Two Patented Probiotics Mitigated the Haematological Alterations Induced by a Very Virulent Infectious Bursal Disease Virus in Chicks

Aliyu Danlami Andamin

Ahmadu Bello University

Paul Ayuba Abdu

Ahmadu Bello University

Felix Tersua Akade

College of Agriculture

Ochuko Orakpoghenor (✉ ochuko.orakpoghenor@gmail.com)

Ahmadu Bello University <https://orcid.org/0000-0003-0833-1640>

Tagang Aluwong

Ahmadu Bello University

Research Article

Keywords: Mitigating, Antox®, Bactofort®, blood, ISA Brown, infectious bursal disease

Posted Date: March 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-254420/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Veterinary Research Communications on April 22nd, 2021. See the published version at <https://doi.org/10.1007/s11259-021-09790-7>.

Abstract

The study investigated the mitigating effects of two probiotics on blood parameters of ISA Brown chicks inoculated with a very virulent infectious bursal disease virus (vvIBDV). Two hundred chicks were assigned into four groups of 50 birds each. Groups A and B were administered Antox[®] in water and Bactofort[®] in feed daily from 1 to 42 days of age and inoculated with a vvIBDV at 28 days and C and D served as positive and negative controls, respectively. Blood samples were examined for changes in packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC), total white blood cell (TWBC), heterophil and lymphocyte counts seven days post inoculation. The PCV between groups A and C differed ($P < 0.05$) and in group B it was higher ($P < 0.05$) than that of group C. The Hb concentration between groups A, B and C differed ($P < 0.05$). There was a difference ($P < 0.05$) in RBC counts between groups A, B, C. Differences in TWBC between group A and C were significant ($P < 0.05$) and TWBC in group B was higher ($P < 0.05$) than that of group C. There was a significant difference in heterophil ($P < 0.05$) and lymphocyte ($P < 0.05$) count between group A and C, and B and C. Heterophil/lymphocyte ratio was significantly higher in positive control compared to groups A, B, C. Antox[®] and Bactofort[®] mitigated the deleterious effects of vvIBDV on blood parameters and can assist in the control of IBD.

Introduction

Probiotics are rich in microorganisms that improve host's health (Guillet 1998; Line et al. 1998), proteins, B-complex vitamins, trace minerals and 'Plus Factors' (Glade and Sist 1998). The functions of probiotics include enhancing availability of phosphorus and nutrient utilization, colonization or inhibition pathogens resulting in reduced incidence and duration of disease (Ehrmann et al. 2002; Moreno et al. 2002). Probiotics improve immunity by stimulating subsets of immune cells to produce cytokines, which in turn play a role in the induction and regulation of immune response and enhance epithelial innate immunity-related gene expression through anti-inflammatory cytokine (Fuller 1989; Christensen et al. 2002; Pagnini et al. 2008; Amit-Romach et al. 2010).

Infectious bursal disease (IBD) is an acute, highly contagious, immunosuppressive disease of young chickens (Abdu 2014). It is caused by a member of the genus *Avian birnavirus* in the family *Birnaviridae*, whose genome consists of double-stranded RNA segments, designated A and B, which are enclosed within a non-enveloped icosahedral capsid (Lasher and Shane 1994; Luket and Saif, 2003; Muller et al. 2003). Two serotypes of infectious bursal disease virus (IBDV) are recognized: I and II (Jackwood et al. 1982). Only infection with serotype I viruses in chickens results in clinical disease. Serotype I viruses have been categorized into four based on pathogenicity: Classical, Variants, Attenuated and Very virulent strains (Kibenge et al. 1988). Infectious bursal disease virus (IBDV) produces two forms of disease: The sub-clinical and clinical in susceptible chickens, depending on the age of the bird at the time of infection. Sub-clinical and clinical infections with IBDV may cause immunosuppression (Lukert and Saif 2003). The sub-clinical form is observed in chicks below 3 weeks of age; while the clinical form is reported in 3-8-week old chickens (Sharma et al. 2000; Lukert and Saif 2003). Following infection, IBDV multiplies rapidly in the B-lymphocytes of the BF, leading to immunosuppression, increased susceptibility to other diseases and reduced growth rate of surviving birds (Kibenge et al. 1988; McIlroy et al. 1989; Becht and Muller 1991). The BF is the principal organ of virus replication and peak virus titres in the BF can be detected between 3 and 5 days after IBDV infection (Lukert and Saif 1997). Uncomplicated IBDV infection with original type I isolates results in up to 90% flock morbidity and 20% mortality in susceptible 3–8 weeks-old hybrid Leghorn replacement pullets (Lasher and Shane 1994).

