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Effect of graded dietary levels of Roselle (*Hibiscus sabdariffa*) calyx meal supplementation on sperm profile of rabbit bucks

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Abstract

Oxidative stress has been identified as one of the factors contributing to poor quality semen. Antioxidants however have been found useful in reducing oxidative stress thus protecting spermatozoa from reactive oxygen species (ROS)-induced production of abnormal spermatozoa and prevent deoxyribonucleic acid (DNA) fragmentation, thereby, improving semen quality. Roselle calyx has been identified as a rich source of antioxidants prominent among which is vitamin C (ascorbic acid). This experiment was conducted to evaluate the effect of graded dietary levels of Roselle calyx meal supplementation on sperm profile of mongrel rabbit bucks. Twenty clinically screened mongrel rabbit bucks were used for the study. The rabbits were managed intensively and were provided with feed, water and forages ad-libitum. Four experimental diets were formulated to contain dietary levels of Roselle calyx meal at 0.0% (control), 2.0%, 4.0% and 6.0% and coded as T_1, T_2, T_3 and T_4 respectively. The four treatment groups were assigned to four experimental diets in a completely randomized design (CRD) each replicated three times with two rabbits per replicate. Each replicate received an assigned diet for 56 days. Parameters measured in the were semen volume, semen colour motility, sperm concentration, live sperm percentage, abnormal sperm cells percentage, total sperm cells per ejaculate and reaction time. The semen volume (mL) recorded in this study were 0.47, 1.73, 0.57 and 0.53 for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM,) respectively. Sperm motility was higher in T_2 (2.0% RCM) group with a value of 82.6% while the control group (0.0% RCM) recorded the least value of 68.6%. Rabbit bucks in T_3 (4.0% RCM) and T_4 (6.0% RCM) had higher sperm motility values of 78.3% and 81.6% respectively above those in control group. From the findings of this study Roselle calyx meal supplementation at 2.0% improved sperm characteristics of mongrel rabbit bucks.

Keywords: Roselle, Sperm Profile, Bucks, Mongrel

Effet de l'apport alimentaire progressif de farine de calice de roselle (Hibiscus sabdariffa) sur le profil spermatique de lapins mâles

Résumé

Le stress oxydatif a été identifié comme l'un des facteurs contribuant à la mauvaise qualité du sperme. Les antioxydants se sont toutefois révélés utiles pour réduire ce stress oxydatif, protégeant ainsi les spermatozoïdes de la production de spermatozoïdes anormaux induite par les espèces réactives de l'oxygène (ERO) et empêchant la fragmentation de l'acide désoxyribonucléique (ADN), ce qui améliore la qualité du sperme. Le calice de roselle a été identifié comme une source riche en antioxydants, parmi lesquels la vitamine C (acide ascorbique) occupe une place prépondérante. Cette expérience a été menée afin d'évaluer l'effet de différents niveaux d'apport alimentaire en farine de calice de roselle sur le profil spermatique de lapins mâles de race croisée. Vingt lapins mâles de race croisée, ayant subi un dépistage clinique, ont été utilisés pour cette étude. Les lapins ont été élevés en élevage intensif et ont reçu de la nourriture, de l'eau et du fourrage à volonté. Quatre régimes alimentaires expérimentaux ont été formulés pour contenir des teneurs en farine de calice de roselle de 0,0 % (témoin), 2,0 %, 4,0 % et 6,0 %, et ont été codés respectivement T1, T2, T3 et T4. Les quatre groupes de traitement ont été affectés aux quatre régimes alimentaires expérimentaux selon un plan d'essai complètement aléatoire (CRD), chacun étant répété trois

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fois avec deux lapins par répétition. Chaque réplication a reçu le régime alimentaire qui lui était attribué pendant 56 jours. Les paramètres mesurés dans le cadre de cette étude étaient le volume de sperme, la couleur du sperme, la motilité, la concentration en spermatozoïdes, le pourcentage de spermatozoïdes vivants, le pourcentage de spermatozoïdes anormaux, le nombre total de spermatozoïdes par éjaculat et le temps de réaction. Les volumes de sperme (mL) enregistrés dans cette étude étaient respectivement de 0,47, 1,73, 0,57 et 0,53 pour les groupes T1 (0,0 % de RCM), T2 (2,0 % de RCM), T3 (4,0 % de RCM) et T4 (6,0 % de RCM). La motilité des spermatozoïdes était plus élevée dans le groupe T2 (2,0 % de RCM), avec une valeur de 82,6 %, tandis que le groupe témoin (0,0 % de RCM) a enregistré la valeur la plus faible, soit 68,6 %. Les mâles de lapin des groupes T3 (4,0 % de farine de calice de roselle) et T4 (6,0 % de farine de calice de roselle) présentaient des valeurs de motilité des spermatozoïdes supérieures à celles du groupe témoin, s'élevant respectivement à 78,3 % et 81,6 %. D'après les résultats de cette étude, une supplémentation en farine de calice de roselle à hauteur de 2,0 % a amélioré les caractéristiques spermatiques des mâles de lapin croisés.

