



# The Effects of Discarded Cocoa (*Theobroma cacao*) Seed Meal Based Diets on Semen Traits, Testicular Morphometry and Histomorphology of Rabbit Bucks

LUKE CHUKWUDI ALI\*, NNANNA EPHRAIM IKEH, BRIGHT CHIGOZIE AMAEFULE, AMARACHI LINDA OBINNA, NDUBUISI SAMUEL MACHEBE

Department of Animal Science, Faculty of Agriculture, University of Nigeria, Nsukka

**Abstract** | A 10-weeks study was carried out to evaluate the effect of dietary intake of discarded cocoa seed meal (DCSM) on reproductive characteristics of rabbit bucks. A total of 36 mixed-bred rabbit bucks of 20 weeks old and having a mean weight of  $1384.34 \pm 283.34$ g were randomly assigned to 4 treatment (T) groups of 9 rabbit bucks each replicated 3 times using a one-way analysis of variance in a completely randomized design (CRD). Water and feed were made available ad-libitum to the bucks. Diets containing 0, 15, 30 and 45% DCSM were compounded and fed to bucks in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively. Prior to semen collection, rabbit bucks were trained three times per week for semen collection with an artificial vagina (AV). At the end of the 10 weeks, semen collection and evaluation were carried out. Thirty six (36) rabbit bucks were slaughtered and testes collected and dissected for testicular morphometry and histomorphology evaluation. Semen colour showed milky white in all groups although T<sub>4</sub> appeared creamy white. There were significant ( $P < 0.01$ ) differences among treatment groups of rabbits in all the semen traits except in semen volume. Semen pH showed significant ( $P < 0.01$ ) variations with semen of bucks in T<sub>4</sub> having the highest value (8.67). Sperm motility, sperm concentration, live and normal sperms in decreased with an increase in dietary DCSM whereas non-motile, dead and abnormal sperms increased with an increase in dietary DCSM. Testicular morphometric measurements and histomorphological evaluations were similar in all treatments ( $P > 0.05$ ). This investigation depicts that DCSM inclusion above 15% affected semen quality negatively even though results on testicular morphometric traits and histology appeared non-significant.

**Keywords** | Rabbit bucks, Semen traits, Sperm characters, Testicular morphometry, Histomorphology, Discarded cocoa seed meal

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**\*Correspondence** | Ali LC, Department of Animal Science, Faculty of Agriculture, University of Nigeria, Nsukka; **Email:** aliluke230@gmail.com

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## INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) have the potential to fill the gap in human protein supply, particularly in developing and underdeveloped countries. They are effective feed converters, because they can withstand up to 30 percent crude fiber compared to 10 percent in most poultry species (Egbo et al., 2001). While rabbits can be raised on a forage-based diet, according to Amao and Showunmi (2016), deficient and unbalanced grass

quality, as well as seasonal availability of forage, are major constraints to successful rabbit farming. Other immense potentials of rabbit include proverbial prolificacy, high growth rate, short gestation period, high genetic selection ability, relatively low cost of production, economic utilization of space and high nutritional quality (Biobaku and Oguntola, 1997; Hassan et al., 2012). In addition, the consumption of rabbit meat is free from religious and cultural biases.

Nutrition has a significant impact on the reproductive efficiency of rabbit bucks (Amao and Showunmi, 2016). Over the years, animal scientists has primarily focused on identifying less expensive alternative feedstuff that are readily available and do not compete with the dietary requirements of man (Egbunike, 1997). In a study of rabbit development on tropical feed sources, it was reported that feeding only grasses to rabbits was unsuitable due to its low digestibility of about 10% (Cheeke and Raharjo, 1988). Also, the availability of conventional feed ingredients of protein and energy sources as concentrates for rabbit diets is heavily limited. Grain production in both developed and developing countries are primarily for human consumption, resulting in extremely high feed prices (Ogbuewu et al., 2008). For instance, in developing countries like Nigeria, the key restriction to livestock development is the inconsistency in the availability of good quality feed in large amount throughout the year. As a result, the use of unconventional feed resources becomes extremely important and is promoted (Amao and Showunmi, 2016). In line with the afore-mentioned, Onifade and Tewe (1993) noted that, tropical agricultural extraction industries provide a large number of by-products with untapped feeding potential. So far, certain agricultural by-products and plants have been used in animal production with varying degrees of success.

