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Effect of Moringa Leaf Aqueous Extract Feeding on Humoral Response in Chicks

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ABSTRACT

A study was conducted to evaluate the humoral immune modulating effects of aqueous *Moringa oleifera* leaf extract supplementation in chicks by measuring antibody titre against Newcastle disease virus (NDV). Two treatment groups consisting of T1 and T2 were offered aqueous *M. oleifera* leaf extract supplementation in drinking water at the rate of 60ml/litre and 90ml/litre, respectively. Aqueous extract in drinking water were supplemented daily for two weeks starting from the age of four weeks and continued till the age of six weeks. Feeding of moringa leaf extract had profound impact on antibody response against NDV. Increase in antibody titre was recorded in both the treatment groups, but group treated with 90ml moringa leave extract supplementation in drinking water recorded significantly (<0.05) higher antibody titre than the non-supplemented control group.

Keywords: *Moringa oleifera*, aqueous extract, chicks, antibody titre, Newcastle disease virus.

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INTRODUCTION

Poultry industry is regarded as one of the important segments among agriculture sector and plays a significant role in economic development of the country (Nath *et al*., 2012). Diseases and poor health conditions of the poultry adversely affect its productivity and profitability. Thus, improvement of immunity for prevention of infectious diseases along with minimizing the effects of immunosuppression could be important strategies for successful poultry farming (Hashemi and Davoodi, 2012). Since long times, antibiotics are being used as growth promoters in poultry feed and these have dramatically increased the efficiency of commercial poultry production (Kim and Lillehoj, 2019), but concerns regarding the inappropriate use of antibiotics in animals and the emergence of multidrug-resistant superbugs are rising. Therefore, now much focus is given for the antibiotic-free methods of poultry raising and alternatives like phytochemicals, herbs and their extracts, adjuvants, prebiotics, probiotics etc. that augment non-specific host immunity (Faixova and Faix, 2008; Lillehoj and Lee, 2012).

Moringa oleifera is a useful plant known for its nutritional, medicinal and economical values. Several researches have reported beneficial effects of different parts of moringa *viz.* leaves, seeds, pods etc. on immune response, meat quality and growth performance of farm animals and poultry (Akhouri *et al*., 2013; AbouSekken, 2015; Mahfuz and Piao, 2019). Moringa can be added in poultry feed as leaf meal as well as supplemented in drinking water in extract form. The aqueous leaf extract of *M. oleifera* has shown immune modulation activities due to its phytochemical constituents like alkaloids and saponins present in aqueous extract (Oyewo *et al*., 2013).

Newcastle disease (ND) is an economically important, highly contagious and infectious viral disease of poultry that causes severe morbidity, mortality and acts as a major threat to poultry industry (Rehman *et al*., 2020). Vaccination is considered as the main method to control this disease. But in spite of advancement in immunization programs, ND remains a significant threat to poultry industries worldwide. *M. oleifera* contains natural immune modifying substances and can be considered as complementary means to control Newcastle disease virus (NDV). Therefore, this work was planned to study the effect of aqueous extract of *Moringa oleifera* leaves on humoral immune response and to determine the serum NDV antibody titres against ND virus in chicks at different concentrations.

MATERIALS AND METHODS

Chickens and Experimental design

A total of 50 day-old aged chicks were procured from Poultry Seed Project, Bihar Animal Sciences University, Patna and were reared for a period of six weeks. The chicks were reared on deep litter using all routine management practices and vaccinated against Newcastle disease and Infectious bursal disease following standard poultry vaccination protocol. The commercially available feed and clean drinking water were provided *ad libitum* throughout the study period. After 4 weeks of rearing, chicks were randomly divided into three groups of 15 chicks each, consisting of 01 control groups (C) and 02 different treatment groups (T1 and T2) to offer aqueous *M. oleifera* leaf extract supplementation in per litre (l) of drinking water at the rate of 60ml/litre and 90ml/litre, respectively.

Preparation of aqueous *Moringa oleifera* leaf extract

For the preparation of aqueous moringa leaf extract, fresh leaves of this plant were harvested from the Bihar Veterinary College campus, air-dried and grounded into fine particles using a grinder. Aqueous extract preparation of moringa leaves was done as per the protocol of Alabi *et al*. (2017). For this, 60 g of the ground particles were soaked in one litre of

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distilled water for 24 hours, then the preparation was filtered using a muslin cloth and the extract was stored in clean container.

Blood collection

After two weeks of supplementation, blood was collected aseptically from the jugular vein of randomly selected six birds from each group for serum isolation. The collected sera samples were stored in micro-centrifuge tubes at -20^0 C with proper labelling until analysis.

