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Effect of Dietary Energy Level on Some Reproductive Characteristics of *Clarias jaensis* Female (Boulenger, 1909), an Endogenous Species from Cameroon

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Research Article

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Abstract

To determine the level of food energy necessary for a good sexual maturation of Clarias jaensis females in captivity, 180 post fingerlings with average weight of 96 ± 7g were submitted in triplicates to four experimental isoproteic (40 % crude protein) diet with 3000, 3100, 3200 and 3300 Kcal/kg of energy. The study took place at the Application and Research Farm of the University of Dschang for 4 months. At the end of the test, 12 females presenting the sexual maturity characteristics (bloated belly containing mature oocytes) from each group were sacrificed. The results showed that FSH (0.81 ± 0.35 mlU/ml) and LH (0.66 ± 0.23 mlU/ml) were higher at 3100 Kcal/kg while the higher concentration of estradiol (450,00 ± 109,86 pg/ml), the higher weight of ovaries (31,88 ± 6,86g) and the higher rate of mature female (75%) were recorded with 3000 Kcal/kg. Other reproductive characteristics such as ovarian biochemical characteristics, GSI, HSI and oocyte characteristics were also better with lower energy diets. In conclusion, females with the best reproductive characteristics were obtained with low energy rations.

Keywords: Energy Level; Clarias jaensis; Female; Reproductive Characteristics

Abbreviations

FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; TGS: Triglycerids; LDL: Low Density Lipoprotein Cholesterol; HDLC: High Density Lipoprotein Cholesterol; GSI: Gonado Somatic Index; HSI: Hepatho-Somatic Index.

Introduction

The disappearance of fishery resources is linked to the fact that most of the products of aquatic origin consumed by the population are taken from the natural environment. Indeed, 50% of the proteins of animal origin consumed come from fishing, which unfortunately has already reached its limits due to overexploitation of resources. Hence, the need to find an alternative such as aquaculture which has not stopped growing in recent years [1]. To be efficient and sustainable, aquaculture must be based on the breeding of endogenous species able of easily resisting environmental constraints and producing better.

On the other hand, the breeding of endogenous species contributes to preserving and restoring biodiversity through repopulation [2,3]. Among the endogenous species likely to be farmed are some of the Clariidae family, also called catfish. They represent almost a third of the freshwater fish known in the world [4,5]. Their interest in fish farming was summarized by Ducarme C, et al. [6] and is linked to their ability to easily adapt to climate change, their omnivorous diet, their rapid growth correlated with the ability to efficiently transform compound feed and to their highly valued flesh [7,8]. In Cameroon, *Clarias gariepinus* and *Heterobranchus longifilis*, non-native species, are the most cultivated catfish [9,10]. In the Mbô floodplain of the West region of Cameroon, there is an endogenous Clariidae named *Clarias jaensis* which has proven to be a good candidate for aquaculture. The first works to our knowledge on this species are those of Pouomogne V [11], Zango P [12], Mfossa MD [13], Zango P, et al. [14], Etaba AD, et al. [15] and Efole ET, et al. [16] who worked on various aspects of its reproduction and growth. However, for its production to be effective, it would be necessary to determine all the standards for its breeding. It would therefore be interesting to initiate investigations relating to nutrition and their effects on the growth and reproduction characteristics of *Clarias jaensis*.

Nutrition fundamentally affects the production performance of fish through its levels of proteins, minerals, vitamins and energy [17,18]. According to Barnabe G [19], the growth and maturation of the gonads does not occur in lean fish that do not have sufficient mobilizable energy reserves. Some studies on *Clarias jaensis* feeding have revealed that the energy and protein level of the ration influences the survival and growth [20,21]. The work of Tsoupou KSG, et al. [22] revealed that the level of dietary energy has a significant impact on the reproductive characteristics of *Clarias jaensis* males. However, according to the literature, no study has yet been carried out on the effect of feed energy on the reproductive characteristics of the female of *Clarias jaensis*.

The general objective of this work is therefore to contribute to the preservation and enhancement of fisheries biodiversity in general and Clariidae in particular by determining their nutritional needs. More specifically, the aim was to evaluate the effect of the optimum energy level of the ration on the reproductive characteristics of *Clarias jaensis* female.

Material and Method

Area of the Study

The study carried out in the west region of Cameroon and at the Application and Research Farm of the University of Dschang (LN 5°44’-5°36’ and 5°44’-5°37’; LE 10°06’- 9°94’ and 10°06’-9°85’ at an altitude of 1392 -1396 m.

Animal Material

180 post fingerlings female of *Clarias jaensis* with average weight and average total length of 96 ± 7g and 22.0 ± 1.7 cm were captured from the wild in Nkam river flood plain nearby Santchou settlemen. They were transported in oxygenated buckets and stocked in a breeding pond at the study site. The fish were acclimatized for two weeks and were fed ad libitum with a standard diet (3A) containing 40% of protein and consisting of wheat bran, soybean meal, and fishmeal [7].

Experimental Diet

Four isoproteic experimental diets 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* and the principal source of energy was palm oil [20]. The chemical compositions of different diets were analyzed according to AOAC methods [23].