The rabbit buck's reproductive system produces spermatozoa that are deposited into the female genital system. Spermatozoa are formed in the testes' seminiferous tubules and then transferred to the epididymides via the rete testes, where they are deposited until maturity (Amao et al., 2019). Spermatogenesis in rabbits takes 48 days (Amann, 1982) and includes a sequence of successive mitotic divisions, two meiotic divisions, and spermiogenesis. However, some chemical factors like reactive oxygen species or chemotherapeutic agents can interrupt this process, resulting in changes in the consistency of the sperm or the structure of the testicles (Iyama and Wilson, 2013; Chenoweth and Lorton, 2014). Cocoa and its derivatives (chocolate, cocoa cake or butter) are currently investigated as possible livestock feed ingredients.

Discarded cocoa seed meal (DCSM) are agricultural by-products that are often discarded in or around cocoa factories or communities in Southern Nigeria, resulting in environmental contamination as a result of inappropriate composting (Sukha, 2003; Nwanna and Fashae, 2010). The use of cocoa by-products as a feed option for animal feeding has alleviated the disposal problems faced by cocoa factories (Figueroa et al., 2019). When used as alternative feed ingredient, feed formulation cost is reduced, and the negative effects of climate change caused by pollution is properly mitigated.

Investigations on the benefit of the use of cocoa has revealed that it has some therapeutic effect because of their high contents of antioxidants primarily flavonoids, epicatechin, catechin, and procyanids (Visioli et al., 2000). Other contents are nitrogenous compounds, minerals, and methylxanthine (Greer et al., 2001). Even though, methylxanthine (caffeine and theobromine) are considered as anti-nutritional factors, available report has shown that it depends on the processing method and the quantity fed to the animal (Rios et al., 2003). Cocoa powder has been shown to have a variety of physiological and biological effects in vitro, such as antioxidant, anti-inflammatory, antiviral, antibacterial, vasodilatory, anticancer, and anti-ischemic effects, as well as improved endothelial cell functions, which enhance cardiovascular processes (Steinberg et al., 2003; Prochazkova et al., 2011). The feeding of dietary antioxidants to male animals have been reported to aid in minimizing the damaging effect of free radicals to sperm cells and other biological functions in the body system. Thus, chocolate (a cocoa product) has been suggested for males with fertility problems (Yildirim et al., 2014). Theobromine is of primary concern in the use of cocoa and its products as animal feed components. It has been shown to have a detrimental effect on reproduction in a variety of animal species, including rats (Tarka et al., 1981), egg laying birds (Odunsi and Longe, 1995) and rabbits (Adeyina et al., 2010).

Considering the benefit of using cocoa by products/waste as alternative feed materials, this experiment was therefore carried out to evaluate the effect of feeding discarded cocoa (*Theobroma cacao*) seed meal diets on semen traits, testicular morphometry and histomorphology of rabbit bucks.

## MATERIALS AND METHODS

### ETHICAL APPROVAL

This study was conducted noting the recommended research ethics for scientific researches involving animal subjects: code 5.4.2 and rabbit bucks were handled according the principles of Animal Experimentation Ethics Committee University of Nigeria, Nsukka (2013).

### EXPERIMENTAL SITE

This research was carried out at the Rabbit Unit of the Animal Science Teaching and Research Farm, University of Nigeria, Nsukka in Enugu State, South Eastern Nigeria. Nsukka lies in the derived Savannah region and is located at longitude 7° 21'39"E and latitude 6° 51'39"N (Ihinegbu et al., 2019) at an elevation of 456m above sea level (Onyenuchey and Nnamchi, 2018). The climate is a humid tropical setting with temperature ranging from 22.9 to 27.0 °C (Phil-Eze, 2012) and a relative humidity range of 54.5 to 84.32%. In September, the peak of rainfall is 108.55mm and January and December being the lowest

with no observed rainfall (Onyenucheya and Nnamchi, 2018). The light cycle of the study area was 11 hours day light.

