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# Effects of some antioxidants on antibody response to infectious bursal disease vaccine, morbidity and mortality rates in cockerels infected with infectious bursal disease virus

## Abstract

Infectious bursal disease is an acute and highly contagious viral disease of chickens. it is one of the most economically important viral diseases that threatens the survival of the poultry industry in Nigeria. Clinico-pathological changes that could lead to high morbidity and mortality caused by infectious bursal disease have been linked to changes associated with oxidative stress. This study investigated the effects of selected antioxidants on antibody response, morbidity and mortality rates in chickens infected with infectious bursal disease. An experiment was conducted to determine the combined effects of vitamin E-selenium complex, vitamin C and, E-selenium complex and vitamin C on the antibody responses of Novagen cockerels to intermediate infectious bursal disease vaccine; investigate the effects of the antioxidants on clinical signs, morbidity and mortality rates in infectious bursal disease-vaccinated and non-vaccinated birds experimentally infected with a very virulent infectious bursal disease virus. Results showed that, cumulative mean antibody titre of birds supplemented with vitamin C (8,612.6 ± 2370.28) was significantly ($p < 0.05$) higher than group supplemented with vitamin E-selenium (8,053.7 ± 3053.07), positive control group (7,769.5 ± 3044.70) and group supplemented with their combination (7,509.6 ± 1155.08). Morbidity rates in infectious bursal disease vaccinated, and non-vaccinated birds in group supplemented with vitamin E-selenium was 38%, and 74%, respectively; vitamin C, 36%, and 68%, respectively and vit E-Se combined with vitamin C, 30%, and 66%, respectively, was lower when compared to positive control groups 40%, and 82%, respectively. Mortality rates in infectious bursal disease vaccinated and non-vaccinated birds in group supplemented with vit E-Se was 8%, and 54%, respectively; vitamin C, 6%, and 46%, respectively, vit E-Se combined with vitamin C, 6% and 50%, respectively, was lower when compared to positive control groups 8%, and 62%, respectively. It was concluded that vitamin C supplement used in drinking water of Novagen cockerels improves antibody titre to intermediate infectious bursal disease vaccines, reduced morbidity and mortality rates in cockerels infected with a very virulent infectious bursal disease virus. It is therefore recommended that vitamin C should be supplemented in poultry drinking water from day 1-50 of age to improve antibody response to infectious bursal disease vaccine, reduce morbidity and mortality rates associated with a very virulent infectious bursal disease virus infection in chickens.

**Keywords:** cockerels, antioxidants, infectious bursal disease virus, antibody, morbidity, mortality

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Audu Shekaro,[1] Sunday Blessing Oladele,[2] Paul Ayuba Abdu,[3] Muhammed Yakasai Fatihu[2]

[1]National Veterinary Research Institute Vom, Nigeria
[2]Department of Veterinary Pathology, Ahmadu Bello University Zaria, Nigeria
[3]Department of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria

**Correspondence:** Audu Shekaro, National Veterinary Research Institute Vom, Nigeria

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**Abbreviations:** AGDP, agricultural gross domestic product; IBDV, infectious bursal disease virus; IBD, infectious bursal disease; vvIBDV, very virulent infectious bursal virus; CMT, cumulative mean titres; Se, selenium; ANOVA, analysis of variance; SEM, standard error of mean; DMRT, Duncan's multiple range test

