IMMUNOLOGIC AND HAEMATOLOGIC EFFECTS OF METHANOLIC STEM BARK EXTRACT OF *AZADIHIRACTA INDICA* ON CHICKENS EXPERIMENTALLY INFECTED WITH VELOGENIC NEWCASTLE DISEASE VIRUS (KUDU 113) STRAIN

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ABSTRACT

The study was aimed at evaluating the weight, haematologic and immunologic effects of crude methanolic stem bark extract of Azadihiracta indica stem bark on vaccinated chickens experimentally challenged with the velogenic Newcastle disease virus. One hundred day old cockerels were randomly divided into four equal groups (I, II, III and IV). Birds in all groups were vaccinated with La Sota strain of Newcastle disease vaccine. The birds were challenged with the velogenic strain of Newcastle disease virus (VNDV) at 42 days of age. Following challenge, groups I, II and III were given oral treatment of methanolic stem bark extract of Azadihiracta indica at 200mg/kg, 400mg/kg and 600mg/kg, respectively until day 56 of age, while group IV was not given the treatment. Thereafter, birds in all the groups were assessed for onset of clinical signs, changes in live body weight, humoral immune responses and haematologic changes. The birds in groups I, II and III showed no sign of Newcastle disease while birds in group IV exhibited mild depression and huddling. The mean body weight of the vaccinated-treated groups were significantly (p<0.05) higher than that of the vaccinated-untreated group on day 56 of age. On days 63 and 70 of age, the mean body weight of group III was significantly higher than groups I and II. The mean haemagglutination inhibition titres of group III was significantly higher (p<0.05) than groups I, II and IV. The mean PCV, Hb and RBC values of the vaccinated-treated group was significantly (p>0.05) higher than the vaccinated-untreated group on day 49 of age. The WBC count of groups I and II were significantly higher (p>0.05) than groups III and IV on days 49 and 56 of age. The mean absolute heterophil counts of vaccinated-treated groups was significantly (p>0.05) higher than the vaccinated-untreated group on days 56 and 63 of age. The mean absolute lymphocyte counts of the vaccinated-treated increased significantly (p>0.05) than that of vaccinated-untreated birds days 49 of age till the end of the experiment.

Keywords: *Azadihiracta indica,* Stem bark extract, Chickens, Newcastle disease, Immune response, Haematological changes

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease affecting wild and domestic avian species (Seal *et al.,* 2000, Alexander, 2003). ND is caused by an avian paramyxovirus serotype of the genus Avulavirus belonging to the family paramyxoviridae. The disease is worldwide in distribution (Alexander *et al.,* 1997). Since its first outbreak in Ibadan, Nigeria in 1952, the

disease is still one of the most important diseases of chickens in Nigeria (Hill *et al*., 1953; Ezeokoli *et al*., 1984; Ezema *et al,.* 2009).

The impact of ND is most notable in domestic poultry due to high susceptibility of poultry and the severe consequences of virulent strains on the poultry industries (Alexander, 2003). ND continues to be of serious economic threat to the poultry industry resulting in increased morbidity and mortality rates and loss of eggs for both breeding and human consumption (Abdu *et al*., 1992). ND vaccination of poultry provides an excellent means to lessen clinical signs of infection caused by virus (Alexander, 2003; Senne *et al* 2004; Kapezynzki, 2005) and in response to the threat presented by ND, several countries have put in place vaccination campaigns to prevent the epizootics. Outbreaks have been reported in vaccinated populations despite the fact that vaccination is widely applied (Burridge *et al.*, 1975) therefore; there is an increasing awareness on the use of various plants in treatment and control of animal diseases (Atawodi and Spiegelhalder, 1994).

Neem (*Azadirachta indica)* a Meliaceae family tree, is a hardy evergreen tree commonly found in South Asia and parts of Africa including Nigeria. All parts of the neem plant have been found useful in the treatment of various ailments (Subapriya and Nagini, 2005). It has been demonstrated to exhibit anti-inflammatory, antipyretic, antiarthritic (Kaur*et al.,* 2004), antihyperglycaemic (Murthy *et al*., 1978), diuretic (Binde *et al.,* 1958) and immunomodulatory properties (Arivazhagan *et al*., 2000). Neem oil has been reported to have anti-fertility properties and immune stimulating effects (Upadhyay *et al.,* 1992).

