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Daily sperm production, gonadal and extragonadal sperm reserves in rabbit bucks fed dietary ascorbic acid supplementation

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Target Audience: *Feed producers and rabbit farmers*

Abstract

A study was conducted for 20 weeks to investigate the effect of dietary ascorbic acid supplementation levels on daily sperm production, gonadal and extragonadal sperm reserves of crossbred rabbit bucks. A total of 20, matured rabbit bucks of 18 to 20 weeks old with average weight of 2.75 kg were used. Ascorbic acid was included in the diets of the rabbits at levels of 0 (control), 100, 200, 300 and 400 mg/kg feed. The rabbits were randomly allotted to the diets (n=4 rabbits/treatments) and housed individually. Each rabbit was a replicate. At the end of the experiment, two bucks per treatment were sacrificed and their testes and epididymides were carefully sampled, weighed and processed for determination of gonadal and epididymal spermatozoa reserves. All data were subjected to general linear model while the significant differences in means were separated using pairwise-difference. Results showed that gonadal sperm reserves of the right testis of bucks fed ascorbic acid supplementation were significantly (P<0.05) higher (288.75, 287.15, 299.98, 296.00 vs. 263.10 x10^6/ml) than their counterparts fed the control diet. Bucks fed ascorbic acid supplemented diet had significantly (P<0.05) higher (98.76, 98.00, 115.72, 112.67 vs. 83.55) daily sperm production of the right testis compared to bucks fed the control diet. The results of this study suggest that feed grade ascorbic acid supplementation could improve gonadal and extragonadal sperm reserves and daily sperm production of rabbit bucks.

Key words: Ascorbic acid, rabbits, sperm reserves.

Description of problem

High ambient temperature affects reproductive performance of rabbit bucks (1). Heat stress (due to high ambient temperature) coupled with excessive production of pro-oxidants, causes imbalance between pro-oxidants and antioxidants, resulting in oxidative stress (2). These stress are damaging agents which have been reported to induce an adaptive response in rabbits (3). (4) suggested that heat stress is induced under high ambient temperature coupled with very high relative humidity. When rabbits are exposed to heat/oxidative stress, a series of remarkable changes in their biological functions are effected which ends with reduction in productivity, libido, fertility and embryonic survival. Other effects may include facilitated disease processes from the resulting cellular damage and impairment of production and reproduction performance (5). Antioxidant

vitamins such as ascorbic acid among others is a substance that interact with and protect cells/soft tissues against damaging effect of unstable free radical molecules (6). Ascorbic acid is essential in maintaining homeostasis such as alleviation of heat stress in rabbits. It is also essential for maturation of gonads, maintenance of tubular structure of the testes and gonadal tissue restoration (7).

This investigation was to assess the gonadal and epididymal sperm reserves and sperm production of rabbit bucks fed ascorbic acid supplementation.

Materials and methods

The study was conducted in the Rabbit Unit of the National Animal Production Research Institute (NAPRI), Shika, Zaria, situated on latitude 11° and 12°N and between longitude 7° and 8°E with an altitude of 691m above sea level (8). The mean maximum temperature varies from 19^{o}C to 38^{o}C depending on season, while the mean relative humidity during dry and wet season is 21% and 72% respectively. Morning temperature range between 19ºC and 32ºC while afternoon temperature ranges between 20ºC and 40ºC (9). A total of 20 sexually-mature crossbred rabbit bucks aged 18 to 20 weeks old with an average weight of 2.75 kg were used. The rabbits were weighed and randomly allotted to five dietary groups in a completely randomized design. Each group constituted a treatment and each rabbit within a group was a replicate (four rabbits per treatment). The rabbits were equally assigned to one of the following levels of ascorbic acid (0, 100, 200, 300, 400 mg ascorbic acid/kg diet).

At the end of the feeding trial, two bucks were anesthetized and sacrificed and their reproductive tracts were dissected. The testes and epididymides were carefully removed and weighed. The right testis, the left testis and the different segments of epididymides (caput, corpus, cauda) were homogenized separately in 0.154 \M NaCl (physiological saline) at the dilution rate of 5ml/g testis. The suspensions were mixed and filtered through a double layer of sterile gauze into clean glass test tubes and the sperm concentrations therein were determined by direct haemocytometric count after proper dilution (1:20 v/v) in 0.154 M NaCl (10). The concentration of sperm cells per gram of testis parenchyma, gonadal sperm reserves, daily sperm production and daily sperm production per gram parenchyma (testis) per animal were calculated as:

Concentration per gram = number of sperm cells x volume used/weight of sample
Gonadal sperm reserves = concentration per gram testis x total weight of right testis ($x10^{6}$)
Daily sperm production = testes sperm count (gonadal sperm reserve)/time divisor. Time divisor for rabbits = 3.43

Daily sperm production/g parenchyma (testis) per animal = Gonadal sperm reserve/gross testes weight–tunica albuginea x 1/3.43 (11).

All data obtained from this study were subjected to analysis of variance (ANOVA) of (12) package and the significant differences in means were separated using pairwise-difference (P-DIFF, 12) of the same software.

Results and discussion

The effect of ascorbic acid (AA) supplementation on gonadal and epididymal sperm reserve in rabbit bucks is presented in Table 1. Gonadal sperm reserves of right testis per g testis, per testis and per paired testes as well as epididymal sperm reserves of right and left caput and caudal epididymides of bucks fed 100, 200, 300 and 400 mg/kg diets were significantly ($P<0.05$) higher than their counterparts fed 0 mg

AA/kg diet. Gonadal sperm reserves of left testis per g testis, per testis and paired testes/g testis as well as epididymal sperm reserves of right and left corpus epididymides were statistically (P>0.05) similar for all levels of ascorbic acid supplementation. Higher gonadal sperm reserves were observed in bucks fed dietary ascorbic acid supplementation compared to their counterparts fed the control diet.

