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Semen characteristics and reproductive performance of rabbit bucks administered carrot seed powder

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Abstract

Semen quality and quantity can adversely be affected by nutrition. The use of biomolecules containing antioxidants and nutrients essential for growth and reproduction in rabbits have been established. However, information on the effect of carrot seed meal (CSM) on semen qualitative and quantitative characteristics of rabbit bucks is scanty and thus investigated. A 10-week investigation was conducted in order to assess the effects of orally administered CSM on reproductive performance and semen characteristics of rabbit bucks. A total of 24 mixed breed, adult rabbit bucks were randomly allotted to four treatments with six replicates each (T1-control, T2-0.2mg CSM/kg body weight, T3-0.4mg CSM/kg body weight and T4-0.6mg CSM/kg body weight) in a completely randomised design. On weeks 8, 9 and 10, semen samples were collected from replicate bucks for semen quality assessment using standard procedures. Data were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. *Semen colour and mass activity were not influenced by CSM levels. Sperm cell livability was higher in bucks on T3 (100.00±0.00 %), T4 (100.00±0.00%) 'and T2 (98.67±2.31%) compared to T1 (94.67±1.53%). Spermatozoa motility observed at weeks 9 and 10 was significantly (P<0.05) lower in bucks in the control group (80.33±1.53% and 68.00±10.00% respectively compared to those on CSM administration. Epididymal weight and epididymal sperm reserves were significantly (P<0.05) higher in bucks on T2, T3 and T4 than bucks on the control (T1). The average paired weights (125.83±29.51g) and sperm reserves (*$9.43\pm2.21\times10^{8}$*) of epididymis of bucks administered 0.40mg CSM were the highest among the treatments. Testicular weight, sperm reserves per gram testis, daily sperm production and sperm production efficiency were not significantly (P>0.05) affected by varying levels of CSM. Reaction time observed in control bucks (11.50±3.55 seconds) was significantly higher compared to those on CSM administration. However, libido score was apparently lower in control bucks (8.00±1.83) compared to bucks administered CSM. Highest conception rate of 75% was obtained in rabbit does mated with T3 (75.00±6.25%) compared to other treatments. A 0.40mg carrot seed meal administered to rabbit bucks enhanced spermatozoa motility, livability, libido score and fertility.*

Keywords: Carrot seed meal, Conception rate, Rabbit bucks, Rabbit does Spermatozoa motility and Livability.

Caractéristiques du sperme et performances de reproduction des lapins mâles ayant reçu de la poudre de graines de carotte

Résumé

La qualité et la quantité de sperme peuvent être affectées négativement par la nutrition. L'utilisation de biomolécules contenant des antioxydants et des nutriments essentiels à la croissance et à la reproduction chez le lapin a été établie. Cependant, les informations sur l'effet de la farine de graines de carotte (FGC) sur les caractéristiques qualitatives et quantitatives du sperme des lapins mâles sont rares et donc étudiées. Une enquête de 10 semaines a été menée afin d'évaluer les effets du FGC administré par voie orale sur les

