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Full length Research

Mitigative Effects of Molasses, Antox® and EN-FLORAX® on Haematological Parameters in Commercial Pullets Infected with Infectious Bursal Disease Virus

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ABSTRACT: The study evaluated the mitigative effects of Molasses, Antox® and EN-FLORAX® on haematological parameters of ISA brown chicks inoculated with a very virulent infectious bursal disease (vvIBDV). Two hundred and fifty chicks were assigned into five groups (A, B, C, D and E) of 50 chicks each. Groups A, B and C were supplemented with Molasses, Antox® and EN-FLORAX®, respectively daily through drinking water from 1 to 49 days while D and E were not administered supplements and served as positive and negative controls, respectively. Groups A, B, C and D were inoculated with a vvIBDV at 28 days while E was not inoculated. Blood samples were collected from all the groups at 1, 7, 14, 21, 28, 35, 42 and 49 days, and processed using standard laboratory procedures. Results revealed significantly ($p \leq 0.05$) higher packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and thrombocyte counts in Groups A, B and C compared to D at 35, 42 and 49 days. Total white blood cell (TWBC), heterophils and lymphocyte counts were significantly ($p \leq 0.05$) higher in groups A, B and C than in group D at 35, 42 and 49 days. The heterophil/lymphocyte ratio (H/L ratio) between groups A, B, C and D differed significantly ($p \leq 0.05$). The haematological changes induced by vvIBDV were mitigated by the supplements in this study. Therefore, Molasses, Antox® and EN-FLORAX® could be administered to mitigate haematological alterations due to vvIBDV infection in poultry.

Keywords: Supplements; molasses, Antox®, EN-FLORAX®, blood, ISA Brown, vvIBDV.

INTRODUCTION

Infectious Bursal Disease (IBD) or Gumboro Disease (GD) is an acute, highly contagious viral disease of young chickens. It is caused by infectious bursal disease virus (IBDV). The disease is characterised by increased immunosuppression and mortality in 3 to 6-week-old chickens (Abdu, 1986; Andamin *et al.,* 2023). Two serotypes (1 and 2) of IBDV have been identified, with serotype 1 considered to be virulent while serotype 2 is avirulent (Orakpoghenor *et al.,* 2021b; Abdu *et al.,* 2023). Faeco-oral route constitutes the predominant mode of

transmission of IBD (Andamin, 2021). The IBDV is extremely lymphocidal and shows selective tropism for the bursa of Fabricious (BF) where it attacks immature B lymphocytes thereby inducing bursal lesions (Orakpoghenor *et al.,* 2021b). However, other lymphoid organs such as the thymus, spleen, caecal tonsils, Peyer's pactches, Harderian gland and bone marrow have been reported to be affected by IBDV (Liang *et al.,* 2015; Orakpoghenor *et al.,* 2021b). The virus is resistant to many disinfectants and environmental factors and remains infectious for at least four months in contaminated poorly sanitized poultry houses thus the disease reoccurs in subsequent flocks (Gary and Richard, 2015; Aliyu *et al.,* 2016). The infectious bursal disease has been reported to cause alterations in haematological parameters in poultry (Andamin *et al.,* 2022; Andamin *et al.,* 2023). There is no effective treatment documented against IBD, but there are speculations on the use of supplements such as Molasses, Antox® and EN-FLORAX® (Andamin *et al.,* 2022; Andamin *et al.,* 2023).

Molasses are prebiotics that are selectively fermented and constituents that cause specific changes in gastro-intestinal microbiota composition and/or activity, resulting in host's benefits (Davani-Davari *et al.,* 2019; Andamin, 2021; Andamin *et al.,* 2022). Antox® are probiotics that are viable, defined microorganisms in sufficient numbers and can alter the gastrointestinal tract (GIT) microflora to exert beneficial health effects (Guillot, 1998; Schrezenmeir and De Vrese, 2001; Andamin *et al.,* 2022). EN-FLORAX® are synbiotics with appropriate mixtures of prebiotics and probiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the GIT of the host (de Vrese and Schrezenmeir, 2008; Bandyopadhyay and Narayan, 2014; Mohammed *et al.,* 2018; Andamin *et al.,* 2022).

Haematological values of avian species are significantly influenced by diseases, such as IBD (Panigraphy *et al.,* 1986; Juranova *et al.,* 2001). Zeryehun *et al.* (2012) and Andamin *et al.* (2022) reported that IBDV causes alterations in different haematological parameters of poultry. The diagnostic application of haematology in veterinary medicine is a well-established procedure (Ross *et al.,* 1976). There is a paucity of information on the effects of prebiotics and synbiotics on haematological parameters to a very virulent IBD virus (vvIBDV) infection in commercial pullets. Therefore, the study aimed to evaluate the mitigative effects of Molasses, Antox® and EN-FLORAX® in commercial pullets inoculated with a vvIBDV on haematological parameters.

MATERIALS AND METHODS

Ethical consideration

The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), with the approval number: ABUCAUC/2019/19.

