



# Supplements Administration during Infectious Bursal Disease Virus Infection in Poultry: Evaluation of the Effects of Prebiotic, Probiotic and Synbiotic on the Haematological Alterations in Commercial Pullets

Andamin AD<sup>1</sup>, Orakpoghenor O<sup>2,6\*</sup>, Markus TP<sup>3,6</sup>, Abdu PA<sup>1</sup>, Akade FT<sup>4</sup>, OladeleSB<sup>2</sup> and Aluwong T<sup>5</sup>

<sup>1</sup>Department of Veterinary Medicine, Ahmadu Bello University, Nigeria

<sup>2</sup>Department of Veterinary Pathology, Ahmadu Bello University, Nigeria

<sup>3</sup>Department of Veterinary Microbiology, Ahmadu Bello University, Nigeria

<sup>4</sup>Department of Animal Health Technology, Taraba State College of Agriculture, Nigeria

<sup>5</sup>Department of Veterinary Physiology, Ahmadu Bello University, Nigeria

<sup>6</sup>Regional Disease Surveillance Systems Enhancement (REDISSE) Project, Nigeria

## Research Article

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**\*Corresponding author:** Ochuko Orakpoghenor, Department of Veterinary Pathology, Ahmadu Bello University, Nigeria; Tel: +2347067522037; Email: [ochuko.orakpoghenor@gmail.com](mailto:ochuko.orakpoghenor@gmail.com)

## Abstract

The administration of supplements during viral infections in poultry is gaining more attention. This study evaluated the effects of supplements (prebiotic, probiotic and synbiotic) administration on haematological parameters of ISA Brown chicks inoculated with a very virulent infectious bursal disease (vvIBDV). Two hundred and fifty one-day-old chicks were assigned into five groups (A, B, C, D and E) of 50 chicks each. Groups A, B and C were administered molasses, Antox<sup>®</sup> and EN-FLORAX<sup>®</sup>, respectively daily via drinking water from 1 to 49 days of age (doa) while D and E were not administered any supplement. Groups A, B, C and D were inoculated with a vvIBDV at 28 doa while E was not inoculated. Blood was collected from all groups at 1, 28, 35, 42 and 49 doa, and processed for haematology using standard laboratory procedures. Results revealed significantly ( $P < 0.05$ ) higher packed cell volume (PCV), haemoglobin concentration, total red blood cells and thrombocyte count in groups A, B and C compared to group D at 35, 42 and 49 doa. Total white blood cells, heterophils and lymphocyte counts were significantly ( $P < 0.05$ ) higher in groups A, B and C than in group D at 35, 42 and 49 doa. The PCV between groups A, B, C and D differed significantly ( $P < 0.05$ ). The haematological changes induced by vvIBDV were mitigated by the supplements in this study. Therefore, molasses, Antox<sup>®</sup> and EN-FLORAX<sup>®</sup> could be administered to ameliorate haematological alterations due to vvIBDV infection in poultry.

**Keywords:** Supplements; Molasses; Antox<sup>®</sup>; EN-FLORAX<sup>®</sup>; Haematology; Infectious Bursal Disease

## Introduction

Infectious bursal disease (IBD), also known as Gumboro disease (GD), is a highly contagious viral disease that affects young chickens. It is caused by infectious bursal disease virus (IBDV). In 3- to 6-week-old chickens, the disease causes immunosuppression and mortality [1,2]. The IBDV is extremely lymphocidal and shows selective tropism for the bursa of Fabricius (BF) where it targets immature B lymphocytes and induces bursal lesions [2]. Also, the virus affects other lymphoid organs such as the thymus, spleen, caecal tonsils, Peyer's patches, Harderian gland and bone marrow [2-4]. Infectious bursal disease has been documented to cause hematological alterations in poultry [5,6]. Although no effective treatments exist against IBD, there are speculations on the use of supplements such as pre-, pro- and synbiotic. Prebiotics are feed ingredients that have the ability to stimulate the growth and metabolic activity of specific gut micro flora. They are significant because intestinal luminal bacteria play an important role in maintaining the homeostatic immune functions of the host's intestinal immune compartment [7,8]. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. They are live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance [9,10]. Synbiotics are appropriate mixtures of pre- and probiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (GIT) of the host [9,10].