## Introduction

In Nigeria the poultry industry is one of the fastest growing segments of the agricultural sub-sector. The industry in Nigeria is a private sector driven subsector of the Nigeria economy, contributing over 25 percent to the Agricultural Gross Domestic Product (AGDP) of the national economy, with the industry worth of over ten trillion naira.[1] Infectious bursal disease is caused by infectious bursal disease virus (IBDV), a non-enveloped, double-stranded RNA virus of the Birnaviridae family, genus Avibirnavirus.[2] Infectious bursal disease poses a significant and ongoing threat to Nigeria's poultry industry, causing severe economic losses that was estimated to be over three billion Nigerian Naira (approximately 7.3 million USD) between 2009 and 2011 due to outbreaks alone.[3-5] Despite widespread vaccination efforts, control of IBD remains problematic, with outbreaks frequently occurring even on farms employing various vaccination programs and vaccine strains,[6,5] thereby provide incomplete protection against very virulent infectious bursal virus (vvIBDV) strains and fail to address the underlying oxidative stress mechanisms that contribute to disease pathogenesis.[7] This failure is partly attributed to the immunosuppressive nature of IBDV, which compromises the immune system of infected chickens, leading to poor responses to vaccines against other pathogens and increased susceptibility to secondary infections.[8,9] Additionally, the disease is associated with oxidative stress marked by elevated pro-inflammatory cytokines, chemokines, and nitric oxide production, which further impair immune organ development and function,[9] hence poor antibody response to the IBDV, resulting to high morbidity and mortality. Many concoctions and therapeutic agents are in use against IBD, but so far none of them is reliable.[10,11] Clinically, IBD causes high morbidity and mortality

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rates in both vaccinated and unvaccinated flocks, severely impacting production through reduced growth and increased mortality.[8] The virus targets the bursa of Fabricius, a key immune organ, and replicates in lymphoid and visceral organs such as the thymus, liver, kidneys, and spleen, causing extensive tissue damage and immunosuppression.[10]

The aim of this study was to investigate the ameliorative effects of some antioxidants on the antibody response, morbidity and mortality rates in Novagen cockerels infected with a vvIBDV.

## Materials and methods

### Ethical approval

Ethical approval was obtained from the Animal Ethical Committee of the Ahmadu Bello University, Zaria, Committee on Animal Use and Care (approval number: ABUCAUC/2024/043).

In this experiment 225-day-old Novagen cockerel chicks were reared. The study was conducted at the poultry farm under the animal experimental unit of the National veterinary research institute Vom, Nigeria. The chicks were assigned to 9 groups (A-I) for 50 days following a randomized design. Groups A and F were given vitamin E-Selenium (vit. E-Se) complex as supplement in drinking water at a dose of 100 mg vit. E and 0.05 mg of Se/L, groups B and G were given vitamin C supplement in drinking water at a dose of 250mg/L, groups C and H were given vit. E-Se complex combined with vitamin C as supplement in drinking water at a dose of 100 mg vit. E and 0.05 mg of Se/L and 250 mg/L respectively, while groups D and I were not given any supplement in their drinking water (Table 1).

**Table 1** Experimental design for assessing the effects of some antioxidants on vaccine-induced antibody response, clinical signs, morbidity and mortality rates in Novagen cockerels infected with a very virulent infectious bursal disease virus

| | | | | | Age in days | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 7 | 14 | 21 | 28 | 35 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
| Group | Antioxidant | No. of birds | Age (days) of IBD vaccination | Age (days) challenged with vvIBDV | No. of birds bled | | | | | | Observation of clinical signs, morbidity and mortality rates | | | | | | | |
| A | Vit. E/Se | 25 | 7 and 21 | 42 | 5 | 5 | 5 | 5 | 5 | 5 | DO | DO | DO | DO | DO | DO | DO | DO |
| B | Vit. C | 25 | 7 and 21 | 42 | 5 | 5 | 5 | 5 | 5 | 5 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| C | Vit. E + Vit. C | 25 | 7 and 21 | 42 | 5 | 5 | 5 | 5 | 5 | 5 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| D | Nil | 25 | 7 and 21 | 42 | 5 | 5 | 5 | 5 | 5 | 5 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| E | Nil | 25 | Nil | Nil | 5 | 5 | 5 | 5 | 5 | 5 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| F | Vit. E/Se | 25 | Nil | 42 | 0 | 0 | 0 | 0 | 0 | 0 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| G | Vit. C | 25 | Nil | 42 | 0 | 0 | 0 | 0 | 0 | 0 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| H | Vit. E + Vit. C | 25 | Nil | 42 | 0 | 0 | 0 | 0 | 0 | 0 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |
| I | Nil | 25 | Nil | 42 | 0 | 0 | 0 | 0 | 0 | 0 | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- | -DO- |

Vit. E/Se = vitamin E/selenium complex; Vit. C = vitamin C; vvIBDV = very virulent infectious bursal disease virus

The dosages of the antioxidants (vit. E-Se complex and vitamin C) employed in this experiment were based on the recommendation of the manufacturers. Vitamin E/Selenium were provided as Vit. E-Se100/50(R), a solution of vitamin E and sodium selenite manufactured by MAVIT GmbH Germany. Vitamin C was provided in powdered form, manufactured by Aether Centre Biology Co. LTD Beijing, China. The antioxidants were administered in drinking water throughout the experimental period.

