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ORIGINAL ARTICLE

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Effect of Honey on Haematology, Plasma Biochemistry and Liver Enzymes in Broiler Chickens Administered Dietary Corticosterone

Monsuru Oladimeji Abioja, Obafemi Foluso Akinjute, Samuel Iyanuoluwa Balogun, Michael Segun Oguntimehin and Timothy Olanrewaju Oluwasola

Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, PMB 2240 Abeokuta, Nigeria

*E-Mail: ***dimejiabioj@yahoo.com; abiojamo@funaab.edu.ng***

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Effect of honey on blood parameters in broiler chickens fed diet containing corticosterone (CORT) was examined. Arbor acres broiler chickens aged 28d were allotted to four groups. The birds received 30mg CORT/kg feed plus either 0 (C0H), 5 (C5H), 10 (C10H) or 15ml honey/l drinking water (C15H) for 7d. Blood sampling was carried out in five birds per treatment. Honey had significant ($p < 0.01$) effect on PCV, RBC and haemoglobin concentration (HB). Birds on honey treatment had higher PCV and RBC than the CONTROL. For HB, the same pattern was observed except that C0H and C15H were similar. Birds on C5H recorded highest values for the 3 parameters respectively. There were no significant ($p > 0.05$) differences in MCV, MCH, MCHC, WBC, leukocyte differentials and H:L ratio, except for oesinophil (EOS; $p < 0.05$). C5H birds recorded higher EOS than C10H and C15H, but not significantly different from C0H. Total protein, albumin, globulin, uric acid, creatinine, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were not significantly ($p > 0.05$) affected by treatment. Honey in drinking water of could be of help in improving the welfare of broiler chickens during stress episode by increasing the PCV, RBC and HB.

Key words: anti-oxidant, honey, blood, poultry, tropics

Stress is a constant menace in broiler production (Lin *et al.*, 2004a; Virden *and* Kidd, 2009), lowering welfare and productivity in birds. Pawlak *and* Kontecka (1995) described stressors as all factors that disturb the capability of living organisms for maintenance of physiological and biochemical characteristics at a stable level called homeostasis, and response to stressor is referred to as stress. Broiler chickens are under constant challenges of varying degrees and varieties of stressors from day-old till the market age. Extreme environmental temperature, humidity, vaccination, feed and/or water deprivation, mycotoxins in feed, dust, ammonia, radiation, hyperoxia, bacterial or viral exposure, overstocking and transportation (catching, crating, loading, motion, off-loading, etc.) are some of the stressful situations faced by broiler chickens (Hangalapura *et al*., 2004; Viola *et al*., 2009; Beloor *et al*., 2010; Abioja *et al*., 2013; Chikumba *and* Chimonyo, 2014; Olukomaiya *et al*., 2015). Stress triggers hypothalamic-pituitary-adrenal axis (Vandenborne *et al*., 2005), leading to cascade of events involved in fight/flight syndrome of sympathetic nervous system in birds. Corticosterone is one of the final hormones released by the axis, with multifunctional roles in chickens (Freeman, 1987; Carsia *and* Harvey, 2000; Shini *et al*., 2009).

Exogenous administration of corticosterone intramuscularly or orally had been recognized as a method of inducing stress in living animals instead of subjection to physical stressors (Hu *and* Guo, 2008). The target is to increase circulating plasma corticosterone (Vahdatpour *et al*., 2009; Moberg and Mench, 2000). Blood corticosterone level was reported to increase linearly as the dosage of dietary corticosterone increases. Increase in circulating corticosterone leads to increase in incidence of oxidative stress in living organisms (Orzechowski *et al*., 2000; Lin *et al*., 2004a). All tissues in animals undergo oxidative metabolism under normal situation, which naturally controlled by balance in pro-oxidants and anti-oxidants in the system. During the normal oxidative metabolic process, various reactive oxygen species and reactive nitrogen species, which are harmful, are produced. Several specialized metabolic mechanisms producing scavengers normally remove these harmful reactive species (Panda *and* Cherian, 2014; Rengaraj *and* Hong, 2015). The problem of oxidative perturbations comes into play when presence of pro-oxidants outnumbers and/or overwhelms the anti-oxidants (Sies, 1991). Oxidative stress leading to lipid peroxidation of cellular and mitochondrial membranes is implicated in deterioration of heart function in fast growing commercial broilers (Nain *et al*., 2008). Fast growth and high metabolic rate in modern birds can stimulate free radical generation and enhance oxidative stress. Besides, stress responses in chickens affect blood cells and plasma metabolites. Altan *et al* (2000) reported that heat exposure of broiler chickens caused an increase in heterophil, basophil and heterophyl/lymphocyte ratio while lymphocyte and monocyte decreased. Ferrante *et al.* (2016) identified H/L ratio as a positive adaptation indicator in chickens.

