Faculty of Natural and Applied Sciences Journal of Applied Biological Sciences
Print ISSN: 3027-0685, e-ISSN: 3043-6494
www.fnasjournals.com
Volume 2; Issue 4; December 2025; Page No. 8-19.
DOI: https://doi.org/10.63561/jabs.v2i4.1002

Comparative Evaluation of Serum Oxidative Stress in Indigenous Chickens Administered Newcastle Disease (Lasota) Vaccine With and Without Multivitamin–Mineral Supplementation

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Abstract
This study investigated the hematological and biochemical responses of three strains of exotic birds (Isa brown, Broiler and Noiler) subjected to vaccination errors induced by the ND-LASOTA vaccine. The research was conducted over a period of eight weeks at the Poultry Unit of the Teaching and Research Farm, Faculty of Agriculture, Niger Delta University. The experiment lasted for eight weeks following a stabilization period of two weeks. A total of 75 day-old unsexed birds were purchased from Federal University of Agriculture Abeokuta Ogun State. The birds were subjected to a range of hematological and biochemical analyses at various time points after the vaccination. The experiment followed a Completely Randomized Block Design, with three treatment groups and three control groups representing different strains of birds. The results revealed variations in hematological parameters, including hemoglobin levels, packed cell volume, red blood cell count, white blood cell count, and different white blood cell percentages following vaccination errors. The study also identified significant strain-specific differences in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicating potential stress-induced liver damage due to vaccination errors. The findings suggest that vaccination errors can impact the hematological and biochemical responses of exotic birds, leading to strain-specific variations and potential liver stress. It is recommended that vaccination protocols be refined to minimize errors, strain-specific responses be investigated further, stress management strategies be implemented during vaccination, and long-term monitoring be conducted to assess the prolonged effects of vaccination errors. Additionally, further research is needed to elucidate the mechanisms underlying strain-specific variations and stress-related impacts on bird health.

Keywords: Comparative, Supplementation, Evaluation, Multivitamin, Haematological

Introduction

In Nigeria, the National Animal Production Research Institute located in Zaria first started poultry breeding (Adebambo,1992), were reports on research about local chickens had started with comprehensive information on the local chickens. They are small in size and grow slowly (Ajayi, 2010, and have been reports on its potential for egg and meat production (Nwosu, 1979: Adebambo, 2005). Indigenous chickens (*Gallus gallus domesticus*) serve as a source for generating economic for households (Ekei *et al*., 2019). There has been an increase in the demand for local chickens over time due to their protein source via meat and egg consumption, which is affordable (Al-Garib et al., 2003; Apunu et al., 2011). 90% of Nigerians poultry are chickens, which in the world are the most important poultry species (Barrow, 2000).

Poultry chickens are about 98% of the entire poultry population (ducks, turkeys and Guinea fowls) kept in the African region (Gueye, 2005). Out of the 150 million chickens in Nigeria, 120 million are local and 30 million are exotic (RIM, 1992). Indigenous chickens are found everywhere in the rural environments of Nigeria where they are kept by the majority of the rural poor individuals. A lot of investigations made by (Adene, 1990; Dafwang, 1990 and Nwosu, 1990) reported that each rural household have an average of 11 to 34 indigenous

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chickens within their living environment. RIM, 1992 discovered that the frequency distribution of the normal feathered chicken variety is about 91.81% while those of frizzled and naked neck varieties are about 5.2% and 3.0% respectively. Their meat and produce of the local chickens are preferred by the majority of Nigerians because of the pigmentation, taste, leanness and suitability for special dishes, Adebambo et al., 2015. Their products (eggs and meat) are the major sources of protein and also serve as sources of income for the rural, urban and semi-urban dwellers (Horst, 1989). Studies (Udoh et al., 2012b) reveal the varying egg laying characteristics of these varieties, with the naked neck variety performing best in egg weight, egg number, pause length and feed efficiency. There is need to improve the productivity of the Nigerian chicken varieties that are up till now characterized by small body weight, small egg size and few egg numbers (Udoh et al., 2012b). Nigerian indigenous chickens are very important reservoirs of useful genes and they have possessed a number of adaptive genetic traits (Horst, 1989). They are widely produced and distributed in the rural areas where they are kept by the majority of the rural dwellers and the local communities. They are generally hardy, adaptive to rural environments, survive on little or no inputs and adjust to fluctuations in feed availability. Nigerian indigenous chickens are found scattered in hamlets, villages, towns and cities (Oni, 1999). Nigerian indigenous chickens lay 8-9 eggs/clutch within a laying period of 12-14 days. Rearing period is 64-70 days and the age at point of lay is 32-36 weeks (Ikeobi et al., 1996).