performances de reproduction et les caractéristiques du sperme des lapins mâles. Un total de 24 lapins mâles adultes de races mixtes ont été répartis au hasard dans quatre traitements avec six répétitions chacun (témoin T1, T2-0,2 mg de FGC/kg de poids corporel, T3-0,4 mg de FGC/kg de poids corporel et T4-0,6 mg de FGC /kg de poids corporel) dans une étude entièrement randomisée. Aux semaines 8, 9 et 10, des échantillons de sperme ont été prélevés sur des boucs répétés pour l'évaluation de la qualité du sperme en utilisant des procédures standard. Les données ont été analysées à l'aide de statistiques descriptives et d'ANOVA à a 0,05. La couleur et l'activité de la masse du sperme n'ont pas été influencées par les niveaux de FGC. La vivabilité des spermatozoïdes était plus élevée chez les mâles en T3 (100,00 ± 0,00 %), T4 (100,00 ± 0,00 %) et T2 (98,67 ± 2,31 %) par rapport à T1 (94,67 ± 1,53 %). La motilité des spermatozoïdes observée aux semaines 9 et 10 était significativement (P < 0,05) plus faible chez les mâles du groupe témoin (80,33 ± 1,53 % et 68,00 ± 10,00 % respectivement par rapport à ceux sous administration de FGC. Le poids de l'épididyme et les réserves de sperme de l'épididyme étaient significativement (P <0,05) plus élevé chez les boucs en T2, T3 et T4 que chez les boucs du témoin (T1).Les poids appariés moyens (125,83±29,51g) et les réserves de sperme (9,43±2,21×108) de l'épididyme des boucs ayant reçu 0,40mg de FGC étaient le plus élevé parmi les traitements. Le poids testiculaire, les réserves de sperme par gramme de testicule, la production quotidienne de sperme et l'efficacité de la production de sperme n'étaient pas significativement (P>0,05) affectés par les différents niveaux de FGC. Le temps de réaction observé chez les mâles témoins (11,50 ± 3,55 secondes) était significativement plus élevé par rapport à ceux sous administration de FGC Cependant, le score de libido était apparemment inférieur chez les mâles témoins (8,00 ± 1,83) par rapport aux mâles ayant reçu du FGC Le taux de conception le plus élevé de 75 % a été obtenu chez les lapines accouplées avec T3 (75,00 ± 6,25 %) composition sont assujettis à d'autres traitements. Une farine de graines de carotte de 0,40 mg administrée à des lapins mâles a amélioré la motilité, l'habitabilité, le score de libido et la fertilité des spermatozoïdes.

Mots-clés : Farine de graines de carotte, taux de conception, lapins, la motilité et habitabilité des spermatozoïdes de lapin.

Introduction

Rabbit has been widely adopted as a species of choice by researchers as animal model for experimental research and also by many famers for rearing in order to meet the increasing demand for animal protein. Rabbit nutrition is a key factor in growth performance and reproduction as it complements the expression of the hereditary make-up for attaining maximum growth which in turn increase farmer profit. Oyedipe *et al. (*1982); Vincent *et al. (*1985) reported that nutrition exerts great effects on reproductive performance of farm animals.

Several medicinal plants have been used as dietary adjuncts and in the treatment of numerous diseases without proper knowledge on how they affect other body systems. Carrot (*Daucus carota)* was said to be among the medicinal plants which affected male and female fertility (Al-Snafi, 2016). It belongs to the family 'Apiaceae' and also one of the important root vegetables with high antioxidant capabilities. Over the years, pharmacological studies have shown that carrot seeds exhibit antifertility properties in females (Majumder *et al*., 1997; Keenan *et al*., 1997). Pandiarajan *et a*l. (2012) reported that the petroleum ether extract and fatty acids of carrot seeds were able to arrest the normal estrus cycle of adult mouse and reduced the weight of ovaries significantly. Nouri *et al*. (2009) also reported that the extract could protect the

reproductive system against gentamicin-induced toxicity and induced spermatogenesis possibly through elevating testosterone levels in male rat.

Carrot seed extracts and its essential oil have been reported in experimental studies to have cardio- and hepato-protective, cognitive dysfunction, cholesterol lowering, anti-bacterial, anti-fungal, anti-inflammatory, analgesic, and wound healing benefits (Silva Dias, 2014). Also, ethnobotanical investigations have documented the use of carrot seed as a method of fertility control by women in India (Maurya *et al*., 2004)

With references to previous researches, carrot seed has been tested to ascertain the possibilities of suppressing fertility in some female animals. However, little has been reported concerning its effects on reproduction in male animals. Therefore, the present study was designed to investigate the effect of carrot seed meal on reproductive performance and semen characteristics in rabbit bucks.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Rabbit Unit of the Teaching and Research Farm and the Physiology Laboratory of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria. The site is located on the latitude 7° 20‟N and longitude 3° 50‟E, southwestern agro-ecological zone of Nigeria with annual rainfall between 1200mm and 1900mm.