Supplements were reported to ameliorate oxidative changes following very virulent IBDV (vvIBDV) infection in chickens [11]. Also, a report of pre-, pro- and synbiotics on changes induced by parasitic infection in broiler chickens has been documented [12]. However, there is paucity of information on the effects of pre-, pro- and synbiotic on hematological parameters of chickens following vvIBDV infection. Hence, in this study, the mitigative effects of pre-, pro- and synbiotic on vvIBDV-induced hematological changes in commercial pullets were evaluated.

## Materials and Methods

### Ethical considerations

The use chicken in this study was approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) - ABUCAUC/2019/19.

### Experimental Chickens and housing

Two hundred and fifty one-day-old ISA Brown pullet chicks were obtained from a commercial hatchery and

housed on deep litter. The pen was cleaned, washed and disinfected with adequate rodent and insect control prior to 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.

### Pre-Pro- and Synbiotic used

The prebiotic was liquid molasses (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).

The probiotic supplement was liquid Antox® (Montajat Pharmaceuticals, Bioscience Division, Dammam 31491, Saudi Arabia). It contained *Saccharomyces cerevisiae* ( $4.125 \times 10^6$  cfu/mL), citric acid (6 g), lactic acid (2 g), vitamin B1 (100 mg), vitamin B2 (7.5 mg), vitamin B6 (80 mg), vitamin B12 (0.6 mg), biotin (1.5 mg), nicotinamide (1 g), calcium chlorine (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).

The synbiotic was powdered EN-FLORAX® (EKSPOL s.c, ul, Romana Maya 1, 62-030 lubań, Poland). It contained inulin (45%), malto-dextrin (55%), dextrose (60%), fructo-oligosaccharide (45%), oligo-fructose (35%), *Enterococcus faecium* ( $1.5 \times 10^{11}$  cfu/kg), *Lactobacillus casei* ( $1.5 \times 10^{11}$  cfu/kg), *Lactobacillus plantarum* ( $1.5 \times 10^{11}$  cfu/kg), *Paicoccus acidilactici* ( $1.5 \times 10^{11}$  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 B1 (350 mg), vitamin B2 (250 mg), nicotinamide (2000 mg), vitamin B6 (320 mg), vitamin B12 (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 Kwass foliowy (3,000 mg).

## Monitoring of Chicks for Maternal Antibodies against IBDV

The chicks were monitored for maternal antibodies using indirect enzyme-linked immunosorbent assay (ELISA) (IDEXX Laboratory, Incorporate, Westbrook, Maine 04,092, USA). However, the chicks were not vaccinated against IBD. At 28 days of age (doa) when the maternal antibodies had waned below protective level, the chicks were inoculated.

## Inoculation of chicks with IBDV

A characterized vvIBDV (Nigerian isolate) suspension (109.76 CID50/mL) was used to inoculate the birds at 28 doa. The birds were inoculated with 0.05 mL of the suspension via oral route.

## Grouping of Birds

The two hundred and fifty one-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 was administered molasses at 2 mL/L, group B Antox® at 1.5 mL/L and group C EN-FLORAX® at 1 g/L in drinking water daily from one-day-old to 49 doa and inoculated at 28 doa. No supplement was administered to chicks in group D (Positive control) but were inoculated at 28 doa, while group E (Negative control) were neither administered supplements nor inoculated.

## Clinical Signs Observation and Confirmation of Challenge Outcome

Following inoculation, the chicks were observed for clinical signs due to IBDV. Also, the challenge outcome was confirmed by collection of cloacal swabs which were

tested for the presence of the virus by reverse transcriptase polymerase chain reaction (RT-PCR).

