Effects of Moringa oleifera methanolic leaf extract on the morbidity and mortality of chickens experimentally infected with Newcastle disease virus (Kudu 113) strain

ARTICLE *in* JOURNAL OF MEDICINAL PLANT RESEARCH · JANUARY 2012

Impact Factor: 0.88

5 AUTHORS, INCLUDING:

Eze Didacus C

University of Nigeria

31 PUBLICATIONS **54** CITATIONS

SEE PROFILE

Emmanuel Chukwudi Okwor

University of Nigeria

35 PUBLICATIONS **40** CITATIONS

SEE PROFILE

John Osita Okoye

University of Nigeria

71 PUBLICATIONS **213** CITATIONS

SEE PROFILE

Denis Nnabuike Onah

University of Nigeria

31 PUBLICATIONS **422** CITATIONS

SEE PROFILE

Journal of Medicinal Plants Research Vol. 6(27), pp. 4443-4449, 18 July, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.792
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Effects of *Moringa oleifera* methanolic leaf extract on the morbidity and mortality of chickens experimentally infected with Newcastle disease virus (Kudu 113) strain

Didacus Chukwuemeka Eze[1]*, Emmanuel Chukwudi Okwor[1], Okoye John Osita A.[1], Onah Denis Nnabuike[2] and Shoyinka S. Vincent Olu[1]

[1]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Accepted 19 June, 2012

Newcastle Disease (ND) is an important disease of poultry worldwide. Its economic impact is high because mortality may reach 100% in affected poultry farms. This study was aimed at evaluating the protective properties of crude methanolic extract of *Moringa oleifera* in chickens. Forty two-day old chicks were randomly divided into four groups: I, II, III and IV. Groups I and II were given daily oral treatment of methanolic extract of *M. oleifera* at 200 mg/kg body weight until day 56 of age. Groups II and III at 42 days of age were vaccinated with the La Sota strain of ND vaccine. Group I was not vaccinated, while IV was left as untreated/unvaccinated control. All the groups were challenged with the velogenic strain of ND virus on day 56 of age. Feed intake and weight gain were evaluated. Following challenge, the birds were monitored for clinical signs, morbidity and mortality. Results of feed intake and weight gain were analysed using the statistical package for social sciences (SPSS). Survival was 0, 32, 88 and 100% in groups IV, I, II and III animals, respectively. Therefore, *M. oleifera* can protect birds from ND and can be used prophylactically against ND.

Key words: Velogenic Newcastle disease, chickens, *Moringa oleifera*, morbidity, mortality.

INTRODUCTION

Newcastle disease (ND) is a serious threat to the aviculturists and the commercial poultry industry worldwide. According to the Office International des Epizooties/World Organization for Animal Health (OIE), ND belongs to List A Group of diseases which have the following characteristics: "a transmissible disease that has the potential for very serious and rapid spread irrespective of national borders, that is of serious socio-economic or public health consequence, and that is of major importance in the international trade of animals and animal products (OIE, 2005)". The economic impact of ND outbreaks is characterized by high mortality in commercial flocks, condemnation of other infected flocks and trade restrictions associated with quarantine and surveillance of affected areas within individual states where outbreaks have been detected (Pandey, 1992). ND virus (NDV) is an Avian *Paramyxovirus* serotype 1 (APMV-1) that belongs to the genus *Avulavirus* in the family *Paramyxoviridae* (Alexander, 2003). The most effective means of controlling NDV has been through vaccination and biosecurity. The most common routes of inoculation of the vaccine are oral, ocular, and intranasal (Spradbrow, 1994).

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated and has become naturalized in many locations in the tropics. It is

*Corresponding author. E-mail: didacus.eze@unn.edu.ng. Tel: +2348037292020.

a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses (Morton, 1991). It is already an important crop in India, Ethiopia, the Philippines and the Sudan, and is being grown in West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands. All parts of the *M. oleifera* tree are edible and have long been consumed by humans. *M. oleifera* is a natural anthelmintic, mild antibiotic, detoxifier and outstanding immune builder (Dahot, 1998). It is used in many countries to treat malnutrition and malaria (Dahot, 1998). *M. oleifera* is widely regarded by water purification experts as one of the best hopes for reducing the incidence of waterborne diseases.

