0.24	4.28 ± 0.18	3.57 ± 0.19
24	3.60 ± 0.22	3.82 ± 0.13	3.47 ± 0.19	3.70 ± 0.21

**Table 8:** Red blood cells ( $10^6/\mu\text{l}$ ) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Packed cell volume (%)

The results of the mean Packed Cell Volume (PCV) were presented on Table 9. There were no significant ( $P \geq 0.05$ ) differences in the PCV among groups A, B, C and D. On day 5 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the PCV among groups A, B, C and D. On day 10 post challenge, the PCV of chicks in group A was significantly ( $P \leq 0.05$ ) higher than that of group B but not significant ( $P \geq 0.05$ ) different from those of groups C and D chicks.

There were no significant ( $P \geq 0.05$ ) differences in the PCV among groups B, C and D. On day 17 post challenge, the PCV of chicks in group C was significantly ( $P \leq 0.05$ ) higher than those of groups A, B and D. There were no significant ( $P \geq 0.05$ ) differences in the PCV among groups A, B and D. On day 24 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the PCV among groups A, B, C and D.

	A	B	C	D
0	27.80 ± 0.96 <sup>a</sup>	29.80 ± 1.27 <sup>a</sup>	29.20 ± 0.82 <sup>a</sup>	29.50 ± 1.35 <sup>a</sup>
5	25.40 ± 1.91 <sup>a</sup>	27.30 ± 1.49 <sup>a</sup>	28.90 ± 1.57 <sup>a</sup>	29.70 ± 1.51 <sup>a</sup>
10	32.60 ± 1.44 <sup>a</sup>	28.50 ± 0.50 <sup>b</sup>	31.20 ± 0.72 <sup>ab</sup>	30.30 ± 0.66 <sup>ab</sup>
17	28.40 ± 1.36 <sup>a</sup>	29.40 ± 0.94 <sup>a</sup>	34.20 ± 1.52 <sup>b</sup>	29.80 ± 0.70 <sup>a</sup>
24	30.40 ± 1.16 <sup>a</sup>	31.90 ± 1.17 <sup>a</sup>	29.20 ± 0.78 <sup>a</sup>	30.90 ± 1.10 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 9:** Packed cell volume (%) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Haemoglobin concentration (g/dl)

The results of the Mean Haemoglobin Concentration (MHC) were presented on Table 10. There were no significant ( $P \geq 0.05$ ) differences in the MHC among groups A, B, C and D. On day 5 post challenge, the MHC of chicks in group B chicks was significantly ( $P \leq 0.05$ ) higher than that of groups A, but not significantly ( $P \geq 0.05$ ) different from MHC of those of groups C and D chicks. There were no significant ( $P \geq 0.05$ ) differences in the MHC among groups A, C and D.

On day 10 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the MHC among groups A, B, C and D. On day 17 post challenge, MHC of chicks in group C chicks was significantly ( $P \leq 0.05$ ) higher than that of groups A, but not significant ( $P \geq 0.05$ ) different from MHC of those of groups B and D chicks. On day 24 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the MHC among groups A, B, C and D.

	A	B	C	D
0	8.32 ± 0.51 <sup>a</sup>	8.23 ± 0.18 <sup>a</sup>	8.47 ± 0.40 <sup>a</sup>	8.69 ± 0.13 <sup>a</sup>
5	8.18 ± 0.15 <sup>a</sup>	9.06 ± 0.29 <sup>b</sup>	8.75 ± 0.22 <sup>ab</sup>	8.58 ± 0.30 <sup>ab</sup>
10	7.80 ± 0.44 <sup>a</sup>	8.08 ± 0.48 <sup>a</sup>	8.60 ± 0.52 <sup>a</sup>	9.21 ± 0.41 <sup>a</sup>
17	7.64 ± 0.47 <sup>a</sup>	8.78 ± 0.43 <sup>ab</sup>	10.19 ± 1.09 <sup>b</sup>	8.89 ± 0.33 <sup>ab</sup>
24	9.05 ± 0.44 <sup>a</sup>	10.07 ± 0.40 <sup>a</sup>	9.02 ± 0.25 <sup>a</sup>	10.05 ± 0.51 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 10:** Haemoglobin (g/dl) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Serum proteins

