Volume 7 • Issue 1 • 2025

Research Article

Archives of Veterinary and Animal Sciences

Antibodies, Haematocrit and leukocytic-index Responses to Prophylactic administration of some Selected Nutraceuticals in Broilers Infected with Gumboro Virus

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Received: November 26, 2024; **Published:** February 06, 2025

Abstract

Infectious bursal disease (IBD) is an acute, viral infection associated with immunosuppression of chickens infected at an early age, and a major setback to productivity and profitability in poultry industries. The study evaluated the prophylactic effects of some of the nutraceuticals to a very virulent IBD virus (vvIBDV). Sixty-five-week-old Arbor Acre Plus broilers were divided into seven groups, A1, A2, B1, B2, C, D and E of nine birds each. The nutraceuticals administered to the birds for five days were Chick-on®(A1), King-Herb oral solution® (A2), antimicrobial cocktail (B1), Vitalyte extra®(B2), and Khaya senegalensis (KS) leaves(C) while groups D (inoculated with vvIBDV and no nutraceuticals given) and E(not inoculated with vvIBDV and no nutraceuticals given) served as positive and negative controls, respectively. Blood samples were obtained at day 0 and 7 dpi. The PCV (34.6 %) and TRBC (5.96×1013/L) of the group administered Vitalyte extra® (B2) were higher than those of other groups (A1, A2, B1, D and E). Heterophil/lymphocyte ratio of 0.12 was the lowest in the group administered King-herb oral solution® (A2). For King-herb oral solution® (A2) and Vitalyte extra® (B2) all the nine birds in the groups had higher antibody titre level against IBDV than other groups. Assay for antibody titre against Newcastle disease which is used to evaluate immunosuppression revealed higher (8.33 ± 0.33) antibody titre level against Newcastle disease in the group administered with King-herb oral solution® than other groups. King-herb oral solution® and Vitalyte extra® can be used during IBD outbreak and before vaccination against ND and IBD to boost immune response.

Key words: *Chick-on®; King-Herb® oral solution®; Antimicrobial cocktail; Vitalyte extra®; And Khaya senegalensis leaves*

Introduction

Infectious bursal disease (IBD) is an acute, viral infection associated with immunosuppression of chickens infected at an early age, and a major setback to productivity and profitability in poultry industries. The IBDV primarily infects immature B cells causing pathological changes in the bursa of Fabricius (BF) of chickens, which in turn facilitates secondary infections and poor immune response to pathogens and vaccines (OIE, 2012). Recovery from

Infectious bursal disease (IBD) is an acute, viral infection associated with immunosuppression of chickens infected at an early age, and a major setback to productivity and profitability in poultry industries. The IBDV primarily infects immature B cells causing pathological changes in the bursa of Fabricius (BF) of chickens, which in turn facilitates secondary infections and poor immune response to pathogens and vaccines (OIE, 2012). Recovery from IBD or subclinical infection will be followed by immunosuppression with more

severe consequences if the strain is very virulent and infection occurs early in life (Yao, 1998). Infectious bursal disease causes economic losses through morbidity, mortality, and increased susceptibility to other diseases and poor response to vaccination as a result of its immunosuppressive effect. King-herb oral solution was found to reduce immunosuppression in broilers infected with IBDV. Haematology and biochemical values of avian species are significantly influenced by poultry diseases, such as IBD (Juranova et al., 2001). The control of IBD depends on appropriate immunization schedules and maintenance of good hygiene on the farm (Farooq et al., 2003). In spite of the extensive use of vaccines, farmers still have to contend with IBD (Besseboua et al., 2015). To date, there is no known chemotherapeutic agent that is effective in the prevention and control of IBD. However, farmers are being misled by drug vendors to stop vaccination against IBD and thus causing serious loss to the poultry industry through explosive outbreak of IBD (Abdu, 1986). Farmers are losing financially and mentally are suffering whenever there is an outbreak of IBD. Although some farmers and veterinarians have claimed IBD cure using some nutraceuticals such as Cleome gynandra (Yasmin et al., 2020), only few researches on herbal and chemical products have been carried out to validate such claims. Due to its hardy nature, IBDV persist in poultry houses despite thorough cleaning and disinfection (Alexander and Chettle, 1998). There are products in the market that claim to modulate immune response for example livol and immunotone (Khan et al., 2017), King-herb oral solution, immunoIBD, vitalyte extra, and plant extract like Khaya senegalensis available in the Nigeria markets. To find nutraceuticals that are effective and of cost benefit to the farmers and veterinarians (Musa et al., 2012). Consequently, nutraceuticals found effective will increase farmer' s and veterinarian's choices on which of the nutraceuticals to use for the control of IBD. Effective nutraceuticals will reduce the economic losses due to IBD infection in the poultry industry. This study was to determine the antibodies, haematocrit and leukocytic-index response to prophylactic administration of some selected nutraceuticals in broilers infected with Gumboro virus.