Recently, there has been an increased interest in the utilization of the *M. oleifera*, as a protein source for livestock (Makkar and Becker, 1997; Sarwatt et al., 2002). It is a multipurpose tree of significant economic importance with industrial and medicinal uses (Morton, 1991). There is little information available on the use of the leaves of this tree as an immunomodulator, especially in reducing the mortality rate in chickens infected with NDV and also as an adjuvant to vaccination. Therefore, in this project, the effects of administration of leaf extract of *M. oleifera* on mortality and morbidity with ND was investigated.

MATERIALS AND METHODS

The green leaves of *M. oleifera* were collected during the months of March and April 2010, at Ibagwa-Aka, Nsukka, Enugu State, Nigeria. The plant was identified by Mr. Ozioko of Bioresources Development and Conservation Programme, Nsukka. Extraction of the dried leaves was performed by soaking the plant material in absolute methanol (98%) for 24 h at room temperature (28°C). The resulting extract was concentrated *in vacuo* and subsequently air dried in a shade. The extract was solubilized in 5% Tween 80 and tested for protective activity in chickens experimentally infected with Velogenic Newcastle disease virus (VNDV). Phytochemical tests were carried out on the absolute methanolic extract using standard method (Trease and Evans, 1972).

Experimental animals

One hundred and fifty day-old White Harco cockerels were purchased from Zartec Ltd, a commercial breeder farm based at Ibadan, South West Nigeria. The birds were housed in an isolation pen at the Poultry Disease Research Unit of the Department of Veterinary Pathology and Microbiology University of Nigeria, Nsukka. The poultry house was of the open sided type and deep litter floor. Brooding was by kerosene stove and electric bulbs for the first two weeks. The birds were given feed and water *ad-libitum*, and were not vaccinated against any disease.

Experimental challenge

The birds were randomly divided into four groups- I, II, III, and IV, of twenty-five chicks each on day 42 of age. The group VI birds were kept in isolation from the other groups. Groups I and II were drenched orally with 1 ml 200 mg/kg body weight of *M. oleifera* daily between 42 to 56 days of age. Groups II and III were vaccinated with La Sota® vaccine at 42 days of age. At 56 days of age, all the groups were inoculated intramuscularly with 0.2 ml challenge dose of VNDV strain (Kudu 113) with titre of $10^{9.5}$ EID_{50} per ml of the inoculum.

Clinical signs

The birds in all groups were weighed weekly on days 42, 49, 56, 63, 70 and 77 of age to determine the mean body weights of the birds in the groups. The feed intake was also evaluated by weighing the left-over feed every morning before feeding the birds to determine the mean feed intake per bird in for the groups. The birds were observed daily for clinical signs. Morbidity and mortality were recorded. Necropsies were carried out on the birds that died during the experiment.

Statistical analyses

Body weights and feed intake data were subjected to analysis of variance (ANOVA) statistics using the Statistical Package for the Social Science (SPSS). Significant means were separated using the Duncan's new multiple range test and tests were considered significant at a probability of $P < 0.05$.

RESULTS

Phytochemical analysis

Qualitative phytochemical tests carried out on the extract showed the presence of alkaloid, saponin, glycoside, flavonoid, steroid, fats and oil, and reducing sugar (Table 1). The concentrations of saponin, glycoside, steroid and reducing sugars were higher in the methanolic extract of *M. oleifera* when compared to other chemical agents. However, tannins and terpenes were not detectable in the extract (Table 1).

