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RESEARCH ARTICLE

Effect of Molasses Supplementation on Live Weight Gain, Haematologic Parameters and Erythrocyte Osmotic Fragility of Broiler Chickens in the Hot-dry Season

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ABSTRACT

The use of haematology as an index of health status is well known, just like the use of erythrocyte osmotic fragility and heterophil-lymphocyte ratio (H:L) as biomarker of oxidative stress. The aim of this study was to investigate the effect of molasses supplementation through drinking water on live weight gain, haematologic parameters and erythrocyte osmotic fragility in 7-week-old broiler chickens during the heat stress of the hot-dry season. Thirty unsexed broiler chickens of Arbor acres strain were used for this study. At 4-week-old, the birds were randomly allotted to three groups of 10 birds each. Group I chickens served as control and were given only drinking water. Group II chickens were given 5 mL molasses and group III chickens 7 mL molasses per litre of drinking water. The percentage weight gain was higher ($P<0.01$) in group II and III chickens than control. Group III chickens had significantly higher mean corpuscular haemoglobin concentration ($P<0.05$) and mean corpuscular volume ($P<0.01$). Lymphocyte counts were higher in group II ($P<0.01$) and group III chickens ($P<0.01$), while neutrophil counts and H:L were lower in the treatment groups ($P<0.01$) as compared with control chickens. Group III chickens had significantly higher platelet count than control. At 0.1% NaCl, erythrocytes of both control and group III chickens were more fragile ($P<0.01$) than those of group II chickens. While, at 0.7% NaCl erythrocytes of control chickens were significantly more fragile than that of group III. Therefore, it was concluded that molasses increased live weight gain, enhanced haemopoiesis and ameliorated the effect of oxidative stress induced by the heat stress of the hot-dry season in 7-week-old broiler chickens.

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INTRODUCTION

The poultry industry has mainly two branches, which are; egg and meat production. Studies have indicated that broiler enterprise has great potential for increasing protein supply in Nigeria (Ezeh *et al*., 2012). Of the three seasons in the northern Guinea savannah zone of Nigeria (Igono and Aliu, 1982; Dzenda *et al*., 2011), the hot-dry season is known to have the highest ambient temperature, long duration of sunshine and high relative humidity, making it thermally stressful to animals (Igono and Aliu, 1982; Oladele *et al*., 2001). Heat stress in birds increase oxidative damage to cell evidenced by decrease in weight gain, feed intake and feed efficiency (Del Vesco *et al*., 2014).

Molasses is the final effluent obtained in the preparation of sucrose by repeated evaporation, crystallization and centrifugation of juices from sugar cane or sugar beets (Curtin, 1983). Cane molasses is a viscous and dark coloured liquid which is rich in soluble carbohydrate, vitamins, minerals and other materials (Olbrich, 1963). Some of the mineral contents are; iron, zinc, copper, manganese, potassium, sodium and calcium. The vitamins belong to vitamin B complex including: thiamine, riboflavin, niacin, panthothenic acid, biotin and choline. But molasses lacks vitamin C and has very low content of phosphorus (Curtin, 1983). Sugarcane molasses has several important roles in livestock feeding, due to the nutritive, appetizing and physical properties of its sugar content. However, toxicity of molasses has been reported

when given in large dose (Pérez, 1995). In poultry, molasses is commonly used as a binder in dry poultry diets and as an energy source. Similarly, its administration to chickens through drinking water has been reported (Reddy *et al*., 1998; Ndelekwute *et al*., 2010).

Haematology is important in evaluating the health and nutritional status of an animal (Gupta *et al*., 2007). Also, the use of heterophil-lymphocyte (H:L) ratio as a sensitive haematological indicator of stress response in chickens (Rajalekshmi *et al*., 2014) and as a general biomarker relevant to immune function (Dietert *et al*., 1996) has been demonstrated.

