

## Vaccination of Chickens with Thermostable Newcastle Disease Vaccine I<sub>2</sub> Coated on Processed Grains and Offals

Abah H.O<sup>1</sup>, Abdu P.A<sup>2</sup>, Sa'idu L<sup>3</sup>

<sup>1</sup>Department of Veterinary Medicine, College of Veterinary Medicine, University of Agriculture Makurdi, Benue State, Nigeria

<sup>2</sup>Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria

<sup>3</sup>Veterinary Teaching Hospital, Ahmadu Bello University, Zaria

### \*Corresponding Author

Name: Abah H.O

Email: [helenabah505@gmail.com](mailto:helenabah505@gmail.com)

**Abstract:** Vaccination experiment was conducted with thermostable Newcastle disease (ND) vaccine strain I<sub>2</sub> (NDVI<sub>2</sub>) to investigate its efficacy as feed based vaccine in chickens using cracked treated maize (TMZ) cracked treated sorghum (TSG), treated maize coated with gum Arabic (TMZG), treated sorghum with gum Arabic (TSGG) and their offals: untreated maize offal (MO) and sorghum offal (SGO) as vehicles. Immune response to vaccination and resistance to challenge were assessed by the haemagglutination inhibition (HI) test. Following vaccination at three and six weeks of age, sera was collected and analysed to determine the antibody titre in the different groups. All vaccinated birds developed HI antibodies to Newcastle disease virus (NDV) ranging from 0.0 log<sub>2</sub> to 8.7 log<sub>2</sub>. In all the groups, the mean HI antibody titre peaked two weeks after second vaccination but declined prior to challenge at nine weeks of age. The highest mean antibody titre of 7.39 ± 0.42 was recorded when the vaccine was administered through (TSGG). Seventy eight (78%) per cent mortality was observed after challenge with NDV Kudu 113 strain in birds vaccinated with NDVI<sub>2</sub> through TMZ and TSGG. No protection was observed in the unvaccinated control group, SGO and TMZG groups. Protection rate in all the groups was low with the highest rate (14%) when the vaccine was administered in TMZ and TSGG. There was no significant difference (P>0.05) between the NDV HI antibody titre in the different feed carriers and the offals while a significant difference (P<0.05) was observed between all feed groups at five weeks of age. From the study it was concluded that the grains and their offals were not suitable vaccine carriers for NDVI<sub>2</sub>. Further research need to be conducted on different methods of processing maize, sorghum and other locally available grains to remove possible antiviral properties in them to make them suitable vaccine carriers for protection of village poultry against ND.

**Keywords:** Newcastle disease, antibody titer, chickens, vaccination, thermostable vaccine

### INTRODUCTION

Newcastle disease is one of the most important infectious viral diseases of poultry due to its potential for devastating losses [24]. The disease can produce mortality of up to 100% among infected populations of birds [5, 18]. The causative agent is NDV, an enveloped, non-segmented, negative-stranded RNA virus and belongs to the genus Avulavirus in the family Paramyxoviridae [22]. Vaccination is currently the most effective method of controlling endemic ND in both commercial and village chickens, but is rarely given priority in rural communities in Nigeria where majority of poultry are kept [1, 28]. Newcastle disease vaccine strains, such as LaSota and Hitchner B1, have been used widely in commercial flocks. However, these vaccines are not generally suitable for village flocks [3]. The main problem associated with these vaccines is their thermo instability and subsequent requirement of a cold chain for the delivery of viable vaccines to villages [16]. Conventional vaccination methods for ND are impracticable for the village farmer that has very small

flock due to large dose presentation, transportation and lack of electricity supply for maintenance of cold chain [35]. Thermostable NDV vaccines have been used widely to control ND for village poultry flocks, due to their independence of cold chains for delivery and storage [16]. Avirulent NDV4 and NDVI<sub>2</sub> strains of ND vaccines have been reported to give varying degrees of successes in village chickens populations in many countries in Asia and Africa including Nigeria in both laboratory and field trials [39, 25, 7, 14, 27].

In an attempt to make delivery of the ND vaccine easier to village chickens, many types of feed stuff have already been tested as carriers for the vaccine [3]. Not all feed stuffs were found to be suitable and some staple foods such as sorghum, millet and other grains produced in many areas of Nigeria have not yet been studied in detail [39, 26]. The major problem with this method is that most food grains possess antiviral agents that often inactivate such coated vaccine viruses [10]. Several workers have suggested various

treatments to be given to carrier feeds so as to enhance the viability of the vaccine. These include washing, boiling or heating of the chosen or available feed before coating with vaccine virus [10, 19, 21, 37]. The control of ND in village chickens can make a vital contribution to the improvement of household food security and poverty reduction in Nigeria. Intensive commercial poultry farmers vaccinate chickens routinely, but village chicken farmers do not [11]. In the current study, two cereal grain species in different forms were evaluated for suitability and efficacy as a carrier for the NDV<sub>2</sub> vaccine to target chickens in the laboratory for adoption in the field as a way forward for developing suitable vaccine delivery system for village chicken production system.

