Study of Bacterial Loading in Macrophage Cells of Hybrid Catfish

T. Somsiri [1], M. Crumlish [2], K. Thompson[2], Y. Dumrongphol[1] and A. Panbanpeaw[1]

[1] : Aquatic Animal Health Research Institute, Bangkok Thailand

[2] : Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland

Abstract

Hybrid catfish (*Clarias macrocephalus* x *Clarias gariepinus*) farming has developed very successfully in Thailand over the last 10 years. Farmers, however, are still encountering disease problems and as a result, face economic loss. Some disease problems are associated with inadequate farm management practices. This study was carried out to investigate the presence of bacteria in macrophage cells isolated from hybrid catfish cultured in different environmental conditions. Results from farm investigations showed that the percentage of macrophages containing intracellular bacteria was related to particular water quality parameters.

Experimental trials were conducted using high stocking density as stressor and bath challenge with pathogenic *Aeromonas hydrophila* . The results showed that the fish kept in high density and bath challenged with bacteria had a higher percentage of macrophages containing bacteria than those kept under normal conditions. The presence of bacterial loading in macrophage cells is therefore considered to be an indication of the health of the fish.

Key words : Macrophage, hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*).

Introduction

Presently one of the most commonly cultured fresh water fish species in Thailand is hybrid catfish (*Clarias macrocephalus x Clarias gariepinus*). Catfish are particularly popular due to their short culture cycle, high growth rate and comparatively high tolerance to low water quality and low water volumes. They give a high yield and command high market price. (Wattanutchariya *et al.* 1982) However, disease problems and high mortality reduces production efficiency. High mortality and disease may well be related to the deterioration of water quality due to the presence of uneaten decaying feed, which is commonly found in *Clarias* ponds (Tonguthai *et al.* 1993).

This study was carried out to investigate the presence of bacteria in macrophage cells isolated from hybrid catfish cultured in different environments. It was hypothesized that the presence of intracellular bacteria may be associated with the health of the fish, and could be a useful indicator of fish health.

Materials and Methods

Fish

Hybrid catfish (*Clarias macrocephalus x Clarias gariepinus)*, weight 80 grams, were collected from farms with poor management (poor water quality including high level of ammonia and hydrogen sulfide, no water change and fed with chicken carcass daily). Macrophage cell (T_0) was examined immediately at the time of fish had arrived laboratory. Bacteria had been found in macrophage cell in head kidney taken from sample fish. Fish were kept in clean water tanks at $28^{O}C$ for 1 week, and were not fed during the experimental period.

Macrophage Separation

Monolayers of macrophages were prepared from suspensions of macerated whole head kidneys of one fish (filtered through a 100 μm mesh) using L-15 medium supplemented with heparin and 2% catfish serum. The kidney homogenate suspensions were dropped onto cleaned microscope slides. After 30 minutes the slides were rinsed with 0.85% NaCl and immediately fixed with 80% ethanol. Then they were stained with Diff-Quick Solution (Clinag Co., Ltd.). The cells were observed using compound microscope. Two hundred cells were counted from each fish sampled.

Bacteria Challenge

Fish were divided into 4 groups with 4 replications and placed in 10 liter glass tanks.

Group 1	5 fish not challenged with bacteria
Group 2	5 fish challenged with bacteria
Group 3	10 fish not challenged with bacteria
Group 4	10 fish challenged with bacteria

Group 1 and 2 represented low stocking density and group 3 and 4 represented high stocking density pattern. Experimental fish in group 1 and 3 were bath challenged with pathogenic *Aeromonas hydrophila* at the dose 1 x 10^5 cfu/ml for 1 hour, then moved to clean water. Fish were observed daily for 7 days and mortality was recorded. Two fish were sampled for macrophage preparation from each group on day 4 and 7.

Farm Investigation

Hybrid catfish were sampled from 4 different types of commercial fish farms :

Farm 1	
Culture type :	Monoculture
Stocking density :	60 fishes per square metre
Feed :	Commercial pellets occasionally, chicken carcass daily
Water exchange :	No water change during entire culture period
Farm 2	
Culture type :	Monoculture
Stocking density :	45 fishes per square metre
Feed :	Commercial pellets occasionally, waste food from canteen daily
Water exchange :	No water change during entire culture period
Farm 3	
Culture type :	Monoculture
Stocking density :	50 fishes per square metre
Feed :	Commercial pellets for larval fish, chicken carcass and commercial pellets daily
Water exchange :	No water change during entire culture period
Farm 4	
Culture type :	Integrated farm-chicken and fish
Stocking density :	50 fishes per square metre
Feed :	Commercial pellets, chicken manure and chicken feed
Water exchange :	No water change during entire culture period

Six fish from each farm were sampled for macrophage examination once a month for 4 months before harvest.

Water quality analysis

Pond water samples were taken from these 4 farms once a month for 4 months at the time of fish sampling. Water temperature and pH level were measured using a thermometer and pH meter. Turbidity, nitrite and ammonia were determined using a spectrophotometer. Total alkalinity was analyzed by titration. Total suspended solid was analyzed by passing water sampled through a fine filter and weighing the dried material (APHA, 1989)

Statistical analysis

Statistical analysis was performed using analysis of variance and Duncan 's multiple-range test (where $P < 0.05$ was considered statistically significant).