Haematology evaluates the health, physiological and biological status of birds (Ross et al., 1976). It checks for haemoglobin and haematocrit (evaluation of anaemia), rate of leucocytes or white blood cells (infection indicators) and heterophil/lymphocyte ratio (stress indicator) (Oladele et al. 2005). Blood components may be influenced by physiological factors, such as age and species, and pathological factors (Cheneme and Cho, 1984). There is no

information on the effects of probiotics on haematological parameters of commercial chickens inoculated with a vvIBDV.

Materials And Methods

Experimental chicks and housing

Two hundred day-old ISA Brown pullet chicks were purchased 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 twice one week apart.

Feeds and feeding

Chick mash was purchased from a commercial feed distributor in Zaria, Nigeria and proximate analyses carried out. The feed contained the following nutrients: % DM 97.20, % ASH 13.96, % EE 7.41, % CF 6.49, % N 3.60 and % CP 22.50. All the chicks were fed with for up to 42 days of age. The chicks were allowed access to feed and water *ad libitum*.

Probiotics

Liquid Antox[®] containing, *Saccharomyces cerevisiae* (4.125×10^6 cfu/mL), Citric acid-6 g, Lactic acid-2 g, Vitamins B₁-100 mg, B₂-7.5 mg, B₆-80 mg, and 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, manufactured by (Montajat Pharmaceuticals, Bioscience Division, Dammam 31491, Saudi Arabia) was used. Powdered Bactofort[®] containing, *Lactobacillus acidophilus* (77×10^9 cfu/kg), *Enterococcus faecium* (44×10^9 cfu/kg), *Saccharomyces cerevisiae* ($5,000 \times 10^9$ cells/kg), *Bacillus subtilis* (2.2×10^9 cfu/kg), manufactured by (Biofeed Technology Inc., Brossand, QC, Canada) was used.

Inoculation of chicks with infectious bursal disease virus

A characterised vvIBDV [24] was obtained from the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Each chick in the test and positive groups was inoculated with 0.05 ml of a vvIBDV suspension ($10^{-9.76}$ CID₅₀/mL) via conjunctival instillation at 28 days of age.

Experimental design

Two hundred day old ISA Brown chicks were assigned randomly into four groups, A, B, C and D with 50 chicks each. Group A was administered Anthox[®] at 1.5 mL/L in drinking water daily from day-old to 42 days of age and inoculated with a vvIBDV at 28 days of age (0 dpi). Group B chicks were administered Bactofort[®] at 12.5 g/25 kg in feed daily from day-old to 42 days of age and inoculated with a vvIBDV at 28 days of age. No probiotic was administered to chicks in group C but were inoculated with a vvIBDV at 28 days of age and. chicks in group D were not administered any probiotic and not inoculated with vvIBDV.

Collection of blood

Blood was collected through the heart from each bird at weekly interval from day-old to 42 days of age. The blood was emptied into a heparinised universal bottle. Blood samples were labeled and processed for haematology (Campbell and Ellis 2007).

Data analyses

Data collected were presented as means $\pm$ standard errors of the mean (Mean $\pm$ SEM). Two-way analysis of variance (ANOVA) was used in the analyses of the data. Bonferoni's multiple comparison *post-hoc* tests was used to analysed the level of significance at $P < 0.05$. GraphPad Prism 4.0 for windows (GraphPad Software, San Diego, California USA) was used to conduct the analyses.

Results

Packed cell volume

The PCV in groups A and B decreased from a value of 30.40 ± 0.11 %, and 28.20 ± 0.58 % at 0-day post inoculation (0 dpi) with vvIBDV to 19.30 ± 0.37 %, and 17.00 ± 0.35 % at 7 dpi, respectively but the decrease was lower compared to values for group C (25.80 ± 0.69 %, and 10.20 ± 0.37 %). There was a significant difference ($P < 0.05$) in the PCV between A, B and C at 7 dpi (Table 1).

