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# Heat Stress Control of Chickens: Effects of *Griffonia simplicifolia* (DC.) Baill. Seed on Zootechnical and Blood Parameters

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## ABSTRACT

**Background:** Heat stress is one of the main causes of mortality and reduced productivity in poultry. The current study aims to control heat stress of chickens by incorporating the seed of *G. simplicifolia* in their feed.

**Methods:** This study was carried out in a poultry farm located at Badja (Togo) for three months on 210-day-old chicks of Sasso strain, divided into five groups. The birds of groups T0, T2, T3 and T4 received *G. Simplicifolia* seed in their feed at doses of 0.0; 2.5; 5.0 and 10.0 g /kg of feed respectively. The chickens of group T0 served as negative control group. Chickens of group T1 were fed 0.5 g of aspirin /kg of feed, served as a positive control group.

**Result:** The result showed that the supplementation of *G. simplicifolia* seed in the feed improved the zootechnical and physiological parameters of the Sasso broilers. The presence of 5-HTP, fats, phenolic compounds and carotenoids in the seed improved the bird performances. In conclusion, *G. simplicifolia* seed can be used as an alternative to reduce heat stress of chickens in tropical regions.

**Key words:** Food safety, *Griffonia simplicifolia* powder, Sasso chickens, Thermal stress, Zootechnical and blood parameters.

## INTRODUCTION

In Africa, poultry farming is expanding rapidly due to short-cycle poultry farming, job creation for local and urban youth. In 2017, the National Investment and Food Security Programme (PNIASA) and the Agricultural Sector Support Project (PASA) led to a strong increase in the avian production sector in Togo. Indeed, over the past six years, poultry production has tripled or more (Togo First, 2022), from 8 million heads of poultry in 2011 to 30.67 million in 2021. Consequently, poultry farming is considered in Togo as the main lever for boosting the togolese economy and ensuring food self-sufficiency for the population in animal proteins. However, the intensification of poultry production in the tropical areas in general and in Togo in particular, faces many difficulties, especially the high temperature recorded during some seasons of the year. In fact, during hot periods, birds are subjected to very high heat stress, resulting in a drop in their performance. Heat stress arises when the amount of heat produced by an animal is more than its ability to dissipate heat into its environment (Akbarian *et al.*, 2016).

Heat stress causes great economic losses in poultry industry due to increasing climate change globally (Korkmaz, 2023; Tugiyanti *et al.*, 2023). Heat stress has negative impacts on physiological response, growth and laying performance, resulting in reduced feed consumption, body weight gain, egg production, feed efficiency, meat quality, egg quality and immune response (Abdel-Moneim *et al.*, 2021). This situation sometimes leads to high mortality (Abo Ghanima *et al.*, 2020) with heavy economic losses for poultry farmers (Bayraktar and Tekce, 2019).

Heat in the tropical region normally triggers thermoregulatory processes in birds through the mechanism of decreased thermogenesis and increased thermolysis. This heat stress causes birds to lose their appetite and energy, resulting in a decrease in their zootechnical performances. If the ambient temperature is between 18°C and 32°C, the birds' food intake decreases by 1.72% for each 1°C increase in temperature. More importantly, when the ambient temperature is between 32°C and 38°C, the appetite of the birds decreases by 5% for each additional degree Celsius (Rao *et al.*, 2002).

To fight against heat stress in poultry, nutritional alternatives (Rao *et al.*, 2002; Abdel-Moneim *et al.*, 2021) and food additives (Sahin *et al.*, 2005) have been developed in recent years to reduce heat stress in poultry.

According to literature, *G. simplicifolia* seed contains fat up to a level of 28.40±1.20% (Novidzro *et al.*, 2019a), 8.11±0.25% of 5-hydroxytryptophan (5-HTP), carotenoids and chlorophylls A and B (Novidzro *et al.*, 2019b), significant

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amounts of mineral elements and some essential amino acids (Novidzro *et al.,* 2019c). These findings may be helpful to reduce heat stress in poultry birds.

This study aimed to investigate the effects of *G. simplicifolia* seed to reduce heat stress in poultry birds.

## MATERIALS AND METHODS

### Study framework

"AYODÉLÉ" farm (6°22′15.65″N and 0°58′07.35″E), situated near Badja village (Togo), was the experimental site where the annual average temperature ranged from 25°C to 32°C and the medium humidity level close to 75%. The egg brooding and rearing for a fortnight were carried out at CERSA (*Centre d'Excellence Régional sur les Sciences Aviaires*). LAGEPREN research laboratory provided its technical supports for phytochemical characterizations of *G. simplicifolia* seeds. The biochemical and hematological parameter analyses was carried out at INH (*Institut National d'Hygiène*) at Lomé, Togo.

### Experimental animals and design of experiment

After hatching, 210 chicks were weighed before transfer to the brooder where they were reared for a fortnight. Then, they were transferred to a poultry house where they were divided into five groups:

- ✓ Group T0 (Negative control) was fed the basic feed alone;
- ✓ Group T1 (Positive control) was fed the basic feed + 0.5 g of aspirin powder per kg of feed.
- ✓ Groups T2, T3 and T4 were respectively fed the basic feed plus: 2.5 g; 5.0 g and 10.0 g of *G. simplicifolia* seed powder per kg of feed.