## Collection of Blood and Haematological Analyses

Blood was collected from each bird at 1, 28, 35, 42 and 49 doa in a labeled sample bottle containing ethylenediaminetetraacetic acid (EDTA). The blood was processed for haematological analyses using standard laboratory procedures [13].

## Data Analyses

Data collected were presented as mean  $\pm$  standard error of the mean (Mean  $\pm$  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 no significant ( $P > 0.05$ ) difference in all groups of birds at 1, 14 and 28 doa (Table 1). At 35 doa (7 days post-inoculation - dpi), PCV was significantly ( $P < 0.05$ ) lower in group D (13.56  $\pm$  0.05%) than in A (22.29  $\pm$  0.12%), B (19.26  $\pm$  0.08%) and C (20.28  $\pm$  0.10%). There were significantly ( $P < 0.05$ ) lower PCV in group D (19.20  $\pm$  0.09; 23.25  $\pm$  0.13%) compared to groups A (26.32  $\pm$  0.16; 31.46  $\pm$  0.19%), B (23.28  $\pm$  0.13; 26.30  $\pm$  0.15%) and C (24.30  $\pm$  0.14; 29.34  $\pm$  0.17%) at 42 (14 dpi) and 49 (21 dpi) doa (Table 1).

Group	Treatment	Age (days)					
		1	14	28	35	42	49
		Mean ( $\pm$ SE) packed cell volume (%)					
A	Molasses	15.25 $\pm$ 0.07	21.32 $\pm$ 0.11	27.46 $\pm$ 0.16	22.29 $\pm$ 0.12 <sup>c</sup>	26.32 $\pm$ 0.16 <sup>c</sup>	31.46 $\pm$ 0.19 <sup>d</sup>
B	Antox®	16.26 $\pm$ 0.07	22.33 $\pm$ 0.12	28.47 $\pm$ 0.17	19.26 $\pm$ 0.08 <sup>b</sup>	23.28 $\pm$ 0.13 <sup>b</sup>	26.30 $\pm$ 0.15 <sup>b</sup>
C	EN-FLORAX®	15.26 $\pm$ 0.06	23.35 $\pm$ 0.13	29.48 $\pm$ 0.18	20.28 $\pm$ 0.10 <sup>b</sup>	24.30 $\pm$ 0.14 <sup>b</sup>	29.34 $\pm$ 0.17 <sup>c</sup>
D	Positive control	16.27 $\pm$ 0.07	19.30 $\pm$ 0.09	24.37 $\pm$ 0.14	13.56 $\pm$ 0.05 <sup>a</sup>	19.20 $\pm$ 0.09 <sup>a</sup>	23.25 $\pm$ 0.13 <sup>a</sup>
E	Negative control	15.26 $\pm$ 0.07	20.32 $\pm$ 0.10	25.37 $\pm$ 0.15	29.48 $\pm$ 0.18 <sup>d</sup>	35.55 $\pm$ 0.20 <sup>d</sup>	39.63 $\pm$ 0.22 <sup>e</sup>

Values with different superscript letters down the same column differ significantly at  $P < 0.05$ .

**Table 1:** Mean ( $\pm$  SE) packed cell volume (%) of ISA Brown chicks administered molasses, Antox® and EN- FLORAX® from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

No significant ( $P > 0.05$ ) difference existed in the haemoglobin (Hb) concentration of all groups of chicks at 1, 14 and 28 doa (Table 2). There was significantly ( $P < 0.05$ ) higher Hb concentration in groups A (7.44  $\pm$  0.05 g/dL),

B (5.89  $\pm$  0.03 g/dL) and C (6.98  $\pm$  0.04 g/dL) than in D (3.59  $\pm$  0.01 g/dL) at 35 doa (7dpi). At 42 and 49 doa, Hb concentration was significantly ( $P < 0.05$ ) lower in group D compared to A, B and C (Table 2).

