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Immunostimulating potentials of methanolic extract of *Plectranthus parviflorus* in chickens vaccinated against Newcastle disease and Infectious bursal disease

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Abstract

Outbreaks of Newcastle disease (ND) and Infectious Bursal disease (IBD) have been reported in vaccinated poultry flocks in many countries highlighting the need for the administration of immunostimulants to improve immunity in such birds. The immunomodulatory effects of methanol leaf extract of *plectranthus parviflorus* on ND and IBD antibody titre was investigated. One hundred day-old cockerels were randomly assigned into five groups each, A-E and A1-E1 for Newcastle disease and Infectious bursal disease studies respectively. Groups A and A1 were control groups and received water only while groups B and B1 received LaSota and IBD intermediate plus ® vaccine respectively. Groups C and C1, D and D1 were administered 100mg/kg and 200mg/kg of *Plectranthus parviflorus* orally for three days before vaccination respectively, while groups E and E1were given 100mg/kg of the extract orally three days post vaccination. Blood samples were obtained via jugular vein at day-old and 14 days as well as on days 7, 14 and 21 post vaccination. Haemagglutination-inhibition (HI) test and enzyme linked immunosorbent assay (ELISA) were used to assay the antibody titre against ND and IBD respectively. A decline in maternal antibody over 14 days was observed for antibody titre against Newcastle disease and infectious bursal disease from 6.1 to 1.8 geometric mean (log_2) and (1043.37±2.1) to (524.48±2.7) respectively. Group D was observed to have a significant titre value of 2.3 and 3.7 geometric mean (log_2) 14 and 21 days post ND vaccination while group D1 also had a significant increase (1037.90±3.6) titre 14 days post-vaccination. This study showed that *plectranthus parviflorus* extract had a significant effect on Newcastle disease and Infectious bursal disease humoral response when administered orally at 200mg/kg before vaccination with ND and IBD vaccines respectively.

Keywords: ELISA, Haemagglutination-inhibition, Infectious bursal disease, Newcastle disease, *Plectranthus parviflorus*

Introduction

The challenges of food insecurity worldwide particularly in developing countries like Nigeria have continued to receive attention from governments and experts (Esan *et al.,* 2016). The Nigerian population had mostly inadequate protein intake especially of animal origin compared with the most recent protein recommendations by the Food and Agricultural organization (FAO, 2013). The addition of animal-source foods bridges the protein quality gap created by the predominance of plant-based foods in

the Nigerian diet. (Heise *et al.*, 2015; De Vries-Ten Have *et al.,* 2020). To increase protein intake in Nigeria, there is an urgent need to increase poultry production at both household and commercial holdings due to its rapid growth rate and acceptability across different religious and ethnic backgrounds (Matemilola, 2017). Eggs, one of the major products of poultry production, are more affordable for the common person than other sources of animal protein (Ojo, 2003; Aboki *et al.*, 2013; Knoema, 2019).

However, poultry farming in Nigeria has been prone to a heavy risk of increased disease incidences especially Newcastle disease (ND) and Infectious bursal disease (IBD) causing high morbidity and mortality, even when scheduled vaccination programmes are implemented (Esan *et al.*, 2016). Newcastle disease is a highly contagious viral disease affecting many domestic and wild avian species and it is transmissible to humans (Tagesu & Tolera, 2017). Newcastle disease (ND) is one of the highly pathogenic viral diseases of avian species characterized by major economic losses due to huge morbidity and mortality. The disease is endemic in major parts of the world especially in third-world countries where agriculture serves as the primary source of income for the rural and peri-urban dwellers. Newcastle disease virus (NDV) belongs to the family *Paramyxoviridae* and is a well-characterized member among the avian paramyxovirus serotypes (Ganar *et al*., 2014).

