

Spermatozoa production efficiency of rabbit bucks administered *Azanza garckeana* during heat stress conditions

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ABSTRACT

This study assesses the impacts of oral administration of *Azanza garckeana* (*Goron Tula*) syrup on semen quality, reproductive hormone profile, sperm production and storage efficiency in rabbit bucks during heat stress scenarios. Ripe *Azanza garckeana* fruits were procured, air-dried, weighed and processed into syrup by aqueous extraction 30%w/v for 12 hours. The gelatinous fluid obtained was referred to as *Goron Tula* syrup (GTS). Sixty mixed-breed rabbit bucks divided into four treatment groups receiving 0mL, 1mL, 2mL, and 3mL/kg BW GTS daily for 16 weeks. Semen samples were collected and analyzed using computer-assisted sperm analysis. At the end of the study, blood samples were collected and all animals slaughtered, and reproductive organs excised, weights and sperm reserves assessed. Results obtained showed that bucks administered 1mL/kg BW GTS exhibited significantly ($p < 0.05$) higher semen volume, progressive motility, curvilinear velocity, and semen concentration compared to other groups. Epididymal sperm reserves of bucks on control and 3mL/kg BW GTS were significantly ($p < 0.05$) higher than other groups. The daily sperm production and sperm production efficiency were highest in bucks receiving 1mL/kg BW GTS, and these bucks had higher testicular sperm reserves than the others. In

conclusion, a dose of 1mL/kg BW *Azanza garkeana* enhance reproductive performance of bucks under heat stress conditions.

Keywords: anti-mullerian hormone, heat stress, spermatozoa, sperm production efficiency, testicular function

INTRODUCTION

Sexual health reflects an individual's overall quality of life and well-being (Jimoh et al., 2021a). Male infertility has emerged as a significant global issue, with poor semen quality and low sperm count being responsible for over 90% of male infertility cases (Jimoh et al., 2021b). Heat stress, affecting both animals and humans, disrupts normal physiological processes and can damage multiple organs (Jimoh and Ewuola, 2016; Jimoh et al., 2023a; Oni et al., 2023), leading to serious consequences for health, fertility, and overall well-being (Abdelnour et al., 2020).

Heat stress stands out as one of the most significant stressors, often leading to reduced productivity and reproductive inefficiency (Jimoh and Ewuola, 2018a; Jimoh et al., 2023c; Oke et al., 2024). In mammals, maintaining a scrotal temperature that is 2–8°C below body temperature is crucial for reproductive function; even a slight increase in this temperature can disrupt spermatogenesis and lead to infertility (Pei et al., 2012).

Heat stress negatively affects reproductive functions, contributing to embryonic loss and impairing sperm quality (Ngoula et al., 2020; Onagbesan et al., 2023). High temperatures are known to increase oxidative stress and lower reproductive hormone levels (Jimoh et al., 2023c; Kpomasse et al., 2023a; 2023b). The testis, due to its rapid cellular turnover and high polyunsaturated fatty acid content, is particularly vulnerable to damage from free radicals (Ji et al., 2016).

Medicinal herbs have the potential to combat free radicals by creating a protective antioxidative environment within cells, which can mitigate oxidative damage to testicular DNA and improve sperm production and quality (Ji et al., 2016). Medicinal plants have been employed for the treatment of a wide range of human diseases, including those associated with oxidative stress, such as infectious diseases, inflammation, diabetes, malaria, and hepatorenal disorders (Yusuf et al., 2020).

Developing antioxidant and herbal supplementation strategies to enhance sperm characteristics during periods of high heat stress is essential for producing higher-quality semen and improving fertility over extended periods (Jimoh and Ewuola, 2018b; Jimoh et al., 2023a). The use of phytogetic feed additives to boost reproductive efficiency in livestock has gained significant interest due to their antioxidant and anti-inflammatory properties, which are generally less toxic compared to synthetic alternatives (Jimoh and Ewuola, 2018b; Jimoh et al., 2023a).

Phytogenic antioxidants are increasingly recognized for their potential as growth enhancers and modulators of oxidative stress, with global interest in their use for reproductive improvements (Jimoh et al., 2021a). Davoodi et al. (2020) noted that around 80% of people in developing nations and about one-third of the U.S. population use herbs for their free radical-neutralizing properties.

In Nigeria, various plants have been evaluated for their medicinal and nutritional benefits aimed at improving fertility in both male and female animals (Jimoh et al., 2021a; Jimoh et al., 2023d). Reactive oxygen species (ROS) and lipid peroxides are known to contribute to infertility, with high seminal oxidative stress being common in infertile animal groups (Jimoh and Ewuola, 2019). Recent advancements have seen serum Anti-mullerian hormone (AMH) and Inhibin B used as indicators for semen quality and male reproductive health (Deng et al., 2023). Dietary antioxidants supplementation is a proven tool for reducing oxidative stress-induced reproductive disorders, hence making plant extracts a potent strategy for boosting sustainable animal production. Following this is the heightened demand for different antioxidants capable of improving reproductive activities.

Azanza garkeana, commonly referred to as 'Goron Tula' in Hausa, Nigeria, is also known by various names such as snot apple, Azanza, tree hibiscus, quarters, wild hibiscus, and African chewing gum in English (Itodo et al., 2022). The fruit of *Azanza garkeana* is rich in antioxidants, essential nutrients, minerals, and phytochemicals including flavonoids, steroids, triterpenes, saponins, phenols, and tannins, all of which contribute to both human and animal health (Itodo et al., 2022). In traditional medicine, Goron Tula is renowned for its purported benefits on male reproductive health. Nevertheless, further scientific studies are required to validate the role of Goron Tula in the diet of male rabbits and to substantiate its traditional medicinal uses (Itodo et al., 2022). According to Yusuf et al. (2020) and Itodo et al. (2022), the metabolites of Goron Tula possess substantial antioxidant properties that have been shown to enhance the spermiogram of male rabbits treated with its extracts. Goron Tula is commonly processed into syrup due to its gel-like texture when its powder is mixed with water. The syrup is often retailed in the market, fertility centres and online vendors due to its folklore attributes. Hence, *Azanza garkeana* is a potential candidate for mitigating heat stress-induced reproductive pathologies in farm animals. This study aims to investigate the effects of Goron Tula syrup on semen quality, sperm production, reproductive hormone, and organ characteristics in rabbit bucks during heat stress.