Materials and Methods

The experiment was conducted at the Experimental Poultry unit, of the Veterinary Teaching Hospital Ahmadu Bello University Zaria Nigeria. The study was carried out during the dry season (November-April), which is characterized by low temprature (13.8°C) (Iloeje, 2004). The area has a tropical Savannah climate with annual total rainfall of about 1,099 mm (Iloeje, 2004).

Ethical approval

Ethical clearance to conduct the study was sought and approved by the Ahmadu Bello University Committee on Animal Use Care (ABUCAUC/2019/27).

Experimental chickens

Sixty-five-week-old broilers non-vaccinated against infectious bursal disease were purchased from a reputable hatchery in Ibadan, Nigeria. The breed of the birds was Arbor Acre plus, broilers. Birds were randomly assigned into seven groups of nine chicks each and housed on deep litter with a floor space of 0.14 m2 per bird. The chicks were kept in ventilated in open-sided pens poultry research pens.

Housing

The pens were washed with detergent and disinfected with Diskol® (5% benzalkonium chloride, 7.5% glutaraldehyde, 7.5% formaldehyde, stabilizers and antioxidants 9.5). Formalin 10% was used to sprayed the pen and the poultry premises (Abdu and Musa, 2014). Strict biosecurity was adhered to throughout the period of research.

Feeding and watering

Feed was purchased from an accredited large distributor of a reputable commercial feed mill (Olam Company). It was provided ad libitum using galvanized feeders. Fresh clean water from a borehole was provided ad libitum in plastic drinkers.

Brooding and lighting

A charcoal brooding improvised-pot, was used per compartment, for brooding the chicks for a period of one week to provide the 28-34oC brooding temperature for the chicks when there was power outage. One two- hundred-watt bulbs was used as source of illumination and heat per compartment when there was power supply.

Challenge virus

A very virulent IBDV was obtained from the Department of Veterinary Medicine, ABU Zaria. The vvIBDV came from a previously vaccinated commercial layers that died of a natural outbreak of IBD (characterized strain). Seventy-five per cent (75%) of commercial cockerels inoculated with the vvIBDV at 30 days of age with 50 µL of bursal suspension (v/w) in phosphate buffered saline (PBS) (pH 7.4) died. One millilitre of the bursal suspension (v/w) in PBS (pH 7.4) contained 109.76 CID50 of IBDV (Abdu et al., 2007).

Commonly used Nutraceuticals in Kaduna metropolis in control of Gumboro disease

Chick-on® is an herbal nutraceutical produced in Nigeria by First Fish Poultry Drug Treatment Limited 6/7 Kaduna, Nigeria, with the following composition: Oxen base, Kamallory base, vitamin C, vitamin A, and calcium base. Four millitres (4 ml) of chick-on was administered to 4 litres of drinking water. The nutraceuticals are commonly used by poultry farmer within Kaduna metropolis as a substitute to vaccination of poultry against IBDV (oral interviews of farmers and the manufacturer of the nutraceuticals 2017-2018).