The continuous reoccurrence of Newcastle disease even in vaccinated flocks, is of the greatest challenges facing the poultry industry in Nigeria, hence the need for an alternative remedy to prevent the devastating activity of this disease on the poultry industry. In south-east Nigeria, during the harramattan period, the stem of neem is traditionally used in prevention of Newcastle disease by soaking in the drinking water of birds and it is believed that neem bark has both protective and curative property against Newcastle Disease hence the need to study the immunomodulatory effects of neem especially as an adjuvant to ND vaccination, with an objective to evaluate the effects of neem stem bark extract on the haematological indices and immune responses of chickens experimentally infected with the velogenic strain (Kudu 113) of NDV.

MATERIALS AND METHODS

Plant material: The barks of *Azadirachta indica* were collected during the months of March 2009, at Nsukka, in Enugu State, Nigeria. The plant was authenticated at the Bio-resources development and conservation programme, Nsukka, Enugu state, Nigeria. Extraction of the dried bark was performed by soaking into 80% methanol for 48 hours with intermittent shaking at room temperature (28^{o} C). The resultant extract was concentrated using a rotatory evaporator. The extract was solubilized in 5% Tween 80 and acute toxicity test of the extract was done.

Acute Toxicity Test: 20 birds were randomly divided into 4 groups (A, B, C and D) of 5 birds each. The birds in each group (A, B, C and D) were given different doses of the extract (150 mg/kg, 300 mg/kg, 600 mg/kg and 1,200 mg/kg), respectively. The birds were observed for 24hrs for signs of acute toxicity which includes depression, weakness, nervous signs, excitability and death.

Experimental Birds: A flock of 100 day old cockerels were procured from CHI hatchery in Nigeria. They were housed in an isolated pen in the Poultry Disease Research unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The birds were given hitchner B1 at day old, gumboro vaccine on day 10 and 21 while La Sota vaccine was given on day 28. The birds were fed with commercial poultry feed ad libitum and provided with drinking water.

Viral Inoculum: The viral inoculum was obtained from National Veterinary Research Institute Vom, Jos, Plateau State, Nigeria. Viral

content of an ampoule was reconstituted with 0.5ml of phosphate buffered saline (PBS). This was added to 9.5ml of PBS (1/10) and later to 10ml of PBS and then double diluted to 20ml ($ELD_{50} = 10^{5.32}$).

Experimental Challenge: The birds were randomly divided into 4 equal groups (groups I, II and III) of twenty chicks each on 28 day of age. By day 42 of age, birds in all the groups were inoculated intramuscularly with 0.2ml challenge dose of VNDV strain (Kudu 113) with titre $10^{9.5}$ per milliliter of the inoculum. Following challenge with VNDV strain, birds in groups I, II and III were given oral treatment with 200, 400 and 600mg/kg body weight of the plant extract, respectively daily for two weeks, while group IV was not treated. On days 28, 42, 49, 56 and 63 of age, blood samples were collected from each group for serology and haematology. Sera from the blood samples were stored at -20^0C until used.

Clinical Signs: The birds were clinically monitored twice daily for clinical signs of ND from day 42 till the end of the experiment. Ten birds in each group was randomly selected and live body weight taken on days 42, 49, 56, 63 and 70 of age.

Haemagglutination (HA) and Haema-gglutination Inhibition (HI) Tests: Two milliter of blood was collected from each of birds in a test tube containing EDTA as anticoagulant. The blood was washed in phosphate buffered saline (PBS) and centrifuged at 3000 rpm for 5 minutes. This was repeated until a clear supernatant was obtained. The packed red blood cells (RBC) were re-suspended in a measured volume of PBS solution to make 0.5% RBC suspension (Beard, 1989).

The antigen titre for running HI tests was determined by standard HA technique using La Sota as antigen (Alexander, 2003). The reciprocal of the highest dilution of La Sota ND antigen causing 100% agglutination of an equal volume of standardized RBCs was taken as the HA titre of the antigen. The HI titres were determined by the method of Beard (1989).