Infectious Bursal disease (IBD) commonly known as Gumboro disease is an acute, highly contagious viral infection in chickens manifested by inflammation followed by atrophy of the bursa of Fabricius. The IBD virus belongs to the *Birnaviridae* family, genus *Avibirnavirus* of double-stranded RNA viruses (Adamu *et al.,* 2013). Infectious bursal disease (IBD) is an acute, highly contagious and immunosuppressive poultry disease and the most important immunosuppressive disease threatening the poultry industry worldwide (Fan *et al.,* 2020). IBDV-pathogenesis studies have focused mainly on primary lymphoid organs. (Li *et al.*, 2018).

Vaccination is the only effective method of disease control for Newcastle Disease (ND) and Infectious Bursal Disease (IBD), which have no approved medications for treatment. Despite vaccinations, there are reports worldwide of birds dying or still showing clinical infections (Aldous & Alexander, 2001). This could be due to improper handling of vaccines, wrong timing of vaccinations or interference of vaccine antigen with the maternal antibody, or the inability of the birds to maintain immunity after vaccination (Esan *et al.,* 2016).

Hashemi & Davoodi (2012) assert that a key strategy for success in poultry production is to increase immunity by reducing immunosuppression and its effects. The use of immunostimulants in poultry production has been discovered to be crucial for enhancing avian immunity. Some herbal products are efficient immunopotentiating agents, but their exact mechanisms of action are unknown (Adedeji *et al.,* 2013; Esan *et al.,* 2016).

Plectranthus is the largest genus of the mint family, *Lamiaceae* with over three hundred and fifty (350) genus and seven thousand (7000) species and is commonly referred to as fork leaf. It is widespread throughout Africa, in the wild and under cultivation as an annual plant (Kujeke *et al*., 2019). They are also found in abundant quantities in South Africa's eastern part and most parts of Africa (Rice *et al*., 2011). It has been reported that the plant has an antibacterial and antiseptic property due to the presence of carvacrol, codeine, flavones, phenols, tannins, and aromatic acid (Lukhoba *et al.,* 2006; Roshan *et al*., 2010; Valdivieso-Ugarte *et al*., 2019). It has been reported also to have antiviral activity against the herpes simplex virus and has been shown to contain compounds that inhibit HIV-1 integrase (Lukhoba *et al.,* 2006).

Thus, this study investigated the immunomodulating effects of *plectranthus parviflorus* on the level of antibody titre in cockerel birds vaccinated against Newcastle disease virus and infectious bursal disease (Gumboro).

Materials and Methods

Experimental chickens

One hundred cockerel chickens obtained from CHI Limited Hatchery, Ibadan, with prior request that the birds should not be vaccinated against any disease at the hatchery were housed in open-sided tropical cages at the avian experimental unit, Department of Veterinary Medicine, University of Ibadan, Nigeria. Brooding was done for two weeks while feed and water were provided as often as needed. Strict biosecurity measures were imposed and necessary Veterinary attention was given as required. The chickens had no prior vaccinations for Newcastle disease (ND) and Infectious Bursa disease (IBD) from the hatchery as requested for this study. Maternally derived antibodies against ND and IBD were assayed weekly until the decline of maternal antibody to 1.8 similar to the recommended range of between 1.5± 06 and 4.33±2.33 geometric mean titre respectively

as reported by Abdi *et al*. (2016); Oni & Adedipe (2012) for Newcastle disease while Deventer formula as described by Śmiałek *et al*. (2016) was used to predict the appropriate timing for IBD vaccine administration which was similar to the value 524.48 ELISA unit obtained at day 14 in this study. The study was conducted in accordance with the provision and procedure set out by the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC) with assigned number UI-ACUREC/052-0623/16 and National Health Research Ethics Committee, Nigeria.

Vaccine antigens and vaccination

Newcastle disease vaccine (LaSota) was obtained from ABIC Biological Laboratories Ltd, Israel with minimum infective dose 8.2 Log 10. The vaccine was administered to groups B, C, D, and E through drinking water at day 14 while the infectious bursal disease vaccine (Intermediate plus strain vaccine) containing at least $\geq 10^3 TCID_{50}$ of virus per dose was obtained from Living BP. VET, India and was administered to groups B1, C1, D1, and E1 through drinking water at 14 days of age.