King-Herbs oral solution® is a greenish mixture of herbal extracts produced by GMP certified company Aether Centre (Beijing) Biology Co. Ltd. It is indicated for prevention and treatment of viral diseases such as IBD, ND. King-Herbs oral solution®, is to induce production of interferon, enhance body's immunity, promote formation of antibody and improve the activity of macrophages. It is also to enhance antiviral and antibacterial effects to infectious diseases. The extract adjusts intestinal function, improve the growth of probiotics, protect intestinal mucosa, increase feed intake and promote growth. The KH was administered at 1 ml to 1 litre of drinking water for 5 days.

Antimicrobial Cocktail: The antimicrobial cocktail was made by streptomycin 5 g, hypoclorite 1 ml, Fortified Procaine Penicillin 3,000,000 IU and Groundnut oil. 1 ml was dissolved in 8 litres of drinking water and administered to the experimental broiler chickens for 5 days.

Vitalyte Extra® produced by Anupco group of company, obtained from the open market in Kaduna. It is a combination of vitamins, electrolytes and amino-acids and was administered at 4 grams to 4 litres of drinking water i.e. 1g/L. It was administered to the experimental broiler chickens for 5 days.

***Khaya senegalensis* (KS):** The fresh leaves of KS used in this study were collected in December, 2015 in the morning hours in a forest at Anchau village, Kubau Local Government Area (LGA) of Kaduna State, Nigeria. The plant was validated to specie level by a Plant Taxonomist in the Herbarium Unit of the Department of Biological Sciences, ABU Zaria and the specimen voucher numbers; 900181 for KS was deposited with the Herbarium for reference purposes. The fresh leaves of KS were dried using the sun rays and crush using electric blender. The pulverized leaves of (KS) was obtained from the Department of Veterinary Medicine Zaria and administered at 100 g in 8 litres of drinking water. It was administered to the experimental broiler chickens for 5 days.

How to access immunosuppression Gumboro in disease: Vaccine and Vaccination

Newcastle disease vaccine La Sota was purchased from the National Veterinary Research Institute (NVRI) outlet in College of Animal Science Mando Kaduna. It was reconstituted according to manufacturer (National veterinary research institute NVRI vom) recommendation) 2 litres of water to 200 doses of the vaccine. The vaccine was reconstituted and administered to the chicken in drinking water at 49 days of age.

Collection of blood sample

Blood sample 4 ml collected via the brachial vein was divided into two; one part was poured into a test-tube containing ethylene diamine tetra acetic acid (EDTA) for determination of haemograme. The other part was poured into a plain test tube (without anticoagulant) and allowed to clot to produce sera according to the methods described by Okeudo et al. (2003).

Determination of the presence of precipitin antibodies against infectious bursal disease virus

Agar gel precipitation test (AGPT) as modified by Abdu, 2007 was performed to detect the IBDV antigen and precipitin antibody in the bursal homogenates and sera respectively. The central wells were filled with 0.2 ml of the hyper immune serum (positive serum) and 0.02 ml of the bursal homogenate (test antigen) was dispensed into the wells in the parallel vertical columns. The Petri dishes was incubated in humid chamber at room temperature and is observed after 24 and 48 h for lines of precipitation. Twenty-five (25µL) microlitres of antigen sera was dispensed in central well. While the chickens' sample to be tested for antibody was placed in the peripheral wells. The plates were incubated for 48-72 h in a humid chamber after appropriate labeling for easy identification. The test plates were incubated at room temperature and was observed after 48-72 h for lines of precipitation Abdu 2007, Lukert and Saif 1997.

Determination of Antibodies against Newcastle Disease Virus which is use to estimate immunosuppression in Gumboro Disease outbreak.

To determine Virus concentration (HA) and to calculate 4HAU needed to perform the HAI test Microtitres plate was used and 0.025µL of PBS was dispense into well 2 to 12. Then followed by 0.025 µL of LaSota vaccine (prepared by diluting 200 doses to 2 ml of PBS) was dispense into well 1 and 2. Then a serial dilution was carried from well 2 to 11. Every well 0.025 µL of 1% RBC was dispense from 1 to 12 well. This was allowed for 15 minutes before

reading Haemagglutination unit (HAU) to know how to prepare antigen. The standard usually is 4 HAU was used to make dilution for antigen Grimes, 2002.