## MATERIALS AND METHODS

### Study location

The study was carried out at the Nutrition laboratory of the Veterinary medicine department, Ahmadu Bello University Zaria, Nigeria.

### Experimental Birds

Two hundred day-old unvaccinated cockerels obtained from the Poultry Research Farm, National Veterinary Research Institute (NVRI), Vom were used for the experiment. The chicks were housed in a brooding room that was cleaned, washed, disinfected and fumigated. They were fed commercial chick mash and provided with water *ad libitum*. The birds were brooded, raised to three weeks and screened for maternal HI antibody.

### Experimental design

The chicks were divided into 4 groups (A, B, C and D) at 3 weeks of age. Each group was subdivided into 2 subgroups each consisting of 18 birds. All birds in subgroups A to C were vaccinated and challenged; groups: A1 (treated maize) A2 (treated sorghum) B1 (treated maize plus treated gum Arabic) B2 (treated sorghum plus treated gum Arabic) C1 (maize offal) C2 (sorghum offal). Birds in subgroup D1 were not vaccinated but challenged and D2 were unvaccinated and unchallenged and served as positive and negative controls respectively.

### Source of NDV<sub>2</sub> vaccine and challenge virus

The NDV<sub>2</sub> vaccine was obtained from the Viral Research Department, National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. The vials of the vaccines were 50 dose vials meant to be reconstituted in 50 ml of chlorine free water and to be giving orally at 1 ml/ bird. The virus strain used for the challenge experiment was the NDV (Kudu 113 strain) isolated and characterized in a previous study [12] with EID<sub>50</sub> titre of 10<sup>7.5</sup>. The virus was obtained from the Virology Division of the NVRI, Vom.

### Vaccine Carriers

Five kilograms each of maize, sorghum and their offals and four kilograms of gum Arabic purchased from a local market in Zaria, Kaduna State were used as vaccine carriers.

### Preparation and coating of food carrier with vaccine virus

The maize and sorghum were cracked and polished ('surfe') to remove the husk and then crushed into a gritty mash. These were soaked in clean chlorine free water for 72 hours, while changing the water daily. The soaked grains were then washed, sieved and placed to dry in the sun. They were then weighed and packaged in polythene bags of 1 kg/package and stored at room temperature until used. The maize and sorghum offals were not subjected to any treatment; they were dried, packaged and kept at room temperature until used. About 2kg of gum Arabic (used as additive) was soaked to dissolve overnight in 1,000 ml of distilled water. The gum Arabic was then boiled for an hour, allowed to cool and then autoclaved at 121°C for 15 minutes.

The method described by Alders and Spradbrow [2] was used for coating the feed with the vaccine virus. The quantity of grains or offals consumed by 18 birds (10 g per bird) was measured. Three vials of the 50 doses of NDV<sub>2</sub> vaccines were reconstituted in 100 ml of PBS (pH 7.4) Then 50 ml of the treated diluted gum Arabic was thoroughly mixed with the reconstituted vaccine and then mixed with the feed (at a ratio of 1 ml to 10 g of the dried grain or offal) in a bowl and then spread on trays and kept at room temperature for 30 minutes before administering to the birds. The time taken to consume the vaccine feed was noted.

### Vaccination/Serology

The birds were vaccinated at 3 (1<sup>st</sup> dose) and 6 weeks (2<sup>nd</sup> dose) of age with the NDV<sub>2</sub> coated on the treated grains or offals. About one to two milli litres of blood was collected through the wing vein with a 2 ml syringe and 21 G needle from each bird at days 7, 14 and 21 before primary vaccination and at 2 and 3 weeks post vaccination. The blood samples were deposited into sterile test tubes and sera were separated by allowing the blood to clot in the test tubes slanted in racks at room temperature for one to two hours. All sera collected were tested for NDV specific antibody by the haemagglutination inhibition (HI) test by methods described by OIE [31].

### Challenge of experimental birds

Vaccinated birds were challenged at 9 weeks of age (3 weeks after second vaccination) with NDV Kudu 113 strain. Each bird was inoculated with 0.20 ml of the NDV through the ocular route. All birds inoculated were observed for clinical signs and the number of sick and dead in each group were recorded.