Feed intake

On day 49 of age, the mean daily feed intake in groups I, II and IV in the study was not significantly (P> 0.05) different from one another, but were significantly (P < 0.05) lower than that of group III, the vaccinated and untreated group (Figure 1). On day 56, there was significant (P< 0.05) difference in the mean daily feed intake in groups I, II and III and the mean daily feed intake in group I was significantly (P< 0.05) lower than those in groups II and III, but not significantly (P>0.05) different from those of groups IV. Moreover, on day 63, the mean daily feed intake in group I was significantly (P< 0.05) lower that those in groups II and III, but there was no significant (P> 0.05) difference between groups I and IV. There was no significant (P> 0.05) difference among the vaccinated groups II and III (Figure 1). On day 70 and 77, the mean daily feed intake in group I was significantly (P<0.05) lower than those of other groups. However,

Table 1. Results of phytochemical analyses of *M. oleifera.*

Compounds	Presence in methanol extract
Alkaloid	+
Saponin	++
Tannins	-
Glycoside	++
Flavonoid	+
Steroid	+++
Terpenes	-
Fats and Oil	+
Reducing Sugar	**++**

- Absent; + present in trace concentration; ++ present in moderately low concentration; +++ present in very high concentration.

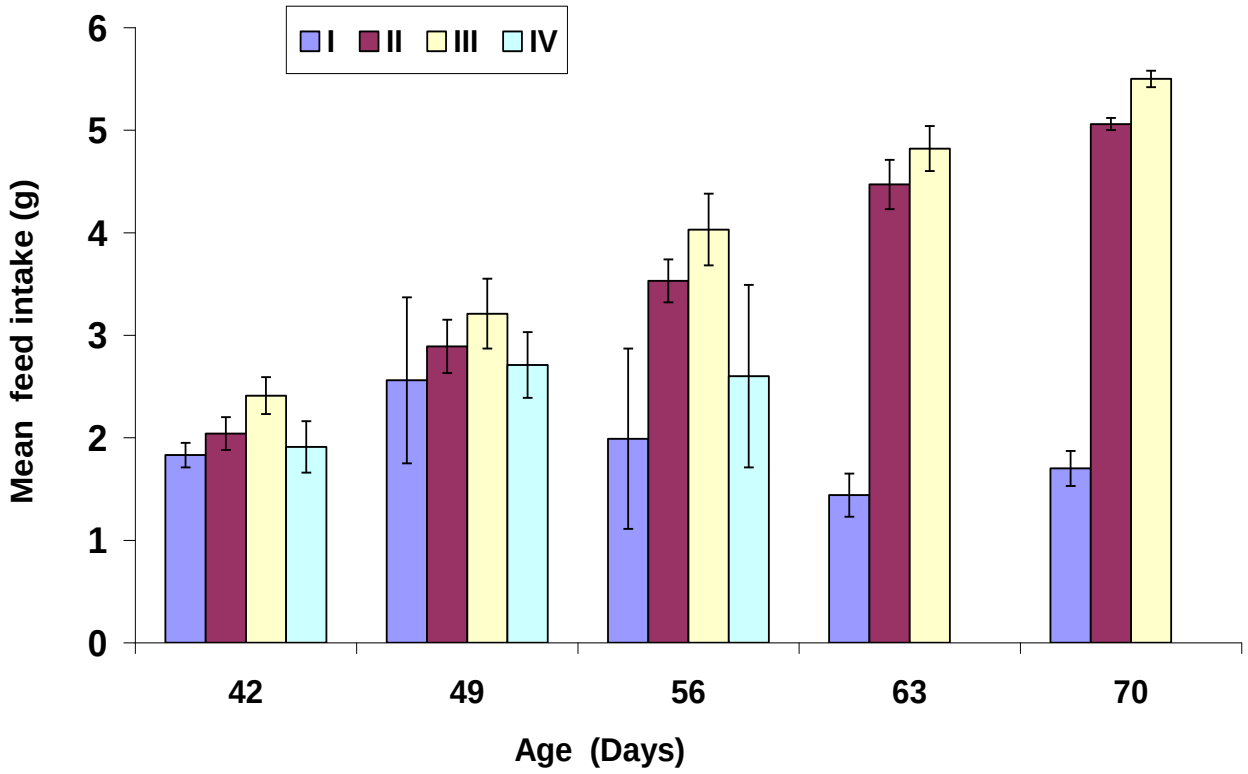

Figure 1. Feed intake (g) of the different groups of the birds treated with *M. oleifera* and or NDV vaccination.

there was significant (P<0.05) difference in the mean daily feed intake in groups II and III. The recorded mean daily feed intake in group II in this study was significantly (P< 0.05) lower than that of group III (Figure 1).

