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# Effects of the Aqueous Extract of *Solanum aethiopicum* Shum Fruit on the Growth Performance, Hematological and Biochemical Parameters of Broilers Exposed to High Heat Index

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**Abstract** | Phytoadditives have been identified as a promising solution to mitigate the deleterious effects of heat stress. *Solanum aethiopicum* has been demonstrated to be a rich source of stress-reducing agents, including zinc, vitamins C and E. Furthermore, studies have reported the presence of substantial antioxidant activities in *Solanum aethiopicum* leaves and fruits. These properties make *Solanum aethiopicum* ~~as~~ a promising candidate for use as an anti-stress agent. The objective of this study was to evaluate the effects of the aqueous extract of *Solanum aethiopicum* shum fruit on the zootechnical performance and physiological responses of heat-stressed broiler chickens. A total of 200 Cobb 500 broiler chicks, aged eight days and with a weight range of 165.55 ± 33.23 g, were utilised in the study. The chicks were randomly assigned to 20 pens (4 groups × 5 replicates, with each replicate containing 10 chicks). Group 1 received water devoid of supplements (control); Group 2, 3 and 4 received drinking water fortified with 200 mg/L of vitamin C (Vit C), 200 mg/L of an aqueous extract of *Solanum aethiopicum* fruit (S.a200), and 400 mg/L of an aqueous extract of *Solanum aethiopicum* fruit (S.a400), respectively. The supplementation of water with vitamin C or S.a200 led to a significant decrease in the feed conversion ratio ($P < 0.05$) and an increase in serum uric acid content ($P < 0.01$) in heat-stressed broilers. In comparison to the control and S.a400 groups, vitamin C significantly increased mean corpuscular haemoglobin ($P < 0.05$). The supplementation of water with vitamin C or S.a200 resulted in a 14% reduction in mortality. It can be concluded that the aqueous extract of *Solanum aethiopicum* fruit at a concentration of 200 mg/L in drinking water has the potential to enhance the zootechnical performance of heat-stressed broilers by increasing their feed efficiency and livability.

**Keywords** | Broiler, Growth performance, Heat stress, Liveability, Physiological response, *Solanum aethiopicum*

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# INTRODUCTION

Broiler production faces the challenge of heat stress, which increases animal morbidity and mortality, reduces production performance and leads to huge economic losses (Hu *et al*., 2019). It has been demonstrated that heat stress exerts a substantial influence on various physiological parameters, including growth performance, immune response, nutrient digestibility, meat quality and cellular oxidative stress status (Abdel-Moneim *et al*., 2021). As posited by the authors, chronic stress has the potential to exert a detrimental effect on fat metabolism, muscle growth, and meat quality. This is attributable to a disturbance in electrolyte balance and the activation of lipid peroxidation (Qaid and Al Garadi, 2021; Apalowo *et al*., 2024). A number of studies have demonstrated alterations in various blood parameters, including uric acid, glucose, potassium, sodium, haematocrit and haemoglobin (see Senay *et al*., 2019; Badakshan and Barazandeh, 2022; Livingston *et al*., 2022). Heat stress has been shown to diminish feed intake by decreasing gastrointestinal motility and prolonging gastric emptying. Moreover, an increase in water intake and peripheral blood flow in response to heat stress has been shown to decrease food digestion and absorption (Hu *et al*., 2019). A decrease in feed intake, food digestion and absorption leads to limited nutrient utilisation, thus reducing poultry production performance and feed conversion efficiency (Kwakye *et al*., 2023).

A plethora of strategies have been developed for the purpose of managing heat stress in poultry and reducing its global impact. These strategies can be categorised into two main groups: nutritional and non-nutritional. However, it has been determined that nutritional strategies are more economically viable than non-nutritional strategies (Saracila *et al*., 2021). Apalowo *et al.* (2024) state that nutritional techniques which prioritise the inclusion of immune-enhancing components and antioxidants in broiler diets represent a promising alternative to minimise the physiological consequences of heat stress. Furthermore, Mahasneh *et al.* (2024) demonstrate that the nutritional addition of medicinal plants has been shown to improve growth performance, antioxidant status, immunity, and intestinal health in heat-stressed chickens. Phytoadditives have been demonstrated to exert their function by means of promoting health and enhancing the antioxidant and immune systems (Abo Ghanima *et al*., 2023). It has been determined that a number of plant species are capable of producing bioactive compounds, including flavonoids and polyphenols, which have been shown to possess significant antioxidant properties (Crozier *et al*., 2009; Hu *et al*., 2019; Senay *et al*., 2019; Mahasneh *et al*., 2024). Hassan *et al.* (2016) demonstrated that the incorporation of 0.3% *Moringa Oleifera* leaves into the diet of broiler chickens resulted in enhanced performance and physiological parameters, as well as an augmented thermotolerance capacity. In a separate study conducted using the Cobb 500 model, Abo Ghanima *et al.* (2023) observed that the administration of a chicory extract at a dosage of 3 g/kg led to an increase in body weight gain, a reduction in feed intake and feed conversion ratio, and a decrease in mortality rate. D'Alex *et al.* (2024) demonstrated that a diet supplemented with 1% *Balanites aegyptiaca* leaves enhanced the antioxidant status of the liver and liveability of broilers during periods of hot fattening. According to Rheman *et al.* (2018), the supplementation of ginger at a level of 2% in the feed of broilers experiencing heat stress resulted in improved immunity and growth performance.

