

## Phytochemical properties of *Hibiscus sabdariffa* Calyx and the effects of its aqueous extract supplementation on haematological and serum biochemical indices of broiler birds

<sup>1</sup>Ugwu D.O., <sup>2</sup>Jiwuba P.C., <sup>2</sup>Ubogu V.R. and <sup>2</sup>Akazue R.C.

<sup>1</sup>Department of Science Laboratory Technology, Federal College of Agriculture, Ishiagu, Ebonyi State

<sup>2</sup>Department of Animal Production Technology, Federal College of Agriculture, Ishiagu, Ebonyi State

**Corresponding author:** [ugwudaniel2016@gmail.com](mailto:ugwudaniel2016@gmail.com); Phone: +2348065671192

### Abstract

The study was carried out to examine the effect of dietary supplementation of *Hibiscus sabdariffa* aqueous extract on the haematological and serum biochemistry indices of broiler birds. The experiment lasted for eight weeks. Ninety six broiler birds of agro-tech strain were randomly assigned to four treatments each replicated three times with twenty four birds per treatment. The treatment groups are: T1 control (anti-biotic), T2 (50 ml of *Hibiscus* extract into 1litre of water), T3 (100 ml of *Hibiscus* extract into 1 litre of water), T4 (150ml of *Hibiscus* extract into 1 litre of water) respectively. Results showed that there were significant differences ( $P<0.05$ ) among the four treatment groups for all the haematological parameters studied except for PCV. However, all the values fall within the normal range for healthy chickens. The serum total protein, AST, ALP, ALT, creatinine and glucose values showed no significant difference ( $P<0.05$ ) among the treatment groups. There was a significant difference ( $P<0.05$ ) in the serum urea in T4 compared to T2. *Hibiscus sabdariffa* aqueous extract from the present research have no harmful effect on the internal organs such as the livers and the kidneys.

**Keywords:** *Hibiscus sabdariffa*, blood profile, poultry, phytogetic compound and alternative antibiotics

### Description of Problem

Achieving sustainable and rapid production of high quality animal proteins have become a global concern due to the difficulty in meeting the protein need of an ever increasing human population. Poultry meat from broiler production is a very good source of high quality and readily digestible proteins. Antibiotics have been used for decades as growth promoters and as feed supplements in broiler production (1). However, there are concerns in recent years about the continued use of antibiotics as supplements at sub therapeutic level in poultry feed due to the emergence of multiple drug resistant bacteria (2). The use of antibiotics in livestock production are currently being replaced with phytogetic compounds from plant materials which serve as natural sources of growth promoters and are more

environment friendly and pose less or no threat to humans and animals (3). One of such plants with known medicinal and nutritional values is *Hibiscus sabdariffa*.

*Hibiscus sabdariffa* belongs to the super order Malvaceae and it is believed to originate from East Africa (4). The plant is widely cultivated for its strong fibers and it is well known for its edibility and medicinal properties, though the calyx is the most frequently used portion of the plant, the leaves and seeds are often made into salads, curries and potherbs (5). *Hibiscus sabdariffa* is known as 'roselle' in Australia and Ivory Coast and Zobo in Nigeria (6). The plant is used traditionally in many folk medicines (7). It is reported to be antihypertensive, antiseptic, sedative, diuretic, digestive, purgative, emollient, demulcent and astringent (8). The leaves and calyces of *Hibiscus sabdaiffa* are

used as vegetables in many tropical countries as they are rich in vitamins A and C, natural carbohydrates, protein and other antioxidants including minerals (9, 10). The calyx also contains anthocyanins, flavonoids and polyphenols which act as antioxidants and have cardioprotective and hepatoprotective actions (11).

Blood and serum parameters are major indices of the physiological, pathological and nutritional status of the animal and they also help to ascertain animal feed quality and toxicity (12, 13). Inadequate intake of protein and energy decreases packed cell volume (PCV) and haemoglobin (Hb) concentrations

which indicates anaemia (14, 15). Increased level of serum urea could be due an indication of the lowering of the protein quality of animal diet caused by the presence of anti-nutritional factors in such diets (16). Kidney malfunction could also lead to an elevated level of serum urea level (17).