Haematology: Blood samples were collected were used in determining the haematological profile of the birds which includes; PCV was determined using the microhaematocrit method (Coles, 1986), HbC determined by the cyanomethamoglobin method (Kachamar, 1970), RBC count determined by the haemocytometer method (Schalm *et al.,* 1975) and total and differential WBC count determined using the improved Neubaur counting chamber (Campbell and Coles, 1986).

RESULTS

Toxicity of Methanolic Stem Bark Extract of *Azadihiracta indica:* The result of the acute toxicity tests showed that the extract is safe even at dose of 1200mg/kg. At all the dose levels tested, there was no death recorded within 24 hours post treatment.

Clinical Signs Induced by Methanolic Stem Bark Extract of *Azadihiracta indica:* They birds in all the groups did not show any typical sign of ND however, slight depression was observed in 2 birds on days 45 till 48 of age in group IV.

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on Body Weight of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: There was a decrease in the mean body weight of both the treated and untreated vaccinated groups on days 45 and 56 of age, however the mean body weight of the vaccinated-treated groups were significantly ($p<0.05$) higher than that of the vaccinated-untreated group on day 56 of age. On days 63 and 70 of age, the mean body weight of group III was significantly higher than groups I and II (Table I).

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on Immune Response of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: The mean haemagglutination inhibition titres (log^2) of all the groups increased on days 49 – 63 of age of the birds in all the experimental groups (Table 2), however on days 56 and 63 of age, the mean haemagglutination inhibition titres (log^2) of group III was significantly higher ($p<0.05$) than group I, II and IV.

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on PCV, Haemoglobin Concentration and RBC count of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: There was a decrease in the mean packed cell volume, haemoglobin concentration and red blood cell count in all the groups on day 49 of age, however the mean PCV of the vaccinated-treated group was significantly ($p>0.05$) higher than the vaccinated-untreated group on day 49 of age, whereas there was no significant difference ($p<0.05$) among the vaccinated-treated group till end of the experiment (Table 3). The mean haemoglobin concentration of the vaccinated-treated group was significantly ($p>0.05$) higher than the vaccinated-untreated group, however there was no significant difference on the mean haemoglobin concentration among the vaccinated-treated groups on day 49 of age. On day 63 of age the mean haemoglobin concentration of group III was higher than group I and II as well as group IV (Table 4). The mean red blood cell count of the vaccinated-untreated group were significantly ($p>0.05$) higher than the vaccinated-untreated group on day 49 of age, whereas on days 56 and 63 of age, the mean red blood cell count of group III was significantly ($p>0.05$) higher than groups I, II and IV.

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on Total leucocyte Count of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: There was an increase in the mean White blood cell count for both vaccinated-treated and vaccinated-untreated groups on days 49 and 56 of age, however the mean WBC count of group I and II were significantly higher ($p>0.05$) than groups III and IV on days 49 and 56 of age (Table 6).

Effect of Methanolic Stem Bark Extract of *Azadihiracta indica* on Differential leucocyte count of Chickens Experimentally Infected with Velogenic Newcastle Disease Virus (Kudu 113) Strain: The mean absolute heterophil counts increased in all the groups on days 49 and 56 of age, however the mean absolute heterophil counts of vaccinated-treated groups was significantly ($p>0.05$) higher than the vaccinated-untreated group on days 56 and 63 of age. The mean absolute lymphocyte counts of the vaccinated-treated increased significantly ($p>0.05$) than that of vaccinated-untreated birds days 49 of age till the end of the experiment, however the mean absolute lymphocyte count of group I of the treatment group were significantly ($p>0.05$) higher than that of group III on day 49 of age, group II and III on day 56 and II on day 63 of age (Table 8).

DISCUSSION

The significant decrease in weight observed in both the vaccinated-treated and vaccinated-untreated groups on days 49 and 56 of age are similar to reduction in weight reported by Okoye *et al.* (2000) in birds challenged with velogenic ND virus, the decrease in weight is a common occurrence in septicaemic or viraemic diseases due to reduction in feed and water intake. Decrease in weight was notably more severe in the vaccinated-untreated group than the treated vaccinated group and the group III of the vaccinated-treated group with the highest dose of the extract had the least reduction in weight, this may be due to the inhibitory property of neem on viruses observed by Waafa *et al.* (2010) in an in vitro study with neem leaf and fruit extract on ND virus, the inhibitory property of neem on the virus may have led to slight reduction in feed intake, ability of the birds to overcome the infection, and a return of feed/water intake.