There was no significant ( $P>0.05$ ) difference in total red blood cells (TRBC) in all groups of chicks before inoculation (at 1, 14 and 28 doa) (Table 3). At 7 dpi (35 doa), TRBC was significantly ( $P<0.05$ ) lower in group D than in A, B and C. The TRBC was significantly ( $P<0.05$ ) higher in groups A

( $2.34 \pm 0.02$ ;  $2.99 \pm 0.03 \times 10^{12}/L$ ), B ( $1.87 \pm 0.01$ ;  $2.25 \pm 0.02 \times 10^{12}/L$ ) and C ( $1.92 \pm 0.02$ ;  $2.35 \pm 0.02 \times 10^{12}/L$ ) than in D ( $1.27 \pm 0.01$ ;  $1.66 \pm 0.01 \times 10^{12}/L$ ) at 14 and 21 dpi (Table 3).

		Age (days)					
		1	14	28	35	42	49
Group	Treatment	Mean ( $\pm$ SE) haemoglobin concentration (g/dL)					
A	Molasses	$3.59 \pm 0.01$	$7.79 \pm 0.05$	$10.78 \pm 0.09$	$7.44 \pm 0.05^c$	$8.67 \pm 0.06^c$	$9.59 \pm 0.08^d$
B	Antox <sup>®</sup>	$3.58 \pm 0.01$	$7.99 \pm 0.05$	$10.99 \pm 0.08$	$5.89 \pm 0.03^b$	$6.58 \pm 0.04^b$	$7.68 \pm 0.06^b$
C	EN-FLORAX <sup>®</sup>	$3.68 \pm 0.02$	$8.52 \pm 0.07$	$11.61 \pm 0.10$	$6.98 \pm 0.04^b$	$7.91 \pm 0.05^b$	$8.89 \pm 0.07^c$
D	Positive control	$3.71 \pm 0.02$	$5.31 \pm 0.03$	$7.65 \pm 0.05$	$3.59 \pm 0.01^a$	$4.48 \pm 0.02^a$	$5.37 \pm 0.03^a$
E	Negative control	$3.68 \pm 0.02$	$5.33 \pm 0.03$	$7.64 \pm 0.05$	$8.77 \pm 0.07^d$	$9.86 \pm 0.08^d$	$10.99 \pm 0.10^e$

Values with different superscript letters down the same column differ significantly at  $P<0.05$ .

**Table 2:** Mean ( $\pm$  SE) haemoglobin concentration of ISA Brown chicks administered molasses, Antox<sup>®</sup> and EN-FLORAX<sup>®</sup> from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

		Age (days)					
		1	14	28	35	42	49
Group	Treatment	Mean ( $\pm$ SE) red blood cells ( $\times 10^{12}/L$ )					
A	Molasses	$0.95 \pm 0.00$	$1.79 \pm 0.01$	$2.74 \pm 0.02$	$1.95 \pm 0.01^c$	$2.34 \pm 0.02^c$	$2.99 \pm 0.03^c$
B	Antox <sup>®</sup>	$0.96 \pm 0.01$	$1.83 \pm 0.02$	$2.76 \pm 0.03$	$1.46 \pm 0.00^b$	$1.87 \pm 0.01^b$	$2.25 \pm 0.02^b$
C	EN-FLORAX <sup>®</sup>	$0.95 \pm 0.00$	$1.85 \pm 0.02$	$2.79 \pm 0.03$	$1.59 \pm 0.02^b$	$1.92 \pm 0.02^b$	$2.35 \pm 0.02^b$
D	Positive control	$0.96 \pm 0.01$	$1.46 \pm 0.01$	$2.22 \pm 0.02$	$0.92 \pm 0.00^a$	$1.27 \pm 0.01^a$	$1.66 \pm 0.01^a$
E	Negative control	$0.95 \pm 0.00$	$1.47 \pm 0.01$	$2.23 \pm 0.02$	$2.77 \pm 0.02^d$	$3.33 \pm 0.03^d$	$3.98 \pm 0.03^d$

Values with different superscript letters down the same column differ significantly at  $P<0.05$ .

