



EFFECT OF SUPPLEMENTATION OF PHYTO-ANTIOXIDANT (BAOBAB FRUIT PULP MEAL) ON REPRODUCTIVE PERFORMANCE OF RABBIT DOES DURING HEAT STRESS

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 Supporting Information

ABSTRACT: A study was conducted to evaluate the influence of a phyto-antioxidant (baobab fruit pulp meal; BFPM) on the reproductive performance of heat-stressed rabbits does. A total of 50 New Zealand White crosses adult rabbit does of 12 months old were used in the present study. The rabbits were randomly allotted into five experimental treatment groups, with ten rabbits per treatment in a completely randomized design. The rabbits were fed diets supplemented with graded levels (0.0%, 2.5%, 3.5%, 4.5% and 5.5%) of BFPM. Parameters monitored were thyroxin and progesterone secretion, serum metabolites, reproductive traits, and physiological performance of the kitten. Initial thyroxin, progesterone, and serum metabolite levels were low in all the treatment groups, and significantly increased during and after pregnancy. BFPM significantly improved litter size and weight of litter and reduced rectal temperature of the kitten. Gestation period, kit weight at weaning, weight gain, and heart rates of kitten did not show any significant difference. It was concluded that reproductive performance of does during heat stress is enhanced by the supplementation of phyto-antioxidants and performance was higher in 5.5% inclusion level of BFPM.

Keywords: Antioxidant, Gestation, Heat stress, Rabbit, Reproductive hormones.

INTRODUCTION

The recent trend in the changes of food preferences of many citizens as a way of a healthy life style is increasing the demand for Rabbit meat (Petrescu and Petrescu-Mag, 2018). In Nigeria, farmers are constraint to meeting with this demand because of reproduction inefficiency of the rabbits as a result of heat stress (Mailafia et al., 2010; Oladimeji et al., 2021). Rabbits are vulnerable to heat stress because of their poor functional sweat gland. Hence rabbits can only tolerate narrow temperature range for effective reproduction (Marai et al., 2001). The expression of the growth and reproductive potentials is only possible when animals develop and establish a homeostatic equilibrium with their external environment (Marai et al., 2001).

Previous studies observed that high temperature and heat stress were significantly detrimental to rabbits and their reproduction efficiency. It has been shown that heat stress alters TSH secretion and triiodothyronine (T₃) and thyroxin (T₄) concentrations in serum, in rabbits (Marai et al., 2004), poultry (He et al., 2018; Vandana et al., 2020), and sheep (Todini, 2007). Heat stress has been implicated in promoting oxidative stress either through excessive production of reactive oxygen species (ROS) or decreased antioxidant defenses, including vitamin C (Chauhan, et al., 2014). Thyroid hormone (free T₃ and free T₄) levels were decreased in heat-stressed goats in an attempt to reduce metabolic rate and heat production (Sivakumar et al., 2010). Heat stress also reduces the level of progesterone and causes a loss of LH surge in sheep (Sejian, 2014). It has been reported that exposure to hyperthermia during pregnancy caused marked growth retardation of the adrenal cortex and a decreased population of somatotropin in the adenohypophysis in the off-springs (Ross et al., 1985). Exposing females to heat stress after fertilization caused decreases in the quality and quantity of embryos in cows (Hussin and Al-Taay, 2009; Luceño et al., 2020) and mice (Kahl et al., 2015) after superovulation, and caused decreases in fetal growth in pigs (Omid, 2015), and beef cows (Kumar, et al., 2015). It has been demonstrated that heat stress induces oxidative stress and vitamin C as an important water-soluble antioxidant might reduce the adverse effects of heat stress (Oloruntola, et al., 2015). Based on my knowledge, the vitamin C and heat stress impact on thyroid hormone levels in rabbits has not been clarified.