### Bird's management

The birds were raised in a semi open sided building ideal for poultry rearing, with strict biosecurity measures put in place and adhered to. The birds were fed with chick mash (ME 2896 Kcal and crude protein 13.5%) ad-libitum throughout the experimental period as well as drinking water. The birds were vaccinated against Newcastle disease using laSota and Komarov strain at days 14 and 35 of life respectively, while fowl pox vaccine was administered at day 40 0f life. Birds in groups A, B, C and D were vaccinated against IBD at days 7 and 21 using freeze dried live attenuated intermediate strain IBD vaccine (Abic laboratory ltd). The vaccine was administered at a dose recommended by the manufacturer.

Birds in all the experimental groups (except those of group E, which served as negative control) were challenged with a very virulent infectious bursal disease virus (vvIBDV) orally at a dose of 0.05 ml per bird[12] at day 42 of age. The IBDV inoculum used for the challenge was a field strain of vvIBDV obtained from layer birds that died of natural outbreak of IBD. Sixty-five per cent of commercial cockerels inoculated at 30 days of age with 50 µL of bursal suspension (v/w) in PBS (pH 7.4) died. One millilitre of bursal suspension (v/w) in PBS (pH 7.4) contained 10-976 CID50 of IBDV.[12]

### Collection of blood sample

Five birds were randomly selected from groups A, B, C, D and E at days 7, 14, 21, 28, 35 and 42 of age. The birds were restrained between the index finger and thumb. 1ml of blood sample was aseptically collected from each bird through the brachial vein using a sterile 23G hypodermic needle and 5 ml syringe. The blood in the syringes were kept slanted at room temperature (RT) for two hours to allow the sera to separate. The serum from each bird was transferred into clean sterile tubes, labelled accordingly, and stored at -20 OC until used for the determination of antibody titre to IBD vaccine.

### Determination of antibody titre against infectious bursal disease vaccine

The ELISA antibody titres against IBD were measured using commercial ELISA kits (IDVET Innovative Diagnostics from Pasteur-Grabels, France) as described by Zahid et al.,[13] with manufacturer protocol duly followed. Mean ELISA titres of each group were

calculated each week and cumulative mean titres (CMT) of each group were calculated after 42 days.

### Clinical observations

After infection with vvIBDV at 42 days of age, all the birds in different groups were observed at six hours interval for clinical signs, morbidity and mortality rates for 8 days pi. Clinical signs, morbidity and mortality rates observed were presented in percentages, graded and scored from 1 to 5, with 1 = mild (1 to 20%), 2 = moderate (21 to 40%), 3 = severe (41 to 60%), 4 = very severe (61 to 80%) and 5 = grave (81 to 100%) as described by previous authors.[10, 14]

### Determination of clinical signs

The number of birds that shows any of the clinical signs such as anorexia, depression, recumbency, huddling and prostration per day in each group was recorded in percentages (%).[15]

### Determination of morbidity and mortality rates

Morbidity and mortality rates were calculated using the formulae described by Babiker and Tawfeeg[15]

$$\text{Morbidity}=\frac{\text{Number of birds inoculated}}{\text{Number of sick birds}}\text{X100}$$

$$\text{Morbidity}=\frac{\text{Number of birds inoculated}}{\text{Number of birds that died}}\text{X100}$$

### Data analyses

The ELISA antibody titres were expressed as means ± standard error of mean (SEM). They were further subjected to analysis of variance (ANOVA), using the Statistical Package for Social Sciences (SPSS 15) software. Significant mean difference between treatments groups were determined at a 5% significance level ($p \leq 0.05$) using Duncan's Multiple Range Test (DMRT). [16] Clinical signs, morbidity and mortality rates were presented in percentages.[17] Data were presented using Tables and Charts.

