

# Coconut water enhances immunity of cockerels before and after vaccination against Newcastle disease virus

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**ABSTRACT:** Coconut water contains cytokinins and lauric acid which have potentials to enhance immune response. Poor immune response after vaccination is a common problem in poultry industry. Due to this challenge, this experimental study was carried out to evaluate the effects of coconut water on cell mediated and humoral immunity in cockerel chicks. Seventy five day-old cockerel chicks were randomly divided into three groups (A, B and C) and each group contained 25 birds. Group A (control) received ordinary water for 32 days while birds in group B received 100 ml of coconut water/1 L of water for 32 days. Group C received 100 mL of coconut water/1 L of water till day 19 of age and ordinary water from day 20 to day 32 of age. The birds were vaccinated against Newcastle disease (NDV) on day 20. Blood samples were collected on day 19 and 32 to evaluate the leukogram, antibody titer against NDV, total proteins and cytokines. Intestinal samples were also collected for immunoglobulin A analysis following the euthanasia of the birds. Means  $\pm$  SD of the parameters were calculated and compared for significance differences using one-way ANOVA ( $p < 0.05$ ). A significantly lower total protein and globulin levels were recorded in groups B and C on day 19. Contrarily, there was an increase in total protein and globulin levels in groups B and C, and an increase in monocytes count in group B on day 32. The serum levels of gamma interferon, interleukin 2 and Newcastle disease antibody titre increased significantly in groups B and C on day 32. Immunoglobulin G and A concentrations were not significantly different across the three groups on day 32. These results showed that coconut water supplementation before and after vaccination improved immune response to Newcastle disease vaccine.

**Keywords:** Coconut water, cytokines, leukocyte, Newcastle disease vaccine, total protein.

## INTRODUCTION

Poultry birds like other animals are susceptible to infections which consequently caused diseases and attendant economic losses to poultry sector (Abdullahi et al., 2009). In Nigeria like other developing countries, there is predominance of Gumboro and Newcastle disease (ND) which continue to have negative effects on poultry production globally in spite of advancement made on vaccination (Alexander et al., 2012). Biosecurity and

vaccination of birds remain the cheapest and effective means of preventing viral diseases in poultry flocks (Bosha and Nongo, 2012; Ramirez et al., 2013). Aside specific antibody, IgY (IgG in mammals), IgA and IgM antibodies are usually produced by birds as part of humoral immune response to vaccination (Jeurissen et al., 2000). The roles of these antibodies in immunity were associated with their capacity to neutralize Newcastle disease virus (NDV) (Al-

Garib et al., 2003).

Apart from the humoral antibody, cell mediated immunity (CMI) is also a vital part of immunity. T lymphocytes are the principal cells of the CMI response and gamma interferon released by these cells played an important role in controlling the maturation and differentiation of numerous immune cells (Fensterl and Sen, 2009). Measurement of gamma interferon level in chicken is very useful in assessing CMI after vaccination or infection (Karaca et al., 1996; Breed et al., 1999). Interleukin 2 is also secreted by activated Th-1 cells and dendritic cells (Nelson et al., 2004). Its role in CMI by enhancing activity of natural killer cells (Sakaguchi et al., 2008) and formation of immune memory cells (Malek and Raya, 2004) have been reported. The increased secretion of these antibodies and cytokines could alter serum total protein level. However, the importance of plasma protein in assessing immunity development against infections has been demonstrated (Oladele et al., 2005). There are reports on vaccine failure due to inability of birds to develop sufficient immunity after vaccination against diseases especially Newcastle disease virus (NDV). The common causes of vaccine failure as shown by previous studies include vaccine handling, storage (Bosha and Nongo, 2012), immune suppression, stress (Butcher and Yegani, 2008). In order to get sufficient immune response to NDV vaccines, adjuvants are commonly added to both inactivated (Yin et al., 2006) and live NDV vaccines (Hilton et al., 2002; Zhang et al., 2007). Also, the use of locally available dietary supplements such as lactobacillus-based probiotics (Sohail et al., 2010), Astragalus polysaccharide (Huang et al., 2008) and glycyrrhetic acid liposome (Zhao et al., 2011) to boost immune response in chicken after vaccination has been established.