Preparation and collection of plant extract

The leaves of the plant of *plectranthus parviflorus* were identified and procured at the University of Ibadan botanical garden. The leaves were carefully removed from the stem and air-dried for three weeks. The dried leaves were ground into powder before extraction. A total of 365.8g of powdered *plectranthus parviflorus* leaves was soaked in 5 litres of methanol for 72 hours while turning once daily. The resulting suspension was filtered and then concentrated with a rotary evaporator. The concentrated suspension yield was 148ml, which was dried in a hot air oven and yielded 15g of extract. The extract was mixed with corn oil to obtain a homogenous mixture which was administered to each bird orally.

Experimental design

The birds were randomly assigned into five groups each, A-E and A1-E1 for Newcastle disease and Infectious bursal disease studies respectively. Each group was reared separately in individual house to prevent cross infection for the period of the experiment but had adequate access to clean water and feed and were reared under strict biosecurity conditions.

Cockerels in group A (control group) were unvaccinated and were not treated with *plectranthus parviflorus* extract. Group B were vaccinated against ND at day 14 after sufficient decline in maternal antibody but were not treated with the plant extract. Groups C and D were vaccinated against ND (LaSota) and received 100mg/kg and 200 mg/kg of *plectranthus parviflorus* extract orally for 3 days prior to vaccine administration respectively. Group E received ND vaccine at day 14 and 100mg/kg of *plectranthus parviflorus* extract for 3 consecutive days post vaccine administration. Similarly, group A1 (control group) was unvaccinated and not treated with *plectranthus parviflorus* extract. Group B1 were vaccinated against IBD at day 14 after sufficient decline in maternal antibody but were not treated with the plant extract. Groups C1 and D1 were vaccinated against IBD and received 100mg/kg and 200 mg/kg of *plectranthus parviflorus* extract orally for 3 days prior to vaccine administration respectively. Group E1 received IBD vaccine at day 14 and 100mg/kg of *plectranthus parviflorus* extract for 3 consecutive days post vaccine administration.

Evaluation of Newcastle disease antibodies and infectious bursal disease antibodies

About 2 ml of blood were collected from all the cockerel chickens in the five groups (A-E) via the jugular vein on days zero and 14, respectively, to determine the maternal antibody and subsequently on day 21 (7 days post vaccination), day 28 (14 days post vaccination) and day 35 (21 days post vaccination) into plain bottles for ND antibody assay. Sera were obtained from the clotted blood and were stored at -20°C until tested. Similarly, about 2 ml of blood were collected from all the cockerel chickens in groups (A1-E1) via the jugular vein on day 14 to determine the maternal antibody and subsequently on day 21 (7 days post vaccination), day 28 (14 days post vaccination) and into plain bottles for IBD antibody assay.

Haemagglutination inhibition test (HI test) and commercial Enzyme linked immunosorbent assay (ELISA) kit (Idexx, France) were used to evaluate ND and IBD antibody titres in collected sera from the experimental birds. The HI test and ELISA procedure was carried out following standard protocol and the manufacturer's instructions.

Statistical analysis

Mean ± standard deviation (SD) of data obtained per group was determined using the statistical package SPSS (San Diego version 22). Comparison between groups was carried out by one-way analysis of variance (ANOVA) and Tukey's multiple comparisons post-test for multiple comparisons at $P<0.05$.

Results

The geometric mean maternal antibody titre at day 1 for ND was 6.1 log_2 and this decreased substantially to 1.8 log_2 at day 14 due to the natural decline of the passive immunity as shown in table 1 below. The antibody titre for the unvaccinated (Group A) decreased from 1.8 to 0.4 log_2 over a period of 21 days while the group that received vaccine only (group B) showed an increase in the antibody titre from 1.8 log_2 to 1.9 log_2 and 2.6 log_2 at 7 days and 14 post vaccination respectively post vaccination as presented in table 1.The results obtained for groups C and D showed increased antibody titre 2.0 log_2 and 2.3 log_2, 2.8 log_2 and 3.7 log_2 respectively at days 7 and 14 post vaccination which was observed to rapidly decay to 2.0 log_2 and 1.8 log_2 after 21 days post vaccine administration as shown in table 1. The titre in group E picked from 2.1 log_2 form day 7 post-vaccination to 4.8 log_2 21 days after vaccination.

