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REGULAR ARTICLE

Comparative Evaluation of *Moringa oleifera* and Vacci-Boost Immunomodulators in Chickens experimentally infected with Newcastle Disease Virus (Kudu 113 Strain)

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ABSTRACT

This study compared the immunomodulatory effects of methanolic leaf extract of *Moringa oleifera* and Vacci-Boost® in chickens experimentally challenged with velogenic Newcastle Disease virus (vNDV) strain. One hundred 21 days old chicks were randomly divided into five equal groups designated A, B, C, D and E. Groups B and D were given 200mg/kg dose of *M. oleifera* extract from day 21 to day 35 of age in drinking water while group C was treated once with Vacci-Boost® during vaccination on day 21 of age according to the manufacturer's instructions. Groups A, C and D chicks were vaccinated on day 21 of age with Newcastle disease (ND) vaccine La Sota while groups B and E were not vaccinated. All the groups were challenged on day 35 of age. Haemagglutination Inhibition titer, relative organ weights, body weight and Packed Cell Volume were assessed. On days 28 and 35, HI titer of the immunomodulators supplemented groups were significantly ($p \leq 0.05$) higher than those of the unsupplemented groups. The relative weights of the lymphoid organs of the supplemented groups were significantly ($p \leq 0.05$) higher than those of the unsupplemented group. Also, Vacci-Boost® supplemented group had higher mean body weight although they were not significantly ($p \geq 0.05$) higher than those of the unsupplemented groups. There were no significant ($p \geq 0.05$) differences in the body weight of the *M. oleifera* treated and those of untreated groups.

1. Introduction

Birds are susceptible to many infectious diseases. These include bacterial, fungal and viral infections. One of the most economically important of all these diseases in poultry is Newcastle disease (ND). This disease causes tremendous loss in both commercial and village chickens (FA0, 2005). ND easily spreads leading to death in quick succession. It affects domestic poultry, aviary, caged and wild birds and is characterized by digestive, respiratory and nervous signs (Gueye, 2002). Newcastle disease virus (NDV) occurs worldwide and has a considerable economic impact on the world poultry industry, ranging from losses due to disease to the expenses of vaccination and cost of diagnostic laboratory investigation (Jorgensen et al., 1999). ND is currently one of Nigeria's most problematic diseases in the poultry industry (Adu et al., 1986). The major control measure for ND in Nigeria is vaccina-

tion (Ugochukwu, 1982). Despite various vaccinaiton programs, ND outbreaks have continued to occur in vaccinated as well as unvaccinated flocks (Ugochukwu, 1982). Lack of potent vaccines against ND (Spadbrow, 1988) and infections with endoparasites can reduce the immune response towards ND vaccines (Bhopal et al., 1998). Also, vaccination will protect birds from more serious consequences of NDV infections, since clinical signs can be greatly reduced in relation to increased antibody level achieved. However virulent strain of NDV may still infect, replicate, and be excreted from vaccinated birds (Capua et al., 1993). In view of these circumstances, there is need to enhance the immunity of birds with nutrients and drug extracts that would aid the birds in combating the disease situations (Linge, 2005). Stimulating the immune system of chickens with thymus extract in feed, drinking water and by aerosol enhances B and T-cells performance in vaccinated chickens (Qureshi, 1992; Hakkerain et al., 1994). Administration of immunomodulating compounds can prevent losses due to diseases and poor vaccination response (Ogbe et al., 2008).

Dry leaf powder of *Moringa oleifera* is a valuable nutrient for the poor communities of Africa because it helps the immune system to fight infections in HIV positive patients (Burger et al., 2002). Hitherto, not much work has been done on *M. oleifera* with respect to enhancement of immune responses in chickens. Vacci-Boost® is a commercially available feed supplement designed to achieve most efficient 'take' of vaccines. It contains special herbal extracts that have strong immunnomodulating activity, and also have the capacity to neutralize residual chlorine in drinking water. It also imparts blue colour to reconstituted vaccine water to help in monitoring uniform vaccine administration as it remains visible on beaks of vaccinated birds for at least 3 to 4 hours post vaccination. This study compared the immunomodulatory effects of methanolic leaf extract of *M. oleifera* and Vacci-Boost® in chickens experimentally challenged with velogenic Newcastle Disease virus (vNDV) strain.

2. Materials and Methods

2.1. Plant Material

The green leaves of *M. oleifera* were collected from Ibagwa-Aka, Nsukka, Enugu State, Nigeria. The plant was identified at the Bioresources Development and Conservation Programme, Nsukka. Extraction was performed on the dried leaves by soaking in absolute methanol (98%) for 24 h at room temperature (28^0C). The resulting extract was concentrated *in vacuo* and subsequently air dried in a shade. The extract was solubilized in 5% Tween 80. Phytochemical analyses of the extracts were performed using standard methods (Evans, 2002).