HAI test was performed to determine the antibody titre before describing the procedure. Microtitre plate was used and 0.025 µL of PBS was dispensed from well 2 to 12. Then followed by 0.025 µL of serum of chicken whose antibody titre to Newcastle disease virus to be determined was added to the first well and 2-fold serial dilution of 25 µl volume of the serum made across to 11 wells in the plate. The last serial dilution was discarded. Then allowed for 30 minutes, diluted antigen was added from well 1 to 11. It was allowed to stay for 30 min. Then 0.025 µL of 1% RBC was dispensed from well 1 to 11 which was allow for 30 min and then read on an inverted mirror. The inhibition was assessed by tilting the plates so that those wells in which the RBCs of absence streamed tear-shape RBCs were considered positive Rehman *et al*., 2003.

Determination of effect of Gumboro on haematocrit and leukocytic index

The packed cell volume (PCV) was determined using standard technique as described by Rehman et al. (2003) using non-heparinized capillary tube, TG12MX® Microhaematocrit centrifuge machine, and Hawksley® Micro-haematocrit reader. Red blood cells (RBCs) and total white blood cells (WBCs) counts were evaluated using Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer Schalm, 1975. The differential WBCs was determined using Leishman technique Coles, 1986.

Total white blood cells (WBCs) counts were evaluated using Natt-Herrick solution (1:200 dilution) and the improved Neubauer haemocytometer Campbel and Ellis, 2007. The cells were viewed using a light microscope (Olympus XSZ-107BN, Wincom Company Limited, Changsha, China) at a low power magnification (×40) and counted by means of a tally counter.

N/20 = WBC X 10^9/L (Campbell and Ellis, 2007) Where N = number of cells counted in the four outer large squares (or in 64 small squares).

Data analyses

Data were entered into and stored in Microsoft Excel 2007. They were exported to Graph pad Prism version 5.03 data base where descriptive statistics were carried out. One-way ANOVA was used to compare data between groups while variations between groups was determined using Tukey's comparative test, result were expressed as mean (±SD). The difference between groups were considered to be significant if P values were less than equal to 0.05

Results

Pack Cell Volume

On day 0 pi the PCV was higher in groups B2 (32.25 ± 2.02), B1 (31.50 ± 0.87) and A2 (30.75 ± 3. 54) administered vitalyte extra®, antimicrobial cocktail and King-herb oral solution® when compare to group D which was inoculated with Gumboro virus but not given nutraceuticals (positive control) (29.50 ± 1.56). 7 days pi the PCV was higher in group B2 (34.60 ± 2.11) administered Vitalyte extra® when compare to other groups A1, A2, B1, C, D and E administered chick-on®, King-herb oral solution®, antimicrobial cocktail Khaya senegalensis leaves positive control and negative control and were statistically significant (P ≤ 0.05) (Table 1).

Total Red Blood Cell Count

The TRBC at day 0 pi in groups A1 (4.80 ± 0.31), A2 (5.23 ± 0.64), B1 (5.30 ± 0.31), B2 (5.55 ± 0.22), C (5.00 ± 0.21) and D (4.90 ± 0.28) administered chick-on®, King-herb oral solution®, antimicrobial cocktail, vitalyte extra®, Khaya senegalensis leaves and that which was inoculated with Gumboro virus but not given nutraceuticals were lower than the negative control (which was not inoculated with Gumboro virus and not given nutraceuticals group) E (5.34 ± 0.39) when compared. 7 dpi the TRBC in groups A1 (5.04 ± 0.29), A2 (5.42 ± 0.39), B1 (5.28 ± 0.30), B2 (5.96 ± 0.27) and C (5.38 ± 0.43) administered chick-on®, King-herb oral solution®, antimicrobial cocktail, vitalyte extra® and Khaya senegalensis leaves were higher though not statistically significant compare to (positive control D) (4.94 ± 0.14) (P ≤ 0.05) (which was inoculated with Gumboro virus but not given nutraceuticals) (Table 2).

