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Erythrocyte Osmotic Fragility and Excitability Score in Rabbit fed *Hibiscus Sabdariffa* in Graded Level

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Summary: This study was conducted for 10 weeks with the aim of investigating the erythrocyte membrane integrity as measured by erythrocyte osmotic fragility and excitability scores of rabbits fed graded level of *Hibiscus sabdariffa* calyx (HSC). Twenty weaners' rabbit of both sexes were used for the study and were placed on four experimental diets which contain the following percentages of HSC 0 %, 25 %, 50 %, 75 %, as feed additive and were added at 0 g, 62.5 g, 125 g, 187.5 g designated as T_1, T_2, T_3 and T_4 experimental diets. Excitability scores were measured weekly as described by Voisnet *et al*. (1997). At the end of the experiment, the rabbits were slaughtered by severing the jungular vein. A Blood sample (2 ml) was collected from each rabbit into sampled bottles, containing the Na EDTA as anticoagulant for hematological analysis. Packed cell volume (PCV) Haemoglobin concentration (Hb), Total red blood cell (RBC) count, Total leukocyte count as well as differential leukocyte was determined using standard method. The percentage haemolysis recorded at 0.3 % to 0.8 % was significantly ($P < 0.05$) higher in rabbits in T_1 compared to the remaining 3 diets. The result of excitability score shows that rabbit on diet 1 and 2 had a lower value which was significantly ($P < 0.05$) lower than rabbits on diets 3 and 4 with a value of 65.5 ± 5.0 and 70.00 ± 5.50 % respectively. In conclusion this study demonstrated for the first time that chronic administration of HSC improves haematological parameters, brain mood and function as well as maintaining erythrocyte membrane integrity.

Keywords: Erythrocyte osmotic fragility, Excitability score, *Hibiscus sabdariffa*, Rabbits, Haematological parameters.

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INTRODUCTION

Hibiscus sabdarifa calyces (HSC) is a common beverage drink in Nigeria, and it possesses some medicinal properties, the extract has been reported to contain 17 amino acid and possess antibacterial (Oboh, 2004) and antipyretic activities (Ali *et al*., 20005). 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; Ismail *et al*., 2008). The medicinal and nutritive values of calyces of HSC suggest that it could be used in ameliorating the adverse effect on heat stress in poultry (Minka *et al*. (2007) because it was reported by Wang *et al.* (2000) and Essa *et al.* (2006) that it contain flavonoid anthocyanin and vitamin C and there are indications as postulated by several authors that extracts from the calyces of HSC contain antioxidant principles (Wang *et al*., 2000; Tee *et al*., 2002; Ologundudu and Obi, 2005; Ologundudu *et al*., 2009). The extract has been demonstrated to protect cells against oxidative stress in rats (Wang *et al*., 2000) and against oxidative tissue damage (Wolff *et al.*, 1986). Haematological and behavioural parameters have been used to assess the degree of stress and also the health status in livestock (Adenkola and Ayo, 2010).

This study was therefore design to investigate the excitability and erythrocyte osmotic fragility of rabbits fed graded level of HSC.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at Federal Housing Estate in North Bank Makurdi (07^0 $41^/$ N, 08^0 $37^/$ N) in the Southern Guinea Savannah Zone of Nigeria.

Experimental Animals and Management

The study was conducted using a total number of twenty weaners' rabbit of both sexes which were obtained within Makurdi metropolis in a study which lasted 10 weeks. The animals were randomly allocated to four dietary treatments groups with five rabbits per treatment in a completely randomized design. The cages were kept in an open sided building for easy and effective cross-ventilation, and the cages were washed thoroughly and disinfected at least two weeks before the animals were brought in. The rabbits were kept for seven days prior to the commencement of the study, during which the animals were accustomed to routine handling, and

acclimatize to the new environment, as well as to stabilize them from the stressful effect of road transportation (Adenkola *et al*, 2011) which they may have been subjected to from where they were purchased to the experimental site.

