

EFFECT OF INCLUSION LEVEL OF VITAMIN C ON REPRODUCTION AND SEMEN QUALITY CHARACTERISTICS OF RABBITS IN A HOT CLIMATE

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ABSTRACT

The current study was conducted to determine the effect of inclusion of vitamin C on reproduction and semen quality characteristics of rabbits (Buck and Doe). A total of 32 growing rabbits aged 3 to 4 months, 16 buck and 16 doe were used in the study. The rabbits were randomly allocated to four (4) dietary treatments of 8 rabbits each per treatment and replicated four times with 2 rabbits per replicate in a Randomized Complete Block Design (RCBD). Vitamins C were included at 0, 100, 150 and 200mg/kg representing T₁, T₂, T₃ and T₄ respectively. All parameters considered under reproductive performance in this study for the first gestation period showed no significant ($P>0.05$) difference across the treatments. Doe mating weight and the doe kindling weight indicates significant ($P<0.05$) difference among the treatments. Similarly second gestation length showed no significant ($P>0.05$) difference across the treatments, except for doe mating weight, doe kindling weight and average litter weight at kindling were significant ($P<0.05$) across the treatments. Semen characteristic differ ($P<0.05$) significantly across the treatments, except for density of the semen which showed no significant ($P>0.05$) difference. It could therefore be concluded that vitamin C had no adverse effect on the rabbits, but rather have positive significant effect on reproduction and semen quality characteristics of buck and doe. It could therefore be recommended that vitamin C could be included in their diet up to 200mg/kg without detrimental effects.

Keywords: Reproduction, Semen, Rabbit Buck and Doe, Vitamin C, Reproduction

INTRODUCTION

Environmental condition such as heat stress, if pronounced could reduced reproductive performance of rabbits when the temperature-humidity index is elevated at 28°C and heat stress may found when the temperature-humidity index is up to 38.9°C. This manifest clearly in male rabbits as significant reduction in the semen quality such as decreases libido and biological semen characteristics, number of spermatozoa, motility, abnormality, acrosome integrity (Marai *et al.*, 2002). Reproduction is impaired as a result of the drastic changes in biological functions caused by heat stress. The use of antioxidant agent to improve fertility during environmental stress becomes necessary (Marai *et al.*, 2002). Dietary supplementation with vitamin C has been proved to be a simple and convenient strategy to introduce a natural antioxidant that may effectively inhibit the oxidation reactions

(Botsoglou *et al.*, 2004). It is a major chain-breaking antioxidant present both in seminal plasma and in the membrane, It reacts directly with free radicals such as the peroxy radical (ROO) yielding lipid hydro-peroxides which can be removed by phospholipase GSH-Px systems and interrupt the lipid peroxidation process, that is why it is called a chain breaking antioxidant (Wang *et al.*, 2002).

MATERIALS AND METHODS

Experimental Site

The study was conducted at rabbitry unit of Modibbo Adama University of Technology, Yola, Girei Local Government Area, Adamawa State. It is located within the Guinea Savanna Zone. It is between Latitude 9° and 11° North and Longitude 11° and 14° East. The climatic is tropical with distinct dry and wet season. The rainfall starts in April and ends around late

October, November to April marks the dry season. It has annual rainfall of about 700mm-1600mm and relative humidity that ranges from 5 to 42% and average maximum temperature of 39^oc (Adebayo and Tukur 2000).

Experimental Design and Management

Vitamins C were included at 0, 100, 150 and 200mg/kg representing T₁, T₂, T₃ and T₄ respectively. The feed composition was same across the treatments. The difference was just the varying levels of vitamin C inclusion at mg/kg. A Randomized Complete Block Design (RCBD) was used for the experiment. The study was conducted using a total of 32 growing rabbits

aged 3 to 4 months 16 does and 16 bucks. The rabbits were stabilized for a week, weighed and randomly allocated to four dietary treatments with eight rabbit per treatment with 4 replicates of 2 rabbits each. The experiment lasted for a period of three and half months. The does were introduced in to the pen of the buck only when mating is needed.

The rabbits were given anti-stress (vitalyte) supplement on arrival and housed in a well ventilated cages equipped with feeders and drinkers with clean water and feed was provided for a week to get them acclimatized with the new environment. Ivermectin and tetracycline injections were administered to treat for endo and ecto parasites.

