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Technical note

Effects of GnRHa (D-Ala^6, Pro^9-NEt) combined with domperidone on ovulation induction in wild loach *Misgurnus anguillicaudatus*

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ABSTRACT

The effects of a single intramuscular injection of gonadotropin releasing hormone analogue (GnRHa) alone or in combination with the dopamine antagonist domperidone (DOM) on ovulation induction of loach *Misgurnus anguillicaudatus* broodstocks collected from the wild were tested under routine hatchery conditions. The ovulation ratio, latency period, ovulation index, fertilization success and hatching rate were evaluated. The following hormone treatments were tested: 2 mg kg^{-1} BW of CPE as a positive control (PC), GnRHa alone at doses of 10 μg (G10), 20 μg (G20), 40 μg (G40) and 60 μg (G60) kg^{-1} BW and combinations of GnRHa and DOM at doses of 5 μg + 2.5 mg (GD5), 10 μg + 5 mg (GD10), 20 μg + 10 mg (GD20) and 40 μg + 20 mg (GD40) kg^{-1}, respectively. Physiological saline injected fish were used as a negative control (NC). The results showed that the combination of 20 μg + 10 mg (GD20) and 40 μg + 20 mg (GD40) kg^{-1} of GnRHa and DOM, respectively, injection led to higher ovulation ratio and shorter latency periods in comparison with the control and the other hormone treatments ($P<0.05$), and there was no significant difference between the two ovulating groups with respect to ovulation ratio and latency period ($P>0.05$). There was a significant difference between the GnRHa alone groups and the GnRHa + DOM combined groups on the ovulation index (the former < the latter, $P<0.05$), while no significant differences in the fertilization success and hatching rate were found in any of the hormone treatments ($P>0.05$). Only 20% of the fish ovulated in group G10 and G20, and no fish ovulated in group NC, suggesting a dopaminergic inhibitory action on gonadotropin (GtH) secretion in this fish at the preovulatory stage. Therefore, it can be concluded that like many other cyprinids, dopamine inhibitory action was operating in loach and it was necessary to combine GnRHa with a dopamine antagonist for ovulation induction. As a result, ovulation can be induced successfully in loach broodstocks with 20 μg kg^{-1} GnRHa + 10 mg kg^{-1} DOM treatment in a single injection without any negative effect on egg quality. Application of this combination could be beneficial for hatchery and broodstock management in loach culture.

1. Introduction

The loach *Misgurnus anguillicaudatus*, is a valuable fish native to Russia, Korea, Japan, China, Vietnam and Burma (Berg, 1962), but populations have been decreasing rapidly. In native habitats, the loach inhabits both lotic and lentic systems and are particularly prevalent in rice paddies where the loach activity to uproot weeds is considered beneficial for rice farmers (Kim et al., 1994). This species is a multiple spawner that migrates from rivers to paddy fields for spawning from mid May to August (Saitoh et al., 1988; Tanaka, 1999; Fujimoto et al., 2008). A fecundity range of 10,000 to 24,000 for individuals with total length 12–20 cm has been found, and the egg diameter was 1.2–1.5 mm (Liu, 2008). The good taste and high medical value (Kimura et al., 1994; Park et al., 1997; Qin et al., 2002; Dong et al., 2002; Zhang and Huang, 2005, 2006) has created a high year round demand for loach and the annual demand of loach in Korea and Japan was over 100,000 t in 2004 (Jiangsu Meteorological Bureau, 2004). It is anticipated that an expanded and consistent requirement of loach production will ultimately require the development of all kinds of culture systems. Reliable cultured fry production of loach and rice–fish farming system (Lu and Li, 2006) are necessary in south China, where environmental degradation has reduced wild fry yields (Beveridge et al., 1997; Li, 1999), and a similar situation has been reported in Japan (Fujimoto et al., 2008).