Haemoglobin concentration

The mean Hb concentration of groups A and B decreased from mean value of 9.76 ± 0.71 g/dl, and 9.02 ± 0.19 g/dl to 6.16 ± 0.14 g/dl, and 6.00 ± 0.27 g/dl, respectively, compared to group C (4.20 ± 0.25 g/dl) at 7 dpi. There was a significant difference ($P < 0.05$) in Hb value between groups A, B and C at 7 dpi (Table 2).

Red blood cells and leucocyte counts

The RBC count of groups A and B decreased from $7.55 \pm 0.62 \times 10^{12}/L$ and $6.95 \pm 0.16 \times 10^{12}/L$ at 0 dpi to $5.40 \pm 0.29 \times 10^{12}/L$ and $4.10 \pm 0.43 \times 10^{12}/L$ at 7 dpi but the decrease was significantly ($P < 0.05$) lower compared to group C ($3.40 \pm 0.43 \times 10^{12}/L$) at 7 dpi. There was a significant difference ($P < 0.05$) in the RBC count between A, B and C at 7 dpi (Table 3). The TWBC count of groups A and B decreased from $13.88 \pm 0.24 \times 10^9/L$ and $12.58 \pm 0.32 \times 10^9/L$ at 0 dpi to $8.90 \pm 0.29 \times 10^9/L$ and $7.00 \pm 0.42 \times 10^9/L$ at 7 dpi compared to group C ($4.30 \pm 0.37 \times 10^9/L$) at 7 dpi. There was a significant difference ($P < 0.001$) in the TWBC counts between A, B and C at 7 dpi (Table 4).

Differential leucocyte counts

The heterophil counts of the groups (A and B) administered probiotics and inoculated with vvIBDV decreased from $4.09 \pm 0.02 \times 10^9/l$, and $4.05 \pm 0.02 \times 10^9/l$, at 0 dpi to $3.04 \pm 0.02 \times 10^9/l$ and $3.00 \pm 0.03 \times 10^9/l$ at 7 dpi, respectively but the decrease was significantly lower compared to positive control (group C) ($2.98 \pm 0.02 \times 10^9/l$) at 7 dpi. There was a significant difference ($P < 0.001$) in the heterophils count between groups A, B and C at 7 dpi (Table 5). Lymphocyte counts of groups A and B decreased from $7.05 \pm 0.01 \times 10^9/l$, and $7.02 \pm 0.02 \times 10^9/l$ at 0 dpi to $5.05 \pm 0.00 \times 10^9/l$ and $4.55 \pm 0.00 \times 10^9/l$ at 7 dpi, respectively but the decreases were significantly lower compared to positive control group C ($3.04 \pm 0.02 \times 10^9/l$) at 7 dpi. There was a significant difference ($P < 0.05$) in the lymphocyte count between groups A, B and C at 7 dpi (Table 6).

There were increases in heterophil/lymphocyte ratios in groups A, B, and C (0.75 ± 0.00 , 0.84 ± 0.00 and 0.98 ± 0.10) at 7 dpi with vvIBDV. However, the increase was significantly lower in the groups A and B compared to positive control group (C). There was statistical significant difference ($P < 0.05$) in the heterophil/lymphocyte ratios between group A, B and C at 7 dpi (Table 7).

Table 1: Mean ($\pm$ SE) packed cell volume (%) of ISA Brown chicks ($n = 5$) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

		Age in days							Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean ($\pm$ SE) packed cell volume (%)									% decrease (-) or increase (+)	
A	Antox [®]	20.40 $\pm$ 2.44	24.40 $\pm$ 0.44	27.20 $\pm$ 0.66	28.22 $\pm$ 0.34	30.40 $\pm$ 0.11	19.30 $\pm$ 0.37***	21.00 $\pm$ 0.58***	-36.51	+30.92
B	Bactofort [®]	21.40 $\pm$ 0.68	23.80 $\pm$ 0.97	25.00 $\pm$ 0.34	26.01 $\pm$ 0.21	28.20 $\pm$ 0.58	17.00 $\pm$ 0.35**	20.00 $\pm$ 0.00**	-39.72	+29.08
C	Positive control	20.80 $\pm$ 0.58	22.10 $\pm$ 0.40	23.60 $\pm$ 0.54	24.10 $\pm$ 0.20	25.80 $\pm$ 0.69	10.20 $\pm$ 0.37*	15.50 $\pm$ 0.50*	-60.47	+39.92
D	Negative control	21.00 $\pm$ 0.87	22.40 $\pm$ 0.75	24.60 $\pm$ 0.78	24.88 $\pm$ 0.82	25.90 $\pm$ 0.56	26.60 $\pm$ 0.93	27.40 $\pm$ 0.60	+2.70	+5.79

