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Recent Comparative Studies on the Performance and Survival Rate of African Catfish (*Clarias gariepinus*) Larval Produced under Natural and Synthetics Hormones: A Review

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Abstract

Induced breeding in African catfish (*C. gariepinus*) became germane in addressing unreliable seed supply due to low fertilization and egg hatchability, improper selection and use of natural or synthetic hormones to induce ovulation and spawning. The proper use of hormones promote reproduction in fish which is controlled by several factors such as sex steroids in the regulation of reproductive processes which are controlled through the brain-pituitary gonadal axis. A review was conducted on recent comparative studies on the performance and survival rate of African catfish (*C. gariepinus*) larval produced under natural and synthetics hormones. More than 20 journal published papers on inducing ovulation and spawning in African catfish (*C. gariepinus*) either by using natural or synthetic hormones, mostly from West Africa were reviewed. Literatures suggested that, using synthetic or non- synthetic hormones ensure availability of matured quality eggs, and ensuring good and viable milt production for commercial fish farming. Among reviewed papers revealed that, higher fertilization, hatching and survival rate of African catfish larval were achieved at 0.4-0.5 ml/kg of Ovaprim compared to Ovatide, Ovulin and Pituitary Gland Extract. In ovarian lavage with combination of sperm and hormone method, the simplicity of induced spawning can be combined, with a less time dependent delivery of sperm than with conventio na l, *in-vitro* fertilization. Therefore, this review suggests that African catfish seeds production can be encouraged through the use of Ovaprim. However, Catfish Pituitary Gland Extract (CPE) which is more readily available at farm level can also be used *in-vitro* fertilization in case there will be any shortage of Ovaprim whose supply varies with changes in import duties. In addition, to optimize fertilization and larval rearing pituitary gland extract in combination with disinfectants such as FA and MG can be used.

Keywords: Synthetic hormones; Pituitary gland extract; Induced breeding; African catfish; Larval performance and survival rate

Introduction

The African catfish, *Clarias gariepinus* Burchell 1822, is the favorite fish for aquaculture in West Africa [1] and other parties of the African continent. The species is dominant in freshwater environments including lakes, rivers and dams. *C. gariepinus* has been preferably in aquaculture due to its ease of culturing, fast growth rate, high resistance to disease, tolerance of a wide range of temperature, low dissolved oxygen as well as high salinity levels and most importantly high commercial value [2,3]. In East Africa, its demand has increased as food, control of over-population in tilapia ponds and as a bait for the Nile perch fishery [4]. In Tanzania, it has gained popularity in the aquaculture sector in the recent past times [5].

This species is not readily breed in captivity [6] all year round, therefore most farmers depend on fingerlings collected from the wild.

However, due to problems associated with wild fish seed such as seasonality in availability, uncertainty of species of fish seed collected, disease infestation and limited quality of harvestable fish seed [7,8], wild sources are unreliable and hence the need for seeds production using hormones. The hormones promote reproduction in fish which is controlled by several factors such as sex steroids in the regulation of reproductive processes [9]. These reproductive processes are controlled through the brain-pituitary gonadal axis. The brain is stimulated by environmental cues (water rise, temperature, feeding, rainfall, and photoperiod) to release gonadotropin releasing hormones [10]. Then, ovulation and spermiation are affected as a result of the sex steroids that have been produced [11]. Administration of these hormones to induce ovulation and spawning in fish is achieved through artificial propagation with either natural or synthetic hormones [12,13].

Artificial propagation of African catfish *(C. gariepinus*) is the most promising and reliable way of ensuring large scale production of quality fish seeds throughout the year and sustainability of the aquaculture sector [14]. Induced breeding in African catfish became germane in addressing unreliable seed supply due to low fertilization and egg hatchability, and improper selection and use of hormones to induce ovulation and spawning. Therefore, this study reviewed on recent comparative studies on the performance and survival rate of African catfish (*C. gariepinus*) larval produced under natural and synthetics hormones.

Literature Review

Natural hormones

A large number of natural spawning agents for induced breeding of the African catfish *C. gariepinus* are available and these include Deoxycorticosterone Acetate (DOCA), Human Chorionic Gonadotropin (HCG) and Pituitary Gland Extract (PGE) [15]. These hormones are known to be used successfully, however they are deficient in various ways, such as Deoxycorticosteroid Acetate (DOCA) causes severe ulcer on the

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injected female, Human Chronic Gonadotropin (HCG) is expensive [16], and Pituitary Gland Extract (PGE) is difficult to quantify [17,18].