2.2. Experimental Animals

One hundred day-old White Harco cockerels were used for the study. The chicks were not vaccinated against any disease. The birds were housed in an isolated pen at the Poultry Disease Research Unit of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka and were brooded for 3 weeks. They were fed with commercial poultry feed *ad-libitum* and provided with drinking water.

2.3. Experimental Procedure

On day 0 of the study, the birds were randomly divided into five equal groups of 20 chicks each designated A, B, C, D and E. Groups B and D were given *M. oleifera* extract at the dose of 200mg/kg from day 0 to day 14 of the study in drinking water while group C was treated once with Vacci-Boost® during vaccination on day 0 of the study according to the manufacturer's instructions (Polchem Hygiene Laboratories (P) Ltd , India). Groups A, C and D chicks were vaccinated on day 0 of the study with Newcastle disease (ND) vaccine La Sota while groups B and E were not vaccinated. All the groups were inoculated intramuscularly with 0.2ml challenge dose of vNDV strain (Kudu 113) with titre $10^{8.32}$ embryo infective dose (EID_{50}) per ml of the inoculums on day 14 of the study.

2.4. Serology

On weekly basis, ten birds from each group were chosen at random and blood samples were collected from the ulnar vein aseptically for serum. Serum samples were separated by centrifugation at 3000g for 15 min and the harvested sera stored at – 40^0C until used.

2.5. Haemagglutination Inhibition Test for ND

The HI titer was conducted according to standard procedures (Beard, 1989). Briefly, twofold serial dilution of serum, after inactivated at 56^0C for 30min, was made in a 96-well, V-shaped bottom microtitre plate containing 50µl of PBS in all wells and then 50µl of NDV antigen (4 HA units) were

added into all wells. The antigen–serum mixture was incubated at 37^{0}C for 30 min. Then, 50µl of 1% chicken erythrocytes suspension were added into each well and re-incubated for 30min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest dilutions of serum causing complete inhibition were considered the endpoints. The geometric mean titers were expressed as reciprocal log2 values of the highest dilutions that displayed HI as described by Villegas and Purchase (1989).

2.6. Body Weight

Chickens in each group were weighed at weekly intervals to monitor growth rates. At the end of the experiment five birds from each group were sacrificed humanely and the liver, proventriculus, thymus, spleen, and Bursa of Fabricius were weighed in order to calculate organ/body weight ratio according to Lecui et al. (1998).

2.7. Packed Cell Volume determination

The Packed Cell Volume (PCV) values were determined by micro-heamatocrit method of Benjamin (1986). Blood samples containing EDTA were aspirated into a set of plain capillary tubes. The tubes were sealed and centrifuge at 3000 x g for 15 min., the results were read as a percentage using the hematocrit reader.

2.8. Statistical Analysis

The geometric mean titre (GMT) was calculated using the Tube Number Method and Table (Villegas and Purchase 1989). Body weights and feed intake data were subjected to Analysis of Variance (ANOVA). Significant means were separated using the Duncan's New Multiple Range Test and tests were considered significant at a probability of $p \leq 0.05$.

3. Results

3.1. Heamagglutination

The HI antibody titers are shown in figure 1. On day 7 of the study (post vaccination), the HI titer of the Vacci-Boost® treated ND vaccinated group C was significantly ($p \leq 0.05$) higher than that of the *M. oleifera* treated and ND vaccinated group D. The titers of the immunomodulators treated groups C and D did not vary significantly ($p \geq 0.05$) on days 14 and 21 of the study, but the HI titers of group C birds were significantly ($p \leq 0.05$) higher than those of group D birds on days 28, 35 and 42 of the study;

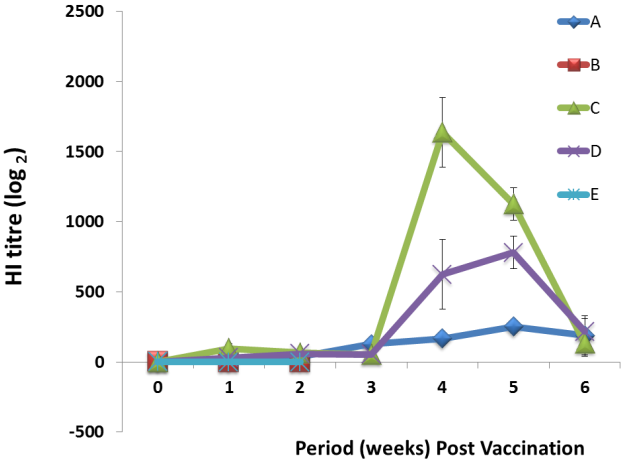

Figure 1: Effect of *M. oleifera* and Vacci-Boost supplementation on antibody titers against Newcastle disease in Broiler.
Group A = Vaccinated only and challenged
Group B = Unvaccinated, *M. oleifera* treated and challenged
Group C = Vaccinated, Vacci-Boost treated and challenged
Group D = Vaccinated, *M. oleifera* treated and challenged
Group E = Unvaccinated, untreated and challenged

