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# Coconut Water Can Prevent Cardiopulmonary Problems in Broiler by Improving Maternal Antibody, Antioxidant, Haematological and Lipid Parameters

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## ABSTRACT

*Coconut water is useful in the management of cardiovascular disturbance. Its effects on haematological parameters, relevant organs, antioxidant enzyme, lipid profile and antibody against Newcastle disease virus were assessed in broiler chicks. Control, CW100 and CW200 groups received water, 100 ml and 200 ml of coconut water /1 of water for 28 days respectively. Samples were collected to evaluate CBC, antioxidant enzymes level, lipid profile, antibody against NDV and histological changes. Mean ± SEM values were calculated and compared for significance differences using one-way ANOVA. MCV increased in CW100 while MCH and platelets count were higher in CW100 and CW200. Serum total cholesterol and triglyceride levels decreased while SOD and GPx levels increased in CW100. The antibody against NDV in CW100 and CW200 increased at day 12 but reduced in CW100 at day 28. Pulmonary oedema, congestion of hepatic central vein and cardiac hypertrophy were mild in CW100 and CW200. These results showed that coconut water had potential to prevent cardiopulmonary problems in broilers.*

**Key words**: Coconut Water, Antioxidants, Lipid Profile, Haematological Parameters, Newcastle Disease Antibody Titre

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## INTRODUCTION

In mammals, the right ventricle of heart propels the entire cardiac output through the lungs at relatively low pulmonary arterial pressure inside the pulmonary circulation. This low pressure, which is sustained by low pulmonary vascular resistance, minimizes threat of fluid infiltration into gas exchange space [1]. Contrarily, broilers possess high pulmonary vascular resistance due to functional predominance of pulmonary vasoconstrictors over vasodilators [2, 3]. Therefore, broilers possessing the most restrictive pulmonary vascular capacity are severely susceptible to cardiopulmonary problems such as pulmonary hypertension symptom (PHS) [4]. Studies also associated predisposition of broilers to cardiopulmonary problems to genetic selection for fast growth[5, 6]. Fast growth in broilers was assumed to facilitate PHS by amplifying hypoxaemia in the birds. Also, high metabolic rate of current lines of broiler render them susceptible to PHS by causing hypoxaemia due to inability of relatively underdeveloped cardio-respiratory system of these birds to provide the required oxygen needed [7]. Genetic selection for fast growth causes high incidence of infections due to decreased antibody response [8]. Adiposity that accompanies this fast growth could be the central factor linking fast growth in broilers and to susceptibility to infection since high adiposity caused gastrointestinal leakiness and low grade systemic infection[9]. However, respiratory infection has also been implicated as one of the factors contributing to cardiopulmonary problems in broilers [10]. Newcastle disease, one of these respiratory infections, was ranked first among other diseases ravaging poultry industry in Nigeria [11]. This prevalent disease was associated with lung damage thus contributed to severity of respiratory problem in broilers[12].

Free radicals are generated in biological systems as a result of normal cellular aerobic metabolism. The increased usage of oxygen and high metabolic rate in broiler chicken due to their fast growth lead to increased generation of free radicals. High free radical generation can induce cardiopulmonary problems because its role in pulmonary vascular endothelium damage and attendant reduction in the amount of endothelial nitric oxide synthase has been shown [13]. Increase in pulmonary vascular resistance, a condition that drives pulmonary hypertension syndrome, was shown to develop in broilers with depleted antioxidant enzyme levels [14, 15]. Lipids are important components of chicken feed and alteration in their body level known as dyslipidemia was characterized by hypertryglyceridaemia, hypercholesterolaemia, low serum high density lipoprotein (HDL-c) and high serum low density lipoprotein (LDL-c). The emerging body of evidence showed positive correlation between dyslipidemia and pulmonary vascular disease[16, 17]. This implies that dyslipidemia can also facilitate development of cardiopulmonary problems in broilers

Coconut water was a good source of antioxidant precursors and non-enzymatic antioxidants such as vitamin C, methionine and L-arginine [18]. Findings had shown its antioxidant properties and effectiveness in the management of cardiovascular diseases [19, 20]. Coconut water also possessed hypolipidemic activity due to its ability to reduce serum cholesterol level, triglyceride, LDL-c and increase blood HDL-c level [21]. Due to chemical components of coconut water and inability of chickens to synthesize arginine denovo, it was hypothesized that coconut water has potential to arrest some reported pathophysiologic factors that predispose

broilers to cardiopulmonary problems. Hence, this research work was aimed at evaluating the effects of coconut water supplementation on antioxidant enzymes level, haematological parameters, lipid profile, antibody titer against Newcastle disease and histopathology of relevant organs in broiler chicks

## MATERIALS AND METHODS

### Experimental Location and Duration

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Oyo State College of Agriculture and Technology (OYSCATECH), Igbo Ora, Nigeria.

