Original Research Article

Effect of the type of pituitary extracts and dose of synthetic hormones HCG and Ovaprim on some reproductive characteristics of the endogenous catfish of Cameroon *Clariasjaensis* (Boulanger, 1909) in a controlled environment

ABSTRACT

Aims: Improve the domestication of *Clariasjaensis* throughfry production in acontrolled environment.

Place and duration of the study: From April to August 2020 in Integrated Aquaculture Common Initiative Group of West in Batie (LN: 5°17'0"-5°18'53" and LE: 10°17'0"-10°19'31"), Sudano-Guinean altitude zone of West Cameroon.

Study Design:42 females and 15 males of respective weight and size: 177±40.46g, 347.62±111.72g, 29.02±1.74cm and 39.52±12.27cm were used and divided into 7 batches of comparable weights and sizes. Two randomly selected batches were subjected to the female pituitary extract of *Clariasjaensis* and Toads, five others to the synthetic hormones HCG (doses 3800 and 4000 IU/kg live weight) and Ovaprim (doses of 0.4, 0.5 and 0.6 ml/kg) respectively.

Methodology:eggs were enumerated by direct observation after fertilization. At the end of the hatching (D1) and of vitellineresorption (D3), thelarvae of each experimental lot were counted andmeasured.

Results: The mean diameter of the oocytes after induction, the relative and absolute fecundities and the fertility rate were higher in batches treated with the hormone HCG at a dose of 4000 IU/kg (2.12±0.1mm; 29,584±5,19 oocytes/g; 4694±620 oocytes and 80.14±9.78%), the lowest values were obtained with HCG at 3800 IU /kg, Ovaprim at 0.4ml/kg, pituitary toad extract and Ovaprim at 0.5 ml/kg (1.93±0.06mm; 15.29 ± 4.22 oocytes/g; 1410±106 oocytes and 55.34±1.51%). The hatchability rate was higher with Ovaprim at 0.6ml/kg, followed by HCG at 4000 IU/kg, However, no significant differences were observed between treatments (P>0.05)."

Conclusion: In a fish farm of intensive production, the use of the hormone Ovaprim dose 0.6 ml/kg or HCG at 4000IU/kgwill lead to high results.

Keys words:hormones, *Clariasjaensis*, fecundity, fertility rate, hatchability rate.

1. INTRODUCTION

Fisheries and aquaculture remain a major resource for many countries around the world, particularly those in sub-Saharan Africa, as they not only provide a diet rich in animal protein but also income for millions of people [1]. Indeed, the global supply of fish has reached a record figure of 20 kg per inhabitant, thanks to the strong growth of aquaculture, which now supplies half of the fish for human consumption. This growth could be explained by the gradual valorization of indigenous species in some regions of the world [1]. The use of preferably endogenous fishery resources that can be more resilient to the effects of climate change, therefore, contributes to the efficiency of aquaculture. A better knowledge of endogenous species for aquaculture valorization purposes is then necessary. This is the case, for example, of studies conducted on certainCarp and African -catfish particularly, *Labeosenegalensis*[2] and *Chrysichthysauratus*[3] in Benin.

Cameroon has great geo-climatic diversity, thus corresponding to the existence of a great potential in endogenous fishery resources, of which only a small proportion is currently known and valued. This is the case, for example,of *Clariasjaensis*, whose studies on its artificial reproduction have been successfully conducted in recent years [4]. In these studies, female pituitary extracts of *Clariasjaensis* and the synthetic hormone HCGdose of 4000 IU/kg were used to obtain the larvae of *Clariasjaensis*[5]. Since there are also other natural hormones such as pituitary extracts of toads and cheap synthetic hormones such as Ovaprim, for artificial reproduction in Clariidae[6 and 7], it would be important to know which of the hormones could further improve artificial reproduction in *Clariasjaensis*and at what dose?

This work aims to contribute to the improvement of knowledge on artificial reproduction in *Clariasjaensis*to preserveand enhancethe biodiversity of endogenous species, more specifically, to determine the effects of synthetic hormones (HCG at doses 3800 and 4000 IU/kg of female and Ovaprim at doses of 0.4; 0.5 and 0.6 ml/kg female) and pituitary extracts (from toads and females of *Clariasjaensis*) on some reproductive characteristics in a controlled environment such as oocyte diameter, fecundity (relative and absolute), fertility and hatchability rates.

2. MATERIALS AND METHODS

2.1 Study area

The study took place from April to August 2020 in the group farm of Integrated Aquaculture Common Initiative Group of West (GIC AIO) located in "Batié" (LN: 5°17'0"- 5° 18'53" and LE: 10°17'0" – 10° 19'31") Sudano-Guinean altitude zone of West Cameroon.

2.2 Data collection

A total of 42 females and 15 males of *Clariasjaensis* (donor's milt) weights and sizesof177±40.46g, 347.62±111.72g, 29.02±1.74cm and 39.52±12.27cm respectively were used and divided into 7 batches with 3 repetitions each, of comparable weights and sizes. Two randomly selected batches were subjected to the female pituitary extract of *Clariasjaensis* and Toad, five others to the synthetic hormones HCG (doses 3800 and 4000 IU/kg live weight) and Ovaprim (0.4; 0.5 and 0.6 ml/kg) respectively (Figure 1).

