USE OF CHEMOPROPHYLACTIC AGENTS IN THE MANAGEMENT OF *Clarias gariepinus* SEED PRODUCTION

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Abstract
Some chemotherapeutic agents and procedures haveshown toindividually enhance fish egg hatchability. This paper reviews the use of such disinfectants (iodine, formaldehyde and hydrogen peroxide), antibiotics (ciprofloxacin-metronidazole), procedure (egg washing) and their combinations on Clarias gariepinus hatchability. One thousand eight hundred each of washed and unwashed fertilised eggs obtained from both parenteral, prophylactic ciprofloxacin-metronidazole treated (each 10mg/kg) and untreated broodstock, were disinfected by bath for 15 minutes in three recommended disinfectants: hydrogen peroxide (H_2O_2; 83.3, 166.7 and 250 ppm), povidone-iodine (33.3, 66.7 and 100 ppm) and formaldehyde (333, 667 and 1,000 ppm), incubated at 26°C and hatchability determined. Appropriate controls were set up, data analysed using standard tests at $\alpha_{0.05}$. Hatchability significantly improved with egg washing (40.1%), antibiotics (39.8%), 250 ppm H_2O_2 disinfection (23.5%), over the control. The combination of egg washing and 166.7 ppm H_2O_2 disinfection, antibiotics and 83.3 ppm H_2O_2 disinfection; antibiotics and egg washing improved hatchability by 53.4%, 50.8% and 46.7% over the control, respectively. Antibiotics, egg washing, disinfection with 83.3 ppm H_2O_2 combination gave the highest hatching rate of 76.8%. To prevent drug resistance, egg washing and166.7 ppm H_2O_2 disinfection combination is recommended for routine Clarias gariepinus egg management.
Keywords: *Clarias gariepinus,* egg management, hydrogen peroxide, egg washing

Introduction

*Clarias gariepinus***,** a major freshwater fish in Nigeria, is of growing economic value in African aquaculture industry (FDF, 2007, Goda*et al*., 2007; Osman *et al.,* 2007).According to Macharia *et al*.,(2005), the hatching rates of *Clarias gariepinus* eggs in many hatcheries in Africa are erratic, ranging from 8.0 to 70%, depending on the degree of sophistication of the management of the hatcheries. Since hatchery production is the fundamental means towards sustainable fish production, it is tenable that tackling hatchery-related diseases will boost fish production.

There are four principal means of managing fish diseases vis-à-vis the use of antibiotics (Zaki *et al.*, 2012), essential oils (Bakkali *et al.*, 2008), probiotics (Chen and Hoover, 2006; Arioleand Okpokwasili, 2012) and phage therapy (Nakai *et al.*, 1999; Almeida *et al*, 2009). Chemicals such as common egg disinfectants may also be engaged in hatchery egg management (Small, 2006; Rasowo *et al.*, 2007). Quite a number of anti-bacterial chemicals (antibiotics and disinfectants) and procedures have been identified for egg disease management (Small, 2006; Adeyemo *et al*., 2012). However, there are scanty studies that compared the use of such chemicals and their combinations in fish egg management.

Consequently, a study was carried out to determine the single and combined effects of some common hatchery disinfectants (povidone-iodine, hydrogen peroxide and formaldehyde), parenteral prophylactic antibiotics (ciprofloxacin-metronidazole) and egg-washing in the management of *Clarias gariepinus* eggs.

Methodology

A study was carried out to evaluate the effects of parenteral prophylactic antibiotics on broodstock used for larval production, the use of topical egg disinfectants and egg washing on fertilized eggs. These studies were considered singly and jointly, and the effects assessed on percentage hatchability.

Chemo-prophylactic management of *Clarias gariepinus*

Two sets of fertilized eggs were obtained for the experiment from the two pairs of broodstock used – prophylactically treated (10mg/kg ciprofloxacin and 10mg/kg metronidazole intra-muscularly) and