Key: n = number of birds sampled, SE = standard error of mean, means with asterics (*) ($P < 0.05$), (**) ($P < 0.01$), (***), ($P < 0.001$) in the same column differed significantly

Table 2: Mean ($\pm$ SE) haemoglobin concentration (g/dl) of ISA Brown chicks ($n = 5$) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

		Age in days							Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean ($\pm$ SE) haemoglobin concentration (g/dl)									% decrease (-) or increase (+)	
A	Antox [®]	7.04 $\pm$ 0.81	7.74 $\pm$ 0.81	8.80 $\pm$ 0.30	9.38 $\pm$ 0.42	9.76 $\pm$ 0.71	6.16 $\pm$ 0.14***	7.37 $\pm$ 0.52***	-36.89	+24.49
B	Bactofort [®]	7.05 $\pm$ 0.23	7.76 $\pm$ 0.23	8.62 $\pm$ 0.44	8.90 $\pm$ 0.55	9.02 $\pm$ 0.19	6.00 $\pm$ 0.27**	7.00 $\pm$ 0.00**	-33.48	+22.39
C	Positive control	7.04 $\pm$ 0.17	7.10 $\pm$ 0.29	7.16 $\pm$ 0.52	7.25 $\pm$ 0.62	7.36 $\pm$ 0.56	4.20 $\pm$ 0.25*	5.75 $\pm$ 0.25*	-42.93	+21.88
D	Negative control	7.03 $\pm$ 0.61	7.08 $\pm$ 0.55	7.16 $\pm$ 0.29	7.38 $\pm$ 0.30	7.46 $\pm$ 0.21	7.66 $\pm$ 0.31	7.88 $\pm$ 0.53	+2.62	+5.63

Key: n = number of birds sampled, SE = standard error of mean, Means with asterics (*) ($P < 0.05$), (**) ($P < 0.01$), (***), ($P < 0.001$) in the same column differed significantly

Table 3: Mean ($\pm$ SE) red blood cell count ($\times 10^{12}/l$) of ISA Brown chicks ($n = 5$) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

		Age in days							Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean ($\pm$ SE) red blood cell count ($\times 10^{12}/l$)									% decrease (-) or increase (+)	
A	Antox [®]	4.33 $\pm$ 0.48	6.80 $\pm$ 0.62	6.99 $\pm$ 0.27	7.10 $\pm$ 0.26	7.55 $\pm$ 0.62	5.40 $\pm$ 0.29***	7.00 $\pm$ 0.29***	-28.48	+7.28
B	Bactofort [®]	4.32 $\pm$ 0.86	6.50 $\pm$ 0.35	6.75 $\pm$ 0.19	6.85 $\pm$ 0.21	6.95 $\pm$ 0.16	4.10 $\pm$ 0.43**	6.00 $\pm$ 0.00**	-41.01	+13.67
C	Positive control	4.31 $\pm$ 0.54	5.40 $\pm$ 0.29	5.50 $\pm$ 0.51	5.65 $\pm$ 0.75	5.78 $\pm$ 0.63	3.40 $\pm$ 0.43*	4.25 $\pm$ 0.25*	-41.18	+26.47
D	Negative control	4.32 $\pm$ 0.29	5.30 $\pm$ 0.25	5.40 $\pm$ 0.94	5.58 $\pm$ 0.99	5.70 $\pm$ 0.60	6.30 $\pm$ 0.12	6.52 $\pm$ 0.37	+10.53	+14.39

Key: n = number of birds sampled, SE = standard error of the mean, means with asterics (*) ($P < 0.05$), (**) ($P < 0.01$), (***) ($P < 0.001$) in the same column differed significantly

Table 4: Mean ($\pm$ SE) total white blood cell count ($\times 10^9/l$) of ISA Brown chicks ($n = 5$) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

