Mal. J. Anim. Sci. 23(1): 1-10 December 2020 Malaysian Society of Animal Production

Baobab fruit pulp meal as an antioxidant: effect on physiological performance and semen quality of heat stressed rabbits in the tropics

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Abstract

The aim of the study was to evaluate the ameliorative effects of Baobab fruit pulp meal (BFPM) as an antioxidant on physiological performance and semen quality of rabbit bucks in an experiment that lasted for 90 days. A total of twenty-five (25) adult rabbit bucks of 12 months old were used. The rabbits were randomly allotted into five experimental treatment groups, with five (5) rabbits per treatment in a completely randomized design (CRD). The animals were fed diets containing graded levels [0.0% (Control), 2.5%, 3.5%, 4.5% and 5.5%] of BFPM. Temperature humidity index (THI) was calculated, rectal temperature, heart rate was recorded; feed intake and weight gain were determined and semen quality was evaluated. The results of the values of the THI of March to May showed that environmental conditions were stressful in these months to the animals. BFPM significantly ($P<0.05$) decrease rectal temperature and heart rate, increased feed intake and weight gain, and improved semen quality of the rabbit bucks. It was concluded that Baobab Fruit Pulp Meal had a positive influence in ameliorating heat stress and improving physiological conditions and semen quality of rabbits under a hot tropical environment.

Keywords: Heat Stress, Semen quality, Organic Antioxidants

Introduction

Bernard (1878), described that the body has the ability to maintain a stable internal environment to challenges from widely variable externals environments; the concept was later referred to as homeostasis. Bernard, (1878) also discovered that diverse detrimental (extraneous) stimuli (stressors) such as pain, hunger, thirst, severe climatic conditions, or noxious agents cause physiological changes in animals that may lead to a pathological state.

High ambient temperature has been shown to increase the free radicals and other reactive oxygen (ROS) species production in body fluids and tissues (El-Ghaffar *et al.* 2000; Akiyama, 1999). Although, low levels of ROS are essential for many biochemical processes, their accumulation due to over-production or a decreased antioxidant defense leads to damage of biological macromolecules and disruption of normal cell metabolism (Spurlock, and Savage, 1993). Heat stress is responsible for the increased production of ROS (Greco *et al.*, 2005), decrease feed intake and weight gain (Marai *et al.*, 2001) increase rectal temperature and heart rate (Marai *et al.*, 2002; 2001) and also increases the scrotal temperature which disrupt spermatogenesis and ultimately cause infertility (Paul *et al.*, 2008; Jung *et al.*, 2005 Lue *et al.*, 2002). Heat stress has been reported to negatively affect testicular function and also lead to low sperm quality and viability (Rasooli *et al.*, 2010).

Normally available antioxidants in the body are vitamin C, vitamin E, folic acid, zinc, and chromium (Thomas and Reed 1989), they play a vital role in protecting cellular damage from the harmful effects of ROS (Amakye-Anim *et al.,* 2000 and El-Ghaffar *et al.* 2000). High ambient temperature depletes such as antioxidants and induces oxidative stress (Abou-Ashour *et al*., 2004). In recent time attention is shifting from the use of synthetic antioxidants in animal production, this brought about a renewed interest in the use of organic products that are viable and physiologically safe. Moringa has been used as a source of plant antioxidants on semen quality (El-Desoky *et al* 2017; Prabsattroo *et al*., 2015; 2012) and was effective in the enhancement of libido and sperm quality, and also improved sexual activity and testosterone level in immobilization-stressed male rats and rabbit bucks. Baobab has been described by (Sidibé and Williams, 2002; Bosch *et al*., 2004). The fruit pulp was reported to contain a high amount of vitamin C (PhytoTradeAfrica, 2009; Sena, 1998), it was reported to increase feed intake, weight gain and was effective in alleviating heat stress in rabbits (Anoh, 2017).

The aim of this study is to evaluate the ameliorative effects of Baobab fruit pulp meal (BFPM) on semen quality of rabbit bucks

Materials and methods

Experimental site

This study was carried out at the Rabbit unit of the National Animal Production Research Institute (NAPRI), Shika-Zaria, Nigeria. Shika-Zaria lies between 11° 12' 42" N and 7° 33' 14" E at an altitude of 691 m above sea level (Ovimaps, 2014). 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 ºC – 25 ºC and 20 – 41% respectively during the cold period (Mornings and August – Feb) and 20 ºC – 39 ºC and 25% - 60% during the hot periods (Afternoons and March – July).