Cyprinid fry are produced by artificial breeding using hormonal treatment to induce ovulation (Dorafshan and Heyrati, 2006). The development of the cyprinid culture worldwide has led to a shortage of carp pituitary extract (CPE) to aquaculturists. This has prompted the development of new approaches for inducing ovulation in cyprinid fishes. The presence of a dopaminergic inhibitory tone in

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many cyprinid fish requires the addition of a DA antagonist to facilitate the release of LH response to GnRHa. Such a combined treatment is considered as the "Linpe Method"(Lin et al., 1986; Peter et al., 1988). These methods stimulate secretion of endogenous gonadotropin (GtH) (Zohar, 1989; Zohar and Mylonas, 2001). The addition of a dopamine receptor antagonist (DA) potentiates the response to GnRHa by reducing the dopaminergic inhibitory action in the target species (Peter et al., 1988; Zohar, 1989; Heyrati et al., 2007). Many cyprinid fish exhibit this dopaminergic inhibitory action and, therefore, require the addition of a DA antagonist to facilitate the release of LH in response to GnRHa (Peter et al., 1988; Heyrati et al., 2007).

The GnRHa and domperidone are the most popular compounds for induction of ovulation and spermiation in various fish species (Donaldson and Hunter, 1983; Donaldson, 1996, 2003; Heyrati et al., 2007). The effectiveness of the above treatments was found to vary depending on species and local conditions (Zohar and Mylonas, 2001). Therefore, it is necessary to determine the optimal therapy condition in each species under local conditions.

The aim of this study was to evaluate the minimal effective dose of GnRHa alone or in combination with domperidone in wild loach under hatchery conditions during its normal reproductive season. The study includes ovulation ratio, ovulation index, latency period, fertilization success and hatching rate.

2. Materials and methods

2.1. Broodstock selection and maintenance

Experiments were conducted at the aquaculture research center of Huazhong Agricultural University, Wuhan City, China. Adult fish were captured from the ditches around rice paddies in the outskirts of Wuhan City on May 10, 2007 (water temperature 20–23 °C). After 3 days, ovarian biopsies were taken with a polyethylene cannula, clarified and examined as described by Levavi-Zermonsky & Yaron (1986). Only fish having more than 60% of the oocytes with a migrating germinal vesicle were selected for the ovulation experiment.

One hundred female fish weighing 30–80 g body weight (BW) were selected according to the criterion mentioned above. Prior to injection, fish were individually weighed and randomly assigned to ten experimental groups.

2.2. Preparation of hormones

The GnRHa D-Ala^6, Pro^9-NEt mGnRHa ethylamide and domperidone (DOM) were purchased from Ningbo Renjian Pharmaceutical Co., Ltd, Ningbo, China. CPE stock solution was prepared by mixing 4 mg dry carp pituitary provided by ARGENT (Argent Chemical Laboratories, Inc. Redmond, WA, USA) with 10 ml of 0.7% saline (NaCl). The solution was centrifuged for 10 min at 2500 rpm. Each ml of the resultant supernatant contained the extract from 0.4 mg of acetone-dried carp pituitary, and GnRHa and DOM were dissolved in physiological saline and dimethyl sulfoxide respectively (Omeljaniuk et al., 1987), to achieve a concentration of 8, 4 and 2 μg ml^{-1} (GnRH) in order to adjust the injected volume to 0.15–0.4 ml fish^{-1} in all treatments with the same concentration of dimethyl sulfoxide.

2.3. Experimental design

All groups (10 fish per group) were injected as follows: 2 mg kg^{-1} BW of CPE as a positive control (PC), 10 (G10), 20 (G20), 40 (G40) and 60 (G60) μg kg^{-1} BW of GnRHa alone and the following combination of GnRHa with DOM: 5 μg + 2.5 mg (GD5), 10 μg + 5 mg (GD10), 20 μg + 10 mg (GD20) and 40 μg + 20 mg (GD40) kg^{-1} BW. Physiological saline (0.7% NaCl) was injected as negative control (NC). The substances were injected intraperitoneally in a single injection (Table 1).

