THE INFLUENCE OF SYNTHETIC AND NATURAL ANTIOXIDANTS ON THE PERFORMANCE OF HEAT-STRESSED RABBITS DOES REPRODUCTIVE FUNCTION IN A TROPICAL CLIMATE

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ABSTRACT

A total of thirty-six matured rabbit Does were used to study the impact of natural and synthetic antioxidants on the ability of heat-stressed rabbits to reproduce in a tropical climate. Using a completely randomized design, the rabbits were divided into four treatment groups, each consisting of 9 rabbits (6 Does and 3 bucks): (T1) control diet; (T2) diet supplemented with sodium bicarbonate; (T3) diet containing synthetic Vitamin C; and (T4) diet supplemented with Baobab fruit pulp meal (BFPM), as a natural antioxidant. The Does were served feed and water ad libitum. Five ml of blood samples were collected from the ear veins of rabbits chosen randomly from each treatment of rabbit treatments, respectively, before, during, and after pregnancy, for the evaluation of thyroxine hormone concentrations and serum metabolite. The rabbits' ability to reproduce was also evaluated. Thyroxine secretion and serum glucose increased significantly($p<0.05$) after kindling. The vitamin antioxidants improved the reproductive performance of the Does ($p<0.05$). The rectal temperature significantly ($p<0.05$) decreased while the serum metabolites of the kits increased in the BFPM treatments. Supplementing with BFPM, a naturally occurring antioxidant can help reduce stress by heat in reproductive Does. BFPM might be a part of a reproductive diet in the tropics for efficient reproduction.

Keywords: Antioxidants, Heat Stress, Hormones, Rabbit, Reproduction, Tropics

INTRODUCTION

Rabbit production has the potential to alleviate micronutrient and protein malnutrition while positively contributing to the sustainable increase in smallholder agriculture. Various factors such as genetics, nutrition, environment, and overall health influence rabbit breeding and reproduction. Previous studies reported that hyperthermia and heat stress significantly affected rabbits and their reproduction efficiency (Anoh *et al*., 2018). Cows exposed to heat stress after fertilization have been reported to have a decrease in the quality and number of embryos in cows (Hussin and Al-Taay, 2009; Luceno *et al*., 2020) and also a negative effect on intrauterine development in mice (Silva *et al*., 2024), and a decrease of thyroid stimulating hormone and thyroid hormones in lambs (Omidi *et al*., 2015), and variation in hormonal concentration at various gestation stages of black Bengal goat (Kumar *et al*., 2015). Heat stress has been reported to cause oxidative stress in animals through the excessive production of reactive oxygen species (ROS) or by lowering the potency of the antioxidant defences, including Vitamin C (Chauhan *et al.,* 2014; Yin *et al*., 2018). Increased free radicals in animals can damage reproductive cells, affecting fertility, embryo development, and overall reproductive success (Ruder *et al.*, 2009).

Thyroxine hormone concentration is an indicator of stress. Thyroxine levels in the serum of rabbits (Marai *et al.*, 2004), poultry (He *et al.*, 2018; Vandana *et al.*, 2021), as well as sheep (Todini, 2007), have been reported to be altered due to heat stress. Sivakumar *et al.* (2010) reported that thyroid hormone concentration decreased in heat-stressed goats, as a response to reducing the metabolic rate and heat production in goats exposed to antioxidants.

The use of antioxidants to alleviate heat stress in rabbits may be physiologically safer compared to other methods. Antioxidants can help reduce oxidative stress which is induced by heat stress by neutralizing free radicals, thereby protecting reproductive cells and supporting reproductive health. Supplementing rabbits' diets with antioxidants can positively impact reproductive parameters (Oloruntola *et al.*, 2015; Anoh *et al.*, 2017) including litter size. Vitamin C can dissolve in blood and cytosol and can act quickly before cell damage occurs. Sodium bicarbonate (Na_2HCO_3) was found to improve oxidative stress and heat tolerance by immune modulation (Peng *et al.*, 2013). Increase performances of heat-stressed poultry birds and rabbits treated with sodium bicarbonate have been reported in growth (Ahmad *et al.*, 2005; Khattak *et al.*, 2012; Peng *et al.*, 2013), egg quality (Kaya *et al.*, 2004; Jiang *et al.*, 2015) and improved blood profile (Kurtoglu *et al.*, 2007).

There is a prospect for the use of organic products in livestock production because synthetic products may be hazardous. This inspired our interest in the use of organic products for rabbit production, that are viable and physiologically safe. Plants and their parts have served as phytobiotics and antioxidants in livestock production (Dhama *et al.*, 2015; Valenzuela-Grijalva *et al.*, 2017) because of the presence of Vitamin C and other phytochemicals in them (Anoh *et al.,* 2018; 2022). Some phytochemicals in plants improve antioxidant, anti-microbial, feed flavour, and palatability, which can increase feed intake and animal performance (Valenzuela-Grijalva *et al.*, 2017). These tropical plants are available because of their rapid growth which is enhanced by favourable climatic and environmental factors. Baobab has been well described by Asogwa *et al.* (2021). The fruit pulp meal contains high amounts of Vitamin C (Stadlmayr *et al.*, 2020), can increase growth performance, and alleviates heat stress in rabbits (Anoh *et al.*, 2017).

