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Reproductive performance of male broodstock *Clarias jaensis* (Boulenger, 1909) fed various dietary energy

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Abstract

This study aims to investigate the effects of four diets with different energy contents; 3000, 3100, 3200 and 3300 Kcal/kg, on reproductive characteristics of male broodstock *Clarias jaensis*. 360 male and female (97 ± 9 g) were divided into four groups, corresponding to the different diet and were breed during 5 months. Except the FSH and Hepatho somatic index, reproductive characteristics were comparable between diets ($P > 0.05$). The higher FSH (1.15 ± 0.27 mIU/ml), LH (1.03 ± 0.18 mIU/ml), testosterone (179.67 ± 5.37 ng/ml), Gonado somatic index ($0.33 \pm 0.05\%$) and mature male (87.5%) were recorded at 3000 Kcal/kg followed by 3100 Kcal/kg, while Hepatho somatic index were higher ($1.11 \pm 0.14\%$) at 3100 Kcal/kg. Histological inspections of gonads did reveal that fish fed at 3000 Kcal/kg had heavier and more developed gonads compared to other group. Therefore; 3000 Kcal/kg was found to be optimal for healthy gonad development.

Keywords: *Clarias jaensis*, energy, reproduction, broodstock

1. Introduction

Cameroon has an aquatic biodiversity rich in Claridae, represented in many rivers by different species. Nevertheless, in the rivers of the Mbô Plain, located in the Western Region, there are endogenous species of this family still little known like *Clarias jaensis* [1]. This species is currently suffering from uncontrolled exploitation by peasants for profit and food purposes. However, its domestication could help to preserve it and support sustainable aquaculture development like any other local species [2]. To this end, developing aquatic resource management strategies adapted to this species is important [3, 4]. Preliminary studies have been carried out on the reproduction of *Clarias jaensis* in the wild [5]. However, its use in aquaculture would be a benefit comparable to that of introduced sister species, particularly for the supply of broodstock and fry. However, knowledge of its nutrition and reproduction technics, which are essential for breeding and the factors that influence them are still limited. The reproduction of *Clarias jaensis* as other Claridae could be influenced by nutrition through its nutritional needs such as energy. Indeed, the sperm quality of fish species may be improved through the nutritional quality of broodstock diets [6]. Different researchers have revealed that reproductive performance (egg and sperm quality) are influenced by nutrients like protein, lipids, and rations size in fish such as tilapia, *Oreochromis niloticus* [7, 8], common carp [9] and rohu, *Labeo rohita* [10]. In addition, the work of çek and Yilmaz [11] and Khan *et al* [12] showed that the energy level in the feed affects the reproductive performance of *Clarias gariepinus* and *Rhamdia quelen*. Characteristics reproduction are regulated by gonadotrophins (LH and FSH) [13, 14] and gonadal hormone (testosterone) [14, 15] who can be affected by energy level on food. There has been a few work on the optimal dietary energy for gonad development and growth and that is why the general objective of this work, is to contribute to a better knowledge of claridae by determining their nutritional needs for their preservation and enhancement. More specifically, evaluate the effect of energy level on the reproductive performance of male *Clarias jaensis*.

2. Materials and methods

2.1 Period and zone of the study

The study took place between May and July 2016 in the west region of Cameroon and at the Application and Research Farm of the University of Dschang (LN $5^{\circ} 44' - 5^{\circ} 36'$ and $5^{\circ} 44' - 5^{\circ} 37'$; LE $10^{\circ} 06' - 9^{\circ} 94'$ and $10^{\circ} 06' - 9^{\circ} 85'$ at an altitude of 1392 -1396 m.

2.2 Animal material

A number of 360 post fingerlings of male and female *Clarias jaensis* with an average weight of 97 ± 9 g were fished in the natural environment in the Santchou Area Rivers, located in the western Cameroon region and transported in nurseries to the FAR of the University of Dschang. The fish were immediately acclimatized for two weeks in the 1 m^3 concrete tanks. During the acclimation period, a 3A feed containing 40% of protein and consisting of wheat bran, soybean meal and fishmeal (Lacroix 2004) was distributed to them twice a day.