	Energy level (MJ/kg)			
Ingredients (g/Kg)	**3000**	**3100**	**3200**	**3300**
Maize	28	24.5	22.5	20.3
Wheat bran	6	5.1	3.5	3.8
Cottonseed cake	1.5	1.3	1	1.5
Soybean meal	3	4.4	6.0	4.5

Fish meal	56.7	57.0	56.5	57.0
Shell meal	0.1	0.1	0.1	0.1
Bone meal	0.1	0.1	0.1	0.1
Palm oil	2.6	5.5	8.3	10.7
Premix 2%	2	2	2	2
Total	100	100	100	100
Chemical Composition				
Crude Protein (%)	39.76 ± 0.49	40.06 ± 0.75	40.66 ± 0.37	40.56 ± 0.50
Crude Energy (MJ/kg) (kcal/kg)	13.75 ± 0.10	14.04 ± 0.16	15.82 ± 0.09	16.11 ± 0,11
Crude 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

Table 1: Composition of experimental diets. (Source: Adapted from [21]).

Experimental Design

Fish were randomly divided into four comparable groups repeated 3 times with 15 females per repetition (Figure 1). Each group was randomly assigned to one of the experimental rations Rg1 (3000 Kcal/kg), Rg2 (3100 Kcal/kg), Rg3 (3200 Kcal/kg) and Rg4 (3300 Kcal/kg).

Rg1, Rg2, Rg3 and Rg4: ration containing respectively 3000, 3100, 3200 and 3300 Kcal/kg of energy, N=number of females per repetition.

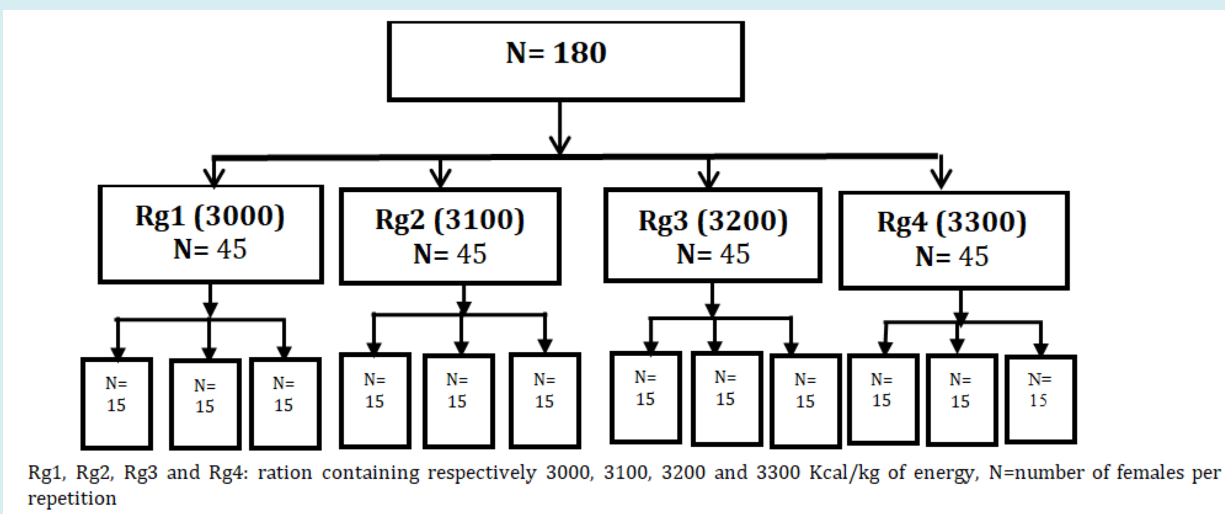


Figure 1: Experimental design.

Conduct of the Test

The trial was conducted in 12 polystyrene happa (like cages) of $1m^3$, built in a pond of $100m^2$ and depth 0.90 m. This pond was filled with water from a lake located 150m away. The meshes of the happa were cleaned once a week to facilitate the flow of water. The food was distributed twice a day (8 am and 6 pm) in pellet form during 4 months and representing 3% of the ichtyobiomasse. 20% of fish from each group were randomly taken each month, weighed individually using an ichtyometer (0.1cm precision) and SF-400 balance (1g precision) in order to adjust the amount of food to distribute.

At the end of the test, 12 females presenting the characteristics of sexual maturity (bloated belly containing mature oocytes) from each group were sacrificed.