Mots-clés : Roselle, profil spermatique, mâles, lapins croisés

Introduction

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). Gametes are susceptible to reactive oxygen species (ROS) attack (Bansal and Bilaspuri, 2011). Several factors are known to affect semen quality, one of which is level of antioxidants such as vitamins C and E. Antioxidants are compounds capable of scavenging and inhibiting the formation of reactive oxygen species (ROS) (Palani and Alahmar, 2020). ROS have critical role in male reproductive function, and these active molecules are required in small quantities for normal sperm function (Hsieh, 2006), while high quantities could decrease semen quality and impair sperm fertilization capability (Kefer *et al.*, 2009). Hence, antioxidants are critical to inactivate ROS continuously to maintain only the small amount required for normal sperm function (Agarwal and Saleh, 2002).

Studies have shown that antioxidants protect spermatozoa from ROS-induced production of abnormal spermatozoa by scavenging ROS produced by leukocytes, prevent deoxyribonucleic acid (DNA) fragmentation, improve semen quality, reduce cryodamage to spermatozoa, block premature sperm maturation and stimulate spermatozoa (Fraga *et al.*, 1991). Low seminal plasma antioxidants have also been found to be involved in reducing sperm concentration, motility and morphology (Roychoudhury *et al.*, 2016).

Vitamin C is considered a major antioxidant in the testis (Augustine *et al.*, 2005). Vitamin C (ascorbic acid) as an antioxidant has been associated with fertility (Millar, 1992) and has also been known to play significant roles in reproduction in several other mammalian species (Luck *et al.*, 1995). It neutralizes ROS and prevents sperm agglutination (Dawson *et al.*, 1992). Vitamin C is present at a high concentration in seminal fluid as compared to plasma (400 vs 60 mM), but present in low levels in the infertile seminal plasma (Lewis *et al.*, 1997). Roselle is considered as a great source of natural antioxidants (Hertog *et al.*, 1993). Studies has shown that the calyx of Roselle is rich in phenolic compounds and anthocyanins (Mourtzinos *et al.*, 2008).

Roselle or sorrel (*Hibiscus sabdariffa*), belonging to the family Malvacea, is one of the most common flower plants grown worldwide. According to Akanbi *et al.* (2009), Roselle is cultivated in many tropical and subtropical

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regions of the world. It is also an economically important plant, particularly in the Sahel zone of West Africa. The leaves, seeds and calyces according to D'Heureux and Badrie, (2004) are valued for their nutritional and medicinal uses. However, the calyces of Roselle are reported (Schippers, 2000) to be the most exploited part of the plant and may be green, red or dark red. Amin *et al*. (2008) reported that the Calyces of Roselle contain nine times more vitamin C than citrus. The objective of this study is to evaluate the effect of dietary levels of Roselle calyx meal supplementation on sperm profile of mongrel rabbit bucks.

Materials and methods

Experimental site

The study was carried out at the Rabbitry unit of Teaching and Research Farm of the Department of Animal Science, University of Uyo, Uyo, Akwa-Ibom State. Uyo is located at $5^0$2'N; $7^0$55'E with a mean annual temperature of between 26 ^{0}C and 28 ^{0}C while the mean annual rainfall ranges from 2000mm – 3000mm (Solomon and Udoh, 2017).

Experimental animals and management

Twenty healthy mongrel rabbit bucks were purchased and used for this study. The rabbit bucks were allowed for two weeks of acclimatization period during which they were fed with commercial feed. Prior to the commencement of the experiment, the rabbits were treated against internal and external parasites by administering ivermectin injection at 0.1mL/rabbit subcutaneously and a broad-spectrum antibiotic (Oxytetracycline L.A) was given at 0.2 mL/rabbit using 2ml disposable syringe and needle. The rabbits were managed intensively in a wired rabbit hutch. The experimental period was 56 days (8 weeks).