The slight reduction in weight observed in the vaccinated-treated group than in the vaccinated-untreated group was also reported by Eze *et al.* (2012) in birds challenged with velogenic NDV and treated with methanolic leaf extract of *Moringa oleifera* than those challenged with the VNDV but not treated with the extract.

Following vaccination with La Sota vaccine and challenge with the VNDV strain of Kudu 113, high NDV antibody titre were observed in all the groups, this is similar to what was reported that following challenge with VNDV, that NDV HI titers are usually high (Illango and Olaho-munkini, 2005; Kakenji *et al*., 2007). The presence of high NDV antibody titres is necessary to provide long term protection against ND (Ritchie *et al*., 1994; Sa'idu *et al*., 2006; Ruwaan *et al*., 2009).

Table 1: Effect of different doses of *Azadihiracta indica* extract on body weight of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Days	I 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	393.80 ± 17.58	393.00 ± 11.59	369.00 ± 10.35	385.8 ±15.65
45	347.70 ± 12.60	348.10 ± 14.69	332.00 ± 6.60	330.80 ± 11.81
49	343.50 ±16.85	350.70 ± 16.27	340.50 ± 17.07	327.00 ± 24.38
56	480.50 ± 27.87^{b}	481.00 ± 19.13^{b}	497.00 ± 24.14^{a}	437.50 ± 29.87^{c}
63	544.00 ± 28.74^{c}	597.50 ± 19.51^{b}	620.00 ± 20.00^{a}	598.78 ± 24.18^{b}
70	607.00 ± 28.74^{c}	638.50 ± 18.64^{b}	658.00 ± 18.56^{a}	639.44 ± 17.57^{b}

**Different superscripts in a row indicate significant difference between the groups ($p<0.05$).*

Table 2: Effect of different doses of *Azadihiracta indica* extract on immune response of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Groups Days	I 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	8.00 ± 0.32	8.20 ± 0.49	8.60 ± 0.93	8.00 ± 0.32
49	8.80 ± 0.20	8.70 ± 0.32	8.90 ± 0.45	8.40 ± 0.60
56	10.00 ± 0.49^{b}	10.80 ± 0.37^{b}	11.87 ± 0.49^{a}	8.80 ± 0.32^{c}
63	11.77 ± 0.24^{b}	11.87 ± 0.37^{b}	12.60 ± 0.68^{a}	9.00 ± 0.34^{c}

*The data are given as mean haemagglutination inhibition titre (log_{2}) ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p<0.05$).*

Table 3: Effect of different doses of *Azadihiracta indica* extract on PCV of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group Days	I 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	27.88 ± 0.23	28.25 ± 0.44	28.50 ± 0.54	27.68 ± 0.54
49	27.00 ± 0.58^{a}	27.67 ± 0.88^{a}	27.33 ± 0.88^{a}	26.67 ± 0.38^{b}
56	27.67 ± 2.33	27.33 ± 0.33	27.00 ± 1.53	26.67 ± 1.45
63	27.33 ± 1.81	28.33 ± 0.33	28.33 ± 1.20	27.00 ± 1.53

*The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p<0.05$).*

Table 4: Effect of different doses of *Azadihiracta indica* on haemoglobin concentration of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group Days	II 200mg/kg	II 400mg/kg	III 600mg/kg	IV 0mg/kg
42	8.79 ± 0.02^{a}	8.70 ± 0.23^{a}	8.82 ± 0.15^{a}	8.92 ± 0.19^{a}
49	8.47 ± 0.23^{a}	8.51 ± 1.26^{a}	8.53 ± 1.11^{a}	8.27 ± 1.32^{b}
56	8.83 ± 0.33	8.86 ± 0.47	8.87 ± 0.55	8.97 ± 0.61
63	8.87^{bc} ± 1.77	8.97 ± 0.67^{bc}	9.23 ± 0.42^{a}	8.93 ± 0.26^{c}