*Solanum aethiopicum* is a plant that has a long history of use in traditional African medicine for the treatment of a wide range of health concerns, including constipation, weight management, obesity, respiratory conditions such as asthma, inflammatory diseases, diabetes, eye conditions like glaucoma, rheumatic diseases, joint pain, and colic (Eze and Kanu, 2014; Edeke *et al*., 2021; Han *et al*., 2021; Eze *et al*., 2023) The pharmacological properties of the plants under investigation have been attributed to the presence of certain chemical substances, including alkaloids, flavonoids, saponins, tannins, phenols and glycosides (Eze *et al*., 2023; Michel *et al*., 2024). *Solanum aethiopicum* has also been shown to be rich in vitamins (A, B, C, D and E) and essential minerals such as calcium, magnesium, potassium, sodium, manganese, iron, copper, phosphorus and zinc. These minerals play a crucial role in various physiological processes, including acid-base balance, regulation of cellular metabolism, and the catalysis of enzymatic reactions (Edeke *et al*., 2021; Djaomanwe *et al*., 2023; Eze *et al*., 2023). A number of studies have reported the antioxidant, antibacterial, antiviral and anti-inflammatory properties of *Solanum aethiopicum* leaves and fruits (Tuem *et al*., 2022; Djaomanwe *et al*., 2023). These properties render *Solanum aethiopicum* a serious candidate as an anti-stress agent. In a study on broiler chickens, Adeyemi *et al.* (2021) demonstrated that a diet containing 5 g/kg of *Solanum aethiopicum* leaves was able to mitigate the adverse consequences of heat stress. In male Wistar rats, Iheagwam and Chinedu (2024) revealed antioxidants activities of the ethanol extract of S. aethiopicum fruit at high concentrations (2000mg/kg). Additionally, Djaomanwe *et al.* (2023) reported that the fruit of *Solanum aethiopicum* shum is traditionally employed as an anti-stress agent in poultry during the hot season in the Sahelian region of Cameroon. Nevertheless, scientific research on the anti-stress effect of *Solanum aethiopicum* Shum fruit remains inconclusive. The aim of this study was to evaluate the effect of aqueous extract of *Solanum aethiopicum* Shum fruit on growth performance and physiological responses of heat-stressed broilers.

## MATERIALS AND METHODS

### Study Area

The present study was conducted at the application and research farm of the National Advanced School of Engineering of Maroua, situated in the Far North Region of Cameroon (see Figure 1). The geographical location of the farm is specified by the following coordinates: 10°35'27.4"N, 14°18'57.3"E, and the altitude of the site is 384 metres. The experimental period occurred from 25 April to 9 June 2024. The mean ambient temperature recorded in the study area was 35°C, while the relative humidity ranged between 11-23%.

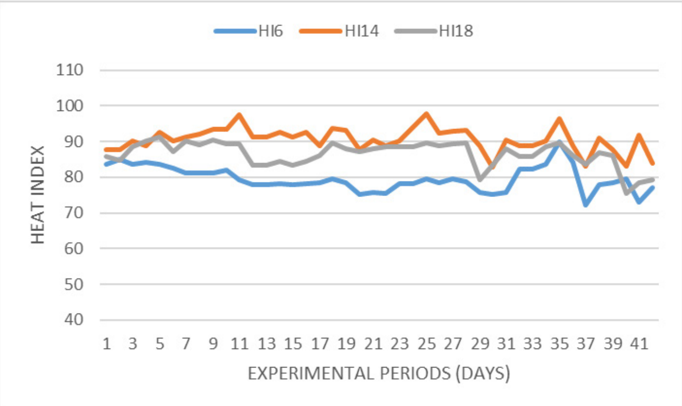


**Figure 1:** Heat index inside the breeding room during the experiment.

### Bird Management and Housing

A total of 200 one-day-old Cobb 500 broiler chicks were procured from a commercial hatchery (Agrocam, Yaoundé, Cameroon) for the experiment. The study was conducted in accordance with the animal welfare requirements approved by the Cameroonian Bioethics Committee (Reg. No. FWA-IRB00001945/2024) and the HIN-care and use of laboratory animals manual (8th edition).

Upon arrival, the chicks were weighed and acclimatised for a period of seven days. On the eighth day, the chicks were reweighed (165.55 ± 33.23 g) and randomly distributed into 20 cages (10 chicks/cage). All birds were housed in floor cages with wood shavings as bedding material (5 cm deep) at a density of 8 birds/m$^2$. The lighting schedule was continuous for the initial 24-hour period, followed by a 4-hour period of darkness and 20 hours of light until the conclusion of the experiment. Throughout the experiment, the broiler chickens were housed in an open-sided poultry housing system where the environmental elements were not controlled during the hot season. Feed and water were provided ad libitum in adapted facilities. Standard health and vaccination programmes against Newcastle and Gumboro diseases were carried out. The chicks were observed and examined on a daily basis for any syndromes throughout the experiment. The birds were fed commercial diets (see Table 1) throughout the experiment.