### Source of Coconut Water

Ripe coconut fruits were bought at commercial market in Igbo Ora, Oyo State, Nigeria. The juice was manually extracted by puncturing the fruit with nail and was used for this experiment

### Experimental Chicken and Management

Forty five day-old broiler chicks were purchased from a commercial hatchery in Ibadan, Nigeria. They were reared in open-sided cages in three separate groups of 15 chicks each. The birds were fed with commercially prepared chick mash (Top feed) *ad libitum*. The control group was given ordinary water *ad libitum* while groups CW100 and CW200 were given 100 ml of coconut water/ L of water and 200ml of coconut water/ L of water *ad libitum* respectively. 200 dose of Newcastle disease vaccine-LaSota strains (Biovac®) was dissolved in 200 ml of water and each of the birds was vaccinated at day 14 against Newcastle disease by receiving 1 ml of the solution orally. The experiment was terminated at day 28. All experimental protocols were in compliance with internationally accepted principles for animal use and care

### Collection of Blood and Serum Samples

Ten chicks were randomly selected at day 12 from the fifteen birds from each group and were bled via wing vein into heparinized bottles for analysis of maternally derived antibody against NDV. At the end of the 28th week, ten birds from each group were bled via wing vein puncture into heparinized bottles and plain bottles. The blood samples in the plain bottles were allowed to clot in order to get sera and the sera were separated by centrifugation at 2000-3000 RPM for 20 minutes [22]. All the samples were taken to the laboratory for determination of haematological indices, antioxidant enzymes, lipid profiles, and antibody titer against NDV.

### Chicken Sacrifice and Organs Collection

Five chicks from each group were euthanasized at day 28 by cervical dislocation. Carcasses were dissected and organs (lung, heart and the liver) were harvested and immediately fixed in 10% formalin for histopathological evaluation.

### Haematological Analysis

The packed cell volume (PCV) and haemoglobin concentration were determined by the microhematocrit and cyanmethaemoglobin methods respectively [23]. Erythrocyte count was carried out (Cheesbrough, 2000). Counting of total white blood cell (WBC) and differential leucocytes was determined using Neubauer ruled chamber [24]. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were calculated from the values recorded for red blood cell count and PCV [25]. Platelet count was done according to the visual method[26].

### Serum Lipid Profile and Antioxidant Enzymes Analysis

The enzymatic method for determination of cholesterol concentration was used to determine total cholesterol in this study [27],

while the levels of HDL and triglycerides were determined [28]. Superoxide dismutase activity was analysed in the serum using SOD Assay Kit-WST (SOD-19160water-soluble tetrazolium salt, Sigma-Aldrich, USA) following manufacturer's instruction. Glutathione peroxidase (GPx) activity in the serum was evaluated using the Fortress Diagnostic glutathione peroxidase assay kits protocol BXC0551A (Antrim, UK) [29]. Total antioxidant capacity was assayed by using ferric reducing ability of the serum[30].

**Quantification of Serum Antibody Titre against Newcastle Disease Virus**

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 was 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 is recorded as log base 2 [31].

**Histopathological Evaluation of Visceral organs**

Five chicks from each group were euthanasized at day 28 by cervical dislocation. Lung, heart and liver samples were collected and immediately fixed in 10% neutral buffered formalin, dehydrated in descending grade of alcohol, cleared in chloroform and impregnated in paraffin. Then 5-6 μm sections were collected into the grease free slide, de-paraffinized in xylene and stained with Haematoxyline and Eosin

**Statistical Analysis**

Data collected from this randomized experiment were statistically analyzed with SPSS (Version16.0, 2007). Differences between the means were tested with one-way ANOVA. The results from each group were expressed as mean ± standard error of mean (S.E.M) and means were compared with Duncan's multiple range test for significant differences at $P<0.05$