The use of plants in different forms as alternatives to synthetic products which are relatively expensive and hazardous (because of their residual effect) as antioxidants and in alleviating heat stress is becoming more popular in the tropics (Dhama, et al., 2015; Valenzuela-Grijalva, 2017; Ogunwole and Mosuro, 2020). Plants and their parts could serve

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as phytobiotics and antioxidants to the livestock (Dhama et al., 2015; Valenzuela-Grijalva, 2017). Some phytochemicals in plants improve antioxidant, anti-microbial, feed flavour, and palatability which could result in increased feed intake and performance in animals (Valenzuela-Grijalva, 2017). These tropical plants are available because of their rapid growth which is enhanced by the prevailing and environmental factors (Valenzuela-Grijalva, 2017). Baobab has been described by (Sidibe and Williams, 2002; Bosch et al., 2004). The fruit pulp was reported to contain a high amount of vitamin C (Sena, 1998; PhytoTrade-Africa, 2009), it was reported to increase feed intake, weight gain and was effective in alleviating heat stress in rabbits (Anoh, 2017).

This study was designed to evaluate the effects of baobab fruit pulp meal supplement (as a phyto-antioxidant) on the reproductive performance of heat-stressed rabbit does.

MATERIALS AND METHODS

Ethical regulation

The procedures for care and handling of animals in this study were strictly followed in accordance with the code of ethics for animal experiment as stated in http://ec.europa.eu/environment/chemicals/lab_animal/legislation_en.htm.

Experimental site

This study was carried out at the Rabbit unit of the National Animal Production Research Institute (NAPRI), Shika-Zaria, Nigeria in March – April 2015. Shika-Zaria lies between 11° 12' 42" N and 7° 33' 14" E at an altitude of 691 m above sea level. Zaria is about 245 KM from Abuja Nigeria's capital. The area is situated in the Northern Guinea Savannah Zone of Nigeria having an average annual rainfall of 1100 mm, which starts from May to September, and average ambient temperature and relative humidity of 17 °C – 25 °C and 20 – 41% respectively during the cold period (Mornings and August – Feb 2015) and 20 °C – 39 °C and 25% - 60% during the hot periods (afternoons of March – July 2015).

Housing

The animals were housed in perforated metallic hutches measuring 75 × 75 × 75 cm and raised 80 cm from the floor level. The hutches were thoroughly washed and disinfected with a locally made disinfectant and allowed to dry for one week before the animals were brought. Feed and watering troughs which were made of bunt clay were provided in each hutch. The rabbits were placed individually in clearly labeled cells.

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. (2001) as follows: $THI = t - [(0.31 - 0.31 \times RH) (t - 14.4)]$

Where RH = relative humidity /100; and 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.

Experimental animals, diet and design

A total of 50 adult rabbit does (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 and five replicates. They were ten rabbits per treatment and each rabbit in a treatment served as replicate in a completely randomized design. Rabbits in group 1 were not supplemented and served as the control 0%; while those in groups 2–5 were fed diets, containing graded levels of BFPM, 2.5%, 3.5%, 4.5% and 5.5%, respectively. The baobab fruit pulp meal which was in powdered form was purchased from a local market in Zaria-Nigeria. The basal diet composition (kg) was as follows: Maize 35, Groundnut haulm 20, 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 NRC-1995. The nutrient composition of the basal diet is shown in Table 1. Feed and water were served *ad libitum*. All recommended managerial practices were duly observed.

Table 1 - Nutrient composition of the experimental diets.

	Control	2.5%	3.5%	4.5%	5.5%
Metabolizable Energy (kcal/kg)	2200	2200	2200	2200	2200
Crude Protein (%)	16	16	16	16	16
Crude fiber (%)	10	10	10	10	10
BFPM Supplementation	0.00g	250g	350g	450g	550g

Hormonal assay and serum metabolite evaluation

Blood samples (5 ml) were collected at 10 h through the ear vein of 5 rabbits in the treatment groups before mating, during pregnancy, and after kindling, respectively into a bottle without anticoagulant and allowed to clot. The clotted blood samples were centrifuged at 3000 rounds/minute for 15 minutes. The serum harvested was stored at -10°C until when analyzed. Serum thyroxine and progesterone concentrations were determined for does with the use of commercially-available ELISA Kits (Diagnostic Procedure Corp®, Los Angeles, CA, USA) according to the manufacturer's instructions. Serum glucose, total protein, albumin, and cholesterol concentrations were evaluated using an auto-analyzer and Chemical Commercial Kits from Stanbio Laboratory Inc®, San Antonio, Texas, USA.

