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Ameliorative effects of Bi-carbonate buffer, Vitamin C and Baobab fruit pulp meal on Progesterone and Testosterone Secretion in Heat stressed Rabbits

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Abstract

The aim of the study was to evaluate the progesterone and testosterone secretion of heat stressed rabbits ameliorated with bi-carbonate buffer, vitamin C and baobab fruit pulp meal. A total of 50 adult rabbits were used comprising 25 males and 25 females. The rabbits were randomly allotted into five experimental treatment groups, with ten (10) rabbits per treatment in a completely randomized design. Rabbits in the first group (T_1) were the control, animals in the treatment 2 and treatment 3 (T_2) and (T_3) were fed with diets as in the control and given potassium bicarbonate ($KHCO_3$) and sodium bicarbonate ($NaHCO_3$) buffer respectively. Rabbits in treatment four (T_4) were fed diet containing synthetic vitamin C and the fifth group (T_5) was fed diet-containing BFPM (Baobab Fruit Pulp Meal). Rabbits were given access to feed and water ad libitum. All recommended managerial practices were dully observed. Microclimate parameters of ambient temperature (AT) and relative humidity (RH) of the rabbitary were taken daily from February through June. The values were used to calculate temperature-humidity index (THI). Blood samples (5 ml) were collected from the ear vein at 10.00 h from four animals chosen randomly from each group of rabbits respectively before, and at the end of the experiment and progesterone and testosterone secretions were evaluated. THI values showed that environmental conditions were stressful to the animals. Vitamin C and BFPM significantly ($P<0.05$) increased progesterone and testosterone secretions compared to the buffers supplements. It was concluded that BFPM and vitamin C should be used in rabbit diets during hot season.

Key Words: Reproduction, Hormones, Heat stress, Supplements.

Description of Problem

Multiplying and breeding rabbits will be a quick remedy to meeting the increasing demand for meat and livestock products because of their fast growth rate and short generation interval. Progesterone and testosterone are two important hormones that are required for effective reproduction. Progesterone can be used to evaluate the number of implantation sites per doe, and number of viable embryos per doe. Progesterone is known to promote secretory changes in the uterine endometrium during the latter half of the monthly female sexual cycle, thus preparing the uterus for implantation of the fertilized ovum [1]. In addition to this effect on the endometrium, progesterone decreases the frequency and intensity of uterine contractions, thereby helping to prevent expulsion of the implanted ovum.

Among the hormones secreted by the testis, testosterone is more abundant than the others that can be consider being the significant testicular hormone. Testosterone is formed by the *interstitial cells of Leydig*, which lie in the interstices between the

seminiferous tubules and constitute about 20 per cent of the mass of the adult testes [1].

Heat stress has been a major constraint to the adequate secretion of these hormones. For instance, heat stress has been reported to cause the degeneration of most cells like the leydig cells, degeneration of the germinal epithelium and partial atrophy of seminiferous tubules [2] which is responsible for testosterone production. Ameliorating heat stress with vitamins and other supplements have been proven to improve rabbit's production. Much has not been reported on the use of bi-carbonate buffers and BFPM on reproductive hormones secretion in rabbits.

Adansonia digitata is a massive deciduous tree, up to 20-30 m tall with a diameter up to 2-10 m at adult age [3]. The endocarp consists of a white mealy pulp, completely dry at maturity. The white, powdery pulp is reported to have a high content of vitamin C [4]. When compared with synthetic vitamin C on a trial involving broilers chicken, it was reported that baobab improved feed intake, reduced rectal temperature and respiratory rate during hot season in Nigeria.

This study was designed to evaluate the progesterone and testosterone secretion of heat stressed rabbits ameliorated with bi-carbonate buffer, vitamin C and baobab fruit pulp meal.

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 11o 12' 42" N and 7o 33' 14" E atan altitude of 691m above sea level [6].

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 [5] as follows: THI = t - [(0.31 – 0.31 x 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:

a. < 27.8 = Absence of heat stress,
b. 2). 27.8 - 28.9 = Moderate heat stress,
c. 28.9 – 30 = Severe heat stress
d. above 30 = Very severe heat stress.

Experimental Animals and Design

A total of 50 adult rabbits consisting of 25 males and 25 females were used in this study. The rabbits were divided into five experimental groups with 10 rabbits (5 Bucks and5 Does) per group. Each group was randomly assigned to one of five experimental treatments in a completely randomized design. Rabbits were given access to feed and water *ad libitum*. All recommended managerial practices were dully observed.

Progesterone and testosterone hormonal assay

Blood samples were collected from bucks before and after the experiment and from the does before mating, during and after pregnancy. 5 ml of blood samples were collected through the ear vein, randomly selected from the treatment groups, from each group of rabbits, respectively into a bottle without anticoagulant and allowed to clot. The blood samples were centrifuged at 3000 rounds/minute for 15 minutes. Serum was collected and stored until analyzed. The concentrations of progesterone and

testosterone in blood serum were determined using commercially available ELISA kits (Diagnostic Procedure Corp., Los Angeles, CA, USA) according to the manufacturer's instructions.