This study evaluated the influence of synthetic and natural antioxidants on the performance of heat-stressed rabbits Does reproductive function in Nigeria, a country in the tropics.

MATERIAL AND METHODS

Study Location: This study was carried out at the National Animal Production Research Institute (NAPRI), Shika, Zaria, Nigeria (11° 12′ 42″ N and 7° 33′ 14″ E) at an altitude of about 691 m above sea level (Achi *et al.*, 2021). Zaria has the following annual average conditions: rainfall of 1100 ± 50 mm, which starts from late April and early May to mid-October, temperature of 37 ± 5 ^{0}C, and relative humidity (RH) of 75 ± 5%.

Housing: The rabbits were raised in perforated metallic cages measuring 75 x 75 x 75 cm^3, raised 85 cm above the ground. The hutches were properly cleansed using a disinfectant manufactured locally; and left to dry for a week before the rabbits were introduced. Each hutch had feeding and watering troughs constructed of burnt clay. Each rabbit was placed in its cell with a legible label.

Chemicals, Drugs and their Preparation: The sodium bicarbonate and the carbonate anhydrous salts were purchased from Mike-Ventures equipment and chemicals stores in Samaru-Zaria Nigeria. The Vitamin C used in this study was the feed grade Vitamin C, it was purchased from a feed additive vendor in Samaru-Zaria Nigeria. The buffer solution was produced according to the methods of (Mohan, 2003).

Baobab Fruit Pulp Meal: Baobab fruit pulp meal is a white, powdery substance obtained from the endocarp of Baobab. The pulp completely dry at maturity is obtained from the endocarp of the Baobab and after that crushed and sieved to produce the powdery substance. Two hundred grams (200 g) of the powdery Baobab were included in a kg of feed in this study.

Experimental Animals, Design, and Diets: A total of 36 New Zealand White Cross adult rabbits with an average weight of 2600 ± 32 g were used in this study. Using a completely randomized design, the rabbits were randomly distributed into the four treatment groups, replicated thrice with each replicate having three rabbits (two Does and one Buck), totalling nine rabbits (six Does and three Bucks) per treatment group. Group A - control rabbits were fed the standard diet and water only, Group B – rabbits were given the standard diet and sodium bicarbonate buffer, Group C – rabbits were given 250 g/kg of Vitamin C mixed in the standard diet, and Group D – rabbits were fed 200 g/kg of BFPM mixed in the diet. All rabbits in the different treatments were fed their respective diets and water *ad libitum.* The standard diet used was commercial feed (Vital Feed Broiler Starter, 18.00 ± 0.50 g/100g crude protein, and 2106.00 kcal/kg metabolizable energy, Vital Feed, Grand Cereals Limited, Jos, Plateau State, Nigeria). The sodium bicarbonate ($NaHCO_3$) solution and water were changed daily in the morning. The study lasted for 20 weeks (February 21 - June 30, 2024).

Estimation of the Temperature-Humidity Index: The readings of the micro-climate (temperature and relative humidity) of the pen were taken at 08:00 and 15.00 hours daily during the study period, from February through June. The temperature of the pens was measured using a Cocet Digital Thermometer Iris (KFT-04 Hard Head) (Cocet, Shenzhen, Guangdong, China). The humidity of the rabbit pens was measured using a 736710 Sling Psychrometer, Sper Scientific, USA. A modified version of the temperature-humidity index (THI) for rabbits, which serves as a measurement of the animals' degree of thermal comfort in the enclosures: $THI = t - [(0.31 - 0.31 \times RH) (t - 14.4)]$ was used to determine the THI of the pen. The values of THI obtained for the tropical regions are classified as < 27.8°C = absence of heat stress, 27.8 – 28.9°C = moderate, 29.0 – 30°C = severe, and > 30°C = highly severe (Asemota *et al.*, 2017).

Determination of Thyroxine (T4) Concentration and Serum Metabolites: Blood samples (5 ml) were collected from the ear vein of the Does, before (24 hours before mating), during (14^{th} day of pregnancy), and after gestation (24 hours after kindling); and from the three weeks old kittens for serum metabolite evaluation. The samples were collected into a tube without anticoagulant and allowed to clot and then centrifuged at over 2500 rpm for 14 minutes. The sera obtained was stored at -12°C pending use for assay of the thyroxine and serum metabolite levels. The serum metabolites assayed for in this study (glucose, total protein, albumin, and cholesterol concentrations) were evaluated using an auto-analyzer and specific biochemical commercial test kits that were purchased from Stanbio Laboratory Incorporated, USA whereas the thyroxine concentration was determined for the Does and Kittens using ELISA kits (thyroxine) obtained from Liaison, Byk-Sangtec Diagnostica, Dietzenbach, Germany according to the manufacturer's instructions. The sensitivity of this assay, or Lower Limit of Detection (LLD) was set at 10 ng/ml.

The Reproductive Performance of Rabbits Does: Mating was done after four weeks after the start of the study. Does were brought to the buck for mating at a ratio of 1 buck: 2 Does/replicate. 7^{th}-day weight increment and abdominal palpation were used to confirm pregnancy. Does who were not pregnant were re-serviced. The parameters evaluated included the date of the Kindle, litter size, litter weight, kit weight, and kit weight at weaning (Di Meo *et al.*, 2004). Data were obtained from two parities and the interval between these two parities was four weeks. The experiment lasted for 20 weeks.

