COMPARATIVE STUDY OF SYNTHETIC HORMONE AND CARP PITUITARY GLAND IN INDUCED BREEDING OF AFRICAN CATFISH (*Clarias gariepinus*) IN HATCHERY

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ABSTRACT

The study was initiated to evaluate the effectiveness of carp pituitary gland (PG), and synthetic hormone ovaprim (OV) on spawning success of *Clarias gariepinus* in a hatchery. In total, 21 female and 9 male mature catfish were used for the study. The study examined the mean fecundity, fertility rate, egg hatching and survival of *C.gariepinus* using different hormones. The mean fecundity was calculated from the total weight of eggs released. The percentage of fertilization was determined based on the surviving embryos 45 second after fertilization. The hatching percentage was calculated from the total number of fertilized eggs, while the percentage of survival rate was determined by physical counting after 14 days feeding with *Artemia nauplii*. The mean number of eggs ovulated was higher in pituitary gland injected catfish than ovaprim treated catfish. However, in the control group, one brood fish was ovulated poor quality eggs out of three female fish but was not hatched at all. The highest rate of fertilization, 73.2 %, was observed in ovaprim treated catfish compared to 72 % in carp pituitary treated catfish. On the other hand, ovaprim treated fish showed much higher hatching rate (89%) than the pituitary treated catfish (69%). Mean survival rate of fry was higher in Ovaprim (85 %) than in carp pituitary gland treated fish (79 %). However, Ovaprim treated fish showed slightly higher hatching and survival success as compared to carp pituitary gland but no significant differences in stimulating effect ($P > 0.05$) was obsereved in all parameters investigated.

Key Words: Fecundity, fertilization, hatching, ovaprim, carp pituitary gland, *Clarias gariepinus*

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Introduction

Aquaculture in Africa is predominantly rural and orientated principally to the immediate needs of the farmers and their families. In many instances the fish are consumed directly by the farmers' family, as much as 50% of the harvest in Kenya, Ivory Coast and Rwanda (FAO, 1994). In Ethiopia fish farming is still at its infant stage despite favorable physical conditions that support its development. The present agricultural policy encourages aquaculture production and sustainable utilization of water resources in general (MoA, 2008). However, the country still depends mainly on capture fisheries from natural water bodies. The annual fish demand is increasing rapidly with population growth. The fish resources from the natural water bodies have failed to meet the growing demand which indicated that there is a strong need to develop aquaculture to close the gap between fish demand and supply (Moehl et al., 2005). Developing commercial aquaculture also demonstrates promising opportunities based on the current demand.

The African sharp tooth catfish *C. gariepinus* is considered as a suitable aquaculture species in accelerating Ethiopian aquaculture development. The biological, ecological attributes and life history of *C. gariepinus* are well documented scientifically. Since 1996, the *Clariids* and *B agrids* are attracting vast interest within African aquaculture sectors. *Clarias* species rearing has recently undergone major development throughout Africa. They respond efficiently to induce breeding and artificial propagation. The growth is directly related to temperature, as they can attain a total length of 22-32 cm after one year. The rearing is carried out typically in lakes and ponds; both mono-cultural and poly-cultural environments are considered suitable. In some cases the species also functions as predators in tilapia fingerling

control in poly culture (Babiker, 1984). The marketable size could be achieved within six months in small-scale rural fish farmers under poly cultural conditions (Graaf et al., 1996). In rural poly cultural ponds production varies from 0.2-0.35 kg /m^3/year. Both catfish and tilapia normally reach their marketable sizes of 120-150 g and 250-450 g, respectively, after 10-12 months (FAO, 1994).

However, several possible problems may occur during the production period. The cases of low reproduction success, aggressive behavior and cannibalism may cause injuries that lead to infection and create obstacles for transportation (Babike, 1984). The research and development efforts have not yet met the ever-rising demand of improved seeds. In natural habitat, *C. gariepinus* do not spawn all-year round and hence fry production is required for regular supply to the farmers. This study was, therefore, conducted to evaluate the effect of natural and synthetic hormones on African catfish (*C. gariepinus*) seed production performance. The study output would be significant in improving the rural area supply of *C. gariepinus* seed through integration with irrigation and livestock farming practices.

