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Experimental study of the coccidial infection on growth performance of juvenile of *Clarias gariepinus* Burchell, 1822 (Poisson, Siluriformes)

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Abstract

The study focused on the characterization and quantification of the effects of coccidiosis on intensive rearing of Clarias gariepinus production. The first phase of works, performed on 117 specimens of C. gariepinus consisted of the collection of oocysts of Coccidia which served as inoculum. The experimental phase was conducted on 81 juvenile of C. gariepinus divided into three groups (T, I and II) including 27 fish specimens per group. Group T served as control group; groups I and II were infected orally with respectively 600 and 500 oocysts from the inoculum. The results show a high parasite load in adult specimens of C. gariepinus harvested in the wild. The collection of oocysts has allowed to identify four (04) species of Cyclospora and two (02) species of Eimeria. After infection of juvenile of C. gariepinus, the presence of lesions including intestinal congestion and stomach distension indicates the pathogenic effects of the infestation. The performance analysis showed that the weight of juvenile from control group is significantly higher ($p < 0.001$) than those from infected groups I and II at D7, D14 and D21. Groups I and II also show weight loss at D7 and D21, characterizing negative specific growth rate (SGR), particular for group II. Infestation rates of groups I and II are respectively 33.33% and 29.62%. These results show a real impact of coccidiosis on juvenile of C. gariepinus bred in captivity, including the effectiveness of heavy infestation and deteriorating of growth performance of fish.

Keywords: *Clarias gariepinus, Cyclospora spp, Eimeria spp, Lesions, Growth performance, Mortality rate.*

1. Introduction

In a world in economic crisis, Africa is one of the few areas where the countries growth rate is excess. This growth is materializing by the significant investment dedicate to the development of the primary sector, including agriculture, livestock and fisheries. The case of fisheries is indicative of this situation. Indeed, to the gradual depletion of catches in water plans and watercourse, substitutes the domestication and breeding of different species of fish. The growing magnitude of tropical aquaculture production led to an intensification and profound changes in traditional farming systems, particularly in the countries of Southeast Asia and sub-Saharan Africa (Caruso, 2009). The Republic of Benin does not remain on the sidelines of this development of aquaculture and fish farming in particular. According to data from INSAE (2006), the total of the fisheries sector provides (fisheries and aquaculture) amounted in 2006 to over 93,700 tons against 72,670 tons in 2003; an increase of nearly 30%. Fish production consisting mainly of Tilapia (*Oreochromis niloticus*) and Catfish (*Clarias gariepinus*).

This intensification of fish production remains quite critical to control because with the high density of breeding fish and low water renewal, the conditions exist for the frequent occurrence of animal diseases in livestock. Despite the consequences running into hundreds of millions of US dollars each year (FAO, 2007), estimation of the socioeconomic and environmental impact of epizootic and enzootic diseases in tropical fish farming is difficult to assess (Caruso, 2009); the health control by farmer are almost exclusively based on chemotherapy with a risk of production tailings impoundment.

The work achieved by Siko (2010) on the identification of gastrointestinal Coccidia of *Clarias gariepinus* in the Ouémé valley revealed the presence of several genera of Coccidia. Coccidia are among the most common parasites in fish (Dykova & Lom, 1983). They infest different organs,

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sometimes causing significant damage to their hosts (& Toguebaye Diouf, 1993). The productivity of *C. gariepinus* farms may be compromised. This study sign up in the characterization and quantification of the real effects of coccidiosis on production in intensive rearing of *Clarias gariepinus*. The main objectives of this work are to assess the effects of infection by digestive Coccidia in order to contribute to improving the catfish productivity and solve a problem of socio-economic development.

2. Materials and methods

2.1. Study area, animals and research of Coccidia oocysts

The biological material (fish) was collected in the delta of the Ouémé River (6° 21' 11" N, 2° 26' 39" E). It has a sub-equatorial climate with two (02) dry seasons and two (02) rainy seasons (a long dry season from December to March, a long rainy season from April to July, a small dry season from August to September and a small rainy season from October to November). The highest species richness in fish fauna of the river Ouémé were observed in Delta, according to a study by Lalèyè *et al.* (2004). This study has identified 122 fish species distributed in 50 families and 87 genera.

At the delta of the Ouémé, collecting fish was conducted in the locality of Agonli-Lowé (06° 39' 378'' N, 02° 28' 571'' E) (Fig. 1). Specimens of *Clarias gariepinus* Burchell, 1822 were collected, from local fishermen and fish merchants, from September to December 2012 and brought back alive to the laboratory where, before being dissected, the weight and the total length of each specimen were taken. After dissection, the gastrointestinal tract is removed and placed in saline. For each dissected fish, wet smear between blades and blades of different portions of the intestine were performed and observed by light microscopy for the detection of oocysts.