Pituitary Gland Extract (PGE)

Pituitary gland is the main source of the major hormones responsible for reproduction in catfish and other farmed species. The hormonal induction involves the injection of pituitary gland extract from the donor fish of equivalent weight [19,20] or from other species to the female spawned. Pituitary gland secretes the gonadotropins such as Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH). FSH causes growth and maturation of ovarian follicles in females and spermatogenesis in the testes of males. LH cause Luteinisation in females and promote the production of testosterone in males [21]. The pituitary cells have been reported to undergo continuous mitotic process [22]. It can be cultured and proliferated *in-vitro* in order to use their secretions for induction of spawning in catfish. The extracted gland which contain hormones are grinded in a motor and pestle and mixed with normal saline solution (0.9% NaCl). 1 ml is administered to the female brood stocks in between the dorsal fin and the lateral line towards the abdominal region using syringe and needle [23].

The pituitary gland extract has advantages of better rate of fertilization and hatching, better conditions for growth and survival of larvae [24]. In addition, it enhances free release of eggs from the genital papilla of female broodstock, and stimulation the release of hormones into the blood circulatory system which induced ovulation and production of mature eggs [3]. Also, African catfish pituitary hormone is said to be readily available and cheaper than any other hormone [25] and can be prepared in a suspension [19]. However, the extraction of the pituitary gland from other donor species is a challenge. For instance, Commoncarp (Cyprinus carpio) pituitary gland materials are not easily accessible [16].

Synthetics hormones

The use of synthetic hormones in African catfish particularly a female fish is now popular as a means of artificially inducing the female fish in order to ovulate. These includes synthetic gonadotrophin releasing hormone analogues (GNRH-a) which are administered to the female brood stocks [23]. Gonadotropin Releasing Hormone analogue (GnRHa) is now the best available biotechnological tool for the induced breeding of fish. These includes the following which have been used to induce breeding successfully, Ovaprim, Ovatide, Ovaryprim, Ovopel, Ovupin-L, Ovulin, Dagin and Aquaspawn [12,13,26-28]. Among Gonadotropin Releasing Hormone analogue (GnRHa), Ovaprim and Ovatide containing salmon gonadotropin releasing hormone analogue and domperidone (SGnRHa + Domperidone) which are usually used for spawning induction in catfishes to get quality seed [29]. In addition, for large scale production in hatcheries, the use of hormones may be the solution for massive production. However, some of these hormones particularly Ovaprim is usually expensive [17,18,30].

Ovaprim

Ovaprim consists of GnRH-a and dopamine receptor antagonist, domperidone. It is developed by Dr. Lin of China and Dr. Peter of Canada [31]. It is marketed in liquid form and does not require any special preparation [32]. Ovaprim reported to significa ntly increase ovulation in African catfish [33] and aid spawning in the matured female Catfish. According to Watson et al. [32], the average success rates of 50% ovulation, 54% spermiation and 1.3% mortality were recorded after injection of different species with Ovaprim. Also, it has been used successfully for hypophysation in different families of fish like cyprinidae [34], Characidae and Cobitiidae [22].

Ovatide

Synthetic compound contains GnRH analogue with dopamine antagonist, pimozide and marketed in a pelleted form Indian major carp [31]. During administering, pellet should be powdered and mixed with saline solution (0.65% Nacl) 12 hour before injecting the female fish to stimulate ovulation and spawning.

Other substances used for induced breeding

Other substances like LH-RH analogues, steroids, HCG and clomiphene can used for inducing breeding [31]. During breeding induction, environmental factors such as temperature, DO and light are very important factors for the success since they control the reproduction of fish.

Comparative Studies

Among research papers reviewed, Ovaprim, Pituitary Gland Extract (PGE), Ovatide and Ovulin are the hormones which were mostly used.

Ovaprim (0.4 ml/kg) and Pituitary Gland Extract (1 ml/kg)

Induced breeding of African catfish (*C. gariepinus*) by using Ovaprim or Pituitary Gland Extract (PGE) ensures availability of fish seeds in hatcheries for commercial fish farming. These two hormones can be used separately or in a combination. Krol et al. [35] reported that, using a combination of these two hormones bring about quick ovulation and higher percentage of hatched fish, though Ovaprim hormone gives higher yield than the natural hormones (PGE). In addition, Oyeleye et al. [3], reported higher fertilization rates and eggs spawned when Ovaprim (0.4 ml/kg) used compared to Fresh Pituitary Gland (1 ml) administered to matured catfish female after 10 hours of inducement at 27°C. While, there was no significant difference in hatchability rates among eggs ovulated using Ovaprim (Table 1). The similar results reported by Fagbenro, Nagahama and Yamashita [19,36,37].