		Age in days							Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean ($\pm$ SE) total white blood cell count ($\times 10^9/l$)									% decrease (-) or increase (+)	
A	Antox [®]	8.06 $\pm$ 0.61	10.95 $\pm$ 0.94	11.96 $\pm$ 0.74	12.96 $\pm$ 0.85	13.88 $\pm$ 0.24	8.90 $\pm$ 0.29***	10.00 $\pm$ 0.29***	-35.88	+27.95
B	Bactofort [®]	8.07 $\pm$ 0.59	9.75 $\pm$ 0.74	10.85 $\pm$ 0.35	11.62 $\pm$ 0.22	12.58 $\pm$ 0.32	7.00 $\pm$ 0.42**	9.00 $\pm$ 0.00**	-44.35	+28.46
C	Positive control	8.06 $\pm$ 0.94	8.23 $\pm$ 0.69	8.44 $\pm$ 0.73	8.98 $\pm$ 0.25	9.20 $\pm$ 0.54	4.30 $\pm$ 0.37*	6.00 $\pm$ 0.50*	-53.26	+34.78
D	Negative control	8.06 $\pm$ 0.38	8.22 $\pm$ 0.58	8.42 $\pm$ 0.54	8.99 $\pm$ 0.64	9.18 $\pm$ 0.44	9.48 $\pm$ 0.25	9.80 $\pm$ 0.07	+3.27	+6.75

Key: n = number of birds sampled, SE = standard error of the mean, means with asterics (*) ($P < 0.05$), (**) ($P < 0.01$), (***) ($P < 0.001$) in the same column differed significantly

Table 5: Mean ($\pm$ SE) heterophil count ($\times 10^9/l$) of ISA Brown chicks ($n = 5$) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

Age in days									Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean (± SE) heterophil count (x 10 ⁹ /l)									% decrease (-) or increase (+)	
A	Antox®	2.05 ± 0.01	2.95 ± 0.02	3.33 ± 0.00	3.75 ± 0.02	4.09 ± 0.02	3.04 ± 0.02***	3.45 ± 0.01***	-25.67	+15.65
B	Bactofort®	2.06 ± 0.02	3.88 ± 0.01	3.25 ± 0.02	3.70 ± 0.00	4.05 ± 0.02	3.00 ± 0.03**	3.20 ± 0.01**	-25.93	+20.99
C	Positive control	2.05 ± 0.02	2.51 ± 0.03	2.88 ± 0.01	3.26 ± 0.03	3.56 ± 0.02	2.98 ± 0.02*	3.05 ± 0.03*	-16.29	+14.33
D	Negative control	2.06 ± 0.01	2.52 ± 0.01	2.81 ± 0.02	3.25 ± 0.03	3.55 ± 0.01	3.82 ± 0.00	4.14 ± 0.02	+7.61	+16.62

Key: n = number of birds sampled, SE = standard error of the mean, means with asterics (*) (P < 0.05), (**) (P < 0.01), (***) (P < 0.001) in the same column differed significantly

Table 6: Mean ($\pm$ SE) lymphocyte count ($\times 10^9$ /l) of ISA Brown chicks (n = 5) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

		Age in days							Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean (± SE) lymphocyte count (x 10 ⁹ /l)									% decrease (-) or increase (+)	
A	Antox®	3.77 ± 0.00	4.99 ± 0.01	5.77 ± 0.00	6.65 ± 0.01	7.05 ± 0.01	5.05 ± 0.00***	4.85 ± 0.03***	-42.55	+31.21
B	Bactofort®	3.78 ± 0.03	4.85 ± 0.04	5.65 ± 0.01	6.59 ± 0.02	7.02 ± 0.02	4.55 ± 0.00**	3.99 ± 0.00**	-49.43	+43.16
C	Positive control	3.77 ± 0.01	3.99 ± 0.01	4.57 ± 0.03	4.86 ± 0.03	5.73 ± 0.01	3.04 ± 0.02*	3.25 ± 0.00*	-46.95	+43.28
D	Negative control	3.78 ± 0.00	3.98 ± 0.04	4.61 ± 0.01	4.95 ± 0.01	5.82 ± 0.01	6.54 ± 0.02	7.32 ± 0.01	+12.37	+25.77