MATERIALS AND METHODS

Ethical approval

The institutional research and ethics committee of the Federal Polytechnic Ado Ekiti approved the experimental protocols of this study (AP/REC/2024/00389), in conformity with NRC guidelines and the ARRIVE 2.0. The experiment was performed in line with guidelines of University Animal Scientific procedures [Animal (Scientific procedures) Act 1986]. Adequate management practices were followed to reduce discomfort on the laboratory animals.

Experimental Site

This study was performed from December to March 2023 in Nigeria's south-western agro-ecological belt, a period recognized for inducing heat stress in rabbits, especially from February to March (Jimoh et al., 2021b). Temperature and relative humidity data were collected daily at 08:00, 12:00, and 18:00 hours using a Thermo-Hygrometer, and the monthly average Temperature Humidity Index (THI) was calculated and displayed in Fig. 1. Based on the heat stress conditions defined by Jimoh et al. (2023c) Briefly, $THI = t - [(0.31 - 0.31 \times RH) (t - 14.4)]$.

where RH = relative humidity /100. t = ambient temperature.

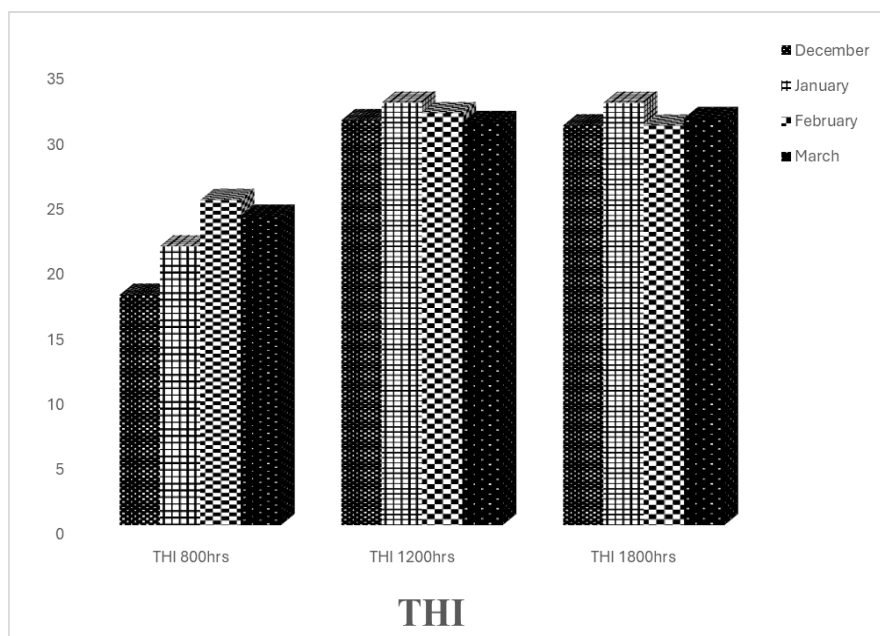


Figure 1. Temperature Humidity Index of the rabbit house during the study

THI are classified as: $<27.8^{\circ}\text{C}$ = absence of heat stress, $27.8 - 28.9^{\circ}\text{C}$ = moderate heat stress, $28.9 - 30^{\circ}\text{C}$ = severe heat stress and above 30°C = very severe heat stress

The average daily THI values— 26.48 ± 0.65 for December, 28.74 ± 1.28 for January, 29.08 ± 0.95 for February, and 28.63 ± 2.54 for March - indicate that the rabbits experienced moderate to severe heat stress throughout the study period.

Experimental Animals and Management

Ripe *Azanza garkeana* fruits were procured at a market in Tula, Kaltungo local government Area of Gombe State, Northern Nigeria. They were air-dried, weighed and processed into syrup by aqueous extraction 30%w/v for 12 hours. The gelatinous fluid obtained was referred to as Goron Tula syrup and stored at -40°C . *Azanza garkeana* was extracted using aqueous solution because antioxidant and free-radical scavenging activities were found to be more effective in extracts obtained using polar solvents compared to those extracted with non-polar solvents (Itodo et al., 2022).

Table 1. Gross composition of experimental diets

Ingredient	Percentage
Maize	38
Wheat Offal	26
Palm kernel cake	21
Soyabean meal	11.2
Methionine	0.1
Lysine	0.2
Bone Meal	3
premix	0.25
Salt	0.25
Total	100
Calculated nutrient	
Dry matter, %	85.14
Crude Protein, %	16.704
Digestible Energy, kcal/kg	2550.27
Ether Extract, %	4.082
Crude Fibre, %	6.218
Lysine, %	0.965
Methionine, %	0.38138
Calcium, %	1.2063
Phosphorus, %	0.663

The experiment utilized sixty mixed breed rabbit bucks (New Zealand White X California X Chinchilla) at 8 weeks of age, averaging 1.02 ± 0.26 kg,

obtained from the Teaching and Research Farm of the Federal Polytechnic, Ado Ekiti. The bucks were randomly allocated to four different treatment groups, with two bucks per replicate in a completely randomized design. The treatment consisted of Goron Tula syrup administered at 0mL/kg BW (control; Treatment 1), 1mL/kg BW; Treatment 2, 2mL/kg BW; Treatment 3 and 3mL/kg BW; Treatment 4. The rabbit bucks were given a standard diet, detailed in Table 1, and had unlimited access to clean water throughout the study. The bucks were gavage orally GTS at 0mL/kg BW (control), 1mL/kg BW, 2mL/kg BW and 3mL/kg BW once daily for treatment 1, treatment 2, treatment 3 and Treatment 4, respectively over 16 weeks.

Semen Collection and Evaluation

At 12 weeks, the bucks were trained to utilize an artificial vagina for semen collection. Following the training, semen samples were collected from each buck twice weekly, with volume recorded and subsequently analysed for sperm kinetics following the protocol established by Jimoh et al. (2021a). Semen volume was measured to the nearest 0.1 mL using tuberculin syringes. Sperm kinetics were assessed using a computer-assisted sperm analyser (SpermAnalyzeWin7, Xuzhon City, China) configured according to the 5th WHO manual: x10 magnification, 51 sperm tracks, 60 frames per second. The analysis included metrics such as motility percentages, progressive versus non-progressive motility, curvilinear velocity ($\mu\text{m/s}$), average path velocity ($\mu\text{m/s}$), straight-line velocity ($\mu\text{m/s}$), linearity, straightness, amplitude of lateral head (μm), beat cross frequency (Hz), and wobble. For sperm concentration and livability, standard methods were used: concentration was measured with a Neubauer hemocytometer (TH-100; Hecht Assistant, Sondheim, Germany) and reported as spermatozoa $\times 10^8/\text{mL}$, while livability was assessed by staining with eosin-nigrosin, preparing a slide, air-drying, and viewing at x400 magnification.