Table 1: Ingredients Composition (%) and Calculated Analysis of Different levels of vitamin C

Levels of Vitamins C	T1(0.00mg/kg)	T2 (100mg/kg)	T3(150mg/kg)	T4(200mg/kg)
Ingredients				
Maize	22.3	22.3	22.3	22.3
Maize brand	16.0	16.0	16.0	16.0
Rice brand	39.0	39.0	39.0	39.0
Soya meal	19.0	19.0	19.0	19.0
Bone meal	3.0	3.0	3.0	3.0
Methionine	0.1	0.1	0.1	0.1
Lysine	0.1	0.1	0.1	0.1
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated Analysis				
ME kcal/kg	2703.98	2703.98	2703.98	2703.98
Crude protein	17.04	17.04	17.04	17.04
Crude fiber	14.0	14.0	14.0	14.0
Phosphorus	0.74	0.74	0.74	0.74
Lysine	0.94	0.94	0.94	0.94
Methionine	0.4	0.4	0.4	0.4

Vitamin premix 1.25kg contains; Vitamin A,10,000,000 IU; Vitamin D3, 2,000,000 IU; Vitamin E, 10,000mg; Vitamin K3,2,000mg; Folic Acid, 1,000mg; Naicin, 20,000mg; Calpan, 8,000mg;VitaminB2,5000mg; Vitamin B12, 15m; VitaminB1,1,500mg; VitaminB6,3,000mg; Biotin,50mg; Antioxidant, 125,000mg

Vitamin premix 1.25kg contains ; Cobalt 200mg; Selenium, 200mg; Iodine, 1,000mg; Iron,20,000mg; Manganese, 60,000mg; Copper,5000mg; Zinc, 50,000mg; Choline Chloride, 100,000mg

Data Collection

Reproductive Performance

Does fed the dietary treatments were used for the mating trial. Natural mating was carried out. Does were presented to a buck fed similar diet, minimum of two times to ensure successful mating at a ratio of 1 doe to 1 buck and the following parameters were taken. Weight of the does before mating and at parturition

Semen Sample Collection

Semen from the rabbit bucks were obtained from the epididymis. The rabbits were anesthetized using chloroform and the epididymis were removed completely and placed into a container with a wide bore containing 1.2mls lactate ringer's solution to maintain osmolality and preserves the spermatozoa until examination. The specimen is kept at ambient temperature of 37 °C, to avoid large changes in temperature that may affect the spermatozoa after collection. (Ahmed, 2013).

Semen Analysis

Sperm Motility

Sperm sample was placed on Neubauer chamber and progressive sperm motility was determined by the method reported by (Belsey *et al.*, 1980). The whole process was performed within 5 minutes following their isolation from caudal epididymis. Sperm motility was scored in 20 separate fields. First non-motile sperms were counted followed by motile sperms. Sperm motility was expressed as a percent of total sperm counted.

Sperm Viability

Sperm viability was estimated by assessing the membrane integrity of the cells. The percentage of live spermatozoa was assessed by identifying those with an intact cell membrane, from dye exclusion (Belsey *et al.*, 1980; Ahmed *et al.*, 2009). The ratio of live to dead spermatozoa was determined using 1% trypan blue solution by the method of (Talbot and Chacon, 1981). About 0.2 ml of sperm sample was incubated with 1% trypan blue stain for 10 min at 37°. A drop of the suspension was placed in a Neubaur haemocytometer chamber under a cover slip, allowed to settle for 1 minute and observed under an Olympus microscope. The number of stained (blue) and unstained (colourless) spermatozoa were scored in 20 separate fields. The spermatozoa, which were not

stained with trypan blue were considered as viable. Sperm viability was expressed as a percent of total sperm counted. The dye exclusion method is based on the principle that damaged plasma membranes, such as those found in non-vital (dead) cells, allow entry of membrane-impermeable stains. The hypo-osmotic swelling test presumes that only cells with intact membranes (live cells) will swell in hypotonic solutions (Srinivasulu and Changamma, 2017).

Sperm Numbers

The number of spermatozoa in the appropriate squares of the haemocytometer was counted under an Olympus microscope. The number of spermatozoa per ml epididymal plasma derived employing the following equation: $\text{sperm count} = \frac{\text{number of spermatozoa} \times \text{dilution factor} \times \text{depth factor}}{\text{number of areas counted}}$ (Belsey *et al.* 1980).

Semen pH

The pH of semen reflects the balance between the pH values of the different accessory gland secretions, mainly the alkaline seminal vesicular secretion and the acidic prostatic secretion. pH paper in the range 5.0 to 10.0 was used. The semen sample was thoroughly mixed; spread a drop of the semen evenly onto the pH paper. Wait for the color of the impregnated zone to become uniform (<30 seconds), the compared the color with the calibration strip of the pH meter (Ahmed *et al.*, 2013).

STATISTICAL ANALYSIS

All data generated were analyzed using statistical package for social science SPSS Version 24. Differences between the treatment means were separated using Duncan Multiple Range Test.