Reproductive performance of Rabbit Does

The rabbits were allowed to adjust to the treatment for four weeks before mating. Does were brought individually to be serviced by the buck (1 buck: 1doe / treatment). 7th day weight increment and abdominal palpation was used to confirm pregnancy. Does that were not pregnant were re-mated. Parameters monitored included, date of kindle, litter size, weight of litter, weight of kitten, survivability (%) of kitten at weaning and kitten weight at weaning. Data were obtained from 2 parities and the experiment lasted for 20 weeks.

Physiological performance of Kittens

When the kittens were 3 weeks old, their rectal temperature (RT) and heart rate (HR) were measured. The readings were taken at 14.00 h to 15.00 twice a week for 3 weeks. Rectal temperature was measured with a digital thermometer, and HR was measured by counting the heartbeat of each rabbit representing their treatment for one minute with the help of a stethoscope. Weekly weight gain was also determined.

Statistical Analysis

Data obtained from all the experiments were subjected to analysis of variance, using the General Liner Model Procedure of (SAS version 2.8, 2002). Significant differences among treatment means were separated using the pair wise difference (Pdiff) in the SAS package. Values of P < 0.05 were considered significant

RESULTS

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°C while the Afternoon THI averaged 28.74°C. The THI values kept increasing from the month of February with a peak in May. There was a decline in THI in June. The values indicated that the month of February had absence of heat stress in the rabbit house.

Initial thyroxine was low across the treatments (Figure 2) and significantly increased during pregnancy. Thyroxine levels increased (60.9 (ng/ml), 69.87(ng/ml), 76.88(ng/ml) and 80.1(ng/ml) for 2.5%, 3.5%, 4.5% and 5.5% respectively) as the levels of BFPM increases in the diets with 4.5% and 5.5% recording the highest values. After kindling, thyroxine concentration decreased. Thyroxine concentration after pregnancy did not show any significant difference among the treatment groups. Progesterone concentration (Figure 3) were generally low before pregnancy and significantly (P<0.05) increased during and after pregnancy in BFPM treated rabbits. Rabbits in the BFPM treatments recorded significantly higher progesterone during pregnancy (21.67 ng/mL, 20.12, ng/mL 19.68 ng/mL and 19.25 ng/mL) compared to the control (18.04 ng/mL). Progesterone decreased as the levels of supplementation of BFPM in the diets increases. After kindling, it was observed that the control recorded a higher progesterone compared to the BFPM treated does.

During pregnancy, BFPM treatments recorded a significantly (P<0.05) high glucose, calcium and phosphorous compared to the control. Total protein was low in 2.5% group and phosphorous was low in 3.5% group. After kindling, total protein, albumin, triglyceride and phosphorous showed significant (P<0.05) difference among the treatment groups with the control having a significantly higher values than the BFPM treated groups except in triglyceride which was low. In the treatments with BFPM, treatments 3.4% and 4.5% performed better than treatments 2.5% and 5.5%

Litter size and weight of litter were significantly (P<0.05) increased in 3.5% and 4.5% BFPM inclusions (Table 3). Weight of kitten increased in 2.5% and 4.5%, while litter size at weaning was larger in 4.5 and 5.5% BFPM. Gestation period and weight of kitten at weaning did not show any significant difference.

The BFPM significantly (P<0.05) reduced rectal temperature; rectal temperature decreases as the inclusion of BFPM in the diet increases. Heart rate and weight gain did not show any difference.

