Original Article

Reduced stress and increased immune responses in Nile tilapia kept under self-feeding conditions

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ABSTRACT: Stress and immune responses were studied in Nile tilapia Oreochromis niloticus under both conditions of self-feeding and scheduled feeding. Self-feeding fish had a significantly lower cortisol level in their blood plasma than that of scheduled feeding fish. Furthermore, skin color was found to be paler in the self-feeding fish compared with that of the scheduled feeding fish. Thus, the self-feeding fish had a less stressful physiological status than the other experimental fish. Immunological analyses revealed that the self-feeding fish significantly exceeded the scheduled feeding fish in the phagocytic activity of their macrophages, antibody production, and the number of blood-circulating lymphocytes. The higher immune responses of self-feeding fish seem to be attained through a less stressful environment. This self-feeding behavior could be exploited to create reduced stress for disease control in fish farming.

KEY WORDS: antibody production, cortisol, immunity, phagocytosis, self-feeding, stress.

INTRODUCTION

A large number of studies have been conducted on the qualities and quantities of diets, whereas there has been only a limited number of studies on feeding skill, despite its significance and importance.¹² In most commercial fish farms at present, scheduled hand-feeding or intermittent feeding with an automatic feeder, based on a feeding chart or farmer’s experience, has been employed to supply the diet. Alternatively, some studies have recently introduced self-feeding to fish farming.³⁵ It is a new and novel approach to feeding from the perspective that the feeding of fish is dependent on their own appetite.⁶⁷

Among the past studies done on the self-feeding of fish, Sánchez-Vázquez et al.⁷ and Kohbara et al.⁸ have reported that fish show daily and individual variations in their feed consumption and timing of feeding. Sánchez-Vázquez et al. further revealed the selection of nutrients by fish themselves.⁹ Adron and Mackie have used the self-feeding device to study feeding stimulants.¹⁰ In terms of fish farming, self-feeding leads to better feed conversion and growth rates, and reduces the amount of uneaten feed pellets.³¹¹ Disease control has become one of the major challenges for fish farming today. Stress and immunity are tightly linked with disease control; however, there have been no reports on the effect of feeding skill on stress and immunity. The present study was designed to compare the stress and immune responses in fish fed by self-feeding and hand-feeding at a scheduled timing.

MATERIALS AND METHODS

Fish and holding conditions

Nile tilapia Oreochromis niloticus (36–60 g) was used in the study. Fish were kept in a 10-L tank with a water flow-through system under the following conditions: water temperature 24.0–26.0°C, pH 6.5–7.3, and dissolved oxygen 6.5–7.5 p.p.m. All experiments were performed using both self-feeding fish and scheduled feeding fish. The self-feeding procedure used was identical to that...
described by Kohbara et al. In brief, an electronic switch-feed box apparatus (Adocom Electronic, Shiga, Japan) supplied the fish their diets when the fish pulled or pushed the switch, the tip of which was placed 2 cm below the water surface. The feed container was set above the tank. During the testing period, the daily feed consumption was recorded, which was determined by calculating the difference between the initial feed amount and the remaining feed found in the container. During scheduled feeding, fish were hand-fed once a day at 11:00 hours, whereby an identical amount of feed to that fed daily to the self-feeding fish was supplied. A commercial pellet (Nosan, Tokyo, Japan) was used during all experimental diets. All the experiments were carried out for 12 days.

### Experimental design

The study consisted of three experiments. The first experiment was performed to analyse the cortisol levels in the blood plasma of self-feeding and scheduled feeding fish as an indicator of stress. In the second experiment, functions of phagocytic cells were investigated in the self-feeding and scheduled feeding fish. The third experiment was conducted to examine for vaccination and hematology to confirm antibody production in the self-feeding and scheduled feeding fish.

### Skin color observations

As another parameter of stress, the skin color of all tested fish were checked once a day at random times throughout the whole experimental period.

### Experiment 1: Cortisol level

At 11:00 hours of the final test day, the self-feeding and scheduled fish were anesthetized using 0.02% FA-100 (Tanabe, Tokyo, Japan). The scheduled feeding fish were anesthetized without a final feeding. After anesthetization, blood samples were obtained from the caudal vessels using heparinized syringes and then centrifuged at 3000 ×g for 10 min. The plasma was stored at −25°C until required for analysis. Plasma cortisol levels were analysed using a commercial kit (Enzaplate cortisol, Ciba Corning Diagnostics, Tokyo, Japan) according to the kit’s manual. This experiment was repeated four times. The self-feeding fish consumed 3.92 g (0.43–0.22 g/day) of feed in the first test, 6.30 g (0.79–0.31 g/day) in the second test, 10.90 g (1.66–0.00 g/day) in the third test, and 3.15 g (0.55–0.00 g/day) in the fourth test throughout the experimental period.