### Plant material

The seeds used as raw material were harvested in the botanical garden of Université de Lomé (Togo), in January 2021. In Fig 1, are shown pictures of seeds and powder of *G. simplicifolia,* used as a feed additive.

### Composition of feed

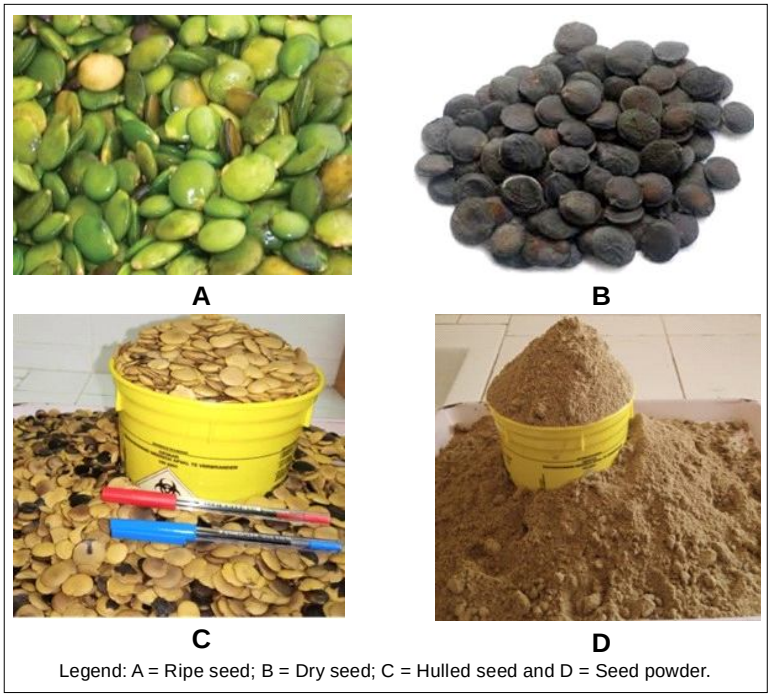
The composition of basal feed is presented in Table 1.

### Management of experimental chicken

The chickens were reared for 12 weeks. During this period, the chickens were given water and feed *ad libitum*.

**Table 1:** Composition of basal feed.

| Ingredients | Start-up phase | Growing phase |
|---|---|---|
| Maize (%) | 51.2 | 62.2 |
| Bran cubed (%) | 16.0 | 10.0 |
| Roasted soybeans (%) | 25.0 | 23.0 |
| Spent grains (%) | 0.0 | 0.0 |
| Lysine (%) | 0.3 | 0.3 |
| Methionine (%) | 0.3 | 0.2 |
| Concentrated Flesh (%) | 5.0 | 2.0 |
| Shells (%) | 2.0 | 2.0 |
| Salt (%) | 0.2 | 0.0 |
| **Nutritional composition** | | |
| Metabolizable energy (kcal/kg) | 2889.99 | 3015.64 |
| Crude protein (%) | 20.33 | 18.16 |
| Crude fat (%) | 7.91 | 7.58 |
| Crude fiber (%) | 5.36 | 5.04 |
| Calcium (%) | 0.97 | 0.89 |
| Phosphorus (%) | 0.64 | 0.49 |




Legend: A = Ripe seed; B = Dry seed; C = Hulled seed and D = Seed powder.

**Fig 1:** Pictures of *G. simplicifolia* seeds (A, B and C) and its powder (D) used as a feed additive.

The prophylactic plan set out in the Table 2 was used during the investigation. Mortalities were counted and recorded daily throughout the experiment.

**Ambient temperature, relative humidity and rectal temperature measurements**

The ambient temperature (AT) and relative humidity (RH) were taken at the three different times of the day, *i.e.* 7 a.m., 10 a.m. and 12 p.m. using a thermo-hygrometer.

Twenty (20) chickens were selected, 4 per group, to have rectal temperatures taken in the morning and at midday, twice a week, using a digital thermometer.

**Determination of zootechnical parameters**

The zootechnical parameters determined by evaluating feed consumption and weighing the chickens were: daily feed consumption DFC (Formula 1); weekly average weight WAW (Formula 2); daily average gain DAG (Formula 3), Consumption index CI (Formula 4).

$$DFC\ (g) = \frac{\text{Amount of feed distributed - Amount of feed remaining}}{\text{Number of topics} \times 7} \quad (1)$$

$$WAW\ (g) = \frac{\text{Sum of the chicks weight}}{\text{Total number of chicken weighed}} \quad (2)$$

$$DAG = \frac{WAW\ (i+1)\ (g) - WAW\ (g)}{7} \quad (3)$$

$$CI = \frac{DFC}{DAG} \quad (4)$$

**Mortality rate determination**

Mortality rate (MR) of the chickens was calculated using formula 5.

$$TM\ (\%) = \frac{NDC}{TNC} \times 100\% \quad (5)$$

With:
NDC = Number of dead chickens.
TNC = Total number of chickens at the beginning of breeding.

**Monitoring of blood parameters**

Two types of blood parameters were assessed as following.