**Table 1:** Nutrient composition of experimental diets.

| Nutrients | Starter diet | Grower diet | Finisher diet |
|---|---|---|---|
| M E (Kcal/kg) | 2850 | 2900.52 | 2900.43 |
| Crude Protein (%) | 21.7 | 20 | 18.55 |
| Phosphore (%) | 0.8 | 0.9 | 1 |
| Calcium (%) | 2 | 2.3 | 2.6 |
| Lysine (%) | 1 | 0.9 | 0.8 |
| Methionine (%) | 0.4 | 0.4 | 0.5 |

*Premix 10%: Crude protein: 40%; Lysine: min. 3.00%; Methionine: min. 1.70%; Calcium: min. 7.00%; Phosphorus: min. 1.80%; EM: 2100 kcal/kg. ME: Metabolizable Energy.

### Supplements

***Solanum aethiopicum* Shum:** In February 2024, fresh fruits of *Solanum aethiopicum* Shum were procured from a producer in the town of Maroua. The taxonomic identity of the plant was confirmed at the Department of Biological Sciences, Faculty of Science, the University of Maroua, Cameroon. The purchased fruits were meticulously washed, meticulously cut into small pieces, and then air dried to crispness in the laboratory (at a prevailing room temperature of 35 ± 6°C) for a period of one week. Thereafter, the dried materials were ground to fine particles using a pestle and mortar. The crude aqueous extract was prepared by decoction; 100 g of dried fruit powder was added to 1.5 L of distilled water, and the mixture was boiled at 60°C for 30 minutes, to prevent protein denaturation. The mixture was decanted and filtered through sterile Whatman No. 2 paper. The filtrate was then evaporated to dryness using a freeze dryer at 60°C, after which the crude extract was stored in a glass vial at room conditions (35 ± 6°C, and 29±9% of relative humidity). The dosage for administration of this crude extract was determined to be 200 and 400 mg/L of drinking water.

**Vitamin C:** Ascorbic acid or Vitamin C was procured from a reputable pharmacy in Maroua and administered to the birds in their drinking water. The dosage administered was 200 mg/L of water.

### Experimental Design

At eight days of age, the chicks were randomly allocated into four treatment groups, each with five replicates (consisting of ten birds per replicate). Group 1 was administered water without additional supplements (control); Group 2 was given drinking water with 200 mg/L of vitamin C (Vit C); Group 3 was given drinking water with 200 mg/L of an aqueous extract of *Solanum aethiopicum* fruit (Sa200); and Group 4 was given drinking water with 400 mg/L of an aqueous extract of *Solanum aethiopicum* fruit (Sa400). The experiment was conducted under non-controlled temperature and humidity conditions. The broilers were exposed to the natural and arid conditions of the hot dry season in Maroua. The ambient temperature recorded in the experimental room varied between 24 and 43°C, while the relative humidity ranged between 40 and 67%.

## Data Collection

In the course of the experiment, the consumption of food and the live body weight of the birds were recorded on a weekly basis. At the age of 42 days, two birds per pen replicate, i.e. ten per treatment, were randomly selected and fasted for 12 hours. They were weighed and blood samples were taken for haematological and biochemical analyses. The blood samples collected were kept on ice until they were transported to the laboratory for analysis.

The manipulation of animals in this study was in accordance with the recommendations of the institutional guidelines for the care and use of laboratory animals. Chicks were handled humanely in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments.

**Heat index (HI):** The ambient temperature and relative humidity were measured on a daily basis at 6 am, 2 pm and 6 pm using a Taylor brand temperature and humidity sensor (FCC ID: WEC-1502) positioned at the chickens' back level in the centre of the room. The heat index was calculated using the formula described by National Research Council (1971), cited by Kendal and Webster (2009).

*The humidity Index (HI) is calculated as follows:* $HI = (1.8\ Tmax + 32) - [(0.55 - 0.0055\ Hmin) \times (1.8\ Tmax - 26)]$

In this equation, T denotes the maximum ambient temperature (°C) and H represents the minimum relative humidity (%).

Table 2 described the heat stress classes as "bird comfort", according to the recommendation of Wasti *et al.* (2020).

**Table 2:** Heat stress classification for poultry (adapted from Wasti *et al.*, 2020).

| THI value (°F) | Description |
|---|---|
| ≤72 | Absolute comfort (no heat stress) |
| 73 to 76 | Light discomfort (mild heat stress) |
| 77 to 80 | Moderate discomfort (moderate heat stress) |
| 81 to 87 | Severe discomfort (severe heat stress) |
| ≥88 | Life threatening (extreme heat stress) |

**Performance parameters:** Throughout the experiment, a range of metrics were meticulously monitored for each treatment in each replicate, including body weights, feed intakes, feed conversion ratios and mortality rates. The weighing of subjects was conducted using a balance of the model SF-400.