Body weight

The mean body weights in all the groups in this study were not significantly (P>0.05) different on day 42 (Figure 2). On the day 49, the mean body weight in group I was significantly (P< 0.05) higher than that of group IV, while the mean body weight in group II was also significantly (P< 0.05) higher than that of group III. On day 56, the mean body weight per bird in group I was also significantly (P< 0.05) higher than that of the group IV, while the mean body weight per bird in group II was equally significantly (P< 0.05) higher than that of group III. On days 70 and 77 of age, the mean body weight in group II was significantly (P< 0.05) higher than that of

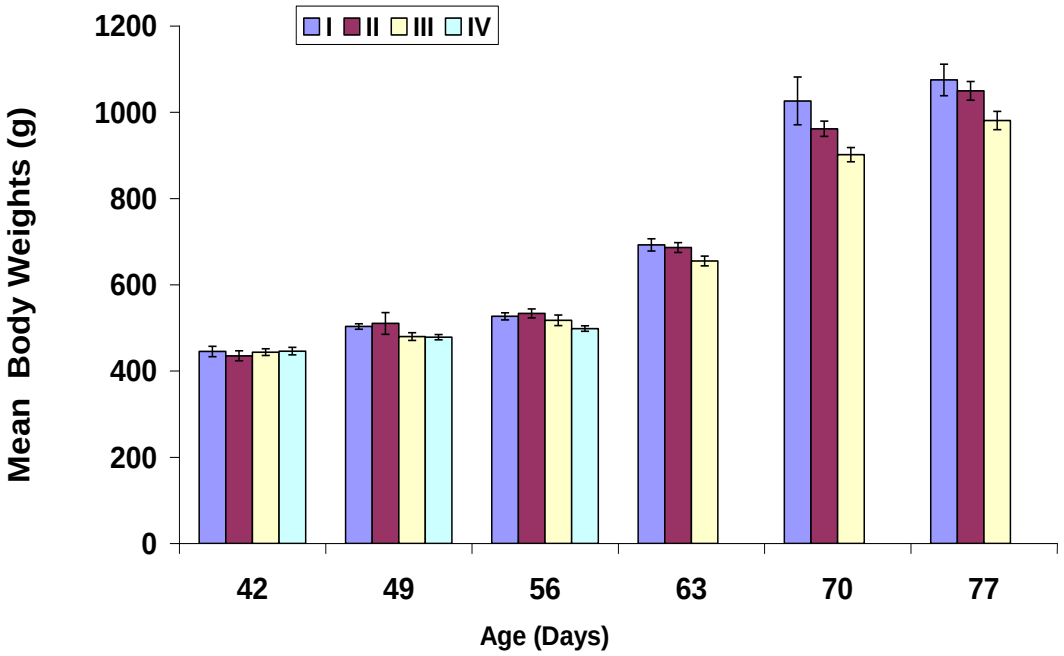

Figure 2. Body weights (g) of the different groups of the birds treated with *M. oleifera* and/or NDV vaccination.

group III. However, throughout the study, there was no significant (P> 0.05) difference in the mean body weight in the treated groups I and II. Meanwhile, significant (P< 0.05) differences were observed between the treated groups I and II, which was higher than those of the untreated groups III and IV from days 49 - 77. There were equally no significant (P> 0.05) differences among the untreated groups III and IV (Figure 2).

