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Antox® and Bactofort® Improved Alterations in Oxidative Stress Biomarkers Induced by a Very Virulent Infectious Bursal Disease Virus in ISA Brown Chicks

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Abstract

This study investigated the ameliorative effects of Antox® and Bactofort® on some oxidative stress biomarkers in ISA Brown chicks inoculated with a vvIBDV. Two hundred one-day-old ISA Brown chicks were assigned into four groups of 50 birds each. Groups A and B were supplemented with Antox®, and Bactofort® respectively from 1 to 42 days of age, and inoculated with vvIBDV at 28 days of age, while C and D served as positive and negative controls. Sera collected from chicks at 1, 14, 28, 35, and 42 days of age were analyzed for activities of superoxide dismutase (SOD), catalase (CAT), glutathione perioxidase (GPx), malondialdehyde (MDA) concentration, and erythrocyte osmotic fragility (EOF). Results revealed significantly ($p < 0.05$) increased SOD activity, decreased CAT and GPx activities, decreased MDA concentration and EOF in groups administered Antox®, and Bactofort® compared to positive control at 7 (day 35) and 14 (day 42) days post-inoculation. In conclusion**,** Antox® and Bactofort® ameliorated the deleterious effects of vvIBDV on SOD, CAT and GPx activities, MDA concentration, and EOF. Antox® and Bactofort® could be used to ameliorate vvIBDV-induced oxidative changes and further studies should be carried out to determine their possible adverse effects when administered for preventive purposes.

Keywords: Antox®, Bactofort®, Infectious bursal disease, Oxidative stress biomarkers

Introduction

Infectious bursal disease (IBD) is caused by *Avian birnavirus*, a double-stranded RNA virus with a non-enveloped icosahedral capsid (Lukert & Saif, 2003; Orakpoghenor et al., 2020). The disease affects young chickens and manifests as an acute immunosuppressive infection (Abdu, 2014; Orakpoghenor et al., 2021). Following infection, there is the multiplication of IBD virus (IBDV) in the B-lymphocytes of the bursa of Fabricius (BF), leading to immunosuppression, increased susceptibility to other diseases, and decreased growth of birds that survive (Arafat et al., 2017; Eladl et al., 2020). Two serotypes, I and II, of IBD virus (IBDV) are recognized but clinical signs are reported following infection with only serotype I in chickens (Mosad et al., 2020; Sikandar et al., 2022). Based on virulence, the 4 pathogenic groups of serotype I IBD viruses identified are classical strains, variants, attenuated strains, and very virulent strains (Sikandar et al., 2022).

Probiotics comprise non-pathogenic and non-toxic microorganisms with the capacity to improve the health of the host following administration (Line et al., 1998; Sikander, 2021). In commercial supplement form, they contain proteins, vitamins, trace minerals, and several unique 'Plus Factors' (Glade & Sist, 1998). They play a significant role in nutrition by enhancing the availability of phosphorus, nutrient utilization, pathogen inhibition, and decreasing in occurrence and course of diseases (Ehrmann et al., 2000; Pagnini et al., 2010). In addition, there is the indirect enhancement of immune response by probiotics via anti-inflammatory cytokine-mediated enhanced immune gene expression (Revolledo et al., 2006; Amit-Romach et al., 2010).

Oxidative stress constitutes an imbalance between free radicals and antioxidants in the body which results in lipid peroxidation of cell membranes and organelles (Ji, 1999; Bartsch & Nair, 2006; Kalpacioglu & Senel, 2008). The lipid peroxidation results in the generation of end-products such as malondialdehyde (MDA) as well as cause erythrocyte osmotic fragility (EOF) which are indirect indices of oxidative stress (Montuschi et al., 2004; Adenkola et al., 2010). During viral infection, there are a decrease in superoxide dismutase (SOD), and an increase in catalase (CAT) and glutathione peroxidase (GPx) activities (Rehman et al., 2018). There are no documented reports on the ameliorative effects of Antox® and Bactofort® on oxidative stress biomarkers in commercial chickens inoculated with a vvIBDV. Hence in this study, the ameliorative effects of Antox® and Bactofort® on changes in oxidative stress biomarkers in chickens inoculated with a vvIBDV were investigated.