**Feed intake:** The quantity of food consumed by the birds was measured at the conclusion of each week. The birds were provided with a known amount of food, and the remainder was measured for each replicate.

$$\textit{Total feed intake (g)} = \textit{Total feed given to birds (g)} - \textit{feed left-over (g)}$$

$$Average\ feed\ intake = \frac{Total\ feed\ intake\ (g) - Total\ feed\ leftover\ (g)}{Number\ of\ birds}$$

**Body weight and weight gain:** The body weights of the birds were recorded at the commencement of the experiment using a balance, and then repeated at the end of each week until the experiment's conclusion. The weight gain of the birds was determined by the difference in their weekly weights.

$$\textit{Weekly body weight gain} = \textit{live body weight of week (n+1)} - \textit{live body weight of week (n)}$$

$$Average\ body\ weight\ gain = \frac{weekly\ body\ weight\ gain}{7}$$

**Feed conversion ratio:** The feed conversion ratio (FCR) was calculated using records of weekly feed consumption and weight gain.

$$Feed\ conversion\ ratio = \frac{Weekly\ Feed\ intake\ (g)}{Weekly\ body\ weight\ gain\ (g)}$$

**Mortality:** Mortality records were meticulously maintained throughout the experimental period. These records were expressed as the ratio of the number of deceased birds to the total number of birds in each pen at the commencement of the study, and were subsequently expressed as a percentage. This calculation was performed on a replicate basis. The mortality rate (MR) was determined as follows:

$$MR = \frac{Number\ of\ death}{total\ number\ of\ animals} \times 100$$

**Hematological profile:** In order to assess the haematological parameters, two birds were selected from each replicate, and blood samples were collected by venipuncture (wing vein). The 3-ml syringe with a 25G needle was used to collect the blood, which was then transferred to ethylenediaminetetraacetic acid (EDTA) tubes (0.5 ml). The blood samples were then stored in a refrigerator at 8°C for a period of 4 hours. The samples were then subjected to haematological analysis in the


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laboratory. This analysis included the measurement of red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The erythrocyte count was determined after dilution of the blood sample with Natt and Herrick's solution (Campbell and Ellis, 2007). Haemoglobin and packed cell volume were determined using an automated analyser, the SNFS BC30S. The erythrocyte indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) were calculated according to the following formulae:

$$MCHC\ (g/L) = \frac{Hb}{PCV}$$

$$MCV\ (fL) = \frac{PCV \times 10}{RBC}$$

$$MCH\ (pg) = \frac{Hb}{RBC}$$

**Biochemical analysis:** For the purpose of serum biochemistry analysis, 2 mL of blood was collected from two birds per replicate during the sixth week of the experiment. Collection of the blood sample was undertaken using a 3 mL syringe with a 25 G needle. The blood was collected in a plain tube and allowed to settle to its characteristic components for approximately fifteen minutes to separate the serum. The serum was then separated from the residual cellular components by using a pipette to aspirate the serum from the plain tubes for serum biochemical parameter analysis. The total protein, albumin, globulin, glucose, uric acid, potassium and sodium concentrations were determined using a spectrophotometer (URIT-810) in accordance with the protocol outlined in the manufacturer's kits (HUMAN commercial kit). The calculation of globulins was performed as follows:

$$Globulins = Total\ protein - Albumin$$

### Data Analyses

Data were statistically analysed using Graphpad Prism 8.4.3. The collected data were expressed as the mean ± standard deviation and then subjected to one-way analysis of variance (ANOVA) (type of supplement). Statistical significance was considered at P<0.05. The Tukey multiple test was employed as a post-test to ascertain the means.

## RESULTS

### Heat Index

The evolution of the ambient heat index in the room during the experiment is demonstrated in Figure 1. Throughout the experiment, the ambient temperature and the relative humidity in the breeding room were measured and recorded. Their values ranged between 24-36°C and 57-68% at 6 a.m., 35-43°C and 40-59% at 2 p.m., and 27-39°C and 49-60% at 6 p.m., respectively. The combination of these parameters revealed that the lowest values of the heat index were recorded at 6 a.m. (72–86°F) and the highest values were obtained around 2 p.m. (83–97°F).

**Table 3:** Growth parameters of heat stressed broilers receiving water supplemented with vitamin C or the aqueous extract of *Solanum aethiopicum* fruit.

| Parameters | Control | Vit C | Sa200 | Sa400 | P value |
|---|---|---|---|---|---|
| DFI | 104.1± 6.168[a] | 87.77± 2.997[b] | 94.01± 5.128[bc] | 100.8± 6.729[ac] | 0.0010 |
| DBWG | 45.51± 3.828 | 45.57± 2.595 | 46.96± 4.927 | 46.70± 4.642 | 0.6571 |
| FCR | 2.284± 0.090[a] | 1.924± 0.059[b] | 2.006± 0.109[bc] | 2.164± 0.132[ac] | 0.0002 |
| Mortality | 28.57 ±14.29 | 14.28 ±10.10 | 14.28 ±10.10 | 20.00± 7.827 | 0.1636 |

Values in the same line not sharing a common superscript differ significantly (P<0.05). DFI: daily feed intake; DBWG: daily body weight gain; FCR: feed conversion ratio.

### Growth Parameters

As demonstrated in Table 3, the growth parameters of heat-stressed broilers receiving water with added vitamin C (200 mg/L) or the aqueous extract of *Solanum aethiopicum* fruits (200 mg/L and 400 mg/L) were comparable, irrespective of the treatment. No statistically significant differences (P > 0.05) in body weight gain (BWG) were observed among the groups. However, water supplementation with ascorbic acid (Vit C) or Sa200 led to a significant (P<0.01) decrease in daily feed intake (DFI) and feed conversion ratio (FCR) in comparison to the control group. Furthermore, as compared to Sa400, the DFI and FCR were significantly lower (P<0.05) with Vit C. Statistical analysis demonstrated that the mortality rate was not significantly different (P>0.05) between treatments. However, the value obtained in the control group was found to be two times higher than that obtained with Sa200 and Vit C (P>0.05).