Materials and Methods

The study was conducted between October 2014 and January 2015 at Sebeta National Fisheries and other Aquatic Life Research Centre (NFALRC) which is located approximately 25 km southwest of the capital, Addis Ababa, Ethiopia. The center is situated at an altitude of 2200 m above sea level, 08 ^{o} 54' N latitude and 38^{o}38' E longitude. *C.gariepius* brood fish were collected from Koka hydroelectric dam. All collected brood fish were stocked and acclimatized in a fish pond for two weeks before the start of the experiment. The female fish were kept separated from the male fish in

concrete ponds ($5\times10\times1.5m^3$). They were fed with formulated feed at 5% of their total body weight twice a day with locally available feed composed of 29.3% crude protein. The brood stock were checked regularly based on external morphological features associated to ripeness, e.g. swollen reddish genital papilla, soft abdominal region, and comparatively larger in size.

The synthetic hormone, ovaprim (OV) is a commercial product that contains 10 mg/ml of a salmon gonadotrophin releasing hormone (GnRHa). It was purchased from the Aqua life syndial laboratories LTD. Nanaimo, B.C. Canada.

The carp pituitary gland (PG) was collected from the fishermen catch in Lake Koka reservoirs. All pituitary glands were preserved in alcohol or with acetone immediately after collection then transferred to vials. The acetone was decanted after 8 hours and then refilled with fresh acetone. This was kept in a cool place for 24 hours after which the acetone was completely emptied, dried by evaporation and stored in a sealed vial for use (Woynarovich and Horvath, 1980).

Experimental Design

The experiment has three treatments: ovaprim (OV), pituitary gland (PG) and control which was kept without treatment. Each treatment has three replicates where ovaprim and carp pituitary gland each has three levels of injection. The injection levels were one time total dose at once, two times (10% and 90% at 6 hours time interval between the first and second injection), and three times injection (10%; for the first injection, 10% 4 hours later and 80% two hours after the second injection) and the control has no injection.

Twenty one females and nine male fish were used for this study. The first nine gravid females were each weighed and injected with ovaprim at a dose rate of 0.5 ml kg^{-1} of their total weight. Three levels of injection were once, twice and three times at different time intervals. In the second group another nine gravid females were weighed individually and injected with acetone dried carp pituitary gland (PG) at a dosage of 3 mg kg^{-1} of their total weight. After injection each fish was finger rubbed to avoid the back flow of fluid. The injected fish were then kept in water tanks until they were stripped. The control group, which contains three similar size female catfish were weighed and kept in 25 to 28 ºc water temperature without hormone injection.

The injected fish were regularly checked for ovulation every time by pressing their abdomen. Striping took place 14 to16 hours after injection in a water temperature between 25 to 28^{o}C. The ovulated eggs were squeezed into a plastic bowl frequently and stopped with the appearance of blood. The male was sacrificed to obtain their sperm and dissected; the milt sac was removed before artificial spawning. The stripped eggs were weighed, and gently mixed with the creamy milt by hands using a feather. The bowl was gently swirled to mix the eggs and milt. In order to activate fertilization of the egg 0.9% (NaCl) saline solution was added to enhance movement of sperms. The fertilized eggs were then spread over the nylon net in each incubation plastic basin. Number of eggs released in each tray was estimated using gravimetric methods (Bagenal and Braum, 1978). Total weight of the oocyte was measured using electronic balance and small subsample of ovary was taken from the total and weighed. The eggs were preserved with Gilson's fluid and then number of eggs was counted. Thus fecundity was calculated using the following formula.

$$F = \frac{\text{Total Oocytes Weight} \times N}{\text{Weight of subsample Oocytes}}$$

Where F represent fish fecundity and N represent number of eggs counted in the subsample.

The fertilization of eggs was calculated through random sampling and examined under light microscope from each breeding trails. The eggs which were translucent were considered as fertilized while opaque eggs as unfertilized. About two hundred to five hundred eggs were microscopically examined for each trial. The fertilized eggs were then counted in order to calculate the percentage of fertilization following formula below (Alam et al., 2006)

$$\text{Fertilization rate } (\%) = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs sampled}} \times 100$$

(Alam et.al)

Immediately after the fertilization, the fertilized eggs were spread over in a single layer of nylon mosquito net (mesh size 0.5mm) that was submerged in water, but suspended a few centimeters above the base of the tray. Incubation followed in well aerated flow water in plastic basin filled with 35 L of water. Water flows gently in and out of the basin to obtain well oxygenated medium. The outer flow of the basin was covered with meshed net to prevent escape of young larvae. Incubated eggs were kept in separate plastic basin with water temperature of 25 to 28^{o}c. After 24 hours of incubation, the nylon mesh was removed. Spoiled or dead eggs attached to the net, were discarded. The hatched larvae were clustered at the dark corners of the incubation basin. After the larvae hatched, the percentage of hatched larvae of each basin was calculated. The hatching rate was calculated using the following formula.