Haemagglutination inhibition (HI) test

All the sera samples were analysed for NDV antibodies by HI test. HI test was done according to the procedure of OIE (2018). Haemagglutination test was performed to calculate the 4HA unit of NDV. Two-fold serial dilution of 25µl serum was made with PBS in U-bottomed microtiter plate and 25 µl of 4HA units of NDV was added to each dilution. Subsequently, after 30 minutes of incubation, 25 µL of 1% chicken red blood cells was added to each well and incubated for 45 min at room temperature. Hemagglutination inhibition end points were scored and the titre was recorded as reciprocal log2 values of the highest dilution showing complete haemagglutination.

Further, animal use and the procedures used in this study was approved by the Institutional Animal Ethics Committee of Bihar Veterinary College, Bihar Animal Sciences University, Patna (IAEC/BVC/20/14).

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine statistical differences in mean values and the significance differences between the mean values were calculated using Tukey's HSD procedure. Significant differences between treatment means were identified at a level of $P < 0.05$.

RESULTS AND DISCUSSION

Newcastle disease is a highly contagious viral disease caused by the Newcastle disease virus and severely affects poultry health. Despite vaccination and therapy programmers, it causes heavy morbidity and mortality and accounts for almost 40–60% average losses in poultry (Bello et al., 2018). So, in this research, we studied the effect of *Moringa oleifera* to assess the effect of its aqueous extract on body's humoral defence against the Newcastle disease virus. The antibody titre which gets elicited due to Newcastle disease vaccinations is a measure of the humoral immune response in the experimental birds. In present study, the mean values of ND antibody titre under different treatment groups C (control), T1(60ml/l) and T2 (90ml/l) were 5.2222, 6.5556 and 7.5556, respectively. The maximum value of ND antibody titre was found in treatment group T2 followed by T1 and control, respectively (Table 1).

Increase in antibody titre was recorded with both treatment groups, but statistical analysis of the data indicated significant ($P < 0.05$) differences in the means of HI titres between T2 and the control and thus group (T2) supplemented with 90ml moringa leave extract per litre of drinking water (Table 2) showed better immunological response than T1 in higher antibody titre.

Table 1: Effect of feeding different levels of *Moringa oleifera* leaf aqueous extract on antibody titres against Newcastle disease (log2 HI titre)

Experimental group with detail of moringa treatment	HI titre (mean)	Standard Deviation	Standard Error	*f*-ratio value	*p*-value
Group 1 (C) No supplementation	5.2222	1.8005	.42438	7.3139	.00161
Group 2 (T1) 60 ml /litre of drinking water	6.5556	1.977	.46598		
Group 3 (T2) 90 ml/litre of drinking water	7.5556	1.7226	.40602		

(The result is significant at $P < 0.05$)

Table 2: Pairwise comparisons of groups (Post Hoc Tukey HSD, beta)

Pairwise Comparisons		HSD.05= 1.4777 HSD.01= 1.8664	Q.05 = 3.4139 Q.01 = 4.3119
C: T1	M1 = 5.22 M2 = 6.56	1.33	Q = 3.08 (p =.08466)
C: T2	M1 = 5.22 M3 = 7.56	2.33	Q = 5.39* (p =.00107)
T1:T2	M2 = 6.56 M3 = 7.56	1.00	Q = 2.31 (p =.24100)

Several investigations have reported beneficial effects of *M. oleifera* in boosting the immune systems (Olugbemi *et al*., 2010; Oyewo *et al*., 2013). The study of Eze *et al*. (2013) recommended moringa supplementation as a prophylactic treatment as an immune-booster against ND in non-vaccinated birds. This study is in line with the work of Oyewo *et al*. (2013) which suggested that the immune modulation activities of *Moringa oleifera* might be due to the phytochemical constituents like alkaloids and saponins present in aqueous extracts of the plant. Similar to this study, Younis and Elbestawy (2017) indicated that the water supplementation of *M. oleifera* leaves aqueous extract had increased immune response to NDV vaccine. In contrast to the present study, with similar preparation of aqueous extract, Khan *et al.* (2021) reported significant ($p < 0.05$) increase in antibody titre against ND with 60 ml/l *Moringa oleifera* leaf extract.

CONCLUSION

Moringa oleifera leaf extract may be useful in the protection against Newcastle virus when used along with the vaccine as moringa leaf extract has profound impact on antibody response against NDV. Group supplemented with 90ml moringa leave aqueous extract per litre of drinking water recorded significantly higher antibody titre than the control group.

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