Honey is becoming a popular means of ameliorating the adverse effects of heat stress in chickens, especially under tropical conditions (Abioja *et al*., 2012; 2016; Oke *et al*., 2016; Adekunle *et al*., 2017). Though there is a dearth of information on its usefulness in stress induced with dietary corticosterone in poultry species. Ashoori *et al* (2015) concluded that early feeding with honey may improve weight gain and FCR in broiler chickens at different ages. Honey is reported to contain phyto-chemicals, phenols and other natural antioxidants that could help scavenge pro-oxidants generated in the body system under stress. Its protective effect in amelioration of oxidative stress in GIT, liver, kidney, pancreas, eye, plasma, red blood cells and reproductive organs in rats is reputed (Al-Mazrooa *and* Sulaiman, 1999; Gharzouli *et al*., 2002; Al-Waili *et al*., 2006; Erejuwa *et al*., 2010; Mohammed *et al*., 2011; Zaid *et al*., 2011; Erejuwa *et al*., 2012). It is not clear if similar effect can be elicited with honey in chickens fed dietary corticosterone. Therefore, the present study aimed at determining the effect of honey on hematology, plasma biochemistry and liver enzymes in broiler chickens treated with corticosterone in feed.

MATERIALS AND METHODS

Experimental location:

The study took place at the Poultry Unit of University Farms, Federal University of Agriculture, Abeokuta, Nigeria (latitude 7o 13'N; longitude 3o 26'E (Google Earth, 2017) and altitude 76 m above sea level). The location falls within the rain forest of the south-western Nigeria.

Animals and management:

Arbor acres broiler chickens aged 28d were allotted to four dietary treatment groups. Birds in Treatment I received diet containing no CORT plus no honey in drinking (NC0H). All the other 4 groups (Treatments II-V) received 30mg CORT/kg feed (Malheiros *et al.,* 2003) each plus either 0 (C0H), 10 (C10H), 15 (C15H) or 20ml/l drinking water (C20H). Corticosterone used was sourced from Sigma-Aldrich®, USA. The treatment lasted for 7 days.

Data collection:

Haematological parameters

Blood samples were obtained from the chickens after 7d dietary treatment via the wing web into heparinized and blank tubes for haematology and plasma biochemistry respectively. Plasma from the heparinized tubes was harvested using centrifuge. The samples were centrifuged and the plasma stored at -20oC for analyses. Wintrobes microhaematocrit and colorimetry methods (Lamb, 1991) were used to determine packed cell volume (PCV), haemoglobin concentration (Hb) and white blood cell count (WBC). Blood collected into labeled EDTA bottles were placed in the micro-haematocrit centrifuge and spun for 5 minutes at 2000 g. The PCV values were subsequently determined by measuring the height of the red cell column and expressing this as a ratio of the height of the total blood column using microhaematocrit reader. Red blood cell count was done by diluting the blood sample with 0.9% NaCl and shaking well. The diluted blood was mounted on a haemocytometer and the number of erythrocytes counted microscopically. Four Blood smears were stained using May-Grunwald and Giemsa stains approximately 4h after preparation with methyl alcohol fixation. Leucocyte differentials (heterophil, lymphocyte, eosinophil, monocyte and basophil) were counted for each smear and heterophil:lymphocyte ratio was calculated according to Yalcin *et al.* (2005). Total erythrocytic counts and total leukocytic counts were determined with the aid of Neubaur counting chamber (Haemocytometer) and Hb concentration was determined by Sahl's (acid haematin) method (Benjamin, 1985). MCV, MCH and MCHC values were calculated from PCV, Hb and RBC values (Jain, 1986).

