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Immune regulatory potentials of coconut water and Vitamin C in broiler chicken exposed to varying housing temperature

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The ambient temperature has been found to affect the performance and immune response of broiler, hence the need for determination of immunoglobulin level routinely to evaluate their state of immunity call for concern. This study determines the effects of housing temperature and coconut water (CW) on serum immuglobulins of broiler chickens. A total of 600 *Marshall* broiler chickens were used, with 200 birds in different housing temperature: Cold (CHT, 18.3-22.1°C), Natural (NHT, 26.3-26.6°C), and Hot (HHT, 34.9-36.1). Each housing temperature was partitioned into five treatment groups: Ordinary water (T_1), 0.5 g of Vitamin C/L of water (T_2), 0.5% CW/L of water (T_3), 1% CW/L of water (T_4) and 1.5% CW/L of water (T_5), with four replicates and 10 birds per replicate. Immunoglobulin IgG, IgM and IgA were determined. Data collected were subjected to factorial arrangements with one-way Analysis of Variance. Housing temperature significantly ($p<0.05$) influenced immunoglobulin value for IgG and IgM. Birds under natural housing temperature had the highest IgG (1.62±0.07 mg/dl) compared to others under cold housing temperature (1.39±0.15 mg/dl) and hot housing temperature (0.38±0.07 mg/dl). The IgM level showed a negatively changing trend with birds under hot housing temperature having the highest value (1.95±0.07 mg/dl), followed by cold housing temperature (1.49±0.01 mg/dl), and natural housing temperature (1.06±0.11 mg/dl). Upon administration of coconut water and vitamin C to birds, there is a significant ($p<0.05$) influence on immunoglobulin values (IgG, IgM and IgA) across the three housing temperatures. It was therefore, concluded that change in ambient temperature can cause variation in immune response of birds and immune responses could be ensured by regulated administration of coconut water and vitamin C in broiler production.

Key words: Immunoglobins, coconut water, vitamin C, housing temperature, broiler.

INTRODUCTION

The immune system protects the body from disease producing organisms and foreign bodies by producing antibodies like IgG, IgM and IgA with the help of antigen specific T helper cell (Megha and Mohanan, 2021; Maheshwari et al., 2022). When exposed to antigens, the thymus, bursa of Fabricius, bone marrow, and spleen of birds generate immunoglobulins (also known as antibodies), which are a primary effector of humoral

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immunity Leslie and Clem 1969; Tiwari et al., 2020).These immunoglobulins are utilized in a variety of diagnostic and scientific research procedures, including immuno-diffusion, ELISA, and Western blotting, to detect or quantify antibodies and specific antigens linked to a variety of diseases (Colombo et al., 2015; Tiwari et al., 2020; Morgan, 2021). Both performance and immune response of broiler could be affected by ambient temperature. Heat stress has also been reported to have detrimental effects on immunoglobulins (Hind et al., 2012). Barlett and Smith (2003) stated that heat stress reduced the total circulating antibodies, and titer values of IgG and IgM during primary and secondary humoral response in broiler chicken. Tabidi et al. (2004) reported that weight of bursa, spleen, thymus, and liver reduced significantly during heat stress periods. Modern commercial broilers appeared to have weakened immune systems, greater mortality, and decreased resistance because of stress, according to Khanet et al. (2012). The first isotype of IgM expressed during embryonic development is chicken. It is the main isotype that develops following initial contact with a new antigen (Davison, 2008). According to studies (Figueiredo et al., 2017; Li et al., 2020), specific IgM levels have been shown to rise following primary infection, with secondary exposures producing lesser responses. As in mammals, this humoral response is usually transient (Kamle et al., 2022; Jiminez et al*.,* 2015), although after chronic bacterial infection, such as *Bordetella avium* in turkeys.

Avian immunoglobulin G predominates in sera and is the principal isotype in the secondary response, produced following IgM in the original antibody response (Dasilva et al., 1991). It is thought to be the main immunoglobulin providing defense against infectious pathogens because it is primarily systemic rather than a secretory antibody and can also be discovered in duodenal contents, tracheal washings, and seminal plasma in addition to the egg yolk (Keyt et al., 2020; Härtle et al., 2022). IgG is the major avian systemic antibody active in infections (Davison, 2008).

