

A Comparison of the Potency of Dopamine Antagonists on Spawning Induction in Thai Carp (*Puntius gonionotus* Bleeker)

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ABSTRACT

The potency of three dopamine antagonists (DA) i.e., domperidone (DOM), sulpiride (SUL), and metoclopramide (MET) on spawning induction in Thai carp was compared. Sexually mature female Thai carp were injected with DOM, SUL or MET at concentrations of 5, 10, 20 or 40 mg/kg alone or in combination with the LHRH analog Buserelin (BUS) at 5 or 20 µg/kg and were allowed to spawn naturally. The results of the study showed that the three DA had the same potency when used alone. When used in combination with BUS at either low or high dosage, the order of potency of the three DA was 5 mg/kg DOM = 20 mg/kg MET = 40 mg/kg SUL. It was concluded that DOM had the highest potency on the spawning induction of the Thai carp. However, it was recommended to use 20 mg/kg MET in combination with 5 µg/kg BUS to induce spawning of mature female Thai carp, when both potency and cost were considered.

Key words : dopamine antagonist, induced spawning, Thai carp, domperidone, metoclopramide, sulpiride, LHRH

INTRODUCTION

Gonadotropin (GtH) secretion in most teleost fish is regulated by a dual hypothalamic control of gonadotropin releasing hormone (GnRH) and dopamine, which acts as a gonadotropin release inhibitory factor (Peter *et al.*, 1986). Final maturation and ovulation in fish is normally initiated by a surge of GtH II. Spawning in cultivated fish can be induced by increasing the output of the endogenous GtH using GnRH or its superactive analogs in combination with a dopamine antagonist (DA) (Peter *et al.*, 1988). This method has proven to be very effective and reliable in induced spawning of several freshwater fish species in Thailand (Sukumasavin and Leelapatra, 1988). Recently, the potency of several forms of GnRH and its

superactive analogs (GnRHa) on the induction of spawning of the Thai carp has been compared, and Buserelin (BUS), a mammalian GnRHa (or LHRHa), has proven to be the most potent analog (Sukumasavin and Leelapatra, 1993). The only limitation on using this technique for induced spawning of freshwater fish in Thailand is the lack of knowledge on DA. To date domperidone (DOM) is the only DA that has been studied and used for induced spawning of freshwater fish in Thailand. Recent studies have shown that there are several DA such as sulpiride (SUL) and metoclopramide (MET) which can stimulate the secretion of GtH II and induce ovulation in fish when used alone or in combination with GnRH, for example, in goldfish (Lin and Peter, 1986; Omeljaniuk *et al.*, 1987), bream (Glubokov *et al.*, 1991), tilapia (Gissis *et al.*,

1991) and common carp (Drori *et al.*, 1994). The objective of this study is to compare the potency of 3 DA i.e., DOM, SUL and MET on the spawning induction of Thai carp (*Puntius gonionotus*) when used alone or in combination with BUS at low dosage (5 µg/kg) or high dosage (20 µg/kg).

MATERIALS AND METHODS

This study was carried out at Surin Inland Fisheries Station in July 1998.

Experimental fish

Thai carp (*Puntius gonionotus* Bleeker) were reared in earthen ponds at a stocking density of 1 kg/4 m² and were fed once daily with pellets containing 15% protein at 2% body weight. Females that had 80% of oocytes at the germinal vesicle migration stage were used in the study. Males were selected by the appearance of milt from gentle pressure on the abdomen. Water temperature during the study was 29±1°C.

Hormone and drugs preparation

Buserelin (Suprefact, Hoechst) and metoclopramide (Science Medic) were dissolved in distilled water at concentrations of 100 µg/ml and 25 mg/ml, respectively. Domperidone (Sigma) and sulpiride (Sigma) were dissolved in N,N-dimethylformamide (DMFM; Sigma) at a concentration of 25 mg/ml.

Experiment 1: Comparison of the potency of DOM, SUL and MET when used alone

Sexually mature female Thai carp with an average body weight of 201.78±43.69 g (mean±SD) were divided into 14 groups of 15 fish. Each group received an intraperitoneal injection (IP) of DOM, SUL or MET at 5, 10, 20, or 40 mg/kg. Control groups received an IP injection of distilled water or DMFM as shown in Table 1. After injection,

females in each group together with the same number of untreated sexually mature males were transferred to 2.5 m³ round fiberglass tanks, supplied with running water. The fish were allowed to spawn naturally, and the number of fish that spawned during the 8-h period following injection was recorded. After spawning, the eggs from each group were transferred to hatching jars for determination of fertilization and hatching rates.