Proximate Analysis

The chemical analysis of the experimental diet was carried out to determine the dry matter (DM) content, crude protein (CP), crude fiber (CF), ether extract (EE), according to AOAC (2000) methods. The dry matter was determined by drying the samples at 80°C for 24hours until constant weight. The nitrogen free extract (NFE) was calculated using the values obtained from the proximate composition and metabolisable energy (ME) was calculated according to the procedure of Ponzenga (1985)

RESULT AND DISCUSSIONS

The proximate composition in Table 2 indicates that the crude fibre (CP) and crude protein (CF) contents of the diet were within the recommended range of 17-20% and 6-16 % as reported by Sheldon and Williams, (2000), while ether extract values fell within the range of 5-7 %

as reported by NRC (1996). The energy levels at the experimental diet were however higher than the 2500kcal/kg and 2900kcal/kg ME as recommended by Esonu (2006) for growing rabbits. All the diets met the dietary requirement of growing rabbits.

Table 2: Proximate Composition of the Experimental Diets

Nutrient	Composition
Moisture (%)	6.60
Dry matter (%)	93.2
Crude Protein (%)	17.02
Crude fiber (%)	14
Ash (%)	18.6
Nitrogen free extract (%)	35.28
Ether Extract (%)	8.5
Metabolizable energy (ME) (kcal/kg)	2814.98

ME(kcal/kg) = 37x CP (%) + 81.8 x EE (%) +35.5 x NFE (%) (Pauzenga, 1985)

Effect of Vitamin C Levels on Reproductive Performance of Rabbit Does

The result indicates the beneficial effects of ascorbic acid treatment on reproductive performance of does and their bunnies as compared to the control diet. The first and second gestation length considered in the study falls within 31-32days was the same with 31-32 days reported by Abu El-Hamd *et al.* (2013) and close to the range of 28-31days reported by Nguyen *et al.* (2006). Diet with various inclusion levels of vitamin C did not affect the gestation period for all groups. Conception rate were recorded higher in does fed various levels of vitamin C than the control in both the two gestation phase, the animals fed the control diet ,therefore requires more than one service to conceive, this is in line with the findings of Abu El-Hamd *et al.* (2013) who reported lower conception rate in control diets without vitamin C. The improved conception rate could probably be due to increase

in vitamin C concentration in their diet, thereby prevent tissue depletion (Naidu, 2003). As an effective antioxidant, has an important metabolic role as reducing agent and functions as an electron carrier, Neurons use ascorbic acid for many different chemical and enzymatic reactions, including the synthesis of neurotransmitters and hormones (Arrigoni and De-Tulio, 2000). The litter size at birth and at weaning and average litter weight at birth and at weaning shows no significant ($P>0.0$) difference across the treatment groups, although numerically the animals on vitamin c diet recorded higher values than the control groups, this might be due to its protective action against lipid oxidation in the cell membrane, it is important for newborns which exhibits a greater sensitivity to oxidative damage than adults, and for the development of the immune system in young animals (Debie *et al.*, 2005). The overall mortality rate during the suckling period was higher in control group.

Table 3: Effect of Various Inclusion Levels of Vitamin C on Does first Gestation Period

Vitamin C	0mg/kg	100mg/kg	150mg/kg	200mg/kg	
Parameters	T ₁	T ₂	T ₃	T ₄	SEM
DMW (g)	1016.20 ^c	1019.50 ^{bc}	1021.80 ^{ab}	1026.00 ^a	1.5*
1 st Gestation length (days)	31.00	32.00	31.50	31.50	0.2 ^{NS}
1 st conception rate	43.75	81.25	81.25	81.25	11.9 ^{NS}
Doe kindling weight (g)	1222.50 ^b	1322.80 ^a	1332.50 ^a	1349.50 ^a	13.1*
Litter size at kindling	1.75,m	2.00	2.50	3.50	0.5 ^{NS}
ALWAK (g)	26.25	27.00	33.00	36.25	4.3 ^{NS}
LSAW	0.75	1.25	1.25	2.75	0.6 ^{NS}
ALWAW (g)	176.25	256.50	283.75	395.25	85.2 ^{NS}

Table 4: Effect of Various Inclusion Levels of Vitamin C on Does Second Gestation Period

Vitamin C	0mg/kg	100mg/kg	150mg/kg	200mg/kg	
Parameters	T ₁	T ₂	T ₃	T ₄	SEM
DMW (g)	1234.80 ^c	1286.20 ^b	1319.50 ^{ab}	1336.80 ^a	12.7*
2 nd Gestation length (days)	32.00	32.00	31.75	31.5	0.2 ^{NS}
2 nd conception rate	93.75	93.75	100	100	4.6 ^{NS}
Doe kindling weight (g)	1342.00 ^c	1368.80 ^b	1381.20 ^b	1401.20 ^a	4.1*
Litter size at kindling	4.25	4.00	5.00	5.00	0.8 ^{NS}
ALWAK (g)	34.50 ^b	33.50 ^b	36.00 ^b	40.00 ^a	1.1*
LSAW	2.50	2.50	3.75	4.25	0.9 ^{NS}
ALWAW (g)	257.50	249.75	374.00	400.00	63.3 ^{NS}

