Comparison of the effects of buserelin in combination with various forms of domperidone and pituitary gland on the induction of spawning and gonadal development in the Thai carp, *Barbonymus gonionotus*

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ABSTRACT

This study set compares the effects of buserelin (BUS) in combination with various forms of domperidone (DOM) to the effects of extract of pituitary gland (PG) on the induction of spawning and gonadal development in the Thai carp, *Barbonymus gonionotus*. The results indicated that injection of 10 mg/kg BUS in combination with 10 mg/kg DOM (in the form of the human preparation, Motilium) dissolved in distilled water, dimethylsulfoxide (DMSO) or N,N-dimethylformamide (DMFM) had similar effects on the number of fish spawned, fertilization, hatching and survival rates of seven-day-old fry ($p>0.05$) to those seen after the injection of one dose of pituitary gland. Repeated treatments with BUS in combination with all forms of DOM used in this study had no negative effect on gonad development in females, which was completed within three weeks of each spawning. Fish were induced to spawn at least three times during the season and the same was true of fish that were induced to spawn by repeated use of pituitary gland.

KEY WORDS: *Barbonymus gonionotus*, *Puntius gonionotus*, induced spawning, buserelin, domperidone

INTRODUCTION

In most teleost fish, including the Thai carp (*Barbonymus gonionotus*), a surge of gonadotropin (GtH II) secreted by the pituitary initiates the final maturation of gonads and ovulation, and triggers the onset of spawning. The hypothalamus regulates this surge through the interaction of two other hormones, gonadotropin-releasing hormone (GnRH) and dopamine (an agent that inhibits the release of GtH).

Fish reared artificially, will spawn if dosed with GnRH, or its one of its analogues (GnRHa), in combination with a dopamine antagonist (DA) (Peter *et al*. 1988). In Thailand, this proved a very effective, and reliable, way of inducing several freshwater fish species to spawn (Sukumasavin and Leelapatra 1988). Sukumasavin and Leelapatra (1993) also found that buserelin (BUS), a mammalian luteinising hormone, is the most effective of the several forms of GnRHa.

To date, domperidone (DOM) is the only DA used in spawning experiments. DOM is insoluble in water but dissolves in organic solvents such as DMSO (Gissis *et al*. 1991) and DMFM (Gissis *et al*. 1991). It is also partly soluble in propylene glycol (PROP). However, the most common practice is to use Motilium suspension (MOT), a DOM preparation designed for use in humans. However, the effect of repeated use of a BUS-DOM combination on the development the Thai carp's gonads is unknown. In this study, therefore, we set out to investigate the consequences of using BUS-DOM (in various

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preparations), and extract of pituitary gland (PG), over several spawning cycles.

MATERIALS AND METHODS

The study was carried out at Surin Inland Fisheries Station from February to September 2001.

Experimental fish

Thai carp were reared in earthen ponds at a stocking density of 1 kg/4 m^2 and fed once daily with pellets containing 30% protein at 1.5% body weight. The fish used in this experiment were ripe to spawn, i.e. females with 80% of their oocytes at the germinal vesicle migration stage and males that produced milt when gentle pressure is applied to the abdomen.

Hormone and drug preparation

BUS: Buserelin (Hoechst) was diluted in distilled water at a concentration of 100µg/ml.

DOM: Two types of DOM were used i.e. the pure chemical (Sigma) and the human drug, MOT (Jenssen Pharmaceutica). Pure DOM was dissolved in:

- DMSO (Sigma) at a concentration of 25 mg/ml (DMSO-DOM)
- DMFM (Sigma) at a concentration of 25 mg/ml (DMFM-DOM)
- PROP (Sigma) at a concentration of 25 mg/ml (PROP-DOM)

MOT: MOT was ground and dissolved in distilled water at a concentration of 25 mg/ml (MOT-DOM).

PG: PG, collected from mature common carp (average body weight 500 g) about one hour prior to injection was homogenised and mixed with distilled water. The injection volume was adjusted to 0.5 ml/ kg of fish. The dosage was determined using the following formula:

- 1 dose = weight of donor/weight of recipient

Experiments

Experiment 1: Effects of various forms of DOM in combination with BUS on inducing Thai carp to spawn.

Sexually mature female Thai carp weighing an average of 300 g were divided into five groups of 80 fish each. Fish in each group were identified using spaghetti tags. Each group received an intra-peritoneal injection of BUS and DOM as follows:

Group 1: 1 dose of PG

Group 2: 10µg/kg BUS in combination with 10 mg/kg MOT-DOM

Group 3: 10µg/kg BUS in combination with 10 mg/kg DMSO-DOM

Group 4: 10µg/kg BUS in combination with 10 mg/kg DMFM-DOM

Group 5: 10µg/kg BUS in combination with 10 mg/kg PROP-DOM

Following this treatment, the groups of female fish were transferred to 10 m^3 cement tanks containing the same number of untreated males. The fish then spawned naturally. The resultant eggs incubated in hatching jars and the rates of fertilisation and hatching were counted. Newly hatched larvae were moved to cement tanks. The following data were collected:

- spawning time
- number of fish that spawning during the 8 hr induction period
- fertilization rate
- hatching rate
- survival rate of larvae at seven days old

Experiment 2: Effects of various forms of DOM in combination with BUS on gonadal development in the Thai carp.