Experimental diets

Red Roselle calyx variety was purchased from Itam market in Uyo metropolis, dried, milled and used in this study as Roselle calyx meal (RCM). Four experimental diets were formulated to contain dietary levels of Roselle calyx meal at 0.0%, 2.0%, 4.0% and 6.0% and coded as T_1, T_2, T_3 and T_4respectively. The T_1 contained 0.0% of the test ingredients and hence served as the control diet. The diets were fortified with bone meal, vitamin premix and salt.

Table 1: Composition of experimental diet

Ingredients	Diets			
	T_1 (0.0% RCM)	T_2 (2.0% RCM)	T_3 (4.00% RCM)	T_4 (6.0% RCM)
Maize	38.20	38.20	38.20	38.20
RCM*	0.00	2.00	4.00	6.00
Soybean cake	31.80	31.80	31.80	31.80
Wheat Offal	26.00	26.00	26.00	26.00
Bone meal	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25
Vit-Premix	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**
Calculated composition				

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Metabolizable Energy (Kcal/Kg)	2751.70	2751.70	2751.70	2751.70
Crude Protein (%)	16.08	16.08	16.08	16.08
Crude fibre (%)	5.11	5.11	5.11	5.11
Ether Extract (%)	7.84	7.84	7.84	7.84

RCM – Roselle calyx meal, * - supplement

Experimental design

The four treatment groups were assigned to the four experimental diets in a completely randomized design (CRD). Each treatment was replicated three times with two rabbits per replicate each received an assigned diet for eight weeks of the experiment.

Semen collection

The bucks were trained for semen collection during the acclimatization period according to the method of Shinkut (2015). Semen collection was done using a specially designed artificial vagina for rabbits. To collect the semen from the bucks, a rabbit doe was introduced to the buck's cage to serve as a teaser. The buck was watched closely and as it mounted the doe, the AV was placed gently at the vulva of the doe, so as to direct the penis into the AV for penetration and eventual ejaculation.

Semen evaluation

The ejaculates obtained were evaluated according to the method of Zemjanis (1970) adopted by Shinkut (2015). This include 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.

Semen volume: volume of semen was measured directly from the calibrated tube used for the collection.

Semen colour*:* The three colour categories of milky, creamy and watery were used for scoring the semen as described by Zemjanis (1970) and adopted by Shinkut (2015).

Sperm motility: Sperm motility was examined as quickly as possible aftercollection, by placing a drop of the semen sample on a pre-warmed glass slide, cover slipped and examined at ×10 magnification (Shinkut, 2015). One hundred motile and nonmotile cells were counted. Motility was calculated as follows:

$$\text{Sperm Motility (\%)} = \frac{\text{Total sperm cells counted} - \text{Non motile sperm}}{\text{Total sperm counted}} \times 100$$

Spermatozoa concentration: This was determined using Neubauer haemocytometer as described by Azawi and Imaeel (2012) and adopted by Shinkut (2015). 0.02mL of semen was aspirated and diluted with 0.38 ml of semen diluting fluid in a test tube, dilution factor of 1000 (1:20). The exterior of the pipette was wiped to remove any adhering semen. A cover slip was placed on the haemocytometer and two drops of the diluted semen was placed under the cover slip on each side of the haemocytometer. The haemocytometer was left for five minutes. It was then examined using a light microscope at ×40 magnification and the sperm cells were counted in five Thoma squares of the chamber (four corners and the centre squares). The semen concentration was calculated as follows:

Concentration (sperm cells/mL) = Number of sperm cells counted × dilution factor/volume x 1000mm^3.

Percentage live sperm cells*:* This was determined as described by Esteso *et al.* (2006) and adopted by Shinkut (2015). A thin smear of the semen was made on a clean grease free slide and stained with eosin-nigrosin stain. This technique is based on the principle that eosin-nigrosin penetrates and stains dead sperm cells while live spermcells repel the stain. Dead spermatozoa stained pinkish or reddish while

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live spermatozoa remained colourless. One hundred stained and unstained sperm cells were counted when the slides were dried, using light microscopy at ×40 magnification and percentage of eachestimated (Esteso *et al.*, 2006). Live sperm percentage was calculated as follows:

$$\text{Live Sperm (\%)} = \frac{\text{Total Sperm Cells Counted} - \text{Dead Sperm Cells}}{\text{Total sperm counted}} \times 100$$

Sperm abnormalities: Sperm abnormalities was determined by making a thin smear of the semen sample, on clean grease-free glass slide and stained with eosin-negrosin. One hundred sperm cells were counted per slide using hand counter under light microscopy at ×40 magnification as described by Shinkut (2015). Abnormal sperm percentage was calculated as follows:

$$\text{Abnormal cells (\%)} = \frac{\text{Total sperm cells counted} - \text{Normal sperm cells}}{\text{Total sperm counted}} \times 100$$

Reaction time: Reaction time was obtained by measuring the time in seconds from the time of introducing the rabbit doe into the buck's hutch to the time the buck first mounts the doe.

