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*Research article*

## **Humoral Immune Response of Broilers fed with *Moringa oleifera* Supplemented feed and Vaccinated with an Inactivated Infectious Bursal Disease Vaccine**

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### **ABSTRACT**

Infectious bursal disease virus has been reported to be one of the very important immunosuppressive agents in poultry. Therefore, the present study was conducted to evaluate the immune modulating effect of *Moringa oleifera* leaf (MOL) supplemented feed in broilers. Two-hundred-and-forty-day old Ross 308 hybrid broiler chicks were assigned into groups A, B, C and D of 60 chicks each in deep litter type housing. Groups A and B were fed with formulated broiler starter and finisher feeds, containing 5% MOL for 4 and 3 weeks, respectively, while groups C and D were fed with formulated broiler starter and finisher feed without MOL for 4 and 3 weeks, respectively. Broilers in groups A and C were vaccinated intramuscularly with 0.5 ml of inactivated intermediate strain of infectious bursal disease (IBD) vaccine at 14 and 21 days of age, respectively and inactivated Newcastle disease virus (NDV) vaccine (Komorov strain) at 18 days of age, while those in groups B and D served as positive and negative control. Blood was collected from 10 broilers in each group via the wing vein on 14, 21, 35, and 38 days of age to determine the antibody titres of the various groups of broilers using Enzyme linked immunosorbent assay (ELISA). Significant decrease ( $p = 0.001$ ) was observed in the mean IBD antibody titre of birds in group A at 21 days of age and significant increase at 35 days of age. Birds in group B showed a significant decrease ( $p = 0.000$ ) in antibody mean titre at 21 days of age and a significant ( $P = 0.002$ ) increase at 35, 38 and 42 days of age. Significant decrease ( $p = 0.000$ ) was observed in the mean antibody titre of broilers in group C at 21 days of age and a significant increase ( $p = 0.022$ ) was observed at 35 days of age. The antibody mean titre of broilers in group D showed a significant decrease ( $p = 0.000$ ) at 21 days of age, and a significant ( $p = 0.000$ ) increase at 35 days of age. Broilers fed with MOL supplemented diet need to be vaccinated to have a high antibody titre against IBD.

**Keywords:** *Broilers, Moringa oleifera leaf, IBD, Antibody*

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### **INTRODUCTION**

Infectious bursal disease virus has been reported to be one of the very important immunosuppressive agents in modern poultry production and infection with IBDV may induce a temporary or permanent destruction of the bursa cloaca and other lymphoid tissues (Sharma *et al.*, 2000; Lukert and Saif, 2003; Khatri *et al.*, 2005). Therefore, the main targets of the IBD virus are the lymphoid organs and the immune cells (Faragher, 1972). The disease is characterized by immune-

deficiency and high mortality in chicks that are between 3 and 6 weeks old.

Studies have shown that, IBD has acquired an endemic status in poultry farms in Nigeria (Nawathe *et al.*, 1978; Durojaiye *et al.*, 1984; Abdu 2007). The prevention of IBD in Nigeria is largely dependent on vaccination with single prototype indigenous and different types of imported IBD vaccines (Okoye and Uzoukwu, 2001). Regrettably, severe outbreaks still take place with high mortality rates both in vaccinated and unvaccinated flocks (Okoye, 1983; Abdu, 1986; Sainbury, 2000; Musa *et al.*, 2010), and these makes the

control of IBD to virtually be impossible in most of the farms (Musa, 2009; Musa *et al.*, 2010).

Infectious bursal disease has been reported as a disease of economic importance to the poultry industry worldwide (Hamoud *et al.*, 2007) due to the high mortality, reduced weight gain and condemnation of carcasses as a result of marked haemorrhage in the skeletal muscle (Van den Berg, 2000) and secondary losses due to immunosuppression (Anderson *et al.*, 1977; Lukert and Saif, 2003).