## Results

### Effects of Some Antioxidants Supplement on Vaccine-induced Antibody Titres of Novagen Cockerels Vaccinated with an Intermediate Infectious Bursal Disease Vaccine.

Data from ELISA titre showed, at one week of age (Pre-vaccination), there was no significant difference ($p > 0.05$) in mean antibody titres to IBD vaccine between groups A, B, C, D, and E. At 14 days of age, after the first vaccination with the IBD vaccine, the mean antibody titre (3,864.6 ± 540.3) in birds from group A and the mean antibody titre (3,711.6 ± 570.3) in birds from group D were significantly ($p < 0.05$) higher than the mean antibody titre (3,230.2 ± 245.3) in birds from group B and the mean antibody titre (3,022.4 ± 159.1) in birds from group C. All vaccinated groups (A, B, C, and D) had significantly ($p < 0.05$) higher mean antibody titres compared to group E (735 ± 469.4). At day 21 of age, group C and group D had significantly ($p < 0.05$) higher antibody titres (8,490.80 ± 655.58 and 8,411.80 ± 2,471.38, respectively) than group A (7,763.20 ± 3,073.09) and group B (6,337.40 ± 1,769.07;). At day 28 of age, the antibody titre (12,992.4 ± 3,895.06) in group A was significantly ($p < 0.05$) higher than those in groups B (11,342.4 ± 2,094.76), C (10,200.2 ± 1,641.10), and D (8,577.4 ± 3,560.34). At days 35 and 42 of age, group B had significantly ($p < 0.05$) higher antibody titres (13,440.4 ± 1,876.94 and 16,156.2 ± 2,504.27, respectively) than groups D (10,179.8 ± 4,040.14 and 14,588.8 ± 3,986.48), C (10,473.8 ± 2,534.14 and 11,699.2 ± 2,462.50), and A (12,100.0 ± 3,749.68 and 10,439.8 ± 4,394.62). All groups, except group A and E, showed steady increases in antibody titre levels from day 7 to day 42. The antibody titre in group A dropped sharply from day 35 to day 42 (Table 2 and figure 1). The cumulative mean ELISA antibody titre from 7 to 42 days of age showed that, group B (vaccinated and given vitamin C supplement) had significantly ($p < 0.05$) higher antibody titre (8,612.6 ± 2,370.28) than birds in group A (8,053.7 ± 3,053.07), group D (7,769.5 ± 3,044.70), and group C (7,509.6 ± 1,155.08). Group B also maintained a steady increase in antibody titre from day 7 to day 42 (Figure 1 and 2).

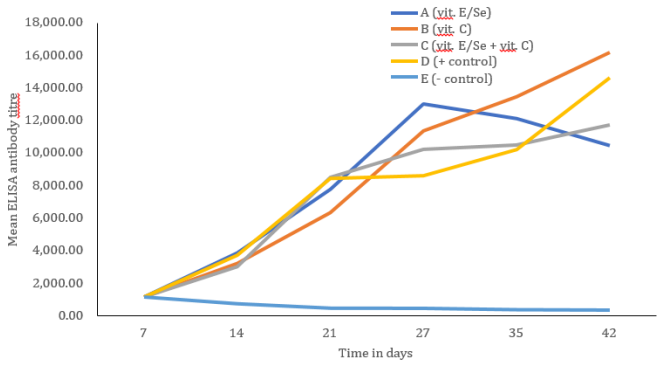


**Figure 1** ELISA antibody titres of Novagen cockerels administered antioxidants supplements and vaccinated with an intermediate Infectious Bursal Disease Vaccine. A: were supplemented with vitamin E-Selenium complex, B: were supplemented with vitamin C, C: were supplemented with vitamin E-Selenium complex in combination with vitamin C, D: were not supplemented with any of the antioxidants, E: were not supplemented with any of the antioxidants. All the groups except group E were vaccinated with intermediate infectious bursal disease vaccines at days 7 and 21 of age.