Experimental chickens and housing

Two hundred and fifty ISA Brown day-old pullets were obtained from a commercial hatchery, housed on deep litter and provided a floor space of 0.10 square metres per bird. Before stocking the house was cleaned, washed and disinfected. Rodent and insect control was achieved using a rodenticide and insecticide, respectively prior to the arrival of the chicks.

Feeds and feeding

The chicks were fed with chick mash that contained the following nutrients: % DM 97.20, % ASH 13.96, % EE 7.41, % CF 6.49, % N 3.60 and % CP 22.50. The chicks were allowed access to feed and water *ad libitum*.

Molasses, Antox® and EN-FLORAX®

The molasses was liquid prebiotic (Manufactured by Savannah Sugar Company, Yola Road, Gyewana, Lamurde Local Government of Adamawa State, Nigeria). It contained water (17-2%), sucrose (30-40%), glucose (4-9%), fructose (5-12%), potassium oxide (30-50%), calcium oxide (7-15%), magnesium oxide (2-14%), sodium oxide (0.3-9%), metal oxide (0.4-2.7%), sulfur trioxide (7-27%), chloride (12-20%), silicate and insoluble (1-7%), nitrogenous compounds (1.5-3.0%), protein (0.5-1.5%), amino-acids (0.3-0.5%), non-nitrogenous compound (1-5%), thiamine (2-10 ppm), riboflavin (1-6 ppm), pyridoxine (1-10 ppm), nicotinamide (1-25 ppm), pantothenic acid (2-25 ppm), folic acid (10-50 ppm) and biotin (0.1-2 ppm), was used.

The Antox® was a liquid probiotic (Manufactured by Montajat Pharmaceuticals, Bioscience Division, Dammam 31491, Saudi Arabia). It contained *Saccharomyces cerevisiae* (4.125×10^{6}cfu/mL), citric acid (6 g), lactic acid (2 g), vitamin B_1 (100 mg), vitamin B_2 (7.5 mg), vitamin B_6 (80 mg), vitamin B_{12} (0.6 mg), biotin (1.5 mg), nicotinamide (1 g), calcium chrorine (300 mg) potassium iodide (4.6 mg), sodium selenite (78.8 mg), zinc chloride (320 mg), iron chloride (300 mg), magnesium chloride hexahydrate (250 mg), manganese chloride (631 mg), copper sulphate (32 mg), cobalt chloride (3.08 mg), was used.

The EN-FLORAX® was a powdered synbiotic (Manufactured by EKSPOL s.c, ul, Romana Maya 1, 62-030 lubañ, Poland). It contained inulin (45%), malto-dextrin (55%), dextrose (60%), fructo-oligosaccharide (45%), oligo-fructose (35%), *Enterococcus faecium* (1.5×10^{-11}

cfu/kg), *Lactobacillus casei* (1.5 × 10^{-11} cfu/kg), *Lactobacillus plantarum* (1.5 × 10^{-11} cfu/kg), *Paiococcus acidilactici* (1.5 × 10^{-11} cfu/kg), crude protein (0.04 mg), crude fibre (0.02 mg), crude fat (0.01 mg), crude ash (0.5 mg), colloidal silico (4600 mg**),** vitamin B_1 (350 mg), vitamin B_2 (250 mg), nicotinamide (2000 mg), vitamin B_6 (320 mg), vitamin B_{12} (1000 mg), calcium pantothenate (1,200 mg), calcium (30,000 mg), potassium (3,000 mg), sodium chloride (3.9 mg), phosphorus (0.01 mg), magnesium (0.01 mg), lysine (0.01 mg), methionine (0.01 mg) and Kwas foliowy (3,000 mg), was used.

Inoculation of chicks with an Infectious Bursal Disease Virus

A characterised vvIBDV (Nigerian isolate) contained ($10^{9.76}$ CID_{50}/mL) suspension with a GenBank accession number: IBDVJX424067 (Adamu *et al.,* 2013) was used to inoculate the birds at 28 days. Each chick in the test and positive control groups were inoculated with 0.05 mL ($10^{8.46}$ CID_{50}/0.05 mL) of a vvIBDV suspension via the oral route.

Experimental design

The two hundred and fifty-day-old ISA Brown chicks were assigned randomly into five groups, A, B, C, D, and E with 50 chicks each. Chicks in group A were administered Molasses at 1 mL/L, group B Antox® at 1 mL/L and group C EN-FLORAX® at 1 g/L in drinking water daily from day-old to 49 days and inoculated at 28 days. No supplements were administered to chicks in group D (positive control) but were inoculated at 28 days, while group E (negative control) were neither administered supplements nor inoculated.

Collection of blood and haematological analyses

Blood was collected from each chick at 1, 7, 14, 21, 28, 35, 42 and 49 days in a labelled sample bottle containing ethylenediaminetetra acetic acid (EDTA). The blood was processed for haematological analyses using standard laboratory procedures (Campbell and Ellis, 2007).

Data analyses

Data collected were presented as mean ± standard error of the mean (Mean ± SEM). One-way analysis of variance (ANOVA) was used in the analysis of the data followed by Tukey's post-hoc test. GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego, California USA) was used for the analyses. Values of $p \leq 0.05$ were considered significant.