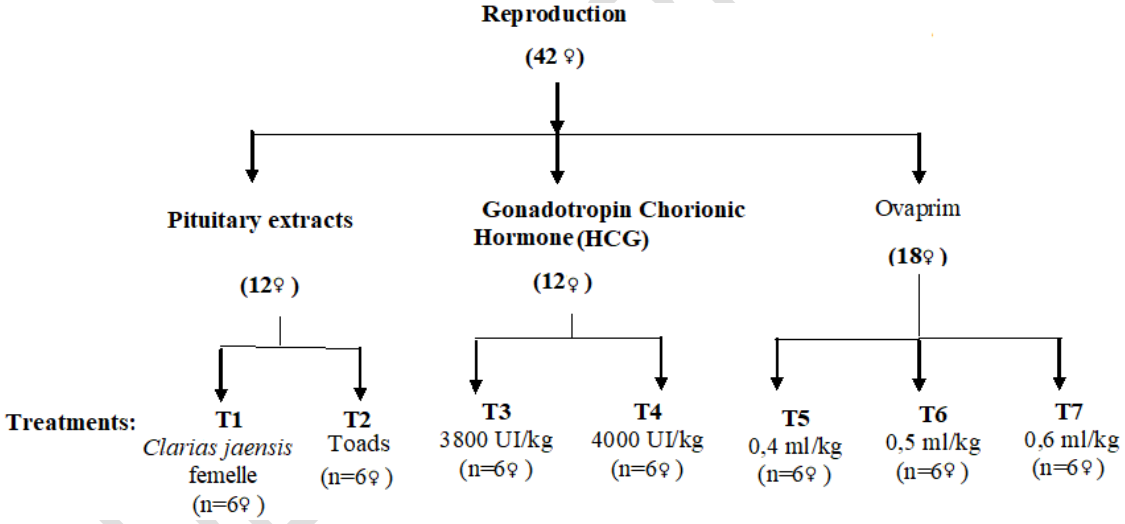


Figure 1: Flow diagram for reproduction

2.3 Realization of the trial

To determine the effects of synthetic hormones (HCG at doses 3800 and 4000 IU/kg female and ovaprim at doses of 0.4; 0.5 and 0.6 ml/kg female) and pituitary extracts (toads and females of *Clariasjaensis*) on some reproductive characteristics, 45 females and 15 males of *Clariasjaensis* of weights and sizes were177±40.46g, 347.62±111.72g, 29.02±1.74cm and 39.52±12.27cmrespectively. Females had a bloated, soft belly and emittedyellowish-colored eggs at least 1.5 mm in diameter obtained by slight abdominal pressure, while males had a live

weight greater than or equal to 200 g. These reproducers were divided into 7 batches of comparable weights and sizes. Two randomly selected batches were subjected to the female pituitary extract of *Clariasjaensis* and Toad, five others to the synthetic hormones HCG (doses 3800 and 4000 IU/kg live weight) and Ovaprim (doses of 0.4; 0.5 and 0.6 ml/kg) respectively.

2.3.1 Hormonal injection

The pituitary extract came from females of *C. jaensis* having a weight greater than or equal to that of the female to be injected and also from Toads whose sum of live weights was greater than or equal to that of the female to be injected. The pituitary extracts were prepared and administered [8, 9and10].

The synthetic hormones HCG (at doses of 3800 and 4000 IU/kg live weight) and Ovaprim (at doses of 0.4; 0.5 and 0.6 ml/kg) were administered into the animal's dorsal muscle[9].

For both pituitary extracts and synthetic hormones, the females received two intramuscular injections respectively 1/3 and 6 hours and 2/3 of the hormone wasinjected into the back muscles.

Each female thus treated was stored individually in a 20-liter plastic perforated gallon, numbered and immersed in a concrete tank (water height = 0.65 m and water flow = 7.16 l / s). The females thus conditioned did not receive food.

2.3.2 Egg harvesting and testicular sample collections

Oocyte maturation took place 28 hours after the second injection. The eggs of each female were collected after top-down abdominal pressure [11], in pre-weighed and numbered plastic bowls and after harvest, each bowl and its contents (eggs) were weighed using a Kitchen scale SF-400 electronic scale with a sensitivity of 0.1 g and the weight of the eggs was obtained by the difference between the weightings, while the oocyte diameter was obtained by the average distance over 10 oocytes measured on graph paper.

To obtain the milt for fertilization of the eggs, the testes were taken entirely after sacrificing each male that fulfilled theabove-mentioned weight criterion, according to the technique [11and12]. Thetestis were cut with blade, and pressed into a mortar to extract milt for fertilization of oocytes.

2.3.3 Egg fertilization and incubation

The addition of one milliliter of the physiological solution made it possible to dilute the milt before being mixed with the eggs until a homogeneous mixture was obtained. Then a fertilization solution (6 g of urea + 3 g of salt in 1.5 liters of water) was used to rinse the eggs for 60 seconds. This mixture was spread over the labeledracks arranged beforehand in the incubation tank (water height = 0.37 m and water flow = 5.18 ml / s).

A sample of 1g of eggs was taken from each bowl and placed in the incubation tank at the same time as the racks, as well as another bowl containing 1g of unfertilized eggs previously taken before mixing with milt, to assess the fertilization and hatching rates of each female. The incubation tank was covered with a black tarpaulin. A multi-parameter was previously installed in the incubation tank to note the physicochemical parameters of the water during the incubation of the eggs. The larvae after hatching were kept in the incubation tank. No food intake was made for 4 days (yolk resorption).

