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Protective Influence of Calyces of *Hibiscus sabdariffa* Against Heat Stress in Laying Hens During the Hot-Dry Season

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Abstract: The study investigated the effect of ethanol extract and ethyl acetate soluble fraction of Calyces of *Hibiscus sabdariffa* (C.H.S) on hyperthermia, hematology and behaviour of laying hens during the hot-dry season. Experimental layers administered orally ethanol extract or ethyl soluble fraction of C.H.S at doses of 200 mg kg^{-1}, respectively, maintained a normal rectal temperature value and lower Heterophil Lymphocyte ($H L^{-1}$) ratio. They did not exhibit any stressful behaviours of excess water intake, wing fluffing or panting. The control layers not administered with any extract had higher ($p<0.01$) rectal temperature values, $H L^{-1}$ ratio and exhibited stressful behaviours, including 10% mortality rate. The correlated results between the atmospheric temperature and rectal temperature and behaviour of panting were significant and negative ($p<0.05$) in experimental layers. In control layers the correlation was significant and positive ($p<0.05$). The results showed that C.H.S reduced or eliminated the effect of heat stress in the layers. The calyces might have direct or indirect inhibitory and immuno-modulating effects on the nervous system, apart from its antioxidant and antibacterial property. Hence, could be used in ameliorating the negative effect of heat stress on laying hens predominantly reared under hot climatic conditions.

Key words: Behaviour, heat stress, *hibiscus sabdariffa*, layers, rectal temperature

INTRODUCTION

It has been established that heat stress adversely affects poultry productivity, especially in tropical countries with hot- humid climatic conditions (Anthony, 1992). Heat stress is aggravated in birds not only by excessive high ambient temperature and relative humidity, but also by the wide fluctuations in the ambient temperature between the day and night time (Anthony, 1992; Leeson, 2000). The responses of birds to heat stress virtually affect all parts of the body and they are manifested in physical, mental and emotional activities. Behavioural changes are often the first signs of disease and an indicator of welfare status (Ayo *et al*., 2002; Minka and Ayo, 2006). Stressful behaviour due to high ambient temperature and relative humidity, occurring during the hot-hours of the day are difficult to manage, especially during the hot-dry season when the cumbersome usage of modified housing designs is recommended. Even if sufficient ventilation is provided, the hot air entering and circulating within the poultry house creates a serious discomfort to birds.

It is conceivable that heat stress in poultry may be reduced by dietary supplementation of antioxidants, amino acids and mineral elements that prevent cellular dysfunction and the accumulation of oxidants. The need to sort for more effective, cheaper and readily available supplement that can combat the effect of heat stress and at the same time improve the productivity and health status of poultry became paramount.

Calyces of *Hibiscus sabdariffa* Linn. (family Malvaceae) is a common beverage drink in Nigeria, popularly referred to as 'sobo'. In many parts of the world, it is used as food and it possesses some medicinal properties. The extract of Calyces of *Hibiscus sabdariffa* (C.H.S) is reported to contain 17 amino acid and posses antibacterial (Oboh, 2004), antinociceptoic (Wang *et al*., 2000) and antipyretic activities (Daffala and Al-musatapha, 1996; Ali *et al*., 2005). It has also been shown to protect cells against oxidative stress in rats (Wang *et al*., 2000) and increases immune-modulating factors (Muller and Franz, 1992). In spite of the numerous researches in laboratory animals and the importance of C.H.S in humans, there are still limited studies that

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translate the results of these studies and the effect of C.H.S in poultry production. The medicinal and nutritive values of C.H.S suggest that is could be used in ameliorating the adverse effect of heat stress in poultry, especially in countries with hot climatic conditions.

Measurement of Rectal Temperature (RT) behaviour and Heterophil Lymphocyte (H L^{-1}) ratio in animals has been used as reliable index in the evaluation of the health and adaptability of animals to various environmental conditions.

The aim of the study was to evaluate the effects of extract of C.H.S against heat stress in laying hens during the hot-dry season.

