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Antioxidant Activities of Vitamin C and Bi-carbonate Buffers on Hormones Secretion and Serum Metabolites of Heat-stressed Rabbit Buck

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Authors' contributions

This work was carried out in collaboration among all authors. Author KUA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EEN and EEA managed the analyses of the study. Authors ECU and NPJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antioxidant activities of vitamin C and bicarbonate buffers on hormones secretion and serum metabolites of heat-stressed rabbit buck.
Methodology: A total of twenty-five (25) Adult rabbit bucks (New Zealand White crosses) of 12 months old were used in this study. The rabbits were randomly allotted into the experimental treatments of five treatment groups with five (5) rabbits per treatment in a Completely Randomized Design (CRD). The treatment groups consisted of Control, Sodium Bicarbonate (Na_2HCO_3) and Potassium bicarbonate (KNO_3), Vitamin C, and baobab fruit pulp meal (BFPM) as supplements respectively. The experiment lasted for 9 weeks. Temperature humidity of the pen house and thermoregulatory condition of the rabbits were evaluated. Blood samples (5 ml) were collected from the ear vein at 10.00 h from four rabbits chosen randomly from each group of rabbits respectively before and the end of the experiment for serum metabolite, thyroxine and testosterone hormones evaluation.
Results: It was found that Vitamin C and BFPM significantly (P<0.05) reduced thermoregulatory parameters and increased hormonal secretions. The pattern of secretion of serum metabolites was not significantly (P<0.05) consistent.
Conclusion: It was concluded that Vitamin antioxidants were more effective to ameliorate heat stress than bicarbonate buffers.

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Keywords: Antioxidants; heat stress; hormones; thermoregulation.

1. INTRODUCTION

Rabbits are hoped to play an important role in solving meat production deficiency, particularly in Nigeria. Nigeria is localized in the tropical regions; rabbits reared in Nigeria are suffered from a variety of problems related to heat stress. Poor reproductive efficiency is one of the factors militating against the increase in the production output of rabbits. The rabbit reproductive bucks are prone to heat stress because of their poor functional sweat gland. The formation of free radicals and other reactive oxygen (ROS) species in bodily fluids and tissues has been demonstrated to increase when the ambient temperature is high [1,2]. Damage to biological macromolecules and disruption of normal cell metabolism result from the accumulation of these free radicals as a result of overproduction or a reduced antioxidant defence [3]. Heat stress is responsible for the increased production of ROS [4], decrease feed intake and weight gain [5] increase rectal temperature and heart rate [5,6], and also increases scrotal temperature which disrupts spermatogenesis and ultimately causes infertility [7-9]. Heat stress has been reported to negatively affect the testicular function and also lead to low sperm quality and viability [10] this has led to a decrease in the rate of reproduction of young rabbits.

There have been several strategies established to ameliorate the harmful effects of high environmental temperature on rabbit performance, including dietary and physiological approaches [11]. Studies [12-14] have shown that antioxidant nutrient supplementation, especially vitamin C, E, and A, zinc, and chromium, can be used to mitigate the negative effects of environmental stress. Vitamin C is also present in some plants; plants and their parts could serve as probiotics and antioxidants to the livestock [15,16]. Some phytochemicals in plants improve antioxidant, anti-microbial, feed flavor, and palatability which could result in increased feed intake and performance in animals [16]. $NaHCO_3$ was found to improve oxidative stress and heat tolerance by immunomodulation. Sodium bicarbonate in feed or water has shown potential benefits on production performance [17-19], egg characteristics [20-21], and blood profile [22] in poultry birds and rabbits exposed to heat stress. It is cheap, easily available, and easy to handle, therefore, can be safely used to ameliorate the adverse effects caused by heat stress.

The influence of antioxidants on hormone secretion and serum metabolites of heat-stressed reproductive bucks was therefore investigated.