RESULTS

Erythrocytic parameters

Packed Cell Volume (PCV) showed a significant ($p \leq 0.05$) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days being highest in group C. The PCV in groups A, B and C decreased from 27.46 ± 0.16%, 28.47 ± 0.17% and 29.48 ± 0.18% at 28 days to 22.29 ± 0.12%, 19.26 ± 0.08% and 20.28 ± 0.10% at 35 days, but the decrease was significantly lower when compared to that of positive control group D (13.56 ± 0.05%), at 35 days. The PCV in A, B, C and D were significantly ($p \leq 0.05$) different at 42 and 49 days (Table 1).

Haemoglobin (Hb) concentration showed a significant ($p \leq 0.05$) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days being highest in group C. The mean Hb concentration in groups A, B, and C decreased from 10.78 ± 0.09 g/dL, 10.99 ± 0.08 g/dL and 11.61 ± 0.10 g/dL at 28 days to 7.44 ± 0.05 g/dL, 5.89 ± 0.03 g/dL and 6.98 ± 0.04 g/dL at 35 days, but the decrease was significantly lower when compared to that of positive control group D (3.59 ± 0.01 g/dL), at 35 days. The Hb concentrations in A, B, C, and D were significantly ($p \leq 0.05$) different at 42 and 49 days (Table 2).

Red Blood Cell (RBC) counts showed a significant ($p \leq 0.05$) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days being highest in group C. The RBC count in groups A, B, and C decreased from 2.74 ± 0.02 ×10^{12}/L, 2.76 ± 0.03 ×10^{12}/L and 2.79 ± 0.03 ×10^{12}/L at 28 days to 1.95 ± 0.01 ×10^{12}/L, 1.46 ± 0.00 ×10^{12}/L and 1.59 ± 0.02 ×10^{12}/L at 35 days, but the decrease was significantly lower when compared to that of positive control group D (0.92 ± 0.00 ×10^{12}/L), at 35 days. The RBC count in A, B, C, and D were significantly ($p \leq 0.05$) different at 42 and 49 days (Table 3).

Thrombocytes count

Before inoculation with vvIBDV, thrombocyte count increased as chicks grew older with values from 7 to 28 days being highest in group C. Thrombocytes count in groups A, B, and C decreased from 9.77 ± 0.07 ×10^9/L, 9.85 ± 0.08 ×10^9/L and 9.97 ± 0.08 ×10^9/L at 28 days to 7.84 ± 0.05 ×10^9/L, 6.78 ± 0.04 ×10^9/L and 6.88 ± 0.04 ×10^9/L at 35 days, but the decrease was significantly lower when compared to that of positive control group D (4.96 ± 0.02 ×10^9/L), at 35 days. The thrombocyte counts in A, B, C, and D were significantly ($p \leq 0.05$) different at 42 and 49 days (Table 4).

Leucocytic parameters

Total white blood cell (TWBC) count showed a significant

Table 1. Mean (± SE) packed cell volume (%) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) packed cell volume (%)]							
		1	7	14	21	28	35	42	49
A	Molasses	15.25 ± 0.07	19.30 ± 0.09	21.32 ± 0.11	23.35 ± 0.13	27.46 ± 0.16	22.29 ± 0.12****	26.32 ± 0.16****	31.46 ± 0.19****
B	Antox®	16.26 ± 0.07	20.31 ± 0.10	22.33 ± 0.12	24.36 ± 0.14	28.47 ± 0.17	19.26 ± 0.08**	23.28 ± 0.13**	26.30 ± 0.15**
C	EN-FLORAX®	15.26 ± 0.06	21.32 ± 0.11	23.35 ± 0.13	25.37 ± 0.15	29.48 ± 0.18	20.28 ± 0.10***	24.30 ± 0.14***	29.34 ± 0.17***
D	Positive control	16.27 ± 0.07	17.28 ± 0.08	19.30 ± 0.09	21.32 ± 0.11	24.37 ± 0.14	13.56 ± 0.05*	19.20 ± 0.09*	23.25 ± 0.13*
E	Negative control	15.26 ± 0.07	18.29 ± 0.09	20.32 ± 0.10	22.33 ± 0.12	25.37 ± 0.15	29.48 ± 0.18*****	35.55 ± 0.20*****	39.63 ± 0.22*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

Table 2. Mean (± SE) haemoglobin concentration (g/dL) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) haemoglobin concentration (g/dL)]							
		1	7	14	21	28	35	42	49
A	Molasses	3.59 ± 0.01	5.57 ± 0.02	7.79 ± 0.05	9.66 ± 0.08	10.78 ± 0.09	7.44 ± 0.05****	8.67 ± 0.06****	9.59 ± 0.08****
B	Antox®	3.58 ± 0.01	5.89 ± 0.03	7.99 ± 0.05	9.89 ± 0.07	10.99 ± 0.08	5.89 ± 0.03**	6.58 ± 0.04**	7.68 ± 0.06**
C	EN-FLORAX®	3.68 ± 0.02	6.31 ± 0.04	8.52 ± 0.07	10.45 ± 0.09	11.61 ± 0.10	6.98 ± 0.04***	7.91 ± 0.05***	8.89 ± 0.07***
D	Positive control	3.71 ± 0.02	4.65 ± 0.02	5.31 ± 0.03	6.55 ± 0.04	7.65 ± 0.05	3.59 ± 0.01*	4.48 ± 0.02*	5.37 ± 0.03*
E	Negative control	3.68 ± 0.02	4.67 ± 0.02	5.33 ± 0.03	6.56 ± 0.04	7.64 ± 0.05	8.77 ± 0.07*****	9.86 ± 0.08*****	10.99 ± 0.10*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

