Vol. 11: 45–48, 1991 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published June 20

Anorexia in goldfish *Carassius auratus* infected with *Trypanosoma danilewskyi*

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ABSTRACT: *Trypanosoma danilewskyi* Laveran & Mesnil, 1904 caused anorexia in experimentally infected goldfish *Carassius auratus*. This was most evident when the parasitemias were high. Some anorexic fish died of high parasitemias but those that survived the crisis returned to normal feeding.

INTRODUCTION

Trypanosoma danilewskyi Laveran & Mesnil, 1904 is a pathogenic haemoflagellate which is not host specific (Woo & Black 1984). The parasite has been isolated from common carp *Caprinus carpio*, crucian carp *Carassius auratus gibelio*, tench *Tinca tinca* and eel *Anguilla* sp. (Lom 1979). Its morphology and development in fish have been relatively well studied (Lom 1979, Lom et al. 1980, Paterson & Woo 1984, Paulin et al. 1980, Skarlato et al. 1987, Woo 1981a).

Anorexia, which is decrease or cessation of food consumption (Morris et al. 1982, Symons 1985), has been associated with a number of gastrointestinal parasite infections (Morris et al. 1982, Crompton 1984, Symons 1985, Holmes 1987). It has also been reported in mammalian trypanosomiasis (Koberle 1968, Holmes 1987) and piscine cryptobiosis (Li & Woo 1991, Thomas & Woo 1991). In contrast, there was increased food intake in laboratory rats inoculated with *Trypanosoma lewisi* homogenate (Lincicome 1971). Little is known about the effects of trypanosome infection on food consumption in fish. The present study was undertaken to carefully examine this relationship.

MATERIALS AND METHODS

The strain of *Trypanosoma danilewskyi* used was initially isolated from crucian carp. It was morphologically characterized, cryopreserved and maintained in goldfish *Carassius auratus* by blood inoculation (Woo 1981a, b). Goldfish (average 7.35 g; 4.70 to 10.65 g) were bought from a local supplier and maintained at 20 ± 0.5 °C in tap water in 50 l glass aquaria (maximum 35 fish per aquarium). Fish were acclimatized to laboratory conditions for at least 3 to 4 wk before being used.

Fish were fed daily at about the same time with Martin's 83G salmonid pellets until satiated. Small pellets (ca 0.0025 g each) were prepared by grinding the pellets and grading them through sieves of 1.70 and 0.95 mm mesh. The fine portion (< 0.9 mm; which floats) was discarded using the second sieve (0.95 mm mesh). A pilot study indicated this pellet size (i.e. between 0.9 and 1.7 mm) was acceptable to the fish. Food was presented to goldfish in an aquarium with a clean bottom. Pellets were first soaked in water for 5 to 10 s so that they sank readily. Fish were allowed 3 min to pick up the pellets. Feeding was stopped when 1 or 2 pellets remained on the bottom for more than 3 min.

The caudal peduncle of an infected fish was severed after being killed by exposure to an overdose of MS 222. Blood was collected in heparinized haematocrit centrifuge tubes or in heparinized 1 ml syringes. The number of parasites in the blood was determined using a Bright Line Haemocytometer (Archer 1965). If the number was too low for the leucocyte-count method, blood was drawn into a microhaematocrit centrifuge tube, sealed at one end with Critoseal and centrifuged at $13\,000 \times g$ for 4 min in a microhaematocrit centrifuge. The parasites at the junction of the plasma and buffy coat were counted under a microscope (Woo 1969).

Experiments were designed to investigate the effects of infection on food consumption. Parasitemias in experimental groups were inferred by bleeding fish from another infected group maintained under similar

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0177-5103/91/0011/0045/$ 03.00

conditions to the 2 experimental groups (infected and uninfected). For analysis, total food consumed per day was converted to food consumed per g body weight of fish per day. Student's *t*-tests were used to compare food consumed by infected and uninfected fish. In the second experiment regression was used to determine the relationship between parasitemias and food consumption.

Experiment I. 30 fish of approximately similar size and weight were divided into 2 groups (15 fish each; Group A: total weight 91.72 g, Group B: total weight 89.5 g). Each group was maintained in a separate aquarium, fed at approximately the same time every day and amount of food consumed recorded. A third group (Group C with 60 fish) was kept in 2 aquaria and fed as above. The amount of food consumed was not recorded in Group C. After 3 wk each fish in Groups A and C was inoculated intraperitoneally with 20 000 parasites; fish in Group B were inoculated with similar volume of saline. Five fish from Group C were killed each week and parasitemias determined. Total weights (Groups A and B) were recorded each week for 9 wk.