Experiment 2: Comparison of the potency of DOM, SUL and MET in combination with a low dosage of BUS (5 µg/kg)

Sexually mature female Thai carp with an average body weight of 206.43±43.66 g were divided into 14 groups of 15 fish. Each group received an IP injection of DOM, SUL or MET at 5, 10, 20, or 40 mg/kg in combination with BUS at 5 µg/kg. Control groups received an IP injection of distilled water or DMFM as shown in Table 2. After injection, fish were treated as in Experiment 1.

Experiment 3: Comparison on the potency of DOM, SUL and MET in combination with a high dosage of BUS (20 µg/kg)

Sexually mature female Thai carp with average body weight of 217.74±49.42 g were divided into 14 groups of 15 fish each. Each group received an IP injection of DOM, SUL or MET at 5, 10, 20, or 40 mg/kg in combination with BUS at 20 µg/kg. Control groups received an IP injection of distilled water or DMFM as shown in Table 3. After injection, fish were treated as in Experiment 1.

Data analysis

The proportion of fish that spawned in each group were analyzed non-parametrically by Fisher's exact probability test. Fertilization and hatching rates were analyzed by Mann Whitney U test.

Table 1 Effects of domperidone (DOM), sulpiride (SUL) and metoclopramide (MET) on the number of fish spawned, fertilization rate and hatching rate in Thai carp (*Puntius gonionotus* Bleeker).

Group	Buserelin (µg/kg)	Dopamine antagonist (mg/kg)	No. of fish injected	No. of fish spawned	Fertilization rate (%)	Hatching rate (%)
1	0	Dist. Water	15	0	-	-
2	0	DMFM*	15	0	-	-
3	0	DOM 5	15	0	-	-
4	0	DOM 10	15	0	-	-
5	0	DOM 20	15	0	-	-
6	0	DOM 40	15	1	88.00±1.00	84.67±6.11
7	0	SUL 5	15	0	-	-
8	0	SUL 10	15	0	-	-
9	0	SUL 20	15	0	-	-
10	0	SUL 40	15	0	-	-
11	0	MET 5	15	0	-	-
12	0	MET 10	15	0	-	-
13	0	MET 20	15	0	-	-
14	0	MET 40	15	0	-	-

* DMFM = N,N-dimethylformamide

RESULTS AND DISCUSSION

As shown in Table 1, injection of SUL and MET at 5 to 40 mg/kg or DOM at 5 to 20 mg/kg alone did not induce sexually mature Thai carp to spawn. However, in the DOM 40 mg/kg group 1 of 15 fish was induced to spawn 4–5 h after injection. This coincides with the finding that DOM alone at low concentrations can not induce sexually mature female Thai carp to spawn, but DOM at 25 mg/kg can induce up to 40% of Thai carp to spawn (Sukumasavin and Leelapatra, 1993). The lower percentage of spawned fish in this study, though the dosage was higher, may be due to the timing of the study. The present study was conducted in July, which is almost the end of the spawning season, while the earlier study was conducted in May, which is in the middle of the spawning season of

Thai carp. It has been shown in the goldfish that there is a seasonal variation in responsiveness to injection of pimozide (PIM), a dopamine receptor antagonist, alone or in combination with GnRHa (Solowska *et al.*, 1985). PIM was most effective on GtH release in fish in the late stage of ovarian recrudescence; fish that were sexually regressed were the least responsive. From the results of experiment 1, it was concluded that when used alone, at the dosages tested, DOM, SUL and MET had either no effect or only a slight effect on spawning induction of the Thai carp.

When used in combination with a low dosage of BUS (5 µg/kg) in experiment 2, spawning occurred in all DA treated groups, 4–5 h after injection (Table 2). However, only DOM induced all the treated fish to spawn at all concentrations. In SUL treated groups, the numbers of fish that

Table 2 Effects of domperidone (DOM), sulphiride (SUL) and metoclopramide (MET) in combination with Buserelin at 5 mg/kg on the number of fish spawned, fertilization rate and hatching rate in Thai carp (*Puntius gonionotus* Bleeker).