Physiological Performance of Kits: The evaluation rectal temperature (RT) and heart rate (HR) of the kits were measured when they were 3 weeks old. The readings were taken between 14.00 15.00 hours twice a week for 3 weeks. A digital thermometer was used to measure the rectal temperature, while the heart rate (HR) was determined by counting the heartbeat of each rabbit in their respective treatments for one minute with the help of a stethoscope. Weekly weight (g) gain was also determined for 6 weeks from the first day of kindling using a digital weighing balance.

Statistical Analysis: Data from the study were subjected to a one-way analysis of variance (ANOVA) using the general linear model (SAS, 2002). Least Square Difference (LSD) was used to separate the differences in significant means at $p<0.05$. The following model was used: $Yij = \mu + Ti + Eij$, where Yij is the observed value, μ, is the overall mean value, Ti is the random effect of the natural and synthetic antioxidants, and Eij is the random error.

RESULTS

Temperature Humidity Index of the Rabbit House: The monthly THI inside the rabbit house during the experimental period is shown in Figure 1. THI in the mornings averaged 26.44 ± 1.03°C while in the afternoon THI averaged 28.74 ± 1.63°C. This graph also shows that the THI values kept increasing from February with a peak in May. There was a decline in THI in June 2024.

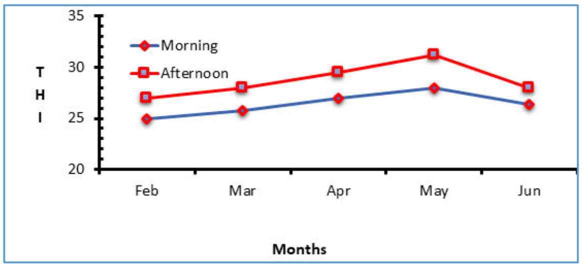

Figure 1: Monthly temperature-humidity index of the rabbit pens

Thyroxine Concentration of Does: The thyroxine concentrations of the Does obtained 24 hours before mating (Table 1) were generally low and were not significant amongst the treatment group. During pregnancy (14th day of pregnancy), the rabbits fed Vitamin C and BFPM significantly ($p<0.05$) increased thyroxine concentration compared to the buffer. The values were slightly above the values that were recorded 24 hours before mating. Thyroxine concentration was significantly ($p<0.05$) high in the rabbits fed Vitamin C and BFPM diet.

Serum Metabolite of Does: The effect of buffers, Vitamin C and BFPM on serum metabolites of the Does (Table 2) shows that apart from calcium, the initial serum metabolites like glucose, total protein, albumin, cholesterol, triglyceride, and phosphorous did not show any significant difference across the treatments. During pregnancy, the Does administered Na_2CO_3 had significantly increased ($p<0.05$) phosphorous, compared to the control, Vitamin C and BFPM treatments. The values of albumin and triglycerides were statistically similar ($p>0.05$) across the treatments. After kindling, the values of the serum metabolites did not follow any particular pattern. Serum glucose and total protein respectively, were significantly increased ($p<0.05$) in the Vitamin C and BFPM diets. Albumin, phosphorous as well as triglyceride, and calcium did not show any significant difference ($p>0.05$). It was also observed that most of the serum metabolites were reduced during pregnancy compared to before and after pregnancy.

Reproductive Performance of Does: There was a significant increase ($p<0.05$) in the average weight of litter size at birth (Table 3) in Does fed Vitamin C and BFPM diets. The BFPM diet significantly increased ($p<0.05$) litter size and weight at weaning compared to the Na_2CO_3 and Vitamin C treatments but similar to the control group. However, Na_2CO_3 treatment showed a significantly lower ($p<0.05$) average litter size at birth but a higher average weight of kits than the rest of the groups.

Physiological Performance of Kits: The result of the physiological performance of the kits (Table 4) shows that rectal and ear temperatures were significantly decreased ($p<0.05$) by the antioxidants. The values of the rectal temperature were however lower in the Vitamin C and BFPM treatments than kits administered Na_2CO_3. The Na_2CO_3 buffer increased heart rate (139.94 ± 0.56) more than the groups treated with Vitamin C (135.75 ± 0.56) and BFPM (136.10 ± 0.56), but was lower than the control (140.44 ± 0.56).

Table 1: Thyroxine concentration of heat-stressed does treated with synthetic and natural antioxidants in a tropical climate

Parameters	Group A (Control)	Group B (Na_2CO_3)	Group C (Vitamin C)	Group D (BFPM)
	Thyroxine concentration (ng/ml)			
Initial (24 hours before mating)	56.00 ± 1.20^{a}	55.33± 1.20^{a}	62.67 ± 1.20^{b}	64.67 ± 1.20^{b}
During pregnancy (14th Day)	66.67 ± 0.57^{c}	68.33 ± 0.57^{bc}	76.35 ± 0.57^{ab}	82.42 ± 0.57^{a}
After (24 hours after kindling)	68.50 ± 0.43^{b}	70.00 ± 0.43^{a}	70.33 ± 0.43^{a}	71.00 ± 0.43^{a}

abc = Means within a row with different superscript letters are significantly different (p<0.05), BFPM = Baobab fruit pulp meal, Na_2CO_3 = Sodium bicarbonate buffer

Table 2: Serum metabolites of heat-stressed does treated with synthetic and natural antioxidants in a tropical climate