*The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups ($p<0.05$).*

Table 5: Effect of different doses of *Azadihiracta indica* on RBC count of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	2.54 ± 0.03	2.54 ±0.03	2.50 ± 0.02	2.52 ± 0.03
49	2.38 ± 0.27	2.48 ± 0.26	2.48 ± 0.45	2.23 ± 0.62
56	2.41 ± 0.78^{b}	2.54 ± 0.07^{a}	2.62 ± 0.25^{a}	2.40 ± 0.40^{b}
63	2.55 ± 0.78^{b}	2.55 ± 0.48^{b}	2.62 ± 0.19^{a}	2.50 ± 0.14^{b}

*The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups (p<0.05)*

Table 6: The Effect of different doses of *Azadihiracta indica* extract on total leucocyte count (10³/µl of blood) of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	15.23± 0.44	15.23± 0.43	15.25± 0.24	15.22± 0.22
49	17.93 ± 2.00^{a}	17.47 ± 1.74^{b}	16.67 ± 1.57^{b}	16.53 ± 1.66^{b}
56	18.47 ± 2.06^{a}	18.07 ± 2.03^{a}	17.53 ± 1.35^{b}	17.40 ± 2.21^{b}
63	16.20 ± 1.44	16.53 ± 1.95	15.07 ± 1.79	15.30 ± 0.64

*The data are given as mean ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups (p<0.05)*

Table 7: Effect of different doses of *Azadihiracta indica* extract on heterophil count (10³/µl of blood) of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	2.87± 0.44	2.75± 0.13	2.72± 0.26	2.78±0.22
49	4.68 ± 0.65	4.94 ± 0.10	4.51 ± 0.73	4.62 ± 0.90
56	5.01 ± 1.17^{a}	4.90± 12.74^{ab}	4.63 ± 0.18^{b}	4.07 ± 1.39^{c}
63	4.34 ± 0.60^{a}	4.26 ± 0.30^{a}	4.02 ± 1.53^{b}	3.57 ± 0.56^{c}

*The data are given as mean haemagglutination inhibition titre (log_{2}) ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups (p<0.05)*

Table 8: Effect of different doses of *Azadihiracta indica* extract on absolute lymphocyte counts (10³/µl of blood) of chickens experimentally infected with velogenic Newcastle disease virus (Kudu 113) strain

Group	I	II	III	IV
Days	200mg/kg	400mg/kg	600mg/kg	0mg/kg
42	9.41 ± 0.23	9.44 ± 0.11	9.40 ± 0.34	9.41 ± 0.23
49	10.79 ± 1.23^{a}	10.53 ± 0.50^{a}	10.12 ± 0.39^{b}	9.23 ± 0.44^{c}
56	10.27 ± 0.99^{a}	10.05 ± 0.48^{b}	10.03 ± 0.83^{b}	9.16 ± 0.41^{c}
63	9.77 ± 1.67^{a}	9.57 ± 0.86^{b}	9.73 ± 0.41^{ab}	8.98 ± 1.65^{c}

*The data are given as mean haemagglutination inhibition titre (log_{2}) ± standard error of the mean *Different superscripts in a row indicate significant difference between the groups (p<0.05)*

It is also important to note that NDV HI antibody titre was significantly higher in the vaccinated-treated group than the vaccinated-untreated group, this shows that the extract has immune stimulating effects (Upadhyay *et al.,* 1993).

The gradual increase in the NDV antibody titres observed in the vaccinated-treated groups is an indication that the birds had more protection against ND, this increase has also been reported by Ahsan *et al.* (1991), Sadekar *et al.* (1998) and Kwakwukwe *et al.* (2013) following

challenge with VNDV and treatment with *Azadirachta indica* extract.

The slight decreased in the mean PCV, Hb and RBC observed in both the vaccinated-treated groups and vaccinated/ untreated group has also been noted by other researchers who reported that decrease in blood parameters following challenge with VNDV occurs as a result of the destruction of the RBC by the ND virus (Caldron *et al.,* 2005; Ruwaan *et al.,* 2009). The decrease was more severe in the vaccinated-untreated group than in the vaccinated-treated group this shows that the vaccination to a certain degree could not prevent the destructive capabilities of the NDV virus on the RBC, that maybe why ND outbreaks have been reported in chicken flock despite vaccination (Alexander, 2003; Senne *et al*., 2004; Ezema *et al*., 2009), however in the treated groups *Azadirachta indica* extract at the different doses enhanced the immune system enabling the birds to overcome the destructive effects of the ND virus.