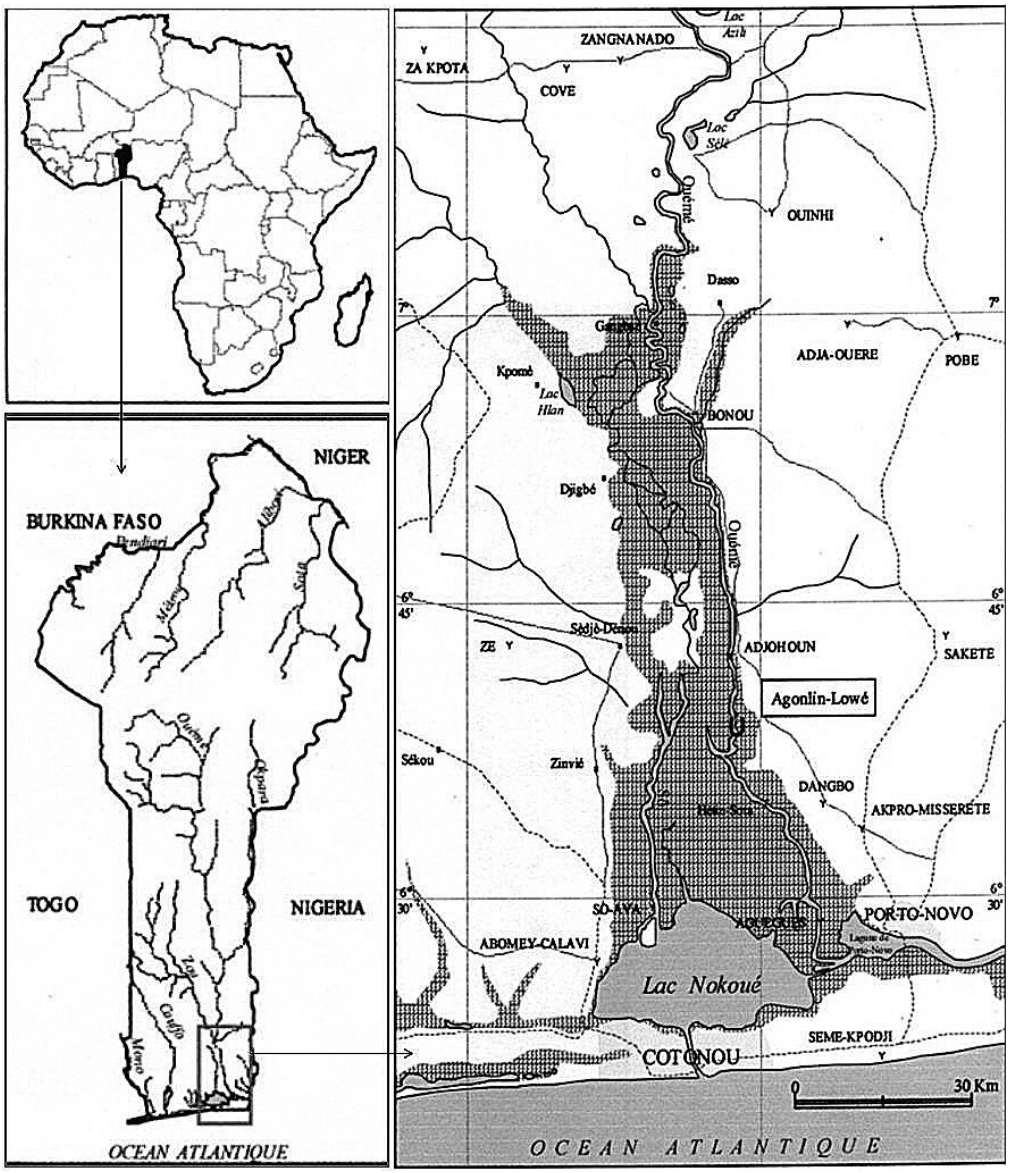

Figure 1: Oueme Delta River with the sampled area (Agonli-Lowé)

2.2. Measurements and identification of oocysts

Drawings and measurements of the different Coccidia oocysts encountered were obtained using the scale line by making a rule of three. The sizes (length, width or diameter) of Coccidia were measured on the image after magnification to the microscope and zoom by photography. For each oocyst observed, magnification and zoom were noted and the product of their factorization was calculated; thus constituting the scale. Real measurements of oocysts were obtained by dividing for each oocyst sizes measured on the image to their scale of enlargement. All measurements are given in micrometers as the range followed by the mean and standard deviation. The identification of Coccidia was conducted in accordance with the criteria defined by Coudert (1989).

2.3. Collection, sporulation and storage of oocysts

The collection of oocysts for the preparation of the inoculum was carried from infested intestinal tissue. Material was homogenized in a blender and filtered with water through a fine mesh sieve (100 µm) in a beaker using a spatula to stir the suspension. Oocysts were separated from the tissue homogenate into a saturated solution of sodium chloride and filtered through a fine mesh sieve (100 µm). The semi-solid oocysts suspension was distributed into centrifuge tubes; and centrifuged at moderate speed (1500 rpm) for 10-15 minutes to pellet the solids and allow the oocysts remain suspended in the top of the supernatant. Oocysts were removed from the upper layer of liquid with a pipette and were re-suspended in water.

Oocysts were subjected to sporulation before being infectious. This process occurred in Petri dishes, in a well ventilated environment at 30 °C, during a period of 24 to 72 hours. Storage of oocysts was done in a potassium dichromate solution.

2.4. Constitution of lots, infestation and parameters studied

For the experimental phase, 81 laboratory-bred juveniles of *C. gariepinus*, average of 4 g were randomly divided into three (3) groups of 27 individuals each, of which one (01) control group (T) and two (02) infested groups (I and II). Each group was divided into three (03) sub-groups of nine (09) juveniles. These various sub-groups were distributed uniformly in the experimental chamber (Fig. 2) in rearing tanks of identical dimensions, of which the filling level in water was the same.

- group T was formed of sub-groups T_1, T_2, et T_3 ;
- group I was formed of sub-groups I_1, I_2 et I_3 ;
- group T was formed of sub-groups II_1, II_2 et II_3.

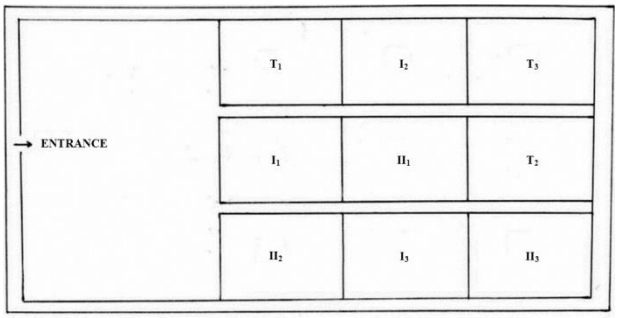

Figure 2: Groups layout from the experimental laboratory

The duration of the experimental phase was three (03) weeks. A conditioning period of one (01) week was carried out prior to the adjustment of subjects. Juveniles of groups I and II were then infested with oocysts of Coccidia, orally as described by Ahmed *et al.* (2010). Infestation rates were 600 oocysts per ml and 500 oocysts per ml of solution for the group II and group I respectively. Group T, served as control group, is considered uninfected.

During the three (03) weeks of experimentation, samples were carried out on dead individuals. An autopsy was performed and the gut of each dead fry was taken to determine the pathogenesis of Coccidia and types of lesions. The feed for each group was weighed and dispensed *ad libitum* daily from the 1st to 21st day. For each lot, the rest of food was collected in jars siphoned, dried and weighed daily. The quantities of food consumed per group have been estimated weekly, as the difference between the quantities distributed and refusal weekend (Aba *et al.*, 2012). Juveniles were weighed and measured at the beginning of the experiment; the mean weight and mean size at startup were calculated for each group. Thereafter, the weights and measurements were made once a week until the end of the experiment.

3. Data analysis

The main indexes measuring changes, in the host, of coccidial population are prevalence and parasite load. The prevalence rate is given by the following formula:

$$\text{Prevalence} = \frac{Number\ of\ infested\ fish}{Number\ of\ fish\ examined} \times 100$$

The parasitic load is expressed by the number of oocysts per gram of feces (OPG). This method for the enumeration of oocysts required the use of a McMaster cell, generally used in the counting procedures of the oocysts in the litter. The McMaster method was that used by Hodgson (1970) and Long *et al.* (1976).