2. MATERIALS AND METHODS

2.1 Experimental Site

This study was carried out at the Rabbit Unit of the National Animal Production Research Institute (NAPRI) Shika, Zaria. Shika lies between 11^o 12' 42" N and 7^o 33' 14" E at an altitude of 691 m above sea level [23]. Zaria has an average rainfall of 1100mm which starts from late April and early May to mid-October and an average temperature of 37^oC and average relative humidity of 75%

2.2 Preparation of Buffer Solution

Potassium bicarbonate, sodium bicarbonate, and carbonate anhydrous salts were purchased from a laboratory equipment and chemicals vendor in Samaru-Zaria Nigeria. Distilled water was prepared in the Multiuser Laboratory of the Department of Chemistry, Ahmadu Bello University Zaria. The buffer solution was prepared according to the methods of [24] at a pH of 7.5 in the Department of Biochemistry, Ahmadu Bello University Zaria.

2.3 Experimental Animals, Diets, and Design

A total of thirty (25) Rabbit Bucks (New Zealand White crosses) of 12 months old were used in this study. The rabbits were randomly allotted into the experimental treatments of five treatment groups with five (5) rabbits per treatment in a Completely Randomized Design (CRD). The treatment groups consisted of Control (Basal diet with no supplementation), Sodium Bicarbonate ($NaHCO_3$) and Potassium bicarbonate (KNO_3) solution with basal diet respectively, Basal diet-Vitamin C, and BFPM as supplement respectively (designated T1, T2, T3, T4, and T5 respectively). The basal diet composition (kg) was as follows: Maize 30, Groundnut haulm 25, Groundnut cake 10, soybean meal 10, rice bran 15, Bone meal 9.2, common salt 0.35, mineral-vitamin premix 0.25, dl-methionine 0.1, and lysine 0.1. The basal diet was formulated to meet the nutrient requirements of growing rabbits according to the recommendations of [25]. The

calculated analysis of the nutrient composition of the basal diet is shown in Table 1. The water was offered ad libitum but changed daily in the morning. All recommended managerial practices were dully observed and the study lasted for 9 weeks.

Table 1. Calculated composition of the experimental diets

Nutrients	Composition
Metabolizable Energy (kcal/kg)	2300
Crude Protein (%)	17
Crude fiber (%)	14

2.4 Housing

In a naturally ventilated facility, the animals were housed in perforated metallic hutches of 75 X 75 X 75 cm and raised 80 cm above the floor level. Before the animals were transported, the hutches were properly scrubbed and disinfected using a locally manufactured disinfectant, then allowed to dry for one week. Each hutch had its own set of feed and watering troughs constructed of burnt clay. Individually tagged compartments were placed in which the rabbits were placed.

2.5 Meteorological Data of Rabbit Microclimate

The microclimate (ambient temperature and relative humidity values) within the rabbit house were recorded twice daily at 08:00 h and 15.00 h during the study period using a digital thermometer (Cocet, Shenzhen-Guangdong, China). The data collected was used to compute the temperature-humidity index (THI), an indicator of the thermal comfort level of the rabbits. The THI was calculated using the modified formula for the rabbit by Marai et al. [5] as follows: $THI = t - [(0.31 – 0.31 \times RH) (t – 14.4)]$

Where

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

The values of THI obtained were compared to that classified for tropical regions as shown below:

1) < 27.8 = Absence of heat stress, 2). 27.8 - 28.9 = Moderate heat stress, 3)28.9 – 30 = Severe heat stress and 4) above 30 = Very severe heat stress.

2.6 Measurement of Thermoregulatory Parameters

Parameters measured included rectal temperature (RT), ear temperature (ET), respiratory rate (RR), and heart rate (HR). Measurements were taken at 14.00 h to 15.00 h of the day. Rectal and ear temperature were measured with a digital thermometer. The ear temperature was measured by placing the digital thermometer in direct contact with the central area of the auricle. RR was measured by visually counting the flank movements for one minute with the help of a stopped clock. HR was measured by counting the heartbeat for one minute with the help of a stethoscope.