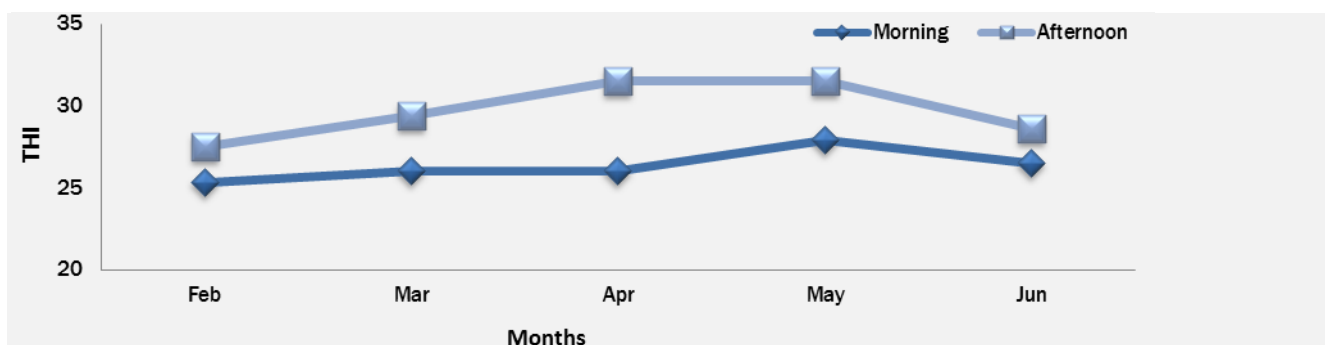


Figure 1 - Monthly temperature humidity index inside the rabbit house

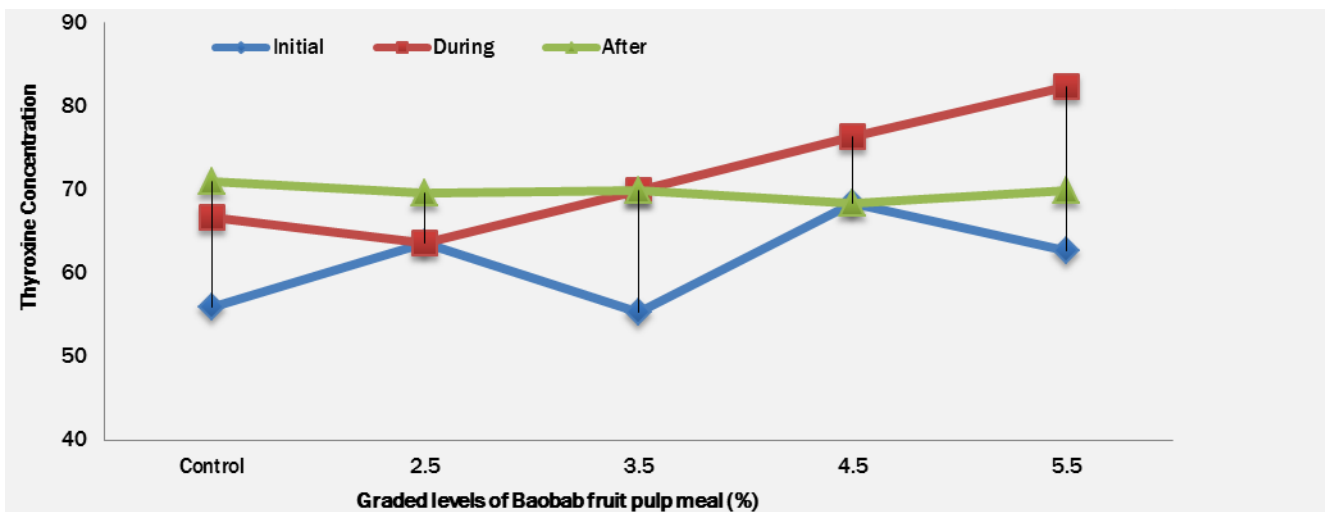


Figure 2 - Effect of graded levels of BFPM on Thyroxine concentrations (ng/ml) in adult rabbit does

