Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
GUASNR

Comparative Induction of Oxidative Stress and Antioxidant Defense in Infectious Bursal Disease Virus-Infected Chickens, Turkeys and Ducks

Omolade A. Oladele[1], Precious J. Adedoyin[1], Oluwaseun O. Esan[1] & Ademola A. Oyagbemi[2]

[1] Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[2] Department of Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Nigeria

Poultry Science Journal 2025, 13(2): 171-180

Keywords
Antioxidant
Poultry species
Oxidative stress
Infectious bursal disease

Corresponding author
Omolade A. Oladele
lade.oladele@gmail.com

Article history
Received: July 14, 2024
Revised: January 27, 2025
Accepted: February 02, 2025

Abstract

Infectious bursal disease (IBD) is an immunosuppressive disease of chickens. The mechanism of tolerance in turkeys and ducks is not yet deciphered. This study investigated the pathogenesis of IBD in chickens, turkeys and ducks by assessing the severity of oxidative stress and antioxidant response in infected birds. Forty chicks and 35 turkey poults and ducklings at 4 weeks were separated into two groups/bird species (A and B), i.e., chickens, CA and CB; turkeys, TA and TB; and ducks, DA and DB. Each bird in group A was inoculated with $1LD_{50}$ IBD virus, while group B served as control. Infected birds were bled, and liver, kidney, and bursa of Fabricius (BF) were harvested at 5 days post-infection and assayed for MDA, H_2O_2, NO, MPO, GSH, GPx, SOD and GST using standard methods. Results indicated that MDA level (µmol/mg protein) was higher in liver (13.11 ± 1.77) and kidney (11.10 ± 0.16) of CA than in DA (2.74 ± 0.01; 7.62 ± 0.37) and TA (1.42 ± 0.09; 6.79 ± 0.27). H_2O_2 level (µmol/min/mg protein) in BF of CA (49.10 ± 3.99) was higher than DA (35.89 ± 1.54) and TA (36.07 ± 2.18). MPO level in CA was higher than in DA and TA. Superoxide dismutase activity (unit/mg protein) in BF of CA (11.56 ± 1.85) was higher than DA (8.59 ± 0.15) and TA (7.00 ± 0.42) and GSH level (µmol/mg protein) was higher in BF of DA and TA (105.81 ± 1.59 and 93.89 ± 7.93) than in CA (92.30 ± 3.12). GPx level (unit/mg protein) was higher in the liver of DA and TA (26.81 ± 2.45 and 30.01 ± 2.18) than CA (24.21 ± 0.94) as well as in BF of TA (92.79 ± 3.38) than in CA and DA (83.63 ± 4.62, 81.85 ± 4.42). Thus, chickens undergo a higher degree of oxidative stress post-IBD virus infection than ducks and turkeys. Simultaneously, the antioxidant defense system was generally more active in infected ducks and turkeys than in chickens.

Introduction

Infectious bursal disease (IBD) is caused by a highly contagious virus, the Birnavirus. The disease is acute and usually causes immunosuppression and death of young chicks. It causes significant economic loss in the poultry industry globally. The bursa of Fabricius has been established as the predilection site for the virus. Changes have also been seen in other organs like the spleen, liver, thymus, kidney, and cecal tonsils. Most chicks that come down with clinical signs are between 3 and 6 weeks of age (Ley *et al.,* 1983). The common clinical signs associated with IBD include diarrhea, ruffled feathers, unthriftiness, and anorexia.

While it is well known that chickens are highly susceptible to this disease, other poultry birds like ducks and turkeys show little or no susceptibility and rarely come down with clinical signs (Oladele *et al.,* 2005; Kegne and Chanie, 2014), despite the presence of the bursa of Fabricius, the predilection organ, in them. Also, IBD virus has been isolated from healthy-looking turkey poults showing no clinical sign, thus establishing the fact that these poultry species have varying levels of susceptibility to the virus, with chickens being the most susceptible (Oladele *et al.,* 2009; Kegne and Chanie, 2014).

According to Migdal and Serres (2011), oxidative stress occurs when there's an imbalance between the

Please cite this article as Omolade A. Oladele, Precious J. Adedoyin, Oluwaseun O. Esan & Ademola A. Oyagbemi. 2025. Comparative Induction of Oxidative Stress and Antioxidant Defense in Infectious Bursal Disease Virus-Infected Chickens, Turkeys and Ducks. Poult. Sci. J. 13(2) 171-180.

DOI: 10.22069/psj.2025.22716.2124

generation of reactive oxygen species (ROS) and the cell's antioxidant defenses. Earlier, ROS were considered harmful by-products of normal aerobic metabolism in the mitochondria and were linked to various diseases. However, increasing evidence suggests that regulated ROS production also plays important physiological roles in maintaining cell redox homeostasis and cell signaling. It is believed that differences in cellular mechanisms of IBD progression post-infection amongst poultry species, which can be determined through the assessment of ROS and antioxidant defenses, might further explain the susceptibility differential that exists between them.

The aim of the study was to compare induced oxidative stress and antioxidant defense mechanisms in infectious bursal disease virus-infected chickens, turkeys and ducks in order further to elucidate its pathogenesis in the three poultry species and advance knowledge regarding the differential susceptibility to the virus that exists between the species.

