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The Effect of Very Virulent Infectious Bursal Disease Virus on Immune Organs of Broilers Fed *Moringa Oleifera* Supplemented Feed

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ABSTRACT

A study was conducted to evaluate the immune modulating effect of *Moringa Oleifera* Leave (MOL) feed supplementation in broilers. 240 day old Ross 308 hybrid broiler chicks were assigned into four groups (A, B, C and D) of 60 chicks each in a deep litter house. Groups A and B were fed with formulated broiler starter and broiler finisher feeds containing 5% MOL for 28 and 21 days, respectively, while groups C and D were fed with formulated broiler starter and broiler finisher feed without MOL for 28 and 21 days, respectively. Broilers in groups A and C were vaccinated intramuscularly with 0.5 ml of an inactivated vaccine of intermediate strain of Infectious Bursal Disease (IBD) at 14 and 21 days of age, respectively. Broilers in groups A, B and C were challenged intraocularly at 35 days of age with 0.05 ml of a live very virulent Infectious Bursal Disease Virus (vvIBDV). The Thymus to Body Index (TBI) of birds in group A was 1.09, 1.05 and 1.03 at 35, 38 and 42 days of age respectively, while those in group B had a TBI of 0.84, 1.02 and 0.89 at 35, 38 and 42 days of age respectively. The TBI of birds in group C were 1.04, 1.22 and 1.29 at 35, 38 and 42 days of age respectively, however, there was significant difference between group B and C ($P < 0.02$). *Moringa oleifera* leaves feed supplementation improved the bursa, spleen, harderian and thymus to body weight index of broilers. The MOL feed supplementation and inactivated vaccine did not prevent the atrophy of bursa, spleen and harderian gland against the negative effect of vvIBDV 7 days post infection.

Keywords: Broilers, Immune organs, Infectious bursal disease virus, Organ to body weight index, *Moringa oleifera*

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INTRODUCTION

Infectious Bursal Disease Virus (IBDV) has been reported to be one of the very important immunosuppressive agents in modern poultry production. 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 and Khatri et al., 2005). Therefore, the main targets of the IBD virus are the lymphoid organs and the immune cells (Faragher, 1972). IBD has been reported as a disease of economic significant 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 (Kaufer and Weiss, 1976 and Van den Berg, 2000) and secondary losses due to immunosuppression (Anderson et al., 1977 and Lukert and Saif, 1997).

The measurement of the lymphoid organ weight plays an important role in providing information on the body's ability to provide lymphoid cells in time of immune response (Heckert et al., 2002). Therefore, one of the measures of immunity that have been commonly used in assessing poultry health is the lymphoid organ weights (Pope, 1991). Any change that may occur during the developmental stages in these organs in response to possible lymphotrophic agent will result in the alteration of the immune function that is associated with lymphoid cells (Cooper et al., 1966 and Boehm and Bleul, 2007).

Bioactive constituents of feed that interact with the immune response have considerable potential of reducing susceptibility to infectious diseases (Kogut, 2009). Due to the growing population of the poultry industry, the use of nutritional dietary intervention strategies may be a cost effective means of preventing specific infectious diseases and

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maintaining the health status of poultry (Kogut, 2009). 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 other to fight infections (Conroy, 2005).

Interestingly, *Moringa Oleifera* Leave (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 and Odura et al., 2008). *Moringa oleifera is* in high demand for their medicinal values as they have been reported to have the potential of boosting the immune system (Ramachandran et al., 1980; Atawodi et al., 2008; Sreelatha and Padma, 2009). The absence of available literature on evaluating the bursa of Fabricius, spleen, thymus and harderian gland to body weight index of broilers fed with MOL feed supplementation, vaccinated with an inactivated IBD vaccine and challenged with a very virulent IBDV was the reason for this study.

MATERIALS AND METHODS

Study location

The study was conducted at the poultry research unit of the faculty of veterinary medicine, Ahmadu Bello University Zaria, Nigeria.

Collection and processing of *Moringa oleifera* leaves

Moringa oleifera leaves were harvested from orchards at an early flowering stage in Potiskum, Yobe State, Nigeria. The stem and branches were cut from the Moringa trees and spreads out to shade dry under room temperature for five days. The MOL were then removed manually by hand and grounded into powder using a milling machine.

Mineral analysis

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

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 phlobatannins.