Free radicals are reactive oxygen, nitrogen and chlorine species that are continuously produced in the animal body. Lipid peroxidation is a highly destructive free radical phenomenon that results in alteration of membrane structures and consequently, cell damage and destruction (Evans and Halliwell, 2001; Singh *et al*., 2013). Erythrocyte osmotic fragility (EOF) which measures the resistance of erythrocyte to intracellular pressure is known to be a biomarker of oxidative stress or lipid peroxidation (Brzezinska-Slebodzinska, 2001; Adenkola *et al*., 2010; Asala *et al*., 2011). High ambient temperature is a very important stressor in hot regions of the world (Atlan *et al*., 2003) and it is known to activate the body stress mechanism (Gaughan *et al*., 2013) which adversely affects production and health of livestock (Sharma *et al*., 2013). During heat stress, large quantity of free radicals is generated in the body such that the natural antioxidant defence systems of the body are overwhelmed. Through enzymatic (mainly involving super oxide dismutase (SOD), catalase, glutathione (GSH) peroxidise and reductase) and non-enzymatic (mainly involving GSH) activities, the antioxidant defence mechanism can eliminate free radicals from the biological system (Noori, 2012).

Molasses is commonly used in Nigeria, particularly by small scale poultry farmers. This patronage is likely because its use in drinking water is relatively cheap and easy to administer. Although information on the effect of molasses on weight gain, feed and water consumption is available (Ndelekwute *et al*., 2010), few or no studies exist on haematology and antioxidant effect of molasses in chickens. Similarly, data on the physiologic response of chickens to different doses of molasses is scanty. Such information may serve as important input in the design of a versatile poultry feed ration that could ameliorate the effect of heat stress on broiler production during the hot-dry season. Amelioration of heat stress may improve performance and reduce economic loss in broiler chickens. Therefore, the aim of this study is to evaluate the live weight gain, haematologic changes and EOF as a biomarker of oxidative stress following the administration of molasses in drinking water during the heat stress of the hot-dry season.

MATERIALS AND METHODS

Study location and chicken management

The study was carried out in the Livestock Unit of the Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, Nigeria in May 2013. The chicks used for this study were of the Arbor acres strain, obtained from a commercial farm in Nigeria. The birds were housed in deep litre pen and fed Hybrid® starter and finisher feeds for 0-4 weeks and 4-8 weeks, respectively (Table 1). Water was provided *ad libitum* using a manual poultry drinker and the drinker was washed daily using sponge and water. The birds were vaccinated routinely. Molasses used in the experiment was obtained from Dangote Sugar Refinery, Nigeria. The techniques used to determine the nutrient composition of molasses were wet digestion method (for the elements) and proximate analysis (Table 2). Meteorological parameters of ambient temperature and relative humidity were obtained from the Meteorological Unit, Institute of Agricultural Research, Ahmadu Bello University, Zaria located about less than one km away from the experimental site. The minimum and maximum values of ambient temperature and relative humidity were 22.03-35.32^{o}C and 47.23-64.48%, respectively.

Experimental design

At 4-week-old, thirty (n = 30) unsexed broiler chickens were randomly allotted to three groups of 10 birds each. Group I chickens served as control and were given only drinking water. Group II chickens were given 5 mL molasses per litre of drinking water (Fayomi *et al.,* 2007), that is 1.10 mL of molasses in 220 mL of water per bird, while group III chickens were given 7 mL molasses per litre of drinking water (i.e. 1.54 mL of molasses in 220 mL of water per bird).

Blood samples were collected at 7-week-old through the wing vein into vacutainer tubes containing ethylenedeiamine-tetraacetic acid (K_3EDTA) for EOF test and complete blood count. Heamatologic parameters of Packed cell volume (PCV), Haemoglobin concentration (Hb), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular heamoglobin (MCH), mean corpuscular heamoglobin concentration (MCHC), RBC distribution width coefficient of variability (RDW-cv), RBC distribution width standard deviation (RDW-sd), platelet count (PLA), platelet distribution width (PDW), mean platelet volume (MPV) and platelet large cell ratio (P-LCR) were obtained using an automated analyser for haematology (Sysmex, KX-21N, Japan) and was subsequently crosschecked using manual complete blood count as described by Jain (1986). The total leucocyte count (TLC) and relative differential leucocytes count (lymphocytes and neutrophils) were obtained manually, using a hemocytometer and a stained slide, respectively. The heterophil-lymphocyte ratio (H:L) was then calculated. Weight of the chickens was taken individually at 4- and 7-week-old to obtain the initial and final live weights, respectively using Metlar MT-5000D Electronic Balance. Percentage weight gain was then calculated from the initial and final live weights. The daily feed given to each group of birds was weighed and the amount left in the feeders was also weighed the following day to determine the daily feed intake.