Materials and Methods

Experimental birds

Forty 1-day-old chicks, 35 turkey poults, and 35 ducklings were used for this study. The birds were purchased from a reputable hatchery in Ibadan, Nigeria. They were reared for three weeks at the Experimental Animal Unit, Department of Veterinary Medicine, University of Ibadan, following institutional ethical approval from the University of Ibadan, Animal Care and Use Research Ethics Committee with approval number UI-ACUREC/054-1222/09. Each bird species was placed in a separate cage compartment for rearing. The birds were fed daily and provided with water *ad libitum.*

Infectious Bursal Disease Virus (IBDV) infection of experimental birds and sampling

At four weeks of age, each bird species was separated into two subgroups of approximately equal number of birds, A and B, i.e., CA and CB for chickens, TA and TB for turkeys and DA and DB for ducks. Birds in subgroup A were inoculated with $1LD_{50}$ IBDV, via conjunctival instillation, while subgroup B served as uninoculated control. At 5 days post-infection, five birds were randomly selected from each subgroup, and 5 mL of blood was collected from each bird via jugular venipuncture into plain bottles, allowed to clot and centrifuged at 2000 g for 5 minutes to harvest serum. The selected birds were euthanized using cervical dislocation (AVMA, 2020) and tissue samples from the kidney, liver and bursa of Fabricius were harvested. The samples were labeled as CA1 - CA5 for infected chickens, TA1-TA5 for infected turkeys and DA1 - DA5 for infected ducks. Also, CB1- CB5 for the control group of chickens, TB1 - TB5 for the control turkeys and DB1 - DB5 for the control ducks. The tissue samples were measured, rinsed in distilled water, and put into sample bottles labeled appropriately for each sample type and stored at -20ºC.

Samples of the liver, kidney, and bursa of Fabricius were assayed for markers of oxidative stress, i.e., malondialdehyde and hydrogen peroxide, as well as elements of the antioxidant defense system, i.e., reduced glutathione, glutathione peroxidase, superoxide dismutase and glutathione S-transferase. Also, nitric oxide levels and myeloperoxidase activity in serum samples were assayed as additional markers of oxidative stress.

Tissue preparation

Harvested tissues of the kidney, liver and bursa of Fabricius were allowed to thaw on ice and were carefully chopped into bits. Phosphate buffer (0.1M), pH 7.4, was added to the chopped tissues and homogenized using a Teflon homogenizer. Homogenate was centrifuged at 10,000 *g* for 10 minutes using a cold centrifuge at -4˚C to obtain post-mitochondrial fractions (PMFs). The supernatant was aliquoted and stored at -20ºC until used for biochemical assays.

Biochemical assays

Total protein concentration in samples (tissue supernatant and serum) was determined using the biuret method (Gornall *et al.,* 1949). Malondialdehyde (MDA) concentration in tissue supernatant as an index of lipid peroxidation was determined as described by Farombi *et al.* (2000) while Hydrogen peroxide (H_2O_2) generation was determined by the method of Wolff (1994). The method described by Jollow *et al.* (1974) was used to assay reduced glutathione (GSH) concentration. Glutathione peroxidase (GPx) activity was measured as described by Rotruck *et al*. (1973), while the Superoxide dismutase (SOD) assay was carried out as described by Misra and Fridovich (1972) and Oyagbemi *et al.* (2016). Glutathione S-transferase (GST) activity was estimated by the method of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene as substrate. In serum, Nitrite (NO) content and Myeloperoxidase (MPO) activity were determined using the methods of Olaleye *et al*. (2007) and Xia and Zweier (1997), respectively.

Statistical analysis

Results were expressed as the mean ± standard error of the mean. Data generated were subjected to One-way analysis of variance (ANOVA) using Graph Pad Prism and were compared for significant differences by the Tukey Multiple Range Test at P less than 0.05 ($P < 0.05$).

Results

Markers of oxidative stress

Malondialdehyde

In the liver, mean ± SEM MDA levels were 13.11 ± 1.77 µmol/mg protein, 2.74 ± 0.01 µmol/mg protein and 1.42 ± 0.09 µmol/mg protein in infected chickens, ducks and turkeys, respectively, which are significantly higher ($P < 0.05$) than values in their respective control subgroups i.e., 2.81 ± 0.39 µmol/mg protein, 2.17 ± 0.12 µmol/mg protein and 0.82 ± 0.02 µmol/mg protein (Figure 1). In the kidney, MDA levels were 11.10 ± 0.16 µmol/mg protein, 7.62 ± 0.37 µmol/mg protein and 6.79 ± 0.27 µmol/mg protein, in infected chickens, ducks and turkeys, respectively, which are significantly higher ($P < 0.05$) than values in their respective control subgroups i.e., 4.85 ± 0.28 µmol/mg protein, 4.64 ± 0.22 µmol/mg protein and 4.47 ± 0.27 µmol/mg protein. Also, MDA levels in the bursa of Fabricius of infected chickens, ducks and turkeys were 15.41 ± 1.17 µmol/mg protein, 13.70 ± 1.17 µmol/mg protein and 18.10 ± 3.21 µmol/mg protein, which are significantly higher ($P < 0.05$) than values in their respective control subgroups, i.e., 7.27 ± 0.29 µmol/mg protein, 7.08 ± 0.46 µmol/mg protein and 5.11 ± 0.23 µmol/mg protein. Comparing MDA levels between infected species, chickens had significantly higher ($P < 0.05$) levels in the liver and kidneys than in those of ducks and turkeys.

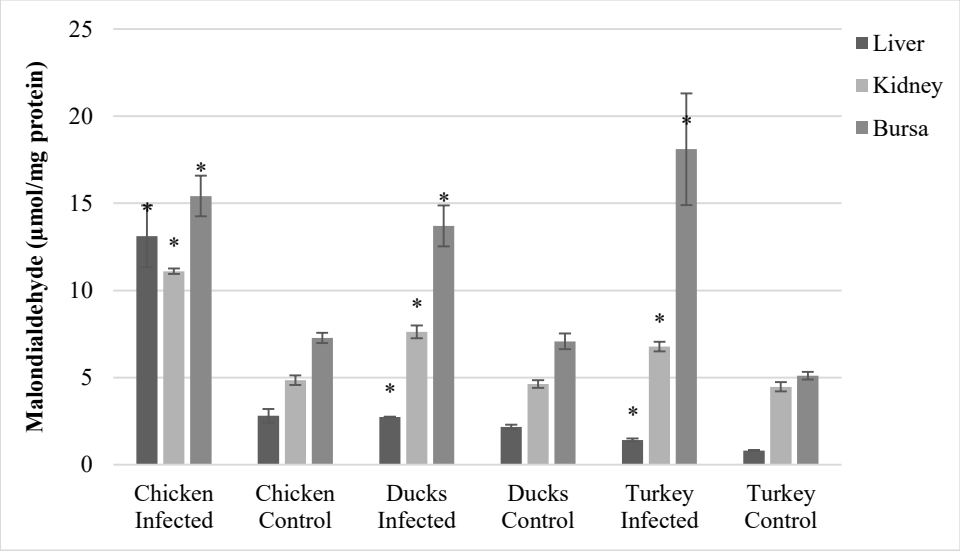

Figure 1. Concentrations (mean ± *SEM*) of malondialdehyde (µmol/mg protein) in liver, kidney and bursa of Fabricius of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