Group	Buserelin (µg/kg)	Dopamine antagonist (mg/kg)	No. of fish injected	No. of fish spawned	Fertilization rate (%)	Hatching rate (%)
1	5	Dist. Water	15	0 ^a	-	-
2	5	DMFM*	15	0 ^a	-	-
3	5	DOM 5	15	15 ^d	93.67±1.53	81.33±2.52
4	5	DOM 10	15	15 ^d	89.67±4.58	79.33±0.58
5	5	DOM 20	15	15 ^d	90.00±4.58	80.67±1.53
6	5	DOM 40	15	15 ^d	87.67±1.15	81.00±2.64
7	5	SUL 5	15	2 ^{ab}	90.00±1.73	83.00±1.00
8	5	SUL 10	15	1 ^a	95.00±2.00	77.67±1.53
9	5	SUL 20	15	11 ^{bc}	86.00±3.00	79.67±0.58
10	5	SUL 40	15	11 ^{bc}	89.00±4.36	80.33±2.52
11	5	MET 5	15	2 ^{ab}	89.33±5.51	80.67±4.16
12	5	MET 10	15	6 ^b	88.67±3.78	79.00±1.00
13	5	MET 20	15	13 ^{cd}	93.00±7.00	81.33±2.31
14	5	MET 40	15	12 ^{cd}	93.33±3.78	79.33±0.58

* DMFM = N,N-dimethylformamide

Groups which are similar in number of fish spawned are identified by the same letter (p>0.05).

spawned when injected with SUL at 5 or 10 mg/kg were not significantly different from control groups. When the concentration of SUL was increased to 20 and 40 mg/kg, the number of fish that spawned was significantly higher than that of controls but was significantly lower than that of DOM treated groups. In MET treated groups, on the other hand, MET at 10 mg/kg and over when used in combination with BUS at 5 µg/kg increased the number of fish that spawned significantly when compared with controls. Furthermore, the numbers of fish that spawned in the MET 20 and 40 mg/kg injected groups were not significantly different from DOM treated groups. The results of this experiment indicated that when using in combination with a low dosage of BUS, DOM had

the highest potency on spawning induction in Thai carp. Using DOM at 5 mg/kg could induce all fish to spawn. While 20 mg/kg MET could not induce all the fish to spawn though the number of fish that spawned was not significantly different from DOM treated groups. Furthermore, the number of fish that spawn when treated with SUL at the highest dosage used in this study was lower than that of DOM treated groups. The rank of potency of these DA on spawning induction of Thai carp, in combination with 5 µg/kg BUS can be summarized as DOM 5 µg/kg > SUL 40 mg/kg = MET 10. Omeljaniuk *et al.* (1987) also reported a similar result in goldfish that the rank of potency of DA, in combination with GnRHa on serum GtH release in goldfish was DOM > MET > SUL.

Table 3 Effects of domperidone, sulphiride and metoclopramide in combination with Buserelin at 20 mg/kg on the number of fish spawned, fertilization rate and hatching rate in Thai carp (*Puntius gonionotus* Bleeker).

Group	Buserelin (µg/kg)	Dopamine antagonist (mg/kg)	No. of fish injected	No. of fish spawned	Fertilization rate (%)	Hatching rate (%)
1	20	Dist. Water	15	0 ^a	-	-
2	20	DMFM*	15	0 ^a	-	-
3	20	DOM 5	15	15 ^d	88.00±1.73	80.00±1.00
4	20	DOM 10	15	15 ^d	91.67±5.86	78.67±2.31
5	20	DOM 20	15	15 ^d	92.67±3.21	79.67±1.15
6	20	DOM 40	15	15 ^d	88.33±3.21	78.33±0.58
7	20	SUL 5	15	3 ^{ab}	90.00±1.00	79.67±1.53
8	20	SUL 10	15	4 ^{ab}	92.00±1.00	80.67±3.05
9	20	SUL 20	15	8 ^{bc}	90.33±4.16	75.33±0.58
10	20	SUL 40	15	12 ^{cd}	87.00±1.00	81.00±1.00
11	20	MET 5	15	4 ^{ab}	90.00±1.00	80.67±3.78
12	20	MET 10	15	4 ^{ab}	92.67±3.21	79.33±4.16
13	20	MET 20	15	12 ^{cd}	92.00±1.00	79.67±4.04
14	20	MET 40	15	15 ^d	90.33±3.21	79.00±1.00

* DMFM = N,N-dimethylformamide

Groups which are similar in number of fish spawned are identified by the same letter (p>0.05).