According to research by Davison (2008), immunoglobulin A (IgA) serves as a first line of defense against several infections. Therefore, it is more plausible that secretory IgA antibodies are involved in the resistance to parasites that infect the intestinal mucosa. According to Moazzam et al. (2012), certain nutritional elements can strengthen chicken broilers' immune systems while simultaneously reducing the harmful effects of heat stress. Inclusion of antioxidant is thought to influence immunity positively and aid normal metabolic function of the body. These substances include sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), calcium chloride ($CaCl_2$), and ammonium chloride (NH_3Cl). Sodium zeolite and aspirin are also beneficial in reducing the effects of heat stress in chicken (John, 2008). Vitamin C and $NaHCO_3$ appeared to be the most popular electrolytes used in tropical and subtropical poultry production (John, 2008).

Coconut water is known to be used to alleviate the negative effects of heat stress. The antioxidant ability of coconut water was studied by some authors in human (Rethinam and Kumar, 2001; Saat et al., 2002; Smith et al., 1993). Coconut water is rich in vitamin C and some of the metallo elements that help in antioxidant activity. Thus, the study aimed at evaluating the effect of vitamin C and coconut water on serum immunoglobulins of broiler chickens in varying the housing temperature.

MATERIALS AND METHODS

Experimental site

This experiment was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria with latitude 7°13’49.46”N; longitude 3°26’11.98”E and altitude 76 m above sea level (Google Earth, 2015). The climate of the experimental site is humid, located in the rain forest vegetation zone of western Nigeria.

Meteorological observations

The daily temperatures and relative humidity of the pen were monitored using digital thermo-hygrometer throughout the experimental period.

Experimental birds and management

Six hundred *Oba Marshall* broiler chickens were purchased. Proper disinfection and fumigation of the poultry house was done. Brooding was carried out for a period of two weeks. Prescribed medication and vaccination were administered to the chickens as prophylactics and curative measure. At the end of the 4th week, the birds were weighed and randomly allotted to five (5) different treatments and three housing units (Hot, Cold, and Natural). Each treatment was replicated four times under each housing condition with 10 birds per replicates and placed on treatment till 7 weeks of age. Throughout the brooding phase, birds were reared in deep litter inside the open sided poultry house. Broiler starter was fed to the birds for the first four weeks, while broiler finisher was fed to them for the last 3 weeks of the experiment *ad libitum*. Descriptions of different housing condition used in this experiment are: Hot housing temperature (34.9-36.1°C), this was maintained with the use of charcoal; Natural open-sided pen (25.2-33.5°C), kept open-sided with temperature cycling; Cold (18.3-22.1°C), housing temperature was maintained within the thermo-neutral zone of the birds with the use of air-housing temperature.

Source and processing of coconut water

The coconut fruit from which coconut water used for the purpose of this research work was purchased from Oja Odan, in Yewa South Local Government area of Ogun State, Nigeria. Coconut water was collected by breaking the coconut shell. Coconut water was then, drained directly into a bowl covered with clean cloth as a sieve after which it was given fresh to the birds.

Treatments

The group treatments used during for the experiment are as

follows: T_1: Ordinary water without vitamin C or coconut water (control); T_2: 0.5 g of Vitamin C in 1 L of water; T_3: 0.5% of coconut water in 1 L of water; T_4: 1% of coconut water in 1 L of water; T_5: 1.5% of coconut water in 1 L of water.

Data collection and analysis

Blood collection

Five milliliters of blood was collected on day 49 from 2 birds in each replicate via the brachial vein using 5 ml syringe and needle. Blood was collected in EDTA and plain bottles. The blood samples in the plain bottle were spun for 5 min at 4000 revolutions per minute (r.p.m) to separate the serum.

Determination of immunoglobulins titre values

Determination of IgG titre value

DIALAB kits manufactured in Austria were used for chemical analysis of IgG, IgM and IgA. Six separate test tubes were used to serve as calibrator, each tube contains diluted protein calibrator of 20 µL with the following 1:10, 1:20, 1:40, 1:80, 1:160, and 0.9% normal saline to serve as blank (to zero absorbance). Each tube was added with 900 µL buffer solution and one tube as test sample, while another tube as control. Into other tubes, 20 µL of sample and 20 µL of control were added. All the tubes were gently mixed and incubated for 5 min at 37°C. The absorbance 1(A_1) of calibrators, control and sample were read at 340 nm. Then anti-body reagent of specific for IgG 100 µL was added to calibrators, samples and control, then mixed together and incubated for another 5 min at 37°C. Absorbance 2(A_2) was then read at 340 nm (A_2-A_1). After changes in absorbance have been calculated, changes in calibrator absorbance versus assigned concentration were plotted on graph where real titre values were determined.

