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Original Research Article

Effects of dietary energy level on reproductive characteristics in male African giant rat *Cricetomys gambianus*

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ABSTRACT

Background: The study was conducted to assess the effects of the digestible dietary energy level on some reproductive characteristics in African giant rat.

Methods: Sixteen young males were randomly distributed into 4 groups of 4 animals each. To each group was attributed randomly one of the 4 dietary energy levels (3600 Kcal/kg, 3800 Kcal/kg, 4000 Kcal/kg or 4200 Kcal/kg). The daily distribution of experimental diets last six months, ie ended when cricetoma were 8 months old. At the end of that period, all animals were sacrificed.

Results: Results showed an increase in testes weight with the augmentation of dietary digestible energy level (0.79±0.13, 0.88±0.17, 1.02±0.28 and 1.02±0.16 respectively for 3600 Kcal/kg, 3800 Kcal/kg, 4000 Kcal/kg and 4200 Kcal/kg). The serum testosterone level, the sperm mobility (76.67, 62, 63 and 57%) and count per cauda epididymis (18.25±3.75, 16.38±4.19, 10.83±2.02 and 10.13±2.9) and per gram cauda epididymis (39.09±11.82, 27.01±4.23, 15.41±3.31 and 17.40±7.28) significantly ($p < 0.05$) decreased with the increasing level of digestible energy in the feed.

Conclusions: The dietary digestible energy level that gave the higher reproductive performances in male African giant rat was 3600 Kcal/kg DM.

Keywords: African giant rat, Cauda epididymal sperm characteristics dietary energy level, Genital organs, Testosterone concentration

INTRODUCTION

Cricetoma is the biggest rat all over the world and is found in more than 29 countries. Because it is an appreciated source of meat for the population, it is the most hunted among the Sub-Saharan Africa games of small size.^{1,2} Its breeding would be a solution against overhunting. Unfortunately, in captivity, the African giant rat reproduces rarely, and if any, gives very low litters. Results of the studies conducted at the teaching

and research farm of the University of Dschang, and those from private farms are perfect illustrations. Contrarily, when acquired from the wild life in pregnant status, the litter size can reach 7-8 at the kidding. The poor performances from the captivity are indicators of the lack of mastery of breeding techniques.

Cricetoma is an omnivorous and in the bush, feeds on various sources.^{2,3} It is possible that its nutritional requirements be not covered in captivity.

Nutrition is one of the main factors involved in sexual development and reproductive function in mammals.^{4,5} Most studies indicate that a better nutrition at the beginning of life increases body and testes weights, blood testosterone level and epididymis reserve of sperm.⁶ In adult males for example, a high energy diet increases libido, seminiferous tubules and testes size hence, improves sperm production.⁷

Energy is the major element in feed responsible for the ingestion and utilization of different nutrients, and consequently animal productivity. Indeed, inadequate feed, particularly in energy in most breeding animals would negatively affect productive parameters as life weight and body fat which will have repercussions on cholesterol level and possibly on circulating LH and testosterone levels and semen quality in male.⁸⁻¹¹ That is why 9 reported that energy excess or deficit in feed is detrimental to reproductive performances. Besides, 11 showed a decrease in sperm mobility and consequently fertility in male rats given high-fat diet. It is consequently necessary to know the optimal concentration of energy that allows the best productive performances in each animal species. To the best of our knowledge the energy

needs of cricetoma are not known, hence the present study.

METHODS

Animal material

Sixteen young males' rats (*Cricetomys gambianus*) aged 2 months and averaging 293.41±19.21 g, bred at the Teaching and Research Farm of the University of Dschang, Cameroon were used. They were housed individually in cages of 100 cm x 80 cm x 60 cm (length, width and height) under natural photoperiod.

Inclusion criteria

- All males in this study weighing between 285-295 g and the age of data collection of reproductive parameters were considered at 8 months old which represents in this study.

Exclusion criteria

- Animals with growth delay; animals with aggressive behaviour.

Table 1: Centesimal composition and bromatological characteristics of diets.