### MANAGEMENT OF EXPERIMENTAL ANIMAL

The discarded cocoa seed meal (DCSM) used in the diet formulation was collected in and around cocoa powder manufacturing factories in Osun, Ogun and Oyo states in Nigeria. The percentage and proximate composition of the experimental ration is presented in Table 1. A total number of thirty six (36) mixed-bred rabbit bucks of an average weight of  $1384.34 \pm 283.34\text{g}$  were randomly assigned to 4 treatments (T) diets according to the level of inclusion of discarded cocoa seed meal in the diet. Each treatment contained 9 rabbit bucks and replicated three times with each replicates containing 3 bucks. Bucks were held in individually washed and disinfected hutches with a dimension of 4ft x 2ft x 2ft and a wire-screened floor that allowed faeces and urine to pass through. Feeders and water troughs containing feed and water, respectively were provided to the bucks and allowed *ad-libitum* method of intake. On arrival, bucks were allowed a 1-week period to undergo acclimatization followed by an 8 weeks of feeding of the test ingredients. The rabbit bucks were also provided with equal supplemental forages plus the experimental concentrates.

**Table 1:** Percentage (%) and proximate composition of the experimental diets containing DCSM at various levels.

Feedstuff	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Maize	39.84	29.48	19.16	8.84
Wheat offal	26.56	19.66	12.77	5.89
Soya bean meal	11.84	12.74	13.63	14.51
Palm kernel cake	17.76	19.12	20.44	21.76
Cocoa seed meal	0.00	15.00	30.00	45.00
Limestone	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Iodize salt	0.25	0.25	0.25	0.25
Vit-Min premixes	0.25	0.25	0.25	0.25
<b>Total proximate</b>	100.00	100.00	100.00	100.00
Moisture	5.65	4.80	4.85	4.85
Crude protein	15.78	15.92	16.02	16.51
Crude fibre	9.55	10.21	10.39	10.43
Ash	9.90	9.50	9.30	9.25
Ether extract	4.63	4.84	5.02	5.15
Nitrogen free extract	54.49	54.73	54.42	53.81
Metabolizable energy (Kcal/kg)	2570.24	2514.75	2491.69	2490.54

### SEMEN EVALUATION

Thirty six (36) semen samples were collected after 10 weeks for evaluation from bucks in all the treatments. Prior to semen collection, bucks were all trained for using an artificial vagina (AV). The AV used was constructed locally with rubber rings, graduated collection tube, rubber funnel and latex inner liner (Figure 1). The consistency of the collected semen showed normal viscosity according to the method of Comhaire and Vermeulen (1995). The colour of the semen was determined by a careful visual appraisal according to Campos et al. (2014); and the volume was recorded via the use of calibrated tube attached as part of the artificial vagina. Sperm concentration was determined in a counting chamber with diluting fluid containing sodium bicarbonate, formalin and distilled water in a ratio of 1:20 using the improved Neubauer haemocytometer slide according to the method of Ochei and Kolhatkar (2000). The pH was measured using a specially treated calibrated paper blot that changes colour according to the pH of the semen (Comhaire and Vermeulen, 1995). Sperm viability was estimated using the improved one step eosin-nigrosin staining techniques according to Bjorndahl et al. (2003). Thus, live sperms will exude the eosin-nigrosin while dead sperm cells will not. Motility was determining according to World Health Organization (1992) methods. Samples from different treatments were dropped on a glass slide, covered with a slip and viewed under a microscope using x10 and x40 objectives. A minimum of 5 microscopic fields were assessed to evaluate sperm motility. The % of sperm motility was analyzed for actively motile and non-motile distinguished by the movement of the sperm cells. Sperm morphology determined by making a smear as blood film by placing a small drop of well mixed seminal fluid on a clean glass slide smearing it to a feather edge and quickly fixed (while still wet) in a cytological fixative such as 90% alcohol. The smear was stained with Giemsa. The stained smear was examined under microscope using x100 objective with immersion oil. About 100 spermatozoa with tail was counted and the percentage of abnormal forms was noted (Ochei and Kolhatkar, 2000).