Plasma biochemistry

Total plasma protein was determined according to Colowick and Kaplan (1955) while serum albumin and globulin was determined using bromocresol purple method of Varley *et al*. (1980). Plasma concentration of uric acid (UA) was measured by commercial colorimetric diagnostic kits (Lin *et al.,* 2004a). Creatinine was analyzed as described by Ochei and Kolhatkar (2000).

Liver enzymes

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were analysed spectrophotometrically by using commercially available diagnostic kits (RANDOX® Test Kits).

Statistical analyses:

Data collected were subjected to analysis of variance using SAS (2002) computer statistical package. Means were separated with Tukey. Means were considered significantly different at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of honey on the packed cell volume, red blood cell count and haemoglobin concentration in broiler chickens given corticosterone in feed is presented in Figures 1 – 3 respectively. Honey had significant ($p < 0.01$) effect on PCV, RBC and HB. Birds on honey treatment had higher PCV and RBC than those in CONTROL. For haemoglobin concentration, the same pattern was observed except that the values for C0H and C15H were similar. Birds on C5H recorded highest values for the 3 parameters respectively. Table 1 shows the erythrocytic indices, leukocyte and differential counts in broiler chickens offered corticosterone and different dosage of honey in drinking water. There was no ($p > 0.05$) differences in erythrocytic indices, WBC, leukocyte

differentials and H:L ratio, except for EOS ($p < 0.05$). C5H birds recorded higher EOS than C10H and C15H, but not significantly different from C0H. Plasma biochemical parameters and liver enzymes in broiler chickens offered corticosterone and different dosage of honey in drinking water are presented in Tables 2 and 3 respectively. Total protein, albumin, globulin, uric acid, creatinine, ALT, AST and ALP were not significantly ($p > 0.05$) affected by treatment.

Stress factors generally cause reduction in haematocrit, erythrocyte count and haemoglobin in poultry species (Yahav *and* Hurwitz 1996; Yahav 1999). Addition of honey to drinking water of physiologically stressed broiler chickens between 5 and 15ml per litre however improved the packed cell volume, red blood cell count and haemoglobin concentration in the present study. In consonance with the present findings, Obun *et al*. (2008) reported efficacy of honey-flavoured diet in improving haematological parameters (PCV, RBC and Hb concentration) in broiler chickens. Though the chickens used were not subjected to stressful conditions as in the present study. In another report, PCV was elevated by both 20ml honey and 200mg ascorbic acid per litre water in similar proportion in laying chickens over the control birds that receive only water (Osakwe *and* Igwe, 2015). However, the present finding is in avarice with the report of Oke *et al.* (2016). The authors found no effect of honey on PCV, RBC and Hb concentration in broiler chickens raised during dry season. Unlike in the present study where the study took 7 days, birds were raised for 84 days. Possibility of having the effects of honey on haematological parameters masked over time is there with blood sampling done weekly for 8 weeks and the data analyzed together. In the same vein, Adekunle *et al.* (2017) reported no change in PCV of heat-stressed laying pullets monitored for 16 weeks even when honey was offered up to 20ml per litre water. This might be due to the fact that circulating corticosterone in the heat-stressed laying birds was not enough to trigger enough responses as found in the relatively younger broiler chickens in the present study.