Clinical signs

The clinical signs started on day 3 post infection (PI) in groups I, II, and IV. The clinical signs were characterized by a progressive depression, drop in feed and water consumption, nasal discharge of muco-fibrinous fluid, listlessness and somnolence, dullness, droopy wing and tail feathers, ruffled feathers, facial edema, coughing and sneezing were observed in groups II and IV. Greenish-yellow diarrhea, torticollis and paralysis of the legs, sitting on the hock, in-coordination, and muscular twitching were seen on day 4 PI in the chickens in group IV. Morbidity occurred at day 3 PI up to day 6 PI. Morbidity on day 3PI was up to 32% in group I, 12% in group II, 0% in group III, and 32% in group IV. On day 6 PI, the morbidity was 32% in group I, 4% in group II and 0% in group III (Figure 3). Mortality started from day 4 PI and the total mortality by day 6 PI was 68% in group I, 12% in group II, 0% in group III, and 100 % in group IV (Figure 4). At the end of the study, survivability of the chickens 32% in group I, 88% in group II, 100% in group III and 0 % in group IV (Figure 5).

Lesion

Grossly, the post mortem examination showed congested breast and the thigh muscles which occurred in (100%) in group I, (66.67%) in group II, and (100%) in group IV. There were haemorrhages on the proventricular mucosa in (50%) in group I, (100%) in group II, and (50%) in group IV. Severe intestinal haemorrhagic enteritis and ulcers were in (20%) in group I, and nil in groups II and IV. The cecal tonsils were also enlarged and haemorrhagic in (30%) in group IV, and non in groups I and II. The bursa was atrophic in 100, 66.67% and (100% in groups I, II, and IV, respectively. Atrophy of the thymus was 100% in group IV and nil in groups I and II. Splenic atrophy was 80%, 30% and non in groups I, IV, and II, respectively. The kidneys were haemorrhagic and swollen and mottle in 40% of group I, 100% of group IV and non in group II, while the liver was equally congested in groups I, II, and IV birds (Table 2).

DISCUSSION

In this study, the incubation period of experimental ND was three days, which is in agreement with the report of Okoye et al. (2000). Hamid et al. (1991) reported an incubation period of 2 to 16 days. The morbidity rates observed were higher among birds in the unvaccinated

