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Adoption of pelleted *Digitalia iburua* grain as carrier for heat stable Newcastle disease vaccine virus for village poultry

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The development of pelleted food–based vaccine against Newcastle disease (ND) using a thermostable $NDVI_2$ strain in Acha (*Digitalia iburua)* is reported. The Acha cereal was subjected to rigorous processing of washing, soaking, boiling, roasting and grinding into fine powder prior to virus incorporation. The mixture which was passed through a locally fabricated pelleting machine resulted in fine pellets. The virus content in the pellets determined by inoculation of susceptible embryonated chicken eggs showed appreciable titres above 10^8 Egg Infective Dose 50% end points (EID_{50}) per g of feed. These titres appear to be higher than the recommended minimum immunizing dose of $10^{5.5}$ EID_{50} per ml of vaccine. Further *in-vitro* and *in-vivo* assessment of the pelleted vaccine is advocated.

Key words: Pelleted, $NDVI_2$, food vaccine, *Digitalia iburua*, high virus titres.

INTRODUCTION

Newcastle disease (ND) is endemic in poultry in Nigeria (Abdu et al., 1992; Sa'idu et al., 1994; Halle et al., 1999). The maintenance of the causative virus in the country is largely by the scavenging poultry in villages, which act as reservoirs for themselves and the more susceptible exotic flocks in commercial farms (Gomwalk et al., 1985).

The most cost effective control strategy for ND is by vaccination (Lurthu Reetha et al., 2016). Current conventional vaccines in use are formulated in multiple dose units targeted towards commercial flocks. Such vaccines have little relevance in village poultry which are often small, scattered, multi-aged, and free- roaming with

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Table 1. Proximate analysis of untreated Acha (*Digitaria iburua*).

Moisture	Crude protein (g/100 g of sample)	Crude fibre (g/100 g of sample)	Crude fat (g/100 g of sample)	Ash (g/100 g of sample)	NFE (g/100 g of sample)	Calcium (mg/100 g of sample)	Phosphorous (g/100 g of sample)
Raw Acha	4.60	10.49	7.23	2.88	14.15	65.25	0.14

NFE, Nitrogen – free extract.

Table 2. Phytochemical analysis of untreated Acha (*Digitaria iburua*).

Phytochemical analysis	Phytic acid (mg/ 100 g of sample)	Oxalate (mg/100 g of sample)	Tanins (mg/100 g of sample)
Raw Acha	49.34	20.00	0.827

minimal control.

A viable solution to this problem is the formulation of pelleted feed – based vaccine using a thermostable virus strain in small doses. The search for a suitable feed material is a continuous one. This has been attempted in several countries including Nigeria with limited successes (Iroegbu and Nchinda, 1999; Wambura et al., 2007; Echeonwu et al., 2008).

In the present study, Acha (*Digitalia iburua*), a protein–rich cereal obtained from Jos plateau in central Nigeria, was processed and used as a carrier of heat stable $NDVI_2$ vaccine virus. The vaccine-incorporated carrier was pelleted and the virus content assessed for virus viability in susceptible embryonated chicken eggs.

MATERIAL AND METHODS

Proximate and phytochemical analysis of Acha (*D. iburua*) grains

The untreated Acha grains were subjected to proximate and phytochemical analysis using 100 g of sample according to the method of the Association of official analytical chemists (AOAC, 1990).

Treatment of Acha grains

Approximately, 1 kg of Acha grains was weighed out, washed in clean water, sieved of sand particles and soaked for 24 h. Thereafter, the soaked Acha was re-washed and per-boiled for 10 min with continues stirring during the boiling process. Per- boiled Acha was then air – dried for 10 min, at room temperature in aluminum pans.

The air- dried grains were spread in an oven (100°C) to roast for 4 h. The roasted grains were grinded to a fine powder using a manual hand blender. Once this process was completed, the Acha was ready for vaccine incorporation.

Preparation of pelleted vaccine

The thermostable $NDVI_2$ vaccine stock was obtained from the virus vaccine production division of the National Veterinary Research Institute, Vom, Nigeria. The vaccine virus was propagated and titrated in 10 days – old embryonated chicken eggs, according to standard methods (OIE, 2013).The egg infective dose 50% end point (EID_{50}) was calculated using the Karber formular (Muthannan, 2016).

The prepared $NDVI_2$ vaccine, consisting of a minimum titre of $10^{8.5}$ EID_{50} per ml was taken unto a sterile conical flask. To this was added 100 ml peptone consisting of 2,180 ml wet virus harvest water, 100 ml antibiotics made up to five times the normal working strength of penicillin, streptomycin, gentamycin, and amphotericin B (5xPSGA). This virus / antibiotic / antifungal mixture was added unto 2000 g of processed Acha powder in a glass trough. The mixture was allowed to stand for 8 min for adsorption to take place. The adsorbed mixture was placed in a pelleting chamber of a locally fabricated pelleting machine. The resultant pellets were stored at -80°C.