Total White Blood Cell Count

The TWBC at day 0 pi in groups A1 (15.24 ± 1.64), A2 (14.83 ± 2.52), B1 (13.80 ± 1.33), B2 (15.63 ± 2.86), administered chick-on®, King-herb oral solution®, antimicrobial cocktail, vitalyte extra®, D (16.83 ± 1.36) positive control and E (12.95 ± 1.65) negative control were significantly higher compare to C (11.28 ± 0.86) administered *Khaya senegalensis* leaves. 7 days pi there were high in all the groups A1 (13.98 ± 1.20), A2 (12.54 ± 0.86), B1 (9.42 ± 1.36), administered chick-on®, King-herb oral solution®, antimicrobial cocktail, vitalyte extra®, D (11.78 ± 1.18) positive control and E (11.56 ± 1.46) negative control compare to C (10.60 ± 0.89) administered Khaya senegalensis leaves.

Days post inoculation	A1	A2	B1	B2	C	D	E
0	29.20 ± 18.05	30.75 ± 27.42	31.50 ± 6.74	32.25 ± 15.65	29.60 ± 9.06	29.50 ± 12.08	30.20 ± 10.53
7	29.80 ± 9.91	31.60 ± 16.34	31.40 ± 12.63	34.60 ± 16.34	30.60 ± 12.86	29.80 ± 6.20	31.20 ± 8.99

Table 1: *Mean (± SD) Pack cell volume (%) of broilers (n = 5) administered unconventional remedies and inoculated with a very virulent infectious bursal disease virus, days 0 and 7 post inoculation.*

Days post inoculation	A1	A2	B1	B2	C	D	E
0	4.80 ± 2.40	5.23 ± 4.95	5.30 ± 2.0	5.55 ± 1.70	5.00 ± 1.63	4.90 ± 2.17	5.34 ± 3.02
7	5.04 ± 2.25	5.42 ± 3.02	5.28 ± 2.32	5.96 ± 2.09	5.38 ± 3.33	4.94 ± 1.08	5.22 ± 2.01

Table 2: *Mean (± SD) Total red blood cell Count ($\times 10^{13}$/L) of broilers (n = 5) administered with unconventional remedies and inoculated with a very virulent infectious bursal disease virus, 0- and 7-days post inoculation.*

Days post inoculation	A1	A2	B1	B2	C	D	E
0	15.24 ± 12.70	14.83 ± 19.52	13.80 ± 10.30	15.63 ± 22.15	11.28 ± 6.66	16.83 ± 10.53	12.95 ± 12.78
7	13.98 ± 9.30	12.54 ± 6.66	9.420 ± 10.53	12.52 ± 12.39	10.60 ± 6.89	11.78 ± 9.14	11.56 ± 11.31

Table 3: *Mean (± SD) Total white blood cell count ($\times 10^{9}$/L) of broilers (n = 5) administered with unconventional remedies and inoculated with a very virulent infectious bursal disease virus, 0- and 7-days post inoculation.*

Lymphocytes count

There were high lymphocytes count at 0 pi in groups A1 (12.27 ± 1.18), A2 (11.62 ± 2.26), B1 (11.34 ± 1.15), B2 (11.65 ± 2.76), administered chick-on®, King-herb oral solution®, antimicrobial cocktail, vitalyte extra®, D (13.57 ± 1.22) positive control and E (10.85 ± 1.01) negative control compare to C (8.83 ± 0.88) administered *Khaya senegalensis* leaves. 7 dpi when there were high lymphocytes count in groups A1 (11.46 ± 0.77), A2 (10.59 ± 0.70), B1 (7.78 ± 1.03), B2 (10.45 ± 1.27), administered chick-on®, King-herb oral solution®, antimicrobial cocktail, vitalyte extra®, D (9.79 ± 0.92) positive control and E (9.46 ± 1.24) negative control compare to group C (8.34 ± 074) administered Khaya senegalensis leaves.