**Total protein concentrations (g/dl):** The results of the mean total protein concentrations were presented on Table 11. On day 0 post challenge, the results of the mean total protein concentrations were  $2.95 \pm 0.07$ ,  $3.03 \pm 0.13$ ,  $3.26 \pm 0.08$  and  $3.16 \pm 0.15$  for groups A, B,

C and D respectively. There were no significant ( $P \geq 0.05$ ) differences in the mean total protein concentrations among groups A, B, C and D on day 0, 5, 10, 17 and 24 post challenges.

	A	B	C	D
0	$2.95 \pm 0.07^a$	$3.03 \pm 0.13^a$	$3.26 \pm 0.08^a$	$3.16 \pm 0.15^a$
5	$3.21 \pm 0.06^a$	$3.20 \pm 0.27^a$	$3.29 \pm 0.15^a$	$3.19 \pm 0.21^a$
10	$4.40 \pm 0.13^a$	$5.09 \pm 0.19^a$	$4.95 \pm 0.34^a$	$4.45 \pm 0.32^a$
17	$3.58 \pm 0.23^a$	$3.86 \pm 0.14^a$	$4.26 \pm 0.20^a$	$3.85 \pm 0.25^a$
24	$3.93 \pm 0.21^a$	$3.79 \pm 0.14^a$	$3.93 \pm 0.22^a$	$3.76 \pm 0.10^a$

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 11:** Total protein concentrations (g/dl) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Serum albumin concentration (g/dl):** The results of the mean albumin concentrations were presented on Table 12. On day 0 post challenge, the results of the mean albumin concentrations were  $2.61 \pm 0.08$ ,  $2.57 \pm 0.04$ ,  $2.63 \pm 0.08$  and  $2.72 \pm 0.12$  for groups A, B, C and

D respectively. There were no significant ( $P \geq 0.05$ ) differences in the mean total albumin concentrations among groups A, B, C and D on day 0, 5, 10, 17 and 24 post challenges.

	A	B	C	D
0	$2.61 \pm 0.08^a$	$2.57 \pm 0.04^a$	$2.63 \pm 0.08^a$	$2.72 \pm 0.12^a$
5	$2.42 \pm 0.09^a$	$2.38 \pm 0.14^a$	$2.41 \pm 0.13^a$	$2.37 \pm 0.13^a$
10	$2.28 \pm 0.07^a$	$2.34 \pm 0.05^a$	$2.32 \pm 0.07^a$	$2.22 \pm 0.10^a$
17	$2.38 \pm 0.08^a$	$2.40 \pm 0.09^a$	$2.47 \pm 0.06^a$	$2.49 \pm 0.10^a$
24	$2.74 \pm 0.08^a$	$2.74 \pm 0.06^a$	$2.72 \pm 0.05^a$	$2.71 \pm 0.08^a$

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 12:** Albumins concentration (g/dl) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Serum globulins concentration (g/dl):** The results of the mean globulin concentrations were presented on Table 13. On day 0 post challenge, the results of the mean globulin concentrations were  $0.34 \pm 0.04$ ,  $0.45 \pm 0.15$ ,  $0.63 \pm 0.11$  and  $0.44 \pm 0.04$  for groups A, B, C and D respectively. There were no significant ( $P \geq 0.05$ ) differences in the

mean total albumin concentrations among groups A, B, C and D on day 0, 5, 10, and 17 post challenges. However, the mean globulin concentration of chicks in group C chicks was significantly ( $P \leq 0.05$ ) higher than that of group A at day 17 post challenge. On day 24 post challenge, there were no significant ( $P \geq 0.05$ ) differences in the mean globulin concentrations among groups A, B, C and D chicks.