Determination of erythrocyte osmotic fragility

Erythrocyte osmotic fragility was determined according to the method described by Oyewale (1992). Briefly, 1% NaCl stock solution was prepared with phosphate buffer (3.22 g/L) at a pH of 7.4. Five millilitres

(5 mL) of varying concentrations of NaCl solutions (0.0, 0.1, 0.3, 0.5, 0.7 and 0.9%) were prepared in a set each of 6 centrifuge tubes. Blood (0.02 mL) was added to each concentration of the test solution in each tube. The contents were mixed and incubated at room temperature for 30 min and then centrifuged at 3000 g for 10 min. The concentration of haemoglobin in the supernatant solution of each tube was measured at 540 nm using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, England) by reading the absorbance. By assuming the haemoglobin concentration in 0.0% NaCl solution was 100% haemolysis, haemolysis percentage and haemolytic increment values were calculated and their curves were drawn. The NaCl solution which has 90% haemolysis was taken as maximum osmotic fragility limit and the NaCl solution which has the minimum haemolysis was accepted as the minimum osmotic fragility limit on the haemolysis percentage curves. The haemolytic increment curves were drawn from the appropriate NaCl concentration and percentage haemolysis difference values as described by Dariyerli *et al*. (2004). Haemolytic increment values were determined to find out the actual haemolysis in each tube. This calculation was performed by starting from the maximum haemolysis limit and subtracting the haemolysis amount of each tube from the next tube value.

Data analysis

Values obtained were expressed as mean (± SEM) and subjected to one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. The statistical package used was GraphPad Prism version 4.0 for windows (2003), San Diego California, USA (www.graphpad.com). Values of $P<0.05$ were considered significant.

Table 1: Nutrient Composition of the Diet (Starter and Finisher) Fed to Broiler Chickens

Feed Ingredients	Starter	Finisher
Crud protein (%)	22	21
Fat (%)	7.9	6.8
Crude Fibre (%)	4.3	3.0
Calcium (%)	2.0	2.0
Available Phosphorus (%)	0.8	0.7
Methionine (%)	0.56	0.50
Lysine (%)	1.2	1.2
Metabolizable Energy (Kcal/Kg)	2900	2980

Source: Hybrid feed®, Kaduna, Nigeria

Table 2: Nutrient Composition of Molasses

Ingredients	Amount
Moisture (%)	19.21
Crude protein (%)	5.18
Crude fibre (%)	0.0
Nitrogen Free Extract (%)	67.86
Fat (%)	3.91
Ash (%)	3.84
Calcium (mg/kg)	7962.00
Iron (mg/kg)	1718.35
Zinc (mg/kg)	14.93
Copper (mg/kg)	6.60
Manganese (mg/kg)	7.70

Table 3: Composition of Vitamins in Molasses

Vitamins	Amount (mg/kg)
Biotin	0.36
Choline	745.0
Pantothenic Acid	21.0
Riboflavin	1.8
Thiamine	0.9
Niacin	800.0

Source: Curtin LV 1983. Molasses - general considerations. National Feed Ingredients Association, West Des Moines, Iowa, USA.

RESULTS

Live weight gain

Results of initial and final live weights, percentage weight gain and daily feed consumption were presented in Table 4. No significant difference ($P>0.05$) in daily feed consumption, initial and final live weights was observed among groups, however, the percentage weight gain was significantly higher in Group II ($P<0.001$) and Group III ($P<0.01$) chickens compared to control.

Haematology

Proximate analysis of the molasses used in the current study indicates high concentration of minerals particularly calcium and iron (Table 2). Result on the effect of treatment on haematologic parameters is presented on Table 5. The PCV values of Groups I, II and III chickens were 24.4±0.77, 27.37±1.60 and 29.00±2.34, respectively. Although these values were not statistically significant, Group III birds showed a PCV value that was 15.86% higher than that of the control. Erythrocytes of group III chickens had higher MCV ($P<0.01$) and MCHC ($P<0.05$) than those of control chickens. There was no significant difference in TLC among the three groups. However, values of percentage lymphocyte counts were higher in Group II ($P<0.01$) and Group III chickens ($P<0.001$) while values of percentage neutrophil counts were lower in the treatment Groups as ($P<0.01$) compared with the control. The H:L ratio was higher in control chickens in comparison to the treatment groups ($P<0.01$). Platelet count was higher in Group III than control chickens ($P<0.01$). While variations in other parameter were not statistically significant, higher platelet count was accompanied by lower MPV and vice versa.