Parameters	Group A (Control)	Group B (Na_2CO_3)	Group C (Vitamin C)	Group D (BFPM)
Before pregnancy (24 hours before mating)				
Glucose (mg/dl)	4.77 ± 0.08	4.63 ± 0.08	4.47 ± 0.08	5.0 ± 0.08
Total protein (mg/dl)	63.63 ± 0.39	66.00 ± 0.39	66.67± 0.39	67.00 ± 0.39
Albumin (mg/dl)	35.67 ± 0.20	37.00 ± 0.20	37.33 ± 0.20	36.00 ± 0.20
Cholesterol (mg/dl)	1.60 ± 0.02	1.60 ± 0.02	1.43 ± 0.02	1.30 ± 0.02
Triglyceride (mg/dl)	0.93 ± 0.02	0.86 ± 0.02	0.83 ± 0.02	0.87 ± 0.02
Calcium	2.34 ± 0.02^{ab}	2.21 ± 0.02^{a}	2.39 ± 0.02^{b}	2.34 ± 0.02^{ab}
Phosphorous	1.05 ± 0.02	1.00 ± 0.02	1.10 ± 0.02	1.11 ± 0.02
During gestation (14th day of pregnancy)				
Glucose (mg/dl)	4.30 ± 0.05	4.47 ± 0.05	5.00 ± 0.05	5.22 ± 0.05
Total protein (mg/dl)	61.67 ± 0.37	64.34 ± 0.37	64.00 ± 0.37	63.33 ± 0.37
Albumin (mg/dl)	37.33 ± 0.28	39.00 ± 0.28	38.08 ± 0.28	41.17 ± 0.28
Cholesterol (mg/dl)	1.70 ± 0.02	1.63 ± 0.02	1.57 ± 0.02	1.57 ± 0.02
Triglyceride (mg/dl)	1.03 ± 0.03	1.43 ± 0.03	1.20 ± 0.03	1.30 ± 0.03
Calcium	2.28 ± 0.01	2.36 ± 0.01	2.41 ± 0.01	2.35 ± 0.01
Phosphorous	0.95 ± 0.02	0.52 ± 0.02	1.14 ± 0.02	1.17 ± 0.02
After kindling (24 hours after kindling)				
Glucose (mg/dl)	4.13 ± 0.04^{a}	4.57 ± 0.04^{ab}	4.73 ± 0.04^{b}	5.27 ± 0.04^{c}
Total protein (mg/dl)	67.33 ± 0.20^{ab}	68.00 ± 0.20^{b}	69.33 ± 0.20^{c}	64.33 ± 0.20^{a}
Albumin (mg/dl)	37.17 ± 0.15	40.35 ± 0.15	41.33 ± 0.15	38.00 ± 0.15
Cholesterol (mg/dl)	1.46 ± 0.01	1.47 ± 0.01	1.47 ± 0.01	1.37 ± 0.01
Triglyceride (mg/dl)	0.87 ± 0.02	0.86 ± 0.02	0.77 ± 0.02	0.92 ± 0.02
Calcium	2.43 ± 0.01	2.21 ± 0.01	2.34 ± 0.01	2.19 ± 0.01
Phosphorous	1.01 ± 0.01	1.08 ± 0.01	1.07 ± 0.01	1.09 ± 0.01

abc = Means within a row with different superscript letters are significantly different (p<0.05), BFPM = Baobab fruit pulp meal, Na_2CO_3 = Sodium bicarbonate buffer

Table 3: Reproductive performance of heat-stressed does treated with synthetic and natural antioxidants in a tropical climate

Parameters	Group A (Control)	Group B (Na_2CO_3)	Group C (Vitamin C)	Group D (BFPM)
Number of Does	6	6	6	6
Average litter size at birth	5.67 ± 0.06^{b}	4.00 ± 0.06^{a}	6.00 ± 0.06^{c}	6.00 ± 0.06^{c}
Average weight of litter (g)	236.67 ± 1.67^{b}	180.00 ± 1.67^{a}	200.00 ± 1.67^{ab}	200.00 ± 1.67^{ab}
Average weight of kit (g)	41.78 ± 0.02^{ab}	45.00 ± 0.02^{b}	40.00 ± 0.02^{a}	40.00 ± 0.02^{a}
Average litter size at weaning	4.00 ± 0.07^{bc}	2.67 ± 0.07^{a}	3.67 ± 0.07^{b}	4.67 ± 0.07^{c}
Average weight at weaning (g)	500.00 ± 3.08^{a}	550.00 ± 3.08^{ab}	540.00 ± 3.08^{ab}	600.45 ± 3.08^{b}

abc = Means within a row with different superscript letters are significantly different (p<0.05), BFPM = Baobab fruit pulp meal, Na_2CO_3 = Sodium bicarbonate buffer

Table 4: Physiological performance of kittens from heat-stressed does treated with synthetic and natural antioxidants in a tropical climate

Parameters	Group A (Control)	Group B (Na_2CO_3)	Group C (Vitamin C)	Group D (BFPM)
Rectal temperature (°C)	38.51 ± 0.04^{b}	37.62b ± 0.04^{ab}	37.20 ± 0.04^{ab}	36.90 ± 0.04^{a}
Heart rate (beat/min)	140.44 ± 0.56^{b}	139.94 ± 0.56^{b}	135.75 ± 0.56^{a}	136.10 ± 0.56^{a}
Thyroxine levels (ng/ml)	66.10 ± 0.5	66.78 ± 0.5	65.00 ± 0.5	65.34 ± 0.5
Weight gain (g/day)	11.04 ± 0.24	11.68 ± 0.24	12.86 ± 0.24	12.12 ± 0.24

abc = Means within a row with different superscript letters are significantly different (p<0.05), BFPM = Baobab fruit pulp meal, Na_2CO_3 = Sodium bicarbonate buffer

The values of thyroxine obtained from the serum show that Vitamin C significantly reduced (p<0.05) the thyroxine levels compared to other treatments, with the buffer treatments recording the highest values. Daily weight gain did not show any significant difference.