2.4 Characteristics studied

- Oocyte diameter (OD) in mm

OD = average distance over 10 oocytes measured on millimeter paper

- Absolute and relative fecundities (Fa and Fr)

Fa= number of oocyte/laying gram× total laying weight (g)

Fr= total number of oocytes per individual/body weight

- Fertility Rate (FR)

$$\text{FR}\ (\%) = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs collected}} * 100$$

- Hatchability rate (HR)

$$\text{HR}\ (\%) = \frac{\text{Number of larvaes hatched}}{\text{Number of eggs fertilized}} * 100$$

2.5 Statistical analysis

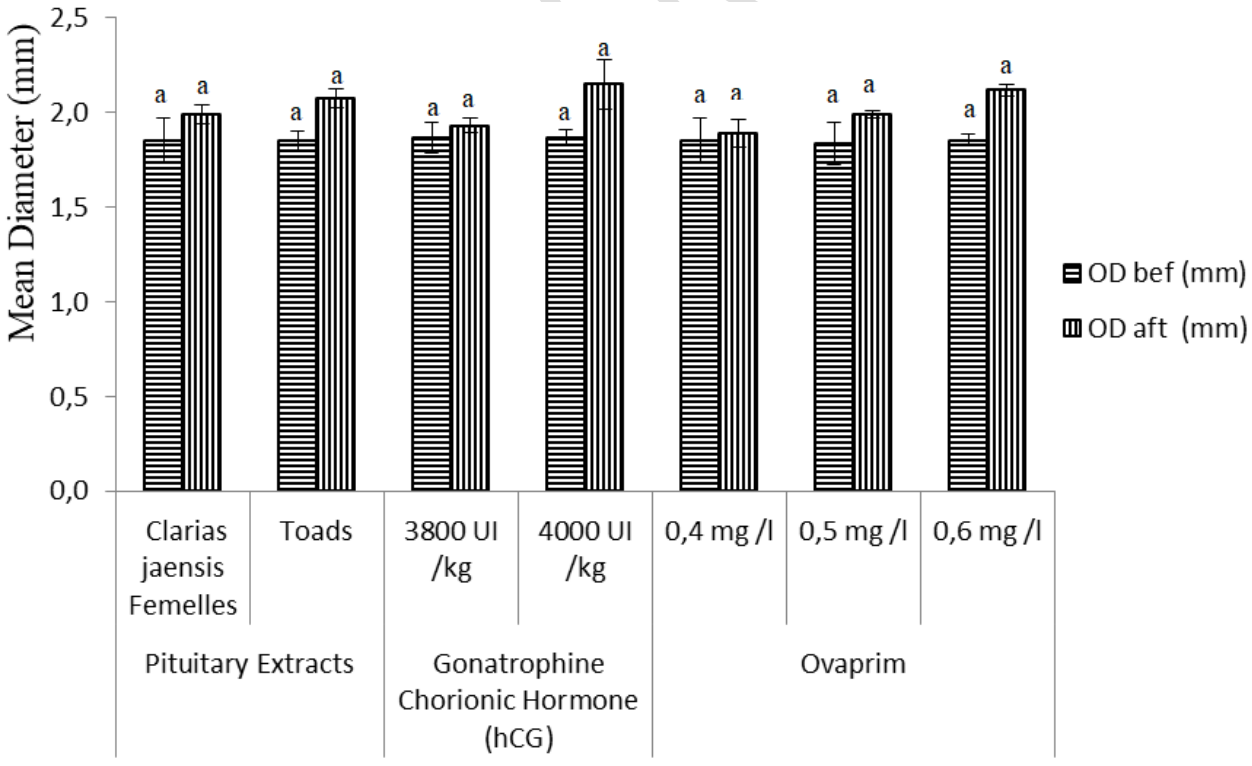
The results obtained were subjected to the *one-way analysis of variance ANOVA*(oocyte diameter, fertility and hatchability rates) followed by theStudent's T-test

(fecundities among types of hormones). Duncan's test separated the averages at the 5% threshold. SPSS version 20 software and EXCEL workbook were used in data processing.

3. RESULTS

3.1 Effects of hormone type and dose on the diameter and average weight of the oocyte before and after hormonal induction

The effects of the type and dose of hormones on the diameter and mean weight of the oocyte before and after hormonal induction are recorded in Figures2 and 3. It follows that regardless of the type or dose of hormone, both the diameter and the average weight of the oocyte increased after hormonal treatment. Thus, the highest diameter was obtained on oocytes from females treated with HCG hormones at 4000 Ui/kg and Ovaprim at a dose of 0.6 ml/kg followed by those from the pituitary extract of frogs. The smallest oocyte diameter is recorded with adose of 0.4 ml/kg and with HCG at 3800 IU/kg. The mean weight of female oocytes was higher with HCG hormones at 4000 IU/kg and Ovaprim at 0.6 ml/kg and lower at 3800 IU/kg HCG and Toads extracts. However, no significant difference ($P > 0.05$) was observed between hormone types and doses after hormone treatment.