Erythrocyte osmotic fragility

Results of this study indicated that molasses significantly increased erythrocyte resistance to osmotic stress (Fig. 1 and 2). From the standard haemolytic curves (Fig. 1), the minimum EOF level of groups II and III was 0.7% NaCl, which is lower than that of the control chickens (0.9% NaCl). The maximum EOF level of Groups II (0% NaCl) is lower than that of Group III and control (0.1% NaCl) chickens. At 0.7% NaCl, the EOF was higher ($P<0.01$) in the control than group III chickens. In addition to the higher maximum EOF level of the control and group III chickens, they also had higher ($P<0.01$) percentage haemolysis compared with group II chickens at 0.1% NaCl concentration. Erythrocytes of control chickens commenced their haemolysis earlier (in 0.9% NaCl concentration) and completed it earlier in a higher percentage NaCl concentration (0.1% NaCl) in comparison to other groups. From the haemolytic

Table 4: Mean (±SEM) values of live weights, percentage weight gain and daily feed intake in control and chickens administered 5mL (group II) and 7 mL (group III) molasses per litre of drinking water

Parameters	Control	Group II	Group III
Initial Live Weight (Kg)	0.80±0.040	0.76±0.051	0.80±0.045
Final Live Weight (Kg)	1.56±0.051	1.87±0.048	1.78±0.037
Live Weight Gain (%)	44.44±1.16	58.46±3.27***	55.02±2.46**
Daily Feed Intake (Kg/Bird)	0.134±0.090	0.138±0.035	0.137±0.076

Mean values with superscripts ** (P<0.01) and *** (P<0.001) within rows differ significantly in comparison to control.

Table 5: Mean (±SEM) values of haematological parameters of control and chickens administered 5mL (group II) and 7 mL (group III) molasses per litre of drinking water

Parameters	Control	Group II	Group III
TLC ($x10^3$/µL)	19.78±0.96	20.09±0.48	20.78±0.31
PCV (%)	24.40±0.77	27.37±1.60	29.00±2.34
RBC ($x10^6$/ µL)	1.81±0.11	1.85±0.14	2.09±0.37
Hb (g/dL)	7.88±0.322	8.87±0.46	8.03±0.36
MCV (fL)	122.80±0.98	131.10±2.06	139.60±5.49**
MCH (pg)	38.19±0.39	42.27±1.21	41.85±2.02
MCHC (g/dL)	31.30±0.34	32.14±0.48	37.48±3.28*
RDW-cv (%)	24.12±1.38	23.41±1.50	23.37±2.39
RDW- sd (fL)	40.87±1.81	45.29±3.59	39.98±1.65
LYMP (%)	77.14±0.28	78.69±0.45**	78.95±0.21***
NEUT (%)	22.86±0.28	21.31±0.45**	21.05±0.21**
H:L	0.31±0.0073	0.27±0.0060**	0.27±0.0040**
PLA ($x10^3$/µL)	39.43±1.82	61.00±9.69	101.50±6.15**
PDW (fL)	7.35±0.15	7.43±0.64	7.81±1.18
MPV (fL)	8.10±0.00	6.96±0.33	6.88±0.35
P-LCR (%)	12.80±5.50	9.67±1.80	11.02±2.45

Mean values with superscripts * (P<0.05), ** (P<0.01) and *** (P<0.001) within rows differ significantly in comparison to control. TLC = total leucocyte count, PCV = packed cell volume, RBC = red blood count, Hb = haemoglobin concentration, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration, RDW-cv = red blood cell distribution width coefficient of variability, RDW-sd = red blood cell distribution width standard deviation, LYMP (%) = percentage lymphocyte count, NEUT (%) = percentage neutrophil count, H:L= heterophil-lymphocyte ratio, PLA = platelet count, PDW = platelet distribution width, MPV = mean platelet volume and P-LCR= platelet large cell ratio.

increment curves (Fig. 2), the control group had significantly higher percentage haemolysis difference at 0.3% NaCl compared to group II chickens. Also, a slight right-shift was noticed in the increments curve of the control chickens.