**DATA ANALYSIS**

The mean HI antibody titre and percentage of birds with detectable ND antibody were calculated. Data collected were analyzed using Statistical Package for Social Sciences (SPSS) version 17 program. One way analysis of variance (ANOVA) was performed with Tukey post hoc multiple comparison, which determined statistical significant difference between subgroups at 95% confidence interval with  $P \leq 0.05$  considered as significant. Mortality and protection rates were also calculated.

**RESULTS**

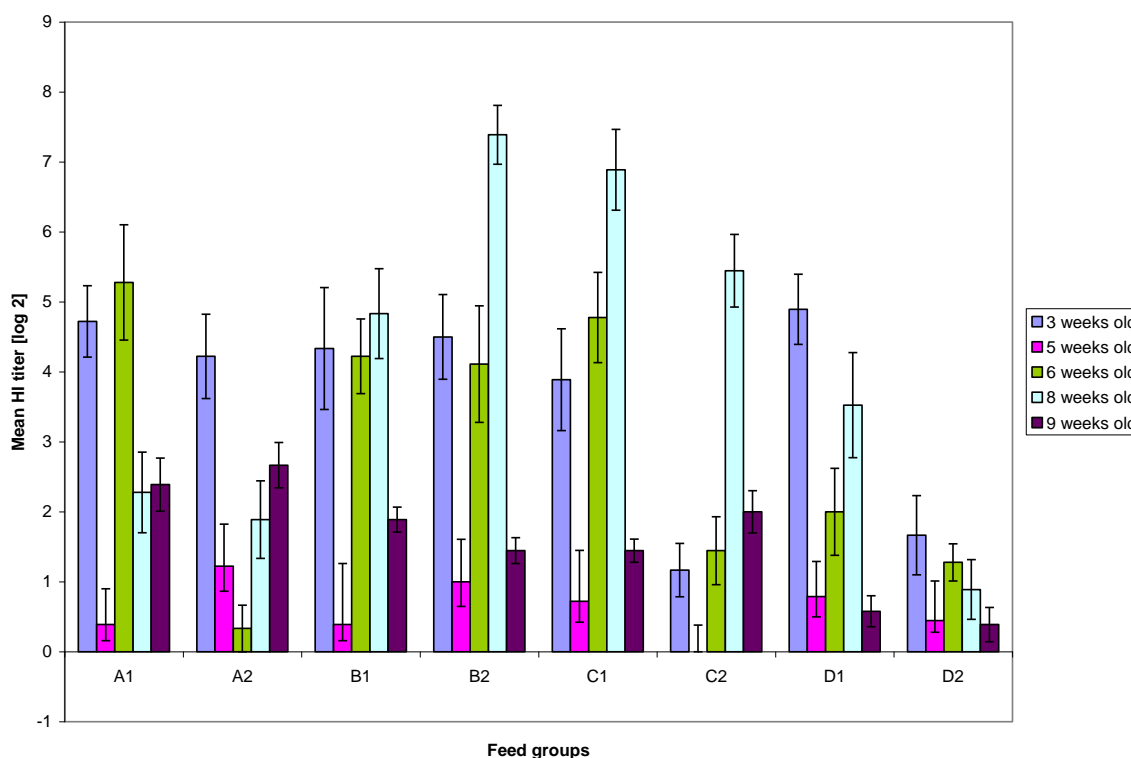
**Response of Birds to Primary Vaccination**

All birds screened prior to administration of NDVI<sub>2</sub> vaccine at three weeks of age had a mean HI antibody titre of  $\geq 3 \log_2$  except those in group C2 ( $1.17 \pm 0.38 \log_2$ ) and D2 ( $1.67 \pm 0.56 \log_2$ ). Two weeks after primary vaccination (at five weeks of age) the HI ND

antibody titre dropped in all groups with group A1 and B1 having the lowest mean HI ND antibody titre of  $0.39 \pm 0.23 \log_2$ . At six weeks of age the lowest mean HI ND antibody titre was recorded in group A2 ( $0.33 \pm 0.33 \log_2$ ), followed by groups D2 ( $1.27 \pm 0.27 \log_2$ ), C2 ( $1.44 \pm 0.49 \log_2$ ) and D1 ( $2.00 \pm 0.62 \log_2$ ) while the mean HI ND antibody titre of  $\geq 3 \log_2$  was recorded in groups A1 ( $5.27 \pm 0.82 \log_2$ ), B1 ( $4.22 \pm 0.53 \log_2$ ), B2 ( $4.11 \pm 0.83 \log_2$ ) and C1 ( $4.78 \pm 0.64 \log_2$ ) (Figure 1).