2.7 Serum Evaluation

Blood sampling was done at the beginning and the end of the experiment; the sampling was done at 10.00 h. Four rabbits were randomly selected from each treatment group and 5 ml of blood was collected from their ear veins into sample bottles without anticoagulants. The blood sample was allowed to clot and the serum was harvested after centrifuging the samples at 3000 rounds/minute for 15 minutes. The serum harvested was stored at -10^{o}C until when analyzed. Serum thyroxine and testosterone concentrations were determined by using commercially-available ELISA Kits (Diagnostic Procedure Corp., Los Angeles, CA; The USA) according to the manufacturer's instructions. Serum glucose, total protein, albumin, and cholesterol concentration were evaluated using an auto-analyzer and Chemical Commercial Kits from Stanbio Laboratory Inc. San Antonio, Texas, USA, according to the manufacturer's instruction. The detection ranges of the T_4 were 26-58.4 g/mg.

2.8 Statistical Analysis

Data obtained from the study were subjected to analysis of variance using the general linear model procedure of SAS [26]. Significant differences among treatment means were separated using the pair-wise difference (Pdiff) in the SAS package.

3. RESULTS AND DISCUSSION

3.1 Monthly Temperature-humidity Index (THI)

The monthly temperature-humidity index (THI) inside the rabbitry during the experimental period

is shown in Figure 1. THI in the mornings averaged 26.44^{o}C while the Afternoon THI averaged 28.74^{o}C. The graph also shows that the THI values kept increasing from the month of February with a peak in May. There was a decline in THI in the month of June. The THI value of 27^{o}C (Feb) indicated that there was no heat stress in the month of February, while the THI values of 28^{o}C (March), 29.5^{o}C (April), 31.2^{o}C (May), and 28^{o}C (June) are indications that the rabbit house was moderately thermally stressful, severely thermally stressful and very severely thermally stressful [5] in these months. The averaged THI 28.74^{o}C during the experimental period indicated that the rabbit house was thermally stressful and may have had adverse effects on the rabbits [5]. Overall data obtained indicated that THI in the afternoon was higher by 1.24 % than THI in the morning.

3.2 Thermoregulatory Response of Adult Rabbit Bucks

The result of the thermoregulatory response (Table 2) shows that Vitamin C and BFPM significantly ($P < 0.05$) reduced heart rate, rectal, and ear temperature in adult bucks compared to $KHCO_3$ and Na_2CO_3 or the control. Antioxidant vitamins were reported to be effective to alleviate heat load in rabbits [27,13-14]. The administration of ascorbic acid [28], glutathione supplementation [12], during exposure to high environmental temperatures reduces the body temperature in chickens. In heat-stressed sheep, selenium injection decreased rectal temperature and body weight loss [29]. The BFPM treatments gave the best performance, which agrees with the fact that the presence of phytochemical compounds in plants may facilitate the ability of animals to maintain their body homeostasis including body temperature by provoking endogenous cellular defense mechanisms to cope with oxidative stress and inflammation induced by heat stress [30]. $NaHCO_3$ could increase respiratory alkalosis severity [31], which may be responsible for the high values of heart rate, rectal and ear temperature recorded in rabbits treated with the buffers.

3.3 Serum Metabolites of Adult Rabbit Bucks

Serum metabolites of rabbits administered the antioxidants (Table 3) showed that triglyceride and phosphorous were significantly ($P<0.05$) different among the treatment groups. The final serum values in BFPM-treated rabbits significantly ($P < 0.05$) improved albumin; the control recorded significantly higher serum glucose while Na_2CO_3 significantly increased serum calcium level. The significant increase in final serum albumin in male rabbits administered vitamin C and BFPM, may be due to the fact that vitamins are essential parts of some of the enzymes or co-enzymes [32] and enzymes are important in food digestion, making micronutrients to be available for growth and other body functions. The significant increase in final serum calcium in the treatment with $NaHCO_3$ may be due to the increase in body metabolism and excitation caused by the buffers, thereby increasing blood calcium [32]. The trend in serum glucose recorded in this experiment agrees with the reports of Ondruska et al. [33]. It was attributed to being due to an increase in glucose utilization during muscular movements required for high respiratory activity [34,35], or due to increases in corticosteroid concentrations [36].