DISCUSSION

Live weight gain

Administration of molasses through drinking water did not affect feed consumption, but significantly increase percentage weight gain in broiler chickens, with the increase being marked in those that had lower dose of molasses (group II). This finding agrees with the study of Hildalgo *et al.* (2009) in vinasse (a molasses fermentation byproduct). Contrary to the result of the current study, administration of molasses has been reported to decrease feed intake, but increase live weight gain in chickens (Rahim *et al*., 1999; Ndelekwute *et al.,* 2010). The increase in percentage weight gain in the molasses treated chickens in the current study may be due to the nutritive and appetizing qualities of molasses (Pérez, 1995) which enhanced feed consumption and increased weight gain. Furthermore, molasses supplement in feed has been reported to increase the production of short chain fatty acid (SCFA) such as acetic and propionic acids in the caecum of chickens (Gultemirian *et al*., 2014) which must have contributed to the increase in weight gain (Hwangbo *et al*., 2013).

Haematology

A significant influence of molasses on erythrocyte indices (MCV and MCHC), differential leucocyte count and platelet was established. In agreement with the findings of Njidda *et al.* (2006) and Fayemi *et al.* (2007), no significant difference in PCV and RBC between control and molasses treated chickens was observed in the present study. In contrast, Fayemi *et al.* (2007) reported an increase in all other hematologic parameters besides PCV and RBC in control chickens. This difference may be because the latter study was conducted in cockerels and the molasses was not administered continuously to the cockerels.

MCV refers to the average size of individual erythrocyte (Dacie and Lewis, 1995) and younger erythrocyte are larger than older ones (Nash and Wyard, 1981). Therefore, the presence of high MCV may indicate an active erythropoiesis. The significantly higher MCV and MCHC coupled with the slight increase in PCV demonstrate a more efficient erythropoiesis in group III as compared with control chickens. Also, since results of this study indicate no significant difference in values of RWD-cv and –sd across the groups, it could be concluded that this enhanced erythropoiesis is physiological, not pathological. The improved nutrient availability due to higher dose of molasses in group III chickens may be responsible for the enhanced erythropoiesis in this group.

The iron, copper and zinc components in molasses are important nutrient requirement for the erythropoiesis. The combination of molasses and oligofructose as supplement in chicken feed is known to increase SCFA production which in turn improves absorption of minerals in the colonic epithelium (Gultemirian *et al*., 2014). Functional iron deficiency has been shown to occur in experimental copper deficiency, because copper-containing proteins hephaestin and ceruloplasmin are required for normal iron transport (Lee *et al*., 1968; Wessling-Resnick, 2006). Zinc deficiency has been reported to cause mild to moderate anaemia in growing rats (El Hendy *et al*., 2001), which is probably due to impeded erythropoiesis and protein synthesis (Shakoori *et al*., 1994). This is not unconnected to the involvement of zinc in a wide spectrum of biological activities. The current study neither demonstrated anaemia nor impeded erythropoiesis in any of the groups, but the group III chickens that were exposed to high amount of iron, copper and zinc showed more efficient erythropoiesis evident by the high MCV, MCHC and slight increase in PCV. Also, since erythrocytes that are larger (with larger MCV) are

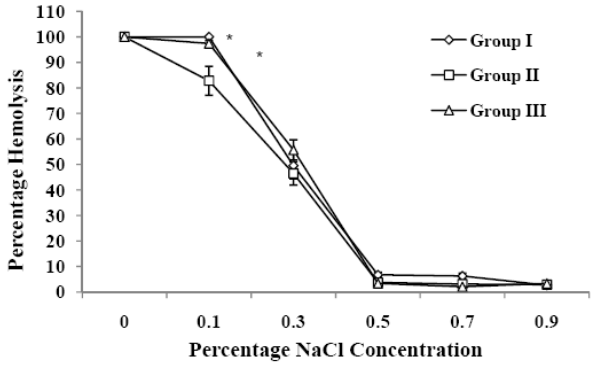

Fig. 1: Standard EOF curves of control (group I) and chickens administered 5mL (group II) and 7 mL (group III) molasses per litre of drinking water. Asterisks (*) indicate significant difference between control and the lower curve (P<0.01).