Newcastle Disease (ND) also known as pseudo-fowl plague, is an infectious disease of poultry species that is caused by virulent strains of Avian Paramyxovirus -1, which is a single strand non segmented negative sense RNA virus. The ND is an economically important disease and also a major threat to poultry industry. It is a highly contagious viral disease that can affect a large number of avian species and cause severe economic losses (Dzogbema et al., 2021). The Newcastle disease can infect more than two hundred different species of birds (Rauw et al., 2009). This disease is capable of causing 100% rate of mortality and morbidity (Patti, 2016). Newcastle disease has been identified as a major constraint to the development of poultry farming (Dzogbema et al., 2021).

Clinical diagnosis based on history, signs and lesions in addition hemagglutination and hemagglutination inhibition test, virus neutralization test, Enzyme linked immune-sorbent assay, plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of the ND virus. The transmission of NDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing (Patti, 2016). Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhea, depression and in appetence, partial or complete drop in egg production and an increased production of deformed eggs. Gross and microscopic lesions as with clinical signs, the organs affected in birds infected with NDV are dependent on the strain and pathotype of the infecting virus, in addition to the host and all the other factors that may affect the severity of the disease (Patti, 2016).

The redox equilibrium is very important in sustaining or preserving the correct functionality of cellular vital functions (Valko et al., 2007). Oxidative stress is expressed as an imbalance in the redox characteristics of some cellular environmental locations which can be the result of either biochemical processes leading to the production of reactive species, exposure to damaging agents (i.e., environmental pollutants and radiations), or limited capabilities of endogenous antioxidant systems (Bickers and Athar, 2006; Hodjat et al., 2015). Oxidative stress leads to lipid peroxidation, protein nitration, DNA damages and apoptosis. Oxidative stress is linked with heat stress, which is a combination of high environmental temperature and humidity hindering proper thermoregulation processes. Reactive oxygen and nitrogen species (ROS/RNS) produced under oxidative stress are known to damage all cellular biomolecules (lipids, sugars, proteins, and polynucleotides) (Negre-Salvayre et al., 2010; Roberts et al., 2010). Thus, several defense systems have been involved within the cells to prevent uncontrolled ROS increase. These systems include nonenzymatic molecules (glutathione, vitamins A, C, and E, and several antioxidants present in foods) as well as enzymatic scavengers of ROS, with superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) being the best-known defense systems (Valko et al., 2007). Mitochondria are the predominant source of ROS in all cell types (Musatov & Robinson, 2012). Superoxide (O_2^-) is mainly generated at the level of the mitochondrial electron transport chain and it can also be converted to hydrogen peroxide (H_2O_2) by SOD or undergo spontaneous dismutation (Valko et al., 2007). In the presence of transition metal ions, for example, iron and copper ions, H_2O_2 can generate via Fenton reaction the highly reactive hydroxyl radical ($HO^{\bullet}$). Reactive species may also be enzymatically produced by xanthine

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oxidase (XO), uncoupled nitric oxide synthases (NOS), and NADPH oxidase (NOX). ROS production is related not only to cell damage or death, but physiological and signaling roles for ROS have also been ascertained. Reactive species are the principal source of defensive pro-oxidants generated in the respiratory burst of neutrophils (El-Benna et al., 2016; Jaganjac et al., 2016). Over the years, stress is used to describe detrimental effects of a variety of conditions surrounding animal on health performance. Stress in poultry results from elements of transportation, alteration of atmospheric temperature, poor handling by man, removal of feed and water (starvation), relative humidity (RH), ambient temperature (AT), and other factors (vaccination and disease condition), which falls under these factors, environmental, nutritional, microbiological and management factors. These factors negatively affect poultry health and production (Ahmadu et al., 2015). Oxidative stress markers are important tools to assess the biological redox status, disease state and progression, and the health-enhancing effects of antioxidants in humans. However, for some of stress markers, there is a lack of consensus concerning validation, standardization, and reproducibility (RIM, 1992).

Materials and Methods

Experimental Site and Duration of Study

This experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Niger Delta University, Wilberforce Island Bayelsa state of Nigeria. The Wilberforce Island is geographically located with latitude 4°51N and 5°23S and longitude 5°22W and 6°45E. The experiment lasted for ten (10) weeks, with four (4) weeks brooding, one (1) week acclimatization period and five (5) weeks of experiments.

Experimental Bird and Management

90-day old chicks where purchased from Federal University of Agriculture (FUNNAB) Abeokuta, Ogun States, Nigeria, in which were, thirty (30) Frizzled feather (FF), thirty (30) Normal feather (NF) and thirty (30) Naked neck (NN). The birds were assigned into Split split plot Design, distributed into three (3) treatment groups, T1=Frizzled feather, T2= Normal feather, T3= Naked neck. 30 birds for each treatment, with subunits of 10 birds given multivitamins, 10birds with no vitamin and 10 control. This experiment lasted for a period of 10weeks.