Heterophil/ lymphocyte Ratio

The heterophil/lymphocyte ratio on day 0 pi was high in group B2 (0.33 ± 0.11) administered vitalyte extra® compare to groups A1 (0.20 ± 0.04), A2 (0.23 ± 0.05), B1 (0.22 ± 0.05), C (0.28 ± 0.05), D (0.24 ± 0.04) and E (0.14 ± 0.02) administered chick-on®, King-herb oral solution®, antimicrobial cocktail, *Khaya senegalensis* leaves, positive control D and negative control E. Heterophil/lymphocyte ratio were higher 7 days pi in groups A1 (0.17 ± 0.05), A2 (0.12 ± 0.02), B1 (0.19 ± 0.05), C (0.14 ± 0.02), D (0.16 ± 0.02) and E (0.18 ± 0.03) administered chick-on®, King-herb oral solution®, antimicrobial cocktail, Khaya senegalensis leaves, positive control D and negative control E compare to group B2 (0.15 ± 0.01) administered vitalyte extra® (Table 5).

Days post inoculation	A1	A2	B1	B2	C	D	E
0	12.27 ± 9.14	11.62 ± 17.51	11.34 ± 8.91	11.65 ± 21.38	8.829 ± 6.82	13.57 ± 9.45	10.85 ± 7.82
7	11.46 ± 5.96	10.59 ± 5.42	7.78 ± 7.98	10.43 ± 9.84	8.844 ± 5.73	9.793 ± 7.13	9.460 ± 9.60

Table 4: *Lymphocytes ($\times 10^{9}$/L) of broilers (n = 5) administered unconventional remedies and inoculated with very virulent infectious bursal disease virus, 0- and 7-days post inoculation (Mean ± SD).*

Days post inoculation	A1	A2	B1	B2	C	D	E
0	0.20 ± 0.31	0.23 ± 0.39	0.22 ± 0.39	0.33 ± 0.85	0.28 ± 0.39	0.24 ± 0.31	0.14 ± 0.15
7	0.17 ± 0.39	0.12 ± 0.15	0.19 ± 0.39	0.15 ± 0.08	0.14 ± 0.15	0.16 ± 0.15	0.18 ± 0.23

Table 5: *Mean (± SD) Heterophil/lymphocyte ratio of broilers (n = 5) administered with unconventional remedies and inoculated with very virulent infectious bursal disease virus, 0- and 7-days post inoculation.*

Response to vvIBDV

At day 0 there was no antibody titre to infectious bursal disease virus. At 7 dpi the antibody titre to infectious bursal disease virus for groups A2 (100%) and B2 (100%) were the higher compare to groups A1(60%), B1(80%), C (40%) and D (80%) E (0%) (Table 6).

Antibody Response of Newcastle Disease Vaccine La Sota

At day 0 pi the antibody titre against Newcastle disease of broilers for groups A1 (0.00 ± 0.00), B2 (0.0 ± 0.0), and E (0.0 ± 0.0) administered chick-on®, vitalyte extra were lower compare to A2 (0.2 ± 0.20), B1 (1.00 ± 0.78), C (1.20 ± 0.73) and D (0.60 ± 0.40) administered King-herb oral solution®, antimicrobial cocktail, Khaya senegalensis leaves and positive control D. There was an increase after one week in all the groups. However statistically significant increase was observed day 21 in group A2 (8.33 ± 0.33) administered with King-herb® after 3 weeks of vaccination when compared with group D (5.25 ± 1.03) positive control (Table 7).