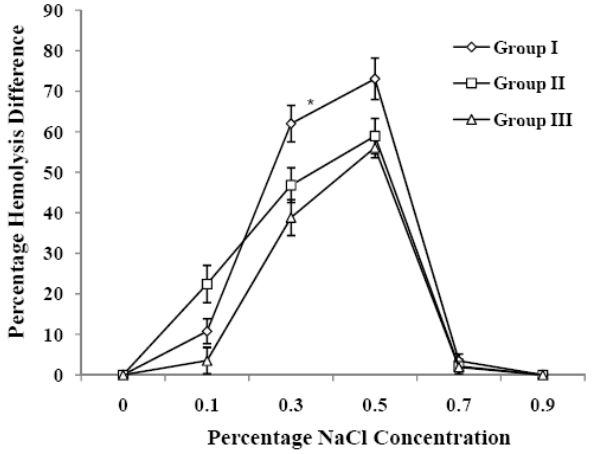

Fig. 2: Haemolytic increment curves of control (group I) and chickens administered 5mL (group II) and 7 mL (group III) molasses per litre of drinking water. Asterisk (*) indicates significant difference between control and the lowest curve (P<0.05).

more deformable (Harvey, 2010), and erythrocytes need to be deformed to pass through capillaries which are usually smaller than their size (Henquell *et al*., 1976), the higher MCV in group III chicken may allow for better oxygen distribution to tissues.

The results of H:L ratio in the present study is in agreement with the findings of Fayemi *et al.* (2007). During stressful environmental conditions, the decrease in lymphocyte and increase heterophil numbers results to an increase in H:L ratio (al-Murrani *et al*., 1997). In the present study, the heat stress of the hot-dry season decreased the lymphocyte count, but increased heterophil count and H:L ratio in the control as compared with treated chickens. Therefore, the administration of molasses classically reduced heterophil count and H:L ratio, but increase lymphocyte count, thus enhancing the immunity of the molasses treated chickens. In a pattern similar to that observed in the current study, antioxidants such as melatonin, vitamins A and C have been shown to ameliorate the haematologic change induced by heat stress in chickens (Ajakaiye *et al*., 2010; Rajalekshmi *et al*., 2014). Similarly, in broiler chickens exposed to heat stress and continuous lighting, diet with low amount of molasses (2%) and vitamin C have been shown to be more beneficial (Hwangbo *et al*., 2013) than diet with high amount of molasses (5%) and vitamin C (Park *et al*., 2013). In the chickens that were supplemented with low amount of molasses (2%), but exposed to extreme heat stress and continuous lighting, both weight gain and immune status were reported to improve (Hwangbo *et al*., 2013).

Alterations in P-LCR, MPV and PDW occur in thrombogenic disorders (Babu and Basu, 2004; Boos and Lip, 2007). In the present study, the increase in platelet count in group III chickens was not accompanied by significant changes in values of P-LCR, MPV and PDW; hence, the increased platelet count is physiological. Also, agreeing with the findings of Bozkurt *et al.* (2006), the current study observed that an increase in platelet count was accompanied by a decrease in MPV and vice versa.