Honey contains numerous phyto-chemicals that possess anti-oxidant properties (Erejuwa *et al.,* 2012). Many researchers had identified honey as one of the promising sources of natural anti-stress/anti-oxidant (Gheldof *et al.,* 2003; Aljadi *and* Kamaruddin, 2004; Wasagu *et al.,* 2013). Blood is a good way of assessing the health status of an animal. Proportion of red cells in the blood enables the functioning of blood. The red blood cells are among the cellular part of the blood which are biconcave in shape and transport oxygen and carbon dioxide from one part of the body to another (Guyton *and* Hall, 2006). They are synthesized in the bone marrow (Ganong, 2003). In vertebrates, the most occurring pigment protein circulating in blood is haemoglobin. It functions in transportation of inhaled oxygen from lungs to the heart before being pumped to different target cells in the system by forming oxyhaemoglobin. About 200 million haemoglobin molecules are found in the cell membrane of erythrocytes (Sanyal *et al.,* 2013). It has been found in almost all domains of life. Phenols and other anti-oxidant components of honey may be involved in the development of red blood cells in the marrow of long bones in chickens.

Red blood indices, leukocytic count and differentials were not affected by honey in stressed chickens in the present findings. This agrees with the work of Oke *et al.* (2016) that reported that these parameters were not affected by honey in drinking water of heat-stressed broiler but Adekunle *et al*. (2017) stated that honey increased lymphocyte count but decrease basophil in laying pullets. It was postulated that the avian basophil may be associated with stress response in poultry species. It was noted that in feed-restricted 4-20 week old broilers recorded higher incidence of basophils compared with the control birds. This response was anchored to the increase in circulating adrenocorticotropic hormone levels (Maxwell *et al.,* 1992). Obun *et al*. (2008) reported that the WBC and its differential counts of birds on honey flavoured diets produced better immune status compared to the control dietary birds. Heterophyl/lymphocyte ratio was not affected by honey treatment in chickens (Adekunle *et al.,* 2017). Reference values for the heterophil to lymphocyte ratio of about 0.2, 0.5 and 0.8 were suggested as low, optimal and high degrees of stress, respectively in chickens (Gross *and* Siegel, 1983). All the birds in the current study are therefore to be under

optimal level of stress (0.52-0.60). Though there was gradual decrease in H/L as the level of honey increased in drinking water, the difference was not statistically different. Altan *et al*. (2000) reported that when broiler chickens are exposed to acute heat stress, the result is a decrease in monocyte and lymphocyte proportions while the proportion of eosinophil remained unaffected.

Circulating corticosteroids increase causes elevations in blood glucose, non-protein nitrogen and uric acid in stressed chickens (Lin *et al.,* 2004a,b). Blood proteins are lowered in the circulating extracellular fluid because of catabolic effects of corticosterone on the protein molecules. Obun *et al.* (2008) reported that serum biochemical components (serum protein, albumin and creatinine) of birds improved with increasing dietary honey inclusion in feed. Liver enzymes were not affected by honey in the present study. Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase are enzymes commonly found in the liver and leaks out into the general circulation when liver cells are injured. It has also been observed that a high value of ALP suggests increased activity of the liver due to the presence of toxic substances (Daramola *et al.,* 2015).