## RESULTS

**Haematological Parameters**

The haematological indices of broiler chicks in control group and groups on coconut water supplementation were presented in Table 1. The packed cell volume, erythrocyte counts, haemoglobin concentration and mean corpuscular haemoglobin concentration of broiler chicks in groups CW100 and CW200 showed no significant difference when compared with the control group. The values of mean corpuscular haemoglobin (MCH) recorded for groups CW100 and CW200 respectively were significantly higher ($p<0.05$) than the value recorded for control group. The value of mean corpuscular volume (MCV) observed in group CW100 was significantly increased ($p<0.05$) when compared with the control group. Platelet counts recorded for groups CW100 and CW200 were significantly higher ($p <0.05$) compared with the control group. Group CW200 had significantly lower neutrophil count when compared with the control group.

**TABLE 1. Haematological Parameters of Broiler Chicks in Control a nd Coconut Water Supplemented Groups**

| PARAMETERS | CONTROL | CW100 | CW200 |
|---|---|---|---|
| **PCV (%)** | 24.20 ± 2.41 | 29.00 ± 2.88 | 29.00 ± 2.88 |
| **RBC Count (x $10^{12}$/L)** | 5.15 ± 0.83 | 3.81 ± 0.42 | 4.35 ± 0.39 |
| **Hb Conc (g/dL)** | 8.06 ± 0.80 | 9.68 ± 0.96 | 9.75 ± 0.52 |
| **MCV (FL)** | 45.78 ± 7.94 | 80.67 ± 9.16 * | 70.34 ± 3.90 |
| **MCH (pg)** | 11.85 ± 3.41 | 26.89 ± 3.07* | 23.26 ± 1.29 # |
| **Total White Blood Cell Count (x $10^3$ $mm^3$)** | 3.67 ± 0.61 | 4.20 ± 0.80 | 3.70 ± 0.69 |
| **Neutrophil Count (x $10^3$ $mm^3$)** | 1.77 ± 0.33 | 0.96 ± 0.19 | 0.88 ± 0.16# |
| **Lymphocyte Count (x $10^3$ $mm^3$)** | 1.79 ± 0.34 | 2.93 ± 0.51 | 2.61 ± 0.58 |
| **Platelets Count (x $10^3$/$mm^3$)** | 5.42 ± 0.90 | 50.00 ± 5.16* | 34.80 ± 5.42# |

* indicates significant difference when group CW100 is compared with control (P<0.05)

# indicates significant difference when group CW200 is compared with control (P<0.05)

**Serum Lipid Profile and Antioxidant Enzymes Level**

The serum total cholesterol level recorded for group CW100 was significantly lower (p<0.05) when compared with the control group. Group CW100 had significantly (p<0.05) lower level of serum triglyceride and HDL-c when compared with the control group. Groups CW100 and CW200 showed no significant difference in their serum low density protein levels when compared with control group. Serum glutathione peroxidase (GPx) and superoxide dismutase levels recorded in group CW100 were significantly higher (p<0.05) when compared with the control group.

**Table 2. Serum Lipid Profile and Antioxidant Enzymes Level of Broiler Chicks in Control and Coconut Water Supplemented Groups**

| PARAMETER | CONTROL | CW100 | CW200 |
|---|---|---|---|
| **Total Cholesterol (mg/dL)** | 108.23 ± 6.4576 | 78.33 ± 12.61 | 131.07 ± 2.81 |
| **Triglyceride (mg/dL)** | 39.00 ± 2.75 | 29.50 ± 2.80 * | 37.60 ± 1.87 |
| **HDL-c (mg/dL)** | 74.13 ± 5.85 | 49.63 ± 10.99* | 91.25 ± 2.49 |
| **LDL-c (mg/dL)** | 26.30 ± 1.05 | 22.80 ± 2.24 | 32.30 ± 1.29 |
| **GPx (U/L)** | 22.33 ± 4.57 | 65.94 ± 11.31* | 17.42 ± 6.46 |
| **SOD (U/L)** | 836.44 ± 534.52 | 2263.90 ± 200.81* | 290.91 ± 37.66 |
| **Total Antioxidant Capacity (mmol/ml)** | 6.35 ± 1.54 | 4.80 ± 1.11 | 6.50 ± 0.99 |

* indicates significant difference when group CW100 is compared with control (P<0.05)
# indicates significant difference when group CW200 is compared with control (P<0.05)

**Geometric Mean Titer of Antibody against NDV**

The geometric mean titer (GMT) of maternal antibody against NDV observed in groups CW100 and CW200 respectively were significantly higher when compared with the control group at day 12. GMT of maternal antibody against NDV recorded for group CW100 was significantly lower (p<0.05) when compared with the control group at day 28. The GMT of antibody against NDV in group CW200 showed no significant difference when compared with control group at day 28.