**Response of Birds to Booster Vaccination**

When administered with a booster dose of the feed base NDVI<sub>2</sub> vaccine at 6 weeks of age, the birds further seroconverted with the peak mean HI ND antibody titre of  $7.39 \pm 0.42 \log_2$  recorded for group B2 at eight weeks of age followed by group C1 ( $6.89 \pm 0.58 \log_2$ ) and C2 ( $5.44 \pm 0.52 \log_2$ ). (Figure 1).



**Fig 1: Haemagglutination inhibition antibody titres in serum of birds vaccinated at three and six weeks of age with Newcastle disease vaccine (NDVI<sub>2</sub>) through different grains and their offals. Groups: A1 (treated maize) A2 (treated sorghum) B1 (treated maize plus gum Arabic) B2 (treated sorghum plus gum Arabic) C1 (maize offal) C2 (sorghum offal) D1 and D2 (positive and negative controls not vaccinated).**

Three weeks after booster vaccination (at nine weeks of age), there was a sharp drop in mean antibody ND HI titre in all the groups especially in groups B2 and C1 which had the highest mean HI ND antibody titre at eight weeks of age. The lowest mean HI ND antibody titre at nine weeks of age was recorded in the control groups, D1 ( $0.58 \pm 0.22 \log_2$ ) and D2 ( $0.39 \pm 0.24 \log_2$ ).

**Percentage of Birds with ND Antibody HI Titres of  $\geq 3 \log_2$**

The percentage of birds with NDV HI titres of  $\geq 3 \log_2$  are presented in Table 1. At three weeks of age prior to primary vaccination 88% of the birds in group A1 and 94% of birds in group D1 had HI antibody titre of  $\geq 3 \log_2$ . At five weeks of age 33% of birds in group A2 and 27% in group B2 had HI antibody titre of  $\geq 3 \log_2$ . At six weeks of age groups A1 and C1 had 77% of birds with HI antibody titre  $\geq 3 \log_2$ . At eight weeks

of age 83% of the birds in group B1, 100% of birds in group B2, 94% of birds in group C1 and 88% of birds in group C2 had HI antibody titre  $\geq 3 \log_2$ . Prior to challenge at nine weeks of age 44% of the birds in groups A1, 50% in group A2 and 38% in group C2 had HI antibody titres  $\geq 3 \log_2$ .

**Mortality and protection Rate**

The mortality and protection rates of birds challenged with NDV Kudu 113 are presented in

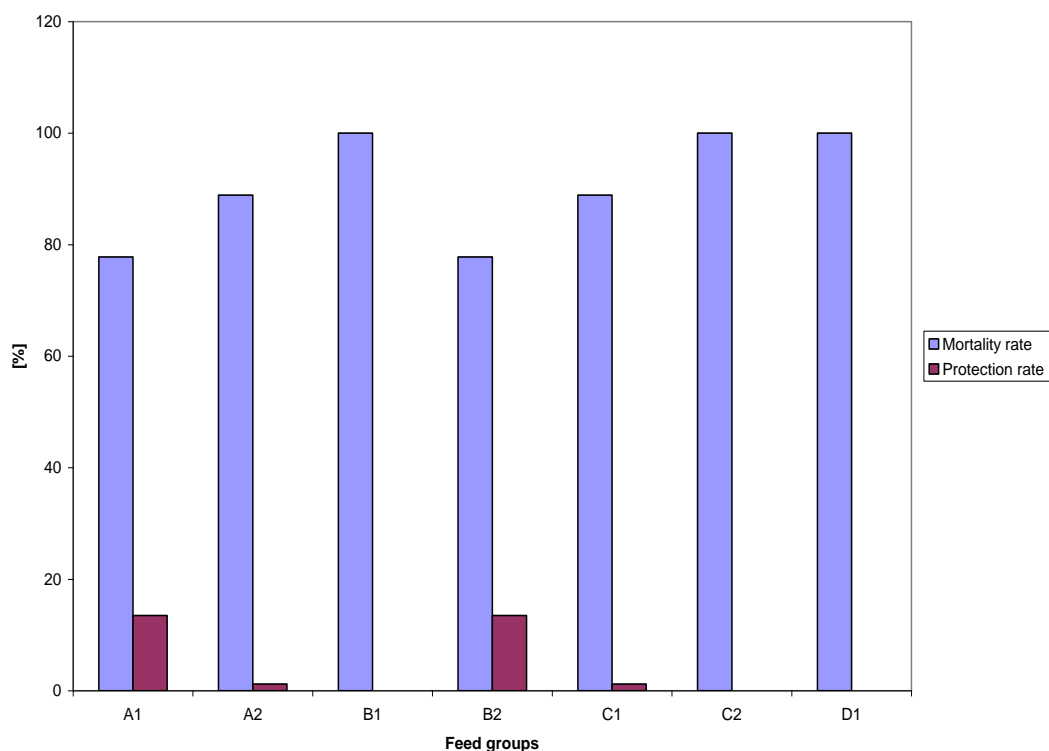
Figures 2. The highest mortality rate (100%) was recorded in groups B1 and the control group, while the lowest (78%) was recorded in groups A1 and B2. Groups A2 and C1 had 89% mortality rate (Figure 2).