### TESTICULAR MORPHOMETRY OF RABBIT BUCKS

After semen collection, bucks were slaughtered using the procedure described by Blasco and Ouhayoun (1996). Through the ventral region of the abdomen, the sacrificed bucks were dissected using a sharp blade to expose both right and left testes. The organ were freed off other connective tissues leaving behind only the testis and the attached epididymis. Testes weight and paired testes weight were determined using a Mettler Toledo sensitive scale. Testes length and circumference were measured with the use of vernier caliper and a tape. The volume of the testes was determined using Archimedes' principle of water displacement with a measuring cylinder in milliliter



(ml). Relative testes weight (%) and testes density (g/ml) were calculated using the formulas described by Amao et al. (2013).

$$\text{Relative Testes Weight} = \frac{\text{Paired Testes Weight} \times 100}{\text{Body Weight}}$$

$$\text{Testes Density} = \frac{\text{Testes Weight}}{\text{Testes Volume}}$$



**Figure 1:** The constructed artificial vagina for rabbit bucks.

## HISTOMORPHOLOGICAL EXAMINATION OF RABBIT BUCKS

For histomorphological test, 0.4g part of the harvested testes were collected. The samples were fixed in 10% phosphate buffered formalin for a minimum of 48 hours. The tissues were subsequently trimmed, dehydrated in four grades of alcohol (70%, 80%, 90% and absolute alcohol), cleared in three grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in water bath and incubated at 60°C for 30 minutes. The 5µm thick sectioned tissues were subsequently cleared in three grades of xylene and rehydrated in three grades of alcohol (90%, 80% and 70%). The sections were then stained with Haematoxylin for 15 minutes. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX. The prepared slides were examined with a Motic™ compound light microscope using x40 objective lens. The photomicrographs were randomly taken using a Motic™ 5.0 megapixels microscope camera at x400 magnifications. The method applied was according to Zahid et al. (2002) and Ansa et al. (2017).

## STATISTICAL ANALYSIS

All data were analyzed by a one-way analysis of variance (ANOVA) in a completely randomized design (CRD). Statistically ( $P < 0.05$ ) different means among treatment

groups were separated according to the procedures of Duncan's New Multiple Range Test (Duncan, 1955).

## RESULTS AND DISCUSSION

### SEMEN TRAITS OF RABBIT BUCKS

The results of the effect of varying dietary levels of discarded cocoa (*Theobroma cacao*) seed meal on semen traits of rabbit bucks are presented in Table 2.

An evaluation of the semen appearance showed that the inclusion of DCSM did not change the colour of the semen as there were milky white except for those in  $T_4$  that appeared creamy white. Semen pH was significantly ( $P < 0.01$ ) higher for the DCSM treatment groups compared with semen pH of those in the control group. Higher semen pH was shown for rabbit bucks in  $T_4$  (45% DCSM) diet and it varied from those of rabbit bucks in  $T_2$  and  $T_3$  groups. However, semen volume of rabbit bucks were similar ( $P < 0.05$ ) among treatment groups. Motility of spermatozoa and percentage live sperm in semen in the control,  $T_2$  and  $T_3$  were higher and did not vary ( $P > 0.05$ ) among treatments. Thus, at 45% DCSM dietary inclusion, the motility of sperm declined significantly ( $P < 0.05$ ) compared with others. Sperm concentration and percentage normal sperm were higher and similar ( $P > 0.05$ ) at 0 and 15% DCSM inclusion. A significant decline in these parameters were shown at 30% and 45% levels of DCSM. The percentage of non-motile and dead sperms in the semen had no significant ( $P > 0.05$ ) changes as the level of DCSM was increased from 0 to 30% inclusion in the diet, except at 45% where it significantly increased ( $P < 0.05$ ). The percentage of abnormal sperm showed no significant ( $P > 0.05$ ) difference at 0 and 15% DCSM, however, the number of abnormal sperm began to rise significantly from 30% to 45% DCSM dietary inclusion ( $P < 0.05$ ).