When used with BUS at a higher concentration (20 µg/kg), spawning occurred in all DA treated groups (Table 3). Thus, DOM at all concentrations, in combination with 20 µg/kg BUS, induced all the fish to spawn. SUL and MET at 5 and 10 mg/kg did not significantly increase the number of fish that spawned compared to the control groups. When the concentration of SUL and MET was increased to 20 mg/kg, the number of fish that spawned was significantly higher than that of the control groups. SUL at the highest concentration used in this study, in combination with 20 µg/kg BUS, still did not induce all the fish to spawn. However, in contrast to experiment 2, the number of fish that spawned when injected with SUL at 40 mg/kg was not significantly different

from that of the DOM treated groups. The number of fish that spawned when injected with MET at 20 and 40 mg/kg was not significantly different from that of the DOM treated groups. Furthermore, MET at 40 mg/kg, in combination with 20 µg/kg BUS induced all the treated fish to spawn. The results from Experiment 3 provided information on the rank order of potency of these DA for the induced spawning of Thai carp as follows: DOM 5 mg/kg = SUL 40 mg/kg = MET 20 mg/kg.

Fertilization and hatching rate were not significantly different among the spawning groups and were not different from those reported earlier for the Thai carp (Sukumasavin and Leelapatra, 1992; 1993). The fertilization rates were between 95.00±2.00 to 86.00±3.00%, and the hatching rate

were between 84.67 ± 6.11 to $75.33 \pm 0.58\%$.

From the results of this study, it was concluded that, in Thai carp as in goldfish (Omeljaniuk *et al.*, 1987) and in African catfish (Goos *et al.*, 1987), DOM had the highest potency for spawning induction. The optimum dosage for each DA for spawning induction in Thai carp was summarized as follows: for DOM, BUS 5 µg/kg in combination with DOM 5 mg/kg, for MET, BUS 5 µg/kg in combination with MET 20 mg/kg, and for SUL, BUS 20 µg/kg in combination with SUL 40 mg/kg.

The cost of dopamine antagonists and GnRHa used in this study and the costs of using each DA at the optimum dosage were shown in Tables 4 and 5, respectively. Using MET had the lowest cost of 1.105 Baht/kg. Using DOM costed 1.150 Baht/kg, but if using Motilium (MOT) which

was a form of DOM that was available in 10-mg tablets as a human pharmaceutical, the cost was 2.325 Baht/kg. Using SUL had the highest cost of 6.3 Baht/kg.

It is well established that DOM is the preferred DA for spawning induction of fish because DOM does not cross the blood-brain barrier (Omeljaniuk *et al.*, 1987), and DOM can cause a long-lasting, dose-dependent depletion of dopamine in the pituitary (Sloley *et al.*, 1991). However, DOM is a water-insoluble drug, and normally must be dissolved in an organic solvent such as dimethylsulfoxide (Gissis *et al.*, 1991) or DMFM used in this study. In Thailand, DOM is normally administered in the form of MOT crushed and injected as an aqueous suspension, which is less effective than in the dissolved form (Gissis *et al.*, 1991).

Table 4 Price of drugs and chemicals used in the study.

Drug/chemical	Unit	price (Baht/unit)
Buserelin	µg	0.145
Domperidone	mg	0.065
Sulpiride	mg	0.065
Metoclopramide	mg	0.019
N,N-dimethylformamide	mg	0.500
Motilium	mg	0.320

Table 5 Cost of effective dosages of Buserelin (BUS) in combination with Domperidone (DOM), Sulpiride (SUL), Metoclopramide (MET) or Motilium (MOT) for induced spawning 1 kg Thai carp.

Dosages	Cost (Baht/kg)
BUS 5 µg /kg + DOM 5 mg/kg	1.150
BUS 5 µg /kg + MET 20 mg/kg	1.105
BUS 20 µg /kg + SUL 40 mg/kg	6.300
BUS 5 µg /kg + MOT 5 mg/kg	2.325

For formulation of a protocol to be used by fish breeders, it would be preferable to avoid as much as possible both the use of organic solvents and the need to prepare an aqueous suspension. It would thus appear that the water-soluble MET, which showed a potency similar to DOM, and was less expensive than DOM would be the DA of choice for spawning induction of freshwater fish of Thailand.

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