2.3 Livestock structures

Twelve polystyrene happas of 1 m^3 , attached to four sides chinese bamboo were built in a pond of 100 m^2 area and depth

0.90 m. This pond was supplied with water from a dam pond located at 150 m. The height of water in each happa was 0.80 m. The food was served in a circular floating frame 30 cm in diameter, placed on the surface water of each happa. Below this frame was placed a basin 33 cm in diameter to collect feed refusals use to estimate food consumption.

2.4 Experimental diet

Four isoproteic experimental diet (Table 1) were formulated with different energy levels 3000, 3100, 3200 and 3300 kcal/kg of food. The chosen energy levels refer to those of *Clarias gariepinus*. The calculated and analyzed chemical composition of different diet were obtained respectively by a formulation table and AOAC ^[16] method.

Table 1: Composition of experimental diets

Ingredients (Kg)	R1 (3000 kcal/kg)	R2 (3100 kcal/kg)	R3 (3200 kcal/kg)	R4 (3300 kcal/kg)
Maize	28.00	24.50	22.50	20.30
Wheat bran	6.00	5.10	3.50	3.80
Cottonseed cake	1.50	1.30	1.00	1.50
Soybean meal	3.00	4.40	6.00	4.50
Fish meal	56.70	57.00	56.50	57.00
Shell meal	0.10	0.10	0.10	0.10
Bone meal	0.10	0.10	0.10	0.10
Palm oil	2.60	5.50	8.30	10.70
Premix 2%	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00
Calculated chemical composition (% /MS)				
CP (%)	40.12	40.45	40.06	40.00
ME (kcal/kg) (kcal/kg)	3000.21	3102.69	3200.62	3303.65
Calcium (%)	3.80	3.82	3.65	3.82
Phosphorus (%)	2.12	2.12	2.07	2.11
P/E	0.01	0.01	0.01	0.01
Ca/P	1.80	1.80	1.80	1.80
Analyzed chemical composition (% /MS)				
CP (%)	39.76 ± 0.49	40.06 ± 0.75	40.66 ± 0.37	40.56 ± 0.50
CE (kcal/kg) (kcal/kg)	3284.67 ± 25.40	3354.67 ± 39.50	3780.00 ± 22.09	3848.00 ± 28.35
Lipid (%)	7.00 ± 0.00	11.33 ± 0.28	12.00 ± 0.00	15.33 ± 0.57
Ash (%)	2.93 ± 0.05	1.96 ± 0.05	2.70 ± 0.17	2.06 ± 0.05

CP= Crude protein, ME= Metabolize energy, CE= Crude energy, P/E= Protein/energy ratio, Ca/P= Calcium/phosphorus ratio

2.5 Experimental set up

Post fingerlings of *Clarias jaensis* were randomly divided into four comparable groups (45 ♂ and 45 ♀/group) of size and weight with three replication. At each group was randomly assigned one of the experimental diet R1 (3000 kcal / kg), R2 (3100 kcal / kg), R3 (3200 kcal / kg) and R4 (3300 kcal / kg of energy) previously formulated.

2.6 Conduct of the test

The food was distributed twice a day (8 am and 18 pm) at 3% of the ichtyobiomasse. Each month, a check fishery was conducted during which 20% of the fish in each group were individually weighed using a 0.1gram precision electronic scale (OHAUS) and measured with an ichthyometer to evaluate growth characteristics. The physico-chemical parameters of the water (temperature, dissolved oxygen, pH and conductivity) were measured weekly just to have the state of water. At the end of the experiment, 12 males from each group were sacrificed to determine the reproductive characteristics.

2.7. Studied Characteristics

Stage of gonad maturity: After fish dissection, maturity stages were determined by eye examination of the gonads based on the maturity scale used by Lalève ^[17], inspired by Micha ^[18].