Housing

The animals were housed in perforated metallic hutches measuring 75 X 75 X 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.
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, diets and design

A total of twenty five Adult rabbits (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). Rabbits in the first group (T1) served as the control; rabbits in treatments 2 to 5 were fed diets formulated in the research institute containing graded levels of Baobab Fruit Pulp Meal (BFPM). The baobab fruit pulp meal which was in powder form was purchased from a local market in Zaria-Nigeria. Feed and water were served *ad libitum*. All recommended managerial practices were duly observed.

Physiological performance evaluation

Measurements of rectal temperature (RT) and heart rate (HR) were taken at 14.00 h to 15.00 h of the day. Rectal temperature was measured with a digital thermometer, and HR was measured by counting the heart beat of each rabbit representing their treatment for one minute with the help of a stethoscope. Weight gain and feed intake were determined weekly. Blood sampling was done every two weeks 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 anticoagulant. The blood sample was allowed to clot and the serum was harvested after centrifuging the samples at 3000 rounds/minute for 15 minutes. The harvested serum was analyzed for serum glucose total protein, albumin and cholesterol concentration using an auto analyzer and Chemical Commercial Kits from Stanbio Laboratory Inc. San Antonio, Texas, USA.

Semen quality evaluation:

The bucks were allowed to get adjusted to experimental diet for four weeks and were thereafter trained for semen released and collection via an artificial vagina, while attempting to mount a teaser doe over a two-week period prior to the collection of semen. During this period the bucks were tested in order to be sure that they were reproductively normal based on their libido and semen characteristics, and were allowed to adjust to the twice-weekly schedule of experimental semen collection. Ejaculate volume (ml), semen pH, semen colour, sperm motility (%), sperm concentration (x 10^6 /ml) were determined at the Fertility Laboratory of the Artificial Insemination Unit, of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria Nigeria.

Evaluation of epididymal and gonadal sperm reserves

Three bucks were selected from each treatment group and slaughtered. The two testes of each of the rabbits were carefully removed and evaluated for their gonadal sperm and/or spermatid reserves. The determination of sperm and spermatid reserves was done according to the standard method of Igboeli and Rakha (1971) and Rekwot *et al*. (1994). Briefly, each testis was homogenized in 20 ml of saline with antibiotic and centrifuged for about two minutes. After rinsing the blender container with 50 ml of saline and adding this to the effluent, the volume of the homogenate was measured and 5 ml of the homogenate was transferred to a conical flask and further diluted with 30 ml of saline. The homogenate was then stored overnight at 5^{o}C to allow sperm cells to ooze out of the tissues. Gonadal sperm/spermatid concentration was determined with a haemocytometer using the erythrocyte counting chamber of a haemocytometer that was crossed with microscopic grids containing small squares (Cole, 1974; Rekwot *et al.*, 1994).

Statistical Analysis

Data obtained from the study were subjected to analysis of variance using the general liner model procedure of SAS (2002). Significant differences among treatment means were separated using the pair wise difference (Pdiff) in the SAS package.

Results and Discussion

The monthly THI inside the rabbitry during the experimental period is shown in Figure 1. THI in the mornings averaged 26.4^{o}C while the Afternoon THI averaged 28.7^{o}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. The THI values of 29.4 (March), 30 (April), 31.5 (May) and 28.49 (June) indicated that the rabbit house was thermally severely stressful and very severely stressful (Marai *et al.*, 2001) in these months. The averaged THI of 29.85 during the experimental period showed that the rabbit house was thermally stressful and may exert adverse effects on the rabbits (Marai *et al.*, 2001). Overall, data obtained indicated that THI in the afternoon was higher by 1.45 % than THI in the morning.

The 2.5% and 3.5% BFPM significantly ($P < 0.05$) increased weight gain compared to 4.5% and 5.5%. This result agrees with Adeosun (2012). The author reported an increase in the weight gain of broilers as the levels of BFPM in the diet increases and a declined at 4% inclusion. However, Oladunjoye *et al.* (2014) reported an increased weight gain of baobab treated rabbits beyond 3.5% inclusion. The mass of BFPM may have a tendency of diluting other nutrients that are responsible for weight gain. Feed intake significantly ($P < 0.05$) increased as the inclusion of BFPM increases. BFPM might have stimulated the increase in feed intake by improving the palatability of the feed. BFPM ($P < 0.05$) significantly reduced rectal temperature and heart rate. Rectal temperature reduced from 39.3^{o}C to 37.8^{o}C and heart rate reduced from 154.60 (bpm) in the control to 137.6 (bpm). It is worthy to note that baobab contains high amount of vitamin C and other phyto-chemicals (PhytoTradeAfrica, 2009; Sena, 1998), an antioxidant vitamin has been known to alleviate heat load in rabbits (El-Desoky *et al.* 2017; Al-Shanty, 2003) and poultry (In-Surk *et al.*, 2014). 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).