❖ **Biochemical parameter analysis**

Biochemical analysis (glucose, creatinine, cholesterol, triglycerides and uric acid) and catalytic activities of enzymes (alanine amino transferase (ALAT) and aspartate amino transferase (ASAT), were carried out using Mindray BS-200 automated instrument.

Total protein assay was performed using Cypress Diagnostic kits with a Mindray BA-88A spectrophotometer reading against a control serum for analytical range. The analytical methods used with references are recorded in Table 3.

❖**Haematological parameter analysis**

Haematological parameters were measured by SYSMEX XN-550 five-differential system for quantification of white blood cells (WBC), red blood cells (RBC), haemoglobin (Hb), haematocrit (Ht), lymphocyte and heterophils.

**Phytochemical characterisations of *G. simplicifolia* seed**

❖**Powder extraction**

Extraction by maceration method, using water and hydromethanolic mixture (50%-50%) for 48 h, were adopted after delipidation of the seed powder with hexane, using Soxhlet method.

❖**Qualitative phytochemical analyses**

Different techniques described in the literature were used to analyse qualitatively the phytoconstituents contained in the aqueous and hydromethanolic extract, such as: alkaloids (Tiwari *et al.*, 2011); tannins (Eke *et al.*, 2014); Saponins **(**Eke *et al.*, 2014); flavonoids (Tiwari *et al.*, 2011); coumarins (by UV lamp observation at 365 nm); carbohydrates (Elzagheid, 2018); reducing sugars or glucides (Tiwari *et al.*, 2011); cardiac glycosides (Dominique *et al.*, 2018); triterpenic phytosterols (Yadav *et al.*, 2019).

❖ **Quantitative phytochemical analyses**

**Total phenolic compound determination**

Total phenols in the hydromethanolic extract were determined by the UV-Visible spectrophotometric method using the Folin Ciocalteu reagent (Singleton *et al.*, 1999). The optical density of the solutions was read at 760 nm. Total phenolic contents were calculated using calibration curve (Fig 2).

**Antioxidant activity quantification**

This analysis was carried out on the hydromethanolic extract by UV-Visible spectrophotometric measurement with FRAP reagent (Benzie and Strain, 1999). The optical density was read at 593 nm. The calibration curve (Fig 3) was constructed with $FeSO_4(7H_2O)$ solution.

**Antiradical activity assessment**

The antiradical activity of the extract was assessed by the UV-Visible spectrophotometric method which involves reacting DPPH• radical (2,2'-Diphenyl-1-picrylhydrazyl) with an antioxidant molecule (AH) present in the extract (Fig 4). The reduction test was monitored by measuring absorbance at 517 nm. Gallic acid was used for calibration curve (Fig 5).

**Statistical analyses of the data**

Data collected were analysed with Graph Pad Prism 8 software. ANOVA One Way test was applied to evaluate the differences. Means were compared with Tukey's test and the probability $P < 0.05$ was taken as the significance level.

**Table 2:** Vaccination and supplemental feeding plan for experimental.

| Age (in days) | Treatments carried out | Method of administration |
|---|---|---|
| D2 | Newcastle vaccine + Infectious Bronchitis | Beak dipping |
| D3 | Gumboro vaccine | Beak dipping |
| D4 - D7 | Vitamins | Oral administration |
| D9 | Newcastle vaccine + Infectious Bronchitis | Oral administration |
| D10 | Gumboro vaccine | Oral administration |
| D16, D17 and D18 | Vitaminized antibiotics | Oral administration |
| D19 | Newcastle vaccine + Infectious Bronchitis | Oral administration |
| D20 | Gumboro vaccine | Oral administration |
| D21, D22 and D23 | Amprol + Vitamins | Oral administration |
| D30 | Newcastle vaccine + Infectious Bronchitis | Oral administration |
| D31 | Gumboro vaccine | Oral administration |
| D32 | Transfer from brooder to chicken house | Oral administration |
| D32-D34 | Vitamins | Oral administration |
| D35, D36 and D37 | Vitaminized antibiotics | Oral administration |
| D38, D39 and D40 | Vitaminized antibiotics | Oral administration |
| D41 | Internal parasite control | Oral administration |
| D42 | Avian pox + Ita News | Injection |
| D55, D56 and D57 | Vitaminized antibiotics | Oral administration |
| D58, D59 and D60 | Amprol + Vitamins | Oral administration |
| D71 | Internal parasite control | Oral administration |