### Haematological Parameters

The data pertaining to the erythrocyte count, haemoglobin content, packed cell volume and erythrocyte indices of broilers subjected to heat stress and provided with water augmented with Vit C or Sa200 and Sa400 is presented in Table 4. The statistical analyses demonstrated that, with the exception of the mean corpuscular haemoglobin (MCH), all the haematological parameters analysed were not influenced by the treatments (P>0.05). In comparison with the control and Sa400 groups, Vit C significantly increased (P<0.05) the MCH of heat-stressed broilers.

**Table 4:** Hematological parameters of heat-stressed broilers receiving water supplemented Vitamin C or the aqueous extract of *Solanum aethiopicum* fruit.

| Parameters | Control | Vit C | Sa200 | Sa400 | P value |
|---|---|---|---|---|---|
| RBC ($10^6$/ µL) | 1.366± 0.096 | 1.464± 0.347 | 1.358± 0.192 | 1.301± 0.164 | 0.4306 |
| HB (g/dL) | 9.660± 0.804 | 9.980± 0.962 | 9.340± 0.766 | 9.120± 1.022 | 0.1718 |
| PCV (%) | 18.54± 1.432 | 19.96± 3.724 | 18.71± 1.814 | 17.66± 1.771 | 0.2047 |
| MCV (g/ dL/µm$^3$) | 136.3± 3.020 | 142.2± 4.566 | 141.1± 12.43 | 136.5± 6.433 | 0.1812 |
| MCH (g/ dL/µm$^3$) | 70.94± 5.175$^b$ | 79.81± 7.099$^a$ | 72.83± 7.479$^{ab}$ | 70.14± 4.531$^b$ | 0.0051 |
| MCHC (g/ dL) | 52.08± 3.764 | 49.30± 8.755 | 50.47± 6.852 | 51.38± 2.538 | 0.7539 |

Values in the same line not sharing a common superscript differ significantly (P<0.05). **RBC:** red blood cell; HB: Hemoglobin; **PCV:** pack cell volume; **MCV:** mean corpuscular volume; **MCH:** mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

### Biochemical Parameters

As demonstrated in Table 5, the impact of water supplementation with ascorbic acid or *Solanum aethiopicum* fruit on the biochemical parameters of serum from heat-stressed broilers is illustrated. The serum concentrations of potassium, sodium, total protein, albumin, globulin and glucose remained unaltered in the presence of ascorbic acid or *Solanum aethiopicum* fruit. However, the serum uric acid content was found to be significantly increased (P<0.01) by the addition of Vit C or Sa200.

**Table 5:** Biochemical parameters of serum of heat-stressed broilers receiving water supplemented with Vitamin C or the aqueous extract of *Solanum aethiopicum* fruit.

| Parameters | Control | Vit C | Sa200 | Sa400 | P value |
|---|---|---|---|---|---|
| Potassium (mEq/L) | 4.410± 0.738 | 3.960 ±0.688 | 3.970± 0.718 | 4.000± 0.763 | 0.4544 |
| Sodium (mE-q/L) | 144.3± 5.250 | 143.3± 4.270 | 141.3± 4.832 | 142.1± 4.508 | 0.5132 |
| Total protein (g/L) | 93.90± 3.843 | 93.20± 4.211 | 93.40± 4.671 | 92.90± 4.122 | 0.9600 |
| Albumin (g/L) | 43.90± 3.479 | 44.90± 4.280 | 45.70± 4.322 | 46.70± 4.572 | 0.4980 |
| Globulin (g/L) | 50.00± 3.432 | 48.30± 5.417 | 47.70± 6.865 | 45.20± 5.574 | 0.2812 |
| Glucose (g/L) | 0.784± 0.135 | 0.842± 0.066 | 0.774± 0.091 | 0.845± 0.092 | 0.2548 |
| Uric acid (g/L) | 38.50± 8.48$^b$ | 51.30± 10.25$^{ac}$ | 58.30± 6.80$^a$ | 42.00± 6.960$^{bc}$ | 0.0063 |

Values in the same line not sharing a common superscript differ significantly (P<0.05).

### Correlation

The correlation between growth, haematological and biochemical parameters, and mortality is demonstrated in the matrix below (Figure 2). A statistical analysis was conducted, which revealed a significant and positive correlation between mortality and the feed conversion ratio (P = 0.008); as well as a correlation between feed intake and the feed conversion ratio (P = 0.019). However, a negative and significant correlation was identified between mortality and red blood cell count (P = 0.009); feed intake and mean corpuscular volume (P = 0.036); and feed conversion ratio and uric acid (P = 0.020).