Values of serum metabolites recorded in this result did not follow a particular pattern; however, serum metabolites were significantly high in BFPM treated groups. The 2.5% BFPM significantly increase (1.67) cholesterol, 4.5% improved triglyceride (1.03) while 2.5 and 3.5% treatments recorded the highest calcium. Plasma cholesterol concentrations have been reported to decrease due to increases in total body water, resulting from exposure to elevated temperature (Marai *et al*., 1995; Habeeb *et al*., 1996).

BFPM significantly (P< 0.05) improved semen characteristics of rabbit bucks, especially at 4.5% inclusion. At 4.5% inclusion BFPM increased semen concentration to about 102.50 x 10^6, from 6.61 x 10^6 in the control, improved semen pH, motility, colour and volume. Heat stress was responsible for the poor semen quality recorded in the control (Rai *et al*., 2004) and could be explained by the degeneration of the germinal epithelium, and to the partial atrophy of seminiferous tubules (Marai *et al.*, 2002). Theau-Clement *et al.* (1995); Marai *et al.* (2002) and Roca *et al.* (2005) have attributed the increased abnormal sperms rate in the summer ambient conditions to defects of spermatogenesis, particularly in the last stage of differentiation of spermatids. BFPM improved semen characteristics up to 4.5% inclusion level in this study because of the antioxidant properties BFPM contains. Administration of antioxidants such as vitamin E, selenium, vitamin C, and carotenoids may reduce oxidative stress and improve sperm motility (Castellini 2008; Castellini *et al.* 2003; 1999). The role of antioxidants is to counteract the spermatic cell membrane lipid peroxidation and sperm DNA fragmentation, caused by reactive oxygen species and responsible for male infertility in animals and man (Aitken *et al.*, 1989; Baker *et al.*, 1996; Greco *et al.*, 2005).

Testicular volume and sperm concentration were observed to significantly (P < 0.05) increased in BFPM diets, compared to the control treatment. Epididymal sperm concentration did not follow any pattern. The poor sperm characteristics recorded in the control may be attributed to heat stress. Increasing the temperature of the testes can prevent spermatogenesis by causing degeneration of most cells of the seminiferous tubules besides the spermatogonia (de Kretser 2004). Marai *et al.* (1991) attributed the increase in the abnormal sperms rate in the summer ambient conditions to defects in the spermatogenesis, particularly in the last stage of differentiation of spermatids. Thus, the improvement in quality of sperm cells observed in the present study could be attributed to the enrichment of BFPM with vitamin C; this attribute makes it an excellent organic antioxidant. Antioxidants protect cells from oxidation of substrates such as proteins, fatty acids, and DNA (Pincemail *et al*., 1998). Generally, previous studies showed that natural products with antioxidant activity such as Moringa leave extract (Desoky *et al.* 2017), royal jelly (Elnagar, 2010) and propolis (Hashem *et al*., 2013) could improve sperm concentration in rabbits under the heat stress conditions.

Conclusion and recommendation

The study revealed that heat stress adversely affected physiological performance and semen quality of rabbits. Using organic antioxidants is a non expensive and hazardous method of ameliorating heat stress in rabbits. BFPM is recommended to be used in rabbit's diets during hot periods for effective reproduction.