During the experimental phase, the following parameters were measured:

- weekly average weight = $\frac{Total\ weight\ at\ the\ end\ of\ the\ week}{Number\ of\ fish\ living\ at\ the\ end\ of\ the\ week}$

- weekly average size = $\frac{Sum\ of\ the\ total\ length\ of\ each\ fish}{Number\ of\ fish\ living\ at\ the\ end\ of\ the\ week}$
- feed efficiency = $\frac{Amount\ of\ food\ consumed\ per\ week}{Average\ weight\ gain\ per\ week}$
- specific growth rate (SGR) = $\frac{ln\ (Final\ weight\ -Initial\ weight)}{Test\ period\ (days)} \times 100$

Statistical analyzes were performed using SPSS (v.18) for Windows and the results are considered significant at 95% ($p < 0.05$). The χ^2 test was used to assess differences in infestation rates, by Coccidia species observed among males and females of host populations. The possible relationship between the physico-chemical parameters and the parasitic infestation rates were evaluated using the Pearson correlation coefficient. Total length (cm) of adult specimens of *C. gariepinus* were grouped into five (05) different classes ([25-30[, [30-35[, [35-40[, [40-45[and [45 and + [). The number of oocysts per gram of feces (OPG) calculated for each individual examined has been reported to the size class and averages for each size class were compared using the z test. The amount of food consumed per week, the feed efficiency, the weight performance and weekly size average of juveniles were calculated by lot and compared by the Student t test. The group effect was used for analysis of variance.

4. Results

4.1. Description of the Coccidia species

Six (06) different forms of Coccidia (Table 1), belonging to the family of Eimeridae and subfamily of Eimeriinae, were determined and identified to genus level. They have been grouped into two genera. The genus *Cyclospora*, comprised four (04) species and the genus *Eimeria* with two (02) species.

Genus *Cyclospora* Schneider, 1881

- *Cyclospora* sp.1

The oocyst is egg-shaped, thin-walled and measure 9.16 ± 0.58 µm long and 7.08 ± 0.59 µm wide. The polar granules are absent. Several refractile granules form the oocyst residuum. Each oocyst contains two ellipsoidal sporocysts of 4.37 ± 0.29 µm x 2.49 ± 0.58 µm. The presence of a relatively small micropyle is noted at the apical end of the oocyst. The observation of fresh specimens showed an endogenous sporulation.

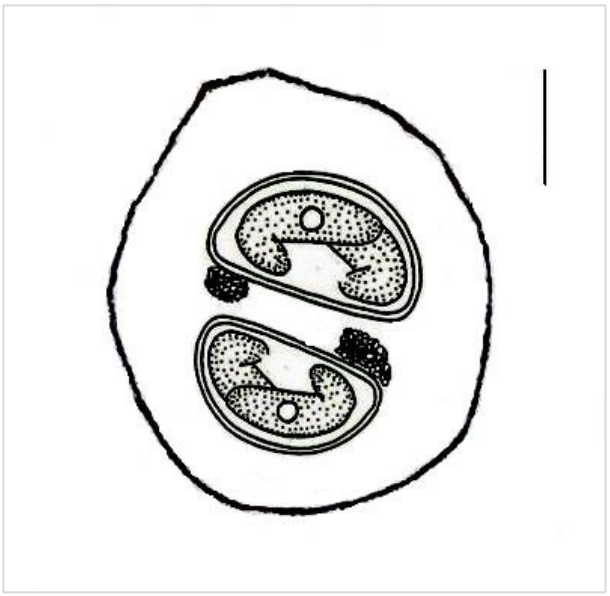

Figure 3: Schematic representation of the *Cyclospora* sp.1 oocyst (bar: 4 µm)

- *Cyclospora* sp.2

The oocyst wall is very thin. Oocyst is elongated ovoid shape and measure 12.7 ± 0.88 µm in length and 8.54 ± 0.29 µm in width. The presence of polar granules and oocyst residuum were not recorded inner the oocyst; whereas sporocyst present a residuum. In each oocyst there are two large and ovoid sporocysts composed each one of two sporozoites. Sporocysts dimensions were 8.75 ± 1.76µm (length) × 7.49 ± 0.58µm (width).

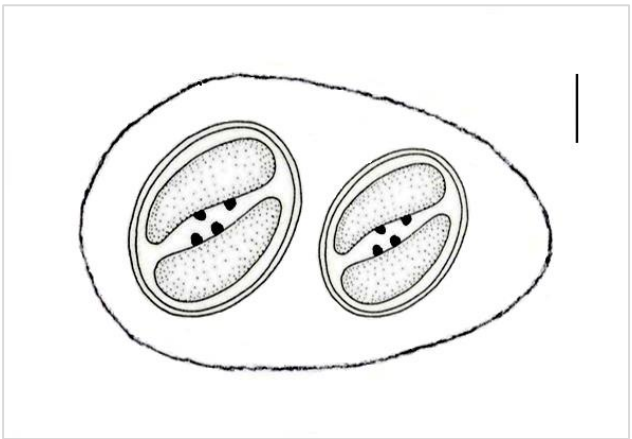

Figure 4: Schematic representation of the *Cyclospora* sp.2 oocyst (bar: 4 µm)

- *Cyclospora* sp.3

The oocyst wall is thick slightly. Oocyst has an ovoid shape, is small in size and measures 5.53 ± 0.63 µm × 3.83 ± 0.26 µm. The presence of oocyst residuum and the absence of polar granules were noted. Inside each oocyst two oviform sporocysts of size 2.08 ± 0.59 µm × 1.45 ±

0.28 µm are present. Inside each sporocyst stand out clearly two elongate, almost vermiform sporozoites.

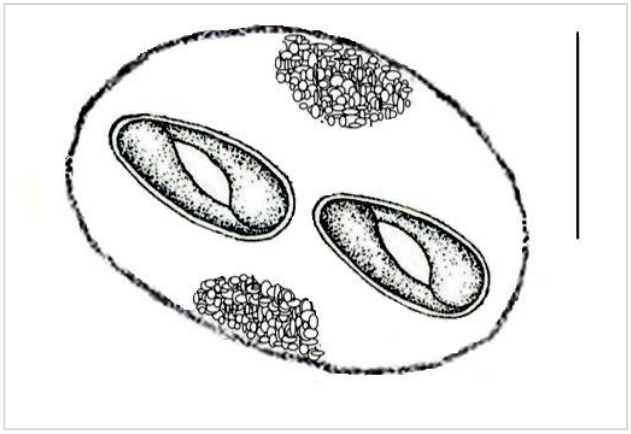

Figure 5: Schematic representation of the *Cyclospora* sp.3 oocyst (bar: 4 µm)

- *Cyclospora* sp.4

The oocysts are spherical and measure 6.24 ± 0.37 µm in diameter. A polar granule was observed; the oocyst residuum is contrariwise missing. In each oocyst, it is located two ovoid sporocysts of 2.29 ± 0.29 µm × 1.45 ± 0.28 µm. Each sporocyst contains two sporozoites with sporocyst residuum in formation.