Similarly, the maternal-derived antibody titre at day 1 for IBD was 1043.37EU and decreased over 14 days to 524.48EU. The antibody titre value for group A1 (IBD unvaccinated group) significantly reduced from 458.20±127.7 to 326.80±78.3 over 14 days post-vaccination. Group B1 that received IBD vaccine only had a titre of 696.80±226.1 day 7 post vaccination but declined significantly to 447.40±114.1-day 14 post-vaccination as shown in table 2. Also, groups C1 and D1 had titer values of 621.50±104.1 and 498.50±105.6 which increased significantly to 732.30±338.7 and 1037.90±306.5 respectively 7 and 14. days post vaccination. There was also a significant increase in the antibody titre values obtained in group E1 from 466.90 ± 161.3 on day 7 post-vaccination and 700.20±233.6, day 14 post-vaccination respectively.

Discussion

Naturally occurring or synthetic compounds capable of modulating immune responses in order to confer greater protection against infectious agents have been of immense interest in human and veterinary medicine (Kehrli & Roth, 1990). Hasselquist & Nilsson (2009), reported that maternal transfer of antibodies via egg yolk is very important to protect newly hatched chicks from common pathogens before their immune systems mature, which was supported by the findings of this study.

Crude extracts of *plectranthus parviflorus* have been used in ethno-medicine for the management of various disease conditions, such as bacterial and fungal infections in both humans and animals (Musila, 2017, Valdivieso-Ugarte *et al*., 2019). *plectranthus* spp has been shown to possess a wide safety margin and has been used widely in managing animal diseases such as east coast fever and some bacterial infections at a continuous dosage of 200mg/kg without any sign of toxicity (Ole-Miaron, 2003). Ezeonwumelu *et al.* (2019) also demonstrated the safety of *Plectranthus spp* when it was administered at a dose of 10,000mg/kg in Wistar rats without any obvious sign of toxicity just as no sign of toxicity was observed in this study.

Table 1: Geometric Mean haemagglutination antibody titre (log_2) for Newcastle disease at Pre-vaccination and post vaccination

Age (days)	Post vacc. period (days)	Group A	Group B	Group C	Group D	Group E
0	0			6.1		
14				1.8		
14				NCD Vaccination		
21	7	1.8	1.9	2.0	2.3	2.1
28	14	1.8	2.6	2.8	3.7	2.6
35	21	0.4	1.0	2.0	1.8	4.8

Table 2: Mean ELISA IBD antibody titres pre-vaccination and post vaccination periods

Age (days)	Post vacc. period (days)	Group A1	Group B1	Group C1	Group D1	Group E1
0				1043.37		
14	0			524.48		
				IBD Vaccination		
21	7	458.20±127.7	696.80±226.1^{a}	621.50±104.1^{ab}	498.50±105.6^{bc}	466.90±161.3^{c}
28	14	326.80±78.3^{c}	447.40±114.1^{c}	732.30±338.7^{b}	1037.90±306.5^{a}	700.20±233.6^{a}

Values are expressed as mean ± SD

Means with different superscripts within rows differ significantly ($p<0.05$).

Newcastle disease (ND) seriously affects food security and source of livelihood of poultry farmers due to heavy mortalities encountered in many countries (Amanfu, 2011). To control ND, the use of vaccines is necessary and effective vaccination depends on some critical factors including immune stimulating agents (Banu *et al*., 2009) The antibody titre for ND of the day-old chicks had a uniform level of maternally derived antibody (MDA) for ND with a geometric mean 6.1 $\log_2$, this was similar to the finding of Ahmed (2015) who reported that maternally derived antibody is expected to decline to non-protective level within two weeks steadily. Siwek & Knoll (2005) reported that chicks break down maternal antibodies within 14 days after hatching and that maternal antibodies provide early protection from diseases and it is passed from parent to offspring through the egg yolk. Once the titre is mopped up or interfered with by vaccines, it reduces the immune response and makes the birds susceptible to diseases (Faulkner *et al.,* 2013).