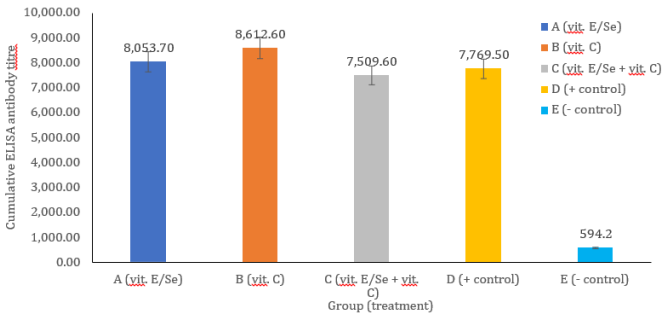


**Figure 2** Cumulative mean ELISA antibody titres of Novagen cockerels administered antioxidants and vaccinated with intermediate infectious bursal disease vaccine at days 7 and 21 old, A: were given vitamin E-selenium complex supplement; B: were given vitamin C supplement; C: were given vitamin E-selenium complex in combination with vitamin C supplement; D: were not given any of the antioxidants supplement; E: were not given any of the antioxidants supplements and not vaccinated.

### Effects of some antioxidants supplement on clinical signs observed in white novagen cockerels infected with a very virulent infectious bursal disease virus

Clinical signs observed in cockerels from groups administered vitamin E-Se complex, vitamin C, the combination of vitamin E-Se complex and vitamin C supplements, and those not administered antioxidants but vaccinated against IBD at 7 and 21 days of age included depression, prostration, recumbency, huddling, and anorexia. Cockerels from the negative control group (E) appeared healthy (Plate

1). The severity of clinical signs was moderate (score 2) in groups administered vitamin E-Se complex, vitamin C, and the combination of vitamin E-Se complex plus vitamin C supplements, with values of 38%, 36%, and 40%, respectively. The group not administered any antioxidant (group D) showed severe (score 3) clinical signs with a value of 48% (Figure 3).

From the non-IBD vaccinated groups, very severe clinical signs (score 4) which included depression, prostration, recumbency, huddling, anorexia and death were observed in all experimental groups (F, G, H and I), however, group H administered vitamin E-Se complex and vitamin C supplements, had the lowest percentage (66%), clinical signs, followed by Group C were administered vitamin C supplement (68%), then group F administered vitamin E-Se complex (74%), while group I, not administered any antioxidant supplement (positive control) had the highest percentage of clinical signs (80%) (Figure 3). Plates I - IV showed birds in different treatment groups manifesting clinical signs of IBD.

**Plate 1:** White Novagen cockerels from group E (negative control), showing apparently healthy birds.

**Plate 2:** White Novagen cockerels from group F (administered vit E- Se complex) infected with a very virulent infectious bursal disease virus at 42 days of age, showing depression (black arrows), recumbency (red arrows), prostration (blue arrows), death (yellow arrows) 3 days post infection.

**Plate 3:** White Novagen cockerels from group G (administered vitamin C) infected with a very virulent infectious bursal disease virus at 42 days of age, showing depression (black arrow), recumbency (red arrow), prostration (blue arrows), death (yellow arrow) 3 days post infection.

**Plate 4:** White Novagen cockerels from group H (administered vit E-Se complex plus vitamin C) infected with very virulent infectious bursal disease virus at 42 days of age, showing depression (black arrow), prostration (blue arrow), recumbency (red arrows), death (yellow arrows) 3 days post infection.

**Plate 5:** White Novagen cockerels from group I (positive control) infected with very virulent infectious bursal disease virus at 42 of age, showing depression (black arrow), recumbency (red arrow), prostration (blue arrow), huddling (double black arrows), death (yellow arrows) 3 days post infection.