Blood Sample Collection and Hormonal assay

At the expiration of the study on the 16th week, blood samples were collected using the ear vein from all bucks into plain vacutainer bottles. The samples were centrifuged to obtain serum and assayed for hormonal profile. The hormones assessed were follicle stimulating hormone (FSH), Luteinising Hormone (LH), Anti-mullerian hormone (AMH), Testosterone and Inhibin B with commercial ELISA kits and its protocol for each assay, with inter-intra assay covariance of 5.7%.

Assays were performed on serum samples using enzyme-linked immunosorbent assay (ELISA) with proprietary kits. The procedure for each assay was followed as outlined in the respective kit instructions.

- Luteinizing hormone (LH): ELISA Kit, Catalog No LH231F. Calbiotech Inc, El Cajon CA

- Folicle stimulating hormone (FSH): ELISA Kit, Catalog No FS232F. Calbiotech Inc, El Cajon CA
- Testosterone: ELISA Kit, Catalog No TE187S, Calbiotech Inc, El Cajon CA
- Anti mullerian hormone (AMH): ELISA Kit, Catalog No AM448T. Calbiotech Inc, El Cajon CA
- Inhibin B: ELISA Kit, Catalog No. QS0366Ra. Sunlong Biotech Inc, China.

Thereafter, values obtained were used to compute AMH/FSH ratio, Inhibin B/FSH ratio, FSH/LH ratio and testosterone/LH ratio.

Reproductive Organ Characteristics

At the expiration of the study, all bucks were euthanized, slaughtered and their reproductive tract were carefully removed and separated anatomically. The gonads, testis, epididymis, penis, vas deferens and spermatic cord were weighed accordingly. The relative weights were calculated by dividing each organ's weight by live weight and expressed in percentage.

Sperm Reserve and Production Efficiency

To evaluate gonadal and extra-gonadal sperm reserves as well as sperm production efficiency, epididymis and testis were first cleaned of connective tissues (Jimoh et al., 2021a). The testes, caput, corpus and caudal epididymis were homogenized separately with normal saline solution (0.154M NaCl). The suspensions were mixed, filtered through a double layer cheese cloth and the filtrate transferred into test tubes. Sperm count was done haemocytometrically using the improved Neubauer haemocytometer.

The gonadal sperm reserve was estimated as the total number of late spermatids and spermatozoa in the homogenised testicular tissues expressed in millions.

Extra gonadal sperm reserves were determined by direct counting of sperm cells in haemocytometer after 1:2 v/v dilutions. The extra-gonadal sperm reserve was the total number of spermatozoa in all the sections of epididymal tissues expressed in millions.

The daily sperm production was determined by direct measurement from testicular sperm reserves. The number of late spermatids and spermatozoa in homogenised testes were determined by haemocytometric counts using time divisor of 3.43 proposed by Amann, (1970) as estimated from the 48 days duration of one cycle of the seminiferous epithelium. The daily sperm production was then calculated with the formula as outlined in Ewuola and Egbunike (2010). $DSP = \text{Testicular Sperm Count} / \text{Time Divisor}$

Time divisor is 3.43 calculated by length of one cycle of the seminiferous epithelium x Percentage of cycle represented by spermatids counted.

The spermatogenic efficiency or the efficiency of sperm production was determined by dividing the daily sperm production by the paired testes weight.

Statistical Analysis

The data were analyzed using a general linear model to assess reproductive characteristics, semen kinetics, hormonal profiles, sperm production, and sperm storage potentials as described in the statistical model:

$$Y_{ijl} = \mu + B_i + e_{ijl}$$

In this model, Y_{ijl} denotes the measurement of reproductive characteristics for the l th animal, μ represents the overall mean for each trait, B_i indicates the fixed effect of the i th herbal supplement administered, and e_{ijl} signifies the random residual effect. Statistical analyses were performed using the general linear model procedure in Analysis of Variance (ANOVA) with SAS software (2003) at a significance level of $p = 0.05$. Mean differences were assessed using Duncan's Multiple Range Test (DMRT) in the same software.

RESULTS

The semen kinetics of rabbit bucks treated with GTS under heat stress conditions is presented in Table 2. The administration of GTS did not significantly affect non-progressive motility, amplitude of lateral head movement, or spermatozoa livability ($p > 0.05$). However, the semen volume was significantly greater in bucks given 1 mL and 3 mL/kg BW GTS compared to those receiving 0 mL, with the 2 mL/kg BW GTS group showing the lowest semen volume ($p < 0.05$). Bucks administered 1 mL/kg BW GTS exhibited significantly higher percentage sperm motility than those treated with 2 mL/kg BW GTS ($p < 0.05$). Furthermore, progressive sperm motility was significantly greater in the 1 mL/kg BW GTS group compared to the 0 mL, 2 mL, and 3 mL/kg BW GTS groups, which had statistically similar motility values ($p < 0.05$). The average path velocity of semen from bucks treated with 0 mL and 1 mL/kg BW GTS was significantly higher than that from the 2 mL and 3 mL/kg BW GTS groups ($p < 0.05$). In terms of curvilinear velocity, bucks administered 1 mL/kg BW GTS had higher values compared to those on 2 mL and 3 mL/kg BW GTS, which showed similar results ($p < 0.05$). Straight-line velocity, linearity, straightness, and beat cross frequency were significantly lower in the 0 mL, 2 mL, and 3 mL/kg BW GTS groups compared to the 1 mL/kg BW GTS group ($p < 0.05$). Additionally, semen concentration was significantly higher in bucks receiving 3 mL/kg BW GTS compared to those receiving 0 mL/kg BW GTS, while concentrations in the 1 mL and 2 mL/kg BW GTS groups were similar to other treatments ($p < 0.05$).