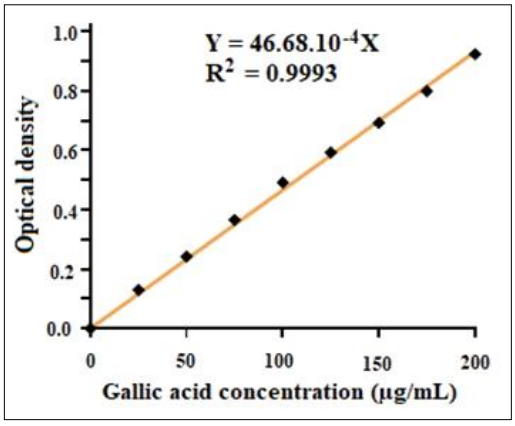



**Fig 2:** Gallic acid calibration curve for the determination of total phenols.

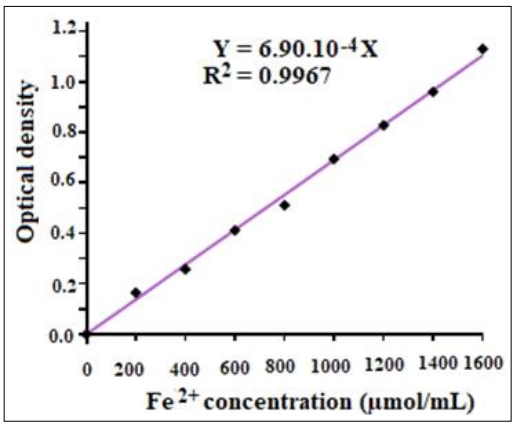



**Fig 3:** Ferrous ion calibration curve for the FRAP test.

## RESULTS AND DISCUSSION

### Variations in ambient conditions during the experiment

This investigation indicated that the ambient temperature of the chicken house rose gradually from 31.1°C at 7:00 a.m. to a maximum value of 31.57°C at around 12:00 p.m., before falling down to a value of 29.27°C at around 4:00 p.m, while the relative humidity decreased from 46.33% at 7:00 a.m. to a minimum value of 32.63% at around 12:00 p.m., before increasing to 38.67% at around 4:00 p.m. (Fig 6).

### Impact of the seed on the rectal temperature of chickens

The effect of *G. simplicifolia* seed on rectal temperature of chickens was illustrated in Fig 7. In the morning time, no significant difference was observed between the control group (T0) and the others. However, in the midday, a slight decrease in rectal temperature was detected between the control group (T0) and the others.

### Impact of the seed on feed consumption and live weight of Sasso chicken

The feed consumption of Sasso chickens was impacted by the use of the seed as shown in Fig 8. A significant decrease ($p<0.05$) in feed consumption was noted in groups T1, T3 and T4 compared to groups T0 and T2.

### Impact of the seed on live weight and daily average gain of Sasso chickens

The average live weight of the chickens has changed over time and depending on the various treatments received by the Sasso chickens. Chickens of groups T0 and T2 had significantly higher live weight values ($P < 0.05$) (Fig 9).

**Table 3:** Analytical methods of biochemical parameters.

| Parameters | Analytical methods | |
|---|---|---|
| Blood glucose level | Enzymatic, Glucose oxidase | "Mindray" Ref: GLU 0102 |
| Uricemia | Enzymatic degradation | "Mindray" Ref: UA 0102 |
| Creatinine level | Kinetic, Colorimetric, Jaffe | "LaBKit" Ref: 30210 |
| Cholesterol | Enzymatic, Colorimetric | "Mindray" Ref: TC 0102 |
| Cholesterol-HDL | Enzymatic, Colorimetric | "Mindray" Ref: TC 0102 |
| Cholesterol-LDL | **By calculation** | |
| Triglycerides | Enzymatic, Colorimetric | "Mindray" Ref: TG 0102 |
| ALAT | UV Kinetic - 37°C | "Mindray" Ref: ALT 0102 |
| ASAT | UV Kinetic - 37°C | "Mindray" Ref: AST 0102 |