MATERIALS AND METHODS

Experimental site, birds and management: The study was conducted at the College of Agriculture and Animal Science (CAAS) of the Ahmadu Bello University, Mando-Kaduna (11° 10 N$^{/}$; 07° 38$^{/}$ E) located in the Northern Guinea Savannah zone of Nigeria.

A total of 180, 42- week-old layers, belonging to the Shika Brown breed served as subjects. The layers were raised in a standard battery cage system and each cage contained two layers, occupying an area of 0.3 m^2 per bird. The layers were fed twice per day with recommended standard layer mash compounded by the College. Clean drinking water was provided *ad libitum.* All vaccination procedures were fully administered as scheduled. There was no medication carried out on the layers two weeks before and during the experimental period. The pen was kept clean and well ventilated. The birds were pre-conditioned to the experimental procedures 2 weeks to the day of commencement. During this period the birds were screened for evidence of diseases. Only healthy layers served as subject of the study.

Through out the study period the birds were humanely handled and treated in adherence to the International standard for animal welfare.

Experiment design: The birds were divided into three groups of 60 layers each. Group 1 served as control layers (CG) while groups 2 and 3 served as experimental 1 and 2 (E 1 and 2), respectively. Birds in CG were orally administered 2ml kg^{-1} bodyweight of distilled water. E 1 layers were orally administered ethanol extract of dried C.H.S at a dose of 200 mg kg^{-1} bodyweight. While E 2 layers were orally administered ethyl acetate soluble fraction of the dried C.H.S at a dose of 200 mg kg^{-1} bodyweight dissolved in 2 mL of distilled water. In all the groups, the water and extracts were g iven daily to individual layers at 7:00 h, thereafter drinking water and feed were provided *ad libitum.* The experiment lasted 12 weeks.

Plant material and its extraction: Calyces of *Hibiscus sabdariffa* were obtained and identified by a botanist CAAS. The calyces were shade dried. Two different methods of preparation were used to obtain the extracts. Preliminary investigation reveled that C.H.S used in this present study contained flavonoids, anthocyanin, protocatechuic acid, vitamin C and glycosides. The first extract, ethanol extract, was prepared as described by Wang *et al.* (2000) and Essa *et al.* (2006). Briefly, the shade-dried granded powder of C.H.S was subjected to extraction under reflux for 8 h with 70% ethanol. Thereafter, the extract was concentrated to a solid mass under reduced pressure using a rotavapour apparatus (Buchi Labertechnik AH, Switzerland). The obtained residual extract, 28% (w w^{-1}) was kept until used. The second extract, which was an ethyl acetate soluble fraction of C.H.S, was prepared as described by Tseng *et al.* (1997). The extracts were dissolved in distilled water and used in the study. Other chemical analysis required was performed according to the standard methods described by AOAC (1990).

Measurement of rectal temperature: The RT of 40 layers from each group was recorded at 06:00, 14:00 and 18:00 h. The RT was recorded using a digital clinical thermometer (Cocet, China). The clinical thermometer was inserted at a depth of 2-3 cm into the cloaca of each layer and kept as such until the sound of the alarm was heard indicating end of reading. The RT values were recorded 7 days before the experiment commenced and subsequently, for the 12 weeks of the experimental period three times per week.

Measurement of behavioural activities: The behavioural activities of the layers were recorded by direct observation during the experimental period, as described by Stone *et al.* (1984) and Seigel (1993) with slight modification. Briefly, the number of layers and the frequency and time spent by the layers in performing different behavioural activities were recorded from 08:00 h to 18:00 h with 2 h of observation and 2 h of pause three times per week. The timing and days of observation were interchanged to cover the entire hours of the day both during feeding and non-feeding periods for the 12 weeks of the experiment. The behavioural activities of standing, eating, drinking, lying down, wing fluffing, beak opening/panting (hyperventilation) pecking and fighting were analyzed.