$$\text{Hatching rate } (\%) = \frac{\text{No. of hatchlings}}{\text{Total no. of fertilized eggs}} \times 100$$

After 14 days of rearing, the percentage of survived embryo was obtained through visual counting at the end of experimental period. Siphoning of uneaten food and dead fry was removed daily to determine the mortality rate.

$$\text{Survival } (\%) = \frac{\text{No. of larvae at the end of study}}{\text{No. of larvae at the beginning of study}} \times 100$$

Water is important parameters which regulates fish body temperature and growth. The physico-chemical parameters like pH, dissolved oxygen (DO), conductivity and temperature were monitored and recorded daily during the trials using a multi-meter (HQ) electronic probe.

Results

In the present study the highest mean ovulated (36,829) eggs was found in pituitary gland twice injected brood fish and lowest number of (22,353) eggs was recorded in brood fish injected once with ovaprim (Fig. 1). There was no significant difference ($p>0.05$) in the mean number of eggs released with different hormone administration rate.

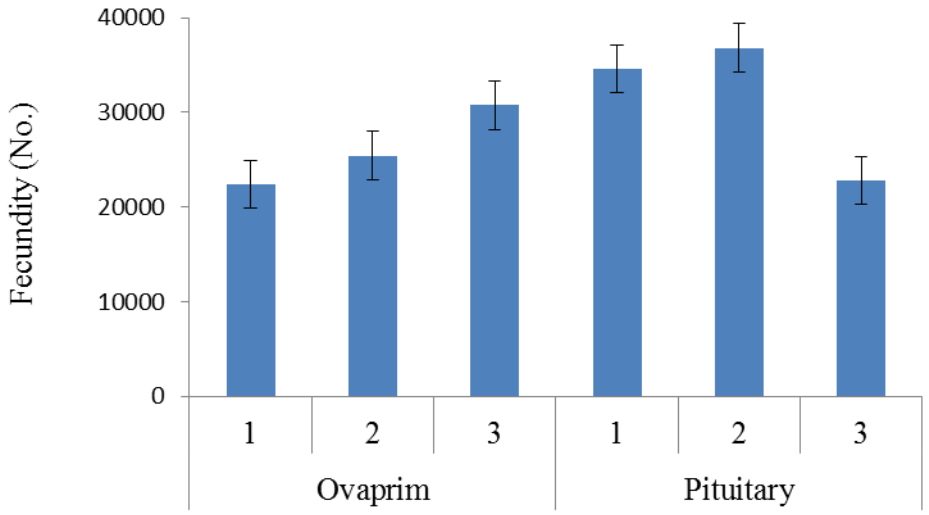

Figure 1: Mean number of ovulated eggs of *C. gariepinus* injected with Ovaprim and carp pituitary glands with different administration rate.

Fertilization and hatching rate

The fertilization rate of brood fish (*C.gariepinus)* injected with carp pituitary gland ranged between 61.3- 72.2 % while catfish treated with synthetic hormone (Ovaprim) had mean fertilization rate ranging from 63.6 to 72.6%. Both Ovaprim and pituitary gland hormones were found to be of equal effect on fertility successes of catfish induced breeding (Fig. 2). However, fertilization rate was slightly higher in (72.6%) fish which were injected two times with ovaprim in different doses with 6 hours time intervals between the first and second injection (fig. 2). Significant hatching rates (89%) was recorded in OV2 (ovaprim two times injected) fish as compared to all other treatments except pituitary gland 1 (one time injected) fish. Similarly, pituitary gland 1 has significantly higher hatching rates than Ovaprim 1, Ovaprim 3 and Pituitary gland 2 treated catfish. (Fig. 2).