**Table 3:** Mean ( $\pm$  SE) red blood cells of ISA Brown chicks administered molasses, Antox<sup>®</sup> and EN-FLORAX<sup>®</sup> from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

### Thrombocytes counts

Before inoculation (at 1, 14 and 28 doa), no significant ( $P>0.05$ ) differences existed in thrombocyte count of all groups of chicks (Table 4). At 7 and 14 dpi (35 and 42 doa)

thrombocyte counts were significantly higher in groups A, B and C than D. There was significantly higher thrombocyte count at 21 dpi (49 doa) in group A ( $9.69 \pm 0.08 \times 10^9/L$ ), B ( $8.68 \pm 0.07 \times 10^9/L$ ) and C ( $8.97 \pm 0.07 \times 10^9/L$ ) than in D ( $6.95 \pm 0.04 \times 10^9/L$ ) (Table 4).

		Age (days)					
		1	14	28	35	42	49
Group	Treatment	Mean ( $\pm$ SE) thrombocyte count ( $\times 10^9/L$ )					
A	Molasses	$4.66 \pm 0.02$	$7.62 \pm 0.04$	$9.77 \pm 0.07$	$7.84 \pm 0.05^c$	$8.87 \pm 0.07^c$	$9.69 \pm 0.08^c$
B	Antox <sup>®</sup>	$4.69 \pm 0.03$	$7.87 \pm 0.05$	$9.85 \pm 0.08$	$6.78 \pm 0.04^b$	$7.66 \pm 0.06^b$	$8.68 \pm 0.07^b$
C	EN-FLORAX <sup>®</sup>	$4.68 \pm 0.02$	$7.95 \pm 0.05$	$9.97 \pm 0.08$	$6.88 \pm 0.04^b$	$7.95 \pm 0.05^b$	$8.97 \pm 0.07^b$
D	Positive control	$4.69 \pm 0.03$	$6.64 \pm 0.04$	$8.83 \pm 0.06$	$4.96 \pm 0.02^a$	$5.87 \pm 0.03^a$	$6.95 \pm 0.04^a$
E	Negative control	$4.65 \pm 0.02$	$6.65 \pm 0.04$	$8.82 \pm 0.06$	$9.96 \pm 0.08^d$	$10.94 \pm 0.04^d$	$11.89 \pm 0.09^d$

Values with different superscript letters down the same column differ significantly at  $P<0.05$ .

**Table 4:** Mean ( $\pm$  SE) thrombocyte count of ISA Brown chicks administered molasses, Antox<sup>®</sup> and EN FLORAX<sup>®</sup> from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

### Leukocytic Parameters

Total white blood cells (TWBC) showed no significant ( $P>0.05$ ) difference in all groups of birds at 1, 14 and 28 doa (before inoculation) (Table 5). At 35 doa (7 dpi), TWBC was significantly ( $P<0.05$ ) lower in group D ( $1.95 \pm 0.01 \times 10^9/L$ ) compared to A ( $3.95 \pm 0.03 \times 10^9/L$ ), B ( $3.19 \pm 0.03 \times 10^9/L$ ) and C ( $3.33 \pm 0.03 \times 10^9/L$ ). There were significantly ( $P<0.05$ ) lower PCV in group D than in groups A, B and C at 42 and 49 doa (Table 5).

No significant ( $P>0.05$ ) difference existed for heterophil counts in all groups of chicks at 1, 14 and 28 doa (Table 6). There was significantly ( $P<0.05$ ) higher heterophil count in groups A ( $2.00 \pm 0.02 \times 10^9/L$ ), B ( $1.33 \pm 0.01 \times 10^9/L$ )

and C ( $1.52 \pm 0.01 \times 10^9/L$ ) than in D ( $0.29 \pm 0.00 \times 10^9/L$ ) at 35 doa (7dpi). At 42 and 49 doa, heterophil counts were significantly ( $P<0.05$ ) lower in group D compared to A, B and C (Table 6).