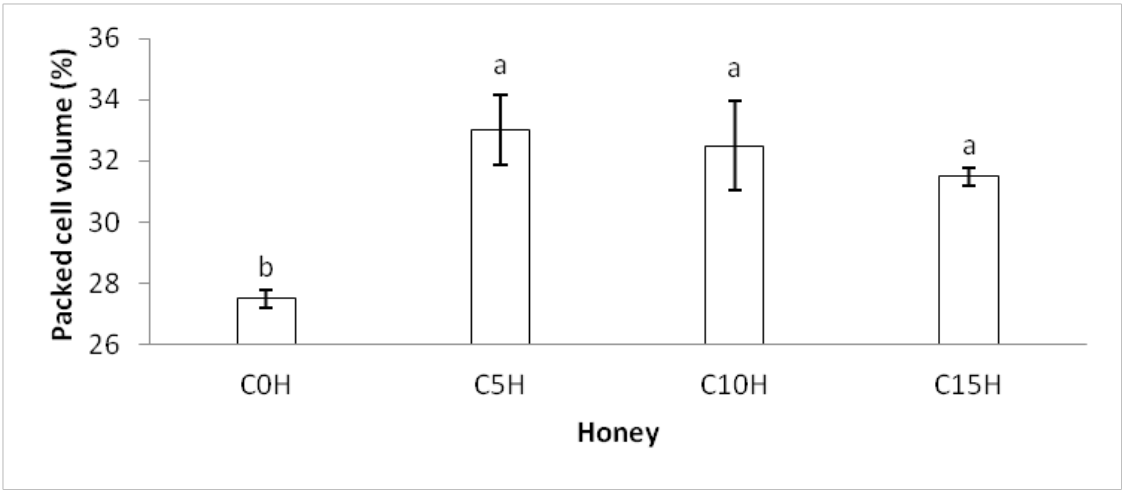

Figure 1. Effect of honey on the PCV in broiler chickens given corticosterone in feed. M ± m (n=5)

[a,b] Bars with different letters have means that differ significantly ($p < 0.01$)

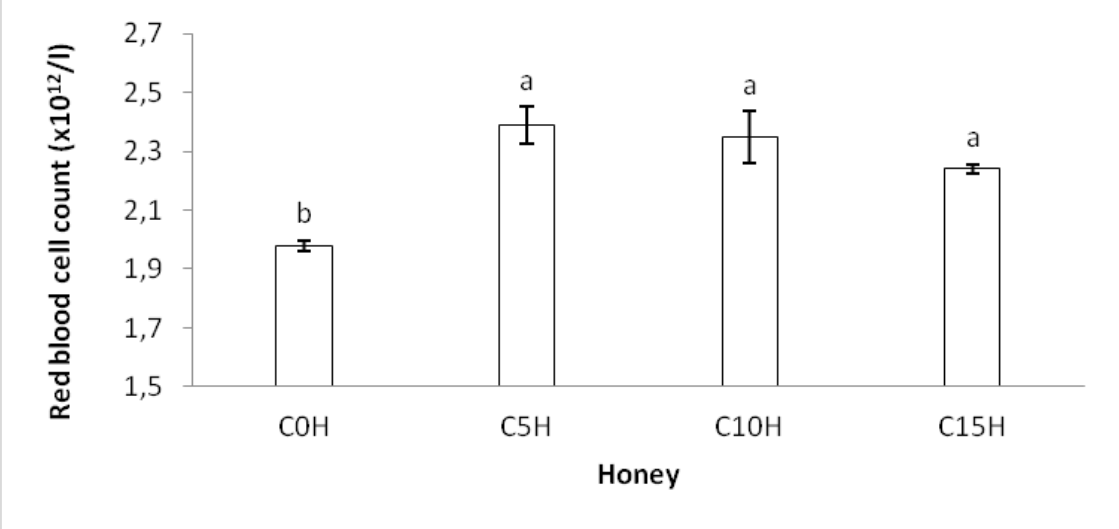

Figure 2. Effect of honey on the RBC in broiler chickens given corticosterone in feed. M ± m (n=5)

[a,b] Bars with different letters have means that differ significantly ($p < 0.01$)