Table 1: Rectal temperature responses of layers (n = 40 for each group) administered ethanol extract and ethyl acetate fraction of calyces of *Hibiscus sabdariffa*

	Rectal temperature °C											
	Mean±SEM			Maximum			Minimum			Range		
Hour	CG	E1	E2	CG	E1	E2	CG	E1	E2	CG	E1	E2
07:00	41.9±0.04	41.0±0.03	40.9±0.0	40.8	41.7	42.1	40.0	40.1	40.2	2.2	1.6	1.9
14:00	42.3±0.04	41.3±0.04	41.5±0.03	43.0	42.2	42.0	41.6	40.9	40.3	1.4	1.3	1.7
18:00	42.5±0.02	41.8 ±0.02	41.7±0.02	43.1	42.2	42.2	41.6	40.4	41.0	1.5	1.8	1.2
Mean± SEM	42.3± 0.01[a]	41.4±0.01[b]	41.4±0.03[b]	43.0±0.02[a]	42.0±0.01[b]	42.1±0.1[b]	41.3±0.03[a]	40.5±0.04[b]	40.5±0.02[b]	1.7±0.01[a]	1.6±0.01[a]	1.6±0.04[a]

Mean values along the same column with different superscript alphabets are significantly ($p<0.05$) different.

Table 2: Influence of Calyces of *Hibiscus sabdarrifa* on the percent number of layers, mean frequency and time taken by the layers performing different behavioural activities

	Control (n = 40)			Experimental 1 (n = 40)			Experimental 2 (n=40)		
Behavioural Activity	No (%)	Frequency	Time %	No (%)	Frequency	Time %	No (%)	Frequency	Time %
Stand of eating	18.4 (40)[a]	7.7±0.18[a]	50[a]	10.8 (49)[a]	4.3±1.07[a]	45.8[a]	16.8 (42)[a]	3.9±0.91[a]	41.7[a]
Lying down (rest)	12 (30)[a]	7.4±1.06[a]	18[a]	10.8(27)[a]	3.0±2.01[a]	25[b]	14 (35)[a]	4.0±1.72[a]	28[b]
Drinking	12 (30)[a]	9.7±0.71[a]	1.7[a]	7.6 (19)[b]	3.8±1.02[b]	0.2b	5.2 (13)[b]	4.2±0.82[b]	0.3[b]
Wing fluffing	10.5 (25)[a]	6±1.25[a]	2.0[a]	0 (0)[b]	0.0±0.00[b]	0.0[b]	1 (2.5)[b]	0.0±0.00[b]	0.0[b]
Pecking	5 (12.5)[a]	5.5±0.25[a]	1.6[a]	2 (5)[b]	2.0±0.20[b]	0. 0[b]	1(2.5)[b]	2.4±1.20[b]	0.2[b]
Beak opening	27 (67.5)[a]	19.8±0.92[a]	1.7[a]	5.5 (13.8)[b]	4.0±1.22[b]	0.5[b]	7.1(17.8)[b]	6.2±0.61[b]	0.3[b]
Fighting	3 (7.5)[a]	4.5±0.56[a]	2.1[a]	5(12.5)[a]	5.6±1.41[a]	1.4[a]	4.6 (11.5)[a]	4.2±1.41[a]	1.7[a]

Mean values with the same parameters along the same row having difference superscript alphabets are significantly different ($p<0.05$)

Blood analysis: Five milliliters of blood were collected from the wing vein of 20 layers from each group into heparinized test tubes. The blood samples were collected before the experiment to obtained baseline values and after every 4 weeks of the study. The samples were analyzed for PCV, Hb, TP and leucocytes count (Schalm *et al*., 1975).

Measurement of fecal water content: Fecal water content was determined by collecting feces from each group of layers three times a week for 12 weeks. After each collection, 20 g of feces were weighed from each sample and dried in an ovum. The samples were then reweighed to determine the water content.

Statistical analysis: All data obtained were subjected to student's t-test and correlation analysis. Data were expressed as mean standard error of the mean. Values of $p<0.05$ were considered significant.