Biochemical Characteristics

To determine the biochemical characteristics, the pituitary glands (Figure 2a) and the ovaries (Figure 2b) of the fish sacrificed from each batch at the end of the test were weighed, then crushed in a mortar placed on a piece of ice. The ground materials obtained were mixed with physiological fluid (NaCl) to obtain homogenates 1% for the pituitaries and 15% for the gonads [24]. The different homogenates were centrifuged at 3000 rpm for 30 min and the supernatant obtained was stored at – 20°C in tubes labeled for the determination of FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) in the pituitary glands, estradiol, total proteins, total cholesterol, HDLC (High density lipoprotein cholesterol) and TGS (Triglycerids) in the gonads using the methods described in the instructions for the following kits:

- The FSH: DIALAB commercial kit (Reference Z05302)
- The LH: DIALAB commercial kit (Reference Z02301)
- Testosterone: GOLDEN BIO TECHNOLOGIES commercial kit (Reference GBE - 2722)
- Estradiol: OMEGA DIAGNOSTICS commercial kit (Reference OD477)
- Total proteins and cholesterol, HDL and tissue TGS: enzymatic colorimetric method following the commercial kit instructions
- LDL (Low density lipoprotein cholesterol) were obtained using the formula below:

  LDLC = total cholesterol (HDLC + TGS/5)

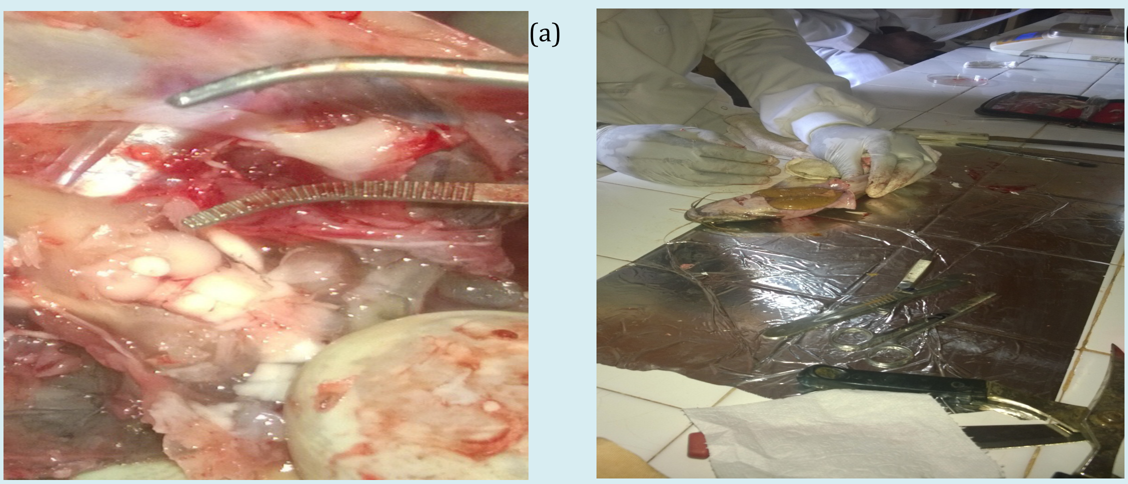


Figure 2: Pictures of pituitary gland (a) and ovaries (b) of *Clarias jaensis*.

Reproductive Characteristics

- Absolute fecundity (Af) = Number of oocytes per female
- Relative fertility (Rf) = Number of oocytes per g of female
- Oocyte diameter: measured on graph paper
- Gonado somatic index (GSI) = Gonad weight (g) / Total weight (g) X 100
- Hepatho-somatic index (HSI) = Liver weight (g) / Total weight (g) X 100

Histological Sections of the Gonads

For histological sections, the ovaries of the fish from each previously sacrificed batch were immersed in a fixative (Bouin's solution) contained in labeled jars, then analyzed in the Animal Physiology laboratory of the University of Yaoundé I, according to the method described by Ngoula F [24].

Statistical Analysis

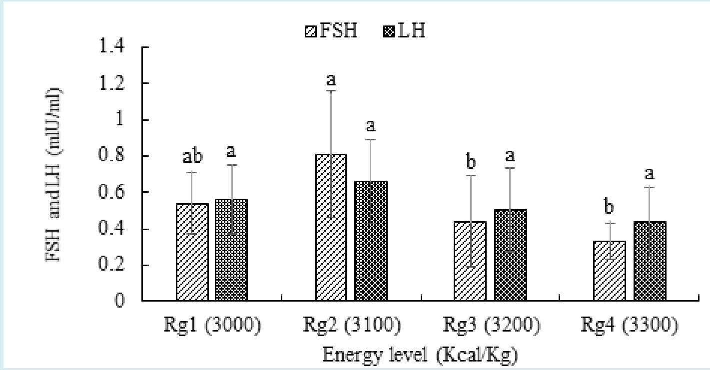
The data on FSH, LH, estradiol and biochemical characteristics of the ovaries were subjected to one-way ANOVA (energy level). When there were significant differences between the means, they were separated by the Duncan test at 5% significance level. SPSS 20.0 statistical software was used for these analyses.

Results

FSH and LH Concentration

FSH and LH concentrations of the pituitary gland tended to decrease with energy level (Figure 3). FSH was significantly higher (0.81 ± 0.35 mlU/ml) with the Rg2 ration containing 3100 Kcal/kg of energy.