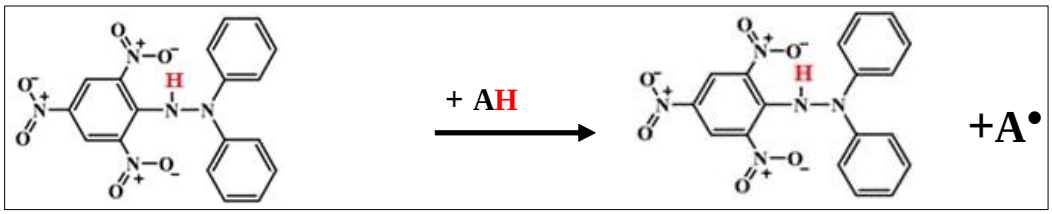



**Fig 4:** Balance sheet equation for DPPH• reaction of with AH antioxidant (Assoti *et al.*, 2019).

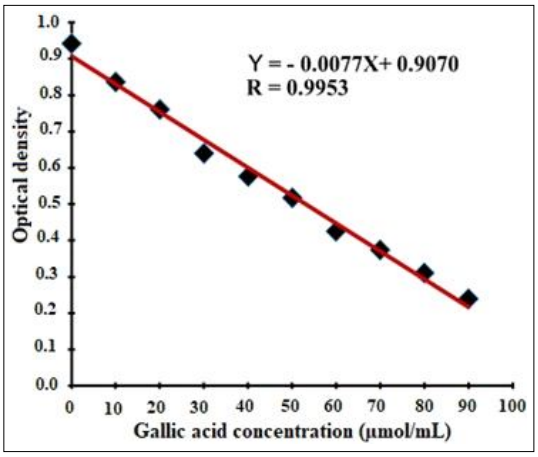



**Fig 5:** Gallic acid calibration curve for anti-free radical analysis with DPPH• reagent.

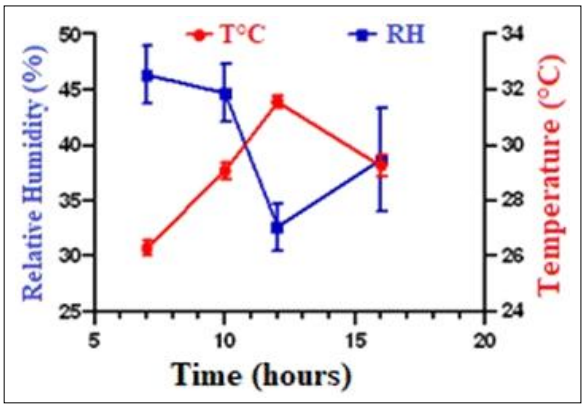



**Fig 6:** Variations in ambient temperature and relative humidity inside the poultry house.

In Fig 10, the influence of the seed on the daily average gain of Sasso chickens was presented. Compared to groups T0 and T2, the DAG decreased significantly ($P<0.05$) in groups T3, T4 and T1.

**Influence of the seed on the consumption index of Sasso chickens**

The influence of the seed on the CI of the chickens was shown in Fig 11. The consumption index was almost similar in all groups, except for group T1 which showed a significantly lower value ($p<0.05$).

**Influence of the seed on the mortality rate of Sasso chickens**

Fig 12 showed that MR of chickens in groups T2 and T4 was almost similar, but was very low compared other groups (T0, T2 and T3).

**Effects of the seed on biochemical parameters of Sasso chickens**

Table 4 showed the effects of the seed on: plasma concentrations of total protein, creatinine, glucose, triglycerides, uric acid, total cholesterol and transaminases.

Compared to the control group, total protein and triglyceride values increased ($p<0.05$) with the use of 2.5% of the seed, while creatinine and uric acid values decreased ($p<0.05$). The use of 10% of the seed resulted in a significant increase ($p<0.05$) in blood glucose. For transaminases (ALAT and ASAT) and total cholesterol, their values were similar ($P>0.05$) for different groups.

**Effects of seeds on haematological parameters of Sasso chickens**

The data shown in Table 5 were the haematological parameters of Sasso chickens.

**Table 4:** Powder effects on biochemical parameters of Sasso chickens.

| Parameters | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T4 | *p-value* |
| Protein levels (g/L) | 39.73±0.63$^{c}$ | 45.50±0.93$^{a}$ | 46.10±0.16$^{a}$ | 43.33±1.30$^{a}$ | 40.97±0.64$^{bc}$ | 0.0001 |
| Blood glucose (mmol/L) | 1.66±0.01$^{bc}$ | 1.95±0.05$^{ab}$ | 1.78±0.03$^{b}$ | 1.80±0.15$^{b}$ | 2.19±0.06$^{a}$ | 0.0013 |
| Triglycerides (g/L) | 0.87±0.01$^{b}$ | 0.99±0.08$^{b}$ | 1.31±0.03$^{a}$ | 0.87±0.05$^{b}$ | 1.08±0.04$^{b}$ | 0.0001 |
| Creatinine (mg/dL) | 2.4±0.06$^{a}$ | 2.26±0.09$^{ab}$ | 1.63±0.14$^{c}$ | 2.30±0.08$^{a}$ | 1.83±0.09$^{bc}$ | 0.0001 |
| Uric acid (mg/L) | 56.1±2.22$^{a}$ | 53.14±1.73$^{ab}$ | 40.07±1.38$^{bc}$ | 38.19±0.21$^{c}$ | 44.68±1.12$^{ab}$ | 0.0060 |
| Total cholesterol (g/L) | 1.09±0.02 | 1.18±0.04 | 1.160±0.01 | 1.26±0.05 | 1.23±0.04 | 0.0600 |
| ASAT (IU/L) | 212.00±14.41 | 182.60±8.15 | 216.10±24.66 | 192.20±19.76 | 214.80±26.98 | 0.6700 |
| ALAT (IU/L) | 4.07±0.06 | 3.96±0.26 | 4.03±0.20 | 4.36±0.63 | 3.23±0.43 | 0.3400 |

The averages on the same line not sharing the same letters are significantly different (P<0.05); ASAT = Aspartate aminotransferase; ALAT = Alanine aminotransferase.