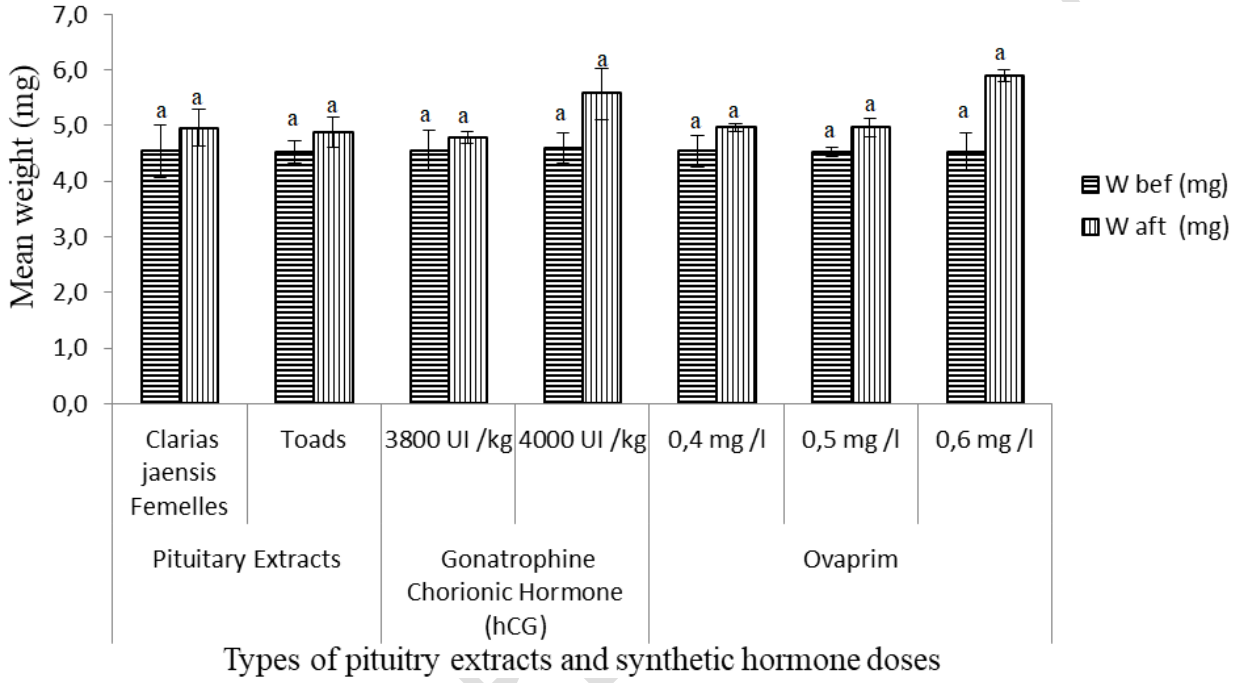


Types of Pituitary Extracts and synthétic Hormone Doses

OD bef: oocyte diameter before hormonal induction, OD aft: oocyte diameter after hormonal induction,

a: vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

Figure 2: Average oocyte diameter in *Clariasjaensis*before and after stripping aboutthe type of pituitary extracts and synthetic hormones



W bef: average weight of oocytes before hormone injection, W aft: average weight of oocytes after hormone injection,

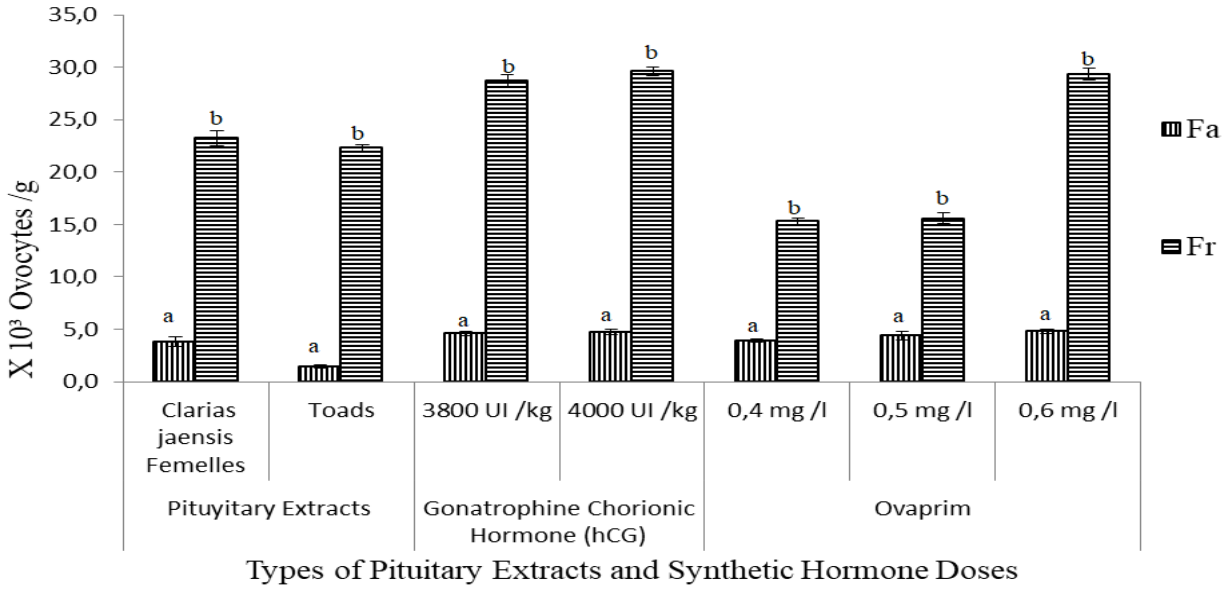
a: vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

Figure 3: Average weight (mg) of oocytes in *Clariasjaensis*according to the type of extracts Pituitary gland and hormone doses

3.2 Effects of hormone type and dose on absolute and relative fecundities, latency time, fertility and hatchability rates in *Clariasjaensis*

The effects of hormone type and dose on reproductive characteristics are illustrated by figures 4 to 7. It shows that absolute and relative fecundity (Fig.4) as well as fertilityrate (Fig.6) and hatchability rate (Fig.7) were higher in Ovaprim-induced females at a dose of 0.6 ml/kg followed by females treated with the hormone HCG at 4000 IU/kg. The latency duration (Fig.5) was higher with ovaprim followed by HCG at 4000 UI/kg and lower with female pituitary extracts.