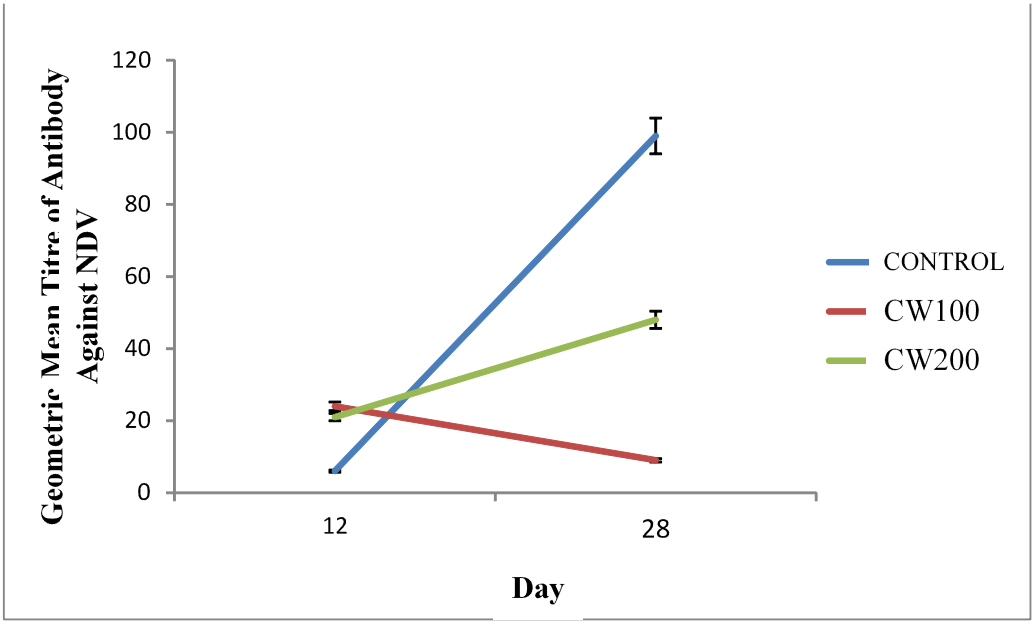
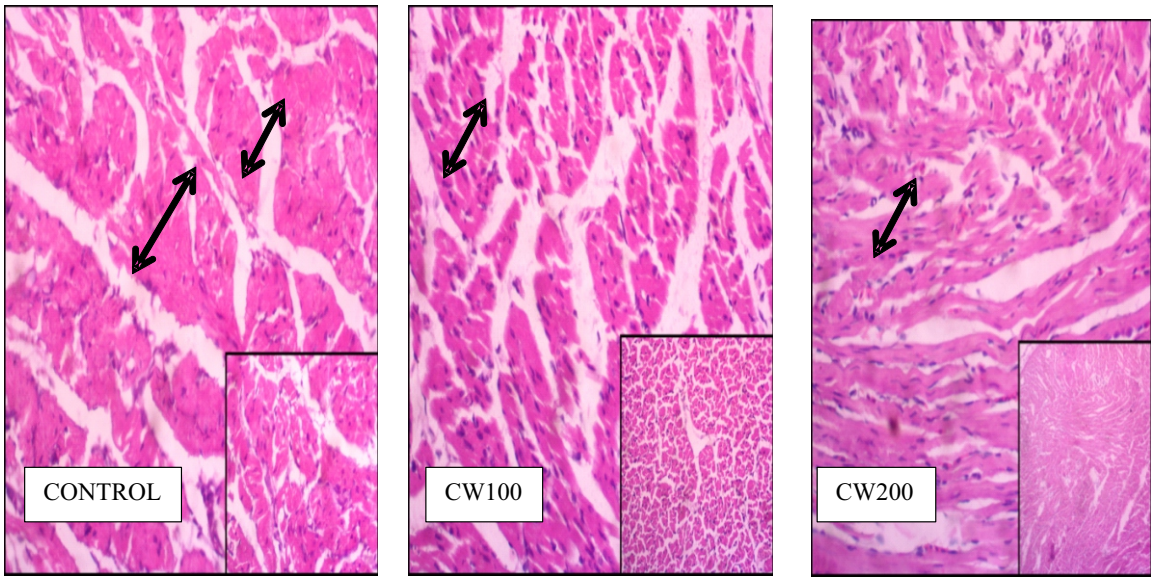