3.4 Thyroxine Levels in Adult Rabbit Bucks

The effect of buffer, vitamin C and BFPM on thyroxine levels of adult male rabbits (Fig. 2) revealed that initial thyroxine levels of the rabbits did not differ significantly when compared to those of the treatment groups. The control group and Na_2CO_3 group recorded the lowest thyroxine levels compared to the rest of the treatments. On termination of the experiment, the values of thyroxine obtained from the serum showed that vitamin C and BFPM increased significantly ($P<0.05$) thyroxine levels compared to other treatments, and the control group recorded the least value. The lower thyroxine levels recorded in the control reveal that the control group was heat-stressed and metabolic activities may have been lowered. Previous studies reported a decrease in thyroid hormones with increasing temperature [37,38], a decrease in T3 level, and a concurrent increase in T4 level [39] during heat stress. Yahav et al. [40] reported reduced concentrations of thyroid hormones (triiodothyronine and thyroxine) in the blood plasma and a decreased metabolic rate of pullets and cocks reared under a high ambient temperature. The reduction in thyroxine levels after the experiment compared to the initial values can be attributed to two factors; acclimation of the animals for the control and amelioration of heat stress by the buffers and the vitamin. The values are similar to the report of [41]. Acclimation increases the adaptive ability of

birds to subsequent thermal stress by reducing the level of triiodothyronine. The buffers especially $KCHO_3$, may have triggered the stimulation of the thyroid glands leading to the secretion of more thyroxine compared to the control while the exogenous antioxidant vitamin sources (vitamin C and BFPM) may have reduced oxidative stress and improved body metabolism, leading to the increase in serum thyroxine [42]. Vitamin C synthesized in rabbit liver has been demonstrated to protect the animal from heat stress and improve disease resistance in rabbits by optimizing the function of the immune system [43], but during stress vitamin C produced is rapidly consumed and the amount synthesized falls below animal requirements. Supplemental vitamin C may therefore reinstate normal metabolic functions during heat stress. Serum concentrations of T4 increased by increasing dietary VC or VE levels of heat-stressed Japanese quails [44] and rabbits [42]. Thyroid hormones are the key hormones in the regulation of metabolism and adaptation of animals to stress [45].

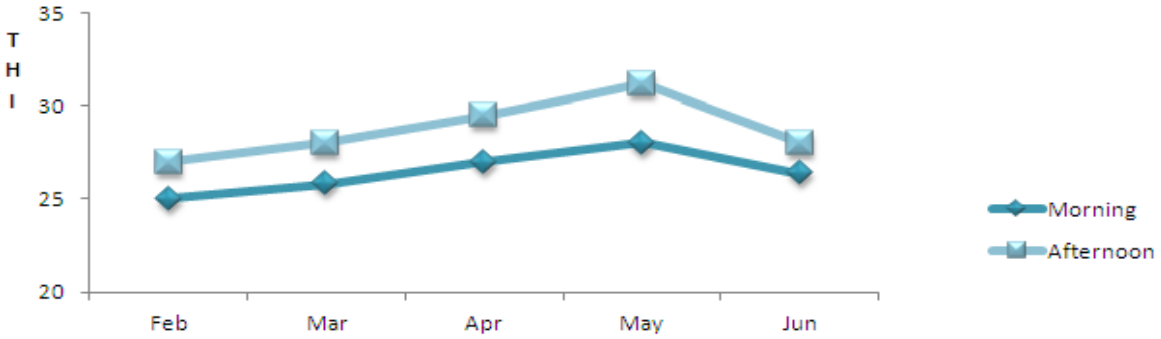

Fig. 1. Monthly Temperature Humidity Index of the Pen House

Table 2. Effect of Bicarbonate Buffers, Vit C and BFPM on Thermoregulatory Response of Adult Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Respiratory Rate (counts/min)	158.34	154.02	148.91	150.58	141.03	3.78
Heart Rate (beat/min)	141.03^{b}	143.23^{ab}	143.53^{a}	140.99^{b}	141.03^{ab}	0.67
Rectal Temperature (°C)	38.73^{a}	38.04^{a}	38.29^{a}	37.97^{ab}	37.04^{b}	0.28
Ear Temperature (°C)	36.79^{a}	35.59^{ab}	36.13^{ab}	35.23^{ab}	34.99^{b}	0.47