Table 3. Mean (± SE) red blood cell count (x 10^{12}/L) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) red blood cell count (x 10^{12}/L)]							
		1	7	14	21	28	35	42	49
A	Molasses	0.95 ± 0.00	1.48 ± 0.01	1.79 ± 0.01	2.19 ± 0.02	2.74 ± 0.02	1.95 ± 0.01****	2.34 ± 0.02****	2.99 ± 0.03****
B	Antox®	0.96 ± 0.01	1.53 ± 0.02	1.83 ± 0.02	2.21 ± 0.03	2.76 ± 0.03	1.46 ± 0.00**	1.87 ± 0.01**	2.25 ± 0.02**
C	EN-FLORAX®	0.95 ± 0.00	1.55 ± 0.02	1.85 ± 0.02	2. 24 ± 0.03	2.79 ± 0.03	1.59 ± 0.02***	1.92 ± 0.02***	2.35 ± 0.02***
D	Positive control	0.96 ± 0.01	1.19 ± 0.00	1.46 ± 0.01	1.89 ± 0.01	2.22 ± 0.02	0.92 ± 0.00*	1.27 ± 0.01*	1.66 ± 0.01*
E	Negative control	0.95 ± 0.00	1.18 ± 0.00	1.47 ± 0.01	1. 88 ± 0.01	2.23 ± 0.02	2.77 ± 0.02*****	3.33 ± 0.03*****	3.98 ± 0.03*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05) or (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

Table 4. Mean (± SE) thrombocyte count (x 10^9/L) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) thrombocyte count (x 10^9/L)]							
		1	7	14	21	28	35	42	49
A	Molasses	4.66 ± 0.02	6.55 ± 0.03	7.62 ± 0.04	8.53 ± 0.05	9.77 ± 0.07	7.84 ± 0.05****	8.87 ± 0.07****	9.69 ± 0.08****
B	Antox®	4.69 ± 0.03	6.85 ± 0.04	7.87 ± 0.05	8.88 ± 0.06	9.85 ± 0.08	6.78 ± 0.04**	7.66 ± 0.06**	8.68 ± 0.07**
C	EN-FLORAX®	4.68 ± 0.02	6.99 ± 0.04	7.95 ± 0.05	8.98 ± 0.06	9.97 ± 0.08	6.88 ± 0.04***	7.95 ± 0.05***	8.97 ± 0.07***
D	Positive control	4.69 ± 0.03	5.59 ± 0.03	6.64 ± 0.04	7.79 ± 0.05	8.83 ± 0.06	4.96 ± 0.02*	5.87 ± 0.03*	6.95 ± 0.04*
E	Negative control	4.65 ± 0.02	5.57 ± 0.03	6.65 ± 0.04	7 78 ± 0.05	8.82 ± 0.06	9.96 ± 0.08*****	10.94 ± 0.04*****	11.89 ± 0.09*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

Table 5. Mean (± SE) total white blood cell count (x 10^9/L) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) total white blood cell count (x 10^9/L)]							
		1	7	14	21	28	35	42	49
A	Molasses	1.83 ± 0.01	2.93 ± 0.02	3.75 ± 0.03	4.36 ± 0.04	4.97 ± 0.04	3.95 ± 0.03****	4.87 ± 0.04****	5.57 ± 0.05****
B	Antox®	1.84 ± 0.01	2.96 ± 0.02	3.85 ± 0.03	4.88 ± 0.04	5.35 ± 0.05	3.19 ± 0.03**	3.55 ± 0.02**	3.99 ± 0.03**
C	EN-FLORAX®	1.83 ± 0.01	2.99 ± 0.02	3.95 ± 0.03	4.97 ± 0.04	5.45 ± 0.05	3.33 ± 0.03***	3.89 ± 0.03***	4.36 ± 0.04***
D	Positive control	1.84 ± 0.01	2.33 ± 0.02	2.89 ± 0.02	3.39 ± 0.03	3.93 ± 0.03	1.95 ± 0.01*	2.37 ± 0.02*	2.98 ± 0.02*
E	Negative control	1.83 ± 0.01	2.34 ± 0.02	2.88 ± 0.02	3.38 ± 0.03	3.94 ± 0.03	4.59 ± 0.04*****	5.68 ± 0.05*****	6.71 ± 0.06*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

(p ≤ 0.05) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days being highest in group C. The TWBC count in groups A, B, and C decreased from 4.97 ± 0.04 ×10^9/L, 5.35 ± 0.05 ×10^9/L and 5.45 ± 0.05 ×10^9/L at 28 days to 3.95 ± 0.03 ×10^9/L, 3.19 ± 0.03 ×10^9/L and 3.33 ± 0.03 ×10^9/L at 35 days, but the decrease was significantly lower when compared to that of positive control group D (1.95 ± 0.01 ×10^9/L), at 35 days. The TWBC count in A, B, C, and D were significantly (p ≤ 0.05) different at 42 and 49 days (Table 5).