### TESTICULAR MORPHOMETRY AND HISTOMORPHOLOGY OF RABBIT BUCK

The results of the effect of varying dietary levels of discarded cocoa (*Theobroma cacao*) seed meal on the testicular morphometry of rabbit bucks are presented in Table 3. The results on testicular morphometry of rabbit bucks fed DCSM diet were not significantly ( $P > 0.05$ ) different among the treatment groups except in  $T_4$  with spermatogonial disruption. Figure 2 presents the micrograph of the histomorphology of rabbit buck testes fed varying dietary levels of discarded cocoa (*Theobroma cacao*) seed meal. It showed that no testicular histomorphological differences were observed among different treatments exposed to DCSM. The sections of the testes presented in all the experimental treatments showed normal testicular histomorphology. Thus, the magnified sections of the testes

**Table 2:** Semen evaluation of rabbit bucks fed discarded cocoa seed meal based diets.

Parameters	T <sub>1</sub> (0% DCSM)	T <sub>2</sub> (15% DCSM)	T <sub>3</sub> (30% DCSM)	T <sub>4</sub> (45% DCSM)	P. value
Semen appearance	Milky white	Milky white	Milky white	Creamy white	
Semen pH	6.17 ± 0.17 <sup>c</sup>	7.00 ± 0.00 <sup>b</sup>	6.83 ± 0.17 <sup>b</sup>	8.67 ± 0.33 <sup>a</sup>	0.000**
Semen volume (ml)	0.83 ± 0.09	0.77 ± 0.07	1.10 ± 0.15	0.77 ± 0.12	0.190 <sup>NS</sup>
Sperm motility (%)	76.67 ± 3.33 <sup>a</sup>	76.67 ± 3.33 <sup>a</sup>	70.00 ± 0.00 <sup>a</sup>	46.67 ± 3.33 <sup>b</sup>	0.000**
Sperm Con. (x10 <sup>6</sup> /ml)	84.67 ± 9.26 <sup>a</sup>	89.33 ± 5.21 <sup>a</sup>	57.67 ± 1.45 <sup>b</sup>	39.67 ± 9.35 <sup>b</sup>	0.003**
Non-motile sperm (%)	23.33 ± 3.33 <sup>b</sup>	23.33 ± 3.33 <sup>b</sup>	30.00 ± 3.33 <sup>b</sup>	53.33 ± 5.77 <sup>a</sup>	0.002**
Live sperm (%)	86.67 ± 3.33 <sup>a</sup>	86.67 ± 3.33 <sup>a</sup>	80.00 ± 0.00 <sup>a</sup>	53.33 ± 3.33 <sup>b</sup>	0.000**
Dead sperm (%)	13.33 ± 3.33 <sup>b</sup>	13.33 ± 3.33 <sup>b</sup>	20.00 ± 0.00 <sup>b</sup>	46.67 ± 3.33 <sup>a</sup>	0.000**
Normal sperm (%)	76.67 ± 3.33 <sup>a</sup>	76.67 ± 3.33 <sup>a</sup>	63.33 ± 3.33 <sup>b</sup>	36.67 ± 3.33 <sup>c</sup>	0.000**
Abnormal sperm (%)	23.33 ± 3.33 <sup>c</sup>	23.33 ± 3.33 <sup>c</sup>	36.67 ± 3.33 <sup>b</sup>	63.33 ± 3.33 <sup>a</sup>	0.000**

<sup>abc</sup>: Means ± SEM on the same row with different superscripts are significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ); Con.: Concentration.

