Nig. J. Anim. Prod. 2023, 49(5):162 - 173 https://doi.org/10.51791/njap.v49i5.3530.
Nigerian Journal of Animal Production

Effect of frequency of ejaculation and graded dietary levels of Roselle (*Hibiscussabdariffa*) calyx meal supplementation on sperm profile of mongrel rabbit bucks

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Abstract

Changes in semen quality occasioned by ejaculation frequency is one of the important yet neglected factor considered in artificial insemination. Several factors have been shown to influence semen parameters, one of which is sexual abstinence. Rabbits have a very short interval between successive ejaculations hence, semen can easily be collected for quality examination. This experiment was carried out to evaluate the effect of frequency of ejaculation and graded dietary levels of Roselle calyx meal supplementation on sperm profile of mongrel rabbit bucks. Twenty healthy 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 0.00% (control), 2.00%, 4.00% and 6.00% 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 (2) rabbits per replicate. Each replicate received an assigned diet for 8 weeks. The experimental data obtained were subjected to analysis of variance (ANOVA) procedure. Semen volume, colour, motility, concentration, live sperm percentage, abnormal sperm percentage, total sperm cells per ejaculate and reaction time were evaluated in this study. From the results obtained, semen volume, sperm concentration, live sperm cells, reaction time and total cells per ejaculate were significantly ($p<0.05$) influenced by frequency of ejaculation while sperm motility and abnormal cells did not show ($p>0.05$) statistical variation. Roselle calyx meal supplementation in mongrel rabbit bucks' diet influenced all sperm indices except sperm motility and reaction time. The highest semen volume was observed in once per week frequency of ejaculation with a value of 1.01ml while twice per week frequency of ejaculation recorded a lower value of 0.85ml. There was interaction ($p<0.05$) between frequency of ejaculation and graded dietary levels of Roselle calyx meal on semen volume of bucks in this study. Live sperm cells were higher (82.51%) in once per week frequency of ejaculation than in twice per week (77.08%). From the findings of this study, once a week frequency of ejaculation and Roselle calyx meal supplementation at 2.0% improved sperm characteristics of mongrel rabbit bucks.

Keywords: Frequency of ejaculation, Roselle, sperm profile, mongrel rabbit

Effet de la fréquence de l'éjaculation et des niveaux diététiques de Roselle (Hibiscus sabdariffa) Calyx Supplémentation de repas sur le profil de sperme de Mongrel Lapin Daims

Résumé

Les changements de qualité de sperme occasionnés par la fréquence d'éjaculation sont l'un des facteurs importants mais négligés considérés dans l'insémination artificielle. Il a été démontré que plusieurs facteurs influencent les paramètres de

sperme, dont l'une abstinence sexuelle. Les lapins ont un très court intervalle entre éjaculations successives donc, le sperme peut facilement être collecté pour un examen de qualité. Cette expérience a été réalisée pour évaluer l'effet de la fréquence de l'éjaculation et des niveaux diététiques de RoselleCalyx Supplémentation des repas sur le profil de sperme de Daims de lapin de Mongrel. Vingt daims de lapin de Son ont été utilisés pour l'étude. Les lapins ont été gérés de manière intensive et ont été fournis avec des aliments pour animaux, de l'eau et des forages ad-libitum. Quatre régimes expérimentaux ont été formulés pour contenir des niveaux diététiques de farine de CalyxRoselle à 0,00% (contrôle), de 2,00%, 4,00% et 6,00% et codés comme T_1, T_2, T_3 et T^4 respectivement. Les quatre groupes de traitement ont été attribués à quatre régimes expérimentaux dans une conception entièrement randomisée (CER) répliquée à trois reprises avec deux (2) lapins par réplication. Chaque réplique a reçu une alimentation assignée pendant 8 semaines. Les données expérimentales obtenues ont été soumises à une analyse de la procédure de variance (ANOVA). Volume de sperme, couleur, motilité, concentration, pourcentage de sperme en direct, pourcentage de sperme anormal, cellules de sperme total par éjaculation et temps de réaction ont été évalués dans cette étude. Des résultats obtenus, le volume de sperme, la concentration de sperme, les spermatozoïdes en direct, le temps de réaction et les cellules totales par éjaculation ont été significativement ($p < 0,05$) influencés par la fréquence d'éjaculation tandis que la motilité des spermatozoïdes et les cellules anormales n'ont pas montré ($p > 0,05$) variation statistique. RoselleCalyx Supplément repas dans le régime de Daims de lapin de Mongrel influencé tous les indices de sperme sauf la motilité du sperme et le temps de réaction. Le volume de sperme le plus élevé a été observé en fréquence d'éjaculation une fois par semaine avec une valeur de 1,01 ml, tandis que la fréquence d'éjaculation de deux fois par semaine enregistrait une valeur inférieure de 0,85 ml. Il y avait une interaction ($p < 0,05$) entre la fréquence d'éjaculation et les niveaux diététiques gradués du repas RoselleCalyx sur le volume de sperme de dollars dans cette étude. Les spermatozoïdes en direct étaient plus élevés (82,51%) en fréquence d'éjaculation une fois par semaine qu'à deux fois par semaine (77,08%). Des conclusions de cette étude, une fois par semaine, une fréquence d'éjaculation et une supplémentation de repas RoselleCalyx à 2,0% des caractéristiques de sperme améliorées de Daims de lapin de Mongrel.