Materials and Methods

Ethics approval

The approval for use of chickens in this study was granted by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2017/013.

Experimental chickens

Two hundred one-day-old ISA Brown pullet chicks were acquired from a commercial hatchery, all chicks were fed with chick mash and allowed access to water *ad libitum*.

Supplements

Antox®, a liquid from 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), Vitamins B_1 (100 mg), B_2 (7.5 mg), B_6 (80 mg), and B_{12} (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 sulfate (32 mg), Cobalt chloride (3.08 mg).

Powdered Bactofort® (Biofeed Technology Inc., Brossard, QC, Canada) containing, *Lactobacillus acidophilus* (77×10^9 cfu/kg), *Enterococcus faecium* (44×10^9 cfu/kg), *Saccharomyces cerevisiae* ($5{,}000 \times 10^9$ cells/kg), and *Bacillus subtilis* (2.2×10^9 cfu/kg).

Experimental design

The chicks were randomly divided into 4 groups (A-D) of 50 chicks each. Group A was administered Anthox® at the dose of 1.5 mL/L in drinking water from 1 day old to 42 days. Group B were administered Bactofort® at the dose of 12.5 g/25 kg in the feed from 1 day old (day 1) to 42 days of age. Groups A, B, and C were inoculated with a vvIBDV at 28 days of age at the rate of 0.05 mL/bird intra-occular. Group D chicks were neither supplemented nor inoculated. On days 1, 14, 28, 35, and 42 of age, blood was collected from birds and divided into 2 parts. One part was emptied into plain bottles and serum was harvested; the other part was dispensed into bottles containing anticoagulants and analyzed for erythrocyte osmotic fragility (EOF).

Serum biochemical analyses

The sera were analyzed for the activity of superoxide dismutase (SOD) using the Northwest Life Science Specialties Vancouver, Canada, SOD kit (NWLSS™ NWK-SOD02). Catalase (CAT) activity was determined using catalase kits (Abcam Plc, Cambridge, United Kingdom) according to the manufacturer's instructions. The activity of glutathione peroxidase (GPx) was determined using a standard procedure for microtitre plate assay (Flohê and Gunzler, 1984). Malondialdehyde (MDA) concentration was determined using thiobarbituric acid (TBA) reactive substance using the double heating method of Draper and Hadley (1990) as modified by Yavuz et al. (2004). The EOF test was performed as described by Oyewale (1992).

Data analyses

Data were expressed as mean ± standard error of the mean (Mean ± SEM) and subjected to one-way analysis of variance (ANOVA) with Tukey's *post-hoc* test. GraphPad Prism 8.0 for windows (GraphPad Software, San Diego, California USA) was used for the analyses. Values of $P < 0.05$ were considered significant. Also, % changes in mean values were calculated.

Results

The clinical signs of ruffled feathers, inappetence, huddling, watery diarrhea, prostration, somnolence, and mortality were severe in group C but mild in groups administered Antox® and Bactofort®.

The SOD activity increased as birds aged (from days 1 to 28) in all groups with higher values recorded in supplemented groups than in controls. At 7 days post-inoculation (day 35), SOD activity significantly ($P < 0.05$) decreased in all infected groups but was higher in groups administered Antox® (22.16 ± 0.17 U/mL) and Bactofort® (19.13 ± 0.15 U/mL) than in positive control (10.07 ± 0.11 U/mL). This was followed by a significant increase at 14 days post-inoculation (day 42) with higher values in groups administered Antox® (27.20 ± 0.22 U/mL) and Bactofort® (23.18 ± 0.20 U/mL) than in positive control (15.10 ± 0.16 U/mL) (Table 1). The % increase at day 42 was lower in groups administered Antox® (22.7%) and Bactofort® (21.2%) than in positive control (50.0%) (Table 1)