Proximate analysis

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

Feed formulation and analysis

Following shade drying of MOL, it was milled with a hammer mill and sieved with 3 mm mesh to obtain *Moringa oleifera* leaf meal. 22% and 20% of crude protein for broiler starter and broiler finisher mash respectively were formulated [with 5% MOL (Olugbemi et al., 2010a) forming part of the feeds ingredients for group A and B] using pearson square and milled in a toll mill in Zaria, Nigeria. The feed was subjected to 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 reputable commercial hatchery located in Yola, Nigeria. The chicks were brood in a conventional open-sided house which was properly disinfected before the arrival of the chicks (deep litter system of management with wood shavings as litter material, feeders and drinkers were provided) with cyclic temperatures. 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 (each pen has a floor space of 3 × 4 m). A 100 watt bulb was provided in each of the compartment to supply light and heat during brooding. The broilers were fed with broiler starter for 28 days and broiler finisher for 21 days. Feed and water were provided ad libitum (Table 1).

Vaccines and vaccination

Inactivated vaccines against IBD (Virsin 122, oil emulsion, Biovac limited, Isreal, Batch 1- 382222) and inactivated killed vaccines against Newcastle Disease (ND) (oil emulsion Komorov strain, Biovac limited, Isreal, Batch 1- 422222)

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were obtained from a reputable veterinary pharmaceutical supplier in Jos, Nigeria. Broilers in groups A and C were vaccinated intramuscularly with 0.5 ml of killed IBD vaccine on 14 and 21 days of age respectively, while vaccination against ND was done with an inactivated ND vaccine (0.5 ml) on the thigh muscles intramuscularly on 18 days of age (Table 1).

Challenge with infectious bursal disease virus

At 35 days of age, all the broilers in groups A, B and C were challenged intra ocularly with 0.05 ml of a live vvIBD virus. The IBD virus used for the challenge was a field strain of vvIBDV obtained from previously vaccinated layers that died of natural outbreak of IBD. One millilitre of bursal suspension (v/w) in phosphate buffered saline (pH 7.4) contained 10^{-976} chick infective dose (CID_{50}) of IBDV.

Collection of immune organs

Five birds were randomly selected from each group on the 35, 38 and 42 days of age (Table 1). The selected birds were euthanized and the bursa of fabricius, thymus, spleen and harderian gland were removed for the evaluation of organ body weight index (Lucio and Hitchner, 1979). The organ body index was obtained by employing the formula:

$$\text{Organ: body index} = \frac{\text{Organ to body weight ratio of groups}}{\text{Mean organ to body weight ratio of control}}$$

$$\text{Where, organ to body weight ratio} = \frac{\text{organ weight in grams}}{\text{body weight in grams}} \times 100$$

Table 1. Experimental design for evaluating the effect of *Moringa oleifera* feed supplementation on immune organs of broilers challenged with a very virulent infectious bursal disease virus at 35 days of age.

Group (MOL%)	Age (days)					
	14	**18**	**21**	**35**	**38**	**42**
A (5%)	IBD killed vaccine	NDV killed vaccine	IBD killed vaccine	Collection of Immune organs, Challenged with IBD virus	Collection of Immune organs	Collection of Immune organs
B (5%)	No vaccination	No vaccination	No vaccination	Collection of Immune organs, Challenged with IBD virus	Collection of Immune organs	Collection of Immune organs
C (0%)	IBD killed vaccine	NDV killed vaccine	IBD killed vaccine	Collection of Immune organs, Challenged with IBD virus	Collection of Immune organs	Collection of Immune organs
D (0%)	No vaccination	No vaccination	No vaccination	No challenge with IBD virus	Collection of Immune organs	Collection of Immune organs

MOL = *Moringa Oleifera* Leave; IBD = Infectious Bursal Disease; NDV = Newcastle Disease Virus

Data analyses

The values of the immune organs were expressed as organ to body index and compared with the organ to body index of the control. Values obtained were further subjected to one way analysis of variance (ANOVA) followed by tukeys post-hoc test for multiple comparism. Values of $p < 0.05$ were considered significant using GraphPad Prism version 4.0 for windows.

RESULTS

The Bursa to Body Index (BBI) of broilers in group A was 1.4, 1.57 and 0.71 at 35, 38 and 42 days of age respectively. Group B had a BBI of 1.4, 0.71 and 0.86 at 35, 38 and 42 days of age respectively. Birds in group C had a BBI of 0.86, 1 and 0.71 at 35, 38 and 42 days of age respectively, though no statistical significance between all the groups ($P > 0.05$) (Table 2).