Determination of IgM titre value

Six separate test tubes were used to serve as calibrator, each tube contains diluted protein calibrator of 20 µL with the following 1:10, 1:20, 1:40, 1:80, 1:160, and 0.9% normal saline to serve as blank (to zero absorbance). Each tube was added with 900 µL buffer solution and one tube as test sample, while another tube as control. Into other tubes, 25 µL of sample and 25 µL of control were added. All the tubes were gently mixed and incubated for 5 min at 37°C. The absorbance 1(A_1) of calibrators, control and sample was read at 340 nm. Then, anti-body reagent of specific for IgG 60 µL was added to calibrators, samples and control, then mixed together and incubated for another 5 min at 37°C. Absorbance 2(A_2) was then read at 340 nm (A_2-A_1). After changes in absorbance have been calculated, changes in calibrator absorbance versus assigned concentration were plotted on graph where real titre values were determined.

Determination of IgA titre value

Six separate test tubes were used to serve as calibrator, each tube contains diluted protein calibrator of 20 µL with the following: 1:10, 1:20, 1:40, 1:80, 1:160, and 0.9% normal saline to serve as blank (to zero absorbance). Each tube was added with 900 µL buffer solution and one tube as test sample, while another tube as control. Into other tubes, 20 µL of sample and 20 µL of control was added. All the tubes were gently mixed and incubated for 5 min at 37°C. The absorbance 1(A_1) of calibrators, control and sample were read at 340 nm. Then anti-body reagent of specific for IgG 60 µL was added to calibrators, samples and control, then mixed together and incubated for another 5 min at 37°C. Absorbance 2(A_2) was then read at 340 nm (A_2-A_1). After changes in absorbance have been calculated, changes in calibrator absorbance versus assigned concentration were plotted on graph where real titre values were determined.

RESULTS

Effects of housing temperature and coconut water on immunoglobulins of broiler chickens

Table 1 shows effect of housing temperature on serum immunoglobulin (IgG, IgM and IgA) of broiler chickens. Housing temperature had significant ($P<0.05$) effect on serum immunoglobulin (IgG and IgM). However, IgA was not significantly ($P>0.05$) affected by housing temperature. Both natural and cold housing temperatures (1.62 and 1.39 mg/dl, respectively) had higher IgG values compared to hot housing temperature (0.38 mg/dl). For IgM, hot housing temperature had the highest value (1.95 mg/dl) followed by cold housing temperature (1.49 mg/dl), while natural housing temperature had the least value (1.06 mg/dl).

The effect of coconut water on serum immunoglobulin (IgG, IgM and IgA) of broiler chicken is shown in Table 2. It was obvious that coconut water had significant ($P<0.05$) effect on IgG and IgA of broiler chickens. IgG value of birds given 0.5, 1.0 and 1.5% coconut water were similar and higher than those birds offered ordinary water and Vitamin C. IgA value of broiler chickens offered ordinary water, Vitamin C, 0.5 and 1.0% coconut water were significantly ($P<0.05$) higher than birds administered 1.5% coconut water

Interactive effect of housing temperature and coconut water on immunoglobulin of broiler chickens

The interactive effect of housing temperature and coconut water on immunoglobulins of broiler chickens is presented in Figures 1 to 3. Figure 1 shows the interactive effect of housing temperature and coconut water on immunoglobulin G (IgG). IgG was high in birds under natural housing temperature (offered 0.5 and 1% CW), cold housing temperature (offered 0.5, 1 and 1.5% CW), while IgG was lower in birds in hot housing temperature (offered water, vitamin C, 0.5, 1 and 1.5% coconut water). The interactive effect of housing temperature and coconut water on Immunoglobulin M (IgM) is as shown in Figure 2. IgM was highest in birds in hot housing temperature (administered water, vitamin C, 0.5, 1.0 and 1.5% coconut water), while lowest observed IgM value was recorded in birds offered ordinary water under cold housing temperature. Birds in natural (offered water, vitamin C, 0.5, 1 and 1.5%), in cold and given (water, vitamin C, 0.5, 1.0 and 1.5% coconut water) was

Table 1. Effects of housing temperature on specific immunoglobulin titre value of broiler chicken.