There was an observable increase in WBC count in both the untreated and treated vaccinated groups, however the mean WBC was significantly higher ($p<0.05$) in group I and II of the vaccinated-treated group than group IV of the vaccinated-untreatedon days 49 and 56 of age. The leukocytosis may be attributed to increased production of leucocyte in the haemopoietic tissues (Yongola *et al*., 2006; Ravindraa *et al.,* 2009). Low leucocyte count in stressed chickens is a primary consequence of suppression of the immune system and increased susceptibility to disease (Wambura, 2009). This increase in WBC is a finding consistent with that of Kwawukume *et al*. (2013) in chickens challenged with NDV and treated with neem leaf extract, this is attributable to the neems ability to boost the macrophage response in the body which stimulates the lymphocytic system and boost the production of WBC (Sadekar *et al*., 1998). Ruwaan *et al.* (2012) also observed increased WBC on VNDV challenged birds treated with the root extract of *Anorthosis nobilis.* The higher WBC count noted in low dose treatment groups (Group I, II) as against the higher dose treatment group (III) is a finding consistent with the report of Kwawukume *et al.* (2013), who also observed a higher WBC count in birds fed with 5% neem leaf extract than those fed with 10% neem leaf extract this he attributed to probable toxicity of neem in higher concentration (Kwawukume *et al.,* 2013).

The mean absolute heterophil counts of vaccinated-treated group was significantly higher than the vaccinated-untreated group, that might have resulted from the fact that heterophils exhibit high level of apoptosis when infected by NDV (Ravindraa *et al.,* 2009). The level of the heterophils usually indicates the severity of the initial immune response; therefore their high values in the vaccinated-treated groups showed that *Azadirachta indica* possibly protected them from apoptosis.

The mean absolute lymphocyte counts increased in the vaccinated-treated group on day 49 of age and decrease subsequently till the end of the experiment this is in agreement with the report that the increase in lymphocytes might be physiologic, reactive, proliferative in disease conditions (Wambura, 2009). Birds that normally have high circulating lymphocytes in the initial response to infective pathogens might develop leucopenia due to lymphopenia. While the low level of the mean lymphocyte counts in group IV is in agreement with the report that NDV has the ability to cause agglutination and lyses of lymphocytes of affected birds thereby reducing the no of circulating lymphocyte (Bennet *et al.,* 2003; Khesorn, 2009).

REFERENCES

ABDU, P. A., MERA, U. M. and SAIDU, L. (1992). A study of chicken mortality in Zaria, Nigeria. Pages 51 – 55. *In: Proceeding of National Workshop on Livestock,* Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria.

AHSAN-UL-HAQ, K., AKHTAR, M. and RASOOLL, R. (1991). Effect of supplementing *Allium sativum* (garlic) and *A. indica* (neem) leaves in broiler feeds on their blood cholesterol, triglycerides and antibody titre. *International Journal of Agriculture and Biology*, 1(3): 125 – 127.

ALEXANDER, D. J. (1997). Newcastle disease and other paramyxovirus infections. Pages 541 – 569. *In:* CALNEK, B. W., BARNES, H. B., BEARD, C. W., MCDOUGLAD, L. R. and SAIF, Y. M. (Editors) *Diseases of Poultry,* 10th Edition, Iowa State University Press, Ames, IA, USA.

ALEXANDER, D. J. (2003). Newcastle disease and other avian paramyxoviridae infection. Pages 63 – 87. *In:* SAIF, Y. M., BARNES, H. J., GLISSON, J. R., FADLY, A. M., MCDOUGALD, L. R. and SWAYNE, D. E. (Editors), *Diseases of Poultry,* 11th Edition, Iowa State University Press, Ames, 1A, USA.

ATAWODI, S. E. and SPIEGELHALDER, B. (1994). Precursors of N-nitrous compounds in some Nigerian medicinal plants. *Cancer Letters,* 79(1): 107 – 115.

ARIVAZHAGAN, S., BALASENTHIL, S. and NAGINI, S. (2000). Modulatory effects of garlic and neem leaf extracts on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced oxidative stress in Wistar rats. *Cell Biochemical Function,* 18(1): 17 – 21.