- Gonado somatic index (GSI) = gonad weight (g) / total weight (g) X 100
- Hépatho somatic index (HSI) = liver weight (g) / total weight (g) X 100

After sacrifice of fish, the pituitary glands and one of the testicle were crushed in a mortar placed on an ice block and containing 0.9% NaCl solution, so as to obtain homogenates 1% for the pituitary gland and 15% for the testicle. The crushed material obtained was centrifuged at 3000 rpm for 30 min. The supernatant was removed and stored in labeled tubes at -20°C and were used for the determination of FSH and LH concentration in the pituitary gland and testosterone in the gonads. These assays were performed using kits sold and ready for use, at the Laboratory of Physiology and Animal Health of the University of Dschang.

2.8. Gonad histology

Fish testicles were taken from individuals in each group and then, immersed in a fixative (Bouin's solution) contained in labeled tube. These organs were used to perform histological sections at the Animal Physiology Laboratory of the University of Yaoundé I.

2.9. Statistical analysis

The one-way ANOVA was used to test the effect of energy level on reproductive characteristics. When there were significant differences between the means, they were

separated by the Duncan test at 5% significance level. SPSS 20.0 statistical software was used for these analyzes.

3. Results

3.1. Effect of dietary energy on weight, total length and weight gain of male *Clarias jaensis*

Table 2 presents some growth characteristics of male of *Clarias jaensis*. It appears that, dietary energy has significantly affect body weight, total length and weight gain. These characteristics were highest at 3100 Kcal/kg and lowest with ration at 3300 Kcal/kg except total length ($P < 0.05$).

Table 2: Mean weight, total length and weight gain of male *Clarias jaensis* in function of energy level

characteristics	Energy level (Kcal/kg)			
	3000	3100	3200	3300
W (g)	197,75 ± 37,87 ^a	263,00 ± 29,70 ^b	206,61±33,14 ^a	194,50 ± 10,00 ^a
TL (cm)	28,71 ± 3,69 ^{Aa}	32,63 ± 0,85 ^b	30,55 ± 1,89 ^c	29,43 ± 0,83 ^{ca}
WG (g)	96,36 ± 40,03 ^a	160,27 ± 32,17 ^b	103,88 ± 36,46 ^a	92,25 ± 12,47 ^a

W= weight, TL= Total length, WG= weight gain

3.2. Effect of energy level on concentrations of FSH and LH

Figure 1 shows that the energy level had a significant effect on FSH ($P < 0.05$) but not significant on LH ($P > 0.05$). Males fed the ration containing 3000 Kcal/kg of energy recorded

higher concentrations of FSH (1.15 ± 0.27 mlU/ml) and LH (1.03 ± 0.18 mlU/ml). On the other hand, these characteristics were lower (0.5 ± 0.16 mlU/ml) and (0.83 ± 0.23 mlU/ml) respectively for FSH and LH in group that received 3200 Kcal/kg of energy.

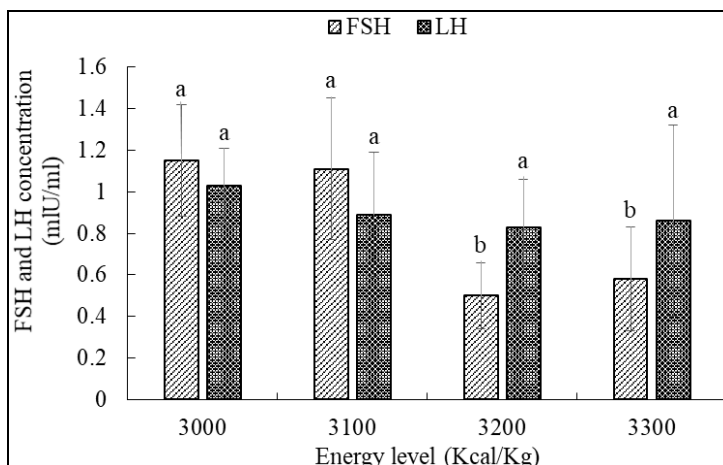


Fig 1: Concentration of FSH and LH in function of energy level

3.3. Effect of energy level on testosterone concentration

The effect of energy level on testosterone concentration in fish is shown in figure 2. It appears that although comparable ($P > 0.05$), the males of the group fed at 3200 Kcal/kg

recorded lower testosterone concentration (171.09 ± 3.93 ng/ml). However, this hormone was higher (179.67 ± 5.37 ng/ml) with the ration containing 3000 Kcal / kg of energy.