Ovaprim (0.5 ml/kg) and pituitary gland extract (1 ml/kg)

High concentration of synthetic hormones reported to increase the egg hatchability and survival rate of larval over natural hormones. Olaniyi and Akinbola [16], reported that, the values obtained for the spawning response when female (average weight of 1160 g) induced with 0.5 ml/kg of ovaprim and 1 ml/kg of African Catfish Pituitary Gland (ACPG) hormone treatments and kept for 9.05 hours after injection at a constant temperature of 27°C were significantly ($p<0.05$) higher in ovaprim than in ACPE (Table 2). The similar results reported by Saidin [38].

However, Shourbela et al. [2] reported the lower hatching rates ranged between 51.1% to 73% in the different hormone treatments. Also, Nagahama and Yamashita [36] observed that, yolk absorption was faster in fries produced from ACPE and was completed after 3days while fries produced from ovaprim took about 5 days to be completed. In addition, faster growth rate and survival rate of fries after 4 weeks were also observed in ACPE compared to ovaprim. Similarly, Adebayo and Popoola [25] observed survival rate of greater than 60% after 30 days of rearing.

Ovaprim (0.5 ml/kg) and Ovatide (0.3 ml/kg)

Most of the literatures suggest that, Ovaprim produces best results in inducing ovulation and spawning in African catfish than Ovatide. This might be due to a higher level of the hypothala mus pituitary gonad axis than gonadotropins resulted from GnRHa. Consequently, GnRH can provide a more balanced stimulation of reproductive events and presumably a better integration of these events with other physiological

Inducer	Weight of egg spawned (g/kg)	Fertilization (%)	Hatchability (%)
N	4	4	4
FPG	127.5 ± 11.54[b]	81.54 ± 2.42[b]	75.43 ± 3.34[a]
Ovaprim	157 ± 13.66[a]	87.34 ± 1.32[a]	76.54 ± 1.21[a]

Means in the same column with the same letter are not significantly different p>0.05.
FPG: Fresh Pituitary Gland; Ovaprim: GnRH analogue. N: Sample Size [3].

Table 1: Induced spawning with fresh and cultured cell solutions.

Parameters	Hormones		
	Ovaprim	ACPE	SEM
ABW(g)	ABW (g)	**1160.00**	0.01
MEW(g)	116.75[b]	177.98[a]	0.11
TNE (g)	35901.50[b]	90861.00[a]	32.46
Relative fecundity	30.95[b]	78.33[a]	0.19
Hatchability (%)	46.30[a]	25.99[b]	1.01
Survival (%)	50.14[b]	82.89[a]	0.04
Hatching periods (hrs)	48.00	48.00	0.00
Latency (hrs)	9.05	9.05	0.03

Means in the same row with different superscripts (a,b) are significantly different (p<0.05)
ABW: Average Body Weight, MEW: Mean Egg Weight, SEM: Standard Error of Mean TNE; Total Number of Eggs, ACPE: African Catfish Pituitary Extract. Source: [16].

Table 2: Induced ovulation and spawning of *Clarias gariepinus* using synthetic (ovaprim) and non-synthetic (ACPE) hormones.

functions, by directly or indirectly affecting the release of other hormones necessary for successful spermiation [39]. In case of males Gbemisola and Adebayo [37] reported that, milt from males administered with ovaprim (0.5 ml/kg) had higher percentage fertilization, hatchability, and survival than ovatide (0.3 ml/kg) induced hormone (Table 3). The similar results have been reported by other researches such as Mylonas et al., Clearwater, Crim, Ndimele, Owodeinde and Adebiyi et al. [40-43].

Combined hormones (Ovaprim +Ovulin)

Among papers reviewed on a synthetic hormones combination, Ovaprim and Ovulin seemed to be featured as the best when treated with others. According to Kutwal et al. [15], the effect of hormone manipulation on fecundity indicated that combined hormonal treatment 3 (75% Ovaprim + 25% Ovulin) had significantly ($p<0.05$) higher weight of eggs produced, fertilization, hatchability, and survival rate than other treatments (Table 4). The similar results are reported by Audu and Yisa et al. [44,45].