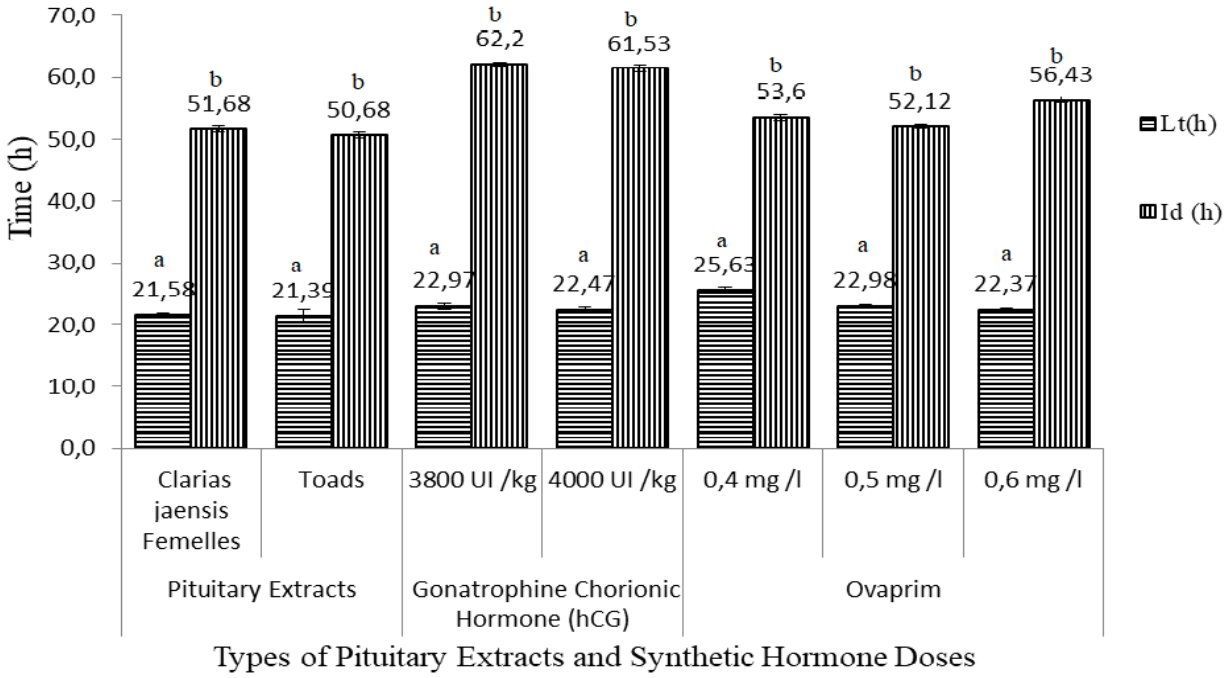
On the other side, incubation duration was higher with HCG hormone at 4000 UI/kg and lower with Pituitary extracts. However, no significant difference (P> 0.05) was observed regardless of the type and dose of hormones used.



Fa: absolute fecundity; Fr : relative fecundity

(a, b): vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

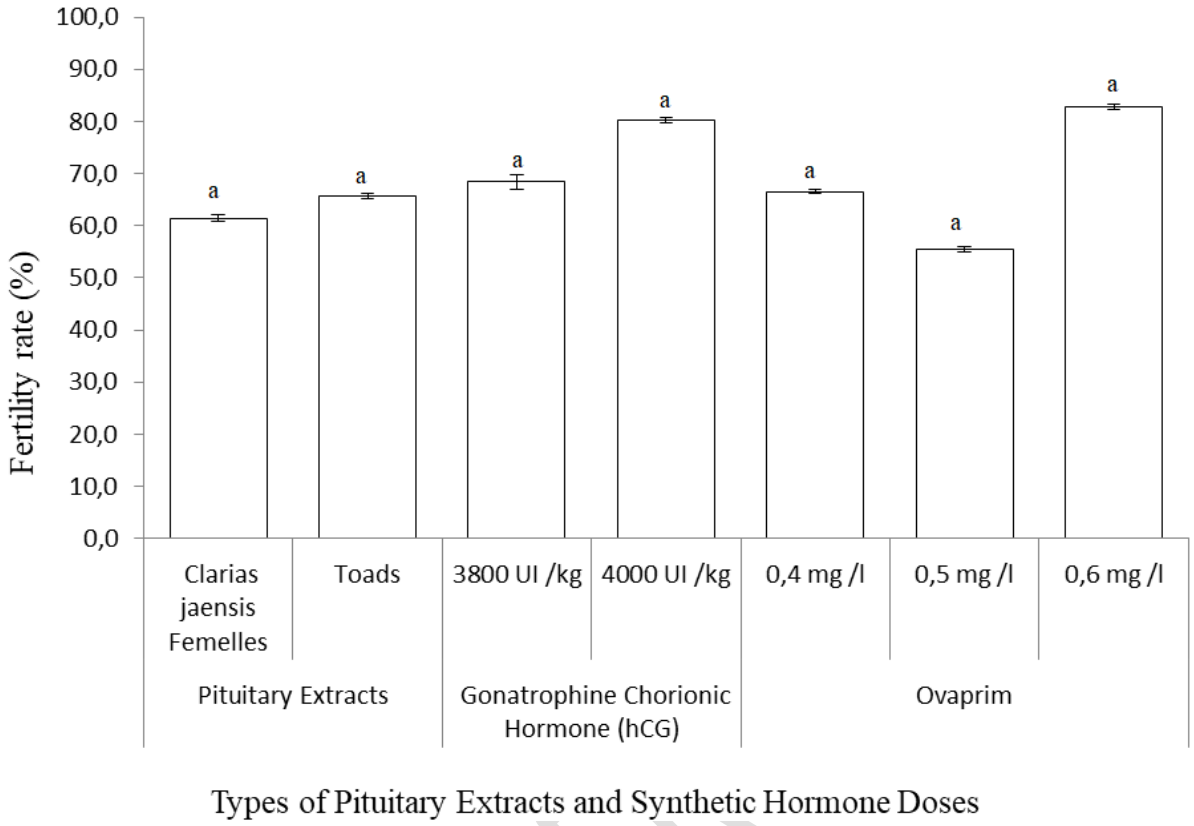
Figure 4: Absolute and relative fecundities in *Clarias jaensis* according to the type of extracts pituitary and synthetic hormones