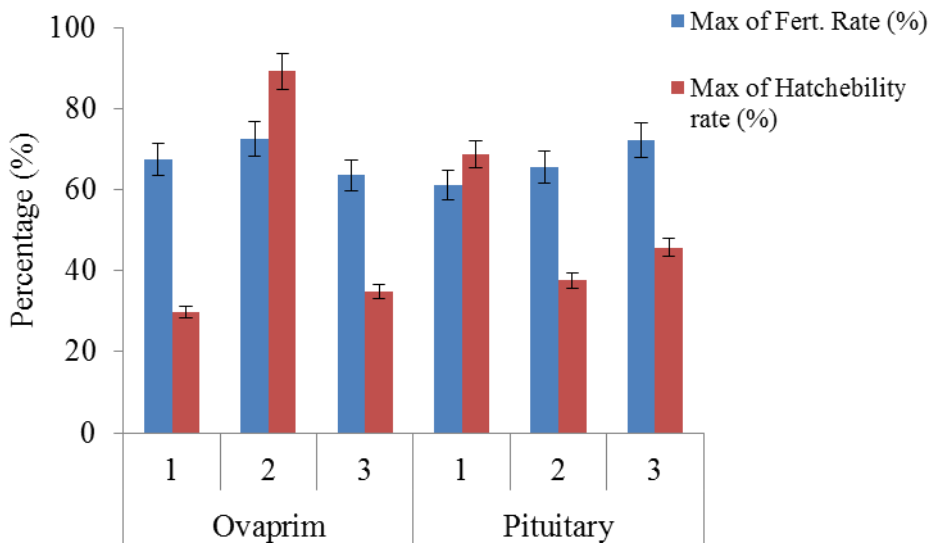

Figure 2: Percentage of fertilization and hatchability of *C.gariepinus* eggs in different levels of injections using ovaprim and carp pituitary glands.

Survival

Dead fry were counted every day and the survival rate was calculated for each treatment at the end of the 14 days. The lowest survival of fry (67.8 %) in Ovaprim 2 (twice) injected catfish and the higher survival (85.5%) of fry was found in the Ovaprim 1 (one time) injected (Fig. 3). However, there was no significant variation on survival rate of fry among treatments ($P>0.05$).

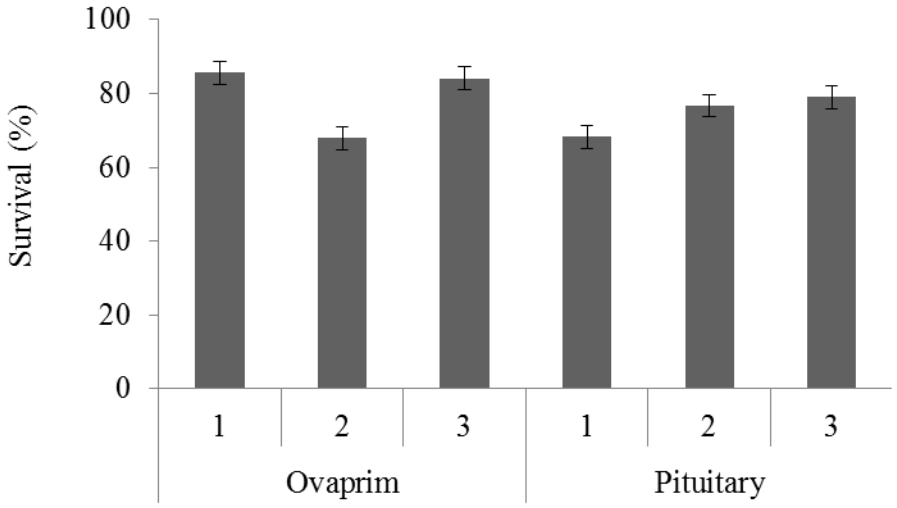

Figure 3: Percentage survival rate of artificially reared fry of *C. gariepinus* treated with ovaprim and carp pituitary glands.

Water quality

The water quality parameters of experimental basins are given in table 1.

The mean water temperature were (25.12±1.54) and (25.1 ± 1.37) for carp pituitary treated basin and ovaprim hormone treated basin, respectively.

Water pH ranged between 7.77 and 7.60 while conductivity ranged from 287.9 µs cm^{-1} to 285.4 µs cm^{-1}.

Table 1: Physico-chemical parameter and ammonia concentration of water measured at different treatments over 14 days. Values are mean ± SE of three replicates

Water quality parameters	carp pituitary gland treated basins	ovaprim hormone treated basins
Temperature (ºC)	25.12±1.54	25.1 ± 1.37
pH	7.77 ± 0.4	7.6 ± 0.19
Oxygen (mg L^{-1})	4.95 ± 0.59	5.43 ± 0.57
Oxygen Saturation (%)	78.38 ±0.19	85.97 ± 0.52
Conductivity (µs cm^{-1})	287.9 ±27.33	285.4 ± 17.08
Ammonia-Nitrogen (NH_4-N) (mg L^{-1})	0.104 ± 0.026	0.12 ± 0.025

Discussion

The mean fecundity of PG (pituitary gland) injected *C.gariepinus* ranged between 22838 and 33799 eggs. The overall Ovaprim injected *C. gariepinus* mean fecundity was in the range of 22352 - 29440 eggs.