Ovarian lavage with combination of sperm and hormone (Sperm + Pituitary gland extract)

Ovarian lavage is a technology in breeding induction for finfish where African catfish (*Clarias gariepinus*) are used as a model fish [14] in most of experiments. It involves the injection of a sperm and pituitary gland extract mixture into the ovary through the oviduct. Both catfish and carp pituitary extract glands with a specific fish's sperm have been reported to be used successfully. Müller et al. [46] reported that, sperm injection into the ovary through the oviduct leads to successful recovery of developing embryos after delivering sperm to eggs in carp. This result indicates that spermatozoa are stored in the ovary and oviduct for up to 12 hours without loss of biological activity and that ovulated eggs could be fertilized after being released from the body cavity. In recent study, Müller et al. [46] reported significantly ($p<0.05$) higher fertilization percentages in both G1 and G2 groups of African catfish (1366 ± 164.1 g bodyweight) when sperm samples were treated with 3 mg CPE/ 1 ml and 0.9%.

NaCl solution/kg bodyweight injected intraperitoneally, 20 hours prior to the egg stripping of females (Table 5). Similarly, Watson et al. [32] published that *Tetraodon nigroviridis* was used to develop an alternative method for application of hCG via direct lavage into the ovaries. In addition, Németh et al. [47] presented that a non-invasive method of ovarian lavage is, as equally suitable method for the induction of ovulation in pikeperch (*Sander lucioperca*), as the traditionally used intramuscular injection of hormonal products using CPE.

Combining hormones and disinfectants (Pituitary gland + Formalin and Malachite green)

The optimization of fertilization and larval survival rate can be achieved through controlling of infections at the early stage of development. In farming catfish, Saprolegnia fungal infection have been reported to cause egg mortality and reduces hatchability of viable fertilized fish eggs, leading to a huge loss to aquaculture industry [2]. Therefore, several chemotherapeutics have been examined and used as curative agents for external ciliated protozoan parasites in food fishes [48,49]. Among these, Formalin (FA), malachite green (MG) and sodium chloride are very good examples of the therapeutants applied in aquaculture. Shourbela et al. [2] reported that, the ovulation percentage for the FA-treated groups was greater than the MG-treated broodfish when CPE used at the same latency periods. Similar results reported by Hossain et al., Sahoo et al., and El-Hawarry et al., [50-52]. In contrary, Adeyemo et al. [53,54] found that, the fertilized eggs of the FA and MG treated groups did not hatch and did not develop as normally as those eggs collected from the non-treated broodfish.

Discussion

Basing on the reviewed research papers, Ovaprim (0.4-0.5 ml/kg) has been known to have better results because of its potency and quality as well as its relatively cheaper cost, ease handling and better survival of hatchlings [12]. However, Orji et al., [55] stated its limitations on induced spawning thus farmers encounter shortage of fingerlings with which to stock their ponds irrespective of many hatcheries in different locations. Failure in fertilization and hatching during induced spawning may also be due to environmental and physico-chemical factor such as salinity. In addition, the price of Ovaprim increases indiscriminately due to import duties. Therefore, to reduce the cost of production arising from purpose of Ovaprim, there is need to find an alternative

Parameters	Ovatide	Ovaprim
Fertilization (%)	87.73 ± 3.38[b]	94.40 ± 2.03[b]
Hatchability (%)	59.7 ± 0.88[b]	66.37 ± 0.74[c]
Survival (%)	63.56 ± 13.56[a]	77.38 ± 5.95[a]
Hatching time	23.5 ± 0.50[a]	24 ± 00[a]
Hatching period	72 ± 00	72 ± 00
*Mean values in the same row having the same superscript are not significantly different. Source: [37]		

Table 3: Reproductive performance of induced male sperm on fertility, hatchability and survival percentage of *C. gariepinus* egg.

TRT	Wt of eggs (g)	% Fertilization	% Hatchability	% Survival
TRT1	161	72.50	76.22	38.12
TRT2	159	75.40	81.25	36.63
TRT3	175	87.41	93.90	58.90
TRT4	169	75.20	84.75	48.82
TRT5	165	76.70	83.35	36.93
Mean	166 ± 2	75083 ± 7645.4		44(4.44)
Where TRT-Treatment; TRT1-(100% Ovaprim); TRT2-(100% Ovulin); TRT3-(75% Ovaprim + 25% Ovulin); TRT4-(50% Ovaprim + 50% Ovulin) and TRT5-(25% Ovaprim + 75% Ovulin) Source: [15].				