Statistical Analysis

Data obtained from the experiment were subjected to analysis of variance, using the General Liner Model Procedure of [7]. 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 and Discussion

Monthly Temperature Humidity Index in Rabbit Pen

The 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 it averaged 28.74^{o}C. THI values progressively increased from the month of February and peaked in May, but declined in the month of June. The average THI of 28.74^{o}C during the experimental period indicated that the rabbit house was thermally stressful and may have adverse effects on the rabbits [6]. Overall data obtained indicated that THI in the afternoon was higher by 1.24 % than THI in the morning.

Testosterone concentration in Adult Rabbit Bucks

The initial testosterone concentration of rabbits treated with buffer, vitamin C and BFPM in Fig. 2 did not show any significant difference among the treatments. It was observed that testosterone concentration was significantly ($P < 0.05$) higher in rabbits fed vitamin C and BFPM supplemented diets compared to the rest of the treatments whose values were lower than those 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 may affect the Leydig cells. Jelodar and Zare [8] 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 [9]. 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 imparing the activity of key regulatory enzymes in steroidogenesis [10]. The significant improvement recorded in rabbits treated with vitamin C and BFPM could be the vitamins counteracted free radicals that may limit normal functioning of the Leydig cells from functioning properly. Vitamin C has been reported to be an effective antioxidant.

Progesterone concentration in Adult Rabbit Bucks

The effects of buffer, vitamin C and BFPM on progesterone levels of adult rabbit does are shown in Fig. 3 Initial progesterone concentration of the rabbits were low across the treatment group compared to the values obtained during and after pregnancy. The values did not show any significant difference among the treatment groups. During pregnancy, progesterone significantly ($P<0.05$) increased in vitamin C and BFPM treatment groups. After kindling, vitamin C and BFPM significantly ($P < 0.05$) reduced the values of

progesterone while the values in the control, $KHCO_3$ and Na_2CO3 groups increased above the initial progesterone values and those recorded during pregnancy. The trend in progesterone secretion and concentration observed in this study during and after pregnancy cannot be attributed to heat stress but to the physiological status of the rabbits. Maria [2] reported that the effect of heat stress on plasma progesterone and LH concentration is controversial. Some studies found that heat stress has no effect on progesterone levels [11], although other studies have reported reduced [12, 13] increased [14] or unchanged [15] concentration during summer in dairy cows. Increased progesterone levels during pregnancy by the treatments with vitamin C and BFPM may be attributed to the fact that vitamin C and BFPM stimulated the follicles to shed more ova, creating more CL sites, which were responsible for more progesterone secretion. The CL is a transient endocrine gland that secretes progesterone to support pregnancy in rabbits, the CL, is maintained throughout the gestation, a characteristic that differentiate the rabbits from other species [16]. The CL are formed from ovulated follicles in a process that involves angiogenesis and tissue remolding under the influence of several endothelial-derived factors, including vascular endothelial growth factor, transforming growth factor and fibroblast growth factor acting locally in a paracrine/autocrine manner [16]. Two factors may have been responsible for the increase in progesterone after pregnancy in the control and the treatments with bi-carbonate buffers. For the control and $NaHCO_3$, at the time of collection of blood samples, only about 40% of the rabbits had kindled while the remaining 60% were yet to kindle and were in their last trimester of pregnancy this is in contrast to the treatments with vitamin C and BFPM with about 90% of rabbits that had already kindled. For the treatment with KHO_3 buffer, the rabbits could have been pseudopregnant, because no rabbit in this group kindled even after three consecutive mating trials. Progesterone is believed to be high during pseudo pregnancy and reduces at the end of pseudopregnancy [16]. The high progesterone level during pseudo pregnancy may arise as a result of the alteration in dominant follicles, failure of implantation and early embryonic death. It is expected that when this happens, there will be active sites of CL hence more progesterone secreted before luteolysis.

Conclusion and Applications

1. Heat stress as well as using buffers was has negative effect on secretion of testosterone in bucks whereas in the does, there was an indirect effect of heat stress by inhibiting follicular growth and development and by the buffers.
2. Ameliorating heat stress with vitamin C and BFPM proved effective in increasing testosterone and progesterone secretion directly or indirectly.
3. Vitamin C and BFPM, preferably BFPM should be included in rabbit breeder diets during hot period for optimum reproductive performance.