Ingredients	Centesimal composition			
	D1	D2	D3	D4
Corn	52.00	60.50	60.00	60.00
Brewers chaffs	15.00	08.00	11.50	09.50
Cottonseed meal	08.00	05.00	02.00	00.00
Soya bean meal	15.50	14.00	09.00	08.00
Fish flour	2.00	04.00	05.00	06.00
Bone flour	4.00	03.00	03.00	02.50
Palm oil	2.00	03.00	05.50	08.00
Blood flour	1.00	02.00	05.00	06.00
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Bromatological characteristics of diets				
Crude Protein (% DM)	19.30	19.30	19.10	19.16
Digestible energy (Kcal/kg DM)	3620.00	3816.50	4018.78	4208.41

Feeding

The composition and chemical characteristics of experimental feeds are presented in Table 1.

Experimental design

Animals were randomly allocated to 4 groups, each corresponding to one the 4 dietary energy levels (3600 Kcal/kg, 3800 Kcal/kg, 4000 Kcal/kg and 4200 Kcal/kg). Each group was made up of 4 male cricetoma. The

distribution of experimental feed last six month. At the end of that period, animals were sacrificed in order to evaluate the development of reproductive organs and epididymal sperm characteristics.

Studied parameters and data collection

Organ weight

At the end of treatment, the live weight was registered and animals were sacrificed. Reproductive organs (testes, epididymides, seminal vesicle, vas deferens and prostate)

were removed, weighed and the average weight of each organ calculated as follow:

$$\text{Organ weight} = \frac{\Sigma (\text{individual organ weight} / \text{animal life weight} \times 100)}{\text{number of individuals}}$$

Histology of testes

One testis of each animal was preserved for histological studies. The testis was fixed in 10% formol, washed with tap water, dehydrated gradually in alcohol solution (80, 90 and 100°), clarified in xylen baths, hardened in paraffin, sectioned and stained with haematoxylin and eosin. The sectioned tissues were observed and filmed under a light microscope at 400X magnification, using the camera of a microsystem Leica.

Cauda epididymal sperm characteristics

One of caudal epididymis was weighed and shredded in 10 ml of 0.9% NaCl solution, incubated at 37°C.

1. Mobility

The mobility of sperm was appreciated by direct examination of the solution. Thus, 20 µl of the previously prepared solution were placed on a slide, and then observed under a light microscope at magnification 400. Sperm mobility was evaluated by attributing a score of 0-5.¹²

2. Concentration

0.1 ml of the mother solution (10 ml) was diluted with 2.4 ml of formol (35%) solution to kill and stabilize sperm during counting. A drop of the diluted solution was placed on the Thoma cell chamber and sperms counted under the light microscope at magnification of 400. To

determine the sperm number in a tail of epididymis, the following formula was used

$$\text{Number of sperm cells/tail} = \frac{(N \times 4 \times V \times DF \times 1000/0.1)}{2}$$

N: number of sperm in 4 big squares of the thoma cell.

V: volume of stock solution (10 ml),

DF: Dilution factor (25),

1000: convection factor of mm³ to ml,

0.1: volume of chamber (mm³),

2: number of caudal epididymides shredded.

Sperm number was also expressed per gram of epididymis. Serum level of LH, FSH and testosterone. Serum was collected and used measure the concentration LH, FSH and testosterone in male using Omega Diagnostics (Scotland, United Kingdom).

Ethic

Animals were handled according to ethical guidelines of the cameroon national veterinary laboratory.

Statistical analysis

Results were expressed as mean±standard deviation. Differences between groups were assessed using one-way ANOVA followed by the Duncan's test at 5% significance.

RESULTS

Table 2 summarize the variation of genital organs weights based on dietary digestive energy level. It indicates that testes, epididymis, vas deferens and accessory sex glands weight seemed to increase with the dietary digestible energy level, although not significantly (p > 0.05).

Table 2: Effects of dietary energy level on sexual organ weight in cricetoma.

Sexual organs (g/100 g bw)	Dietary energy level (Kcal/kg DM)				p-value
	3600	3800	4000	4200	
Testes	0.79±0.13	0.88±0.17	1.02±0.28	1.02±0.16	0.27
Caudal epididymides	0.07±0.02	0.09±0.02	0.11±0.03	0.10±0.02	0.23
Epididymis head	0.06±0.01	0.07±0.02	0.07±0.01	0.07±0.02	0.57
Epididymis	0.13±0.02	0.15±0.04	0.18±0.04	0.17±0.04	0.33
Vas deferens	0.03±0.01	0.04±0.02	0.03±0.00	0.03±0.01	0.68
Accessory sex glands	0.52±0.18	0.76±0.23	0.89±0.34	0.51±0.11	0.37