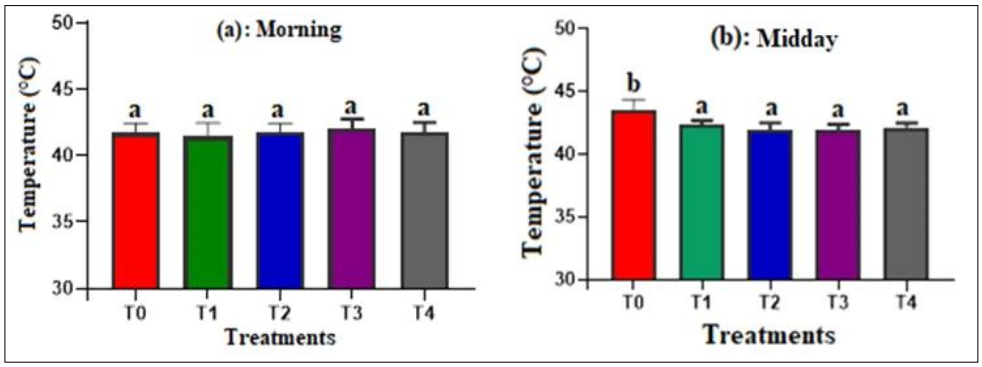



**Fig 7:** Impact of *G. simplicifolia* seed on rectal temperature of chickens.

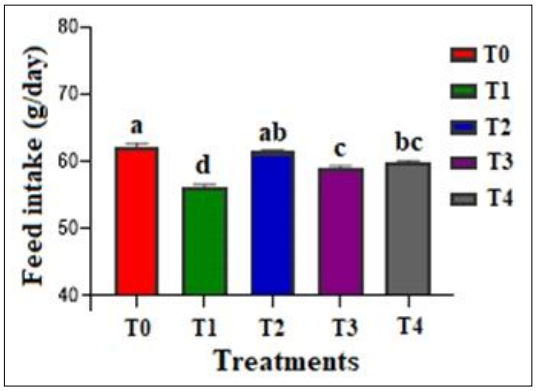



**Fig 8:** Impact of the seed on Sasso chicken feed consumption.

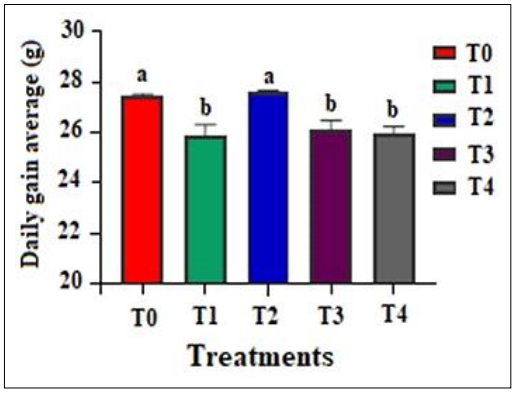



**Fig 10:** Influence of powder on the DAG of Sasso chickens.

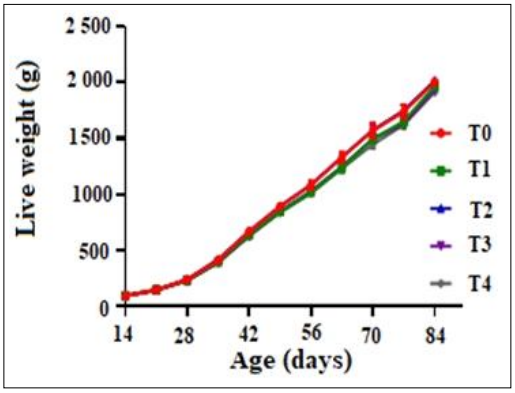



**Fig 9:** Impact of seed on live weight of Sasso chickens.

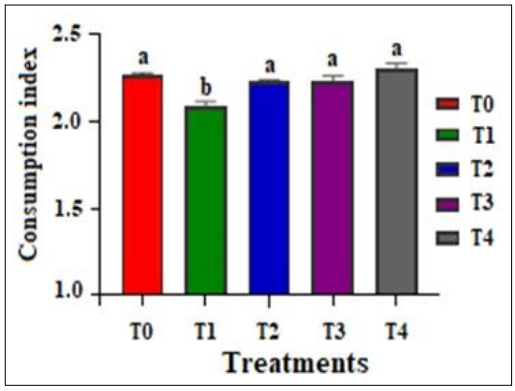



**Fig 11:** Influence of the seed on the CI of Sasso chickens.

**Table 5:** Effects of seeds on hematological parameters of Sasso chickens.

| Parameters | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T4 | *p-Value* |
| White blood cell | 129.80±2.177$^{ab}$ | 142.80±27.5$^{b}$ | 151.10±10.47$^{a}$ | 134.6±3.78$^{ab}$ | 134.00±6.77$^{ab}$ | 0.0320 |
| Lymphocyte (%) | 60.00±1.73$^{d}$ | 66.66±0.57$^{c}$ | 85.00±1.00$^{a}$ | 73.33±3.06$^{b}$ | 75.30±6.81$^{b}$ | 0.0001 |
| Heterophile (%) | 40.00±1.73$^{a}$ | 33.00±0.00$^{ab}$ | 15.00±1.00$^{c}$ | 27.67±3.06$^{b}$ | 24.67±6.81$^{b}$ | 0.0001 |
| Hemoglobin (%) | 10.80±0.29 | 11.60±0.27 | 11.93±0.65 | 11.23±0.26 | 11.30±0.29 | 0.3260 |
| Red blood cell | 2.25±0.02 | 2.41±0.01 | 2.51±0.20 | 2.39±0.08 | 2.28±0.03 | 0.3813 |
| Hematocrit (%) | 29.30±0.62$^{b}$ | 31.40±0.05$^{b}$ | 36.80±0.45$^{a}$ | 30.60±0.80$^{b}$ | 30.13±0.68$^{b}$ | 0.0001 |
| H/L | 0.68±0.05$^{a}$ | 0.50±0.01$^{b}$ | 0.17±0.01$^{c}$ | 0.38±0.06$^{b}$ | 0.33±0.11$^{bc}$ | 0.0001 |

Means on the same line not sharing the same letters are significantly different (P<0.05); H/L = Heterophile divided by Lymphocyte.