Housing management

The chicks were brooded for four weeks in a deep litter system of fresh wood shavings measuring 1.50m X 1.50m. The poultry house was properly cleaned with Izal, germicide and water prior to the arrival of the birds. Electric bulbs of 200 watts were used on each of the pen. In addition, charcoal stoves were also used during power failure as source of heat for the birds. All birds were fed the same starter diet containing 2.80/kg ME and 23.75% crude protein. Prior to the commencement of the experiment, birds were weighed to obtain their initial body weight and subsequently in weekly basis. Feeds were given *ad-libitum*, vaccination and other routine practices, includes daily inspection of birds for symptoms of diseases, mortality, cleaning of troughs and supply of feed and fresh clean water were maintained.

Feeding

Birds were fed with starter ration (20%CP and 2800Kcal ME/kg) from day old to fourth(4^{th}) week of age while the grower ration (16%CP and 2750Kcal ME/Kg) from fifth (5^{th}) to seventh (7^{th}) week of age and the finisher ration were given from the eighth (8^{th}) to ninth (9^{th}) week of age (18-19%CP and 3200Kcal/kg). All feeds were purchased from Feed house located in Yenagoa, Bayelsa State. Feeding and water were supplied *ad-libitum* for all birds both the experimental and control.

Experimental bird preparation and method at which Newcastle disease vaccine and multivitamin was administered

A 150g Vitamix was purchased from veterinary shop in Yenagoa, Bayelsa State and was administered at the 5^{th} week.2g of the Vitamix was added to 1L of drinking water daily and given to the birds under multivitamin treatment throughout the 5^{th} week. At the 6^{th} week 100 dose LaSota Newcastle vaccine was purchased from veterinary shop in Yenagoa, Bayelsa State and was stored in a cool place. The 100 dose of LaSota vaccine was mixed in a one liter of pure drinking water and about 10g of powdered milk was also added in to the water, this was to enhance the vaccine and thereafter was shared equally into the drinkers and served to the experimental birds. Multi-vitamin (Vitamix) was given at the 5^{th} week and all experimental birds, excluding the controls were vaccinated on the 6^{th} week.

Blood Collection

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Initially, at the 5th week blood samples were collected from chickens selected randomly before vaccination was conducted and also on the 6th week of age, blood sample were collected from the jugular vein, through a venepunture, using 2ml syringes and hypodermic needles. The blood collected were about 1-2ml from individual birds weekly. This was done for both control and experimental birds. The blood samples were collected at the 5th week, 6th week, 7th week, 8th week, 9th week and 10th week respectively and all samples were labelled correctly.

Blood sample for serum collection

Blood samples were collected in plain bottles and were allowed to rest overnight for the blood to coagulate and the serum was collected the following day and transferred to new plain bottles and store in a freezer until ready to use. Serum samples were collected at the 5thweek, 6thweek, 7th week, 8th week 9th week and 10th week for both experimental birds and control.

Statistical Analysis

All data collected from the experiment were analyzed using one-way analysis of variance procedure of Statistical Analysis System.

Results

Serum oxidative stress in three strains of Nigerian indigenous chickens given Newcastle Disease vaccine (Lasota) with or without multivitamin and mineral supplementation for week 1

The level of GSH was high in the three local chicken in week one. While the other pro-oxidants and anti-oxidant were generally low under vitamin supplementation. Similar trend was observed in the local chickens with no vitamin supplementation and control at week 1 (Fig 4.1).

Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (Lasota) with or without multivitamin and mineral supplementation for week 2

There is increase in the level of reduced glutathione (GSH) and H2O2 in the FF local chicken at week 2 with vitamin supplementation (Fig 4.2). Similar observation was observed for increased GSH level in the local chicken (NF) than in NN and FF. The antioxidant levels were very low in the local chickens with no vitamin supplementation and control at week 2 (Fig 4.2).

Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (Lasota) with or without multivitamin and mineral supplementation for week 3

The activity of reduced glutathione was significantly high in the three local chicken with vitamin supplementation than with no vitamin supplementation and control at week 3 and a balance between the pro-oxidant and antioxidant was maintained (Fig 4.3).

Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation for week 4

The activity of glutathione (GSH) was high in the NF local chickens with vitamin supplementation than with no vitamin and control at week 4. There was slight increase in H2O2in the three local chicken more without vitamin supplementation and control (Fig 4.4).

Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation for week 5

The activity of glutathione (GSH) was remarkable high in the local chickens with vitamin supplementation than with no vitamin supplementation and control at week 5. There was also a slight increase in the frizzle feather (FF) local chicken in the control.