Little information is available on the effect of the use of *Hibiscus sabdariffa* as a natural growth promoter and supplement on the blood and serum profile of broiler finishers; hence this study. This study was aimed at evaluating the effect of *Hibiscus sabdariffa* flower extract on the heamatology and serum biochemistry of broiler finishers.

**Table 1: Gross Composition of Experimental Broiler Finisher Diet**

Ingredients	Diet
Maize	54
Soyabean meal	15
Groundnut cake	12
Corn offal	9
Palm kernel cake	4
Fish meal	2
Oyster shell	1
Salt	0.25
Premix	0.25
Methionine	0.25
Lysine	0.25
<b>Total</b>	<b>100</b>

**Calculated values**

Crude protein (CP) (%) = 21.10, Metabolizable energy (Kcal/kg) = 2841.67, Crude fiber (%) = 4.70  
 Ether extract (%) = 5.65, Calcium (%) = 1.60, Phosphorus (%) = 0.58, Methionine (%) = 0.64,  
 Lysine (%) = 2.26

**Table 2: Phytochemical composition of *Hibiscus sabdariffa* calyx**

Composition	Values (mg/100g)
Flavonoids	17.02 ± 0.01
Anthocyanin	1.72 ± 0.01
Phenol	8.77 ± 0.02
Tannin	16.23 ± 0.01
Saponin	1.32 ± 0.01
Alkaloid	3.18 ± 0.01

Each value is expressed as mean ± standard deviation (n = 2)

## Materials and Methods

**Experimental Site:** The experiment was carried out at the Poultry Unit of Federal College of Agriculture Ishiagu, which is located on latitude 5° 55' North and Longitude 7° 31' East with the mean annual rainfall of 1655 mm, mean annual temperature of 28.8°C and relative humidity of 65% (18).

**Sourcing and Preparation of experimental materials:** Air dried samples of *Hibiscus sabdariffa* were sourced from Ishiagu, Ebonyi State, Nigeria. The flower was washed to keep them free from dust. Other foreign materials were removed to make it as clean as possible. Fifty grams (50g) of *Hibiscus sabdariffa* flower were soaked in one liter (1 liter) of hot boiling water for 15 minutes prior to usage. The collected aqueous extract was then added to the drinking water.

**Experimental Diet:** The birds were fed with a formulated finisher diet. The diet was fed *ad lib*. (Table 1)

**Experimental Birds, Management and design:** A total of ninety-six (96) day old broiler chicks were sourced from a reputable commercial poultry outlet in Enugu, Enugu state. The birds were divided into four (4) treatments of three (3) replicates and each replicate contained eight (8) birds in a Completely Randomized Design (CRD). *Hibiscus sabdariffa* flower extract was added at 0, 50, 100 and 150ml/liter of drinking water representing T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> respectively. Birds on treatment one received synthetic antibiotics instead of the leaf extract. Before the arrival of the birds, the brooder house was thoroughly swept, cleaned, disinfected and littered with wood shaving. The brooder house was heated to 32.3°C - 33.3°C. Fresh water containing glucose was given to combat stress and enable them gain instant energy. Feed and water was supplied *ad-libitum* to the birds. The

birds were subjected to routine vaccination and broiler management procedure.

**Blood analysis:** At the end of the experiment (48 days) four birds from each replicates were randomly selected for the blood through the marginal wing vein for haematological and serum biochemical studies. The samples were separated into two lots and used for biochemical and haematological determinations as described by (19). Four milliliters of blood sample was collected from each sample in labeled sterile universal bottle containing ethylene diamine tetraacetic acid (EDTA) and used for haematological analysis. Another 4 ml was collected over anticoagulant free bottle and used for serum biochemical determination. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyser and Bayer DCA 2000+ HbA1c analyser, respectively. Mean cells haemoglobin (MCH), mean cell volume (MCV) and mean cell haemoglobin concentrations (MCHC) were calculated.

**Chemical Analysis:** The phytochemical analysis of the test sample (*Hibiscus sabdariffa* flower) was carried out using the procedure of (20).