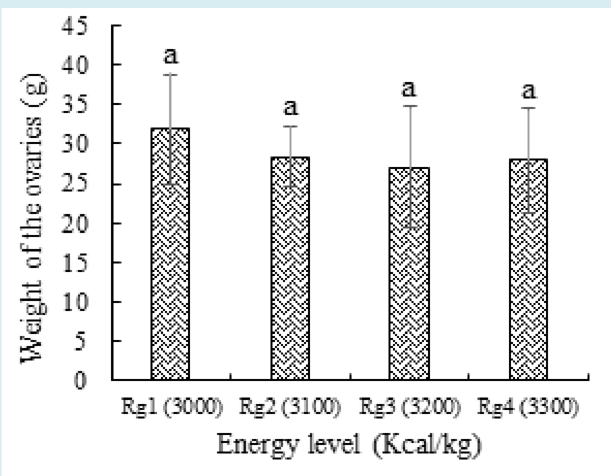
Similarly, LH concentrations were highest (0.66 ± 0.23 mlU/ml) with the Rg2 treatment, but comparable to the other three energy levels. The lowest values (0.33 ± 0.10 mlU/ml) and (0.66 ± 0.23 mlU/ml) for FSH and LH respectively, were obtained with the ration most concentrated in energy Rg4 (3300 Kcal/kg).



a, b: histograms bearing the same letters are not significantly different ($p > 0.05$).
Figure 3: Effect of the energy level of the ration on the FSH and LH concentrations of the pituitary gland.

Weight of the Ovaries

The weight of the ovaries (Figure 4), although comparable between the different dietary energy levels, was the highest (31.88 ± 6.86g) with the Rg1 treatment (3000 Kcal/kg) and the lowest (27.05 ± 7.64) with the Rg3 ration 3200 Kcal/kg.



a: histograms bearing the same letters are not significantly different ($p > 0.05$).
Figure 4: Weight of *Clarias jaensis* ovaries depending on the energy level of the ration.

Estradiol Concentration of the Ovaries

The concentration of estradiol in the ovaries decreased significantly with increasing energy level in the diet (Figure 5). It was the highest (450.00 ± 109.86 pg/ml) with the Rg1 ration containing 3000 Kcal/kg but comparable with the concentration recorded with the energy level 3100 Kcal/kg (398.89 ± 142. 16 pg/ml). The lowest values (301.25 ± 99.02 pg/ml) were obtained in females fed with the Rg4 ration (3300 Kcal/kg). The relationship between estradiol concentration and energy level was very strong ($R^2 = 0.9996$).

a, b, c : histograms bearing the same letters are not significantly different ($p > 0.05$).
Figure 5: Effect of the energy level of the diet on the estradiol concentrations of the ovaries.

Biochemical Characteristics of the Ovaries

The biochemical characteristics of *Clarias jaensis* ovaries are summarized in Table 2.

Total Protein: The significantly highest total protein concentration was obtained with the Rg2 treatment (3100 Kcal/kg) followed by the value observed with the least energy concentrated ration (3000 Kcal/kg). The lowest concentration of total protein was obtained with the most concentrated energy treatment (3300 Kcal/kg) and comparable with the value recorded in batch Rg3 (3200 Kcal/kg).

Total Cholesterol: The lowest total ovarian cholesterol concentrations were recorded with the Rg2 ration (3100 Kcal/kg) and were comparable to the values observed in the Rg3 and Rg4 treatments, the highest values being obtained with the lowest level of ovarian cholesterol energy (3000 Kcal/kg).

HDLC (HDL density lipoprotein cholesterol): Ovarian HDLC concentrations tend to decrease with increasing energy levels. The significantly highest values were recorded with the Rg1 ration (3000 Kcal/kg). The lowest concentrations ($P<0.05$) were observed with the Rg2 ration.

LDLC (Low density lipoprotein cholesterol): LDLC concentrations tended to increase significantly with increasing energy level. The highest values ($P<0.05$) were recorded with the Rg3 treatment (3200 Kcal/kg) followed by Rg4 (3300 Kcal/kg). The lowest concentrations were obtained with the energy level 3100 Kcal/kg.

TGS (Triglycerids): The highest ovarian TGS concentrations were recorded with the Rg2 treatment (3100 Kcal/kg) followed by the value obtained with the Rg1 ration (3000 Kcal/kg). The lowest concentrations were observed with the treatment at 3300 Kcal/kg. However, no significant differences were recorded between treatments in females.

Biochemical Characteristics	Energy Level (Kcal/kg)				
	Rg1 (3000)	Rg2 (3100)	Rg3 (3200)	Rg4 (3300)	P
T Prot (g/dl)	7.83 ± 1.67[a]	8.00 ± 1.35[a]	4.50 ± 1.91[b]	4.46 ± 2.09[b]	0.00
T Chol (mg/dl)	59.90 ± 11.66[a]	35.12 ± 6.33[b]	37.01 ± 11.90[b]	38.29 ± 14.28[b]	0.04
HDLC (mg/dl)	79.66 ± 12.13[a]	36.54 ± 10.8[b]	51.13 ± 8.76[b]	51.32 ± 1.87[b]	0.01
LDLC (mg/dl)	79.47 ± 0.41[a]	69.32 ± 0.58[b]	109.16 ± 0.14[c]	84.85 ± 0.20[d]	0.00
TGS (mg/dl)	300.15 ± 67.62 [a]	345.24 ± 49.98 [a]	220.41 ± 56.38 [a]	211.64 ± 54.35 [a]	0.12

a, b and c: means with the same letter on the same line are not significantly different (P>0.05) for the same parameter. N=12, T prot= Total proteins, T Chol = Total cholesterol, HDLC = High density lipoprotein cholesterol, LDLC = Low density lipoprotein cholesterol, TGS = Triglycerids, P = probability

Table 2: Biochemical characteristics of Clarias jaensis ovaries depending on the energy level of the diet.