There was no significant ( $P>0.05$ ) difference in lymphocyte count in all groups of chicks before inoculation (Table 7). At 7 and 14 dpi (35 and 42 doa), lymphocyte counts were significantly ( $P<0.05$ ) lower in group D than in A, B and C. The TRBC was significantly ( $P<0.05$ ) higher in groups A ( $4.58 \pm 0.04 \times 10^9/L$ ), B ( $3.08 \pm 0.03 \times 10^9/L$ ) and C ( $3.57 \pm 0.03 \times 10^9/L$ ) than in D ( $2.38 \pm 0.02 \times 10^9/L$ ) at 21 dpi (Table 7).

		Age (days)					
		1	14	28	35	42	49
Group	Treatment	Mean ( $\pm$ SE) total white blood cells ( $\times 10^9/L$ )					
A	Molasses	$1.83 \pm 0.01$	$3.75 \pm 0.03$	$4.97 \pm 0.04$	$3.95 \pm 0.03^c$	$4.87 \pm 0.04^c$	$5.57 \pm 0.05^c$
B	Antox®	$1.84 \pm 0.01$	$3.85 \pm 0.03$	$5.35 \pm 0.05$	$3.19 \pm 0.03^b$	$3.55 \pm 0.02^b$	$3.99 \pm 0.03^b$
C	EN-FLORAX®	$1.83 \pm 0.01$	$3.95 \pm 0.03$	$5.45 \pm 0.05$	$3.33 \pm 0.03^b$	$3.89 \pm 0.03^b$	$4.36 \pm 0.04^b$
D	Positive control	$1.84 \pm 0.01$	$2.89 \pm 0.02$	$3.93 \pm 0.03$	$1.95 \pm 0.01^a$	$2.37 \pm 0.02^a$	$2.98 \pm 0.02^a$
E	Negative control	$1.83 \pm 0.01$	$2.88 \pm 0.02$	$3.94 \pm 0.03$	$4.59 \pm 0.04^d$	$5.68 \pm 0.05^d$	$6.71 \pm 0.06^d$

Values with different superscript letters down the same column differ significantly at  $P<0.05$ .

**Table 5:** Mean ( $\pm$  SE) total white blood cells of ISA Brown chicks administered molasses, Antox® and EN- FLORAX® from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

		Age (days)					
		1	14	28	35	42	49
Group	Treatment	Mean ( $\pm$ SE) heterophils count ( $\times 10^9/L$ )					
A	Molasses	$0.53 \pm 0.00$	$1.86 \pm 0.01$	$2.85 \pm 0.02$	$2.00 \pm 0.02^c$	$2.55 \pm 0.02^c$	$3.17 \pm 0.03^c$
B	Antox®	$0.52 \pm 0.00$	$1.99 \pm 0.01$	$2.97 \pm 0.02$	$1.33 \pm 0.01^b$	$1.87 \pm 0.01^b$	$2.19 \pm 0.02^b$
C	EN-FLORAX®	$0.53 \pm 0.00$	$2.26 \pm 0.02$	$3.24 \pm 0.03$	$1.52 \pm 0.01^b$	$2.33 \pm 0.02^c$	$2.89 \pm 0.02^c$
D	Positive control	$0.52 \pm 0.00$	$1.59 \pm 0.01$	$2.38 \pm 0.02$	$0.29 \pm 0.00^a$	$0.89 \pm 0.00^a$	$1.15 \pm 0.01^a$
E	Negative control	$0.53 \pm 0.00$	$1.58 \pm 0.01$	$2.39 \pm 0.02$	$2.98 \pm 0.02^d$	$3.88 \pm 0.03^d$	$4.47 \pm 0.04^d$

Values with different superscript letters down the same column differ significantly at  $P<0.05$ .