**Table 3:** Testicular morphometry of rabbit bucks fed discarded cocoa seed meal based diets.

Parameters	T <sub>1</sub> (0% DCSM)	T <sub>2</sub> (15% DCSM)	T <sub>3</sub> (30% DCSM)	T <sub>4</sub> (45% DCSM)	P. Value
Live weight (kg)	2.10 ± 0.10	2.00 ± 0.20	2.05 ± 0.15	1.8 ± 0.20	0.641 <sup>NS</sup>
<b>Testicular characteristics</b>					
Right testis length (cm)	3.20 ± 0.00	3.25 ± 0.05	3.20 ± 0.10	3.10 ± 0.20	0.824 <sup>NS</sup>
Left testis length (cm)	3.30 ± 0.00	3.25 ± 0.05	3.25 ± 0.15	3.10 ± 0.20	0.724 <sup>NS</sup>
Right testis circumference (cm)	3.90 ± 0.30	3.95 ± 0.35	4.15 ± 0.05	3.65 ± 0.05	0.565 <sup>NS</sup>
Left testis circumference (cm)	3.95 ± 0.25	4.05 ± 0.35	4.20 ± 0.00	3.65 ± 0.15	0.465 <sup>NS</sup>
Right testis weight (g)	2.75 ± 0.25	2.95 ± 0.25	2.45 ± 0.05	2.10 ± 0.40	0.269 <sup>NS</sup>
Left testis weight (g)	2.55 ± 0.15	2.35 ± 0.35	2.60 ± 0.10	2.10 ± 0.50	0.696 <sup>NS</sup>
Paired testes weight (g)	5.25 ± 0.35	4.80 ± 0.60	5.25 ± 0.05	4.25 ± 0.85	0.578 <sup>NS</sup>
Right testis volume (ml)	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	2.25 ± 0.50	0.479 <sup>NS</sup>
Left testis volume (ml)	2.50 ± 0.50	2.50 ± 0.50	3.50 ± 0.05	2.50 ± 0.50	0.479 <sup>NS</sup>
Paired testes volume (ml)	5.50 ± 0.50	5.50 ± 0.50	6.50 ± 0.50	5.00 ± 0.00	0.242 <sup>NS</sup>
Relative testes weight (%)	0.25 ± 0.01	0.24 ± 0.01	0.26 ± 0.02	0.23 ± 0.02	0.586 <sup>NS</sup>
Testes density (g/ml)	0.96 ± 0.03	0.87 ± 0.03	0.81 ± 0.07	0.85 ± 0.17	0.747 <sup>NS</sup>

<sup>abc</sup>: Means ± SEM on the same row with different superscripts are significantly different ( $P \leq 0.05$  or  $P \leq 0.01$ ).

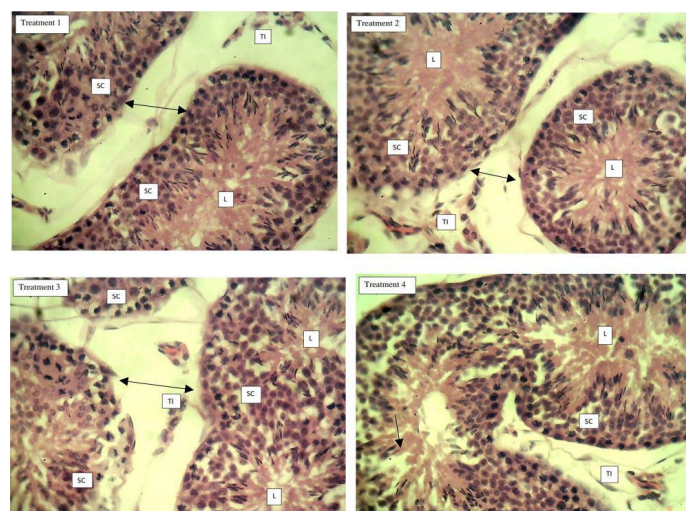
showed that a normal architecture were observed in control and treated groups and a clear and well separated seminiferous tubules surrounded by the basement membrane were shown. The only exception is found in T<sub>4</sub> containing 45% DCSM with some degrees of disruption of sperm cells.