Stages of Sexual Maturity of Females

Females of stages III and IV of sexual maturity are found in all treatments (Figure 6). Thus, stage IV was the most represented with rates of 75 and 62.5% respectively in treatments Rg1 (3000 Kcal/kg) and Rg2 (3100 Kcal/kg). The females having received the most concentrated energy rations (3200 and 3300 Kcal/kg) were mainly (87.5%) at stage III of sexual maturity.

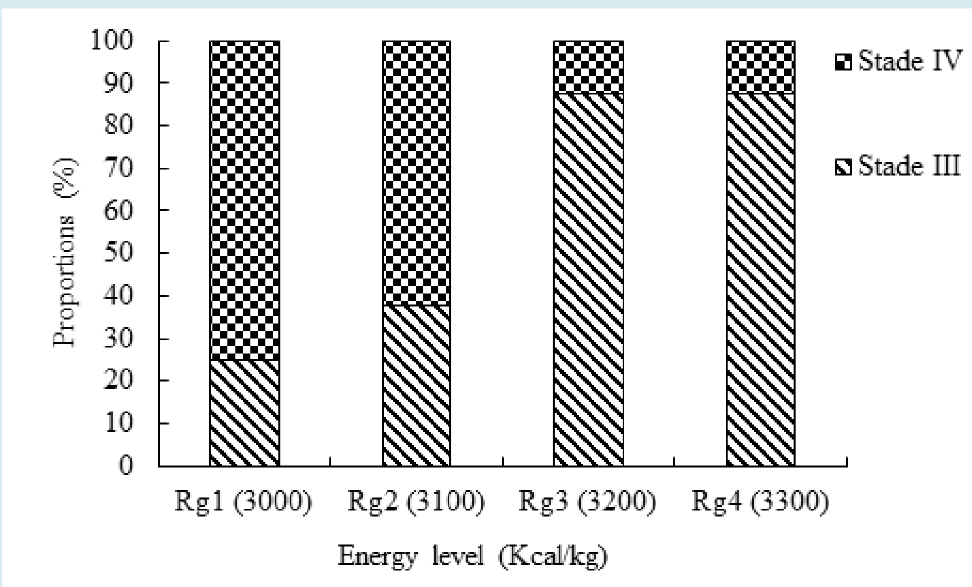


Figure 6: Stage of sexual maturity of the ovaries of *Clarias jaensis* depending on the energy level of the ration.

Fecundity (Absolute and Relative), Oocyte Diameter and Weight

The effect of the energy level of the ration on absolute fertility, relative fertility, oocyte weight and diameter is summarized in Table 3. It appears that these characteristics tended to decrease with the increasing level of energy of the ration except relative fertility which has rather increased.

Absolute Fertility and Relative Fertility

Absolute fertility (Af) tended to decrease with increasing energy levels. The highest value was recorded with 3000 Kcal/kg of energy followed by the value obtained with the highest level of energy (3300 Kcal/kg), the lowest being obtained with the Rg3 ration (3200 Kcal/kg). Relative fertility (Rf), on the other hand, increased with increasing energy levels and the highest value was recorded with the highest energy level (3300 Kcal/kg). It was lower with the Rg1 ration (3000 Kcal/kg). However, no significant difference was observed between treatments for either absolute fertility or relative fertility.

Oocyte Weight and Diameter

The increase of the energy level in the ration tends to reduce oocyte weight and diameter. The highest values were recorded with the ration with the least concentrated energy (3000 Kcal/kg) and the lowest with the three other treatments in terms of oocyte weight and the Rg2 treatment (3100 Kcal/kg) for the diameter. However, the treatments showed no significant difference for either weight or oocyte diameter (P>0.05).