**Statistical Analysis:** Data collected was analysed by one-way analysis of variance using (SAS 2000) means were compared using Duncan's Multiple Range Test of the same package.

## Results and Discussion

The result of the phytochemical evaluation (Table 2) of the *Hibiscus sabdariffa* calyx showed the presence of phytochemical compounds such as phenols, flavonoids, saponins, alkaloids, anthocyanins and tannins and these could be responsible for the medicinal and biological properties of *Hibiscus*

*sabdariffa*. Flavonoids, phenols and anthocyanins are known to have antioxidant and medicinal properties (11). Flavonoids show anti allergic, anti inflammatory, anti microbial and anti cancer activity (21). Phenols show anti carcinogen, anti inflammation, cardiovascular and hepato protection activities in animals (21). Anthocyanins possess a high thermostability and contribute towards antioxidative, antiinflammatory, cardioprotective and hepatoprotective activities (22). Tannins have been reported to enhance

physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, wide range of anti-infective actions and to cure or prevent variety of sicknesses (23); hence functions in the inactivate microbial adhesins, enzymes, cell envelope transport proteins (24). Saponins have been reported to have antimicrobial ability (25); thus the pharmacological ability of the test ingredient. Alkaloids though reported to be toxic, but are used as basic medicinal agents for their bactericidal effects (26).

**Table 3: Effect of *Hibiscus sabdariffa* calyx extract on the haematological parameters of Broiler Finisher**

Parameters	T1	T2	T3	T4	SEM
PCV (%)	49.15 <sup>a</sup>	49.40 <sup>a</sup>	48.85 <sup>a</sup>	40.80 <sup>a</sup>	1.37
HB (g/dl)	13.50 <sup>a</sup>	13.35 <sup>a</sup>	12.95 <sup>b</sup>	10.95 <sup>c</sup>	0.40
RBC (x10 <sup>12/l</sup> )	4.20 <sup>a</sup>	4.05 <sup>ab</sup>	3.52 <sup>b</sup>	2.78 <sup>c</sup>	0.22
Platelets x10 <sup>9/l</sup>	35.00	37.00	38.00	32.00	0.13
MCHC (%)	264.50 <sup>b</sup>	262.00 <sup>b</sup>	256.00 <sup>c</sup>	268.00 <sup>a</sup>	0.85
MCH (pg)	40.20 <sup>a</sup>	40.10 <sup>ab</sup>	39.70 <sup>b</sup>	39.15 <sup>c</sup>	0.16
MCV (fl)	149.45 <sup>a</sup>	149.05 <sup>ab</sup>	148.70 <sup>ab</sup>	146.55 <sup>b</sup>	0.50
WBC (x10 <sup>9/l</sup> )	205.00 <sup>ab</sup>	201.00 <sup>ab</sup>	217.70 <sup>a</sup>	194.00 <sup>b</sup>	3.62

<sup>a,b,c ab</sup>, Means values within the same row but with different superscripts are statistically (P<0.05) significant. PCV, Packed cell volume, HB, Haemoglobin, RBC= Red Blood Cell, MCV= Mean Corpuscular Volume, WBC=White blood cell, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin concentration.

**Table 4: Effect of *Hibiscus sabdariffa* Calyx Extract on Serum Biochemistry of Broiler Finisher chickens**

Parameter	T1	T2	T3	T4	SEM
Total protein(g/l)	4.88	4.17	4.82	3.27	0.3
Glucose(mg/dl)	139.50	133.50	136.00	172.50	7.30
Urea(mg/dl)	22.11 <sup>ab</sup>	23.04 <sup>a</sup>	19.75 <sup>ab</sup>	17.82 <sup>b</sup>	0.88
Creatinine(mg/dl)	0.86	0.81	0.93	0.81	0.02
AST(U/L)	29.00	33.00	31.50	30.50	0.85
ALT(U/L)	22.50	16.50	22.50	24.50	1.36
ALP(U/L)	66.62	70.58	73.89	73.82	1.47

<sup>Abc</sup> means a row with different superscript differ significantly (p<0.05) from one another