Table 2. Semen kinetics of rabbit bucks administered Goron Tula syrup during heat stress conditions

	0mL/kg BW GTS	1mL/kg BW GTS	2mL/kg BW GTS	3mL/kg BW GTS	SEM
Volume, mL	0.17 ^b	0.19 ^a	0.14 ^c	0.20 ^a	0.01
Percentage motility, %	87.62 ^{ab}	95.10 ^a	83.48 ^b	86.89 ^{ab}	2.01
Non-progressive motility, %	15.16	16.28	12.76	13.72	1.41
Progressive motility, %	72.47 ^b	78.82 ^a	70.72 ^b	73.34 ^b	1.68
Average path velocity, µm/s	6.06 ^a	6.58 ^a	5.67 ^b	5.64 ^b	0.26
Curvilinear velocity (VCL), µm/s	6.95 ^{ab}	7.33 ^a	6.41 ^b	6.40 ^b	0.29
Straight line velocity, µm/s	3.88 ^b	4.61 ^a	3.75 ^b	3.54 ^b	0.22
Amplitude of lateral head, µm	0.37	0.38	0.36	0.36	0.02
Beat cross frequency, Hz	0.62 ^b	0.78 ^a	0.57 ^b	0.53 ^b	0.14
Linearity, %	56.55 ^b	65.41 ^a	58.85 ^b	59.14 ^b	2.08
Straightness, %	63.95 ^b	71.89 ^a	65.19 ^b	66.22 ^b	1.99
Liveability, %	87.29	90.03	89.03	88.36	3.76
Semen Concentration, x 10 ⁸ /mL	3.98 ^b	6.90 ^{ab}	7.75 ^{ab}	9.93 ^a	0.75

^{abc} Means along the same row with different superscripts, differ significantly ($P < 0.05$), SEM: standard error of mean.

The effects of GTS on the reproductive hormones of rabbit bucks under heat stress is shown in Table 3. FSH levels were significantly higher in bucks treated with 2 mL/kg BW GTS compared to those administered 0 mL, 1 mL, and 3 mL/kg BW GTS, which had similar values ($p < 0.05$). LH levels were significantly higher in bucks given 1 mL/kg BW GTS compared to those on 2 mL and 3 mL/kg BW GTS, with the lowest levels observed in the 0 mL/kg BW GTS group ($p < 0.05$). Testosterone levels were significantly greater in the 0 mL and 2 mL/kg BW GTS groups compared to the 1 mL and 3 mL/kg BW GTS groups, which had similar values ($p < 0.05$). Anti-Müllerian hormone (AMH) was significantly higher in bucks treated with 1 mL/kg BW GTS compared to those receiving 2 mL/kg BW GTS, with the lowest AMH values recorded in the 0 mL and 3 mL/kg BW GTS groups ($p < 0.05$). Inhibin B levels were significantly lower in bucks administered GTS compared to those in the control group ($p < 0.05$). The AMH/FSH ratio was higher in the 1 mL/kg BW GTS group compared to the 2 mL/kg BW GTS group ($p < 0.05$). Inhibin B/FSH ratios were significantly lower in the 1 mL, 2 mL, and 3 mL/kg BW GTS groups compared to the control ($p < 0.05$). The FSH/LH and Testosterone/LH ratios were significantly higher in the 0 mL and 2 mL/kg BW GTS groups compared

to those in the 1 mL and 3 mL/kg BW GTS groups, which were similar ($p < 0.05$).

Table 3. Reproductive hormone of bucks administered Goron Tula syrup during heat stress conditions

	0mL/kg BW GTS	1mL/kg BW GTS	2mL/kg BW GTS	3mL/kg BW GTS	SEM
FSH, $\times 10^{-3}$ mIU/mL	42.00 ^b	41.00 ^b	58.83 ^a	38.00 ^b	3.85
LH, mIU/mL	5.09 ^c	6.45 ^a	5.94 ^b	5.99 ^b	0.30
Testosterone, ng/mL	2.56 ^a	1.69 ^b	2.52 ^a	1.61 ^b	0.21
AMH, ng/mL	0.70 ^c	1.57 ^a	0.92 ^b	0.66 ^c	0.18
Inhibin B, pg/mL	13.47 ^a	4.42 ^d	9.54 ^b	6.42 ^c	1.94
AMH/FSH ratio	17.36 ^{ab}	36.35 ^a	15.98 ^b	17.94 ^{ab}	3.53
Inhibin B/FSH ratio	356.70 ^a	119.80 ^b	158.42 ^b	190.07 ^b	54.27
FSH/LH ratio, $\times 10^{-3}$	8.56 ^a	6.76 ^b	9.90 ^a	6.37 ^b	0.80
Testosterone/LH ratio	0.50 ^a	0.28 ^b	0.42 ^a	0.30 ^b	0.04

FSH: follicle stimulating hormone; LH: Luteinising Hormone; AMH: Anti-mullerian hormone;

^{abc} Means along the same row with different superscripts, differ significantly ($P < 0.05$);

SEM: standard error of mean.

The relative reproductive organ characteristics of rabbit bucks treated with GTS during heat stress is summarized in Table 4. Gonadal weights in the 1 mL and 3 mL/kg BW GTS groups were similar to those of the other treatment groups, but bucks administered 2 mL/kg BW GTS had significantly higher gonadal weights than those receiving 0 mL/kg BW GTS ($p < 0.05$). The right gonad weight was highest in the 3 mL/kg BW GTS group compared to other treatments, which showed similar results ($p < 0.05$). The left gonad weight was significantly higher in the 1 mL and 2 mL/kg BW GTS groups compared to those on 0 mL and 3 mL/kg BW GTS ($p < 0.05$). The right epididymis weight was highest in the 2 mL/kg BW GTS group compared to the 1 mL/kg BW GTS group ($p < 0.05$). For the left epididymis, bucks on 2 mL/kg BW GTS had significantly higher weights than those on the control and 3 mL/kg BW GTS ($p < 0.05$). The penis weight was highest in the 2 mL/kg BW GTS group compared to those administered 1 mL and 3 mL/kg BW GTS, with the lowest value observed in the control group ($p < 0.05$). The right vas deferens weight was highest in the 3 mL/kg BW GTS group compared to the 0 mL/kg BW GTS group ($p < 0.05$). The left vas deferens weights were similar among the 0 mL, 2 mL, and 3 mL/kg BW GTS groups, but higher than in the 1 mL/kg BW GTS group ($p < 0.05$). The weights of the right spermatic cord, right testis, and left testis were not significantly different across treatment groups. The left spermatic cord weight was highest in the 2 mL/kg BW GTS group compared to the 0 mL/kg BW GTS group, with the 1 mL and 3 mL/kg BW GTS groups showing the lowest values ($p < 0.05$). Paired testis weights in the 0 mL

and 2 mL/kg BW GTS groups were significantly higher than in the 1 mL and 3 mL/kg BW GTS groups ($p < 0.05$). Paired epididymal weights were significantly higher in the 2 mL/kg BW GTS group compared to those administered 0 mL, 1 mL, and 3 mL/kg BW GTS ($p < 0.05$).