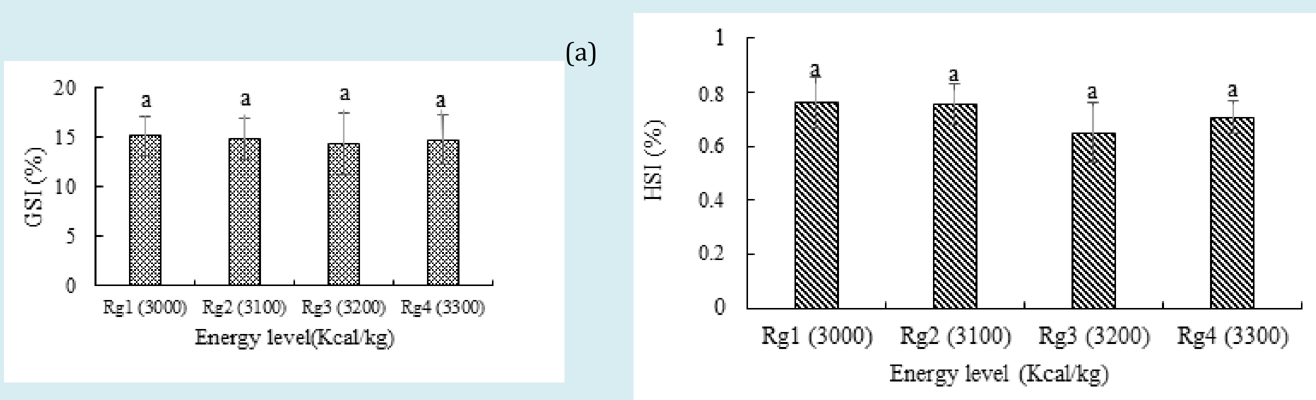
Oocytes Characteristics N=12	Energy level (Kcal/kg)				
	Rg1 (3000)	Rg2 (3100)	Rg3 (3200)	Rg4 (3300)	P
Af	7234.68 ± 1730.98	6887.37 ± 275.67	6819.06 ± 1690.16	7027.89 ± 1504.84	0.938
Rf	35.00 ± 8.93	36.21 ± 3.33	36.78 ± 8.14	40.09 ± 7.52	0.579
Oocytes weight (g)	0.005 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.656
Oocytes diameter (mm)	0.66 ± 0.04	0.60 ± 0.35	0.63 ± 0.05	0.62 ± 0.04	0.326

Af = Absolute fertility, Rf = Relative fertility, P = probability, N = number of observations

Table 3: Oocyte characteristics of Clarias jaensis according to energy level.

Gonado Somatic Index (GSI) and Hepato Somatic Index

The GSI Figure 7a was highest (15.18 ± 1.99%) in the Rg1 ration, with the lowest value (14.38 ± 3.11%) recorded in the Rg3 treatment. The hepato-somatic index Figure 7b was higher (0.076 ± 0.09%) in females with the Rg1 ration containing 3000 Kcal/kg of energy and lower (0.70 ± 0.06%) with the Rg3 treatment (3200 Kcal/kg). However, no significant differences were recorded between the treatments. However, no significant difference was noted between the treatment.

