Induced ovulation and spawning of African catfish *Clarias gariepinus* (Bloch) using ovaprim

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ABSTRACT

Experiments were conducted to study the effects of different doses of ovaprim in stimulating ovulation and spawning response of African catfish, *Clarias gariepinus*. Nine matured female and 9 male fishes weighing from 1.46 to 2.80 kg were randomly selected for three different doses of hormonal injection. Both male and female fishes from all the three replicate groups (3 females and 3 males per replicate) were administered a single dose of 0.4, 0.5 and 0.6 mL of ovaprim/kg body weight (BW), respectively. After 12 h of post-injection, the females were stripped individually into dry and pre weighed plastic containers to record the stripped egg weight per female. The breeding performance was assessed on the basis of total egg mass and stripping response. To assess the egg quality of hormone induced fishes, the fertilization rate and hatching rates were examined among the three doses administered. Complete ovulation was observed in all the three doses of hormonal injection. The results indicated that the total weight of stripped eggs were significantly highest (P < 0.05) at the individuals received 0.4 mL ovaprim/kg BW compared to those injected with other higher doses. Highest fertilization (97.88%) and hatching (93.66%) were also recorded in the individuals received 0.4 mL ovaprim/kg BW. However, breeding performance and egg quality were not statistically significant among the three hormone doses tested in this study. Hence, it was evidenced that ovaprim, the synthetic gonadotropin-releasing hormone with a dopamine antagonist at a dose of 0.4 mL/kg BW could be used as an appropriate spawning dose for *C. gariepinus*.

KEYWORDS

African catfish; *Clarias gariepinus*; hormonal treatment; induced breeding; seed production

1. INTRODUCTION

African catfish, *Clarias gariepinus* is one among the highly demanded freshwater food fish and cultivar species in Malaysia and other Asian and African countries due to its higher resistance to diseases, ability to tolerate a wide range of environmental parameters and high stocking culture conditions, relatively fast growth rate and good quality meat (Hogendoorn, 1980; Huisman and Richter, 1987; Haylor, 1991; Goos and Richter, 1996). It is one of the popular native fish species of African countries and has been introduced and commercially cultured in several countries in Europe (Netherlands, Germany and Belgium) and Asian countries (Indonesia, Thailand and Malaysia) and South America (Brazil). *C. gariepinus* inhabits
a wide range of water bodies like swamps, lakes and rivers. It is a hardy fish and is able to thrive in harsh environmental conditions such as muddy, turbid and oxygen depleted water bodies with the help of accessory air-breathing organ which allows them to breathe oxygen from the atmospheric air. Generally, *C. gariepinus* is omnivorous in nature and usually feeds on insects, plankton, snails and plant matters in the natural water bodies (Bruton, 1979; Uys, 1989). However, this species is highly cannibalistic when substantial differences occur in size (Baras and Jobling, 2002).

African catfish breeds naturally during the rainy season in flooded rivers, inundated paddy fields and earthen ponds. The seed collection of this species from the wild is unreliable, time consuming and uneconomical for large-scale culture of this fish. To overcome these problems, induced spawning is thought to be the only alternative method for seed production and supply. Further, the rearing of the wild collected broodstock in captive conditions may not receive appropriate environmental cues for gonad maturation and spawning and it can cause reproductive development to be arrested in late vitellogenesis stage. Hence, matured females are induced to spawn by hormonal manipulation (Zohar and Mylonas, 2001).

Over the last few decades, hormonal administration techniques have been used to induce final oocyte maturation and spawning in fishes which allowed the reproduction in controlled conditions (Marimuthu et al., 2007 & 2009; Marimuthu and Haniffa 2010). Moreover, induced breeding techniques have significantly contributed a lot to the expansion and diversification of the aquaculture industry (Zohar and Mylonas, 2001). The injection of different spawning agents in fish is adopted for successful ovulation and collection of eggs. Traditional methods of induced spawning in fish are based on the injection of GtH-II from different sources, including extract of carp pituitary gland, partially purified fish GtH-II and mammalian GtH, especially human chorionic gonadotropin (HCG) (Lam, 1982; Donaldson and Hunter, 1983; Peter et al., 1988; Zairin et al., 1992; Goswami and Sharma, 1997). The GnRHa and domperidone are the most popular compounds for induction of ovulation and spermiation in various fish species. The introduction of GnRH analogues has been proven to be efficient in inducing maturation and spawning in many fish species (Tamaru et al., 1988; Thomas and Boyle, 1988; Zohar, 1988; Slater et al., 1995; Berlinsky et al., 1996; Larsson et al., 1997; Mylonas et al., 1997 & 1998). Similarly, an antidopaminergic drug, pimozide has also been found to be highly effective for stimulating the spawning process of fishes mainly in cyprinids and catfishes (Billard et al., 1984; Tan-Fermin, 1997). These hormones are used to stimulate the secretion of endogenous gonadotropin (Zohar, 1989 & 2001).