Experiment II. The study was repeated with (a slightly different experimental design) 2 groups of fish; one group consisted of 15 and the other 30 fish (Group D). The group of 15 fish was divided into 6 subgroups, 3 of which consisted of 3 fish each and 3 of 2 fish each. The subgroups consisting of 3 fish each were infected (A/ex, B/ex, and C/ex) and those with 2 fish were uninfected controls (A/co, B/co, and C/co). A substitute fish was added to the infected group to replace any fish which died. Three 50 l aquaria were partitioned into 2 compartments with a net. One compartment contained control fish and the other infected fish. Fish were allowed to adjust to the system and technique of food presentation for 1 wk. Then fish from A/ex, B/ex, and C/ex and Group D were each inoculated intraperitoneally with trypanosomes (30 000 parasites per fish). Control groups (A/co, B/co, and C/co) were inoculated with Ringer's solution. Fish in all groups were fed until they stopped taking food. The experiment lasted 38 d and amount of food consumed and body weight of the fish were recorded at 7, 12, 17, 22, 27, 32, and 38 d after infection. Three fish from Group D were bled each day to determine parasitemias.

RESULTS

Experiment I

Food consumption of the 2 groups (A and B) was not significantly different for 3 wk before fish were inoculated. After inoculation the infected group (A) consumed less food (mg g^{-1} body wt d^{-1}) than the uninfected group (B) (Fig. 1). Decreased food consumption was related to the parasitemia which increased until the 6th wk (significant at $p < 0.05$ when compared with 1st wk parasitemia) and decreased through the last 3 wk post-infection (parasitemia not significantly different from 1st wk) (Fig. 1).

Food consumption of infected fish during the first 6 wk post-infection was significantly lower ($p < 0.01$) than that in the uninfected fish. The anorexia coincided with parasitemias during that period. There was no significant difference in food consumption during the last 3 wk of infection (7th, 8th, and 9th wk post-infection) between these 2 groups.

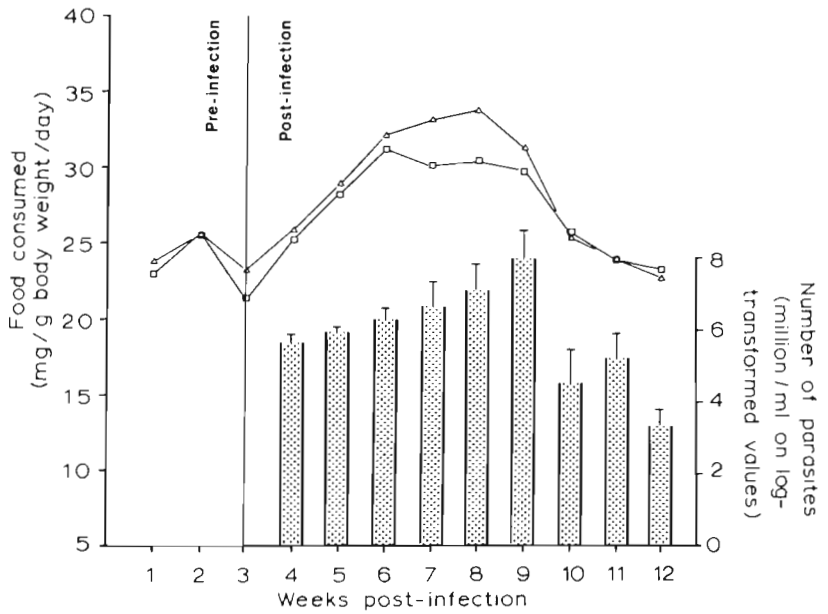

Fig. 1 *Carassius auratus*. Food consumption in uninfected and *Trypanosoma danilewskyi* infected goldfish. Parasitemias (mean with standard error of the mean) in another group of infected fish are shown by bars (Expt I). (△) Food consumed by uninfected fish; (□) food consumed by infected fish

Experiment II

As a group, infected fish consumed less food than uninfected fish (Fig. 2, Table 1). At the 17th and 22nd days post-infection, infected fish consumed significantly less food than uninfected fish (Fig. 2, Table 1). The decline in food consumption in infected fish coincided with high parasitemias. Parasitemias were significantly higher at the 12th, 17th, and 22nd days post-infection when compared with that on the 7th day (Fig. 2).

Weight gain in the infected fish (percent weight gain per fish) was less than uninfected fish, but was not statistically significant compared to that in uninfected fish. Cessation or reduction in food intake was observed in some fish during high parasitemias (12 to 22 d post-infection). These fish usually isolated themselves from the group and some of them died (with massive parasitemias) 12 h to 7 d later. Fish that survived the infection resumed normal feeding.