**Citation:** Shekaro A, Oladele SB, Abdu PA. et al. Effects of some antioxidants on antibody response to infectious bursal disease vaccine, morbidity and mortality rates in cockerels infected with infectious bursal disease virus. *J Dairy Vet Anim Res.* 2026;14(1):47–53. DOI: 10.15406/jdvar.2026.14.00361

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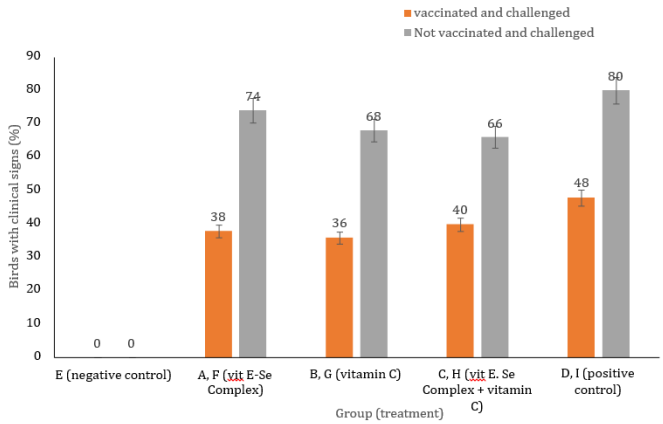


**Figure 3** Effects of vitamin E-selenium complex, vitamin C and, vitamin E-selenium complex and vitamin C on clinical signs in percentage, observed in Novagen cockerels infected with a very virulent infectious bursal disease virus. A and F were administered vitamin E- Selenium complex, B and G: were administered vitamin C, C and H were administered vitamin E- Selenium complex and vitamin C, D and I: were not administered any of the antioxidants, E: were not administered any of the antioxidants. All the groups except group E were infected with a very virulent infectious bursal disease virus at day 42 of age.

## Effects of some antioxidants supplement on morbidity rates of novagen cockerels infected with a very virulent infectious bursal disease virus

Morbidity rates observed in cockerels from groups A, B, C, and D, which were vaccinated against IBD at 7 and 21 days of age, showed moderate (score 2) morbidity rates. However, group B (administered vitamin C supplement), had the lowest morbidity rate of 30%, group C (administered vitamin E-Se complex and vitamin C supplement) had a morbidity rate of 36%, group A (administered vitamin E-Se complex supplement) had 38%, while group D (positive control) had the highest morbidity rate of 40 %. Groups not vaccinated against IBD (F, G, H, and 1), the lowest morbidity rate (66%) was observed in group H (administered vitamin E-Se complex and vitamin C supplements), while group G (administered vitamin C supplement) (68%), group F (administered vitamin E-Se complex supplement) (74%) and group I (positive control) had grave morbidity rate (82%) (Figure 4).

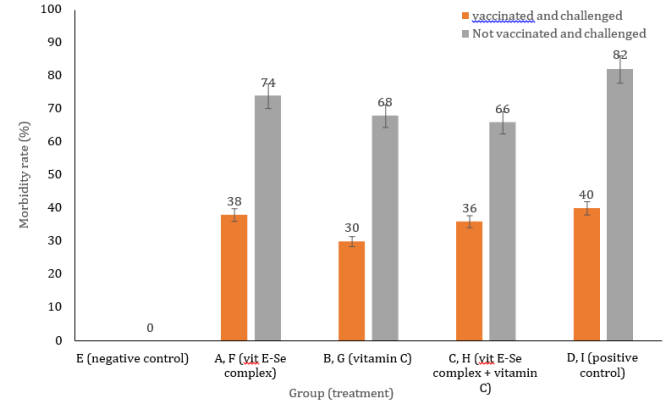


**Figure 4** Effects of vitamin E-selenium complex, vitamin C and a vitamin E-selenium complex and vitamin C on morbidity rates of Novagen cockerels infected with a very virulent infectious bursal disease virus. A and F were administered vitamin E-Selenium complex, B and G: were administered vitamin C, C and H were administered vitamin E-Selenium complex and vitamin C, D and I: were not administered any of the antioxidants, E: were not administered any of the antioxidants. All the groups except group E were infected with a very virulent infectious bursal disease virus at days 42 of age.

