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SHORT PAPER

Comparison of the Efficiency of Common Carp and Silver Carp Pituitary in the Breeding of Common Carp (*Cyprinus carpio*) and Northern Pike (*Esox lucius*)

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Abstract

Hormone preparations are used by fish farmers for artificial propagation of fish. In practice, acetone-dried common carp pituitary is the most commonly used agent to induce ovulation. In the last two decades the supply of glands has reduced and the market price has soared. The aim of our research was to compare the effects of silver carp pituitary (SCP) to those of common carp pituitary (CCP) on different reproductive traits. Experiments were performed over the large-scale breeding of northern pike (*Esox lucius*) and common carp (*Cyprinus carpio*). Ovulation rate of northern pike females for both groups was 70.4% in the first experimental year. In the second year, ovulation rates were 94.4% and 87% for the CCP and SCP groups, respectively. The relative amounts of stripped eggs (PGSI) for the two groups were similar (14.3%) in the first year. In the second year PGSI values were 18.7±3.43% and 20.1±3.92% for the CCP and SCP groups, respectively. Fertilization rates for the two groups were similar in both experimental years. Ovulation rates for common carp females induced by CCP and SCP were 66.7% and 100%, respectively. In the CCP group PGSI was 13.7±5.49%, while in the SCP group is was 14.5±2.32%. With the exception of ovulation rate of common carp, all reproductive traits for the two treatments were statistically similar. According to our research, SCP could be suitable to have wide application for induced ovulation of fish in the future.

Keywords: Fish pituitary, induced breeding, common carp, northern pike.

Introduction

For many years fish farmers and scientists have been using hormone preparations for the artificial propagation of carps and other fish species. In practice, acetone-dried common carp (*Cyprinus carpio*) pituitary is the most commonly used agent to induce ovulation (i.e. hypophysation technique). This small organ, which contains the active hormone (gonadotropin), is collected from mixed populations of marketable carp in temperate climate (usually 3^+ year old fish).

The pituitaries (weighing around 3.0 mg each) are marketed in different forms. In the simplest form, the freshly dissected and dehydrated glands are stored whole at room temperature until required (Woynárovich and Horváth, 1980). Additional forms of the hormone include partially to fully purified gonadotropins prepared from pituitary glands (Donaldson and Hunter, 1983). However, these preparations are poorly suited to direct application in fish culture as they are packaged in given weights. Once the preparation has been dissolved in water or saline, the active ingredient is only active for a few hours. Therefore breeders have to adapt their breeding project around the packaged amounts. Other disadvantages of purified preparations are their restricted availability and relatively high price.

Apart from these difficulties, it has become increasingly difficult to purchase pituitary glands. In recent years, following the political and economic changes in Eastern Europe, many big fish processing factories have closed or been divided up. This has reduced the supply of glands from the region; world supply has been reduced, and the market price has soared.

A relatively new development in the technology of induced breeding is the stimulation of endogenous gonadotropin release from the pituitary of the treated fish by the use of a synthetic analogue of gonadotropin releasing hormone (GnRH) (Mylonas *et al.*, 2010). However, the injection of GnRH analogues alone is generally ineffective in inducing ovulation in cyprinid fish species where there is a strong dopamine inhibitory tone on gonadotropin secretion (Chang and Peter, 1983). To facilitate the gonadotropin releasing

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activity of the GnRH analogue, it is combined with a dopamine receptor antagonist (Peter *et al.*, 1988).

This method is gaining acceptance throughout the world, still lots of fish breeders refuse to adopt this new method because application techniques differ from hypophysation that has been used for decades. These differences include: 1) how the dosage for the brood stock is calculated, 2) how the administered agent is prepared and 3) the occasional combination of GnRH analogue-treatment with a dopamine receptor antagonist injection. It should also be noted that the latency period between treatment and ovulation is shorter and the time of ovulation is more predictable after the pituitary treatment (Brzuska, 2005; Kucharczyk *et al.*, 2005). In contrast, ovulation in females is rather unsynchronized after GnRH analogue treatment which makes difficult to organize work in the hatchery.

The culture of Chinese major carps, namely the silver carp (*Hypophthalmichthys molitrix*), the bighead carp (*Aristichthys nobilis*) and the grass carp (*Ctenopharyngodon idella*) has expanded steadily over the last three decades. According to the 2012 FAO database, silver carp has the largest annual production in freshwater aquaculture globally exceeding 5.3 million tons in 2011.