Table 4: Mean induced ovulation and spawning of *Clarias gariepinus* using two synthetic hormones (Ovaprim and Ovulin) and effect of the hormones on weight of eggs, fertilization, hatchlings and survival rate.

Exp.	Samp.no	Treatment	BW(g)	PGSI(%)	Fertilization rate (%)	
					No additional sperm	With additional fresh sperm
G1	1	CPE + sperm	1244	11.1	73.4 ± 0.6	–
G2	2		890	10.8	66.9 ± 5.1	–
	3		1134	8.6	68.7 ± 6.9	–
	4	NaCl dissolved CPE to right ovary	1078	10.2	94.9 ± 0.8	–
	5	Sperm without CPE to left ovary	780	10.0	80.5 ± 4.1	–
	6	CPE + sperm	1322	7.3	91.1 ± 2.6	90.3 ± 4.1
	7		518	13.0	76.7 ± 3.9	72.7 ± 6.3
	8		640	9.2	93.7 ± 0.8	87.8 ± 2.2
	9		1000	11.3	78.7 ± 2.0	60.6 ± 4.5
	10		464	10.1	83.7 ± 2.3	71.7 ± 2.7
	11		778	9.0	57.8 ± 9.7	76.7 ± 10
	12		1598	6.1	41 ± 4.4	65.7 ± 8.8
Sum. G2			902.9 ± 427	9.4 ± 2.3	74.7 ± 18.4	75.1 ± 11.5
Data in rows marked without did not differ statistically ($p<0.05$). Abbreviations: CPE: Carp Pituitary Extract, BW: Body Weight, PGSI: Pseudogonadosomatic Index. Source [5]						

Table 5: Summarized data which represent means and standard deviations (Means ± SD).

cheaper spawning aid [16]. Several techniques have been developed, such as multiple ovaprim dilution technique. This technique involves using diluted Ovaprim. Several studies have been conducted and promised results to have been reported with the same time achieving a high spawning, hatchability and survival success of the fish. Olumuji and Mustapha [8] reported that normal saline diluted Ovaprim at 75% and 50% is effective in induced breeding of *Clarias gariepinus*. Also, he found that, production and hatchability of eggs as well as survival of fry which compared well with undiluted Ovaprim. Therefore, the normal saline could enhance the production, hatchability of eggs and survival of fry. The similar results reported by Shepherd and Bromage [7,17]. However, Olumuji and Mustapha [8] found the percentage survival was relatively lower compared to results obtained by Nwokoye et al. study [12]. This could be attributable to the size of the receptacle (tank) in which the experiment was conducted which was relatively smaller and also more eggs were fertilized, and the tank was not power-aerated as reported by Olumuji and Mustapha [8]. On the other hand, natural hormone (ACPE) which is more readily available can also be used in case there will be shortage of Ovaprim whose supply varies with changes in import duties.

In-vitro fertilization of fish, some advances has been reported by several scientists. Müller et al. [46] suggested that, it may be possible to propagate without the presence of males in spontaneous spawning fish species, which release ovulated eggs spontaneously [24,56-61].

Therefore, the method can be applicable in the field of aquaculture management where it is important to increase genetic diversity. For instance, in induced spawning of paired spawning fish species (such as *Sander lucioperca, Silurus glanis, Umbra krameri* etc.) 5–10 sperm samples can be used from different males, for fertilization [46]. In addition, during fertilization care should be taken to prevent infection in the eggs. Several chemotherapeutics such as Formalin (FA), malachite green (MG) and sodium chloride [49] have been examined [48,49-61].

Conclusion

Generally, induce breeding in African catfish *(C. gariepinus)* by using synthetic or non- synthetic hormones ensure availability of matured quality eggs and high survival rate of larval for commercial fish farming. For males, it helps in boosting reproductive performance and ensuring good and viable milt production. *In-vitro* fertilization, ovarian lavage with combination of sperm and hormone method can be used since simplify the induction of spawning with a less time dependent delivery of sperm. In addition, the review revealed that higher fertilization, hatching and survival rate were achieved when Ovaprim used at 0.4-0.5 ml/kg of fish compared to Ovatide and Pituitary Gland Extract (PGE). Therefore, the review suggests that African catfish seeds production can be encouraged through the use of synthetic hormone particularly Ovaprim for better results in ovulation and pawning of eggs. Also, to optimize fertilization and larval rearing pituitary extracted gland in combination of disinfectants such as FA and MG can be used.

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