Experimental Animals Design and Management

A total of 24 sexually mature rabbit bucks of mixed breed (New Zealand white, Dutch and Chinchilla) with average weight of 1.79kg were used. The rabiits were randomly distributed into four treatments (Table 1) of different levels of carrot seed meal Each treatment was allotted six animals in a completely ramdomised design. The rabbits were housed individually in a well ventilated wooden hutch-box. The experiment lasted for 10 weeks and the bucks were provided with drinking water and feeds ad-libitum. All rabbits were kept under necessary management practices and hygienic condition throughout the duration of the experiment.

Administration of Carrot Seed Meal (CSM) and Semen Collection

Dried carrot seeds were ground into powder using a milling machine and stored in an air-tight container at room temperature. The mixture of a known weight of CSM and distilled water was orally administered to the bucks by drenching with the aid of syringe at every-other day interval. Two weeks prior to semen collection, the rabbit bucks were trained to serve an Artificial vagina and it was used in collecting semen in the 8^{th}, 9^{th} and 10^{th} week of the experiment with the aid of a teaser doe.

Semen Characteristics

Volume of semen for each buck was measured using tuberculin syringe to the nearest 0.1 mL. The mass activity was scored subjectively according to the intensity of the wave motion seen in the medium by the collective activities of spermatozoa, from the absence of wave motion (+) to very turbulent motions (+++) as described by Jimoh and Ewuola. (2018). *Sperm cell livability* was determined as described by (Esteso *et al.,* 2006). A thin smear of semen stained with eosin-nigrosin stain was examined under light microscope. The live spermatozoa appear to be transparent while the dead cell absorbed the stain.

Sperm motility was assessed by placing a drop of individual semen and a drop of diluent (sodium citrate) with the aid of micropipette on a pre-warmed microscope slide, then covered with a cover side before it was examined under a light microscope of ×400 magnification. At least 3 microscopic

Table 1.Treatment layout showing treatment group and dosage of carrot seed received per group

Treatment	CSM Dose (mgkg) of Buck
1 (control)	0.0
2	0.2
3	0.4
4	0.6

CSM: Carrot seed meal

fields were examined for each semen sample and progressive motility was estimated in percentage as described by Ewuola and Egbunike (2010). The Sperm cell concentration was estimated by the direct sperm cell count method using an improved Neubauer haemocytometer slide. Formal saline (1%) was used to dilute to immobilize the cells and was subsequently enumerated under a light microscope. The concentration of spermatozoa per volume was determined using the standard formula of Hafez (1985).

$C = 50{,}000 \times N \times D$, where C is the concentration of the sperm cell per milligram of the semen, N is the number of spermatozoa counted and D is the dilution factor.

Fertility, Reaction Time and Libido Evaluation

In the 8 week, three bucks were randomly selected from each treatment and were allowed to mate two does successfully, the resulting conception was used to adjudge the effect of CSM on fertility of bucks. During the 8th week of the experiment, the reaction time was observed from the time the rabbit doe was introduced to the buck for sniffing and grooming to the time of mounting with the use of a stopwatch. While the libido was scored as the number of times the buck attempted to mount the doe within a minute as described by Chibundu (2005).

Determination of Gonadal and Extra-Gonadal Sperm Reserves.

At the terminal of the experiment, three bucks were sacrificed from each treatment and their reproductive tracts were dissected. The testes and epididymis were carefully harvested and weighed. The right and left of the testis and the epididymis were homogenized separately in 3 mL normal saline solution and then filtered through a doubled layer cheese cloth into a clean glass test tubes. The resulting filtrate was further diluted into ratio 1:20 with normal saline according to the method of (Igboeli and Rakha, 1971; Egbunike *et al*., 1975). The sperm cell concentrations therein determined by direct haemocytometric count.

Determination of daily sperm production (DSP)

The daily sperm production was estimated from the testicular sperm reserves. The DSP of the rabbits was therefore calculated with the formula proposed by Amann (1970) as follows:

$$DSP = \frac{\text{Testicular Sperm Count}}{\text{Time Divisor (3.43)}}$$

Statistical Analysis

All data were subjected to descriptive statistics and one-way analysis of variance (ANOVA) using Statistical Analysis System software version 9.3 (SAS, 2011). The means were separated using Duncan Multiple Range Test of the same software.

