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Effect of garlic and neem leaf aqueous extracts on immune response of broilers to live Newcastle disease vaccine

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ABSTRACT

This study was designed to determine the effects of Garlic and Neem leaf aqueous extracts on their individual basis and/ or mixture on humoral immune response of broilers against NDV (Newcastle Disease Virus) Vaccine. One hundred and sixty day old broiler chicks were randomly allocated into four groups A, B, C and D. All the birds were raised in individual cages beneath the same house operating under conventional deep litter system. Group A was regarded as control received neither of the extracts. Group B, C and D were treated with 5g Garlic, 5g Neem leaf and a mixture of 2.5g Garlic and 2.5g Neem leaf extracts respectively mixed per liter of drinking water for the first 3 weeks and the experimental birds lasted for 8 weeks. The birds from all the groups were vaccinated with ND- La Sota vaccine orally at 21 day old. Ten blood samples were taken from each group on day 1, 7, 14 and 21 after the vaccination. The serum antibody level against NDV was measured by HI test. Better immune response ($p< 0.05$) was found in group B, C and D than the control but no significant difference ($p> 0.05$) between the treated groups. Present findings suggest that aqueous extracts of garlic and neem leaf enhanced the immunomodulatory response to live ND vaccine.

1. Introduction

Newcastle disease is of profound economic significance to poultry farmers in Nigeria. This is because of its overall negative effect on performance and disease resistance (Abdu *et al.*, 2001). Newcastle disease (ND) poses a serious threat as it has economic and ecological impact on pet, free-living, as well as domestic birds. An average of 200-250 outbreaks of the disease is reported in Nigeria annually (Okeke and Lamorde, 1988). The virus strains prevalent in nature are highly virulent (velogenic) and are kept in circulation by vast populations of local chickens, apparently normal ducks and free-flying birds (Abdu *et al.,* 1985; Ibu *et al.,* 2000). Immunosuppression has been recorded, resulting in poor response to Newcastle disease vaccine and high susceptibility to some protozoan and bacterial infections (Abdu *et al.*, 2001; Lukert and Saif, 2002). The only known method of controlling the disease is through vaccination and proper biosecurity (Abdu *et al.*, 2001; Okoye, 2005). Outbreaks of the disease have been reported in vaccinated flocks (Okoye and Shoyinka, 1983). The vaccination failures may be as a result of neutralisation of the vaccine virus at the time of vaccination by maternal antibodies (Okoye, 1984; Abdu, 1986). Another difficulty encountered with prevention of ND infection is the presence of antigenic variants in the field that are kept in circulation by vast populations of local chickens, apparently normal ducks and free-flying birds (Nawathe *et al.,* 1975; Abdu *et al.,* 1985; Ibu *et al.,* 2000). Researches have therefore been targeted at improving the immunogenicity of the vaccines by using antioxidants such as Vitamin C (Pardue, 1987; Okoye *et al.*, 2000). Reports on their effectiveness or otherwise are conflicting and therefore inconclusive, possibly due to difference in the virulence of the virus strains or weather conditions (Okoye, 2005).

Nigeria has a wide range of medicinal herbs due to the favorable climatic conditions. Inclusion of Garlic and Neem in feed has been shown to upgrade the immune performance against infectious bursal disease (IBD) and ND in poultry (Ahsan *et al*., 1991). In vitro studies have shown that various preparations of garlic have antibacterial (Bakri and Douglas, 2005), antiviral (Webes *et al*., 1992), antifungal (Lawson,1996) and antiparasitic (Ankri *et al*., 1997) properties against human's pathogens. The beneficial effects of Garlic oil on renal function and calcium metabolism have been reported (Ohaeri, 1991). It has also been reported as an expectorant and its oil is considered as an important remedy for wounds treatment (Ohaeri, 2001). Neem is the most useful traditional medicinal plant and a valuable natural product for the development of medicinal recipes against various diseases (Biswas *et al*., 2002). Chickens treated with full fat neem kernel cake exhibit less mortality rates due to IBD when compared to the groundnut cake fed group during the natural outbreak (Uko, 2003). Sadekar *et al*., (1998) also reported that dry leaves of Neem are beneficial in IBD affected broilers. Therefore, the objective of this study was to further study the effects of Garlic and Neem leaf extracts on serological response of broilers to live NDV vaccine in Sokoto, Nigeria.

2. Materials and methods

The study was spread over a period of 8 weeks and was carried out in an open sided deep litter system at the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.

2.1. Experimental design

The experiment was carried out in randomized completely block design. A total of one hundred and sixty broiler chicks obtained from a hatchery (Zartech^{R}), were divided into four groups. Group A was the control while groups B, C and D received 5g garlic aqueous extract, 5g neem leaf aqueous extract and a mixture of 2.5g garlic and 2.5g neem leaf aqueous extracts mixed per litre of drinking water for three weeks respectively. All the chicks were fed with a commercial feed (Vital feed^{R}) and the feed and water were provided ad libitum. The birds from all the groups were vaccinated against ND via drinking water with ND- La Sota vaccine at 21 days old.