The fish resulting from the spawning during Experiment 1 were reared together in a 400 m^2 earthen pond. Every day each fish was fed with 30% protein pellets equivalent to 1.5% its body weight. On the first day, five fish were taken from each group; these were weighed, killed and their gonads removed. This procedure was repeated every seven days. The gonadosomatic index (GSI = gonad weight*100/ body weight) was calculated for each fish. After four weeks, the surviving fish were used to repeat Experiment 1. After the fish had spawned, Experiment 2 was repeated for another cycle.

RESULTS

Experiment 1: Effects of various forms of DOM in combination with BUS on the induction of spawning of the Thai carp (Appendix I, Tables 1, 2 and 3).

The form of DOM is not a critical factor in determining the number of fish that spawn (Table 1). The difference in the number of fish that spawned following injections of PG, of DOM in the form of MOT, or various preparations of DOM, is not statistically significant. This is true for all three induction-cycles. In each cycle, latency periods were all between 4.15 - 6.15 hours and there is no significant difference

($p>0.05$) in the rates of fertilisation and hatching, and the proportion of larvae that survived to seven days old.

Experiment 2: Effects of various forms of DOM in combination with BUS on gonad development in the Thai carp (Appendix I, Table 4)

Within spawning cycles, the GCI varied little between groups of fish that had been injected with PG, different preparations of DOM and DOM in the form of MOT. At the time of injection, the index varied within the range 17.02±0.42% (MOT-DOM) and 21.13±3.99% (PG). In all instances the index fell sharply after spawning (to within the range 5.56±0.68% (PG) to 7.8±1.8% (DMSO-DOM)); thereafter it increased until, by week three, the index returned to the levels recorded at the time of the first injection. This pattern repeated during the second and third spawning cycles. Overall, there was no statistically significant variation in the levels of the GSI within, or between, induction-cycles.

DISCUSSION

The results of this study show that BUS in combination with DOM is as efficient in inducing spawning in the Thai carp as PG (c.f. Sukumasavin and Leelapatra, 1988). Furthermore, BUS in combination with DOM dissolved in DMSO, DMFM or PROP did not affect the number of fish that spawned, fertilisation rate, hatching rate or survival rate of seven-day-old larvae when compared with PG or DOM in the form of the human preparation, MOT (Sukumasavin 1994).

Sirikul (1987) demonstrated that Thai carp induced to spawn with PG would, if induced again, spawn for a second time one month later and three or more times in a spawning season. In this study, Thai carp induced to spawn with BUS in combination with DOM, if induced again, also spawned at least three times in the spawning season at intervals of one month.

This study uses GSI as an indicator of gonadal development because in the Thai carp GSI is highly correlated with mature oocytes ($r=0.9$, $p<0.01$) (Sukumasavin and Leelapatra 1994). GSI has been used as an indicator for gonadal development in several cyprinids, including goldfish (Clemens and Reed 1967; Munkittrick and Leatherland 1984). The peak GSI at the beginning of the study was between 17% and 21%, similar to that of fish reared in earthen ponds reported by Sukumasavin (1992). Furthermore, three weeks after spawning the GSI of fish treated with all forms of DOM in combination with BUS were not significantly different. This indicates that the gonadal development in the Thai carp can complete within three weeks under culture conditions. In addition, there were no significant differences in GSI between the two reproductive cycles ($p>0.05$). The two sets experiments demonstrate repeated use of DOM in combination with BUS has no negative effect on the induction of spawning or gonadal development in the Thai carp.

Table 5 (Appendix I) gives the costs of using PG and BUS in all forms of DOM to induce spawning of 1 kg Thai carp. PG is the most expensive at 10 baht/kg. BUS and MOT-DOM cost 4.65 baht/kg while the costs for DMSO-DOM, DMFM-DOM or PROP-DOM are lower. PROP-DOM is the cheapest overall at

2.27 baht/kg. These results show that PROP-DOM is the most cost effective method of inducing the Thai carp to spawn.

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Table 1. *Effects of using 1 dose of PG and 10 µg/kg BUS in combination with 10 mg/kg DOM as MOT-DOM, DMSO-DOM, PROP-DOM, and DMFM-DOM on spawning induction in Thai carp at first spawning induction*

Hormone	N° of fish	Mean body weight (g±SD)	Latency period (hr)	N° of fish spawned	Fertilization rate (%)	Hatching rate (%)	Survival rate of 7-day-old fry (%)
PG	80	*294.63±23.38*	4.15	75	98.57±0.91	97.58±2.26	39.27±14.66
BUS+MOT-DOM	80	*315.11±07.15*	4.45	80	99.17±0.85	97.85±1.71	34.97±09.64
BUS+DMSO-DOM	80	*343.23±00.64*	4.45	80	98.42±1.39	97.83±1.14	52.33±12.79
BUS+PROP-DOM	80	*336.36±37.67*	4.45	80	98.65±0.35	96.65±2.05	35.50±09.99
BUS+DMFM-DOM	80	*341.47±02.08*	4.45	80	99.26±0.72	98.80±3.03	33.67±08.75