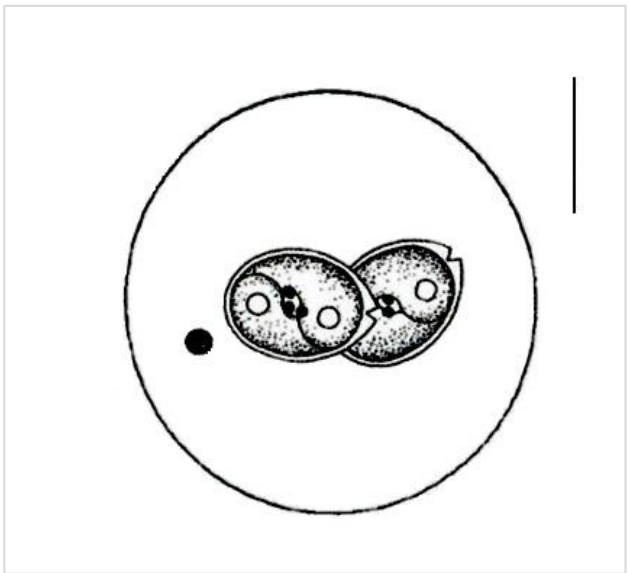

Figure 6: Schematic representation of the *Cyclospora* sp.4 oocyst (bar: 4 µm)

Genus *Eimeria* Schneider, 1875

- *Eimeria* sp.1

Oocysts are ovoid in shape and measured 5.62 ± 1.47 µm long and 4.58 ± 0.59 µm wide. Their wall is thick enough. Inside, the oocyst residuum is observed while the polar granules are absent. Four sporocysts 1.93 ± 0.63µm × 1.11 ± 0.24 µm in size were observed within the oocyst. The packaged form of sporocysts does not allow to observe clearly the Stieda body.

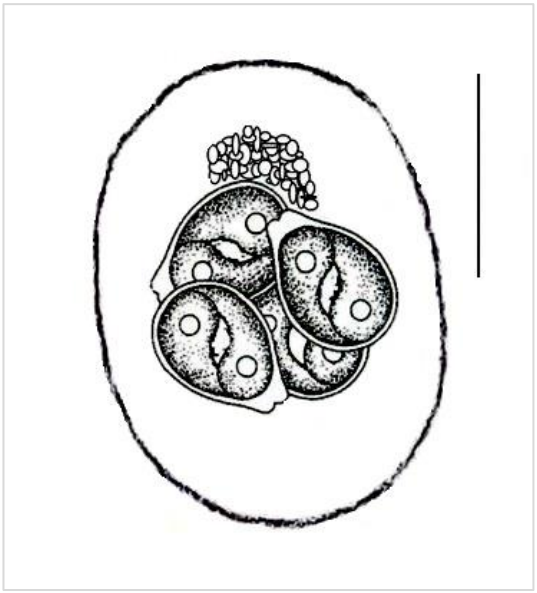

Figure 7: Schematic representation of the *Eimeria* sp.1 oocyst (bar: 4 µm)

- *Eimeria* sp.2

This species is represented by oocyst with varied dimensions. The oocyst wall is thin. Its shape is spherical with a diameter of 7.33 ± 1.11 µm. There is no polar granules. The oocyst residuum is formed by the refracting granules. Each oocyst contains four ovoid sporocysts (1.1 ± 0.2 µm x 0.9 ± 0.1 µm), with a Stieda body composed of papillae which is found at their narrowest end. These very small dimensions sporocysts occupy only a portion, sometimes eccentric, of the volume of oocyst.

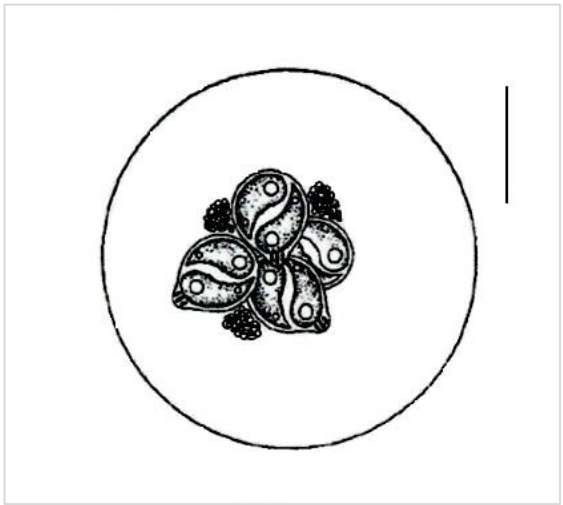

Figure 8: Schematic representation of the *Eimeria* sp.2 oocyst (bar: 4 µm)

Table 1: Summary of morphological characteristics and taxonomic affinities of different forms of Coccidia observed

Parasite	Morphologic characteristics			
	Cyst		Sporocyst	
	Shape	Size (µm)	Shape	Size (µm)
Cyclospora sp.1*	Ovoid	9,1 ± 0,6 × 7,1 ± 0,6	Ellipsoidal	4,4 ± 0,3 × 2,5 ± 0,6
C. sciaenae Diouf, 1993**	Ovoid	7,3 ± 0,7 × 6,8 ± 0,5	Ovoid	5,2 ± 0,6 × 3,5 ± 0,5
Cyclospora sp.2*	Ovoid	12,7 ± 0,9 × 8,5 ± 0,3	Ovoid	8,8 ± 1,7 × 7,5 ± 0,6
C. elopsi Diouf, 1993**	Ovoid	11,9 ± 1,0 × 8,0 ± 0,6	Ellipsoidal	8,3 ± 0,7 × 5,0 ± 0,6
Cyclospora sp.3*	Ovoid	5,5 ± 0,6 × 3,8 ± 0,2	Ovoid	2,1 ± 0,6 × 1,4 ± 0,3
-	-	-	-	-
Cyclospora sp.4*	Spherical	6,24 ± 0,37	Ovoid	2,3 ± 0,3 × 1,5 ± 0,2
-	-	-	-	-
Eimeria sp.1*	Ellipsoidal	5,6 ± 1,4 × 4,6 ± 0,6	Ovoid	1,9 ± 0,6 × 1,1 ± 0,2
E. kayarensis Diouf & Toguebaye, 1994**	Ellipsoidal	16,7 ± 1,1 × 13,9 ± 0,9	Ovoid	7,9 ± 0,7 × 6,0 ± 0,6
Eimeria sp.2*	Spherical	7,33 ± 1,11	Ovoid	1,1 ± 0,2 × 0,9 ± 0,1
E. ethmalosae Diouf & Toguebaye, 1994**	Spherical	22 (20 - 25)	Ellipsoidal	9,2 (8-10,5) × 6,7 (5-8)