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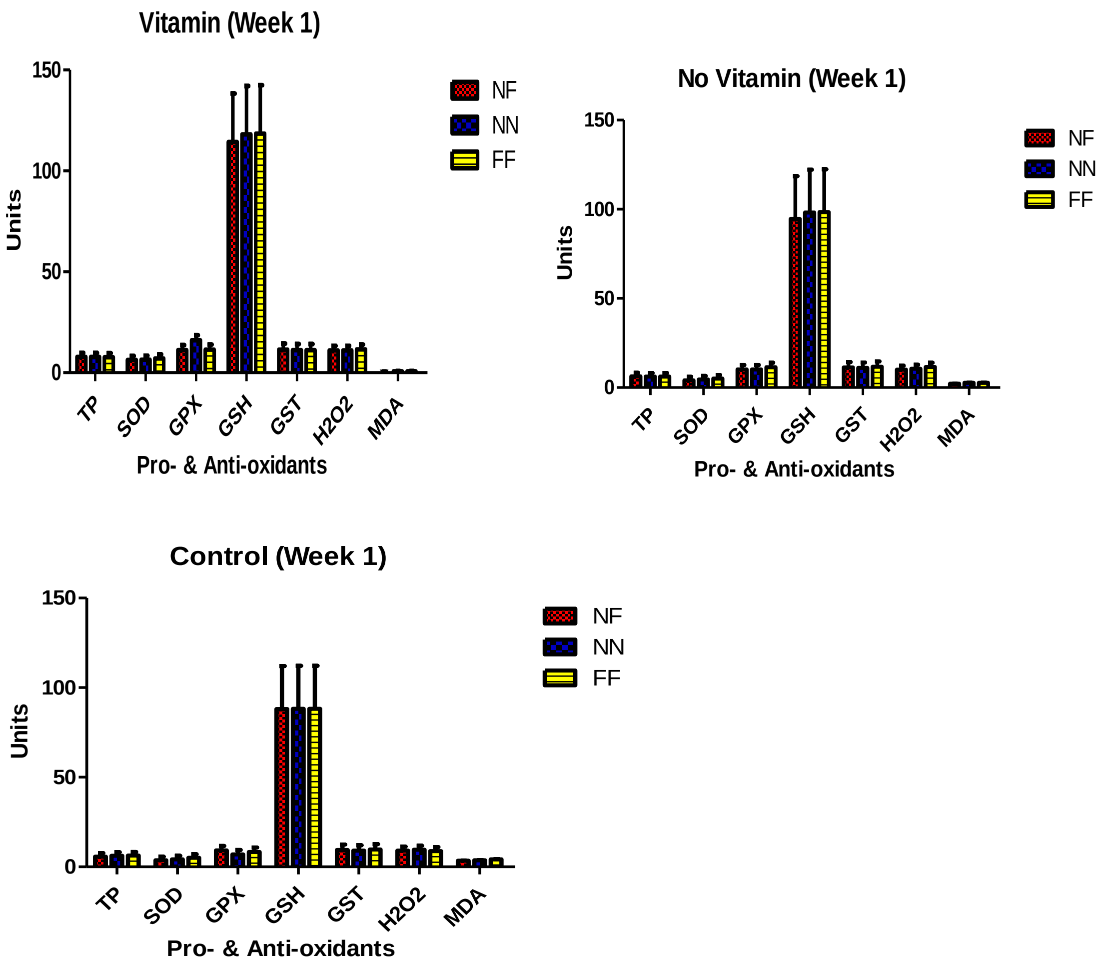
Figure 1: Showing serum oxidative stress in three strains of Nigerian indigenous chickens given Newcastle Disease vaccine (Lasota) with or without multivitamin and mineral supplementation for week 1



NN: Normal feather

FF: Frizzle feather

NF: Normal feather

- TP - Testosterone propionate
- SOD - Superoxide dismutase
- GPx - Glutathione peroxidase
- GSH - Glutathione
- GST- Glutathione S-transferance
- H_2O_2 - Hydrogen peroxide
- MDA - Malondialdehyde