Heterophils count showed a significant (p ≤ 0.05) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days highest in group C. The heterophils count in groups A, B, and C decreased from 2.85 ± 0.02 ×10^9/L, 2.97 ± 0.02 ×10^9/L and 3.24 ± 0.03 ×10^9/L at 28 days to 2.00 ± 0.02 ×10^9/L, 1.33 ± 0.01 ×10^9/L and 1.52 ± 0.01 ×10^9/L at 35 days, but the decrease was significantly lower when compared to that of positive control group D (0.29 ± 0.00 ×10^9/L), at 35 days. The heterophils counts in A, B, C, and D were significantly (p ≤ 0.05) different at 42 and 49 days (Table 6).

Lymphocyte count showed a significant (p ≤ 0.05) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days being highest in group C. The lymphocytes count in groups A, B, and C decreased from values of 4.85 ± 0.04 ×10^9/L, 4.99 ± 0.04 ×10^9/L and 5.23 ± 0.05 ×10^9/L at 28 days to 3.34 ± 0.03 ×10^9/L, 2.29 ± 0.02 ×10^9/L and 2.53 ± 0.02 ×10^9/L at 35 days, but the decrease was significantly lower when compared to that of positive control group D (1.33 ± 0.01 ×10^9/L), at 35 days. The lymphocyte counts

Table 6. Mean (± SE) heterophils count (x 10^9/L) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) heterophils count (x 10^9/L)]							
		1	7	14	21	28	35	42	49
A	Molasses	0.53 ± 0.00	1.45 ± 0.01	1.86 ± 0.01	2.34 ± 0.02	2.85 ± 0.02	2.00 ± 0.02****	2.55 ± 0.02****	3.17 ± 0.03****
B	Antox®	0.52 ± 0.00	1.65 ± 0.01	1.99 ± 0.01	2.52 ± 0.02	2.97 ± 0.02	1.33 ± 0.01**	1.87 ± 0.01**	2.19 ± 0.02**
C	EN-FLORAX®	0.53 ± 0.00	1.85 ± 0.01	2.26 ± 0.02	2. 87 ± 0.02	3.24 ± 0.03	1.52 ± 0.01***	2.33 ± 0.02***	2.89 ± 0.02***
D	Positive control	0.52 ± 0.00	0.98 ± 0.00	1.59 ± 0.01	1.93 ± 0.01	2.38 ± 0.02	0.29 ± 0.00*	0.89 ± 0.00*	1.15 ± 0.01*
E	Negative control	0.53 ± 0.00	0.99 ± 0.00	1.58 ± 0.01	1.92 ± 0.01	2.39 ± 0.02	2.98 ± 0.02*****	3.88 ± 0.03*****	4.47 ± 0.04*****

Key: n = total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterics (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

Table 7. Mean (± SE) lymphocyte counts (x 10^9/L) of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) lymphocytes count (x 10^9/L)]							
		1	7	14	21	28	35	42	49
A	Molasses	1.42 ± 0.01	2.55 ± 0.02	3.17 ± 0.03	4.37 ± 0.04	4.85 ± 0.04	3.34 ± 0.03****	3.98 ± 0.03****	4.58 ± 0.04****
B	Antox®	1.43 ± 0.01	2.62 ± 0.02	3.38 ± 0.03	4.55 ± 0.04	4.99 ± 0.04	2.29 ± 0.02**	2.84 ± 0.02**	3.08 ± 0.03**
C	EN-FLORAX®	1.42 ± 0.01	2.78 ± 0.02	3.63 ± 0.03	4.96 ± 0.04	5.23 ± 0.05	2.53 ± 0.02***	2.98 ± 0.02***	3.57 ± 0.03***
D	Positive control	1.43 ± 0.01	2.25 ± 0.02	2.64 ± 0.02	3.29 ± 0.03	3.79 ± 0.03	1.33 ± 0.01*	1.95 ± 0.01*	2.38 ± 0.02*
E	Negative control	1.42 ± 0.01	2.26 ± 0.02	2.65 ± 0.02	3.28 ± 0.03	3.80 ± 0.03	4.32 ± 0.04*****	4.84 ± 0.04*****	5.43 ± 0.05*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

in A, B, C, and D were significantly ($p \leq 0.05$) different at 42 and 49 days (Table 7).

The heterophil/lymphocyte (H/L) ratio showed a significant ($p \leq 0.05$) increase as chicks grew older before inoculation with vvIBDV, with values from 7 to 28 days being highest in group C. There was a decrease in H/L ratio in groups A, B, and C from values of 0.59 ± 0.05 ×10^9/L, 0.60 ± 0.05 ×10^9/L and 0.62 ± 0.06 ×10^9/L at 28 days to 0.60 ± 0.06 ×10^9/L, 0.58 ± 0.05 ×10^9/L and 0.60 ± 0.05 ×10^9/L at 35 days, but the increases were significantly lower compared to that of positive control group D (0.22 ± 0.00 ×10^9/L.), at 35 days. The H/L ratios in A, B, C, and D were significantly ($p \leq 0.05$) different at 42 and 49 days (Table 8).