Days post inoculation	A1	A2	B1	B2	C	D	E
0	0	0	0	0	0	0	0
7	60	100	80	100	40	80	0

Table 6: *Antibody against Infectious bursal disease virus of broilers (n = 5) administered with unconventional remedies and inoculated with a very virulent infectious bursal disease virus, 0- and 7-days post inoculation.*

Days post inoculation	A1	A2	B1	B2	C	D	E
0	0.0 ± 0.0	0.2 ± 1.55	1.00 ± 6.04	0.0 ± 0.0	1.20 ± 5.65	0.60 ± 3.10	0.0 ± 0.0
7	0.0 ± 0.0	0.40 ± 3.10	1.80 ± 7.13	0.20 ± 1.55	1.80 ± 7.51	1.80 ± 5.73	0.0 ± 0.0
21	8.00 ± 0.00	8.33 ± 2.56	7.50± 11.62	5.00 ± 0.00	5.67 ± 6.82	5.25 ± 7.98	0.0 ± 0.0

Table 7: *Antibody titre level (log2) against Newcastle disease virus of broilers (n = 5) administered unconventional remedies and inoculated with very virulent infectious bursal disease virus.*

Discussion

At 7 pi the nutraceuticals administered to groups A2, B2, groups administered king-herb oral solution®, vitalyte extra® had immunomadulatory effect resulting in antibodies to vvIBDV 100%, 100%, while 80% in B1 could be antibiotics was able reduce secondary bacterial infection observed in viral infection. The 80% observe in the positive control could be due anamnestic respone to infection of birds having antibodies to vvIBDV. It has been reported that, after vaccination, antibodies to vvIBDV are produced between 7-10 days in the body of pullets Bublot et al, 2007. Antibodies were detected using AGPT Lukert and Saif 2003 which is less sentitive than RT-PCR which detect viral antigen Musa et al, 2012, Islam and Samad 2004. This is similar with work of Islam and Samad 2004, Juranova et al, 2001 who reported low sensitivity using AGPT compared to RT-PCR. The administration of chick-on and king-herb oral solution might have led to the antibodies in groups A2 which is similar to the studies of Rauw who reported that administration of King-herb oral solution led to the production of interferon which reduce vvIBDV infection Rauw et al, 2007. Andamin also reported the presence of MDA could be attributed to transfer of antibodies from parent to chicks and as well as supplementation of nutraceuticals (Andamin et al., 2021). The nutaceuticals probably contain substances that

stimulated the immune system to produce more immunoglobulins (Kabir et al., 2004; Świątkiewicz and Korelski, 2007; Janardhana et al., 2010; Alireza et al., 2015). Also, the findings of Khaksefidi and Ghoorchi (2006), who reported that, the antibody titre level in nutraceuticals supplemented group was significantly higher at 5 and 10 dpi with vvIBDV when compared to positive control.

At day of inoculation Log_2 HI titre of antibodies to Newcastle diseases were A1, A2, B1, B2, C, D and E were generally low (4 Log_2) meaning that the broilers are susceptible to Newcastle disease virus which was below protection level in birds (Allan et al., 1978; Verma et al., 1985; OIE, 1996). Three weeks after A2 was 8.33 ± 0.33 possibly because of interferon present in King-herb oral solution help reduce immunosuppression cause by IBDV (Joseph et al., 2024) and group D2 was 5.25 ± 1.03 observed to have mean ND antibody titre greater than 4 Log_2 possibly because post exposure to IBDV the birds develop immunity to infectious diseases.

The significant increased mean HI titre obtained at first week for all the experimental groups could be attributed to the MDAs inherited from their parent at day-old. This is because specific immunity against ND develops within a week of age or older (Kapczynski et al., 2013). Moreover, the mean antibodies titre for groups A, B, C, 4.50 ± 0.03 log_2, 4.80 ± 0.03 log_2, 5.00 ± 0.04 log2 respectively were significantly higher than that of positive control group, D 3.50 ± 0.03 log2. This could be due the supplementation of nutraceuticals to the groups. This is in agreement with the findings of Świątkiewicz and Korelski, (2007), Kabir et al. (2004) and Alireza et al. (2015) who concluded that administration of nutraceuticals will stimulate the production of antibodies against ND

At 7 days Pi the PCV and TRBC in A2 and B2 administered king-herb oral solution and vitalyte extra® possibly prevented the destruction of precursor cells in the bone marrow or the haemorrhages usually seen in IBD (Kabir *et al*., 2004). This agrees with the report of Andamin *et al*., 2021 who reported increase in PCV, Hb concentration, TRBC of chicks in groups supplemented with nutraceuticals, which could have probably enhanced the production of erythropoietin, prevented the destruction of precursor cells in the bone marrow or the haemorrhages usually seen in IBD. Hence the higher values seen in the two groups. This could be due to antioxidant effects of vitalyte extra® (Igene et al., 2022). A, B and C was less severe when compared to that of positive control at 7 dpi.