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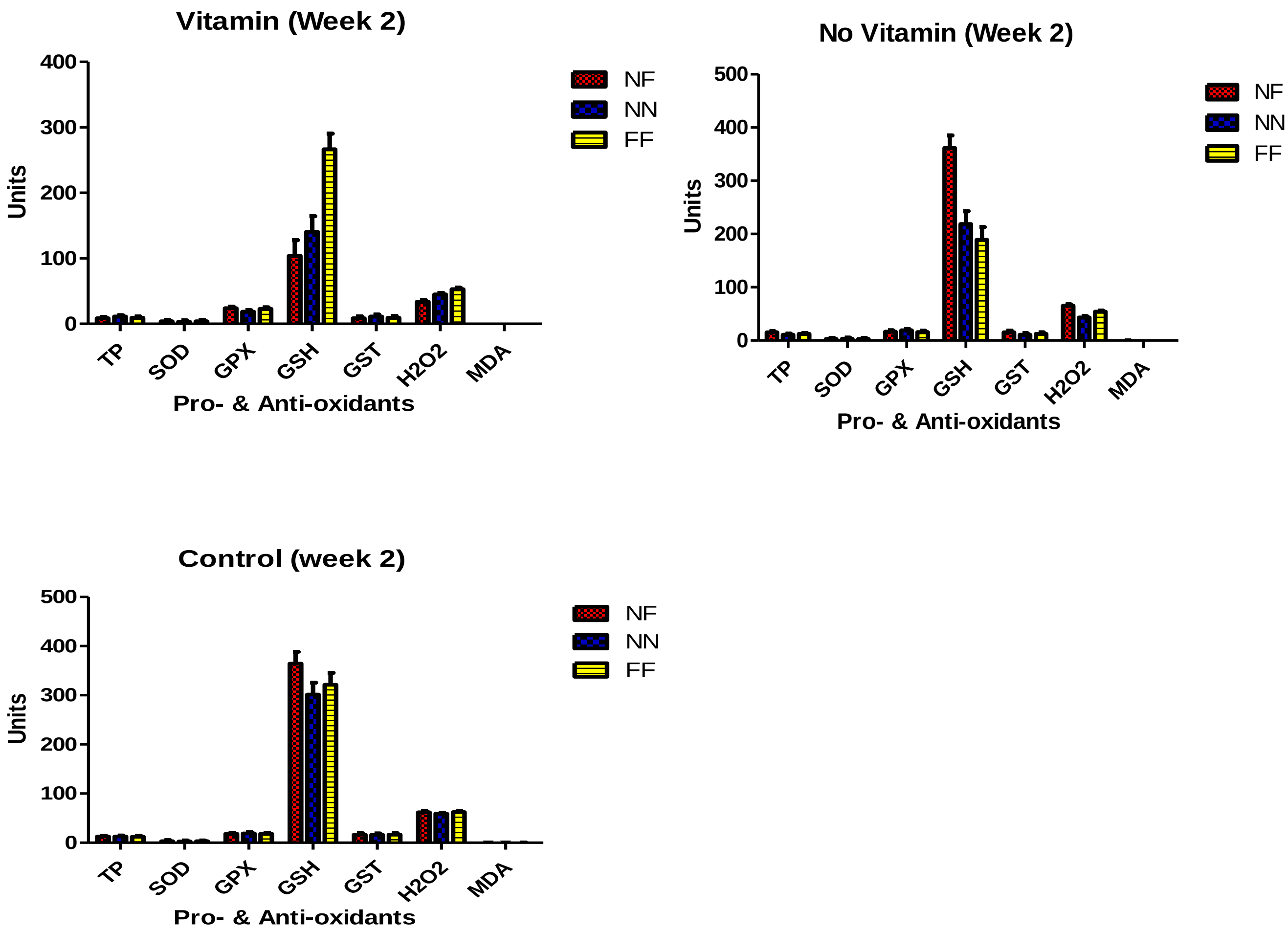
Figure 2: Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation



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- MDA - Malondialdehyde

Key	
NF	Normal feather
NN	Naked neck
FF	Frizzled feather

Figure 3: Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation

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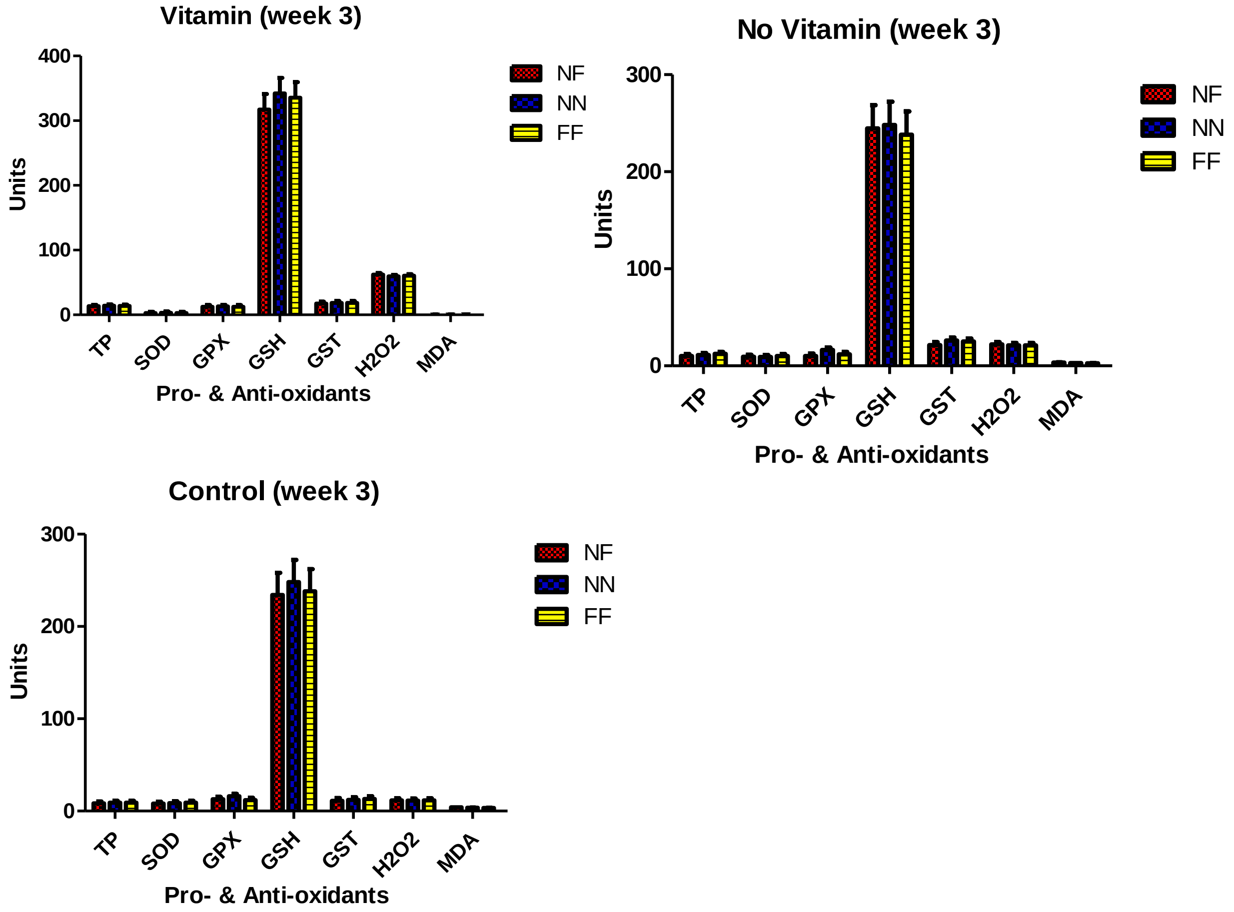

Key	
NF	Normal feather
NN	Naked neck
FF	Frizzled feather

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- SOD - Superoxide dismutase
- GPx - Glutathione peroxidase
- GSH - Glutathione
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Figure 4: Showing serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation

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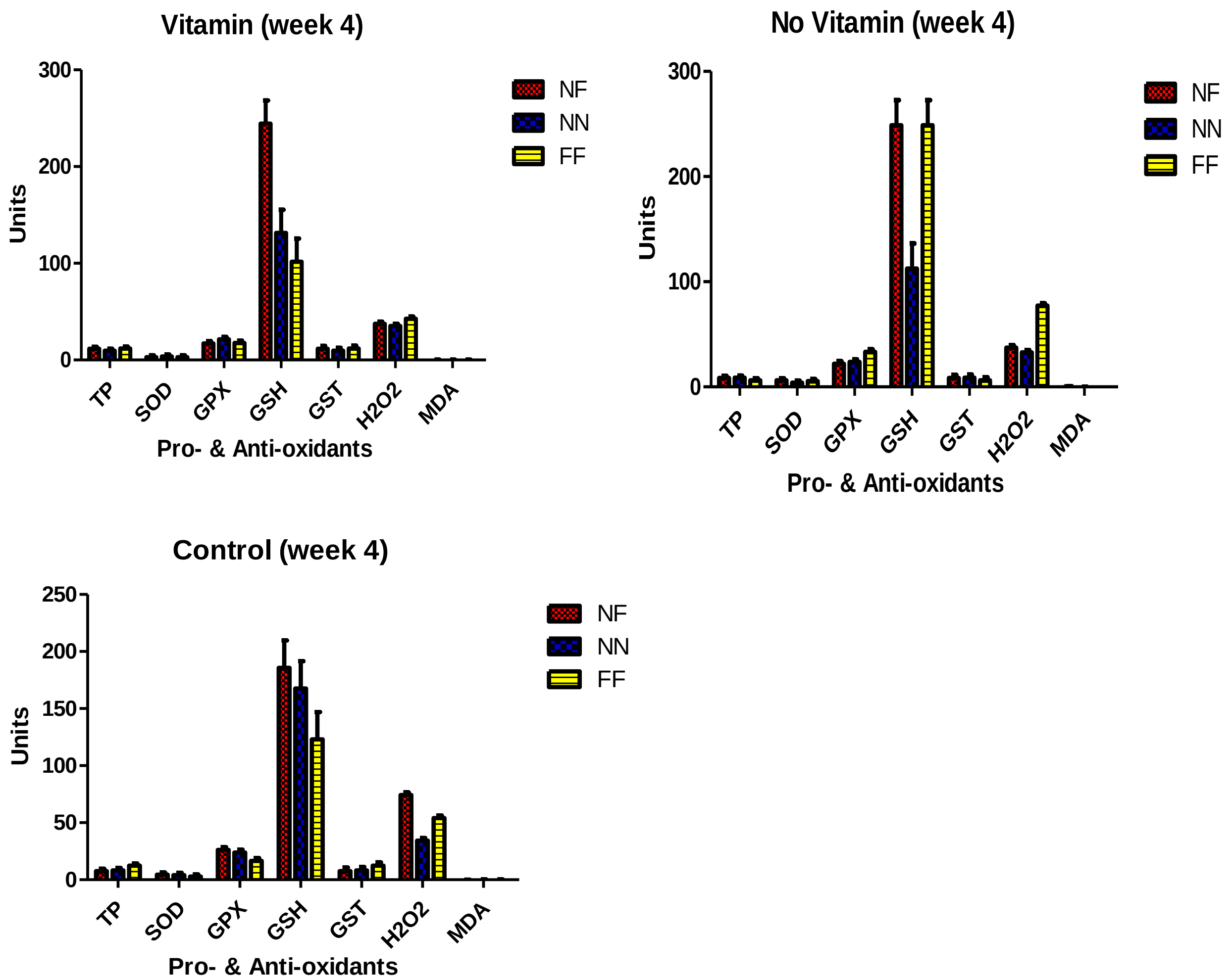