DISCUSSION

The erythrocytic (PCV, Hb and RBC), leucocytic (TWBC, heterophils and lymphocyte counts) parameters and thrombocyte counts increased before inoculation (from 7 to 28 days) in all the groups of chicks in this study. These increases were a result of the increase in the physiological demands of the chicks often associated with increase in age in terms of metabolism and immune response to pathogens. Although there were significant ($p \leq 0.05$) differences in these parameters across all the groups each week, the supplemented groups (A, B and C) had higher values compared to the groups not administered supplements (D and E). This suggests that the supplements might have contributed to the enhancement production through the actions of their constituents. After inoculation, there was a

Table 8. Mean (± SE) heterophil/lymphocyte ratios of ISA Brown chicks (n = 5) administered Molasses, Antox® and EN-FLORAX® from day-old and inoculated with a very virulent infectious bursal disease virus at 28-day-old.

Group	Treatment	Age of chicks in days [Mean (± SE) heterophil/lymphocyte ratio]							
		1	7	14	21	28	35	42	49
A	Molasses	0.37 ± 0.00	0.57 ± 0.05	0.59 ± 0.03	0.54 ± 0.05	0.59 ± 0.05	0.60 ± 0.06****	0.64 ± 0.06**	0.69 ± 0.08**
B	Antox®	0.36 ± 0.00	0.63 ± 0.05	0.59 ± 0.03	0.55 ± 0.05	0.60 ± 0.05	0.58 ± 0.05**	0.82 ± 0.05****	0.71 ± 0.06***
C	EN-FLORAX®	0.37 ± 0.00	0.67 ± 0.05	0.62 ± 0.03	0.58 ± 0.05	0.62 ± 0.06	0.60 ± 0.05***	0.78 ± 0.01***	0.68 ± 0.06****
D	Positive control	0.36 ± 0.00	0.44 ± 0.00	0.60 ± 0.05	0.59 ± 0.03	0.63 ± 0.03	0.92 ± 0.07*	0.86 ± 0.00*	0.78 ± 0.00*
E	Negative control	0.37 ± 0.00	0.44 ± 0.00	0.60 ± 0.05	0.59 ± 0.03	0.63 ± 0.06	0.69 ± 0.00*****	0.80 ± 0.08*****	0.82 ± 0.08*****

Key: n = Total number of birds sampled, Mean ± SE = standard error of the means, Means values with asterisks (*) (P ≤ 0.05), (**) (P ≤ 0.01), (***) (P ≤ 0.001), (****) (P ≤ 0.0001) or (*****) (P ≤ 0.00001) in the same column differed significantly.

decrease in these parameters in the inoculated groups (A, B, C and D) compared to the non-inoculated group (E).

The decrease in the erythrocytic parameters and thrombocyte counts observed in the vvIBDV-inoculated groups in this study might be associated with anaemia due to haemorrhages, destruction of haemopoietic organs and/or viraemia (Oladele *et al.,* 2005; Andamin *et al*., 2021; Orakpohenor *et al.,* 2021a). In the groups administered supplements, the decreases were less severe compared to the positive control. This effect might be due to decreased destruction of erythroid cells in the bone marrow and/ or endothelial cells in the blood vessels. The significant amount of minerals, vitamins, electrolytes and irons found in the supplements used, enhanced the production of erythropoietin which might be another possible mechanism (Pimentel *et al.,* 1998; Kabir *et al.,* 2004; Andamin *et al*., 2021).

The decrease observed in the leucocytic parameters in the inoculated groups in this study is consistent with the findings of Cheville (1967); Jain (1986); Andamin *et al*. (2021; 2022) whose reported severe panleucopaenia during the severe inflammatory stage of IBD. The possibility might be linked with the destruction of myeloid cells in the bone marrow and/or the mature cells within circulation (Weiss and Kaufer-Weiss, 1994) by the vvIBDV. The lymphopaenia might also be associated with vvIBDV multiplication in lymphocytes and subsequent necrosis of bursal lymphocytes (Ley *et al.,* 2007). The leucopaenia in this study were however less severe in the supplemented groups compared to the positive control. This might be due to direct and/or indirect enhancement of immune response by the supplements leading to significant immunoglobulin production and subsequently neutralization of the vvIBDV. This virus neutralization might have led to a decrease in leucocyte destruction (Cakır *et al.,* 2008).

Heterophil/lymphocyte (H/L) ratio is the index of immune system tension as a result of oxidative stress as well as the status of immunosuppressive diseases (Moreno *et al.,* 2002; El-Lethey *et al.,* 2003; Clinchy *et al.,* 2004; Andamin *et al.,* 2021). The H/L ratio was significantly higher in positive control when compared to the groups administered supplements. This could be probable because the supplements contained significant antioxidant properties that mitigated oxidative stress as reported by Andamin *et al.* (2021). Scope *et al.* (2002) observed a considerable increase in the H/L ratio following stress associated with transportation, handling and viral infection of birds. Acute stress is known to increase the H/L ratio (El-Lethey *et al.,* 2003). Therefore, the higher H/L ratio observed in this study could result from the destruction of lymphocytes caused by vvIBDV infection.