Titration of pelleted feed vaccine virus

Each batch of the pelleted feed vaccine virus was titrated as follows: 1 g of the pellets was weighed out and placed in each of three sterile universal bottles containing 9 ml of 5xPSGA and votexed. The votexing was repeated at 15 min intervals for 1 h to dissolve the pellets.

Similarly, 1 ml of wet $NDVI_2$ virus only was added to 9 ml of 5xPSGA as control. Thereafter, 0.5 ml of the feed pellet suspension was added to 4.5 ml phosphate buffered saline (PBS) to make a 1:10 feed/ virus suspension. The virus suspension was titrated in embryonated chicken eggs as earlier described (OIE, 2013).

RESULTS

The result of proximate analysis of untreated Acha (*D. iburua*) showed that it contained the following per 100 g of grains (Table 1): Crude protein 4.6 g; crude fiber 10.49 g; crude fat 7.23 g; ash 2.88 g; NFE 14.15 g; Ca 65.25 mg; and P 0.14 g. The following phytochemical compounds were also detected per 100 g Acha grains (Table 2): Phytic acid 49.34 mg; Oxalate 20.00 mg; and Tannins 0.827 mg.

The results of virus concentrations in pelleted feed

Table 3. Results of virus concentrations in $NDVI_2$ pelleted feed vaccine batches.

Vaccine batch	Pellet set	End point titres (log_{10}EID50)	Mean titres
1	A	8.45	-
1	B	8.50	8.48
2	A	8.10	-
2	B	8.50	8.30

showed a range between $10^{8.1}$ EID_{50} and $10^{8.5}$ EID_{50} per g of feed (Table 3). The positive $NDVI_2$ (only) controls for batches A and B were $10^{8.8}$ EID_{50} per ml and $10^{8.30}$ EID_{50} per ml, respectively.

DISCUSSION

The development of a pelleted $NDVI_2$ vaccine is to provide a viable alternative for the immunization and protection of the scavenging (village) poultry flocks against the most dreaded disease, the ND. This need arises from the fact that, village poultry accounts for approximately 84% of the entire poultry population in Nigeria (Sonaiya, 2007), contributing between 68.5 and 72.7% national poultry meat and egg supply, respectively (David-West, 1972). These birds serve as economic, social, ritual, pest control, and waste disposal functions as well as sources of organic fertilizer for rural farmers.

Village poultry has the potential of providing bulk of the much needed animal protein, for majority of Nigerians who live in rural areas. The productivity of village poultry is constrained by annual outbreaks of ND among others. ND is the most important infectious disease affecting village poultry with mortality rates of up to 90% in susceptible flocks (Janviriyasopak et al., 1989; Cumming, 1992; Echeonwu et al., 1993).

Immunization of this group of birds using feed - based thermostable virus vaccine has been advocated as a viable alternative for their protection. The selection and use of $NDVI_2$ virus vaccine in this study is based on its thermostability profile reported previously (Ibu et al., 2009; Guoyuan et al., 2016).

The formulation and maintenance of viable virus content in infected feed pellets for easy vaccine administration has been the greatest challenge to this immunization alternative. This challenge occurs due to the presence of anti-viral elements inherent in the grains as a natural defensive strategy (Egbuna and Ifemeje, 2015). Some of these antiviral elements as contained in the phytochemical analysis of *D. iburua* (Acha) grain elicited the rigorous processing of the grains, to reduce their concentrations prior to incorporation of the vaccine virus.

The determination of virus content in the treated Acha grain is an effective way of assessing the concentration and viable virus retention. The Egg Infective Dose 50% end point (EID_{50}) virus concentration calculated for the pellets showed appreciable viral titre retention in the feed.

A mean virus titre of $10^{8.10}$ to $10^{8.50}$ EID_{50} per g of feed obtained in batches 1 and 2 pelleted vaccine obtained herewith, seems to exceed the minimum immunizing dose threshold of $10^{5.5}$$EID_{50}$ per ml required to vaccinate birds to achieve protection (OIE, 2013).

Conclusion

Further work is required for quality assessment of this pelleted vaccine *in-vitro* and *in-vivo* to determine potency, safety, shelve life, as well as challenge studies among other requirements prior to recommendation for its use.

It should also be noted that the use of a simple locally fabricated easily reproducible pelleting machine used in this work, enables this process to be assessible to less sophisticated laboratories in the third world.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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