untreated broodstock. Using a graded dropper, 0.2ml of (~50) eggs obtained from each setof fertilized eggs was transferred into 40 Petri-dishes i.e. 40dishes of fertilized eggs each for treated and untreated broodstock. Each of the two sets of 40 Petri-disheswere arranged in duplicates of three disinfectant treatments of three doses each, and controls (that is 36 Petri-dishes for treatments and 4 Petri-dishes for control). Each treatment contained duplicates (those washed and unwashed after fertilization) of three disinfectant doses of a third, two-thirds and full recommended dose of each disinfectant (that is 33.3%, 66.7% and 100% of 100ppm of povidone-iodine, 250ppm of hydrogen peroxide and 1,000ppm of formaldehyde), as illustrated in figure 1. The fertilized eggs were disinfected for 15minutes, washed with adjusted (pH, 7.2 and water hardness, 60mg/l) distilled water and incubated within the Petri-dishes at 26ºC for 24 hours. Percentage hatchability of each test factor and their combinations were compared with the controls (that is non-disinfected eggs obtained from treated and untreated broodstock).

Statistical analysis

The data obtained from the results were subjected to statistical analysis, using appropriate tests that included ANOVA and Tukey-Kramer Multiple Comparisons Test. This was performed with the aid of an online software tagged GraphPadInstat 3 (GraphPad software Inc., La Jolia, CA, USA).

P values less than 5% (<0.05) were considered significant, and means were all reported with a standard error of the mean (SEM).