RESULTS

Meteorological data: The ambient temperature had minimum and maximum values of 25.2°C and 38.8°C, recorded at 07: 00 h and 14:00 h, respectively. The mean DBT was 36.7±0.6°C, with a range value of 2.4±0.0°C. The Relative Humidity (RH) was 77.7±7.8% with a wide range of 20%. The sunshine duration was 11.15 h and the wind direction was predominantly South-east.

Rectal Temperature: The RT value recorded during the experimental period is shown in Table 1. The minimum and maximum RT values of 40.0 and 43.1°C in control layers were recorded at 0.6:00 h and 18:00 h, respectively. The control layers had a mean RT value of 42. 3±0.01°C and a range value of 1.7±0.01°C. These values were significant ($p<0.05$) higher than the values recorded in E 1 and 2 layers (41.4°C), respectively. In E 1 and 2 layers there were no significant ($p<0.05$) differences in the RT values throughout the measurement period. In all the groups the RT values recorded at 18:00 h were significantly higher than the values recorded at any time of the day. The correlated parameters between the AT and RT values were positive and significant in CG ($r = 0.8680$, $p<0.001$) while in E 1 and 2, the values were significant but negatively correlated ($r = - 0.5780$, $p<0.05$).

Behavioural activities: Table 2 shows the effect of extract of C.H.S on the number of birds found performing different behavioural activities. The number of layers, frequency and time taken performing stressful behaviours of excess water intake, wing fluffing, pecking and beak opening (hyperventilation) recorded in CG (not administered with any extract) were significantly ($p<0.05$) higher than the values recorded for E 1 and 2 layers. The result was not statistically ($p>0.05$) different between E 1 and 2. However, the behavioural activities were affected earlier in layers administered ethyl acetate fraction.

Hematological results: The result of the blood analyzed showed no significant different statistically in PCV, Hb

and TP in all the groups, however the Hb was higher in E1 and 2. The Heterophil/Lymphocyte (H L^{-1}) ratio recorded in CG was not different from their baseline values. In E 1 and 2 layers the H L^{-1} ratio was significantly ($p<0.01$) lower than the baseline values and than those recorded in CG during the study period.

Fecal water and mortality rate: The fecal water content in all the groups was not statistically different ($p>0.05$) from each other. However, feces from E1 had higher value of water than CG and E2. Mortality rate of 10% was recorded in CG. In E 1 and 2 layers no mortality recorded.

DISCUSSION

The DBT recorded during the study period was higher than the established (22-28°C) zone of comfort for poultry in the tropics (Donkoh, 1992). This indicated that the season was stressful and was characterized by a wide fluctuation in the AT. During heat stress the natural antioxidants responsible for eliminating harmful free radicals or Reactive Oxygen Species (ROS) are overwhelmed or exhausted and the first response of the body to this effect is increase in RT, above normal (42.0°C) and a shift in H L^{-1} ratio as observed in CG not administered with any extract.

The normal RT value and lower H L^{-1} ratio recorded in E 1 and 2 layers administered extracts of C.H.S suggested that the extracts ameliorated the negative effect of the high AT and enhanced the immune system of the layers by increasing lymphocyte counts. Also, C.H.S ameliorated the effect of the wide fluctuations in AT recorded between day and night time, known to be more stressful for bird than a constant high AT (Leeson, 2000). This is true because the C.H.S contain anthocyanin, flavonols, glycosides, protocatechuic acid, vitamin C and other substances, which are powerful antioxidants. The antioxidants prevent cells and tissue from oxidative damage, lipid perioxidation and also improve immunity response and productivity against stress, including challenges of diseases (Tseng *et al*., 1997; Ali *et al*., 2003; Tsai *et al*., 2002; Essa *et al*., 2006). The ameliorating mechanism was through the ability of the hydroxyl groups and other features of the antioxidants found in C.H.S in scavenging the harmful free radicals and Reactive Oxygen Species (ROS) generated in the layers as a result of high AT induced heat stress.