Serum Metabolite of the Newly Weaned Rabbits: The serum metabolite of the newly weaned rabbits (Table 5) did not show any significant difference (p>0.05).

Table 5: Serum metabolite of the newly weaned rabbits from heat-stressed Does treated with synthetic and natural antioxidants in a tropical climate

Parameters	Group A (Control)	Group B (Na_2CO_3)
Glucose (mg/dl)	4.50 ± 0.04	4.60 ± 0.04
Total Protein (mg/dl)	69.67 ± 0.37	67.00 ± 0.37
Albumin (mg/dl)	34.50 ± 0.35	34.74 ± 0.35
Cholesterol (mg/dl)	1.00 ± 0.01	1.24 ± 0.01
Triglyceride (mg/dl)	1.00 ± 0.02	1.00 ± 0.02
Calcium	2.00 ± 0.01	2.20 ± 0.01
Phosphorous	1.21 ± 0.01	1.29 ± 0.01
	Group C (Vitamin C)	**Group D (BFPM)**
Glucose (mg/dl)	4.80 ± 0.04	5.03 ± 0.04
Total Protein (mg/dl)	69.57 ± 0.37	69.67 ± 0.37
Albumin (mg/dl)	36.43 ± 0.35	36.50 ± 0.35
Cholesterol (mg/dl)	1.33 ± 0.01	1.27 ± 0.01
Triglyceride (mg/dl)	1.10 ± 0.02	1.20 ± 0.02
Calcium	2.43 ± 0.01	2.31 ± 0.01
Phosphorous	1.18 ± 0.01	1.11 ± 0.01

abc = Means within a row with different superscript letters are significantly different (p<0.05), BFPM = Baobab fruit pulp meal, Na_2CO_3 = Sodium bicarbonate buffer

DISCUSSION

Temperature Humidity Index: The average value of THI (28.74 ± 1.63°C) during the experimental period indicated that the rabbit house was thermally stressful. Marai *et al.* (2001) had previously reported that this value may adversely affect the rabbits' performance. The values of THI obtained at 14 – 15 hours were higher by 1.23% than THI values in the morning.

Thyroxine Concentration of Does: In the present study, thyroxine was evaluated as a stress indicator. Thyroid hormones are the key hormones involved in regulating metabolism and in the adaption of animals to stress (Brecchia *et al.*, 2010). The reason, for the increase in thyroxine value during pregnancy, may be due to the importance of the hormone in body metabolism, leading to an increase in serum protein, fat, and carbohydrates to fulfil the high demand of the foetus from glucose, amino acids, vitamins, and other nutrients (Brecchia *et al.*, 2010). Thyroxine is considered necessary for cellular metabolism of the mammary gland and energy utilization which is an important factor in milk biosynthesis. The noted significant decrease in thyroxine levels in the control and concurrent increase in the antioxidant treatments after kindling is similar to the reported by Liang *et al.* (2022). Thyroxine hormone decreased significantly in growing male rabbits by about 16% when the rabbits were exposed to 35°C for six hours daily (Liang *et al.,* 2022). BFPM was reported to be high in Vitamin C this antioxidant property is known to reduce oxidative stress and improve body metabolism, leading to an increase in serum thyroxine (Lin *et al.*, 2004). Hypothyroidism is related to oxidative stress and cellular damage (Cano-Europa *et al.*, 2012). Thyroxine concentration increases when the quantity of Vitamin C or E in Japanese quails reared under heat stress increases (Sahin *et al.*, 2002).

Serum Metabolites of Does: The increase in serum glucose after pregnancy is contrary to previous reports on cattle (Abbas *et al.*, 2020), rabbits (Marai *et al.*, 2007), and quails Sahin *et al.* (2002). Liang *et al.* (2022) reported a decrease in glucose during pregnancy. This was attributed to the reduction in feed intake of dams, an increase in water retention, and the high demand for the foetus at late stages of pregnancy. In the present study, the vitamin antioxidants (Vitamin C and BFPM) significantly increase glucose and protein levels after kindling in rabbits probably because of their suppressive effect on heat stress by counteracting the free radical species (Liang *et al.,* 2022) and stimulating the peripheral thermal receptors by the environmental temperature to transmit suppressive nerve impulses to the appetite centre in the hypothalamus to trigger appetite (Guyton and Hall, 2006) leading to increased feed intake. This phenomenon can lead to increased serum glucose, albumin, and triglyceride.