	A	B	C	D
0	$0.34 \pm 0.04^a$	$0.45 \pm 0.15^a$	$0.63 \pm 0.11^a$	$0.44 \pm 0.04^a$
5	$0.78 \pm 0.10^a$	$0.82 \pm 0.15^a$	$0.89 \pm 0.04^a$	$0.82 \pm 0.10^a$
10	$2.11 \pm 0.07^a$	$2.76 \pm 0.19^a$	$2.63 \pm 0.29^a$	$2.23 \pm 0.25^a$
17	$1.21 \pm 0.18^a$	$1.46 \pm 0.15^{ab}$	$1.79 \pm 0.19^b$	$1.40 \pm 0.16^{ab}$
4	$1.19 \pm 0.21^a$	$1.05 \pm 0.12^a$	$1.21 \pm 0.20^a$	$1.28 \pm 0.02^a$

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 13:** Globulins concentration (g/dl) of chickens treated with clove essential oil and experimental challenge with vNDV.

### Serum enzymes

**Alkaline Phosphatase (ALP) IU/L:** The results of the mean alkaline phosphatase concentrations were presented on Table 14.

There were no significant ( $P \geq 0.05$ ) differences in the mean alkaline phosphatase concentrations among groups A, B, C and D chicks on day 0, 5, 10, 17 and 24 post challenges.

	A	B	C	D
0	376.02 ± 4.48 <sup>a</sup>	380.50 ± 7.03 <sup>a</sup>	386.23 ± 3.29 <sup>a</sup>	385.00 ± 2.73 <sup>a</sup>
5	520.26 ± 7.05 <sup>a</sup>	496.17 ± 32.69 <sup>a</sup>	509.01 ± 24.82 <sup>a</sup>	524.11 ± 12.22 <sup>a</sup>
10	332.31 ± 38.09 <sup>a</sup>	392.31 ± 19.91 <sup>a</sup>	350.55 ± 38.41 <sup>a</sup>	348.22 ± 44.91 <sup>a</sup>
17	383.78 ± 8.14 <sup>a</sup>	395.44 ± 1.37 <sup>a</sup>	375.36 ± 8.42 <sup>a</sup>	374.13 ± 16.35 <sup>a</sup>
24	475.85 ± 29.16 <sup>a</sup>	519.02 ± 7.02 <sup>a</sup>	473.28 ± 21.55 <sup>a</sup>	416.55 ± 52.87 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05. Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 14:** Alkaline phosphatase (IU/L) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Alanine Aminotransferase (ALT) IU/L:** The results of the Mean Alanine Aminotransferase Concentrations (MAAC) were presented on Table 15. The results of the MAAC were 2.92 ± 0.85, 3.81 ± 0.57, 2.57 ± 0.88 and 1.21 ± 0.50 for groups A, B, C and D respectively. The MAAC concentration of chicks in group B chicks was significantly (P ≤ 0.05) higher than that of group D on day 0 post challenge. On day 10 post challenge, the MAAC of chicks in group B chicks was

significantly (P ≤ 0.05) higher than those of groups C and D chicks, but not significant (P ≥ 0.05) different from MAAC of that of group A chicks. On day 17 post challenge, there were no significant (P ≥ 0.05) differences in the MAAC among groups A, B, C and D. On day 24 post challenge, the MAAC of chicks in group A chicks was significantly (P ≤ 0.05) higher than that of groups D, but not significant (P ≥ 0.05) different from the MAAC of those of groups B and C chicks.