Table 4. Relative reproductive organ characteristics of rabbit bucks administered Goron Tula syrup during heat stress conditions

Relative weight $\times 10^{-2}$	0mL/kg BW GTS	1mL/kg BW GTS	2mL/kg BW GTS	3mL/kg BW GTS	SEM
Organ weight, %	56.36 ^b	69.62 ^{ab}	76.00 ^a	69.53 ^{ab}	2.82
Right Organ, %	20.64 ^b	20.59 ^b	24.57 ^b	26.45 ^a	1.54
Left Organ, %	24.40 ^b	31.01 ^a	28.87 ^a	25.03 ^b	1.16
Right epididymis, %	3.68 ^{ab}	3.43 ^b	4.29 ^a	3.68 ^{ab}	0.13
Left Epididymis, %	3.68 ^b	3.78 ^{ab}	4.21 ^a	3.35 ^b	0.11
Penis, %	11.43 ^c	17.12 ^b	22.50 ^a	18.08 ^b	1.25
Right vas deferens, %	0.54 ^b	0.69 ^{ab}	0.67 ^{ab}	0.81 ^a	0.04
Left vas deferens, $\times 10^{-2}$	0.69 ^a	0.55 ^b	0.72 ^a	0.70 ^a	0.03
Right spermatic cord, %	3.12	4.04	4.23	3.48	0.30
Left spermatic cord, %	3.61 ^b	2.96 ^c	4.31 ^a	2.17 ^c	0.38
Right Testis, %	9.31	8.77	8.89	8.11	1.40
Left testis, %	9.07	7.70	8.94	8.45	1.46
Paired testis weight, %	18.38 ^a	16.47 ^b	17.83 ^a	16.56 ^b	0.84
Paired epididymal weight, %	7.36 ^b	7.21 ^b	8.51 ^a	7.03 ^b	0.22

^{abc} Means along the same row with different superscripts, differ significantly ($P < 0.05$);

SEM: standard error of mean.

The epididymal sperm reserves in rabbit bucks treated with GTS during heat stress is detailed in Table 5. The right caput sperm reserve was significantly higher in the 3 mL/kg BW GTS group compared to the 1 mL and 2 mL/kg BW GTS groups ($p < 0.05$). The right corpus sperm reserve, right caudal sperm reserve, and left caput sperm reserve were significantly higher in the 0 mL/kg BW GTS group compared to the 1 mL, 2 mL, and 3 mL/kg BW GTS groups ($p < 0.05$). The left corpus sperm reserve was highest in the 3 mL/kg BW GTS group, while those on 1 mL and 2 mL/kg BW GTS had similar reserves to the 0 mL group but lower than the 3 mL/kg BW GTS group ($p < 0.05$). The left caudal sperm reserve, total caput sperm reserve, total corpus sperm reserve, total caudal sperm reserve, and right epididymal sperm reserve were all significantly higher in the 0 mL and 3 mL/kg BW GTS groups compared to the 1 mL and 2 mL/kg BW GTS groups, which showed similar values ($p < 0.05$). The left epididymal sperm reserve was highest in the 3 mL/kg BW GTS group compared to all other treatments, while the 2 mL/kg BW GTS group had similar values to the 0 mL and 1 mL/kg BW GTS groups ($p < 0.05$).

Table 5. Epididymal sperm reserves of rabbit bucks administered Goron Tula syrup during heat stress conditions

	0mL/kgBW (control)	1mL/kgBW GTS	2mL/kgBW GTS	3mL/kgBW GTS	SEM
Right caput Sperm reserve, x10 ⁷ spz/mL	4.11 ^{ab}	1.33 ^b	0.90 ^b	14.03 ^a	2.18
Right corpus Sperm reserve, x10 ⁷ spz/mL	36.50 ^a	17.72 ^b	11.57 ^b	19.42 ^b	7.88
Right caudal Sperm reserve, x10 ⁷ spz/mL	26.28 ^a	1.86 ^c	1.69 ^c	14.62 ^b	5.07
Left caput Sperm reserve, x10 ⁷ spz/mL	8.87 ^a	1.12 ^b	2.36 ^b	2.04 ^b	1.46
Left corpus Sperm reserve, x10 ⁷ spz/mL	2.25 ^c	7.07 ^b	6.94 ^b	24.29 ^a	4.49
Left cauda Sperm reserve, x10 ⁷ spz/mL	16.88 ^a	3.84 ^b	7.55 ^b	20.39 ^a	4.59
Total caput sperm reserve, x10 ⁷ spz/mL	12.98 ^a	2.45 ^b	3.26 ^b	11.98 ^a	2.33
Total corpus sperm reserve, x10 ⁷ spz/mL	38.75 ^a	24.79 ^b	18.51 ^b	43.70 ^a	8.81
Total caudal sperm reserve, 10 ⁷ spz/mL	43.13 ^a	5.70 ^b	9.24 ^b	35.01 ^a	6.37
Right Epididymal sperm reserve, 10 ⁷ spz/mL	66.86 ^a	20.92 ^b	14.16 ^b	43.98 ^a	10.02
Left Epididymal sperm reserve, x10 ⁷ spz/mL	27.99 ^b	12.03 ^c	16.85 ^{bc}	46.71 ^a	8.47

^{abc} Means along the same row with different superscripts, differ significantly ($P < 0.05$); SEM: standard error of mean.

The sperm storage potential and sperm production efficiency of rabbit bucks administered GTS during heat stress is illustrated in Table 6. The right testis sperm reserve/testis was significantly higher in the 1 mL/kg BW GTS group compared to the 0 mL, 2 mL, and 3 mL/kg BW GTS groups, which had similar values ($p < 0.05$). Bucks in the 1 mL/kg BW GTS group also had significantly higher left testis sperm reserve/testis, right testis sperm reserve/g testis, left testis sperm reserve/g testis, testicular sperm reserve/testis, and testicular sperm reserve/g testis compared to all other treatment groups, with the lowest values recorded in the control group ($p < 0.05$). The daily sperm production was highest in the 1 mL/kg BW GTS group, with the lowest production observed in the control group ($p < 0.05$). Sperm production efficiency was significantly lower in the 2 mL and 3 mL/kg BW GTS groups compared to the 1 mL/kg BW GTS group, with the lowest efficiency found in the control group ($p < 0.05$).