Parameter	Housing Temperature		
	Hot	Natural	Cold
IgG (mg/dl)	0.38±0.072[b]	1.62±0.265[a]	1.39±0.152[a]
Ig M (mg/dl)	1.95±0.071[a]	1.06±0.106[c]	1.49±0.066[b]
Ig A (mg/dl)	4.48±0.055	4.23±0.092	4.52±0.192

[a,b]Means± SEM with different superscripts in the same row differ significantly (P<0.05).

Table 2. Effect of coconut water on specific immunoglobulin titre value of broiler chicken.

Parameter	Treatment				
	Water	Vitamin C	0.5% CW	1.0% CW	1.5% CW
IgG (mg/dl)	0.19±0.037[b]	0.68±0.168[b]	1.65±0.286[a]	1.53±0.221[a]	1.52±0.269[a]
IgM (mg/dl)	1.32±0.157	1.57±0.131	1.58±0.115	1.53±0.117	1.52±0.135
IgA (mg/dl)	4.45±0.110[ab]	4.51±0.095[ab]	4.63±0.181[a]	4.33±0.073[ab]	4.11±0.244[b]

[a,b]Means ± SEM with different superscripts in the same row differ significantly (P<0.05). CW=Coconut water, IgG =immunoglobulin G, IgM= immunoglobulin M, IgA = immunoglobulin A.