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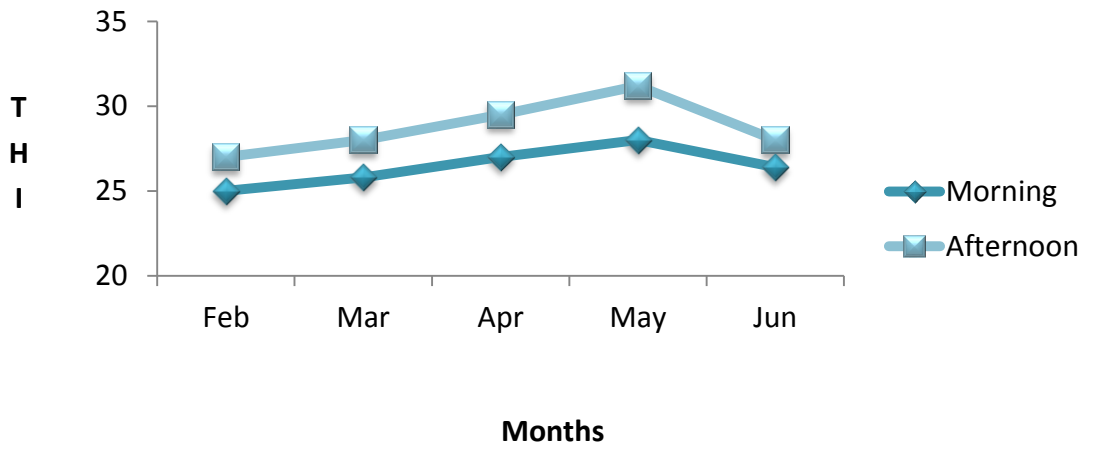
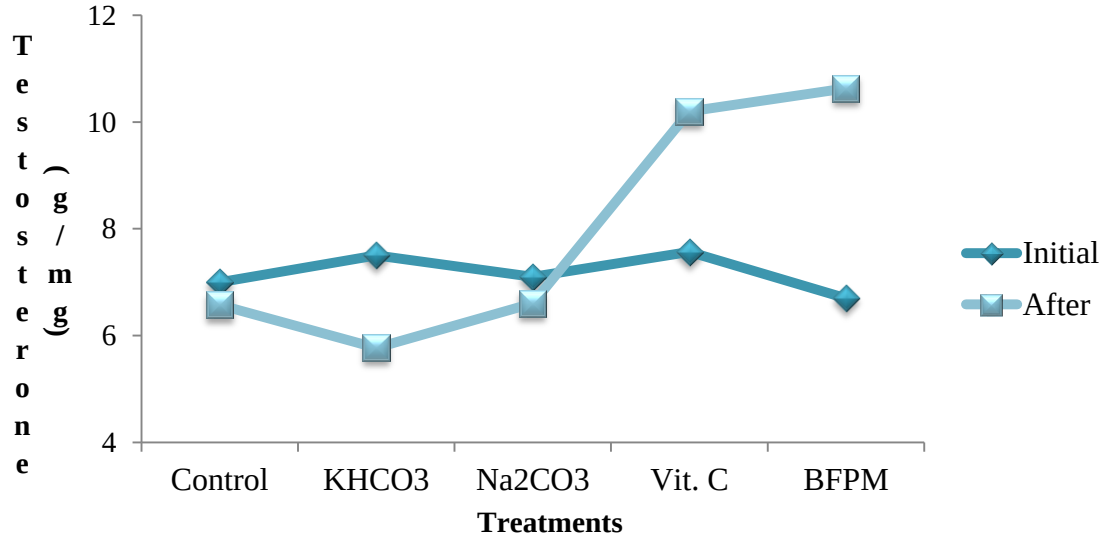
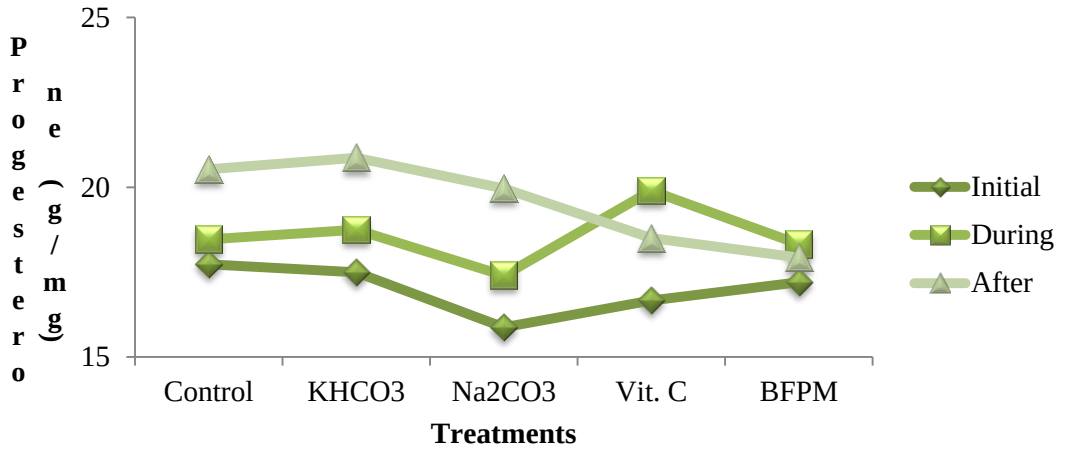


Figure 1: Monthly Temperature Humidity Index of the Rabbit Pen



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

Fig. 2. Effects of Bicarbonate Buffers, Vit C and BFPM on Testosterone 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 Progesterone Levels in Adult Rabbit Does