Hydrogen peroxide

In the liver, mean ± SEM H_2O_2 levels were 45.70 ± 1.01 µmol/mg protein, 47.32 ± 1.73 µmol/mg protein and 47.01 ± 1.77 µmol/mg protein in infected chickens, ducks and turkeys, respectively, which are significantly higher ($P < 0.05$) than values in respective control chicken and duck subgroups i.e. 42.57 ± 0.98 µmol/mg protein and 42.80 ± 1.54 µmol/mg protein (Figure 2). However, there was no significant difference between infected turkeys and the control subgroup (46.55 ± 0.1 µmol/mg protein). In the kidney, H_2O_2 levels were 66.62 ± 1.76 µmol/mg protein, 71.19 ± 3.54 µmol/mg protein and 59.51 ± 2.99 µmol/mg protein in infected chickens, ducks and turkeys, respectively, which are significantly higher ($P < 0.05$) than values in control chickens (56.79 ± 1.5 µmol/mg protein) and ducks (63.48 ± 3.06 µmol/mg protein) subgroups but not between infected turkeys and control (56.65 ± 1.43 µmol/mg protein). Levels in the bursa of Fabricius of infected chickens, ducks and turkeys were 49.10 ± 3.99 µmol/mg protein, 35.89 ± 1.54 µmol/mg protein and 36.07 ± 2.18 µmol/mg protein, respectively, while the values were 32.49 ± 1.2 µmol/mg protein, 32.68 ± 1.63 µmol/mg protein and 30.89 ± 1.73 µmol/mg protein in control chickens, ducks and turkeys groups, respectively. The difference between infected and respective control subgroups was significant ($P < 0.05$) only in chickens. Also, the level in the bursa of Fabricius of infected chickens was significantly higher ($P < 0.05$) than in infected ducks and turkeys.

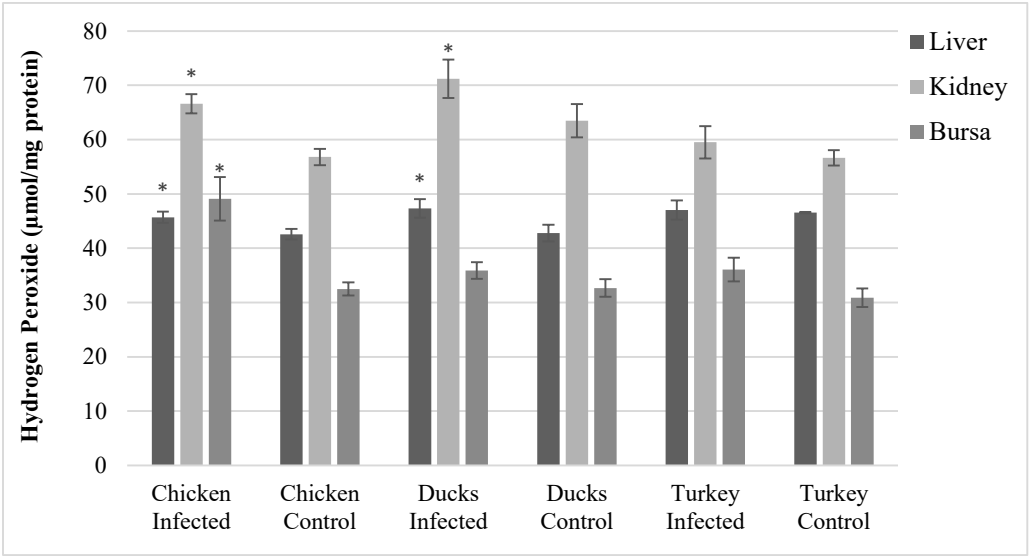

Figure 2. Concentrations (mean ± *SEM*) of hydrogen peroxide (µmol/mg protein) in liver, kidney and bursa of Fabricius of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

Nitric oxide and myeloperoxidase

Mean ± *SEM* serum levels of Nitric Oxide (NO) in infected chickens, ducks and turkeys were 5.72 ± 0.23 µmol/L, 4.63 ± 0.12 µmol/L and 6.30 ± 0.42 µmol/L, respectively, which are higher than values in control subgroups i.e., 4.18 ± 0.35 µmol/L, 4.22 ± 0.17 µmol/L and 4.24 ± 0.13 µmol/L, respectively with significant difference ($P < 0.05$) between the infected and control subgroups in chickens and turkeys (Figure 3). Also, mean ± SEM serum activity of myeloperoxidase in infected chickens, ducks and turkeys were 7.74 ± 0.42 unit/L, 5.49 ± 0.18 unit/L and 7.08 ± 0.51 unit/L, respectively, which are significantly higher ($P < 0.05$) than activity levels in control chickens, ducks and turkeys, i.e., 3.49 ± 0.18 unit/L, 2.88 ± 0.21 unit/L and 3.54 ± 0.36 unit/L respectively. A comparison between infected subgroups showed that NO levels in the serum of chickens and turkeys were significantly higher than the level in ducks, while MPO activity level in chickens was significantly higher than in ducks and turkeys.