The behaviour of feeding suggested that the administration of extracts of C.H.S had no effects on the feeding behaviour of the layers. This may be due to the age of the layers or the fact that C.H.S at this current dose did not stimulate appetite.

The increase in the number, time and frequency of layers that drank water recorded in the CG was a clear indication that the CG was more thirsty and stressed than those administered with the extracts. The CG drank more water in order to cool their bodies since the only effective way of heat loss mechanism in birds is by evaporation of water from the birds' lungs (Anthony, 1990; Siegel, 1993). This is confirmed by the significant increased in the number, frequency and time taken by the layers performing stressful behaviour of pecking, wing fluffing and hyperventilation in CG, despite their large water consumption. The results obtained in E 1 and 2 suggested that extracts of C.H.S reduced the abnormal behaviour of layers, often encountered during heat stress and may therefore have some direct or indirect inhibitory effects on the nervous system, apart from its antioxidant property. Specific compounds from C.H.S that might be responsible for eliminating the stressful behaviour of hens in the present study were not isolated.

However, C.H.S has been reported to have neuroprotective, pro-cholinergic, anti-acetylcholinesterase (Joshi and Parle, 2006) and affect mood and behaviour (Minka and Ayo, 2006) and also possess inmmuno-modulating effect (Tseng *et al*., 1997; Buhler and Miranda, 2000). Beside, it is known to be a sedative and inhibit alpha-amylase (Gaet, 1992; Joshi and Parle, 2006). Also, the present of magnesium in C. H. S might have played a role in calming and cooling the nervous system, as it was has been reported to elevate activity of catalase and superoxide dismutase, as such has the capability of reducing oxidation (Guo *et al*., 2003), hence normalized the layers' behaviour. This ameliorating effect was further confirmed by the correlated result, which showed a decrease in RT and stressful behaviour as the AT increased with the hour of the day in E1 and 2 layers. We speculate that one or more of these properties might be responsible for ameliorating the stressful behaviour of the layers.

The result of the fecal water content suggested that C.H.S administered to layers at a dose of 200 mg kg^{-1} body weight had no effect on the motility of the intestine of the layers. Our preliminary findings showed that doubling the current dose used in the present study resulted into watery feces, sluggish and depressed behaviour of the hens. In a study conducted in rats, doses of 400-8000 mg kg^{-1} dried extracts of C.H.S induced cathartic activities of the intestine (Haruna, 1997). Doses more than 1000 mg kg^{-1} produced profuse watery stool in rats (Joshi and Parle, 2006). The present result showed no cathartic effect, apparently, due to the minimal dose of the extract used for the layers and may be due to the physiological peculiarity of the birds' digestive system. More studies are, therefore, recommended to ascertain the threshold dose for the birds.

Even though the causes of mortality in CG were not investigated, the administration of C.H.S was able to eliminate such mortality. This may be due to the immune modulating and the antibacterial effect of C.H.S, particularly its flavonoids and anthocyanin, know to inhibit bacterial growth (Oboh, 2004, Buhler and Miranda, 2000) consequently, the health status of the layers was improved. The ethanol extract and ethyl acetate fraction used in the present study showed no significant ($p>0.05$) difference in ameliorating the effect of heat stress in layers.

In conclusion, the extract of C.H.S could be used in reducing or eliminating the adverse effect of heat stress especially the high AT and its wide fluctuation prevailing during hot dry season, in countries with hot-climatic conditions.

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REFERENCES

Ali, B.H., H.H. Mousa and S. El-Mougy, 2003. The effect of a water extract and anthocyanins of *Hibiscus sabdariffa* L. On paracetamol induced hepatoxicity in rats. Phytother Res., 17: 56-59.

Ali, B.H., N. Wabel and G. Blunden, 2005. Phytochemical and toxicological aspects of H. Sabdariffa L.: A review. Phytother Res., 19: 369-375.

