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Supplementation of *Phyllanthus amarus* and *Cuminum cyminum* Hasten Vaccinal Antibody Response against Newcastle Disease Virus and Increases Gut Absorptive Surface in Backyard Poultry

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ABSTRACT

Newcastle disease (ND) is a highly contagious disease that causes devastating effects in the economy of backyard poultry farmers. Even though, vaccination is highly effective in controlling the disease, the protective antibody titres can only be achieved after second dose of ND vaccine at 3-4 weeks of age. Certain herbs have immune-stimulant property, thus supplementation of herbs can help the chicks to attain the protective antibody titres earlier. In the present study, chicks were fed with *Phyllanthus amarus* as whole plant along with *Cuminum cyminum* (seeds) water to hasten the production of antibodies to ND vaccines. Humoral immune responses were assessed in terms of haemagglutination inhibition (HI) titers. Morphometric analysis small intestinal villi were performed to assess the impact of herbal supplements on gut health. Our results showed that birds supplemented with herbs mounted a better immune response to ND vaccines. Birds received herbs attained HI titres $\geq \log_2 4$ as early as 7 days after the primary vaccination ($P<0.001$) and continued to have protective antibody titres until 60 days-of-hatch. Intestinal morphometry revealed that herbal supplementation significantly improved the length of villi in duodenum ($P<0.01$) and jejunal portions of small intestine. In conclusion, we suggest that feeding of *P. amarus* and *C. cyminum* aids birds to elicit better and earlier protective immune response against ND and improve gut health of backyard poultry.

HIGHLIGHTS

- Feeding of *Phyllanthus amarus* and *Cuminum cyminum* helps backyard poultry to attain protective HI titres faster upon ND vaccination.
- Herbal supplementation increases villi length in the duodenum.

Keywords: *Cuminum cyminum*, Newcastle disease, *Phyllanthus amarus*

The backyard poultry sector plays pivotal role in alleviating poverty in rural parts of India. However, it encounters huge threat in terms of high mortalities caused by Newcastle disease (ND) (Kumar, 2015). Vaccinations against ND using live and inactivated vaccines are practiced in large scale commercial broiler and layer farms, whereas backyard poultry farmers often do not practice vaccination as recommended (Dey *et al*., 2014). Hence, chicks hatched in backyard poultry farms tend to have minimal maternal antibodies, and they are

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highly vulnerable to NDV infection. Antibiotics and other synthetic compounds had been used as immune boosters in poultry feed (Sorum and Sunde, 2001; Suresh *et al*., 2018) to improve the immune status of chicks. However, due to the emergence of antimicrobial resistance in microbes, their use has been prohibited (Cogliani *et al*., 2011). Therefore, the use of natural alternatives like herbs is an increasing trend as poultry feed additive (Dharmaraj *et al*., 2017; Gheisar and Kim, 2018). *Phyllanthus amarus* is a tropical plant, ubiquitous in rural India and found to have immunomodulatory activities (Joseph and Raj, 2011; Patel *et al*., 2011). It also has anti-oxidant, antimicrobial, anti-cancer activities (Sarin *et al*., 2014; Mao *et al*., 2016). Studies suggested that *Cuminum cyminum* seeds have the ability to improve immune status, digestibility and egg quality in birds (Habibi *et al*., 2016; Saleh *et al*., 2019). With this background of information in the present study, we have assessed the immunomodulatory effect of *Phyllanthus amarus* and *Cuminum cyminum* in backyard poultry, with the objective to attain faster and better protective immunity to ND upon vaccination.

MATERIALS AND METHODS

Experimental birds and design

The present study was conducted in a backyard poultry farm located (10.62°N 79.27°E) at Tamil Nadu state, India. Newly hatched ninety day-old chicks of upgraded native chicken (Aseel cross) variety were randomly selected and divided into three groups namely, vaccine, herb and in-contact control each consists of 30 birds (Table 1). The birds were reared in deep-litter system of housing with *ad libitum* potable boiled water and feed. Birds were reared up to 90 days of age and sold for meat purpose.

Collection of samples

Blood samples were collected aseptically from metatarsal vein on 7th, 14th, 21st, 30th, 60th and 90th days post-hatch (DPH) from all the birds. A drop or two of blood were directly collected in a Whatman No. 1 filter paper and allowed to dry in room temperature for 3 hours and stored at refrigerated conditions until further processing for haemagglutination inhibition assay. At the market age (90 days of age) birds were slaughtered and sold for meat purpose. At the time of slaughter, portions of small intestine were randomly collected (each group, n=3) in buffered formalin and processed for morphometric studies and histopathological examination.