Key: n = number of birds sampled, SE = standard error of the mean, means with asterics (*) (P < 0.05), (**) (P < 0.01), (***) (P < 0.001) in the same column differed significantly

Table 7: Mean ($\pm$ SE) heterophil/lymphocyte ratio of ISA Brown chicks (n = 5) administered Antox[®] and Bactofort[®] from day old and inoculated with a very virulent infectious bursal disease virus at 28 days of age

		Age in days							Days post inoculation	
Group	Treatment	1	7	14	21	28	35	42	7	14
Mean ($\pm$ SE) heterophil/lymphocyte ratio									% increase (-) or decrease (+)	
A	Antox [®]	0.53 $\pm$ 0.00	0.59 $\pm$ 0.20	0.57 $\pm$ 0.00	0.56 $\pm$ 0.20	0.58 $\pm$ 0.20	0.75 $\pm$ 0.00*	0.71 $\pm$ 0.33*	+29.31	-22.41
B	Bactofort [®]	0.54 $\pm$ 0.10	0.54 $\pm$ 0.25	0.58 $\pm$ 0.20	0.56 $\pm$ 0.00	0.58 $\pm$ 0.10	0.84 $\pm$ 0.00**	0.80 $\pm$ 0.00**	+44.83	-37.93
C	Positive control	0.53 $\pm$ 0.10	0.63 $\pm$ 0.30	0.63 $\pm$ 0.33	0.67 $\pm$ 0.10	0.62 $\pm$ 0.20	0.98 $\pm$ 0.10***	0.94 $\pm$ 0.00***	+58.06	-51.61
D	Negative control	0.54 $\pm$ 0.00	0.65 $\pm$ 0.25	0.63 $\pm$ 0.20	0.65 $\pm$ 0.30	0.61 $\pm$ 0.10	0.58 $\pm$ 0.00	0.57 $\pm$ 0.20	-4.92	-6.56

Key: n = number of birds sampled, SE = standard error of the mean, means with asterics (*) (P < 0.05), (**) (P < 0.01), (***), (P < 0.001) in the same column differed significantly

Discussion

The decrease in PCV, Hb, and RBC count could be as a result of non-regenerative anaemia which the vvIBDV caused by destruction of haemopoietic organ and viraemia in inoculated chicks (Campbell 1994; Mitchell and Johms 2008). But the decrease in these haematological parameters in chicks administered Antox[®] and Bactofort[®] was less severe, compared to that of positive control. Antox[®] and Bactofort[®] could have either enhanced production of erythropoietin, prevented the destruction of precursor cells in the bone marrow or the haemorrhages usually seen in IBD (Lukert and Saif, 2003; Kabir et al. 2004).

The decrease in TWBC, heterophil and lymphocyte counts which were lower in chicks administered Antox[®] and Bactofort[®] compared to positive control, is an indication that the probiotics possibly elicited the production of significant amount of immunoglobulins that neutralised the vvIBDV and thereby reduced the destruction of leucocytes (Midilli et al. 2008). The leucopaenia observed in positive control due to decrease in heterophils and lymphocytes counts at 7 dpi was the consequence of corresponding heteropaenia and lymphopaenia. This result is in agreement with the findings of Cheville (1967), who also reported severe panleukopaenia during the severe inflammatory stage of IBD. The lymphopaenia observed in positive control at 7 dpi was probably due to multiplication of vvIBDV in lymphocytes and subsequent necrosis of bursal lymphocytes as observed by Ley et al. (2007).

Heterophil/lymphocyte ratios provide important information of immune system tension following prolonged stress as well as infection status for some immunosuppressive diseases (Moreno et al. 2002; El Lethey et al. 2003; Clinchy et al. 2004). Heterophil/lymphocyte ratio was significantly higher in positive control when compared to that of the groups administered Antox[®] and Bactofort[®]. Scope et al. (2002) observed a considerable increase in H/L ratio following stress associated with transporting, handling and viral diseases of birds. Acute stress is known to increase H/L ratio (El Lethey et al. 2003). Therefore, the higher H/L ratio observed in this study could be as a result of the inoculation of the chicks with the vvIBDV.