Results and Discussion

Table 2: Semen Characteristics of Rabbit Bucks administered CSM at week 8

	Treatments				
	T1	**T2**	**T3**	**T4**	**SEM**
Parameters	**(0.0mg CSM)**	**(0.2mg CSM)**	**(**0.4mg **CSM)**	**(0.6mg CSM)**	
Volume (mL)	0.27±0.12^{ab}	0.47±0.17^{a}	0.33±0.07^{ab}	0.19±0.45^{b}	0.41
Colour	Milky white	Milky white	Milky white	Milky white	
Motility (%)	80.33±1.53^{b}	93.00±2.65^{a}	95.00± 0.00^{a}	95.00± 0.00^{a}	1.88
Mass Activity	+++	+++	+++	+++	
Livability (%)	98.00 ± 1.73	99.33 ± 1.15	98.67 ± 2.31	100.00 ±0.00	0.44
SP Conc. ($\times10^8$/mL)	5.15±3.73^{b}	5.98 ± 1.05^{ab}	6.91±2.75^{a}	7.06±1.38^{a}	2.72

$^{a\,b}$ Means with different superscript in the same row are significantly different (P<0.05)
SP Conc. = Sperm concentration

Presented in Table 2 are Semen characteristics of rabbit bucks administered CSM at week 8. The CSM did not have significant effect on semen colour and progressive mass activity throughout the weeks of assessment. However, the mean values for spermatozoa livability and motility of rabbit bucks were significantly (P<0.05) lower in the control group T1 than those in treatments T2, T3 and T4, respectively. The spermatozoa concentration of rabbit bucks was observed to be significantly (P<0.05) higher in bucks on T3 and T4 than bucks in T1 (control group) and T2 as shown in Tables 3 and 4. The reaction time was apparently shorter in rabbit bucks administered CSM, while libido score was not significantly influenced among the treatments (Table 5). The does mated with bucks on T3 recorded the higher (75%) conception rate, T4 had 50% conception rate while each of T1 and T2 had 25% conception rate as shown in Figure 1.

Table 3: Semen characteristics of rabbit bucks administered CSM at week 9

	T1	**T2**	**T3**	**T4**	**SEM**
	(0mg CSM)	**(0.2mgCSM)**	**(**0.4mg**CSM)**	**(0.6mg CSM)**	
Parameters					
Volume (mL)	0.44± 0.23^{ab}	0.39 ± 0.82^{ab}	0.20±0.00^{b}	0.48±0.69^{a}	0.44
Colour	Milky white	Milky white	Milky white	Milky white	
Motility (%)	82.00±2.64^{b}	93.33±3.06^{a}	95.00±0.00^{a}	94.33± 0.58^{a}	1.68
Mass Activity	+++	+++	+++	+++	
Livability (%)	94.67±1.53^{b}	98.67±2.31^{a}	100.00±0.00^{a}	100.00±0.00^{a}	0.75
Spermatozoa Conc ($\times10^8$/mL)	7.24 ± 5.40	8.47 ± 5.40	7.74 ± 7.65	8.04± 1.02	0.27

$^{a\,b}$ Means with different superscript in the same row are significantly different (P<0.05)

Table 4: Semen characteristics of rabbit bucks administered CSM in week 10

	T1	**T2**	**T3**	**T4**	SEM
Parameters	**(0mg CSM)**	**(0.2mg CSM)**	**(0.4mg CSM)**	**(0.6mg CSM)**	
Volume (mL)	0.44±0.12	0.42±0.14	0.36±0.19	0.23±0.95	0.43
Colour	Milky white	Milky white	Milky white	Milky white	
Motility (%)	68.00±10.00^{b}	88.00±5.29^{a}	91.00±3.60^{a}	94.33±1.15^{a}	3.42
Mass Activity	+++	+++	+++	+++	
Livability (%)	97.00±2.64	98.33±1.53	98.67±2.31	100.00±0.00	0.57
Sperm Conc ($\times10^8$/mL)	6.18±2.24^{b}	6.26±1.61^{b}	7.03± 2.67^{a}	7.06±3.88^{a}	1.42

$^{a\,b}$ Means with different superscript in the same row are significantly different (P<0.05)