Haeamagglutination Inhibition (HI) test

Serum was eluted from the filter paper as per the protocol described by Roy *et al*. (1992). Fifty microlitre of eluted sera were used to determine the Haemagglutination inhibition titres against 4 HA units of Newcastle disease virus as per the standard protocol (OIE, 2019). Serum samples were tested in duplicates, against thermostable D58 strain of NDV (Ananth *et al*., 2008) available in the Department of Veterinary Microbiology, Madras Veterinary College to determine the HI titre. Positive and negative sera available in the department were used as controls.

Morphometry of intestinal villi

Tissue sections of intestinal loops (each group, n=3) were stained using haematoxylin and eosin staining method and lengths of villi in duodenum, jejunum, ileum regions were measured using micrometry. On the basis of intactness of lamina propria, six villi were selected for the measurement

Table 1: Details of the experimental groups

Treatments	Groups (n=30)		
	Vaccine	Herb	Control
Vaccination schedule (Newcastle Disease)			
• Days post hatch, DPH 7 – 'F' strain instilled on eye • DPH 14 – '*La Sota*' strain given through drinking water • DPH 28 – 'R_2B' strain given subcutaneously	Yes	Yes	No
Herbal supplementation			
• Boiled water with *Cuminum cyminum* seeds @ 10 g / litre • Feed – Freshly plucked whole *Phyllanthus amarus* @ 1 per cent / kg of feed	No	Yes	No

of length of villi for each region. The villus length was determined by calculating the distance between the apex of the villus to the junction of the villus and crypt (Prakatur *et al*., 2019).

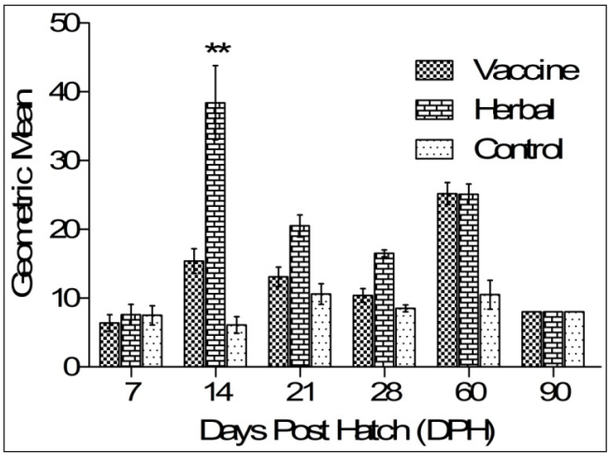
STATISTICAL ANALYSIS

All the data were analyzed using GraphPad prim 5.01 (GraphPad Software Inc. USA). The comparison among the groups was done using Two-way ANOVA with Bonferroni post-hoc test. The data represented are either as geometric mean or average with standard error.

RESULTS AND DISCUSSION

Haemagglutination inhibition titres

Humoral immune response against Newcastle disease was determined by Haemagglutination inhibition assay. In our study, we found that prevaccinal HI titres (DPH 7) against NDV were less than $\log_2 3$ (Fig. 1).



Each bar represents geometric mean of HI tires of a particular group along with standard error. **, *P<0.001* compared to control.

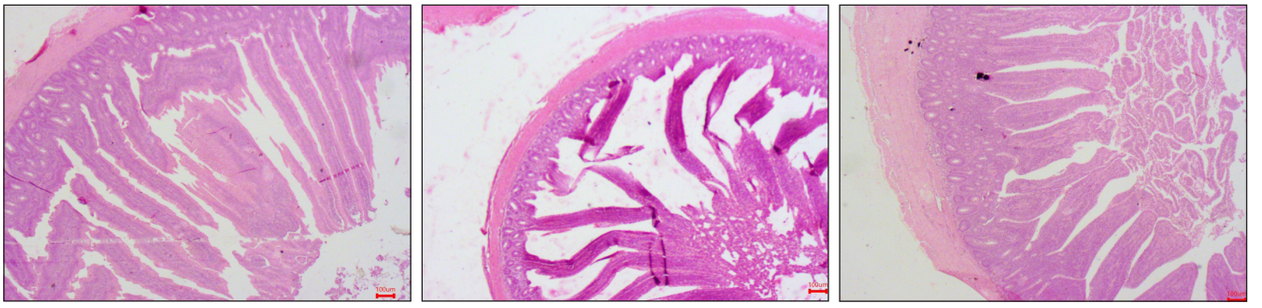
Fig. 1: Haemagglutination Inhibition titres