Protection rate after challenge was low for all the groups, A1 had (14%), A2 (1.2%), B2 (14%), C1 (1.2%) while B1 and C2 were not protected (Figure 2).

**Table 1: Percentage of birds with NDVI<sub>2</sub> vaccine haemagglutination inhibition antibody titers of  $\geq 3 \log_2$  following vaccination at 3 and 6 weeks of age**

Group (n=18)	Age in weeks				
	3	5	6	8	9
A1	88.9	11.1	77.8	38.9	44.4
A2	72.2	33.3	5.56	33.3	50.0
B1	61.1	11.1	72.2	83.3	22.2
B2	77.8	27.8	55.6	100	5.56
C1	66.7	11.1	77.8	94.4	5.56
C2	22.2	0.0	22.2	88.9	38.9
D1	94.4	22.2	38.9	61.1	5.56
D2	11.1	0.0	11.1	16.7	11.1

Groups: A1 (treated maize) A2 (treated sorghum) B1 (treated maize plus gum Arabic) B2 (treated sorghum plus gum Arabic) C1 (maize offal) C2 (sorghum offal) D1 and D2 (positive and negative controls not vaccinated).



**Fig 2: Mortality and protection rate in birds after challenge with Kudu 113 Newcastle disease virus at three weeks after second vaccination with NDVI<sub>2</sub> administered through different grains and their offals. Groups: A1 (treated maize) A2 (treated sorghum) B1 (treated maize plus gum Arabic) B2 (treated sorghum plus gum Arabic) C1 (maize offal) C2 (sorghum offal) and D1 (unvaccinated challenge).**

## DISCUSSION

The results from the study showed that the mean HI ND antibody titre was high before primary vaccination in all the groups. Since the birds had no previous vaccination at day-old, these results might be due to the presence of maternal antibodies in the chicks. This finding is similar to the report of Hamal [17] who observed that maternally derived antibodies in newly hatched chicks if present might last up to 14 days of age. Two weeks after primary vaccination (at five weeks of age), very low level of ND antibody titre was recorded in all the groups. This sudden drop could be due to interference from the maternal antibodies in the chicks as reported by Jalil [20]. It is believed that maternal antibodies neutralize the introduced vaccine, rendering the vaccine ineffective during primary vaccination [4, 6]. There was a general increase in the HI ND antibody titre two weeks after secondary vaccination in all the groups except group A1, A2 and D2 where the antibody level declined. Similar results were reported by other workers [34] that secondary vaccination yielded HI titres that were significantly higher than the antibody titres after single vaccination. This delayed response might also be explained by the varying amounts of feed vaccine eaten by the chicks and the time required to consume the feed since the amount of vaccine virus taken per bird is a critical factor [27]. It was observed that during the first vaccination it took young chicks more than two hours to consume the vaccine treated feed, while during the second vaccination it was consumed in less than 30 minutes. This shows that the time of vaccine feed consumption decreases with age. The prolonged exposure of the vaccine/feed mix at first vaccination might affect the survivability of the vaccine virus, particularly under extreme environmental conditions [27]. The results of this experiment also showed that booster dose of the vaccine given three weeks after the first vaccination only led to a temporary rise in antibody titres, which declined gradually until the birds were challenged three weeks post booster vaccination. Similar findings were reported by Nasser [7] and Baba [7] that titres among vaccinated birds generally peaked by day 21 post vaccination and declined subsequently. Previous studies on dose response with NDV4 revealed peak response occurring at two or four weeks after vaccination [36].

The reported protective antibody titre for ND vaccines are  $HI \geq 4 \log_2$  (OIE, 2000) with reference to conventional ND vaccine designed for intensively reared commercial chickens. However, HI ND antibody titre of  $\geq 3 \log_2$  was considered to be adequate for food-based vaccines orally administered to scavenging chickens [13]. The percentage of vaccinated birds with HI titres  $\geq 3 \log_2$  showed a marked increase at six and eight weeks of age. Flock immunity reported by Boven [9] as the only means to prevent the transmission of NDV can only be achieved when  $\geq 85\%$  of vaccinated

birds have antibody titres of  $\geq 3 \log_2$ . In the present study this was achieved in groups B2, C1 and C2 at eight weeks. However, prior to challenge at nine week of age, the percentage dropped with none of the groups having percentage mean HI antibody titre sufficient to protect the birds from challenge.