The highest and least mean number fecundity was recorded in fish injected with pituitary 2 (two times injection) and Ovaprim 1 (One time injection), respectively. The maximum number of spawned eggs (75,220) was found in fish that were injected twice with PG and minimum amount of eggs (4899) was recorded in fish that were injected twice with OV. In this study, the brood fish injected with PG solution gives the highest mean number of eggs (36,829) followed by fish injected with OV hormone (30,730). In both PG and OV injected catfish, all ovulated with varied number of eggs. In the control group, among three treated brood catfish, two of them did not

demonstrate ovary development, the one left ovulated eggs that were poor in quality and the eggs were attached together by muscle tissue, which results in no hatching during incubation period. From this study we conclude that catfish (*C.gariepinus)* would not spawn in captivity unless it is induced by different hormones in the hatchery. The overall fecundity pattern has shown that there is wide variation in the number of eggs released. The higher mean number of ovulated egg was recorded in PG treated fish than those injected with OV. There is no significant differences observed on the ovulation stimulator ($p>0.05$) in the number of ovulated eggs from all treatments.

In the study conducted by Britz and Hecht (1988) using PG extract, catfish spawned 76,500 eggs, which is comparable to the finding of this study of 75,220 eggs. The recent studies conducted by Khan et al. (2006) and Shano et al. (2005) reported fecundity on *C.gariepinus, C.batrachus*, and *Labeo rohita* when OV was induced at the rate of 0.2 mg kg^{-1}. This result indicates that the dose and administration rate of synthetic hormone and pituitary gland do not influence in mean number of eggs released. This result however disagrees with the report of Hill, et al. (2009) that increasing in dose and administration rate of breeding hormones results in more eggs being produced. The variation in fecundity within a common trial of similar-sized fish species could be attributed to hormone administration rate, breeding history, maturity stage, and other external environmental factors (Lager, 1986; Schulz et.al., 2007; Ataguba et .al., 2009). Use of hormones may produce poor results if the brood fish are not well conditioned. Under such conditions a partial spawn or no spawning at all may occur, and others may not respond to hormone treatment even if they are in relatively good condition (Piper et.al., 1982).

The results of this study showed that the higher percentage of fertilization rate were in OV 2 (fish injected twice) with a value of 72.6 %, and the lowest rate 61.3 % was recorded in PG 1(fish injected once). There was still no significant difference ($p>0.05$) in fertilization rate in all treatments. This study agreed with the finding of other studies for instance, Nandeesha et.al. (1990); More et.al. (2010); Adebayo and Afagbenro (2004), which reported that OV injected *C. gariepinus* had higher fertilization rate followed by the PG injected group. Haniffa and Sridhar (2002) reported of 70% fertilization rate of *Heteropneustes fossilis* (Stinging Catfish, *Clariidae*) treated also with synthetic hormone Ovaprim at dose rate of 0.3 ml/kg^{-1} body weight, the same size of our study targets. The highest fertilization rate (98%) was recorded in *H.fossilis* with a dose of 75 mg/kg^{-1} PG injection. This number is much higher than the study result of Begun et.al. (2001). The variation in fertilization rate might be attributed to varied egg and sperm quality, physiological difference of brood stocks, seasonal variation, as well as difference in hormone dosage (Gheyas et.al., 2002; Haniffa and sridhar, 2002; Nwokoye et.al., 2007). Environmental factors, water quality parameters (pH, oxygen concentration, hardness and temperature of water) and handling procedure of the brood fish also are determining factors (Khan et.al., 2006). Artificial breeding of African catfish or Salmon fish prolonged exposure of both sperm and eggs to the water reduces the fertility and hatching potential (Piper et.al., 1982). The present study shows that, the mean hatching rate has generally lowered in all treatments. The maximum hatching rate was (89%) and the minimum hatching rate was (30%) recorded in *C.gariepinus* OV 2 (injected twice) and OV 1 (one time injection) study groups, respectively. The OV 2 group had shown a significant lead in maximum ($p<0.05$) hatching rate not only than both OV 1 and OV 3 (one time and three time injection), but also ahead of group PG 1

and PG 3 (one time and three times injection). The mean hatching rate of OV 1 demonstrated significantly lower ($p<0.05$) value than OV 2 and OV 3. The poor hatching rate of spawned eggs could be linked to the exposure of fungus infection, where amount of dead eggs risked at being nutritional bases for fungal growth (Tucker and Robinson, 1990). Additional interferences that would affect the hatching rate are breeding history, age of fish and hatchery water quality according to Ataguba et al. (2009). Other disturbances to hatching rate would be water temperature fluctuation and egg hatching lengths (Piper et al., 1982). The higher fertilization rate and the consequent lower hatching rate could be an outcome of the over-ripped eggs, which might have result in egg mortality post fertilization. This was similar to the finding of Sahoo et al. (2005).