Apart from haemoglobin and red blood cells, the haematological parameters in the different groups varied significantly (P<0.05). The seed at did not affect (P>0.05) the white blood cell number; whereas the lymphocyte number increased (P<0.05). In contrast, the heterophil and heterophil/lymphocyte ratio (H/L) values decreased (P<0.05) with the seed used.

### Results of phytochemical screening of *G. simplicifolia* seed

#### ❖ Results of qualitative phytochemical analyses

The results of qualitative phytochemical revealed the presence of various secondary metabolites in the two extracts of *G. simplicifolia* seed (Table 6).

#### ❖ Results of quantitative phytochemical analyses

Total phenol and fat contents and antiradical and antioxidant activity of the extract are provided in Table 7.

The increase in ambient temperatures due to global warming and climate change is one of the main obstacles facing the poultry sector, causing heat stress, which has a negative impact on the welfare and health of animals, also involving considerable economic losses (Bayraktar and Tekce, 2019). In fact, heat stress is one of the most important factors affecting poultry productivity in warm-climate regions as noted by Abdel-Moneim *et al.* (2021).

In the current study, data collected at the rearing site indicated that the temperature and relative humidity varied between 26.32°C and 31.57°C, and 32.63% to 46.33%, respectively (Fig 4). The change in sunlight intensity was the main factor influencing the both parameters. These results were similar to those of Hammouche (2011) who obtained values between 28.63°C and 33.07°C. However, relative humidity result was lower than those reported by Hammouche (2011), *i.e.* 61.82°C-70.58%. This difference could be due to disparities between the climatic conditions of the two studies. However, the thermal and hygrometric conditions in the current work were not conform to rearing guide (Arbor, 2007) which recommended that average ambient temperature and relative humidity of chicken house must be between 21.0°C - 24.5°C and 70%, respectively.

In the morning time, the rectal temperature of the chickens (Fig 7) were almost constant, demonstrating that

**Table 6:** Results of qualitative phytochemical analyses of *G. simplicifolia* seed.

| Phytoconstituents and Identification reagents | Results | |
|---|---|---|
| | AE 100% | HE 50%-50% ( v/v) |
| **Alkaloids** | | |
| Mayer's reagent | + | + |
| Dragendorff reagent | + | + |
| **Tannins** | | |
| 1% $FeCl_3$ | + | + |
| 10% $Pb(CH_3COO)_2$ | + | + |
| **Saponins** | | |
| Foam Test | + | + |
| **Flavonoids** | | |
| 1% NaOH + diluted HCl | + | + |
| **Coumarins** | | |
| 10%NaOH, UV lamp (365 nm) | + | + |
| **Reducing sugars** | | |
| Molisch reagent | + | + |
| Fehling reagent | + | + |
| **Cardiac glycosides** | | |
| $HCCl_3$ + concentrated $H_2SO_4$ | + | + |
| **Terpenoids** | | |
| $H_2SO_4$ (1 M) | + | + |

+ = Presence of tested compounds and - = Absence of tested compounds. AE- Aqueous extract; and HE- Hydromethanolic extract.

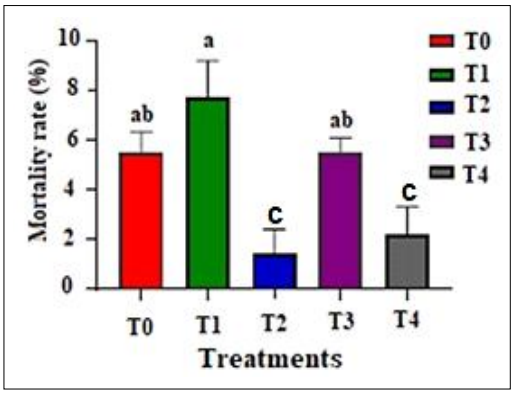



**Fig 12:** Influence of the seed on the MR of Sasso chickens.

**Table 7:** Total phenol and fat contents and anti-free radical and antioxidant activities of *G. simplicifolia* seed.

| Phytochemical parameters quantified | Hydromethanolic extracts |
|---|---|
| Total phenols (mg GAE/g of dry extracts) | 189.39±3.35 |
| *Folin Ciocalteu reagent* | |
| Antioxidant activity (µmol Eq $FeSO_4$/g of dry extracts) | 2,135.75±15.99 |
| *FRAP test reagent* | |
| Antiradical activity (mg EAG/g of dry extracts) | 205.91±6.11 |
| *DPPH• reagent* | |
| Fats (%) | 27.40±2.49 |
| *Soxhlet extraction with hexane* | |

the chickens had not been subjected to heat stroke. By contrast, the relatively high rectal temperature of the chickens in group T0 indicated that the chickens had been exposed to the heat stroke. Yahav (2009) and De Basilio *et al.* (2001) reported that an initial heat stress, with exposure to 36°C for 24 hours, caused a 1°C rise in body temperature. Decrease in rectal temperature of chickens in groups T1, T2, T3 and T4, would probably be justified by the feed supplementation with aspirin and *G. simplicifolia* seed.

Among the biomolecules revealed in *G. simplicifolia* seed, phenolic compounds such as tannins, flavonoids and coumarins are well known for their antioxidant and antiradical scavenging capacities, so this allowed the chickens to fight against heat stress (Attia *et al.*, 2020).