Coconut water is the liquid endosperm found at the centre of coconuts. It contains chemical constituents such as vitamins, amino acids, selenium, cytokinins (kinetin), minerals (Bhagya et al., 2010) and lauric acid. Antimicrobial properties of coconut water have been shown by different studies (Rajan et al., 2016; Rukmini et al., 2017). Kinetin was reported to have pro-proliferative effect on cells (Lee et al., 2006) and immune cells (Li et al., 2017). The immune-stimulatory (Prabhu et al., 2014) and anti-inflammatory (Christina et al., 2015) properties of coconut water have also been established.

Due to the poor immune response to vaccines (Xiao et al., 2009) and high prevalence of immunosuppressive diseases in Nigeria, there is need to look for locally available supplements that can boost immune response to vaccination. Coconut water is readily available in Nigeria and seems to be a promising immune booster because of its chemical constituents especially kinetin which can enhance immune response to vaccination. Unfortunately, the usage of coconut water as a supplement in poultry industry is still poorly harnessed.

Therefore, this study was carried out to determine the immunity of chicken on coconut water supplementation

before and after vaccination against NDV by assessing their leukogram, serum level of total proteins, immunoglobulins, HI antibody titers and cytokines.

## MATERIAL AND METHODS

### Study design, place and duration

This randomized control experimental study was carried out at the Poultry Unit of Teaching and Research Farm, Oyo State College of Agriculture and Technology between November 2019 and January 2020.

### Experimental birds

Seventy-five day old cockerel chicks were obtained from a commercial farm in Ibadan, Nigeria. They were housed in clean and well-ventilated cages. The birds were given water and fed *ad libitum*.

### Source of coconut water

Ripe coconut fruits (*Cocos nucifera*) were obtained from Igboora market, Oyo state and the fluid inside them was manually extracted for this study.

### Experimental design

The birds were randomly divided into three groups (A, B and C) of twenty five birds each. Group A (control) received ordinary water for 32 days while birds in group B received 100 ml of coconut water / 1 L of water for 32 days. The birds in group C received 100 ml of coconut water / 1 L of water till day 19 of age and ordinary water from day 20 to day 32 of age. All the birds were placed on the same feed regimen throughout the period of the experiment. At day 20 of age, the birds were vaccinated against Newcastle disease with LaSota strain (live attenuated vaccine) via their drinking water. The experimental protocols used in this study were in compliance with internationally accepted principles for animal use and care.

### Collection of blood and serum samples

Fifteen birds in each group were bled on day 19 and 32 via wing vein puncture and the blood samples collected were transferred into EDTA vacuum tubes and plain vacuum tubes. Each EDTA tube containing blood was gently inverted 3 to 5 times to guarantee mixing of the sample with the anticoagulant, while the blood samples collected inside plain tubes were allowed to clot in order to get sera (Tuck et al., 2009). Thereafter, the sera were separated by centrifuge at 2000 to 3000 RPM for 20 minutes.

### Bird sacrifice and intestinal sample

At day 32 of the experiment, fifteen birds from each group were humanely slaughtered by euthanasia. To achieve euthanasia, 500 mg/kg body weight of phenobarbitone was administered to each bird via intravenous route (Igwebuike and Eze, 2010). The birds were carefully dissected with a scalpel blade to access the abdominal cavity and sections of duodenum were cut. The contents inside the intestinal samples were squeezed out and the samples were put inside separate organ bottles containing phosphate buffer solution (PBS). The tissues were homogenized in 1 ml of PBS/100 mg of intestinal tissue.

### Leukogram, total protein and antibody titer against NDV analysis

The procedure described by Samour (2006) was adopted to determine total and differential leucocyte counts. The total protein level in the serum was measured using biuret reaction, while colorimetric estimation of albumin level was determined using sigma diagnostic reagent (Sigma Diagnostic, UK) which contained Bromocresol Green (BCG). Serum globulin concentration was determined as the difference of total proteins and albumin (Van Wyk et al., 1998). The serum samples were tested for antibodies against NDV using standard haemagglutination inhibition test method. The antigen used was prepared from reconstituted commercial NDV Lasota vaccine. The principle of the test is based on the ability of serum samples in which antigen has been added to haemagglutinate washed red blood cells. The settling pattern of each serum was observed and antibody level of each serum sample was recorded as log base 2.