Anthony, J.C., 1992. The tropical Agriculture, Macmillan, London and Bashintone, pp: 60.

AOAC,1990. Official Methods of Analysis. Association of Official Analytical Chemists, (15th Edn.), Washington DC.

Ayo, J.O., S.B. Oladele and A. Fayomi, 2002. Behavoural reaction of cattle to stress situation: A review. J. Agric. Technol., 8: 15-20.

Buhler, D.R. and C. Miranda, 2000. Antioxidant Activities of Flavonoids. Department of Environmental and Molecular Toxicology Oregon State University. The Linus Pauling Institute.

Daffala, A.A., Z. Al-Musatafa, 1996. Investigation of the anti inflammatory action of Acacianilotica and Hibiscus sabdariffa. Amer. J. Chinese Med., 24: 263-269.

Donkoh, A., 1992. Ambient temperature a factor affecting performance and physiological responses of broiler chicken. Int. J. Biometeorol, 33: 259-265.

Essa, M.M., P. Subrammanian, G. Suthakar, S. Subash, T. Manivasagam, K.B. Dakshayani, R. Sivaperu Mul and G. Vinothini, 2006. Influence of Hibiscus sabdariffa (Gangura) on the levels of circulatory lipid perioxidation products and liver marker enzymes in experimental hyperammonemia. J. Appl. Biomed., 4: 53-58.

Gaet, N., 1992. *Habiscus sabdariffa* L In Medicinal plants of the World, Ira A (Ed). Human press, New York.

Guo, Z.G., J. Yuan and W. Nie, 2003. Effects and level of magnessium and Vitamin E on prevention of hepatic peroxidation and oxidative deterioration of broiler meat. Animal Feed Sci. Technol., 107: 143-150.

Haruna, A.K., 1997. Cathartic activity of Soborodo: The aqueous extract of calyx of *Hisbiscus sabdariffa* L. Phytother. Res., 11: 307-308.

Joshi, H. and M. Parle, 2006. Nootropic Activity of calyces of *Hibiscus sabdariffa* Linn. Iranian J. pharmacol. Therapeut. 5: 1-10.

Leeson, S., 2000. Broiler Feed Management During Stress, Development of Animals and Poultry Science. Geulph, Ontario

Minka, N.S. and J.O. Ayo, 2006. Behavioural responses of laying hens administered calyces of *Hibiscus sabdariffa* during the hot-dry season. Book of Abstract. The 9th Ann Conf Neurosci Soc Nig. Held in Jos, Nigeria.

Muller, B.M. and G. Franz, 1992. Chemical Structure and biological activity of polysaccharides from *Hibiscus sabdariffa*. Planta Med., 58: 60-67.

Oboh, G., 2004. Nutrient composition and anti-microbial activity of Sorrel Drinks (Soborodo). J. Med. Food 7: 340-342.

Schalm, O.W., N.C. Jain and E.J. Carroll, 1975. Veterinary Hematology. 3rd Edn. Lea and Ferbger, Philidephia. pp: 12-18.

Siegel, P.B., 1993. Behaviors-genetic analysis and poultry. Poult. Sci., 72: 1-6.

Stone, N.N., P.B. Siegel, C.S. Ackinson,W.N.B. Gross, 1984. Vocalization and behaviour of two commercial stocks of chicken. Poult. Sci., 51: 1928-1937.

Tsai, P.J., J. Mcintosh and P. Pearce, 2002. Anthocyanum and antioxidant capacity in Roselle *Hibiscus Sabdarfiffa* L extract. Food Res. Int., 35: 251-356.

Tseng, T.H., E.S. Kao, C.Y. Chu, F.P. Chou, W. Lin, C.J. Wangi, 1997. Protective effect of dried flower extract of *Hibiscus Sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food Chem. Toxicol., 35: 1159-1164.

Wang. C.T., J.M. Wang and W.L. Lin, 2000. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxidase induced hepatic toxicity in rats. Food Chem. Toxicol., 51: 411-416.