Experimental Design

The animals were allocated to four dietary treatments. The four experimental diets were formulated contain the following percentages of HSC 0 %, 25 %, 50 %, 75 %, was added at 0 g, 62.5 g, 125 g, 187.5 g for T_1, T_2, T_3 and T_4 experimental feed (Table 1).

Determination of Haematological Parameters

At the end of the experiment, the rabbits were slaughtered by severing the jungular vein. Blood samples (2ml) was collected as the rabbits bled into sampled bottles, containing the anticoagulant, sodium salt of ethylene, diaminetetraacetic acid at the rate of 2 mg/ml of blood (Adenkola and Ayo, 2009). After collection, the blood samples were taken to the physiology laboratory, in the department of veterinary physiology and pharmacology, University of Agriculture Makurdi, for hematological analysis, in which packed cell volume (PCV) was determined using microhaematocrit method, haemoglobin concentration (Hb) using the cyanomethaemoglobin method, total red blood cell (RBC) count, total leukocyte (WBC) count and differential leukocyte was also determined using the haemocytometric method (Schalm *et al* 1975).

Erythrocyte Osmotic Fragility Determination

Sodium chloride solution was prepared according to Faulkner and King (1970) in volume of 200ml for each of the samples in concentration ranging from 0.1 to 0.85 at pH 7.4. a set of 10 test tubes, each containing 5 ml of sodium chloride solution of concentration ranging from 0.1 to 0.85 %, where arranged serially in a test tube rack. One set of the test tube was used to analyze each sample. The test tubes were labeled with corresponding sodium chloride concentrations. A drop of blood was dropped into each of the ten test tubes using a syringe. The content was then mixed by gently inverting the test tubes for about 3 times. The test tubes were allowed to stand at room temperature (26 - 27^0 C) for 20 minutes. The contents of the test tubes were maintained at pH 7.5 thereafter the contents of the test tube were centrifuged at 1,500 g for 20 minutes. The supernatant of each test tube was transferred into a cuvette. The concentration of haemoglobin in the supernatant solution was measured at 540 nm using a spectrophotometer by reading the absorbance. The same procedure was repeated for every blood sample used for the study. The percent haemolysis was then calculated using the formula (Faulkner and King, 1970):

$$\text{Percent Haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} X\ 100$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the sodium chloride concentrations.

Measurement of Excitability Scores

Excitability scores were recorded weekly. They were measured as described by Voisnet *et al*. (1997), Kannan *et al.* (2002), and Maria *et al*. (2004). While weighing, a score of one to four was allocated to each rabbit by a single observer; a higher score representing a greater level of excitability. A score of one was allocated to a rabbit that was calm, and made little movement during the handling. Two was allocated to a rabbit that occasionally shook itself in an attempt to escape, while three was assigned to a rabbit that continuously attempted to free itself. A score of four was given to those that struggled violently throughout the entire weighing period.

Statistical Analysis

All the data obtained were subjected to analysis of variance and where significant difference exists, the Means were separated using Duncan multiple test. Data were expressed as mean of standard error of mean. Values of $P < 0.05$ were considered significant.

RESULTS

The recorded PCV and haemoglobin concentration in rabbits in diet 1 was significantly ($P < 0.001$) lower than rabbits in the remaining 3 diets, similarly total RBC of 5.68 ± 0.18 and 5.43 ± in diet 3 and 4 respectively was significantly ($P < 0.05$) higher than rabbit on diet 1 and 2. Total WBC and differential has no specific pattern and they are not significantly ($P > 0.05$) different.