while the HI titers of the untreated and ND vaccinated group A were significantly ($p \leq 0.05$) higher than those of the untreated and ND unvaccinated group E throughout the period post vaccination.

3.2. Weight

Over the course of the experiment, it was observed that there were no significant ($p \geq 0.05$) difference in the mean body weight of the *M. oleifera* treated and ND vaccinated group D and those of the Vacci-Boost® treated and ND vaccinated group C on days 7 and 28 of the study. On days 21 and 35 of the study, the mean body weight of group D birds were significantly ($p \leq 0.05$) higher than those of group C birds and those of group A birds. At the end of the study, group D birds had higher mean body weight than that of group C birds, though not significant ($p \geq 0.05$).

3.3. Relative Organ Weight

The relative organ weights are shown on table 1. There were no significant ($p \geq 0.05$) difference in the relative organ weights of liver, gizzard, and proventriculus in all the groups. The relative weight of the thymus in *M. oleifera* treated and ND vaccinated group D was significantly ($p \leq 0.05$) higher than those of the Vacci-Boost® treated and ND vaccinated group C and the untreated but ND vaccinated group A while the relative weights of the bursa in group C birds were significantly ($p \leq 0.05$) higher than those of group D birds and group A birds. There were no significant ($p \geq 0.05$) difference in the relative weights of the spleen and heart in

Weights	A	C	D
Terminal wt (g)	1340± 48.48[a]	1400 ± 63.25[b]	1420 ± 37.42[b]
Proven-triculus (g)	4.162± 0.06[a]	4.21 ± 2.08[a]	4.22 ± 0.31[a]
Gizzard (g)	43.77 ±1.57[a]	47.15± 2.37[a]	47.40 ± 3.6[a]
Liver (g)	23.46 ± 0.99[a]	23.98± 1.65[a]	27.52 ± 1.62[a]
Spleen (g)	1.13 ± 0.15[a]	1.59 ± 0.16[b]	1.7 ± 0.19[b]
Thymus (g)	3.34 ± 0.20[a]	3.88 ±0.34[b]	4.46 ± 0.30[c]
Bursa (g)	0.51 ± 0.09[a]	1.34 ± 0.61[b]	0.92 ± 0.33[c]
Heart (g)	1.34 ± 0.46[a]	3.95 ± 0.23[b]	4.07±0.13[b]

Table 1: Effect of *M. oleifera* and Vacci-Boost supplementation on the Mean Relative Organ Weight antibody of Broiler.
[ab]Different superscripts in a row indicates the significant difference between a group at (p≤0.05)
Group A = Vaccinated only and challenged
Group C = Vaccinated, Vacci-Boost treated and challenged
Group D = Vaccinated, *M. oleifera* treated challenged

group D birds and those of group C birds which were significantly ($p \leq 0.05$) higher than those of group A birds.

3.4. Packed Cell Volume

The packed cell volumes of group D birds were significantly ($p \leq 0.05$) higher than those group C birds as well as those of group A through the study. The PCV of group C birds on weeks 3 (748 ± 32.62%[b]) and 7 (1400 ± 63.25% [b]) were significantly ($p \leq 0.05$) higher than those of group A birds on weeks 3 (663 ± 11.79%[a]) and (1340 ± 48.48% [a]) respectively. There was no significant ($p \geq 0.05$) difference in the PCV group A birds and those of group E birds day 7 to day 21 of study (figure 2).