The percentage of survival rate was higher (85%) in group OV 1. In a similar study, where Ovaprim was also used for breeding the stinging catfish *H. fossilis,* Haniffa and Sridhar (2002) stated that Ovaprim treated fish had better survival than other hormonal materials used. In this study group, PG 1 had a lower fry survival of 68.2 % recorded at the end of 14 days indoor experimental period. In a similar *Heteropneustes longifilis* induced breeding study by Nwadukwe (1993), the use of frog pituitary extract had a lower fry survival rate. The survival rate ranging between 84- 85 % was recorded in Ovaprim treated catfish (*C.gariepinus*) after the 14-day experimental period. The higher survival rate of *C.gariepinus* fry might occur due to the appropriate water management during the experimental period. The overall results showed comparatively higher (68.2-85%) survival rates in all treatments after 14 days. However, there were no statistically significant variations ($p> 0.05$) among the treatments. Physical observation during the study had indicated that yolk sacs disappear within the first three to four

days after hatching. During this period no mortality occurred, the reason might be limited food seeking activities and social competitions within groups. However, mortality begun on the 5th day of the experiment, where live feed (*Artemia nauplii*) was given. The reason of mortality could be physical injury, over feeding, competition for food and high movement in confined spaces. From 10 to 14 days cannibalism surfaced and attacks among the same size begun. The mostly attacked parts were tails, broken with body wounds, eventually death. Baras and Almeida (2001) stated that by grading catfish fry every 3 days it resulted in better survival rate. During the study, separating and providing pure live feed (*Artemia nauplii)* was difficult, due to the amount of cysts and shell mixed in feed which requires a lot of time and effort to remove. Microscope examination from the laboratory revealed that some *C.gariepinus* fry had died of eventual digestion failure from accidental consumption of cysts.

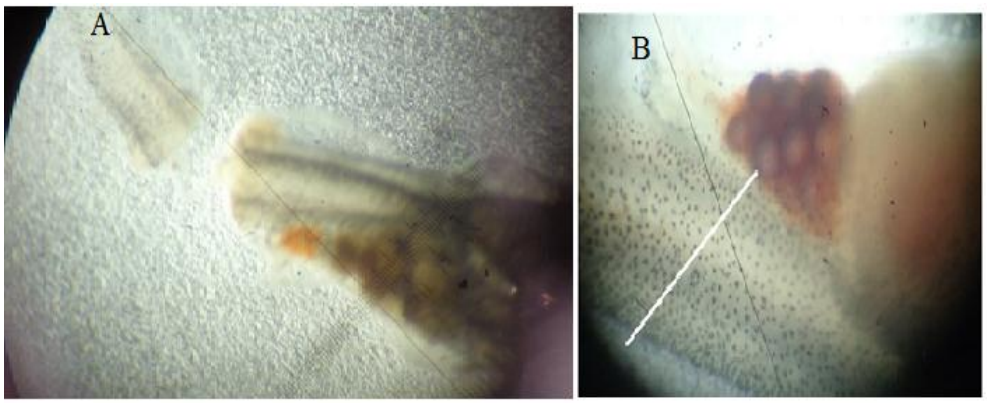

Figure 4: Picture showing (A) cannibalism and (B) cysts blocking fry stomach observed under a microscope in the laboratory.

The physico-chemical parameters (pH, dissolved oxygen and ammonia were maintained at desired range while temperature was slightly below the

acceptable range for fresh water fish culture (Auburn, 1979). According to Akinyemi (1988), water temperature between 27-30ºC is suitable for *Clarias garipinus* larvae rearing.

Conclusion

Even though the experiment was carried out with limited facilities, the result obtained was promising. The study result has the potential to be a guide for further researches to improve artificial breeding of African catfish in Ethiopia. It is concluded that, the fertilization and hatchability rate of catfish using carp pituitary gralnd and ovaprim hormone are similar. In terms of accessibility, ovaprim being imported could be expensive, while pituitary extract is relatively affordable and available locally. Hence, carp pituitary gland (PG) is highly recommended for the benefit of small scale aquaculture farming and commercial hatchery uses.

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