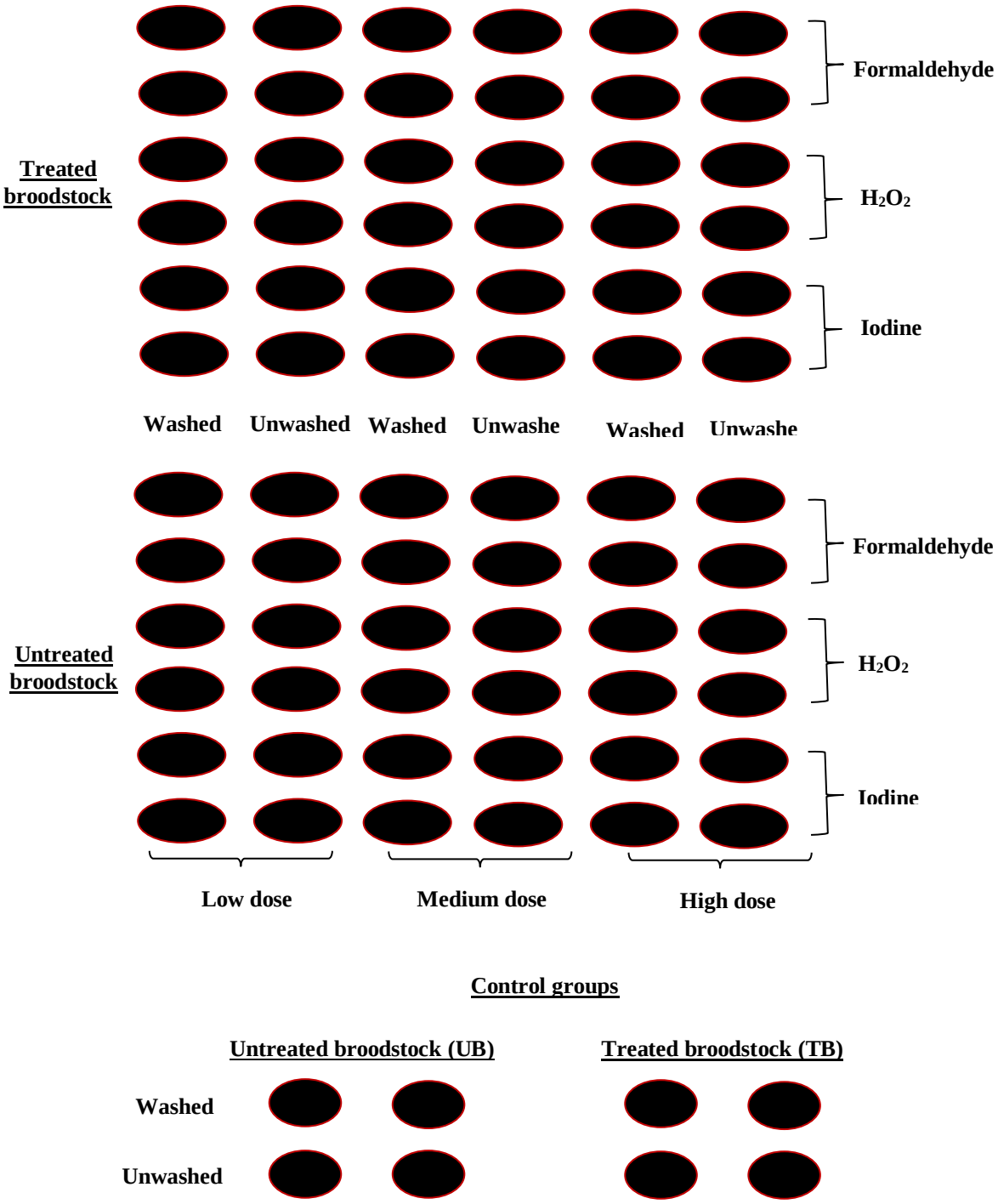

Figure 1: Illustration showing the application of various treatments on egg hatchability

Results

The results of the effects of washing fertilized egg immediately after fertilization, egg disinfection using varied concentrations of recommended disinfectants (povidone-iodine, hydrogen peroxide and formaldehyde), prophylactic treatment of broodstock prior to spawning, and their combinations on egg hatchability, are presented in Tables 1 and 2.

The mean percentage hatchability of the control (UB) was observed to be significantly higher ($P < 0.05$) than those of iodine-treated and formalin-treated eggs, with or without the eggs being washed. With hydrogen peroxide, a high level of significance ($P < 0.05$) exists over the use of iodine and formaldehyde as egg disinfectant. The mean effect of graded doses (83.3ppm, 166.7ppm and 250ppm) of hydrogen peroxide as a single treatment of *Clarias gariepinus* eggs on hatchability was observed to boost hatching by 13.7%, 8.6% and 23.5% over the control (UB C1). When washed eggs were disinfected with the varying concentrations of disinfectant (83.3ppm, 166.7ppm and 250ppm of hydrogen peroxide), increment of 18.4%, 41.2% and 14.0% was recorded over those that were unwashed but disinfected, and 34.5%, 53.4% and 40.8% over the untreated and unwashed control (UB C1), The percentage increase on hatchability of hydrogen peroxide-treated eggs was significant over the control (UB C1), iodine-treated and formaldehyde-treated eggs but insignificantly different ($P > 0.05$) from that of washed eggs (UB C2).

Prophylactic antibiotictreatment of broodstock (AB C1) produced an increase of 39.8% hatchability over the untreated broodstock (UB C1). However, the combination of antibiotic treatment and egg washing resulted in an increment of 46.7% over the control UB C1 (untreated, not disinfected and unwashed control), but mean hatchability increased inconsequentially by 4.7% and 5.0% above control UB C2 (untreated but washed) and control AB C1 (antibiotic treated broodstock and unwashed eggs). The combinations of broodstock antibiotic therapy and 83.3 ppm hydrogen peroxide disinfection, and antibiotic therapy and 33.3 ppm povidone-iodine disinfection brought about 50.8% and 49.8% rise in mean hatchability, over the control UB C1, respectively. An increment of 53.8% and 54.2% was recorded when the above combinations were further pooled with egg-washing (that is broodstock antibiotic therapy, egg disinfection and egg-washing) over control UB C1, as presented in Tables 1.

Washing of fertilized eggs, as observed in the controlled experiment, significantly ($P < 0.05$) improved percentage hatchability over the unwashed counterpart (control, UB C1) by 40.1%, but hatchability was slightly increased over that of the unwashed eggs obtained from treated broodstock (AB C1) by 5%.

The results of the varied treatment combinations when compared with the control (UB C1) are given as: hydrogen peroxidedisinfection and egg-washing; antibiotic treatment and hydrogen peroxidedisinfection; antibiotic treatment and egg-washing, and antibiotic treatment, hydrogen peroxidedisinfection and egg-washing improved hatchability by 53.4%, 50.8%; 46.7% and 54.2%, respectively (Table 2).