KEY: a, b, c = Means within the same row bearing different superscripts differ significantly ($p < 0.05$), * = Significant ($P < 0.05$), NS = Not significant,

SEM = Standard Error of Mean, ALWAK = Average litter weight at kindling, LSAW = Litter size at weaning, ALWAW = Average litter weight at weaning, DMW = Doe mating weight

Effect of Inclusion Levels of Vitamin C on Semen Characteristics of Rabbit Buck

The study shows the beneficial effects of ascorbic acid treatment on semen characteristics of Rabbit Bucks. All the parameters were significantly ($P < 0.05$) different across the treatment groups except for sperm density. Active sperm ranged from 18.33 to 51.67 %, the highest value 51.67 % was presented in treatment four, while the least 18.33 % was indicated in treatment one, this could be due to antioxidant properties of Ascorbic Acid which is essential to maintain membranes and the genetic integrity of sperm cells by preventing oxidative damage to sperm DNA Yahaya *et al.* (2015). Non motility ranged from 20.00 to 41.67 %, the highest value was indicated in treatment one while the least was recorded in treatment four, followed by three and two, were also lower than the value in treatment one, which means motility was higher in other groups than the control group (0mg/kg vitamin c), this could be due to positive influence of Vitamin C by reducing excessive levels of reactive oxygen species which is prevalent during the hot dry season on spermatozoa motility, thereby increasing sperm motility and viability (Marai *et*

al., 2002), Asadpour *et al.* (2011) also reported that semen motility was significantly higher in vitamin C compared with the control group. Ezzat *et al.* (2011) found that supplementation of vitamin C in diets of animals under hot dry condition significantly increased sperm motility, though the effect appears to be variable with level of supplementation. Metwally and Fouad (2009) reported that the higher the vitamin C level of inclusion in diet the higher the sperm motility obtained. Hassan *et al.* (2012) reported an increase in sperm motility of rabbit bucks fed 250mg/kg vitamin C and low semen motility in bucks without vitamin C supplementation. Fazeli *et al.* (2010) reported a significant increase in sperm motility in animals fed both 20mg/kg and 40mg/kg vitamin C over the non-vitamin C treatment.

Semen viability, pH and Semen concentration all showed significant difference with there values similar to reports by (Khalil *et al.*, 2008, Fazeli *et al.*, 2010) . Yousef *et al.*, 2003 reported that addition of Vit C improved significantly ejaculate volume, pH, and motility of sperm, than control group in male rabbits and can alleviate the effect of thermal heat stress.

Table 5: Effect of Inclusion Levels of Vitamin C on Semen Characteristics of Rabbit Bucks

Vitamin C	0mg/kg	100mg/kg	150mg/kg	200mg/kg	
Parameter	T ₁	T ₂	T ₃	T ₄	SEM
Active %	18.33 ^c	26.67 ^{bc}	40.00 ^{ab}	51.67 ^a	3.98*
Slow %	40.00 ^a	36.67 ^{ab}	31.67 ^{bc}	28.33 ^c	1.59*
Non motile %	41.67 ^a	36.67 ^a	28.33 ^{ab}	20.00 ^b	3.89*
Viability %	33.33 ^b	36.66 ^b	43.33 ^{ab}	53.33 ^a	4.4*
Morphology (Normal %)	90.00 ^b	92.66 ^a	95.00 ^a	95.00 ^a	0.7*
Ph	6.500 ^b	6.66 ^{ab}	6.51 ^b	7.16 ^a	0.1*
Semen concentration (x10 ⁶ cell/ml)	3.17 ^b	6.80 ^a	6.66 ^a	7.90 ^a	0.9*
Semen density (cell/ejaculation)	86.66	224	277	371	103.4 ^{NS}

KEY: a, b, c = Means within the same raw bearing different superscripts differ significantly (P<0.05), * = Significant (P<0.05), NS = Not significant,

SEM=Standard Error of Mean

CONCLUSION

From the result of this experimental research, it was concluded that vitamin C had no adverse effect on the rabbits, but rather have positive significant effect on reproduction and semen quality characteristics of Buck and Doe Rabbits. Based on the result of these findings, vitamin C can be included in the diet of rabbits up to 200mg/kg in diets without any detrimental effect.

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