The mechanism by which Molasses, Antox® and EN-FLORAX® mitigated the vvIBDV-induced haematological changes in this study might be due to the actions of their constituent's individually. These supplements contain vitamins and essential minerals which are critical for the formation of blood cells and play critical roles in immune responses (Hochleithner, 1994; Pimental *et al.,* 1998; Abdu *et al.,* 2023). Also, they might have served as a source of nutrients to take care of increased nutritional demand during the IBDV infection (Andamin *et al*., 2021). Thus, these might be possible mechanisms for the mitigative effects of these supplements on the vvIBDV-induced haematological changes in this study. However, molasses exhibited more mitigative effects compared to Antox® and EN-FLORAX®, and this might be associated with the presence of glucose, fructose and sucrose which enhanced its ability to take care of the increased nutritional demand.

Conclusion

Molasses, Antox® and EN-FLORAX® mitigated the haematological changes induced by vvIBDV infection in ISA Brown pullets in this study.

Recommendation

Therefore, these supplements (Molasses, Antox® and EN-FLORAX®) could be administered for prophylactics to mitigate haematological alterations due to vvIBDV infection in poultry.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Abdu, P. A. (1986). Infectious bursal disease immunisation failures in chickens in Nigeria. *Tropical Animal Health and Production*, *18*(2), 123-125

Abdu, P., Andamin, A. D., Orakpoghenor, O., Akade, F. T., Sani, D., Aluwong, T., & Markus, T. P. (2023). Antox® and Bactofort® Improved Alterations in Oxidative Stress Biomarkers Induced by a Very Virulent Infectious Bursal Disease Virus in ISA Brown Chicks. *Scientific Reports in Life Sciences*, *4*(1), 69-84.

Adamu, J., Owoade, A. A., Abdu, P. A., Kazeem, H. M., & Fatihu, M. Y. (2013). Characterization of field and vaccine infectious bursal disease viruses from Nigeria revealing possible virulence and regional markers in the VP2 minor hydrophilic peaks. *Avian Pathology*, *42*(5), 420-433.

Aliyu, H. B., Sa'idu, L., Jamilu, A., Andamin, A. D., & Akpavie, S. O. (2016). Outbreaks of virulent infectious bursal disease in flocks of battery cage brooding system of commercial chickens. *Journal of Veterinary Medicine*, *2016*(1), 8182160.

Andamin, A. D. (2021). Effects of prebiotic, probiotic and synbiotic on clinico-pathological changes, antibody response and performance in commercial pullets experimentally infected with Infectious Bursal Disease Virus. *PhD Thesis;* 2019/2020 Session. Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria, 231p.

Andamin, A. D., Abdu, P. A., Akade, F. T., Orakpoghenor, O., & Aluwong, T. (2021). Antox® and Bactofort® mitigated the haematological alterations induced by a very virulent infectious bursal disease virus in chicks. *Veterinary Research Communications*, *45*, 101-109.

Andamin, A. D., Orakpoghenor, O., Markus, T. P., Akade, F. T., Abdu, P. A., & Aluwong, T. (2023). Supplements Administration during Infectious Bursal Disease Virus Infection in Poultry: Evaluation of the Effects of Prebiotic, Probiotic and synbiotic on the haematoloical alterations in commercial pullets. *Open Access Journal of Veterinary Sciences & Research, 8*(2), 000247.

Andamin, A., Abdu, P., Orakpoghenor, O., Markus, T., Oladele, S., Akade, F., & Aluwong, T. (2022). Molasses, Antox® and EN-FLORAX® decreased antibody decay rate and enhanced response to a very virulent infectious bursal disease virus and Newcastle disease vaccine La Sota in ISA Brown chicks. *Journal of Immunoassay and Immunochemistry*, *43*(5), 546-556.

Bandyopadhyay, B., & Narayan, C. M. (2014). Prebiotics, probiotics and synbiotics -In health improvement by modulating gut microbiota: *International Journal of Current Microbiology and Applied Science,* 3, 410 420.

Cakır, S., Midilli, M., Alp, M., Ylımaz, H., Muglal, O. H., Turan, N., & Kocabaglı, N. (2008). Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. *South African Journal of Animal Science*, *38*(1), 21-27.

Campbell, T. W.. & Ellis, C.K. (2007). Haematology of birds. In: Campbell, T.W., Ellis, C.K. (eds.). *Avian and Exotic Animal Haematology and Cytology* (Third edition) Blackwell Publishing Professional, Ames (I.A), USA. Pp. 3-50.

Cheville, N. F. (1967). Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen, and thymus of the chicken. *The American journal of pathology*, *51*(4), 527-551.