* Present study; ** Similar species

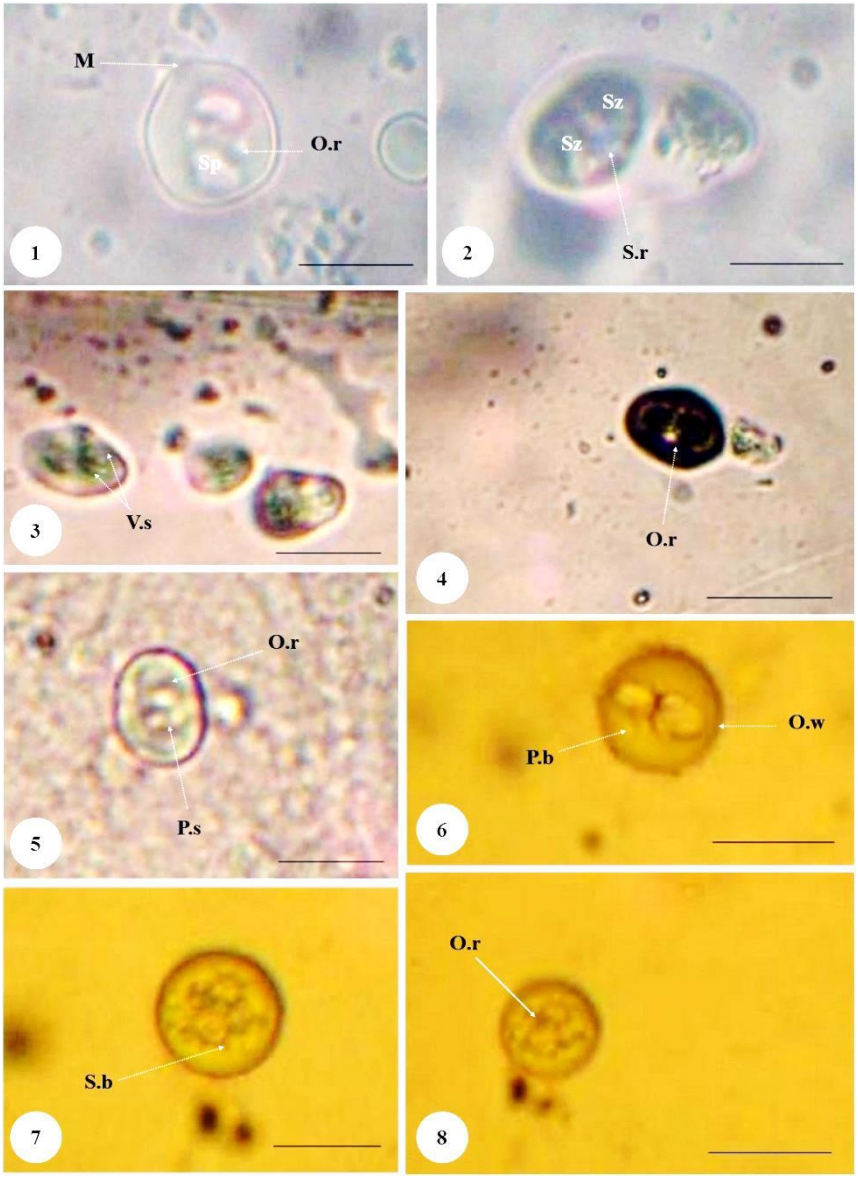

Figure 9: Plate of identified oocysts (bar: 5 µm)

(1) *Cyclospora* sp.1: O.r- oocyst residuum, M- micropyle, Sp- sporocyst; (2) *Cyclospora* sp.2: S.r- sporocyst residuum, Sz- sporozoite; (3-4) *Cyclospora* sp.3: V.s- vermiform sporozoite, O.r- oocyst residuum; (5) *Eimeria* sp.1: O.r- oocyst residuum, P.s- packed sporocyst; (6) *Cyclospora* sp.4: P.b- polar granule, O.w- oocyst wall; (7-8) *Eimeria* sp.2: S.b- Stieda body, O.r- oocyst residuum.

Table 2: Global prevalence and prevalence by gender of each parasite

Parasites	Host (*Clarias gariepinus*)						χ^2
	Total examined	Male		Female		Global prevalence (%)	
		Examined	Infested (%)	Examined	Infested (%)		
Cyclospora sp.1	117	64	44 (68.75)	53	39 (73.58)	70.94	0.329
Cyclospora sp.2			03 (04.68)		03 (05.66)	05.12	0.056
Cyclospora sp.3			16 (25.00)		17 (32.07)	28.20	0.717
Cyclospora sp.4			35 (54.69)		31 (58.49)	56.41	0.171
Eimeria sp.1			09 (14.06)		19 (35.84)	23.92	7.559
Eimeria sp.2			06 (09.37)		13 (24.52)	16.23	4.894
Total	117	64	44 (68.75)	53	39 (73.58)	70.94	-

Table 3 Presents the values, taken monthly (September to December), of different physico-chemical parameters picked up in the sampled water

Table 3: Values for physico-chemical parameters of the sampled water

Month	Temperature (°c)	pH	Dissolved Oxygen (mg/L)	Ammoniac (mg/L)	Nitrates (mg/L)	Nitrites (mg/L)	Phosphates (mg/L)	TDS (mg/L)
September	29.11	7.2	2.78	0.98	17	0.172	1.28	59
October	28.37	7.09	1.21	0.97	8.38	0.047	0.4	42
November	28.71	7.52	3.46	0.44	1.1	0.019	0.57	40
December	29.53	7.01	1.87	1.09	3.2	0.04	0.32	61

Prevalence and water quality

Table 2 shows that 83 of the fish examined are infested by Coccidia (44 males and 39 females); an overall prevalence of 70.94% (68.75% for males and 73.58% for females). *Cyclospora* sp.1 has the highest prevalence rate among both males (68.75%) than females (73.58%), but is also present in the intestine of all infected fish. The lowest prevalence rate (05.12%) is obtained with *Cyclospora* sp.2 both in females (05.66%) than in males (04.68). The data in Table 2 show, moreover, that for each of the identified forms of Coccidia, the proportion of females *Clarias gariepinus* infested is greater than that the proportion of infested males. These differences are not significant for *Cyclospora* sp.1 ($\chi2$ = 0.329; p-value = 0.566), *Cyclospora* sp.2 ($\chi2$ = 0.056; p-value = 0.813), *Cyclospora* sp.3 ($\chi2$ = 0.717; p-value = 0.397) and *Cyclospora* sp.4 ($\chi2$ = 0.171; p-value = 0.679). On the other side, these differences are significant for *Eimeria* sp.1 ($\chi2$ = 7.559; p-value = 0.006) and *Eimeria* sp.2 ($\chi2$ = 4.894; p-value = 0.026).

The Pearson correlation coefficient (r) and the degree of significance (p) between the prevalence rate for each parasite and the physicochemical parameters are given in Table 4. This table shows that the prevalence rate for each form of Coccidia parasite of *Clarias gariepinus* is not significantly correlated with different physicochemical parameters.

4.2. Parasite load

The various values calculated of OPG has been reported to the size of specimens of *C. gariepinus*. It shows that the strongest OPG levels were obtained for the smallest size classes. Indeed, for the size class [25-30[, it was observed OPG close to 6000 oocysts per gram of feces (5772.17). This value gradually decreases with increasing size and is experiencing a sharp drop in the size class [45 and + [. The number of fish by size classes decreases and as one moves from small to large sizes: the values of 28, 22, 16, 11 and 6 individuals were obtained, respectively for size classes [25-30 [, [30-35 [, [35-40 [, [40-45[, and [45-+[(Fig. 9).