After injection fish were placed in an indoor concrete fish pond with recirculated water and temperature of 20–23 °C. The first examination for ovulation was carried out 6 h after injection and repeated every hour. When the ovulation had occurred, the eggs were stripped manually, weighed and fertilized with milt from at least two males, and 1 to 5 g of fertilized eggs from each female was incubated in jar incubators until they hatched.

In order to determine ovulation index (OI, the weight of stripped egg mass / (weight of stripped egg mass + remnant ovaries) × 100%) (Szabo et al., 2002), females were sacrificed after stripping and the ovaries or ovarian remnants were weighed. The ovulation ratio (number of ovulated females / number of injected × 100%) and the latency period (mean time between injection and ovulation) were calculated (Drori et al., 1994). Fertilization success was determined by examining 100 eggs from each female under a dissecting microscope, when eggs were at the gastrulation stage. Hatching rate was calculated as: number of hatched larvae / number of fertilized eggs × 100%.

2.4. Statistical analysis

Percentage of ovulated females was analysed by χ^2 test (Szabo et al., 2002). The differences in latency period, OI, fertilization success and hatching rate were analysed by one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range test at minimum significant of $P<0.05$. Results are presented as means ± standard deviation (S.D).

3. Results

No fish ovulated in the negative control group after injecting physiological saline. In the positive control group, five out of ten fish ovulated (50%). The lowest ovulation ratio (20%) in the hormone-treated groups was observed in group G10 and G20. The ovulation

Table 1
The effect of different hormone treatments on ovulation ratio, latency period, ovulation index and fertilization success of *M. anguillicaudatus*.

Groups	Treatment	Dosage*	Ovulation ratio (%)	Latency period (h)	OI (%)	Fertilization success (%)	Hatch rate (%)	Hormone cost ($/kg^{-1})**
NC	Saline (0.7% NaCl)	–	0	–	–	–	–	
PC	CPE	2	50^{bc}	15.6 ± 1.5^{b}	73.8 ± 3.9^{b}	78.2 ± 5.9^{a}	92.3 ± 1.9^{a}	0.800
G10	GnRHa	10	20^{a}	23.0 ± 1.4^{d}	61.5 ± 0.7^{a}	77.0 ± 2.8^{a}	90.9 ± 2.2^{a}	0.099
G20	GnRHa	20	20^{a}	19.0 ± 0.0^{c}	61.0 ± 1.4^{a}	79.0 ± 1.4^{a}	89.9 ± 2.7^{a}	0.198
G40	GnRHa	40	40^{b}	17.3 ± 1.0^{bc}	63.3 ± 3.9^{a}	78.8 ± 3.0^{a}	89.7 ± 2.9^{a}	0.396
G60	GnRHa	60	40^{b}	16.8 ± 1.0^{b}	63.3 ± 2.8^{a}	77.5 ± 1.7^{a}	92.2 ± 1.6^{a}	0.594
GD5	GnRHa + DOM	5 + 2.5	40^{b}	16.3 ± 1.0^{b}	75.5 ± 4.8^{b}	81.3 ± 2.2^{a}	88.9 ± 3.0^{a}	0.091
GD10	GnRHa + DOM	10 + 5	60^{c}	15.7 ± 1.2^{b}	77.8 ± 3.2^{b}	79.3 ± 4.0^{a}	91.1 ± 1.9^{a}	0.182
GD20	GnRHa + DOM	20 + 10	100^{d}	10.0 ± 1.0^{a}	77.4 ± 4.1^{b}	82.9 ± 3.6^{a}	90.2 ± 1.5^{a}	0.363
GD40	GnRHa + DOM	40 + 20	100^{d}	10.5 ± 1.8^{a}	77.5 ± 4.7^{b}	81.9 ± 4.9^{a}	90.1 ± 1.7^{a}	0.726

*The dose of GnRHa is μg kg^{-1} BW and that of CPE and DOM is mg kg^{-1} BW.
**The cost of hormones was expressed as cost of hormone to induce the spawning of 1 kg of females (CPE $400/g, GnRHa $9.9/mg, DOM $16.5/g).
Values are means ± S.D. Values in the same row followed by different superscripts are significantly different ($P<0.05$).