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Use of Pineapple Juice for Elimination of Egg Stickiness of Common Carp (*Cyprinus carpio* L.)

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Abstract

Four treatments including three different concentrations of pineapple solution (1%, 3%, 5%) and the traditional method (salt/urea/tannin solutions) were used to eliminate egg stickiness of common carp. Using the traditional method, fertilization and hatching rates were $74.5\% \pm 1.2$ and $70.2\% \pm 1.1$, respectively. The highest fertilization rate ($89.3\% \pm 0.7$) and hatching rate ($86.6\% \pm 1.4$) were found with treatment of 1% pineapple juice solution and was significantly different from those obtained with other treatments ($P < 0.05$). Using pineapple juice for desticking common carp eggs also reduced the time of egg handling from almost one hour required with the traditional method, to about three minutes, as well as increasing fertilization and hatching rate under hatchery conditions.

Introduction

Common carp (*Cyprinus carpio* L.) is one of the most important cultured fish in the world, with an annual production of nearly three million metric tons (FAO 2001). Although artificial propagation methods of common carp have been presented by numerous authors (Rottmann et al. 1991, Horvath et al. 2002, Billar 1999), applying those methods in hatcheries with poor facilities was not always successful.

One problem encountered in hatchery incubations of common carp is egg stickiness, which need to be reduced before eggs can be incubated

successfully (Rottmann et al. 1991, Horvath et al. 2002). Normally, eggs stickiness is reduced by using traditional methods, such as treating with a solution of sodium chloride and carbamide (40 g of urea and 30 g of salt dissolved in 10 l clean water), followed by a tannin solution (5 g tannin in 10 l of clean water; Rottmann et al. 1991, Billard 1999). However, this method requires significant labor and experience, and requires at least one hour.

Pineapple is commonly found in many tropical countries. Pineapple juice contains an abundance of proteolytic enzymes, principally bromalinases which can digest protein, (Michael 2001). Proteolytic enzymes have been used successfully for the elimination of egg stickiness of tench in hatcheries (Linhart et al. 2002).

This paper presents a quick, cheap and a simple method of elimination egg stickiness of common carp, as well as increasing fertilization and hatching rates under specific hatchery conditions.

Material and Methods

The experiment was conducted in the Melinh Freshwater Fish Research Station of the Research Institute for Aquaculture No1 (RIA 1), Vietnam. Artificial propagation methods were performed following those outlined by Billar (1999). Six pairs of mature broodstock were chosen from ponds. Male and female fish were kept in separate tanks.

Component Luteinizing Hormone Release Hormone analogue (LHRHa) and domperidone (DOM) were utilized to stimulate spawning of broodstock with a single injection; 25 μg LHRHa and 5 mg DOM. $\cdot\text{kg}^{-1}$ and 8 μg LHRHa and 1mg DOM. $\cdot\text{kg}^{-1}$. Water temperature and dissolved oxygen concentrations were 26°C and was 6 mg l^{-1} , respectively. Fish were checked for ovulation by a slight pressure to the abdomen. The checking began seven h after the injection and continued every hour up to the time of extrusion of eggs. Eggs and milt were stripped in dry batches at the same time and mixed with 100 μl of milt per 40 g of eggs for 30 sec. After fertilization, eggs from each batch were divided into four independent treatments consisting of 1%, 3%, 5% pineapple solutions and the traditional method of salt/urea/tannin (Table 1).

Pineapple juice was prepared by squeezing peeled fresh fruit. Solutions were made up at 1% (100 ml juice to 10 l clean water), 3% and 5%. A small volume of solution was first poured over the eggs and stirred continuously with a feather for about 1 min. Then while stirring continuously sufficient solution was added to just cover the eggs, for a further 1 min and the supernatant decanted. The procedure was repeated with fresh juice and the eggs washed with clean hatchery water and maintained in hatchery Weiss jars in running water. The traditional treatment methods (salt/urea/ tannin) (Horvath et al. 2002) were carried out for 1 h.

Five hundreds eggs, randomly selected from each treatment were incubated in separate Weiss jars with flow-through water (rate of 9.0 l $\cdot\text{min}^{-1}$, at 26.5°C and 7 $\text{mg}\cdot\text{l}^{-1}$ O₂). Each treatment was triplicated. Fertilization rates

were calculated for all treatment after 12 h incubation period. The developing eggs were counted under a magnifying glass. After three days, larvae were collected from each treatment for estimating hatching rate.

The means values of the data acquired were estimated from three replicates and statistical significances determined using ANOVA. Paired comparisons of mean hatching and fertilization rate were made using LSD test between treatments.

Results

All treatments reduced the stickiness of the eggs. Treatment 2 with 1% pineapple juice showed the highest fertilization rate ($89.3\% \pm 0.7$) and hatching rate ($86.6\% \pm 1.4$), and was significantly different from the other treatments ($P < 0.05$) (Table 2). Fertilization rate ($74.5\% \pm 1.2$) and hatching rate ($70.2\% \pm 1.1$) in the traditional treatment (salt/urea/tannin) were higher than those of treatment 3 (3% of juice) and treatment 4 (5% of juice).

The experiment indicated that the application of pineapple juice for desticking of common carp eggs reduces egg handling time from almost one hour, as necessary for conventional desticking, to just 3 minutes.

Discussion

Reducing the stickiness of eggs is a very important procedure in controlled artificial reproduction in freshwater aquaculture. It improves hatching rate of fish under hatchery conditions (Rottmann et al. 1991). Elimination of egg stickiness of common carp is traditionally carried out by using salt, urea and tannin. However, this method required handling the eggs for one hour

Table 1. The weight of females, mean weight of egg and the approximate number of eggs used in each treatment

Female (g)	Mean egg Wt. (mg)	No. of eggs
1700	1.7	51,000
1250	1.5	34,375
1000	1.2	25,000
1510	1.5	41,148
1100	1.2	28,875

Table 2. Mean (\pm SD) fertilization and hatching rate of common carp egg treated using traditional method and different concentration pineapple juice

Treatment	Fertilization rate (%)	Hatching rate (%)
Sal/urea/tannin	74.5 ± 1.2^a	70.2 ± 1.1^a
Pineapple (1%)	89.3 ± 0.7^b	86.6 ± 1.4^b
Pineapple (3%)	52.4 ± 3.4^c	47.4 ± 1.5^c
Pineapple (5%)	28.1 ± 1.9^d	25.3 ± 2.9^d

Different superscripts within rows indicate significant differences ($P < 0.05$)

and treatment with two different solutions. Moreover, the tannin solution is very toxic to the eggs and contact time is very critical, contact exceeding 20 seconds being toxic (Horvath et al. 2002). Billar (1999) suggested an alternative technique egg and milk mixtures in Zoug jars through which air is bubbled. However, this technique is also disadvantaged as it requires extensive egg handling (> 1 hour).

Desticking of eggs using pineapple juice is quick and simple and required 3 min only. In this experiment, 1% of pineapple juice was the most effective. This technique not only increases fertilization and hatching rates, but has the advantage over conventional procedures by dramatically reducing egg handling period.

Eliminating stickiness of eggs by using pineapple juice solutions is also cost effective; one million eggs can be treated with 400ml pineapple juice at a cost of only \$US 1. This method does not affect growth and survival rates of larvae and fingerlings (Hoang, pers.comm, 1998 to 2003).

In conclusion, pineapple juice solution effectively reduces stickiness of common carp eggs. This method is now popular in hatcheries in Vietnam because of ease of adoptions by rural farmers. However, the influence of temperature on the time of egg treatment needs to be further researched.

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