Gharaibeh and Mahmoud (2013) reported that the half-life of ND maternal antibodies is about 6.3 days, hence chicks hatched from vaccinated hens will have antibodies against NDV only during first week of life. Further, Hamal *et al*. (2006) findings also suggested that the maximum antibody levels were seen up to 3 days of life and then started decreasing thereafter. Therefore, the chicks become susceptible to NDV infection upon disappearance of maternal antibodies until they receive the second dose of vaccine at 4-6 weeks of age. In order to rule out possibility of natural infection to NDV, we kept an in-contact control group without any vaccination or treatment. Haemagglutination titres of control group indicated that the birds had not been exposed to any natural infection throughout the study period but exposed to vaccine virus through horizontal transmission.

Challenge studies conducted on field, suggested that haemagglutination inhibition titers above $\log_2 4$ provide protection against virulent NDV in birds (Kapczynski and King, 2005). Moreover, HI titers above $\log_2 3$ can only be achieved after two doses of vaccination. In India, backyard poultry are immunized with LaSota and R_2B/RDVK during second and six weeks of age respectively. In the present study, we found that birds which received herbal supplementation, fresh *Phyllanthus amarus* whole plant along with feed and cumin water had significantly higher protective HI titres than other groups ($P<0.001$) as early as 7 days after primary vaccination. Moreover, herbal group birds had antibody titres more than $\log_2 4$ until 60 DPH. Highest antibody responses were found on 14^{th} and 21^{st} DPH in herb and vaccine groups respectively. Birds which received vaccine alone developed HI titers above $\log_2 4$ only after 30 days of booster with LaSota. Similarly, Jagadeeswaran *et al*. (2012) reported that feeding of *Phyllanthus niruri* extracts, improved the HI titre against NDV, however no significant difference was observed with control birds. Moreover, they reported that the maximum HI titre was found only after 7 weeks. In our model, induction of antibodies to protective levels as early as 14 DPH may be due to synergistic effects of fresh *P. amarus* and cumin water. Our study, suggested that feeding of *P. amarus* as a whole plant inclusive of roots especially fresh condition, can enhance immune responsiveness in birds against NDV upon vaccination.

Morphometry of intestinal villi

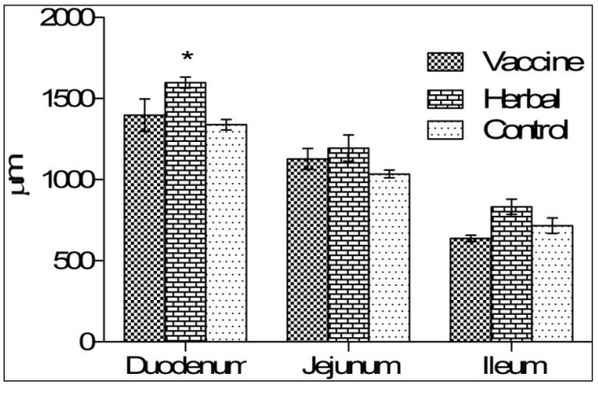
The majority of digestion and absorption processes of carbohydrates, proteins and fats are completed within the duodenal and jejunal regions of small intestine of the birds (Awad *et al*., 2009). Intestinal villi length directly correlates



(a) Duodenum **(b)** Jejunum **(c)** Ileum. (H&E)

Fig. 2: Histology of villi from the small intestinal sections of birds supplemented with herbs

with absorption capacity of the birds, therefore the length of villi in the small intestinal portions were measured (Fig. 2). Birds supplemented with *Phyllanthus amarus* and cumin water had significantly higher villi length ($P<0.01$) in the duodenal region when compared to other groups of birds (Fig. 3). Natsir *et al*. (2013) also reported similar findings that broilers fed with encapsulated form of *Allium sativum* and *Phyllanthus niruri* had improved their performance and villi length. Hence, our findings support the fact that feeding herbs will help the birds to assimilate the feed and herbs fed, which will in turn boost the immune response.



Data represented as mean ± SEM. *, $P<0.01$ compared to control.

Fig. 3: Morphometry of small intestinal villi

CONCLUSION

In conclusion, *P. amarus* and cumin seeds help the birds to achieve protective titres of antibodies against NDV as early as 14 days of hatching. Hence, chicks can be protected from NDV infection in the critical periods of life after the maternal antibodies wane. Further, supplementation of birds with above herbs also increases the intestinal absorptive surface.

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ETHICAL STATEMENT

Experiment was conducted in a backyard poultry farm. The study was conducted after explaining the details of the experiment, sampling procedures to the owner of the farm and consent was obtained for the same.

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