Key	Indigenous chicken
NF	Normal feather
NN	Naked neck
FF	Frizzled feather

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Figure 5: Serum oxidative stress in three strains of indigenous chickens given Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation

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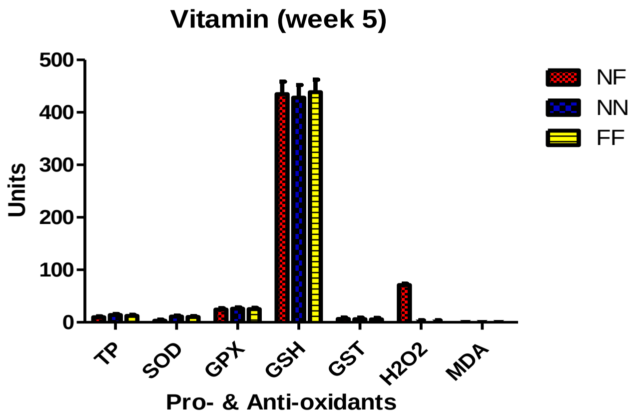

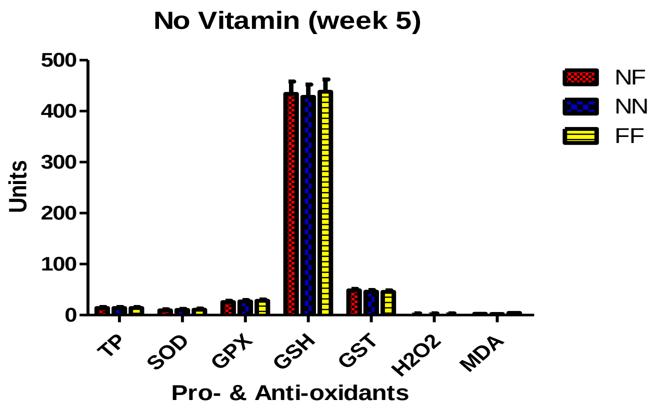

Key	
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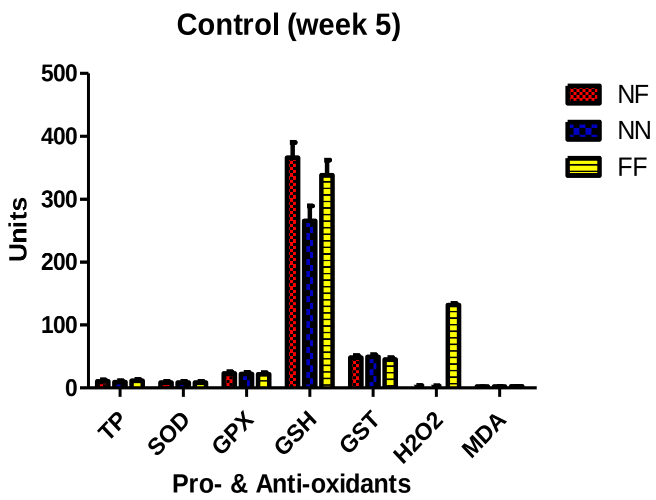
NN	Naked neck
FF	Frizzled feather



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Discussion

The result shown in this study involving three strains of Indigenous chicken (Naked neck, Frizzle Feather and Normal Feather) that were administered the Newcastle Disease vaccine (LaSota) with or without multivitamin and mineral supplementation. The researchers measured serum oxidative stress markers and antioxidant levels in the chickens at different weeks to assess the impact of the vaccine and supplementation.