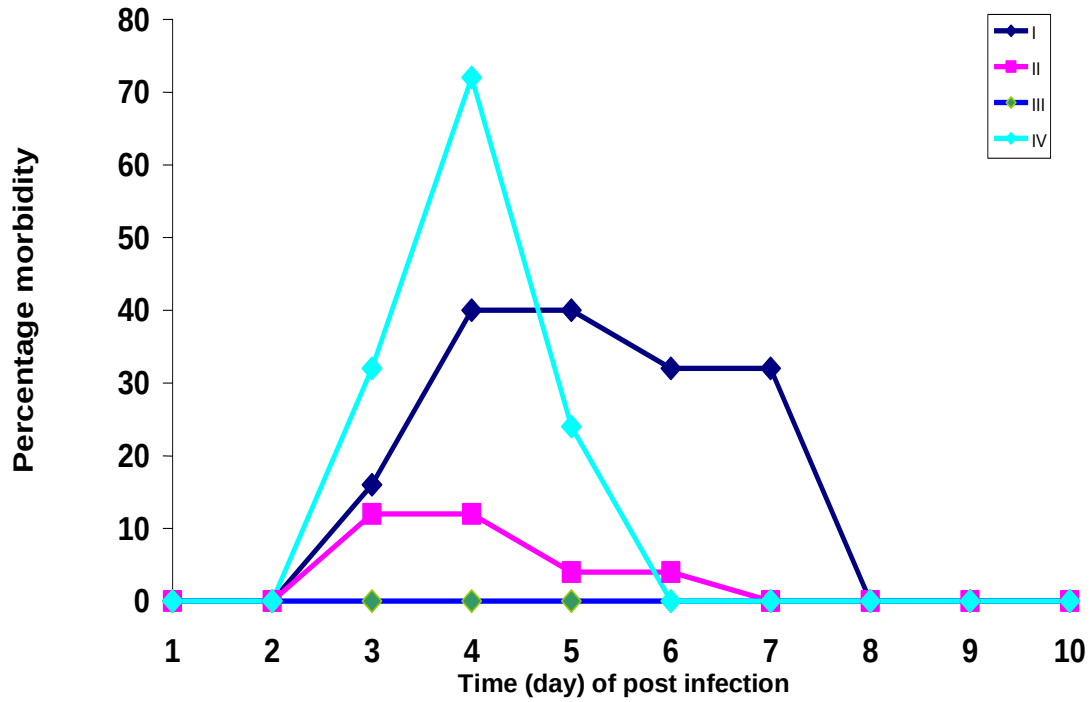

Figure 3. Morbidity profile following inoculation with NDV.

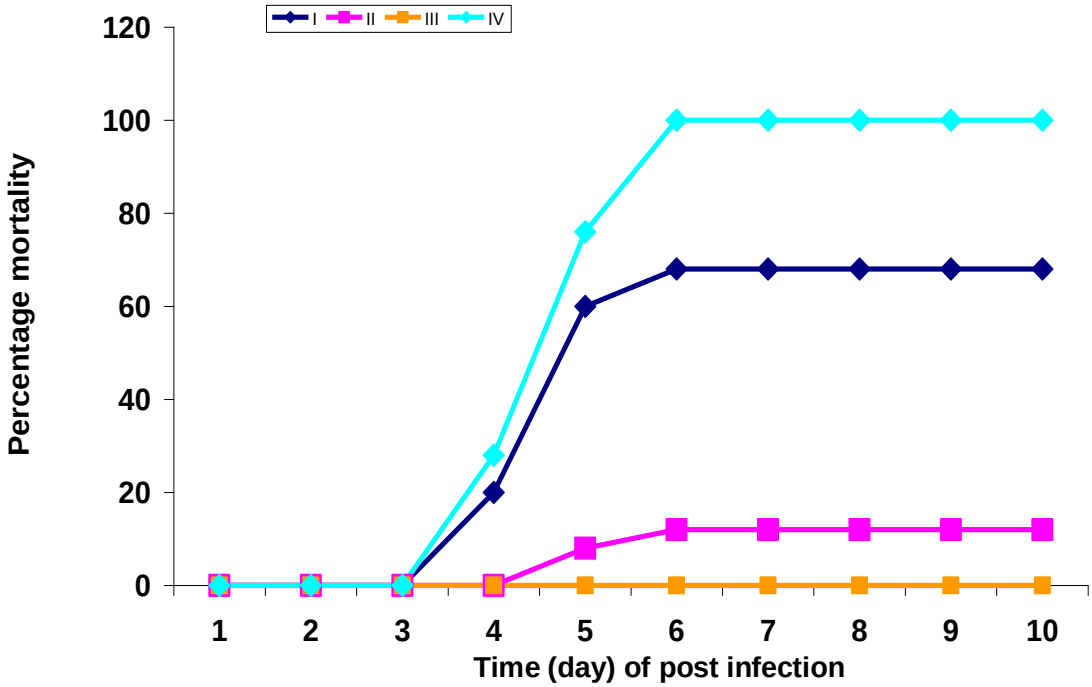

Figure 4. Mortality profile of birds following inoculation with NDV.

and treated groups I and unvaccinated and untreated IV and this agrees with the reports that the morbidity and mortality of VND outbreak could be up to 100% in non-immunized birds (Okoye et al., 2000). While the mortality in this study was 100% in group IV, it was 68% in group I, and also, 12 and 0% in the vaccinated and treated group II, and vaccinated and untreated group III, respectively. The survival rate in group I was 32%, 88% in group II,