In addition to phenolic compounds, other natural substances in the seed (*i.e.* carotenoids and 5-HTP have therapeutic effects as reported by Lemaire and Adosraku (2002). Moreover, according to Novidzro *et al.* (2019b), the seed also contained a large amount of linoleic acid, with a rate of 73.19% of total fat. This unsaturated acid would be involved in the body temperature reduction of the chickens because it was kown that heat stress produced a decrease in triglycerides level in the plasma of chickens (Xie *et al.*, 2015). Concentration of plasma insulin increase in heat-stressed chickens (Lin *et al.*, 2000) for promoting lipogenesis and fat retention, so the high levels of dietary fat contribute to falling dawn heat production.

Because of the less heat increment of dietary fats, it was found that the oxidation of fatty acids is promoted to fulfil energy needs under heat stress conditions (Mujahid, 2011).

Some findings recommended that dietary fats rich in saturated fatty acids have a better effect on heat-stressed broiler chickens than those rich in unsaturated fatty acids. Nevertheless, omega-3 fatty acids combination improved the antioxidative status, decreased lipid peroxidation and improved the antibody responsiveness in laying chickens (Ebeid, 2011) which might be involved in alleviating of heat stress.

Heat stress has negative effects on physiological response, growth performance and laying performance. As results, it appeared reducing feed consumption, body weight gain, egg production, feed efficiency, meat quality, egg quality and immune response (Abdel-Moneim, 2021). According to Habashy *et al.* (2017), feed consumption (FC) in the birds subjected to heat stress was 20% less than the control group, while the body weight gain (BWG) was 22% lower than the control group. However, previous studies showed that lowered FC was not the only aspect that reduced broiler performance and body weight (BW) in hot weather (Daghir, 2008).

Findings of previous studies have reported that heat stress caused an increase in weight gain, performance index and feed conversion ratio in high levels of either poultry fat or metabolizable energy, feed efficiency and rectal temperature values (Ghazalah *et al.*, 2008). However, Attia *et al.* (2017) have reported that heat stress provokes a decrease in live weight and weight gain, an increase in feed efficiency and rectal temperature values.

In the current study, except group T2, all treated chickens showed a lower level of FC compared to group T0 (Fig 8). These results contradicted the work of Ndam (2007).

Chickens of groups T1, T3 and T4 showed a better average gain than the others, due to the effects of supplementation of aspirin or *G. simplicifolia* seed (Fig. 10). Chickens of group T1 had a lower feed conversion ratio than the others, with almost similar values (Fig 11). Ndam (2007) reported that the feed conversion ratio was higher in treated chickens than in control group.

The use of 2.5 g of *G. simplicifolia* seed/kg in feed induced the best growth for broilers (group T2). May be, the beneficial properties of 5-HTP as well as the phenolic compounds and fat, contained in the seed were the endogenous factors which improved the adverse impacts of heat stress.

The MR of the chickens was higher in positive control group T1 and lower in group T2 (Fig 12). Therefore, *G. simplicifolia* seed had the advantage of limiting the MR of the chickens. Mortality was higher in group T3 compared to group T4, so we suggested that there is another factor, not clarified in this study, which was responsible for the survival of the chickens. For example, heat stress can also cause oxidative stress and raise red blood cell susceptibility to peroxidation (Reddy *et al.*, 2017).

The blood analysis results showed that the average of blood protein, blood glucose and triglyceride levels recorded in all treated groups were higher compared to the control

group T0 (Table 4). Gouda (2019) had obtained similar results with ascorbic acid and/or folic acid used to increase total protein levels in serum.

Creatinine and uric acid levels were lower in the treated group, except group T0. *G. simplicifolia* seed would have caused reductions in creatinine and uric acid levels in the chickens. White blood cell and haematocrit levels of the chickens were higher in treated groups than the control group values. Msaid (2017) stated that heat stress leads to an increase in white blood cell and haematocrit.

The lymphocyte rate of the chickens in the treated groups was significantly higher ($P<0.05$) compared to the control group T0, but the case of heterophils opposite was contrary. The H/L ratio was lower in the treated groups. Group T2 was especially distinguished by a lower heterophilic rate, a higher lymphocyte rate and a lower H/L ratio.

In short, the decrease in feed consumption and body weight gain coupled with the improvement in haematological and biochemical parameters have indicates a promotion of bird health by supplementing the birds' feed with aspirin or *G. simplicifolia* seed, resulting in improved performances of the treated chickens.

*In fine*, the decrease in FC and body weight gain with improvement in haematological and biochemical parameters, were the main factors revealing health promotion of the birds by supplementation their feed *G. simplicifolia* seed.

## CONCLUSION

In the present study, the use of *G. simplicifolia* seed in comparison with aspirin to improve zootechnical and physiological parameters in Sasso chickens was investigated. The results indicated that feed supplementation with the seed at the dose of 2.5 g powder/kg appeared to be more successful. Specific phytoconstituents contained in the seed, such as 5-HTP, lipids, phenolic compounds and carotenoids, would therefore be beneficial for improving chicken performances. Applying the findings of this study can help farmers to combat heat stress in poultry.

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### Conflict of interest

All authors declare that there are no known conflicts of interest.

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