2.2. Preparation of garlic and neem leaf aqueous extracts

Fresh Garlic bulbs and Neem leaves were purchased from Sokoto central market. The herbs were trodden into small pieces with the help of metallic grinder and taken into separate non- metallic jar and were added one

litre of hot boiling water, kept at room temperature over night following the procedure mentioned by Liela (1977). Hence, the extracts collected mixed in drinking water.

2.3. Sample collection

Ten blood samples were taken via wing vein from each group at day 1, 7, 14 and 21 post vaccinations. The sera were stored at -20^{0}C until the end of the experiment. The assessment of NDV- specific antibody levels were made by conventional haemaglutination- inhibition test (4 HA unit of Ag) as per Thayer and Beard (1998). The data were analyzed by ANOVA at 95% confidence intervals using Graph Pad statistical soft ware package.

3. Results and discussion

The table below shows a significant difference in the mean ND antibody titre between the control group and treated groups but the treated groups were significantly the same. These findings can be viewed from three perspectives: Firstly, after the administration of *La Sota* vaccine at 21 day old (Day 1 post ND-*La Sota* vaccination), the broilers were not having detectable antibodies against Newcastle disease as shown in table 4.1 and this indicates that there was no MDA and no previous exposure to the field strain of Newcastle disease.The non detectable antibodies at 21 day old in this study is in conformity with the findings of Sa'idu *et al,* (2006) who reported that the maternal antibodies in chicks decline to a non protective level by 2 weeks of age and Halle *et al.* (1999) who reported that chicks 3-4 weeks old are at high risk of suffering from ND which may be due to a decline in maternal antibody levels.This shows that garlic/ neem leaf aqueous extracts administration have on inflence on the MDA to ND. Secondly, at day 7 and 14 post ND la Sota vaccination there was higher antibody titre against Newcastle disease in the garlic/ neem leaf aqueous extracts treated groups than the control group as shown in table 4.1 although the antibody titre produced were not statistically different between the treated groups and the control group. Thirdly, at day 21 post ND-L*a Sota* vaccination both the treated and control groups had a protective antibody titre against ND but those groups (B, C, and D) that received garlic-neem leaves equeous extracts had a significantly higher ($p< 0.05$) antibody titre when compared with the control group but no significant difference between the treated groups. The present finding is in agreement with the findings of Durrani *et al*., 2008 which reported a significant increase in the antibody titre against gumboro disease after the infusion of neem leaves extract at 50 ml/L. Furthermore, Nidullah *et al*., 2010 reported that mixed extracts of garlic, neem and ginger increased the immuno-modulatory response against ND, IB and IBD.

Table 1
Post-vaccination effect of garlic bulb and neem leaf aqueous extracts on serum hi titre (log_2) in broilers vaccinated against newcastle disease virus.

Day	control	Garlic extract	Neem extract	Garlic+Neem
1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
7	0.4±0.50^a	0.9±0.44^a	0.8±0.75^a	0.7±0.99^a
14	1.4±0.55^a	2.0±1.41^a	1.8±0.84^a	1.8±0.86^a
21	4.0±1.87^b	9.8±0.45^a	9.6±0.55^a	9.2±0.84^a

^{a} and ^{b} within row = means without common superscript differ significantly ($P<0.05$).
A= Control; B= Garlic-5g; C= Neem-5g and D= Garlic-Neem extracts mixture-5g.

Gabor *et al*., (1998) observed a significant rise in serological response of broilers to inactivated NDV vaccine after using Garlic and microbial cell extracts at 1mg/ L in drinking water for 17-20 days. However, Jafari *et al*., 2008 did not find any increase on humoral immune response to live NDV vaccine after dietary garlic supplementation at 1% and 3% garlic powder. This discrepancy likely relates to the differences in environmental factors (the later was in Iran), garlic preparations and the strain of the birds. For example, Chowdhury *et al*. (2002) investigated the effects of sun-dried garlic paste on reproduction parameters in different strains of laying hens and found significantly different responses in some traits among the strains. Like the present findings, Neem leaves infusion was found to enhance immunomodulatory response against IBD (Sarang and Durrani, 2005). Also, Sadekar, *et al*., 1998 fed Neem dry leaves to broilers and observed significant effect on the immune performance against IBD. The significant increase recorded in this study could be attributed to the effects of garlic/ neem on B- lymphocyte which differentiate into memory cells and plasma cells. The plasma cells secrete antibodies against antigen and are

capable of producing about 2,000 molecules per second while the memory cells have the ability to rapidly differentiate into antibody- producing plasma cells when they encounter the same antigen in another infection (Richard and Tracey, 2001). It is also known that ND vaccine strains especially the mesogenic ND vaccine cause damage to the bursa of fabricius, leading to reduced immune response (Okoye *et al.,* 2000; Oladele, 2003). It is possible that these extracts overcome the ND vaccinal effects in the bursa of fabricius, thus leading to increased humoral immune response as seen in this result. In conclusion, the result of this study clearly shows that garlic/ neem leaf aqueous extracts increased the humoral immune response against Newcastle disease to *La Sota* vaccine. Research should be done to identify and characterize the active constituents of garlic/ neem leaf that influence avian cell responsiveness and mechanisms that trigger activation of humoral immunity in birds. Further research should be done to study the effects of garlic/ neem leaf aqueous extract at graded doses in poultry in order to ascertain the immunostimulatory dosage.

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