The percentage haemolysis recorded at NaCl concentration of 0.85 % was lowest in all the groups; the median and maximum corpuscular fragility occurred at 0.45 % and 0.1 % NaCl concentration. The percentage haemolysis recorded at 0.3 % to 0.8 % was significantly ($P < 0.05$) higher in rabbits in diet 1 compared to the remaining 3 diets (Figure 1). The results of the excitability in experimental and control rabbits are shown in Figure 2. An excitability score of 4 was recorded in 70.00 ± 5.50 % in rabbit

Table 1: Composition of Experimental Diets

Ingredients	Dietary Treatment			
	T1	T2	T3	T4
Maize (kg)	11.75	11.75	11.75	11.75
Full fat soya beans (kg)	6.38	6.38	6.38	6.38
Rice offal (kg)	4.50	4.50	4.50	4.50
Brewer dry grain (kg)	1.50	1.50	1.50	1.50
Bone Meal (Kg)	0.75	0.75	0.75	0.75
Common Salt (g)	02.5	02.5	02.5	02.5
Mineral and Vitamin (g)	62.5	62.5	62.5	62.5
Additive (g)	0	62.5	125	187.5
Total	25.00	25.00	25.00	25.00

Table 2: Haematological Parameters of Rabbit Fed Graded level of *Hibiscus sabdariffa*

	Dietary Treatments			
Haematological Parameters	T1	T2	T3	T4
Packed Cell Volume	35.33 ± 0.88	42.00 ± 0.58	38.00 ± 1.00	38.67 ± 1.76
Haemoglobin Concentration	11.77 ± 0.30	14.00 ± 0.19	12.67 ± 0.34	12.89 ± 0.59
Total Erythrocyte	3.82 ± 0.54	4.67 ± 0.36	5.68 ± 0.18	5.43 ± 0.48
Total leukocyte	2.13 ± 0.33	2.40 ± 0.61	2.30 ± 0.30	2.73 ± 0.33
Lymphocyte	42.00 ± 10.15	34.33 ± 2.91	43.50 ± 13.50	38.33 ± 0.67
Monocyte	4.00 ± 1.15	5.33 ± 0.33	5.00 ± 1.00	4.33 ± 0.88
Neutrophils	52.33 ± 8.84	57.00 ± 3.61	46.50 ± 15.50	54.00 ± 2.52
Basophils	0.67 ± 0.33	2.00 ± 0.58	2.50 ± 1.50	2.00 ± 1.15
Eosinophils	1.0 ± 0.58	1.67 ± 0.33	2.00 ± 1.00	1.00 ± 1.00

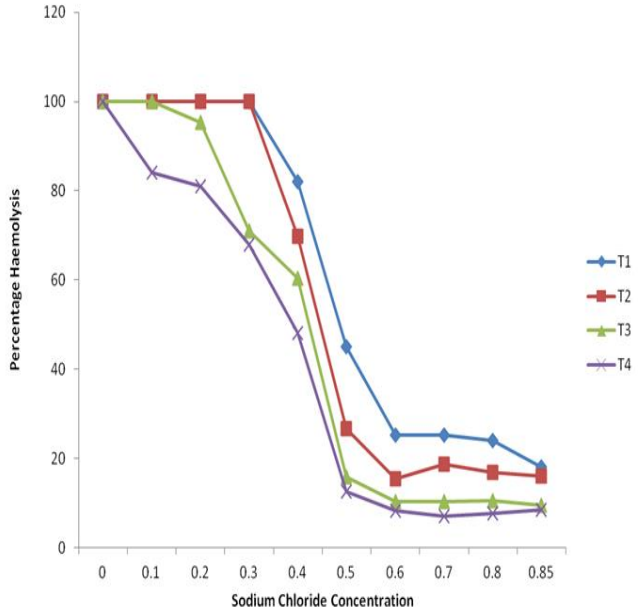

Figure 1: Erythrocyte Osmotic Fragility of Rabbit Fed Graded level of *Hibiscus sabdariffa*