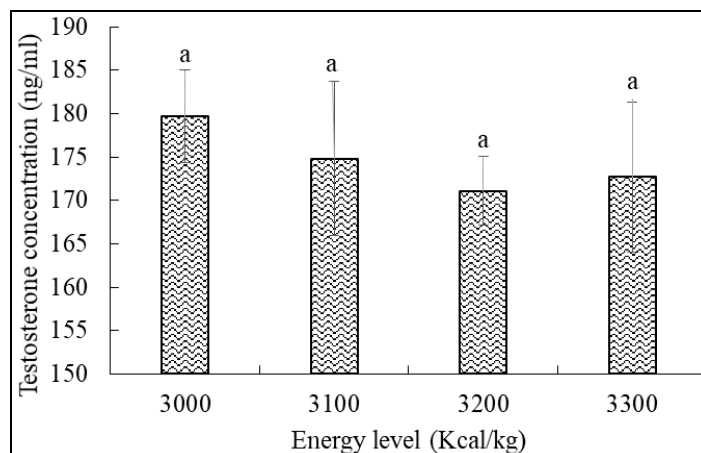


Fig 2: Testosterone concentration in function energy

3.4. Effect of energy level on stage of maturity

The influence of energy level on the maturation of male gonads is illustrated in figure 3. It appears that the majority of males in group fed at 3000 Kcal/kg (87.5%) and 3100 Kcal/kg

(62.5%) are at stage III of sexual maturity. In the opposite, those fed diets containing 3200 Kcal/kg (75%) and 3300 Kcal/kg (87.5%) are mostly in stage II.

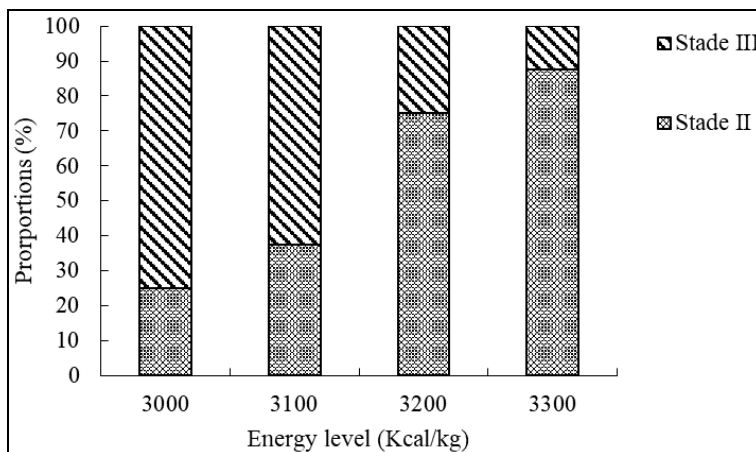


Fig 3: Stage of maturity of gonad in function of energy level.

3.5. Effect of energy level on gonado somatic index (GSI)

Figure 4 show that the effect of energy level on GSI was significant ($P < 0.05$). This characteristic was higher ($0.33 \pm$

0.05%) with the ration containing 3000 Kcal/kg and lower ($0.22 \pm 0.04\%$) with that having 3300 Kcal/kg of energy.