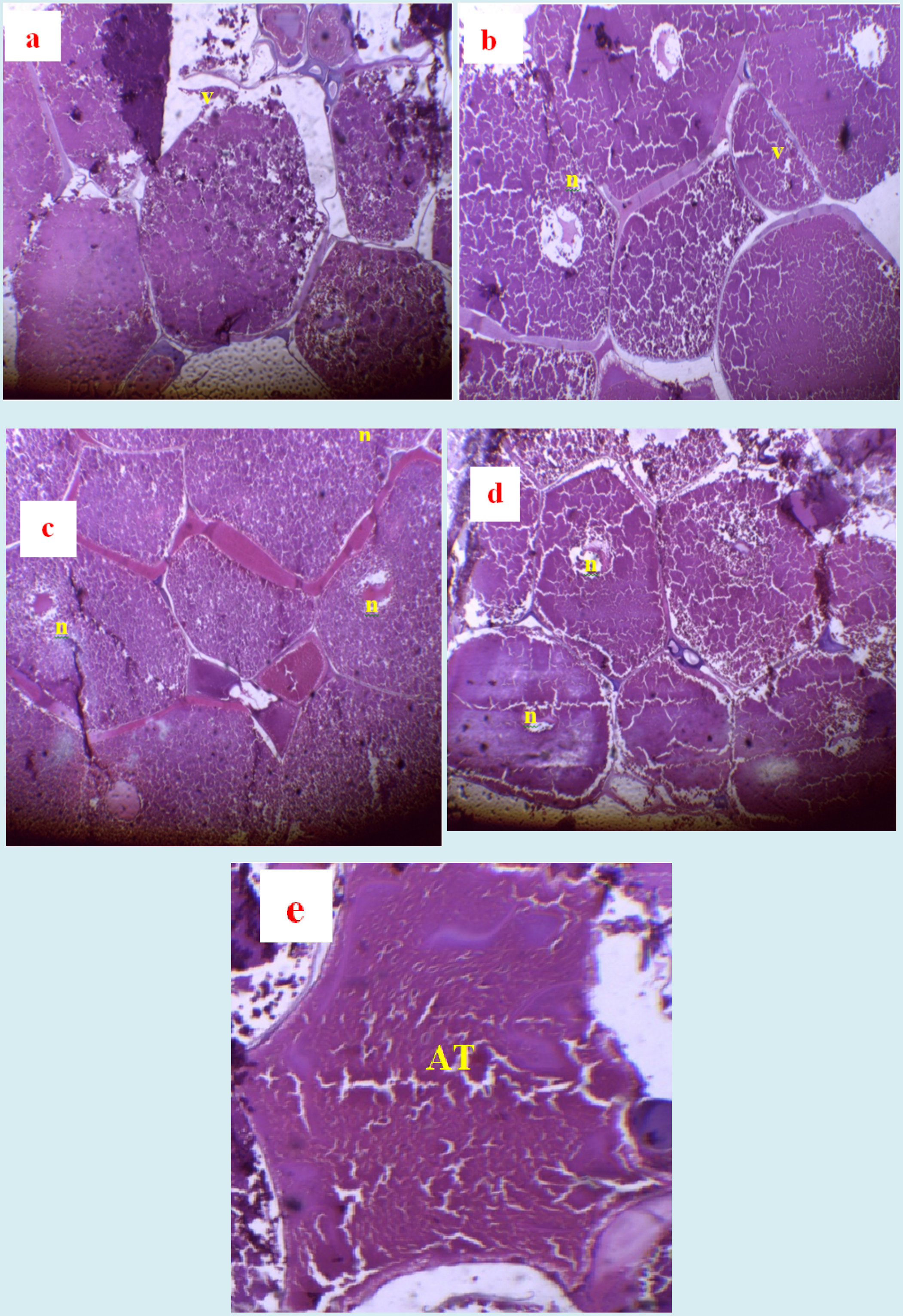


a,b : histograms bearing the same letters are not significantly different (p > 0.05)

Figure 7: Gonado Somatic Index (a) and Hepato somatic Index (b) depending on the energy level of the ration Rapport Hépatho Somatique (RHS).

Histology of the Ovaries

The effect of the energy level of the ration on the structure of the ovaries of *Clarias jaensis* females fed with rations at 3000, 3100, 3200 and 3300 Kcal/kg of energy is illustrated respectively by photos a, b, c, d and e of Figure 8. Regardless of the energy level considered, females presented mature oocytes at stage III and IV whose lumen is filled with yolk. A few atretic oocytes were observed in the batch that received the most dietary energy (Figure 8e).



n=nucleus, v=yolk, AT=atresia

a, b, c and d present the oocytes of females who received rations containing 3000, 3100, 3200 and 3300 Kcal/kg of energy respectively. (e)atretic oocyte present in females fed at 3300 Kcal/kg.

Figure 8: Histological sections of the ovaries of *Clarias jaensis* fed with different levels of food energy.

Discussion

Reproduction consumes energy that the animal obtains from its food. Fish that do not have sufficient mobilizable energy reserves cannot reach sexual maturity [19]. FSH and LH are hormones involved in gonad development, steroidogenesis and ovulation [25]. The FSH and LH concentrations of females were highest with the 3100 Kcal/kg energy ration and lowest with the 3000 Kcal/kg energy ration. However, the concentration of estradiol was higher with the 3000 Kcal/kg ration although those of FSH and LH were low in this batch. This observation could be explained by negative feedback exerted by estradiol on the pituitary gland, which would have led to a reduction in gonadotropin secretion. Furthermore, regardless of energy level, estradiol concentrations were low compared to those recorded by Sabet SS, et al. [26] in the cyprinidae (*Rutilis frisii* kutum) and by Cornish in *Oreochromis mosambicus* in the natural environment. The difference observed between these results would be linked to the species, environmental factors and breeding techniques.

The highest values of total protein concentration in the ovaries were observed with the ration at 3100 Kcal/kg followed by those at 3000 Kcal/kg ($p < 0.05$). We do not have enough information on normal protein values in fish gonads. However, in fish plasma, these values are in the range 3-5 g/dl [27]. The values obtained in the ovaries of *Clarias jaensis* were lower than those observed in *Clarias batrachus* which had not been deprived of food [28]. Low energy levels would have led to strong mobilization of proteins into lipoproteins during gonad maturation [22]. Indeed, protein concentrations increase significantly during the sexual maturation process of fish [27].

According to the work carried out by Barnhart RA [29], the main variation factors for total cholesterol concentrations are species and nutrition. In our experience, total cholesterol varied significantly with energy level, the highest value being recorded with the Rg1 treatment (3000 Kcal/kg) as was estradiol. Females fed at 3000 Kcal/kg would have mobilized more cholesterol which was not entirely used for the production of estradiol. The low concentrations of LDLC in the ovaries of females from the same batch would be linked to their high use in the transport of estradiol. Cholesterol is the major component present in animal tissue [30]. It is modified by an enzymatic system to produce steroid hormones in tissues such as the brain, adrenal glands, ovaries and testes [30,31]. Note that the concentrations of protein, cholesterol and estradiol contained in the gonads of females from the group fed at 3000 Kcal/kg were the highest, which shows that they were more mature and ready to reproduce. According to Yeganeh S [27], these three characteristics increase significantly during sexual maturity.

HDLC concentrations in the ovaries of females from the group fed the low-energy diet were the highest. This result could be explained by the fact that the high concentration of estradiol in this same batch would have stimulated a strong production of HDLC which was used by the growing oocytes for the synthesis of the yolk.

Triglyceride concentrations tended to decrease with increasing energy level, however, no significant difference was recorded between treatments. However, females from the groups that received the least energy recorded the highest values. These females would have stored energy useful for reproduction in the form of triglycerides. Indeed, according to Sujatha K [32], TGS are stored in the body to be used as an energy source.