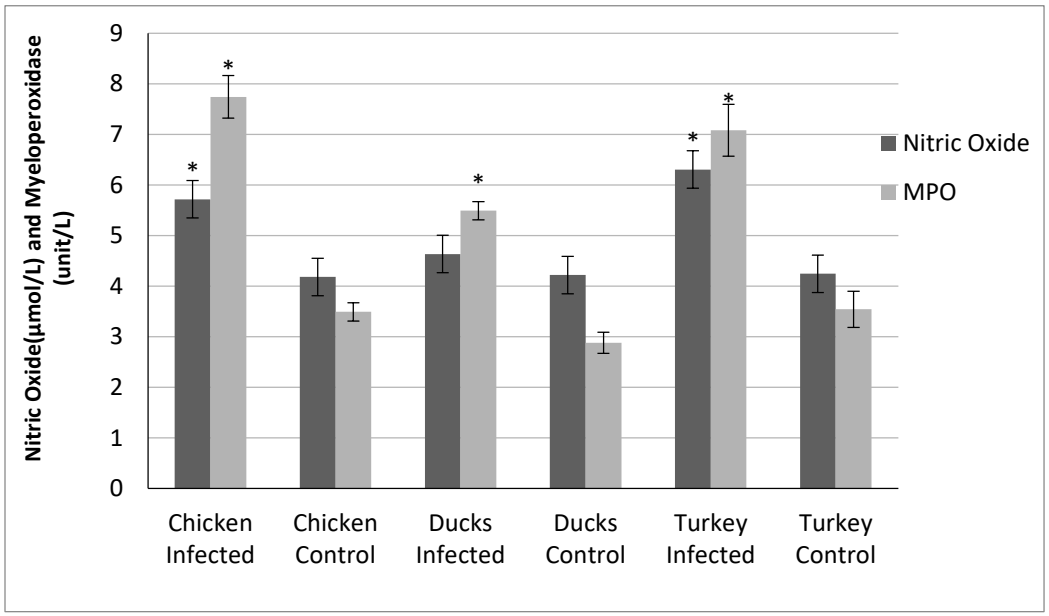

Figure 3. Activity levels (mean ± *SEM*) of Nitric Oxide (µmol/L) and myeloperoxidase (unit/L) in serum of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

Antioxidant defense system

Superoxide dismutase

In the liver, mean ± *SEM* SOD activity levels were 50.96 ± 8.3 unit/mg protein, 8.67 ± 0.22 unit/mg protein and 20.44 ± 1.74 unit/mg protein in infected chickens, ducks and turkeys, respectively, which were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 63.22 ± 2.46 unit/mg protein, 21.56 ± 2.89 unit/mg protein and 70.22 ± 8.0 unit/mg protein (Figure 4). In the kidney, SOD levels were 10.67 ± 1.18 unit/mg protein, 12.07 ± 1.26 unit/mg protein and 11.70 ± 0.77 unit/mg protein in infected chickens, ducks and turkeys, respectively, which were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 20.30 ± 1.63 unit/mg protein, 14.22 ± 1.33 unit/mg protein and 29.42 ± 2.64 unit/mg protein. Also, SOD activity levels in the bursa of Fabricius of infected chickens, ducks and turkeys were 11.56 ± 1.85 unit/mg protein, 8.59 ± 0.15 unit/mg protein and 7.00 ± 0.42 unit/mg protein with values in ducks and turkeys being significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 13.96 ± 1.32 unit/mg protein and 15.11 ± 1.78 unit/mg protein. However, there was no significant difference between the mean value in infected chickens and that of control chickens (13.00 ± 1.62 unit/mg protein). Comparing SOD activity levels in infected birds, values in liver and bursa of Fabricius of chickens were significantly higher ($P < 0.05$) than in ducks and turkeys.

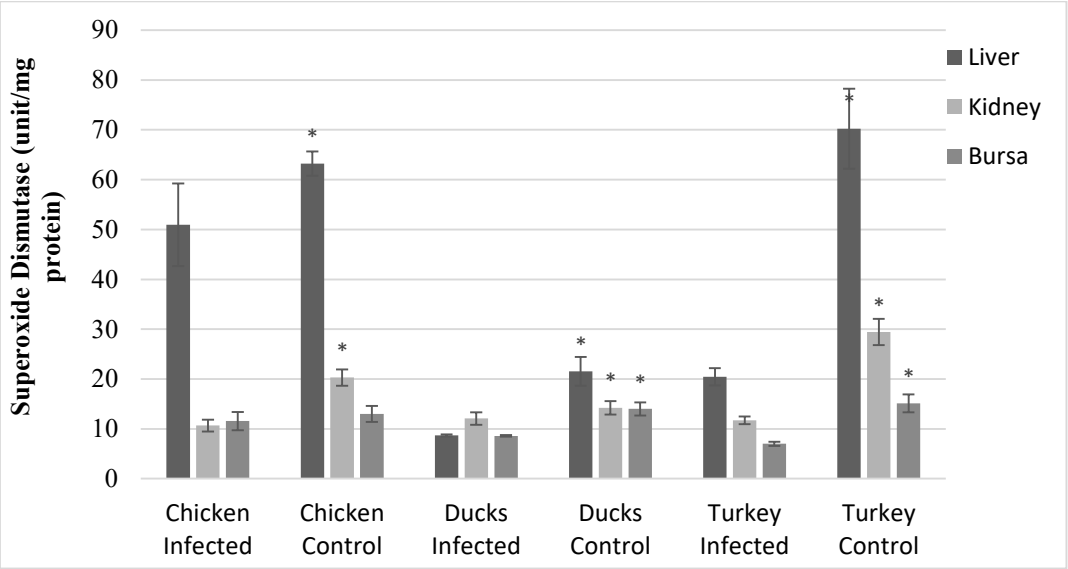

Figure 4. Activity levels (mean ± *SEM*) of superoxide dismutase (unit/mg protein) in liver, kidney and bursa of Fabricius of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