Reproductive Performance of Does: The improvement in reproductive performance of rabbits in BFPM and Vitamin C treatments was similar to previous reports by Fayeye and Ayorinde (2016) which might be due to the protective action of the vitamin antioxidants against lipid oxidation in the cell membrane (Castellini *et al.*, 2003). The vitamin antioxidants were important for young animals because they exhibit a greater sensitivity to oxidative damage than adults, and for developing the immune system in young animals (Debier *et al.*, 2005). Antioxidants can help reduce oxidative stress induced by heat stress by neutralizing free radicals, thereby protecting reproductive cells and supporting reproductive health (Sivakumar *et al.*, 2010; Ganaie *et al.*, 2012). Antioxidants like Vitamin C, vitamin E, selenium, and beta-carotene have been considered for their possible benefits in improving reproductive outcomes in various animal species including rabbits 24 hours after kindling (Xiao *et al.*, 2021). Thyroxine concentration was similar in the Na_2CO_3 treatment on Day 14th and higher 24h after kindling regarding the control group.

Physiological Performance of the Kits: The rectal temperature of the newly weaned rabbits that were exposed to a high environmental temperature beyond the thermoneutral zone of the rabbits did not increase (Marai *et al.*, 2001). This may be a result of their poorly developed adipose tissues. A well-developed adipose tissue has a way of conserving heat which can lead to an increase in heat load by the rabbits when exposed to high temperatures. Even though the rectal temperature of the weaned rabbits was within their thermoneutral zone, the BFPM-treated groups recorded a significantly low rectal temperature and heart rate compared to the rest of the treatment groups. This finding is in agrees with the reports of Qota *et al.* (2008) and Daader *et al.* (2018). Dietary supplementation with mineral and vitamin antioxidants was effective for partial amelioration of the effects of heat stress on performance in growing chicks and rabbits respectively.

Conclusion: Heat stress unfavourably affected thyroxine secretion and reproductive performance of Does. The Na_2CO_3 buffer may reduce reproductive performance in rabbits, whereas the vitamins especially the organic BFPM improved thyroxine concentrations during pregnancy and after kindling impacts positively in rectal temperature, some serum metabolites, and kits weight at weaning. BFPM may be recommended to be used in rabbit diets during hot conditions.

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REFERENCES

ABBAS, Z., SAMMAD, A., HU, L., FANG, H., XU, Q. and WANG, Y. (2020). Glucose metabolism and dynamics of facilitative glucose transporters (GLUTS) under the influence of heat stress in dairy cattle. *Metabolites*, 10(8): 312. https://doi.org/10.3390/metabo10080312

ACHI, J. N., ACHI, N. P., BARJE, P. P., MALLAM, I., WAZIRI, D. L. and MAKAMA, R. S. (2021). Thermoregulatory and growth performance of weaner rabbits fed diets supplemented with brewers dried grain. *Nigerian Journal of Animal Science*, 23(2): 75 – 82.

AHMAD, T., SARWAR, M., NISA, M., HAQ, A. and HASAN, Z., (2005). Influence of varying sources of dietary electrolytes on the performance of broilers reared in a high temperature environment. *Animal Feed Science and Technology*, 120: 277 – 298.

ANOH, K. U., BARJE, P. P., IYEGHE-ERAKPOTOBOR, G. I. and AKPA, G. N. (2017). Influence of Baobab (*Adansonia digitata*) fruit pulp meal on semen characteristics and morphology of rabbit buck during hot season in Nigeria. *Nigerian Journal of Animal Production*, 44(3): 84 – 88.

ANOH, K. U., OLAYINKA, O. I., ABDULAHI, I. and ATTE, P. O. (2018). Ameliorative effects of Bi-carbonate buffer, Vitamin C and Baobab fruit pulp meal on progesterone and testosterone secretion in heat-stressed rabbits. *Nigerian Journal of Animal Science*, 20(4): 459 – 465.

ANOH, K. U., PAUL, J. N. and AMU, U. M. (2022). Effect of Supplementation of phyto-antioxidant (Baobab fruit pulp meal) on reproductive performance of rabbit Does during heat stress. *Online Journal of Animal and Feed Research*, 12(2): 89 – 96.

ASEMOTA, O. D., ADUBA, P., BELLO-ONAGHISE, G. and ORHERUATA, A. M. (2017). Effect of temperature-humidity index (THI) on the performance of rabbits (*Oryctolagus cuniculus*) in the humid tropics. *Archivos de Zootecnia*, 66(254): 257 – 261.

ASOGWA, I. S., IBRAHIM, A. N. and AGBAKA, J. I. (2021). African Baobab: Its role in enhancing nutrition, health, and the environment. *Trees, Forests and People*, 3: 100043. https://doi.org/10.1016/j.tfp.2020.100043

BRECCHIA, G., ZERANI, M., BONANO, A. and BOITI, C. (2010). Metabolic adaptations in neonatal mother-deprived rabbits. *World Rabbit Science*, 18(4): 229 – 236.

CANO-EUROPA, B. V. V., MARGARITA, F. C. and ROCIO, O. B. (2012). The relationship between thyroid states, oxidative stress and cellular damage. Pages 413 – 436. *In:* LUSHCHAK, V. (Ed.). *Oxidative Stress and Diseases*. InTechOpen, London. https://doi.org/10.5772/35082

CASTELLINI, C., LATTAIOLI, P., DAL BOSCO, A., MINELLI, A. and MUGNAI, C. (2003). Oxidative status and semen characteristics of rabbit buck as affected by dietary. *Reproduction Nutrition Development*, 43(1): 91 – 103.