Table 5: Reaction Time and Libido Score of rabbit bucks administered varied levels CSM

Parameters	T1 (0 mg CSM)	T2 (0.2mg CSM)	T3 (0.4mg CSM)	T4 (0.6mg CSM)	SEM
Reaction Time (seconds)	11.50±3.55[a]	6.46±1.71[b]	6.15±2.22[b]	7.55±3.09[ab]	0.82
Libido Score (mounts/minute)	8.00±1.83	11.21±4.12	10.67±1.47	11.83±4.87	0.85

[a b-] Means with different superscript in the same row are significantly different (P<0.05)

Daily sperm production, testicular and epididymis sperm reserves of rabbits administered CSM are shown in Table 6. The weights of testes as well as the testicular sperm reserves were not significantly influenced by the treatments. However, the weights of epididymis and sperm reserves in the epididymis of bucks in the control treatment were significantly (P<0.05) lower to those administered CSM, wherein bucks on T3 had the highest paired epididymis weight and paired epididymal sperm reserve ($125.83\times10^{8/}$g/ml and $9.43\times10^{8/}$mL) respectively. The daily sperm production and sperm production efficiency (SPE) were not significantly affected by the treatments. The sperm count is considered to be an important parameter with which to assess the effects of chemicals on spermatogenesis (Suryavathi *et al*., 2005; Reddy *et al*., 2006).

The results of present findings corroborate the report of Nouri *et al*. (2009) who established the effect of carrot seed extract on spermatogenic activity and the number of developing germ cells which were increased after exposure to carrot seed extract indicating the positive effect of the extract on meiosis which intends increases the spermatozoa counts. Oyeyemi and Okendran (2007) also stated that high concentration of spermatozoa is an indication of possible high fertility rate by increase in the number of spermatozoa available at the time of copulation or insemination. Fertility could be measured for the male by the percentage of successful mating resulting in conception (Devendra and Burns, 1979). The fertility of bucks was observed to be enhanced by CSM, with bucks on T3 recording the highest percentage fertility.

The reaction time was significantly influenced by CSM, bucks on T1 had the highest reaction time while those on T3 had the lowest reaction time across the treatments However, the libido score of bucks was not significantly influenced by the treatments but it was observed to have a relatively inverse relationship with the reaction time. Bucks on control with the highest reaction time had the least libido score compared to the bucks on 0.4mg CSM with the lowest reaction time but had a higher libido score. This result also corroborates the findings of Ahmad *et al*. (2005) who concluded that a bull exhibiting higher libido may carry better semen quality which is a desirable characteristic of an animal for a successful breeding programme.

From the present study, administration of carrot seed extract led to a significant elevation in epididymal sperm reserve. This increment could be as a result of an increase in testicular spermatogenesis which in turn may be indicative of an elevation in the number of differentiated germ cells. The elevation in epididymal sperm reserved observed in this study could be attributed to the antioxidant properties of carrot seed extract, although the role of other factors such as male hormones cannot be ruled out. This corroborate with the findings of (Huynh *et al*., 2000) who reported that there is a direct correlation between both the epididymal sperm count and motility and fertility in animals.