Mobility and concentration of caudal epididymal spermatozoa

The sperm mobility was significantly (p < 0.05) higher in animals fed 3600 Kcal/kg DM compared to diet with of digestible energy 4200 Kcal/kg DM. However, no

significant (p > 0.05) difference was registered between diets at 3800 et 4000 Kcal/kg DM. Sperm count per caudal of epididymis was significantly (p < 0.05) higher in animals fed 3600 Kcal/kg DM compared to diet with digestible energy 4200 Kcal/kg DM. Likewise, Sperm count per caudal of epididymis of animals receiving 3800

Kcal/kg DM was significantly ($p < 0.05$) higher than that of animals fed 4000 Kcal/kg DM. Sperm count per gram of caudal epididymis decreased significantly ($p < 0.05$) with increasing level of energy in feed (Table 3), the diet

with 3800 Kcal/kg DM being comparable firstly with diet containing 3600 Kcal/kg DM and secondly with diet at 4000 and 4200 Kcal/kg DM.

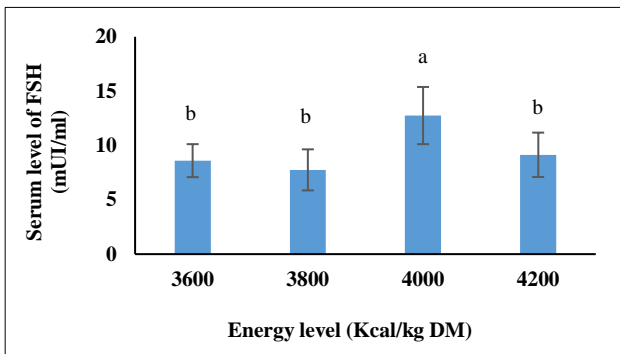
Table 3: Effects of dietary energy level on epididymal sperm characteristics in cricetoma.

Epididymal sperm characteristics	Dietary energy level (Kcal/kgDM)				p-value
	3600	3800	4000	4200	
Motility (%)	76.67±5.77 ^a	62.50±5.00 ^{ab}	63.33±15.28 ^{ab}	57.50±5.00 ^b	0.04
Number/tail ($\times 10^7$)	18.25±3.75 ^a	16.38±4.19 ^{ab}	10.83±2.02 ^{bc}	10.13±2.95 ^c	0.02
Number/g of tail ($\times 10^7$)	39.09±11.82 ^a	27.01±4.23 ^{ab}	15.41±3.31 ^b	17.40±7.28 ^b	0.01

a, b, c: on the same line, values affected with the same letter do not differ significantly ($p > 0.05$).

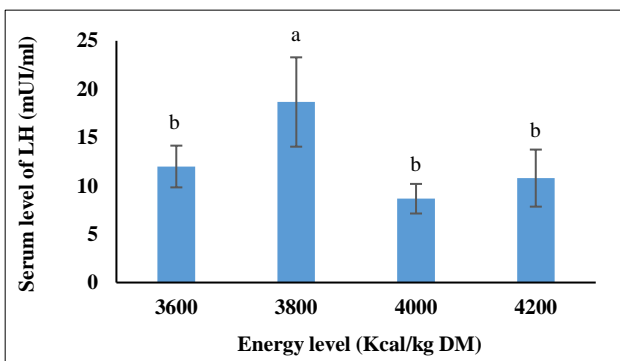
Serum level of LH, FSH and testosterone

The serum levels of FSH (Figure 1) was significantly ($P < 0.05$) higher in animals fed 4000Kcal/kg DM compared to other diets. That of LH (Figure 2) was significantly ($P < 0.05$) higher on rat fed on diet digestible energy 3800 Kcal/kg DM. Concentration of testosterone in the serum (Figure 3) decreased significantly ($p < 0.05$) with increasing level of digestible energy in feed.



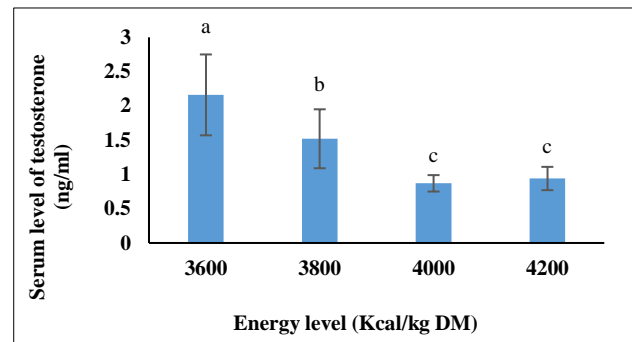
a, b: on the same bar affected with the same letter do not differ significantly ($p > 0.05$).