The differences in the protection and mortality rate after challenge with virulent NDV as observed in this study could be due to the differences in the vehicles used in administering the NDVI<sub>2</sub> vaccine virus. The highest mortality rate (100%) in vaccinated birds was recorded in birds vaccinated with TMZG and SGO. However, birds vaccinated with TMZ (A1) and TSGG (B2) had the lowest mortality of 78% respectively. Also, low protection rate was also recorded in all the groups except the group vaccinated with treated maize and treated sorghum coated with gum Arabic. This is similar to reports by Nasser [27] in vaccination trials in Ethiopia, where untreated and parboiled sorghum used as vaccine carriers for NDVI<sub>2</sub> failed to protect birds after challenge. Similarly, Musa [26] reported that untreated sorghum, parboiled sorghum, sorghum coated with gum Arabic and a commercial feed mash used as feed carriers for NDVI<sub>2</sub> vaccine gave low antibody titre and low protection following challenge with a velogenic NDV. Reports from Alders and Spradbrow [2] and Musa [26] also reported that when NDV4HR and NDVI<sub>2</sub> respectively were administered via eye-drop route it produced higher mean antibody titres and protection on challenge compared to drinking water and feed routes. Failure of some of these feed vaccination trials was attributed to antiviral factors in the seeds or substances introduced as additives or preservatives. The presence of tannins in sorghum and gum Arabic was observed in a similar study reported by Musa [26] as responsible for inactivating the vaccine virus. Samuel [33] and Cumming [10] found that uncooked grains were not entirely satisfactory as vaccine carriers and showed that vaccine washed off immediately after addition to grains and lost at least 90% of its initial virus titre. Oakeley [29] reported that grains grown in different agro-ecology and on different soil characteristics tend to vary in their vaccine virus carrying capacity due to variation in the grains' physico-chemical characteristics, especially their surface properties and chlorine content. Therefore, [10] suggested that short boiling; washing and coarse cracking of the grain might significantly extend the survival of the virus on the grain. This necessitated the cracking, soaking and washing treatments given to the grains investigated in this study. Previous work in Nigeria [14], which showed that the NDVI<sub>2</sub> vaccine mixed with treated maize offal resulted in seroconversion and 100% protection after secondary vaccination, could not be confirmed by the present work. The use of additive was meant to stabilize the virus in dried condition. Although there was evidence that the virus titre was better maintained in the presence of the additive. These results agree with the findings of



[8], who reported that additives (especially gelatin) enhanced the survival of the NDV<sub>12</sub> thermostable vaccine strain after storage for weeks at room temperature.

The use of feed waste (maize and sorghum offals) in this study was meant to reduce the problem of buying feeds by the rural poultry farmer which is difficult in the natural habitat of the village chicken and to demonstrate that food waste may be a good carrier of NDV<sub>12</sub> vaccine if adequately treated. Ordinarily, the grains offal are waste product of household food processing and so would be available at little or no cost to the village chicken farmer [15].

In general, parboiled grains, followed by cracked ones, induced higher serological response and protection level than intact (untreated) grains. Heating, soaking, washing and cracking grains might be useful in developing a successful vaccine carrier feed. Similar findings have been reported from other countries [10, 19]. Cracked maize and treated sorghum were found to be better vaccine carriers in this study though the protection rate was low. Similar work in Nigeria [14, 23] and in Ethiopia [32] showed that Cracked maize and treated sorghum would be promising suitable feed carriers for administration of NDV<sub>12</sub> vaccine under field (village) conditions

## CONCLUSIONS

The immune response of the birds to the NDV<sub>12</sub> vaccine coated on treated maize, sorghum and their untreated offals showed that the carrier feeds sustained virus infectivity and immunogenicity though gave low protection to vaccinated birds after challenge with velogenic NDV. The NDV<sub>12</sub> vaccine could be useful for the protection of village chicken against ND provided the carrier feeds are adequately treated to remove antiviral substances. The study recommends different processing methods should be employed to treat these grains and other locally available feeds such as millet, to test their suitability as ND vaccine feed carriers. The duration of immunity after oral feed vaccination and the frequency of revaccination required to maintain full immunity needs to be subjected to further research.

## ACKNOWLEDGEMENTS

The authors appreciate the contributions of Prof. S.B Oladele of the department of Veterinary Pathology and Microbiology, Ahmadu Bello University Zaria, Dr. Musa U. of the Veterinary Research Institute, Vom and thank all staff of the Nutrition Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, for their technical assistance.

