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SEMEN CHARACTERISTICS OF MONGREL RABBITS BUCKS (*Oryctolagus cuniculus*) ADMINISTERED CITRUS LIMON JUICE

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The study was conducted to assess the effect of oral administration of levels of Citrus limon juice on semen characteristics of mongrel rabbit bucks. The rabbits were randomly allocated into four treatments containing 0% (control), 25%, 50% and 75% fresh citrus lemon juice respectively. A total of 24 mongrel rabbit bucks were used for the study. The bucks were allotted to the four treatments (T1, T2, T3 and T4) with two rabbits per replicate and six rabbits per treatment. The trial lasted for 8 weeks. Effects of the administered levels of Citrus limon on semen characteristics were elicited on the results. Semen was collected using artificial vagina specially designed for rabbits using a doe as a teaser. The result revealed that the semen volume, sperm concentration and live sperm cells percentage differed significantly ($P < 0.05$) while sperm motility and abnormal sperm cells were not significantly affected ($P > 0.05$) by oral administration of levels of Citrus limon juice. Hence, Citrus limon juice can be administered orally up to 75% concentration to rabbit bucks without adverse effect on the semen characteristics.

Keywords: Citrus limon juice, Oxidative Stress, Antioxidant, Semen, mongrel.

Introduction

Citrus limon as a popular fruit has a significant role as a source of natural antioxidants. *Citrus limon* is the third most important species of citrus fruit after orange and mandarin. The presence of bioactive compounds, such as citric acid, ascorbic acid, minerals, flavonoids, carotenoid and essential oils content contribute to the value of *Citrus limon* in terms of it being associated with promoting good health (Hajimahmoodi *et al.*, 2012). *Citrus limon* is known to increase the blood levels of antioxidant enzymes which scavenge the Reactive Oxygen Species (Elwan *et al.*, 2019), thereby protecting the sperm cells against depletion caused by oxidative stress.

One of the most important factors contributing to poor quality semen has been reported to be oxidative stress (Bucak *et al.*, 2010). Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen derived oxidants commonly known as reactive oxygen species (ROS) (Sikka *et al.*, 1995). Uncontrolled production of ROS that exceeds the antioxidant capacity of the seminal plasma leads to oxidative stress (OS) which is harmful to spermatozoa (Desai *et al.*, 2010).

Dietary antioxidant supplements can enhance the physiological and productive statuses of animals by modifying metabolic processes. Antioxidants are the main defense factors against oxidative stress induced by free radicals (Sikka, 1996). Research studies have shown the usage of citrus pulp and peel meal as animal feedstuff without any side effect on the animals. Meanwhile, no significant studies have been carried out on the juice of citrus lemon to establish its beneficial effect and possible toxicity in rabbit models (Solomon *et al.*, 2015). Therefore, this study was carried out to determine the effect of *Citrus limon* juice on the semen characteristics of rabbit bucks.

Materials and Methods

Experimental animals and management

Twenty-four (24) clinically sound mongrel rabbits were purchased and used for this study. The rabbit bucks were allowed for two weeks of acclimatization period before the administration of the treatment (*Citrus lemon* juice). The rabbits were randomly divided into four treatment groups. Each treatment was replicated three times with two (2) rabbits per replicate in a 3-tier rabbit hutch. The experiment was carried out at the rabbitry unit of the Teaching and Research Farm



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of Animal Science Department, University of Uyo, Akwa Ibom State (Solomon *et al.*, 2015).

Experimental plan and Fresh Citrus lemon Juice Administration

Rough *Citrus limon* fruits were bought from a reliable source at Itam Market, Itu, Akwa Ibom state, Nigeria and used for this study. The *Citrus limon* fruits were peeled and the juice squeezed out into a clean container manually. The extract was filtered using a clean sieve and the filtrate collected into clean bottles. After 14 days of acclimatization, all rabbits were administered fresh *Citrus limon* juice orally corresponding to their group as follows for eight (8) weeks:

Treatment 1 – distilled water

Treatment 2 – 25% *Citrus limon* juice + 75% water

Treatment 3 - 50% *Citrus limon* juice + 50% water

Treatment 4 - 75% *Citrus limon* juice + 25% water

Semen Collection and Evaluation

The bucks were trained for semen collection during the acclimatization period and semen collection was done using a specially designed artificial vagina for rabbits using a doe as a teaser. The ejaculates obtained were evaluated as described by Zemjanis (1970). This included the visual or gross evaluation of the ejaculate immediately after collection for volume and colour as well as microscopic examination for motility, concentration, percentage live spermatozoa and morphological abnormalities.

Statistical Analysis

The experimental data was subjected to analysis of variance (ANOVA) procedure in a completely randomized design, using IBM Statistical Package for Social Science (SPSS) version 21. Differences between treatments means were separated using Duncan multiple Range Test (Duncan, 1955).