**Table 6:** Mean ( $\pm$  SE) heterophils count of ISA Brown chicks administered molasses, Antox® and EN- FLORAX® from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

		Age (days)					
		1	14	28	35	42	49
Group	Treatment	Mean ( $\pm$ SE) lymphocytes count ( $\times 10^9/L$ )					
A	Molasses	$1.42 \pm 0.01$	$3.17 \pm 0.03$	$4.85 \pm 0.04$	$3.34 \pm 0.03^c$	$3.98 \pm 0.03^c$	$4.58 \pm 0.04^d$
B	Antox®	$1.43 \pm 0.01$	$3.38 \pm 0.03$	$4.99 \pm 0.04$	$2.29 \pm 0.02^b$	$2.84 \pm 0.02^b$	$3.08 \pm 0.03^b$
C	EN-FLORAX®	$1.42 \pm 0.01$	$3.63 \pm 0.03$	$5.23 \pm 0.05$	$2.53 \pm 0.02^b$	$2.98 \pm 0.02^b$	$3.57 \pm 0.03^c$



D	Positive control	1.43 ± 0.01	2.64 ± 0.02	3.79 ± 0.03	1.33 ± 0.01 <sup>a</sup>	1.95 ± 0.01 <sup>a</sup>	2.38 ± 0.02 <sup>a</sup>
E	Negative control	1.42 ± 0.01	2.65 ± 0.02	3.80 ± 0.03	4.32 ± 0.04 <sup>d</sup>	4.84 ± 0.04 <sup>d</sup>	5.43 ± 0.05 <sup>e</sup>

Values with different superscript letters down the same column differ significantly at  $P < 0.05$ .

**Table 7:** Mean ( $\pm$  SE) lymphocytes count of ISA Brown chicks administered molasses, Antox® and EN-FLORAX® from one-day-old and inoculated with a very virulent infectious bursal disease virus at 28 days of age.

## Discussion

The erythrocytic (PCV, Hb and TRBC), leukocytic (TWBC, heterophil and lymphocyte counts) parameters and thrombocyte counts increased before inoculation (from 1 to 28 doa) in all groups of chicks in this study. These increases were due to increase in the physiological demands of the birds often associated with increase in age in terms of metabolism and immune response to pathogens. Although there were no significant differences in these parameters across all group each day, the supplemented groups (A, B and C) had higher values compared to the groups not administered supplements (D and E). This suggests that these supplements might have contributed to the enhancement production via the actions of their constituents. After inoculation, there was 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 in the vvIBDV-inoculated groups in this study might be associated with haemorrhages with subsequent iron deficiency, destruction of haemopoietic organs and/or viraemia [4,6,14]. 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 enhanced production of erythropoietin might be another possible mechanism [4,15].

The decrease in the leukocytic parameters in the inoculated groups in this study is consistent with findings of Chevillat [16], who reported severe panleukopenia during the severe inflammatory stage of IBD. The possible might be linked with destruction of myeloid cells in the bone marrow and/or the mature cells within circulation [17] by the vvIBDV. The lymphopenia might also be associated with vvIBDV multiplication in lymphocytes and subsequent necrosis of bursal lymphocytes [18]. The leukopenia 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 immunoglobulins production and consequently neutralisation of the vvIBDV. This virus neutralisation might have led to the decrease in

leukocytes destruction [19].

The mechanisms by which Molasses, Antox® and EN-FLORAX® mitigated the vvIBDV-induced haematological changes in this study might be due to the actions of their constituents individually and/or synergistically. These supplements contained vitamins and essential minerals which are critical for the formation of blood cells and play critical roles in immune responses [11,20,21]. Also, they might have served as a source of nutrients to take care of increased nutritional demand during the IBDV infection [4]. Thus, these might be the possible mechanisms for the mitigative effects by 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 cater for the increased nutritional demand.

## Conclusion and Recommendation

Pre-, pro- and synbiotic mitigated the haematological changes induced by vvIBDV infection in ISA Brown pullets in this study. Therefore, these supplements could be administered for prophylaxis to ameliorate haematological alterations due to vvIBDV infection in poultry.

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