Figure 1: Effects of dietary energy level on serum level of FSH on male cricetoma.



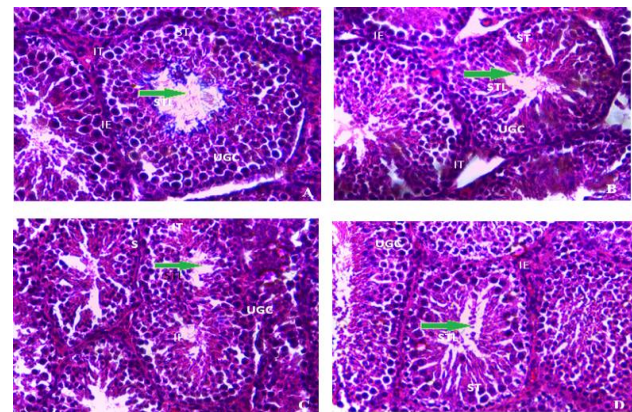
a, b: on the same bars, values affected with the same letter do not differ significantly ($p > 0.05$).

Figure 2: Effects of dietary energy level on serum level of LH on male cricetoma.



a, b, c: on the bar, values affected with the same letter do not differ significantly ($p > 0.05$).

Figure 3: Effects of dietary energy level on serum level of testosterone on male cricetoma.



UGC: undifferentiated germ cell; IE: Interstitial space; STL: Seminiferous tubules light; IT: Interstitial tissue; ST: seminiferous tubules. Plate A: 3600 Kcal/kg DM; Plate B: 3800 Kcal/kg DM; Plate C: 4000 Kcal/kg DM; Plate D: 4200 Kcal/kg DM.

Figure 4: Testes structure of cricetoma fed on digestible various dietary energy level (HE x 400).

Testes histology

Whatever the dietary energy level, the microscopic observation of histological sections of testes (Figure 4) showed well organized, individualized and reduced

interstitials space of seminiferous tubules. From the basal membrane to lumen were observed germinal cells at different stage of their development. The tubules lumen contains many matures sperm regardless of dietary energy level.

DISCUSSION

The reproductive function in animals is influenced by their nutritional requirements.^{5,7} In this study, the reproductive organs weight such as testes and epididymis were higher, although not significantly in animals fed higher level of energy (4200 Kcal/kg DM). Similar results were obtained by in laboratory rat.^{13,14} The absence of the significant difference in testes weight is comforted by the structural integrity of those glands as shown by histological section under different dietary energy levels. Indeed, at the view of testes sections, none of the studied energy levels altered the structure of testes; their observation revealed none of the commonly apparent anomalies shown by altered testes, such as vacuolization of the seminiferous epithelium, depletion, massive presence of immature spermatozoa in the lumen of seminiferous tubules.

The similarity of epididymis, vas deferent and accessory glands weights seemed to be linked to the testis rather than the testosterone concentration that is known to be the hormone controlling their development. Indeed, contrarily to testis weight, testosterone concentration diminished with the increase of energy in the diet.

The low concentration of testosterone in rat fed 4000 kcal/kg. DM and above would be linked to blood LH concentration; this trend is normal because LH is the hormone stimulating its production.

Sperm concentration, mobility and morphology are first criteria used to evaluate male fertility. They are the first markers of testicular spermatogenesis and epididymis maturation.^{15,16} Generally, the higher the sperm count, the higher the mobility. Thus, the weak sperm mobility in cricetoma receiving the highest energy ration could be linked to their weak concentration in the cauda epididymis. Inturn the low sperm count would signify that the dietary energy level was excessive for an optimal production of spermatozoa, because a negative relationship is established between spermatogenesis and dietary energy level.^{10,17}

CONCLUSION

At the end of this study, it can be admitted that the dietary digestible energy requirement that gave the best reproductive performances in male African giant rat was 3600 Kcal/kg DM. However, others study could be done on low energy level to more appreciate the real energy requirement in male African giant rat.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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