Reduced glutathione

In the liver, mean ± *SEM* GSH levels were 75.49 ± 0.76 µmol/mg protein, 72.12 ± 1.02 µmol/mg protein and 71.69 ± 0.46 µmol/mg protein in infected chickens, ducks and turkeys, respectively, which are significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 78.92 ± 0.93 µmol/mg protein, 109.00 ± 4.29 µmol/mg protein and 76.55 ± 1.21 µmol/mg protein (Figure 5). In the kidney, GSH levels were 135.01 ± 7.34 µmol/mg protein, 116.68 ± 4.39 µmol/mg protein and 113.79 ± 2.89 µmol/mg protein in infected chickens, ducks and turkeys, respectively, which are significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 171.38 ± 6.91 µmol/mg protein, 199.45 ± 5.35 µmol/mg protein and 148.09 ± 3.54 µmol/mg protein. Also, GSH levels in the bursa of Fabricius of infected chickens, ducks and turkeys were 92.30 ± 3.12 µmol/mg protein, 105.81 ± 1.59 µmol/mg protein and 93.89 ± 7.93 µmol/mg protein, while those of control subgroups were 109.13 ± 2.56 µmol/mg protein, 110.19 ± 2.98 µmol/mg protein and 102.47 ± 1.45 µmol/mg protein, respectively, being significantly lower ($P < 0.05$). Comparing infected birds, the GSH level was significantly higher ($P < 0.05$) in the kidney of chickens than those of ducks and turkeys, while it was significantly higher ($P < 0.05$) in the bursa of Fabricius of ducks than turkeys and that of chickens.

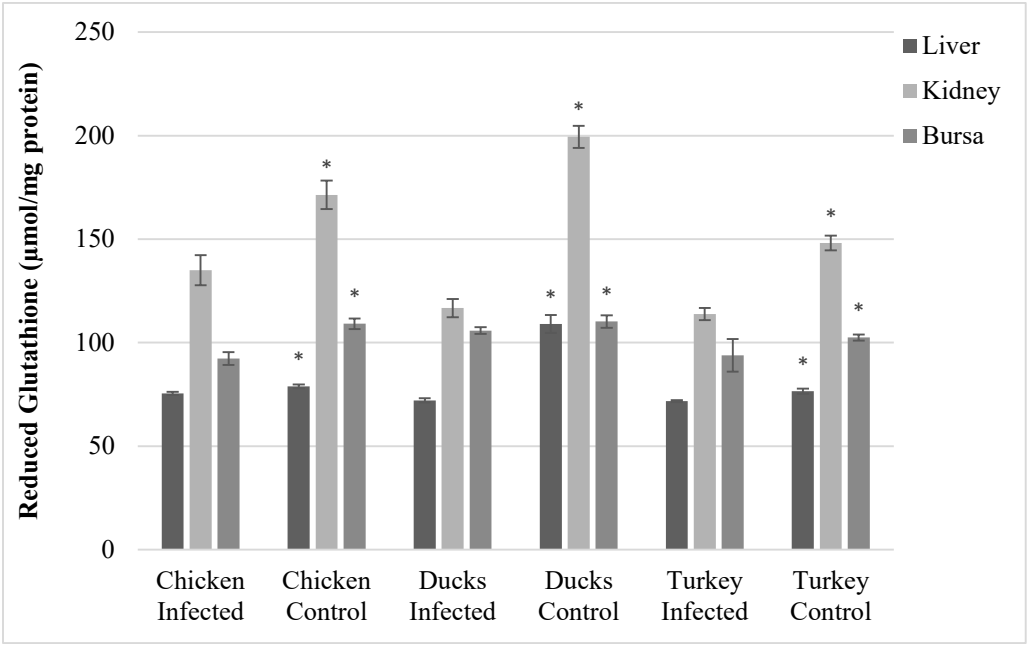

Figure 5. Concentrations (mean ± *SEM*) of reduced glutathione (µmol/mg protein) in liver, kidney and bursa of Fabricius of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

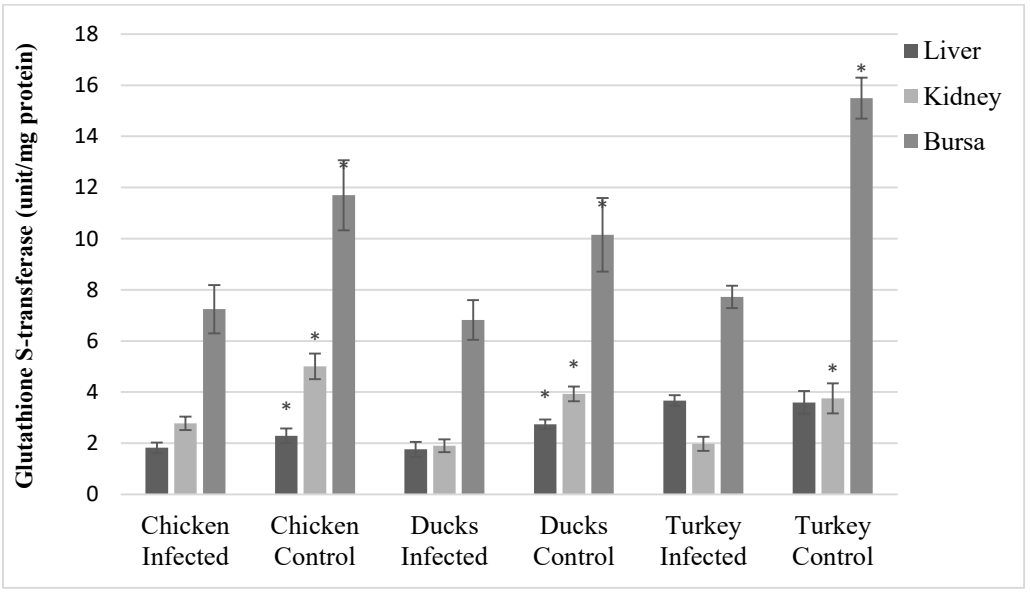

Figure 6: Activity levels (mean ± *SEM*) of glutathione S-transferase (unit/mg protein) in liver, kidney and bursa of Fabricius of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