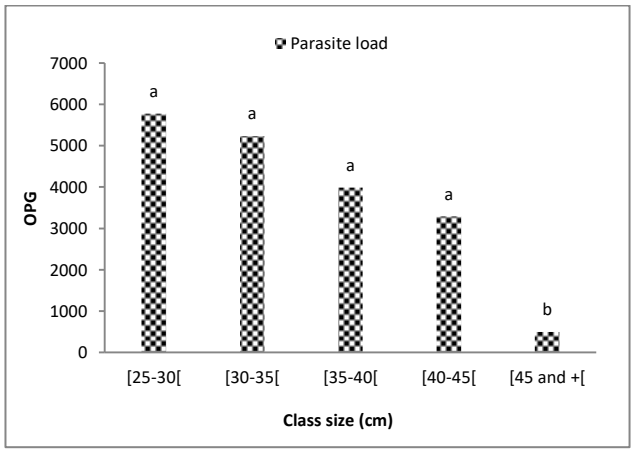

Figure 9: Evolution of number of oocysts per gram of feces as a function of the size

Table 4: Correlation between the prevalence of each parasite and physicochemical parameters

Parasites	Temperature	pH	Dissolved Oxygen	Ammoniac	Nitrates	Nitrites	Phosphates	TDS
Cyclospora sp.1	.847	.164	.565	-.111	-.298	-.002	.086	.561
	.153	.836	.435	.889	.702	.998	.914	.439
Cyclospora sp.2	.220	-.520	-.606	.318	-.674	-.730	-.922	.051
	.780	.480	.394	.682	.326	.270	.078	.949
Cyclospora sp.3	.498	.603	.921	-.473	-.051	.237	.488	.273
	.502	.397	.079	.527	.949	.763	.512	.727
Cyclospora sp.4	.645	-.432	.024	.628	.767	.882	.712	.884
	.355	.568	.976	.372	.233	.118	.288	.116
Eimeria sp.1	-.327	.770	.508	-.893	-.817	-.758	-.448	-.706
	.673	.230	.492	.107	.183	.242	.552	.294
Eimeria sp.2	.142	.726	.938	-.550	.258	.467	.755	.062
	.858	.274	.062	.450	.742	.533	.245	.938

For each parasite, the first line gives the value of the Pearson correlation (r) and the second, the probability value (p)

4.3. Artificial infestation

Health status

The monitoring of fry allowed to identify the different clinical signs and mortality both in the control group than in infected groups. The first deaths occurred on Day 5 (D_5) and were observed in the sub-groups I_1 and I_3. Mortalities were then spread over time in different sub-groups, especially infected sub-groups, with a peak in the third week of the trial period. Group I present the highest mortality at the end of the experiment (9 dead); the first deaths occurred during the first week and continued in the second and third weeks. The dead identified in group II were during the last two weeks with a lower mortality rate than that of group I in the third week. Mortalities recorded in the control group (3 dead) are lower than those observed in the infested groups and intervened in the last week of experience in the sub-group T_1.

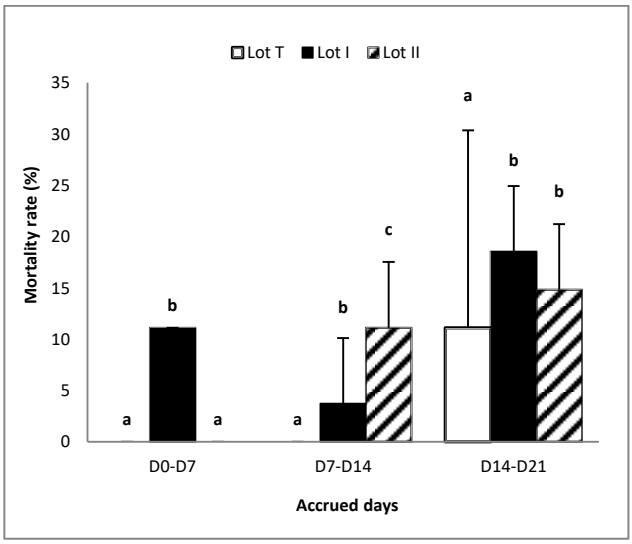

Figure 10: Weekly evolution of mortality (Graphics no bearing at least one letter in common are significantly different (p <0.001))

Gut samples from the dead individuals and fecal analysis digestive tracts revealed a strong infestation of the intestine of individuals from infested groups. The observation of fresh intestinal tissue samples allowed to identify *Cyclospora* sp.4 in sporulation phase. The samples of digestive tubes and fecal analyzes of individuals from group T proved free of Coccidia.

In terms of clinical signs, the appearance of symptoms of the infestation is almost always occurred during the final phase, in dying fry. Before this phase, the presence of infestation in groups could be seen by some behavioral problems, including a reluctance to food intake, a decrease in activity seen mainly in the day. Terminally, we observed two types of symptoms in the abdomen of fry: the appearance of small redness of spots (petechiae) on a good part of the abdomen or swelling of the abdomen (abdomen distended). The autopsy of the dead fry showed congestion of the intestine and stomach distension.

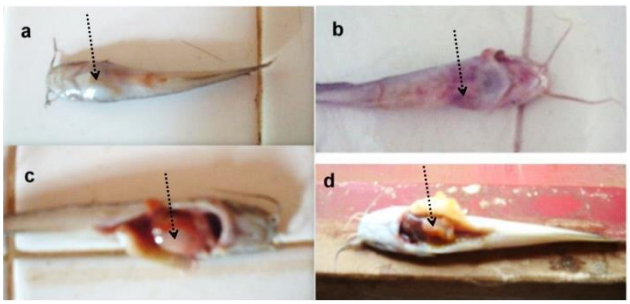

Figure 11: Symptoms and lesions observed at autopsy (arrows). (a) : Swollen abdomen ; (b) : Spot of redness on the abdomen ; (c) : Distension of the stomach (dissection) ; (d) : Congestion (dissection)

Evolution of the weekly food consumption

The evolution of data on food consumption of the different groups show the same profile of the weekly food consumption, moving increasingly. This increase was recorded in the three (03) weeks of testing. Table IV shows the evolution of food consumption over time.

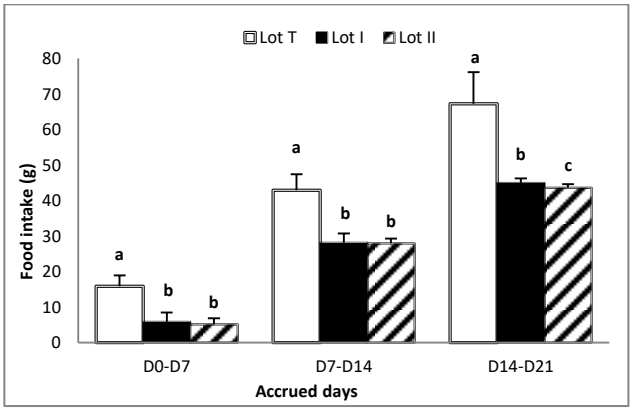

Figure 12: Evolution of food intake (g) of fry with the time. (Graphics not carrying at least one letter in common are significantly different (p <0.001)).