Table 6. Sperm storage potential and sperm production efficiency of rabbit bucks administered Goron Tula syrup during heat stress conditions

	0mL/kgBW (control)	1mL/kg BW GTS	2mL/kg BW GTS	3mL/kg BW GTS	SEM
Right testis sperm reserve, x10 ⁷ spz /testis	9.06 ^b	21.93 ^a	4.64 ^b	12.07 ^b	1.92
Left Testis sperm reserve, x10 ⁷ spz/testis	7.49 ^d	55.57 ^a	33.91 ^b	12.54 ^c	3.96
Right testis sperm reserve, x10 ⁷ spz/g testis	5.34 ^{bc}	14.57 ^a	2.92 ^c	8.02 ^b	0.66
Left Testis sperm reserve, x10 ⁷ spz/g testis	4.85 ^b	41.14 ^a	19.66 ^{ab}	8.51 ^b	2.62
Testisicular sperm reserve, x10 ⁷ spz/ testis	16.55 ^c	77.50 ^a	38.55 ^b	24.61 ^{bc}	4.47
Testicular sperm reserve, x10 ⁷ spz/g testis	10.19 ^c	55.71 ^a	22.58 ^b	16.52 ^{bc}	3.10
Epididymal sperm reserve, x10 ⁷ spz	94.86 ^a	32.95 ^b	31.00 ^b	90.69 ^a	1.20
Daily Sperm Production, x10 ⁷ spz	4.82 ^d	22.59 ^a	11.24 ^b	7.17 ^c	1.30
Sperm Production Efficiency, x10 ⁷ spz	1.48 ^c	7.90 ^a	3.31 ^b	2.39 ^b	0.44

^{abc} Means along the same row with different superscripts, differ significantly (P<0.05); SEM: standard error of mean.

DISCUSSION

Globally, male sexual dysfunction in the form of libido, erection, ejaculation and fertility is growing, largely because sexual function affects quality of life (Alahmadi et al., 2020). The negative effects of heat stress on the productivity and reproductive functions of commercial animals are well-documented (Jimoh et al., 2017; Jimoh, 2019). Therefore, improving semen quality in rabbit bucks exposed to heat stress is a key strategy for boosting reproductive efficiency in rabbits (Jimoh and Ewuola, 2018b). The use of herbal medicine in the treatment of reproductive pathologies is common globally. Interestingly, about 1% of available medicinal plants have been explored, because of the dearth of scientific research on their benefits, despite the belief that natural aphrodisiacs have less or no side effects, have systemic effect, are nutritional and cheaper than synthetic drugs (Alahmadi et al., 2020). In traditional medicine, *Goron Tula* is believed to positively influence male reproductive health. However, more research is needed to establish the importance of *Goron Tula* in the nutritional management of male rabbits, provide some scientifically beneficial to support its traditional claims (Itodo et al., 2022). This study underscores the potential of GTS as a natural supplement to improve reproductive performance and resilience to heat stress in rabbit

bucks, offering practical applications for livestock management in tropical and subtropical regions.

The administration of *Goron Tula* syrup had varying effects on the semen kinetics of rabbit bucks under heat stress. Notably, non-progressive motility, amplitude of the lateral head, and livability were not significantly affected, indicating these parameters were relatively stable across different GTS doses. This could be likened to the claims of Joseph et al. (2019), who reported that methanol extract of *Ocimum gratissimum* leaves did not influence semen quality indices of male albino rats. Bucks administered 1mL/kg BW and 3mL/kg BW GTS had significantly higher semen volume compared to the control and 2mL/kg BW groups. This suggests that moderate (1mL/kg BW) and higher doses (3mL/kg BW) of GTS could enhance semen volume under heat stress. Progressive motility was highest in the 1mL/kg BW group, indicating that this dosage was optimal for improving sperm movement efficiency. The role of progressive motility in enabling sperm to reach and fertilize the egg underscores its potential importance for fertility. Average path velocity and curvilinear velocity were better in the 1mL/kg BW group, and this further supports the benefit of a moderate GTS dosage in enhancing the semen quality metrics, which is critical for successful fertilization. Our findings are consistent with those of Yusuf et al. (2020) and Maroyi and Cheikh-Youssef (2017), who observed that high antioxidant levels in *Goron Tula* metabolites positively affected the spermogram of bucks. Furthermore, Melnikovova et al. (2015) reported that *L. meyenii* was effective in increasing sperm motility, progressive motility, semen volume, and sperm viability.

Bucks treated with 1mL/kgBW GTS showed significant enhancements across multiple semen parameters including volume, percentage of sperm motility, progressive motility, and kinematic properties such as curvilinear and straight-line velocity. These improvements are consistent with the findings of Somphol et al. (2003), who reported that *Boesenbergia pandurata* improved semen volume, spermatozoa concentration, viability, and progressive motility in rabbits. This suggests that a dose of 1mL/kg BW GTS optimizes semen quality under heat stress conditions. Similar herbs such as *Salvia miltiorrhiza* extract were reported to increase progressive motile spermatozoa, sperm concentration, and significant reduction in non-progressive motile spermatozoa (Davoodi et al., 2020). Higher doses of GTS (2mL and 3mL/kg BW) did not yield similar benefits and, in some cases, showed lower values compared to the control. This indicates a potential dose-dependent effect where moderate supplementation (1mL/kg BW) is beneficial, but higher doses may be less effective or even detrimental. Our results align with the study by Attia et al. (2017), which indicated that 10 g/kg of rosemary leaves led to a decline in semen quality, serum testosterone, and fertility, whereas a lower dose of 5 g/kg diet was found to enhance the reproductive function in rabbit bucks. Similarly, Litescu et al. (2010) reported