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**

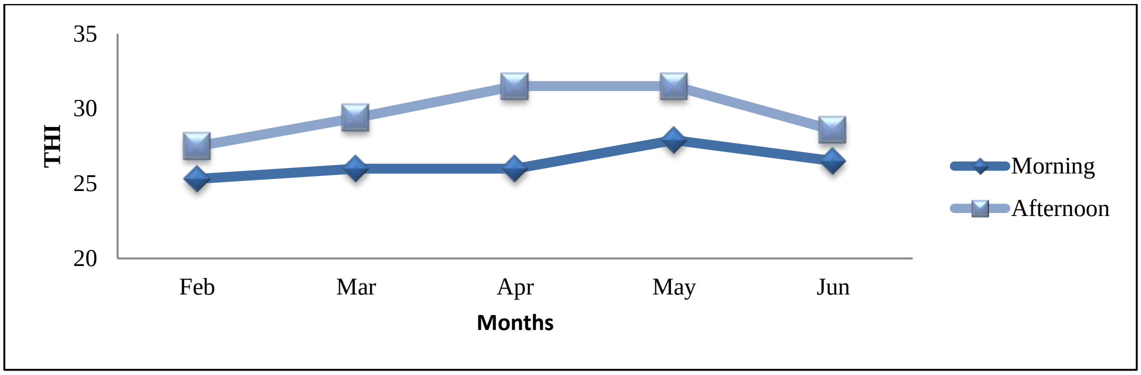

Figure 1. Monthly Temperature Humidity Index inside the Rabbit house

Table 2. Effect of Graded Levels of BFPM on Serum Metabolite of Growing Rabbits

Parameters	**Treatments**					
	0%	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Glucose (mg/dl)	4.30	5.13	5.20	5.40	5.33	0.13
Total Protein (mg/dl)	66.67	64.67	68.33	62.33	70.00	1.75
Albumin (mg/dl)	35.67	35.67	38.57	33.33	38.00	1.75
Cholesterol (mg/dl)	1.47^{ab}	1.67^{a}	1.40^{ab}	1.47^{ab}	1.37^{b}	0.06
Triglyceride (mg/dl)	0.70^{bc}	0.73^{b}	0.90^{ab}	1.03^{a}	0.97^{a}	0.06
Calcium (mg/dl)	2.26^{b}	2.42^{a}	2.45^{a}	2.27^{b}	2.32^{ab}	0.03
Phosphorous (mg/dl)	1.01	1.06	0.93	1.08	1.05	0.04

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

Table 3. Effect of Graded Levels of BFPM on Semen Characteristics of Adult Buck

Parameters	**Treatments**					
	Control	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Volume (ml)	0.55^{b}	0.94^{a}	1.00^{a}	0.96^{a}	1.03^{a}	0.22
Colour	1.50^{ab}	1.93^{a}	1.17^{a}	1.2^{ab}	1.0^{b}	0.18
Mortility (%)	28.33^{c}	65.67^{ab}	66.25^{ab}	77.09^{a}	51.88^{b}	4.13
pH	7.19^{ab}	7.30^{a}	6.75^{ab}	6.75^{ab}	6.71^{b}	0.12
Concentration (x10^{6})	6.61^{c}	82.71^{b}	86.88^{b}	102.50^{a}	71.92^{b}	9.58

Means within rows with different superscript letters are significantly P < 0.05 different; Semen colour = Milky: 1.00 – 1.5, Creamy: 1.51- 2.00, and Watery, 2.10 and above.

Table 4. Effect of Graded Levels of BFPM on Gonadal and Epididymal Sperm Reserve of Adult Rabbit Bucks

Parameters	**Treatments**					
	Control	**2.5%**	**3.5%**	**4.5%**	**5.5%**	**SEM**
Testis sperm volume (ml)						
Right	0.90^{b}	4.50^{ab}	5.00^{ab}	7.67^{a}	2.70^{b}	0.17
Left	0.80^{b}	3.17^{ab}	4.33^{ab}	5.67^{a}	1.97^{b}	0.17
Testis sperm conc. (x 10^6)						
Right	20.33^{bc}	27.33^{ab}	17.67^{c}	33.67^{a}	32.67^{a}	2.13
Left	19.33^{c}	25.00^{b}	16.67^{c}	33.33^{a}	25.33^{b}	1.36
Epididymis						
Caput (x 10^6)						
Right	5.33^{b}	6.00^{b}	20.33^{a}	13.00^{ab}	12.00^{ab}	3.25
Left	7.33^{c}	8.00^{b}	9.67^{abc}	16.00^{ab}	17.33^{a}	1.92
Corpus (x 10^6)						
Right	2.56^{b}	6.00^{a}	4.00^{ab}	6.00^{a}	2.67^{b}	0.55
Left	5.33^{ab}	7.00^{a}	2.00^{b}	5.33^{ab}	3.33^{ab}	1.00
Cauda (x 10^6)						
Right	124.00^{c}	137.33^{ab}	154.33^{a}	143.00^{a}	132.33^{b}	21.26
Left	110.00^{c}	107.67^{c}	173.67^{a}	17.67^{a}	165.33^{b}	22.74

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

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