## Effects of some antioxidants supplement on mortality rates of novagen cockerels infected with a very virulent infectious bursal disease virus

Mortality rates observed in cockerels from groups A, B, C, and D, which were vaccinated against IBD at 7 and 21 days of age, groups B (administered vitamin C supplement) and C (administered vitamin E- Se complex and vitamin C supplement) had mortality rates of 6% each, while groups A (administered vitamin E- Se complex supplement) and D (no supplement) had 8% mortality rates each. For cockerels in groups E, F, G, H, and I, which were not vaccinated with IBD vaccine, after 8 days pi, no mortality was recorded in group E (negative control), group G (administered vitamin C supplement) had 46% mortality rate, group H (administered vitamin E-Se complex and vitamin C supplement) 50%, group F (administered vitamin E-Se complex supplement) 54% while group I (positive control) 62% (Figure 5).

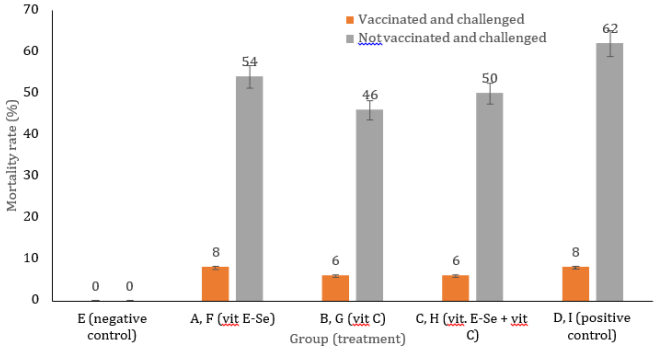


**Figure 5** Effects of vitamin E-selenium complex, vitamin C and vitamin E-selenium complex mixed with vitamin C on mortality rates of Novagen cockerels infected with a very virulent infectious bursal disease virus. A and F: were administered vitamin E-Selenium complex; B and G, were administered vitamin C; C and H, were administered vitamin E-selenium complex and vitamin C; D and I, were not administered any of the antioxidants, E: were not administered any of the antioxidants. All the groups except group E were infected with very virulent infectious bursal disease virus at days 42 of age.

# Discussion

This study demonstrated that using vitamin C and vitamin E-Se complex supplement in the drinking water of Novagen cockerels significantly boosts antibody response following vaccination with an intermediate IBD vaccine. This was shown by the higher ELISA antibody titres observed in supplement administered groups compared to non-supplemented controls, particularly at 42 days post-vaccination. Vitamin C, a potent antioxidant, shields B cells responsible for antibody production from oxidative damage, thereby enhancing antibody output.[18] Selenium, on the other hand, increases antibody production by activating immune cells.[19] Although chickens can synthesize vitamin C endogenously,[20, 21] vaccination with the IBD vaccine leads to its depletion, reducing endogenous antioxidant levels and potentially lowering the humoral response.[20,21] Exogenous vitamin C supplement, as observed in this study, helped to replenish depleted vitamin C, thus boosting the birds' response to the IBD vaccine. This was particularly evident in the higher antibody titres observed in group administered vitamin C supplement compared to controls. These findings are consistent with previous research works, [17, 22- 25] which showed that fortifying chicken diets with antioxidants such as selenium and vitamins A, C, and E enhanced immune responses to viral vaccines, including IBD vaccines. Similarly, Arshad et al., [23] Sanda et al., [25] and Latifat et al.[26] demonstrated that selenium, zinc,

vitamin C, and vitamin E supplements in chicken diets increased serum bioavailability of these micronutrients, decreased oxidative stress, and boosted immune responses.

Unexpectedly, the present study found that birds administered vitamin C supplement alone had significantly higher ELISA antibody titres at days 35 and 42 post vaccination compared to those administered vitamin E-Se complex alone or E-Se complex and vitamin C. This suggests that combining multiple antioxidants does not always leads to additive or synergistic effects; in some cases, the components may rather act antagonistically, reducing overall immune response. This observation aligns with the findings of Latifat et al.,[26] who reported that, use of selenium or zinc supplement singly in the diet of chickens resulted in higher serum bioavailability and concentration of the antioxidants that led to higher antibody titres than when used concurrently. However, Skrivan et al.,[27] found that combining vitamin B6, vitamin C, and selenium improved antioxidant status and cellular immunity compare to using any of the antioxidant singly. In this case vitamin B6 could have likely enhanced the synergistic effect between vitamin C and selenium which was not included in the present study.