Total cell per ejaculate was obtained by multiplying semen volume by sperm concentration.

Proximate composition

Proximate analysis of the experimental diet was carried out according to the method of AOAC (1999) to determine the dry matter, crude protein, crude fibre, ether extract, ash and nitrogen free extract (NFE) contents.

Statistical analysis

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

Results

Table 2. Sperm profile of mongrel rabbits buck fed diets supplemented with Roselle calyx meal

Parameters	**Treatments**				SEM
	T_1 (0.0% RCM)	T_2 (2.0% RCM)	T_3 (4.0% RCM)	T_4 (6.0% RCM)	
Volume (ml)	0.47^{d}	1.73^{a}	0.57^{b}	0.53^{c}	0.16
Colour	Milky	Milky	Milky	Milky	
Motility (%)	68.67^{c}	82.67^{a}	78.33^{b}	81.67^{ab}	1.74
Sperm concentration (x 10^6 /mL)	82.66^{d}	98.33^{a}	90.00^{c}	94.00^{b}	1.81
Live sperm (%)	71.33^{c}	85.67^{a}	81.33^{a}	83.33^{ab}	1.72
Abnormal (%)	24.67^{a}	16.33^{c}	20.33^{b}	16.33^{c}	1.15
Total cells/Ejaculate (x 10^6/mL)	38.83^{c}	170.70^{a}	51.77^{b}	50.13^{b}	16.24
Reaction time (s)	20.35^{a}	5.34^{c}	10.95^{b}	21.26^{a}	2.06

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a b c d – Means in the same row with different superscript are significantly different (P< 0.05); s – second, ND - Not determined.

Sperm profile of mongrel rabbit bucks fed diets supplemented with Roselle calyx meal

The sperm profile of mongrel rabbit bucks fed diets supplemented with levels of Roselle calyx meal is presented in Table 2. From the table, all the sperm parameters evaluated differed (p<0.05) significantly except semen colour.

The semen volume of rabbit bucks fed diets supplemented with Roselle calyx meal varied (p<0.05) significantly among the different treatment groups. Semen volume of the rabbit bucks was highest in treatment group 2 (2.0% RCM) and lowest in the control group (0.0% RCM). The semen volume (mL) recorded in this study were 0.47, 1.73, 0.57 and 0.53for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM) respectively. The semen volume is seen to increase in treatment groups containing the supplemented levels of Roselle calyx meal when compared with the control but decreased with higher levels of Roselle calyx meal in bucks' diets. Semen colour is an important parameter in determining semen quality. The result of this study revealed that semen colour was not affected by levels of Roselle calyx meal supplementation in bucks' diet, hence the constant milky colour observed in all treatment groups.

Roselle calyx meal supplement significantly (p<0.05) improved sperm motility of rabbit bucks. Sperm motility of the rabbit bucks fed supplementary levels of Roselle calyx meal in this study increased when compared with the control group without Roselle calyx meal. Sperm motility was highest in T_2 (2.0% RCM) group with a value of 82.67% while the control group (0.0% RCM) recorded the least value of 68.67%. Rabbit bucks in T_3 (4.0% RCM) and T_4 (6.0% RCM) had higher sperm motility values of 78.33% and 81.67% respectively above those in control group.

Sperm concentration of bucks was positively (p<0.05) influenced by levels of Roselle calyx meal supplementation in bucks' diets. The values observed were 82.66 x 10^6/ml, 98.33 x 10^6/ml, 90.00 x 10^6/ml and 94.00 x 10^6/ml for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM) respectively. The highest value (98.33 x 10^6/ml) was observed in bucks fed T_2 (2.0% RCM) while bucks on T_1 (0.0% RCM) diet recorded the lowest (82.66 x 10^6 /ml) sperm concentration. The result revealed that mean values of sperm concentration obtained in this study increased with Roselle calyx meal supplement in bucks' diets when compared with bucks without Roselle supplementation in their diets.