The activity of catalase decreased as birds aged (from days 1 to 28) in all groups but the values were lower in supplemented groups than in controls. After inoculation with vvIBDV, catalase activity significantly ($P < 0.05$) increased in all infected groups at day 35 followed by a decrease at day 42, with the highest recorded in the positive control (45.22 ± 0.21; 40.18 ± 0.19 U/mL) compared to those administered Antox® (30.10 ± 0.11; 25.09 ± 0.08 U/mL) and Bactofort® (35.12 ± 0.16; 30.10 ± 0.11 U/mL). The % decrease at day 42 was higher in groups administered Antox® (16.7%) and Bactofort® (14.3%) than in positive control (11.1%) (Table 2).

Glutathione peroxidase activity decreased as birds aged (from days 1 to 28) in all groups with the lower values recorded in supplemented groups than in controls. Post-inoculation with vvIBDV, there was significantly ($P < 0.05$) increased GPx activity in all infected groups at day 35 followed by a decrease at day 42, with positive control (45.22 ± 0.21; 40.18 ± 0.19 U/mL) recording the highest compared to those administered Antox® (30.11 ± 0.12; 25.09 ± 0.10 U/mL) and Bactofort® (35.14 ± 0.16; 30.11 ± 0.12 U/mL). The % decrease at day 42 was higher in groups administered Antox® (16.7%) and Bactofort® (14.3%) than in positive control (11.1%) (Table 3).

Malondialdehyde concentration decreased as birds aged (from days 1 to 28) in all groups with the lower values recorded in supplemented groups than in controls. Post-inoculation with vvIBDV, MDA concentration was significantly ($P < 0.05$) increased in all infected groups at day 35 followed by a decrease at day 42, with the highest in the positive control (55.33 ± 0.24; 45.22 ± 0.21 nmol/mL) compared to those administered Antox® (40.25 ± 0.20; 35.18 ± 0.19 nmol/mL) and Bactofort® (45.22 ± 0.21; 40.19 ± 0.20 nmol/mL). The % decrease at day 42 was lower in groups administered Antox® (12.6%) and Bactofort® (11.1%) than in positive control (18.3%) (Table 4). The EOF significantly ($P < 0.05$) increased in all infected groups at day 35 and decreased at day 42. The % decrease at day 42 was higher in groups administered Antox® (28.0%) and Bactofort® (18.3%) compared to the positive control (16.4%) (Table 5).

Table 1: Mean (± SE) superoxide dismutase (U/mL) activity of ISA Brown chicks administered Antox® and Bactofort® from day old and inoculated with a very virulent infectious bursal disease virus at day 28

		Age (days)					Days post-inoculation	
		1	14	28	35	42	7	14
Group	Treatment	Mean (± SE) superoxide dismutase (U/mL) activity					% change in activity	
A	Antox®	12.47 ± 0.05	30.40 ± 0.25	45.31 ± 0.35	22.16 ± 0.17[a]	27.20 ± 0.22[a]	-51.1	+22.7
B	Bactofort®	12.46 ± 0.04	26.39 ± 0.20	40.25 ± 0.30	19.13 ± 0.15[a]	23.18 ± 0.20[b]	-52.3	+21.2
C	Positive control	12.48 ± 0.05	22.38 ± 0.15	32.28 ± 0.25	10.07 ± 0.11[b]	15.10 ± 0.16[d]	-68.8	+50.0
D	Negative control	12.49 ± 0.06	21.39 ± 0.16	31.29 ± 0.26	36.21 ± 0.31[c]	41.16 ± 0.36[c]	+15.7	+13.7

Key: - = decrease; + = increase; values with different superscript letters down the same column differ significantly at $P < 0.05$;

% change refers to the change from the previous measurement, i.e., from days 28 to 35 and from days 35 to 42; 7 and 14 days post-inoculation refer to days 35 and 42, respectively.