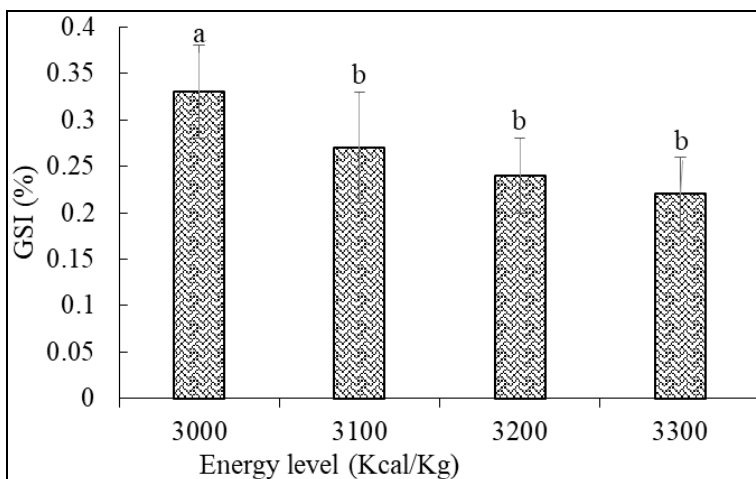


Fig 4: Gonado somatic index (GSI) in function energy.

3.6. Effect of energy level on hepato somatic index (HSI)

The influence of energy level on HSI was not significant ($P > 0.05$) (Figure 5). However, Male receiving 3100 Kcal/kg

of energy had a higher HSI ($1.11 \pm 0.14\%$) and the lower value ($0.92 \pm 0.19\%$) were obtained with the ration at 3300 Kcal/kg.

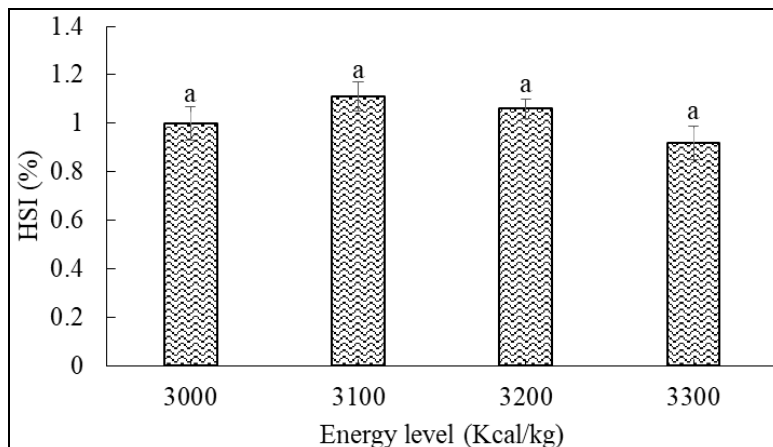


Fig 5: Hepato somatic index (HSI) in function of energy level.

3.7. Histological sections of the gonad of male *Clarias jaensis*

The influence of the energy level on the histology of the gonad of males fed at 3000, 3100, 3200 and 3300 Kcal/kg of energy is illustrated respectively by the pictures a, b, c and d of figure 6. The mature gonad of the male fed at 3000 and 3100 Kcal/kg shows that it consists of a set of seminiferous tubule separated by an interstitial tissue. In these tubes, we note the presence of spermatogonia, spermatocytes and

spermatids. The lumen of each seminiferous tubule of fish fed at 3000 Kcal/kg is filled with mature spermatozoa (Figure 6 a) while in the 3100 Kcal/kg group, few seminiferous tubules contain it (Figure 6 b). In addition, the gonad of immature males fed at 3200 and 3300 Kcal/kg have shrunken and empty seminiferous tubules. There is an abundance of spermatogonia included in the Sertoli cell layer lining the seminiferous tubules, spermatocytes and some spermatids (Figure 6 c and d).

