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ASSESMENT OF VITAMIN AND TRACE ELEMENTS ENRICHED DIET ON SPERM PARAMETERS IN THE GRASSCUTTER (THRYONOMYS SWINDERIANUS)) IN CAPTIVITY IN IVORY COAST

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ABSTRACT

The grasscutter is African rodent which is bred in captivity to protect the species against poarching. The introduction of the industrial-type pellets was a major innovation in grasscutter breeding. The nutritional and minerals played a very important role in male fertility. The aim of this work is to improve the reproductive performance of grasscutters in captivity. Thirty grasscutters (Thryonomys swinderianus) aged seven months were selected. They were divided into two batches of fifteen individuals in order to receive distinctly two types of food. Both batches of grasscutters received forage and pellets respectively for one month. Semen was collected by using the flotation method. Spermogram and spermocytogram tests were then carried out to make a quantitative and qualitative assessment. Sperm analysis showed that grasscutters fed with the pellet had a higher average sperm concentration and a lower percentage of sperm abnormalities than those fed with the forage. The pH, vitality and mobility percentages were not significant in either batch. It should be noted that pellet-fed grasscutter had better body weight gain, testicular weight gain and semen quality. The study showed that the feeding with pellet had increased the quality of the grasscutters sperm quality.

Keywords: Grasscutter, Sperm Parameters, Flotation, Pellet

1. INTRODUCTION

The grasscutter is an African rodent belonging to the order Rodentia and the suborder Hystricomorpha. There are two species of grasscutter: Thryonomys swinderianus (Temminck, 1827) and Thryonomys gregorianus (Thomas, 1894). The breeding of the greater grasscutter (Thryonomys swinderianus) was undertaken with the aim of protecting the species from the pressure of poaching on its natural populations (Soro et al. (2020)). Managing the reproduction of this

species in captivity has been a major challenge. (Okon et al. (2023)). Previous studies have established the anatomical and histological structures of the male and female reproductive tract of the grasscutter. (Mbouga (2011); Okon (2016)). Additionally, methods for collecting sperms from this animal like masturbation, epididymal extraction and testicular sampling have been explored to facilitate their potential use in artificial insemination in grasscutter breeding (Olukole et al. (2010); Adebayo et al. (2019); Okon et al. (2023)).

Diet remains an important parameter in breeding whose influence on the growth and sexual maturity of the grasscutter in captivity has been well-documented (Soro et al., 2014; Aïzoun et al. (2015), Aïzoun et al. (2016)). The introduction of the industrial-type pellets was a major innovation in grasscutter breeding. This development reduced food wastage by cane rats to 24% compared to bulk green fodder and improved the average food consumption index from 3.4:1 to 58.3:1 kg DM/Kg gain PV (Aïzoun et al. (2015),). The use of these pellets also alleviated the challenges of feeding during the dry season which adversely affected the reproduction in grasscutters (Soro et al. (2020)). To sustainably address these issues several studies have focussed on formulating complete feeds from various raw materials. While the impact of pellets has been widely studied, research on other livestock species like (Praag (2016)), Ouled Djellal sheep (Zineddin (2018)) and Muscovy ducks (Korochkina et al. (2014)) has shown that deficiencies in Vit B12 and certain minerals such as selenium, zinc and carnitine play an important role in male fertility (Yapi et al. (2013)). The impact of pellets on grasscutter reproduction has been widely studied. However, it has been observed in certain livestock animals such as male rabbits (Praag (2016)), Ouled Djellal sheep (Zineddin (2018)) and Muscovy ducks (Korochkina et al. (2014)) that deficiencies nutritional vitamin B12 and certain minerals such as selenium, zinc and carnitine play a very important role in male fertility. So what would be the impact of a food enriched with vitamins and trace elements, presented in granulated form, on the quality of male grasscutter sperm?

The main objective of this work is to improve the reproductive performance of male grasscutter in captivity. Specifically, it will be:

1) To determine the variations in body weight and that of the gonads of male grasscutter fed on pellets and fodder.
2) To evaluate the quantitative and qualitative parameters of the seed of grasscutter fed on pellets and fodder.
3) To compare the quality of the semen of cane rats subjected to two diets

2. MATERIAL AND METHODS

2.1. STUDY SITE

Research has been undertaken at the grasscutter farm of Toumodi in the district of Lake. It is located in the centre of Côte d'Ivoire, in the forest-savannah transition zone, between 6°20' and 6°30' North latitude and between 4°55' and 5°10' West longitudes. Toumodi is a transition zone between forest and savannah, with a bimodal tropical rainfall regime. The average annual rainfall is 1200 mm/year. Rainy periods begin from May to August and from October to November with a dry season interspersed in September. The long dry season concerns the period from December to April. The soils are of the Ferralsols, Cambisols type. Hydromorphic soils are also encountered in the lowlands. (Koffi et al. (2023)) (Figure 1).