SEM=Standard error of Means

ALT=Alanine Amino Transferase

AST=Aspartate Amino Transferase

ALP=Alanine Amino Phosphatase

The effects of different treatments of *Hibiscus sabdariffa* calyx extract on the blood parameters of broiler finishers are shown in Table 3. According to (27), blood represents a means of assessing clinical, nutritional and health status of animals. The different treatments of *H. sabdariffa* calyx extract did not have any significant ( $p < 0.05$ ) effect on the PCV and WBC of the birds when compared to the control treatment T1. The PCV values in this study ranged from 40.80 to 49.15% while the WBC ranged from 194.0 to 217.70  $\times 10^9/l$ . The PCV values in T1, T2 and T3 were slightly above the range 25.0-45% reported for apparently healthy birds according to (28, 29). The fact that the WBC did not change significantly across the treatment groups is an indication that the test ingredient did not exact any significant ( $p < 0.05$ ) effect on the bird's leucocytes which are very important components of the birds immune system (30). However, at concentrations of 100ml/l (T3) and 150ml/l (T4), the Hb, RBC and MCH of the birds decreased ( $p < 0.05$ ) when compared to the control treatment T1. The Hb values in the birds treated with the test ingredient remained within the accepted range of 7.0 - 13.0 (g/dl) for broiler chickens (31). The RBC values in T3 and T4 fell within the normal physiological range of 1.5-3.9  $\times 10^6/mm^3$  for healthy chicken as reported by (28). The MCH is the average amount of Hb in each RBC measured and is an indicator of the blood carrying ability of the RBC. Its values in all the treatment groups in this study fall within the normal range of 16 to 53 (pg), respectively as earlier reported by (28) and (31). The values of Hb, RBC and MCH in this study showed that all the birds performed their respiratory functions efficiently and were able to resist any form of respiratory stress (16, 32). The MCV only decreased ( $p < 0.05$ ) in T4 compared to the control. MCHC decreased ( $p < 0.05$ ) in T3 and increased ( $p < 0.05$ ) as the dose of the extract increased in T4. The MCV

values in all the treatment groups in this study were higher than the normal range of 90 to 140(fl) reported by (28, 31)

The effect of different treatments of *Hibiscus sabdariffa* calyx extract on the serum parameters of broiler finishers is shown in Table 4. The serum total protein and glucose of the birds in all the treatment groups did not vary significantly ( $p < 0.05$ ). The stability in the serum total protein is an indication that the diet is adequate for the birds<sup>32</sup> and that the test ingredient may not have interfered with nutrient availability even with increasing dosage level. The total protein values in this study is in agreement with the normal range of 3.31 to 5.39 g/l reported by<sup>27</sup>, but lower than the range 5.00 to 8.00 g/l per bird reported by<sup>30</sup>. The stability in the serum glucose also suggests that the test ingredient did not interfere with glucose intake and subsequent metabolism by the cells of the animals. The liver enzymes: ALP, AST and ALT remained stable and their functions were not significantly ( $p < 0.05$ ) affected by the dose dependent administration of the test ingredient. This observation suggests that the Hibiscus extract at all doses used did not affect the function and integrity of the liver<sup>29</sup> since the activity of these liver enzymes serves as sensitive biomarkers of hepatotoxicity. Serum urea level decreased ( $p < 0.05$ ) in T4 compared to T2. Serum urea comes from the animal diet and the breakdown of tissue proteins. It suggests the quality of dietary proteins<sup>33, 29</sup>. The serum creatinine did not change significantly ( $p < 0.05$ ) across all the treatment groups. Creatinine has been found to be a fairly reliable indicator of kidney function. The stability in the serum creatinine and urea levels in this study suggests that the test ingredients at all the doses administered did not adversely affect the kidney of the animals.

## Conclusion and Applications

### 1. The aqueous extract of *Hibiscus*

*sabdariffa* contains phytochemical compounds with medicinal and biological properties

2. *Hibiscus sabdariffa* had no harmful effect on the internal organs such as the livers and the kidneys of broiler birds, thus *Hibiscus sabdariffa* is safe to be applied as a dietary supplement in broiler production.

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