Based on all the haematological parameters studied, Antox[®] mitigated the deleterious effects of vvIBDV better compared to Bactofort[®] in spite of the fact that it contained only one micro-organism (*Saccharomyces cerevisiae*). However, Antox[®] also contained vitamin B-complex, biotin, cobalt chloride, zinc, copper and iron chloride which are essential elements for the formation of red blood cells (Pimental 1992; Hochleithner 1994). Antox[®] also cannot just be considered as a probiotic only but rather serve as a source of nutritional supplement to take care of any deficiency in the commercial feed and the increase in demand for nutrients during IBDV infection. The efficacy of Bactofort[®] administered through feed may have also been adversely affected by the possible presence of antimicrobial agents in the commercial feed used in the present study.

From this study it was concluded that: Antox[®] and Bactofort[®] mitigated the deleterious effects of the vvIBDV inoculated on PCV, Hb, RBC, TWBC, lymphocyte and heterophil counts. Antox[®] and Bactofort[®] can therefore be used by farmers and veterinarians for the control of IBD.

Declarations

Funding: No funding was received by any of the authors for this research.

Conflict of interest: The authors declare no potential conflict of interest.

Availability of data and material: Not applicable

Code availability: Not applicable

Ethical approval: 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/2017/013.

Author Contributions

Conceptualization, supervision, review and editing - PAA and TA; methodology, investigation, data analyses, original draft preparation - ADA, FTA and OO. All authors have read and agreed to the published version of the manuscript.

Consent to participate: Not applicable

Consent for publication: Not applicable

Acknowledgements

Sampling of chicks and haematology would not have been possible without the kind assistance of Mr. Edima Abaja, Mr. David Leo of the Department of Veterinary Medicine and staff of the Clinical Pathology Laboratory of the Ahmadu Bello University Zaria, respectively.

References

1. Abdu PA (2014) Viral diseases. In *Manual of Important Poultry Diseases in Nigeria* 3rd edn (pp. 2-45). Jos, Nigeria: 6 and 4 Ventures.
2. Adamu J, Owoade AA, Abdu PA, Kazeem HM, Fatihu MY (2015) Characterization of field and vaccine infectious bursal disease viruses from Nigeria revealing possible virulence and regional marker in the VP2 minor hydrophilic peaks. *Avian Pathol* Doi:10.1080/030797.2013.822055.

3. Amit-Romach E, Uni Z, Reifen R (2010) Multi-step mechanism of probiotics bacterium, the effect on innate immune system. *Mol Food Nutr Res* 54:277-284.
4. Becht H, Muller H (1991) Infectious bursal disease B-cell dependent immunodeficiency syndrome in chickens. *Behring Inst Mitt* 89:217-225.
5. Campbell TW (1994) Haematology. In B. W. Ritchie, G.J. Harrison & L. R., Harrison (Eds.). *Avian Medicine: Principles and Application* (176-198). Lake Worth: Wingers Publishing.
6. Campbell TW, Ellis CK (2007) Haematology of birds. In T.W Campbell & C.K. Ellis (Eds.). *Avian and Exotic Animal Haematology and Cytology* 3rd edn (pp. 3-50). Ames: Blackwell Publishing Professional.
7. Cheville NF (1967) Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of the chicken. *Am J Pathol* 51:527-551.
8. Chineme CN, Cho Y (1984) Clinico-pathological and morphological changes in chickens experimentally infected with infectious bursal (Gumboro) disease virus. *Trop Vet* 2:218-224.
9. Christensen HR, Frokiaer H, Pestka JJ (2002) *Lactobacilli* differentially modulate expression of cytokines and maturation surface in murine dendritic cells. *J Immunol* 168:171-178.
10. 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 Sciences* (pp. 2473-2479).
11. Ehrmann MA, Kurzak P, Bauer J, Vogel RF (2002) Characterisation of *Lactobacilli* towards their use as probiotic adjuncts in poultry. *J Appl Microbiol* 92:966-975.
12. El Lethey H, Huber-Eicher B, Jungi TW (2003) Exploration of stress-induced immune-suppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. *Vet Immunol Immunopathol* 95:91-101.
13. Fuller R (1989) Probiotics in man and animals. *J Appl Bacteriol* 66:365-378.
14. Glade MJ, Sist MO (1998) Dietary yeast culture supplementation enhances urea recycling in equine large intestine. *Nutr Reprod Int* 37:11-17.
15. Guillot JF (1998) Les probiotiques en alimentation animale. *Cahiers Agricultures* 7:49-54.
16. Hochleithner M (1994) Avian medicine: principles and application. In B.W. Ritchie, G.J. Harrison and L.R. Harrison (Eds.). *Biochemistries* (pp. 223-245). Wingers Publishing Inc., Lake Worth, FL.
17. Jackwood DJ, Saif YM, Hughes JH (1982) Characteristics and serologic studies of two serotypes of infectious bursal disease virus in turkeys. *Avian Dis* 26:82-871.
18. Kabir SML, Rahman MB, Rahman MM, Ahmed SU (2004) The dynamics of probiotics on growth performance and immune response in broilers. *Int J Poult Sci* 3:361-364.
19. Kibenge FS, Dhillon AS, Rusell RG (1988) Growth of serotypes I and II and variant strains of infectious bursal disease virus in vero cells. *Avian Dis* 32:298-303.
20. Lasher HN, Shane, SM (1994) Infectious bursal disease. *World's Poult Sci J* 50:133-166.
21. Ley K, Laudanna C, Cybulsky MI, Nourshargh S (2007) Getting to the site of information: the leukocyte adhesion cascade updated. *Nature Rev Immunol* 7:678-689.
22. Line EJ, Bailey SJ, Cox NA, Stern NJ, Tompkins T (1998) Effect of yeast –supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. *Poult Sci* 77:405-410.
23. Lukert PD, Saif YM (1997) Infectious bursal disease. In B.W. Calnek, H.J. Barnes, C.W., Beard, L.R. McDougald & Y.M. Saif (Eds.). *Diseases of Poultry*, 10th edn (pp. 721-738). Ames: Iowa State University Press.
24. Lukert PD, Saif YM (2003) Infectious bursal disease. In Y.M. Saif, H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald, & D.E. Swayne (Eds.). *Diseases of Poultry* 11th edn (pp.161-179). Ames: Iowa State Press.