Figure 1

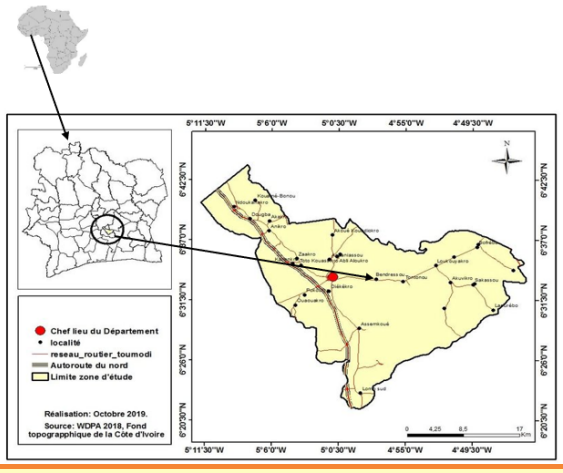

Figure 1 Location of the Grasscutter Center of Toumodi

2.2. STUDY DESIGN

This descriptive and analytical experimental study was carried out over a period of three (3) months. This study was carried out in accordance with Directive 2010/63/EU, supplemented by Resolution No. 493 of 2020 on the protection of animal welfare in the European Union.

2.2.1. ANIMAL SELECTION

Thirty (30) healthy pubescent male grasscutter rats aged 7 months and were used to carry out the study. Was there a method of exclusion?

The animals were divided into two groups A and B. each with 15 animals. Two categories/ forms of food (A and B) were used to feed the cane rats. One type of food was composed of fodder, specifically Pennisetum purpureum, and the other category is an industrial type presented in the form of a granulated food (Figure 2 and B). The nutritional compositions of these feeds are contained in Table 1 and Table 2

Figure 2

Figure 2 a Green Fodders Panicum Maximum- Pennisetum Purpureum **b Pellets**

Table 1

Table 1 Chemical and Energetic Composition of Green Fodders

Nutrients (%)	Nutritional and energy	Panicum maximum	Pennisetum purpureum
Dry matter	Organic matter	26.90	21.90
Total nitrogen material		9.70	8.90
Fat		2.50	2.10
Digestible organic matter		92.48	92.78
Starch		49.37	36.20
NDF		92.46	84.80
Crude cellulose		72.04	61.40
Non-nitrogenous extractive		35.10	33.70
Raw ash		40.80	40.00
Digestible nitrogenous material		6.10	15.20
Raw ash	Mineral and vitamin	11.9	15.2
Calcium		0.43	0.37
Phosphorus		0.37	0.29
Ca/P		1.16	1.27
Raw energy,	Energy	4155.67	1172.2
Metabolizable energy		488.11	500.24

Table 2

Table 2 Chemical composition of Pellets

Composition of pellets	Reproduction food	Growth food (weaning at 4 months)	Finishing food (4 months and over)
Nutrient	Contribution in % of gross product, except energy in kcal/kg		
Dry matter	89.62	89.94	89.75
Crude ash	6.18	7	6.43
Crude protein	16.00	16.00	16
Fat	3.69	3.44	3.53
Crude fiber	15.48	18.26	16.53
Lignocellulose	16	19	17
Lignin	2.5	3.12	2.5
Starch	24.21	16.91	22.17
Lysine	0.71	0.74	0.75
Methionine	0.25	0.26	0.26
Amino acids, total sulfur	0.51	0.52	0.52
Calcium	0.36	0.36	0.35
Phosphorus	0.4	0.47	0.38
Digestible energy	2600	2421	2538

Group A animals (control group) were fed fodder and group B animals were fed with pellets. The animals were watered and fed ad libitum daily in a breeding building with a temperature of 20 to 28°C and lighting for 16 hours/8 hours.