CHAUHAN, S. S., CELI, P., LEURY, B. J., CLARKE, I. J. and DUNSHEA, F. R. (2014). Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *Journal of Animal Science*, 92(8): 3364 – 3374.

DAADER, A. H., AL SAGHEER, A. A., GABR, H. A. and ABD EL MONIEM, E. A. (2018). Alleviation of heat-stress-related physiological perturbations in growing rabbits using natural antioxidants. *Spanish Journal of Agricultural Research*, 16(3): e0610. https://doi.org/10.5424/sjar/2018163-13184

DEBIER, C., POTTIER, J., GOFFE, C. H. and LARONDELLE, Y. (2005). Present knowledge and unexpected behaviours of vitamins A and E in colostrum and milk. *Livestock Production Science*, 98(1-2): 135 – 147.

DI MEO, C., GAZANEO, M. P., RACCA, C., BOVERA, F., PICCOLO, G. and NIZZA, A. (2004). Effect of birth weight and litter size on productive performance of rabbits. *Asian-Australasian Journal of Animal Sciences*, 17(8): 1158 – 1161.

DHAMA, K., LATHEEF, S. K., MANI, S., SAMAD, H. A., KARTHIK, K., TIWARI, R., KHAN, R. U., ALAGAWANY, M., FARAG, M. R., ALAM, G. M. and LAUDADIO, V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and production - a review. *International Journal of Pharmacology*, 11(3): 152 – 176.

FAYEYE, T. R. and AYORINDE, K. L. (2016). Gestation length, litter size at birth and their effects on gestation weight gain, kindling loss, live body weight of kit and survival in domestic rabbit in Nigeria. Pages 201 – 204. *In; Proceedings 11th World Rabbit Congress.* Qingdao, China. June 15 – 18, 2016.

GANAIE, A. H., HOODA, O. K., SINGH, S. V. and UPADHYAY, R. C. (2012). Responses of Vitamin C supplementation on biochemical, hormonal and physiological parameters of pregnant Murrah buffaloes during hot-humid conditions. *Indian Journal of Animal Nutrition*, 29(3): 214 – 221.

GUYTON, A. and HALL, J. (2006). *Textbook of Medical Physiology*, 11th Edition, Saunders, Elsevier, Philadelphia, USA.

HE, S. P., AROWOLO, M. A., MEDRANO, R. F., LI, S., YU, Q. F., CHEN, J. Y. and HE, J. H. (2018). Impact of heat stress and nutritional interventions on poultry production. *World's Poultry Science Journal*, 74(4): 647 – 664.

HUSSIN, A. M. and AL-TAAY, M. M. (2009). Histological study of the thyroid and parathyroid glands in Iraqi buffalo "*Bubalus bubalis*" with referring to the seasonal changes. *Basrah Journal of Veterinary Research*, 8(1): 26 – 38.

JIANG, M. J., ZHAO, J. P., JIAO, H. C., WANG, X. J., ZHANG, Q. and LIN, H. (2015). Dietary supplementation with sodium bicarbonate improves calcium absorption and eggshell quality of laying hens during peak production. *British Poultry Science*, 56(6): 740 – 747.

KAYA, I., KARADEMIR, B. and UCAR, O. (2004). The effects of diet supplemented with sodium bicarbonate upon blood pH, blood gases and eggshell quality in laying geese. *Veterinární Medicína*, 49(6): 201 – 206.

KHATTAK, F. M., ACAMOVIC, T., SPARKS, N., PASHA, T. N., JOIYA, M. H., HAYAT, Z. and ALI, Z. (2012). Comparative efficacy of different supplements used to reduce heat stress in broilers. *Pakistan Journal of Zoology*, 44(1): 31 – 41.

KUMAR, B., ISHWAR, A. K., CHOUDHARY, P. K. and AKHATAR, T. (2015). Effect of temperature variation on hormonal concentration at various gestation stages in black Bengal goat. *Veterinary World*, 8(9): 1137 – 1142.

KURTOGLU, V., KURTOGLU, F. and BALEVI, T. (2007). Effects of sodium bicarbonate, potassium chloride and sodium chloride supplementation on some blood biochemical parameters in laying hens. Pages 189 – 192. *In: Proceedings of the 16th European Symposium on Poultry Nutrition*, August 26 – 30, 2007, Strasbourg, France.

LIANG, Z. L., CHEN, F., PARK, S., BALASUBRAMANIAN, B. and LIU, W. C. (2022). Impacts of heat stress on rabbit immune function, endocrine, blood biochemical changes, antioxidant capacity and production performance, and the potential mitigation strategies of nutritional intervention. *Frontiers in Veterinary Science*, 9: 906084. https://doi.org/10.3389/fvets.2022.906084

LIN, H., MALHEIROS, R. D., MORAES, V. M. B., CAREGHI, C., DECUYPERE, E. and BUYSE, J. (2004). Acclimation of broiler chickens to chronic high environmental temperature. *Archiv für Geflügelkunde,* 68(1): 39 – 46.