Table 1. *Carassius auratus*. Food consumed by uninfected goldfish and goldfish infected with *Trypanosoma danilewskyi* (Expt II)

Days post-infection	Average food consumed[a] (mg g^{-1} body wt d^{-1}) Uninfected	Infected	*t*-value
0	1.5183	1.5639	1.179^{ns}
7	1.5352	1.5612	1.357^{ns}
12	1.5841	1.4341	1.608^{ns}
17	1.5837	1.3950	2.843^{*}
22	1.6059	1.3467	2.887^{*}
27	1.5957	1.4947	0.829^{ns}
32	1.5282	1.5710	0.549^{ns}
38	1.5710	1.5759	0.316^{ns}
0–38	1.5652	1.4725	2.752^{**}

[a] Data are log transformed.
[ns] Not significant; * significant at 5 % level; ** significant at 1 % level

DISCUSSION

In both experiments, food consumption by infected goldfish was greatly reduced when parasitemias were high. Thomas & Woo (1991) reported anorexia in rainbow trout with cryptobiosis and showed that it contributed to immunodepression. Food intake may also be reduced in mammalian trypanosomiasis (Holmes 1987). Several causes of anorexia have been suggested (Crompton 1984, Symons 1985, Holmes 1987) e.g. low motility of food through the intestine, disruption of gut mucosa (usually associated with pathogenic intestinal parasites) thereby impairing digestion and absorption, increase in cholecystokinin (an alimentary hormone that may reduce appetite). It was shown that changes in concentration of cholecystokinin in the brain and plasma elicit satiety (Baile et al. 1986). Changes in plasma level of gastrin in a parasitized animal may also result in altered food intake (Titchen 1982). Like cholecystokinin the gastrin may affect feeding by

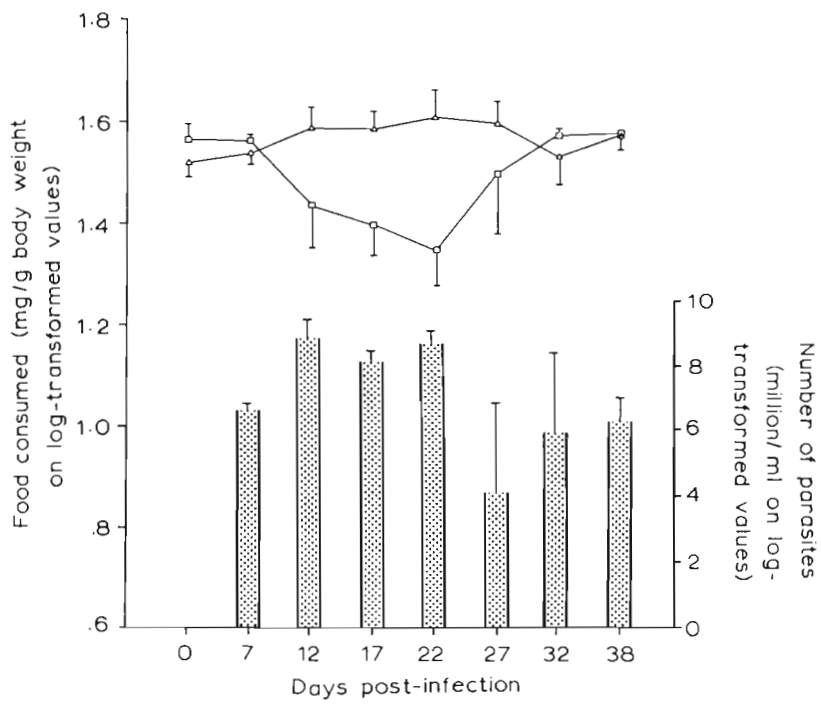

Fig. 2. *Carassius auratus*. Food consumption (mean with SEM) in uninfected and *Trypanosoma danilewskyi* infected goldfish. Parasitemias (mean with SEM) in another group of infected goldfish are shown by bars (Expt II). (△) Food consumed by uninfected fish; (□) food consumed by infected fish

peripheral action or action via the central nervous system (Baile et al. 1986). McCarthy et al. (1985) reported depression in food intake caused by interleukin-1, a protein synthesized and released by phagocytes in response to infection in an immune reaction (Dinarello 1984, Marrack & Kappler 1986). They proposed that this may be due to increased renal excretion and changes in gastric motility caused by interleukin-1. We suggest that interleukin-1 may be an important contributing factor in anorexia-related diseases caused by pathogenic haemoflagellates (e.g. *Trypanosoma, Cryptobia*). *Cryptobia salmositica* of salmonids is engulfed by macrophages during infections (Woo 1979) and it was suggested that phagocytosis plays a vital role in controlling cryptobiosis (Woo 1979, Jones & Woo 1987, Thomas & Woo 1990).

Anorexia retards growth (Crompton 1984, Symons 1985) and was shown to be a contributing factor in retarding growth in trout infected with *Cryptobia salmositica* (Li & Woo 1991). In the present study, however there was no significant decrease in weight in infected fish. Anorexic fish either died from the infection or survived the crisis and returned to normal feeding.

Acknowledgement. This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada to P.T.K.W.

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Responsible Subject Editor: W. Körting, Hannover, Germany

Manuscript first received: March 12, 1990
Revised version accepted: April 10, 1991
(Several months delay due to loss of manuscript in the mail)