Lt: latency time; Id: incubation time when the temperature is 22.16±0.65°C.

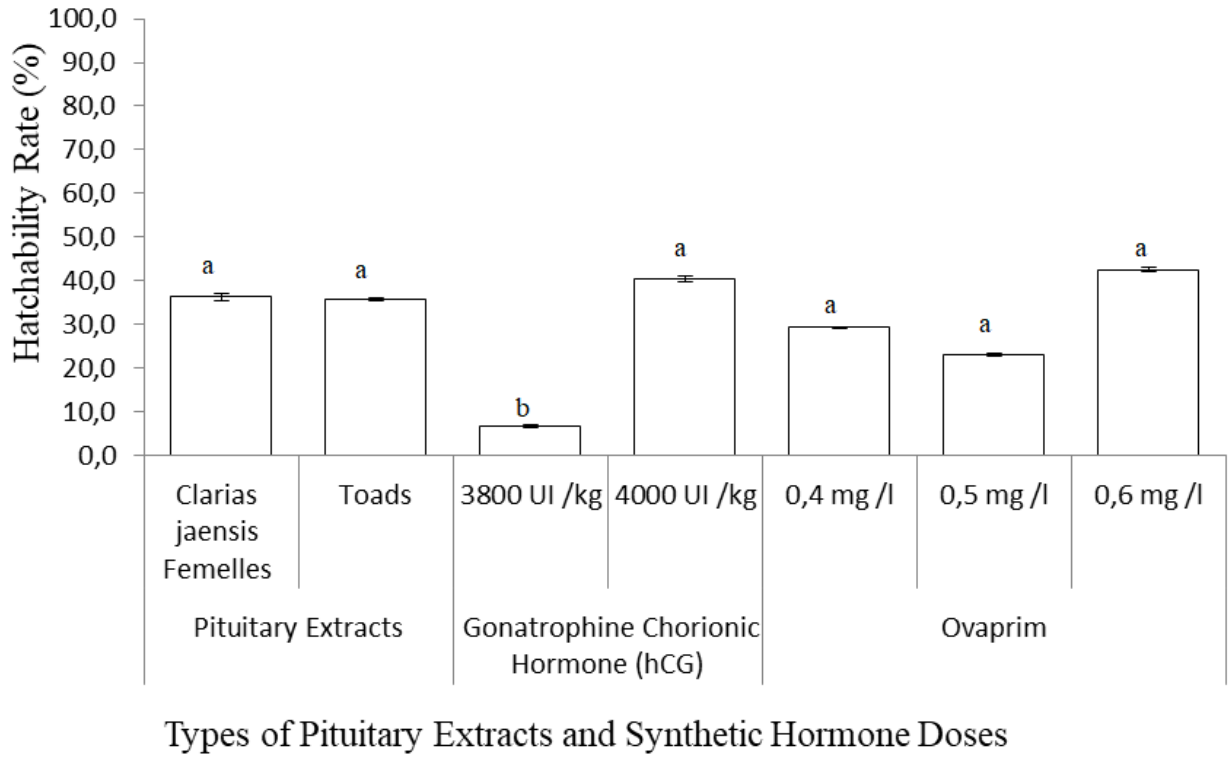
(a, b): vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

Figure 5: Time of latency and duration of incubation in *Clariasjaensis* with regard to the type pituitary extracts and synthetic hormones



a: vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

Figure 6: Fertility rate in *Clariasjaensis* with regard to the Types of Pituitary Extracts and Synthetic hormone Doses