## REFERENCES

1. Abdu PA, Sa'idu L, Bawa EK, Umoh JU. Factors that contribute to Newcastle disease, Infectious bursal disease and Fowl pox

- outbreaks in chickens. In 42nd Annual Congress of the Nigerian Veterinary Medical Association held at university of Maiduguri. 14th-18th November 2005 Nov 18.
2. Alders RG, Inoue S, Katongo JC. Prevalence and evaluation of Hitchner B1 and V4 vaccines for the control of Newcastle disease in village chickens in Zambia. *Preventive Veterinary Medicine*. 1994 Nov 1; 21(2):125-32.
3. Aini I, Ibrahim AL, Spradbrow PB. Field trials of a food-based vaccine to protect village chickens against Newcastle disease. *Research in veterinary science*. 1990 Sep; 49(2):216-9.
4. Al-Natour MQ, Ward LA, Saif YM, Stewart-Brown B, Keck LD. Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. *Avian diseases*. 2004 Jan; 48(1):177-82.
5. Ananth R, Kirubaharan JJ, Priyadarshini ML, Albert A. Isolation of Newcastle disease viruses of high virulence in unvaccinated healthy village chickens in south India. *Intl J Poult Sci*. 2008; 7:368-73.
6. Awang IP, Wan-Ahmed-Kusairy WS, Abdul-Razak J. Detection of maternal antibody against Newcastle disease virus in chicks using an indirect immunoperoxidase test. *Journal of Veterinary Malaysia*. 1992; 4(1):19-23.
7. Baba SS, Iheanacho CC, Idris JM, El-Yaguda AD. Food-Based Newcastle Disease V 4 Vaccine In Guinea Fowl (*Numida Meleagris Galeata Pallas*) In Nigeria. *Tropical Veterinarian*. 2006; 24(3):37-45.
8. Bensink Z, Spradbrow P. Newcastle disease virus strain I 2—a prospective thermostable vaccine for use in developing countries. *Veterinary microbiology*. 1999 Aug 16; 68(1):131-9.
9. Van Boven M, Bouma A, Fabri TH, Katsma E, Hartog L, Koch G. Herd immunity to Newcastle disease virus in poultry by vaccination. *Avian Pathology*. 2008 Feb 1; 37(1):1-5.
10. Cumming RB. Newcastle disease research at the University of New England. In: Spradbrow PB (ed), *Newcastle Disease in Village Chickens, Control with Thermostable Oral Vaccines*. Proceedings no 39. Canberra: Australian Centre for International Agricultural Research, 1992: 48 – 85.
11. Duguma R. Understanding the role of indigenous chicken during the long walk to food security in Ethiopia. *LRRD*. 2009; 21(116): <http://www.lrrd.org/lrrd21/8/dugu2116.htm>. Accessed 24 April, 2017.
12. Echeonwu GO, Iroegbu CU, Emeruwa AC. Recovery of velogenic Newcastle disease virus from dead and healthy free-roaming birds in Nigeria. *Avian Pathology*. 1993 Jun 1; 22(2):383-7.