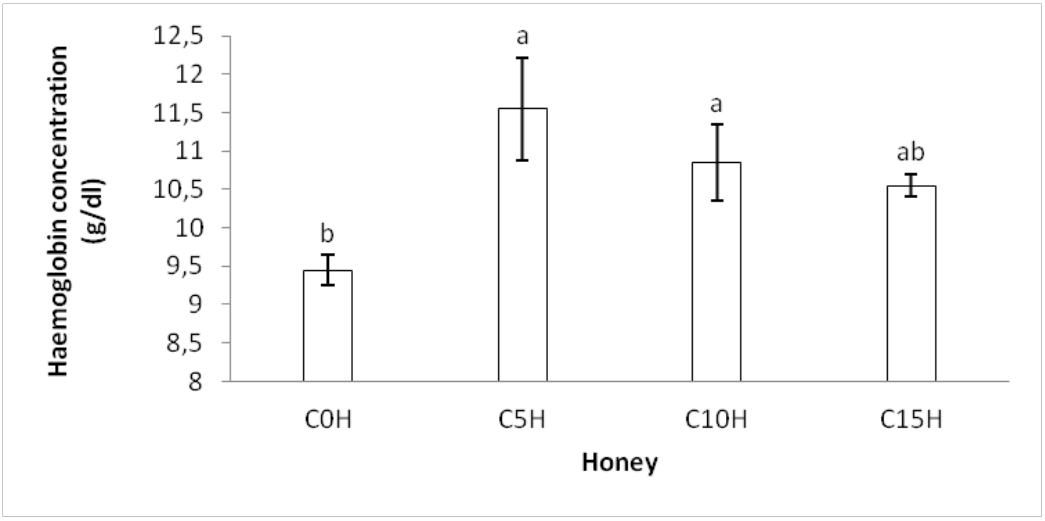

Figure 3. Effect of honey on the hemoglobin concentration in broiler chickens given corticosterone in feed (n=5)

[a,b] Bars with different letters have means that differ significantly ($p < 0.01$)

Table 1. Erythrocyte derivatives, leukocyte and differential counts in broiler chickens offered corticosterone and different dosage of honey in drinking water (n=5)

Parameter	C0H	C5H	C10H	C15H	*p-value*
MCV (μ^3)	138.9±0.24	138.0±1.16	138.5±0.87	140.9±0.38	0.0796
MCH (μμg)	47.7±0.60	48.2±1.50	46.2±0.33	47.2±0.34	0.4227
MCHC (%)	34.4±0.37	34.9±0.79	33.4±0.03	33.5±0.15	0.0913
White blood cell ($x10^9$/l)	11.6±0.14	12.2±0.43	12.1±0.38	12.2±0.06	0.4223
Heterophyl (%)	36.0±1.73	35.5±0.29	34.0±2.31	33.0±0.00	0.4691
Lymphocyte (%)	60.5±1.44	60.5±0.29	63.0±2.31	63.5±0.29	0.3042
Monocyte (%)	0.50±0.289	0.50±0.289	0.50±0.289	1.00±0.00	0.4262
Basophyl (%)	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000	1.0000
Eosinophil (%)	$3.0±0.00^{ab}$	$3.5±0.29^{a}$	$2.5±0.29^{b}$	$2.5±0.29^{b}$	0.0439
Heterophyl/Lymphocyte	0.60±0.043	0.59±0.002	0.55±0.057	0.52±0.002	0.4086

[a,b]Means with different superscripts within the same row differ significantly (*p < 0.05*)

Table 2. Plasma biochemical parameters in broiler chickens offered corticosterone and different dosage of honey in drinking water (n=5)

Parameter	C0H	C5H	C10H	C15H	*p-value*
Plasma total protein (g/dl)	5.8±0.39	5.7±0.10	6.9±0.78	5.9±0.06	0.2665
Plasma albumin (g/dl)	3.3±0.41	2.6±0.13	2.8±0.28	3.0±0.33	0.4937
Plasma globulin (g/dl)	2.5±0.48	3.1±0.09	4.1±0.68	2.9±0.36	0.1517
Plasma uric acid (mg/ml)	5.2±0.75	4.3±1.19	3.9±1.36	4.0±1.49	0.8801
Plasma creatinine (mg/dl)	4.3±0.67	3.7±0.39	4.0±0.59	3.4±0.09	0.6017

Table 3. Liver enzymes in broiler chickens offered corticosterone and different dosage of honey in drinking water (n=5)

Parameter	C0H	C5H	C10H	C15H	*p-value*
Plasma ALT (μ/l)	23.0±5.12	21.3±3.47	22.5±2.06	15.3±2.72	0.4157
Plasma AST (μ/l)	18.8±1.55	20.5±3.57	24.8±5.60	14.8±1.44	0.2868
Plasma ALP (μ/l)	9.9±1.68	9.8±0.78	7.5±1.13	9.3±0.45	0.4301

CONCLUSION

In conclusion, honey in drinking water of drinking could be of help in improving the welfare of broiler chickens during stress episode by increasing the packed cell volume, red blood cell count and haemoglobin concentration.

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