	A	B	C	D
0	2.92 ± 0.85 <sup>ab</sup>	3.81 ± 0.57 <sup>a</sup>	2.57 ± 0.88 <sup>ab</sup>	1.21 ± 0.50 <sup>b</sup>
5	2.46 ± 1.16 <sup>a</sup>	1.72 ± 0.50 <sup>a</sup>	2.85 ± 0.25 <sup>a</sup>	1.49 ± 0.62 <sup>a</sup>
10	5.06 ± 0.76 <sup>ab</sup>	6.22 ± 1.03 <sup>a</sup>	3.59 ± 0.53 <sup>b</sup>	3.69 ± 0.45 <sup>b</sup>
17	7.58 ± 0.68 <sup>a</sup>	7.72 ± 0.79 <sup>a</sup>	7.48 ± 0.37 <sup>a</sup>	6.08 ± 0.87 <sup>a</sup>
24	4.49 ± 0.58 <sup>a</sup>	4.31 ± 0.33 <sup>a</sup>	4.23 ± 0.22 <sup>a</sup>	2.80 ± 0.50 <sup>b</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05. Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 15:** Alanine aminotransferase (IU/L) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Aspartate Aminotransferase (AST) UI/L:** The results of the Mean Aspartate Aminotransferase Concentrations (MAsp) were presented on Table 16. The MAsp of chicks in group C was significantly (P ≤ 0.05) higher than those of group A and B chicks,

but not significant (P ≥ 0.05) different from MAsp of that of group D on day 10 post challenge but there were no significant (P ≥ 0.05) differences among groups A, B, C and D chicks on day 0, 5, 17 and 24 post challenge.

	A	B	C	D
0	47.82 ± 3.12 <sup>a</sup>	51.30 ± 5.65 <sup>a</sup>	45.07 ± 1.30 <sup>a</sup>	43.83 ± 3.18 <sup>a</sup>
5	82.57 ± 4.73 <sup>a</sup>	79.20 ± 5.66 <sup>a</sup>	78.98 ± 4.41 <sup>a</sup>	74.43 ± 5.89 <sup>a</sup>
10	81.98 ± 3.75 <sup>ab</sup>	72.36 ± 5.64 <sup>a</sup>	99.00 ± 3.79 <sup>c</sup>	88.10 ± 4.99 <sup>bc</sup>
17	74.11 ± 2.47 <sup>a</sup>	71.36 ± 1.69 <sup>a</sup>	78.07 ± 3.80 <sup>a</sup>	70.30 ± 4.03 <sup>a</sup>
24	67.79 ± 4.04 <sup>a</sup>	67.14 ± 4.62 <sup>a</sup>	75.73 ± 4.16 <sup>a</sup>	72.72 ± 4.75 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means; P<0.05. Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 16:** Aspartate transaminase (IU/L) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Serum creatinine (µg/dl):** The results of the Mean Serum Creatinine Concentrations (MSCC) were presented on Table 17. On day 0 and 5 post challenge, there were no significant (P ≥ 0.05) differences in the MSCC among groups A, B, C and D chicks. On day 10 post challenge, the MSCC of chicks in group A was significantly

(P ≤ 0.05) higher than that of group D chicks, but not significant (P ≥ 0.05) different from the MSCC of those of groups B and C chicks. On day 17 post challenge, it was significantly (P ≤ 0.05) higher than those of groups B and D chicks, but not significant (P ≥ 0.05) different from

the MSCC of that of group C chicks. On day 24 post challenge, the MSCC of chicks in group B was significantly ( $P \leq 0.05$ ) higher than that of group D chicks, but not significant ( $P \geq 0.05$ ) different from the MSCC of those of groups A and C chicks.