Mots-clés: fréquence de l'éjaculation, Roselle, profil de sperme, lapin de Mongrel

Introduction

Modifications of semen quality related to ejaculation frequency is one of the most important and neglected factors from the standpoint of artificial insemination or sperm competition (Ambriz *et al.*, 2002). Rabbits according to (Ambriz *et al.*, 2002) offer an advantageous experimental model because they have characteristic sexual behavior, they present rapid ejaculation after a single intromission, they have a very short interval between successive ejaculations, and semen can be easily collected. Several factors have been shown to influence semen parameters, one of which is sexual abstinence; a clinical criteria included in the semen evaluation to provide maximum sperm quality

(Mayorga-Torres *et al*., 2015). It has been proposed that recurrent ejaculations could be an approach to improve sperm DNA quality and therefore, reproductive outcome (Mayorga-Torres *et al*., 2015). Rabbits, *Oryctolagus cuniculus* are currently being recommended for countries with low meat production as important meat source (George *et al.* 2017). As a result of its high reproductive advantages, rabbits have been recommended to bridge the protein deficiency gap. In a similar vein, Iyeghe *et al.* (2002) reported that rabbit production be increased. Commercial rabbits employ artificial insemination (A.I) to enhance fertilization in doe and this requires full knowledge of semen metabolism and physiology (Boiti, 2005). 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 calyx is nine time richer in vitamin C than citrus species (Amin *et al.,* 2008) and has been associated with fertility (Millar, 1992) in farm animals. In view of the fact that spermatogenesis is affected by some number of factors including frequency of ejaculation and nutrition, it is therefore crucial to consider the extent to which each of these factors affect semen characteristics. The aim of this study is to evaluate the effect of ejaculation frequency and Roselle calyx meal supplementation on sperm quality 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 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 84 days (12 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 0.00% (control), 2.00%, 4.00% and 6.00% and coded as T_1, T_2, T_3 and T_4 respectively. The diets were fortified with bone meal, vitamin premix and salt.

Experimental design

The four treatment groups were assigned to the four experimental diets in a 2 x 4 factorial experiment design. Each group was replicated three times in the four treatment groups with one rabbit buck per replicate. In the first group, semen collection was carried out once per week (Mondays) with the aid of an artificial vagina specially designed for rabbits while semen was collected twice weekly (Wednesdays and Fridays) from the second group.

