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TESTICULAR AND EPIDIDYMAL SPERM RESERVES OF HEAT STRESSED RABBITS FED DIETS SUPPLEMENTED WITH BI-CARBONATE BUFFER, VITAMIN C AND BAOBAB FRUIT PULP MEAL

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ABSTRACT

A total of twenty five (25) adult male rabbits were used to evaluate effect of heat stress on testicular and epididymal sperm reserves of rabbits treated with bi-carbonate buffer, vitamin C and baobab fruit pulp meal. The rabbits were randomly allotted into five treatment groups with five (5) rabbits per treatment in a completely randomized design. Rabbits in the first group (T_1) was the control, animals in the treatment 2 (T_2) and treatment 3 (T_3) were fed diets as in the control and given potassium bi-carbonate ($KHCO_3$) and sodium bi-carbonate ($NaHCO_3$) buffer respectively. Rabbits in treatment 4 (T_4) were fed diet containing synthetic vitamin C and the fifth group (T_5) was fed diet containing Baobab Fruit Pulp Meal (BFPM). Feed and water were served *ad libitum*. Environmental readings such as Ambient Temperature (AT) and Relative Humidity (RH) of the rabbitry micro-climate was taken daily from February through June. The values were used to calculate temperature-humidity index (THI), an indicator of thermal comfort level for animals in an enclosure. The testicular and epididymal sperm reserves parameters monitored included the testicular and epididymal sperm volume and concentration respectively. It was found that the buffers and BFPM recorded a significantly ($P<0.05$) higher testicular and epididymal sperm reserves compared to the control.

Key Word: Heat stress, Testis, Sperm reserves

INTRODUCTION

The testes are the biological industry in male species involved in spermatogenesis and hormonal secretion. Adequate knowledge of the ability of the testes to produce sperm cells is essential in livestock reproduction. The negative effects of high ambient temperatures on reproductive traits of rabbit bucks are well known, and several works using different breeds reported their susceptibility to heat stress that produces drastic changes in their biological functions (Marai *et al.*, 2002; Theau-Clément *et al.*, 2009).It is also well known that high ambient temperatures decrease semen production and affect fertility by increasing semen pH values and morphological alterations, and also induce a change in sperm motility and libido (Nizza *et al.*, 2003; Safaa *et al.*, 2008). To successfully raise rabbits in a tropical country characterized with high ambient temperature like Nigeria, it is pertinent to ameliorate heat stress in rabbits.

This study was therefore aimed at attempting to alleviate heat stress on testicular and epididymal sperm reserves of adult male rabbits with bi-carbonate buffers, 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 11^{o} 12' 42" N and 7^{o} 33' 14" E atan altitude of 691m above sea level (Ovimaps, 2014).

Experimental Animals and Design

A total of twenty five adult male rabbits 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. Rabbits in the first group (T_1)was the control, animals in treatment 2 (T_2) and treatment 3 (T_3) were fed with diets fed as in the control and given potassium bi-carbonate ($KHCO_3$)and sodium bi-carbonate (Na_2CO_3)buffer respectively. Rabbits in treatment 4 (T_4) were fed diet containing synthetic vitamin C and the fifth group (T_5) were fed diet containing Baobab Fruit Pulp Meal. Feed and water were served *ad libitum*. All recommended managerial practices were dully observed.

Testicular and Epididimal and Sperm Reserves Evaluation

Three rabbits were selected from each treatment group representing their treatments; the rabbits were slaughtered to determine their gonadal sperm/spermatid reserves. The two testes of each of the rabbits were carefully removed and labeled for proper identification. 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 20ml of saline with antibiotics and centrifuged for about two minutes. The homogenate volume, after rinsing the blender container with 50ml of saline and adding the effluent, was measured. About 5ml of the homogenate was transferred to a conical flask and further dilution was made with 30ml of saline and the homogenate was stored overnight at 5^{o}C to allow sperm cells to ooze out of the tissues. The concentration of spermatozoa was determined using the erythrocyte counting chamber of a haemocytometer that was crossed with microscopic grids containing small squares. Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares according to the method of Rekwot *et al*. (1994).

Statistic Analysis

Data generated from the study was analyzed using the general linear model of SAS software (SAS, 2002). Significant differences in means were separated using the pair wise difference (PDIFF) in the SAS package.