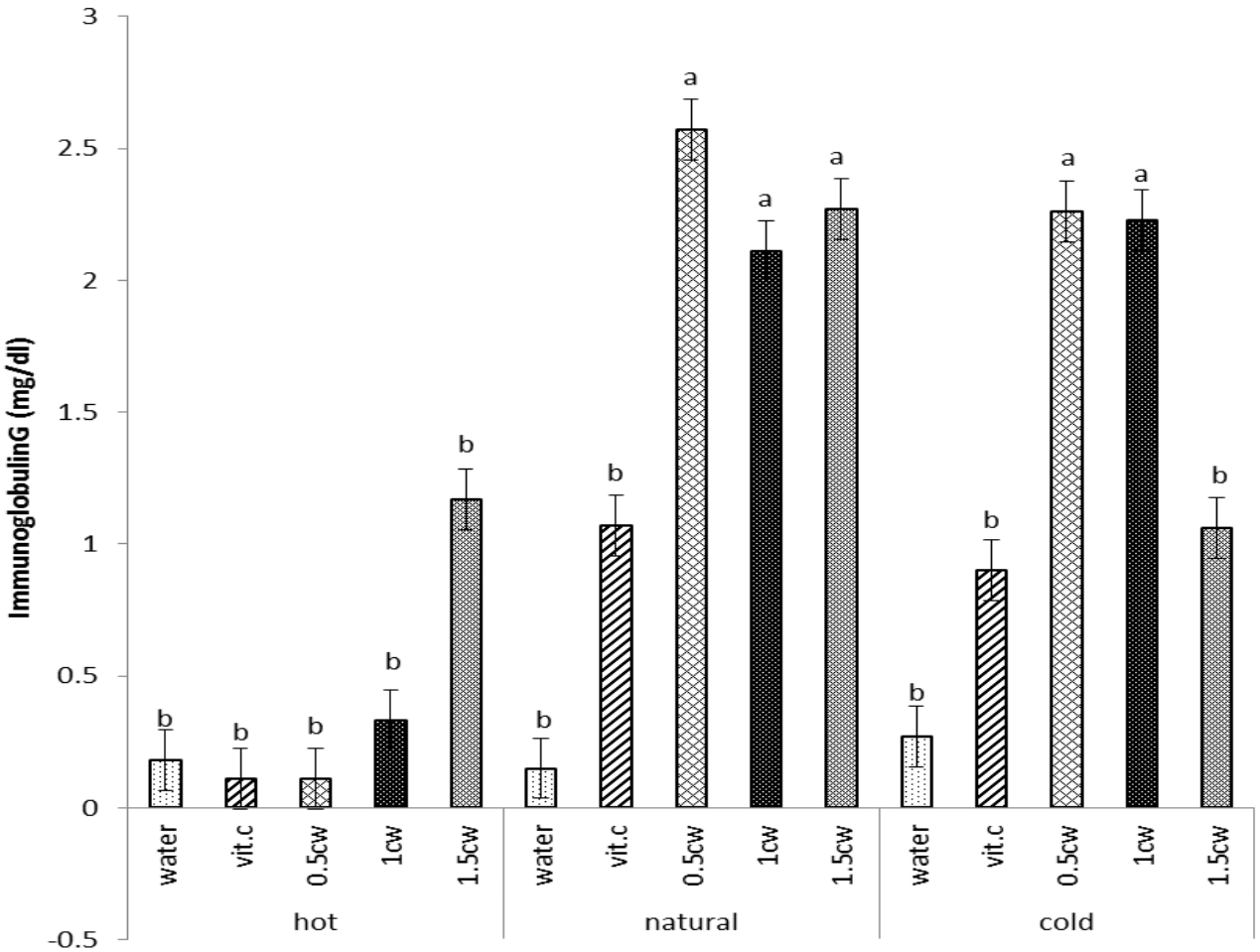

Figure 1. Interactive effect of housing temperature and coconut water on immunoglobulin G (IgG) of broiler chicken (Mean± SEM).