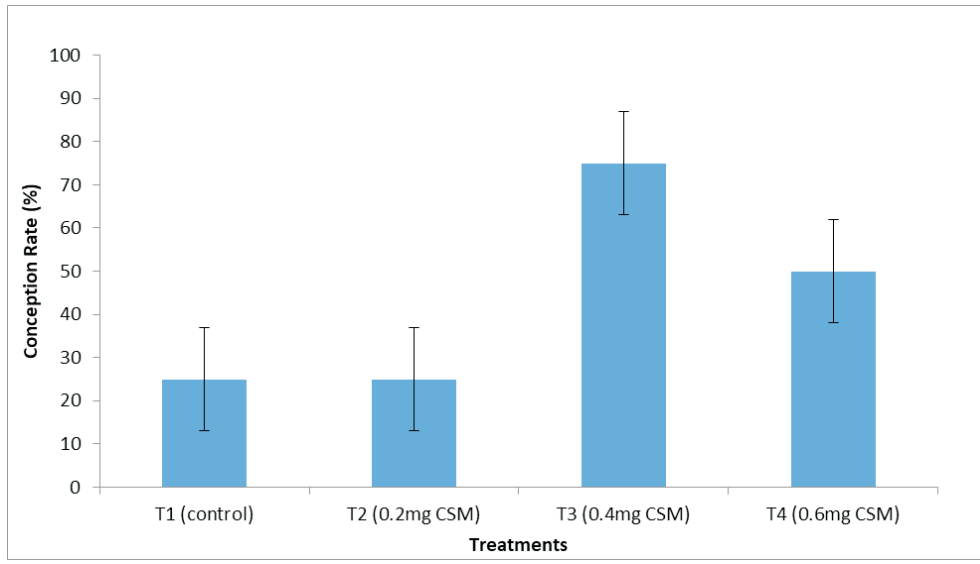

Figure 1:Conception rate of rabbit does mated with bucks administered varied levels of CSM

Table 6: Daily sperm production, gonadal and extra-gonadal assessment of bucks administered varied level of CSM

Parameters	T1 (0 mg CSM)	T2 (0.2mg CSM)	T3 (0.4mg CSM)	T4 (0.6mg CSM)	SEM
Testicular Weight (g)					
Right Testis	1.49±0.27	1.64±0.19	1.58±0.27	1.59±0.41	0.07
Left Testis	1.64±0.17	1.71±0.36	1.59±0.05	1.60±0.44	0.07
Paired Testis	3.13±0.45	3.35±0.55	3.18±0.32	3.19±0.84	0.14
Epididymis Weight (g)					
Right Epididymis	30.40±6.75^{b}	59.83±1.15^{a}	72.00±16.03^{a}	59.50±13.61^{a}	5.35
Left Epididymis	30.56±6.75^{b}	47.67±3.88^{a}	53.83±13.47^{a}	45.50±5.76^{a}	3.28
Paired Epididymis	60.97±11.3^{b}	107.50±4.77^{a}	125.83±29.51^{a}	105.00±8.54^{a}	8.26
Epididymal Sperm Reserve ($\times 10^{8}$)					
Left Epididymis	2.30±0.51^{b}	3.58±0.29^{a}	4.03±1.01^{a}	3.47±0.13^{ab}	0.25
Right Epididymis	2.28±0.50^{b}	4.49±0.87^{a}	5.40±1.02^{a}	4.46±1.02^{a}	0.40
Paired Epididymis	4.57±0.85^{b}	8.06±0.35^{a}	9.43±2.21^{a}	7.87±0.64^{a}	0.62
Testicular Sperm Reserve ($\times 10^{8}$)					
Left Testis per g Testis	0.67±0.13	0.48±0.09	0.93±0.62	0.37±0.07	0.10
Right Testis per g Testis	0.56±0.38	0.90±0.61	0.38±0.10	0.32±0.12	0.11
Paired Testis per g Testis	1.24±0.28	1.38±0.67	1.32±0.73	0.69±0.19	1.16
DSP Testis ($\times 10^{8}$)	1.14±0.54	1.78±1.24	1.07±0.58	0.65±0.09	0.22
SPE (DSP per g Testis ($\times 10^{8}$)	0.76±0.35	1.04±0.63	0.66±0.28	0.43±0.14	0.11

$^{a\ b-}$ Means with different superscript in the same row are significantly different ($P<0.05$)
DSP= Daily Sperm Production; SPE= Sperm Production Efficiency

Conclusion and Recommendation

Administration of carrot seed meal improved the reproductive performance, semen quality and fertility of rabbit bucks. Rabbits bucks administered CSM had significant improvement in the spermatozoa concentration, livability, motility and epididymal sperm reserves when compared to the bucks on the control group. Among the treated bucks, the highest fertility as well as the epididymal sperm reserve were observed in bucks on 0.4mg CSM. Reaction time observed in control bucks was significantly higher compared to those on CSM-supplemented diets. However, libido score was lower in control bucks compared to bucks administered CSM. Therefore, administration of CSM up to 0.4mg per body weight of rabbit bucks can improve the quality of semen with higher and more stable fertilizing ability.

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