In female fish, the average number of oocytes is considered an important criterion for evaluating reproductive performance [33]. The fecundities obtained during this work were not significantly affected by the energy level. This result is different from that observed by El-Sayed FM, et al. [34] in *Oreochromis niloticus*. The differences observed would be due to endogenous and exogenous factors such as age, species, physiological state and rearing conditions. However, absolute fertility was highest with the ration at 3000 Kcal/kg and relative fertility with that at 3300 Kcal/kg of energy. These results are close to those recorded in *Clarias jaensis* induced with HCG at 4000 IU and captured in a natural environment [12,35].

Oocyte diameter and weight help determine egg quality. As well as absolute and relative fecundity, oocyte weight and diameter were highest with the ration with the least energy concentration (3000 Kcal/kg).

Whatever the ration considered, the oocyte diameter was lower than that reported by Zango P [12] in *Clarias jaensis* in a natural environment and the oocyte weight was lower than that recorded in Clariidaes by Le gouessant [36]. The difference observed could be explained by a variation in the size, weight and age of the females. The absolute fecundity of females was the highest with the ration at 3000 Kcal/kg as well as the live weight. This result is similar to that of Alla YL, et al. [37] who reported that the more the size and live weight of *Heterobranchus longifilis* female increase, the higher its fecundity. The fecundity, oocyte diameter and gonad weight recorded in this study show that females fed at 3000 Kcal/kg are more mature than those in other batches. The high estradiol concentrations in females fed rations containing low energy levels would have stimulated high oocyte production and maturation.

The gonado somatic index (GSI) is a characteristic that accounts for the progression of gametogenesis in fish. A high

GSI at a period indicates that the gonads are mature at that period and the broodstock are ready to reproduce [38-40]. The GSI recorded during this study was not significantly affected by energy level. However, it was highest with the ration containing the least energy (3000 Kcal/kg). Çek S, et al. [33] and Reidel A, et al. [41] recorded similar results in females of *Clarias gariepinus* and *Rhamdia quelen*. These results are also similar to those recorded in females of *Oncorhyncus mikiss* fed three foods, containing different energy levels [42]. Estradiol and GSI were highest with the diet containing the lowest energy level. Several studies have shown that the increase in GSI and the development of oocytes are associated with the level of estradiol in plasma [38,40]. The increase in GSI indicates that oocyte maturation progresses with increasing estradiol levels [43,44].

The hepatho-somatic index (HSI) varied significantly with energy level. It generally decreased with increasing energy level and was highest with the 3000 Kcal/kg. Çek S, et al. [33] recorded similar results in *Clarias gairepinus* fed rations with different digestible energy levels. Lincoln RF, et al. [45] reported that the HSI is high when the liver is engaged in the synthesis of vitellogenin, this could explain the presence of a high quantity and quality of oocytes with the 3000 Kcal/kg ration in *Clarias jaensis*. However, the ration containing the lowest energy level recorded the highest GSI and HSI in females. These results do not corroborate those reported by Bedoui RF, et al. [46] who found that in the natural environment, the RGS of liza aurata increases while the RHS decreases. However, they are similar to the results recorded in *Upeneus moluccensis* by Saad A [47] who found that the two relationships evolved synchronously.

According to the other reproductive characteristics cited above, females fed at 3000 and 3100 Kcal/kg of energy were classified as mature compared to those having received higher energy levels. These results are similar to those reported by Çek S, et al. [33] who obtained mature broodstock of *Clarias gariepinus* with rations containing the lowest levels of digestible energy. These results are confirmed by examination of histological sections of the gonads. Whatever the energy level, the histological sections of the ovaries presented oocytes whose cytoplasm was filled with yolk. Some atretic oocytes were recorded with the ration containing the most energy (3300 Kcal/kg). This result is similar to that of Çek S, et al. [33] in *Clarias gariepinus* and Reidel A, et al. [41] in *Rhamdia quelen*. These authors explain that excess energy could have caused follicle atresia by affecting the metabolic activities of the liver, which is the basis of vitellogenin synthesis linked to the growth of vitellogenic follicles. According to Leonardo AFG, et al. [48] atresia is due to the absence of hormonal induction at the time of ovulation, which leads to a reduction in the volume of oocytes, an increase in white oocytes and their reabsorption by phagocytosis. Babin P, et al. [49] rather think that atresia is caused by environmental stress and changes in hormonal concentrations.

Last and not a least explanation, palm oil contains less unsaturated fatty acids. Since overall need in omega 3 and 6 in fish nutrition is well established, using alternate lipid sources could have probably provided more challenging results [50].

Conclusion

Energy level significantly affected reproductive characteristics except LH, ovarian weight, triglycerides, oocyte characteristics, GSI, HSI and K factor. FSH and LH concentrations were higher with the 3100 Kcal/kg treatment while other reproductive characteristics were generally better with the 3000 Kcal/kg treatment. Using alternate lipid source than palm oil for dietary energy may be tried.

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Conflicts of Interest

The authors declare no conflict of interest

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