13. Echeonwu GO, Iroegbu CU, Echeonwu BC, Ngene A, Olabode AO, Okeke OI, Ndako J, Paul G, Onovoh EM, Junaid SA, Nwankiti O. Delivery of thermostable Newcastle disease (ND) vaccine to chickens with broken millet grains as the vehicle. *African Journal of Biotechnology*. 2007; 6(23).
14. Echeonwu BL, Ngele MB, Echeonwu GO, Joannis TM, Onovoh EM, Paul G. Response of chickens to oral vaccination with Newcastle disease virus vaccine strain I 2 coated on maize offal. *African Journal of Biotechnology*. 2008; 7(10).
15. Echeonwu GO, Iroegbu CU, Ngene A, Junaid SA, Ndako J, Echeonwu IE, Okoye JO. Survival of Newcastle disease virus (NDV) strain V4-UPM coated on three grains offal and exposed to room temperature. *African Journal of Biotechnology*. 2008; 7(15).
16. Wen G, Chen C, Guo J, Zhang Z, Shang Y, Shao H, Luo Q, Yang J, Wang H, Wang H, Zhang T. Development of a novel thermostable Newcastle disease virus vaccine vector for expression of a heterologous gene. *Journal of General Virology*. 2015 Jun 1; 96(6):1219-28.
17. Hamal KR, Burgess SC, Pevzner IY, Erf GF. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poultry science*. 2006 Aug 1; 85(8):1364-72.
18. Ibu OJ, Okoye JO, Adulugba EP, Chah KF, Shoyinka SV, Salihu E, Chukwuedo AA, Baba SS. Prevalence of Newcastle disease viruses in wild and captive birds in central Nigeria. *International Journal of Poultry Science*. 2009; 8(6):574-8.
19. Jackson AR. Observations on some difficulties encountered in trials with oral Newcastle disease vaccination. *Newcastle Disease in Village Chickens*. 1991 Oct 6; 6:15.
20. Jalil MA, Samad MA, Islam MT. Evaluation of maternally derived antibodies against Newcastle disease virus and its effect on vaccination in broiler chicks. *Bangladesh Journal of Veterinary Medicine*. 2010 Sep 3; 7(2):296-302.
21. Johnston J, Cumming R, Fontanilla B, Sivanno F. Patterns of NDV activity in village fowls and the measurement of effective field protection following oral vaccination. In: *Newcastle Disease in Village Chickens*. Australian Centre for International Agricultural Research Proceedings 1992; 39: 33-39.
22. Lamb RA, Collins PL, Kolakofsky D, Melero JA, Nagai Y, Oldstone MBA, Pringle CR, Rima BK. Family Paramyxoviridae. In: *Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses*, Edited by Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. London: Academic Press. 2005: 655–668.
23. Lawal JR, El-Yuguda AD, Ibrahim UI. Efficacy of Feed Coated Newcastle Disease I2 Vaccine in Village Chickens in Gombe State, Nigeria. *J Vet Sci Technol* 2016; 7: 349.
24. Miller PJ, Guus K. (2013). Newcastle disease. In *Diseases of Poultry*, Edited by Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, V. Nair V. Ames, IA: Wiley-Blackwell. 13th edn, 2013: 98–107.
25. Msami HM, Young MP. Newcastle disease control using I-2 vaccine in Tanzania: country report. *Village chickens, poverty alleviation and the sustainable control of newcastle disease*. 2009:67.
26. Musa U, Abdu PA, Mera UM, Emmenna PE, Ahmed MS. Vaccination with newcastle disease vaccines strain i 2 and lasota in commercial and local chickens in Plateau State Nigeria. *Nigerian Veterinary Journal*. 2010; 31(1).
27. Nasser M, Lohr JE, Mebratu GY, Zessin KH, Baumann MP, Ademe Z. Oral Newcastle disease vaccination trials in Ethiopia. *Avian Pathology*. 2000 Feb 1; 29(1):27-34.
28. Nwanta JA, Abdu PA, Ezema WS. Epidemiology, challenges and prospects for control of Newcastle disease in village poultry in Nigeria. *World's Poultry Science Journal*. 2008 Mar 1; 64(01):119-27.
29. Oakeley RD. The limitations of a feed/water based heat-stable vaccine delivery system for Newcastle disease-control strategies for backyard poultry flocks in sub-Saharan Africa. *Preventive veterinary medicine*. 2000 Dec 8; 47(4):271-9.
30. Office International Des Epizootics. *Newcastle disease, Manual of Standards for Diagnostics Tests and Vaccines*. 2000: 104-124.
31. Office International Des Epizootics (OIE). *Newcastle disease*. In: *Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals*. 2004.
32. Oakeley RD. The limitations of a feed/water based heat-stable vaccine delivery system for Newcastle disease-control strategies for backyard poultry flocks in sub-Saharan Africa. *Preventive veterinary medicine*. 2000 Dec 8; 47(4):271-9.
33. Samuel JL., Bensik Z, Spradbrow PB. Results of Newcastle disease research projects at the University of Queensland. In: Spradbrow, P.B. (ed). *Newcastle Disease in Village Chickens Control with Thermostable Vaccine*. Proceeding no 39. Canberra: Australian Centre for International Agricultural Research. 1992: 79 – 83.
34. Shuaib M, Ashfaque M, Sajjad-ur R, Mansoor MK, Yousaf I. Comparative immune response

- of broiler chicks to Newcastle disease vaccine (LaSota strain). *Pak. Vet. J.* 2003; 23: 91-93.
35. Spradbrow PB, Ibrahim AL, Mustaffa-Babjee A, Kim SJ. Use of an avirulent Australian strain of Newcastle disease virus as a vaccine. *Avian diseases.* 1978 Apr 1:329-35.
  36. Spradbrow PB, Samuel JL. Oral Newcastle disease vaccination with V4 virus in chickens: Comparison with other routes. *Australian veterinary journal.* 1991 Mar 1; 68(3):114-5.
  37. Spradbrow PB. Newcastle disease in village chicken. Proceeding No. 39, Australian Centre for International Agricultural Research, Canberra, Australia. 1992a.
  38. Spradbrow P. Oral vaccine and mucosal immunity. Australian Centre for International Agriculture Research Proceedings. 1992; 39:56-60.
  39. Wambura PN, Kapaga AM, Hyera JM. Experimental trials with a thermostable Newcastle disease virus (strain I 2) in commercial and village chickens in Tanzania. *Preventive Veterinary Medicine.* 2000 Jan 20; 43(2):75-83.