25. McIlroy SG, Goodall EA, McCracken RM (1989) Economic effects of sub-clinical infectious bursal disease on broiler production. *Avian Pathol* 18:465-480.
26. Midilli M, Alp M, Kocabagli N, Muglali OH, Turan N, Yilmaz H, Cakir S (2008) Effects of dietary probiotic and probiotic supplementation on growth performance and serum IgG concentration of broilers. *South Afr J Animal Sci* 38:21-27.
27. Mitchell EB, Johns J (2008) Avian haematology and related disorders. *Vet Clinics Ex Animals Pract* 11:501-522.
28. Moreno J, Merino S, Martinez J, Sanz JJ, Arriero E (2002) Heterophil/lymphocyte ratios and heat-shock protein levels are related to growth in nestling birds. *Ecoscience* 9:434-439.
29. Oladele OA, Adene DF, Obi TU, Nottidge HO, Aiyedun AI (2005) Sequential haematological study of experimental infectious bursal disease virus infection in chickens, turkeys and ducks. *Revue D'élevage et de Medecine Veterinaire Des Pays Tropicaux* 58:211-215.
30. Pagnini C, Saeed R, Bamias G, Arseneau KO, Pizarro TT, Cominelli F, Amit-Romach E, Uni Z, Reifen R (2010) Probiotics promote gut health through stimulation of epithelial innate immunity. *Proceedings of the National Academy of Sciences* (pp. 454-459),
31. Pimental JM, Greger JL, Cook MF, Stahl JL (1992) Iron metabolism in chicks fed various levels of zinc and copper. *J Nutr Biochem* 13:140-145.
32. Ross JG, Christie G, Halliday WG, Jones RM (1976) Determination of haematology and blood biochemistry values in healthy six weeks old broiler hybrids. *Avian Pathol* 5:273-281.
33. Scope A, Filip T, Gabler C, Resch F (2002) The influence of stress from transport and handling on haematologic and clinical chemistry blood parameters of racing pigeons (*Columba livia domestica*). *Avian Dis* 46:224-229.
34. Sharma JM, Ki LJ, Rautenschlein S, Yeh HY (2000) Infectious bursal disease virus of chicken: Pathogenesis and immunosuppression. *Dev Comp Immunol* 24:223-235.