Semen collection

Semen collection commenced after eight weeks of feeding bucks with Roselle calyx meal as supplement and lasted for four weeks. The bucks were trained for semen

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				
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

collection during the acclimatization period. 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: Semen volume 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 after collection, by placing a drop of the semen sample on a pre-warmed glass slide, cover slipped and examined at ×10 magnification (Shinkut, 2015). 100 motile and nonmotile**cells were counted.** Motility was calculated as follows:

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

Spermatozoa concentration: This was determined using Neubauerhaemocytometer. 0.02mL of semen was aspirated and diluted with 0.38Ml 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 5 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 Estesoet *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 sperm cells repel the stain. Dead spermatozoa stained pinkish or reddish while live spermatozoa remained colourless. One hundred (100) stained and unstained sperm cells were counted when the slides were dried, using light microscopy at ×40 magnification and percentage of each estimated (Estesoet *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: Abnormal sperm cell percentage 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:

Abnormal cells (%) = Total sperm cells counted – Normal sperm cells x 100

Total sperm counted

Reaction time: Reaction time was determined 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: Total cell per ejaculate was obtained by multiplying semen volume by sperm concentration.

Statistical analysis

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

Results

Effect of frequency of ejaculation on sperm profile of mongrel rabbit bucks fed diets supplemented with Roselle calyx meal

The result of the effect of Frequency of Ejaculation on semen characteristics of Mongrel Rabbits is present in Table 2. The result showed that Roselle calyx meal significantly ($p<0.05$) affected the semen volume of mongrel rabbit bucks. It was observed that rabbit bucks fed T_2 (2.0% RCM) yielded higher semen volume (1.71mL) compared (0.93mL) to bucks fed the control diet (0.0% RCM). However, at higher levels of Roselle calyx meal supplementation, the semen volume of the rabbit bucks decreased when compared to those in the control group. Frequency of ejaculation also significantly ($p<0.05$) affected semen volume. The highest semen volume was observed in once per week frequency of ejaculation with value of 1.01mLwhile twice per week frequency of ejaculation recorded lower value of 0.85ml. There was interaction ($p<0.05$) between frequency of ejaculation and graded dietary levels of Roselle calyx meal on semen volume of bucks in this study.

Semen colour did not show colour change in all treatment levels and frequencies of collection, indicating that all treatment levels and frequencies did not influence semen colour negatively. Frequencies of ejaculation failed to show significant influence ($p>0.05$) on the sperm motility of bucks fed diets containing graded dietary levels of Roselle calyx meal. The values though similar was higher in once per week frequency of ejaculation than in twice per

Table 2: Effect of frequency of ejaculation of mongrel rabbit bucks fed diets supplemented with Roselle calyx meal

PARAMETERS	Frequency of Ejaculation (FACTOR A)			Treatments (FACTOR B)					Significance		
	Once weekly	Twice weekly	SEM	T1 (0.0% RCM)	T2 (2.0% RCM)	T3 (4.0% RCM)	T4 (6.0% RCM)	SEM	A	B	AB
Semen Vo lume (ml)	1.01[a]	0.85[b]	0.37	0.93[b]	1.71[a]	0.49[c]	0.61[c]	0.26	*	*	*
Semen colour	Milky	Milky	ND	Milky	Milky	Milky	Milky	ND			
Sperm Motility (%)	79.17	75.25	4.14	74.67	80.67	75.67	77.83	2.93	NS	NS	NS
Sperm Concentration (x10^6/ml)	105.75[a]	89.25[b]	10.29	82.17[b]	108.50[a]	98.17[a]	101.17[a]	7.65	*	*	NS
Live Sperm (%)	82.51[a]	77.08[b]	3.97	73.83[c]	85.50[a]	80.11[b]	79.73[b]	2.81	*	*	*
Abnormal Cells (%)	14.14	15.17	3.10	20.50[a]	12.17[b]	14.44[b]	13.50[b]	2.19	NS	*	NS
Reaction Time (s)	7.98[b]	12.62[a]	2.88	9.76	10.10	10.29	10.25	2.04	*	NS	NS
Total cells/Ejaculate (x10^6/ml)	110.75[a]	75.43[b]	45.83	76.01[b]	188.48[a]	48.13[c]	59.85[c]	32.40	*	*	*