Table 1: Mean percentage hatchability of egg obtained from treated broodstock, egg-washing and disinfection

Group Tx		Untreated broodstock			Treated broodstock		
		Iodine	H_2O_2	Formalin	Iodine	H_2O_2	Formalin
High dose	NR	8.3	61.5	1.9	1.0	-	20.1
	R	13.5	70.1	-	16.3	60.1	36.3
Medium dose	NR	19.8	54.1	9.9	38.0	69.3	63.3
	R	46.6	76.4	29.7	76.0	69.3	75.1
Low dose	NR	38.8	56.6	44.3	74.6	75.1	62.8
	R	49.7	67.0	62.3	76.6	76.8	53.8

Control				
	Untreated broodstock, UB		Antibiotic treated broodstock, AB	
Unwashed control, C1	46.7	52.9	70.0	69.2
Washed control, C2	65.8	73.7	71.1	75.0
Mean control values	49.80 / 69.75 (59.8)		69.60 / 73.05 (71.3)	

Key
Dash – represents clumped eggs with poor hatchability assessment.
NR represents unwashed fertilized eggs
R represents washed fertilized eggs

Table 2: Percentage difference of treatments to control

S/No	Treatment	Increase% hatchability over control
1	Prophylactic antibiotic treatment of broodstock	39.8% increase in hatchability
2	H_2O_2 disinfection	23.5% increase in hatchability
3	Egg-washing	40.1% increase in hatchability
4	Prophylactic antibiotic treatment of broodstock + H_2O_2 disinfection	50.8% increase in hatchability
5	Prophylactic antibiotic treatment of broodstock + egg-washing	46.7% increase in hatchability
6	Hydrogen peroxidedisinfection + egg-washing	53.4% increase in hatchability
7	Prophylactic antibiotic treatment of broodstock + H_2O_2 disinfection + egg-washing	54.2% increase in hatchability

Key
H_2O_2 =hydrogen peroxide

Discussion

Broodstock chemotherapy using broad spectrum antibiotics at the time of hormonal injection was observed to improve percentage egg hatchability by 39.8% over the control. When broodstock antibiotic therapy was combined with egg disinfection, a 50.8% (hydrogen peroxide) and 49.8% (povidone-iodine) rise in hatchability was achieved over the control, and an increment of 53.8% and 54.2% when further combined with egg-washing, respectively. This observation suggests that the use of antibiotics is of importance in fish health management, though its use needs to be guided to avoid drug resistance (Subasinghe, 2009; Efuntoye*et al*., 2012).

Hatchability was observed to be significantly ($P < 0.05$) reduced when common fish egg disinfectants, such as povidone-iodine and formaldehyde, were used to disinfect *Clarias gariepinus*eggs, though recommended for use in catfish (Small, 2006) hatcheries. This observation showed that *Clarias gariepinus*eggs are sensitive to formaldehyde (ilih and Adebayo, 2010; Adeyemo*et al*, 2012) and povidone-iodine (Stuart *et al*., 2010) at concentrations of 333.3 - 1,000ppm and 33.3 - 100ppm, respectively. On the other hand, hydrogen peroxide application as single treatment in *Clarias gariepinus* egg management at 83.3ppm, 166.7ppm and 250ppm produced increment of 13.7%, 8.6% and 23.5% hatchability, respectively. This suggests the positive effect of hydrogen peroxide as a disinfectant, within 83.3 - 250 ppm, on hatchability. Thus, hydrogen peroxide may be considered as an effective egg disinfectant within the specified dosage.

Washing of fertilized fish eggs prior to incubation was observed to significantly ($P < 0.05$) improve egg hatchability when compared to the unwashed control. Washing of fertilized eggs alone increased hatchability by 40.1%, while a 53.4% improvement in hatchability was recorded with hydrogen peroxide disinfection of the washed fertile eggs. The logical reason for such an increase in the hatching rate of washed fertilized eggs could be the reduction in egg-surface microbiota population (Hansen and Olafsen, 1989; Sauteret al., 2006), which is expected to improve the viability of such eggs. This suggests that the act of washing fertilized eggs before incubation should be encouraged to boost productivity.

It was observed that the combination of the three treatments (broodstock antibiotic therapy, egg-washing and disinfection) improved hatchability more than individual applications. The combinations of hydrogen peroxidedisinfection and egg-washing, antibiotic treatment and hydrogen peroxide disinfection, antibiotic treatment and egg-washing and antibiotic treatment, hydrogen peroxide disinfection and egg-washing improved hatchability by 53.4%, 50.8%; 46.7% and 54.2% over the untreated control, respectively. The result suggests that hydrogen peroxide disinfection and egg-washing treatment may be routinely used for preventive hatchery management, while hatchery disease management may be carefully handled using the three combined treatment – broodstock antibiotic treatment and disinfecting washed eggs with hydrogen peroxide.

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