Food consumption was higher in the control group than in the infected groups. This difference was significant (p <0.001) between the control group and the group I the one hand, and between the control group and the group II on the other; during this three week trial. Significant differences in food consumption were noted (p <0.01) during the third week between infested group I and infested group II. This difference resulting into higher food consumption in group I during the three-week trial. As against this difference was not significant during the two (02) weeks.

Evolution of the feed efficiency

We see a very incomplete profile of feed efficiency in infested groups I and II from the first to the third week. Indeed, food consumption index for these two groups was calculated only during the second week of testing; the consumption index of the group I being significantly higher (p <0.001) than the index of consumption of group II.

The consumption index profile of control group is complete and has an increasing trend over time. The consumption index of the group T is significantly lower (p <0.001) than groups I and II during the second week of testing.

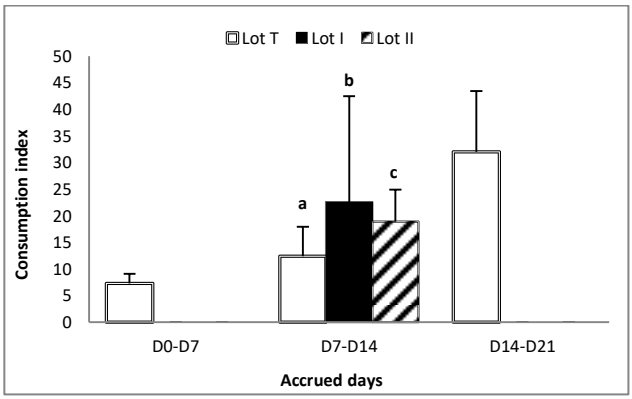

Figure 13: Weekly Evolution of the feed efficiency (Graphics not carrying at least one letter in common are significantly different (p <0.001))

Evolution of weekly average weight of juvenile

The weight of fry from the different groups has varied over time. Table 6 and Figure 7 show the evolution of weekly average weight of fry.

Table 6: Weekly evolution of the average weight (g) of juvenile

Lots	Day			
	D0	D7	D14	D21
Lot T	40.33 ± 7.71a	59.56 ± 16a	90.7 ± 33.99a	108.8 ± 52.53a
Lot I	49.33 ± 6.4b	42.66 ± 2.7b	52.66 ± 6.42b	48.2 ± 9.55b
Lot II	44.6 ± 10.45a	40.43 ± 6.56b	53.73 ± 4.42b	44.73 ± 7.76b
Signification	***	***	***	***

Averages in the same column followed by different letters differ significantly to threshold of 5%. (***) P < 0,001%

At D0, the weight of the control group was significantly lower (p <0.001) than that of the group I while no significant difference in weight was observed between the control group and group II. By against the weight of fry from group I was significantly higher (p <0.05) than that of group II. From D0, group T shows a profile of the average weight of fry evolving increasingly during the three-week trial with a peak growth rate at D14.

The weight of laboratory-bred juveniles from control group was significantly higher (p <0.001) than those of infested groups I and II at D7, D14 and D21. Infested groups I and II have the same profile of the weight of fry evolving saw tooth during the three weeks. It is observed, indeed, a decrease of weight of juveniles of the two groups from D0 to D7 and D14 to D21 while an increase in weight is observed from D7 to D14. No significant difference in weight was observed between infected groups I and II at D7, D14 and D21.

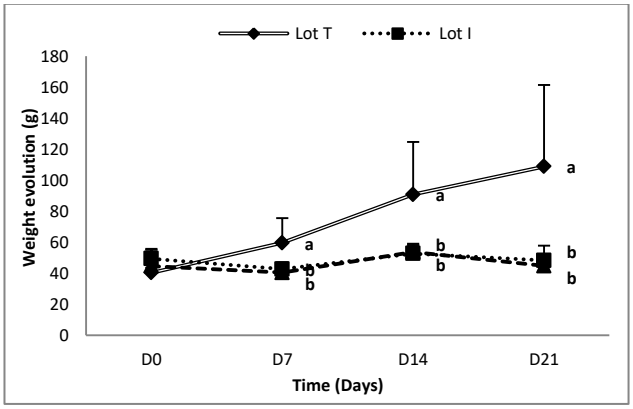

Figure 15: Weekly evolution of average weight. (Graphics not carrying at least one letter in common are significantly different (p <0.001))

Specific growth rate (SGR)

Specific growth rate calculated at the end of the experiment shows that only the juveniles from control

group grown steadily during the three weeks. The final weights of fry in infested groups I and II are quite close starting weight; which results in a negative specific growth rate for the group II or not calculated for the group I.

Table 7: Specific growth rate (SGR)

Groups	SGR (%)
T	24.12
I	-
II	-4.36

5. Discussion

5.1. Taxonomic affinities of Coccidia species

The genus *Cyclospora* belonging to the family of Eimeriidae Minchin, 1903, includes the Coccidia which oocysts contain two sporocysts; and sporocysts, two sporozoïtes. The genus *Eimeria* is composed of Coccidia which of mature oocysts contain four (04) sporocysts having a Stieda body. Each sporocyst consisting of two (02) sporozoites.

- *Cyclospora* sp.1

This species is unlike any Coccidia of the genus *Cyclospora* described in Mediterranean fish and the Gulf of Guinea fish. The presence of a micropyle at the anterior end of the species differentiates our work. The size of the oocyst could bring it closer to *Cyclospora sciaenae* discovered by Diouf (1993) but the irregular shape of *C. sciaenae* and the absence of micropyle confirm that these are two different species.

- *Cyclospora* sp.2

This species is approaching *Cyclospora elopsi* described by Diouf in 1993 in the gut of *Elops senegalensis*. The similarity in the size of the oocyst, the absence of oocyst residue and the presence of sporocyst residue are all common in both species. We can however note the presence of a polar granule in *Elops senegalensis* which tends to disprove this hypothesis especially as the worm morphology of sporozoites contrast with the packed form of *Cyclospora* sp.2 sporozoites.

- *Cyclospora* sp.3

This species differs markedly from *Cyclospora* sp.1, *Cyclospora* sp.2 (present work) and other *Cyclospora* species described to date in fish. Its small size is therefore a peculiarity. However, it's noted the elongated shape of sporozoites that bring him closer to those of *Cyclospora elopsi* (worm).

- *Cyclospora* sp.4

Of *Cyclospora* species described in fish, none has similarities with the species encountered; this is particularly and largely due to the spherical shape of the *Cyclospora* sp.4 oocyst. This is probably a new species of Coccidia named *Cyclospora* sp.4 until other further observations locate exactly the position of the oocyst in systematics.