In groups administered king-herb oral solution® and vitalyte extra® A2 and (B2) there was an increase in TWBC. The report is not similar to report of leukopaenia (Okorie-Kanu et al., 2018 Andamin et al., 2021 and Gana et al., 2019) associated with IBDV. This could possibly be due to antiviral properties of king-herb oral solution and vitalyte extra.

The lymphocyte count was high in A2 and B2 which is not similar to report in IBDV of decrease in lymphocyte count. This could be possibly due to lymphocytosis cause by king-herb oral solution® and vitalyte extra®. It could possibly be that the products elicited the production of significant number of immunoglobulins that neutralized vvIBDV thereby reduced destruction of leucocytes as observed by Midilli et al. (2008) and Liang (2013).

Heterophil/lymphocyte ratio was lowest for group administered King-herb® (A2) and vitalyte extra® indicating that it reduced stress due to IBDV infection (Gross & Siegel, 1983). Increase H/L ratio has been used as an important indicator of stress in birds (Gross and Siegel, (1983). Stress in birds which may vary from water to feeds deprivation, extremes humidity/temperature, constant light or disease condition may usually elevate the number of heterophils and depresses the number of lymphocytes (Gross and Siegel 1983; McFarlanje and Cutis, 1989). Heterophil/lymphocyte ratio was significantly higher in positive control when compared to that of groups A2 and B2 this could be the result of antioxidant properties king-herb oral solution and vitalyte extra® have which reduced the severity of that stress associated with vvIBDV inoculation on these groups. This is similar to work of Davis et al., 2008 who observed increase in H/L in stress condition. Scope et al. (2002) observed a considerable increase in H/L ratio following stress associated with transporting, handling and viral diseases of birds.

Conclusion

At 7 dpi antibodies (%) against Gumboro virus were dictated in broilers administered King-herb oral solution® (100.00), and vitalyte extra® (100.00) and inoculated with vvIBDV while none (0.00) in the negative control. At 7 dpi mean HI antibody titre (log2) to Newcastle disease La Sota vaccine of broilers administered King-herb oral solution® A2 was 8.33 ± 0.33 inoculated with vvIBDV was significantly higher ($P \leq 0.05$) compared to group D2 was 5.25 ± 1.03 that did not received nutraceutical but inoculated. PCV B2 (34.60 ± 2.11) administered Vitalyte extra® and A2 (31.60 ± 16.34) administered King-herb oral solution® were higher compared to positive control D (29.80 ± 6.20). At 7 dpi the mean total white blood cell count (×109/L) group administered King-herb oral solution® (12.54 ± 0.86) were high, compared to positive control (11.78

±1.18). At 7 dpi the mean lymphocyte count (×10^9/L) was high in A2 (10.59 ± 0.70) and B2 (10.45 ± 1.27), administered chick-on®, King-herb oral solution® and vitalyte extra®, compare to D (9.79 ± 0.92) positive control. At 7 dpi the mean Heterophyl/ymphocyte count (×109/L) A2 (0.12 ± 0.02) was lower, in group administered, King-herb oral solution compare to group D (0.16 ± 0.02) positive control. Recommendation to farmers:

Farmers could administer King-herb oral solution® and vitalyte extra® to increase antibodies to Gumboro virus infection. Immunosuppression cause by IBDV could be reduce by administration of King-herb oral solution®. Haematological indices of PCV, TRBC, LC and TWBC were increase administer King-herb oral solution® and vitalyte extra®. Stress often associated with infection in broilers could be reduce by administer King-herb oral solution® and vitalyte extra®. Hence from the study, the significance of the administration of King-herb oral solution® and vitalyte extra® combine with vaccination could be considered in the future, in broilers to help control IBDV infection.

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