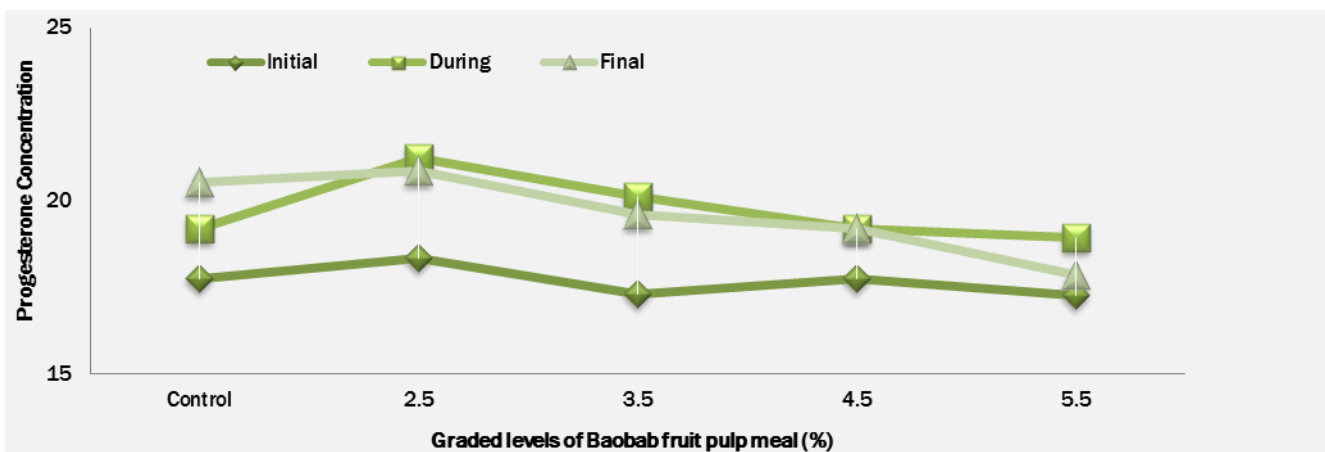


Figure 3 - Effect of graded levels of BFPM on progesterone levels ng/mL in adult rabbit does before, during and after pregnancy

Table 2 - Effect of graded levels of BFPM on serum metabolites of adult rabbit does

Parameters	Treatments	Control	2.5%	3.5%	4.5%	5.5%	SEM
Before Pregnancy							
Glucose (mg/dl)		5.20	4.93	4.77	4.47	5.0	0.35
Total Protein (mg/dl)		63.00	66.67	66.00	67.67	67.33	1.43
Albumin (mg/dl)		36.07	35.67	39.67	37.67	37.33	1.12
Cholesterol (mg/dl)		1.57	1.53	1.47	1.47	1.53	0.11
Triglyceride (mg/dl)		0.93	1.00	0.90	0.87	0.67	0.10
Calcium (mg/dl)		2.33	2.40	2.27	2.56	2.29	0.07
Phosphorous (mg/dl)		1.05	1.02	1.16	1.13	1.01	0.06
During Gestation							
Glucose (mg/dl)		3.20 ^b	4.53 ^a	4.25 ^a	4.83 ^a	4.30 ^a	0.06
Total Protein (mg/dl)		68.33 ^a	62.00 ^b	67.00 ^a	65.00 ^{ab}	68.67 ^a	1.03
Albumin (mg/dl)		37.00	32.00	35.33	36.00	33.00	1.45
Cholesterol (mg/dl)		1.5	1.43	1.35	1.43	1.40	0.05
Triglyceride (mg/dl)		0.93	1.00	0.90	0.87	0.67	0.10
Calcium (mg/dl)		2.31 ^{ab}	2.37 ^{ab}	2.40 ^a	2.40 ^a	2.24 ^b	0.03
Phosphorous (mg/dl)		0.97 ^{ab}	0.93 ^{ab}	0.62 ^b	1.11 ^a	1.16 ^a	0.08
After Kindling							
Glucose (mg/dl)		4.57	5.33	4.50	4.00	4.40	0.14
Total Protein (mg/dl)		68.67 ^a	64.33 ^c	68.00 ^{ab}	68.00 ^{ab}	65.00 ^{bc}	0.79
Albumin (mg/dl)		42.00 ^a	35.33 ^b	40.33 ^a	39.17 ^a	34.33 ^b	0.84
Cholesterol (mg/dl)		1.43	1.50	1.40	1.35	1.50	0.04
Triglyceride (mg/dl)		0.85 ^{bc}	1.10 ^a	0.85 ^b	0.70 ^c	0.94 ^{ab}	0.04
Calcium (mg/dl)		2.17	2.31	2.43	2.41	2.18	0.02
Phosphorous (mg/dl)		1.11 ^a	1.0 ^b	1.06 ^a	1.0 ^b	1.0 ^b	0.01