BEARD, C. W. (1989). Serologic procedures Pages 192 - 200. *In:* FRIEDMAN, H. (Ed.), *A Laboratory Manual for the Isolation and Identification of Avian Pathotypes.* Kendall Hunt Publishing, Dubuque.

BENNETT, R. N., MELLON, F. A., FOIDL, N., PRATT, J. H., DUPONT, M. S., PERKINS, L. and KROON, P. A. (2003). Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and *Moringa stenopetala*. *Journal of Agriculture and Food Chemistry,* 51: 3546 – 3553.

BINDE, N. K., MEHTA, D. J. and LEWIS, R. J. (1958). Diuretic action of sodium nimbinate. *Indian Journal of Medical Sciences*, 12: 141 – 145.

BURRIDGE, M. J., RIEMANN, H. P., UTTERBACK, W. W. and SHARMAN, E. C. (1975). The Newcastle disease epidemic in southern California, 1971 – 1973: descriptive epidemiology and effects of vaccination on the eradication program. *Proceedings of the Annual Meeting of the United States Animal Health Association*, 79: 324 – 333.

CALDRON, N. L., GALINDO-MUNIR, F., ORTIZ, M., LOMNICZI, B., FEHEVARI, T. and PAASCH, L. H. (2005). Thrombocy-potenia in Newcastle disease: Haematological evaluation and historical study of bone marrow. *Acta Veterinaria Hungarica,* 53(4): 507 – 513.

CAMPBELL, T. W. (1994). Avian hematology. *In:* RITCHIE, B. W., HARRISON, G. J. and HARRISON L. R. (Eds). *Avian Medicine: Principles and Application.* Wingers, Lake Worth, Florida, USA.

COLES, E. H. (1986). Determination of packed cell volume. Pages 17 – 19. *In:* Coles E. H. (Ed), *Veterinary Clinical Pathology,* W.B. Saunders Company, Philadelphia.

EZE, D. C., OKWOR, E. C., OKOYE, A. I., ONAH, D. N. and SHOYINKA, S. V. O. (2012). Effects of *Moringa oleifera* methanolic leaf extract on the morbidity and mortality of chickens experimentally infected with Newcastle disease virus (Kudu 113) strain. *Journal of Medicinal Plants Research,* 6(27): 4443 – 4449.

EZEMA, W. S., OKOYE, J. O. A. and NWANTA, J. A. (2009). La Sota vaccination may not protect against the lesions of velogenic Newcastle disease in chickens. *Tropical Animal Health and Production,* 41(4): 477 – 484.

EZEOKOLI, C. D., UMOH, J. U., ADESUYIN, A. A. and ABDU, P. A. (1994). Prevalence of Newcastle disease virus antibodies in local and exotic chicken under different management systems in Nigeria. *Bulletin of Animal Health and Health and Production in Africa,* 32: 253 – 257.

HILL, H. D., DAVIS, O. S. and WILDE, J. K. H. (1953). Newcastle disease in Nigeria. *British Veterinary Journal,* 109: 381 – 385.

ILLANGO, J. and OLAHO-MUKANI, W. (2005) Immunogencity of a locally produced Newcastle disease 1-2 thermostable

vaccine in chickens in Uganda. *Tropical Animal Health and Production,* 37(1): 25 – 31.

KACHMAR, J. F. (1970). Determination of blood haemoglobin by the cyanomethaemaglobin procedure. Pages 268 — 269. *In:* TIETZ, N. W. (Ed.), *Fundamentals of Clinical Chemistry.* W. B. Sanders Company, Philadelphia.

KAKENGI, A., KAIJAGE, J., SARWATT, S., MUTAYOBA, S., SHEM, M. and FUJIHARA, T. (2007). Effect of *Moringa oleifera* leaf meal as a substitute for sunflower seed meal on performance of laying hens in Tanzania. *Livestock Research and Rural Development,* 19: 120.

KAPCZYNSKI, D. R. and KING, D. J. (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine*, 23: 3424 – 3433.

KAUR, G., ALAM, M. S. and ATHAR, M. (2004). Nimbidin suppresses functions of macrophages and neutrophils: relevance to its anti-inflammatory mechanisms. *Phytotherapy Research*, 18(5): 419 – 424.

KHESORN, N. (2009). Antibacterial activity of the capsules of *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology,* 36: 233 – 237.