Glutathione-S-Transferase

In the liver, mean±*SEM* GST activity levels were 1.82 ± 0.2 unit/mg protein, 1.76 ± 0.3 unit/mg protein and 3.67 ± 0.21 unit/mg protein in infected chickens, ducks and turkeys, respectively (Figure 6). Values in infected chickens and ducks were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 2.29 ± 0.29 unit/mg protein and 2.74 ± 0.18 unit/mg protein, while value in infected turkey subgroup was not significantly different ($P > 0.05$) from that of control turkey subgroup (3.59 ± 0.44 unit/mg protein). In the kidney, GST activity levels were 2.78 ± 0.26 unit/mg protein, 1.90 ± 0.25 unit/mg protein and 1.98 ± 0.28 unit/mg protein in infected chickens, ducks and turkeys, respectively, which were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 5.00 ± 0.5 unit/mg protein, 3.93 ± 0.28 unit/mg protein and 3.75 ± 0.59 unit/mg protein. Also, in the bursa of Fabricius, mean GST activity levels were 7.24 ± 0.95 unit/mg

protein, 6.82 ± 0.77 unit/mg protein and 7.72 ± 0.44 unit/mg protein in infected chickens, ducks and turkeys, respectively, which are significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 11.70 ± 1.37 unit/mg protein, 10.15 ± 1.44 unit/mg protein and 15.49 ± 0.8 unit/mg protein. In addition, the GST activity level was significantly higher ($P < 0.05$) in the liver of infected turkeys than in infected chickens and ducks, while the level in the kidney of infected chickens was significantly higher ($P < 0.05$) than in infected ducks and turkeys.

Glutathione peroxidase

In the liver, mean ± *SEM* GPx activity levels were 24.21 ± 0.94 unit/mg protein, 26.81 ± 2.45 unit/mg protein and 30.01 ± 2.18 unit/mg protein in infected chickens, ducks and turkeys, respectively, which were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 37.54 ± 2.72 unit/mg protein, 41.07 ± 1.7 unit/mg protein and 47.89 ± 1.04 unit/mg protein (Figure 7). In the kidney, mean ± SEM GPx activity levels were 48.38 ± 2.51 unit/mg protein, 50.80 ± 2.73 unit/mg protein and 48.05 ± 0.68 unit/mg protein in infected chickens, ducks and turkeys, respectively, which were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 68.69 ± 2.85 unit/mg protein, 70.17 ± 2.67 unit/mg protein and 50.41 ± 1.59 unit/mg protein. Also, in the bursa of Fabricius, mean ± SEM GPx values were 83.63 ± 4.62, unit/mg protein 81.85 ± 4.42 unit/mg protein and 92.79 ± 3.38 unit/mg protein in infected chickens, ducks and turkeys, respectively, which were significantly lower ($P < 0.05$) than values in their respective control subgroups, i.e., 100.12 ± 3.26 unit/mg protein, 89.92 ± 4.01 unit/mg protein and 103.80 ± 3.2 unit/mg protein. Comparing the three species, GPx was significantly higher in the liver of infected ducks and turkeys than in infected chickens. Also, the level is significantly higher in the bursa of Fabricius of turkeys than in ducks and chickens.

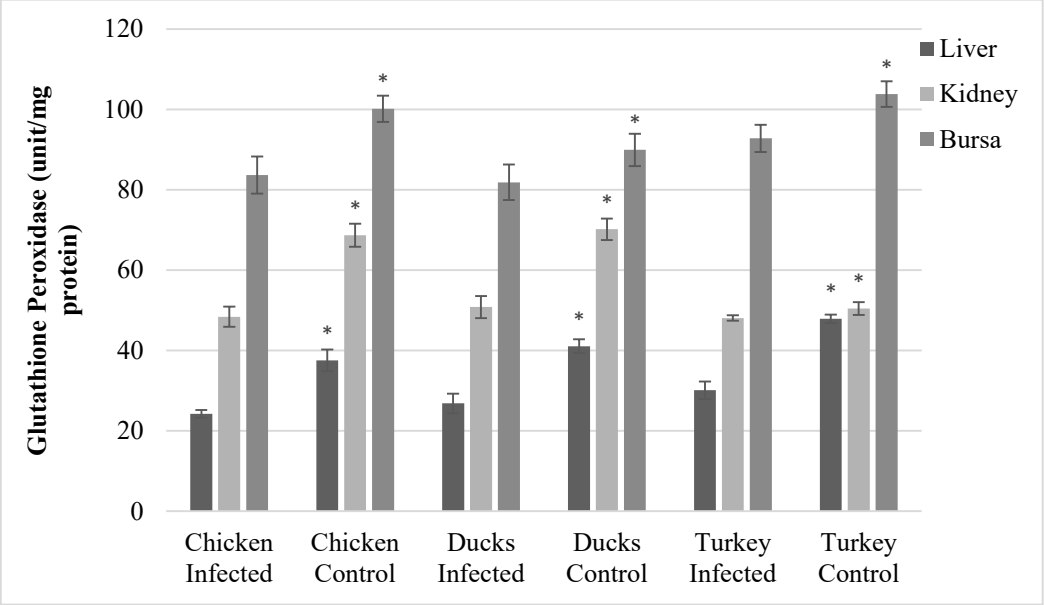

Figure 7: Activity levels (mean ± *SEM*) of glutathione peroxidase (unit/mg protein) in liver, kidney and bursa of Fabricius of chickens, ducks and turkeys infected with infectious bursal disease virus 5 days post-inoculation. (*indicates a significant difference between infected and control subgroups of the same species at $P < 0.05$).