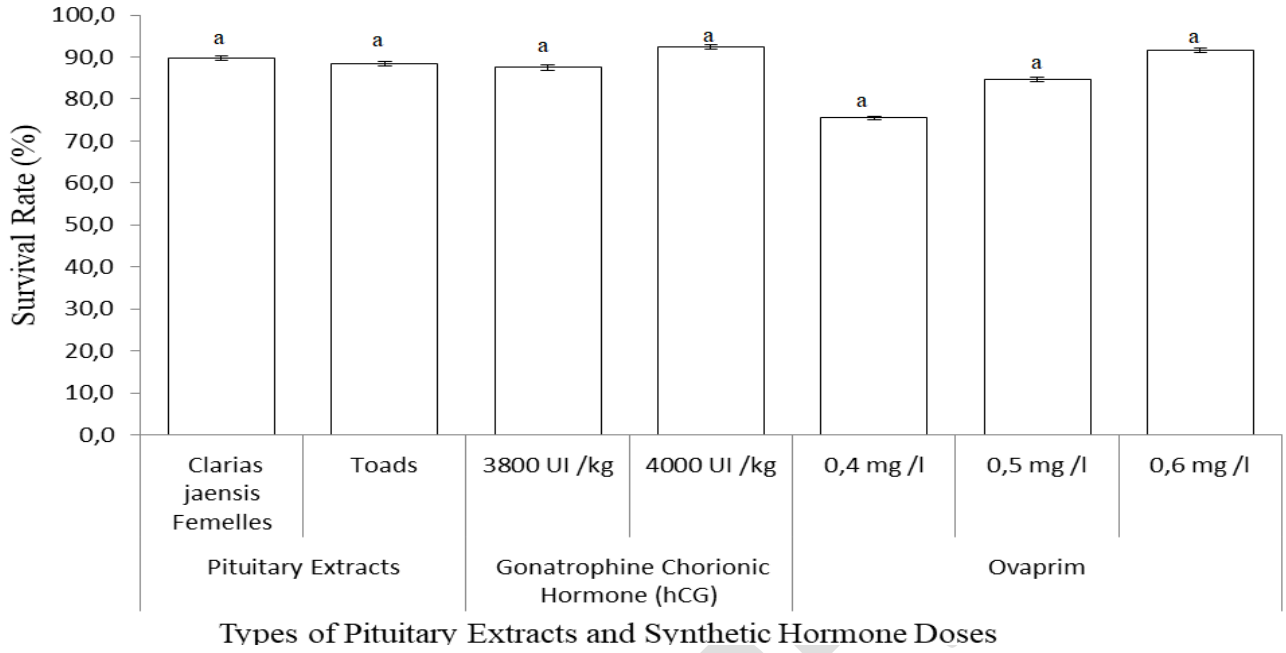


(a, b): vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

Figure 7: Hatchability Rate in *Clariasjaensis*with regard to the type of pituitary extract and synthetic hormones

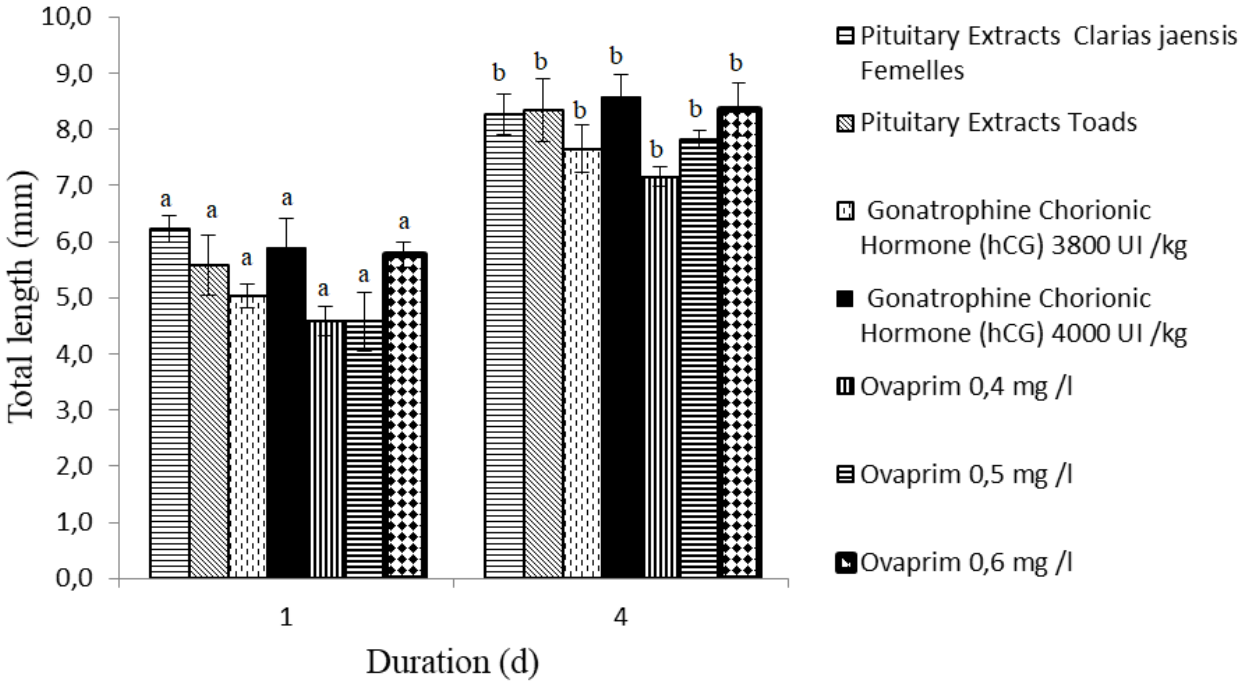
3.3 Effect of hormone types and dose on survival rate and larval size after yolk resorption

Except for higher hatching larval size of females induced with female pituitary extracts (Figures 8 and 9), the survival rate and length of larvae at yolk resorption were higher with hormone HCG at 4000IU/kg, followed by Ovaprim at a dose of 0.6 ml/kg. However, for both the type and dose of hormone. No significant difference (P > 0.05) was found.



a: vertical bars with the same alphabetical letter are not significantly different (P > 0.05)

Figure 8: Survival rate of *Clariasjaensis*larvae after yolk resorption with regard to the type of pituitary extracts and synthetic hormones.



(a; b): vertical bars with the same alphabetical letter in the same duration are not significantly different (P > 0.05)

Figure 9: Evolution of the total length of *Clariasjaensis*larvae with regard to the type of pituitary extracts and synthetic hormones.