LUCENO, N. L., ANGRIMANI, D. D. S. R., DE CÁSSIA BICUDO, L., SZYMAŃSKA, K. J., VAN POUCKE, M., DEMEYERE, K., MEYER, E., PEELMAN, L., MULLAART, E., BROEKHUIJSE, M. L. and VAN SOOM, A. (2020). Exposing dairy bulls to high temperature-humidity index during spermatogenesis compromises subsequent embryo development in vitro. *Theriogenology*, 141: 16 – 25.

MARAI, I. F. M., AYYAT, M. S. and ABD EL-MONEM, U. M. (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Tropical Animal Health and Production*, 33: 451 – 462.

MARAI, I. F. M., HABEEB, A. M. and GAD, A. E. (2004). Reproductive traits of female rabbits as affected by heat stress and lighting regime under subtropical conditions of Egypt. *Animal Science*, 78(1): 119 – 127.

MARAI, I. F. M., HAEEB, A. A. M. and GAD, A. E. (2007). Biological functions in young pregnant rabbit Does as affected by heat stress and lighting regime under subtropical conditions of Egypt. *Tropical and Subtropical Agroecosystems*, 7(3): 165 – 176.

MOHAN, C. (2003). *Buffers a Guide for the Preparation and Use of Buffers in Biological Systems.* Calbiochem, EMD, Merck KGaA, Darmstadt, Germany.

OLORUNTOLA, O. D., DARAMOLA, O. T. and OMONIYI, S. O. (2015). Effect of forages on performance, carcass cuts and haematological profile of weaner rabbits. *Archivos de Zootecnia*, 64(245): 87 – 92.

OMIDI, A., KHEIRIE, M. and SARIR, H. (2015). Impact of Vitamin C on concentrations of thyroid stimulating hormone and thyroid hormones in lambs under short-term acute heat stress. *Veterinary Science Development*, 5(2): 103 – 106.

PENG, Y., WANG, Y., NING, D. and GUO, Y. (2013). Estimation of dietary sodium bicarbonate Does limit in broilers under high ambient temperatures. *The Journal of Poultry Science*, 50(4): 346 – 353.

QOTA, E., HASSAN, R., ALI, M. and ATTIA, Y. (2008). Effect of dietary ascorbic acid and betaine on recovery from heat stress adverse effects in slow-growing chicks in the subtropics. *Egyptian Journal of Animal Production*, 45: 467 – 483.

RUDER, E. H., HARTMAN, T. J. and GOLDMAN, M. B. (2009). Impact of oxidative stress on female fertility. *Current Opinion in Obstetrics and Gynecology*, 21(3): 219 – 222.

SAHIN, K., KUCUK, O., SAHIN, N. and SARI, M. (2002). Effects of Vitamin C and Vitamin E on lipid peroxidation status, serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34ºC). *International Journal for Vitamin and Nutrition Research*, 72(2): 91 – 100.

SAS (2002). *SAS/STAT User's Guide, Version 8.* 6th Edition, Statistical Analysis System (SAS) Institute, Cary, North Carolina, USA. https://books.google.com/books/about/SAS_STAT_User_s_Guide._html?id=5HspAQAAMAAJ

SILVA, J. R., SOUZA-FABJAN, J. M. G., BENTO, T. F. M., SILVA, R. C., MOURA, C. R. F., BARTLEWSKI, P. M., & BATISTA, R. I. T. P. (2024). The effects of heat stress on intrauterine development, reproductive function, and ovarian gene expression of F1 female mice as well as gene expression of F2 embryos†. *Biology of Reproduction*, 110(1): 33 – 47.

SIVAKUMAR, A. V. N., SINGH, G. and VARSHNEY, V. P. (2010). Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Australasian Journal of Animal Sciences*, 23(11): 1462 – 1468.

STADLMAYR, B., WANANGWE, J., WARUHIU, C. G., JAMNADASS, R. and KEHLENBECK, K. (2020). Nutritional composition of Baobab (*Adansonia digitata* L.) fruit pulp sampled at different geographical locations in Kenya. *Journal of Food Composition and Analysis*, 94: 103617. https://doi.org/10.1016/j.jfca.2020.103617

TODINI, L. (2007). Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. *Animal*, 1(7): 997 – 1008.

VALENZUELA-GRIJALVA, N. V., PINELLI-SAAVEDRA, A., MUHLIA-ALMAZAN, A., DOMÍNGUEZ-DÍAZ, D. and GONZÁLEZ-RÍOS, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production. *Journal of Animal Science and Technology*, 59: 8. https://doi.org/10.1186/s40781-017-0133-9

VANDANA, G. D., SEJIAN, V., LEES, A. M., PRAGNA, P., SILPA, M. V. and MALONEY, S. K. (2021). Heat stress and poultry production: impact and amelioration.

International Journal of Biometeorology, 65: 163 – 179.

XIAO, J., KHAN, M. Z., MA, Y., ALUGONGO, G. M., MA, J., CHEN, T., KHAN, A. and CAO, Z. (2021). The Antioxidant properties of Selenium and Vitamin E; Their role in periparturient dairy cattle health regulation. *Antioxidants (Basel, Switzerland)*, 10(10): 1555. https://doi.org/10.3390/antiox10101555

YIN, B., TANG, S., SUN, J., ZHANG, X., XU, J., DI, L., LI, Z., HU, Y. and BAO, E. (2018). Vitamin C and sodium bicarbonate enhance the antioxidant ability of H9C2 cells and induce HSPs to relieve heat stress. *Cell Stress and Chaperones*, 23(4): 735 – 748.