Table 1: Effect of ascorbic acid supplementation on gonadal and epididymal sperm reserves in rabbit bucks

Parameters	Ascorbic acid levels (mg/kg feed)					P value
	0	100	200	300	400	
Gonadal sperm reserves (x10^6/ml)						
Right testis per g testis	219.25±3.28^b	231.00±3.28^a	230.17±3.28^a	235.71±3.28^a	233.00±3.28^a	0.032
Per testis	263.10±3.35^c	288.75±3.35^b	287.15±3.35^b	299.98±3.35^a	296.00±3.35^a	0.040
Left testis per g testis	218.55±8.92	231.80±8.92	233.80±8.92	234.43±8.92	232.60±8.92	0.530
Per testis	260.07±11.92	280.48±10.92	282.89±10.92	286.00±11.92	281.43±10.92	0.199
Paired testes per g testis	218.90±9.70	277.69±9.70	231.72±9.70	234.39±9.70	232.83±9.70	0.650
Per testes	523.17±10.85^b	569.23±10.85^a	570.04±10.85^a	585.98±10.85^a	577.43±10.85^a	0.018
Epididymal sperm reserves (x10^6/ml)						
Right:						
Caput epididymides	6.70±0.39^c	7.81±0.39^b	7.66±0.39^b	9.71±0.39^a	8.96±0.39^a	0.037
Corpus epididymides	1.40±1.52	4.55±1.52	1.95±1.52	3.01±1.52	2.93±1.52	0.282
Cauda epididymides	53.80±1.05^c	98.92±1.05^b	100.00±1.05^b	107.54±1.05^a	102.19±1.05^a	0.010
Left:						
Caput epididymides	5.74±0.40^c	6.60±0.40^b	6.63±0.40^b	7.55±0.40^a	7.49±0.40^a	0.050
Corpus epididymides	1.14±1.80	3.48±1.80	1.63±1.80	1.49±1.80	2.19±1.80	0.243
Cauda epididymides	50.87±0.29^d	90.00±0.29^c	90.60±0.29^b	91.42±0.29^a	90.80±0.29^b	0.010

^{abc}: Means with different superscripts in the same row are significantly (P<0.05) different

Table 2: Effect of ascorbic acid supplementation on daily sperm production and sperm production efficiency in rabbit bucks

Parameters	Ascorbic acid levels (mg/kg feed)					P value
	0	100	200	300	400	
Daily sperm production (x10^5/ml)						
Right testis	83.55±2.14^c	98.76±2.14^b	98.00±2.14^b	115.72±2.14^a	112.67±2.14^a	0.019
Left testis	82.53±8.93	90.55±8.93	94.62±8.93	98.90±8.93	95.59±8.93	0.125
Sperm production efficiency						
Right testis	60.88±2.50^c	67.95±2.50^b	67.25±2.50^b	75.10±2.50^a	73.90±2.50^a	0.043
Left testis	57.40±1.38^d	60.20±1.38^c	62.99±1.38^b	66.99±1.38^a	63.64±1.38^b	0.035

^{abcd}: Means with different superscripts in the same row are significantly (P<0.05) different

The results of the current study suggest that ascorbic acid possibly augmented the highly-concentrated seminal plasma ascorbic acid in the gonads/testes to protect sperm cells from being attacked by reactive oxygen species in the testes of the animals. The scavenging potential in gonads and seminal fluid is normally maintained by adequate levels of antioxidants such as plasma vitamin C (13). Improved epididymal sperm reserves of the right and left caput and cauda epididymides observed in bucks fed ascorbic acid

supplemented diet compared to bucks fed the control diet indicates that ascorbic acid enhanced the epididymide by scavenging reactive oxygen species which deteriorate and reduce sperm reserved within the epididymis. Mammalian spermatozoa are rich in highly-unsaturated fatty acids and are apparently sensitive to oxygen induced damage mediated by lipid peroxidation (14). The effects of ascorbic acid supplementation on daily sperm production and sperm production efficiency in rabbit bucks is presented in Table 2. Daily sperm production of the right testis as well as sperm production efficiency of right and left testes of bucks fed 100, 200, 300 and 400 mg/kg diets were significantly ($P<0.05$) higher than bucks fed 0 mg AA/kg diet. Daily sperm production of the left testis was statistically ($P>0.05$) similar for all levels of ascorbic acid supplementation. Higher daily sperm production was observed in the right testis of bucks fed dietary ascorbic acid supplementation compared to bucks fed the control diet. These results suggest that the testicular germinal epithelium was believed to be protected by ascorbic acid from oxidative stress. Degenerated testicular germinal epithelium due to oxidative stress was reported in scorbutic guinea pigs (7). Recent data suggest that ascorbic acid has defined functions in gamete biosynthesis and protection, gonadal tissue remodeling as well as an important component of the antioxidants in seminal plasma in many species including rabbits (15). Ascorbic acid also plays a vital role in the prevention/reduction of the oxidation of biomolecules induced by reactive oxygen species which cause great damage to sperm cell structures (16).

Conclusion and Application

1. Based on the results obtained from this study, it is concluded that ascorbic acid supplementation improved gonadal and extragonadal sperm reserves as well as daily sperm production and sperm production efficiency of rabbit bucks.

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