### Experiment 2: Functions of phagocytic cells

In order to obtain phagocytic cells from the experimental fish, 0.2 mL of neutrophil lysate (1 ¥ 10⁸ cells/mL) was injected into the swim bladders on the eighth day of the experiment, as described by Matsuyama et al. On the last day of the experimental period, phagocytic cells were collected from the swim bladder lumens of self-feeding and scheduled feeding fish. After collection, the phagocytic cells were suspended in Hank’s balanced salt solution, after being adjusted to 1 ¥ 10⁶ cells/mL. Chemiluminescence was measured in the phagocytic cells using the method of Iida and Wakabayashi, and phagocytic indices were examined separately in the neutrophils and macrophages, as reported by Matsuyama and Iida. This experiment was repeated four times. Throughout the entire period, self-feeding fish consumed 3.15 g (0.55–0.00 g/day) of feed in the first test, 8.84 g (1.05–0.00 g/day) in the second test, 12.13 g (1.34–0.43 g/day) in the third test, and 3.92 g (0.43–0.22 g/day) in the fourth test.

### Experiment 3: Antibody responses and hematological survey

On the fifth day of the experiment, 0.2 mL of formalin-killed bacterial suspensions (Escherichia coli; 0.5 mg/mL) were intraperitoneally injected into the self-feeding and scheduled feeding fish. On the last day of the experiment, blood samples were taken from both groups of fish via the caudal vessels using heparinized syringes, and the blood samples were partially subjected to hematological analyses, during which the red blood cells, neutrophils, macrophages, thrombocytes, and lymphocytes were counted using a hemocytometer and smear preparation with May Grünwald–Giemsa stain, respectively. The remaining blood samples were used to measure antibody agglutinating titers. After the blood sera were collected, their titers were measured using the method of Roberson. This experiment was repeated five times. The self-feeding fish consumed 6.03 g (0.95–0.42 g/day) of feed during the first test, 11.25 g (2.22–0.63 g/day) during the second test, 4.85 g (0.95–0.42 g/day) during the third test, 10.90 g (2.43–0.12 g/day) during the fourth test,
and 5.04 g (0.94–0.54 g/day) during the fifth test throughout the entire experimental period.

Statistics

Student’s *t*-test was used to determine the difference between treatments at the significance level of *P*<0.05.

RESULTS

Skin color and cortisol level

Under self-feeding conditions, it was noticed that the fish generally showed a pale skin color (Fig. 1), whereas the scheduled feeding fish had a dark skin color that was irritated by swimming (Fig. 2). Self-feeding fish had a significantly lower cortisol level than the scheduled feeding fish (Fig. 3).

Functions of phagocytic cells

Phagocytic indices of neutrophils and macrophages were higher in self-feeding fish than in scheduled feeding fish. Self-feeding fish also had higher values for inflammatory cells as determined from chemiluminescence assays when compared with scheduled feeding fish. However, a significant difference between the two groups was seen only for the phagocytic index of macrophages (Fig. 4).

Antibody responses and hematological survey

In all tests, high antibody agglutinating titers were found in the self-feeding fish, whereas the scheduled feeding fish showed lower titers (Fig. 5). The numbers of lymphocytes was larger in the self-feeding fish than in the scheduled-feeding fish (Fig. 6), but there was no significant difference in the numbers of red blood cells, neutrophils, macrophages, and thrombocytes between the self-feeding fish and scheduled feeding fish.

DISCUSSION

Fish show various physiological adaptations to stress. The hypothalamo–pituitary–interrenal axis
adaptation to the stress of hunger. Conversely, judging from their low cortisol levels and their pale skin color, self-feeding fish experienced low levels of stress. Under self-feeding conditions, fish can choose their feed timings and quantities, depending on their individual appetites. Self-feeding works in harmony with a fish's natural appetite rhythm and appears to promote low stress levels.

In fish, stress influences immunity. Various signal molecules are delivered from the hypothalamo–pituitary–interrenal axis under stressful conditions, affecting leukocyte function and numbers via leukocyte receptors. In terms of the impact of stress on phagocytes, two contradictory data sets are currently being presented. One data set has reported a decline or unaffected function, whereas the other has revealed increased function. According to Weyts et al., differences in the duration and severity of the stressor, assay system, and cell population are responsible for the contradictory data. In the present study, however, all parameters analysed in the phagocytic cells tended to show higher values in the self-feeding fish compared with the scheduled feeding fish, although a significant difference was seen only in the phagocytic index of macrophages. In terms of antibody production, a high level of cortisol reduces the antibody agglutinating titer and the number of blood-circulating lymphocytes. In the present study, the agglutinating titer and lymphocyte numbers measured in the self-feeding fish markedly exceeded those measured in the scheduled feeding fish. The self-feeding fish seemed to show a higher antibody production as a result of their low stress levels. Thus, it appears that fish can obtain a higher immune response under self-feeding conditions; however, further studies are required to understand the relationship between the immune response and neuro-hormonal dynamics of self-feeding fish.

Recent fish farming practices have been aimed at sustainable farming because of its economical significance. Disease control is a major consideration. In order to prevent diseases, farmers have recently introduced immunological control methods using immunostimulants and/or vaccines. The self-feeding behavior of fish might facilitate the immunological control of fish diseases through the improvement of fish immune responses because of their reduced stressful physiological status. The present study has demonstrated that the stress status of self-feeding tilapia is reduced and, therefore, we believe such systems have a practical application to commercial fish farming. However, the transfer of experimental investigations to commercial-size farming has yet to be examined.
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REFERENCES