### Cytokines analysis

Serum concentrations of interleukin-2 and gamma interferon were determined by using ELISA- E0003Ch and E0013Ch (Bioassay Technology Laboratory, USA) respectively. The ELISA assay tests operates on the basis of competition between Streptavidin-Horseradish Peroxidase (HRP) conjugate and the interleukins in the sample for a limited number of binding sites on the antibody coated plate. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm  $\pm$  2 nm. The OD value obtained for each sample is proportional to the concentration of IL-2. Serum concentrations of immunoglobulin G and intestinal level of immunoglobulin A were measured using immunoturbidimetric assay-BXC0701 and BXC0721 kits (fortress diagnostics, UK) respectively. The assay principle involved reaction between anti-IgA antibodies and IgA in the homogenized intestinal sample to form antigen-antibody complexes which were then measured turbidmetrically.

### Statistical analysis

Data collected from this experiment were statistically analyzed with SPSS (Version 23) and differences between the means were tested with one-way ANOVA. The means were compared for significant differences at  $p < 0.05$  using Duncan's multiple range test, and the results from each group were presented as means  $\pm$  standard deviation (SD).

## RESULTS

### Leukogram and serum total protein

The total and differential leucocytes count in the birds across the three groups A, B and C at day 19 were not significantly different when compared. Except the monocyte count that increased significantly in group B, the total and other differential leucocyte counts in the three groups were not significantly altered when compared at day 32 (Table 1).

The total protein and globulin levels recorded at day 19 in groups B and C were lower when compared with values observed in group A. The albumin concentrations in groups B and C were not significantly different from the value observed in group A. Contrary to the trend observed at day 19, the total protein and globulin concentrations in groups B and C on day 32 were higher and this increase was only significant when group C was compared with group A. There was no significant difference in albumin levels recorded in groups B and C when compared with group A.

### Cytokine concentration

The treatment groups B and C had significantly higher level of interleukin 2 and gamma interferon when compared with values recorded in group A. Also, the serum NDV antibody titre in groups B and C were significantly elevated when compared with the value observed in group A. Meanwhile, there were no significant change in the intestinal immunoglobulin A level of treatment groups (B and C) as compared with group A (Table 2).

## DISCUSSION

The higher monocyte counts in group B on day 32 indicates the presence of strong cell mediated immunity in the group as a response to vaccination. The exposure of the group to coconut water might be responsible for this finding since kinetin, a major component of coconut water, has pro-proliferative effect on cells (Lee et al., 2006) and immune cells (Li et al., 2014). Reports have also shown

**Table 1.** Leukogram and serum total protein levels in control and treatment groups

Parameter	Group A	Group B	Group C
Day 19			
Total WBC ( $\text{mm}^3 \times 10^2$ )	126.00 $\pm$ 9.69	125.00 $\pm$ 7.79	125.00 $\pm$ 7.27
Heterophil ( $\text{mm}^3 \times 10^2$ )	99.10 $\pm$ 1.10	94.50 $\pm$ 8.39	95.1 $\pm$ 8.58
Lymphocyte ( $\text{mm}^3 \times 10^2$ )	24.90 $\pm$ 2.52	27.10 $\pm$ 3.00	27.30 $\pm$ 2.04
Monocyte ( $\text{mm}^3 \times 10^2$ )	1.94 $\pm$ 0.38	2.26 $\pm$ 0.28	1.70 $\pm$ 0.37
Day 32			
Total WBC ( $\text{mm}^3 \times 10^2$ )	119.00 $\pm$ 5.29	121.00 $\pm$ 9.32	105.00 $\pm$ 4.63
Heterophil ( $\text{mm}^3 \times 10^2$ )	88.70 $\pm$ 5.93	89.00 $\pm$ 6.64	81.8 $\pm$ 3.49
Lymphocyte ( $\text{mm}^3 \times 10^2$ )	29.27 $\pm$ 2.06	29.30 $\pm$ 3.24	21.7 $\pm$ 2.46
Monocyte ( $\text{mm}^3 \times 10^2$ )	1.20 $\pm$ 0.27	2.50 $\pm$ 0.30 <sup>a</sup>	1.83 $\pm$ 0.28
Day 19			
Total Protein (g/L)	68.60 $\pm$ 1.74	59.65 $\pm$ 3.09 <sup>a</sup>	60.04 $\pm$ 3.50 <sup>b</sup>
Albumin (g/L)	28.13 $\pm$ 2.98	31.58 $\pm$ 3.35	30.94 $\pm$ 3.36
Globulin (g/L)	40.47 $\pm$ 3.14	28.07 $\pm$ 2.75 <sup>a</sup>	29.10 $\pm$ 3.09 <sup>b</sup>
Day 32			
Total Protein (g/L)	73.20 $\pm$ 2.00	81.30 $\pm$ 2.20	86.80 $\pm$ 6.30 <sup>b</sup>
Albumin (g/L)	14.30 $\pm$ 0.50	14.30 $\pm$ 0.60	14.70 $\pm$ 0.80
Globulin (g/L)	58.90 $\pm$ 1.60	67.00 $\pm$ 1.80	72.10 $\pm$ 5.60 <sup>b</sup>