2.2.2. SAMPLE COLLECTION AND SPERM ANALYSIS

Semen samples were collected from all cane rats by epididymal puncture under general anesthesia using a solution of ketamine hydrochloride at a dose of 0.1ml per kilogram of live weight (Okon et al. (2023)). The evaluation of the sperm consisted of macroscopic and microscopic examinations within WHO, (2021) recommendations.

Motility was assessed by placing a drop of diluted sperm between slide and coverslip observed at magnification (x40). This examination consisted of assessing the movement of the spermatozoa. It was both quantitative (percentage of motile sperm) and qualitative (type of sperm movement).

Sperm vitality was determined by preparing a smear using the eosin-nigrosin vital stain: a drop of diluted sperm was mixed with a drop of dye, then the mixture was gently spread on a slide using of a coverslip and dried in the open air. Then it was observed under a microscope at magnification (x 40); 100 spermatozoa are counted, from which the percentage of coloured spermatozoa is estimated corresponding to dead spermatozoa whose damaged membrane is permeable to pink colouring, while live spermatozoa with their functional membranes do not allow the dye to diffuse and therefore remain colourless.

The concentration of the sperm, after having carried out a dilution of the sperm using a 0.9% NaCl solution. 1 mL of diluted sperm is introduced into the compartments of the Malassez cell. The sperm count was done at 40× magnification, on at least two strips. The concentration was determined by the formula: Sperm number ×.10×. 1000× dilution denominator. Furthermore, the spermocytogram carried out made it possible to analyze the morphology of the spermatozoa and to detect sperm abnormalities. This examination was carried out by spreading a 0.2 µL drop of sperm on a clean slide using another slide tilted at 45°C. After fixation with alcohol-ether, the smear is dried in the open air before being stained with MGG (May Grunwald Giemsa). The coverslips were then placed on the coloured smear before reading at 100× immersion.

2.2.3. STATISTICAL ANALYSIS

Data was recorded and analysed using Microsoft Excel 2016 software. A student T-test was used to compare mean values for normally distributed data whereas for non-normally distributed data, the mean values were compared by a non-parametric two side Mann-Whitney test. A P value of <0.05 was considered statistically significant.

3. RESULTS AND

3.1. ANALYSIS OF THE BIOMETRIC VALUES OF GRASSCUTTER FED WITH PELLETS AND FODDERS

The weight variations of the grasscutter from batches A and B reported in Table 3 present a highly significant difference ($p \leq 0.01$) using the student test. Animals fed pellets had a significant weight gain 2330±0.52 (n=15) compared to those fed fodder 1870±0.29 (n=15).

Table 3

Table 3 Distribution of Greater Cane Rat by Age Category (n=30)			
Batch	**Value**	**Age (months)**	**Average weight (g) ± standard deviation**

A	15	7	1870±0,29 [1670-2210]
B	15	7	2330±0,52 [1870-2900]

3.2. COMPARATIVE ANALYSIS OF THE SPERMOGRAM-SPERMOCYTOGRAM OF SEMINAL FLUID FROM GRASSCUTTER FED WITH PELLETS AND FODDER

The semen was generally whitish in colour and moderately viscous. The seminal fluid characteristics were mainly related to the volume, pH, vitality, motility, concentration and morphology of the spermatozoa (Table 4).

Table 4

Table 4 Data from Seminal Fluid Analysis of Greater Cane Rat Obtained by Aspiration (N=30)

	Volume	**pH**	**Vitality**	**Mobility**	**Concentration**	**Normal sperm forms (%)**	**Abnormales sperm Forms (%)**		
Lot	(ml)		(%)	(%)	(10^6/ml)		Head	Intermediate piece	Flagellum
A	0,031 ± 0,7	6,5	79 ± 1,4	54 ± 7,6	492,2 ± 0,7	78,6 ± 1	7,13 ± 1,2	12,13 ± 0,9	2,13 ± 0,5

- Volume: The Kruskal-Wallis test showed a no significant difference at P >0.05 between the semen volumes fed with pellets and fodder. The mean volume of semen fed with fodder (0,031 ± 0,7 ml) was substantially equal to that fed with pellets (0.035 ± 0.4 ml).

- pH: The mean pH of the semen collected was 6.8 for semen fed with fodder and 6.5 for the one fed with pellets.

- Vitality: Overall, the Kruskal-Wallis's test did not yield a significant difference P> 0.05 for the comparative analysis of vitality. Mean vitality values were 59.4 ± 8% for fed with fodder and 60.3 ± 4% for those fed with pellets.