Lin and Peter (1996) reported that among the several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (sGnRH) or luteinising hormone releasing hormone (LHRH) analogues in combination with dopamine antagonists were identified as effective agent in fish breeding and seed production. Several practical problems have been reported using exogenous hormones and endogenous hormones, such as weighing of such low quantity, preparation of these analogues and storage of this hormone in prepared solutions. Due to these difficulties, fish breeders and farmers are unwilling to use these hormonal preparations under farm conditions. Commercially available synthetic ovulating agents in ready-made form which contained GnRHa and dopamine antagonist such as ovaprim, ovatide, ovopel, dagin and aquaspawn are becoming very popular nowadays and found to be efficient and successful spawning agents in different fish species (Nandeesha et al., 1990; Peter et al., 1988; Brzuska, 1998; Cheah and Lee, 2000; Das, 2004). Recently, successful spawning through a synthetic analogue of GnRH has been reported in several air breathing fish species including *Clarias batrachus* (Basu et al., 2000; Sahoo et al., 2008), *Heteropeustes fossilis* (Alok et al., 1993 and 1994; Marimuthu et al., 2000), murrel (Haniffa et al., 2003 & 2004; Marimuthu et al., 2001a & 2001b, Marimuthu et al., 2007 & 2009; Marimuthu and Haniffa 2010). However, the information on the induction of spawning and artificial reproduction using synthetic hormones in *C. gariepinus* is limited.

Therefore, the present study was conducted to investigate the efficacy of a synthetic GnRH, with a dopamine antagonist (ovaprim) for the induction of ovulation and spawning performance in *C. gariepinus*; also to determine the minimum effective dose of ovaprim for induced spawning and seed production of the candidate fish species under a controlled condition.

## 2. MATERIALS AND METHODS

### 2.1. Broodstock collection, maintenance and selection

Brood fishes (live body weight ranged between 1.5 and 3 kg) were obtained from a local fish farm at Sungai Petani, Kedah Darul Aman, Malaysia. The collected
fishes were transported to the Aquaculture Research Laboratory, AIMST University, Kedah Darul Aman, Malaysia and maintained in circular cement tanks. They were regularly fed with a commercial pellet feed containing 30% crude protein at 5% of their estimated body weight for two weeks. Nine matured females and 9 males were randomly selected and divided into three treatment groups, each group comprised of three males and three females. The matured male and female fishes were identified based on their external morphological features. Matured male fish was identified by a prominent slightly pointed genital papilla, and mature

Figure 1. Identification of female (left side) and male fish (right side)

Figure 2. Intramuscular injection of hormone ovaprim

Figure 3. Collection of eggs

Figure 4. Egg mass of catfish

Figure 5. Macerate testis and collection of sperm

Figure 6. Mixing sperm suspension and eggs for fertilization
females by a swollen abdomen and a reddish round vent (Figure 1). In addition, maturity of the female was confirmed by gentle pressing on the ventral side of the fish for oozing eggs. The eggs were collected by hand-stripping, and immersed in a solution consists of 70% acetic acid and 30% absolute alcohol for clarification of the cytoplasm. After about three minutes, the position of the oocyte nuclei was determined under microscope. Migration of the nucleus from the center of eggs to the periphery region indicates the readiness of fish for breeding and is the best moment for hormonal stimulation as observed in other fish species. Only those females showing the highest percentage of mature oocytes having germinal vesicle in the center or initial stage of migration were selected for the hormonal treatment (Billard et al., 1984).

2.2. Hormone administration, gamete collection, fertilization and hatching

The selected fishes were randomly assigned to three treatment groups and were injected intramuscularly with 0.4, 0.5 or 0.6 mL ovaprim/kg BW (Figure 2). For each dose, three breeding trials were made to find out the spawning response of the fish and to observe the variation in incubation period, rate of fertilization and percentage of hatching. The hormone-administered males and females were released separately into the circular cement tanks. After 12 h, the females were stripped individually and eggs were collected into dry and pre-weighed plastic containers to record the weight of stripped eggs (Figures 3 and 4). The testes were removed from male fishes, incised and squeezed to get concentrated sperm (Figure 5). The sperms were diluted with 5 mL of physiological saline to prepare a sperm suspension. The eggs were thoroughly mixed with sperm suspension and left for 5 minutes (Figure 6). After 3-4 consecutive washing with water, they were transferred into glass aquaria (40 L) provided with continuous aeration. The breeding performance was assessed on the basis of total egg mass and stripping response. The water quality parameters recorded during the study were as follows: temperature, 27.5 - 29.5 °C; dissolved oxygen, 5.4 - 6.0 mg/L and pH 7.8 - 8.5. The percentage of fertilization and hatching rates were calculated as follows:

Fertilization (%) = number of fertilized eggs/total number of eggs counted x 100  \( \text{(1)} \)

Hatching (%) = number of eggs hatched/ total number of eggs in a batch x 100  \( \text{(2)} \)

2.3. Data analysis

The data for total egg mass, fertilization rate, incubation period and hatching rate were analyzed using one way analysis of variance (ANOVA) followed by Duncan’s New Multiple Range Tests at the significant level, \( P = 0.05 \). All the statistical analyses were performed using the computerized statistical package, SPSS (ver. 13.0).

3. RESULTS AND DISCUSSION

Complete ovulation and spermation was observed in all the three hormone dosages tested in the study. No significant size variations of the male and female fishes were used for breeding among the three hormonal treatments. The results obtained from the induced spawning experiments are summarized in Table 1. The results indicated that the total weight of stripped eggs were significantly highest (\( P < 0.05 \)) in females received 0.4 mL hormonal dose than those females administered with higher doses. Significantly highest percentages (\( P < 0.05 \)) of fertilization rate (97.88%) and hatching rate (93.66%) were also recorded in the females received 0.4 mL ovaprim than those received higher doses. However, both fertilization rate and hatching rates were not statistically significant among the females received 0.5 and 0.6 mL/kg BW. Significantly longer (\( P < 0.05 \)) incubation period was observed in the individuals administered with 0.4 mL ovaprim than those administered with 0.5 and 0.6 mL hormone, the latter two doses did not statistically significant among them.

In this study, a single intramuscular injection of synthetic hormone, ovaprim resulted in successful spawning of *C. gariepinus*. Successful spawning using ovaprim and its analogues have also been reported in several fish species viz., carp (Nandeesh et al., 1990), stinging catfish *H. fossilis* (Vijayakumar et al., 1998), *Ompok bimaculatus* (Sridhar et al., 1998), murrel (Haniffa et al., 2003 and 2004; Francis et al., 2000), catfish *Neolisurus ater* (Cheah and Lee, 2000) and *C. batrachus* (Sahoo et al., 2008). Table 2 summarizes the results of induced spawning and success rate of different fish species using ovaprim. It has been reported that the ability to induce oocyte maturation and ovulation depend on the dose, species and maturation stage of the oocytes (Donaldson and Hunter, 1983; Yaron, 1995; Peter et al., 1988).

In the present study, significantly highest (\( P < 0.05 \)) amount of egg mass was found in *C. gariepinus* when injected with 0.4 mL/kg BW of ovaprim. Further,
Complete spawning was also observed in all the three doses tested. Complete spawning has also been reported using ovaprim in carps (Nandeesha et al., 1990), *Puntius japonicus* (Azad and Shimray, 1991), *N. ater* (Cheah and Lee, 2000), *C. batrachus* (Basu et al., 2000) and using ovatide in *H. fossilis* (Marimuthu et al., 2000) and *Ompok pabo* (Mukherjee and Das, 2001). No significant differences were noticed in the hatching rates between the doses, 0.5 and 0.6 mL/kg BW tested. This observation suggests that inducing ovulation with ovaprim at higher doses did not have any adverse effect on egg viability. However, fish responds differently, when induced with GnRH or their analogue(s) in terms of elevation in the gonadotropin levels (Peter et al., 1985; Breton et al., 1990), steroid levels (Weil et al., 1980; Van Der Kraak et al., 1987), ovulation (Ramos, 1986; Peter et al., 1987; Fermin, 1991), latency period (De Leeuw et al., 1985; Glubokov et al., 1986; Kaul and Rishi, 1986) and spawning (Lee et al., 1986; Nandeesha et al., 1990). The variations in response can be attributed to the fish species, amino acid sequence and purity of the GnRH analogue (Peter et al., 1985 & 1987), dose of the GnRH and based on weak or strong dopaminergic inhibitory mechanism (Fermin, 1991).