The Spleen to Body Index (SBI) of birds in group A was 1.3, 0.9 and 0.8 at 35, 38 and 42 days of age respectively. Birds in group B had a SBI of 1.1, 0.9 and 1.1 at 35, 38 and 42 days of age respectively, while those in group C had a SBI of 0.77, 1, and 0.8 at 35, 38 and 42 days of age respectively, though no statistical significance between all the groups ($P > 0.05$) (Table 3).

Harderian Gland to Body index (HBI) in birds of group A was 2, 2.5 and 0.3 at 35, 38 and 42 days of age respectively. Birds in group B had a HBI of 2, 2 and 0.66 at 35, 38 and 42 days of age respectively, while those in group

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C had a HBI of 1.25, 1.5 and 0.66 at 35, 38 and 42 days of age respectively, though no statistical significance between all the groups ($P > 0.05$) (Table 4).

The Thymus to Body Index (TBI) of birds in group A was 1.09, 1.05 and 1.03 at 35, 38 and 42 days of age respectively, while those in group B had a TBI of 0.84, 1.02 and 0.89 at 35, 38 and 42 days of age respectively. The TBI of birds in group C were 1.04, 1.22 and 1.29 at 35, 38 and 42 days of age respectively, however, there was significant difference between group B and C ($P < 0.02$) (Table 5).

Table 2. Bursa to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	1.4	1.4	0.86	1
38	1.57	0.71	1	1
42	0.71	0.86	0.71	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

Table 3. Spleen to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	1.3	1.1	0.77	1
38	0.9	0.9	1	1
42	0.8	1.1	0.8	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

Table 4. Harderian gland to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus.

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	2	2	1.25	1
38	2.5	2	1.5	1
42	0.33	0.66	0.66	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

Table 5. Thymus to body weight index of broilers fed 5% *Moringa oleifera* leave feed supplement, vaccinated with inactivated infectious bursal disease vaccine at 14 and 21 days old and inoculated at 35 days old with a very virulent infectious bursal disease virus

Groups / Age (days)	A 5% MOL, IBD vaccine, vvIBDV	B 5% MOL, vvIBDV	C 5% MOL, IBD vaccine, vvIBDV	D 0% MOL
35	1.09	0.84	1.04	1
38	1.05	1.02	1.22	1
42	1.03	0.89	1.29	1

MOL= *Moringa oleifera* Leave; IBD = Infectious Bursal Disease; vvIBDV = Infectious Bursal Disease Virus

DISCUSSION

The higher bursal, spleen, harderian and thymus to body weight index observed in the birds of group A and B before the challenge with vvIBDV (35 days of age) could be an affirmation to the immune properties of MOL that has been reported (Jayavardhanan et al., 1994 and Olugbemi et al., 2010a) and could also be that MOL included in the diet of birds in group A and B should have stimulated the infiltration of more lymphoid cells into the various organs. Increase in

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the BBI and HBI observed in group A at 38 days of age indicates the production of more B cells by these organs and also signify the importance of MOL with respect to immune stimulation. The decrease in the BBI and HBI observed at 42 days of age indicates that both the MOL in the diet of birds in group A and B and the inactivated IBD vaccine given to birds in group A and C could not prevent the atrophy of these organs.

An increase observed in the TBI of birds in group A may suggest that both the inactivated IBD vaccine and MOL in the diet of the birds may have been responsible for the increase. This is because of the immune modulatory properties of the MOL (Olugbemi et al., 2010b) and the immune response due to the vaccination with inactivated IBD vaccine. The lower TBI of birds in group B could either be due to the non-vaccination of the birds with inactivated IBD vaccine or that the immune modulatory properties of MOL alone (without vaccination with IBD vaccine) could not cause an increase in the TBI. Very virulent infectious bursal disease virus is known to cause the destruction of the B lymphocytes and has little or no effect of the T lymphocytes and the thymus is responsible for the production of T cells (Cooper et al., 1966; Boehm and Bleul, 2007). This was observed from the findings of present study where the vvIBDV was shown not to cause atrophy of the thymus in birds of group A, B and C.

CONCLUSION

Moringa oleifera leaves feed supplementation improved the bursa, spleen, and harderian to body weight index of broilers of group A and B respectively. The MOL feed supplementation and inactivated vaccine did not prevent the atrophy of bursa, and harderian gland against the negative effect of vvIBDV 7 dpi. The challenge with vvIBDV did not cause a reduction in the TBI of birds in group A, B and C 3 dpi. Supplementing broiler feed with MOL and vaccinating against IBD increased the TBI of birds in group A at 35, 38 and 42 days of age.

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Competing interests

The authors have no competing interest.

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