	A	B	C	D
0	0.37 ± 0.05 <sup>a</sup>	0.38 ± 0.04 <sup>a</sup>	0.39 ± 0.05 <sup>a</sup>	0.34 ± 0.05 <sup>a</sup>
5	0.34 ± 0.04 <sup>a</sup>	0.43 ± 0.02 <sup>a</sup>	0.43 ± 0.05 <sup>a</sup>	0.46 ± 0.06 <sup>a</sup>
10	0.35 ± 0.03 <sup>a</sup>	0.32 ± 0.04 <sup>ab</sup>	0.30 ± 0.01 <sup>ab</sup>	0.26 ± 0.01 <sup>b</sup>
17	0.51 ± 0.03 <sup>a</sup>	0.34 ± 0.05 <sup>b</sup>	0.46 ± 0.04 <sup>ab</sup>	0.37 ± 0.05 <sup>b</sup>
24	0.47 ± 0.03 <sup>a</sup>	0.49 ± 0.05 <sup>a</sup>	0.48 ± 0.01 <sup>a</sup>	0.35 ± 0.03 <sup>b</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 17:** Serum creatinine ( $\mu\text{g/dl}$ ) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Serum uric acid ( $\mu\text{g/dl}$ ):** The results of the Mean Serum Uric Acid concentrations (MSUA) were presented on Table 18. There were no significant ( $P \geq 0.05$ ) differences in the MSUA among groups A, B, C and D chicks on day 0, 17 and 24 post challenges. On day 5 post challenge, the MSUA of chicks in group D was significantly ( $P \leq 0.05$ ) higher than those of groups B and C chicks, but not significant ( $P \geq 0.05$ )

different from the MSUA of that of group A chicks. There were no significant ( $P \geq 0.05$ ) differences in the MSUA among groups A, B and C chicks. On day 10 post challenge, the MSUA of chicks in group D was significantly ( $P \leq 0.05$ ) higher than that of groups B chicks, but not significant ( $P \geq 0.05$ ) different from the MSUA of those of groups A and C chicks. There were no significant ( $P \geq 0.05$ ) differences in the MSUA between groups A and B chicks.

	A	B	C	D
0	6.59 ± 1.38 <sup>a</sup>	6.34 ± 1.12 <sup>a</sup>	7.59 ± 1.48 <sup>a</sup>	4.60 ± 0.37 <sup>a</sup>
5	6.25 ± 0.67 <sup>ab</sup>	5.33 ± 0.50 <sup>a</sup>	4.37 ± 0.89 <sup>a</sup>	8.16 ± 0.85 <sup>b</sup>
10	5.57 ± 1.24 <sup>ab</sup>	4.50 ± 0.30 <sup>a</sup>	12.79 ± 3.71 <sup>b</sup>	13.55 ± 3.35 <sup>b</sup>
17	7.98 ± 1.65 <sup>a</sup>	5.94 ± 0.73 <sup>a</sup>	8.08 ± 1.33 <sup>a</sup>	8.51 ± 0.68 <sup>a</sup>
24	8.43 ± 2.45 <sup>a</sup>	4.34 ± 0.69 <sup>a</sup>	7.06 ± 0.60 <sup>a</sup>	6.75 ± 0.90 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 18:** Serum uric acid ( $\mu\text{g/dl}$ ) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Calcium concentration (mg/dl):** The results of the mean Serum Calcium Concentration (SCC) were presented on Table 19. On day 0 post challenge, the SCC of chicks in group D was significantly ( $P \leq 0.05$ ) higher than that of group A chicks, but not significant ( $P \geq 0.05$ ) different from the SCC of those of groups B and C chicks. On day 5, 10 and 24 post challenges, there were no significant ( $P \geq 0.05$ )

differences in the SCC among groups A, B, C and D chicks. On day 17 post challenge, the SCC of chicks in group B was significantly ( $P \leq 0.05$ ) higher than that of group A chicks, but not significant ( $P \geq 0.05$ ) different from the SCC of those of groups C and D chicks. Also, there were no significant ( $P \geq 0.05$ ) differences in the SCC among groups A, C and D chicks.

	A	B	C	D
0	6.93 ± 0.19 <sup>a</sup>	7.01 ± 0.05 <sup>ab</sup>	7.53 ± 0.27 <sup>b</sup>	7.47 ± 0.09 <sup>b</sup>
5	6.09 ± 0.35 <sup>a</sup>	6.22 ± 0.36 <sup>a</sup>	6.24 ± 0.21 <sup>a</sup>	6.43 ± 0.29 <sup>a</sup>
10	9.57 ± 0.63 <sup>a</sup>	10.04 ± 0.32 <sup>a</sup>	10.48 ± 0.50 <sup>a</sup>	10.07 ± 0.13 <sup>a</sup>
17	7.13 ± 0.25 <sup>a</sup>	8.40 ± 0.55 <sup>b</sup>	7.46 ± 0.11 <sup>ab</sup>	7.74 ± 0.30 <sup>ab</sup>
24	9.87 ± 0.39 <sup>a</sup>	9.62 ± 0.91 <sup>a</sup>	10.65 ± 0.41 <sup>a</sup>	9.62 ± 0.24 <sup>a</sup>

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 19:** Serum calcium concentration (mg/dl) of chickens treated with clove essential oil and experimental challenge with vNDV.