Means within rows with different superscript letters are significantly P < 0.05 different

Table 3 - Effect of graded levels of BFPM on reproductive performance of adult rabbit does

Parameters	Control	2.5%	3.5%	4.5%	5.5%	SEM
Number of Parities/Doe	2	2	2	2	2	-
Gestation Period (days)	30	30	30	30.67	30.33	0.10
Average Litter size at birth	4.00 ^{bc}	3.67 ^c	5.66 ^a	5.33 ^{ab}	5.33 ^{ab}	0.23
Average Weight of litter (g)	186.67 ^b	176.67 ^b	233.33 ^a	218.33 ^{ab}	250.00 ^a	6.68
Average Weight of kitten (g)	47.33 ^{ab}	48.89 ^a	40.00 ^b	40.00 ^b	48.33 ^a	0.06
Average Litter size at weaning	3.67 ^{ab}	3.33 ^b	4.68 ^{ab}	5.00 ^a	5.00 ^a	0.29
Average Litter wt at weaning (g)	502.57	502.00	426.67	504.00	436.67	70.23

Means within rows with different superscript letters are significantly P<0.05 different

Table 4 - Physiological performance of Kittens

Parameters	Control	2.5%	3.5%	4.5%	5.5%	SEM
Rectal Temperature (C°)	38.51 ^a	37.80 ^b	37.62 ^{bc}	37.20 ^c	36.90 ^d	0.14
Heart Rate (beat/min)	140.44	139.16	139.94	135.75	136.10	2.25
Weight gain (g/day)	11.04	12.24	11.68	12.86	12.12	0.95

Means within rows with different superscript letters are significantly P<0.05 different

DISCUSSION

The Temperature humidity index (THI), an indicator of the thermal comfort level of the rabbits was calculated from the values of temperature and relative humidity readings in the pen house using the modified formula for the rabbit by Marai et al. (2001). The average THI value of the pen house was 29.5°C and it is an indication that the rabbit house was severely thermally stressful and had adverse effects on the rabbits (Marai et al., 2001).

Heat stress negatively affected thyroxine secretion in this study (Figure 2.); this finding agrees with (Marai et al., 2004; Todini, 2017). BFPM supplemented groups had an increase thyroxine levels which may be due to the fact that vitamin C and other antioxidant have the ability to reduce oxidative stress and improve body metabolism (Ganaie et al., 2012; Sivakumar et al., 2010; Minuti et al., 2009). Vitamin C antioxidants alleviate retardation in thyroid functions, leading to the increase in serum thyroxine (Usha et al., 2002). Fetal demand for metabolites especially in the control group and 2.5% BFPM supplementation could have been responsible for the significant decrease of T₄ concentration in these treatments during pregnancy. Previous studies reported decrease in thyroid hormones with increasing temperature (Williams and Njoya, 1998; Sokolowicz and Herbut, 1999) decrease T₃ level and a concurrent increase in T₄ level (Marai et al., 2004) during heat stress. The values of thyroxin obtained after kindling is similar to the report of Mahmoud et al. (2014), Acclimation increases the adaptive ability of birds to subsequent thermal stress by reducing the level of T₃.