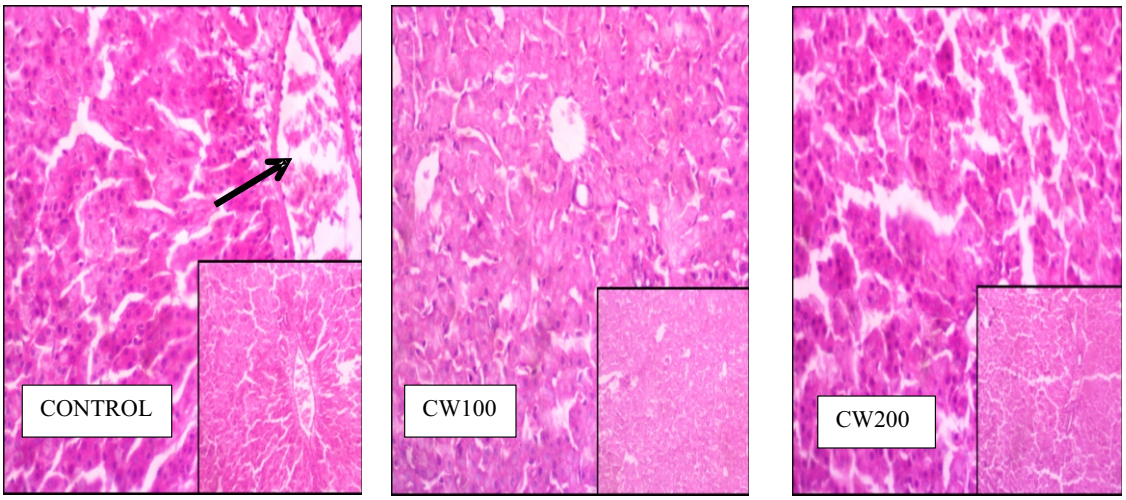

Fig. 1 Geometric Mean Titre against NDV of Broiler Chicks in Control and Coconut Water Supplemented Groups

**Histopathology**

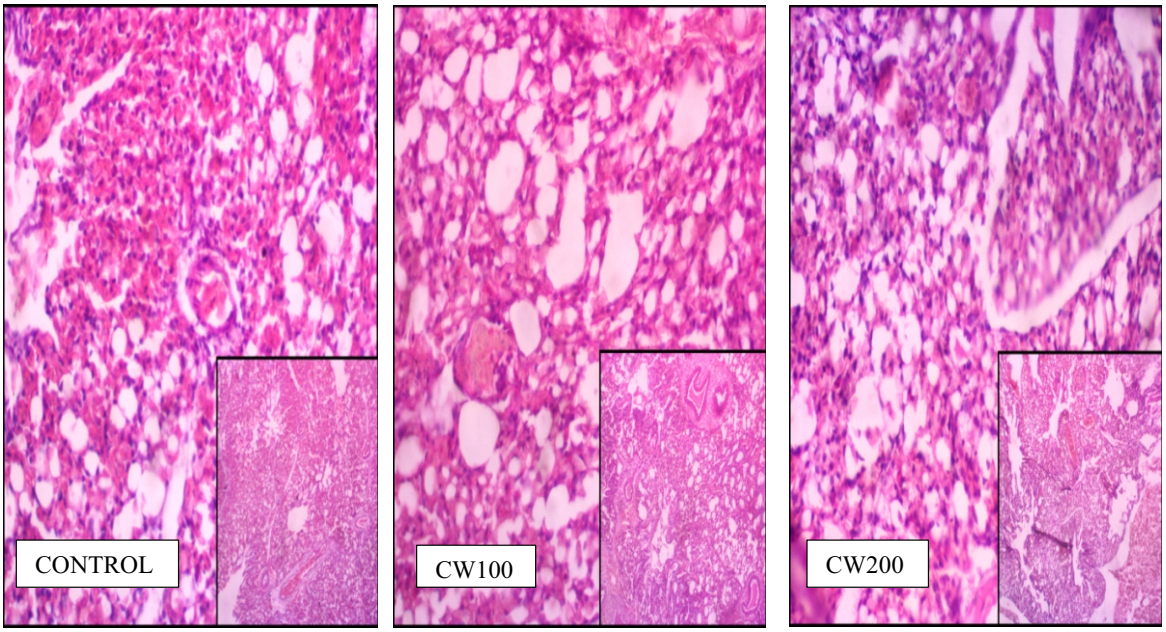
The histological examination of heart (Figure 3) showed that the control group had large myofibres (double edged black arrow) which was suggestive of severe hypertrophy while CW100 and CW200 groups had normal myofibres (double edged black arrow). The histological examination of liver (Figure 4) showed that control group had congested hepatic central vein (black arrow) while CW100 group and CW200 group showed normal hepatic central vein. There was no observable lesion in the histological sections of lungs (Figure 5) in the control, CW100 and CW200 groups