Week 1: the antioxidant glutathione (GSH) level was high in all three strains of Indigenous chickens, despite the different treatments given. GSH is a crucial cellular antioxidant that helps protect cells from oxidative damage by neutralizing harmful free radicals. High levels of GSH can help improve immune function and reduce the risk

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of chronic disease. There was also a similar observation on the chickens with pout vitamin supplementation and control. This is due to the low concentration of vitamin at this time. There was a slight increase in the hydrogen peroxide (H_2O_2) in the vitamin supplementation; this also signifies the presence of stress.

Week 2: the GSH level and was shown to be high in frizzle feather (FF) and H2O2 in vitamin supplementation, this was also observed in the normal feather (NF) compared to Naked neck and frizzle feather (FF), this increase GSH level and H_2O_2 in the FF may be due to intuitive ability of the birds to with stand stress. There was also low level of antioxidant levels in the local chickens with no vitamin supplementation and control.

Week 3: At week three, the activity of GSH was significantly higher NN than the other stains given vitamin supplementation, without vitamin supplementation and control. This could be due to the increasing concentration of vitamin at this time. There was also stress observed the in the vitamin supplementation birds as its seen to also increase in the H_2O_2 level and slightly increased in the birds with no vitamin supplementation.

Week 4: The activity in week five showed also showed a high level of GSH in Normal feather (NF) local chicken with vitamin supplementation compared with no vitamin supplementation and control and also an increase in H_2O_2 level in the three local chickens more without vitamin and control. The NF chicken with vitamin supplementation showed a delay on set in response to stress.

Week 5: the GSH activity was remarkably high in the local chickens with vitamin supplementation compared with birds with no vitamin and control. It also shows an increase in the H_2O_2level of FF local chicken. This signifies the optimized antioxidant activity in the vitamin supplementation birds, which improves immune function. The FF in control shows an increase in H_2O_2level, this signifies stress in the FF strains and light increase in the GST level.

Effect of Vaccine: The Newcastle Disease vaccine (Lasota) could have triggered an immune response in the chickens, leading to increased production of reactive oxygen species (ROS) and subsequent activation of antioxidant defense mechanisms, including the increased synthesis of GSH and GPx.

Multivitamin and Mineral Supplementation: they recommended during vaccination in poultry boosting immune function and improving overall health. They contribute in reduction of oxidative stress. This study is in line with Ekei *et al*., 2019 which study revealed that broilers supplemented with dietary Mn responded to ND and IBD vaccines by producing antibodies at higher levels than chickens fed on the control feed with no Mn supplementation.

Conclusion

The administration vitamix and ND LaSota vaccine to the indigenous chickens, has a level of Glutathione (GSH) shown to be high on chickens which were under the multivitamin supplementation. An increase in hydrogen peroxide (H_2O_2) was also observed on chickens under multivitamin supplementation due to the stress from vaccination, with frizzled feather (FF) stain responding the most compared to the other stains due to the delay in response to stress on some strains resulted in fluctuation among genotypes on each oxidative stress parameter. The strains administered with multivitamin had positive response to GSH and H_2O_2more than their counterparts.

Recommendations

- Multivitamin supplementation (Vitamix) should be administered alongside the ND LaSota vaccine because this study demonstrated that birds receiving both vaccine and Vitamix showed improved antioxidant response, particularly reflected in higher glutathione (GSH) levels across the experimental weeks.
- Normal Feather (NF) and Naked Neck (NN) chickens should be prioritized for management strategies involving oxidative-stress-inducing procedures such as vaccination, as these strains showed more stable and favorable GSH and H_2O_2 responses compared to Frizzle Feather (FF) chickens.
- Frizzle Feather (FF) chickens require additional nutritional or management support during ND vaccination, since they recorded the highest increases in hydrogen peroxide (H_2O_2), indicating greater susceptibility to oxidative stress. Supplementary antioxidants may therefore be necessary when handling this strain.
- ND vaccination should be accompanied by monitoring of oxidative stress biomarkers, especially GSH and H_2O_2, since the present study shows that these parameters fluctuate significantly depending on treatment and genetic group.

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- Multivitamin supplementation should be considered a routine supportive measure during vaccination of indigenous Nigerian chicken strains, as the combined treatment (Vitamix + LaSota) consistently produced more favorable antioxidant responses than vaccination alone.
- Breeding and management programs involving Nigerian indigenous chickens should recognize strain-specific physiological responses particularly the superior GSH response in NF and NN birds and the heightened oxidative sensitivity in FF birds which can guide selection, housing, and feeding strategies.

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Cite this article as:
Yeigba, B. J., Wozi, E. E., Gabriel, E. E., Perekeme, R. T., & John, E. (2025). Comparative evaluation of serum oxidative stress in indigenous chickens administered newcastle disease (lasota) vaccine with and without multivitamin–mineral supplementation. *FNAS Journal of Applied Biological Sciences, 2*(4), 8-19. https://doi.org/10.63561/jabs.v2i4.1002

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