- Motility: The Kruskal-Wallis's test did not yield a significant difference P> 0.05 for the comparative analysis of motility. Mean motility values were 51.26 ± 3.6 % for fed with fodder and 53.4 ± 5.5% for those fed with pellets.

- Sperm concentration: Comparative analysis of semen concentration values showed a significant difference P < 0.05 using the Kruskal-Wallis test. The mean sperm concentration fed with fodder was 154.33 ± 11.44 106/ml. This value is lower than that fed with pellets, which is 512.06 ± 1.1 106/ml.

Sperm shape: Individual sperm analysis revealed normal and abnormal shapes. The average rate of normal sperm shape was 78.4 ± 3.3% versus 21.6 ± 3.3% of abnormal shapes. The Kruskal-Wallis test showed no significant differences P>0.05 for the comparative analysis of the percentages of spermatozoa shapes obtained by the different types of sampling.

3.3. DISCUSSION

The comparative study of the parameters of grasscutter spermatozoa fed with pellets and fodder showed similarities and differences. For instance, the semen was whitish in colour as also observed by Olukole et al. (2014) in grasscutter and Bencheikh (1995) in rabbits. This observation differs from that of Hounzangbe-Adote (2004) and Soro et al. (2009), who observed a yellowish-white colouration of semen collected by masturbation from male greater cane rats. In fact, according to Simons & Ross (2021), the colouring of the semen is caused by a protein of prostate origin called spermine, whose oxidation would cause the yellowing of the semen, very often due to a long period of sexual abstinence. The mean volume of semen of grasscutter fed with pellets and fodder was much lower than the mean volume of semen collected by masturbation (Soro et al. (2009)). This result supports the observations of Boersma et al. (2015), who collected small volumes of semen in mice by percutaneous aspiration of epididymal fluid. Similarly, the mean volume of semen obtained by masturbation is 0.3 ml higher than that obtained by Olukole et al. (2014). In fact, the semen volume of an animal depends on several genetic, nutritional, physiological, pathological and environmental factors Oliveira (2015). The pH values (6.5-6.8) measured on semen samples are approximately the same as those obtained by Hounzangbe-Adote et al. (2004) These pH values, obtained in greater cynomolgus rats between 7 and 13 months of age, confirm those obtained by Bencheikh (1995) in rabbits from 6.68 to 7.06. According to Korochkina et al. (2014), the acidity of semen is due to prostatic secretions. The mean sperm vitality recorded in this study was approximately equal between the two groups. These values are lower than the 95 ± 1.16 obtained by electroejaculation by Olukole et al. (2010) Bencheikh (1995)obtained mean values of 48.9 to 84.5% in rabbits. These different values of sperm vitality in the grasscutters could be related to the sperm collection techniques used, as noted by Cary et al. (2004). The mean values of sperm motility of 53.4 ± 5.5% (pellets) and 51.26 ± 3.6% (fodder) obtained in this study are lower than those obtained by Hounzangbe-Adote et al. (2004)which ranged from 59 to 70% in grasscutters aged 7 to 30 months. Olukole et al. (2014) obtained about 73% motility. This difference in observation could be explained by the method of assessment of semen parameters. The assessment performed in this study is based on the modified David model (WHO, (2021)). In fact, this method requires two measurements to be taken at 1 h and 3 h after collection. However, Hounzangbe-Adote et al. (2004) performed a single reading 1 h after collection, and Soro et al. (2009) and Olukole et al. (2014) did not specify the reading mode for determining sperm motility. The mean sperm concentrations obtained were 154.33 ± 11 106/ml (fodder) and 512.06 ± 1.1 106/ml (pellets), respectively. These mean values are higher than the maximum concentrations obtained by Hounzangbe-Adote et al. (2004) of 143 ± 27 106/ml in the 7–8-month interval and by Soro et al. (2009) of 144 ± 2 106/ml. However, they remain low compared to the values obtained by electroejaculation (136.10 ± 9.15 109/ml) and after testicular biopsy (319.3 109/ml). These different values demonstrate the need to establish reference values for spermiology in the grasscutter.

4. CONCLUSION

This study compared the sperm values of grasscutters fed with pellets and fodder. From the comparative analysis it was concluded that the feeding with pellets provides good vitality and higher sperm concentration. However, a more thorough study involving a large number of animals will allow reference values to be established for grasscutter semen in order to characterise the species.

CONFLICT OF INTERESTS

None.

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