<sup>a</sup>Indicates significant difference when group B was compared with group A.<sup>b</sup>Indicates significant difference when group C was compared with group A.**Table 2.** Concentration of cytokines and antibody against NDV in control and treatment groups at day 32.

Parameters	Group A	Group B	Group C
Serum			
Interleukin 2 (ng/L)	28.09 $\pm$ 0.97	39.32 $\pm$ 3.40 <sup>a</sup>	38.46 $\pm$ 2.44 <sup>b</sup>
IgG (g/l)	1.15 $\pm$ 0.04	1.08 $\pm$ 0.02	1.07 $\pm$ 0.03
Gamma Interferon (ng/L)	1.86 $\pm$ 0.29	4.93 $\pm$ 0.93 <sup>a</sup>	4.66 $\pm$ 0.79 <sup>b</sup>
NDV Antibody titre	56.00 $\pm$ 16.01	100.10 $\pm$ 14.12 <sup>a</sup>	100.60 $\pm$ 10.45 <sup>b</sup>
Intestine			
IgA (g/L)	0.49 $\pm$ 0.02	0.55 $\pm$ 0.04	0.53 $\pm$ 0.09

<sup>a</sup>Indicates significant difference when group B was compared with group A.<sup>b</sup>Indicates significant difference when group C was compared with group A.

that elevated monocyte percentage in birds, especially after vaccination, indicated strong immunity (Green, 1999; Šimpraga et al., 2008).

The low concentrations of serum total protein and globulin in groups B and C, and high albumin level in group C on day 19 (pre-vaccination) is suggestive of lesser degree of infection in the groups relative to group A. The total protein concentration in birds is approximately 40 g/l (Scanes, 2015) and globulin level in 3 days to 20 weeks old turkey has been shown to range between 9.97 to 28.2 g/dl (Szabó et al., 2005). In contrast to the above figures, values of total protein and globulin recorded in this present

study were higher and this is plausible as the birds were vaccinated late hence, they could have been exposed to natural infections. Infections in animals are accompanied by high serum total protein, elevated globulin and low albumin levels resulting from enhanced antibody secretion in response to infection (Orhue et al., 2005; Wamboi et al., 2020). Coconut water supplementation seemingly reduced susceptibility of birds in groups B and C to infection probably as a result of its antimicrobial properties (Rajan et al., 2016; Rukmini et al., 2017).

Generally, there is always an increase in globulin level and serum total protein after vaccination (Šimpraga et al.,

2008; Al-Hussary and Kudair, 2010). Similar trend was also observed across the groups in this current study after vaccination (day 32). However, the higher globulin, total protein and Newcastle disease antibody titre level in groups on coconut water supplementation is indicative of enhanced immune response of the birds in the groups to vaccination. Interleukin 2 is an immune-stimulatory cytokine that promotes production of gamma interferon and proliferation of immune cells (Cha et al., 2010). The significantly higher concentrations of interleukin 2 and gamma interferon in groups B and C signal enhanced cell mediated immunity. These elevated cytokines level alongside with high Newcastle disease antibody titre accounted for the increase in serum total protein observed in the group B and C. Numerous studies in poultry have demonstrated that the measurement of chicken gamma interferon (Karaca et al., 1996; Breed et al., 1999) and antibody titre can be used to evaluate immunity in chicken after vaccination. Similar to the finding in this current study is the report that indicated significant increase in interleukin 2 and gamma interferon concentration in breast cancer cells treated with coconut water vinegar, a fermented product of coconut water (Mohamad et al., 2019).

In conclusion, the results of this study showed that coconut water supplementation strengthened immunity of birds before and after vaccination against NDV by reducing susceptibility to infection and promoting immunity. The addition of coconut water to drinking water of birds before/after vaccination or both is recommended to poultry farmers.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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