Roselle calyx meal supplementation significantly (p<0.05) increased the mean values of live sperm percentage of rabbit bucks in this study higher than the control. From the result, rabbit bucks in T_2 (2.0% RCM) recorded the highest (85.67%) mean value of live sperm percentage followed closely by T_4 (6.0% RCM) with a live sperm percentage of 83.33%. The lowest live sperm percentage (71.33%) was observed in rabbit bucks in the control group (0.0% RCM).

Abnormal sperm cells of rabbit bucks showed significant (p<0.05) variations with Roselle calyx meal supplementation in bucks' diet compared to control group. Abnormal sperm cells decreased in bucks fed Roselle calyx meal as supplement. Mean values of abnormal sperm cell obtained in this study were 24.67%, 16.33%, 20.33% and 16.33% for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM) respectively. T_2 (2.0% RCM) and T_4 (6.0% RCM) recorded the lowest abnormal sperm cells while T_1 (0.0% RCM) recorded the highest mean value of abnormal sperm cells. The result revealed that inclusion of Roselle calyx meal as supplement in bucks' diets significantly

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($p<0.05$) improved the total sperm cells per ejaculate obtained by multiplying semen volume by sperm concentration. Mean values of total sperm cells per ejaculate obtained in the current study were 38.83 x 10^6/ml, 170.70 x 10^6/ml, 51.77 x 10^6/ml and 50.13 x 10^6/ml for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM) respectively.

The result also revealed that total sperm cells per ejaculate was at its peak in bucks fed T_2 (2.0% RCM) diet while those on the control diet (0.0% RCM) recorded the least value.

Reaction time also differed ($p<0.05$) significantly in this study. The reaction time of the rabbit bucks fed diets supplemented with Roselle calyx meal was highest (21.26s) in T_4 (6.0% RCM) just slightly above T_1 (0.0% RCM) which recorded 20.35s. The lowest reaction time (5.34s) was observed in T_2 (2.0% RCM) while T_3 (4.0% RCM) recorded 10.95s.

Discussion

Sperm profile of mongrel rabbits buck fed diets supplemented with Roselle calyx meal

Once semen is collected, it is evaluated for quality (Donoghue, 1998). Evaluation of semen quality is of great economic importance from the point of view of artificial insemination (Siudzinska and Lukaszewicz, 2008). This is because, the male plays a dominant role in fertility rather than the female (Etches, 1996). Thus, most fertility problems have been blamed on the male (Etches, 1996). The semen volume of rabbit bucks fed diets supplemented with Roselle calyx meal varied statistically among the different treatment groups and were within the range of 0.33 ± 0.03ml - 2.17 ± 0.17ml reported by George *et al.* (2017), but slightly lower than 0.80 - 0.94ml reported by Attia and Kamel (2011) except T_2 (2.0% RCM) that recorded 1.73ml. The result revealed that Roselle calyx meal greatly influenced the semen volume of bucks. George *et al.* (2017) opined that the volume of ejaculate varies with species, age, and frequency of ejaculation and mode of collection. This is similar to the earlier report of Johnson (2002) who suggested that total sperm production in species increase with body growth reflecting increased testicular size and increased seminiferous epithelium which explains the highest semen volume observed in T_2 (2.0% RCM).

Gross appearance of ejaculated semen is used to evaluate semen for quality (Bearden *et al.*, 2004). The milky semen colour observed in this study is same with the observation of other authors (George *et al.*, 2017; Ajuogu *et al.*, 2018) for male rabbits. The consistency in semen colour across treatment groups shows that inclusion levels of Roselle calyx meal as supplement in rabbit bucks' diets did not have adverse effect on semen colour. Earlier, Herbert (1992) reported that the appearance of semen is a part of important characteristic of quality. Good quality semen should according to (George *et al.*, 2017) have a uniformly milky appearance which gives the indication of high sperm concentration

Bearden *et al.* (2004) in Ezike (2010) noted that samples with low sperm concentration will appear watery or less opaque. They also attributed pink tinge appearance to indication of blood contamination and added that the use of semen that is discoloured, watery, or contaminated by fecal material, urates, or blood will lead to lowered fertility particularly if the semen is subjected to short-term or long-term storage. All contaminated samples should therefore be discarded.