The aim of our research was to investigate weather the pituitary of silver carp processed in large quantities in fish processing factories is also suitable for the hatchery breeding of fish. Therefore, we compared the effects of silver carp pituitary to those of common carp pituitary on different reproductive traits. Experiments were performed over the large-scale breeding of northern pike (*Esox lucius*) and common carp. Common carp as a domesticated fish species is presently cultured in most parts of Europe, all over Asia, and on a small scale in some countries of Africa and Latin America. Common carp ranks 3rd among all cultured freshwater fish with its production reaching 3.8 million tons in 2012 (2012 FAO database). Northern pike is the most characteristic freshwater piscivorous fish in Europe and play an important ecological role by controlling populations of mixed fish species in natural waters. Northern pike are also one of the most desirable sport fish with high economic value.

Materials and methods

The research were carried out at the Dinnyés Fish Hatchery and Farm in Hungary. Spawning experiments on northern pike were conducted in two consecutive breeding seasons of 2011 and 2012. In 2011 brood fish was bought by the farm management from different sources right before propagation. The size of the fish as well as their conditions were variable among individuals. Before the breeding season of 2012 brood stock were wintered and prepared for propagation for three months on the farm, therefore their general conditions and reproductive stages were similar.

One day before spawning induction northern pike were transferred to the hatchery and placed in 4.0 m^3 concrete tanks with running water at 11±1.0°C. Each season females (2 to 4 kg body weight (BW) were randomly allocated to two treatment groups. In the two treatment groups females received an injection of a crude preparation of dried common carp or silver carp pituitary, respectively, at a dose of 3.5 mg kg^{-1} BW. Pituitaries were administered in a vehicle of a 2.5% aqueous dispersion of Carbopol resin (Szabó, 2008). Injections were applied intraperitoneally at a volume of 0.5 ml kg^{-1} BW. Stripping of females was conducted on the fourth day after injection.

In order to ensure adequate sperm production, all males received a single injection of dried carp pituitary administered in 0.65% NaCl saline at a dose of 3 mg kg^{-1} BW. The testes were surgically removed and sperm was obtained by squeezing the organs through cheesecloth. This required sacrificing the males but enhanced sperm yield.

Experiments on common carp were carried out in the breeding season of 2011. For propagation of common carp, the standard technology of induced spawning has been applied (Woynárovich and Horváth, 1980). In the two treatment groups, each placed in 4.0 m^3 concrete tanks with running water, females received an injection of a crude preparation of dried common carp or silver carp pituitary, respectively. For both groups 4.0 mg kg^{-1} BW of resolving dose of pituitary was preceded by a priming dose of 0.5 mg kg^{-1} BW. The interval between the two doses was approximately 12 hours. The temperature of water was 22±0.5°C during ripening. Stripping of females was conducted twelve hours after the second injection.

Assessment of ovulation for both species was carried out by determining the ovulation ratio (number of ovulated females / number injected) and by the pseudo-gonadosomatic index (PGSI) calculated as follows calculated as follows: (weight of stripped egg mass / body weight before stripping) × 100. The spawning ratio was analyzed by the chi-square test ($P<0.05$). PGSI data were analyzed by one way ANOVA ($P<0.05$).

For northern pike fertilization rate of the stripped eggs were also determined. Eggs from each female were collected into a separate bowl. Eggs in each bowl were evenly mixed with an ample amount of milt. Gametes were activated by adding an appropriate amount of water. An egg batch (about 200 eggs) was taken as a sample to determine the fertilization rate. Egg batches were incubated separately at 11±1.0°C. Fertilization rates were established 12 to 24 hours after fertilization, when eggs were in the morula stage. Fertilization rate data from the two treatment groups were compared with ANOVA.

Results and Discussion

The results from experiments with northern pike are summarized in Table 1. The mean PGSI and mean fertilization rate values were similar in the two treatment groups in both experimental years ($P<0.05$, ANOVA). Ovulation rates as determined by the chi-square test were also similar between the groups treated with common carp or silver carp pituitary ($P<0.05$).