Results and Discussion

Semen Characteristics as Affected by *Citrus Limon* Juice in Rabbit Bucks

The results of Semen characteristics of rabbit administered levels of *Citrus limon* juice is shown on table 1. The result from the table indicates that semen characteristics of rabbit bucks were significantly ($P < 0.05$) affected by *Citrus limon* treatment, except sperm motility (%) and abnormal sperm cells (%) which were not significantly ($P > 0.05$) affected by *Citrus limon* juice.

The mean values of semen volume obtained in this study were 0.50ml, 1.20ml, 0.96ml and 0.60ml for T₁, T₂, T₃ and T₄, respectively. Rabbit bucks administered *Citrus limon* juice recorded higher semen volume than rabbit bucks in the control group (T₁ (0.50ml)). The semen volume observed in this study was within the range (0.50 to 0.66ml) reported by Ogbuwu (2008) for rabbits fed graded levels of Neem (*Azadirachta indica*) leaf meal.

Although there was no significant ($P > 0.05$) difference in sperm motility of bucks across the treatment groups, rabbit bucks administered 50% (T₃ (76.67%)) *Citrus limon* juice recorded the highest value of sperm motility while rabbit bucks administered 25% (T₂ (67.00%)) *Citrus limon* juice recorded the lowest value of sperm motility. The results on sperm motility were below the range (77.00 to 81.00%) reported by Abu *et al.* (2013). Brackett (2004) reported sperm motility of 70% and above for good quality fresh sperm. Pineda (2003) advocated a low score of 30% as a minimally acceptable spermatozoa motility level for fresh ejaculates for breeding organizations. This indicates that sperm motility values in this study were not adversely affected by treatment with *Citrus limon* juice due to the presence of antioxidants such as vitamin C which reduces oxidative stress.

Semen colour for T₁, T₂, T₃ and T₄ appeared milky. According to Campos *et al.* (2014), the ejaculate is mostly milky-white, but the best quality is found in creamy-white semen. Thus, the milky colour observed in this study indicates citric acid present in *Citrus limon* up to 75% of oral administration did not cause inflammation or malfunctioning of the reproductive tract or accessory sex organs and glands.



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The mean sperm concentration obtained in this study were $107.00 \times 10^6/\text{ml}$, $123.66 \times 10^6/\text{ml}$, $108.00 \times 10^6/\text{ml}$ and $125 \times 10^6/\text{ml}$ for T₁, T₂, T₃, and T₄ respectively and were not significantly different ($P>0.05$). The result of sperm concentration was within the range (50 to $350 \times 10^6/\text{ml}$) reported by Brackett (2004) for

rabbit bucks. The range of values observed for live cells in this study, were similar to those of Abu *et al.* (2013) who reported 83.00 to 90.00% for live sperm.

Live sperm cells obtained in this study were 84.00%, 88.59%, 78.00% and 81.65% for T₁, T₂, T₃, and T₄ respectively.

Table 1: Semen Characteristics as Affected by *Citrus Limon* Juice in Rabbit Bucks

PARAMETER	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	SEM*
Semen Volume (ml)	0.50 ^b	1.20 ^a	0.96 ^{ab}	0.60 ^b	0.11
Semen Colour	Milky	Milky	Milky	Milky	
Sperm Motility (%)	72.67	67.00	76.67	76.63	2.19 ^{NS}
Sperm Concentration ($\times 10^6/\text{ml}$)	107.00 ^b	123.66 ^a	108.00 ^b	125.00 ^a	3.08
Live Sperm Cells (%)	84.00 ^b	88.59 ^a	78.00 ^c	81.65 ^b	1.26
Abnormal Sperm cells (%)	12.67	10.33	12.67	10.67	0.54 ^{NS}

a,b,c: means in the same row with different superscripts differ significantly ($P<0.05$); ns= Non significant different ($P>0.05$).

Rabbit bucks administered 25% (T₂ (88.59%)) *Citrus limon* juice recorded the highest live sperm cells. The significant increase in live sperm cells at T₂ suggests viable spermatozoa and possibly higher fertilizing capacity in the rabbit bucks.

Although there were no significant ($P>0.05$) differences in abnormal sperm cells across the different treatment groups, rabbit bucks administered 0% and 50% *Citrus limon* juice recorded the same value of 12.67% and the highest value of abnormal sperm cells while rabbit bucks administered 25% (T₂ (10.33%)) *Citrus limon* juice recorded the lowest value of abnormal sperm cells. The abnormal sperm values obtained in this study were within the values reported by Kuzminsky *et al.* (1996). Ogbuewu (2008) opined that the higher the number of abnormal cells, the lower the percentage of motile sperm cells. The above results suggest that citrus *Citrus limon* juice supplementation may support normal spermatogenesis of rabbit bucks across treatments.

Conclusion

The results obtained in this study suggests that rabbits fed *Citrus limon* juice appeared generally healthy as most semen characteristics were within

the normal range for rabbits suggesting that inclusion of citrus lemon juice up to 75% does not have any negative effect on semen characteristics of rabbit bucks.

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