KWAWUKUME, A. A., ANINGA, A. G., AWUNIB, J. B., OTSYINAC, H. and AWUMBILAA, B. (2013). The effects of *Azadirachta indica* (Neem) leaf extract on white blood cell count and the immune response of chickens vaccinated with Newcastle disease vaccine. *International Journal of Current Sciences,* 7: E23 – E31.

MURTHY, K. S., RAO, D. N., RAO, D. K. and MURTHY, L. B. S. (1978). A preliminary study on hypoglycemic and anti-hyperglycemic effects of *Azadirachta indica. Indian Journal of Pharmacology,* 10: 247 – 250.

OKOYE, J. O. A., AGU. A. O., CHINEME, C. N. and ECHEONWU, G. O. N. (2000). Pathological characterization chicken of a velogenic Newcastle disease virus isolated from guinea fowl. *Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux*, 53(4): 325 - 330.

RAVINDRAA, P. V., ASHOK-TIWARA, K., BARKHA, R., MANISH, V., BAISA, B., UTTARA, C., SUDESH, K. P., BHASKAR, S. and CHAUHANA, R. S. (2009). Time course of Newcastle disease virus – induced apoptotic pathways. *Virus Research,* 144(1 – 2): 350 – 354.

RITCHIE, B. W., HARRISON, J. G., HARRISON, R. L. (1994). *Avian Medicine.* Wingers Publishing Incorporated, Florida.

RUWAAN, J. S., REWOT, P. I., ABDU, P. A., EDUVIE, L. O. and OBIDI, J. A. (2009). Effect of velogenic Newcastle disease virus on packed cell volume total protein and haemagglutination inhibition antibody titres of vaccinated Shika brown cocks. *International Journal of Poultry Science,* 8(12): 1170 – 1173.

RUWAAN, J. S., REWOT, P. I. and OMONTESE, B. O. (2012). Effect of a velogenic new castle disease virus on body and organ weights of vaccinated Shika brown cocks. *Sokoto Journal of Veterinary Sciences,* 10(2): 7 – 12.

SADEKAR, R. D., KOLTE, A. Y., BARMASE, B. S. and DESAI, V. F. (1998). Immuno-potentiating effects of *A. indica* (Neem) dry leaves powder in broilers naturally infected with infectious bursal disease virus. *Indian Journal of Experimental Biology,* 36(11): 1151 – 1153.

SA'IDU, L., BISWALLA, M. and MOUMINI, B. (2006). Response of local breeds of chickens to challenge with Newcastle Disease virus (Kudu 113). *Journal animal Veterinary Advances,* 5(11): 975 – 979.

SEAL, B. S., KING, D. J. and SELLERS, H. S. (2000). The avian response to Newcastle disease virus. *Development*

and Comparative Immunology, 24: 257 – 268.

SENNE, D. A., KING, D. J. and KAPCZYNSKY, D. R. (2004). Control of Newcastle disease by vaccination. *Developments in Biologicals (Basel),* 119: 165 - 170.

SUBAPRIYA, R. and NAGINI, S. (2005). Medicinal properties of neem leaves: a review. *Current Medical Chemical Anticancer Agents,* 5(2): 149 – 156.

UPADHYAY, S. N., DHAWAN, S., GARG, S. and TALWAR, G. P. (1992). Immuno-modulatory effects of neem (Azadirachta indica) oil. *International Journal of Immunopharmacology,* 14: 1187 – 1193.

WAFAA, A. H., HOWAIDA, I., ABD-ALLA, HASSAN, A. and EL-SAFTY, M. M. (2007). Chemical Composition and *in vitro'* Antiviral Activity of *Azadirachta indica* A. Juss (Neem) leaves and fruits against Newcastle disease virus and infectious bursal disease virus. *Australian Journal of Basic and Applied Sciences*, 1(4): 801 – 812.

WAMBURAP, N. (2009). Protective antibody response produced by the chickens vaccinated with green coloured thermostable Newcastle disease virus. *Tropical Animal Health and Production,* 41: 149 – 152.

YONGOLA, M. G. S., MUHAIRA, A. P., MTAMBO, M., MINGA, M. U. and MINJA, M. (2006). Immunogenicity and protection ability of candidate Newcastle disease virus isolated for vaccine production. *Livestock Research and Rural Development,* 18: 10.