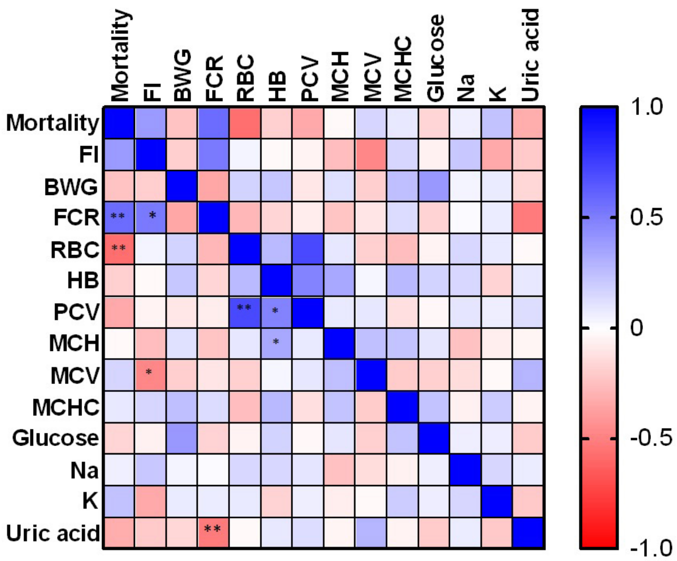


**Figure 2:** Pearson correlation between parameters. (*): P<0.05; (**): P<0.01. RBC: red blood cell; **PCV:** pack cell volume; **MCV:** mean corpuscular volume; **MCH:** mean corpuscular hemoglobin; **MCHC:** mean corpuscular hemoglobin concentration; **DFI:** daily feed intake; **DBWG:** daily body weight gain; **FCR:** feed conversion ratio; Na: Sodium; **K:** Potassium.

## DISCUSSION

The present study evaluated the effects of an aqueous extract of *Solanum aethiopicum* (S.a.) fruits on the growth performance, haematological and biochemical parameters of broiler chickens exposed to elevated heat indices. During the experiment, the heat index was assessed on a daily basis using the thermal-humidity index (THI).

The Thermal-Humidity Index (THI) is a metric of thermal comfort, developed to assess the effect of the thermal environment on the thermoregulatory status of animals (Purswell *et al*., 2012). It has been used as a means of predicting the effects of thermal conditions on broiler performance, and is commonly used to assess the degree of heat stress. In the present study, the daily evaluation of the THI demonstrated that the lowest values were recorded at 6 am

(72 - 86˚F) and the highest values were obtained around 2 pm (83 - 97˚F). These findings suggest that the birds experienced heat stress conditions between 6 am and 6 pm.

As posited by numerous scholars, a consequence of heat stress in poultry is a reduction in feed intake (Wasti *et al*., 2020). However, a comparison between thermal comfort conditions and heat stress conditions was not the focus of the present study. However, the extant literature suggests that the daily feed intake of broilers after 42 days is between 90-110 g/bird (Apolawo *et al*., 2024), which is similar to the value found in our study. The similarity in heat stress conditions may be attributable to early age thermal conditioning, which has been documented to enhance heat resistance in broilers during the finishing period (De Basilio *et al*., 2003). These researchers also demonstrated that following early age thermal conditioning, broiler chickens continued to feed even at a body temperature 0.9°C above the thermoneutral level at 3 to 4 weeks of age. In a separate study, Kang and Shim (2020) reported a mere 6% reduction in feed intake at 35 days of age in broilers exposed to early heat compared to those reared at optimal temperature without heat exposure. In situations of heat stress, chickens increase their energy expenditure by accelerating their metabolism to maintain optimal body temperature (Senay *et al*., 2019). The required energy can be mobilised from lipid stores. In the present study, broilers treated with Vit C or Sa200 showed lower feed intake and feed conversion ratio ($P<0.05$) than untreated broilers (control). These results suggest that these supplements (Vit C or Sa200) improved feed efficiency. As stated in the relevant literature, S.a fruits have been reported to be rich in vitamin C, alkaloids, flavonoids and phenolic compounds. In addition, extracts of the plant have shown a high total antioxidant capacity (Tuem *et al*., 2022; Djaomanwe *et al*., 2023). As posited by several authors, polyphenols have the capacity to enhance intestinal health and absorption, protein digestibility, and thus increase the efficiency of feed utilisation (Omar *et al*., 2020; Hu *et al*., 2019). Moreover, previous authors have demonstrated that supplementation with vitamin C improves nutrient utilisation in heat-stressed broilers (Wasti *et al*., 2020). Vitamin C has been shown to stimulate the thyroid gland, which in turn improves feed efficiency. The findings of this study are consistent with those of Adeyemi *et al*. (2021), who documented enhanced feed efficiency in heat-stressed broilers that were fed with S. a leaves. The treatment group exhibiting the lowest feed conversion ratio demonstrated superior liveability. This observation was corroborated by Pearson's Correlation Analysis, which revealed a substantial and positive correlation between feed conversion ratio and mortality ($P=0.008$).