Clinchy, M., Zanette, L, Boonstra, R., Wingfield, JC., Smith, JNM (2004). Balancing food and predator pressure induces chronic stress in songbirds. In: *Proceedings of the Royal Society of London. Series B*: *Biological Science.* Pp. 2473-2479.

Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J., Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*, *8*(3), 92.

De Vrese, M., & Schrezenmeir, A. J. (2008). Probiotics, prebiotics, and synbiotics. In: *Food biotechnology.* Springer Nature. Pp. 1-66.

El-Lethey, H., Huber-Eicher, B., & Jungi, T. W. (2003). Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. *Veterinary Immunology and Immunopathology*, *95*(3-4), 91-101.

Gary, D. B., & Richard, D. M. (2015). Infectious bursal disease (Gumboro) in commercial broilers. *Bulletin of the Institute of Food and Agricultural Sciences* VM84, University of Florida.

Guillot, J. F. (1998). Les probiotiques en alimentation animale. *Cahiers Agricultures*, *7*, 49-54.

Hochleithner, M. (1994). Avian medicine: Principles and application. In: Ritchie, B. W., Harrison, G. J., & Harrison, L. R. (eds). *Biochemistries*. Wingers Publishing Inc., Lake Worth, FL. Pp. 223-245.

Jain, N. C. (1986). *Schalm's Veterinary Haematology*, 4th Edn, Philadelphia, PA, USA, Lea and Febiger. Pp. 747-748.

Juranova, R., Nga, N. T., Kulikova, L., & Jurajda, V. (2001). Pathogenicity of Czech isolates of infectious bursal disease virus. *Acta Veterinaria Brno*, *70*(4), 425-431.

Kabir, S. L., Rahman, M. M., Rahman, M. B., Rahman, M. M., &

Ahmed, S. U. (2004). The dynamics of probiotics on growth performance and immune response in broilers. *International Journal of Poultry Science*, *3*(5), 361-364.

Ley, K., Laudanna, C., Cybulsky, M. I., & Nourshargh, S. (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nature Reviews Immunology*, *7*(9), 678-689.

Liang, J., Yin, Y., Qin, T., & Yang, Q. (2015). Chicken bone marrow-derived dendritic cells maturation in response to infectious bursal disease virus. *Veterinary Immunology and Immunopathology*, *164*(1-2), 51-55.

Mohammed, A. A., Jacobs, J. A., Murugesan, G. R., & Cheng, H. W. (2018). Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. *Poultry science*, *97*(4), 1101-1108.

Moreno, J., Merino, S., MartÍnez, J., Sanz, J., & Arriero, E. (2002). Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. *Ecoscience*, *9*(4), 434-439.

Oladele, O. A., Adene, D. F., Obi, T. U., Nottidge, H. O., & Aiyedun, A. I. (2005). Sequential hematological study of experimental infectious bursal disease virus infection in chickens, turkeys and ducks. *Revue D'elevage et de Medecine Veterinaire Des Pays Tropicaux*, *58*, 211-215.

Orakpoghenor, O., Oladele, S. B., Abdu, P. A., Markus, T. P., Andamin, A. D., & Esievo, K. A. N. (2021b). Comparative pathological changes induced by very virulent infectious bursal disease virus infection in inoculated, sentinel pigeons and chickens. *Open Veterinary Science*, *2*(1), 55-64.

Orakpoghenor, O., Oladele, S. B., Abdu, P. A., Markus, T. P., Enam, S. J., Andamin, A. D., Muhammed, M. S., Usman, S.G., & Esievo, K. A. N. (2021a). Pigeons (Columba livia domestica) Are Susceptible to Infectious Bursal Disease: A Comparative Study of Their Hematological and Serum Biochemical Alterations. *Frontiers in Veterinary Science*, *8*, 673398.

Panigraphy, B., Rowe, L. D., & Corrier, D. E. (1986). Haematological values and changes in blood chemistry in chickens with infectious bursal disease. *Research in Veterinary Science, 40*(1), 86-88.

Pimentel, J. L., Gerger, J. L., Cook, M. E., & Stahl, L. J. (1998). Iron metabolism in chicks fed various levels of zinc and copper. *Journal of Nutritional Biochemistry*, *3*(3), 140-145.

Ross, J. G., Christie, G., Halliday, W. G., & Jones, R. M. (1976). Determination of haematology and blood chemistry values in healthy six-week old broiler hybrids. *Avian Pathology*, *5*(4), 273-281.

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics—approaching a definition. *The American journal of clinical nutrition*, *73*(2), 361s-364s.

Scope, A., Filip, T., Gabler, C., & Resch, F. (2002). The influence of stress from transport and handling on hematologic and clinical chemistry blood parameters of racing pigeons (Columba livia domestica). *Avian diseases*, *46*(1), 224-229.

Weiss, E., & Kaufer-Weiss, I. (June 1994). Pathology and pathogenesis of infectious bursal disease. In: *Proceedings of the International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia*. Rauischholzhausen, Germany. Pp. 21-24.

Zeryehun, T., Hair-Bejo, M., & Rasedee, A. (2012). Hemorrhagic and clotting abnormalities in infectious bursal disease in specific-pathogen-free chicks. *World Applied Sciences Journal*, *16*(98), 1123-1130.