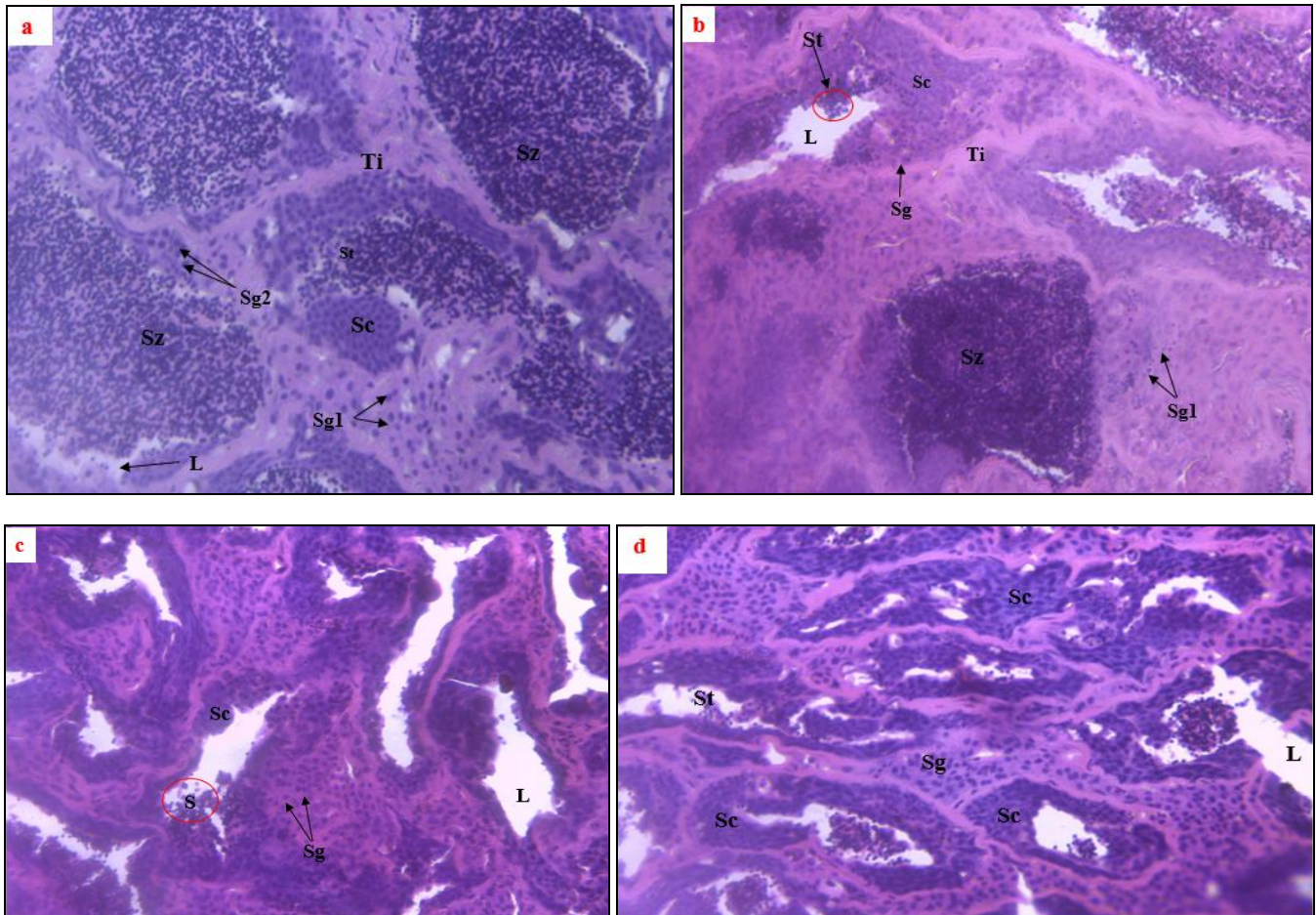


Fig 6: Histological sections of the testicles of *Clarias jaensis*. a, b, c and d present the testicles of the males who received the rations containing respectively 3000, 3100, 3200 and 3300 Kcal/kg of energy. Sg = spermatogonia, Sc = spermatocyte, St = spermatid, Sz = spermatozoa, L = lumen, Ti = seminiferous tube. (Magnification: 40X)

4. Discussion

The higher growth characteristics (weight, total length and weight gain) were recorded with the lowest energy level (3000 Kcal/kg). Çek and Yılmaz^[11] found similar results in *Clarias gariepinus* fed various dietary energy.