The results demonstrated that water supplementation with ascorbic acid (Vit C), Sa200, or Sa400 did not affect body weight gain (BWG) under conditions of heat stress. These data are consistent with those of previous studies that reported no effect of ascorbic acid supplementation on the final body weight of broiler chickens under heat stress or during summer (Abo-Samaha *et al*., 2021). However, the present findings are at odds with those of other studies, which suggest that dietary or drinking water supplementation with ascorbic acid increases body weight gain of broilers under heat stress (Barrio *et al*., 2020; Gouda *et al*., 2020). The discrepancy may be attributable to the dosage of vitamin C used, or the degree or duration of exposure to heat stress. In the present study, the peak intensity (PIC) temperature ranged between 35 and 44°C during the 42-day experiment. In contrast, in the study by Barrio *et al*. (2020), broilers were exposed to 35°C from 8:00 to 13:00 daily. Despite the utilisation of vitamin C at a dosage of 200 mg/L in numerous studies, only a limited number of authors have asserted that the optimal dosage of vitamin C as a dietary supplement is 250 mg/L (Hieu *et al*., 2022).

As demonstrated by earlier researchers, the administration of water with added vitamin C or *Solanum aethiopicum* fruit resulted in a decrease in the mortality rate of heat-stressed broilers. The protective effect of these supplements can be attributed to their antioxidant, anti-inflammatory, and antibacterial properties (Akbarian *et al*., 2016; Hu *et al*., 2019; Adeyemi *et al*., 2021). The hypothesis that improved feed efficiency has led to a reduction in mortality can be substantiated by the improved physiological response of heat-stressed broilers.

The haematopoietic system is considered to be one of the most sensitive targets of xenobiotics (i.e. substances that are not naturally produced in the body), and is recognised as an important marker of the physiological and pathological response of an animal. In this study, the erythrocyte count, haematocrit, haemoglobin and erythrocyte indices, with the exception of mean corpuscular haemoglobin (MCH), were found to be unaffected by water supplementation with vitamin C or aqueous extract of Solanum aethiopicum fruit. In comparison with the standard values documented in the literature concerning broilers, the results of our study indicated low red blood cell (RBC) and haematocrit (HCT) values, while mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were found to be significantly elevated (Odunitan-Wayas *et al*., 2018). It is noteworthy that the present study was conducted under conditions of heat stress, a factor that has been documented to induce increased haemolysis and diminished blood carrying capacity in broilers (Odunitan-Wayas *et al*., 2018). Heat stress has been demonstrated to increase the osmotic fragility of erythrocytes by increasing lipoperoxidative modification of the erythrocyte membrane (Adenkola and Angani, 2017; Saracila *et al*., 2021). Impairment of erythrocyte integrity leads to erythrocyte destruction. Water supplementation with vitamin C significantly increased MCH in heat-stressed broilers when compared to the control and Sa400. Concurrently, the value obtained with vitamin C was analogous to that obtained with Sa200. Elevated levels of MCH have been observed in cases of blood cells that are excessively large, and this has been linked to an increased red blood cell carrying capacity (Adenkola and Angani, 2017). This increased capacity leads to heightened energy levels, which in turn facilitates the body's ability to cope with heat stress. The findings of this study demonstrated a substantial and negative relationship between mortality and RBC count (P=0.009). The primary function of red blood cells is to transport oxygen to animal tissues for the oxidation process, which releases energy and facilitates the transportation of carbon dioxide out of the tissues. The increase in mean corpuscular haemoglobin (MCH) induced by vitamin C can be attributed to its capacity to preserve the integrity of the erythrocyte membrane and its ability to scavenge a significant number of free radicals (Abdel-Moneim *et al*., 2021). The tendency to increase the mean corpuscular volume may also indicate a positive effect of vitamin C on haematopoiesis. Indeed, the highest values of red blood cell (RBC), haemoglobin (Hb) and haematocrit (PCV) were obtained in broilers that had been supplemented with vitamin C. These findings are in agreement with those of Jahejo *et al*. (2019) who reported an increase in RBC in broilers that had been supplemented with vitamin C. In a previous study, Adeyemi *et al*. (2021) showed that dietary supplementation of *Solanum aethiopicum* leaves at 5 g/kg significantly increased RBC and haemoglobin. In their study, broilers were reared at 34°C and 40-50% relative humidity for 6 h/day from 22-42 days. The results obtained in this study differ from those of the aforementioned study, which showed no change in the parameters under investigation. The difference observed between the two studies could be attributed to the duration and intensity of the exposure of the broilers to high temperature.