a b c – Means in the same row with different superscript are significantly different (P<0.05); RCM –Roselle calyx meal; SEM – Standard error of mean; NS – Not significant; * - significant; A – Frequency of Ejaculation; B – levels of Roselle calyx meal; AB – Interaction between frequency of ejaculation and levels of Roselle calyx meal. ND –Not determined

week frequency of collection. The values obtained were 79.17 and 75.25% for once per week and twice per week ejaculation frequencies respectively. The result revealed that Roselle calyx meal supplementation did not ($p>0.05$) influence sperm motility of bucks in this study, however, the highest individual effect (80.67%) of treatment levels on sperm motility was observed in bucks fed T_2 (2.0% RCM) diet. There was also no interaction ($p>0.05$) between frequency of ejaculation and Roselle calyx meal on sperm motility of bucks in this study. Mean values of sperm concentration of mongrel rabbit bucks fed the diets containing varying levels of Roselle calyx meal were positively affected ($p<0.05$) by frequency of ejaculation. The values obtained were 105.75 and 89.25 X 10^6 for once per week and twice per week frequency of ejaculation respectively. Sperm concentration of bucks were also influenced ($p<0.05$) by levels of Roselle calyx meal in bucks' diets. Bucks on T_2 (2.0% RCM) showed superior performance (108.50 x10^6mL) followed closely (101.17 x10^6/mL) by those onT_3 (4.0% RCM). Although sperm concentration was statically similar ($p>0.05$) at all levels of Roselle in this study, it was however better ($p<0.05$) than the control (0.0% RCM) group which recorded the lowest (82.17 x$10^{6)}$) value. The result however failed to show ($p>0.05$) significant interaction between frequency of ejaculation and levels of Roselle calyx meal supplementation in bucks' diets on sperm concentration. Live sperm percentage was also influenced ($p<0.05$) by Roselle calyx meal supplementation in rabbit bucks' diets. The values obtained were 73.83, 85.50, 80.11 and 79.73% for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM) respectively. Highest percentage was observed with Roselle calyx meal supplementation at 2.0%. Bucks fed diets containing Roselle calyx meal as supplement performed better than those without Roselle calyx in their diets. This study revealed that there was interactive main effect ($p<0.05$) between frequency of ejaculation and graded levels of Roselle calyx meal supplementation on live sperm percentage of mongrel rabbit bucks. The result of this study also revealed that Roselle calyx meal supplementation significantly ($p<0.05$) influenced abnormal sperm cells in mongrel rabbit bucks. The highest values of abnormal sperm cells were observed in T_1 (0.0% RCM). The mean values of abnormal sperm cells decrease with supplementation of Roselle calyx meal in the diets of mongrel rabbit bucks when compared to the control (without Roselle calyx meal as supplement). The least value (12.17%) was observed in rabbit bucks fed 2.0% RCM as supplement in their diet. The pattern however did not continue with increased levels of Roselle calyx meal in bucks' diets. Frequency of ejaculation statistically influenced ($p<0.05$) live sperm percentage of rabbit bucks in this study. Live sperm cells were higher (82.51%) in once per week frequency of ejaculation than in twice per week (77.08%). Frequency of ejaculation in this study failed to show significant ($p>0.05$) effect on abnormal sperm cells percentage in all treatments although twice per week semen collection recorded higher mean values of abnormal sperm cells than once per week frequency of ejaculation. The values obtained were 14.14 and 15.17 % for once per week and twice per week frequencies of ejaculation respectively. This study however did not show ($p>0.05$) interactively main effect between frequency of ejaculation and varying levels of Roselle calyx meal supplement on abnormal sperm cells. Reaction time of mongrel rabbit bucks was not ($p>0.05$) statistically influenced by graded levels of Roselle calyx meal. There was no individual main effect of Roselle calyx meal on the bucks in this study. The