Table 2: Mean (± SE) catalase (U/mL) activity of ISA Brown chicks administered Antox® and Bactofort® from day old and inoculated with a very virulent infectious bursal disease virus at day 28

		Age (days)					Days post inoculation	
		1	14	28	35	42	7	14
Group	Treatment	Mean (± SE) catalase (U/mL) activity					% change in activity	
A	Antox®	49.23 ± 0.15	25.10 ± 0.10	15.05 ± 0.06	30.10 ± 0.11[a]	25.09 ± 0.08[a]	+100	-16.7
B	Bactofort®	50.24 ± 0.16	29.11 ± 0.11	19.07 ± 0.08	35.12 ± 0.16[b]	30.10 ± 0.11[b]	+84.2	-14.3
C	Positive control	49.23 ± 0.15	39.17 ± 0.18	29.10 ± 0.11	45.22 ± 0.21[c]	40.18 ± 0.19[c]	+55.4	-11.1
D	Negative control	50.24 ± 0.16	40.18 ± 0.19	30.11 ± 0.12	25.09 ± 0.10[d]	20.05 ± 0.07[d]	-16.7	-20.1

Key: - = decrease; + = increase; values with different superscript letters down the same column differ significantly at $P < 0.05$

% change refers to the change from the previous measurement, i.e., from days 28 to 35 and from days 35 to 42; 7 and 14 days post-inoculation refer to days 35 and 42, respectively.

Table 3: Mean (± SE) glutathione peroxidase (U/mL) activity of ISA Brown chicks (n = 5) Antox® and Bactofort® from day old and inoculated with a very virulent infectious bursal disease virus at day 28

		Age (days)					Days post-inoculation	
		1	14	28	35	42	7	14
Group	Treatment	Mean (± SE) glutathione peroxidase (U/mL) activity					% change in activity	
A	Antox®	49.23 ± 0.15	30.11 ± 0.12	20.06 ± 0.07	30.11 ± 0.12[a]	25.09 ± 0.10[a]	+50.1	-16.7
B	Bactofort®	48.22 ± 0.14	35.14 ± 0.16	25.09 ± 0.10	35.14 ± 0.16[b]	30.11 ± 0.12[b]	+40.1	-14.3
C	Positive control	49.23 ± 0.15	40.18 ± 0.19	30.11 ± 0.12	45.22 ± 0.21[c]	40.18 ± 0.19[c]	+50.2	-11.1
D	Negative control	48.22 ± 0.14	39.17 ± 0.18	29.10 ± 0.11	24.08 ± 0.09[d]	19.07 ± 0.08[d]	-17.3	-20.8

Key: - = decrease; + = increase; values with different superscript letters down the same column differ significantly at $P < 0.05$

% change refers to the change from the previous measurement, i.e., from days 28 to 35 and from days 35 to 42; 7 and 14 days post-inoculation refer to days 35 and 42, respectively.

Table 4: Mean (± SE) malondialdehyde (nmols/mL) concentration of ISA Brown chicks administered Antox® and Bactofort® from day old and inoculated with a very virulent infectious bursal disease virus at day 28

		Age (days)					Days post-inoculation	
		1	14	28	35	42	7	14
Group	Treatment	Mean (± SE) malondialdehyde (nmols/mL) concentration					% change in concentration	
A	Antox®	82.45 ± 0.34	41.25 ± 0.20	21.13 ± 0.15	40.25 ± 0.20[a]	35.18 ± 0.19[a]	+90.5	-12.6
B	Bactofort®	83.46 ± 0.35	45.26 ± 0.21	25.15 ± 0.16	45.22 ± 0.21[b]	40.19 ± 0.20[b]	+79.8	-11.1
C	Positive control	82.45 ± 0.34	62.30 ± 0.25	42.20 ± 0.19	55.33 ± 0.24[c]	45.22 ± 0.21[c]	+31.1	-18.3
D	Negative control	83.46 ± 0.35	63.31 ± 0.26	43.21 ± 0.20	33.20 ± 0.15[d]	28.15 ± 0.11[d]	-23.2	-15.2

Key: - = decrease; + = increase; values with different superscript letters down the same column differ significantly at $P < 0.05$

% change refers to the change from the previous measurement, i.e., from days 28 to 35 and from days 35 to 42; 7 and 14 days post-inoculation refer to days 35 and 42, respectively.