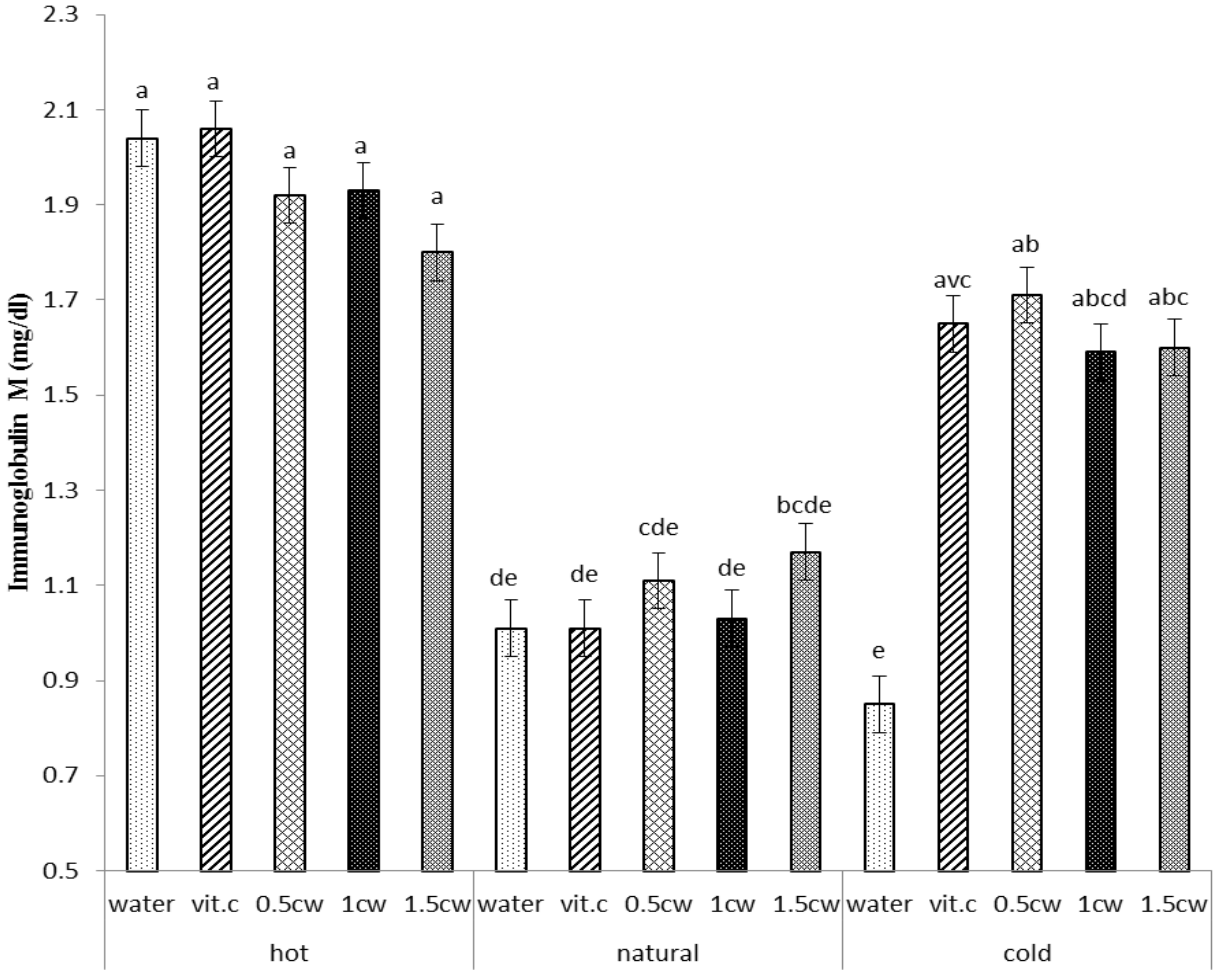

Figure 2. Interactive effect of housing temperature and coconut water on Immunoglobulin M (IgM) of broiler chickens (Mean± SEM).

in between highest and lowest recorded values. Figure 3 shows the interactive effect of housing temperature and coconut water on Immunoglobulin A of broiler chickens. Housing temperature and coconut water had a significant ($P<0.05$) effect on Immunoglobulin A of broiler chickens. Highest IgA value was recorded in birds administered 0.5% coconut water in cold housing temperature, while lowest value was recorded in birds offered 1.5% coconut water under natural housing temperature.

DISCUSSION

Serum immunoglobulin levels are determined routinely in clinical practice because they provide key information on the humoral immune status. Low immunoglobulin levels define some humoral immunodeficiencies (Gonzalez-Quintela et al., 2007). It was noticed that IgG value of broiler chickens in this research work was reduced by the effect of hot housing temperature (Smith et al., 1993). IgG is the major avian systemic antibody active in infections (Davison, 2008). This finding is in agreement with the work of Lucas et al. (2013) and Bartlett and Smith (2003) who affirmed that broilers subjected to heat stress recorded reduced IgG value. Scott et al. (1976) also reported reduced serum IgG in calves as a result of increased cortisol (corticosterone in poultry) count under heat stress. Motasem (2012) attested to lower IgG at hot ambient temperature of 40°C; suggested that natural and cold housing temperature favored the immune status of the broiler chicken compared to birds under hot housing temperature.

For IgM in this study the value recorded was high under hot housing temperature. This observation is line with the report of Gomes et al. (2014) who recorded increased IgM in broiler chickens subjected to overcrowding. On other hand, this research work does not support the findings of Lucas et al. (2013) and Bartlett and Smith (2003) who reported that birds exposed to high temperature had a reduced IgM. Herbert and Cohen