To gain immunity, the animal needs energy and proteins for the manufacture of antibodies and cells, minerals (zinc, copper, iron and selenium) and vitamins (A and E) for communicating messages in parts of the animal's body in order to fight infections (Conroy, 2005). Interestingly, MOL has been reported to possess all the above-mentioned carbohydrate, proteins, minerals, vitamins and amino acids (Makkar and Becker, 1999; Kakengi *et al.*, 2003; Odura *et al.*, 2008). *Moringa oleifera* are in high demand for their medicinal values as they have been reported to have the potential of boosting the immune systems (Ramachandran *et al.*, 1980; Atawodi *et al.*, 2008; Sreelatha and Padma, 2009). The absence of available literature on evaluating the humoral immune response of broilers fed with *Moringa oleifera* feed supplement and vaccinated with an inactivated IBD vaccine necessitate this studies. Therefore, this study aimed at evaluating the antibody response of broilers fed with MOL feed supplementation without vaccinating against IBDV.

## MATERIALS AND METHODS

**Study Location:** The study was conducted at the Poultry Research Unit of the Faculty of Veterinary Medicine, Ahmadu Bello University Samaru, Zaria, Nigeria.

**Collection and processing of *Moringa oleifera* leaf:** *Moringa oleifera* leaf (MOL) was harvested (between the months of August and September) from an orchard at an early flowering stage. The stem and branches were cut from the *Moringa* trees and spread out to dry under shade at room temperature for five days. The MOL were then removed manually by hand and grounded into powder using a locally manufactured milling machine.

**Mineral Analysis of *Moringa oleifera* Leaf:** Mineral analysis of MOL was carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990) to determine the calcium, phosphorus, magnesium, iron, sodium, zinc, copper, selenium, potassium, and manganese components.

**Phytochemical Analysis of *Moringa oleifera* Leaf:** Qualitative and quantitative analysis of MOL was carried out, according to the method described by Sofowora (1993), to determine the presence of tannins, phytates, saponins, oxalates, cyanides, alkaloids, carbohydrates, flavonoids, steroids, terpenoids, phenols and phylobatanins.

**Proximate Analysis of *Moringa oleifera* Leaf:** The standard methods of the Association of Official Analytical Chemists

(AOAC, 1990) for the proximate analysis of the MOL was used to determine the percentage carbohydrates, crude protein, fats, fibre, ash, moisture and metabolizable energy.

**Feed Formulation and Analyses:** The dried MOL was milled with a hammer mill and sieved with 3 mm mesh sieve to obtain *Moringa oleifera* leaf meal. Broiler starter (22% crude protein) and broiler finisher (20% crude protein) were formulated using Pearson square with 5% MOL inclusion as described by the methods of Olugbemi *et al.* (2010). The feed was subjected to proximate and mineral analysis based on the method described by the AOAC (1990) in the Feed Analysis Laboratory of the Department of Animal Science, Ahmadu Bello University Zaria, to determine the level of metabolizable energy, crude protein, crude fibre, moisture, ash content, and dry matter.

**Experimental Chicks and Housing:** A total of 240-day old Ross 308 hybrid broiler chicks were obtained from a commercial hatchery located in Yola, Nigeria. The chicks were brooded in a deep litter house which was properly cleaned and disinfected before the arrival of the chicks with wood shavings as litter material and feeders and drinkers were provided. The chicks were individually weighed and assigned in a complete randomised design into four different groups A, B, C and D of 60 chicks each. A 100-watt bulb was provided in each of the compartment to supply light and heat during brooding.

**Feeds and Feeding:** All the broilers were fed with broiler starter for 28 days (from 0 to 4 weeks of age) and broiler finisher for 21 days (from 5 weeks to 7 weeks). Feed and water were provided *ad libitum* (using plastic drinkers and galvanised feeders).

**Experimental Design:** Groups A and B were fed with broiler starter and finisher diets each containing 5% MOL, while groups C and D were fed with broiler starter and finisher feed without MOL. All the groups were fed for 49 days (7 weeks).