Means within rows with different superscripts are significantly different: P<=0.05
Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

Table 3. Effects of Bicarbonate Buffers, Vit C and BFPM on Serum Metabolite of Adult Rabbit Bucks

			Treatments			
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Initial						
Glucose (mg/dl)	4.17	5.03	4.12	4.60	4.70	0.33
Total Protein (mg/dl)	65.00	67.00	63.37	66.80	67.23	1.67
Albumin (mg/dl)	35.00	36.67	39.00	37.33	40.00	1.53
Cholesterol (mg/dl)	1.37	1.10	1.23	1.20	1.30	0.56
Triglyceride (mg/dl)	0.70^{b}	1.07^{ab}	0.98^{ab}	0.87^{ab}	1.17^{a}	0.09
Calcium (mg/dl)	2.38	2.32	2.37	2.38	2.32	0.06
Phosphorous (mg/dl)	0.87^{b}	1.15^{a}	1.07^{ab}	1.00^{ab}	1.00^{ab}	0.04
Final						
Glucose (mg/dl)	4.70^{a}	4.13^{b}	4.20^{b}	4.47^{b}	4.50^{b}	3.57
Total Protein (mg/dl)	64.00	64.00	67.33	69.33	70.67	1.58
Albumin (mg/dl)	33.67^{b}	32.67^{c}	33.33^{b}	38.66^{ab}	40.00^{a}	1.33

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Cholesterol (mg/dl)	1.50	1.37	1.38	1.55	1.53	0.07
Triglyceride (mg/dl)	2.90	1.33	1.27	1.27	1.37	0.71
Calcium (mg/dl)	2.30^{b}	2.29^{bc}	2.44^{a}	2.25^{c}	2.42^{ab}	0.03
Phosphorous (mg/dl)	1.00	1.07	0.90	1.00	1.06	0.04

Means within rows with different superscripts are significantly different: P<=0.05
Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

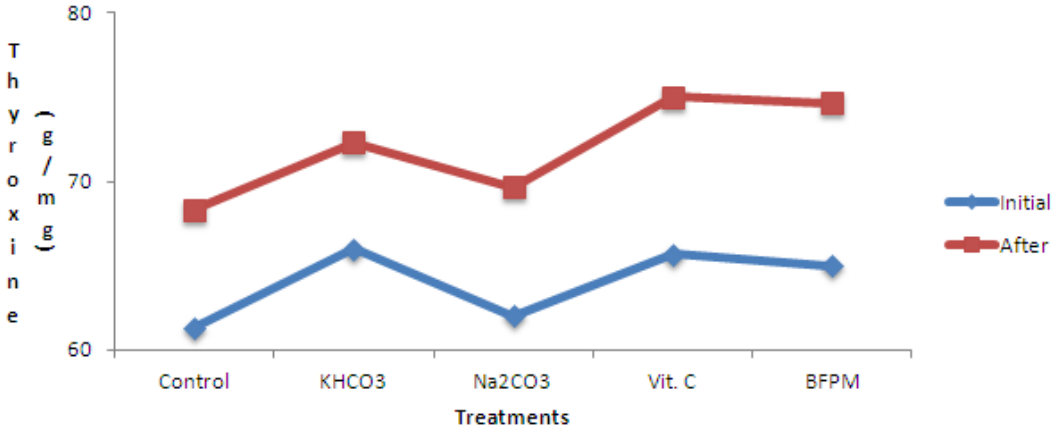

Fig. 2. Effects of Bicarbonate Buffers, Vit C and BFPM on Thyroxine Levels in Adult Rabbit Bucks