Notwithstanding the nature of the treatment administered, the uric acid levels in chickens were found to be within the normal range (19-125 mg/L) (Odunitan-Wayas *et al*., 2018). Elevated blood uric acid levels have been observed to be associated with high protein intake, increased protein metabolism, stress or dehydration (Odunitan-Wayas *et al*., 2018; Abdel-Moneim *et al*., 2021). In birds, uric acid is the end product of purine metabolism, and purines are derived from the breakdown of proteins. The impact of heat stress on protein metabolism in hyperthermic animals appears to be contingent on the duration and intensity of the stress. Short-term heat stress has been observed to increase protein catabolism, as evidenced by elevated plasma uric acid levels, while concurrently diminishing protein synthesis and N retention. Conversely, chronic heat stress has been shown to reduce protein synthesis in multiple muscles, decrease protein catabolism, and maintain uric acid levels within normal parameters (Nawaz *et al*., 2021; Livingston *et al*., 2022). As demonstrated by other researchers, exposure to elevated temperatures has been shown to result in a decrease in blood uric acid levels. This phenomenon may be attributed to a reduction in dietary amino acid intake (Ognik and Sembratowicz, 2012; Senay *et al*., 2019; Qaid and Al-Garadi, 2021). Sharifi *et al*. (2016) have proposed that plasma uric acid plays a crucial role in the scavenging of reactive oxygen species from tissues, thereby contributing to their protection during periods of heat stress. The present study demonstrated a significant increase in serum uric acid levels in response to water supplementation with vitamin C or Sa200. This finding suggests an increased bioavailability of the amino acid from the diet. This result is consistent with the findings of Imik *et al*. (2013), who demonstrated increased plasma uric acid levels with dietary vitamin C supplementation in heat-stressed broilers. Moreover, earlier research has demonstrated that vitamin C or *Solanum aethiopicum* can enhance amino acid digestibility, intestinal absorption, and overall health (Omar *et al*., 2020). This finding aligns with the results of the present study, which revealed a substantial and negative relationship between the feed conversion ratio and blood uric acid levels (P=0.020). In contrast, Livingston *et al*. (2022) found no effect of dietary vitamin C and E supplementation on blood uric acid in heat-stressed and non-stressed broilers. The discrepancy between the findings of the two studies may be explained by the duration and intensity of the heat stress experienced by the birds in each study.

As stated by Borges *et al*. (2003), sodium and potassium levels are essential for the synthesis of tissue proteins, the maintenance of intracellular and extracellular homeostasis, the electrical potential of cell membranes, enzymatic reactions, osmotic pressure and acid-base balance. In the present study, serum levels of Na and K were not statistically different between treatments, but were within the normal range for chickens (Livingston *et al*., 2022; Oni *et al*., 2024). Moreover, the administration of vitamin C, Sa200 or Sa400 did not result in alterations to serum total protein, albumin, globulin and glucose levels. These observations can be ascribed to the adaptation of the chicks, as prolonged exposure of birds to elevated temperatures has been demonstrated to promote adaptive responses (Borges *et al*., 2003). Furthermore, the administration of vitamin C or Sa did not appear to affect electrolyte balance or metabolism. The present findings are consistent with other reports demonstrating no effect of dietary vitamin C supplementation on plasma levels of K and Na (Livingston *et al*., 2022; Ferronato *et al*., 2024) or on serum levels of protein, albumin, globulin and glucose (Hajati *et al*., 2015; Ferronato *et al*., 2024). However, the present data differ from those of Adeyemi *et al*. (2021), who demonstrated that dietary supplementation of *Solanum aethiopicum* leaves at 5 g/kg significantly reduced blood glucose levels in broilers reared at 34°C and 40-50% relative humidity for 6 h/day from

22-42 days. It is important to note that the experimental conditions in this study differ from those previously employed, in which chicks were exposed to elevated temperatures from the first day of life till day 42. In a study on broilers chicken, Siddiqui *et al*. (2021) showed significant decrease of blood glucose of birds exposed to 14 days of heat stress as compare to those exposed to 7 days of exposure. Moreover, authors reported some differences in the phytochemicals and nutrients composition of *S. aethiopicum* fruit and leaves (Choi and Choi, 2024).

## CONCLUSIONS AND RECOMMENDATIONS

The objective of the present study was to evaluate the anti-stress effects of the aqueous extract of *Solanum aethiopicum* fruit on broilers. The results demonstrated that water supplementation with vitamin C or *Solanum aethiopicum* (200 mg/L) enhanced the liveability of broilers by increasing feed efficiency and blood-carrying capacity of red blood cells. The findings of this study demonstrated that the administration of *Solanum aethiopicum* to heat-stressed broilers might enhanced antioxidant status as it increase blood uric acid level. Despite the broilers exhibiting adaptive capacity in response to prolonged exposure to elevated thermal humidity indices, the incorporation of vitamin C or an aqueous extract of *Solanum aethiopicum* fruit at a concentration of 200 mg/L served to mitigate the adverse consequences of heat stress in broilers reared under hot environmental conditions. Further studies are required to elucidate the mechanisms involved and the anti-stress effects of *Solanum aethiopicum* in controlled conditions. It may be also interesting to identify active compounds and testing synergistic effects with vitamins and electrolytes.

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## NOVELTY STATEMENTS

The novelty of our work entitled "Effects of *Solanum aethiopicum* fruit extract on heat-stressed broilers" can be summarized as the aqueous extract of *Solanum aethiopicum* at 200mg/L of drinking water, improved feed efficiency and reduced mortality. The study suggest *S. aethiopicum* as a potential natural anti-stress agent for poultry in hot climates.

## AUTHOR'S CONTRIBUTIONS

Tadondjou Tchingo Cyrille d'Alex: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; writing – original draft; writing – review and editing. Oumarou Souleymanou: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; software; writing – original draft; writing – review and editing. Mube Kuietche Hervé: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; resources; software; supervision; validation; visualization; writing – review and editing. Toukala Jean Paul: Conceptualization; data curation; formal analysis; funding acquisition; investigation; software. Ferdinand Ngoula: Supervision; validation; visualization; writing – original draft; writing – review and editing. Alexis Teguia: Project administration; supervision; validation; visualization; writing – original draft; writing – review and editing.

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No potential conflict of interest is reported by the authors.

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### Conflict of Interest

The authors have declared no conflict of interest.

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