**Vaccines and Vaccination:** Inactivated killed vaccine against IBD (inactivated intermediate strain, Virsin 122, oil emulsion, Biovac Limited, Isreal, Batch 1- 382222) and inactivated killed vaccine against Newcastle disease (ND) (oil emulsion Komorov strain, Biovac Limited, Isreal, Batch 1-422222) were obtained from a Veterinary Pharmaceutical store in Jos, Nigeria. Broilers in groups A and C were vaccinated through the thigh muscles intramuscularly with 0.5 ml of the killed IBD vaccine at 14 and 21 days of age, while vaccination against ND was done with the killed ND vaccine (0.5 ml) through the thigh muscles intramuscularly at 18 days of age.

**Collection and processing of Blood:** Blood samples for serology were collected when broilers were 14, 21, 35, and 38 days of age. On each day of blood collection, 10 birds from each group were randomly selected and bled via the brachial vein using a 25 gauge sterile needle on a 5 ml syringe. Each blood sample collected was emptied into plain (without anticoagulant) test tubes and allowed to coagulate to produce sera according to the methods described by Okeudo *et al.*

(2003). Serum was separated by centrifugation at 447.2 g for 10 min and stored at -20°C until analysed for antibody. Each of the sample bottles was labelled using a permanent marker.

### Serology

**Enzyme-linked immunosorbent assay:** The enzyme linked immunosorbent assay (ELISA) technique was carried out according to the methods described by IDEXX laboratories, USA. Briefly the antigen coated plates and the ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted to five hundred folds (1:500) with sample diluents prior to the assay. A 100 µl of diluted sample was then added into each well of the plate. This is followed by 100µl of undiluted negative control into the well A1 and A2, 100 µl of undiluted positive control was also dropped into well A3 and A4. The plate was then incubated for 30 minutes at room temperature. Each well was then washed with approximately 350 µl of distilled water 3 times. Goat anti-chicken conjugate (100 µl) was dispensed into each well. The plate was again incubated for another 30 minutes, followed by washing each well with 350 µl of distilled water 3 times. Tetramethylbenzidine (TBM) solution (100 µl) was dispensed into each well. The plate was then incubated at room temperature for 15 minutes. Finally, 100 µl of stop solution was dispensed into each well to stop the reaction. The absorbance values were measured and recorded at 650 nm using ELISA reader. Infectious bursal disease antibody titre was calculated automatically, using software by Blankford and Silk (1989).

Data obtained from the results of the biochemical analyses were expressed as means ( $\pm$  standard deviation). The data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's Control test as post-hoc test for multiple comparison. The analyses are considered

significant at  $p < 0.05$  using Statistical Package for Social Science (SPSS) version 20 for windows.

### RESULTS

There was a significant decrease ( $p = 0.001$ ) in the mean IBD antibody titre of birds in group A at 21 days of age and significant increase at 35 days of age. Birds in group B showed a significant decrease ( $p = 0.000$ ) in ELISA antibody mean titre at 21 days of age and a significant ( $P = 0.002$ ) increase at 35, 38 and 42 days of age. A significant decrease ( $p = 0.000$ ) was observed in the ELISA mean antibody titre of broilers in group C at 21 days of age and a significant increase ( $p = 0.022$ ) was observed at 35 days of age. The ELISA antibody mean titre of broilers in group D showed a significant decrease ( $p = 0.000$ ) at 21 days of age, and a significant ( $p = 0.000$ ) increase at 35 days of age (Table 1).

### DISCUSSION

The significant decrease observed in the ELISA antibody titre in birds of groups A, B, C and D at 21 days of age indicates a decline in the maternally derive antibody (MDA) level in the birds. Hair-Bejo *et al.* (2004) and Babiker *et al.* (2008) reported a decline in the MDA level of broilers at 14 and 17 days. Another reason for the decline observed could be that the inactivated IBD vaccine used in vaccinating the birds of groups A and C may have delayed in inducing an immune response. This is in agreement with the experimental findings of Faragher (1972) and Phatek (2000), who in their separate studies reported that inactivated vaccine induces an antibody response more slowly than a live vaccine

**Table 1:**

Changes in enzyme linked immunosorbent assay infectious bursal disease antibody titre level of broilers fed 5% *Moringa oleifera* leaf supplemented feed.