In earlier studies, neither mammalian GnRH analogue ([D-Ala^6, Pro^{9}Net]-mGnRH) nor salmon GnRH analogue ([D-Arg^6,Pro^9-NEt]-sGnRH) administered alone or together with dopamine receptor antagonists induced ovulation in northern pike females (Szabó, 2003). Human chorionic gonadotropin (hCG) treatments were also ineffective (personal observations). At present, carp pituitary is the only reliable agent to induce ovulation in northern pike females.

In this study, the results of the investigated traits are comparable to those from earlier ovulatory experiments on northern pike (Szabó, 2001; 2008). However, differences between the two breeding seasons are remarkable. All investigated traits characterizing the efficacy of induced breeding were higher in 2012 than in 2011. The reason for that was probably the differences between the conditions of brood fish used for propagation in the two breeding seasons. These results proved that professional wintering is essential for successful propagation of northern pike.

Data on ovulation ratio and egg production of common carp are shown in Table 2. In the group receiving silver carp pituitary all the fish ovulated, while 66.7 % of females responded after the common carp pituitary treatment. The mean PGSI values were similar between the two groups. These results are comparable to long-term data on the induced spawning of common carp at a large-scale hatchery production (Szabó *et al.*, 2000). They are also similar to the data from ovulatory experiments on common carp carried out in the spawning season period (Kucharczyk *et al.*, 2008). In the technology of induced breeding of common carp, GnRH analogue treatmen is gaining acceptance throughout the world. Different GnRH analogues combined with dopamine receptor antagonists proved to be effective in inducing final oocyte maturation and ovulation (Peter *et al.*, 1988). Different commercial preparations containing GnRH analogues and dopamine receptor antagonists were also successfuly tested (Horváth *et al.*, 1997; Brzuska, 2001; Yaron *et al*., 2009). hCG, however, is only marginally effective in inducing ovulation, requiring repeated injections of relatively large doses (Yaron *et al*., 2009).

Table 1. Effects on ovulation and fertilization rate in northern pike following intraperitoneal injection with a crude preparation of dried common carp or silver carp pituitary. Pituitary was administered in a 2.5% aqueous dispersion of Carbopol resin at a dose of 3.5 mg kg^{-1} BW for both groups. All females received a single injection at a volume of 0.5 ml kg^{-1} BW

	2011		2012	
	Common carp pituitary	Silver carp pituitary	Common carp pituitary	Silver carp pituitary
BW (mean ± SD)	2192±926	1623±630	2491±869	2617±810
Ovulation rate	19/27	19/27	18/19	20/23
PGSI (mean±SD)	14.5±5.20	14.3±5.54	18.7±3.43	20.1±3.92
Fertilization rate (mean±SD)	53.4±8.87	43.7±8.43	57.5±7.83	63.6±17.0

BW: body weight of females at the time of injection (grams)
Ovulation rate: number of ovulated females / number injected
PGSI: (weight of stripped egg mass / BW of the female before stripping) × 100
The mean PGSI and mean fertilization rate values were similar in the two treatment groups in both experimental year ($P<0.05$, ANOVA).
Ovulation rates as determined by the chi-square test were also similar in the treatment groups ($P<0.05$)

Table 2. Effects on ovulation and fertilization rate in common carp following intraperitoneal injection with a crude preparation of dried common carp or silver carp pituitary. Pituitary was administered in a saline at a dose of 3.5 mg kg^{-1} BW for both groups. All females received a single injection at a volume of 0.5 mL kg^{-1} BW

	Common carp pituitary	Silver carp pituitary
BW (mean ± SD)	7037±2457	7286±3402
Ovulation rate	18/27	7/7
PGSI (mean±SD)	13.7±5.49	14.5±2.32

BW: body weight of females at the time of injection (grams)
Ovulation rate: number of ovulated females / number injected
PGSI: (weight of stripped egg mass / BW of the female before stripping) × 100
Ovulation rates as determined by the chi-square test were higher for the group treated with silver carp pituitary ($P < 0.05$). The mean PGSI values were similar in the two treatment groups ($P<0.05$, ANOVA).

In our study, with the exception of ovulation rate of common carp, all reproductive traits for the two treatments were statistically similar in both northern pike and common carp. The most dominant fish species in freshwater aquaculture is silver carp and the production yield of this species continue to increase steadily (Olsen, 2011). By combining silver carp processing in fish factories with pituitary collection, the supply of glands could be increased securing an inexpensive source for induced breeding. According to our research, silver carp pituitary could be suitable to have wide application for induced ovulation of fish in the future.

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