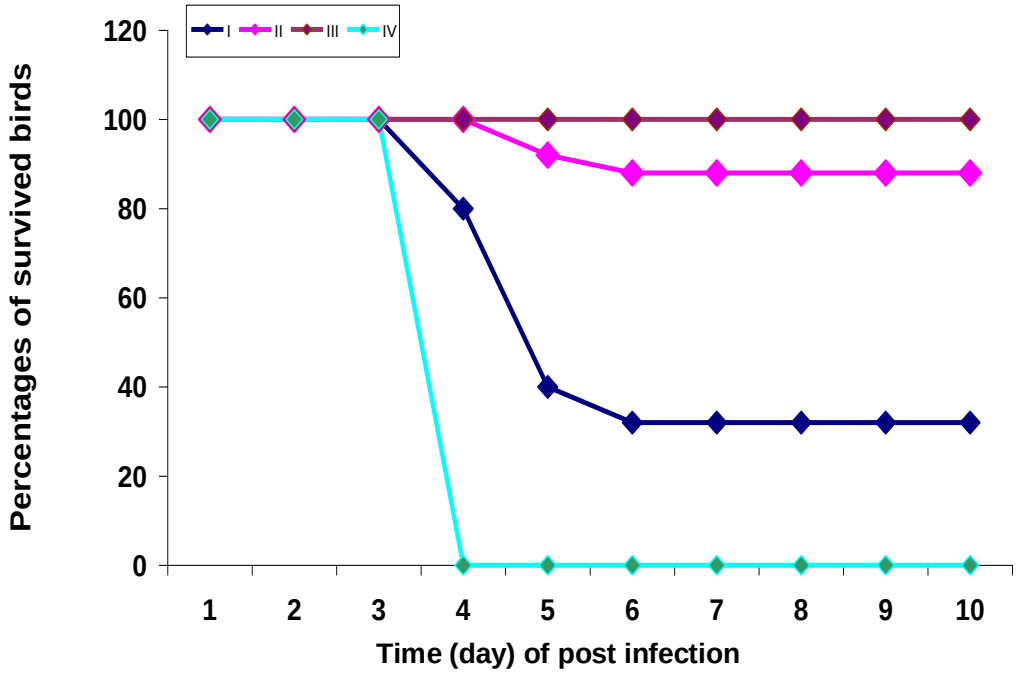

Figure 5. Survival profile of the birds following inoculation with NDV.

Table 2. Type and frequency of post mortem lesions found in the different groups.

Group	Total number of chickens in group after challenge	Number of chickens posted	Enlarged cecal tonsils	Intestinal haemorrhagic ulcer	Proventricular haemorrhage	Atrophic thymus	Atrophic spleen	Congestion of breast and thigh muscle	Atrophic bursa	Intestinal ulcer	Hemorrhagic kidney
I	25	10	-	2/10	5/10	10/10	8/10	10/10	10/10	6/10	4/10
II	25	3	-	-	3/3	-	-	3/3	2/3	3/3	-
III	-	-	-	-	-	-	-	-	-	-	-
IV	25	10	3/10	-	5/10	10/10	3/10	10/10	10/10	6/10	10/10

100% IN group III, and nil in group IV. Mortality in ND depends on the nature of the virus, susceptibility of the host, age and immune status of the host (Hamid et al., 1991). The clinical signs observed in groups I, II and IV birds in this study were similar to those already described for VVND by Alders and Spradbrow (2001) who observed depression, partial and/or complete inappetence, listlessness, and huddling. Following this was greenish diarrhea, which is an indication of gastrointestinal lesion. This is also in agreement with what has been described by Okoye et al. (2000) and Hamid et al. (1991). Severe nervous signs were observed prominent of which were ataxia, paralysis and torticollis. The nervous

involvement according to Okoye et al. (2000) was due to the intramuscular route of inoculation of the virus. The post mortem lesion seen in the challenged birds were those of the VVND, but were more severe in group I, followed by those of the group IV as reported by Okoye et al. (2000).

The post mortem lesion seen in the challenged birds were similar to those reported for VVND by Okoye et al. (2000). This is due to the tropism and virulence of the virus strain, target species, and their immunity (Alexander, 2003). These strains are typically associated with hemorrhagic intestinal lesions. The lesions were more severe in group IV, followed by groups I and then II. There was atrophy of the lymphoid organs such as the thymus, spleen, and bursa of Fabricius. While there was severe atrophy of the thymus in group IV, the lesion was moderate in group I. This was due to the immunosuppressive nature of the virus strain which the extract reduced in group I. Also observed in the birds was enlargement of the caecal tonsils and bursa which was due to inflammatory exudates. Intestinal haemorrhagic ulcers were equally observed.