**Serum phosphorus concentrations (mg/dl):** The results of the mean Serum Phosphorus Concentration (SPC) were presented on Table 20. On day 0 post challenge, the SPC of chicks in group A was

significantly ( $P \leq 0.05$ ) higher than that of group D chicks, but not significant ( $P \geq 0.05$ ) different from the SPC of those of groups B and C chicks. On day 5 post challenge, the SPC in group C chicks was

significantly ( $P \leq 0.05$ ) higher than those of groups A and B chicks, but not significant ( $P \geq 0.05$ ) different from the SPC of that of group D chicks. There were no significant ( $P \geq 0.05$ ) differences in the SPC among groups A, B and C chicks. On day 10 post challenge, the SPC of chicks in group B was significantly ( $P \leq 0.05$ ) higher than that of group A chicks. On day 17 post challenge, the SPC of group C chicks

was significantly ( $P \leq 0.05$ ) higher than that of group D chicks. On day 24 post challenge, the results of the SPC were  $3.82 \pm 0.23$ ,  $4.61 \pm 0.29$ ,  $4.48 \pm 0.27$  and  $4.67 \pm 0.33$  for groups A, B, C and D respectively. There were no significant ( $P \geq 0.05$ ) differences in SPC among groups A, B, C and D chicks.

	A	B	C	D
0	$4.47 \pm 0.17^a$	$4.22 \pm 0.24^{ab}$	$4.32 \pm 0.28^{ab}$	$3.69 \pm 0.20^b$
5	$4.66 \pm 0.27^a$	$4.92 \pm 0.34^a$	$6.24 \pm 0.23^b$	$5.30 \pm 0.39^{ab}$
10	$5.73 \pm 0.49^a$	$7.47 \pm 0.09^b$	$6.27 \pm 0.48^{ab}$	$6.22 \pm 0.43^{ab}$
17	$5.77 \pm 0.48^{ab}$	$6.08 \pm 0.60^{ab}$	$6.65 \pm 0.26^a$	$4.36 \pm 1.02^b$
24	$3.82 \pm 0.23^a$	$4.61 \pm 0.29^a$	$4.48 \pm 0.27^a$	$4.67 \pm 0.33^a$

<sup>a,b,c</sup>: Different alphabetical superscripts in a row indicate significant differences between the means;  $P < 0.05$ . Group A: Treated for 2 weeks and challenged; Group B: Treated for 2 weeks challenged and treated for another 2 weeks; Group C: Challenged and treated for two (2) weeks; Group D: Untreated and challenged.

**Table 20:** Serum phosphorus concentrations (mg/dl) of chickens treated with clove essential oil and experimental challenge with vNDV

## Discussion

The results from this study revealed that antibody responses of chicks to vvNDV challenge were unaffected ( $P > 0.05$ ) by the treatments between days 0 and 17 PC. The results also indicated that on day 24 PC with vvNDV; the mean haemagglutination inhibition titres were improved significantly in the groups which were treated with clove essential oil after challenge with vvNDV. This finding is in line with what Hashemi, et al. and Wang, et al. who revealed that clove essential oils has an antibacterial characteristic and also enhances immune system by improving the IgG synthesis in the body and IgA in the saliva. However, they are also in line with results recorded by Awaad, et al. who documented that eucalyptus and peppermint essential oils can apply to both innate-cell mediated and humoral immune response in chickens. They also reported that the administration of these volatile oils has a potent immunomodulatory effect and evoke the immune response in chickens.