toxic effects at high concentrations of curcumin (100 μM); whereas it conferred protective and antioxidative functions at lower concentration (1–50 μM). The typical explanation for decreased sperm motility is the uncoupling of oxidative phosphorylation in mitochondria, which results in decreased ATP production, impaired mitochondrial electron transport, and elevated levels of superoxide that are toxic to spermatozoa (Anandakumar et al., 2006). During the process of ejaculation, sperm cells are suspended in the seminal plasma, and variations in the biochemical properties of this plasma can have a significant impact on sperm cell quality and viability (Huang et al., 2023). Fruits possess a variety of natural antioxidant compounds such as carotenoids, vitamins, phenolic compounds, and flavonoids, these agents are recognized for their ability to quench singlet and triplet oxygen, scavenge free radicals, and decompose hydrogen peroxide (Gardner et al., 2000). Antioxidants, nutrients, minerals, and phytochemicals such as flavonoids, steroids, triterpenes, saponins, phenols, and tannins found in Snot Apple fruit provide significant health benefits for humans and animals (Itodo et al., 2022). This might be a contributing factor to the results observed in this study, showing the efficacy of Goron Tula syrup in mitigating the effects of heat stress in rabbit semen. Improvement in sperm motility in heat stressed bucks administered royal jelly was attributed to the impact of antioxidant protein compounds and micronutrients on enhancing buck health and metabolic functions (Park et al., 2019). Khadr et al. (2015) indicated that administration of 200 mg/kg Royal Jelly fortnightly over 6 weeks enhanced progressive motility percentage in buck rabbits during the pre-puberty stage of development. The sperm motility enhancement could be attributed to the increase in testosterone (Abdelnour et al., 2020). Reports of Abdelnour et al. (2020) revealed that Royal Jelly increased seminal fluid production and nutritional enrichment plays an essential role in optimizing sperm motility and viability. This could explain the result on semen quality obtained in bucks administered GTS in the present study. Propolis has a favorable impact on libido and semen characteristics in rabbit bucks during the summer season, was reported by El-Sherbiny (2015) and aligns with reference to the outcomes of this study. Likewise, Hashim et al. (2013) also observed that including propolis in male rabbit diets during hot weather conditions effectively mitigates the negative impacts of high temperatures on semen quality. Phytochemicals promote fertility by enhancing the biosynthesis of steroid hormones and the secretion of testosterone, thereby improving sexual activity (Swelum, et al., 2021). FSH concentrations were highest in the 2mL/kg BW group according to the findings of this study. This might indicate an optimal stimulation of spermatogenesis at this dose. Contrariwise, in Yang et al. (2012) it was reported that serum FSH levels decreased in rats following exposure to royal jelly. However, bucks on 1mL/kg BW had the highest LH levels, which are essential for testosterone production and overall reproductive function. Again, this points to the 1mL/kg BW dosage

being beneficial for maintaining hormonal balance under heat stress. The increased levels of reproductive hormones in rabbit bucks treated with Goron Tula can also be attributed to the flavonoids, phenols, saponins, and tannins found in the fruit, which have been documented to boost testosterone levels through stimulation of the hypothalamo-pituitary-testicular axis (Yusuf et al., 2020; Ahangarpour et al., 2017).

Our observation on the higher testosterone levels in the rabbits on control and 2mL/kg BW groups in this study supports the results reported in previous studies, which revealed that oral administration of royal jelly in rabbits enhanced serum testosterone (Khadr et al., 2015). This might suggest that while 1mL/kg BW GTS optimizes sperm motility and other kinetics, the higher doses might be stimulating testosterone without the corresponding benefit in sperm quality. According to research, antioxidants improve steroidogenesis by boosting Leydig cell activity and endocrine function, which results in increased testosterone levels that stimulate spermatogenic processes. The bucks administered 2mL/kg BW GTS in this study had the highest FSH levels, while 1mL/kg BW GTS led to the highest LH levels. This indicates that GTS impacts different hormones at different doses, which may reflect complex regulatory effects on the endocrine system. When the hypothalamus releases GnRH in adequate amounts, it effectively stimulates the secretion of FSH, LH, and testosterone, thereby increasing their serum concentrations. Additionally, this observation supports the claims that natural compounds are used as alternatives for treatment of hormone deficiency (Alahmadi et al., 2020), and have the capacity to increase testosterone and enhance fertility (Tahvilzadeh et al., 2015).

Bucks on 0mL and 2mL/kg BW GTS had higher testosterone levels than those on 1mL and 3mL/kg BW, suggesting that moderate GTS doses might not favour testosterone production compared to no GTS or higher doses. Testosterone is responsible for maintaining the conditions necessary for spermiogenesis, facilitating the transport of spermatozoa, and ensuring that semen is properly deposited at the site of fertilization in females, suggesting that optimal level of testosterone was secreted in bucks on 1mL/kg BW GTS led to its superior semen quality.

Hypothalamic GnRH stimulates the anterior pituitary to secrete FSH and LH, which then promote the production of Inhibin B, Anti-Müllerian Hormone, and testosterone in the testes (Deng et al., 2023). The evidence from this study suggests that 1mL/kg BW dose of GTS was efficient in promoting better hormonal balance and testicular function compared to other doses. This dosage resulted in higher AMH levels and a favourable AMH/FSH ratio, which are indicative of better testicular health and spermatogenesis. Testicular support cells secrete Inhibin B and AMH, which could be more reliable than FSH for predicting normal spermatogenesis in the testis (Deng et al., 2023). While GTS at 2mL/kg BW and higher doses might not confer the same benefits

and could potentially lead to less favourable hormonal profiles. AMH is an indicator of Sertoli cell function, which is crucial for sperm production. The higher AMH levels observed at 1mL/kg BW GTS suggests that this dosage might be more effective in supporting spermatogenesis and overall testicular function under heat stress. While Inhibin B levels were lower across all GTS treatments compared to the control. Lower Inhibin B in GTS-treated bucks indicates a potential reduction in spermatogenic activity compared to the control. However, this could also mean a feedback mechanism regulating FSH levels, which could help in managing heat stress by preventing overstimulation of the testes. In adult males, elevated serum Inhibin B levels are negatively correlated with FSH, which acts as a negative feedback regulator of FSH. The AMH/FSH ratio was significantly higher in bucks administered 1mL/kg BW GTS compared to those on 2mL/kg BW. The higher AMH/FSH ratio recorded in the 1mL/kg BW group suggests a better testicular efficiency and potentially better adaptation to heat stress conditions. Bucks administered GTS had significantly lower Inhibin B/FSH ratios compared to the control. This lower ratio indicates a shift in the hormonal balance that might be an adaptive response to heat stress, reducing FSH inhibition to sustain spermatogenesis under stressful conditions. Similarly, the bucks on 0mL and 2mL/kg BW GTS had significantly higher FSH/LH and Testosterone/LH ratios compared to those on 1mL/kg BW. The higher ratios could suggest that these dosages might not be as effective in mitigating heat stress as the 1mL/kg BW dosage, which shows a more balanced hormonal profile.