4. DISCUSSION

After hormonal induction, the diameter obtained was comparable to 1.5-2.4 mm[4] in the same species and greater than 1.2-1.5 mm[11]at *Clariasgariepinus*, it was also greater than 1.21 – 1.45 mmat *Clariasgariepinus* induced with Ovaprim, hCG and GnRHhormons[12] and greater than 0.3 – 1.4 mm obtained from *Clarias gariepinus* in the natural milieu [13], similarly greater than 1.0 – 1.3 mm from *Clarias gariepinus* induced with pituitary extract [14]. These differencesmay be linked to the species and hormone types and doses, the results showed that *Clariasjaensis* has oocytes with great diameters. This result could be attributed to endogenous factors (size or age of females).In the other way, the oocytes diameter of *Clariasjaensis* was greater than 1.21 – 1.55 mm reported in *Clarias gariepinus* females using oocyte developer hormone and astaxanthin addition diet [15]. It was also greater than 1.0 – 1.3 mm[14] using Pituitaryextract and Ovaprim at 0.4 mg/kg of fish in *Clarias gariepinus*. It impliesthat theooocytes diameter varies among species. The relative fecundity of Ovaprim-induced females at 0.6 ml/kg and HCG 4000IU/kg (31.77 – 91.12 oocytes /g) was lower than 1055.4 ova/g[16] in *Clariasgariepinus* induced at the same dose, lower than 26.75 – 149.70 oocytes /g in *Clarias gariepinus* induced with pituitary extracts [17]. It was comparable with 28.18 – 98.05 oocytes /g obtained in *Heterobranchusbidorsalis*[18]. These results show that relative fecundity varied among species.

The absolute fecundityobtained varied with treatments (1516 – 15570 oocytes).This situation can be attributed to the nature of the hormone and the factors related to the female used. Indeed the fecundity of a female is most often with regard to her size [19]. The larger her size, the greater the number of eggs laid and the number of eggs produced by a female increases with age.Fecundity rates of batches treated with female pituitary gland extracts and HCG hormone (4000 IU/kg) were 27.84% and 26.57% lower, respectively;the absolute fecundityobtained is lower than 55355oocytes [20] from *Clariasgariepinus* in thenatural milieu.The incubation duration (51.70 –61.44 hours at 22,16±0,65°C)was different from 24 hours at 26°C -28°C[21] in *Clariasgariepinus* using different dosages of pituitary extracts.

The hatchability rate was significantly lower than 96 – 98 % (19) while using Ovaprime and homoplastic hormone, and also lower than 18.00 – 69.06% with the use of HCG [18and 22] in *Heterobranchusbidorsalis*. These differences could be attributed to the species and hormone types. The results are also 1.9% lower than with *Clariasgariepinus*[10and 23]. The intra-specific difference could be related to individual variations.

5. CONCLUSION

The reproduction of *Clariasjaensis* by using different types of hormones has shown that the diameter and mean weight of the oocyte, absolute and relative fecundity, fertilization and hatching rates were not significantly affected ($P > 0.05$) by the type and dose of hormones. Thus, the highest value was recorded in individuals treated with the hormone HCG (4000 IU/kg) and those whoreceived the dose of 0.6 ml/kg of Ovaprim. However, the highest hatching rate was observed with Ovaprim at a dose of 0.6 ml/kg followed by HCG (4000 IU/kg) and female pituitary extract. In a fish farm of intensive production of *Clariasjaensis*which is anendogenous fish in Cameroon and one of the new species for aquaculture, the use of the hormone Ovaprim dose 0.6 ml/kg or HCG at 4000IU/kg is more interesting.

Acknowledgement : The authors would like to thank **Mr. Michel Diogne**, Chief of the group farm Integrated Aquaculture Common Initiative Group of West Cameroon for allowing them to use all the necessary facilities to conduct their research work.

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APPENDIX

Table 1: some physicochemical characteristics of water in function of the hormones types and doses during the experiment

Hormones		Physicochemicalcharacteristics				
Types	**Doses**	**Temperature (°c)**	**pH (I.U)**	**NH_4^+ (mg/l)**	**NO_2^-(mg/l)**	**NO_3^{2-} (mg/l)**
PituitaryExtracts	♀*Clarias jaensis*	20,98 ± 0,39 [a]	6,81±0,37[a]	0.26 ± 0.031[a]	0.01 ± 0.009[a]	0.02 ± 0.008[a]
	Toads	22,47 ± 1,06 [a]	6,81±0,37[a]	0.26 ± 0.031[a]	0.01 ± 0.009[a]	0.02 ± 0.002[a]
hCG	3800 IU /kg	23,07 ± 1,96 [a]	6,91±0,03[a]	0.21 ± 0.027[a]	0.01 ± 0.006[a]	0.01 ± 0.003[a]
	4000 IU /kg	21,38 ± 1,09 [a]	6,11±0,76[a]	0.16 ± 0.030[a]	0.01 ± 0.005[a]	0.01 ± 0.004[a]
Ovaprim	0,4 mg /l	21,47 ± 0,96 [a]	6,01±0,97[a]	0.18 ± 0.022[a]	0.01 ± 0.004[a]	0.01 ± 0.001[a]
	0,5 mg /l	22,12 ± 0,43 [a]	6,21±0,11[a]	0.16 ± 0.011[a]	0.01 ± 0.007[a]	0.02 ± 0.001[a]
	0,6 mg /l	20,99 ± 0,34 [a]	6,35±0,83[a]	0.21 ± 0.010[a]	0.01 ± 0.008[a]	0.01 ± 0.005[a]

hCG: Gonatrophine Chorionic Hormone; I.U: International Unit;

a: values in the same column with the same alphabetical letter are not significantly different (P > 05)