The current study was carried out to determine various significant effect of DCSM on the reproductive aspect of rabbit bucks especially on semen traits, testicular morphometry and histomorphology. Results on semen evaluation showed that the test ingredients had various effects on the semen quality indices. The similar milky whitish appearance observed across T<sub>1</sub> to T<sub>3</sub> are indications that DCSM up to 30% inclusion did not cause any malfunctioning or inflammation of the reproductive tract

or the accessory sex glands (Banerjee, 2010). The only difference was experienced in T<sub>4</sub> with a creamy whitish appearance which might be as a result of the dark colour of the DCSM. Kostic (1997) stated that the alkalization process during cocoa processing will cause a darker colour of cocoa powder than the natural state. Thus, Jon (2020) reported that dietary changes may alter the semen colour in some cases. Semen pH and volume recorded in this work are within the normal range according to International Rabbit Reproduction Group (2005) and similar to values observed by Ajuogu et al. (2018) in rabbit bucks semen. The increase in semen pH with an increase in DCSM is in line with the report of Li et al. (2012) that the nature of increase in pH can be explained by the fact that the manufacturing process of cocoa powder involves an alkalization process which generally increases the pH of natural product from



5.2 and 5.6 to between 6.8 and 8.0 depending on the condition of alkalization. The non-significant differences observed for semen volume in this research is the same as those reported by Ojezele et al. (2016).



**Figure 2:** Photomicrograph of the section of the testes from T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. Note: seminiferous tubules are clearly separated and defined with thick layered spermatogenic cells (SC); testicular interstitium (TI) are clearly separated from the spermatogenic cells; spermatogenic cells are clearly distributed within the relatively spacious lumen (L); double arrow shows widened interstitial areas; arrow shows spermatogonial disruption (H & E x 400).

The results of sperm concentration, active, live and normal sperms showed that with an increasing concentration of DCSM above 15%, there exist a negative effect on the semen quality. Parameters for poor semen traits (non-motile, dead and abnormal sperm cells) increased with an increase in DCSM above 15% inclusion level. Thus, this is an indication that DCSM should be included in a minimal level to avoid detrimental effect on the semen quality. The results in this study is similar with the findings obtained by Okunola et al. (2017). According to Munier (2018), the addition of fermented cocoa product up to 20% level led to a decrease in both microscopic and macroscopic semen quality of goat. In addition, our result does not corroborate what Collodel et al. (2014) reported, that both chocolate (cocoa product) and propofenol exerted a protective effect on spermatogenesis and sperm characteristics of rabbit bucks. Spermatogenesis is under the control of the pituitary-hypothalamic-gonadal axis, it is possible that bioactive constituents bound in cocoa powder might have debilitating effects at a certain level on this axis or the testis (Friedman et al., 1979; Minji et al., 2015). It has been reported that theobromine in cocoa powder or the extract showed a negative effect on the sertoli cell leading to failed release of spermatids (Wang et al., 1992), reduction in the prostate and seminal vesicle (Funabashi et al., 2000), hyperplasia of the atrophied Leydig cells (Weinberger et

al., 1978), decrease in the fluid volume of the seminiferous tubules (Wang and Waller, 1994) in rats.

Results on testicular morphometry showed no significant ( $P>0.05$ ) variations across treatment groups. Similar non-significant ( $P>0.05$ ) observations were obtained by Amao and Showunmi (2016) and Velasquez-Pereira et al. (1998). Results from this research contradicts the significant ( $P<0.05$ ) different results reported by Ozung et al. (2019) on rabbit bucks reproductive tract morphometry on some parameters after dietary cocoa husk meal intake. Amao et al. (2013) also reported significant ( $P<0.05$ ) differences when rabbit bucks were fed diets containing neem (*Azadirachta Indica* A. Juss) plant meal. Although the present study observed no significant ( $P>0.05$ ) differences in all parameters, but are within similar range of values reported by Abu et al. (2013); Olarotimi et al. (2015); Abu et al. (2016); Amao et al. (2019); Ozung et al. (2019). According to Coulter (1980), testicular circumference is positively related to testicular weight for species like buck and ram and this report is similar with result of this study. Furthermore, Ahemen et al. (2016) reported that, testis length is among the indicators for the assessment of spermatogenesis, also Perry and Petterson (2001) and Akpa et al. (2013) noted that, length of the testis is a measure of the size of that testis and all these are positively related to sperm production.