Results and Discussion

1. Bacteria challenge

Table 1 show the percentage of macrophages containing bacteria on days 4 and 7 of the tank experiment.

No fish mortality was observed during experimental period. The initial average percentage of macrophages containing intracellular bacteria (T_0) of fish from

farms with inadequate management practices was 0.5683. The average percentage of bacterial macrophages in the group 1 which is the control group on day 4 was the lowest recorded at 0.2500%, but this did not differ significantly ($P < 0.05$) from the initial group (T_0). The average percentages of macrophages containing intracellular bacteria were very high in the other three groups, and differed significantly ($P < 0.05$) compared to initial and control groups. Hepher (1981) reported that polluted water could stress fish, increase their susceptibility to diseases and reduce their growth rate. Potential stressors can result from high organic content in the water and high stocking density, which may affect growth and health as in the present study. By day 7 the percentage of bacterial macrophages in every group was similar to the initial group (T_0), and did not differ significantly. In bacteria challenged fish, the numbers of macrophages containing intracellular bacteria cell was very high on day 4 but had reduced by day 7. Phagocytosis may reduce the number of bacteria rapidly after exposure. Tizard (1977) mentioned that macrophages can be stimulated to become activated macrophages as a result of exposure to bacterial products. These activated macrophages have an enhanced ability to kill bacteria through the secretion of oxygen metabolites. Daly et al. (1994) studied the bactericidal activity of brook trout *(Salvelinus fontinalis)* peritoneal macrophages against avirulent strains of *Aeromonas salmonicida,* and found that killing was dependent on the concentration of bacteria used to infect macrophages. If the bacteria concentration was too low, no killing was observed and if the bacteria concentration was high, maximum killing was detected early in the experiment.

Table 1 The average percentage of macrophages containing intracellular bacteria from the different experimental groups of the tank trial.

Day	Group 1 Low density No bact.	Group 2 Low density With bact.	Group 3 High density No bact.	Group 4 High density With bact.
4	0.2500 ± 0.2297^{a}	1.4167 ± 0.4802^{b}	2.2500 ± 0.4048^{bc}	1.8611 ± 0.4524^{c}
7	0.5000 ± 0.2981^{a}	0.3611 ± 0.4002^{a}	0.6111 ± 0.4792^{a}	0.3889 ± 0.2919^{a}

2. Fish health from commercial farm investigation.

Fish from four hybrid catfish farms with different farm management practices were sampled for macrophages containing intracellular bacteria (table 2). Farm number 2 had harvested fish after the second of sampling time. The percentage of macrophages containing bacteria was generally low, with levels typically between 0 and 3%. Water quality parameters of these 4 farms are shown in Table 3.

Table 2 The average percentage of macrophages containing intracellular bacteria of hybrid catfish sampled from 4 farms.

Sampling Time (month)	Farm 1	Farm 2	Farm 3	Farm 4
1	2.8611 ± 0.4915	2.7220 ± 0.4569	0.2667 ± 0.2978	0.3611 ± 0.3541
2	2.000 ± 0.4831	1.0555 ± 0.4837	0.2084 ± 0.3108	0.3056 ± 0.2546
3	1.6528 ± 0.5098	-	1.0833 ± 0.4949	0.2639 ± 0.2406
4	1.4236 ± 0.4707	-	0.2316 ± 0.2969	2.4167 ± 0.5050
average	1.9840 ± 0.7314[a]	1.8889 ± 0.9677[a]	0.4486 ± 0.5088[a]	0.8368 ± 0.9842[a]

Table 3 The average water quality of 4 farms.

Water parameter	Farm 1	Farm 2	Farm 3	Farm 4
Temperature (°C)	30.5	28.5	30.5	30
Alkalinity (mg/l)	310.5	282.5	552.0	470.3
pH	7	7.25	7.16	7.13
TSS (mg/l)	180.25	154	77	78.25
NO_2 (mg/l)	0.116	0.004	0.111	0.154
Ammonia (mg/l)	2.395	4.421	1.065	3.350
Turbidity	553.25	52.69	174.33	27.18

Alkalinity, TSS and turbidity differed greatly among the 4 farms. However alkalinity and TSS levels of farm 1 were similar to farm 2 and farm 3 were similar to farm 4. Likewise, the average percentage of macrophages containing intracellular bacteria cells in fish from farm 1 were similar to farm 2 and farm 3 levels were closer to farm 4. Both TSS and alkalinity appear to have an association with the number of macrophages with bacteria.

Normally, ponds with total alkalinity of 200-300 mg/l. are successful for fish culture. (Wetzel, 1975). Weeks and Warinner (1986) reported that the two functional aspects of macrophage activity (phagocytosis and chemotaxis) are compromised in fish exposed in the wild to contaminated sediment. This may be the reason why the values of macrophages containing intracellular bacteria in fish from farm 1 and 2 were higher than those from farms 3 and 4.

Conclusion

Particular water quality parameters and fish population density can be associated with the number of macrophages containing with intracellular bacteria.

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