Table 2. *Effects of using 1 dose of PG and 10 µg/kg BUS in combination with 10 mg/kg DOM as MOT-DOM, DMSO-DOM, PROP-DOM and DMFM-DOM on spawning induction in Thai carp at second spawning induction*

Hormone	N° of fish	Mean body weight (g±SD)	Latency period (hr)	N° of fish spawned	Fertilization rate (%)	Hatching rate (%)	Survival rate of 7-day-old fry (%)
PG	40	367.47±53.41	4.15	35	95.01±1.06	91.88±2.75	37.60±05.34
BUS+MOT-DOM	40	382.03±59.78	4.50	40	96.59±2.48	92.59±2.38	37.30±10.08
BUS+DMSO-DOM	40	326.80±33.33	4.55	40	96.98±0.30	91.28±1.90	35.07±16.10
BUS+PROP-DOM	40	402.18±15.57	5.30	39	98.30±0.66	95.84±2.64	28.77±03.46
BUS+DMFM-DOM	40	359.84±37.37	4.50	39	95.01±3.79	94.89±3.03	33.77±04.31

Table 3. *Effects of using 1 dose of PG and 10 µg/kg BUS in combination with 10 mg/kg DOM as MOT-DOM, DMSO-DOM, PROP-DOM and DMFM-DOM on spawning induction in Thai carp at third spawning induction*

Hormone	N° of fish h	Mean body weight (g±SD)	Latency period (hr)	N° of fish spawned	Fertilization rate (%)	Hatching rate (%)	Survival rate of 7-day-old fry (%)
PG	5	373.49±73.45	5.50	4	95.67±1.15	99.67±0.58	42.03±16.10
BUS+MOT-DOM	5	371.03±40.79	5.55	5	96.59±2.48	99.17±1.04	35.40±05.01
BUS+DMSO-DOM	5	399.91±45.57	5.40	5	96.98±0.30	99.50±0.50	35.30±06.55
BUS+PROP-DOM	5	366.17±20.73	6.15	5	97.50±0.87	100.00±0.00	37.00±03.63
BUS+DMFM-DOM	5	410.91±50.62	6.15	5	98.80±0.34	100.00±0.00	30.83±01.26

Table 4. *Effects of using 1 dose of PG and 10 µg/kg BUS in combination with 10 mg/kg DOM as MOT-DOM, DMSO-DOM, PROP-DOM and DMFM-DOM on weekly changes in GSI during the three spawning cycles*

Time	PG	MOT-DOM	DMSO-DOM	PROP-DOM	DMFM-DOM
At 1st spawning induction	21.13±3.99^{d}	17.21± 0.42^{def}	20.01±0.18^{e}	19.99±3.32^{ef}	18.10±0.82b
After 1st spawning	5.56±0.68^{a}	5.84±0.72^{a}	7.48±1.76^{a}	6.43±1.51^{ab}	7.33±1.17a
1 week after 1st spawning	8.29±1.09^{a}	7.00±1.47^{ab}	9.46±1.07^{ab}	9.17±1.85^{bc}	8.34±2.02a
2 weeks after 1st spawning	13.78±1.27^{b}	12.06±1.94^{c}	13.17±1.68^{bc}	13.60±1.96^{d}	14.50±2.10b
3 weeks after 1st spawning	19.77±5.46^{cd}	17.67±2.75^{def}	18.52±2.22^{de}	16.68±2.00^{de}	14.79±4.19b
4 weeks after 1st spawning	20.84±3.25^{d}	20.71±2.44^{f}	20.60±4.80^{e}	18.93±2.09^{ef}	19.60±3.09b
At 2nd spawning induction					
After 2nd spawning	5.64±0.38^{a}	5.65±0.59^{a}	7.29±1.89^{a}	4.94±0.03^{a}	4.86±0.04a
1 week after 2nd spawning	8.80±2.19^{a}	8.47±1.38^{b}	10.06±1.47^{ab}	9.80±2.40^{c}	7.14±0.42a
2 weeks after 2nd spawning	17.13±2.30^{bcd}	15.83±1.35^{de}	14.47±2.72^{cd}	14.06±2.64^{d}	14.42±6.53b
3 weeks after 2nd spawning	14.58±4.36^{bc}	15.54±1.21^{d}	15.25±3.63^{cd}	19.08±4.19^{ef}	14.83±1.71b
4 weeks after 2nd spawning	21.55±2.78^{d}	19.28±4.02^{ef}	20.78±1.29^{e}	22.15±2.64^{f}	19.56±5.45b
At 3rd spawning induction					
After 3rd spawning	7.22±3.60^{a}	5.60± 0.59^{a}	7.02±2.53^{a}	7.64±1.99^{abc}	8.51±2.58a

Table 5. *Cost of induced spawning 1 kg of female Thai carp*

Hormone	Cost (baht/kg)
PG	10.00
BUS+MOT-DOM	4.65
BUS+DMSO-DOM	2.41
BUS+PROP-DOM	2.27
BUS+DMFM-DOM	2.30