The slight non-significant increased values observed in this study might be as a result of cocoa polyphenols. Reactive oxygen species (ROSs) are important components in reproductive mechanisms and the production of ROS is a normal physiological process in several organs including the testes; however, excessive production can appear deleterious to the testis or male fertility (Akiyama, 1999; Al-Gubory et al., 2010). Thus, polyphenols as a strong antioxidant tends to ameliorate oxidative damages of ROS (Rucker, 2009). From all indications, the non-significant ( $P>0.05$ ) differences reported based on the effect of DCSM on the reproductive organ of rabbit buck testis, probably may not have been observed morphometrically at the duration of this study. The effect of DCSM did not cause hypertrophy or hyperplasia in the testis of rabbit bucks throughout the duration of this study and Zar (1996) stated that increased sizes and weights of body organs are as a result of hypertrophy and hyperplasia. Gans et al. (1980) and Tarka et al. (1981) reported that the effect of theobromine on the reproductive organs of male animals were found to be both time and dose dependent.

The photomicrograph on the testes histology obtained were similar with the findings of Abdul-Rahman (2015). The anatomy and histology of the reproductive systems of rabbits according to Herrera et al. (2014) and Zamora et al. (2014) showed a similar morphology as the ones obtained

## AUTHOR'S CONTRIBUTION

Ali LC: Conceptualized the work, prepared first and final drafts. Ikeh NE: Statistical analysis and literature review. Amaefule BC: Literature review and data collection. Obinna AL: Data collection and sorting. Machebe NS: Reviewed manuscript and supervised the field work.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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in this study. Uzochukwu (2016), reported a no testicular histomorphological differences when African catfish (*Clarias gariepinus*) were fed diets containing cocoa bean meal. In other research, non-pathological changes were reported by Shinkut (2015) after feeding rabbit buck with dietary supplemental garlic (*Allium sativum*). At 200mg/kg of diet, it was reported that unsweetened natural cocoa powder had no toxic effect on the testicular histology of Sprague-Dawley rats (Asiedu-Gyekye et al., 2016). Conversely, Oguike et al. (2019) reported a varying degree of degeneration in the histology of rabbit bucks fed *Aspilula africana* leaf meal when compared to the control group; and similar observation was also reported in wistar rats by Asuquo et al. (2015). This outcome probably might be as a result of theobromine inherent in cocoa powder which is capable of causing debilitating effect on the testis (Smit, 2011). This ideology is supported by Eteng et al. (2005); European Food Safety Authority (2008) and Park et al. (2015).

## CONCLUSIONS AND RECOMMENDATIONS

It was concluded from this study that feeding rabbit bucks DCSM diet suppressed semen quality even though results from testicular morphometry showed no significant ( $P>0.05$ ) variations. At 45% inclusion of DCSM, spermatogonial disruption was also reported. Theobromine inherent in DCSM might be the resulting factor leading to these poor result. Theobromine effect could be minimized by practicing detheobromination techniques in other to reduce its content in any sample of cocoa powder. It is recommended that dietary inclusion of DCSM in rabbit bucks feeding must not exceed 15% inclusion level.

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## NOVELTY STATEMENT

To the best of our knowledge, this study is the first to decipher the effects of DCSM (Theobroma cacao) based diet in rabbit bucks with interest on semen, sperm, testicular morphometry and histomorphological traits in a tropical environment, and therefore can be used as alternative feedstuff.



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