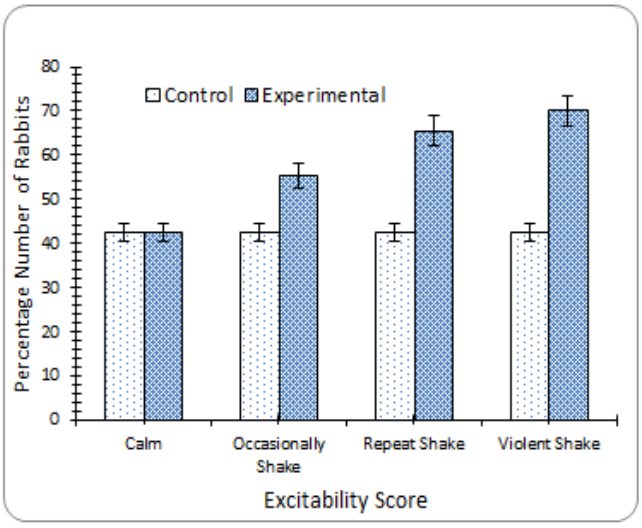

Figure 2: Excitability Score of Rabbit Fed Graded level of *Hibiscus sabdariffa*

on diet 4, while rabbit on diet 1 and 2 had a lower value which was significantly ($P < 0.05$) lower than rabbits on diets 3 and 4 with a value of 65.5 ± 5.0 % and 70.00 ± 5.50 % respectively.

DISCUSSION

In recent time, researchers has focused their attention on the protective biochemical functions of naturally occurring antioxidants in biological systems (Okasha *et al*., 2008; Bako *et al*., 2009 Ahur *et al*., 2010) and most of this plants contain phenolic compounds widely distributed in them and these were considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems (Stanner *et al*., 2004; Olatunji *et al*., 2006). The non-significant in total WBC and differential leukocyte value obtained in this study attributed to the fact that the experimental diets are in no way inferior to other conventional feeds of rabbits and don't pose any nutritional stress to the animals. The obtained values of PCV, Hb and concentration and total RBC in rabbits on diet T_3 and 4 could be attributed to the fact that higher concentration of the HSC possibly supports haematopoesis and HSC has been documented to induce renal secretion of erythropoietin which is needed for proliferative and maturative stages of the erythroblast (pluripotent stem cells) involved in cell formation (Kaur and Kapoor, 2005). The normal function of the erythrocyte is largely hinged on the maintenance of the integrity of its membrane. The lysis of the erythrocyte membrane resulting in increased haemolysis as seen in rabbit on diet 1 (control) may have risen from the increased lipoperoxidative changes which has been documented to cause increase haemolysis (Ambali *et al*., 2010) and this might have led to less haemolysis seen in rabbit on diet 4 in this study.

In contrast the lower percentage of haemolysis recorded in rabbits on experimental diets (2, 3, 4) increases as the percentage of inclusion decreases could possibly be due to the fact that HSC possess and antioxidant property that consolidates the integrity of erythrocyte membranes of and, therefore reduces their oxidative damage. The destruction of erythrocytes observed in this study as evidenced by increase in haemolysis, which act as powerful free radical generators when found as free ions in high concentration (Adenkola, 2010). The extract of HSC has been demonstrated to protect cells against

oxidative stress in rats (Wang *et al*., 2000) and that the antioxidant effect of HSC may be due to the fact that the calyces of the plant contain ascorbic acid and tocopherol as revealed by the preliminary phytochemical screening (Ali *et al.*, 2005; Mohammed *et al*., 2007; Bako *et al*., 2009). This effect seen in this study may be attributed to the possible antioxidant effect of HSC.

Excitability of animals depends on their temperament and temperament in animals is a trait that seems to be stable over time. HSC has been documented to contain vitamin C (Wang *et al*., 2000; Essas *et al*., 2006) and other antioxidant principles (Tee *et al*., 2002; Ologundudu *et al*., 2009). Vitamin C as an antioxidant has been documented to increase excitability scores in animal (Ayo *et al.*, 2006) possibly because it plays a significant role in the synthesis of vitaminergic neurotransmitters in the brain. The findings in this study demonstrated the ability of HSC content of the feed to activate the nervous system especially in rabbit fed higher concentration in their feed. This could possibly explain the higher excitability score seen in rabbits fed higher concentration of HSC in their feed as additives.

In conclusion this study demonstrated for the first time that using of HSC in feed will improves haematological parameters, brain mood and function as well as maintaining erythrocyte membrane integrity.

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