The energy level had an influence on the reproductive characteristics of *Clarias jaensis*. In fact, reproduction consumes energy that the animal obtains from its food. Maturation is therefore not carried out in emaciated fish that do not have sufficient mobilizable reserves^[19]. FSH and LH are key reproductive hormones. They are involved in gonadal development and steroidogenesis^[20]. The FSH and LH concentrations of the males were higher with the diet containing the least energy (3000 Kcal/kg). We don't have information about the effect of dietary energy on the synthesis of sex hormones in fish, but, this result may be explained by the fact that male fish *Clarias jaensis* need a low energy level for the production of FSH and LH. Besides, it is well known that onset of sexual maturity in fish is directly accompanied by elevated energy storage^[21, 22]. However, testosterone was

also higher with the diet ration (3000 Kcal/kg). Regardless of the diet considered, testosterone concentrations were higher than those obtained in *Clarias macrocephalus* (15-25 ng/ml) by Josepha *et al.*^[23] and in *Oreochromis mossambicus* (3.64-12.30 ng/l) by Cornish^[24] in the wild. This difference would be related to the species and the fact that the first author performed their analyzes in the plasma and not in the tissue. High concentrations of LH would have led to a high production of testosterone. According to some authors, the production of androgens including testosterone, which is implicated both in gametogenesis and in the development of secondary sexual characteristics^[25], is reported to be under the control of LH^[26]. The gonado somatic index (GSI) is a characteristic that accounts for the progression of gametogenesis in fish. A high GSI at certain period indicates that the gonads are mature and spawners are ready to breed^[27, 28, 29]. The GSI recorded in this study varied significantly in function of energy level and was higher with the diet containing the least energy level (3000 Kcal/kg). This result is similar to that of Çek and Yılmaz^[11] on *Clarias gariepinus*.

Indeed, the high testosterone concentration of the fish in this group would have favored a rapid maturation of the gonads, hence their high GSI. Regardless of the diet, it was as weak as those recorded by Wango^[5] on *Clarias jaensis* in wild. The hepatic somatic index (HSI) did not significantly change with energy level ($P>0.05$). The general trend shows that it decreased with the increasing level of energy and was higher with the diet at 3100 Kcal/kg. Çek and Yilmaz^[11] reported similar results in *Clarias gariepinus* fed diets with different dietary energy. Note that, the diet containing the lowest level (3000 Kcal/kg) of energy recorded a higher GSI and a lower HIS in this study. So, this could mean that males of this group have drawn the necessary reserves for the maturation of their gonad in the liver. The results observed in males are similar to those reported by Bedoui *et al.*^[30] who found that in the wild, the GSI of *Liza aurata* increases while HSI declines. Fish fed at 3000 and 3100 Kcal/kg of energy were classified as mature compared to those receiving higher energy levels. These results are similar to those reported by Çek and Yilmaz^[11] who obtained mature male of *Clarias gariepinus* with diets containing the lowest energy levels. These results are confirmed by the examination of histological sections of the gonad, who reported that, the diet containing 3000 Kcal/kg yielded mature testicles, whose seminiferous tubules are filled with spermatozoa. In contrast, the diet with the most energy (3300 Kcal/kg) produced immature testicles with empty, shrunken seminiferous tubules. These results are comparable to those reported by Çek and Yilmaz^[11] and Reidel *et al.*^[12], who found that low energy levels favor the maturation of the gonad of *Clarias gariepinus* and *Rhamdia quelen*, in contrast to high levels. Also, mature testicle in diet 3000 Kcal/kg may be due to the high level of LH and testosterone in this group. In fact, Kumar and Panchanan^[31] explain that, the beginning of formation of first spermatocytes in male is regulated by gonadotrophins (LH and FSH)^[13, 14] and testosterone^[14, 15].

5. Conclusion

Growth and some reproductive characteristics were significantly affected by the energy level in the diet. The best body weight, total length and weight gain were observed from the fish fed diet 3100 Kcal/kg. Except the FSH and HSI, other reproductive characteristics were comparable between diets. However, the higher FSH, LH, testosterone, GSI and the high frequency of mature male were recorded at 3000 Kcal/kg and the HSI were higher at 3100 Kcal/kg. Therefore, on the basis of the present results among energy levels tested, 3000 Kcal/kg was found to be optimal for healthy gonad development, despite the fact that the best growth characteristics were obtained with diet at 3100 Kcal/kg.

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