It should be noted that MDA or passive immunity is the device for the prevention of many diseases in newly hatched chicks Thus, MDA could be considered as an effective means of protection of chicks against ND till two weeks of age (Islam *et al*., 2003). The steady decline in ND maternal antibodies in this study agreed with other studies and underscores the need for antibody profiling of day-old birds to accurately predict the appropriate time for vaccination. This signified that the maternal antibody had reduced and the birds are no longer adequately protected against Newcastle disease.

The findings of this study revealed that Groups C and D (100mg/kg and 200mg/kg of the crude extract of *plectranthus parviflorus* for 3 days before vaccination) showed steady increase in the antibody titre until 14 days post vaccination when compared with the positive control (group B) which received ND vaccine only. Group D showed high antibody titres with the peak at 21 days post vaccination. This effect may be due to the presence of essential oils in the plant extract that has been described to improve the cellular and humoral immune responses of chickens in addition to previously described antibacterial, antiviral, antifungal and antioxidant properties (El-Shall *et al*., 2020; Ashaari *et al.,* 2021). A drop in the antibody titre was however observed by day 28 post vaccination when compared to Group E (100mg of the crude extract of *plectranthus parviflorus* for 3 days after vaccination) that had a steady rise in antibody titre up until day 28 post vaccination. This correlates with the findings of Feizi & Nazeri (2011) who reported that an effective protective period could range up to 6 weeks post vaccination.

The average maternally derived antibody (MDA) titre of the day-old birds was 1043.37 which was believed to be high enough for effective protection of the chicks from field IBD virus pending when an appropriate vaccine will be administered. This level of maternally derived antibody agrees with the findings of Jakka *et al.* (2014) and showed that the parent passed very good level of maternally derived antibody to the chicks.

The steady decline in the antibody titre was observed to the end of the experiment in the group that was not vaccinated (group A) which was similar to the findings of Moraes *et al*. (2005). The findings of this study revealed that Group D1 showed higher antibody response to IBD in a dose-dependent manner when compared with groups B1 and C1. This supports the findings of Ezeonwumelu *et al*. (2019) and Oyebanji & Agunloso (2019) that concluded that most of the herbal plants exert their beneficial effects through metabolites such as essential oils, saponins, tannins, alkaloids, terpenoids on animal immune system. Furthermore, natural products such as plant extracts have a long traditional history of use as immunostimulating agents especially in Asia and Africa (Qiu *et al*., 2007). The highest antibody titre against IBD was found in group D1 at day 14 post vaccination agrees with the findings of Shrestha *et al.* (2003) who showed that the chicks vaccinated at around day 14 attain significant level of antibody titre at 29th day of age. The effects of the administration of the plant extract post IBD vaccination were not significantly different from pre-treatment administration as shown in this study and this should be further investigated to determine the mechanism of action of this plant extract.

In conclusion, findings from this study showed that the immunostimulating effects seen were dose dependent as the highest antibody titre for IBD was produced by the administration of 200mg/kg of the extract for three consecutive days before administration of vaccine. Similar effects were observed on the antibody titre from ND vaccinated birds in Group D which peaked at 14 days post vaccination while a prolonged protective titre against ND was obtained in the post vaccination treated group.

We therefore recommend further and expanded trial on the possible inclusion of *plectranthus parviflorus* in drinking water three (3) days prior to IBD and ND vaccination at 200mg/kg body weight as immunostimulating agent, while further work is

recommended to determine the active ingredients in *Plectranthus parviflorus* responsible for the immunostimulating properties seen in this study.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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