The trend in progesterone secretion noticed in this study may be attributed to the differences in the physiological status of the does and not necessarily due to heat stress. The effect of heat stress on plasma progesterone concentration is controversial (Marai et al., 2002; Anoh et al., 2018). In the present study, progesterone was low in the control, increased in 2.5% BFPM and decreases in 4.5% and 5.5% BFPM. However, heat stress may affect the number of mature follicles (Naseer et al., 2017) hence the decrease in progesterone secretion because of less number of *corpus luteum* sites as was noticed in the control. Vitamin antioxidants have been known to alleviate heat stress in rabbits (Prabsattroo et al., 2012, 2015; Anoh 2017; El-Desoky, et al., 2017). The presence of these phyto-chemical compounds may facilitate the ability of animals to rapidly grow follicles and shed more ova, creating more *corpus luteum* (CL) sites and increasing progesterone by counteracting free radicals and other reactive oxygen (ROS) species production in body fluids and tissues due to heat stress. The CL is a transient endocrine gland that secretes progesterone to support pregnancy in rabbits. The CL is maintained throughout the gestation (Theau-Clement, et al., 1995) and P4 level in rabbits remain high throughout pregnancy period (Szendró et al., 2010) a characteristic that differentiate the rabbits from other species.

Values of serum metabolites recorded in this result did not follow a particular pattern; however, serum metabolites were significantly high in BFPM treated groups. Ondruska et al. (2011) reported a decline in plasma TP with rising temperature. The general reduction in most of the serum metabolites during pregnancy compared to what was recorded after pregnancy agrees with the reports of (Marai et al., 2007) During pregnancy, reductions in blood serum metabolite may be due to the decrease in feed intake of dams (Marai et al., 1994) and increase in water retention (Marai et al., 2004), and the high demand of the foetus at the late stages of pregnancy (Marai et al., 1994, 2004). The decrease in glucose in blood may be due to the increase in each glomerular filtration rate (GFR) (Marai et al., 1994) and foetal consumption and conversion of glucose to lactose of milk, and the decrease in each of renal threshold of glucose and capacity of renal tubules to absorb the glucose (Marai et al., 1994).

Heat stress was responsible for the low litter size and conception rate recorded in the control and 2.5% BFPM. Heat stress was attributed to decrease follicular growth and number of ova that were shed as mentioned previously (Naseer et al., 2017). This might have affected conception rate hence reduced litter size. Fertilization failure or early embryonic mortality has been reported to be responsible for poor conception rate and small litter size (Argente, 2016). Heat stress was attributed in previous study for the decrease in receptivity and percentage of voluntary mating which led to a decline in fertility (Marai, et al., 2001) as was noticed in the control and 2.5% BFPM. The values of the litter weight were a reflection of the litter size of the rabbits. Treatments with large litter size had higher litter weight. The increase in the average weight of kits recorded in the control and 2.5% BFPM compared to 3.5% and 5.5% is because of their small litter sizes. Kitten in small litter had the opportunities to receive adequate breast milk compare to kittens in large litter. BFPM might have stimulated the increase in milk production which could have been responsible for the average weight of kitten noticed in 5.5% BFPM. Previous studies have demonstrated that feeding doe rabbits on diets containing vitamin antioxidants increases milk yield of does, litter size, and weight of litter (Argente, 2016; Fayeye and Ayorinde 2016).

The rectal temperature of kittens of BFPM treated does were low compared to the control. It is worthy to note that baobab contains high amount of vitamin C and other phyto-chemicals (Sena, 1998; PhytoTradeAfrica, 2009), antioxidant vitamin has been known to alleviate heat load in rabbits (In-Surk et al., 2014; Prabsaturoo et al., 2012). The presence of these phyto-chemical compounds 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 (Akbarian et al., 2016). Heart rate and weight gain did not show any significant difference.

CONCLUSION

Heat stress reduced fertility in the does, litter size, hormone secretion and rectal temperature of kitten. Ameliorating heat stress with Baobab Fruit Pulp Meal (BFPM) improved reproductive performance, serum metabolites and hormonal concentrations. Baobab fruit pulp meal can be used up to 5.5% in reproductive diets of rabbits during hot periods.

DECLARATIONS

We declare that this research work is original and has not been published elsewhere.

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Authors' contribution

All authors (A. Kevin Usman, J. Nsisong Paul and U. Mary Amu) contributed equally in research and writing process.

Conflict of interests

The authors declare that there is no conflict of interests in this work.

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