Plates 1. Photomicrographs of heart (H&E stain): (x 400). Insert (x100)




Plates 2. Photomicrographs of Liver (H&E stain): (x 400). Insert (x100)




Plates 3. Photomicrographs of lung (H&E stain): (x 400). Insert (x100)

**DISCUSSION**

The significant increase in mean corpuscular volume in group CW100, elevated platelet counts and mean corpuscular haemoglobin in groups CW100 and CW200 was due to enhanced formation of haemoglobin and intact vascular integrity in the groups. Iron and vitamin C components of coconut water could have facilitated the production of haemoglobin. Studies showed that vitamin C facilitated intestinal absorption of iron hence increased the bioavailability of the element for synthesis of haemoglobin[32]. The observation in this study aligned with the findings that demonstrated elevated MCV and MCH in catfish exposed to low dose of coconut; high blood cell and platelets counts at higher dose of coconut water [33].

Neutrophil count that was significantly lower in group CW200 was due to reduced predisposition of the birds to infection and its associated inflammation. The antimicrobial property of coconut water, due to its lauric acid composition, has been shown by previous study [34].

Maternal ND antibody titers that were significantly higher at day 12 in groups CW100 and CW200 indicated protective effect of coconut water on chicks' maternal antibody possibly due to its ability to keep epithelial integrity of mucosal surfaces intact thus prevented conditions, such as early infection, that deplete maternal antibody. Early exposure of chicks to systemic infection caused alteration in antibody protein structure and decreased maternal antibody absorption into systemic circulation from the yolk[35]. The significantly lower ND antibody titer in group CW100 at day 28 suggested lack of active immune response to vaccination. Presumably, high maternal antibody in chicks at the time of vaccination mopped up vaccine that was administered and in turn reduced active immune response. A report showed that vaccination while maternal antibodies were still high led to reduction in efficacy of vaccine[36, 37].

Significantly lower serum total cholesterol and triglyceride levels observed in group CW100 could have resulted from increased conversion of cholesterol to bile acid for excretion and

enhanced lipoprotein lipase activity. A report showed that coconut water reduced total cholesterol level and triglyceride level by enhancing lipoprotein lipase enzyme activity and conversion of cholesterol to bile acid[38].

Although serum HDL-c level was lower in group CW100, but this was unlikely to affect anti-lipidemic property of coconut water since reverse transportation of cholesterol by HDL-c is a function of its serum level and composition. Studies showed that optimal reverse transportation of cholesterol by HDL-c does not only depend on its level in the body but also on its composition which is regulated by many factors such lipolytic enzymes[39].

High serum GPx and SOD levels recorded in group CW100 was due to presence of exogenous antioxidants and their precursors in coconut water. The increased availability of exogenous antioxidants in the group might have complemented the free radical scavenging capacity of endogenously produced GPx and SOD hence increased the systemic accumulation of the enzymes. Recent study had also shown antioxidant property of coconut water and its ability to increase serum SOD, GPx and malondialdehyde levels[20].

Mild congestion of pulmonary interstitial in group CW100 and absence of congestion in hepatic central vein of groups CW100 and CW200 could be due to improved patency of blood vessels resulting from increased synthesis of nitric oxide from exogenous arginine, low total cholesterol and high antioxidant enzymes levels

**Conclusion**

Coconut water could prevent infection of broiler chicks with NDV at early stage of their lives by protecting maternal antibody and also improved oxygen carriage by enhancing hemoglobin formation and cardiovascular function.

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