Gonadal weights were generally higher in GTS-treated bucks compared to the control group, with the 2mL/kg BW dose showing particularly significant effects. Increased testosterone concentration may be responsible for the observed changes, which has been reported to increase testis and epididymis weight in rabbit bucks administered propolis (El-Sherbiny et al., 2015). By supporting spermatogenesis and exerting anabolic effects, testosterone plays a vital role in male reproductive health (Hafez and Hafez, 2000). This suggests that GTS positively influences the development and maintenance of reproductive organs under heat stress conditions. This depicts higher capacity of cells during spermatogenesis due to higher testicular weights (Oyeyemi et al., 2002). Similarly, *Garcinia kola* has been adduced to increase testicular weight of adult male albino rats (Sewani-Rusike et al., 2015), aqueous extract of *M. oleifera* of root has stimulating effect on testes weight and testicular testosterone in male rats (Yakubu et al., 2013). Also, *Phoenix dactylifera* (Malviya et al., 2016), *Lycium barbarum* (Rajender and Akhand Pratap 2012) have been reported to increase epididymis weight. This observation reflects a mitigation of heat stress effects in rabbits, akin to findings by Ngoula et al. (2020), who reported decreased reproductive organ weights, reduced sperm concentration, and compromised sperm membrane integrity in heat-stressed

male cavies. Testosterone, produced by Leydig cells, is crucial for spermatogenesis; a decrease in testosterone can lead to reduced germinative epithelial cell numbers and lower sperm production, while an increase in testosterone can enhance testicular weight and sperm concentration (Ata et al., 2018). Therefore, the increased weights of the testis and epididymis observed in this study suggest a beneficial effect of higher testosterone levels, led to increased sperm concentration and motility obtained in bucks on 3mL/kg BW of GTS, is in line with reports in rabbits orally administered carob bean extract (Ata et al., 2018). Also, as reported by Jasem et al. (2010), *Salvia hypoleuca* increases the motility of spermatozoa, epididymal sperm concentration, and testicular weights in rat.

This study showed that the testicular and epididymal sperm reserves were highest in the 1mL/kg BW group, indicating that this dosage is most effective in maintaining sperm production and storage capacities under heat stress. Also, the 1mL/kg BW group recorded the highest daily sperm production and efficiency, confirming the optimal nature of this dosage for enhancing reproductive output under adverse conditions. Gage and Freckleton (2003) reported that mammalian testicular size is a good predictor of spermatozoa production. Hence, an increase in the relative testis size has been adduced to reflect increased sperm production, due to positive correlation between testicular size and daily sperm production (Kudi et al., 2015). Also, an increase in DSP is an indication that spermatogenesis is positively influenced, and attributable to hormones primarily involved in sperm production (Kudi et al., 2015) due to Incorporating antioxidant molecules into animal diets can enhance health and productivity by mitigating oxidative stress and improving overall well-being (Attia et al., 2017). This points to the potency of Goron Tula syrup's ability to mitigate heat stress-induced sub-fertility in rabbits. This is due to high phenolic and flavonoid in Goron Tula, which was responsible for spermatogenic effects (Itodo et al., 2022). Similarly, Elnagar (2010) reported that the use of Royal Jelly in the diet of heat-stressed male rabbits provides a beneficial intervention to combat summer infertility by improving semen quality and reproductive parameters.

The highest sperm reserves were seen at 3mL/kg BW in some epididymal sections, suggesting that higher doses may still have some beneficial effects on sperm storage. This is an indication of GTS fertility induction in heat stressed bucks, because according to Emmanuel et al. (2019), the fertility of a male animal is primarily determined by its spermatozoa-storing ability. Similarly, Davoodi et al. (2020) reported the effect of *Salvia hypoleuca* to increase epididymal sperm reserves, and testicular weights. And *Salvia haematodes* roots and *I. suffruticosum* leaf extract has been claimed to improve epididymal sperm reserve of male rats in a dose-dependent manner (Bansode et al., 2015). This is in agreement with Kudi et al., (2015) who attributed higher ESR and testicular weights of rabbits on diets supplemental cerium oxide to its

influence sperm production, because of the relationship between sperm production and storage. Moreover, *Nigella sativa* seeds exerted an increase in the weight of reproductive organs and sperm reserves in the cauda epididymis was noted in male albino rats (Mohammad et al., 2009).

Breeders rely on the quantity of viable spermatogenic cells produced by the testis and the epididymis's capability to store spermatozoa as crucial indicators for selecting breeding males (Ewuola, 2013). GTS, particularly at 1mL/kg BW, is effective in enhancing reproductive performance in bucks under heat stress conditions, making it a valuable supplement for rabbit farming in hot climates. The administration of 1mL/kg BW GTS appears to be the optimal dosage for mitigating heat stress effects in rabbit bucks. It significantly improved semen quality, sperm motility, velocity parameters, and daily sperm production while maintaining a balanced hormonal profile in the present study. In the opinion of Oyeyemi et al. (2002), reproductive organ morphometry is a critical factor for determining the breeding value and reproductive success of male breeders. Hence, *Goron Tula* syrup can be used to boost male fertility during heat stress conditions, while higher doses (2mL and 3mL/kg BW) show benefits particularly in hormone levels and organ weights, the 1mL/kg BW dose strikes the best balance between improving sperm quality and maintaining reproductive health without overstressing the animals.

CONCLUSION

The study revealed that *Goron Tula* syrup can mitigate the negative consequences of heat stress for reproductive outcomes in bucks in a dose-dependent manner. 1mL/kgBW appears optimal for improving semen quality, sperm production, optimizes hormonal profile and reproductive organ development. However, 2mL and 3mL/kgBW could provide benefits in ameliorating hormonal deficiency/imbalance. Supplementing rabbit bucks' diets with 1mL/kgBW GTS during heat stress conditions is recommended to enhance semen kinetics, reproductive hormone balance, and overall reproductive organ development. Further studies could explore the long-term impacts and potential mechanisms underlying these effects.

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