Table 5: Mean (± SE) erythrocyte osmotic fragility (%) of ISA Brown chicks administered Antox® and Bactofort® from day old and inoculated with a very virulent infectious bursal disease virus at day 28

		Age (days)					Days post-inoculation	
		1	14	28	35	42	7	14
Group	Treatment	Mean (± SE) erythrocyte osmotic fragility (%) concentration					% change in EOF	
A	Antox®	85.29 ± 0.24	43.28 ± 0.22	23.14 ± 0.17	43.27 ± 0.23[a]	31.15 ± 0.17[a]	+87.0	-28.0
B	Bactofort®	84.28 ± 0.25	48.26 ± 0.25	28.16 ± 0.19	55.32 ± 0.25[b]	45.22 ± 0.21[b]	+96.4	-18.3
C	Positive control	86.31 ± 0.26	63.25 ± 0.29	45.26 ± 0.21	73.29 ± 0.32[c]	61.25 ± 0.29[c]	+61.9	-16.4
D	Negative control	85.30 ± 0.25	62.23 ± 0.28	47.27 ± 0.22	35.18 ± 0.19[d]	25.15 ± 0.17[d]	-25.6	-30.3

Key: - = decrease; + = increase; values with different superscript letters down the same column differ significantly at $P < 0.05$

% change refers to the change from the previous measurement, i.e., from days 28 to 35 and from days 35 to 42; 7 and 14 days post-inoculation refer to days 35 and 42, respectively.

Discussion

This study investigated the ameliorative effects of Antox® and Bactofort® on changes in oxidative stress biomarkers in chickens inoculated with a vvIBDV. In keeping with prior studies of oxidant injury in birds (Bi et al., 2018; Rehman et al., 2018), a protective effect of the supplements reflected by differences in biomarkers between the vvIBDV-infected birds with and without supplements was anticipated. Superoxide dismutase catalyzes the breakdown of superoxide into hydrogen peroxide and water (Landis & Tower, 2005). From the present study, the % decreases in SOD activity observed in groups A, B, and C following vvIBDV infection (day 35) might be due to oxidative stress induced by the viral infection. This is consistent with the studies of Zhang et al. (2011) and Ertekin et al. (2016) who reported a decrease in SOD activity during IBDV infections possibly resulting from depletion of SOD in the face of increased production of reactive oxygen species (ROS). At day 42, Antox® and Bactofort® improved the activity of SOD evident by a lower % increase in SOD activity compared to positive control. Probiotics administration was reported to restore the activity of SOD in broiler chickens reared under heat stress (Bai et al., 2017). In oxidative stress, SOD plays a critical role in controlling ROS damage via direct reaction and/or indirectly by regulating ROS signaling (Wang et al., 2018). Thus, the improved SOD activity by Antox® observed in this study could be linked to the presence of zinc, iron, copper, and manganese as these elements were known to be key components of SOD (Richardson et al., 1975; Tainer et al., 1983; Borgstahl et al., 1992). The production of SOD by the probiotics (Steinman, 1988) could be the possible mechanism by which Bactofort® improved SOD activity in vvIBDV-infected chickens in this study.