	Group			
	A n = 10	B n = 10	C n = 10	D n = 10
<b>Age in days</b>	<b>Mean (<math>\pm</math> SD) IBD antibody titre (%)</b>			
14	3285.71 $\pm$ 920.30 <sup>a</sup>	3363.95 $\pm$ 660.15 <sup>a</sup>	3434.89 $\pm$ 476.89 <sup>a</sup>	2701.24 $\pm$ 829.95 <sup>a</sup>
21	1379.89 $\pm$ 829.98 <sup>b</sup>	1205.94 $\pm$ 612.32 <sup>b</sup>	1576.94 $\pm$ 566.51 <sup>b</sup>	1542.43 $\pm$ 106.80 <sup>b</sup>
35	2836.83 $\pm$ 463.58 <sup>c</sup>	2224.54 $\pm$ 636.35 <sup>c</sup>	2853.42 $\pm$ 544.64 <sup>c</sup>	2953.49 $\pm$ 561.88 <sup>c</sup>
38	2545.13 $\pm$ 1102.17 <sup>d</sup>	3061.87 $\pm$ 617.75 <sup>d</sup>	2722.18 $\pm$ 570.80 <sup>d</sup>	688.16 $\pm$ 821.56 <sup>d</sup>
F statistic	702.947	542.503	751.57	209.391
P value	0.006	0.000	0.000	0.000

Key: n = total number of birds sampled, all values are expressed as Mean  $\pm$  SD = standard deviation of the mean Means having different superscripts within columns differ significantly at  $p < 0.05$

Group A: Fed 5% *Moringa oleifera* leaf supplemented feed and vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old.

Group B: Fed 5% *Moringa oleifera* leaf supplemented feed and not vaccinated against infectious bursal disease virus.

Group C: Fed feed without *Moringa oleifera* leaf supplementation and vaccinated with killed infectious bursal disease vaccine at 14 and 21 days old.

Group D: Fed feed without *Moringa oleifera* leaf supplementation and not vaccinated against infectious bursal disease virus.

However, the significant increase observed in the ELISA antibody titre level of birds in groups A and C at 35 days of age, showed a better sero-conversion and immune response to the second dose of the inactivated IBD vaccine administered at 21 days of age. This finding is in agreement with the report of Ahmed and Akhter (2003) who reported that higher antibody titre level against IBD was achieved by using two doses of inactivated IBD vaccine at 10 and 21 days of age.

The increase in the ELISA antibody titre level in the birds of group B at 35 days of age could suggest that, the MOL inclusion in the diet of broilers in that group may have been responsible for the increase. *Moringa oleifera* leaf has been reported to be an immune modulator in poultry (Jayavardhanan *et al.*, 1994; Olugbemi *et al.*, 2010). Although, there has not been any report on the humoral immune response against IBDV in broilers fed MOL, Didacus *et al.* (2013) reported an increase in the haemagglutination inhibition (HI) titre against Newcastle disease in unvaccinated broilers using methanolic extract of MOL. The significant increase observed in the ELISA antibody titre level in birds of group B at 38 (3 dpi) days of age, could indicate a stimulation of an active immunity (Haddad *et al.*, 1997). Since the birds in group B were not vaccinated against IBD with any of the inactivated IBD vaccines, it can be suggested that, the MOL included in their diet may have aided in stimulating active immunity, since it possess immune modulatory properties as previously described (Jayavardhanan *et al.*, 1994; Olugbemi *et al.*, 2010). The significant decrease observed in the ELISA antibody titre level of birds in group D at 21 and 35 days of age, could be as a result of weaning of the antibody titre against IBD, as the birds in this group were not vaccinated against IBD.

In conclusion, there was an increase in the ELISA Ab titre level of broilers in groups A, B and C up 38 days of age irrespective of whether they were fed with MOL supplemented feed or not.

### Recommendation

Broilers fed with MOL supplemented diet need to be vaccinated to have a high antibody titre against IBD.

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