In general, the response of fish to ovaprim was found to be better, considering the spawning success, egg mass, percentages of fertilization and hatching. Further, the synthetic hormones like ovatide, ovapel and ovaprim are known to act at the pituitary level

<table>
<thead>
<tr>
<th>Species</th>
<th>Ovaprim (mL/kg BW)</th>
<th>Latency period (h)</th>
<th>Spawning success</th>
<th>Fertilization rate (%)</th>
<th>Hatching rate (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acanthopagrus latus</em></td>
<td>0.5</td>
<td>59</td>
<td>-</td>
<td>83</td>
<td>88</td>
<td>Leu and Chou (1996)</td>
</tr>
<tr>
<td><em>Aristichthys nobilis</em></td>
<td>0.6</td>
<td>8</td>
<td>Complete</td>
<td>98</td>
<td>100</td>
<td>Afzal et al. (2008)</td>
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<td><em>Catla catla</em></td>
<td>0.4</td>
<td>9-10</td>
<td>Complete</td>
<td>80</td>
<td>90</td>
<td>Nandeesha et al. (1990)</td>
</tr>
<tr>
<td><em>Channa striatus</em></td>
<td>0.4</td>
<td>21</td>
<td>-</td>
<td>93</td>
<td>-</td>
<td>Francis et al. (2000)</td>
</tr>
<tr>
<td><em>Channa punctatus</em></td>
<td>0.5</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Haniffa et al. (2003)</td>
</tr>
<tr>
<td><em>Cirrhinus mrigala</em></td>
<td>0.35</td>
<td>15</td>
<td>Complete</td>
<td>86</td>
<td>90</td>
<td>Nandeesha et al. (1990)</td>
</tr>
<tr>
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<td>2.0-2.5</td>
<td>16-17</td>
<td>Complete</td>
<td>60-65</td>
<td>-</td>
<td>Mohapatra et al. (2000)</td>
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<td>16-18</td>
<td>Complete</td>
<td>80</td>
<td>60</td>
<td>Basu et al. (2000)</td>
</tr>
<tr>
<td><em>Clarias batrachus</em></td>
<td>1.0-1.5</td>
<td>14-17</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>Sahoo et al. (2008)</td>
</tr>
<tr>
<td><em>Ctenopharyngodon idella</em></td>
<td>0.7</td>
<td>8</td>
<td>Complete</td>
<td>95</td>
<td>100</td>
<td>Nandeesha et al. (1990)</td>
</tr>
<tr>
<td><em>Heteropneustes fossilis</em></td>
<td>0.6</td>
<td>14-12</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>Alok et al. (1993)</td>
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<tr>
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<td>11-12</td>
<td>Complete</td>
<td>80-84</td>
<td>-</td>
<td>Francis (1996)</td>
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<tr>
<td><em>Heteropneustes fossilis</em></td>
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<td>10-24</td>
<td>-</td>
<td>-</td>
<td>80</td>
<td>Vijayakumar et al. (1998)</td>
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<td><em>Hypophthalmichthys molitrix</em></td>
<td>0.5</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Peter et al. (1988)</td>
</tr>
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<td>15</td>
<td>Complete</td>
<td>53</td>
<td>-</td>
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<td><em>Labeo rohita</em></td>
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<td>Complete</td>
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<td>90-95</td>
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</tr>
<tr>
<td><em>Neolisurus ater</em></td>
<td>0.5</td>
<td>17-23</td>
<td>Complete</td>
<td>90</td>
<td>79</td>
<td>Cheah and Lee (2000)</td>
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<td>0.5</td>
<td>5-6</td>
<td>-</td>
<td>75</td>
<td>60</td>
<td>Sridhar et al. (1998)</td>
</tr>
<tr>
<td><em>Puntius japonicus</em></td>
<td>0.4</td>
<td>8</td>
<td>Complete</td>
<td>90</td>
<td>59</td>
<td>Azad and Shimray (1991)</td>
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<td><em>Tor putitora</em></td>
<td>0.2</td>
<td>24</td>
<td>-</td>
<td>70-80</td>
<td>60-65</td>
<td>Pandey et al. (1998)</td>
</tr>
<tr>
<td><em>Channa striatus</em></td>
<td>0.4</td>
<td>23</td>
<td>complete</td>
<td>99</td>
<td>92</td>
<td>Marimuthu et al. (2001b)</td>
</tr>
</tbody>
</table>

Mean values in the each column with the different superscript letter are significantly different (P < 0.05)
leading to the secretion of the fish’s own endogenous gonadotropins, while in the case of hypophysation technique and administration of HCG, exogenous gonadotropins are directly delivered into the body (Habibi et al., 1989; Zairin et al., 1992; Goswami and Sharma, 1997). Endogenous gonadotropins appear to significantly enhance the secretion of the right type of steroids in appropriate quantities, enabling complete maturation of ova.

4. CONCLUSIONS

From the present findings, it is evidenced that the synthetic gonadotropin-releasing hormone with a dopamine antagonist at the low dose of 0.4 mL/kg BW could be used as an appropriate spawning agent for successful breeding and seed production of C. gariepinus. The results emerged from the present study would immensely be helpful for quality seed production in African catfish. Further studies are required to examine the development and growth performances of larvae and fry produced by the induced breeding techniques.

REFERENCES