Catalase is documented to perform antioxidant function via catalyzing of H_2O_2 into water and oxygen in an energy-efficient manner thus preventing the generation of hydroxyl radicals by the Fenton reaction (Le Blanc et al., 2011; Nandi et al., 2019). The increase in CAT activity in vvIBDV-infected groups (A, B, and C) at 7 days post-inoculation in this study might have resulted from increased H_2O_2 production (oxidative stress) induced by the viral infection, as CAT activity was reported to increase during IBDV infections (Balami et al., 2021). The higher % decrease in CAT activity in groups supplemented with Antox® and Bactofort® compared to positive control might be linked to the enhanced antioxidant effects induced by the probiotics. These enhanced antioxidant effects might be due to direct actions on H_2O_2 produced and/or indirect regulation of H_2O_2 production (Wang et al., 2017).

Glutathione peroxidase catalyzes the reaction between the reduced form of glutathione (GSH) and H_2O_2 or lipid peroxides while playing a role in the detoxification of these molecules by creating a glutathione bridge with another glutathione molecule (GSSG) (Dalvi et al., 2012). Increased H_2O_2 production (oxidative stress) induced by the IBDV infection might be responsible for the increased GPx activity in vvIBDV-infected groups (A, B, and C) at 7 days post-inoculation (Balami et al., 2021). However, the % decrease in GPx activity was lower in groups administered Antox® and Bactofort® compared to positive control at 14 days post-inoculation. This might be due to antioxidant effects produced by the probiotic bacteria and/or other constituents in the form of direct actions on H_2O_2 produced and/or indirect regulation of H_2O_2 production (Wang et al., 2017).

Evidence has shown that the concentration of MDA increase during IBDV infections and MDA concentration is an indirect measurement of oxidative stress (Azzam et al., 2019; Balami et al., 2021). The groups administered Antox® and Bactofort® had lower MDA concentrations compared to the positive control in this study. This might be a result of their enhanced antioxidant properties which prevented lipid peroxidation of cells caused by the vvIBDV infection (Birben et al., 2012).

The administration of Antox® and Bactofort® decreased EOF and thus, maintained the integrity of the erythrocyte membrane possibly as a result of their antioxidant properties (Wang et al., 2017). The finding shows that Antox® and Bactofort® exerted antioxidant effects on the cell membrane (Bai et al., 2017), thus protecting the cells from the deleterious effects of oxidative stress-induced lipid peroxidation. This was so as probiotics have been shown to decrease the negative effects of oxidative stress (Kim et al., 2017) and promote the activities of antioxidant enzymes by scavenging excess ROS that may cause cell damage, and consequent improvement in the host's health status (Mishra et al., 2015). The enhanced antioxidant effects induced by Antox® and Bactofort® in vvIBDV infection in this study might be due to metallic ion chelation, increased antioxidants, and antioxidant metabolites production, host antioxidase activities up-regulation, signaling pathways regulation, down-regulation of ROS-producing enzymes activities and intestinal microbiota regulation (Wang et al., 2017).

Based on the activities and concentrations of oxidative stress biomarkers studied, the administration of Antox® mitigated the negative effects of vvIBDV better compared to Bactofort® although it contained only one microorganism (*Saccharomyces cerevisiae*). It is however important to note that Antox® contained vitamins B-complex, biotin, cobalt, zinc, copper, manganese, and iron which are essential elements for the formation of antioxidants

(Borgstahl et al., 1992) and red blood cells (Hochleithner, 1994). Another possible reason might be an adverse alteration of the potency of Bactofort® by the possible presence of antimicrobial agents in the commercial feed used in the present study. This study is limited by the absence of postmortem examination of dead birds.

In conclusion, Antox® and Bactofort® ameliorated the deleterious effects of vvIBDV on the activities of SOD, CAT, and GPx, and decreased the production of MDA and EOF. It is therefore recommended that Antox® and Bactofort® be used by farmers and poultry veterinarians for the prevention of deleterious effects of vvIBDV infection. Also, further studies should be carried out to evaluate different dose regimens of Antox® and Bactofort® in IBD and determine their possible adverse effects when administered for prevention purposes.

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