Discussion

The results of this study showed significantly higher levels of MDA in the liver, kidney and bursa of Fabricius of infected chickens, turkeys and ducks in comparison to control birds which is expected, as viral infections are known to bring about oxidative stress (Rehman *et al.,* 2018; Amini *et al.,* 2022). MDA is one of the final products of polyunsaturated fatty acids peroxidation in cells. Once free radicals are increased, there is over production of MDA (a marker of oxidative stress). A significantly higher level of MDA in the liver and kidney of infected chickens than in infected ducks and turkeys and a significantly higher level of H_2O_2 in the bursa of Fabricius of infected chickens than in infected ducks and turkeys signify a higher level of oxidative stress in infected chickens (Gawel *et al.,* 2004). It was established by Oladele *et al.* (2009) that the liver and kidneys are affected during IBD virus infection in chickens. Thus, the detection of significantly higher levels of MDA in these organs in infected chickens than in infected ducks and turkeys signifies cellular damage, which occurred at lower severity in ducks and turkeys. At inappropriate concentrations, H_2O_2

being an oxidizing agent, triggers apoptosis by causing arrest during cell cycle proliferation, which eventually results in cell death (Heo *et al.,* 2020). Previous studies have shown that IBD virus infection causes apoptosis, which is responsible for the rapid depletion of lymphocytes in the bursa of Fabricius, resulting in immunosuppression (Khatri and Sharma, 2009; Qin *et al.,* 2017). Thus, the significantly higher level of H_2O_2 in the tissues of infected chickens in comparison to those of infected ducks and turkeys is in sync with the pathogenesis of IBD in chickens.

Nitric oxide (NO) concentration and MPO activity in the blood of infected chickens was significantly higher than in ducks in this study. Nitric oxide and MPO are signaling molecules that play key roles in the pathogenesis of inflammation (Nicholls and Hazen, 2005; Sharma *et al.,* 2008). Although NO is considered a pro-inflammatory mediator due to overproduction in abnormal situations, it gives an anti-inflammatory effect under normal physiological conditions (Sharma *et al.,* 2007). The significantly lower concentration in IBD virus-infected ducks compared to infected chickens shows that the duck host experienced less inflammation post-infection.

Superoxide dismutase (SOD) is an important enzyme family in living cells that is responsible for maintaining normal physiology despite stress. It, therefore, protects the integrity of cells and tissues when exposed to harmful substances (Otitoju *et al.,* 2008), including during viral infections. It protects oxygen-metabolizing cells against the deleterious effects of superoxide radicals (Madi *et al.,* 2016). The levels of SOD in the liver, kidney, and bursa of Fabricius of IBD virus-infected chickens, ducks and turkeys were found to be significantly lower than in their corresponding control birds which indicates a positive antioxidant defense system in the control birds in comparison with infected ones. However, infected chickens that exhibited typical clinical signs of IBD had significantly higher levels of SOD in the liver and bursa of Fabricius in comparison with infected ducks and turkeys, which did not exhibit clinical signs. A high level of SOD was probably produced in infected chickens in response to high levels of superoxide radicals produced due to infection in an attempt to protect cellular and tissue integrity (Otitoju *et al.,* 2008).

The results of this study showed that IBD virus-infected birds had significantly lower levels of GSH in the studied organs, which is likely to be due to depletion following excessive production of free radicals, post-infection. Also, the bursa of Fabricius, being the known target organ for IBD virus with extensive cellular destruction in infected chickens, had significantly lower levels of GSH than in infected ducks and turkeys which is also believed to be due to the depletion of GSH in the bursa of Fabricius of chickens, post-infection. Depletion of the antioxidant GSH is a major index of oxidative stress which initiates a cascade of events that ultimately leads to cell death (Mytilineou *et al.,* 2002).

Also, it was observed that IBD virus-infected birds generally had significantly lower levels of GST and GPx in comparison to the non-infected groups of the same species. GST and GPx are known to catalyze the detoxification of a variety of endogenous and exogenous electrophilic compounds and peroxides via conjugation of GSH (Townsend *et al*., 2003) to form water-soluble substances. However, the kidney level of GSH was significantly higher in infected chickens than in infected ducks and turkeys, so also was the GST level which could be an innate response to the active IBD virus infection in the experimental chickens, i.e., an attempt by the infected chickens to alleviate the viral-induced oxidative stress to balance the redox status of the body. The significantly higher levels of GPx in the liver of infected ducks and turkeys than in infected chickens, as well as in the bursa of Fabricius of infected turkeys than in infected chickens, probably indicates a higher antioxidant status in infected ducks and turkeys with the resultant protection against clinical IBD virus infection.

Conclusion

This study has shown that chickens undergo a higher degree of oxidative stress post-BD virus infection in comparison to ducks and turkeys, as exhibited by significantly higher levels of MDA, H_2O_2, NO and MPO in the tissues and blood of infected chickens. Concurrently, the antioxidant defense system was found to be generally more active in infected ducks and turkeys than chickens with significantly higher levels of GPx in the liver and bursa of Fabricius. However, there appears to be an attempt by infected chickens to balance the redox status of the body, as shown by the significantly higher levels of SOD in the liver and bursa of Fabricius as well as GST and GSH in kidney tissues. Thus, ducks and turkeys appear to be less susceptible to IBD virus-induced oxidative stress; as such, chickens could benefit from diet supplementation with antioxidant-rich feed inclusions. It is therefore suggested that the inclusion of exogenous antioxidants, such as Vitamins C and E, carotenoids and polyphenols, in the feed of chickens should be considered as an adjunct in the control of infectious bursal disease.

Acknowledgement

The authors acknowledge the support given by I.A. in the care of experimental birds.