Semen concentration observed in the study was below the range of 316.67 ± 158.99 - 866.67 ± 33.33 x 10^6/ml observed by George *et al.* (2017) and 126 to 154 (x 10^6/ml) reported by Abu *et al.* (2013) for rabbit bucks. Semen concentration was statistically improved with inclusion of Roselle calyx meal compared to the control groups and was at its best at T_2 (2.0% RCM). Adeyemi (2014) opined that increasing testosterone production in rabbit bucks might have positive influence on semen quality, quantity and testis size. Sanni *et al.* (2012)

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reported that testosterone is the major androgen secreted by the male gonad and plays an essential role in the development of the normal male and maintenance of many male characteristics including muscle mass and strength, bone mass, libido, potency and spermatogenesis. These are essential attributes that can enhance the ability of a buck to mount successfully and produce viable sperm cells (Adeyemi, 2014).

The results of sperm motility of the rabbit bucks fed the diets supplemented with Roselle calyx meal was significantly influenced by the dietary treatment and similar to those reported by other authors (Attia and Kamel, 2011; Adeyemi, 2014; Ajuogu *et al.* 2018 and Olarotimi and Adu, 2020) for male rabbits. Motility was positively influenced in the treated groups compared with those in the control group. Jimoh and Ewuola (2019) reported motility value range of 82.63 to 88.30% for exotic male rabbits which is not very far from the value range of 68.67 to 82.67% observed in this study. Motility is the movement of spermatozoa in the semen medium during reproduction. Motility of a sample of semen is expressed as the percentage of cells that are motile under their own power (Bearden *et al*., 2004). The percentage motility of an ejaculate of semen can ranges from 0% to 100% according to Bearden *et al*. (2004) in Ezike (2010). The motility percentage recorded in the study suggests that the Roselle calyx meal did not affect the normal fertility of the rabbit bucks. Fertility levels of ejaculates with initial motilities of 50% to 80% as suggested by Ezike (2010) are high if the desired number of motile sperm is present at the time of insemination. Samples with less than 40% initial motility are not suitable for use unless the ejaculate is from an exceptionally superior sire (Bearden *et al.*, 2004).

Motility provides a means of evaluating semen for artificial insemination programme (Shelton, 2000). Motility as a good indicator of viability (Ezike, 2010) as it basically estimates the proportion of active spermatozoa in the semen where fifty percent (50%) or more of the sperm should be moving. However, although motility is essential for fertility as well as an essential feature of good quality spermatozoa, it is not necessarily an indicative of fertilizing capacity (Ezike, 2010).

Live sperm percentage in this study was statistically improved with Roselle calyx meal supplementation. Live sperm percentage recorded in this study ranged from 71.33 to 85.67% and were similar to those reported by Adeyemi (2014) and Shinkurt (2015) for rabbit bucks. The higher live sperm percentage observed in this study suggests that Roselle calyx meal is capable of improving the survival of sperm cells. This result shows that there was significant decrease in dead spermatozoa percentage between rabbits fed varying levels of Roselle calyx meal in T_2 (2.0% RCM), T_3 (4.0% RCM), and T_4 (6.0% RCM), when compared to those in control (0.0% RCM) group. Good semen quality with high percentage live sperm cells and high libido of the buck is essential in rabbit reproduction (Adeyemi, 2014).

Roselle calyx meal also had positive effect on the morphology of sperm cells by decreasing the percentage of abnormal cells in the current study. The abnormal cells percentage observed in the study was however lesser than that reported by Shinkurt (2015) for male rabbits fed diets supplemented with *Allium sativum*.

Reaction time of mongrel rabbit bucks in this study was statistically influenced by Roselle calyx meal supplementation in bucks' diets. However, the range of values obtained in the study (5.34 to 21.26s) was higher than the value range of 6.40 – 7.50s reported by Jimoh and Ewuola (2019) for exotic rabbits. Bahr and Bakst (1985) reported that libido in the male is not necessarily correlated with high fertility and that there is a tendency for the most frequent copulator to produce many aspermic ejaculates.

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The total sperm cells per ejaculate of rabbit bucks fed diets containing supplementary levels of Roselle calyx meal was statistically higher in the treated groups than the control group. Ezike (2010) reported that the volume of ejaculated semen is determined not only for use in processing but also to establish a pattern for individual male. Ejaculates with larger volume, higher concentration, and higher motility will have higher fertility in most cases and more breeding units can be prepared from an ejaculate, thus, reducing the processing time and cost per breeding unit Ezike (2010)

Conclusion

The study revealed that Roselle calyx meal at 2.0% improved semen quality in mongrel rabbit bucks thus enhanced rabbit production.

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