Novagen cockerels administered antioxidants supplements and infected with vvIBDV in both IBD-vaccinated and non-vaccinated birds, consistently showed lower clinical signs morbidity and mortality rates compared to their respective controls. The observed reductions in morbidity and mortality rates can be attributed to several mechanisms of immune enhancement. Selenium is known to boost the immune system of chickens, increasing their natural resistance to antigenic invasion.[28] Additionally, the antioxidative mechanism of selenium shielded the immune cells from oxidative damage by free radicals orchestrated by the IBDV given room to more viable immune cells producing more antibodies against the IBDV, leading to more clearance of the IBDV from the body thereby contributed to the reduced disease severity observed in supplemented groups. The oxidative stress triggered by vvIBDV infection leads to the generation of free radicals, which can damage immune cells and tissues. Vitamin C and E used in this study neutralized free radicals, thereby minimizing oxidative damage to immune cells resulting in more healthier cells producing antibodies against IBDV resulting to decreased clinical signs, morbidity and mortality rates.[25, 26] Selenium does not only boosts antibody production by protecting immune cells from oxidative damage, but also activates immune cells, including macrophages and T-cells, which are critical for mounting an effective immune response against IBDV.[19] This action of Se further limits the impact of the IBDV and reduce disease progression.

In another development, Leng et al.,[29] reported that antioxidants such as Se and vitamin C stimulated cell-mediated immunity and increase CD4+ cell counts in the bursa of Fabricius, thus mitigating the immunosuppressive effects of vvIBDV. They also suggested that Se enhances the ability of immunocompetent cells to respond to antigenic challenges, improving overall disease resistance. Furthermore, Bahram et al.[18] reported that vitamin C supplementation in poultry alleviates lymphocyte damage caused by reactive oxygen species (ROS) by increasing the activities of antioxidant enzymes such as SOD and GPx and enhances resistance to infection. These enzymes limit disease onset and progression, supporting the findings in the present study that showed reduced morbidity and mortality rates in Novagen cockerels given vitamin E-Se complex, vitamin C supplements, or their combination in drinking water, and infected with vvIBDV compared to control.

It was also observed in this study, cockerels vaccinated with IBD vaccine and supplemented with antioxidants had lower clinical signs morbidity and mortality rates following challenge with vvIBDV, compared to non-supplemented control. This effect underscores the importance of both vaccination with intermediate IBD vaccine and antioxidants (vitamin E- Se, vitamin C) nutritional support in IBD management. Notably in this study, birds given vitamin C supplement exhibited a comparatively lower mortality rate than birds given vitamin E-Se complex and birds given vitamin E-Se complex and vitamin C, this discrepancy in mortality may be due to the distinct mechanisms of action between vitamin C and Se, as well as possible interactions between vitamin E-Se complex and vitamin C combined, that could affect their individual efficacy.

## Conclusion

It was concluded that vitamin C supplement in drinking water of Novagen cockerels improves antibody titre to intermediate IBD vaccines, reduced morbidity and mortality rates in cockerels infected with a vvIBDV. It is therefore recommended that vitamin C should be supplemented in poultry drinking water from day 1-50 of age to improve antibody response to IBD vaccine, reduce morbidity and mortality rates associated with a vvIBDV infection in chickens.

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## Authors' contributions

Audu Shekaro contributed to the research, data collection, data analysis, and manuscript preparation. Sunday Blessing Oladele, Paul Ayuba Abdu and Muhammed Yakasai Fatihu supervised and revised the manuscript. The final version of the article was reviewed and approved by all the authors, who also looked over the information submitted in this publication.

## Availability of data and materials

This article incorporates all research data, with additional material attainable upon reasonable request from the corresponding author.

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## Ethical considerations

The manuscript underwent scrutiny for ethical issues, including plagiarism, permission to publish, misconduct, double publishing, and redundancy by all authors.

## Conflicts of interest

The authors declares that there are no conflicts of interest.

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