Comparing the results of the unvaccinated but challenged groups I and IV, the *M. oleifera* treated group I birds had less mortality (68%) than the untreated group IV birds (100%), less feed intake than group IV. They also had higher mean body weight gain. Also, the treated and vaccinated group II birds had higher mortality (12%) than the untreated and vaccinated group III (0%). The group II birds had less feed intake but higher mean body weight gain than the group III birds. *M. oleifera* contains significant amounts of vitamines A, B and C, minerals such as calcum ions, iron, potassium, proteins, as well as traces of carotenoids, saponins, phytates and phenolic constituents (Ferreira et al., 2008; Siddhuraju and Becker, 2003), which may be responsible for the immunomodulation of the immune systemobserved in this study. The mechanism of action of *M. oleifera* in modulating immune responses could be due to an enhanced production of growth factors such as cytokines that activates both the innate and adaptive immunity (Davis and Kuttan, 1998).

Conclusion

It can be concluded that *M. oleifera* extract had advantageous effects on the treated birds; hence, it can be recommended as a prophylactic treatment against ND in non-vaccinated birds because its advantageous effects were compromised in the vaccinated birds.

REFERENCES

Alders R, Spradbrow P (2001). Controlling Newcastle Disease in Village Chickens, a Field Manual-Australian Center for International Agricultural Research (ACIAR), Canberra, Australia.

Alexander DJ (2003). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. Pages 63-99 in Diseases of Poultry. Y.M. Saif ed., Iowa State Press, Ames Iowa.

Dahot MU (1998). Antimicrobial Activity of Small Protein of *Moringa oleifera* leaves. J. Islam. Acad. Sci., 11:1, 27-32.

Davis L, Kuttan G (1998). Suppressive effect of cyclophosphamide-induced toxicity by *Withania somnifera* extract in mice. J. Ethnopharmacol. 62(3):209-214.

Ferreira PMP, Farias DF, Oliveira JTA, Carvalho AFU (2008). *Moringa oleifera*: bioactive compounds and nutritional potential, Rev. Nutr. Campinas. 21:431-437.

Hamid H, Campbell RS, Parede L (1991). Studies of the pathology of velogenic Newcastle disease: Virus infection in non-immune and immune birds. Avian. Pathol. 20:561-575.

Makkar HPS, Becker K (1997). Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. J. Agric. Sci. 128:311-322.

Morton JF (1991). The Horseradish tree, *Moringa pterygosperma* (*Moringaceae*): A plant boon to arid lands? Econ. Bot. 45:318-333.

OIE (2005). (Office International des Epizooties/World Organization for Animal Health). Newcastle disease. In: Manual of standards for diagnostic tests and vaccines. p. 2,1,15.

Okoye JOA, Agu AO, Chineme CN, Echeonwu GON (2000). Pathological Characterization of a Velogenic Newcastle Disease Virus Isolate from Guinea Fowl. Rev. Élev. Méd. Vét. Pays trop. 53(4):325-330.

Pandey VS (1992). Epidemiology and economics of village poultry production in Africa: An overview, pp. 124-128.

Sarwatt SV, Kapange SS, Kakengi AMV (2002). Substituting sunflower seed-cake with *Moringa oleifera* leaves as supplemental goat feed in Tanzania. Agrofor. Sys. 56:241-247.

Siddhuraju P, Becker K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.). J. Agric. Food Chem. 15(8):2144-2155.

Spradbrow PB (1994). Newcastle disease in village chickens. Poult. Sci. Rev. 5:57-96.

Trease G, Evans W (1972). Pharmacognosy, Univ. Press, Aberdeen, Great Britain. pp. 161-163.