- *Eimeria* sp.1

The packed form of cyst is an observed feature in many *Eimeria* oocysts. This species, when it was found, most often cohabiting with *Cyclospora* sp.3. The ovoid shape of the oocyst differs from that of most *Eimeria* oocysts found in fish that are more spherical. This difference increases at the smaller *Eimeria* sp.1. This would be a new species that more advanced means of observations would characterize better.

- *Eimeria* sp.2

Taking into account the shape of the oocyst and sporocyst location (occupied volume) in the oocyst, the species approaches *Eimeria ethmalosae* described by Diouf & Toguebaye (1994) in *Ethmalosa fimbriata*. These two species differ however in the size, greater in *E. ethmalosae*, and by the shape of cyst which is ellipsoidal in *E. ethmalosae*.

5.2. Prevalence rate and parasite load

On the 117 specimens of examined *C. gariepinus*, 83 individuals were infested by the Coccidia; so a prevalence rate of 70.94%. This prevalence rate is sensivetily close of this one recorded by Siko (2010) in a precedent work on specimens of *C. gariepinus* from Agonli-Lowé village, in low Ouémé delta and which is 100%.

Considering the number of fish harvested in the delta of the Ouémé (117), there is a similarity in prevalence rates between the present results and those obtained by Siko (2010); which denotes the endemic nature of coccidia infection of African catfish in this part of the Ouémé valley. In their inventory of protozoan parasites of catfish in the Nile Delta, El-Tantawy & El-Sherbiny (2010) do not mention any species of coccidia parasite of African catfish *C. gariepinus*. The absence of results on coccidia in this study may be explained by the fact that the research of these authors have exclusively brought against protozoan ectoparasites (skin and gills) and blood parasites of *C. gariepinus*. While for the most part, these parasites (Coccidia) are usually found in the wall of the digestive tract or the body musculature.

As regards the parasite load of specimens of *C. gariepinus* harvested, figure 9 shows information about the gradual reduction of the parasitic load, expressed in number of oocysts per gram of feces, with increasing size

of catfish; the parasite load is very low especially for the largest size class [45 and +[and almost reached the 6000 oocysts per gram of feces for the smallest size class [20-25 [This finding shows effectively a vulnerability of young stages of *C. gariepinus* to infection by Coccidia; this vulnerability seems to decrease with age, so with the gradual acquisition of an immunity towards these parasites.

5.3. Diverse forms of Coccidia encountered

Two (02) genera of Coccidia were identified during this work: genera *Cyclospora* and *Eimeria* for we have found respectively 04 and 02 types of oocysts morphologically different; which may be differentiated in species after further investigation by electron microscopy. These results are different from those obtained by Diouf (1993), on the Silurodei fish of Senegalese coast. Indeed, on 10 of these fish examined none Coccidia species could not be identified, despite the large number of species of Coccidia described by this author: about 36 species of Coccidia belonging to the genera *Cyclospora* (02), *Eimeria* (24) and *Goussia* (10) on 8848 individuals examined and spread in 78 fish families.

5.4. Health status of Clarias gariepinus juvenile

According Euzet & Pariselle (1996), the pathogenic effect of parasites in fish, in natural conditions is reduced, as a result of the equilibrium established during the evolution in the host/parasite system. However, in aquaculture, the accumulation in a host, of a foreign organism: virus, bacteria or parasite, causes a pathogenic effect whose gravity is proportional to this accumulation. This assertion seems to be confirmed by the high rate of infestation identified in the gastrointestinal tract of fish died in infected groups I and II. Infestation rates have led in particular by high mortality, especially in the group I, and characteristic lesions. Kueh Gibson *et al.* (2011) in their study of intestinal infection with *Eimeria* of bar juveniles, *Lates calcarifer*, observe that Coccidia infections are often associated with severe pathogenesis and significant mortality in the absence of other pathogens.

5.5. Use and feed efficiency

In general, the results obtained during the essay, show that food consumption in three groups increases with age. Soltner (1983), explains that the amount of feed consumed by an animal, depend inter alia on its live weight. This demonstration can be applied to the control group T but does not correspond to the evolution of the weekly food consumption in infected groups I and II. Food consumption was significantly higher in the control group than in the infected groups during the three-week trial. This finding may be explained firstly by the high mortality observed in infected groups, affecting overall food intake; secondly by the observed symptoms including numbness that affects individual activity and therefore food intake. Under normal conditions, the feed efficiency increases with age of subjects (Boka, 2006). The feed efficiency of control group has increased steadily during the three weeks. This index is significantly lower than those of groups I and II. This finding indicates that juveniles of groups I and II requires much more food to produce as much weight as those of control group. But there is, in fact, a decrease in food consumption due to parasitic infestation which has the effect of weight loss during the first and third week.

5.6. Growth performance

Data on weight changes show a steady increase in the growth of juvenile of control group with age. This growth follows perfectly the curve of evolution of food consumption predicted by Soltner (1983). Contrariwise, weight development of juveniles of infected groups I and II showed a decrease in weight at the end of the first and the third week in two groups. If at the end of the first week, weight loss can be attributed to low food intake (5.83 g on average for group I and 5.10 g on average for group II) compared to the control group (15, 86 g on average), weight loss at the end of the third week may be due to the combination of two factors namely: food consumption still low compared to control group but also and especially the high mortality recorded during the third week in infected groups. Therefore a significantly higher weight gain for the control group than that observed in groups I and II is observed. This significant difference is corroborated by Yvore (1992) which stresses that Coccidia infections depress animal performance of fish by reducing the speed of growth and increasing feed efficiency.

The weight of the control group at the end of the essay has significantly increased from an average of 40,33g to 108,8g, with a specific growth rate (SGR) of 24.12% reflecting the strong growth in this group. As against, absence of SGR at group I and the negative SGR calculated for group II are characteristic of low weight increase (group II) of juveniles after the test, or even the reduced weight (group I) compared to data collected to the beginning of the experiment.

Conclusion

The main concern in this work was to provide evidence of the impact of coccidiosis on the production of fish, including catfish such as *C. gariepinus*. Our prospecting led to the discovery of 6 different forms of Coccidia, all parasite of the digestive tract of the African catfish *Clarias gariepinus*. The high prevalence observed in fish examined resulted in a number of oocysts per gram of feces (OPG) quite high, especially at the younger stages of *C. gariepinus*. The observed results also show an impact of coccidiosis on juvenile of *C. gariepinus*, including the effectiveness of heavy infestation of Coccidia of the genus *Cyclospora*. The continuation of this work, particularly in

the characterization, by better hardware, of different species of Coccidia infecting fish of Benin river; extending this work to other species of fish raised in Benin to ensure their tolerance or not to coccidial infection are all possible perspectives can contribute to improving the quality of fish production in Republic of Benin.

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