RESULT AND DISCUSSION

The effect of buffer, vitamin C and BFPM on testicular and epididymal sperm reserves of adult rabbits (bucks) as shown in the Table below shows that BFPM and vitamin C significantly ($P<0.05$) improved sperm volume of the right testis, and sperm concentration at the caput region of the epididymis, while the treatment with $KHCO_3$ significantly ($P<0.05$) improved sperm concentration in the testis. Sperm concentration which happened to be high in the cauda epididymis was significantly ($P<0.05$) improved in the right cauda epididymis by $KHCO_3$ and the left by Na_2CO_3 respectively, compared to other treatments. The improvement noticed in the gonadal and epididymal sperm reserves in the treatments with BFPM and vitamin C compared to the control is an indication that the sperm concentration of the control was affected by heat

stress. This could be explained by the degeneration of the germinal epithelium and to the partial atrophy of seminiferous tubules (Marai *et al.*, 1991). Increasing the temperature of the testes can prevent spermatogenesis by causing degeneration of most cells of the seminiferous tubules besides the spermatogonia (Guyton and Hall, 2006). Marai *et al.* (1991) attributed the increase of the abnormal sperms rate in the summer ambient conditions to defects of the spermatogenesis, particularly in the last stage of differentiation of spermatids. The cauda epididymis sperm concentration values obtained in this study in the treatment with vitamin C and BFPM range from 123.67 to 185.67x10^6/ml and were comparable to the range of 126.00 to 154.00 x10^6/ml reported by Ajayi (2009). Oyeyemi and Okendran (2007) stated that high concentration of spermatozoa is an indication of possible high fertility rate by the reason of the number of spermatozoa available at the time of copulation or insemination. The improvement noticed in the treatments with vitamin C and BFPM agrees with the report of Mangiagalli *et al*. (2012). Vitamin C and BFPM may have reduced the negative effect of heat stress on these variables by reducing the damage caused by reactive species of oxygen or by sparing the effect of enzymes involved in the antioxidant systems of the testicle. The importance of vitamins in protecting the sperm membrane against lipid peroxidation may be more important in late spermiogenesis when sperm drop most of their cytoplasm, leading to lowered concentrations of cytoplasmic defensive enzymes (Aitken and Fisher, 1994).

Effect of Buffer, Vitamin C and BFPM on Testicular and Epididymal Sperm Reserves of Rabbit Bucks

	Treatments					
Parameters	**Control**	**$KHCO_3$**	**Na_2CO_3**	**Vit. C**	**BFPM**	**SEM**
Testis Sperm volume (ml)						
Right sperm volume	1.00^{b}	3.33^{ab}	2.33^{b}	3.67^{a}	4.00^{a}	0.02
Left sperm volume	0.54^{b}	0.62^{a}	0.61^{a}	0.56^{b}	0.63^{a}	0.08
Testis sperm Reserves (x 10^6)						
Right testis	25.67^{c}	38.67^{a}	21.00^{e}	32.00^{b}	14.33^{d}	1.35
Left testis	22.33^{d}	25.00^{c}	31.00^{b}	34.67^{c}	17.67^{e}	0.49
Epididymis Reserves (x10^6)						
Right caput	9.67^{c}	14.67^{b}	14.33^{b}	13.00^{b}	29.33^{a}	0.46
Left caput	9.33^{d}	15.67^{b}	12.33^{c}	20.33^{a}	11.33^{c}	0.26
Right corpus	3.67	3.67	3.00	2.67	4.67	0.46
Light corpus	0.67	0.67	1.00	0.67	1.67	0.03
Right cauda	150.33^{c}	196^{a}	73.67^{e}	185.67^{b}	123.67^{d}	2.12
Left cauda	105.66^{d}	105.00^{d}	184.67^{a}	152.67^{c}	156.00^{b}	0.80

abc: Means within rows with different superscripts are significantly different: $P < 0.05$
Vit C = Vitamin C, BFPM = Baobab fruit pulp meal
SEM: Standard Error of Mean

CONCLUSION

Heat stress adversely affected testicular and epididymal sperm reserves, ameliorating with bi-carbonate buffer, vitamin C and BFPM improved sperm concentration with vitamin C and BFPM having a superior performance. It was therefore recommended that vitamin C and BFPM should be included in reproductive rabbit diets during hot periods for optimum performance.

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