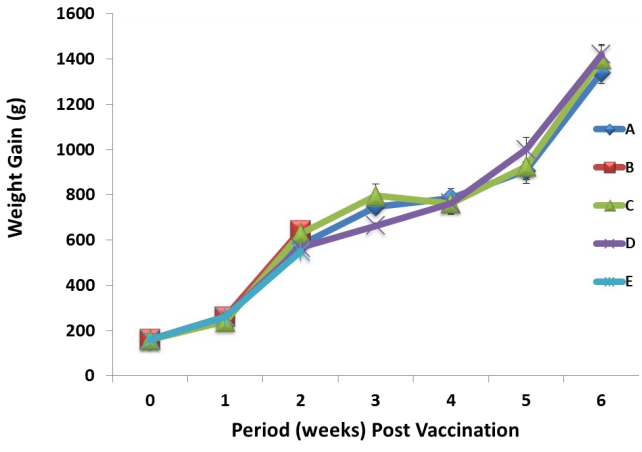

Figure 2: Effect of *M. oleifera* and Vacci-Boost supplementation on the body weight of Broiler.
Group A = Vaccinated only and challenged
Group B = Unvaccinated, *M. oleifera* treated and challenged
Group C = Vaccinated, Vacci-Boost treated and challenged
Group D = Vaccinated, *M. oleifera* treated and challenged
Group E = Unvaccinated, untreated and challenged

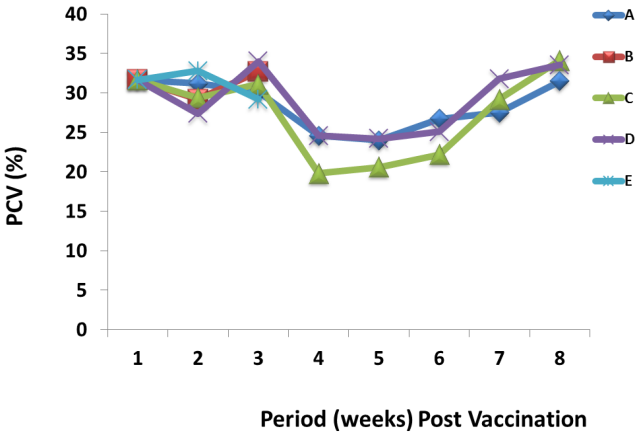

Figure 3: Effect of *M. oleifera* and Vacci-Boost supplementation on the Mean PCV (%) of Broiler.
Group A = Vaccinated only and challenged
Group D = Unvaccinated, *M. oleifera* treated and challenged
Group C = Vaccinated, Vacci-Boost treated and challenged
Group D = Vaccinated, *M. oleifera* treated and challenged
Group D = Unvaccinated, untreated and challenged

4. Discussion

Following vaccination with La Sota vaccine and challenge with velogenic NDV in the course of this study, the HI titer was significantly ($p \leq 0.05$) high in the immunomodulators treated groups. This is similar to the report by Sa'idu et al. (2006), that following challenge with VNDV, the HI titer usually goes high. This high HI titer helps to provide long term protection against ND (Reynolds and Maraqa, 2000; Muhammad et al., 2006). The immunomodulatory effect of *M. oleifera* as observed in this study substantiated what has been reported by Eze et al. (2013). The effects of *M. oleifera* and vacci-boost on the humoral immune responses in the birds could be due to stimulation of activated B cells clones (Wang et al., 2002). Natural products affect the immune system of the different species in different ways (Shabbir, 2008). Nevertheless, the direct effects of *M. oleifera* and vacci-boost might be related to stimulation of the lymphatic tissue; proliferative activity of immunoglobulin production by peripheral blood B-cells (Wang et al., 2002).

Body weights of the birds were not significantly influenced by the immunomodulators. This is in agreement with Alp et al. (1993) and Waldroup et al. (2003) who observed that body weight of birds were not affected by prebiotics and probiotics supplementations. However, Midilli and Turner (2001) and Piray et al. (2007) demonstrated significant increases in body weight gain in broilers fed diets supplemented with probiotics and prehiotics respectively.

The relative weights of liver, gizzard, and proventriculus were not affected in this experiment. This is in accordance with Panda et al. (2008) who reported no significant difference in the organ weights of weights of liver, gizzard, and proventriculus of broilers fed probiotics. However, the size of these organs may not be associated with immune response (Yamamoto and Glick, 1982). Fathi et al. (2003) also reported that the sizes of these non-lymphoid organs may not be associated with higher immune response of chicken. This work observed that the relative weights of lymphoid organs of the birds treated with *M. oleifera* were significantly ($p \leq 0.05$) higher than those of the vacci-boost treated birds as well as those of the untreated birds.

The reduction in PCV values of the untreated group is in line with the results of Oladele et al. (2008) who observed the NDV causes lysis of erythrocytes and consequently reduction in the value of PCV serves as an important indicator of avian heamogram Oladele et al. (2008).

5. Conclusion

M. oleifera and Vacci-Boost® supplementation in drinking water improved significantly the immune response of chickens to Newcastle disease vaccination with Vacci-Boost showing superior effect. The inclusions had lesser and varied effects on the body weight, relative organ weight and packed cell volume of the chickens.

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