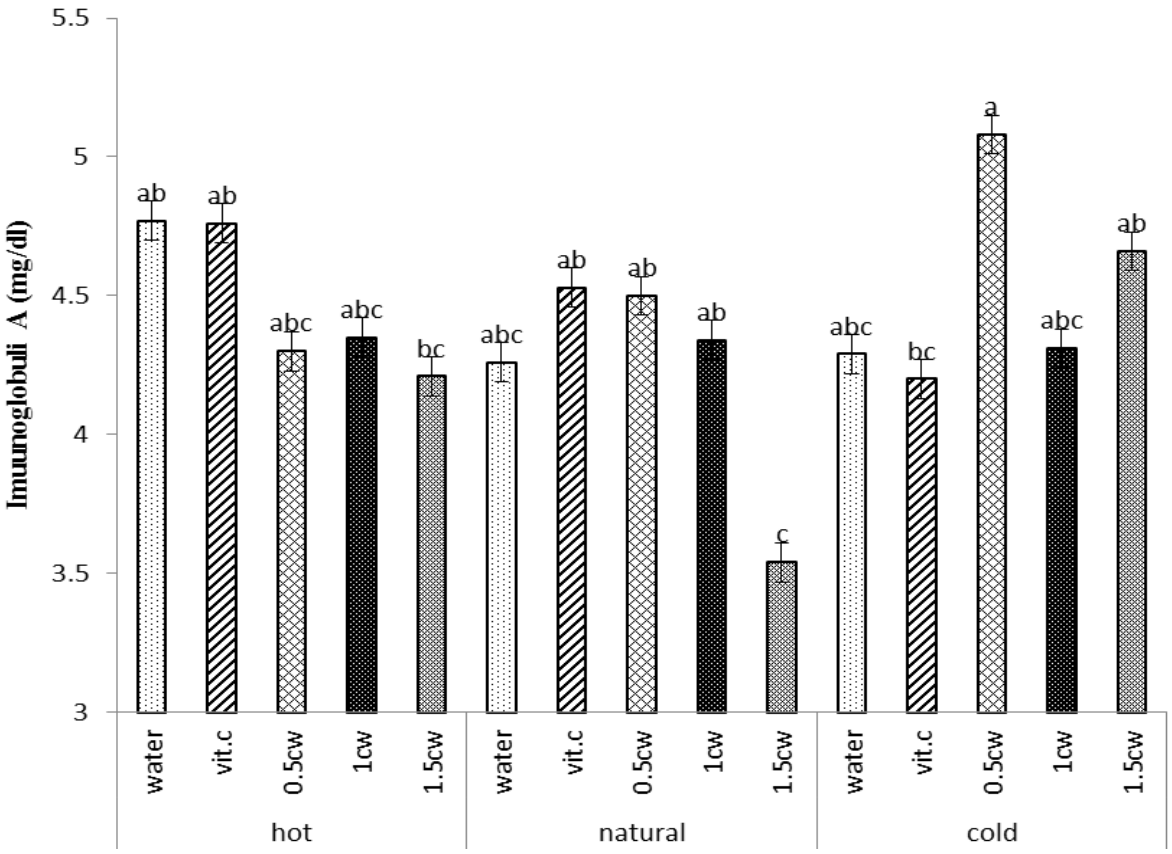

Figure 3. Interactive effect of housing temperature and coconut water on Immunoglobulin A of broiler chickens (Mean± SEM).

(1993) also stated that immunoglobulins A and M decreased by 13 and 24%, respectively on exposure to stress. The higher value of IgM recorded in birds under hot housing temperature indicated that immune system of the birds was challenged on exposure to chronic heat stress through the hot housing temperature. It was this challenge that which triggered the increase in IgM synthesis to cope with the situation being the second immunoglobulin produced by the body in response to challenge.

Diluted coconut water caused an increase on IgG and IgA of birds under this study. This observation is in line with the findings of Motasem (2012) who reported that Vitamin C participates in immunity and also serves as important enzymatic, antioxidant, and regulatory functions in broiler chickens. Research carried out by Motasem (2012) also showed that Vitamin C raises the levels of IgG and IgA in the blood stream. Vitamin C as an antioxidant destroys those phagocytic derived highly reactive oxidant bio-chemicals that are toxic to the tissue cells. A vitamin deficiency in the diet may impair immunity, according to Bourre and Galea (2006). Vitamins are necessary for optimum health and normal physiological functions like growth, development, maintenance, and reproduction. They also act as co-factors in a number of metabolic processes in immune response. According to Rama-Rao et al. (2002), poultry's immune response would be enhanced by usually consuming higher vitamin levels than the present advice for preventing deficient disorders. Motesem (2012) reported an increase in IgG titre of broiler chickens when offered Vitamin C and Zinc. IgG in this study was increased by all levels of diluted coconut water administered, meaning that all the levels of coconut water administered to the birds influenced and aided the maintenance of immune status. Birds offered 0.5% coconut water had a high recorded IgA compared to other groups; may be because low level of coconut water is sufficient to influence IgA improvement. Muir et al. (2002) reported an increase in IgA of broiler chicken administered with vitamin E at 250 and 500 mg. In the same vein, Zdunczyk et al. (2013) and Muir et al. (2002) also reported that vitamin C administration increased IgA concentration in broiler chickens; this corroborates our present research.

It is therefore, concluded that prophylactic administration of mixed coconut water and vitamin E in production of broiler could be beneficial in regulation of immune system of the birds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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