mean reaction time observed in this study were 9.76, 10.10, 10.29 and 10.05s for T_1 (0.0% RCM), T_2 (2.0% RCM), T_3 (4.0% RCM) and T_4 (6.0% RCM) respectively. Frequency of ejaculation on the other hand significantly ($p<0.05$) influenced reaction time (s). Once per week semen collection showed lower (7.98s) reaction time than twice per week frequency of ejaculation. There was no interaction between frequency of ejaculation and graded dietary levels of Roselle calyx meal on reaction time of bucks in this study. Roselle calyx meal in this study had significant ($p<0.05$) main effect on total sperm cells per ejaculate of the mongrel rabbit bucks in this study. The highest value (188.48 $x10^6$/ml) was observed in rabbit bucks fed T_2 (2.0% RCM) diet while T_3 (4.0% RCM) recorded the least value. T_1 (0.0% RCM) and T_4 (6.0% RCM) recorded individual values of 76.01 and 59.85 $x10^6$ cells respectively. Frequency of ejaculation also had positive ($p<0.05$) individual main effect on total sperm cells in this study. Total cells per ejaculate was higher in once per week than in twice per week frequency of ejaculation group. This study revealed that there was interaction ($p<0.05$) between graded levels of Roselle calyx meal supplementation and frequency of ejaculation on total sperm cells per ejaculate.

Discussion

Effect of frequency of ejaculation on semen parameters of mongrel rabbit bucks fed supplementary levels of Roselle calyx meal

Semen volume of rabbit bucks was higher in once per week frequency of ejaculation in all treatment levels than bucks in twice per week frequency of ejaculation group which agrees with the findings of Ambriz *et al.* (2002), who reported that ejaculates volume decreased linearly in relation with the number of ejaculations until ejaculate number six (6). The result hence suggests that Roselle calyx meal supplementation at 2.0% in the diets of mongrel rabbits at once per week frequency of ejaculation would result in better semen volume. Noirault and Brillard (1999) and Nwachukwu *et al.* (2006) observed a decrease in semen volume with increasing frequency of semen collection. Mayorga-Torres *et al.* (2015) observed that during periods of high ejaculation frequency the sperm concentration initially decreases. This was supported by Cecil (1982) who suggested that increasing the frequency of semen collection had negative effects on ejaculate volume without significantly affecting sperm concentration and other sperm physical characteristics. Ezike (2010) suggested that ejaculates with larger volume, higher concentration, and higher motility will have higher fertility in most cases. Semen colour is an important parameter for determining semen quality. Gross appearance of ejaculated semen is used to evaluate semen for quality (Bearden *et al.*, 2004). It was observed that ejaculation frequency did not affect semen colour in this study. Ezike (2010) opined that semen samples with low concentration will appear watery or less opaque and also added that pink tinge appearance is an indication of blood contamination while yellow samples usually have offensive odour. The milky colour suggests good semen quality of bucks in the study. Frequencies of ejaculation did not influence the sperm motility of mongrel rabbit bucks in the current study, the mean values obtained were however similar to range of 82.63 - 88.30% highlighted by Jimoh and Ewuola (2019). This result however, disagrees with the report of Ezike (2010) who reported that frequency of semen collection significantly influenced sperm motility in turkey toms. He also added that fertility levels of ejaculates with initial motilities of 50% to 80% are high if the