The mean haemagglutination inhibition titres against ND were significantly greater in groups B and C that were treated after challenge. The findings were similar to those of Nie, et al. Chen, et al. Guo, et al. Jiang and Yu, et al. who found that supplementation of feed with essential oil extracts improved the humoral immune response against ND. Also, Zho, et al. Ilsley, et al. Kong, et al. Dong, et al. reported that inclusion of plant extracts in broiler feed increased the immune response against IBD. Ziaran, et al. equally found out that smaller doses of essential oil produced a good antibody titer against ND, while Durani, et al. reported that smaller doses of aniseed oil fed to broiler chickens showed a non-significant result on antibody titers against IBD. Nevertheless, these results were not in agreement with the results of Al-Ankari, et al. Durani, et al. Soltan, et al. who reported non-significant increase in mean haemagglutination inhibition titres ND in chicks given feed supplemented with essential oil.

The results obtained for mean red blood cell counts, mean haemoglobin concentration and mean packed cell volume in this study did not give any significant difference ( $P > 0.05$ ) between the control and clove essential oil treated groups. These findings do not agree with the observations made by Nagalakshmi, et al. Gowda, et al. who reported that essential plant oil extracts have a great effect on blood markers especially PCV and Hb of the host, putting the nutritional status into consideration.

According to the hematological findings from this study, there was no differences seen in blood parameters of chickens treated with clove essential oil, and their values were on the same range for healthy chicken. In contrast, Toghyani, et al. reported a significant rise in WBC count, hemoglobin concentration and hematocrit percentage compared to control birds in broilers fed different levels of spice seed (black cumin seed). Elnagar, et al. also observed an increase in RBC counts in pecking ducks fed garlic essential oil. Biricik, et al. recorded a good response from inclusion of myrtle oil to broiler chicken diets at doses above 1000 mg/kg on hematocrit. Toghyani, et al. reported a significant increase in hemoglobin concentration and hematocrit percentage in quails fed with black seeds (*Nigella sativa*). In other studies, there were significant increases in hematocrit values in broiler chickens fed diets supplemented with essential oil extracts gotten from clove and cinnamon, but not with thyme and Ademola, et al. reported significant increase in WBCs count in laying hens fed diet containing 200 mg/kg of dietary garlic oil. Also, Al-Kassie, et al. showed that feeding diets supplemented with thyme and cinnamon oils to broilers, significantly increased RBC, Hb and WBC values.

The results of total serum protein, serum albumin and serum globulin concentrations showed no significant differences when compared to the control in this study. This is in line with the findings of Abdel-Fattah, et al. who observed that supplementation of essential oil and volatile fatty acid blend (biacid) at different levels showed non-significant effect on serum proteins. However, the results obtained from this study, were not in agreement with the reports of AL-Kassie, et al. who observed that the addition of 200 ppm of essential oil xtracted from *C. verum*, et al. to a standard diet of broiler chicks for 42 days gave significant increases in total proteins. Also, it did not agree with the report of Abbas, et al. who recorded that dietary *T. foenum-graecum*, et al. seed extract at 3 g/kg of the diet for 42 days increased numerically the total protein in the chicks. These findings are not in harmony with the results of Abd El-Latif, et al. who stated that adding thyme extract to Japanese quail diets enhanced plasma total protein as well as albumin and globulin.

The insignificant difference in total serum protein level observed in this study was also reported by other studies with spices and herb products.



## Conclusion

Clove essential oil had highly significant effects on antibody responses in cockerels especially at 330 parts per million and had no significant effects on serum creatinine and uric acid concentration, serum enzymes (Alkaline Phosphatase-ALP, Alanine Aminotransferase-ALT and Aspartate Transaminase-AST), serum proteins (total protein, albumins and globulin levels), serum calcium and phosphorus concentrations, total red blood cell, packed cell volume and differential leucocytes counts in the chickens.

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