References

Amini MA, Karimi J, Talebi SS & Piri H. 2022. The association of COVID-19 and reactive oxygen species modulator 1 (ROMO1) with oxidative stress. Chonnam Medical Journal, 58: 1–5. DOI: 10.4068/cmj.2022.58.1.1

AVMA. 2020. American Veterinary Medical Association Guidelines for the euthanasia of animals. 2020 Edition. AVMA. IL. 121 pages. https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf

Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO & Emerole GO. 2000. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron—A Garcinia kola seed extract. Food and Chemical Toxicology, 38(6):535-541. DOI: 10.1016/S0278-6915(00)00039-9

Gawel S, Wardas M, Niedworok E & Wardas P. 2004. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomości Lekarskie Medical Advances, 57(9-10): 453-5. PMID: 15765761

Gornall AG, Bardawill CJ & David MM. 1949. Determination of serum proteins by means of the biuret reaction. Journal of Biological Chemistry, 177:751–766. DOI: 10.1016/S0021-9258(18)57021-6

Habig WH, Pabst MJ & Jakoby WB. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry, 249(22):7130-7139. DOI: 10.1016/S0021-9258(19)42083-8

Heo S, Kim S, Kang D. 2020. The role of hydrogen peroxide and peroxiredoxins throughout the cell cycle. Antioxidants, 9: 280. DOI: 10.3390/antiox9040280

Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology, 11:151–169. DOI: 10.1159/000136485

Kegne T, Chanie M. 2014. Review on the incidence and pathology of infectious bursal disease. British Poultry Science, 3(3): 68-77. DOI: 10.5829/idosi.bjps.2014.3.3.8556

Khatri M & Sharma JM. 2009. Response of embryonic chicken lymphoid cells to infectious bursal disease virus. Veterinary Immunology and Immunopathology, 127: 316–324. DOI: 10.1016/j.vetimm.2008.10.327

Ley DH, Yamamoto R & Bickford AA. 1983. The pathogenesis of infectious bursal disease: Serologic, histopathologic and clinical chemical observations. Avian Diseases, 27(4):1060–85. PMID: 6316894.

Madi M, Babu S, Kumari S, Shetty S, Achalli S, Madiyal A & Bhat M. 2016. Status of Serum and salivary levels of superoxide dismutase in type 2 diabetes mellitus with oral manifestations: A case control study. Ethiopian-Journal of Health Sciences, 26(6): 523-532. DOI: 10.4314/ejhs.v26i6.4

Migdal C & Serres M. 2011. Reactive oxygen species and oxidative stress. Medical Sciences, 27(4):405-12. DOI: 10.1051/medsci/2011274017

Misra HP & Fridovich I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry, 247 (10): 3170-3175. DOI: 10.1016/S0021-9258(19)45228-9

Mytilineou C, Kramer BC & Yabut JA. 2002. Glutathione depletion and oxidative stress. Parkinsonism & Related Disorders, 8(6): 385-7. DOI: 10.1016/s1353-8020(02)00018-4

Nicholls SJ & Hazen SL. 2005. Myeloperoxidase and cardiovascular disease. Arteriosclerosis, Thrombosis, and Vascular Biology, 25: 1102-1111. DOI: 10.1161/01.ATV.0000163262.83456.6d

Oladele OA, Adene DF, Obi TU, Nottidge HO & Aiyedun AI. 2005. Sequential hematological study of experimental infectious bursal disease virus infection in chickens, turkeys and ducks. Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux, 58(4):211-5. DOI : 10.19182/remvt.9914

Oladele OA, Adene DF, Obi TU & Nottidge HO. 2009. Comparative susceptibility of chickens, turkeys and ducks to infectious bursal disease virus using immunohistochemistry. Veterinary Research Communications, 33: 111–121. DOI: 10.1007/s11259-008-9078-2

Olaleye SB, Adaramoye OA, Erigbali PP & Adeniyi OS. 2007. Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats. World Journal of Gastroenterology, 13:5121–6. DOI : 10.3748/wjg.v13.i38.5121

Otitoju O, Onwurah INE, Otitoju GTO & Ugwu CE. 2008. Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin. Biokemistri, 20(2): 93-98. http://www.bioline.org.br/bk

Oyagbemi AA, Omobowale TO, Asenuga ER, Adejumobi AO, Ajibade TO, Ige TM, Ogunpolu BS, Adedapo AA & Yakubu MA. 2016. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Journal of Environmental Toxicology, 10 (2): 1089-1101. DOI: 10.1002/tox.22306

Qin Y, Xu Z, Wang Y, Li X, Cao H & Zheng SJ. 2017. VP2 of infectious bursal disease induces

apoptosis via triggering oral cancer overexpressed 1(ORAOV1) protein degradation. Frontiers in Microbiology, 8: 1-19. DOI: 10.3389/fmicb.2017.01351

Rehman ZU, Meng C, Sun Y, Safdar A, Pasha RH, Munir M & Ding C. 2018. Oxidative Stress in Poultry: Lessons from the viral infections. Oxidative Medicine Cellular Longevity, 2018. DOI: 10.1155/2018/5123147,

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG & Hoekstra WG. 1973. Selenium: Biochemical role as a component of Glutathione peroxidase. Science, 179 (4073):588-590. DOI: 10.1126/science.179.4073.588

Sharma JN, Al-Omran A & Parvathy SS. 2007. Role of nitric oxide in inflammatory diseases. Inflammopharmacology, 15(6): 252-9. DOI: 10.1007/s10787-007-0013-x

Sharma RK, Agrawal M & Marshall F. 2008. Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. Environmental Pollution, 154:254–263. DOI: 10.1016/j.envpol.2007.10.010

Townsend DM, Tew KD & Tapiero H. 2003. The importance of glutathione in human disease. Biomedicine Pharmacotherapy, 57(3-4):145-55. DOI: 10.1016/s0753-3322(03)00043-x

Wolff SP. 1994. Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. Methods in Enzymology, 233:182-189. DOI: 10.1016/S0076-6879(94)33021-2

Xia Y & Zweier JL. Measurement of myeloperoxidase in leukocyte-containing tissues. Analytical Biochemistry, 245:93-96 (1). DOI: 10.1006/abio.1996.9940