Erythrocyte osmotic fragility

Using EOF test as an index of oxidative stress (Brzezinkska-Slebodzinska, 2001) vis-a-vis lipid peroxidation of erythrocyte membranes, it can be inferred from the results of this study that molasses markedly ameliorated the effect of oxidative stress induced by the hot dry season in chickens. To our knowledge, the present study is the first document to demonstrate that supplementation of chickens with molasses through drinking water can ameliorate the high EOF (lipid peroxidation) induced by heat stress of the hot-dry season. Indicating an increase in susceptibility to oxidative damage, erythrocytes of control chickens commenced their haemolysis earlier (in 0.9% NaCl concentration) and completed it earlier in a higher percentage NaCl concentration (0.1% NaCl) in comparison to other groups. The improved antioxidant activity in molasses treated chickens is likely due to the content of zinc, copper and manganese in molasses. Copper and zinc, and manganese are indispensable metals for the activities of Cu, Zn-SOD and Mn-SOD, respectively. Dietary deficiencies of these minerals markedly decrease tissue Cu,Zn-SOD and Mn-SOD activities and result in peroxidative damage and mitochondrial dysfunction (Aruoma, 1998). Severe and moderate zinc deficiency has been shown to cause oxidative damage to lipids, proteins and DNA in rat testes (Oteiza *et al.,* 1995) which is attributed to a reduction in zinc-dependent antioxidant production (Evans and Halliwell, 2001). Increased reactive oxygen species (ROS) generation and intestinal inducible nitric oxide synthase (iNOS) expression have been found in rats that are deficient in copper or zinc (Hammermueller *et al*., 1984; Wepnir, 2000). Such effects render the animal more susceptible to lipid peroxidation (Hammermueller *et al.,* 1984). In addition, zinc promotes cell membrane integrity (Xia *et al*., 1999), and its deficiency is associated with increased EOF (O'Dell *et al*., 1987; Roth and Kirchgessner, 1994).

The riboflavin, niacin and thiamine in molasses may also contribute to its antioxidant effect. FAD and NADPH are cofactors for glutathione reductase, the major enzyme responsible for regeneration of GSH from oxidized glutathione (Sies, 1999). Riboflavin and thiamine which serve as component of FAD/FADH and being essential for NADH generation, respectively, play important roles

in protecting organisms from oxidative stress (Aruoma, 1998; Evans and Halliwill, 2001). Also, through the involvement of niacin in inhibiting the expression of iNOS it reduces the generation of NO which is a reactive nitrogen species (Garcion *et al*., 1997). The improved erythrocyte membrane integrity in molasses treated chickens observed in the current study may have been mediated by the minerals and vitamins in molasses which could have elevated the levels of SOD, GSH, reduced ROS production and promote cell membrane integrity. Similarly, antioxidants such as melatonin (Sinkalu *et al*., 2014), vitamins C and E have been shown to ameliorate the increased EOF induced by oxidative stress (Uzum *et al*., 2006; Ambali *et al*., 2010) in the same pattern molasses ameliorated heat stress in the current study. Also, since heat stress is known to redistribute body's nutrients such as protein and energy and, consequently, interfere with growth and production (Sharma *et al*., 2013), the reduced heat stress in the molasses treated chickens may have contributed to the increase in live weight in the chickens.

The results of the present study indicate that the erythrocytes of the control chickens had the highest EOF which was due to the heat stress of the hot-dry season, while those of group II chicken had the lowest EOF likely due to the ameliorative effect of molasses. However, chickens with the highest amount of molasses (group III) also showed moderately high haemolysis particularly at 0.1% NaCl. High extracellular or intracellular concentration of free iron resulting from dietary overload has been reported to promote production of free radicals, lipid peroxidation and oxidative stress (Dabbagh *et al*., 1994). The molasses used in the current study contains high amount of iron. This may have increased intercellular and extracellular free iron, predisposing the erythrocytes of group III chickens to greater lipid peroxidation and consequently an increase in haemolysis. Notwithstanding, at 0.7% NaCl, erythrocytes of group III chickens were more resistance to hypotonic haemolysis than those of the control. This may suggest that though there was a decrease in susceptibility of erythrocytes of group III chickens as appeared in higher NaCl concentration (0.7% NaCl) due to the ameliorative effect of molasses, lipid peroxidation caused by increased level of free iron may have masked such effect resulting in an increase in EOF in lower NaCl concentration (0.1% NaCl) which is more hypotonic. However, the pattern of EOF between group III and control chickens may require more research using different indices of oxidative damage to further evaluate the ameliorative effect of molasses.

This study has demonstrated that molasses supplementation through drinking water can increase live weight gain and improve haematopoiesis. Using leucocytic parameters and EOF to evaluate stress, it was observed that molasses ameliorated the effect of thermal stress induced by the hot-dry season. Perhaps, its content of minerals and B vitamins may be responsible for the ameliorative effect. The results of this study also suggest that 5 mL molasses per litre of drinking water is optimum for broiler chickens and can be administered as supplement, particularly in poultry farms were manual poultry drinkers are used. However, further studies are required to evaluate the content, benefits and safety of sugar cane molasses administration through drinking water in broiler chickens.

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