desired number of motile sperm is present at the time of insemination. Though the sperm motility of bucks was not affected by frequency of ejaculation, the values were however higher in once per week frequency of ejaculation than in twice per week frequency of ejaculation. The findings of this study indicate that sperm concentration of mongrel rabbit bucks was positively influenced by frequency of ejaculation. Sperm concentration was higher in once per week frequency of ejaculation than in twice per week frequency of ejaculation. This result agrees with the findings of Nwachukwu *et al.* (2006) who had earlier reported that sperm concentration decreases with increasing frequency of semen collection. Accurate determination of the number of spermatozoa per milliliter of semen is extremely important because it is a variable semen quality parameter (Ezike, 2010). When combined with the volume of the ejaculate, sperm concentration not only provides a basis for calculating the number of sperm per insemination dose, but also serves as a measure of semen quality (Bearden *et al*., 2004). Thatohatsi (2009) reported that sperm concentration appears to be the only seminal traits affected by frequency of ejaculation, declining progressively with an increase in ejaculation frequency. Ambriz *et al.* (2002), in his study, also discovered that sperm per milliliter decreased rapidly until ejaculate number six (6). Sperm concentration obtained in this study indicates normal fertility of bucks for both frequencies of ejaculation. This is because ejaculates with low sperm concentration have been associated with low fertility (Bearden *et al.,* 2004). Frequency of ejaculation significantly influenced live sperm percentage of mongrel rabbits fed levels of Roselle calyx meal in their diets which agrees with the result of Ezike (2010) who also reported significant influence of frequency of ejaculation on live sperm percentage of toms under intensive and extensive systems of production. Live sperm counts are highly correlated with visual estimates of progressively motile sperm cells (Bearden *et al.,* 2004). Abnormal sperm percentage was not affected by frequency of ejaculation in this study. The result of this study is in disagreement with Ezike (2010) who reported that frequency of semen collection significantly affected the percentage of abnormal sperm. Ezike (2010) noted that every ejaculate of semen will contain some morphologically abnormal spermatozoa but the expected range of 8% to 10% has no adverse effect on fertility. The abnormal sperm cells percentage observed in this study ranged between 11.67 and 20.67% which is lower than the 25% of Bearden *et al*. (2004). Preston *et al.* (2001) also noted that partial or complete degeneration of the sperm tubules may result to high production of abnormal spermatozoa thereby reducing the proportion of normal spermatozoa. This is important because accelerated transit implies ejaculation of immature spermatozoa and inadequate fertilizing potential of ejaculated sperm (Mayorga-Torres *et al*., 2015). Preston *et al*. (2001) highlighted the fact that ejaculates collected in the latter part of their experiment showed a significantly greater proportion of abnormal sperm cells. The low percentage of abnormal sperm cells with Roselle calyx meal inclusion in bucks' diet shows that frequency of ejaculation did not affect fertility because, according to Lukaszewicz (2003) morphological sperm defects generally affect fertility more than low sperm motility. Reaction time of mongrel rabbit bucks in this study was significantly affected by frequency of ejaculation and was higher in twice per week frequency of ejaculation group than once per week frequency of ejaculation group. 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. The highest total sperm count was observed in once per week frequency of ejaculation and at 2.0% Roselle calyx meal supplementation in the bucks' diet. The results of this study were in agreement with those of Ezike (2010) who also reported significant effect of frequency of ejaculation on total sperm per ejaculate. Santayana (1985) observed a progressive decline in total sperm with increased ejaculation frequency. Nwachukwu *et al.* (2006) also reported significant influence of ejaculation frequency on total sperm in the cock. This is important because Durape (2007) pinpointed that though it takes one single sperm to fertilize an ovum, adequate number of spermatozoa must be available at the site of fertilization to ensure high fertility. Thatohatsi (2009) also reported a positive correlation between total sperm inseminated and fertility.

Effect of interaction between Roselle calyx meal supplementation and frequency of ejaculation on sperm profile of mongrel rabbit bucks

Results obtained in this study indicated that semen volume, live sperm percentage and total sperm cells per ejaculate of bucks were significantly influenced by interactive effect between frequency of ejaculation and Roselle calyx meal supplementation in mongrel rabbit bucks' diet. The best performance was observed in once per week frequency of ejaculation group at 2.0% supplementation of Roselle calyx meal. The results however failed to show significant combined effect of frequency of ejaculation and treatment levels on sperm motility, sperm concentration, abnormal sperm cells percentage and reaction time. The results obtained in this study suggests that though frequency of ejaculation did not have statistical effect on key fertility indices like sperm motility, concentration and abnormal sperm percentage. The values obtained were optimum to maintain fertility in rabbit bucks.

Conclusion

The findings of this study indicated that once per week frequency of ejaculation and Roselle calyx meal supplementation at 2.0% in rabbit bucks' diet improved semen volume, concentration, live and abnormal sperm cells total sperm cells per ejaculate in mongrel rabbits, hence, improving the fertility of the bucks.

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Received: 9th November, 2021
Accepted: 26th February, 2022