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

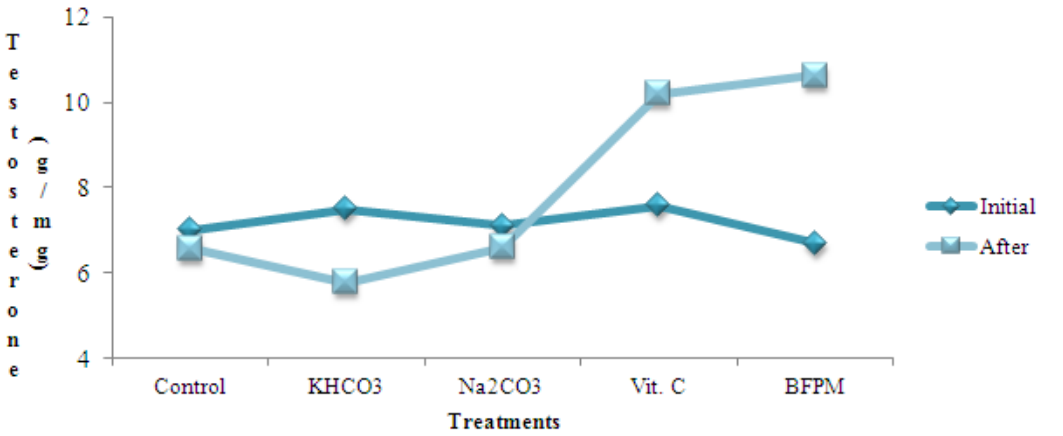

Fig. 3. Effects of Bicarbonate Buffers, Vit C and BFPM on Testosterone Levels in Adult Rabbit Bucks

Vit C = Vitamin C, BFPM = Baobab fruit pulp meal

3.5 Testosterone Levels in Adult Rabbit Bucks

The initial testosterone concentration of rabbits treated with buffer, vitamin C and BFPM in Fig. 3, did not show any significant difference among the treatments. It was observed that vitamin C and BFPM increased significantly ($P < 0.05$) testosterone concentration compared to the rest of the treatments whose values were lower than that recorded initially. The treatment with $KCHO_3$ buffer recorded significantly ($P < 0.05$) lower testosterone levels than the rest of the treatments. The final testosterone values of the control that was lower than the initial values may be attributed to heat stress, which might have affected the Leydig cells. Jelodar and Zare [46] reported a decrease in serum testosterone

concentration of rats exposed to radiation from phones; and attributed the decrease to the effect of radiation on Leydig cells, pituitary, or hypothalamus and alteration of gonadotropin secretion. It is also possible that the buffers, especially $KHO_{3,}$ also had a negative effect on the Leydig cells, responsible for the production of testosterone. Stress can also cause structural and pathological changes in the Leydig cells. Apoptosis associated with nuclear damage of the cells can lead to a decrease in testosterone and estrogen production [47]. It is also possible that the buffers interfered with the transfer of free cholesterol to mitochondria of Leydig cells, which is an important step in steroidogenesis, and also disrupted the conversion of cholesterol to testosterone by impairing the activity of key regulatory enzymes in steroidogenesis [48]. The significant improvement recorded in rabbits treated with vitamin C and BFPM could be the vitamins counteracted free radicals that may limit the normal functioning of the Leydig cells from functioning properly. Vitamin C has been reported to be an effective antioxidant in contracting free radicals especially during heat stress.

4. CONCLUSION

The vitamin antioxidants displayed superiority in ameliorating heat stress on most of the parameters that were evaluated compared to the bicarbonate buffer solution. Baobab fruit pulp meal treatments gave the best performance. The use of a plant source of vitamin C in rabbit diets is therefore encouraged and recommended to be during hot environmental conditions.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

ETHICAL APPROVAL

All authors hereby declare that the “procedures for care and handling of animals in this study were strictly followed in accordance with the code of ethics for animal experiments as stated inhtpp://ec.europa.eu/environment/chemicals/lab_animal/legislation_en.htm. Specific National laws were also followed. All experiments were examined and approved by the appropriate ethics committee”.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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